

# Equine umbilical cord mesenchymal stem cells demonstrate safety and efficacy in the treatment of canine osteoarthritis: a randomized placebo-controlled trial

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## OBJECTIVE

To demonstrate the efficacy and safety of mesenchymal stem cells (MSCs) for xenogeneic use with intra-articular administration in dogs with osteoarthritis.

## ANIMALS

80 client-owned dogs with naturally occurring osteoarthritis in elbow or hip.

## PROCEDURES

A multicentric, double-blinded, parallel, randomized and placebo-controlled clinical trial was performed. After intra-articular injection of equine umbilical cord MSCs, dogs were reexamined at weeks 4, 8, and 12 using a force platform (gait analysis), orthopedic assessment, and validated owner questionnaire. Eighteen months after treatment, a long-term follow-up was done.

## RESULTS

Best results were obtained 8 weeks after treatment, where 63% of the patients showed an improvement in the gait analysis. Also 8 weeks after treatment, 77% of the dogs improved in the orthopedic examination; 65% of the owners considered that the treatment improved their pet's quality of life 8 weeks after treatment. The long-term follow-up revealed that 59% of the owners observed a duration of effect longer than 6 months after a single intra-articular injection of equine umbilical cord MSCs. No systemic or permanent adverse events were detected at any time point.

## CLINICAL RELEVANCE

Results of this study demonstrated the safety and efficacy of intra-articular administration of xenogeneic MSCs for the treatment of canine osteoarthritis.

Osteoarthritis (OA) affects more than 20% of the canine population and is the most common cause of lameness in dogs older than 1 year.<sup>1</sup> This prevalence is significantly increased in senior patients, increasing to almost 80%.<sup>2</sup>

The pathogenesis of canine OA involves changes in all tissues of the synovial joint: cartilage loss, abnormal subchondral bone remodeling, and osteophyte formation.<sup>3</sup>

The most common clinical signs of OA in dogs include pain and lameness.<sup>3</sup> The conventional therapeutic goal for this degenerative disease is the management of that pain with NSAIDs.<sup>4</sup> However, NSAIDs can have deleterious systemic side effects<sup>5</sup> and contraindications, which lead to very complex treatment for

senior animals suffering multiple pathologies like renal disease or liver failure.<sup>6</sup>

In recent years, mesenchymal stem cells (MSCs) have been postulated as a potential treatment for musculoskeletal disorders and systemic diseases in dogs.<sup>7,8</sup> Several publications also support that stem cell therapies are effective for diminishing the symptoms and pain, thereby increasing the quality of life of dogs suffering OA when administered intra-articularly.<sup>9-11</sup> The hypothesis that MSCs are able to promote tissue repair through their immunomodulatory capacity is gaining traction.<sup>12</sup> When transplanted into diseased tissues, MSCs communicate with local cells through the secretion of a wide array of cytokines and growth factors.<sup>13</sup>

MSCs can be considered in 3 categories, depending on the donor source and recipient. These categories include autologous (the patient receives its own MSCs), allogeneic (the patient receives MSCs from a donor of the same species), and xenogeneic (the patient receives MSCs from a donor of a different species).<sup>13</sup>

The autologous use of MSCs has been demonstrated to be safe and efficacious.<sup>14</sup> However, it has the handicap of exposing the patient to 2 different procedures (1 to take the tissue source of MSCs and 1 to administer the MSCs) and also the waiting time in between for the culture and expansion of MSCs. Therefore, the process is more efficient for the recipient if the MSCs are taken from a donor, an important advantage of allogeneic and xenogeneic MSCs.

Several studies<sup>15,16</sup> have demonstrated the possibility of allogeneic and xenogeneic MSCs use. MSCs are classically described as “immune-privileged” because of the absence of major histocompatibility complex class II on their surface. Major histocompatibility complex class II is responsible for the initiation of the antigen-specific immune response.<sup>17-19</sup> This immune-privileged status permits the safe and effective use of allogeneic and xenogeneic treatments.

MSCs can also be differentiated depending on the source. The most common sources for obtaining MSCs are bone marrow,<sup>20</sup> adipose tissue,<sup>13,21</sup> peripheral blood,<sup>11</sup> and umbilical cord (UC).<sup>22,23</sup>

Umbilical cord stem cells are considered the best source of MSC for several reasons: noninvasive source (100% in line with animal welfare), higher proliferation capacity,<sup>24,25</sup> greater immunomodulation capacity,<sup>26</sup> better immune-privileged status,<sup>27</sup> and a more secure profile with fewer risks derived from possible cells mutations, viral agents, parasites agents, or other contaminants.<sup>28</sup>

However, the canine UC is not an ideal source of MSCs. It is almost impossible to obtain the canine UC immediately after natural birth, as the bitch’s instinct prompts her to ingest the placenta and UC; therefore, the only way to obtain the tissue would be after a C-section surgery. Conducting a surgical procedure or C-section solely to obtain UC for pharmaceutical development purposes raises ethical concerns and is not aligned with animal welfare.<sup>29</sup> For these reasons, the allogeneic use of canine MSCs from UC is not a suitable option.

Equine UC (EUC), on the other hand, is a good option because it is a tissue that is discarded after birth, as the mare does not instinctively ingest it. In addition, its size provides a substantial amount of

tissue available for culture while maintaining all the aforementioned advantages of UC as a source of MSCs. Additionally, the immune-privileged status of MSCs may allow the possibility of a xenogeneic use of EUC-MSC in different species, with the advantage of a quick, nonharmful, safe, and effective treatment for canine OA compared with current autologous or allogeneic use of canine-derived MSC.

The xenogeneic use of MSCs is gaining traction because it allows a choice of tissue and donor species without being limited by the recipient species. More and more authors are reporting the safety and efficacy of xenogeneic MSCs<sup>11,13</sup> when administered locally<sup>13,30</sup> and systemically.<sup>15,31</sup>

A force plate was utilized to quantitatively measure lameness and pain.<sup>32</sup> Force plate was selected as primary end point because it allows the evaluation of the treatment’s efficiency in an objective way.<sup>14,33,34</sup> This permits us to measure something as complex as pain as objectively as possible.<sup>3,35-37</sup>

The aim of this study was to assess the efficacy and safety of EUC-MSCs for the treatment of canine OA in a controlled study with regulatory purposes.

