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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for HorStem (EMEA/V/C/004265/0000)

Common name: equine umbilical cord mesenchymal stem cells

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
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Introduction

The applicant EquiCord-Ymas S.L. submitted on 19 May 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for HorStem, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 9 July 2015 as HorStem contains a new active substance (umbilical cord mesenchymal stem cells) not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

The product is an equine umbilical cord mesenchymal stem cell (EUC-MSC) suspension for intraarticular injection in horse, a biological product that has immunomodulatory effects in joints. HorStem suspension for injection contains approximately 15 million EUC-MSC and is presented in packs containing 1 vial.

The applicant applied for the following indication: Treatment of lameness and other clinical symptoms associated with mild to moderate degenerative joint disease (osteoarthritis) in horses.

The applicant is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

The rapporteur appointed is Wilhelm Schlumbohm and the co-rapporteur is Cristina Muñoz Madero.

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

Re-examination

On 25 October 2018, the applicant submitted written notice to the Agency to request a re-examination of the CVMP opinion of 11 October 2018. The applicant requested the involvement of a specific expert group in the re-examination.

The rapporteurs appointed were R. Breathnach and F. Hasslung-Wikström.

The CVMP agreed to the establishment of a specific Ad Hoc Expert Group (AHEG). The AHEG consisted of experts in quality of stem cells, in clinical trials design preferably with expertise in statistics and in osteoarthritis in horses.

In the light of the scientific data available and the scientific discussion within the Committee, the CVMP re-examined its initial assessment concerning the points raised in the grounds for re-examination.

On 21 February 2019, the CVMP adopted a positive opinion, by majority, and on 6 March 2019, the CVMP assessment report.

On 19 June 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for HorStem.

Scientific advice

The applicant received scientific advice from the CVMP on 11 December 2014 (EMA/CVMP/SAWP/610718/2014). The scientific advice pertained to quality of the dossier. Further scientific advice was provided on 11 May 2017 (EMA/CVMP/SAWP/106862/2017) that pertained to quality and clinical development of the dossier and 13 July 2017 (SAWP/300813/2017) pertaining to quality of the dossier.

In general, the applicant followed the scientific advice. However, rather than to the quality part of the dossier, the questions put by the company in the first scientific advice request were related to general Good Manufacturing Practice (GMP) requirements (manufacture in classified/unclassified facilities). After having reviewed the dossier, the CVMP comes to the conclusion that GMP requirements should be implemented prior to the thawing of the cryopreserved cells of the master cell bank (MCB). The establishment of the MCB is a substantial part of the manufacture of the active substance and should be performed under GMP conditions.

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) data requirements would be applied when assessing the application. MUMS/limited market status was granted as target species horse is considered a minor species.

Initial assessment

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated February 2018) 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

Manufacture of the MCB, the active substance and the dosage form, including batch release, takes place at EquiCord-YMAS S.L., Alcorcon, Madrid, Spain. The site has a manufacturing authorisation issued on 10/11/2017 by Agencia Española de Medicamentos y Productos Sanitarios (AEMPS), Spain. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

As the active substance is manufactured at the same site as stated above, a GMP declaration for this manufacturing site was provided from the Qualified Person (QP) at this site. The declaration was based on an on-site audit by a third party.

As all steps subsequent to the collection of the umbilical cord are performed by EquiCord-YMAS and this site has a valid GMP certificate, it can be concluded that the MCB is established under adequate conditions.

MCB quality controls are performed by external companies. The companies perform only laboratory control tests. An updated ISO certificate for one company has been provided. For the other company no ISO or GLP certificate has been provided, however mycoplasma testing is carried out again at the step of the active substance by an external laboratory with GMP certification which CVMP acknowledges to be in compliance with the current CVMP Questions and Answers on allogenic stem cell-based products for veterinary use: specific questions on sterility (EMA/CVMP/ADVENT/751229/2016). The CVMP therefore accepts the applicant's justification for the

GMP status for the site.

Overall conclusions on administrative particulars

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

The GMP status of both the active substance and finished product manufacturing site, EquiCord-YMAS S.L., has been satisfactorily established.

Part 2 - Quality

Composition

The veterinary medicinal product HorStem is a cloudy cell suspension for intraarticular injection which contains the active substance allogeneic mesenchymal stem cells (MSCs) derived from EUC-MSCs after in vitro expansion. The active substance is suspended in excipient media which acts as diluent and cell preservative and is supplied as ready to use.

The product is presented as a single dose of 1 ml volume ($15 \times 10^6 \pm 20\%$ cells) in a sterile 2 ml disposable resin vial.

Containers

HorStem is presented in a 2 ml vial of a cyclic polyolefin polymer (resin) which is closed with a sterile bromobutyl rubber stopper (Type I) and sealed with a 13 mm flip-off aluminium capsule. Adequate information is provided with regard to specifications, testing and sterilisation methods.

Development pharmaceutics

The active substance of the product is in vitro expanded allogeneic EUC-MSCs. A literature review on the effectiveness of MSC treatment in osteoarthritis was done. Based on this review, a relationship between the effective doses and the size of the joint was found and a dosage ($15 \times 10^6 \pm 20\%$ EUC-MSCs) was chosen by the applicant. The efficacy and safety of the chosen dose was assessed in the clinical studies.

A serum-free and protein-free hypothermic (2-8 °C) preservation media was chosen as excipient of the finished product. Stability studies were performed to demonstrate that this excipient improves cell survival and shelf life of the finished product in comparison with other conventional cell therapy suspension diluents. It includes key ions at concentrations that balance the intracellular state at hypothermic temperatures as well as pH buffers, energy substrates, free radical scavengers, antioxidants and osmotic/oncotic stabilisers. Because of its low viscosity and aqueous base, finished product formulated in excipient media should be suitable for undiluted use for intraarticular injection.

Each step of the manufacturing process is considered critical in terms of sterility as it is not possible to terminally sterilise the cell-based product. An alternative sterility testing method is used and consists of a gas production measurement according to European Pharmacopoeia (Ph. Eur.) 5.1.6. BacT/Alert is an automated microbial detection system based on the colorimetric detection of CO_2 produced by growing microorganisms. The use of an alternative method for sterility testing for the MCB, active substance and finished product has been justified. Adequate data is provided regarding the BacT/Alert test method, the validation and detection limits. The results are available 7 days after finished product packaging; however 98% of contaminations are detectable within 72 h. Gram stain

testing is performed to have information on presence of microbiological contamination before batch release.

Sterility assurance of the finished product is critical in light of the fact that product may be administered prior to a final sterility result being obtained. Measures initiated regarding the final product after the detection of a positive sample are described. A control strategy in case a contaminated sample is injected is implemented.

Through stability studies the compatibility of the active substance with the excipient and the compatibility of the active substance and the excipient in the chosen container have been analysed. The excipient, ensures the maintenance of the cell number and cell viability for at least 14 days without altering the properties of the product. The resin vials do not adversely affect cell viability or total cell number. The storage of the product in the resin vials does not lead to interactions.

Overages have been considered in the application. A ready-to-use vial is provided per patient containing the cell dose in 1.05 ml.

Method of manufacture

The manufacturing process includes stages of obtaining the equine umbilical cord, the isolation, amplification and cryopreservation of the MSCs to obtain the MCB, the active substance production by continuous or discontinuous process and the finished product manufacturing. The different stages of the manufacturing process are described in detail in part 2.B and 2.C. Quality and in-process controls are established for the different steps to ensure the reproducibility of the process and batch-to-batch consistency.

The equine umbilical cord collection is performed in a non-classified environment (no GMP).

The concept of the preparation of a MCB is supported. All steps subsequent to the collection of the umbilical cord are performed by EquiCord-YMAS and this site has a valid GMP certificate. It can be concluded that the master cell bank is established under adequate conditions. The manufacture of the active substance and the finished product is performed under GMP conditions.

Allogeneic EUC-MSCs are the active substance of the product HorStem. The cells are isolated from the donor tissue umbilical cord and expanded in vitro after preparation of a master cell bank.

The EUC-MSCs can be obtained by a continuous (fresh) or discontinuous (frozen) manufacturing process.

The finished product manufacturing process starts with the harvested EUC-MSCs pool (active substance) obtained as a result of the continuous active substance manufacturing process or recovered from the discontinuous manufacturing process. The corresponding amount of active substance is taken from the cellular suspension to cover the requested batch size and the corresponding number of vials for reference sample and quality controls. The active substance is centrifuged, the supernatant discarded and the active substance pellet resuspended in the excipient media.

The finished product vials are closed with a rubber stopper and with a flip-off aluminium seal and kept refrigerated (2-8 ºC) until release and shipment. The vials are labelled according to their intended use.

Information is given about remnants of media, which could still be present in the resuspended cell suspension. Importance of homogenisation of the finished product is underlined and measures are described avoiding cellular aggregations and cluster formation. Information is provided regarding the control to detect cellular aggregations and cluster formation after active substance resuspension and

homogenisation. The vial contains 1.05 ml of the finished product to ensure that the dose of 1 ml is extractable for administration to the horse.

Validation data on the manufacturing were provided. Six finished product batches were manufactured. The active substance and finished product batches from three master cell banks from three different donors have been produced to comply with the required quality and in-process controls specifications.

Furthermore, critical key steps of the manufacturing process have been validated to justify the acceptance criteria of the quality and in-process controls. These steps include umbilical cord collection, master cell bank production, container (primary packaging) closure method and product shipment.

Control of starting materials

The following starting materials are described:

- Active substance: MSCs derived from equine umbilical cord.
- Excipients as listed in the section 6.1 in the SPC.
- Container closure system: resin vial, bromobutyl rubber stopper (Type I) and flip-off aluminium caps.
- Substances of biological origin: Equine umbilical cord, MCB, trypsin, foetal bovine serum (FBS), collagenase and other starting materials.

Active substance

The active substance consists of allogeneic MSCs derived from equine umbilical cord (EUC) and is expanded in vitro.

The following in-process and quality controls are described for the active substance:

Active substance identity and purity

Adherence to plastic: The adherence of the cells to plastic is confirmed by the active substance manufacturing process since the expansion of the cells is based on the adherence. The cellular morphology and cellular confluence is frequently monitored microscopically. The MSCs have a specific morphology and way of growth as they are adherent to plastic.

Specific surface antigen expression (EUC-MSCs phenotype): The active substance is analysed for the specific surface antigen expression by testing the cellular phenotype by flow cytometry technique,

Relevance and number of markers used for identity and purity have been further justified. Risks for potential contamination with other cells besides the umbilical cord derived MSCs are properly addressed and acceptable strategies are in place on how to guarantee a finished product free from these contaminants.

Multipotent differentiation potential to osteogenic, chondrogenic and adipogenic lineages: The differentiation capacity of the EUC-MSCs of the active substance is not included as release criteria of the batches because the differentiation protocols need several weeks to get reliable results. A differentiation study has been performed of the EUC-MSCs of the active substance. It has been shown that the cells have the capacity to differentiate.

In order to discard donor-derived genomic alterations, genetic stability analysis in each batch of MCB

was implemented as MCB quality control and will be performed at cryopreservation of the MCB, when a EUC-MSC sample will be taken for karyotype analysis. An overview of the tests performed on EU-MSCs up to MCB has been provided.

To ensure the absence of fibroblasts a quality control test has been implemented.

Active substance potency

Originally the potency of the active substance was only measured as number of total and viable cells and viability per cryovial performed by manual-dye exclusion method according to Ph. Eur. 2.7.29.

A potency test, which is functionally linked to the biological activity of the EUC MSC product, has eventually been developed and implemented on the CVMP's request within MAA procedure. The potency test is based on measurement of the paracrine effect of EUC-MSCs by a quantitative determination of an anti-inflammatory biomarker Prostaglandin E2 (PGE2) by a quantitative determination of PGE2 concentrations in cell culture supernatant. First, it was suggested to perform the potency test at MCB level because the functional test lasts several days. Due to a number of limitations identified for implementing the approach to test only at MCB level (unexpected events during production are not detectable, stability cannot be monitored, loss of secreting activity during manufacture not detected), a modified potency test was established and validated at active substance level (just before starting the final product preparation) that is based on the ability of the EUC-MSC to secrete PGE2 inherently. A scientific rationale has been provided based on literature where it is described that PGE2 is a main cytokine response of the immunomodulation of MSC, responsible for modulating the activation and proliferation of lymphocytes in both human and horses. MSCs secrete PGE2 that provoke a lymphocyte proliferation inhibition and the decrease of inflammatory cytokine production promoting an anti-inflammatory response in the joint due to the down regulation of the inflammatory cytokines (TNF-α, IL-1) and up regulation of the anti-inflammatory cytokines as TGF-β, IL-10.

A specification was established based on a study of the inherent PGE2 paracrine secretion of EUC-MSC at three different manufacturing steps. Potency data provided for active substances and corresponding finished product batches (investigated at day 14 of storage) showed a notable drop off of for each batch. Therefore if an active substance batch is released close to the release specification it could potentially be inefficacious by day 14. To address this concern the potential drop off seen with batches tested to day 14 should have been factored in to the specification set for release at active substance level to ensure that batches released are efficacious over the proposed shelf life of 14 days of the product.

Potency is tested at active substance level and the test takes 4-6 days to be performed. This means that batches are released before information regarding potency values is obtained. A detailed risk evaluation was performed to address potential risks of releasing the final product without having the results from the potency test and measures are implemented for the case of an out-of-specification result.

To simulate detection of an unexpected event occurring in the cells during the production process inhibitor was used to inhibit the inherent cellular PGE2 secretion and to determine the effect on the number of viable cells.

However, the suitability of the proposed potency assay to demonstrate potency and serve as an indicator for stability is not considered appropriately demonstrated. Apart from references to the literature provided PGE2 secretion has not been demonstrated to correlate with biological activity relating to efficacy of the product.

Mycoplasma

Mycoplasma determination is according to Ph. Eur. 2.6.7. Adequate information has been provided regarding the alternative PCR technique used.

Accumulative population doublings (APD)

The number of APD reflects the number of times a culture has doubled the number of initially seeded cells. In each harvest, the PD number is calculated and added to the PD number obtained in the previous passages, giving the number of APD. The APD specification for the active substance has been appropriately justified.

Endotoxins

The active substance is tested following Ph. Eur. 2.6.14. This determination is performed in sample from the culture medium supernatant. The specification has been justified.

Sterility

The active substance used in fresh (not cryopreserved) for the manufacture of finished product is not tested for bacteria and fungi before the finished product manufacture as the result of this test would be available 7 days after the use of the active substance. This test is directly performed in a sample of the finished product formulated with such active substance batch.

A sterility check of the active substance is included in the discontinuous manufacturing process. An alternative sterility testing method (according to Ph. Eur. 5.1.6) is used. Justification, validation and detection limits have been provided regarding the test method.

Genetic stability of active substance

The genetic stability of the active substance EUC-MSCs is important. Cells could have genetic alterations which are derived from the donor itself or from the cell culture process which involves a continuous proliferation and the consequent DNA duplication.

In order to ensure that the cell culture process does not involve genetic alterations, a study was performed. The absence was demonstrated on each of the different batches of equine EUC-MSC. Chromosomal alterations were assessed by cytogenetic study (karyotyping through G-banding). Further growth behaviour was characterised.

Further in-process controls are performed:

- Cell morphology and confluence is monitored periodically during the cell amplification by microscope.
- Determination of the number of viable cells and viability are carried out in each harvest according to Ph. Eur. 2.7.29.
- A range for cellular seeding density is established.

Potential non-cellular impurities: Theoretical calculation indicates that process-related impurities could be present in the final product at very low levels. It is not necessary to set specifications for quantifying these impurities.

Validation of active substance manufacturing process

The continuous manufacturing process and discontinuous manufacturing process were validated using three different MCBs to obtain three active substance batches. The validation of the active substance manufacturing process encompasses the detailed processing of active substance batches which have been manufactured to demonstrate a reliable production process. From the results provided it can be

concluded that the active substance batches produced by the continuous manufacturing process or by the discontinuous manufacturing process are of consistent quality with regard to the performed controls and defined specifications. Certificates of analysis are provided for three active substance batches. The results comply with the acceptance criteria.

Excipients

The excipient is an intracellular-type solution that balances the altered cellular ion concentrations which result from hypothermic temperatures and from nutrient-deprived conditions that exist when cells are without normal culture conditions.

Additional components include pH buffers, energy substrates, free radical scavengers and osmotic/oncotic stabilisers. Main component of the excipient is Dextran-40, a hypertonic colloidal solution is used as plasma volume expander. Dextran-40 10% is used at a dose of 1 ml in the veterinary product.

A summary of the manufacturing process of the excipient is provided as well as information on the quality control tests. The analytical methods are described and acceptance criteria are provided. A certificate of analysis is presented.

The information provided on the excipient is acceptable.

Container closure system

The vial is characterised by the following features: low moisture absorption and water vapour permeability, excellent transparency, high solvent and heat resistance (no deformation after autoclaving at 121 °C), can stand up to a temperature of -196 °C, compatible with wide range of pH (from 2 to 12), no adherence of water and drug preparations in liquid form to the surface of the vial, low extractable.

A declaration confirming that both the vial and the stopper meet pharmacopoeial specifications (Ph. Eur., United States Pharmacopeia (USP) and Japanese Pharmacopoeia (JP)) has been provided.

The information on the packaging materials is acceptable.

