Veterinary Medicines Directorate

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Veterinary Medicines Directorate
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NATIONAL PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

DogStem Suspension for Injection for Dogs

Date Created: November 2022

MODULE 1

PRODUCT SUMMARY

Name, strength and pharmaceutical form	DogStem Suspension for Injection for Dogs, 7.5 x 10^6 cells/ml
Applicant	EquiCord SL C/ Loeches 103-D Alcorcon Madrid 28925 Spain
Active substance	Mesenchymal Stem Cells (EUC-MSCs)
ATC Vetcode	QM09AX90
Target species	Dogs
Indication for use	Improvement in function, reduction of pain and lameness associated with mild to severe osteoarthritis in hip and elbow joints.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Product Information Database of the Veterinary Medicines Directorate.

(www.gov.uk/check-animal-medicine-licensed)

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	National application in accordance with Article 12(3) of Directive 2001/82/EC as amended.
Date of conclusion of the procedure	11/9/2022

I. SCIENTIFIC OVERVIEW

This application is for a GB-National marketing authorisation for a new product, DogStem and is submitted in accordance with Article 12(3) of Directive 2001/82- full application. This application has been submitted in parallel to the EMA following a centralised submission procedure.

II. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

II.A. Composition

The product contains equine umbilical cord mesenchymal stem cells (EUC-MSCs) and the excipients HypoThermosol-FRS.

The container/closure system consists of a cyclo olefin polymer vial closed with rubber stoppers and sealed with aluminium caps. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the formulation and the absence of preservative are justified.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

II.B. Description of the Manufacturing Method

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site. The manufacturing method consists of collection of Equine Umbilical Cord (EUC), isolation and expansion of mesenchymal stem cells (MSCs), preparation of the Master Cell Bank (MCB), cryopreservation of the MCB, thawing of the MCB to produce the active substance, preparation of the active substance, manufacture of the active substance and preparation of the finished product.

Process validation data on the product have been presented in accordance with the relevant European guidelines.

II.C. Control of Starting Materials

The active substance is allogeneic mesenchymal stem cells (MSCs), an established active substance with the specifications being in accordance with the Guideline on 'Test procedures and acceptance criteria for new biotechnological/biological veterinary medicinal products'. The active substance is manufactured in accordance with the principles of good manufacturing practice.

The active substance specification is considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification have been provided.

The excipients meet the requirements of the United States Pharmacopoeia and the National Formulary, the European Pharmacopoeia, the British Pharmacopoeia, and the Japanese Pharmacopoeia.

The packaging complies with Ph. Eur.

II.C.4. Substances of Biological Origin

Scientific data and/or certificates of suitability issued by the EDQM have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

II.D. Control Tests Carried Out at Intermediate Stages of the Manufacturing Process

Not applicable; however, in-process quality controls in the MCB manufacturing process were described.

II.E. Control Tests on the Finished Product

The finished product specification controls the relevant parameters for the pharmaceutical form. The tests in the specification, and their limits, have been justified and are considered appropriate to adequately control the quality of the product. Satisfactory validation data for the analytical methods have been provided. Batch analytical data from the proposed production site have been provided demonstrating compliance with the specification. Control tests on the finished product are those for: cell concentration, viability, endotoxins, gram, sterility, appearance and labelling.

II.F. Stability

Stability data on the discontinuous manufacturing processes of the active substance have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions. No stability studies were performed for the active substance manufactured by the continuous manufacturing process and used

immediately for packaging, since these cells are not subjected to any kind of storage and are used directly to formulate the finished product.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions.

G. Other Information

Shelf life of the veterinary medicinal product as packaged for sale: 21 days.
Shelf life after first opening the immediate packaging: Use immediately.
Store and transport refrigerated (2 °C – 8 °C).
Do not freeze.

