16 February 2022
EMA/131045/2022
Veterinary Medicines Division

## Committee for Veterinary Medicinal Products (CVMP)

# CVMP assessment report for RenuTend (EMEA/V/C/005428/0000)

Common name: equine allogeneic peripheral blood-derived mesenchymal stem cells, tenogenic primed

**Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.**

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# Introduction

On 25 November 2020 the applicant submitted to the European Medicines Agency (The Agency) an application for a marketing authorisation for RenuTend, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope). The initial applicant for the product was Global Stem Cell Technology NV; however, due to the company's acquisition, the applicant changed to Boehringer Ingelheim Vetmedica GmbH during the procedure (17 December 2021).

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 July 2019 as RenuTend contains an active substance (equine allogeneic peripheral blood-derived mesenchymal stem cells, tenogenic primed) which was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

The applicant applied for the following indication: For the treatment of tendon and ligament injuries in horses.

The active substance of RenuTend is tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells. The target species is horses.

RenuTend contains 2.0-3.5 x $10^6$ tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells and is presented as a suspension for injection, for intralesional use, in packs containing 1 vial with one dose (1 ml) of the stem cell suspension.

The rapporteur appointed is Frida Hasslung Wikström and the co-rapporteur is Andrea Christina Golombiewski.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 16 February 2022, the CVMP adopted an opinion and CVMP assessment report.

On 19 April 2022, the European Commission adopted a Commission Decision granting the marketing authorisation for RenuTend.

## *Scientific advice*

The applicant received scientific advice from the CVMP on 16 April 2019. The scientific advice pertained to quality, safety and clinical development of the dossier. In general, the applicant has followed the scientific advice given.

Regarding quality, some identified deviations from the recommendations regarding e.g. specific acceptance criteria and risk evaluations were initially included in the list of questions and were adequately addressed.

Concerning the clinical development, the advice was received after the clinical studies had started. There were some deviations from the given advice (to include a co-primary clinical endpoint and to evaluate efficacy more frequently than every 8 weeks), which are not considered to have had any impact on the outcome of the assessment.

## *MUMS/limited market status*

The applicant requested classification of this application as MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) would be applied when assessing the application.

MUMS/limited market status was granted as horses is considered a minor species.

# Part 1 - Administrative particulars

## *Detailed description of the pharmacovigilance system*

The applicant has provided a detailed description of the pharmacovigilance system (dated January 2021) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

## *Manufacturing authorisations and inspection status*

The application includes a valid GMP certificate for the drug substance and drug product manufacturer, Global Stem cell Technology (GST), issued 4-9-2018 by the Federaal Agentschaap voor Geneesmiddelen en Gezondheidsproducten (BE/GMP/2018/123). The GMP certificate has also been verified in the EUDRA-GMP database. A GMP declaration for the active substance manufacturing site is also provided from the Qualified Person (QP) at the EU batch release site. Lastly, a manufacturing authorisation for GST issued on 2 January 2019 is also attached.

Manufacturing authorisation certificates (MIAs) are provided for all QC-testing laboratories. According to the submitted MIAs, more than three years have passed since the latest inspection for one of the QC-testing laboratories. However, a valid updated certificate exists in the EUDRA-GMP database. No further action is required.

Batch release takes place at Global Stem cell technology NV, Noorwegenstraat 4, 9940 Evergem, Belgium. A valid MIA and GMP certificate are provided.

## *Overall conclusions on administrative particulars*

The GMP status of both the active substance and finished product manufacturing sites has been satisfactorily established and is in line with legal requirements.

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

# Part 2 - Quality

## *Composition*

The veterinary medicinal product RenuTend consists of one vial containing 2.0-3.5 x $10^6$ tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells (tpMSCs; colourless clear suspension) as active substance, resuspended in dimethyl sulfoxide (DMSO) and Dulbecco's Modified Eagle Medium Low Glucose (DMEM LG) as excipients.

## *Containers*

RenuTend is presented in cyclo olefin co-polymer (COC) vials closed with sterile thermoplastic elastomer (TPE) stoppers and sealed with high density polyethylene (HDPE) caps. The material complies with the relevant European Pharmacopoeia (Ph. Eur.). The choice of the container closure system has been sufficiently justified and is considered adequate for the intended use of the product.

The product will be transported in suitable secondary and tertiary containers to maintain the required storage conditions.

## *Development pharmaceutics*

The active substance is the tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells (tpMSCs). A literature review on the mode of action and effectiveness of MSCs in the treatment of tendon injuries was performed. To date, fundamental mechanisms or patterns describing the survival, distribution and homing effects in horses are scarce. However, by tenogenic priming of the MSCs, the mode of action is proposed to be narrowed towards reduction of scar tissue formation and functional repair of the tendon. Moreover, priming MSCs avoids possible formation of ectopic tissues and ensures the presence of the desired tenogenic cell type. The use of tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells as active substance for RenuTend is sufficiently justified based on a comprehensive review of current scientific knowledge as well as on data resulting from relevant studies performed using the intended finished product.

All studies have been conducted using material from pilot batches. The representativity of these batches for the commercial drug product and therefore for the submitted process validation and stability studies has been sufficiently justified.

Evaluation of the genomic stability is performed using shallow whole genome sequencing, which compares the genome of RenuTend equine cells to a reference equine genome, with a mean genome wide resolution of 200 kb. Studies have been performed on different intermediate cell stock (ICS) batches originating from several donor horses and on several batches of tenogenic primed MSCs at passage (P) 10 originating from several donor horses. An additional characterisation study was conducted on several MSC isolates from several donors cultured beyond P10 (the intended final passage in the manufacturing process). Intermediate cell stocks, MSCs tenogenic primed from P9 to P10 and MSCs tenogenic primed from P14 to P15 showed no aberrations. In general, the proposed strategy for the control of genomic stability is considered adequate, based on the provided product-related data and on the current scientific knowledge extensively reviewed in the dossier.

Dimethyl sulfoxide (DMSO) and Dulbecco's Modified Eagle's Medium Low Glucose (DMEM LG) are used as excipients. DMSO is used at a concentration of 10%. This has been justified by a study which showed reduced cell viability at lower amounts. DMEM LG contains amino acids, vitamins, minerals and carbohydrates. It is used for a wide variety of cell culture applications and is reported to be a suitable component for freezing of equine MSCs according to literature.

In the early steps of product manufacture, antibiotics (penicillin, streptomycin and amphotericin B) are added to the culture media. According to theoretical calculations performed by the applicant, the manufacturing process is able to wash out these antibiotics to levels close or below the limit of detection (LOD). These data have been confirmed by an LC-MS/MS study showing that no levels of penicillin, streptomycin and amphotericin B are present in the final product above the LOD limits. Furthermore, the impact of residual antibiotics on the determination of the final product sterility was investigated in a growth promotion test. No interference was observed.

The potential residual level of biological materials used during the production has been adequately calculated and assessed.

In the absence of a defined master cell bank (MCB), the applicant has chosen to introduce an intermediate stage, intermediate cell stock (ICS), to test the characteristics of the isolated cells while providing a sufficient number of cells for release and release testing. This approach is considered acceptable. It is stated that after 10 passages some stem cell isolates demonstrated reduced proliferation rates. A passaging validation study was performed on MSC material demonstrating that the investigated quality attributes remained within the proposed specifications for all samples analysed at P10 and for 75% of samples analysed at P15.

Terminal sterilisation or filtration steps are not considered feasible due to the nature of the product and therefore maintenance of sterility throughout the production is essential. The sterility of the drug product (DP) is assured amongst others by manufacturing under GMP conditions, including the use of environmental checks, aseptic procedures, in-process and finished product sterility controls. The approach is endorsed.

**Potency assay**

The company proposed the decrease in ACTA2 gene expression as a surrogate marker for the tenogenic priming process and therefore for potency determination. The ACTA2 gene encodes for the protein alpha-smooth muscle actin (SMA) which is highly expressed in the cartilage oligomeric matrix protein, which is found in the extracellular matrix and is expressed by the (myo)fibroblastic phenotype of native MSCs. The relative decrease in ACTA2 expression levels of the cells is determined by PCR and the results are depicted as "fold change" values. Within the test, a tenogenic primed sample is compared to the non-primed, native control of the same sample and the level of decrease of the ACTA2 gene expression is analysed.