Substances of biological origin

Equine Umbilical Cord (EUC)

The MSCs used for the manufacture of HorStem are derived from EUC. The stud farm from where the EUCs are collected after birth of a foal has to follow the regulation of the responsible Spanish Regional Agricultural Office for equine life welfare and reproduction use. Permanent veterinary control, vaccination and de-worming plans are established. A quarantine program for the donors is in place to exclude any transmission of infectious diseases. This program has been created to certify the health status of the mares and foals from which the umbilical cords are collected for cell isolation.

The horses are clinically monitored, in particular regarding a spectrum of viral infections. Final diagnosis of an infection is performed during the quarantine period by clinical monitoring.

A ranking which indicates when and what kind of specific diagnostic measures should be chosen thus ensuring that only EUC and corresponding cells of healthy mares/foals are used. Within the first days of the foal's life, biochemistry, whole blood count, exclusion of equine infectious anaemia, and absence of clinical symptoms are investigated.

After the birth of a foal the umbilical cord is collected and handled according to a standard operation

procedure using a defined extraction kit. The umbilical cord is sent to the manufacturer (EquiCord-YMAS) refrigerated accompanied by health certificates of the foal and the mare.

The shipment follows Good Distribution Practises (GDP). A qualification report of each package is provided. Storage conditions for the extraction kit are defined. Furthermore serum and blood samples are collected from the foal and investigated.

Master cell bank (MCB)

The MCB is produced by EquiCord-YMAS and this site has a valid GMP certificate. It can be concluded that the MCB is established under adequate conditions.

After receipt, an inspection of the umbilical cord is carried out including visual control for length, colour, odour, humidity and completeness. The preparation of the umbilical cord to obtain pure Wharton's Jelly for further processing is described in detail.

The cells are seeded in cell culture flasks containing cell culture medium.

To increase the amount of MSCs, several cell culture media changes and one further passage (passage 1 of which the cells are frozen and tested by quality control) are performed.

Before freezing, cells are tested for viability, number of viable cells, number of population doublings, the absence of equine viruses and mycoplasma as well as sterility; the phenotype of EUC-MSC is also determined. The morphology of the EUC-MSCs is evaluated by microscopy in culture to exclude fibroblasts. However, the pure cell morphology evaluation should be completed by the routine use of other methods at MCB level to confirm the absence of fibroblasts.

Subsequently, the cells are cryopreserved as MCB. A tabulated overview of the controls during the processing from EUC-MSCs to MCB starting with the specifications of the umbilical cord up to the stage of passage-1 seeding is provided. The controls are also described.

Extraneous agents testing at MCB

A thorough risk assessment on extraneous viruses to be tested on EUC-MSCs at MCB level is provided. The absence of equine viruses is ensured by the quarantine program and specific analytical methods.

This approach is generally considered acceptable. The PCR methods and their suitability are described. The MCB is tested for parasites.

Validation of the MCB manufacturing process

The validation MSC-MCB manufacturing process encompasses the detailed processing of the three MCB batches which have been manufactured to demonstrate a reliable production process. From the results provided it can be concluded that the MCB batches are of consistent quality with regard to the performed controls and defined specifications. Three certificates of analysis representing the test results of three MSC-MCB passage-1 batches are provided. The batches are stored cryopreserved in liquid nitrogen at -196 °C. The results correspond to the specifications set.

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

In general, the starting materials of biological origin comply with the 'Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathies agents via human and veterinary medicinal products' (EMEA/410/01-Rev.3). The overall TSE risk associated with the product is considered negligible.

For FBS a TSE certificate of suitability (CEP) has been provided.

Control tests on the finished product

The following tests are proposed for the finished product specification:

Appearance and label of the finished product

All finished product vials are visually inspected to ensure that the vials are closed and sealed, the labelling is correct and readable and the packaging is correct.

The finished product is described as a cloudy colourless cellular suspension. The appearance of the finished product is checked by visual inspection and recorded.

Identification and assay of active substance

For the cellular characterisation of the active substance in the finished product the CHMP Guideline "Human Cell-Based Medicinal Product for Human Use" (EMEA/CHMP/410869/2006) and the "Reflection Paper on Stem Cell-Based Medicinal Products" (EMA/CAT/571134/2009) have been considered.

The finished product is not tested for specific surface antigen expression and multipotent differentiation potential. These parameters are tested on the active substance. Relevance and number of markers used for identity and purity have been justified.

Cellular morphology

The adherence to plastic of the EUC-MSCs is confirmed by cellular morphology.

The morphology of the EUC-MSCs is evaluated at active substance by inverse microscopy in culture to exclude fibroblasts.

Active substance potency in the finished product

Initially the potency assay was based on the manual-dye exclusion method according to Ph. Eur. 2.7.29.

As these potency tests are not fully linked with the biological activity of the product (functionality), originally a potency test based on measurement of PGE2 by a quantitative determination of PGE2 concentrations in cell culture supernatant was established at MCB level.

Due to a number of limitations identified for implementing the originally proposed approach to test only at MCB level (unexpected events during production are not detectable, stability cannot be monitored, loss of secreting activity during manufacture is not detected) the potency test was revised. It is now performed at active substance level and is based on the ability of the EUC-MSC to secrete PGE2 inherently.

Safety tests

Freedom of the finished product from adventitious microbial agents (viruses, mycoplasma, bacteria, fungi) is addressed by the applicant.

Bacteria, bacterial endotoxins and fungi

Tests are done according to Ph. Eur. monographs, except for the Gram staining.

- Endotoxin test: according to Ph. Eur. 2.6.14. It has been justified that this amount of endotoxins is safe in the target species.

- Test for bacteria and fungi: Alternative method according to Ph. Eur. 5.1.6.
- Test for bacteria: Gram staining method.

Validation/suitability studies for the methods in compliance with the requirements of Ph. Eur. 5.1.6 are provided.

Product release is prior to obtaining final sterility test results and therefore the product will be administered before final sterility test results are available. The actions to be taken in the event that the sterility test result is "not sterile" after the product is administered to the patient (e.g. notifying the treating veterinarian) are described.

Mycoplasma determination

Mycoplasma determination is performed in one sample from the culture medium supernatant just before the cellular harvest at passage 4 in the continuous process or at active substance recovery phase in the discontinuous process. The pool is analysed by PCR using according to Ph. Eur. 2.6.7.

Virus contamination

A test for virus contamination is not carried out in the finished product batch. The reasons for not testing the finished product (testing was done for donor tissues, as well as media and reagents used during production; manufacture was done under GMP conditions) were provided and are acceptable.

Impurities at finished product

Cellular impurities

Dead cells: The cellular viability is determined by two quantifications using the manual-dye exclusion method.

Non-cellular impurities

In the finished product potential impurities deriving from the manufacturing process are present as in the active substance. Theoretical calculation indicates that process-related impurities could be present in the final product at very low levels. It is not necessary to set specifications for quantifying these impurities.

Quality control reports

Quality control reports for six batches of the finished product are presented. The batches were manufactured using fresh active substance from the continuous process (3 batches) or active substance recovered from the discontinuous process (3 batches). From each of these 6 finished product batches 15 vials (i.e. large batch size) were produced. The results correspond to the specifications set.

Stability

Stability studies were performed on the MCB, the cryopreserved active substance and the finished product. The following recommendations and guidelines are taken into consideration "Human Cell-Based Medicinal Product for Human Use" (EMEA/CHMP/4108869/2006), "Stability Testing of Biotechnological/Biological Products" (ICH Q5C) and "Quality data requirements for veterinary medicinal products intended for minor uses or minor species" (EMEA/CVMP/QWP/128710/2004).

Active substance

MCB stability

MCB stability of EUC-MSCs frozen at -196 ºC in liquid nitrogen was shown for 3 MCB batches at

24 months post MCB freezing by determining viable cellular number and viability using the manual-dye exclusion method (Ph. Eur. 2.7.29). PGE2 secretion of MCB was also tested at 24 months post MCB freezing.

The data presented show that investigated MCB batches fulfil the acceptance criteria related to the number of viable cells and viability at 24 months post MCB freezing. Therefore a minimum shelf life of 24 months is acceptable for MCB, stored in liquid nitrogen at a temperature of -196 ºC.

A new stability study for further MCB produced at EquiCord-YMAS S.L. is currently ongoing and the results will be provided when available.

Active substance stability

Stability of the active substance stored at -196 ºC in liquid nitrogen was demonstrated for 24 months by determining cellular recuperation (equivalent to viable cellular number) and viability using the manual-dye exclusion method (Ph. Eur. 2.7.29).

The data presented show that investigated active substance batches stored in liquid nitrogen for 24 months fulfil the acceptance criteria corresponding to the in-process controls for cellular recuperation and viability, established for the active substance after thawing and recovery in the discontinuous manufacturing process.

A new stability study to revalidate the manufacturing process of the active substance at the manufacturing site EquiCord YMAS has been initiated and results will be provided when available.

A test to measure the potency at active substance level has been established. This test will be included in the new stability studies of the active substance and finished product currently ongoing at EquiCord-YMAS.

Finished product

Finished product stability

Finished product stability under refrigerated conditions (2-8 °C) is shown for 14 days post-packaging.

Three batches manufactured according to the standard operating procedures established for the finished product manufacture and filled into vials were placed in stability chambers on up-right and inverted positions. Cellular viability and number of cells per vial (total cell concentration) are determined. All the batches met the acceptance criteria at all the time-points.

In a new stability study at final product level, potency using the PGE2 assay was tested after 14 days of storage. The finished product batches were produced by using the active substance from three different donors (a total of three final products, one final product vial per active substance donor). The potency value established for active substance batch release was taken as reference. It could be shown that the secretory activity of the cells remains over 14 days after storage of the finished product although a notable drop off was observed. As the batches were not tested at D0, the drop off over 14 days resulted from the value obtained for the active substance used in each finished product tested before final formulation. To address this concern the potential drop off seen with batches tested to day 14 should have been factored in to the specification set for release at active substance level to ensure that batches released are efficacious over the proposed shelf life of 14 days of the product.

As the suitability of the proposed potency assay to serve as an indicator for stability is not considered appropriately demonstrated, stability has not been sufficiently shown.

The compatibility of the active substance with the excipient and the compatibility of the active substance and the excipient in the chosen container have been analysed and are satisfactory.

Overall conclusions on quality

The manufacturing process comprises: obtaining the EUC, isolation, amplification and cryopreservation of the MSCs to obtain the MCB, active substance production and finished product manufacturing. Different stages of the manufacturing process are described in detail. Validations were carried out of the critical key steps of the process to justify the acceptance criteria of quality and in-process controls.

The starting/raw materials involved in the production of the active substance are of biological and non-biological origin. Basic description and certificates of analysis are provided. The characterisation of the active substance and its impurities are presented. The TSE risk is considered negligible. The TSE certificate of suitability for the FBS has been provided. The information on the excipients and packaging materials is acceptable.

A number of quality and in-process controls are established for the different manufacturing steps to ensure the reproducibility of the process and batch-to-batch consistency. The control methods are briefly described and specifications are set.

Identity and purity are controlled and the relevance and number of markers used are justified. An additional marker complementing those already selected was chosen; its relevance was shown.

To ensure the absence of fibroblasts a quality control test based on the morphological differences of EUC-MSC and fibroblasts has been implemented at MCB and active substance level. The pure cell morphology evaluation at MCB level should be completed by the routine use of immunocytostaining assay to confirm the absence of fibroblasts.

Risks for potential contamination are properly addressed and acceptable strategies are in place to guarantee a finished product free from contaminants. Safety is ensured by testing for sterility, mycoplasma, endotoxins and viruses.

Potency has been originally defined as the amount of active substance and the cellular viability present in the finished product and was expressed quantitatively by cell number and cell viability. In addition, the link between the results of the proposed potency test and the biological activity relating to efficacy of the product has not been demonstrated.

The newly proposed potency test is based on measurement of PGE2 by a quantitative determination of PGE2 concentrations in cell culture supernatant. Initially it was performed at MCB level because the functional test lasts several days due to the stimulation process, but it was not able to show consistency of production and to detect if unexpected events during active substance production occurred. Therefore, a potency test at active substance level has been established and validated based on the inherent secretion of PGE2. A specification was set. Potency data provided for active substances and corresponding finished product batches (investigated at day 14 of storage) show a notable drop-off. A possibility to counteract this effect could be factoring it in to the specification set for release at active substance level to ensure that batches released are efficacious over the proposed shelf life of the product.

However, the suitability of the proposed potency assay to demonstrate potency and serve as an indicator for stability is not considered appropriately demonstrated. Furthermore, PGE2 secretion has not been demonstrated to correlate with biological activity relating to efficacy of the product. Genetic and phenotypic stability of the active substance is investigated. Furthermore, sterility assurance of the finished product is critical in light of the fact that the product may be administered prior to a final sterility result being obtained. Therefore the use of an alternative method for sterility testing for the MCB, active substance and finished product has been justified. Data is provided regarding the test method, the validation and detection limits.

The manufacturing process was validated using batches of EUC-MCS from the continuous and discontinuous manufacturing processes. Six batches of the finished product manufactured using fresh active substance from the continuous process (3 batches) or active substance recovered from the discontinuous process (3 batches) have been produced. From each of these 6 finished product batches, 15 vials were produced for the validation of the manufacturing process. The active substance batches were received from three master cell banks from three different donors. The batches complied with the required quality and in-process controls specifications.

Stability studies are performed on the MCB, the active substance and the finished product. MCB has been demonstrated to be stable for 24 months when stored in liquid nitrogen. Active substance stability has been shown for 24 months when stored in liquid nitrogen. Finished product stability has been investigated under refrigerated conditions (2-8 ºC) and a shelf life period of 14 days after packaging has been established. However, in the absence of an assay determined to be relevant as a potency indicator, the proposed shelf life of the active substance and drug product are not considered fully demonstrated.

It could be shown that the secretory activity of the cells remains over 14 days of storage of the finished product although a notable drop off was observed. However, the notable drop off should have been factored in to the specification set for release at active substance level.

In conclusion, concerns remain regarding the establishment of an appropriate test to measure the potency of the active substance and at finished product level (test should correlate to biological activity) to show consistency of production, stability of active substance and finished product, and to detect if unexpected events during active substance production occurred.

Part 3 – Safety

HorStem product is composed of a sterile vial containing EUC-MSCs in a concentration of 15 x 10^6 cells (± 20%) in 1 ml of excipient.

The excipient is a serum/protein-free hypothermic (2-8 ºC) preservation media that enables improved and extended preservation of cells.

Safety documentation

Pharmacodynamics

Please refer to Part 4 - Efficacy.

Pharmacokinetics

Conventional absorption-distribution-metabolism-excretion (ADME) studies with HorStem are not available but are considered not appropriate to determine the fate of administered stem cells in the body. Some limited information on *in vivo* distribution/disposition and migration/persistence of stem cells in laboratory animals was available and the applicant used extrapolations from these data to derive conclusions on "pharmacokinetics" of the product HorStem.

In order to demonstrate the fate of MSCs after intraarticular administration, which is the intended use of the product, three publications on biodistribution of human MSCs in immunodeficient (Shim et al, 2015, Toupet et al, 2013) and immunocompetent mice (Toupet et al, 2015) were submitted. After xenogeneic, intraarticular (i.a) transplantation of MSCs to immune deficient mice small amounts of MSCs resided in the joints, with only 15% of the i.a-injected adipose derived (AD)MSCs determined in the joint for the first month and 1.5% of the AD-MSCs engrafted over the long term, at least 6

months (Toupet et al., 2013). In another investigation in immune deficient mice, human bone marrow derived clonal mesenchymal stem cells (hcMSC) levels were mainly distributed to the joint tissue after intra-articular transplantation (application site) at 8 hours after injection. The levels in joint tissue decreased over time, but hcMSC could still be detected at 120 days post-dose (Shim et. al., 2015). After intraarticular transplantation to immune-competent mice, human adipose derived mesenchymal stem cells (hAD-MSC) were detected in the joints of all mice at day 1, and still remained in three of ten mice at day 10 (less than 10% of the dose). No hAD-MSCs could be detected after 30 days in immune-competent mice (Toupet et al., 2015).

Limited data show that adipose and bone marrow derived human MSCs were initially distributed to the lungs after intravenous administration where fast decrease occurs.

The available data on "pharmacokinetics" are generally equivocal and results are largely dependent on the immune status of the animals. All available information on biodistribution is derived from xenogeneic (human) MSCs in mice and some general information on allogeneic MSCs in humans.

Toxicological studies

Single dose toxicity

No specific studies were conducted to investigate the acute toxicity of HorStem in laboratory animals.

Administering a higher dose of cells than recommended may (or may not) result in increased observation of adverse events and faster onset of certain events. Published literature was submitted to assess the acute toxic potential of MSCs in order to substantiate the statement that MSCs in general are assumed to be safe. The single dose toxicity data described in the literature and presented in the dossier include xenogeneic joint injection of human adipose MSC in mice (Toupet et al., 2013; 2015) or EUC-MSC in rabbits (Saulnier et al., 2014), intravenous injection of human adipose MSC in SCID mice (Ra et al. 2011). Overall, results indicate a relative low acute toxicity of MSC in laboratory animals after single administration. However, it is to be noted that after intravenous (i.v) injection of a relative high cell number (250 x 10^6 cells/kg bw) a few animals died due to thrombosis.

Repeated dose toxicity

No repeated dose toxicity studies with HorStem in laboratory animals were conducted.

