24 January 2019
EMA/805150/2018
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

Final CVMP assessment report for Horse Allo 20 (EMEA/V/C/004328/0000)

Common name: allogeneic mesenchymal stem cells from horse adipose tissue

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

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Introduction

The applicant Centauri Biotech SL submitted on 30 November 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Horse Allo 20, 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 6 November 2015 as Horse Allo 20 contains a new active substance (allogeneic equine adipose derived mesenchymal stem cells) which was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

Horse Allo 20 is a biological product; it is a stem cell suspension for intraarticular injection in horses. Horse Allo 20 contains allogeneic equine adipose derived mesenchymal stem cells (EA-MSC) in a concentration of 10 million cells/ml of and is presented in packs containing 1 pre-filled syringe or 1 vial of 2 ml (one dose).

The applicant applied for the following indication: For the treatment of lameness in osteoarthritis in adult horses.

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

The rapporteur appointed is Cristina Muñoz Madero and the co-rapporteur is Frida Hasslung Wikström.

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

In the light of the overall data submitted and the scientific discussion within the CVMP, a negative opinion for Horse Allo 20 was adopted, by majority, by the CVMP on 21 June 2018. The applicant submitted written notice to the Agency on 25 June 2018 to request a re-examination of the CVMP opinion of 21 June 2018. The applicant requested the involvement of a specific expert group in the re-examination.

During its meeting of 17-19 July 2018, the CVMP appointed J. Poot as rapporteur and G. Kulcsár as co-rapporteur for the re-examination procedure. The CVMP also agreed to the establishment of a specific Ad Hoc Expert Group (AHEG), its mandate and a re-examination timetable.

The applicant submitted the detailed grounds for the re-examination on 13 August 2018.

The re-examination procedure started on 14 August 2018.

The rapporteur's assessment report and co-rapporteur's critique were circulated to all CVMP members on 28 August 2018.

At their September 2018 meeting the CVMP appointed an AHEG and adopted a list of questions for the AHEG to address. The AHEG consisted of experts on quality of stem cells, clinical trials design and statistics and osteoarthritis in horses.

The AHEG meeting was convened on 4 and 5 October 2018 at EMA to consider the questions and to provide responses to the CVMP. During this meeting the applicant gave an oral explanation. The report from this meeting was forwarded to all CVMP members and the applicant on 5 October 2018.

The applicant submitted written notice to the Agency on 9 October 2018, withdrawing from the procedure. The opinion adopted by CVMP on 21 June 2018 therefore becomes final. The European Commission was informed on 11 October 2018.

On 7 December 2018 the European Commission requested the EMA to reconsider one of the grounds for refusal of the CVMP opinion dated 21 June 2018. The relevant grounds focused on the absence of GMP compliance for one of the manufacturing sites, as described below, in Part 1A of this report.

The revised opinion was adopted by CVMP on 24 January 2019.

Scientific advice

The applicant received scientific advice from the CVMP on 7 November 2013. The scientific advice pertained to quality, safety and clinical development of the dossier.

The applicant has broadly followed the scientific advice (EMA/CVMP/SAWP/531441/2013) recommendations, although not to the full satisfaction of the Committee. The recommendation regarding purity and identity tests was mainly followed, but still additional questions about these tests were included in the list of questions. The advice regarding the EA-MSCs identity was followed with the inclusion of bibliographic support and the applicant added one marker to the ones indicated in the scientific advice (MHCII) for the identification.

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

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (December 2017), which however has a number of deficiencies in terms of:

- Discrepancies on the role and responsibilities performed by Centauri and the contract company which includes expedited electronic notification, flow of safety reports, preparation of PSUR and databases.

Manufacturing authorisations and inspection status

Manufacture of the dosage form, primary and secondary packaging and batch release takes place at the contract company. Good Manufacturing Practice (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.

Quality control of the finished product is performed by contact companies.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release.