## Materials and Methods

### Design

The present study is a multicentric, double-blind (owner and researcher), parallel-group, randomized, and placebo controlled trial. Patients were enrolled in 2 veterinary hospitals in 2 areas of Spain with different climatological and geographical characteristics (Madrid and Barcelona). The same number of dogs was included for both groups in the 2 hospitals. It was carried out following the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products for Good Clinical Practice (VICH guidelines). Approval for this clinical trial (428/ECV) was obtained from the regulatory authority in Spain (Agencia Española de Medicamentos y Productos Sanitarios) and satisfied national regulatory and animal welfare standards and requirements. Informed consent was obtained from dog owners prior to the inclusion.

On administration day (day 0), a screening was done to verify that the animals complied with the inclusion criteria (see Animal selection).

According to protocol (**Table 1**), following treatment administration on day 0, patients were reexam-

**Table 1**—Study outline.

Activity	Day 0 (Screening)	Day 1	Week 4 visit	Week 8 visit	Week 12 visit	Long-term follow-up (18 mo)
Hemogram and biochemistry	x				x	
Clinical assessment	x	x	x	x	x	
Radiographs	x					
Product administration	x					
Orthopedic assessment	x	x	x	x	x	
Gait analysis	x		x	x	x	
QoL owner questionnaire				x	x	
Long-term owner questionnaire						x
Clinical trial completion and blind open					x	

ined on day 1, week 4, week 8, and week 12. Examining veterinarian assessment was performed on every visit. Furthermore, a long-term follow-up was carried out 18 months after the product administration date.

Each visit in every patient included clinical assessment, orthopedic assessment, and gait analysis. Any abnormal health observations irrespective of their nature and severity made by either owner or veterinarian were recorded.

Since it is known that the onset of the efficacy takes time,<sup>10,38</sup> the primary efficacy end point was fixed at 8 weeks after treatment.

According to protocol, at the end of the study, the animals in the placebo group (n = 40) received treatment with EUC-MSCs (unblinded) and followed up in the long-term follow-up study.

## Animal selection

A total of 80 owned dogs (40 receiving EUC-MSC and 40 receiving placebo) were enrolled from March 2019 to December 2020 in 2 Spanish veterinary hospitals, receiving 1 intra-articular administration of EUC-MSCs or placebo in the selected joint.

Before inclusion on day 0, a full investigation was made consisting of an orthopedic assessment, a gait analysis, and radiographs of the affected joint to confirm the diagnosis and evaluate the OA grade in each patient. The radiographic score for OA was attributed to the target joint as described by Moreau et al<sup>4</sup> by a European and RCVS specialist in diagnostic imaging (RS). Blood samples were analyzed to be sure of the good health status of the patients.

The inclusion criteria were that the animals must be healthy apart from the OA, must be more than 15 kg weight, and must be older than 1 year with normal hematology and biochemistry. Dogs also had orthopedic discomfort, uni- or bilateral in the elbow or hip joints without improvement for at least 3 months. Radiographic signs of mild to severe OA (according to Moreau et al<sup>4</sup>) in the coxofemoral or elbow joints were mandatory.

If more than 1 limb was affected, the treatment was administered in the limb with the most severe OA grading. OA grading was determined considering the gait analysis severity, orthopedic assessment, and radiographic signs.

One month prior to enrolment and during the study, no treatments that could interfere were allowed (NSAIDs, corticosteroids, vaccinations, analgesics, intra-articular treatments, etc).

Dogs had water ad libitum and were fed dry food for daily adult requirements according to body weight (g/kg).

## Treatments

Dogs that met the inclusion criteria were allocated to the corresponding group at the time of enrolment by use of a randomized block schedule that was produced in advance of the study. The treatment and placebo proportion was 1:1.

Dogs in the treatment group received  $7.5 \times 10^6$  EUC-MSC (DogStem; EquiCord) in 1 mL of vehicle intra-articular. EUC-MSCs and placebo were manufactured according to good manufacturing practices.

## EUC-MSCs manufacturing

EUCs from 4 different donors were collected from concerted studs after the natural birth of the foal, and they were processed. EUC-MSCs were isolated and expanded in culture and cryopreserved. At this first stage, cells were tested for genetic stability, equine viruses and protozoa, mycoplasma, morphology, accumulative population doublings, and characterization by flow cytometry.

After this process, the vials were thawed and expanded until passage 4. After the expansion of the cells, the final product consisting of  $7.5 \times 10^6$  EUC-MSCs/vial was tested for sterility, cell concentration, viability, morphology, endotoxins, accumulative population doublings, mycoplasma, and potency and characterized by flow cytometry.

## Clinical assessment

To evaluate the systemic safety of the treatment, a physical examination of the affected dog was performed on each visit (day 0, day 1, and weeks 4 and 8). The physical examination included temperature, heart rate, respiratory rate, overall status, color of mucous membranes, lymph nodes, and abdominal palpation.

Apart from the prescheduled visits, owners were aware that in case of an adverse event (AE) or complication during the study, they should contact the researchers. Hospitals had 24/7 emergency service available for the owners if required.

Also, blood samples were taken prior to treatment and at the end of the study to detect any possible abnormalities in the hemogram and biochemistry.

## Gait analysis in plate force

Gait analysis (WE4; Walkway Evolution; Medical Sensor 3150QL; Tekscan) was performed on day 0 (before product administration) and on weeks 4, 8, and 12 to determine values of peak vertical force normalized to body weight from the target limb (PVF[%BW]). The same brand and model of force platform was available with the same calibration in both hospitals at every time point.

Before platform was used in every test, the calibration files were uploaded. Before any data were recorded, the dog was leashed and walked by a trained person at least 3 times, from one end toward the other end of the walkway, to get the dog familiarized with the platform.