Published literature to describe toxic effects of MSC after repeated administration has been submitted:
Wang et al. (2012): Human umbilical cord MSCs were administered by the i.v route to Cynomolgus monkeys over an 8 week period. The findings of this study showed that transplantation of hUC-MSC did not affect the general health of cynomolgus monkeys. No stem cell transplantation - related toxicity was observed.

Beggs et al. (2006) investigated the safety of MSC transplantation in baboons by determining the immunologically consequences and safety of administering multiple doses of allogeneic (i.m and i.v) MSCs to immunocompetent animals. Four weeks after the second injection confocal microscopy of muscle biopsies was performed and no signs of inflammation could be detected. Clinically, no adverse reactions were observed, no pathological signs on haematological blood parameters and urinalysis were found. Repeated administration of MSC did affect the immunologically system of recipients by generating low alloantibodies against donor MSC.

Lee et al. (2010) investigated the protective effects of repeated administration (i.v.) of bone marrow-

derived MSCs on a remnant kidney model in male Sprague–Dawley rats. Effect of weekly administration of MSCs was compared with the effect of once injection of MSCs and mesangial cells (MCs) at 1 and 5 weeks, respectively. Regarding safety the data show that 5 weekly i.v injections did not cause worsening of the observed effects compared to single administration. Repeated administration of MSCs improves the protective effect on remnant kidney injury, but primarily via the paracrine effect rather than differentiation.

Tolerance in the target species of animal

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

Reproductive toxicity

Study of the effect on reproduction

No conventional reproductive dose toxicity studies with HorStem in laboratory animals were conducted.

A few bibliographic references regarding the reproductive toxicity of MSC using different animals, routes of administration and dosages are available.

Monsefi et al. (2013) and Hassan and Allam (2014) investigated the therapeutic effects of MSCs in infertile male rats caused by gonadotoxic busulfan and lead, respectively. Host animals received transplanted bone marrow derived MSCs. The presence of MSCs was found to alleviate the gonadotoxic effects induced by lead and busulfan. The transplanted MSCs could differentiate into germinal cells in testicular seminiferous tubules and Leydig like cells situated between testicular seminiferous tubules.

Sanz-Baro et al. (2015) investigated in a human case study whether treatment with adipose-derived stem cells (ASCs) had any influence on fertility, course of pregnancy, newborn weight, or physical condition of new-borns. Due to the limited number of participants (6) who became pregnant after MSC treatment no final conclusion can be drawn.

Study of developmental toxicity

No studies and no literature references on developmental toxicity of the product are available.

A series of safety/toxicological studies (prenatal developmental toxicity) were performed in rodents and non-rodents using single donor-derived bone marrow MSCs as well as using pooled human bone marrow MSCs. To assess prenatal developmental toxicity 10 pregnant rats per group were administered with 3 different doses (12×10^6, 60×10^6 and 120×10^6 cells/kg bw) or vehicle by i.v infusion at days 5, 12 and 18 of pregnancy. At day 20 a caesarean section was made and the foetuses were evaluated. The results showed that i.v administration of high doses of xenogenic stem cells did not cause mortality in pregnant rats nor any related embryo-foetal toxicity. Maternal necropsy did not show remarkable pathological findings (Rengasamy et al., 2016).

Genotoxicity

No genotoxicity tests with EUC- MSCs according to the standard test battery were conducted. As there are no indications that HorStem or parts of HorStem will directly interact with DNA, conventional genotoxicity tests are not deemed necessary. In fact, it is more appropriate to investigate genetic alterations of the stem cells during the culturing process. For this purpose studies to investigate genetic stability of EUC- MSCs were conducted (see Part 2 Quality).

Carcinogenicity

No data exist about the tumorigenic potential of EUC- MSC of the final product HorStem. Chromosome instability and cellular senescence has been identified as a major concern for the risk of malignant transformation of transplanted MSCs. The occurrence of recurrent cell abnormalities appears to be mainly related to the manufacturing process. Thus, the culture conditions should be chosen to avoid a high proliferative rate (e.g., use of excessive amount of growth factors) because this may potentiate chromosomal abnormalities. The manufacturing process is explained in detail (see Part 2 Quality). Based on these data the safety of the active substance in HorStem with regard to its genetic stability is also discussed in Part 2.

Literature references are available that provide basic information under which *in vitro* culture conditions transformation of cells may occur. It seems to be of particular importance at what passage number cells are harvested and used for transplantation (Prockop et al. 2010; Vidal et al.2012). If cells are harvested well before the cultures reach senescence it can be assumed that there is a very low probability of malignant transformation and tumour formation in patients. The onset of replicative senescence in MSC isolated from equine bone marrow (BMSC), adipose tissue (ASC), and umbilical cord tissue (UCMSC) varies. BMSC ceased proliferation after a little >30 total population doublings, whereas UC-MSC and ASC achieved senescence about 60 to 80 population doublings (Vidal et al. 2012).

Additionally to the experimental investigations regarding chromosomal abnormalities several references addressing specific concern in relation to the treatment with stem cells and their theoretical potential of inducing tumorigenicity in experimental animal models were submitted. The systemic transplantation of human adipose-derived MSC appears to be safe and does not induce tumour development (Ra et al. 2011, human adipose MSC, i.v administration to nude mice). No tumour formation was found in immune deficient mice two months after transplantation of human UC-MSC expanded *in vitro* for up to thirteen passages (Wang et al. 2012 umbilical cord MSC in mice). Data of Rengasamy et al. (2016) demonstrate that single injections of hBMMSCs at various doses by the i.v route did not induce tumour formation in mice. Overall, there was no evidence of tumour formation in the animal models. Lalu et al. 2012 reviewed current human clinical trials in a retrospective meta-analysis with regard to potential adverse reactions, including malignant transformation arising from intravascular administration of MSCs. Malignancy was only observed in studies involving participants with ongoing or previous malignancies; no *de novo* malignancies were observed. The weakness of all these animal model studies and the retrospective human study is that the duration of observation was relatively short.

Studies of other effects

Immunological functions, persistence and immune rejection of transplanted MSC:

EUC-MSCs, which are the active substance of HorStem, are suggested to have immune-modulating and anti-inflammatory properties, which are attributed to the paracrine activity of the cells. In addition, EUC-MSCs are suggested to show ”low immunogenic profile” (Expert Report on Safety and Residues).

These properties have not been demonstrated in experiments with HorStem, but are suggested based on literature and prompted the applicant to conclude that allogeneic MSCs would be able to escape rejection by the recipient and would integrate into the tissue of the recipient transiently. The applicant suggests that even repeated administration would be possible due to the limited generation of alloantibodies (3a4-HorStemV0-other.pdf).

The applicant refers to publications that addressed the mechanisms, by which transplanted MSCs were believed to escape rejection by the recipient (Ryan et al. 2005), the proposed mechanism of action of MSCs (Wannemuehler et al. 2012; see chapter 4 pharmacodynamics), and the safety of MSC transplantation in humans (Lalu et al. 2012), horses (Pigott et al. 2013) and baboons (Beggs et al. 2006).

Based on numerous published experiments with adult bone marrow-derived MSCs reviewed by Ryan et al. the low immunogenicity of these cells was primarily attributed to their hypo-immunogenic nature and their capability to modulate T-cell phenotypes and to create a tolerogenic immunosuppressive local environment.

Lalu et al. (2012) evaluated the safety of cell therapies with MSCs in a variety of human therapeutic settings. The authors reviewed current clinical trials in a retrospective meta-analysis with regard to potential adverse reactions arising from intravascular administration of MSCs. Since autologous MSCs or MSCs from HLA (human lymphocyte antigen)-matched donors were used in the majority of studies, the study is not considered useful in drawing any conclusions on potential immune responses occurring after intra-articular transplantation of allogeneic MSCs to horses.

Pigott et al. (2013) investigated the immune responses in horses after intraarticular transplantation of autologous, allogeneic and xenogeneic MSCs. Due to several flaws in the experimental setup as well as in the data reporting, the findings of this study cannot reliably deduce that no allogeneic transplant related immune reactions would be induced after intra-articular injection of MSCs to horses. Nevertheless, the study indicates that MSCs do not survive long term in joints of horses, since cells could not be determined in the synovium 60 days after injection.

In a non-therapeutic, allogeneic setting Beggs et al. (2006) transplanted repeatedly (first injection i.v., second injection i.m.) MSCs from HLA-mismatched donors into recipient baboons. The authors found T-cell alloreactivity as well as alloantibodies towards transplanted MSCs. Interestingly the study indicated a direct relationship between alloreactivity of T-cells and survival of MSCs in vivo, although individual responses differed markedly between recipient animals.

It is hypothesized that the paracrine activity of MSCs would persist even after cells have disappeared. However, submitted publications (Pigott et al., 2013, Toupet et al., 2013) do not reliably support this hypothesis, and no proprietary studies have been conducted with HorStem, which would substantiate this assumption.

In summary, the publications on the immunogenicity and persistence of MSCs submitted contain some valuable information, but immunogenicity and persistence of HorStem has not finally been assessed. In the clinical studies some horses showed local inflammatory reactions in the treated joints with a potential increase after repeated administration, which might indeed indicate immunological responses. These adverse reactions were mild to moderate and resolved within 15 days with or without NSAID treatment.

Potential interactions of MSCs and other medicinal products

In the submitted publications potential interactions between MSCs and medicinal products, which may be used intraarticularly in horses with degenerative or septic joint diseases, such as corticosteroids, aminoglycosid antimicrobials and hyaluronic acid, have been examined by co-incubation in vitro. Results from these experiments justify the advice not to administer HorStem concurrently with any other intra-articular medication, because of potential toxic effects these substances may have on MSCs.

Observations in humans

Several references (Lalu et al. 2012; Peeters et al.2013; Emadedin et al 2012, Davatchi et al.2011) relevant to safety after intravenous and intra-articular treatment with MSCs in human patients are available. Data demonstrate a relatively good tolerance of transplanted autologous or allogeneic MSCs in humans after i.v. and i.a. administration. A systematic review was unable to detect associations between MSC treatment and the development of acute infusional toxicity, organ system complications, infection or death and no *de novo* malignancies were observed. Concerns for immunogenicity may be arbitrary as 13 studies used unmatched allogeneic MSCs with no reports of acute infusional toxicity. Data on autologous bone marrow-derived intra-articular administered MSCs into patients with cartilage damage or osteoarthritis were reviewed for 21 months. Serious and non-serious adverse effects have been reported with low incidences.

No data on the irritation potential to skin and eyes and no data on the sensitization potential of the final product HorStem are available. However the skin and eye irritating properties of the excipient are addressed in the provided safety data sheet indicating that none of the components in the proportions used are considered hazardous.

Excipient

The excipient contains 17 substances, which either have a "No MRL required" status or are considered to be out of scope of Regulation (EC) No. 470/2009 when used as in this product.

User safety

A user risk assessment for the product HorStem was submitted, which has been conducted in accordance with the CVMP Guideline for User Safety for Pharmaceutical Veterinary Medicinal Products (EMEA/CVMP/543/03-FINAL-rev1, March 2010) including a hazard identification, exposure assessment, risk characterisation and formulation of corresponding warning phrases.

For hazard identification no classical toxicological studies with NOELs and clear dose-effect curves are available either for the product HorStem or for the pharmacological active ingredient EUC-MSCs. Instead, a generalised hazard assessment of MSCs on basis of scientific literature and the current knowledge of immunological and carcinogenic properties of MSCs has been undertaken. Additionally, it was noted in the target animal safety (TAS) study that intraarticular administration of EUC-MSC led to adverse effects like transient pain and swelling at the injection site.

The product will be administered by professionals, i.e. the veterinarian. The routes of exposure can be dermal by spilling onto the skin; or ocular by accidental splashes in the eyes. It is agreed that oral exposure is highly unlikely. The most relevant route of exposure will be parenteral by accidental self-injection. It is noted that after an i.v injection of a high cell number (250 x 10^6 cells/kg bw) in immune-deficient mice, some of these animals died due to thrombosis (Ra et al. 2011). The risk of thrombosis after accidental self-injection cannot be completely ignored. However, it has to be borne in mind that thrombosis occurred only after i.v administration of very high cell counts (mice: 250 x 10^6 MSCs per kg/bw; rabbits: 130 x 10^6 cells/kg bw). In case of accidental self-injection it is assumed that 10% (0.1 ml) of the injection volume corresponding to 1.5 x 10^6 cells per 60 kg bw or 2.5 x 10^4 cells per kg bw will be injected. This is significantly lower than the cell count that led to thrombosis or embolism. Further, it is unlikely that in case of self-injection the complete injection volume will enter blood vessels.

The risk of a severe immunologic response after accidental self-injection cannot be ruled out however in the TAS study intraarticular injection of HorStem led only to transient pain and swelling.

In the TAS studies intraarticular injection of HorStem led to transient pain and swelling. These adverse effects can also be assumed for the user after subcutaneous self-injection. No skin or eye-irritation studies and no sensitisation studies were conducted with the product HorStem. This is considered acceptable since the active substance (stem cells) is not assumed to have irritating properties. The 17 ingredients of the excipient are not considered to represent a concern. In the TAS studies intraarticular injection of HorStem led only to transient pain and swelling, therefore it can be concluded that there is no serious risk regarding user safety after accidental self-injection of a low volume (0.1 ml) of the excipient. In conclusion the provided data (literature references) are sufficient to evaluate user safety. The user safety warnings are considered satisfactory to ensure the safety of the user when the product is handled as recommended.

Environmental risk assessment

HorStem is composed of EUC-MSC which are not genetically modified. The active substance EUC-MSC is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Hence, the environmental risk assessment can stop in phase 1 according to Guideline on environmental impact assessment (EIAS) for veterinary medicinal products – Phase I (CVMP/VICH/592/98-FINAL) and the product is not expected to pose a risk to the environment when used according to the labelling.

Residues documentation

MRLs

The active substance contained in HorStem, EUC-MSCs, is considered as not falling within the scope of the MRL regulation, as it is covered by the entry for stem cells in the list of substances considered as not falling within the scope of Regulation (EC) No 470/2009, with regard to residues of veterinary medicinal products in foodstuffs of animal origin (EMA/CVMP/519714/2009-Rev.34). The only excipient, , contains 17 substances, which either have "No MRL required" classifications or are considered as being out of scope of Regulation (EC) No. 470/2009 when used as in this product.

Residue studies

No residue depletion studies were provided.

Withdrawal periods

The requested withdrawal period of zero days is considered acceptable to ensure consumer safety.

Overall conclusions on the safety and residues documentation

No specific studies were conducted to investigate the acute toxicity of HorStem in laboratory animals. Data from published literature indicate a relative low acute toxicity of human adipose and EUC-MSCs in laboratory animals after single administration (i.v. or intraarticular). However, after i.v. injection of a relative high cell number (250 x 10^6 cells/kg bw) a few animals died due to thrombosis.

No data from repeated dose toxicity studies with HorStem in laboratory animals are available. Data from published literature describe no or only mild adverse effects after repeated administration of MSCs to cynomolgus monkeys, baboons and rats. EUC-MSCs were not included. Repeated administration of MSCs affects the immune system of recipients by generating low alloantibodies against donor MSCs.

No conventional reproduction toxicity or developmental toxicity studies with HorStem in laboratory animals were conducted. Bibliographic references using different species, routes of administration and dosages are available. The results showed that i.v. administration of high doses of xenogenic stem cells did not cause mortality in pregnant rats nor any related embryo-foetal toxicity. Maternal necropsy did not show remarkable pathological findings. No genotoxicity tests with umbilical cord MSCs according to the standard test battery were conducted. As there are no indications that HorStem or parts of HorStem will directly interact with DNA, conventional genotoxicity tests are not deemed necessary.

No data exist on the tumorigenic potential of EUC-MSCs. Literature references provide basic information on in vitro culture conditions under which transformation of cells may occur. The passage number at which cells are harvested and used for transplantation seems to be of particular importance. If cells are harvested well before the cultures reach senescence it can be assumed that there is a very low probability of malignant transformation and tumour formation in patients. The onset of replicative senescence in MSCs isolated from equine bone marrow (BMSC), adipose tissue (ASC), and umbilical cord tissue (UC-MSC) varies. BMSC ceased proliferation after slightly over 30 total population doublings, whereas UC-MSC and ASC achieved senescence after about 60 to 80 population doublings.

Additionally, several references were submitted addressing specific concerns in relation to the treatment with stem cells and their theoretical potential to induce tumorigenicity in experimental animal models. After systemic transplantation of MSCs, there was no evidence of tumour formation in the animal models. Data from a retrospective meta-analysis of current human clinical trials with regard to potential adverse reactions, including malignant transformation demonstrate that malignancy was only observed in studies involving subjects with ongoing or previous malignancies; no *de novo* malignancies were observed.

Publications on the immunogenicity and persistence of MSCs were submitted but findings of literature data cannot necessarily be transferred to HorStem since the origin, isolation and culture conditions as well as the microenvironment of MSCs are believed to influence these properties. As no eligible product specific data has been provided, immunogenicity and persistence of HorStem cannot be finally described. However, sufficient information regarding safety of treatment was presented from the clinical trials and further data relating to immunogenicity and persistence of HorStem is not considered necessary.