In order to support the link between the selected potency assay and clinical efficacy of RenuTend, the applicant introduces bibliographical references, (i) an *in vitro* study in which tenogenic primed RenuTend cells displaying various levels of ACTA2 decrease and related native MSCs were analysed for the expression of SMA, collagen (Col) I and Col III, (ii) a functional assay on tendon explants and (iii) a clinical proof-of-concept study.

i. The *in vitro* study performed on seven RenuTend batches with different values of ACTA2 decrease demonstrates a sustained relationship between the decrease of ACTA2 gene expression and the decrease of SMA and between the decrease of ACTA2 gene expression and the increase of Col I expression. Although not as pronounced as for Col I, a similar relationship was observed between ACTA2 gene expression and Col III protein expression. The presented results support the use of ACTA2 as a surrogate marker for the tenogenic priming of MSCs.

ii. An additional experiment designed to support the proposed potency test was performed using RenuTend batches with different values of ACTA2 fold were compared for the level of cell adhesion in a tendon explant assay. Scores for cellularity (cell adhesion) at the lesion site have been appropriately defined and representative pictures have been provided. Based on these scores, it can be agreed that RenuTend batches with high ACTA2 fold decrease levels promote sustained cell adhesion in explanted tendon biopsies when compared to batches with low ACTA2 fold decrease. Cell viability and seeding efficiency does not directly correlate with the levels of tenogenic priming as determined by ACTA2 fold decrease levels.

iii. In a proof-of-concept clinical study, blinded, randomised, placebo-controlled, statistically significant differences were observed between the test group and the placebo group in

terms of SMA distribution (0.5% in RenuTend-treated limbs vs 9.2% in placebo-treated limbs), collagen type I distribution (83% in RenuTend-treated limbs vs 50% in placebo-treated limbs), collagen type III distribution (0.5% in the RenuTend-treated limbs vs 11% in placebo-treated limbs) and *Von Willebrand* factor distribution (8.7% in the RenuTend-treated limbs vs 1.2% in placebo-treated limbs). These *in vivo* data support the results obtained in the above discussed *in vitro* studies in terms of SMA, collagen type I and collagen type III protein distribution at the treatment site and suggest increased vascularisation in the recovering tendons treated with the finished product.

The provided *in vitro* and *in vivo* data collectively indicate a biological effect of RenuTend and support the PCR-based ACTA2 assay as potency indicator for reduction of myofibroblast properties on the one hand and increase of tendon-specific properties on the other hand. The lower limit for the potency assay is supported by clinical data and *in vitro* results, indicating sustained biological activity.

**Immunophenotyping**

Following the recommendations of the International Society for Cellular Therapy (ISCT), flow cytometry is used for testing of purity and impurity for RenuTend. Three positive markers and three negative markers were used in order to immunophenotypically characterise equine MSCs. The markers as well as their limits for release are picked from the literature and are supported by batch data. Based on the limited number of batches analysed for RenuTend, these limits are considered to be relevant.

**Population doubling time**

Population doubling time (PDT) for each new intermediate cell stock is determined (from P1 to P10) in order to qualify these cells as suitable for culture up to passage 10 and tpMSC production. Additionally, PDT testing is included in the release of the final product, calculated during tenogenic priming, and the proposed release limits are adequately justified based on clinical and developmental data. The strategy is considered adequate.

## *Method of manufacture*

### *Manufacturers*

The applicant has listed DS/DP manufacturer, QC-testing facilities and infectious disease testing laboratories.

### *Manufacturing process*

The applicant has provided a brief summary of the production process for RenuTend including flow charts, in-process controls and final product control tests used to ensure the consistency and reproducibility of RenuTend. The manufacturing descriptions are generally considered brief but sufficiently detailed to grasp the process.

### *Process controls*

In-process controls (IPCs), tests on intermediate cell stocks and batch release tests are

implemented to ensure manufacturing consistency and reproducibility. The overall strategy is shown in the dossier in two manufacturing flow charts. In total, these controls include health status on donor horses, visual check of incoming blood, cell viability measurements, cell numbers, morphology, proliferation, trilineage differentiation, identity, purity, impurities, appearance, filling volume, packaging, sterility, endotoxins, and mycoplasma at different stages of manufacturing. In general, the process controls and limits are considered to include relevant tests to ensure consistent production. Specifications for intermediate cell stocks and batch release are provided and discussed elsewhere in the dossier.

***Environmental conditions***

The environmental conditions are only very briefly described. The applicant states that monitoring of the production process is performed according to GMP and a sufficiently detailed description of the aseptic process with results from the last three media fills has been provided.

***Process validation***

The manufacturing process has been validated using three pilot-scale batches obtained from different intermediate cell stocks and commercial batches. All process controls and final product testing met the listed acceptance criteria and the microbiological monitoring showed satisfactory results. A validation of relevant specification parameters has been performed for the filling process.

## *Control of starting materials*

## *Active substance*

The active substance in this product is tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells originating from peripheral blood of donor horses.

The origin of the donor horse(s) and their appropriate control is of importance with regard to the quality and safety of the product. For the selection and control, the applicant has taken the requirements for donor horses as specified in Ph. Eur. 0030 (immunosera for veterinary use) into account. The horses are tested in sufficient intervals for general health and diseases with a specific focus on relevant transmissible diseases. Testing for infectious diseases follows the EMA Guideline on the production and control of immunological veterinary medicinal products (Annex 2) (EMA/CVMP/IWP/206555/2010-Rev.1) and 'Questions and Answers on allogenic stem cell-based products for veterinary use: Specific questions on extraneous agents' (EMA/CVMP/ADVENT/ 803494/2016-Rev.1).

A risk assessment for potential contamination with extraneous agents according to the Ph. Eur. chapter 5.1.7 is included in the dossier. Equine encephalitis virus is not tested since it has been identified and isolated only in South Africa, which is deemed acceptable.

**Starting materials for the cultivation media**

Starting materials of animal origin used for the culture of the cells are tested for the presence of extraneous agents and especially extraneous viruses in line with the CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (Annex2) (EMA/CVMP/IWP/206555/2010-Rev.1) and Questions and Answers on allogenic stem cell-based products for veterinary use: Specific questions on extraneous agents

(EMA/CVMP/ADVENT/803494/2016) as well as the Ph. Eur. monograph 5.2.5. Substances of animal origin for the production of immunological veterinary medicinal products.

**Concluding remarks**

For most of the starting materials there is no Ph. Eur. monograph available. During the manufacturing process of RenuTend microbiological contamination is controlled in addition to testing for sterility, endotoxins and mycoplasma at batch release of the ICS and the final product.

For all starting materials, specifications and certificates of analyses are provided.

For the donor horses and the starting materials of biological origin, a risk assessment for potential contamination with extraneous agents according to the Ph. Eur. chapter 5.1.7 is included in the dossier. Justification is provided in case of omission of testing of specific extraneous agents as per CVMP guidance for extraneous agents testing.

Sufficient data are available and necessary measures have been taken to control the materials used during manufacturing of the active substance.

## *Excipients*

DMSO specifications are according to Ph. Eur. monograph 0763, current edition. Vendor specifications are tested for compliance according to Ph. Eur. monograph, which is deemed acceptable.

## *Excipients not described in a Pharmacopoeia*

DMEM LG control tests are performed by the manufacturer, including bacterial, fungal, pH and osmolality testing according to Ph. Eur. Appearance is tested by the manufacturer according to an internal company specification. Data for three batches are provided and in line with set specifications.

The choice and quality of excipients are deemed acceptable for their purposes.

## *Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies*

There are ingredients with biological origin used in the manufacture of RenuTend including mesenchymal stem cells (equine).

The components used for the manufacture of RenuTend are considered safe with regard to extraneous agents. Data have been presented, and measures have been implemented to support the safety of the product.

A scientific assessment to estimate the risk of transmission of TSE is provided in accordance with section 4 of Ph. Eur. Chapter 5.2.8 and found acceptable.

All components used for the manufacture of Renutend are from countries which are considered safe with regard to TSE. Sufficient data is available to support the safety of the substances. Furthermore, the target animal (horse) is not considered susceptible for TSE.

Where applicable, valid certificates of suitability (CEP) have been provided.

It can be concluded that the risk of transmission of TSE due to the administration of RenuTend to horses is negligible.

## *Control tests during production*

Control tests for the intermediate cell stock have been established at step 4 and 5 of the manufacturing process. Testing before freezing includes trilineage differentiation, cell morphology, total cell number, viability, proliferation, appearance and packaging. Tests after freezing include identity and purity, impurity, sterility, endotoxins and mycoplasma. For all tests an appropriate description is provided. Since the same tests are performed for the final batch release, validation results are discussed in part 2E of the dossier.

The choice of methods used to control the intermediate cell stock is acceptable, although only briefly described.

## *Control tests on the finished product*

The final product consists of one vial containing the stem cell suspension tested before and after freezing. Testing before freezing includes cell morphology, total cell number, viability, proliferation, packaging, filling volume and appearance. Tests after freezing and thawing include identity and purity, impurity, potency, sterility, endotoxin testing and testing for mycoplasma.

The specifications and release limits are addressed in the studies presented and overall are considered adequate. Data from clinical and developmental batches support the proposed acceptance criteria for the quality attributes included in the release specifications.

The information provided in part 2E of the dossier, although rather limited for many of the methods, is complemented by relevant method descriptions included in standard operation procedure annexes. The totality of the documentation, including the attached validation reports, is considered acceptable.

## *Stability*

### *Intermediate cell stock:*

Data have been provided for 3 batches of intermediate cell stocks stored at -80 °C. One batch has been stored for up to 64 months, one batch for 53 months and one batch has been stored for 48 months. The claimed shelf life of the intermediate cell stock is 64 months.

The test panel includes appearance, cell count, viability, morphology, population doubling time, and sterility including endotoxins and mycoplasma and all acceptance criteria have been met in the studies.

Effects of freezing on cell identity, impurities and trilineage differentiation have been evaluated for one intermediate batch after freezing.

The applicant is recommended to include a tpMSC batch produced from the ICS which is the closest to the current proposed shelf life in the first on going stability study.