On day 0, dogs were allowed to move at the velocity that was most comfortable for them. The accepted velocity range was between 0.7 and 1.8 m/s. For the following visits, dogs had to keep the same velocity as on day 0  $\pm$  0.3 m/s.

To consider a trial valid, dogs had to walk (never trot) in a straight line, without hesitation or noticeable distraction, with no overt head movement or visible acceleration or deceleration, and with no resistance or pull against the handler. A total of 10 valid passes were obtained during each visit.

According to Conzemius et al,<sup>39</sup> the therapeutic success for the primary endpoint has been defined as the percentage of dogs that have an increase for

PVF(%BW)  $\geq$  5% compared with day 0 in both treatment and placebo groups.

### Orthopedic assessment

Orthopedic examination in each dog was performed by the same veterinary surgeon on day 0 before the administration of the treatment (considered baseline values), 24 hours after treatment, and after 4, 8, and 12 weeks.

The scale used was based on the work from McCarthy et al.<sup>40</sup>

For animal welfare, no animals were included with 5 points in any of the parameters.

For the evaluation of the efficacy, only the parameters “pain on palpation” and “lameness” were assessed for being the most clinically significant.

The therapeutic success of this parameter was defined as a decrease of at least 1 point on the total score (pain on palpation + lameness) compared with day 0.

### Quality-of-life questionnaire

The owner’s opinion was registered using a questionnaire validated for reliability and validity (data not shown).

The questionnaire consisted of only 3 questions that were easy for the owner to understand and that they had to evaluate on an intuitive scale from 1 to 10. The questions are shown (Table 2). Owners filled the survey out only at 8 and 12 weeks after treatment.

The therapeutic success of this parameter was defined as a punctuation of at least 7 points for each question.

### Long term follow-up

Eighteen months after EUC-MSc administration, owners were contacted by the researchers. This follow-up study was a nonblind and noncontrolled study that allowed us to obtain efficacy and safety data from a large dog population.

An online owner’s survey was completed to assess long-term safety and efficacy. Owners indicated whether the treatment was effective, how long the effect lasted (if applicable), and in which aspects of the dog’s life they noticed improvement. They were also asked about the presence of any AEs not detected during the trial.

The survey was sent digitally to the owners of all the dogs included in the treatment group plus the owners of the dogs that originally received placebo

and were thereafter treated with EUC-MSc. A total of 70 surveys were sent.

### Statistical methods

Statistical analyses were carried out based on guidance from the European Medicines Agency.<sup>41</sup>

Data are presented as the percentage of dogs classified as therapeutic successes in both groups (EUC-MSc vs placebo).

All statistical decisions were performed considering 2-sided tests, and values of  $P < .05$  were regarded as significant for all tests.

Differences between groups were tested by means of  $t$  tests or Mann-Whitney tests when the normality assumption was not met. For qualitative variables, differences between groups were tested by means of the  $\chi^2$  test or Fischer exact test when the Cochran rule was not satisfied. Correlation between efficacy end points has been measured by means of Spearman correlation.

The sample size in this study was conducted based on superiority design with a significance level of 5% and a statistical power of 80%.

The hypothesis assumes a percentage of therapeutic success of 50% in the group treated with mesenchymal cells versus 15% in the control group.

Based on the above assumptions, a minimum of 33 evaluable cases in each group must be obtained.

Therefore, and taking into account the duration of the study and the potential number of non-evaluable cases (eg, deviations, withdrawals by concomitant diseases, concomitant therapies unauthorized) it is proposed to include a total of 40 patients/group.

## Results

### Animals

Baseline homogeneity was seen in all the variables investigated (age, weight, sex, breed, treated joint, OA grade, orthopedic score, lameness, and PVF[%BW]) and is summarized (Table 3). Moreover, the majority of dogs enrolled were Retrievers (41%) and German Shepherds (13.8%), but dogs of 18 different breeds and also mixed breeds were enrolled. This was also homogenous in both groups ( $P$  value = .4128).

The effect of potential epidemiological variables was evaluated (age, gender, OA grade, joint treated,

**Table 2**—Validated quality-of-life survey for owners.

Quality-of-life questionnaire										
Rate from 1–10 the response to treatment in the following terms (as of today) where 1 is “no improvement” and 10 is “complete improvement” (circle the value chosen):										
<b>Q1:</b> Improvement in the quality of life of your animal (general condition, appetite, mood, activity).	1	2	3	4	5	6	7	8	9	10
<b>Q2:</b> Improvement in your perception of your animal’s general pain (lameness, difficulty in moving, inactivity, or discomfort).	1	2	3	4	5	6	7	8	9	10
<b>Q3:</b> Improvement in mobility (degree of activity, desire to play, difficulty getting on and off the sofa, stairs, etc).	1	2	3	4	5	6	7	8	9	10

**Table 3**—Patient demographics at day 0. All the parameters showed basal homogeneity between equine umbilical cord mesenchymal stem cells (EUC-MSCs) and placebo groups.

Variable	EUC-MSCs group	Placebo group	P value
Mean age (y)	7.5	7.6	.6101
Mean weight (kg)	33.6	31.7	.1248
Percentage of senior dogs ( $\geq 8$ y)	47.5%	55%	.4317
Males/females (%)	63/37%	50/50%	.2056
Severe OA grade according to Rx (4; %)	60%	45%	.2337
Elbows/hips (%)	47.5/52.5%	52.5/47.5%	.5727
Orthopedic score (mean)	10.95	10.80	.6573
Lameness grade (40)	Grade 1: 15.4% Grade 2: 38.5% Grade 3: 41.0% Grade 4: 5.1%	Grade 1: 12.5% Grade 2: 55.0% Grade 3: 27.5% Grade 4: 5.0%	.5045
PVF(%BW) (mean)	34.188	37.035	.3268

PVF(%BW) = Peak vertical force normalized to body weight from the target limb.

and severity [pain and lameness]). None of the covariates investigated demonstrated a statistical significance in the prognosis of the treatment success (data not shown).