Results from submitted publications on potential interactions between MSC and medicinal products justify the advice not to administer HorStem concurrently with any other intra-articular medication, because of potential toxic effects these substances may have on MSCs. Data demonstrate a relative good tolerance of transplanted autologous or allogeneic MSCs in humans after i.v. or i.a. administration. A systematic review was unable to detect associations between MSC treatment and the development of acute toxicity, organ system complications, infection or death, no *de novo* malignancies were observed. Concerns for immunogenicity may be arbitrary as 13 studies used unmatched allogeneic MSCs with no reports of acute toxicity. Data about autologous bone marrow-derived MSCs administered intraarticularly to patients with cartilage damage or osteoarthritis demonstrate that serious or other adverse effects occur with low incidences.

No data on the irritation potential to skin and eyes and no data on the sensitisation potential of the final product HorStem are available. This is considered acceptable since the active substance (stem cells) is not expected to have irritating properties. The 17 ingredients of the excipient are not considered to represent a concern, and it can be concluded that there is no serious risk regarding user safety after accidental self-injection of a low volume (0.1 ml corresponding to 10% of the label dose) of the excipient. A user safety assessment in line with the relevant guidance document on user

safety (EMEA/CVMP/543/03-FINAL-rev1, March 2010) has been presented.

The worst-case scenario for user safety is accidental self-injection. The user safety warnings are considered satisfactory to ensure the safety of the user when the product is handled as recommended. An appropriate environmental risk assessment was provided. The active substance of the proposed product is a natural substance which will not alter the concentration or distribution of the substance in the environment. The product is not expected to pose a risk for the environment.

HorStem's active substance, EUC-MSCs, is considered as not falling within the scope of the MRL requirements. Stem cells are included in the list of substances considered as not falling within the scope of Regulation (EC) No. 470/2009, with regard to residues of veterinary medicinal products in foodstuffs of animal origin (EMA/CVMP/519714/2009-Rev.34). The excipient contains 17 substances, which either have "No MRL required" entries in Regulation 37/2010 or are considered as not falling within the scope of Regulation (EC) No. 470/2009 when used as in this product. The requested withdrawal period of zero days is considered acceptable to ensure consumer safety.

Part 4 – Efficacy

Pharmacodynamics

Published references and one proprietary in vitro study were submitted to describe the pharmacodynamics effects of MSCs.

McIlwraith, 2001 summarised joint diseases in horses and reviewed the pathogenesis of osteoarthritis. Inflammatory mediators associated with destruction of hyaluronan (HA) in synovial fluid as well as articular cartilage degradation were described including: the cytokines IL-1 and TNF-alpha; metalloproteinases and aggrecanase; prostaglandins E2; and free radicals.

Wannemuehler, et al., 2012 debates the possible use of MSCs as therapeutic adjuncts in treatment of sepsis in humans. The authors refer MSCs as non-hematopoietic, self-renewing, undifferentiated precursor cells having the potential to differentiate into multiple cell types. Next to that, a range of beneficial abilities of MSCs are described including: (i) homing to sites of injury, (ii) paracrine signalling by a balanced decrease of pro-inflammatory and increase of anti-inflammatory cytokine production, prevention of apoptosis in threatened tissues, promotion of neoangiogenesis, activation of resident stem cells, modulation of the activity of multiple immune cell types, and (iii) antimicrobial effects.

Saulnier et al., 2014 investigated anti-inflammatory and anti-catabolic effects of equine umbilical cord mesenchymal stromal cells (UC-MSC) in a xenogeneic model of mild osteoarthritis (OA). The authors could demonstrate that early intraarticular injection of equine UC-MSC was effective in preventing OA signs in rabbit knees following meniscal release by a lower cartilage fibrillation, reduced cartilage alterations and by persistent synovial reactivity. Lymphoplasmacytic infiltrates were identified in the synovium as well as a significant molecular response of inflammatory and catabolic genes, indicating a strong reactivity of the synovial tissue. After UC-MSC injection in rabbits no local or systemic adverse reactions were observed. In vitro, UC-MSC conditioned medium exerted anti-inflammatory and anti-catabolic effects on synoviocytes exposed to pro-inflammatory stimulus. The results suggest that the synovium is a major target and mediator of MSC therapy.

The results of a proprietary in vitro "Treatment Concept Study" indicate the potential of EUC-MSCs to secrete PGE2 even without stimulation. After stimulation with synovial fluid taken from a horse with osteoarthritis, PGE2 secretion of EUC-MSCs was significantly increased when compared to the negative controls.

Carrade et al., 2014 found in an in vitro mixed leukocyte reaction test that blocking of PGE2 production of stimulated equine MSCs from adipose tissue (AT), bone marrow (BM), cord blood (CB), and cord tissue (CT) significantly restored T-cell proliferation and this was accompanied by restored inflammatory cytokine production including TNF-α, IFN-γ as well as IL-10.

Williams, 2015 investigated the effect of allogeneic equine umbilical cord blood mesenchymal stromal cells (ECB-MSC) in a LPS induced synovitis model in 6 horses. 8 hours after simultaneous injection of LPS and ECB-MSC total nucleated (neutrophil and mononuclear) cell numbers in synovial fluid were significantly reduced compared to contralateral joints treated with LPS, only.

Additional bibliographic references (Barrachina et al., 2016; Barrachina et al., 2017; Schnabel et al., 2014; Crisostomo et al., 2006; and Leijs et al., 2012) were submitted, dealing with in vitro studies conducted with non-product related equine, murine and humane bone marrow derived MSCs. The results of these studies indicate the potential of MSCs for cartilage repair as well as their immunomodulatory properties. It was further stated that many authors suppose that the therapeutic potential of MSCs is mainly based on the paracrine release of cytokines that interact with pro-inflammatory immune cell subsets.

In conclusion general pharmacodynamic properties of MSCs including EUC-MSCs have been reviewed. A distinct mode of action related to the pharmacological, immunological and/or metabolic effect or regenerative, repair and/or replacement effect was not postulated. Based on further relevant information provided with studies of other effects in part 3 MSCs were suggested to have immune-modulating and anti-inflammatory properties attributed to their paracrine activity. Product related investigations in vitro revealed the potential of EUC-MSCs to secrete PGE2 with and without stimulation by synovial fluid. The product's innate mechanism of action presumably underlying the therapeutic effects of EUC-MSCs when transplanted intraarticularly to horses with osteoarthritis has not been demonstrated.

Pharmacokinetics

No proprietary studies were conducted to investigate the pharmacokinetics of HorStem. Two published references were submitted to describe the biodistribution of MSCs in horses.

Becerra et al. (2013) investigated the biodistribution of equine radiolabeled bone marrow derived MSCs after allogeneic transplantation (intravenous, intra-lesional, regional perfusion) into horses with naturally occurring tendon injuries. Biodistribution was determined for up to 48 h by using planar gamma scintigraphy. Low cell retention rates were found at the sites of implantation with highest rates of 24% retained intra-lesional at 24 h. MSCs were determined in lungs after administration by all application routes and in the thyroid after regional perfusion. After intravascular administration, homing of MSCs to damaged tendons did not occur in large numbers.

Valdes, M.A. (2015) provided an oral communication on the biodistribution of equine radiolabelled bone marrow derived MSCs after allogeneic transplantation (intraarticular) into horses without musculoskeletal injury and horses with naturally developed arthropathy. Biodistribution was determined by using planar gamma scintigraphy. The intra-articular retention rates of MSCs during the first 24 hours were reported to be approximately 40%. However, material and methods as well as results were fragmentary reported and discussed. Thus, no firm conclusions can be drawn from this oral communication.

In conclusion, sparse data was provided to describe the biodistribution of MSCs in horses. In two publications radiolabelled MSCs were tracked in horses by use of gamma scintigraphy. This detection method allows for short observation periods (48 hours), only and does not trace small amounts of MSCs. In only one of these studies MSCs were administered according the claimed intraarticular route

but that study could not be properly evaluated due to shortcomings. Thus, the significance of these studies with regard to biodistribution is limited.

Irrespectively, the following can be concluded: Low retention rates of MSCs were determined after 24h: the intra-lesional retention rate was 24% and the intra-articular retention rate was approximately 40%. MSCs were detected in lungs of horses after administration by intravenous, intra-lesional, regional perfusion and in the thyroid after regional perfusion. For detection of MSCs in lungs and thyroid using the intraarticular application route, no results were reported.

The omission of biodistribution studies with HorStem in the target animal species horse was justified by the lack of appropriate techniques for large animals and was considered acceptable. However, extrapolation of results of biodistribution studies of laboratory/small animals was not accepted because the meaningfulness of extrapolating data of both, homologous and heterologous laboratory/small animal studies to equine UC-MSC in horses was considered limited.

Target animal tolerance

Information on the target animal tolerance of HorStem can be obtained from the following studies presented in the table below. The clinical field studies and the pivotal target animal safety study are GCP compliant, while the other studies do not fully comply with the related requirements.

Dossier part, reference	Author / year	Type of study / main theme	Number of animals treated with EUC-MSC, controls	Use of the final formulation
Part 4a3-tas Annex 5 (d121)	2017	Pivotal target animal safety study at label dose, including repeated treatment.	8 horses (plus 8 horses treated with placebo)	yes
Part 4a3-tas Annex 1	Ripa, 2014	Preliminary safety data*	3 horses	no
Part 4a3-tas Annex 2	Ripa, 2016	Safety of a 2x overdose*	6 horses (the same horses were used throughout)	yes
Part 4a3-tas Annex 3	Ripa, 2016	Safety of a 2nd dose (1x)*		
Part 4a3-tas Annex 4	Ripa, 2016	Safety of a 3rd dose (1x) after a single dose of NSAID*		
Part 3a3–tox Annex1	Anonymous, 2016	Retrospective tumorigenicity (cutaneous or subcutaneous tumours)	41 horses	no (7) yes (34)
Part 4b –clin Annex 1	Ripa, 2014	Pilot efficacy and safety study, dose confirmation	6 horses	no
Part 4b –clin Annex 2	Ripa, 2016	Pilot clinical field study	18 horses (plus 22 horses treated with excipient only)	yes
Part 4b – Annex 4	2017,	Pivotal clinical field study	16 horses (plus 17 horses treated with placebo)	yes

*these studies show various flaws regarding the study design and data presentation and are rated therefore only supportive.

In addition to the studies, literature references that provide information on the use of autologous, allogeneic and/or xenogeneic stem cells in various species, including information on the intra-articular use of allogeneic MSCs in horses are included in the dossier.

MUMS/limited market status has been granted for the product. According to the related CVMP Guideline on the efficacy and target animal safety data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/EWP/117899/2004), a basic controlled study for the demonstration of safety of the final formulation in the target species has been provided in addition to field safety data.

MSCs as an active ingredient of a medicinal product present a type of novel therapy. In consequence, the related guidelines on target animal safety (VICH GL43 Target animal safety: pharmaceuticals, CVMP/VICH/393388/2006 and VICH GL44 Target animal safety for veterinary live and inactivated vaccines, CVMP/VICH/359665/2005) are not fully applicable. They can, however, provide some guidance, especially in relation to local tolerance, but also regarding some aspects of the detection of

potential systemic effects. Additional areas of concern arise from aspects related to specific properties of stem cells. These are described in the public domain and have been identified by the CVMP, e.g. immunogenicity, tumorigenicity, biodistribution to other organs, ectopic tissue formation[1].

The classical approach for the determination of a margin of safety is hampered by the low likelihood of high overdoses in intraarticular injection and the specific properties of MSCs named above, and not considered fully applicable, since cells as active ingredients do not necessarily exert activity in a classical dose-related manner. In literature, describing the use of autologous, allogeneic and xenogenic stem cells in various species, adverse events are more or less restricted to local reactions. It is, however, emphasized in many articles that despite these results, safety issues should be kept in mind (e.g. Mokbel et al., 2011; Broeckx et al., 2014). However, Peeters et al. (2013) conclude in their review that the application of cultured [autologous human] stem cells in joints appears to be safe, and that with continuous caution for potential side effects, it is reasonable to continue with the development of articular stem cell therapies. With regard to biodistribution, tumorigenicity and ectopic tissue formation, Bahr et al. (2015) concluded that long-term engraftment of MSCs appears, at most, to be low and that this may limit the long-term risks of MSC therapy, following their findings on human recipients of allogeneic MSCs via i.v. infusion.

The excipient in HorStem, which is marketed as optimised hypothermic cell preservation medium. In HorStem, it acts as a diluent and cell preservative. The 17 ingredients of the excipient are considered to be of no concern, and, while a contribution of the excipient to the adverse events observed in the treated joint cannot be fully ruled out, it can be concluded that there is no serious risk regarding the intraarticular use of the intended dose (1 ml) per animal in horses.

The local tolerance of HorStem was assessed by the clinical evaluation of orthopaedic adverse events in treated joints (lameness, swelling/effusion, flexion pain). Such transient adverse events were noted in all studies presented and could, if necessary, be ameliorated by the administration of NSAIDs. In the pivotal target animal safety study, HorStem was administered to healthy young horses at the intended label dose in a joint representative for osteoarthritis (front fetlock); the most prominent adverse reaction was lameness (grade 1-2/5), which occurred in 2/8 treated horses compared to 0/8 horses in the placebo group. The events were mild to moderate, self-limiting and of short duration (1-3 days). Additionally, slight joint effusion and/or localized oedema were observed in all HorStem treated horses. Some horses showed signs of slight pain. None of the horses required concomitant treatment.

In the pivotal clinical field study, the final formulation was tested compared to placebo, i.e. 16 horses had received in the investigational product (IVP). In addition, 14 placebo horses that had not improved during the trial were treated with the IVP after completion of the trial. Three out of these 30 horses that had received the IVP developed an acute inflammation with lameness (grade 4-4.5/5), joint effusion and pain. One of these three animals required treatment with NSAID for about 3 days. A fourth treated horse exhibited joint effusion without lameness, and a fifth horse showed mild increase in lameness. All animals showed complete resolution by latest at day 12 after treatment. No clinically relevant differences between batches have been observed.

In another clinical field study, considered as a pilot study, the final formulation was tested in 18 animals and compared to 22 animals treated with the vehicle, only. Three horses treated with the product showed signs of a massive acute inflammation on the day after treatment manifest as lameness (grade 3.5-5/5) that was considered to be product related. They required treatment with phenylbutazone for three days and showed complete resolution of symptoms thereafter. A further horse developed signs of inflammation, synovitis and lameness (grade 4/5) seven days after product

[1] Stem cell -based products for veterinary use: Specific questions on target animal safety to be addressed by ADVENT: EMA/CVMP/ADVENT/193811/2016

administration. Similarly this horse received phenylbutazone for three days and complete resolution of signs was achieved. None of the control animals in the clinical field study showed local reactions, indicating that the joint inflammation is a reaction to the MSCs, and not to the injection itself or the excipient. Notably, the adverse events observed after a single product administration in both clinical studies, particularly the magnitude of lameness, were considerably more severe than those observed in the pivotal target animal safety study.

Results obtained after administration of a second dose indicate that the frequency and severity of local adverse reactions increase after subsequent administration. Three out of eight treated horses developed lameness (grade 3.5-4/5) for 2-3 days after the second product administration, with one horse requiring NSAID treatment. All eight treated horses developed signs of slight joint effusion and/or localised oedema. Although efforts have been undertaken to identify whether the reason for this increase in frequency and severity is of immunogenic character (cellular or humoral response), no reliable information could be generated. In addition, results from other study also strongly indicate that repeated treatment increases the severity of adverse events, although some flaws of the study hamper interpretation. In both studies, symptomatic treatment with NSAID became necessary in individual animals due to an acute inflammation of the treated joint. No data on efficacy related to repeated treatments are available which is accepted as repeated treatment is not claimed.

Systemic clinical adverse events were rarely seen in all studies. Haematology and clinical chemistry parameters following treatment are available from the pivotal target animal safety study and the pivotal field study. They were usually within normal limits and/or in case elevations occurred, these had already been present pre-treatment.

There are specific safety concerns related to stem cell treatment such as biodistribution of cells to other organs, ectopic tissue formation, and tumour induction. Overall, it is acknowledged that following intraarticular administration, the risk of biodistribution via the bloodstream is low and that related adverse events by ectopic tissue formation or tumourigenesis in the horse or any other species remain theoretical; data on this issue are very limited but published literature indicate that the risk may be low. More reliable information on the biodistribution of cells from HorStem could only be generated by additional in vivo studies on biodistribution (meaning additional animal testing) or by complex long-term follow up studies which would require a high number of animals due to the expected low incidence rate. Cells have been adequately characterized with respect to properties which could be of interest in relation to tumour formation. Thus, this does not raise concerns as related risks are considered to be low.

The injection volume of 1 ml/joint is small and thus does not raise concerns.

Dose determination/Dose justification

Currently there is no standardised method for dose determination for stem cell products, since there is no dose-response relationship. With reference to the MUMS/limited market-status of the product, the applicant did not conduct a dose determination study with HorStem, but referred to published experiments with MSC transplantation in laboratory animals. The applicant proposed that a dose of 15 x 10^6 cells would be appropriate for use in those horse joints with an estimated synovial volume of 2-3 ml. In experiments in rats 1 x 10^6 cells in 0.2 ml suspension medium had been administered into the knee (van Buul et al., 2014), and in rabbits 2, 3.5 or 6 x 10^6 cells in 0.4 ml suspension medium (Desando et al., 2013, Saulnier et al., 2014). From this, a number of 4-7 x 10^6 cells per ml synovial fluid were derived as appropriate. This dose was found to be in agreement with doses used in MSC experiments in horses published by Wilke et al. (2006), Nixon et al. (2010), McIlwraith et al. (2011), and Ferris et al. (2014). Williams (2015) presents a preliminary evaluation of equine umbilical cord blood-derived MSCs as treatment for synovitis in an experimental study at a dose of 10 x 10^6 or 30 x

10^6 cells. The findings indicate that further studies are needed to evaluate the effect of the dose and other parameters related to the use of MSCs.