III. SAFETY AND RESIDUES DOCUMENTATION (PHARMACO-TOXICOLOGICAL)

III.A Safety Documentation

Pharmacological Studies

Bibliographical data has been provided which show that mesenchymal stem cells act through the release of soluble factors that modulate the cells and environment of the joint, decreasing levels of pro-inflammatory cytokines and increasing levels of anti-inflammatory cytokines which improve the symptoms of osteoarthritis. They have immunomodulatory properties and can possess tissue regenerative properties. The applicant has also provided bibliographical data which show that following intraarticular administration of MSCs, it can be concluded that the joint space acts as a self-contained niche, that favours cell retention and localised activity of the cells. MSCs can be detected in the joint tissue for a short period of time following administration, but systemic migration to other organs has not been detected or reported. To what extent EUC-MSCs from this product persist after intraarticular administration to dogs is not known as no proprietary biodistribution studies have been conducted with DogStem.

Toxicological Studies

The applicant has conducted laboratory studies which show that the toxicity of MSCs is very low.

Single Dose Toxicity

The applicant carried out several studies in different species to assess the safety of a single dose. The first study was conducted in rats and rabbits and was found to be non-toxic, non-teratogenic and non-tumorigenic. The second study was done in mice where there were no side effects and no tumour development and a NOEL of $2.5x10^8$ cells/kg. A human clinical trial was also conducted with a single

dose and no serious adverse effects were reported. A bibliographic study was provided by the applicant, that was performed in mice to determine if MSCs were detectable in the long term. MSCs were detected in injected joints in 90-100% of animals after 3 months and in 60% of animals after 6 months. MSCs were not detected in the liver, kidneys, lungs or stomach at any time. At any point there were less than 4& of MSCs found outside the joint. In 20% of animals, a small number of MSCs detected in the heart, intestine, brain, blood and testis. However, these were rapidly cleared and, in almost 60% of animals, were not detected by day 11. There was an absence of any lesions or neoplasia. Overall, there are no acute toxic effects and only when very high doses are administered there are rarely severe adverse effects in single dose studies.

Repeated Dose Toxicity

A study in rodents was conducted where the animals received $15x10^6$ cell/kg bodyweight daily for fourteen days. No mortality or abnormal clinical signs were observed. A mild swelling at the injection site was observed in some animals but was reversible.
A bibliographical study was provided in monkeys who were given either $2x10^6$ or $1x10^7$ cells/kg bw every two weeks. The findings showed that MSCs did not affect general health. Some animals developed antibodies, but this appeared to be dose dependent.
In summary, after repeated application, no clinical signs or systemic toxicity was observed.

Reproductive Toxicity, including Teratogenicity

A bibliographic study was presented to determine the effects on reproduction. The study was administered in pregnant rats who received either a low, medium or high dose at days 5, 12 and 18 via IV. MSCs are not found consistently or significantly in the reproductive system and if cells did reach the reproductive system, then safety studies show that there are no maternal safety problems or treatment related embryo-foetal toxicity.

Carcinogenicity

Four studies were provided to assess the safety regarding tumour formation in rodents. The first study tested different application routes with a 3-6 month follow up period and there was no evidence of tumour formation. In studies 2 and 3, the highest dose of $2x10^8$ cells/kg bw were administered with no evidence of tumour development.
Study four found that even with the highest dose of $2.5x10^8$ cells/kg bw, the mice had no side effects. Even at the highest dose of $2x10^8$ MSCs/kg there was no evidence of tumour development. This study observed that there is no tumour formation after application and that MSCs prevent the growth of leukemic tumours.
In conclusion, the risk of tumour development is very unlikely.