### *Drug product – finished product:*

The applicant has provided long-term stability data for three batches of drug product, tenogenic primed mesenchymal stem cells (tpMSCs), at -80 °C for up to 30 months. Three additional batches are currently in ongoing studies with up to 18 months' data. Testing includes appearance, cell count, viability, morphology, cell identity, impurity, potency, population-doubling time and sterility,

endotoxin and mycoplasma. A brief justification for the extent of the test panel has been included. All results were within the acceptance criteria.

In addition to the long-term stability studies at -80 °C, the tpMSCs have also been tested for long-term storage in liquid nitrogen (-196 °C) and the influence of short-term temperature excursions (-20 °C, -60 °C, -80 °C and -196 °C). Inverted storage has not been assessed.

The shelf life of the drug product of 24 months is considered acceptable.

Studies to determine in-use shelf life of RenuTend have not been performed. This is acceptable, as the product is presented as a single dose and administered directly after thawing.

### *New active substance (NAS) status*

The applicant requested the active substance, equine allogeneic peripheral blood-derived mesenchymal stem cells, tenogenic primed, contained in the above medicinal product to be considered a new active substance, and provided justification that at the time of submission of the application there was no other veterinary medicinal product authorised in the EU containing tpMSCs as active substance.

Based on the review of data on the quality-related properties of the active substance, it is considered that equine allogeneic peripheral blood-derived mesenchymal stem cells, tenogenic primed is to be qualified as a new active substance.

### *Overall conclusions on quality*

In general, the dossier submitted for RenuTend is of appropriate quality and provides adequate information on the development, manufacture, and control of the finished product.

### *Recommendations:*

The applicant is recommended to provide the following data post-opinion:

A tpMSC batch produced from the ICS which is the closest to the current proposed shelf life in the first ongoing stability study should be included.

## Part 3 – Safety

The active substance of RenuTend, i.e. tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells (tpMSCs), is a new active substance not authorised for a veterinary medicinal product in the EU before. Safety documentation in accordance with Article 12(3)(j) has been provided. Adequate justifications have been provided as appropriate for any lack of data.

### *Safety documentation*

### *Pharmacodynamics*

See part 4.

## *Pharmacokinetics*

See part 4.

## *Toxicological studies*

No toxicity data for tpMSCs in laboratory animal species were provided. This is considered acceptable, as the general study data requirements for pharmaceutical products do not apply for stem cells, which are the active component contained in RenuTend. No relevant guidelines for stem cells are available, therefore recommendations regarding development plans and evaluation requirements are given on a case-by-case basis for each product by the CVMP via the scientific advice procedure. The major safety concern for a biological product containing stem cells is considered to be related to potentially malignant transformation of the cells and an ensuing tumorigenesis. No adequate *in vivo* models for investigating the tumorigenic potential in stem cells are available, albeit a well-controlled production process with adult mesenchymal stem cells that have been cultured for a limited number of passages and are controlled for identity, purity and genomic stability in terms of population doubling time (PDT) and karyotype is believed to indicate a low risk for tumorigenicity as stated in the relevant CVMP/ADVENT questions and answers document ("Questions and Answers on stem cell-based products for veterinary use: questions and answers guidance on tumorigenicity addressed by CVMP/ADVENT"; EMA/CVMP/ADVENT/791717/2016). For additional information see also part 2.

## *Single dose toxicity*

No single dose toxicity data relating to the active substance were provided. This can be accepted, as target animal safety, user safety and consumer safety are addressed by means of other studies. As stated in the document "Questions and Answers on stem cell-based products for veterinary use: specific question on target animal safety addressed by CVMP/ADVENT" (EMA/CVMP/ADVENT/791717/2016), it is considered that investigation of the safety of an overdose does not provide significant value when the MSC-based product is administered locally.

A summary of $LD_{50}$ values for DMSO, which were estimated based on single dose studies from a set of bibliographic references, was provided. The relevance of these $LD_{50}$ values for the evaluation of target animal safety and user safety is considered to be low. The values reported in various laboratory species, ranging from 2,500 mg/kg bw following intraperitoneal administration in mice, ≤ 40,000 after intravenous administration in the dog to ≤ 50,000 mg/kg bw following cutaneous administration to rats and mice, respectively, do, however, indicate that the acute toxicity of DMSO is low.

## *Repeat dose toxicity*

No repeat dose toxicity data relating to the active substance were provided. This can be accepted, as target animal safety, user safety and consumer safety are addressed by means of other data.

A summary of maximum tolerable doses (MTDs) for DMSO, which were estimated based on repeated dose toxicity studies from bibliographic references, was presented. The relevance of the reported MTDs for DMSO (from 1,200 mg/kg bw/day following 24 days of intravenous administration in the dog to 11,000 mg/kg bw/day following 10 days of oral administration in the rat) for the evaluation of target animal safety and user safety of occasional exposure to DMSO is considered to be low.

The toxicity of DMSO is well known. DMSO has, with regards to residuals in human and veterinary medicinal products, been classified as a solvent with low toxic potential (less toxic in acute or short-term studies and negative in genotoxicity studies) but which, due to lack of long term or carcinogenicity data, should be limited. The permitted daily exposure (PDE) of humans and animals to DMSO via medicinal products should be adequately controlled via GMP or other quality-based systems in order not to exceed the PDE of 50 mg/day (ICH guideline Q3C [EMA/CHMP/ICH/82260/2006] for human medicinal products and VICH guideline 18[R] for veterinary medicinal products [EMA/CVMP/VICH/502/99-Rev.1]). As the user will be exposed to DMSO on single occasions and as a PDE for DMSO has been set for humans, the lack of repeated dose data and established No Observable Adverse Effect Level (NOAEL) for DMSO from laboratory animal species in the submitted dossier is not considered critical for the user risk assessment.

For the target animal, but also the user, information on the safety of the excipient DMSO is obtained from the combined target animal safety (TAS)/biodistribution study together with the field studies. For details and assessment of these studies, see part 4.

## *Tolerance in the target species of animal*

The tolerance in the target animal is described under part 4.

## *Reproductive toxicity*

*Active substance*

Equine allogeneic tenogenic primed mesenchymal stem cells

No data from reproductive toxicity studies of the tpMSCs in laboratory animals or in the target animal species have been provided. This is acceptable.

Although the results of the TAS/biodistribution study of RenuTend do not exclude the possibility of migration of cells from the injection site and formation of ectopic tissue, the risk for pregnant and lactating horses and potential impact on the fertility are considered to be low, provided there is an adequate control of the specificity and genetic stability of the tpMSCs.

For human cell-based medicinal products, there are no standard requirements for reproductive toxicity studies ("Guideline on human cell-based medicinal products" [EMEA/CHMP/410869/2006]). As RenuTend is xenogeneic to humans, the risk for pregnant women accidentally exposed to RenuTend is considered to be negligible. See the user safety assessment for more details.

*Excipients*

DMSO

Regarding the reproductive toxicity of DMSO, the applicant refers to a safety data sheet from a chemical company. The safety data sheet concludes that DMSO is not teratogenic at low levels regardless of the route of administration and that the teratogenicity of DMSO is dependent on the route of administration, the dose level and the gestation stage at exposure. For example, in a mouse teratogenicity study, a No Observable Effect Level (NOEL) of 12 g/kg/day was reported for a 50% DMSO solution given orally. Without access to the data underlying the conclusions of the safety data sheet, the conclusion on the teratogenic potential of DMSO cannot be verified.

Nevertheless, a PDE of 50 mg/day, i.e. 0.83 mg/kg/day, is set for the exposure to DMSO via human medicinal products (ICH guideline Q3C [EMA/CHMP/ICH/82260/2006]), which is also used for the user risk assessment (see repeated dose toxicity), and thus the lack of assessable data on

reproductive toxicity of DMSO can be considered acceptable, on the basis that this limit is not expected to be exceeded, therefore providing reassurance of a low risk for potential reproductive toxicity of DMSO.

This is further supported by the fact that no MRL is required for DMSO with respect to consumer safety according to Commission Regulation (EU) 37/2010.

Other excipients

See relevant paragraph at the end of the section.

## *Genotoxicity*

*Active substance*

Equine allogeneic tenogenic primed mesenchymal stem cells

No data from genotoxicity studies of the tpMSCs were provided. Due to the nature of the product this is considered acceptable.

According to the CVMP scientific advice (EMA/CVMP/SAWP/20042/2019), the tumorigenic potential of RenuTend is best controlled by the quality of the cultured product, i.e. by specifications of identity, purity and genomic stability in terms of PDT and karyotype (see part 2). Furthermore, in the "Guideline on human cell-based medicinal products" (EMEA/CHMP/410869/2006), it is stated that genotoxicity studies are not considered necessary for human cells, unless the nature of any expressed product indicates an interaction directly with DNA or other chromosomal material.

Provided the quality in terms of identity, purity and genomic stability of the stem cells is adequate and the data are sufficient to allow for a well-controlled production process, the risk for tumorigenicity of RenuTend is considered to be low.