Regarding the volume/different sizes of equine joints, Ekman et al (1981) and van Pelt et al (1962) are cited. In the latter, synovial volumes are closer to those cited by the applicant than in Ekman et al. (1981). Still, the volume for fetlock joints does not correspond to 2-3 ml as had been considered after extrapolation from rats, rabbits, and humans.

HorStem contains allogeneic, umbilical cord (Wharton's jelly)-derived native MSCs. For extrapolation of data from published experiments in horses using autologous, bone marrow-derived chondrogenic stem cells to HorStem, i.e. allogeneic umbilical cord stem cells (UC-MSC), the applicant cites Park et al. (2016), a study on allogeneic umbilical cord blood-derived MSCs and hyaluronate hydrogel in humans. Two different dosages were used in 7 patients: Four patients received a low dose (12 x 10^6 cells) and 3 others a high dose (18 x 10^6 cells). Both are described as safe and efficacious which the applicant considers supportive of the dosage established for HorStem.

In the clinical field studies only one dose, i.e. 15 x 10^6 (±20%) equine UC-MSCs, was used.

In conclusion, the information provided on dose determination/dose justification is not fully satisfactory, and the efficacy of the product in the reduction of lameness in horses suffering from mild to moderate osteoarthritis at the recommended dose has not been reliably demonstrated in the pivotal field study due to shortcomings in the design and conduct of this study.

Dose confirmation studies

In the proof of concept-study from I.L. Ripa (2014), the safety and efficacy of MSCs derived from the umbilical cord of horses have been examined in 7 horses with naturally occurring degenerative joint diseases. As donor and recipient horses of MSC were not identical, the type of treatment was an allogeneic MSC transplantation in contrast to the autologous MSC implantations performed in most of the submitted publications. In addition, cells were apparently non-differentiated in contrast to most published experiments, where chondrogenic MSC had been used.

The applicant considered this study provided confirmation of the dose. However, a different suspension medium, i.e. Dulbecco's Modified Eagle Medium (DMEM), was used instead of excipient of the final formulation. Published experiments show that the suspension medium significantly affects treatment success. Thus this study cannot be used for confirmation of the dosage.

Clinical studies

Treatment concept

HorStem is indicated for the reduction of lameness associated with mild to moderate degenerative joint disease (osteoarthritis) in horses.

The recommended treatment dose of HorStem is 1 ml of the product containing 15 x 10^6 MSCs for intraarticular injection into the affected joint.

MSCs for the manufacturing of HorStem are derived from the umbilical cord of donor horses, which are not identical to the recipient horses, thus the treatment type is allogeneic MSC transplantation. Based on published and own experimental data the applicant outlines that the therapeutic potential of MSC in equine joint disease results from their immunomodulatory effects which are based on a paracrine activity of the MSC, i.e. mainly PGE2 secretion. However, although PGE2 secretion has been demonstrated for EUC-MSC in vitro, the product's innate mechanism of action underlying the therapeutic effects of EUC-MSCs when transplanted intraarticularly to horses with osteoarthritis has

not been demonstrated. Moreover it can only be speculated how long EUC-MSC survive after intraarticular injection in equine arthritic joints and the impact this could have on efficacy and safety including potential for immunogenicity. Therefore, the treatment concept described appears reasonable although the mechanism of action underlying the therapeutic effect remains unproven.

Clinical field study

In this clinical field study efficacy and safety of HorStem have been analysed in a Good Clinical Practice (GCP) compliant, multi-centre, blinded, randomised and placebo-controlled clinical study in 40 horses suffering from mild to moderate osteoarthritis mainly in the interphalangeal joints. The horses received either a single intra-articular dose of 15 x 10^6 allogeneic MSCs from umbilical cord diluted in 1 ml of the excipient (18 horses), or 1ml of the vehicle, , for control (22 horses). All horses were clinically observed at regular time intervals for 63 days, and lameness, flexion pain and joint effusion were scored according to current standards. Treatment success was defined as a reduction of the total score by ≥ 3 at day 63 as compared to day 0. The total score was the sum of lameness score (grade 0-5), flexion test score (grade 0-3), and joint effusion score (grade 0-2). Results of the study showed treatment success rates of 44.4% in the test group and 13.6% in the placebo group at day 63±2. Initially, day 35 +/-2 was designated as the primary endpoint, but the primary variable was changed to day 63 +/-2 after an interim analysis had been carried out.

The issue of an unplanned interim analysis and the subsequent change of the primary endpoint is unacceptable and against the principles described in the CVMP guideline on statistical principles for veterinary clinical trials (CVMP/EWP/81976/2010). Irrespectively, after taking into account the shortcomings of the initial analyses, recalculations of the study results showed superiority (p=0.049) of the investigational veterinary product (IVP) compared to placebo in the amended primary variable. Moreover, statistical differences was shown between IVP and the control group with respect to the secondary variable "improvement in lameness" (decrease in lameness score to ≤1 at day 63) (p=0.0032). In conclusion, this study can be considered as a pilot study. Follow-up non-GCP compliant, non-blinded data were provided for up to 24 months after treatment. 67% and 58% of the treated horses that were followed up had not relapsed at 12 and 18 months, respectively. No equivalent data were presented for placebo treated horses.

Clinical field study

Efficacy and safety of HorStem was examined in comparison to placebo in a GCP compliant, multicentric, blinded, randomized study in 10 equestrian centers in Spain. A total of 34 mature horses were enrolled for the study. All animals had been diagnosed with mild to moderate osteoarthritis using different diagnostic procedures including clinical signs (lameness, joint effusion, flexion pain), radiography, perineural blocks and confirmation by intra-articular anaesthetic blocks.

Both treatment groups were comparable with respect to sex, age, breed, and activity level at the time of enrolment.

Enrolled horses were treated once intraarticularly with either 15 x 10^6 EUC-MSC (IVP, 17 horses; one horse was sold and lost to follow-up) of 3 different donor horses or saline solution (CVP, 17 horses). Joints of horses in the treatment group included metacarpophalangeal (fetlock joint), distal interphalangeal (coffin joint), and tarsometatarsal joint.

Horses were examined at days 14, 35, and 63 with respect to lameness, flexion pain, and joint effusion. Primary efficacy variable was the percentage of animals regarded as treatment success, which corresponded to an improvement in the lameness grade to a non-lame or an inconsistent lameness (≤ grade 1 AAEP scale). The treatment success rates were 75% (12/16 horses) in the IVP

group and 23.5% (4/17 horses) in the CVP group at day 63± (p=0.0031). Significant differences between treatment and placebo group were shown only with regard to 'lameness' and 'flexion pain'. Many secondary parameters showed statistically significant differences between treatment and placebo groups. Seven out of 16 horses were free of lameness (score 0) at day 63 according to the veterinary assessment. It is noted that one horse (G02) developed joint flare and acute lameness (4.5/5) and joint flare after administration of HorStem. The horse was treated with phenylbutazone, and, thus should have been included as treatment failure in the statistical analysis. CVMP's own calculations show that the treatment success rate was still statistically significantly different between treated and control group with a success rate of 68.8% in the treatment group if horse G02 was included as treatment failure. Another two horses (K03 and Y02) presented with acute lameness grade 4 and 4.5 after treatment with HorStem. These horses did not receive any NSAIDs, but were prescribed box rest or cold water treatment, since they did not display overall signs of discomfort as did horse G02. Signs resolved in these 3 horses within 2 to 15 days. A further 2 horses (U02, C01) presented AEs (mild lameness and moderate joint effusion, respectively) within 24 h after treatment that was assessed as probably related to treatment. Therapeutic success with a significant difference against placebo group was observed earliest at day 35 (±2) after treatment.

The CVMP noted that the equine practitioners involved in this study used a 10-grade modified AAEP lameness scoring system using fractional scoring with a 10 point scale to grade lameness, different to the AAEP scoring system described in the study protocol which corresponds to 5 integer scores. The applicant failed to provide a protocol amendment. The CVMP considered this as a major protocol violation. Taking into account the inter-assessor variability when using a non-standardised scoring system for the assessment of lameness an impact on the efficacy evaluation cannot be excluded. The applicant submitted references to scientific literature showing evidence that half point scoring is commonly used in the EU and U.S. and, in addition, post-study statements from all ten equine practitioners involved in the pivotal field study confirming that they were aware of and prepared to apply half point (fractional) scoring before the trial had started in order to more accurately characterise lameness. However, this additional information was not considered adequate to resolve the CVMP's concerns.

Furthermore, the Committee noted that the mean activity level during the study in the treatment group was lower than in the placebo group, with 4/16 horses in the treatment group having complete rest/handwalking only, whereas in the placebo group, all 17 horses were ridden. This could have influenced the efficacy evaluation. The applicant calculated that the difference in activity levels during the study was not statistically significant. The treatment success was not correlated to decrease of the activity score, but this analysis was conducted post-hoc and it is not known if the study was powered to show a relevant difference. It has to be noted that horses with a lower activity level showed a lower success rate (50%, i.e. 2/4 horses with a very low activity level) than horses that had a higher activity level (75%, i.e. 9/12 horses with medium to very high activity level).

Batches of three different donor horses have been used for the treatment of the IVP group. Five horses treated with one batch, two horses treated with another batch and 5 horses treated with third batch were considered treatment successes. From the data presented there is no clinical indication that batches from different donors act differently with respect to safety and efficacy although this cannot be verified statistically due to the low number of study animals.

Haematology and serum chemistry revealed no significant differences in most horses at day 63 after treatment. Deviations in individual horses were not related to the administration of HorStem.

In conclusion, although a statistically significant treatment benefit for HorStem was demonstrated in regards to improvement of lameness at D63 when compared to placebo, aspects of the study conduct are considered to impact on the robustness of the findings.

Overall conclusion on efficacy

Pharmacodynamics:

General pharmacodynamic properties of MSCs including EUC-MSCs have been reviewed based on published literature. Product related in vitro investigations revealed the potential of EUC-MSCs to secrete PGE2 with and without stimulation by synovial fluid. The product's innate mechanism of action presumably underlying the therapeutic effects of EUC-MSCs when transplanted intraarticularly to horses with osteoarthritis has not been demonstrated.

Pharmacokinetics:

The biodistribution of MSCs in horses was likewise described based on sparse literature data with limited significance. The omission of biodistribution studies with HorStem in the target animal species horse was justified by the unavailability of appropriate determination techniques for large size animals and was considered acceptable. Extrapolation of results from biodistribution studies of laboratory or small animals was not accepted because the adequacy to extrapolate from data of both, homologous and heterologous laboratory or small animal studies is limited.

Tolerance:

Information on the safety of the product can be gained from two controlled clinical field studies, performed in 73 horses in total (thereof 34 treated with HorStem), and from a placebo controlled target animal safety study, performed with 8 horses per group.

In the clinical trials, very common adverse reactions observed in animals after intraarticular injection of HorStem at the recommended treatment dose mainly comprised of local reactions in the treated joint (lameness, flexion pain, effusion) which required symptomatic therapy with an NSAID in some animals. All affected horses experienced complete resolution within days or up to two weeks. No controlled study using overdoses has been conducted, which is considered acceptable regarding the type of the product. Data available to identify the underlying reason for the observed local reactions are not sufficient to rule out immunological rejection of injected cells. Repeated dosing in a small number (8) of healthy horses led to an increase in the severity and frequency of local adverse reactions.

Specific areas regarding the target animal tolerance of MSCs are the biodistribution potential of these cells and subsequent ectopic tissue or tumor formation. In addition, there may be a theoretical risk that biodistribution of MSCs could, in case of cluster formation, lead to thrombosis, e.g. in the lungs of treated animals. It is, however, acknowledged that it is very challenging to generate reliable data on these issues and that the risks of biodistribution of stem cells and related events are low after intraarticular injection.

Dose determination/ Dose justification:

No dose determination study with HorStem has been provided. With reference to the MUMS/limited market-status of the application, the dose has been derived from published experiments in laboratory animals and in horses by attempting to establish a relationship between the joint volume and the number of cells needed for treatment.

The information provided on dose determination/dose justification is not fully satisfactory, and the efficacy of the recommended dose could not be reliably confirmed in the pivotal clinical field study due to shortcomings in the design and conduct of this study.

Efficacy:

The CVMP concludes that efficacy was not robustly confirmed in a multi-centre, placebo-controlled,

randomised and blinded pivotal field study. Concerns remain relating to issues concerning the design and conduct of the study and it is concluded that the findings of the study do not reliably demonstrate a clinically relevant effect of treatment in the alleviation of lameness associated with mild to moderate degenerative joint disease (osteoarthritis) in horses.

Part 5 – Benefit-risk assessment

Introduction

The proposed product, HorStem is a suspension for intraarticular injection for horses which contains EUC-MSCs in a concentration of 15 million cells/ml. It is a new active substance not authorised as a veterinary medicinal product in the European Union on the date of entry into force of the Regulation (EC) No 726/2004.

The proposed indication is reduction of lameness associated with mild to moderate degenerative joint disease (osteoarthritis) in horses.

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 efficacy of HorStem was evaluated in horses suffering from mild to moderate osteoarthritis in a pivotal randomised controlled clinical field study. Concerns remain relating to the design and conduct of the study and whether the findings of the study reliably demonstrate the clinical efficacy of HorStem in the alleviation of lameness in horses with mild to moderate osteoarthritis for up to 63 days.

Additional benefits

Not applicable.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented. Concerns remain regarding the establishment of an appropriate test to measure the potency at the final product and active substance level to show consistency of production, stability of active substance and finished product and to detect if unexpected events during active substance production occurred.

Safety:

Risks for the target animal:

The safety of HorStem was investigated in a placebo-controlled target animal safety study and in two field studies. Very common adverse reactions observed in animals after intraarticular injection of HorStem at the recommended treatment dose mainly comprised of orthopaedic reactions in the

treated joint (lameness, flexion pain, effusion) which required symptomatic therapy with an NSAID in some individual animals.

Risk for the user:

The user safety warnings are considered satisfactory to ensure the safety of the user when the product is handled as recommended.

Risk for the environment:

The product is not expected to pose a risk for the environment when used as recommended.

Risk for the consumer:

Stem cells are included in the list of substances considered as not falling within the scope of Regulation (EC) No. 470/2009, with regard to residues of veterinary medicinal products in foodstuffs of animal origin (EMA/CVMP/519714/2009-Rev.34). Hence the product is not expected to pose a risk to the consumer of foodstuffs derived from treated animals.

The withdrawal period of zero days is considered acceptable.

Risk management or mitigation measures

Appropriate information has been presented regarding the potential risks of this product relevant to the target animal, user, and the environment and to provide advice on how to prevent or reduce these risks.

The withdrawal period of zero days is considered acceptable.

Evaluation of the benefit-risk balance

Efficacy of the product for the reduction of lameness associated with mild to moderate degenerative joint disease (osteoarthritis) in horses has not been reliably demonstrated. It was noted that adverse events were common in the clinical studies.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the quality of the product has not been appropriately demonstrated.

Therefore, the CVMP considered that the data available would not allow the Committee to conclude on a positive benefit-risk balance.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for HorStem is not approvable since data on quality and efficacy remain inconclusive (i.e. quality and efficacy of the product have not been sufficiently demonstrated by the applicant). Therefore the data do not 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 therefore considers that it is not possible to conclude that the overall benefit-risk balance is positive and, therefore, recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product.

Grounds for refusal

Whereas:

- **Ground for refusal 1 (quality):**

  The suitability of the proposed potency assay to demonstrate potency and serve as an indicator for stability is not considered appropriately demonstrated. Prostaglandin E2 (PGE2) secretion has not been demonstrated to correlate with biological activity relating to efficacy of the product.

  In the absence of a suitable potency assay consistency of production and stability cannot be reliably demonstrated.

- **Ground for refusal 2 (efficacy):**

  In the pivotal field trial, issues concerning the design and conduct of the study remain and it is concluded that the findings of the study do not reliably demonstrate a clinically relevant effect of treatment. Horses in the treatment group were not handled in a similar manner to horses in the placebo group with respect to physical exercise after treatment. This may have significantly impacted on the clinical score of lameness.

  The grading system for efficacy (lameness) measurement is not considered suitable because it is not based on an internationally agreed grading system and original protocols were not followed.

On the basis of the above, the CVMP remains concerned about major outstanding issues in regard to the quality and efficacy data provided to support the indications.

The CVMP concludes, after verification of all the documents submitted, that the applicant has not sufficiently demonstrated the quality and efficacy of the veterinary medicinal product and therefore considers that it is not possible to conclude that the benefit-risk balance is positive.

Therefore, the CVMP recommends the refusal of the granting of the marketing authorisation for HorStem, in accordance with Article 37(1)(a) of Regulation (EC) No 726/2004.

Re-examination of the CVMP opinion of 11 October 2018

Introduction

Following a negative opinion on the 11 October 2018 for HorStem, EquiCord-Ymas S.L. requested the re-examination of the CVMP opinion under Article 34(2) of Regulation (EC) 726/2004. At the request of the applicant, an ad-hoc expert group (AHEG) meeting was held on 7 February 2019. The applicant attended the meeting to give a presentation and answer questions from the AHEG.