### Gait analysis

Four weeks after treatment, 40.74% of the EUC-MSC treated dogs and 20.6% in the placebo treated animals showed a  $\geq 5\%$  improvement in the PVF(%BW). No statistically significant improvement ( $P = .1605$ ) was identified.

Eight weeks after treatment (primary end point), 62.86% of the EUC-MSC treated dogs showed an improvement in the plate-force  $\geq 5\%$  compared with 8.00% in the placebo group, a statically significant ( $P < .0001$ ) difference.

Twelve weeks after treatment, 48.27% of the EUC-MSC treated dogs showed an improvement in the plate-force  $\geq 5\%$  compared with 10.81% in the placebo group, a statically significant ( $P < .0054$ ) difference (**Table 4**).

### Orthopedic assessment

Four weeks after treatment, 69.7% of the EUC-MSC treated dogs and 40.5% in the placebo-treated animals decreased at least 1 point in the orthope-

dic examination; this difference was statistically significant ( $P = .0145$ ).

At week 8 in the treatment group, 77.4% of the dogs improved by at least 1 point in the orthopedic exploration compared with 45.9% in the placebo group ( $P = .0082$ ).

At week 12 in the treatment group, 79.3% of the dogs improved by at least 1 point in the orthopedic exploration compared with 43.2% in the placebo group ( $P = .0031$ ; **Table 4**).

### Quality-of-life questionnaire

A quality-of-life questionnaire (**Table 2**) for owners was designed and validated for this study.

Eight weeks after the product administration, 64.5% (20/31) of dogs in the treatment group compared with 19.4% (7/36) in the placebo group scored 7 points or more, demonstrating improved quality of life ( $P = .0002$ ); 51.6% (16/31) of dogs in the treatment group compared with 16.7% (6/36) in the placebo group expressed a clinically relevant improvement in pain assessment ( $P = .0024$ ); and 64.5% (20/31) compared to 16.7% (6/36) in the placebo group improved 7 points or more in mobility ( $P < .0001$ ). Similar results were obtained 12 weeks after treatment (**Figure 1**).

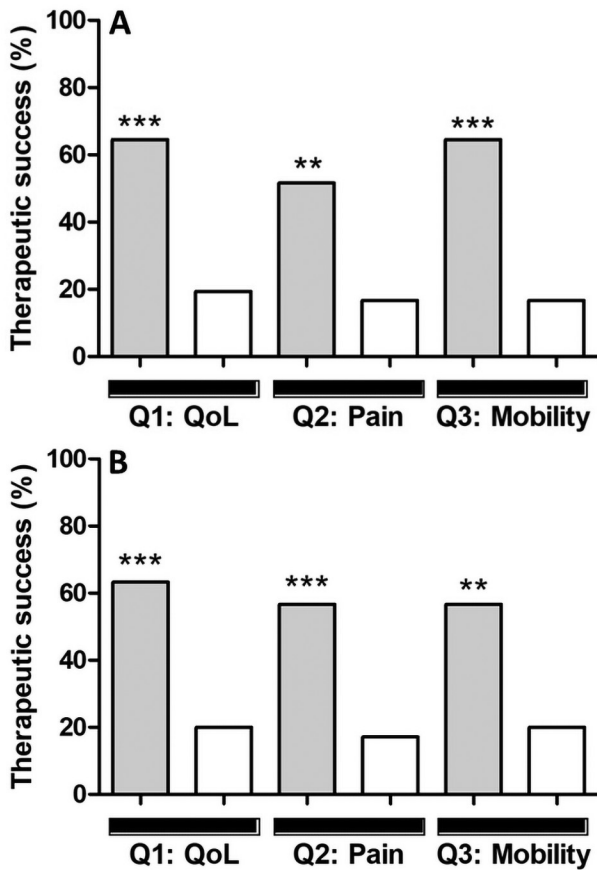
**Table 4**—Results for the gait analysis in force-platform and orthopedic assessment at every time point (4, 8, and 12 weeks after treatment).

Time point	Gait analysis				Orthopedic assessment			
	Variable	EUC-MSCs	Placebo	P value	Variable	EUC-MSCs	Placebo	P value
4 wk	n	27	34	.1605 (NS)	n	33	37	.0145*
	Efficacy	40.74%	20.6%		Efficacy	69.7%	40.5%	
8 wk	n	35	37	< .0001**	n	35	37	.0082***
	Efficacy	62.86%	8.00%		Efficacy	77.4%	45.9%	
12 wk	n	29	37	.0054***	n	29	37	.0031***
	Efficacy	48.27%	10.81%		Efficacy	79.3%	43.2%	

\* $P < .05$ , \*\* $P < .01$ , and \*\*\* $P < .001$ , between EUC-MSCs and placebo groups.

NS = Nonstatistical significance.





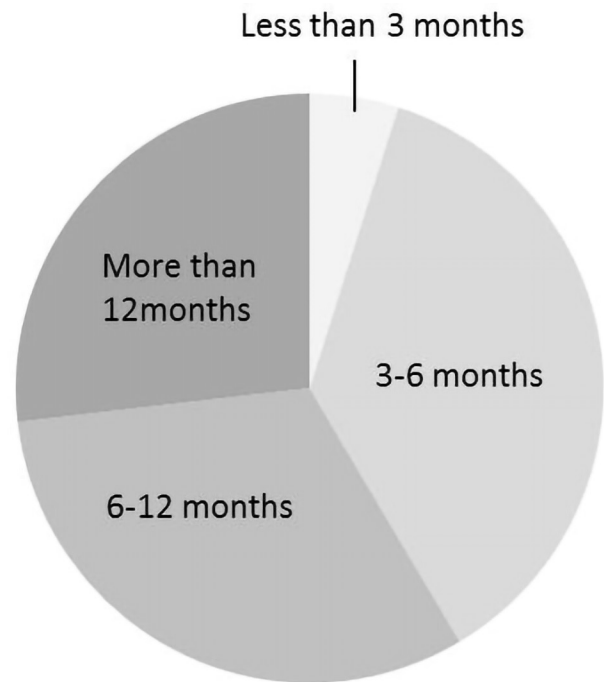
**Figure 1**—Results of a quality-of-life (QOL) questionnaire for owners 8 (A) and 12 (B) weeks after treatment with equine umbilical cord mesenchymal stem cells (gray) or a placebo (white) represented as percentage of therapeutic success in both groups. Therapeutic success is defined as percentage of owners that evaluated as 7 or more the different questions (Q1, Q2 Q3). \*\* $P < .01$  and \*\*\* $P < .001$  with respect to placebo group.