*Excipients*

DMSO

With regards to the genotoxic potential of DMSO, the applicant refers to information or data from publicly available scientific literature. DMSO was reported to be non-mutagenic in *Salmonella*, *Drosophila* and fish cell cultures, whereas a significant increase in femoral bone marrow cells with chromosomal aberrations was reported in an *in vivo* cytogenetic study performed with DMSO (approximately 50 to 5000 mg/kg bw) administered by intraperitoneal injection to male rats. The rationale supporting the applicant's view that the chromosomal effects in femoral bone marrow cells is most likely caused by direct toxicity to the cells was not clear, neither was it confirmed by supporting data. However, as DMSO is classified as a solvent with low toxic potential based on e.g. negative results in genotoxicity studies and for which a PDE of 50 mg/day (0.83 mg/kg bw) has been set (ICH guideline Q3C on residual solvents in human medicinal products [EMA/CHMP/ICH/82260/2006] and VICH guideline 18[R] for veterinary medicinal products [EMA/CVMP/VICH/502/99-Rev.1]), the threshold for potential clastogenic effects of DMSO can be considered to be above 0.83 mg/kg bw, which is significantly higher than any quantity expected to be administered in the target species via single doses or to which a user might be exposed in a worst-case estimate. Further evaluation of the genotoxic effects of DMSO is therefore not considered necessary.

Other excipients

See relevant paragraph at the end of the section.

## *Carcinogenicity*

*Active substance*

Tenogenic primed equine allogeneic mesenchymal stem cells

In line with the CVMP scientific advice (EMA/CVMP/SAWP/80218/2015), no data from tumorigenicity studies were provided. No adequate *in vivo* models for investigating the tumorigenic potential of stem cells are available. The tumorigenic potential of RenuTend is therefore considered to be best controlled by the quality of the cultured product, i.e. by specifications of identity, purity and genomic stability of the stem cells (see part 2).

Provided the quality of the stem cells is adequate, and the data are sufficient to allow for a well-controlled production process, the risk of tumorigenicity emanating from RenuTend is considered to be low.

*Excipients:*

DMSO

The lack of information on the carcinogenic potential of DMSO is considered acceptable based on the fact that DMSO is regarded as a solvent with low toxic potential and of low risk for human health, and for which a PDE via pharmaceutical products has been set as laid out in ICH guideline Q3C on residual solvents in human medicinal products (EMA/CHMP/ICH/82260/2006) and VICH guideline 18(R) for veterinary medicinal products (EMA/CVMP/VICH/502/99-Rev.1). Furthermore, the target animal will be treated with single doses and users are expected to be exposed only on single occasions.

*Other excipients:*

See relevant paragraph at the end of the section.

## *Studies of other effects*

Except for the biodistribution part of the TAS study, no further special studies have been performed.

## *Other excipients*

In addition to DMSO, DMEM LG is also an excipient in RenuTend. DMEM LG was shown to consist of inorganic salts, amino acids and vitamins, which were concluded not to cause any concern for the safety of the target animal, the user or the consumer.

## *User safety*

The applicant has presented a user safety risk assessment which has largely been conducted in accordance with the CVMP "Guideline on user safety of pharmaceutical veterinary medicinal products" (EMA/CVMP/543/03-Rev.1).

The hazard of the product is considered to be related to the tpMSCs and DMSO, whereas DMEM LG, which contains amino acids, vitamins and inorganic salts, is not expected to raise any specific concern with respect to user safety.

Provided cell identity, cell duplication, exponential growth and karyotype are adequately controlled (see part 2), the tumorigenic potential of the tpMSCs is considered to be low. In addition, due to

multiple xenogeneic cell-surface antigens present on these cells, or due to secreted cellular components perceived as foreign, an efficient graft-rejection is expected to occur in exposed users.

The main potential routes of accidental contact with the product have been considered. It was concluded that the most likely routes are those of dermal and/or oral exposure and accidental self-injection, of which the latter is considered to be the worst-case scenario. The major risk in relation to accidental self-injection consists of local immune reactions at the injection site against the foreign cells. Provided the quality of the product is appropriate and adequately controlled, no severe physiologic or pathologic changes, including the potential formation of tumour cells, are expected after potential accidental self-injection. Expected adverse events may include pain, local inflammatory reactions, swelling at the site of injection (which the applicant claims will resolve after a few days, although no data have been presented to support this) and transient fever.

In immunocompromised users, it may be possible that an acute graft-rejection will not occur, although the xenogeneic tpMSCs are unlikely to survive and/or differentiate in the xenogeneic environment due to lack of necessary stimuli. For this reason, accidental self-injection of xenogeneic stem cells is also not considered to pose a risk for pregnant users.

DMSO is not considered to represent a significant risk for the user after single exposure. The PDE of residual levels of DMSO via pharmaceuticals has been established at 50 mg/day. Accidental injection of half the total (1 ml) product volume, i.e. 0.5 ml 10% DMSO, corresponds to the PDE for DMSO via intake of pharmaceuticals for humans. This is considered to provide an acceptable margin of exposure, particularly as the PDE value is established for long term exposure, while users of RenuTend would only be exposed on a single occasion.

Regarding user risk communication, information on expected adverse effects in relation to accidental self-injection, i.e. pain, local inflammatory reactions, swelling at the site of injection which may persist for several weeks, and transient fever has been added to section 4.5 of the SPC, together with standard advice/warnings for the liquid nitrogen container.

As a result of the user safety assessment, the following advice to users/warnings for the user are considered appropriate for section 4.5 of the SPC:

- When the product is stored in liquid nitrogen, direct exposure to liquid nitrogen or cold nitrogen vapours can cause extensive tissue damage or burns. When liquid nitrogen vapourises it can expand to 700-times its volume which may create an explosion hazard in unvented cryovials. Liquid nitrogen containers should be handled by properly trained personnel only. The handling of liquid nitrogen should take place in a well-ventilated area. Before withdrawing the vials from the liquid nitrogen canister, protective equipment consisting of gloves, long sleeves and a facemask or goggles should be worn.
- 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.

Based on the above risk assessment, the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the provisions of the SPC.

## *Environmental risk assessment*

According to VICH GL 6 ("Environmental impact assessment [EIA] for veterinary medicinal products"), the environmental risk assessment (ERA) can stop in phase I and no phase II assessment

is required because the veterinary medicinal product will be used to treat a small number of animals (e.g. not herd treatment), and consequently environmental exposure can be expected to be well below levels that would have an environmental impact.

RenuTend is not expected to pose a risk for the environment when used according to the SPC.

## *Residues documentation*

## *MRLs*

The active substance, tpMSCs, is a "chemical-unlike" biological substance for which an MRL evaluation is not required according to section I.6 of the Annex to Regulation (EU) No 2018/782. Accordingly, tpMSCs are covered by the entry "Stem cells" on the list of "Biological substances considered as not requiring an MRL evaluation as per Regulation (EU) No. 2018/782, with regard to residues of veterinary medicinal products in foodstuffs of animal origin (EMA/CVMP/572629/2019)".

DMEM LG contains amino acids, vitamins, salts and carbohydrates which are relevant nutrients to cells in culture. All components, except for ferric nitrate nonahydrate and sodium pyruvate, are covered by Regulation (EC) 37/2010 (i.e. all vitamins and amino acids as well as remaining salts covered by the entry for food additives with valid E-numbers) or the list of substances considered as not falling within the scope of Regulation (EC) No 470/20091 ("out of scope" list; D-glucose). Ferric nitrate nonahydrate and sodium pyruvate are not considered as being pharmacologically active at the dose administered to the target animal and are thus not considered to fall within the scope of Regulation (EC) 470/2009 when used as in this product. A worst-case consumer exposure estimate supports the view that this exposure would not represent a hazard for the consumer.

## *Residue studies*

## *Pharmacokinetics*

See part 4.

## *Depletion of residues*

Not applicable

## *Withdrawal periods*

As all constituents of the intended product RenuTend are either included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, considered as not falling within the scope of Regulation (EC) No 470/2009 or do not require an MRL evaluation as per section I.6 of the Annex to Regulation (EU) No 2018/782, a zero-day withdrawal period is accepted.

## *Overall conclusions on the safety and residues documentation*

RenuTend contains horse tpMSCs as active substance. Excipients are DMEM LG and DMSO. As the active component in RenuTend consists of stem cells, the general study data requirements for pharmaceutical products do not apply. The safety data package consists, with the exception of a

combined TAS/distribution study and quality data of the horse tpMSCs, of bibliographic data. This is in line with the CVMP scientific advice (EMA/CVMP/SAWP/20042/2019).

The major safety concern for a biological product with stem cells is considered to be related to potentially malignant transformation of the cells and an ensuing tumorigenesis. A well-controlled production process with adult mesenchymal stem cells that have been cultured for a limited number of passages and are controlled for identity, purity and genomic stability in terms of population doubling time (PDT) and karyotype is believed to indicate a low risk for tumorigenicity.

*General toxicity*

No general toxicity data of the tpMSCs or of the final product have been provided. Although the provided $LD_{50}$ and MTD values for DMSO are of limited value for the evaluation of acute and repeated dose toxicity, they do indicate that the acute toxicity of this excipient is low. A permitted daily exposure (PDE) of DMSO of 50 mg/day via pharmaceutical products has been set in relation to medicinal products (ICH guideline Q3C [EMA/CHMP/ICH/82260/2006] for human medicinal products and VICH guideline 18[R] for veterinary medicinal products [EMA/CVMP/VICH/502/99-Rev.1]) and, as the user of RenuTend is expected to be exposed only on single occasions, this PDE is considered a conservative toxicological threshold value for the user risk assessment of DMSO.