The applicant's grounds for re-examination, the AHEG's responses to the questions from CVMP and the CVMP final conclusions are described below.

The CVMP appointed Dr Rory Breathnach as rapporteur and Dr Frida Hasslung-Wikström as co-rapporteur for the re-examination procedure.

On 21 February 2019, the CVMP adopted the final CVMP opinion for HorStem.

On 19 June 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for HorStem.

Grounds for re-examination, AHEG and CVMP considerations

Ground for re-examination 1 (quality)

A. The suitability of the proposed potency assay to demonstrate potency and serve as an indicator for stability is not considered appropriately demonstrated. Prostaglandin E2 (PGE2) secretion has not been demonstrated to correlate with biological activity relating to efficacy of the product.

Applicant's grounds for re-examination

The grounds for refusal were based on the suitability of the proposed potency assay to demonstrate potency and serve as an indicator for stability as it was considered there was a lack of correlation between PGE2 secretion and biological activity relating to efficacy. The proposed potency assay was developed during the procedure in response to CVMP comments, as there was concern over the link between the initial proposed potency test and efficacy of the product. The initial test proposed at the time of submission was a cell viability test with no link to the activity of the cells. Therefore, CVMP requested that the applicant provide a more relevant potency test linked to the mode of action/efficacy of the product. The applicant at this stage (2017) sought scientific advice from CVMP regarding the design of a proposed potency assay to determine PGE2 levels secreted from MSCs. CVMP advised that the potency assay used should correlate to clinically relevant effects of treatment and enable the identification of potent and sub-potent batches. A specific marker selection related to the mode of action is proposed as a useful strategy for development of potency assays for cell therapy products. Furthermore, in line with reflection paper EMA/CAT/51134/2009 'Reflection paper on stem-cell medicinal products' that the assay should be at least semi-quantitative and show correlation with the intended therapeutic effect. Although this document has been adopted in the context of human medicines, it is CVMP's view that scientifically it can be applied also to veterinary medicinal products.

The applicant proposed to develop and validate a potency assay based on the activity of cells by stimulating the cells with synovial fluid and measuring their PGE2 secretion capacity at master cell bank (MCB) level. The applicant proposed a commercial assay to measure PGE2 supported by literature references to show a relationship with the intended therapeutic effect. This was proposed to be linked to MSCs producing PGE2 when they are stimulated by an inflammatory environment, where

the secreted PGE2 would mediate MSC induced immunosuppressive and anti-inflammatory effects (Kyurkchiev et al., 2014 and Wannemuehler at al., 2014). Based on the applicant's position and proposal at that time CVMP advised that 'A PGE2 assay as proposed by the company appears to be functionally linked to the biological activity of the MSCs by measuring PGE2 secretion capacity of the EUC-MSCs'. CVMP further advised that in general the proposed approach seemed to be acceptable to measure relevant cellular characteristics however the test needed to be validated. Ideally, a relationship should be demonstrated between the amounts of PGE2 produced from the EUC-MSC and the number of viable cells in the FP providing a clinically relevant effect of treatment. The applicant therefore followed the advice from CVMP and continued to develop and validate the PGE2 potency assay for HorStem.

The applicant chose to use the PGE2 marker as an indicator of potency, as PGE2 secreted from MSCs is defined as a pro-inflammatory factor with immuno-suppressive/regulatory activity (Kalinski 2012, Krampera et al., 2013) which has been proposed by the applicant to exert an overall anti-inflammatory effect (Manderfinini et al., 2015). It is the immune-regulatory/anti-inflammatory activity that the applicant considers to be the most relevant factor in making PGE2 a potency marker for HorStem. In their justification, based on bibliographical data, the applicant focussed not only on the ability of PGE2 to effect T-cell proliferation but also on the regulation and suppression of different cell types and cytokines involved in the progression of osteoarthritis.

PGE2 has been shown to be secreted from MSCs constitutively and when cells are stimulated with synovial fluid (Leijs et al., 2012), this was confirmed by data provided by the applicant. During the procedure CVMP commented on the appropriateness of the stimulation of MSCs using osteoarthritis synovial fluid (OA-SF) as the results showed a high degree of variability and obtaining batches of SF was not in keeping with the 3R's principles. The applicant therefore proposed to determine constitutive PGE2 secretion from MSCs. Also, further to CVMP comments, the potency assay was to be tested for release at the active substance stage instead of the MCB stage of manufacturing. The testing is completed on the active substance as testing of the finished product would result in a delayed result which would impact on the short shelf life of HorStem (14 days). This is keeping with the reflection paper EMA/CAT/51134/2009 which states that 'functional assays may not always be suitable for release testing, where the time for testing may be limited', although in this case the functional assay performed at the AS level is the product release test.

It is recognised that T-cells and macrophages are predominant cells in an inflamed OA joint. Blocking PGE2 production using indomethacin significantly increased T-cell proliferation *in vitro*, thereby indicating a suppressive effect of PGE2 on T-cell proliferation (Carrade et al., 2014, provided in the RLOQ). Carrade's study showed that PGE2 secreted from MSCs, from equine umbilical cord tissue, inhibit T-cell production through induction of lymphocyte apoptosis.

The PGE2 measured in the potency test has been shown by the applicant to be secreted constitutively from MSCs and this has been described in the literature to have an immuno-regulatory effect on OA joints, in terms of affecting T-cell proliferation, macrophage phenotype and the cytokines effecting the inflammation process. The link to MSC mode of action as exerting an overall anti-inflammatory effect in a pro-inflammatory environment, by way of immune cell regulation/suppression, and measured by the secretion of PGE2 could therefore be considered plausible. This is not without acknowledging that PGE2 is defined as a pro-inflammatory factor of the immune system however more recent publications identify PGE2 as a pro-inflammatory factor with immuno-suppressive/regulatory activity when present in a pro-inflammatory environment.

CVMP assessment and AHEG considerations

HorStem is a MUMS product and therefore in accordance with EMA/CVMP/EWP/117899/2004 Rev 1 it is acceptable to use bibliography data to support the efficacy claim for the product. However, there is no reduction of requirements for quality data as detailed in the "Guideline on quality data requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/limited market" (EMA/CVMP/QWP/128710-Rev.1). Moreover, in this guideline it is stated that "for products containing entirely new active substances, novel therapy products or products representing first in class, the possibilities for data reduction are likely to be limited".

The "Guideline on human-based medicinal products" (EMEA/CHMP/410869/2006) states: "An extensive characterisation of the cellular component should be established in terms of identity, purity, potency, viability and suitability for the intended use, unless justified".

The guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer (CHMP/BWP/271475/06) states that appropriately designed potency assays provide a reliable demonstration of biological activity of the active ingredient. Based on the literature provided during the procedure (Carrade et al., 2014) the applicant has proposed a potential link of measurement of PGE2 to the biological activity of the active substance in HorStem. The guideline recognises the difficulties in designing a potency test for cell based products due to the nature of the active ingredient itself (whole cells) and the unknown complex immune mechanisms exerted by these actives. However, to assure consistent functional activity of the product the guideline states that the potency of the product should be demonstrated with justified limits and based on a defined biological effect as close as possible to the MoA/clinical response. The guideline further states that where a direct measure of potency is not possible surrogates of potency such as secretion factors could be used if they quantify biological activity.

The biological activity of PGE2 in a pro-inflammatory environment has been adequately described in the literature provided in the responses to the list of questions. However, there was substantial debate concerning the pro-inflammatory role versus the anti-inflammatory role of PGE2 in arthritic joints in horses, both by CVMP members and by the AHEG. The CVMP agreed that the guideline recognises the difficulties in designing a potency test for cell based products. In the particular case of HorStem, the applicant has proposed PGE2 secretion as a surrogate marker for potency determination based on the study by Carrade et al., 2014. However, a direct relationship between PGE2 expression and inhibition of T-cell proliferation, although described in the literature, is expected to be very much dependent on the origin of the MSCs (species as well as tissue origin) and on the manufacturing process proposed. Therefore, the suitability of the proposed potency assay although described in the literature would preferentially be supported by product-specific data. Relevant assays designed for this particular purpose, as for example the one presented in the study by Carrade et al., should be conducted using HorStem MSCs to support the PGE2 ELISA method as a quality indicator of potency.

The AHEG considered that the assumption that the measurement of secreted PGE2 correlates to the biological activity of MSCs is a reasonable basis for the proposed potency assay. However, while the ability of the product under investigation to produce PGE2 was considered to be demonstrated, it was not considered that the product itself has been fully linked to a biologically relevant effect in osteoarthritis treatment (that is, product-specific data establishing a clear link to a biologically relevant effect should be provided).

The AHEG concluded that it is accepted that PGE2 could be considered as a suitable marker of potency, providing it is suitably demonstrated for the product in question and that reference to literature data only is not considered sufficient; thus, due to the uniqueness of the manufacturing process/product, the applicant should develop and validate their own in vitro model. The CVMP concluded that a risk

management plan to incorporate this product specific data as an integral component of an authorisation for Horstem is required.

Conclusion

It is concluded that literature data can be used as supportive information to provide evidence of the biological effect of this product; however, due to the particular characteristics of this product (i.e. cell-based product), product-specific data to support the mode of action of PGE2 (and its biological relevance in the target species) will be included as part of the post authorisation risk management plan for Horstem.

B. In the absence of a suitable potency assay consistency of production and stability cannot be reliably demonstrated.

Applicant's grounds for re-examination

The release specification for potency which was originally based on the potency data from 6 batches of AS, 3 of which were used in the pivotal efficacy study, is proposed to be revised. The proposed increase of the release specification is in response to a concern as to whether batches would remain potent over the product shelf life (14 days) as a decrease of ~32% was observed over the 14 day product shelf life. Thus, following the CVMP opinion, the applicant proposed a new release limit, which has been set based on the lowest value for PGE2 in an efficacious batch tested in the pivotal efficacy study (Donor 1502).

CVMP assessment and AHEG considerations

Reflection paper EMA/CAT/51134/2009 states that ideally the potency assay should be semi-quantitative and show correlation with the intended therapeutic effect. The potency assay as a measure of constitutive secreted PGE2 is quantitative with an applied potency limit based on the lowest value of PGE2 shown to be efficacious in the pivotal efficacy study (Donor 1502). The CVMP considers the newly proposed release specification could be acceptable to ensure batches will remain potent (or in this case, will maintain the same or greater levels of PGE2 relative to one of the three batches included in the pivotal clinical trial, which had the lowest PGE2 levels) over the shelf life of the product and therefore that the potency test can serve as an indicator of stability, provided that the decrease observed over the course of the shelf life is taken into account when setting the specification.

The AHEG were requested to consider whether the proposed release limit is adequate to discriminate between batches of sufficient and insufficient biological activity. It was concluded that the applicant's approach appears acceptable and that it is agreed that in this case, the specification can be set as the lowest value of the batch used in the pivotal clinical trial. However, the AHEG considered that the specification should be re-considered when more batch data becomes available.

In addition, the AHEG considered whether the potency test can serve as an indicator of stability. They concluded that if PGE2 were to be used as the only indicator of product stability, it would not be considered sufficient to demonstrate the stability of the product. The AHEG noted that PGE2 has been shown to decrease over time and therefore is shown to be a stability indicating parameter. Therefore, as additional parameters for stability are also monitored for this product, it is considered acceptable that PGE2 could be one such stability marker.

Conclusion

The applicant's proposal to establish the specification limit to correspond with the batch used in the pivotal clinical trial that had the lowest PGE2 value is considered acceptable. However, it is noted that this limit was proposed post-CVMP opinion, is based on limited data, and when more batch data

becomes available, the specification should be re-considered. PGE2 has been shown to decrease over time and therefore is accepted as being suitable as one of the stability indicating parameters.

Conclusions on grounds for re-examination 1 (quality)

The grounds for refusal on quality have been thoroughly reconsidered in the re-examination procedure. An AHEG was convened with the remit to consider the grounds for re-examination in which pivotal questions relating to the original grounds for re-examination were posed to the AHEG and their views sought. This was followed by further CVMP discussions and a final opinion.

While the proposed quality release test to measure PGE2 in active substance is considered acceptable as an indicator of consistency of manufacture and stability, product-specific data have not been provided to support the mode of action of PGE2 as a potency marker. The assumption that the measurement of secreted PGE2 correlates to the biological activity of MSCs was considered as a reasonable basis for the proposed assay, based on published literature submitted. However, further development and validation of the applicant's own quality assay to demonstrate its ability to discriminate between batches of sufficient and insufficient biological activity are required and these data (a product specific in vitro model) should be provided as part of the risk management plan for Horstem. It is considered that the data obtained from the risk management plan needs to further support the link between PGE2 and an anti-inflammatory effect in osteoarthritis treatment.

The proposed specification limit is based on the lowest PGE2 level in the three batches used in the pivotal clinical trial and given that the data obtained in the pivotal clinical trial are considered sufficient to support an effect of treatment, it can be determined that the proposed specification limit would have the ability to discriminate between batches of sufficient and insufficient biological activity.

Overall, it is concluded that PGE2 can be considered a suitable quality marker for further investigation based on the literature; however, in vitro product-specific data are required on future batches as part of the proposed risk management plan for Horstem to further support the link between PGE2 production and a clinically-relevant therapeutic effect.

Ground for re-examination 2 (efficacy)

A. Design and conduct of the pivotal efficacy study and clinically relevant effect of treatment

Applicant's grounds for re-examination

The applicant claimed that the pivotal clinical trial was designed and conducted according to EMA guidelines and CVMP recommendations. It is argued that CVMP Scientific Advice was followed with respect to the planned design of the pivotal field trial, and that the study design and choice of primary efficacy variable was considered acceptable by CVMP. The clinical trial was designed and conducted in accordance with the principles of GCP.

Concerning the scoring system for lameness evaluation in the trial, the applicant highlights that CVMP did not question the adequacy of the scoring system for lameness evaluation. The applicant argued that the study protocol was accurately followed, and that none of the protocol deviations or amendments had an impact on the overall outcome of the study. It is also highlighted that baseline homogeneity of the two groups was statistically demonstrated for 34 out of the 35 variables evaluated (the exception being 'joint selected for treatment'). However, the applicant previously justified the lack of bias between groups attributed to this variable, thus the issue regarding baseline homogeneity did not form part of the grounds for refusal. The results of the primary efficacy parameter demonstrated a statistically significant difference in favour of the treatment group; 75% of treated horses versus

23.5% of placebo horses reached the primary endpoint (defined as horses with an improvement in the lameness grade to a non-lame or an inconsistent lameness (≤ grade 1 AAEP scale by day 63), i.e., 12 out of 16 horses in the treatment group versus 4 out of 17 horses in the placebo group (p=0.0031). This is claimed to represent a clinically relevant improvement that allows a horse to re-enter normal training and sport life. The evaluation of the secondary efficacy variables also demonstrated a clinically relevant effect of treatment. It is therefore claimed that the clinical trial robustly demonstrates the efficacy of HorStem for the reduction of lameness grade in horses with mild to moderate osteoarthritis.

CVMP assessment and AHEG considerations

The CVMP notes that while it is claimed that the applicant has followed the CVMP Scientific Advice with respect to the design of the pivotal clinical trial, two alternative study designs were proposed in the scientific advice, neither of which were considered acceptable as proposed; however, concerns with the two proposed study designs were addressed in the design of the pivotal trial. CVMP agreed that the choice of lameness improvement as primary parameter was acceptable. It should be noted that no specific questions on the AAEP lameness grading were posed to CVMP. In relation to the protocol for pivotal trial, the applicant states that 'the protocol text did not include any specific detailing of the scale, because the AAEP scale was well known by all investigators.' However, the CVMP does not accept this point; on page 11 of the study protocol, definitions of the integer values 0, 1, 2, 3, 4 and 5 of the AAEP Lameness Scale are tabulated.

With the exception of the lameness evaluation, which is considered separately under point C, the study was a GCP-standard study conducted according to a pre-defined protocol with minor deviations and amendments. The AHEG considered the issue whether the design and conduct of the pivotal efficacy trial was robust and sufficiently reliable for evaluation of the efficacy of treatment, in addition to whether the primary efficacy parameter was an appropriate, clinically relevant end-point for efficacy evaluation.

The AHEG thoroughly discussed the efficacy questions, which were considered to be closely related. It was agreed, given the intended indication, that the choice of primary efficacy parameter was an acceptable end-point. While the specific concerns raised by CVMP in the grounds for refusal detailed in the original opinion (differences in activity level, lameness evaluation) were generally considered to be at least partially resolved by the AHEG (refer to points B and C), the AHEG raised concerns on a number of different aspects of the study which were considered to represent an insufficiently robust design of the clinical trial. The lack of appropriate stratification of the factors that inherently have an effect on outcome such as activity level, lower lameness score ratings, different joints treated (low motion versus high motion joints) etc. in combination with a relatively small number of animals included in the study was a cause of concern for the AHEG. While there was no concern with subjective scoring *per se*, the AHEG noted limitations in the area of grading of lameness scores <1.5, (poorer discriminating power at lower levels in the AAEP scale) as documented in the literature. However, it is noted that many of these latter issues had been raised during the original assessment procedure and were considered to have been adequately addressed. For example, the homogeneity of study groups with respect to the distribution of joints affected by OA had been discussed, and as a result the product literature for HorStem includes clear information on the precise joints which were treated in the clinical trial. At inclusion in the trial, horses in both groups (with the exception of one horse in the placebo group with grade 1 lameness) were diagnosed with lameness between grades 2 and 3 on the AAEP scale. It should also be highlighted that the applicant was not aware of any factors or subgroups likely to be clinically relevant, based on the first clinical trial conducted (which was later deemed supportive / exploratory only), that would influence the effect of outcome such that stratified randomisation would be necessary. Finally, regarding the AHEG concern over the limitations in the area of grading lameness

at milder lameness scores (1 – 2), this issue is discussed under point C, and overall, it is accepted that the assessment of lameness was conducted reliably.