This site is not authorised by the Competent Authority in Spain to perform the activities mentioned above and has not yet been inspected for GMP compliance. In its opinion of 21 June 2018 the CVMP concluded that this site did not conform to legal requirements and that consequently it could not be accepted as a manufacturer of this veterinary medicinal product.

Following the adoption of the CVMP opinion on 21 June 2018 the European Commission requested that this position should be reconsidered in light of the published "Questions and Answers on allogenic stem cell based products for veterinary use: Specific questions on extraneous agents" dated 13 July 2017 (EMA/CVMP/ADVENT/803494/2016), which indicates that the principle provisions laid down in Annex 2 of Part I of Eudralex - Volume 4 - GMP requirements for biological medicinal products for human use including Advanced Therapy Medicinal Products (ATMPs) as defined in Regulation (EC) 1394/2007 [currently - Part IV of Eudralex - Volume 4 - GMP requirements for Advanced Therapy Medicinal Products dated 22 November 2017] could be considered applicable to stem cell products for veterinary use, thus foreseeing the possibility that, under specific circumstances, a non GMP-certified site may be accepted for quality control activities. However, the legal basis that allows exceptions to be made to the requirement for manufacturing sites to be GMP-certified applies only to advanced therapy medicinal products for human use and has no parallel relating to veterinary medicinal products. Consequently the exception for veterinary medicinal products has no legal basis.

Having considered that the Questions and Answers document referred to above was publicly available at the time of submission and assessment of the marketing authorisation application and could be considered misleading, it is concluded that, in this specific case, the objection to non-compliance with legal requirements related to good manufacturing practice should not be maintained as a grounds for refusal. Thus, the GMP based grounds for refusal of granting of marketing authorisation for this veterinary medicinal product is removed.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was not considered fully satisfactory with the requirements of directive 2001/82/EC. However, it is considered that the discrepancies could be clarified by the MAH post authorisation if the product were approved.

The GMP status of the manufacturing site, proposed to perform quality control on active substance and finished product is not GMP compliant. The opinion adopted at the June 2018 CVMP meeting included this as a grounds for refusal but following a request received from the European Commission to reconsider the issue it was agreed, in this specific case, not to maintain the objection to non-compliance with legal GMP requirements as a grounds for refusal.

The other manufacturing sites were GMP compliant.

Part 2 - Quality

Composition

Horse Allo 20 is an intraarticular suspension for injection intended for the treatment of osteoarthritis in horses and is composed of equine allogeneic mesenchymal stem cells (EA-MSCs) derived from horse adipose tissue.

Two different presentations were proposed, both of which are single dose containers providing EA-MSC cells as the active substance.

The first presentation is a ready to use ("fresh") single dose 2 ml suspension for injection in a borosilicate glass pre-filled syringe. Each single dose consists of cells in a Dulbecco's Modified Eagle's Medium (DMEM).

The second presentation is a deep frozen suspension for injection (which then has to be thawed before use) in a polypropylene vial. The frozen product provides the same quantity of the active substance again in a Dulbecco's Modified Eagle's Medium (DMEM) but in addition it contains dimethylsulphoxide (DMSO) as a cryopreservative.

Containers

For the ready to use ("fresh") product, the primary packaging consists of a pre-filled, colourless, borosilicate syringe of 2.25 ml of size closed with tamper Evident Luerlock Closure (TELC). TELC comprises a luer lock adapter made of polycarbonate, a tamper evident closure part made of TPE (Thermoplastic Elastomer) and a pre-assembled rubber insert, made of rubber formulation West 7025/65, grey.

For the frozen product, the primary packaging consists of one polypropylene vial of 3.6 ml of size closed with screwcap. Materials of the screwcap are polypropylene and a silicone gasket.

No administration device is supplied with the product.

Appropriate specifications have been proposed for the immediate packaging (containers and closure systems) and in accordance with relevant Ph. Eur. monographs. Certificates of analysis have been supplied demonstrating compliance with the proposed specifications.