*Reproductive toxicity*

No reproductive toxicity data of tpMSCs have been provided. Even though the results of the TAS/biodistribution study of RenuTend do not exclude the possibility of migration of cells from the injection site and formation of ectopic tissue, the risk for pregnant and lactating horses and the potential impact on fertility are considered to be low, provided there is adequate control of the specificity and genetic stability of the tpMSCs. For human cell-based medicinal products, no standard requirements for reproductive toxicity studies (EMEA/CHMP/410869/2006) are available. As the cells are xenogeneic, the risk for pregnant women accidentally exposed to RenuTend is considered to be negligible.

*Genotoxicity and cancer*

Provided the quality of the stem cells is adequate and well controlled, the risk for tumorigenic effects of the tpMSCs is expected to be low.

The genotoxicity and carcinogenicity of DMSO has been adequately addressed.

*User risk assessment*

The main potential routes of accidental contact identified for the user include those of dermal and/or oral exposure and, as the worst-case scenario, accidental self-injection. Provided the quality of the product is adequate and well controlled, no severe physiologic or pathologic changes, including the formation of tumour cells, are expected after accidental self-injection of xenogeneic tpMSCs. As reflected in the risk communication, expected adverse events may include pain, local inflammatory reactions, persistent (for several weeks) swelling at the site of injection, and transient fever.

As the xenogeneic tpMSCs are unlikely to survive and/or differentiate in the xenogeneic environment due to lack of necessary stimuli, the risk for immunocompromised persons or for pregnant users and unborn children in relation to accidental self-injection of xenogeneic stem cells is also considered to be negligible.

DMSO is not expected to result in a significant risk for the user.

*Environmental risk assessment*

The environmental risk assessment stops in phase 1. RenuTend is not expected to pose a risk for the environment when used according to the provisions of the SPC.

*MRLs and withdrawal period*

The MRL status has been confirmed for the active substance, i.e. horse tpMSCs (not falling within the scope of the MRL regulation) and the excipient DMSO (no MRL required as stated in Commission Regulation [EU] 37/2010). The excipient DMEM LG is considered to be out of the scope of Regulation (EC) 470/2009 when used as in this product. A zero-day withdrawal period can therefore be accepted.

# Part 4 – Efficacy

## *Pharmacodynamics*

Published references were provided to describe pharmacodynamic properties of MSCs. Effects of MSCs are thought to result from multiple mechanisms that include anti-inflammatory, angiogenic, homing capacities and/or immunomodulatory effects. Anti-inflammatory effects of MSCs include suppression of both innate and adaptive immune cells such as macrophages, NK-cells and dendritic B-cells, CD8+ T-cells and CD4+ T-cells. Immunomodulatory effects that have been described for stimulated equine MSCs include decreased lymphocyte proliferation, increased prostaglandin E2 (PGE2) and interleukin-2 (IL-2) secretion and decreased secretion of tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). Although MSCs have generally been attributed to be immunomodulatory in the sense that they can reduce immunological activity, allogeneic MSCs in pigs and horses have been demonstrated to elicit immune responses *in vivo* despite a low immunogenic profile *in vitro*. This is thought to be caused by MHC mismatch between donor and recipient. In an *in vitro* assay (mixed leukocyte reaction) to evaluate the immunogenic potential of the MSCs, it was demonstrated that cells of RenuTend had very low immunogenic properties. Fundamental mechanisms or patterns describing the survival, distribution and homing effects of MSCs in horses are lacking in literature, but from studies done using equine MSCs it is indicated that these correspond largely to MSCs from other species in terms of characteristics and mechanism of action.

Alpha-Actin-2 (ACTA2) was selected as potency marker for the allogeneic MSCs, see part 2. The smooth muscle actin (SMA) protein is encoded by the gene ACTA2 and is incorporated in myofibroblasts that facilitate wound contraction and play a role in early post-inflammatory events. Increased SMA levels may result in abundant amounts of collagen type III that leads to formation of scar tissue, reduced blood flow, and chronic tendinopathies. It has been suggested that by tenogenically priming the allogeneic MSCs, the myofibroblastic phenotype is substantially reduced, as confirmed by reduced expression of ACTA2.

Two *in vitro* studies using horse tendon explants were presented. The studies showed that tpMSCs, *in vitro*, adhere to the lesion site after 24 hours of incubation. Cell adhesion to the lesion was higher in batches with ≥2.3-fold decrease in ACTA2 gene expression (so called potent batches), compared to negative controls and to batches with <2.3-fold decrease in ACTA2 gene expression. See part 2 for further information.

In a combined target animal safety (TAS) and proof of concept study, treatment with RenuTend was compared to placebo in horses with surgically induced lesions in the superficial digital flexor tendon (SDFT). Smooth muscle actin distribution in the tendon lesion was determined by immunohistochemistry 112 days after treatment. Results showed that the mean percent distribution

of SMA was lower in tendons treated with RenuTend compared to placebo. Further, it was shown that the mean percent distribution of collagen type I and von Willebrand factor (indicator of vascularisation) was higher in tendon treated with RenuTend, and that distribution of collagen type III was lower, compared to placebo. These results provide support of the mode of action of RenuTend by its beneficial effect on vascularisation and collagen production in the treated tendon.

## *Pharmacokinetics*

Traditional pharmacokinetic studies (absorption, distribution, metabolism, excretion) as requested for pharmaceutical veterinary medicinal products are not relevant for this type of biological product. Instead, it is of value for determining safety of the product to study biodistribution and migration of cells from the injection site to other tissues presenting a possible risk for entrapment, i.e. microvasculature, or ectopic tissue formation.

Biodistribution and ectopic tissue formation was evaluated by immunohistochemistry in the combined target animal safety/proof of concept study. The study evaluated the potential of the tpMSCs to migrate from the tendon to surrounding tissues and the cubital lymph node. Eight healthy horses were included in this study. A lesion in the superficial digital flexor tendon (SDFT) of both front limbs was created using a surgical model. RenuTend was administered intralesionally in one of the front legs of the horse and saline was administered to the other leg. At histopathological examination, no ectopic tissue was observed in any tendon, paratenon nor cubital lymph node of any horse. Biodistribution was investigated using an immunohistochemistry analysis to detect possible persistence of cells at the local injection site and possible biodistribution to tendon, paratenon and cubital lymph nodes. No evidence of biodistribution was demonstrated in any of the samples tested, indicating that biodistribution with persisting cells in the surrounding tissues or cubital lymph node does not occur to any considerable extent after intralesional administration. The expression of the biodistribution marker is considered a relevant marker to test for biodistribution.

## *Dose justification*

No specific dose determination studies were presented, which is acceptable considering that a clear dose-response relationship is not expected for this type of product and that the product has been classified as MUMS. The selection of the dose was based on data from preliminary and pilot studies. The applicant did not clearly present these data; however, since the selected dose was demonstrated to be efficacious and safe in the clinical trials, the dose was accepted. The dose is 2.0–3.5 x $10^6$ tpMSCs. A range is proposed for the final total cell number to account for variation occurring during the final production steps. In the clinical trials, safety and efficacy were studied for four different batches; in these trials, treatment was administered intralesionally at the dose of 3 x $10^6$ tpMSCs in 1 ml.

## *Dose confirmation studies*

No specific dose confirmation studies were presented. However, it is considered that the findings of a GCP-compliant combined target animal safety and proof of concept study and the results of the pivotal field trial provide sufficient support of the proposed dose of 2.0–3.5 x $10^6$ tpMSCs. A dose of 3 x $10^6$ tpMSCs was used for intralesional treatment of surgically induced tendon lesions in the combined target animal safety and proof of concept study. In the pivotal field trial, the same dose of 3 x $10^6$ tpMSCs was used.

The combined TAS/proof of concept study was performed using a model of surgically induced tendinopathy of the superficial digital flexor tendon (SDFT). A surgically induced lesion in the SDFTs of both forelimbs was made in eight horses under general anaesthesia. Seven days after surgery (day 0), RenuTend was administered intralesionally into one of the forelimbs and placebo (NaCl) was administered into the contralateral limb. Two different batches of RenuTend, originating from the same donor horse, were used. Efficacy of treatment was evaluated up to 16 weeks post-treatment (day 112) by tendon assessment (heat, pain, swelling, and limb circumference), lameness assessment (using the American Association of Equine Practitioners (AAEP) scoring), ultrasound assessment (echogenicity, fibre alignment, and anterior-posterior thickness), ultrasound tissue characterisation system (UTC), macroscopic pathology evaluation of tendon, paratenon, and cubital lymph node, histopathology, and immunohistochemistry.

Surgery only induced mild clinical signs and there were no differences in heat and pain between tendons treated with RenuTend compared to placebo. More swelling was observed after administration of RenuTend compared to placebo (see target animal safety section below). Only mild lameness was observed for a period up to 3 weeks after surgery (2 weeks post-treatment), and since horses had lesions in both legs, no conclusion can be drawn with respect to efficacy of treatment on lameness from this study.

Regarding ultrasound assessment, differences in echogenicity scores and fibre alignment scores were observed from 28 days post-treatment and onwards in favour of RenuTend. At day 111, 7 of 8 tendons treated with RenuTend had normal fibre alignment score (≥75% estimated parallel fibre bundle in the lesion) compared to 0 of 8 tendons treated with placebo. Echogenicity was normal in 3 of 8 tendons treated with RenuTend compared to 0 of 8 tendons treated with placebo at day 111. There was an increase in anterior-posterior thickness of the tendon compared to pre-surgery and pre-treatment, from 14 days after treatment and onwards, with no difference between treated limbs until day 84. At day 84 and day 111, the mean anterior-posterior thickness was 0.1 cm less in tendons treated with RenuTend compared to placebo.