Conclusion

The two main issues identified as grounds for refusal concerning the design and conduct of the pivotal clinical trial are considered to have been adequately addressed by the applicant during the re-examination procedure (refer to points B and C). Other concerns on the design and conduct of this study raised by the AHEG during the re-examination procedure had been considered and concluded on by the CVMP during the original procedure and were not specifically included within the scope of the grounds for re-examination.

Overall, it is accepted by the CVMP that the results of the clinical trial were adequate to establish a claim for efficacy of the product.

B. Exercise level of horses in the pivotal efficacy study

Applicant's grounds for re-examination

The applicant justified that the handling of horses in the two groups with respect to physical exercise after treatment was not different in a way that could bias the results. The protocol for the clinical trial specified that the exercise level was required to be unchanged from 4 weeks before treatment to the end of the study (63 days post-treatment); a change in exercise level during the study was one of the withdrawal criteria. The mean exercise levels of horses included in the study (jump, dressage, leisure, equestrian lessons) prior to onset of lameness was 8.125 in the treatment group versus 7.58 in the placebo group (on a scale of 0 to 10, according to the exercise grading scale as defined by Gough *et al*, 2010. Due to the onset of symptoms, the exercise level of horses was reduced in both groups, but it was obligatory to maintain a consistent level of exercise for at least 4 weeks prior to treatment. The exercise level during the trial was 5.1 (mean) in the treatment group and 6.6 (mean) in the placebo group, the differences between groups were claimed as minimal and not statistically significantly different (p=0.07, Kruskal-Wallis test). Concerns over the differences in the exercise level between the treatment vs placebo group primarily arose due to 4 horses in the treatment group with exercise levels of 1 or 2 (very low activity level), while all horses in the placebo group had exercise levels of 3 or higher. The applicant argues that the presumption that lower exercise levels could have positively impacted on the efficacy evaluation in the treatment group is negated by the fact that only 2 of the 4 horses in the treatment group with exercise levels of 1 or 2 were categorised as 'Therapeutic Success', which is a lower success rate (50%) than in that achieved in the treatment group overall (75%). Thus, it is claimed that the inclusion of 4 horses in the test group with exercise levels 1 – 2 did not lead to a more favourable outcome in terms of meeting the primary efficacy variable.

CVMP assessment and AHEG considerations

It is considered that sufficient support has been provided for the claim that the inclusion of 4 horses in the test group with exercise levels of 1 – 2 (while all horses in the placebo group had exercise levels ≥3), did not have a positive effect on the evaluation of efficacy in the trial. If it had been the case that low activity levels were to have had a beneficial effect on the resolution of lameness (as may be expected), it would be expected that the 4 horses in the test group with activity levels of 1 – 2 would be categorised as 'treatment success', however only 2/4 (50%) were 'treatment success', which is a lower success rate than achieved overall in the treatment group (75%). The AHEG were requested to consider this point and to discuss if there were differences in activity level in the two groups included in the pivotal clinical trial which may have impacted on the clinical score of lameness, and, if so, whether this was considered to have biased the study findings (and to what extent) in favour of the HorStem group.

It was the opinion of the AHEG that if the difference in activity level between the test and control groups had been the sole potential bias occurring in the field study, this would have been acceptable based on the information provided during the applicant's oral explanation. Concerning other potential biases identified by the AHEG, as previously discussed, it was noted by CVMP that many of the issues identified had been considered and concluded on by CVMP during the original assessment procedure and had not been identified as grounds for refusal.

Conclusion

The CVMP is of the opinion that it can be accepted that the differences in activity levels between the two study groups did not bias the evaluation of efficacy in the clinical trial.

C. Grading system for lameness evaluation in the pivotal efficacy study

Applicant's grounds for re-examination

The grounds for refusal state that 'The grading system for efficacy (lameness) measurement is not considered suitable because it is not based on an internationally agreed grading system and original protocols were not followed.' The applicant has provided arguments that the AAEP scale, with use of half-points, is an internationally agreed grading system and claims that the original protocol of the clinical trial was followed. The applicant argued that they had not considered that the use of half-points for lameness evaluation would represent an issue and state that no objection had been raised by CVMP concerning the use of half-points for lameness evaluation in the four clinical trials presented in the initial submission (3 safety studies, and one efficacy study). The applicant stated that all considerations, suggestions and improvements stated by the CVMP in the scientific advice were implemented in the new study design and described in the protocol. However, since no comments were raised on the use of half-points for lameness evaluation, this was essentially not considered an issue on the applicant's behalf.

It is claimed that, while the AAEP lameness scale is user friendly and commonly used, the whole number (integer) scale ranging from 0 to 5 does not cover as a linear scale all clinical signs of lameness. For this reason, veterinarians usually use half points inside the AAEP lameness scale, in order to better define the lameness grade of the horse. It is claimed that the use of half-point scoring within the AAEP scale is globally recognised in equine practice, with reference to peer-reviewed scientific articles, PhD theses and chapters in equine textbooks in which half-points were used for AAEP lameness scoring. Furthermore, a signed declaration by the Chairman of the AAEP foundation is provided in which it is stated that in his experience, the AAEP scale with half-points is the most common lameness scale used by equine practitioners worldwide. A similar declaration by the vice president of the Spanish Equine Practitioners Association was also provided.

It is argued that it was not necessary to have specified the use of the AAEP scale including half-points in the protocol text of the pivotal clinical trial, as the grading system for use in lameness scoring was clearly stated to be the internationally agreed AAEP scale. Individual signed statements from each of the participating investigators were provided, declaring that it was known, before starting the trial, that the lameness grade used for evaluation was a practical adaptation of AAEP scale (10-point scale, including half-points). The applicant discussed the issue previously raised as a concern by CVMP, that if the four horses in the placebo group that had been graded as 1.5 points at day 63, had been rounded down to a score of 1.0, these horses would have been classified as 'therapeutic success' (with the consequence that there would not have been a statistically significant difference between groups for the primary efficacy parameter). However, the applicant strongly argues that a lameness grade of 1.5 could not be classified as a therapeutic success given that grade 1.5 lameness would not represent an improvement considered clinically relevant, since horses above grade 1 on the scale have a mild to

consistent lameness grade and are not able to participate in international competitions (Federation Equestre Internacionalle (FEI) guidelines- 2018 Veterinary Regulations).

Overall, with respect to the method used for evaluation of lameness in the pivotal clinical trial, the applicant claims that an internationally agreed and acceptable lameness scale was used without any protocol deviation.

CVMP assessment and AHEG considerations

Concerning the first point regarding if it can be accepted that the use of half point scoring within the 0 – 5 AAEP lameness scale is well-established and widely used in equine practice, robust justification was provided by the applicant to substantiate this claim. The AHEG were requested to provide their opinion on this point and it was concluded that in principle a half-point scoring in the AAEP lameness scale is acceptable and widely used in equine practice.

As referred to in point A 'Design and conduct of the study', it is still considered to represent a deficiency that the study protocol failed to include a detailed description of the method used to score the primary efficacy parameter. However, the investigators involved in the clinical trial confirmed that they regularly use the AAEP scale using half-points in order to accurately describe the grade of lameness, and that they were aware that they should use the scale, including half-points if necessary, prior to starting the trial. The AHEG were requested to address if the justification provided by the applicant was sufficient to overcome this deficiency (i.e. that the half-point scoring was not stated in the protocol), and they considered that sufficient justification had been provided on this point, and that the deficiency and deviation from the protocol did not materially affect the discriminative power of the trial. While it was noted that the AHEG raised concerns regarding the limitations of the subjective evaluation of lameness using the 0 – 5 AAEP score when lameness grades are <1.5/5 (Keegan et al, 2010), the CVMP were further reassured that this particular concern was primarily related to inter-assessor variability, and that in the pivotal trial the same investigator was responsible for evaluating the same horse during the course of the study. Of the participating investigators in the clinical trial, seven investigators used half-points during the trial in their assessment of lameness, and each of their descriptions of the definition of a grade 1.5 and a grade 2.5 was provided. During the re-examination procedure, the CVMP raised concerns over the consistency between investigators in terms of interpretation of a 1.5 grade lameness as the definitions appeared to be subjective. The AHEG were requested to consider if they agreed that the half-point scoring criteria used was applied appropriately, based on the evaluation criteria for grades 1.5 and 2.5 as defined by the investigators in the trial. The AHEG considered this point in detail, with reference to the descriptions provided by the investigators and the definitions of the criteria for the integer AAEP scoring scale, and concluded, based on the information provided in the grounds for re-examination and eluded to during the oral explanation, that they were not convinced that half-point lameness evaluation was applied similarly between the seven investigators. However, the AHEG also stated that they were reassured by individual investigator descriptions of a 1.5 grade lameness and accepted that these scores were more likely to be measured as a 2 if the published 0 to 5 AAEP criteria had been adhered to, and thus the potential treatment effect would remain. Furthermore, the applicant provided additional supportive information during an oral explanation that the descriptions of the 1.5 grade scoring represented valid and acceptable descriptions of the clinical scenario between a score of 1 and 2 on the AAEP scale.

Therefore, taking into account the advice from the AHEG, the key issue with respect to the use of half-points, relating to the grading of 4 horses in the placebo group with lameness grade of 1.5 at Day 63, can be considered resolved. It is accepted that it would not have been appropriate to allocate a score of 1 ('treatment success') for lameness for these animals at Day 63, given that if the degree of lameness had to have been scored according to the integer scale, a score of 2 would have been deemed appropriate in most cases. Thus, the statistically significant difference between the test and

the control group for the primary efficacy parameter is not considered to have been invalidated by the use of half-point scores on the AAEP lameness scale.

Conclusion

It is accepted that the use of half-point scores in the AAEP lameness scale is well-accepted practice and that, while it is a noted deficiency of the study, acceptable justification has been provided for the omission to specify in the clinical trial protocol that half-points within the 0 – 5 AAEP lameness scale could be used. During the re-examination procedure, based on information provided by the applicant, together with the advice of the AHEG, it was concluded by CVMP that the method of lameness evaluation in the study was sufficiently reliable in order to determine an effect of treatment. Therefore, it was concluded that the grounds for refusal relating to the lameness evaluation had been satisfactorily addressed during the re-examination procedure.

Conclusions on grounds for re-examination 2 (efficacy)

The grounds for refusal on efficacy were thoroughly reconsidered in the re-examination procedure. An AHEG was convened, with the remit to consider the grounds for re-examination in which pivotal questions relating to the grounds for re-examination were posed to the AHEG and their views sought.

During the re-examination procedure, the original grounds for refusal were satisfactorily addressed:

- The applicant's justification that the use of half-point scoring on the AAEP scale is well-accepted practice for the evaluation of lameness was considered acceptable.
- The fact that the protocol did not include a clear description of the half-point scoring was still considered to represent a deficiency of the study; however sufficient justification was provided to confirm that all investigators were routinely applying half-points in their evaluation of lameness if needed, when the degree of lameness was between the criteria specified for the integer scoring on the AAEP lameness scale. Thus, this deviation from the study protocol was not a reason *per se* to invalidate the study findings.
- The conclusions of the AHEG were noted regarding the issue that if four horses in the placebo group that had a lameness grade of 1.5 at Day 63 had been scored according to the integer scale: based on the descriptions from the investigators, it was concluded that these horses would likely have been assessed as having a lameness grade of 2 on the 0 – 5 point AAEP scale, rather than a grade of 1.
- Overall, it can be accepted that the grading system for efficacy (lameness) measurement in the pivotal clinical trial was suitable.
- It was accepted that the difference in activity levels between the test and control groups was unlikely to have influenced the study outcome in favour of the test group.

Therefore, during the re-examination procedure, the concerns raised in the original opinion that the pivotal efficacy trial does not reliably demonstrate a clinically relevant effect of treatment due to issues concerning the design and conduct of the study, were considered to have been satisfactorily addressed.

Overall, the CVMP concluded that the original grounds for refusal on efficacy are deemed to have been satisfactorily addressed and that the findings of the pivotal clinical trial reliably demonstrate a clinically relevant effect of treatment. However, due to limitations of the submitted potency assay, the related potential impact on the quality of the batches released to the market after approval and the potential consequences for efficacy, it was agreed that there is a need to gather information on safety and

efficacy of Horstem post-authorisation, when used as intended in the field. These measures have been outlined in the risk management plan, agreed by the CVMP.

Overall conclusions on grounds for re-examination

Following the re-examination for the grounds for refusal 1 (quality) and taking into account the AHEG view, it can be concluded that PGE2 can be considered as an appropriate marker of quality of the active substance. However, due to the uniqueness of the manufacturing process, product-specific data to support the mode of action of PGE2 as secreted from MSCs is required post authorisation by way of a risk management plan proposed by the applicant in accordance with Article 31(1) Regulation 726/2004 and Article 12(3) (k) Directive (No) 2001/82/EC and agreed with CVMP (Annex 1). Reference to literature data supports the proposed mode of action.

The assumption that the measurement of secreted PGE2 correlates to the biological activity of MSCs is a reasonable basis for the proposed PGE2 quality release assay, and the ability of the product under investigation to produce PGE2 was demonstrated. Data obtained from the risk management plan is expected to further support the product's link to an anti-inflammatory effect in osteoarthritis treatment (that is, product-specific data establishing a clear link to an anti-inflammatory effect).

Given that the potency assay will be further developed and validated post-authorisation in the context of a risk management plan, the CVMP concluded that the concerns on quality raised in the grounds for refusal of the original opinion have been satisfactorily addressed.

Following the re-examination for the grounds for refusal 2 (efficacy) and taking into account the input of the AHEG group, it can be considered that the specific issues raised concerning the design and conduct of the pivotal clinical trial in the ground for refusal on efficacy (difference in activity levels, acceptance of the half-point AAEP scale as an internationally recognised grading system) have been resolved. It is therefore accepted that the findings of the study reliably demonstrate a clinically relevant effect of treatment. On this basis, the ground for refusal 2 (efficacy) is addressed and it is concluded that the efficacy of HorStem for the proposed indication has been adequately supported.

Due to limitations of the submitted potency assay, the related potential impact on the testing of the quality of the batches released to the market after approval and the potential consequences for efficacy, it was agreed that the safety and efficacy of Horstem, when used as intended in the field, must be surveyed with greater intensity than normally achieved by a routine pharmacovigilance system. Therefore, a risk management plan has been proposed by the applicant and will include the following risk management measures:

1. To further develop and validate the potency assay, so it can adequately discriminate between potent and non-potent batches of Horstem.
2. To ensure that all released batches have been tested with the above-mentioned validated potency assay and demonstrated to be of adequate quality before release and marketing.
3. To submit the updated and validated potency assay to the CVMP/EMA for evaluation and commenting, and to amend the assay in accordance with comments, if any.
4. To collect further field data, related to both safety and efficacy in horses, from batches that have been released by use of the updated potency assay. This collection of data will be detailed in a post-marketing surveillance study plan, to be drafted by the MAH and agreed by the CVMP, following the principles with the EC guideline - Volume 9B: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-9/vol_9b_2011-10.pdf. The surveillance study will collect safety and efficacy data after use in accordance with the approved product information from a minimum of 800 treated horses. Every reasonable effort will be made

to obtain feedback and collect data from the treating veterinarians and/or the horse-owner, including at least three separate and documented contact attempts by electronic/written means or telephone contact. If a veterinarian indicates that the product was used outside the approved product information (off-label), the safety and presumed efficacy data will still be recorded, but the case is not counted as part of the 800 horses.

Overall, the CVMP is of the opinion that the two grounds on which the CVMP adopted a negative opinion for HorStem have been addressed, and it is concluded that the benefit-risk assessment for the product is positive. There are limitations of the submitted potency assay, related to the level of efficacy in the field. Therefore, the applicant has agreed to a risk management plan, to provide product-specific in vitro data establishing a clear link of HorStem to an anti-inflammatory effect. The potency assay will be further developed and validated post-authorisation. Due to limitations of the submitted potency assay, the related potential impact on the testing of the quality of the batches released to the market after approval and the potential consequences for efficacy, it was agreed that additional safety and efficacy information on Horstem, when used as intended in the field, would be collected. This additional information is product specific data generated to be presented according to a risk management plan.

Final benefit-risk assessment

Introduction

The proposed product, HorStem is a suspension for intraarticular injection for horses which contains equine umbilical cord mesenchymal stem cells (EUC-MSCs) in a concentration of 15 million cells/ml. It is a new active substance not authorised as a veterinary medicinal product in the European Union on the date of entry into force of the Regulation (EC) No 726/2004.

The proposed indication is reduction of lameness associated with mild to moderate degenerative joint disease (osteoarthritis) in horses.