Development pharmaceutics

The active substance is equine allogeneic mesenchymal stem cells (EA-MSCs) expanded *in vitro*.

All the components used during the manufacturing process have been described and justified.

The formulation used during the clinical safety and efficacy studies was the same as the ready to use presentation intended for marketing. However only one clinical safety study was carried out with the frozen product (containing DMSO) and no efficacy studies were conducted with that presentation. Descriptions and certificates of analysis have been provided for the ingredients of both presentations.

The manufacturing process of the product used in clinical trials was similar to that proposed for the product to be marketed. The quality and especially the clinical parts of this application were based on research and development (R&D) batches manufactured in a GMP-like environment. The relevance of GMP-like batches and pre-commercial batches has however still not yet been sufficiently demonstrated in a comparative exercise as requested and no complete specification data set has been submitted for at least one relevant batch.

It is not possible to terminally sterilise a cell-based product and therefore all the steps in the manufacturing process are critical for ensuring the sterility of the product. A test for contamination was proposed to be performed on one passage before release for the fresh product and on the final product (batch release) for the frozen product. This was considered adequate.

In the development pharmaceutics, the proposed composition, constituents, immediate packaging, manufacturing method and control were based almost entirely on published literature which is acceptable. An extensive review of the previously existing bibliographic evidence supporting the choice of identity and purity markers has been provided and was paralleled by sufficient

characterisation of the cell population, performed starting from the stromal vascular fraction (SVF) derived from adipose tissue.

Method of manufacture

The manufacturing process comprises the following stages: adipose tissue extraction from horses, isolation of cells, amplification and cryopreservation of the mesenchymal stem cells to obtain the master cell bank (MCB), active substance production and manufacture of the ready to use ('fresh') and frozen final products.

All data initially provided on the manufacturing process were obtained in GMP-like facilities which cannot be considered acceptable. Therefore, the active substance and the finished product manufacture were transferred to a GMP compliant manufacturing site to ensure the consistency of the batches released. Batch data submitted for the pre-commercial batches demonstrated that the specifications were met for the quality attributes tested. However, these data did not include results from the complete list of the specifications and no complete comparability exercise has been performed, including potency data obtained using an accepted and validated method. The suitability of the newly proposed potency determining method has not been established. A clinical safety study was performed with batches manufactured in compliance with GMP requirements.

For production of the stem cells, horse donors under 2 years of age were selected. The origin of the donor, clinical history and health condition were checked before obtaining the adipose tissue. The tissue was cut and a collagenase digestion performed to isolate the SVF, also called passage 0. After stopping the reaction and changing the media, cells were cultured in complete growth media to obtain the passage 1 which was considered the MCB. The MCB was frozen in foetal bovine serum (FBS) media containing DMSO.

For further production of the active substance, the MCB was thawed and cells were cultured for two more passages, to obtain the active substance which was then frozen in a FBS plus DMSO cryopreservative solution.

From the thawed active substance cells, the two different finished product presentations (ready to use or frozen) were manufactured. Different excipients, primary packaging, storage temperatures and slightly different manufacturing steps were performed to obtain the two different presentations. In the absence of any data from any clinical trials, additional information about the frozen formulation was requested as well as data obtained from a comparability exercise to demonstrate the equivalence between the two proposed formulations. Additional data were submitted but their relevance for this particular exercise is questioned as the formulation of the investigated samples is not clearly stated. A new target animal safety study was provided by the applicant but no data was obtained to support the frozen presentation other than the safety. This issue therefore still remains to be satisfactorily resolved before the quality and efficacy of this presentation could be considered proven.

In-process controls were performed during the production to ensure the homogeneity and reproducibility of the process. Batch to batch consistency has been tested using several batches. Sterility, mycoplasma, endotoxins, genetic stability, purity and potency are the controls proposed to demonstrate batch to batch consistency. However, a complete set of specification data for at least one relevant batch of Horse Allo 20 was not yet provided.