Ultrasound tissue characterisation system (UTC) was used to discriminate between four different echo types generated by: (1) intact and fully aligned fascicles, (2) discontinuous and less aligned fascicles, (3) fibrillary matrix with accumulation of collagen fibrils not yet organised into fascicles, and (4) amorphous matrix and fluid. The results showed that from 6–8 weeks after treatment and onwards, areas with echo type 1 were larger in tendons treated with RenuTend compared to placebo, and that areas with echo types indicative of abnormal alignment of fascicles (types 3 and 4) were larger in tendons treated with placebo compared to RenuTend. Before surgery, 83% of the area generated echo type 1 (normal) in tendons treated with both RenuTend and placebo. At 111 days after treatment, 74% of the area generated echo type 1 (normal) in tendons treated with RenuTend compared to 41% in placebo. At day 112, lesions were still visible macroscopically in all the tendons, with visible discoloration.

All tendons were evaluated by histopathology with regards to fibre structure, fibre arrangement, roundness of nuclei, regional variation in cell density, vascularity, collagen content, glycosaminoglycan content, and presence of inflammatory cells. No differences between groups were observed for any of the parameters. Fibre structure was assessed as normal in 5 of 8 tendons treated with RenuTend compared to 8 of 8 placebo-treated tendons. Fibre arrangement was assessed as normal in 5 of 8 tendons treated with RenuTend compared to 7 of 8 placebo treated tendons. Hence, no treatment-related effect was observed at histopathology and it appeared to be a contradiction between the ultrasound results at day 111 and the histopathology results at day 112. The applicant was asked to discuss this issue. The applicant stated this could have been due to the sample for histopathology not being taken at the maximum injury zone and that the scoring system used might

not have been sensitive enough. Considering that this was an experimental study, not fully representative of the condition to be treated, and that it is not pivotal in terms of efficacy, this issue was not pursued further. However, it should be noted that a treatment effect was not supported by the histopathology findings.

Results of the immunohistochemistry showed that the mean percent distribution of collagen type I was higher, and distribution of collagen type III was lower in limbs treated with RenuTend as compared to placebo. Collagen type I is an indicator of tendon matrix synthesis and part of the healthy tendon, while a healing process is considered abnormal if an increased production of type III and V collagen is involved. It was further shown that the mean percent distribution of von Willebrand factor (an indicator of vascularisation) was higher, and the mean percent distribution of smooth muscle actin (SMA) was lower in tendons treated with RenuTend compared to placebo. Increased SMA levels may result in abundant amounts of collagen type III that leads to formation of scar tissue, reduced blood flow, and chronic tendinopathies. ACTA2, which has been selected by the applicant as the potency marker, codes for the protein SMA.

To summarise the results, differences in favour of RenuTend were observed at ultrasound and immunohistochemistry, which provide some support of efficacy and the mode of action of RenuTend in an experimental setting. Only mild clinical signs, including lameness, were observed after surgery. No difference at histopathology between limbs treated with RenuTend and placebo, and no apparent correlation of histopathology findings with the ultrasound results were observed.

This was an exploratory study with no predefined endpoint. The model is not fully representative of naturally occurring tendinopathy in the horse and results cannot be directly extrapolated to the field. However, it is of benefit that safety and efficacy have been explored under controlled conditions and with detailed follow-up.

### *Target animal tolerance*

One GCP-compliant, randomised, blinded, placebo-controlled combined TAS/proof of concept study was provided to investigate target animal safety of RenuTend, in addition to the safety data obtained from the clinical safety and efficacy trial and supplementary data from a small supportive study. Three additional laboratory studies using RenuTend were performed investigating the immunogenicity of the product with the aim to clarify the cellular and humoral immune response after single and repeated administration of tpMSCs.

The **combined TAS/proof of concept study** was performed using a model of surgically induced tendinopathy in eight healthy horses. A lesion in the superficial digital flexor tendon (SDFT) of both front limbs was created and RenuTend (IVP; two different batches, from the same donor horse) was administered intralesionally in one of the front legs of the horse and the control product (saline) was administered to the other front leg. Local reactions and evaluations of the tendons (circumference, heat, pain, swelling) were compared between IVP- and placebo-treated limbs, and laboratory parameters were compared between animals treated with different batches of the IVP. There were some differences noted between groups regarding the tendon evaluations performed on days 0-14 (circumference, heat, pain, swelling). On days 9 and 10, there was a slight increase in tendon circumference in the IVP-treated limbs compared to the placebo-treated limbs. Temperature at the injection site was slightly increased on days 0-14 for most limbs but there were no differences between treatment groups. No increase of pain to pressure was observed for any limb at any post-baseline day. No increase of swelling at the injection site compared to day 0 was observed for any limb at post-baseline days 1 to 4. Between days 5 and 10, an increase in swelling was observed for some limbs, all in the IVP group.

The mean sum of scores of heat, pain and swelling at the injection site were compared between groups, and no significant differences were found at any time point except for at days 9 and 10, where the sum score in any IVP-treated limb was significantly higher than in the placebo-treated limb. For all other parameters and timepoints there were no statistically significant differences between groups.

There was no increase in lameness score (AAEP) in any of the groups following treatment.

Results were outside the reference range for a number of laboratory parameters at occasional time points during the study. As had been pointed out to the applicant in the Scientific Advice (EMA/CVMP/SAWP/20042/2019), comprehensive evaluation of systemic safety parameters is not possible if the individual horse receives both IVP and CP. In the absence of a negative control group, evaluation of systemic effects based on haematology, serum chemistry, or adverse drug reactions is considered of limited value, since a causality assessment is not possible. However, all deviating values were either only marginally out of range, considered related to physiological processes, present already prior to treatment, or attributed to gastrointestinal parasitosis, and therefore regarded as unlikely to be related to the treatment.

A post-mortem examination was performed on day 112. No ectopic tissue was found at histopathological examination of tendon, paratenon or cubital lymph node. Biodistribution was evaluated by immunohistochemistry and there were no signs of biodistribution observed. At macroscopic evaluation of the tendons, the lesion was still visible in all limbs of all groups, as well as a discoloration of the tendon, indicating an effect related to the surgical model and/or injection rather than to the IVP.

**Supportive systemic safety data** from 18 horses was provided (RenuTend, n=9; *versus* untreated controls, n=9). A wide range of biochemical and haematological parameters were evaluated in this study, and values were within reference range for most of these parameters, or only marginally out of range. Some deviations were present prior to the administration of the tpMSCs or present in both treated and untreated animals. No findings were considered to be related to treatment. Results from this additional small study support the systemic safety of RenuTend in horses with naturally occurring tendon lesions.

The **pivotal field study** was a GCP-compliant, randomised, blinded, placebo-controlled multicentre study including 100 privately-owned horses with first-time unilateral superficial digital flexor tendon (SDFT) or suspensory ligament (SL) lesion by overstrain injury. RenuTend (IVP; two different batches, from two donor horses; groups T1-1, n=34, or T1-2, n=32) or saline (group T2, n=34) was administered intralesionally at the proposed dose. All horses were administered NSAIDs intravenously at the time of treatment which may have reduced inflammatory reactions and this aspect is reflected in the SPC. Safety evaluation consisted of clinical assessments, recording of adverse events and evaluation of local tolerance. Incidence of abnormal clinical signs, adverse events, serious adverse events, and suspected adverse drug reactions were recorded daily and were comparable between groups. None of the adverse events was regarded as related to the study medication (e.g. a few horses with nasal discharge, loose stool). Ectopic tissue formation was not detected by ultrasound or clinical examination on any occasion (up to one-year follow-up) in any of the treated horses.

To assess local tolerance of the intralesional injection, swelling, heat and pain to pressure at the injection site, and tendon circumference were scored at days 1 and 2 and compared to baseline. The percentage of animals with an increase in swelling was significantly higher in the control group than in the IVP group on day 1, and comparable between groups on day 2. The frequencies of scores for heat or pain at the injection site were comparable between groups. For all three signs combined, the incidence of worsening was comparable between groups. The scores for swelling, heat or pain to

pressure at the injection site did not increase by more than 1 score point (i.e. from 0=no pain to 1=slight pain etc.) in any of the cases. The increase of tendon circumference was higher in the control group than in the IVP group at day 1 and comparable at day 2. For all local reactions described in this field study, the frequencies ranged from common (more than 1 but less than 10 animals in 100 animals treated) to very common (more than 1 in 10 animals treated displaying adverse reactions). Local reactions (increased heat, pain at palpation, limb swelling and increased limb circumference) are reflected in SPC section 4.6.

Three studies were performed to investigate potential immunogenicity of RenuTend with the aim to clarify the cellular and humoral immune response after single and repeated administration of tpMSCs. In the first study, **immunogenicity investigation of tpMSCs**, eight horses from the combined TAS/proof of concept study were included. The results from a mixed lymphocyte reaction (MLR) assay, where peripheral blood mononuclear cells (PBMCs) from treated horses were co-cultured with tpMSCs to detect induction of cellular immune response after treatment, showed that the tpMSCs did not elicit a cellular immune response in any of the eight horses. A flow cytometric crossmatch assay (FCCA) was performed to investigate humoral immune response with development of donor-specific alloantibodies following treatment. The results showed that for seven of the eight horses there was no increase in levels of antibody binding to tpMSCs after treatment. One horse, with a pre-existing sarcoid, showed a significantly elevated level of antibody binding after administration of RenuTend. The presence of antibodies in this horse had no impact on the investigated clinical parameters. It was concluded that the administration of tpMSCs did not induce a cellular or humoral response.