Studies of Other Effects

Immunotoxicity

As MSCs are xenogeneic, the potential adverse effects from accidental self-injection in humans is an area of concern. The applicant has provided the following information:
MSCs interact with the immune system to provoke an immunomodulatory and anti-inflammatory effect. They are hypoimmunogenic and prevent T-cell responses both directly and indirectly and induce a suppressive local microenvironment which depletes tryptophan.
Many *in vitro* studies have shown a range of anti-inflammatory/immunomodulatory paracrine effects on adaptive immune system and this ability has been tested in canine-PBMCs. It shows that when EUC-MSCs are in contact with canine-PBMCs, the EUC-MSCs inhibit the exacerbated proliferation of PBMCs. It has been shown that from the repeated allogenic/xenogeneic MSC application, the recipient may develop mild antibody titres to donor cells. Despite this, the response has not been involved in any case with adverse effects of immunological reactions. The humeral response of xenogeneic EUC-MSCs in dogs have been evaluated after both single and repeated administration. The cytotoxicity of EUC-MSCs after a single and a repeated dose in the target species has been evaluated and no cytotoxicity was found.
Xenogeneic MSCs do not generate a specific immune response in recipients and they do not negatively impact the immune system so can be used in repeated doses.

User Safety

A user risk assessment was provided in compliance with the relevant guideline.

Warnings and precautions as listed on the product literature are adequate to ensure safety to users of the product. Therefore the following applicant's user recommendations are appropriate:

There are only limited data available to support the human safety of this product. In particular, women of childbearing age and people with compromised immune systems should take care to avoid contact with the product. It is recommended to wear impermeable gloves at all times whilst handling and administering the product. Wash any spills off exposed skin, eyes, or mucous membranes immediately.
The product contains Dextran-40, which may cause hypersensitivity (allergic) type reactions in some people. Avoid contact with the product if you know you are sensitised to this substance.

Take care not to accidentally self-administer this product. In case of accidental self-injection, this product can cause pain, local inflammatory reactions and swelling at the site of injection, which may persist for several weeks. Transient fever may also occur. Seek medical advice immediately and provide the package leaflet or label to the physician

Environmental Safety

The Environmental Risk Assessment (ERA) was carried out in accordance with VICH and CVMP guidelines.

Phase I:

The product will only be used in non-food animals and as a result environmental exposure will be low. A Phase II ERA was not required.

IV. CLINICAL DOCUMENTATION

IV.I. Pre-Clinical Studies

Pharmacology

Mode of Action

The mode of mesenchymal stem cells is considered to be primarily related to paracrine actions (cell-to-cell and through the secretion of cytokines). Prostaglandin E_2 (PGE_2) secretion is an important immunomodulatory function of MSCs and it has a central role in the ability of EUC-MSCs to suppress the proliferation of activated canine peripheral blood mononuclear cells (PBMCs). PGE_2 influences the pathophysiological processes involved in OA and has effects on macrophages, T-cells and B-cells as well as anti-inflammatory effects on chondrocytes and synoviocytes.

Biodistribution

When tenogenic mesenchymal stem cells are administered by local IA injection into joints, the injected MSCs were not detected outside the joint during the 48 hour follow up period post-administration.

Tolerance in the Target Species

A study to support the safety of product was conducted in young healthy dogs under field conditions. During the study, the safety of single and repeated intra-articular administration of the product was compared to a placebo. The study was a double blinded, randomised, negatively controlled, GCP complaint TAS study.

16 healthy dogs, aged between >1 year and <4 years old, were split into two groups and received a dose of either EUC-MSCs or placebo on days 0 and 28+1.

No significant abnormalities were detected in the blood sample results.
All dogs were measured using the Glasgow pain score on day 29+1 and all but one dog in the control group received the lowest possible score of 0/24 (the lower the score, the less pain). The control group dog had a score of 1.
Nine adverse events were reported and were all considered mild and were all resolved.

In conclusion, the study demonstrated satisfactory safety of the product administered when compared with a placebo.

IV.II. Clinical Documentation

Laboratory Trials

The applicant has provided bibliographical data which shows that multiple injections of MSCs did not affect the overall immune system.

Dose confirmation studies:

The applicant has provided a justification for the selection of a dose of $7.5x10^6$ EUC-MSC per ml of product.