The commercial batch size has been indicated to be about 140 to 310 doses. Two different presentations are proposed:

- Presentation 1: Cardboard box containing one pre-filled, colourless, borosilicate syringe of 2.2 ml

closed with tamper evident Luerlock closure.

- Presentation 2: Cardboard box containing one polypropylene vial of 3.6 ml closed with a screwcap.

Control of starting materials

The following starting materials were described:

- Active substance: EA-MSCs
- Excipients: DMEM (ready to use/'fresh' product) or DMEM plus DMSO (frozen product)
- Substances of biological origin: Equine adipose tissue, MCB, trypsin, FBS, collagenase, penicillin/streptomycin and amphotericin B.

Active substance

The active substance consists of allogeneic mesenchymal stem cells derived from equine adipose tissue (EA-MSCs) that were obtained from the MCB and then stored frozen.

Active substance identity:

The identity of the active substance was based on the recommendations issued by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) and includes:

1) Adherence to plastic: confirmed by frequent monitoring of cellular morphology and cellular confluence.
2) Surface antigen expression for identity and purity control: the choice of the specific markers investigated was initially based mostly on bibliographic evidence. Sufficient characterization of the cellular populations and their variation during the manufacturing process was provided during the evaluation procedure, supporting the relevance of the specific markers included in the control strategy.
3) Multipotent differentiation potential to osteogenic, chondrogenic and adipogenic lineages: the differentiation capacity of the EA-MSCs of the active substance was included as information only.

Active substance potency

The initially proposed potency assay was based on a qualitative determination of the capacity of cultured MSCs to differentiate into adipocytes, osteocytes and chondrocytes. The suitability of this assay as a potency indicator was not established and a new test has been proposed. Although an extensive review of relevant existing literature support a possible mechanistic role in the pathology of osteoarthritis (OA), the capacity of this assay to discriminate between batches with expected biologic activity and those without was not demonstrated. Therefore, another potency test was proposed (ELISA-based). However, it was not clearly stated nor demonstrated how this attribute can identify batches with sufficient biological activity to promote a positive clinical effect. Batch data have been provided to support this potency assay. However, their relevance is not fully established: no finished product batches used in the clinical trials have been tested using this particular potency assay. The active substance batches used in previous studies have been used to produce new finished product. However, no information regarding the period of storage for these three different active substance batches have been described and their stability in terms of potency cannot be determined as they have not been tested with this new method at time 0. Therefore their relevance as supportive for the

finished product batches used in the clinical studies is not yet demonstrated. Additional concerns regarding the suitability of the proposed potency assay are raised under “Control of finished product”.

Genetic stability of active substance

The genetic stability of the cells was tested. No abnormal or malignant cells were observed, beyond the production steps. Genetic stability was tested in the active substance and in the frozen finished product.

A test was included in the specifications for the frozen product but not for the ready to use (‘fresh’) product. This is an important test for the quality of the Horse Allo 20 and therefore is expected to be included as release test for both formulations.

Impurities of the active substance

Impurities can be cellular impurities including contamination with other cell populations and dead cells, and non-cellular impurities including traces of products and media used during the manufacturing process, cell isolation and culture expansion. Possible cellular impurities are sufficiently controlled in the specifications.

Absence of adventitious agents: A risk assessment was performed to evaluate relevant extraneous agents to be tested at active substance and MCB level including also the donor animal. Virus contamination was sufficiently addressed with respect to the donor horse testing strategy. However, a risk assessment for each of the relevant raw materials used in manufacturing as well as a justification for the proposed strategy for viral testing during manufacturing has not been provided.

Bacteria, mycoplasma and bacterial endotoxins are also controlled in the specifications. However, batches found positive after sterility and mycoplasma testing have been used in clinical studies and a root cause analysis was requested. A summary of the performed analysis, although with no clear identified root cause, was provided for one batch only, which is not considered acceptable. In order to demonstrate the microbiological control strategy for Horse Allo 20 and the relevance of the batches used in the clinical studies for the future commercial batches, their microbiological control needs to be established. For this purpose, comprehensive root cause-analysis are needed for all batches identified as contaminated in the clinical trials.