### Long-term follow-up

Eighteen months after administration, a long-term follow-up survey was sent to a total of 70 dog owners; 56 completed this questionnaire. Of these owners, 73% considered that the treatment was effective in treating their dog's OA. The results of the duration of the treatment according to owners are summarized (**Figure 2**). Of the owners, 36% observed a duration of the effect between 3 and 6 months, 32% observed a duration of the effect between 6 and 12 months, 27% observed a duration of the effect > 12 months, and 5% affirm that the effect lasted < 3 months. In addition, the improvement of these dogs was observed by their owners in day-to-day life such as changes in temperature or season (28%), playing with other dogs (27%), walks (60%), stiffness after a nap (42%), running, and stairs and other factors (21%).

### Safety

Some abnormal results in both placebo- and EUC-MSC-treated groups were found in the clinical



**Figure 2**—Long-term follow-up results; 36% of the owners observed a duration of the effect between 3 and 6 months, 32% of the owners observed a duration of the effect between 6 and 12 months, 27% of the owners observed a duration of the effect > 12 months, and 5% of the owners observed a duration of the effect < 3 months.

assessment at different visits, including damage to a nail, low heart rate, mild pain in deep abdominal palpation, subcutaneous lipoma, mild skin infection in the clipped area for injection, and so forth. However, none of these results were considered clinically relevant.

In the laboratory tests performed, some casual findings were identified at both day 0 and the end of the study. Normal values were considered the reference values  $\pm 10\%$ . The clinical impact of abnormal values was investigated case by case. The abnormal findings were mainly an increase in AST or ALT. These findings were present in the placebo and treatment groups and at both time points; therefore, it was considered casual and not related to the treatment.

Adverse events and complications observed in each treatment group during the study were listed and investigated. During the study, some AEs occurred that were classified by the veterinarian as "non-product related" (diarrhea, otitis, allergic outbreak [in an allergic dog], histiocytoma, dermatitis on the neck, etc).

Regarding the product-related AE, 7 dogs in the treatment group and 6 in the placebo group experienced increased lameness after the articular injection. This AE resolved with no need of rescue medication after 3 to 7 days in all patients of the placebo group and 3 patients in the treatment group. Four of the patients in the treatment group suffering from this AE required rescue medication.

## Discussion

As hypothesized, EUC-MSCs have been shown to be safe and effective in xenogeneic use. Due to their mechanism of action through immunomodulation, they not only reduce lameness and pain in dogs suffering from OA, as has been demonstrated in the present report and others,<sup>10,13,14</sup> but also are able to slow down the progression of the disease, enhancing tissue regeneration through their immunomodulatory capacity,<sup>12</sup> something that is not currently offered by any of the treatments available on the market.

Thanks to the use of a completely objective variable as the gait analysis and double-blinding, a very low placebo effect of only 8% on the primary variable has been achieved, allowing clear and objective results and eliminating the subjectivity of visual scales. To the best of our knowledge, this is the first study conducted with regulatory purposes that includes the plate force as an objective variable.

However, the use of the force platform has been challenging due to its high sensitivity. The dogs had to walk at a continuous speed, without sudden movements of the head or trunk, without accelerating or decelerating or without pulling on the lead.

A literature review shows that many research groups carry out the gait analysis trotting.<sup>1,36,37</sup> This way allows detection of more subtle changes in lameness.<sup>39</sup> In our case, this test was performed at walking for 2 reasons: first, according to the experts, studies of dogs with severe lameness are best undertaken at a walk to avoid too many invalid trials and to decrease the coefficient of variation<sup>39</sup>; second, some of the patients included had such severe OA that it was impossible for them to trot.

In addition, a wide range of acceptable walking speeds (0.7 to 1.8 m/s) were used to allow each dog a comfortable speed regardless of the severity of their OA, size, or age. On day 0, the speed of each patient was set, and that same dog had to maintain that same speed ( $\pm 0.3$  m/s) in the following visits as recommended in this type of study to minimize data variance.<sup>39</sup>

The technical error for the force platform is considered to be 2% PVF(%BW). For this reason, some authors establish this cutoff as the minimum value to consider a change clinically relevant.<sup>3,42</sup>

For Gagnon et al,<sup>3</sup> to be considered as “respondent” to a treatment, a patient needed an increase in PVF  $\geq 2.0\%$  body weight, since 2% is considered a technical error of the platform.

The present study was more restrictive, and the cutoff was an increase to 5% PVF(%BW). According to Conzemius et al,<sup>39</sup> in dogs with OA over a period of < 6 months, a change of 3.5% is the minimum value that can be considered clinically important. Conzemius et al established in their review a consensus statement that “[t]he number of dogs in each group that have a change greater than 5% is a good outcome measure.”<sup>39</sup> In line with this international consensus, in the present clinical trial, 5% of PVF increase was chosen.

Apart from the primary variable, other secondary variables were examined to cover as much evidence as possible. The orthopedic scale published by McCarthy

et al<sup>40</sup> was used, and pain and lameness were extracted from it, as these were the most clinically relevant parameters to show if the product was effective.

The cutoff for the therapeutic success was established as a decrease of 1 point, which implies an improvement of at least 12.5%, as no dogs with a score of 5 in any of the parameters were admitted for animal welfare.