In the second study, **immunogenicity after repeated injections of tpMSCs**, six horses suffering from naturally-occurring tendon injuries were treated with tpMSCs twice at 7-9 weeks interval. The results from the MLR showed that the mean PBMC proliferation rate of PBMCs from treated horses was significantly lower than the proliferation rate of the negative control samples, indicating that a cellular immune response was not induced by repeated treatment with tpMSCs. Results from the FCCA showed that no alloantibodies to tpMSCs could be detected, indicating that a repeated administration of tpMSCs did not induce a humoral immune response.

In the third study, **MLR – Field study tpMSCs**, 14 horses from the pivotal clinical trial were selected for an MLR assay. The results showed that the mean PBMC proliferation rate in treated horses was significantly lower compared to the negative control demonstrating that tpMSCs did not induce a cellular immune response in horses with naturally occurring tendon injury.

Taking into account all these results, it is concluded that the product in general is locally well-tolerated at the recommended dose, and that only mild local reactions at the injection site occurred after intralesional administration in the studies provided. Moreover, it is considered that the systemic safety of intralesional treatment with RenuTend has been adequately described and is acceptable.

## *Clinical field trial*

One pivotal multicentre randomised, blinded, placebo-controlled GCP compliant study was conducted to evaluate the efficacy and safety of RenuTend in the treatment of tendinopathy in horses under field conditions.

| ***A multicenter, randomized, blinded and blocked clinical field study to show the efficacy and safety of RenuTend in the treatment of tendinopathy in horses compared to a negative control (saline).*** | |
|---|---|
| Objectives | To evaluate the efficacy and safety of RenuTend in the treatment of |

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| | tendinopathy compared to a negative control in horses under field conditions. |
| Study sites | Multicentre (three sites in Belgium). |
| Study design | Randomised, blinded, placebo-controlled and blocked clinical field study. |
| Compliance with regulatory guidelines | GCP. |
| Interventions: Test product | IVP: RenuTend (two batches), $3x10^6$ tpMSCs in 1 ml. |
| Control product/ Placebo | CP: Vetivex 9 mg/ml (0.9% NaCl), 1 ml. |
| Animals | 100 horses (IVP=66, CP=34). The distribution of age, sex, breed, and discipline was comparable between groups. |
| Outcomes/endpoints | <u>Primary criterion:</u><br>o Relevant improvement (RI) = Fibre alignment score changed from score 2 or 3 on day 0 to score 0 on day 112±3.<br>Fibre alignment was measure by ultrasound at the maximum injury zone (MIZ) and graded according to the estimated percentage of parallel fibre bundles in the lesion: 0=≥75%, 1=50–74%, 2=25–49%, 3=≤25%.<br><u>Secondary criteria:</u><br>o Ultrasound assessment on day 56 ±3 and 112±3: lesion size, echogenicity score (0-3), anterior-posterior thickness.<br>o Tendon assessment: pain, heat, swelling, limb circumference on day 56±3 and day 112±3.<br>o Lameness assessment according to the AAEP score (0-5) on day 56±3 and day 112±3.<br>o Working status and owner's opinion regarding treatment effect on day 112±3.<br>o Re-injury rate.<br>o Question to the owners by phone on days 14±3, 28±3, 42±3, 70±3, 84±3 and 98±3 (only part of the study population): swelling, pain to pressure, heat, lameness, improvement, working status, re-injury, compliance to exercise scheme. |
| Method | Horses were sedated before administration. Administration of treatment was performed by ultrasound guided injection into the lesion. Horses received one concomitant treatment with NSAID intravenously.<br>All horses followed a pre-specified exercise scheme with stall rest day 0–3, and 5 minutes of walk x 3 per day between day 4 and 84. In horses that showed ultrasonographic improvement at day 56±3, exercise was gradually increased from day 85, to 30 minutes of walk |

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| | and 15 minutes of trot at day 112. If no improvement was seen at day 56±3, horses continued to be walked by hand for 5 minutes X 3 until the end of the study.<br><br>Study ended on day 112 ± 3, but follow-up was performed at day 168 ± 5 and 336 ± 5. |
| ***Results*** | |
| Outcomes for endpoints | Of the 100 included animals, 99 completed the study. One animal in the control group was prematurely removed at day 102. A modified ITT population (n=99) was used as the primary population for efficacy analysis at day 112 ± 3.<br><br>Primary criterion: Relevant improvement - a change in fibre alignment score from 2 or 3 at day 0 to 0 on day 112 ± 3:<br><br>IVP: 43/66 (65%)<br><br>CP: 3/33 (9%).<br><br>Difference (95% CI): 56.1 (40.9–71.2), p<0.001<br><br>Secondary criteria:<br><br>At ultrasound assessment, lesions were smaller and echogenicity scores and fibre alignment scores were lower in IVP compared to CP at day 56±3 and day 112±3 (p<0.001). There was no significant difference in anterior-posterior thickness. Results day 112±3: lesion size (area in $mm^2$): IVP=12.9, CP=35.2; lesion size (% of tendon): IVP=7.6, CP=19.1; normal echogenicity score: IVP=38/66 (58%), CP=4/33 (12%); normal fibre alignment score: IVP=43/66 (65%), CP=3/33 (9%); anterior-posterior thickness (mm): IVP=11.4, CP=11.6.<br><br>At tendon assessment, scores for heat, pain, and swelling were lower in IVP compared to CP on day 56±3 and day 112±3 (p≤0.002). Horses with normal scores day 112±3: heat: IVP=63/66 (96%), CP=21/33 (64%); pain: IVP=53/66 (80%), CP=12/33 (36%); swelling: IVP=24/66 (36%), CP=3/33 (9%). Limb circumference, compared to baseline, decreased more in IVP group than in CP group.<br><br>Lameness assessment scores were lower in IVP group compared to CP group at day 56±3 and 112±3 (p<0.001). Horses with no lameness on day 112±3: IVP=47/66 (71%), CP=8/33 (24%).<br><br>On day 112±3, 33/66 (50%) of horses in IVP group had returned to previous work level or returned to work compared to 1/33 (3%) in CP group (p<0.001).<br><br>On day 112±3, owners reported no more discomfort or remarkable improvement in 52/66 (79%) of horses in IVP group compared to 4/33 (12%) in CP group (p<0.001).<br><br>Observations from phone calls with owners: less pain, heat, lameness, and discomfort observed in IVP group compared to CP group at some of the time points. No difference in swelling. On day 98±3 one owner of |

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|---|---|
| | a horse treated with CP reported re-injury.<br><br>Follow up results for study days 168±5 and 336±5:<br><br>On day 168±5, 55/66 (83%) horses in the IVP group and 21/34 (62%) in the CP group were followed-up by veterinary assessment. On day 336±5, the equivalent numbers were 32/66 (49%) and 14/34 (41%). Overall, data were in line with results observed at day 112±3, although differences were not statistically significant for all parameters. Normal fibre alignment score was observed in 48/55 (87%) horses in the IVP group and in 4/21 (19%) of horses in the CP group on day 168±5 ($p<0.001$). The equivalent numbers on day 336±5 were 25/32 (78%) and 2/14 (14%) ($p<0.001$). |
| Adverse events | There were no systemic adverse events associated with treatment.<br><br>Mild injection site reactions, such as increased heat, pain at palpation, limb swelling, and increased limb circumference occurred very commonly during the first 10 days after administration in both treatment groups. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | The study was well designed and conducted, and the results support a treatment effect as significant differences were observed between horses treated with RenuTend compared to placebo for the primary variable and most of the secondary variables. The applicant has justified that ultrasound is a relevant method to study effect of treatment and this is the standard method to assess tendon healing over time. Efficacy is also supported by secondary parameters, including clinical parameters. Potential treatment with RenuTend can only be seen as a part of a multimodal treatment strategy as rehabilitation is expected to have a large effect on the healing of the lesion. |

Horses with first time overstrain lesions in the superficial digital flexor tendon of the front leg, or the suspensory ligament in the back or front leg were included. The parameters tested are considered relevant for demonstrating an effect of treatment on tendon/ligament injuries. Since the outcome of the evaluation of these parameters was positive, it shows that treatment has a beneficial effect on the tendon/ligament. The following indication is therefore proposed: "To improve healing of injuries of tendons or suspensory ligaments in horses". The exact duration of the injuries in relation to when treatment was administered is unknown, since this information was not collected at time of inclusion. It is expected that efficacy could differ depending on when treatment is administered, in relation to when injury occurred. The applicant retrospectively evaluated ultrasound images and found that the majority of horses had ultrasound findings that indicated an acute or subacute injury. However, a number of horses, equally distributed between groups, had ultrasound findings indicative of a more chronic injury. Data did not indicate reduced efficacy in these cases compared to acute/subacute cases. It is therefore accepted that the indication does not need to be restricted in terms of duration of the injury.

Horses from different equestrian principles were included, mainly show jumping and dressage, performing at training level or competition level at the time when injury occurred.