Theoretical calculations indicate that process-related impurities (collagenase, antibiotics, trypsin and FBS) could be present in the final product at very low concentrations.

Validation of active substance manufacturing process

According to the CVMP guideline (EMA/CVMP/QWP/128710/2004-Rev.1), complete data from at a minimum one batch of at least pilot scale should be submitted. Data provided to support the validation of the manufacturing process include results obtained from R&D batches and pre-commercial batches. Although the differences between the R&D batches, pre-commercial batches and commercial batches have been clarified, release data according to the full specification (including the proposed potency assay) on at least one relevant batch have not been provided. Furthermore, a comparative exercise between the R&D (clinical) batches and the commercial batches, including at a minimum a full set of specifications, has not been submitted. Therefore the validation of the manufacturing process is not yet supported.

Excipients

Two different presentations are proposed and each of these contains a different excipient solutions.

Ready to use ('fresh') product:

DMEM is the excipient used for the ready to use presentation. A justification for the use of DMEM has been included. The qualitative composition of DMEM has been provided together with valid certificates of analysis.

Frozen product:

DMEM mixed with DMSO: The composition of the frozen finished product includes DMEM and DMSO. All the control tests listed in the Ph. Eur. are included for the DMSO as well as a justification for the use of this component.

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

A risk evaluation of transmission of Bovine Spongiform Encephalopathy (BSE) has been performed by the applicant taking into account potential sources of BSE/TSE and the risk was considered as negligible for the target species (horse) or for human beings.

For DMSO, a certificate of origin (USA) for TSE purposes is included which indicates that no animal sources are used to manufacture the product. Also a TSE declaration for collagenase has been included. The applicant certifies that the product does not contain and is not derived from specified risk material as defined by the Commission Decision 97/5354/EC.

A valid TSE certificate of suitability for foetal bovine serum (FBS) was provided.

Substances of biological origin

Adipose tissue
The adipose tissue was collected following national and regional regulations on equine life and reproduction (R.D. 804/2011). A donor validation document has been provided describing all the parameters tested and documents required for donor selection.

Adipose tissue samples were identified with the passport number of the animal and come together with the veterinary medical history, the biochemistry and haematological analyses, and the negative results for the pathogen tests. Samples to perform the extraneous agents tests to certify their absence in the donor were directly submitted to a national reference laboratory.

Upon arrival, the adipose tissue was exhaustively rinsed with washing solution, minced and then digested. The stromal vascular fraction containing the cells was resuspended and the cells were seeded.

Master Cell Bank (MCB)

The MCBs used for the batches included in the application were produced under controlled conditions (GMP-like) although not in compliance with GMP. The description of how the SVF was obtained was included. A complete study for the identity and purity of the cell population from the SVF of adipose tissue has been performed in order to characterise the cells from the starting material to the final product. The data submitted are largely considered sufficient to support an adequate characterization of the MCB although the method used to determine the identity and the purity of MCB is not yet considered validated.

Collagenase (included in the dossier as a non-biological starting material)

This enzyme was used for the digestion of the adipose tissue. It was tested for molecular weight,

appearance and protein content. Relevant certificates of analysis from the manufacturer have been presented. Following the transfer of the manufacturing process to a GMP facility, another collagenase from the same supplier has been introduced to comply with requirements for sterility testing.

Trypsin

In the manufacturing of R&D batches, trypsin was used to detach the cells from the culture plates. For the manufacturing of commercial batches a synthetic trypsin was introduced. Certificates of analysis have been provided as well as an assessment report on its BSE risk.

Foetal bovine serum

Foetal bovine serum was used for *in vitro* culture of mammalian cells. A valid TSE certificate of suitability from EDQM has been provided. Sera were tested for bovine viral diarrhoea (BVD), infectious bovine rhinotracheitis (IBR) and parainfluenza type 3 (PI3). Heat inactivation was performed. An EDQM certificate of suitability from the FBS manufacturer has been submitted that covers also the inactivation tested in 11 different viruses.