Although the scale is perfectly described and the same orthopedic surgeon (blinded) always assessed each dog, the placebo effect showed in this parameter was higher than the one observed in the primary end point. Nevertheless, the orthopedic assessment demonstrated statistically significant superiority of EUC-MSCs at all time points.

In addition, a validated owner survey was included. The survey scores the dog’s improvement after treatment in different aspects from 1 to 10. A score of 7 was considered a significant improvement to establish the cutoff for therapeutic success. It revealed a mean improvement of more than 60% in the 3 questions asked and both time points.

According to the results of this questionnaire, the efficacy profile of EUC-MSCs treatment is superior to other products indicated in the treatment of OA, such as NSAIDs,<sup>43</sup> for which the effectiveness shown in owner surveys was 51%, or monoclonal antibodies,<sup>44</sup> which showed an efficacy of 43.5% in the owners’ survey. The questionnaire used was different in each case, but if we compare the owner questionnaire results in the 3 treatments, it permits an approximate comparison.

Due to the mechanism of action of EUC-MSCs, based on immunomodulation rather than blocking the inflammatory cascade,<sup>12</sup> the biggest drawback of the treatment is the time it takes to show an effect. Based on the results, some dogs started to improve as early as week 4 after treatment, but most demonstrated observable improvement from week 8 onward, which is consistent with the bibliography as previously reported by Song et al, who observed the effects of MSCs at week 12 after implantation.<sup>45</sup> Nicpoń et al<sup>46</sup> make the first time point at day 60 after autologous intra-articular administration.

One of the main advantages of MSC is the long duration of the effect.<sup>45,46</sup> Our study revealed that, according to the owner, the product was effective for more than 6 months in 59% of the patients. For 27%, the effect persisted for greater than 12 months (more than 52 weeks), and for 32%, this effect lasted between 6 and 12 months. It is important to take into account that this long-term study was carried out in an uncontrolled and unmasked manner, which can lead to a certain bias of the results; however, it allows obtaining very long-term data in a large sample, being to the best of our knowledge the longest study with the largest sample size reported on the xenogeneic use of MSCs.

Despite the fact that there are authors who claim that factors such as a younger age or male sex are associated with increased efficacy of MSCs,<sup>47,48</sup> in the present work, the effect of covariates (age, gender, OA grade, joint treated, severity) was evaluated, and none of them showed an effect on efficacy outcome (data not shown).

Four dogs suffered a local product-related side effect. This AE was described as an increase in pain and lameness after intra-articular administration that required rescue medication with anti-inflammatory drugs. However, this does not impair the efficacy of the product. Of the 4 dogs that experienced this AE, 2 showed efficacy at week 8, while 2 did not. This is a very small number to make statistical calculations, but the tendency is the same as in the total population, with efficacy being around 50%.

The mentioned reaction has already been described in the literature associated with MSC administration. In the work published by Song et al, 11.1% of the infiltrated stifle joints suffered localized pain after injection, and 44.4% registered swelling at the injection site.<sup>45</sup> Ferris et al<sup>49</sup> reported joint flare (acute lameness and pain) in 9% of horses after MSC injection; however, this AE did not affect the efficacy of the MSCs in these cases. This is similar to the previous experience of the authors in the allogeneic use of EUC-MSC in equine OA.<sup>50</sup> Finally, no systemic or permanent AEs were recorded at any time point of study in any of the patients included.

This clinical trial was conducted with regulatory purposes and following international guidelines. The study was conducted with minor protocol deviations, the most significant of which was the variable number of dogs in each visit. This was variability occurred for different reasons (dogs owners locked down for COVID, incidence with the calibration files of the force platform at week 4, dogs' withdrawal for reasons outside our study [nonrelated AE]). This made the sample size lower at weeks 4 and 12 compared with week 8. Many dogs that were successful in week 8 were not available in week 4 or 12; this could explain the lower results in these 2 time points compared with the results at 8 weeks.

Withdrawals or dog absences can be important in long-term studies such as the present study. It is recommended to include an excess of at least 15% of animals with respect to those merely resulting from mathematical calculation, to compensate for this type of deviation. The authors would recommend that, in long-term studies, the extra sample size be at least 20%, although it is also true that the context of the COVID-19 pandemic has had an impact on the number of dogs that have lost visits, which may mean that in this specific study the number of absences is higher than would be expected in a normal study.

In conclusion, this clinical trial proves the safety and effective use of xenogeneic stem cells in the treatment of canine OA, representing a new therapeutic alternative with an innovative mechanism of action, superior efficacy to conventional treatments, and a wide safety profile.