Field Trials

Study title	Retrospective study of osteoarthritis in dogs, sponsored.
Objectives	To use a survey methodology to evaluate the effectiveness and safety of the AD-MSC therapy in dogs with chronic OA and to determine the duration of effect, the types of adverse effects associated with AD-MSC and the differences in response when AD-MSC are used with or without other medicines such as the commonly used nonsteroidal anti-inflammatory drugs (NSAIDs)
Test site(s)	Veterinary practices
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	1 ml of AD-MSC containing $7.5x10^6$ ± 20% cells
Control product/placebo	None
Animals	103 dogs With OA or dysplasia
Outcomes/endpoints	Level of improvement following treatment
Randomisation	N/A
Blinding	N/A
Method	Administration of 1 ml of AD-MSC followed by aseptically collected adipose tissue. A standard process for isolation and expansion of cells is described. Following processing, the product is administered back into the affected joint of the same donor animal. Veterinary surgeons were asked to complete a survey.
Statistical method	For the descriptive analysis, quantitative variables were described with measures of central tendency

	and dispersion (mean and standard deviation). For continuous variables, the Kolmogrov-Smirnov test was used to assess normality. In bivariate analyses to compare means, parametric and non-parametric statistical tests were carried out. All analyses were performed with SPSS.
RESULTS	
Participant flow	All dogs received intended treatment in at least one affected joint with 57% receiving a second dose. All participants completed the study protocol and were analysed for the primary outcome.
Duration of follow-up	Ongoing.
Outcomes for endpoints	The improvement score had a mean (SD) score of 7.1 (2.1), which was statistically significant positive correlation with the length of improvement ($p<0.001$) and a trend for increased score with increased length of improvement were found. There was a statistically significant difference between the mean improvement scores in dogs aged ≤2 years compared with dogs >2 years.
Adverse events	23 dogs (22.3%) suffered adverse effects. Inflammation was seen in 13 cases and lameness in the first 48 hours was observed in 11 cases. One dog showed infection on the treated joint and three cases were categorised as 'pain on the joint'.
DISCUSSION	The study conclusions were that overall, dogs with chronic OA and dysplasia that were treated with intraarticular injection of AD-MSCs showed a great recovery in lameness, pain and durability of improvement over time. The veterinary survey scores were highly correlated between the score of improvement and the length of improvement.

Study title	Exploratory evaluation of efficacy and safety in canine osteoarthritis (OA) treated with Equine Umbilical Cord Mesenchymal Stem Cells (EUC-MSC) versus placebo in pilot animals. Sponsored.
Objectives	1) Evaluate the safety and efficacy of a single dose of EUC-MSC in a pilot population of dogs under field conditions. 2) To compare results with this xenogeneic treatment with an allogeneic MSC treatment in dogs with OA. 3) To confirm that the dose of 7.5 x 10^6 EUC-MSC ±20% (medium dose) selected by Equicord based on previous research, is appropriate. 4) To determine whether force plate devices are useful for measuring the treatment response in

	dogs with OA for the purpose of designing a confirmatory study.
Test site(s)	Single centre, veterinary practice, EU country.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	The test and positive control treatments were: • Test treatment o Treated with EUC-MSC in 1 ml of Hypothermosol-FRS o Low dose: 3 x 10^6 (± 20%) o Medium dose: 7.5 x 10^6 (± 20%) o High dose: 15 x 10^6 (± 20%) • Positive control o Treated with 7.5 x 10^6 (± 20%) AD-MSC in 1 ml of Hypothermosol-FRS
Control product/placebo	Negatively controlled. Dose of 1 ml by IA injection.
Animals	20 dogs were enrolled on day 0; however, one dog in the negative control group was not reallocated. Inclusion criteria: • Dogs older than 1 year • Body weight ≥ 20 kg • Diagnosed with OA of at least 1 month duration or longer • No signs of improvement in the last month for OA, and if included in a rehabilitation program it should have lasted more than 3 months • A single joint affected per limb • Radiological signs of OA • Not treated in the past 4 weeks with an NSAID with long or short lasting effect, or with a steroid-based medicine in the past 3 months • Global pain assessment score of above 2 but less than 13 • Orthopaedic examination total score between 5-18, inclusive • Force-plate gate analysis differences between the lamb and contralateral limbs of ≥5% • Owner (LOAD) questionnaire score >10 and ≤30 points Exclusion criteria: • Pregnant or lactating dogs • Signs of articular infection