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

Direct therapeutic benefit

The efficacy of HorStem was evaluated in horses suffering from mild to moderate osteoarthritis in a pivotal, randomised, blinded, controlled clinical field study. The findings of the study demonstrated clinical efficacy of HorStem in the alleviation of lameness in horses with mild to moderate osteoarthritis for up to 63 days.

Additional benefits

Not applicable.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented.

The assumption that the measurement of secreted PGE2 correlates to the biological activity of MSCs is a reasonable basis for the proposed PGE2 quality release assay, and the ability of the product under investigation to produce PGE2 was demonstrated. However, to further demonstrate its ability to discriminate between batches of sufficient and insufficient biological activity, the potency assay will be further developed and validated post-authorisation in the context of a risk management plan.

Safety:

Risks for the target animal:

The safety of HorStem was investigated in a placebo-controlled target animal safety study and in two field studies. Very common adverse reactions observed in animals after intraarticular injection of HorStem at the recommended treatment dose mainly comprised of orthopaedic reactions in the treated joint (lameness, flexion pain, effusion) which required symptomatic therapy with an NSAID in some individual animals.

Risk for the user:

The user safety warnings are considered satisfactory to ensure the safety of the user when the product is handled as recommended.

Risk for the environment:

The product is not expected to pose a risk for the environment when used as recommended.

Risk for the consumer:

Stem cells are included in the list of substances considered as not falling within the scope of Regulation (EC) No. 470/2009, with regard to residues of veterinary medicinal products in foodstuffs of animal origin (EMA/CVMP/519714/2009-Rev.34). Hence the product is not expected to pose a risk to the consumer of foodstuffs derived from treated animals.

The withdrawal period of zero days is considered acceptable.

Risk management or mitigation measures

Appropriate information has been presented regarding the potential risks of this product relevant to the target animal, user, and the environment and to provide advice on how to prevent or reduce these risks. However, due to limitations of the submitted potency assay, the related potential impact on the quality of the batches released to the market after approval and the potential consequences for efficacy, it was agreed that there is a need to gather information on safety and efficacy of HorStem post-authorisation, when used as intended in the field. These measures have been outlined in the risk management plan, agreed by the CVMP.

The risk management plan will be terminated when the risks and issues outlined are considered to have been sufficiently addressed. Updates to the risk management plan should be submitted with PSUR submissions or upon request by the Agency.

Evaluation of the benefit-risk balance

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

Information on development, manufacture and control of the active substance and finished product has been presented and lead 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, the environment and consumers, when used as recommended. Appropriate precautionary measures, including withdrawal period, have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for HorStem 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).

Additional follow up measures have been outlined in a risk management plan, agreed by the CVMP to gather information on safety and efficacy of HorStem post-authorisation, when used as intended in the field.

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

Risk management plan for Horstem

In accordance with Article 31(1) of Regulation (EC) 726/2004, which refers to Art 12(3, k) of Directive (No) 2001/82/EC, and the CVMP Reflection paper on risk management plans for centrally authorised veterinary medicinal products (EMEA/CVMP/126726/2007), the following Risk Management Plan is provided for the centrally authorised product Horstem.

1. Identification of known and potential risks:

The approved summary of product characteristics (SPC) serves as the reference document and outlines the information on known risks and agreed risk management measures.

In addition, the need for extended measures for Horstem have been identified due to limitations of the submitted potency assay and the related potential impact on the testing of the quality of the batches released to the market after approval. Consequently, the safety and efficacy of the product must be surveyed with greater intensity than normally achieved by a routine pharmacovigilance system.

2. Defining those specific activities that complement routine pharmacovigilance and that address the specific issues:

To complement the routine pharmacovigilance system, the following measures are provided by the Marketing Authorisation Holder.

A. To further develop and validate the potency assay, so it can adequately discriminate between potent and non-potent batches of Horstem. An outline of the preliminary approach is described in Annex 1.

B. To ensure that all released batches have been tested with the above-mentioned validated potency assay and demonstrated to be of adequate quality before release and marketing.

C. To submit the updated and validated potency assay to the CVMP/EMA for evaluation and commenting, and to amend the assay in accordance with comments, if any.

D. To collect further field data, related to both safety and efficacy in horses, from batches that have been released by use of the updated potency assay. This collection of data will be detailed in a post-marketing surveillance study plan, to be drafted by the MAH, and follow the principles in the guideline for the conduct of post-marketing surveillance studies of veterinary medicinal products (EMEA/CVMP/044/99 - FINAL). The surveillance study will collect safety and efficacy data after use in accordance with the approved SPC from a minimum of 800 treated horses. Every reasonable effort will be made to obtain feed-back and collect data from the treating veterinarians and/or the horse-owner, including at least three separate and documented contact attempts by electronic/written means or telephone contact. If a veterinarian indicates that the product was used outside the approved SPC (off-label), the safety and presumed efficacy data will still be recorded, but the case is not counted as part of the 800 horses.

   Data to be provided for each treated horse by the treating veterinarian;

   - Batch details
   - Case narrative including;
     - Diagnostic approach for the osteoarthritis (OA)
     - Patient details
     - Prior history, related to the OA lameness

- Prior and concomitant treatments, related to the OA
- Activity level of the horse (low – medium – high)

▪ Treatment outcome evaluated at one and two months following treatment

- Endpoints:
  - Reduced lameness level: yes/no
  - Horse able to return to or maintain previous activity level: yes/no
  - An Owner questionnaire focussing on the activity level of the horse and any potential adverse events. This questionnaire will serve at the same time to inform the owner about the ongoing safety and efficacy surveillance.

E. To submit, every six months, an overall assessment of the experience gained including an evaluation of the safety and efficacy reported from the total number of treated horses. The starting date of the reporting scheme (Day 0) is the date of first release of a batch that has been tested with the updated and validated potency assay. The first report is submitted 6 months after Day 0, and subsequently at 6-month intervals, until it is agreed by the CVMP to stop the reporting.

F. To take action, if at any time point the quality, safety, or efficacy of Horstem is deemed inadequate, or if serious un-expected events occur, that would put the authorisation of Horstem in doubt.

ANNEX 1: Plan for further development and validation of the potency assay

Prostaglandin E2 (PGE2), as the proposed suitable marker for a potency test, was considered acceptable by the Ad-Hoc Expert Group (AHEG) consulted during the MA-procedure for HorStem. The AHEG also considered that the PGE2 correlates with the biological activity.

The current release specification limit is based on the minimum amount of PGE2 detected in *in vivo* efficacious batches and includes the potential drop-off during the shelf-life of the Finished Product. A reconsideration of the release specification limit will be conducted as more batch data becomes available. Reference to literature data is seen as supportive but the applicant was asked to develop their own cell-based *in vitro* model.

Cell- Based *in vitro* model:

EUC-MSCs profoundly affect immune responses through their interaction with the cellular components of the innate and the adaptive immune system, which are typically present in the joint infiltrates of patients with OA (mostly containing macrophages and T-cells). Particularly, this immunoregulatory effect of MSCs on T-cell proliferation has been widely described because of their role as effector cells in many diseases with an immune component, such as OA (Castro-Manrreza et al, 2015).

An abundance of publications has reported the suppression of T-cell proliferation by MSC *in vitro* by performing direct and indirect co-cultures of MSCs with T-cells, commonly named Mixed Lymphocytes Reaction (MLR). This assay and its adaptations analyse the effect of MSC on the proliferation of T-cells by co-culturing MSCs with alloantigen-stimulated peripheral blood mononuclear cells (PBMCs), mainly T-cells.

Such suppression of PBMCs has been clearly confirmed to be PGE2-mediated since the PBMC proliferation is restored after the PGE2 production blockage (Aggarwal et al, 2005; Auletta et al, 2015; Ayalla-Cuellar et al 2017, Carrade et al, 2012; Carrade et al, 2014, Colbath et al, 2016; Nicola et al, 2002, Solchaga et al, 2012, Chen et al, 2010).

Based on the approach published by Carrade et al, 2014 where it was demonstrated that PGE2 mediates the inhibition of the T-cell proliferation produced by EUC-MSC in a classic-direct MLR and on the publication by Paterson et al, 2014, where equine MSC-mediated suppression of PBMC proliferation was also demonstrated, the applicant/MAH proposes:

1. The quantitative correlation between suppression of PBMC proliferation and the concentration of exogenous PGE2 will be demonstrated in a standard assay over a relevant range of PGE2 concentrations.
2. The product-specific characteristics of HorStem, relative to the MSC-mediated suppression of PBMC proliferation, will be demonstrated by co-culturing EUC-MSCs at AS from three different donors with equine PHA-stimulated PBMCs, testing the PBMC proliferation inhibition and determining PGE2 levels in the supernatants of the co-cultures.
3. To demonstrate the central role of the PGE2 in mediating the inhibition, the same assay will be performed adding indomethacin (COX2 inhibitor = PGE2 secretion blockage) to observe the recuperation of the PBMC proliferation.

With this assay it should be demonstrated and confirmed the effect of potent HorStem batches in T-cell proliferation inhibition through PGE2 secretion in a co-culture model.

This cell-based *in vitro* model was considered suitable by the Ad-Hoc Expert Group (AHEG) who encouraged the applicant to develop its own *in vitro* model and who confirmed that the measurement of secreted PGE2 in the proposed potency assay, correlates to the biological activity of MSCs.

Non-Potent batches investigation:

During the development of HorStem, no batches were found to be sub-potent or non-potent. In order to detect the effect of non-potent or sub-potent HorStem batches, PGE2 secretion by EUC-MSC will be blocked with indomethacin (a well-known COX2 inhibitor, Carrade et al, 2014; Colbath et al, 2017; Clark et al, 2017). In other words, the applicant/MAH will create non-potent or sub-potent cells by indomethacin treatment. Once the PGE2 secretion capacity is blocked, their capacity to provoke a T-cell proliferation inhibition will be investigated in an *in vitro* test with PBMCs in order to determine the effect in T-cell proliferation of non- or sub-potent batches and thereby validate the potency assay and possibly optimise the specification limits.

Different concentrations of indomethacin will be used to treat EUC-MSC at AS and the corresponding PGE2 secretion will be quantified. In this manner, it is expected to obtain a dose-response curve, for use in the further development and validation.

With this assay, the MAH will validate that non-potent or sub-potent batches with the blocked capacity for PGE2 secretion will not be able to provoke the T-cell proliferation inhibition, and therefore it will not be efficacious *in vivo*.

The subpotent or non-potent batches will be tested in the applicant's ELISA potency assay to validate this assay and demonstrate that it is able to catch subpotent batches.

Depending on the success of the outlined approach, further development or other approaches may be investigated. The intention by the applicant is to reflect on the views expressed by de Wolf et al. (2017), *Regulatory perspective on in vitro potency assays for human mesenchymal stromal cells used in immunotherapy.*

ANNEX 2: Outline of the post-marketing surveillance study planned for Horstem

An open-label post-marketing surveillance study will be conducted following a written protocol and in line with the guideline on post-marketing surveillance studies (EMEA/CVMP/044/99-FINAL) to establish efficacy in customary practice.

Data from a total of 800 horses treated with HorStem according to the SPC will be recorded. In case data will be collected from horses treated with HorStem for OA, which is not in line with the SPC (for example treatment of the proximal interphalangeal joint), this data will also be analysed and sent to the regulatory authorities, but not as part of the original 800 horses.

Data from horses treated for a diagnosis different from osteoarthritis (off-label), e.g. tendinitis, will also be collected to gain information about safety and presumed efficacy, but will not be analysed as part of the 800 OA horses.

The level of efficacy of the post-marketing study should reflect the efficacy measured in the pivotal clinical study, despite different study designs, and be in line with the approved indication; "Reduction of lameness in horses with mild to moderate OA".

The efficacy endpoint of the post-marketing study to be counted as "successful outcome" is suggested as "Lameness improvement classified as "Moderate" or "Clear" two months after treatment".

In addition, the ability of the horses to perform their normal work, and therefore the efficacy of the treatment to enable return to normal work, will be evaluated.

In the pivotal clinical study, successful therapeutic effect was seen in 75% of treated horses, and a placebo effect was seen in 23% of placebo horses. The post-marketing study will be using similar cut-offs for estimating satisfactory effect in the responding OA-cases, and will take into account that the variability in responses and follow-up normally is greater in a post-marketing study than in a clinical study:

- Below 25% success rate: unsatisfactory effect level,
- Between 25 and 50% success rate: under the expected effect level
- Between 50% and 75% success rate: satisfactory effect level
- Over 75% success rate: above the expected effect level.

There has been discussion on whether box rest or moderate exercise could have an influence on the improvement of clinical signs of OA. To evaluate the influence of the patient's activity level on the outcome of the treatment, the activity level after treatment will be estimated as "low", "moderate" or "high" and this parameter will be analysed in the end of the study.

The SPC will be the basis for the expected safety and efficacy that is to be confirmed by this post-marketing study.

In addition the owner opinion will be asked in terms of satisfaction with the treatment, considering the effect in lameness improvement, the ability to perform normal work and the occurrence of adverse events.

For data collection every reasonable effort will be made to obtain feed-back and collect data from the treating veterinarians, including at least three separate and documented contact attempts by electronic/written means or telephone contact.

A protocol of study following the principles in the guideline for the conduct of post-marketing surveillance studies of veterinary medicinal products (EMEA/CVMP/044/99 - FINAL) will be drafted,

including how the data will be collected, the statistical management of the data and all the relevant aspects of the study, and this will be submitted to the corresponding authorities for commenting.

In the study, the treating vets will be asked to fill in a questionnaire for each horse. It will contain at least the following parameters:

- Vet identification
  - Vet name and surname:
  - Vet country:
- Batch Number: _____ (to be prefilled by MAH/EQC)
- Horse identification (please write name or microchip):
  - Describe:
- Age in years:
- Concomitants treatment (please describe if the animal is given any treatment)
  - No concomitants treatments:
  - Describe:
- Relevant clinical history related to OA (eg: OA in other joints, other pathologies in the same limb, chronicity, etc.)
  - Nothing relevant:
  - Describe:
- Activity level during the 2 months after treatment

Low (rest or hand work)	Moderate (ridden/driven occasionally, up to 2 days week)	High (sport activity, ridden/driven often, more than 2 days per week)

- Is the horse OA diagnosed?

	YES		Initially stage (no clear degenerative change)
			Mild to Moderate (Pointed joint margins, small- moderate spun)
			Severe (large spur cyst, fragments)
	NO	Describe the diagnosis: _____	

- Choose the diagnostic tools used (more than one can be selected)

	Lameness evaluation
	X-ray

	Perineural blocks
	Intra-articular block
	Echography
	None

- Joint treated

			Describe
Fetlock	Coffin	Hock	Other

- Has the horse suffered an adverse event suspected to be related to HorStem?

			Describe
No adverse event occurred	Acute synovitis 24h post administration with increase of lameness and pain	Moderate effusion not associated with pain or lameness increase	Other

- Lameness improvement. The scores "clear and moderate" will be counted as "effectiveness", while poor and none will be counted as not effective.

Please describe the lameness improvement 1 month after treatment	
	Clear
	Moderate
	Poor
	None

Please describe the lameness improvement 2 months after treatment	
	Clear
	Moderate
	Poor
	None

- Is the horse able to perform its normal work (evaluated two months after treatment)?

	YES
	NO

- Can you describe owner satisfaction in terms of effectiveness (lameness reduction and returning to normal work), where 1 is completely unsatisfactory and 10 is completely satisfactory

1	2	3	4	5	6	7	8	9	10

- Other comments:

Describe: _____

Divergent position on a CVMP opinion on the granting of a marketing authorisation for HorStem (EMEA/V/C/004265/0000)

HorStem is a novel veterinary therapy consisting of allogeneic mesenchymal stem cells constituting a new approach for treatment of osteoarthritis in horses. The application for marketing authorisation is, however, not considered acceptable for the following reason:

Regarding the mode of action for HorStem, it is implied that immunomodulatory effects might be involved. PGE2 has been proposed as a surrogate marker and a related ELISA assay has been included as a potency test. However, at this point, the presently proposed potency assay demonstrates only that isolated mesenchymal stromal cells are able to secrete PGE2 *in vitro.* For this PGE2 secretion to be correlated with a relevant biologic activity supporting the intended biological effect of Horstem, product-specific data are required, and these data have not been presented during the procedure. For this particular type of medicinal product, potency is a critical attribute without which the quality and batch-to-batch consistency of the product cannot be guaranteed. Information on this critical attribute cannot be obtained using other tests presently included in the quality control of HorStem. A suitable potency assay needs to be in place at the time for approval of the marketing authorisation. This view is also shared by the experts of the Ad Hoc Expert Group convened to provide scientific input to the CVMP on this specific issue.

In the absence of product-specific data, the suitability of the proposed ELISA assay to demonstrate potency and to identify batches with insufficient biological activity is not established. Furthermore, the suitability of this test as a potency indicator is not conclusively proved, and thus its capacity to serve as an indicator for stability is not considered verified.

In addition, we are of the opinion that a Risk Management Plan (RPM) is not a suitable means to provide the necessary information, within the context of an active marketing authorisation. The data, which the RMP is intended to provide, are considered essential data that need to be presented before a decision to grant a marketing authorisation can be made.

In the light of the absence of a suitable potency assay, efficacy of future batches of the product cannot be guaranteed and the Benefit/Risk balance of HorStem is therefore considered to be negative.

London, 21 February 2019

Hanne Bergendahl

Frida Hasslung Wikström

Ellen-Margrethe Vestergaard

Keith Baptiste