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## References

1. Wanstrath AW, Hettlich BF, Su L, et al. Evaluation of a single intra-articular injection of autologous protein solution for treatment of osteoarthritis in a canine population. *Vet Surg.* 2016;45(6):764–774. doi:10.1111/vsu.12512
2. Moreau M, Lussier B, Pelletier JP, et al. A medicinal herb-based natural health product improves the condition of a canine natural osteoarthritis model: a randomized placebo-controlled trial. *Res Vet Sci.* 2014;97(3):574–581. doi:10.1016/j.rvsc.2014.09.011
3. Gagnon A, Brown D, Moreau M, Lussier B, Otis C, Troncy E. Therapeutic response analysis in dogs with naturally occurring osteoarthritis. *Vet Anaesth Analg.* 2017;44(6):1373–1381. doi:10.1016/j.vaa.2017.07.008
4. Moreau M, Dupuis J, Bonneau NH, Desnoyers M. Clinical evaluation of a nutraceutical, carprofen and meloxicam for the treatment of dogs with osteoarthritis. *Vet Rec.* 2003;152(11):323–329. doi:10.1136/vr.152.11.323
5. Moreau M, Lussier B, Doucet M, Vincent G, Martel-Pelletier J, Pelletier JP. Efficacy of licofelone in dogs with clinical osteoarthritis. *Vet Rec.* 2007;160(17):584–588. doi:10.1136/vr.160.17.584
6. European Medicines Agency. Annex I: summary of product characteristics. Updated August 17, 2021. Accessed September 9, 2022. [https://www.ema.europa.eu/en/documents/product-information/galliprant-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/galliprant-epar-product-information_en.pdf)
7. Dias IE, Pinto PÓ, Barros LC, Viegas CA, Dias IR, Carvalho PP. Mesenchymal stem cells therapy in companion animals: useful for immune-mediated diseases? *BMC Vet Res.* 2019;15(1):358. doi:10.1186/s12917-019-2087-2
8. Kang MH, Park HM. Challenges of stem cell therapies in companion animal practice. *J Vet Sci.* 2020;21(3):e42. doi:10.4142/jvs.2020.21.e42
9. Harman R, Carlson K, Gaynor J, et al. A prospective, randomized, masked, and placebo-controlled efficacy study of intraarticular allogeneic adipose stem cells for the treatment of osteoarthritis in dogs. *Front Vet Sci.* 2016;3:81. doi:10.3389/fvets.2016.00081
10. Cabon Q, Febre M, Gomez N, et al. Long-term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal mesenchymal stromal cells for managing pain and lameness in moderate to severe canine osteoarthritis without anti-inflammatory pharmacological support: pilot clinical study. *Front Vet Sci.* 2019;6:10. doi:10.3389/fvets.2019.00010
11. Daems R, Van Hecke L, Schwarzkopf I, et al. A feasibility study on the use of equine chondrogenic induced mesenchymal stem cells as a treatment for natural occurring osteoarthritis in dogs. *Stem Cells Int.* 2019;2019:4587594. doi:10.1155/2019/4587594
12. Zhao X, Zhao Y, Sun X, Xing Y, Wang X, Yang Q. Immunomodulation of MSCs and MSC-derived extracellular vesicles in osteoarthritis. *Front Bioeng Biotechnol.* 2020;8:575057. doi:10.3389/fbioe.2020.575057
13. Tsai SY, Huang YC, Chueh LL, Yeh LS, Lin CS. Intra-articular transplantation of porcine adipose-derived stem cells for the treatment of canine osteoarthritis: a pilot study. *World J Transplant.* 2014;4(3):196–205. doi:10.5500/wjt.v4.i3.196
14. Vilar JM, Morales M, Santana A, et al. Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells associated to PRGF-Endoret in osteoarthritic dogs. *BMC Vet Res.* 2013;9(1):131. doi:10.1186/1746-6148-9-131
15. Debosschere Y, Depuydt E, Pauwelyn G, et al. Safety and immunomodulatory properties of equine peripheral blood-derived mesenchymal stem cells in healthy cats. *Vet Immunol Immunopathol.* 2020;227:110083. doi:10.1016/j.vetimm.2020.110083
16. Lin CS, Lin G, Lue TF. Allogeneic and xenogeneic transplantation of adipose-derived stem cells in immunocompetent recipients without immunosuppressants.