	• Medical history of general surgery in the last 3 months • Medical history of non-arthroscopic surgery in the affected joint in the last 12 months • Presence of ostheosintesis material in the affected joint • Medical history of intraarticular treatment in the affected joint in the last 3 months • Autoimmune or inflammatory arthritis • Disease of central nervous system or peripheral nerves • Open wound in the affected limb • Other systemic disease or non-orthopaedic disorder that could affect gait and lead to a misdiagnosis • Dogs receiving continuous OA treatment for animal welfare reasons
Outcomes/endpoints	Force-plate gait analysis scores at 8 weeks compared with baseline.
Randomisation	Randomised.
Blinding	Double-blinded.
Method	Study schedule: Treatment was administered on day 0. Clinical examinations were performed on days 0, 24 ± 4 hours an in weeks 4, 8 and 12 ± 3 days. Remote follow up was performed 6 hours after treatment and in weeks 2 and 6 ±3 days.
Statistical method	Comparison between analysis at 3 weeks versus baseline analysis with an improvement of >7%.
RESULTS	
Participant flow	Number of participants in each treatment group. Treatment group: • EUC-MSC low dose: 3 • EUC-MSC medium dose: 8 • EUC-MSC high dose: 4 • AD-MSC: 4 • Placebo: 4 Four dogs that were treated with the negative control product were to be reallocated to a test treatment (EUC-MSC low and high dose groups) at the end of the study. However, only 3 dogs were reallocated since one dog was not reallocated due to an adverse event. Therefore, only 3 dogs received a low dose of EUC-MSC.
Duration of follow-up	3 months.
Outcomes for endpoints	Treatment group: • EUC-MSC low dose: 33.33% • EUC-MSC medium dose: 62.5%

	• EUC-MSC high dose: 50% • AD-MSC: 25% • Placebo: 0% The intention to treat (ITT) population were the 20 dogs entering the study and treated on day 0. The per protocol (PP) population were the 20 dogs entering the study, managed according to the protocol, and finishing the trial without withdrawal.
Adverse events	No severe adverse effects were detected. One dog was observed to have pain and lameness starting 24 hours after treatment with EUC-MSC high dose was seen for the first week and after 2 weeks full recovery still had not occurred.
DISCUSSION	The applicant concludes that the medium dose of EUC-MSC was more effective than the low dose for all studied parameters. Despite the sample size being too small to reach statistical significance, the applicant concludes that the highest dose of EUC-MSC does not have superior efficacy to the medium dose and the only product related adverse effect occurred in the high dose group. Therefore, the applicant concludes that the medium dose of EUC-MSC is optimal. In the medium and high EUC-MSC dose groups and the AD-MSC group, the LOAD questionnaire results were correlated with other variables. The applicant notes the favourable treatment response reflected by LOAD questionnaire results in the placebo group; however, a favourable response was not observed based on other variables. The applicant concludes that the force-gait analysis registered a 0% of placebo effect.