Two different batches of RenuTend (final product) from two donor horses were administered to horses in the IVP group. Data was presented separately for horses treated with different batches. No obvious difference in efficacy between batches was observed.

A single dose of NSAIDs was administered to all horses at the time of treatment and this information is included in the SPC. Further, detailed information about the method of administration of the product has been included in the SPC.

Efficacy was evaluated by ultrasound, lameness assessment, tendon assessment and by different questions to the owner. Horses were examined by veterinarians blinded to treatment. The primary endpoint was the relevant improvement in fibre alignment score (from score 2 or 3 at day 0 to score 0 at day 112 ± 3) based on ultrasound assessment. The choice of ultrasound as a method to primarily evaluate efficacy was based on results from the proof of concept study and published literature. The difference between groups in relevant improvement of fibre alignment score at day 112 ± 3 (primary endpoint) was 56% ($p<0.001$). Hence, the criterion for treatment success was met. In the group treated with RenuTend, 65% of horses showed an improvement from score 2 or 3 to 0 compared to 9% in the placebo group. This is considered to be a substantial difference. Results of secondary clinical parameters support a treatment effect as less lameness, pain, heat, swelling, and limb circumference were observed in horses treated with RenuTend compared to placebo both at days 56 ± 3 and 112 ± 3. The secondary ultrasound assessment criteria also support a treatment effect as lesion sizes were smaller, and higher frequencies of normal scores for echogenicity and fibre alignment score were observed in horses treated with RenuTend compared to controls. The difference between groups in anterior-posterior thickness was small, and not significant.

Results were also presented for the different types of lesions separately. Efficacy was considered comparable between injuries in the SDFT and SL. Overall, the results of the primary and secondary endpoints support a clinically relevant effect of treatment.

Two horses, both in the control group, received concomitant treatment during the study. None of the horses received non-medical treatment during the study (e.g. cooling, shock wave treatment).

Horses followed a pre-defined exercise scheme and were restricted to stall rest for the remaining time. In horses that showed ultrasonographic improvement at day 56 ± 3, exercise was gradually increased from day 85. Exercise was increased in more horses treated with RenuTend compared to placebo-treated horses. The approach to increase exercise in horses showing signs of healing is considered reflective of how the condition is handled in the field and is therefore considered appropriate. Advice on rehabilitation (box rest and increasing exercise) is included in the SPC.

One horse in the placebo group was re-injured during the study period (up to day 112 ± 3). Between day 112 ± 3 and 336 ± 5, six horses re-injured, five in the IVP group (of which one had had re-injury in another limb than treated) and one in the placebo group. Re-injury rate at this time point is likely affected by the fact that more horses treated with RenuTend had returned to work compared to placebo-treated horses.

In conclusion, the efficacy results demonstrated that RenuTend can be used to improve healing of injuries of tendons and suspensory ligaments in horses. Information that box rest and slowly increasing exercise under veterinary guidance is required as part of the rehabilitation is included in the SPC.

## *Overall conclusion on efficacy*

*Pharmacodynamics:*

Literature references describing properties of MSCs were provided in support of pharmacodynamics of RenuTend. Effects of MSCs are thought to result from multiple mechanisms that include anti-inflammatory, angiogenic, homing capacities and/or immunomodulatory effects. Although specific information on pharmacodynamic properties of RenuTend is lacking in the scientific literature, data from studies performed using equine MSCs indicate that these correspond largely to MSCs from other species in terms of characteristics and mechanism of action. The cells of RenuTend are tenogenic primed with the purpose of supressing the myofibroblastic phenotype of the cells, as confirmed by reduced expression of ACTA-2 which is used to determine the potency of the product. Results from a target animal safety/proof of concept study indicate that RenuTend has an effect on vascularisation and collagen production in the treated tendon.

*Pharmacokinetics:*

Biodistribution of RenuTend has been evaluated by detection of the expression of a MSC marker by immunohistochemistry, and results showed that biodistribution with persisting cells in the tendon, paratenon or draining lymph node does not occur to any considerable extent. The expression of the MSC marker is considered a relevant and specific marker to test for biodistribution. Histopathological examination did not reveal ectopic tissue formation in any tendon, paratenon nor draining lymph node.

*Dose determination:*

Dose justification was based on published data from studies using MSCs similar to this product. Safety and efficacy of the selected dose were confirmed in clinical trials.

*Tolerance:*

RenuTend was well-tolerated in a clinical field study at the recommended dose of $3 \times 10^6$ tpMSCs in 1 ml. In the TAS study, RenuTend was well-tolerated following a single administration of the recommended treatment dose. Mild and transient local reactions were seen in both control and treatment groups, and no treatment-related effects on systemic tolerance were demonstrated. Mild injection site reactions such as increased heat, pain at palpation, limb swelling, and increased limb circumference occurred very commonly during the first 10 days after administration and are listed as adverse reactions in the SPC.

Results from three immunogenicity studies showed that a cellular immune response was not induced by single or repeated treatment with tpMSCs in horses, nor was a humoral immune response induced.

*Efficacy:*

Efficacy was evaluated in one proof of concept study and in one pivotal field trial. Results showed that the product can be used to improve healing of injuries of tendons and suspensory ligaments in horses.

# Part 5 – Benefit-risk assessment

## *Introduction*

RenuTend is a tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cell suspension for intralesional administration intended for use in order to improve healing of tendon and suspensory ligament injuries in horses.

One dose of RenuTend contains 2.0-3.5 x $10^6$ tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells. The product is presented in packs containing 1 vial of 1 ml stem cell suspension.

The dossier has been submitted in line with the requirements for submissions under Article 31 of Regulation (EC) No 726/2004 of 31 March 2004. The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

The product has been classified as MUMS/limited market and therefore reduced data requirements apply that have been considered in the assessment.

## *Benefit assessment*

## Direct therapeutic benefit

The benefit of RenuTend is its efficacy to improve healing of injuries of tendons and suspensory ligaments in horses, which was investigated in a well-designed laboratory study and a well-designed field study conducted to acceptable standards.

## Additional benefits

RenuTend provides a new treatment for tendon and suspensory ligament injuries, and a new treatment possibility for a minor species.

## Risk assessment

*Quality:*

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

*Safety:*

*Risks for the target animal:*

Local safety of tenogenic primed equine allogeneic peripheral blood-derived mesenchymal stem cells in horses was confirmed in a target animal safety/proof of concept study and in the pivotal field trial. Mild and transient injection site reactions such as increased heat, pain at palpation, limb swelling, and increased limb circumference occurred commonly or very commonly, and this is adequately described in section 4.6 of the SPC. No treatment related systemic effects occurred. The target animal safety has been sufficiently described and is considered acceptable.

*Risk for the user:*

User safety for this product is acceptable when used according to the SPC recommendations.

*Risk for the environment:*

RenuTend is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

*Risk for the consumer:*

All constituents of the intended product RenuTend are either included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010, considered as not falling within the scope of Regulation (EC) No 470/2009 or do not require an MRL evaluation as per section I.6 of the Annex to Regulation (EU) No 2018/782 (the horse MSCs as the active substance). A zero-day withdrawal period is thus accepted.

## *Risk management or mitigation measures*

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks.

The withdrawal period is set at zero days.

## *Evaluation of the benefit-risk balance*

At the time of submission, the applicant applied for the following indication: "For the treatment of tendon and ligament injuries in horses".

The product has been shown to be efficacious to improve healing of injuries of tendon and suspensory ligaments and therefore the CVMP agreed to the following indication: "To improve healing of injuries of tendons and suspensory ligaments in horses."

Information on development, manufacture and control of the active substance and finished product has been presented and led to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Based on the data presented, the overall benefit-risk is considered positive.

## *Conclusion*

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for RenuTend is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above-mentioned medicinal product.

In addition, based on the review of data on the quality-related properties of the active substance equine allogeneic peripheral blood-derived mesenchymal stem cells, tenogenic primed, the CVMP considers that equine allogeneic peripheral blood-derived mesenchymal stem cells, tenogenic primed is to be qualified as a new active substance.

# Divergent position on a CVMP opinion on the granting of a marketing authorisation for RenuTend (EMEA/V/C/005428/0000)

We the undersigned wish to express a divergent position to the CVMP opinion on the application for the marketing authorisation for RenuTend, a mesenchymal stem cell product to "improve healing of injuries of tendons and suspensory ligaments in horses".

The undersigned is of the opinion that appropriate evidence has not been presented to document that the product can 'improve healing'. None of the endpoints assessed document tendon/ligament healing in terms of return of tensile strength and elasticity (i.e. true indicators of healing and function). To document that a product can improve healing, a long-term clinical trial is necessary, in which a sufficient number of horses (treated and controls) are followed long enough to document the return to full use as well as the rate of re-injury in the two groups. The effects documented with the product, RenuTend, in terms of improvement in various ultrasonographic and clinical parameters within the months after treatment, could all reflect anti-inflammatory actions of the stem cells. Mesenchymal stem cells are generally known to exert anti-inflammatory effects mediated by the production of PGE-2 and other molecules. There are ways in which short-term improvements could result in a worse prognosis in the long term. For instance, if the improvements result in using the horse too much and too soon (resulting in re-injury), or if the treatment changes the tissue matrix in a way that doesn't support full restoration of anatomy/physiology.

Amsterdam, 16 February 2022

Keith Baptiste

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