- Stem Cells Dev.* 2012;21(15):2770–2778. doi:10.1089/scd.2012.0176
17. Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT. Mesenchymal stem cell research in veterinary medicine. *Curr Stem Cell Res Ther.* 2018;13(8):645–657. doi:10.2174/1574888X13666180517074444
  18. Dimarino AM, Caplan AI, Bonfield TL. Mesenchymal stem cells in tissue repair. *Front Immunol.* 2013;4:201. doi:10.3389/fimmu.2013.00201
  19. Whitworth DJ, Banks TA. Stem cell therapies for treating osteoarthritis: prescient or premature? *Vet J.* 2014;202(3):416–424. doi:10.1016/j.tvjl.2014.09.024
  20. Li L, Duan X, Fan Z, et al. Mesenchymal stem cells in combination with hyaluronic acid for articular cartilage defects. *Sci Rep.* 2018;8(1):9900. doi:10.1038/s41598-018-27737-y
  21. Cuervo B, Rubio M, Sopena J, et al. Hip osteoarthritis in dogs: a randomized study using mesenchymal stem cells from adipose tissue and plasma rich in growth factors. *Int J Mol Sci.* 2014;15(8):13437–13460. doi:10.3390/ijms150813437
  22. Kim SE, Pozzi A, Yeh JC, et al. Intra-articular umbilical cord derived mesenchymal stem cell therapy for chronic elbow osteoarthritis in dogs: a double-blinded, placebo-controlled clinical trial. *Front Vet Sci.* 2019;6:474. doi:10.3389/fvets.2019.00474
  23. Zhang BY, Wang BY, Li SC, et al. Evaluation of the curative effect of umbilical cord mesenchymal stem cell therapy for knee arthritis in dogs using imaging technology. *Stem Cells Int.* 2018;2018:1983025. doi:10.1155/2018/1983025
  24. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells.* 2006;24(5):1294–1301. doi:10.1634/stemcells.2005-0342
  25. Kalaszczynska I, Ferdyn K. Wharton's jelly derived mesenchymal stem cells: future of regenerative medicine? Recent findings and clinical significance. *BioMed Res Int.* 2015;2015:430847. doi:10.1155/2015/430847
  26. Li X, Bai J, Ji X, Li R, Xuan Y, Wang Y. Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. *Int J Mol Med.* 2014;34(3):695–704. doi:10.3892/ijmm.2014.1821
  27. Schnabel LV, Fortier LA, McIlwraith CW, Nobert KM. Therapeutic use of stem cells in horses: which type, how, and when? *Vet J.* 2013;197(3):570–577. doi:10.1016/j.tvjl.2013.04.018
  28. Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: their advantages and potential clinical utility. *World J Stem Cells.* 2014;6(2):195–202. doi:10.4252/wjsc.v6.i2.195
  29. Sultana T, Lee S, Yoon HY, Lee JI. Current status of canine umbilical cord blood-derived mesenchymal stem cells in veterinary medicine. *Stem Cells Int.* 2018;2018:8329174. doi:10.1155/2018/8329174
  30. Wang Y, Han ZB, Song YP, Han ZC. Safety of mesenchymal stem cells for clinical application. *Stem Cells Int.* 2012;2012:652034. doi:10.1155/2012/652034
  31. Song WJ, Li Q, Ryu MO, et al. Canine adipose tissue-derived mesenchymal stem cells pre-treated with TNF- $\alpha$  enhance immunomodulatory effects in inflammatory bowel disease in mice. *Res Vet Sci.* 2019;125:176–184. doi:10.1016/j.rvsc.2019.06.012
  32. Gupta RC, Canerdy TD, Lindley J, et al. Comparative therapeutic efficacy and safety of type-II collagen (UC-II), glucosamine and chondroitin in arthritic dogs: pain evaluation by ground force plate. *J Anim Physiol Anim Nutr (Berl).* 2012;96(5):770–777. doi:10.1111/j.1439-0396.2011.01166.x
  33. Lascelles BD, Roe SC, Smith E, et al. Evaluation of a pressure walkway system for measurement of vertical limb forces in clinically normal dogs. *Am J Vet Res.* 2006;67(2):277–282. doi:10.2460/ajvr.67.2.277
  34. Brown DC, Boston RC, Farrar JT. Comparison of force plate gait analysis and owner assessment of pain using the Canine Brief Pain Inventory in dogs with osteoarthritis. *J Vet Intern Med.* 2013;27(1):22–30. doi:10.1111/jvim.12004
  35. Brown DC, Boston RC, Farrar JT. Use of an activity monitor to detect response to treatment in dogs with osteoarthritis. *J Am Vet Med Assoc.* 2010;237(1):66–70. doi:10.2460/javma.237.1.66
  36. Rialland P, Bichot S, Moreau M, et al. Clinical validity of outcome pain measures in naturally occurring canine osteoarthritis. *BMC Vet Res.* 2012;8(1):162. doi:10.1186/1746-6148-8-162
  37. Seibert R, Marcellin-Little DJ, Roe SC, DePuy V, Lascelles BD. Comparison of body weight distribution, peak vertical force, and vertical impulse as measures of hip joint pain and efficacy of total hip replacement. *Vet Surg.* 2012;41(4):443–447. doi:10.1111/j.1532-950X.2012.00957.x
  38. Quimby JM, Borjesson DL. Mesenchymal stem cell therapy in cats: current knowledge and future potential. *J Feline Med Surg.* 2018;20(3):208–216. doi:10.1177/1098612X18758590
  39. Conzemius MG, Torres BT, Muir P, Evans R, Krotscheck U, Budsberg S. Best practices for measuring and reporting ground reaction forces in dogs. *Vet Surg.* 2022;51(3):385–396. doi:10.1111/vsu.13772
  40. McCarthy G, O'Donovan J, Jones B, McAllister H, Seed M, Mooney C. Randomised double-blind, positive-controlled trial to assess the efficacy of glucosamine/chondroitin sulfate for the treatment of dogs with osteoarthritis. *Vet J.* 2007;174(1):54–61. doi:10.1016/j.tvjl.2006.02.015
  41. European Medicines Agency. Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals). January 16, 2012. Accessed September 9, 2022. [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-statistical-principles-clinical-trials-veterinary-medicinal-products-pharmaceuticals\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-statistical-principles-clinical-trials-veterinary-medicinal-products-pharmaceuticals_en.pdf)
  42. Moreau M, Pelletier JP, Lussier B, et al. A posteriori comparison of natural and surgical destabilization models of canine osteoarthritis. *BioMed Res Int.* 2013;2013:180453. doi:10.1155/2013/180453
  43. European Medicines Agency. EPAR summary for the public. Galliprant. Updated November 2017. Accessed September 9, 2022. [https://www.ema.europa.eu/en/documents/overview/galliprant-epar-summary-public\\_en.pdf](https://www.ema.europa.eu/en/documents/overview/galliprant-epar-summary-public_en.pdf)
  44. European Medicines Agency. EPAR summary for the public. Librela. Updated April 2021. Accessed September 9, 2022. [https://www.ema.europa.eu/en/documents/overview/librela-epar-medicine-overview\\_en.pdf](https://www.ema.europa.eu/en/documents/overview/librela-epar-medicine-overview_en.pdf)
  45. Song Y, Du H, Dai C, et al. Human adipose-derived mesenchymal stem cells for osteoarthritis: a pilot study with long-term follow-up and repeated injections. *Regen Med.* 2018;13(3):295–307. doi:10.2217/rme-2017-0152
  46. Nicpoń J, Marycz K, Grzesiak J, Śmieszek A, Toker NY. The advantages of autologous adipose derived mesenchymal stem cells (AdMSCs) over the non-steroidal anti-inflammatory drugs (NSAIDs) application for degenerative elbow joint disease treatment in dogs—twelve cases. *Kafkas Univ Vet Fak Derg.* 2014;20(3):345–350.
  47. Fuggle NR, Cooper C, Oreffo ROC, et al. Alternative and complementary therapies in osteoarthritis and cartilage repair. *Aging Clin Exp Res.* 2020;32(4):547–560. doi:10.1007/s40520-020-01515-1
  48. Kim YS, Suh DS, Tak DH, Chung PK, Koh YG. Mesenchymal stem cell implantation in knee osteoarthritis: mid-term outcomes and survival analysis in 467 patients. *Orthop J Sports Med.* 2020;8(12):2325967120969189. doi:10.1177/2325967120969189
  49. Ferris DJ, Frisbie DD, Kisiday JD, et al. Clinical outcome after intra-articular administration of bone marrow derived mesenchymal stem cells in 33 horses with stifle injury. *Vet Surg.* 2014;43(3):255–265. doi:10.1111/j.1532-950X.2014.12100.x
  50. European Medicines Agency. HorStem (equine umbilical cord mesenchymal stem cells). An overview of HorStem and why it is authorised in the EU. Updated February 2019. Accessed September 9, 2022. [https://www.ema.europa.eu/en/documents/overview/horstem-epar-medicine-overview\\_en.pdf](https://www.ema.europa.eu/en/documents/overview/horstem-epar-medicine-overview_en.pdf)