Study title	Confirmatory safety and efficacy clinical trial in the use of xenogeneic mesenchymal stem cells from equine umbilical cord (EUC-MSC) in dogs with mild to severe osteoarthritis (OA) in field conditions. Sponsored.
Objectives	Confirm the safety and efficacy of EUC-MSC in dogs with mild to severe OA under field conditions.
Test site(s)	Multi-centre, veterinary practices, EU country.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP)
Test Product	7.5×10^6 (± 20%) EUC-MSC in 1 ml Hypothermosol for IA administration
Control product/placebo	Negative control.
Animals	80 dogs. Inclusion criteria:

	• Dogs older than 1 year • Normal haematology and biochemistry results and a negative *Leishmania* spp. Test within the last 3 months. • Body weight ≥15 kg • Orthopaedic discomfort, uni- or bilateral in the elbow or hip joint/s of at least a 3 month duration • No signs of improvement in the last month for OA • A single joint affected per limb • Radiological signs of mild to severe OA • LOAD questionnaire >10 and ≤30 points • Joint Mobility and Pain on palpation ≥2 in the target joint based on the PA clinical score • Peak vertical force normalised to bodyweight in the target joint of below 89.35% for forelimbs and below 61.2% for hindlimbs • Informed owner consent Exclusion criteria: • Pregnant or lactating dogs • Signs of articular infection • Medical history of general surgery in the last 3 months • Medical history of surgery in the affected joint in the last 12 months • More than one joint affected per limb based on pain on palpation during orthopaedic examination • Treated in the previous 4 weeks with NSAIDs or the previous 3 months with steroids • Medical history of intraarticular treatment in an affected joint in the last 3 months • Autoimmune or inflammatory arthritis • *Leishmania* spp. test positive result • Disease of the central nervous system or peripheral nerves • Open wound in the affected limb • Other systemic disease or non-orthopaedic disorder that could affect gait and lead to a misdiagnosis • Score of 5 in any category of the clinical scoring system for evaluation of canine OA • Dogs with a medical condition, as defined by the investigator • Dogs in continuous need of use of OA treatment for animal welfare reasons

Outcomes/endpoints	The percentage of animals considered to be a therapeutic success in the treatment group compared with the percentage in the placebo group at 8 weeks. Therapeutic success is defined as animals with a ≥7% increase compared with baseline. The improvement is normalised for bodyweight.
Randomisation	Randomised. Non-stratified randomisation based on a simple method.
Blinding	Double blinded.
Method	Study schedule: Treatment was administered on day 0. Clinical examinations were performed on days 0, 24 ± 4 hours an in weeks 4, 8 and 12 ± 4 days.
Statistical method	Comparison between analysis at 8 weeks versus baseline analysis with an improvement of >7%.
RESULTS	
Participant flow	40 dogs were allocated to each group with 79 dogs being included in the study (39 receiving the test product and 40 receiving the control product). Seven dogs were withdrawn from the study due to the administration of forbidden treatments and they were all from the test product group
Duration of follow-up	3 months
Outcomes for endpoints	All analyses were performed considering two-sided tests and a significance level set at 0.05. Quantitative variables were summarised using mean, median, standard deviation, minimum and maximum values, and a confidence interval for the mean and sample size. Qualitative variables were summarised using relative and absolute frequencies. Eight weeks after treatment, 51.4% of the dogs were classified as a therapeutic success in the test product group compared with 5.4% from the placebo group.
Adverse events	34 adverse effects were observed, and complete recovery was recorded for 31 of these events. Only 13 of the 34 events were considered to be probably or possibly associated with administration of the proposed product although six events occurred in the placebo group. Lameness with pain was the most common AE described (10/13), with three of the dogs being from the placebo group. Of the seven IVP treated dogs, four required supportive treatment with NSAIDs. All local AEs in the IVP group resolved during the study.
DISCUSSION	The applicant concluded that the clinical trial confirmed the efficacy and safety of a single dose of the product. Treatment with the product has proven to be effective at week 12 post-treatment. The treatment

	has shown to be safe, presenting no serious or permanent adverse effects.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics the benefit/risk profile of the product is favourable.

MODULE 4

POST- AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)

The post-authorisation assessment (PAA) contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)