Amphotericin B

Amphotericin B is an antifungal antibiotic from *Streptomyces* sp. The antibiotic solution after passage 0 contains penicillin-streptomycin-glutamine and no amphotericin B.

Penicillin-Streptomycin- Glutamine solution has been tested for sterility and endotoxins.

Control tests during production

The tables below summarise all the tests (material tested, method, proposed specification and purpose of the test) performed during production and at the two final product presentations:

Test	**MCB**	**Active substance**
Packaging integrity	X	X
Colour rinsing solution	X	
Appearance	X	X
Cell count	X	X
Cell morphology	X	X
Viability	X	X
Media colour and turbidity	X	X
Culture density	X	X
Sterility		X
Mycoplasma	X	X
Identity and purity		X
Genetic stability		X
Potency		X
Endotoxins	X	

Cell morphology, media colour and turbidity and cell density are routinely performed. Viability is tested before seeding the cells for every passage. Cell counts are conducted for the MCB and for the drug substance.

For MCB the following tests are performed: packaging integrity, colour rinsing solution, appearance, cell count, cell morphology, viability, media colour and turbidity and culture density.

For the active substance and final product, the same test as for MCB and also the following are performed: Sterility, mycoplasma, identity and purity, genetic stability and potency.

Other controls performed during production are the mycoplasma, the sterility and endotoxins tests.

All test performed as in-process controls (IPCs) are considered sufficiently described. The tests used for the control of the active substance and finished product are further discussed below.

Control tests on the finished product

A summary table with all the controls proposed for the two presentations, that is, ready to use ('fresh') product and frozen product, is included in the section above. In general, the specifications established for the control of active substance and finished product is considered sufficient. However, at this point, the control strategy for Horse Allo 20 is not considered adequate for several reasons:

- The potency assay as well as the established limits are not considered justified (further discussed below)
- A test has not been included in the specifications of the ready to use ('fresh') formulation
- Accepted limits for cell count have not been defined in the specification list.

Identification and purity tests were performed at the level of the active substance and, following the recommendations provided by ISCT, were based on adherence to plastic, fibroblastic shape and a set of positive and negative cell surface makers.

The choice of markers for identity and purity control was based entirely on bibliographic evidence. Description of the surface markers was provided and their specificity towards relevant cells obtained from equine tissue has been demonstrated for the purity markers only.

The assay used to control the identity and purity of Horse Allo 20 is based on flow cytometry method and is considered central in demonstrating the quality of Horse Allo 20. A comprehensive validation of this method as per VICH GL1/2 has not been performed and submitted.

Potency

As previously discussed under the control of starting materials (active substance testing), several potency assays have been proposed in the course of the evaluation of this application. The currently used potency test is ELISA is based. However, it is not clearly stated nor demonstrated how this attribute can identify batches with sufficient biological activity to promote a positive clinical effect. The current data submitted for the newly proposed ELISA method cannot fully support the suitability of this test as potency indicator since:

- The ELISA method has not been fully validated by the applicant using product specific matrix as required in VICH Topic GL1.
- The relevance of the batch data provided for this attribute is not fully established: No finished product batches used in the clinical trials have been tested using this particular potency assay. Active substance batches used in previous studies have been used to produce

new finished product. However, no information regarding the period of storage for these three different active substance batches have been described and their stability in terms of potency cannot be determined as they have not been tested with this new method at time 0. Therefore their relevance as supportive for the finished product batches used in the clinical studies is not yet demonstrated.

- It is not clear how the specification limits are established. The applicant states that three batches have been tested repeatedly and the limits were established based on the minimum and maximum values obtained. The approach is not justified and the raw data on which these limits were stablished are not provided. Additionally, no data has been submitted to demonstrate that the proposed potency test can identify sub-potent batches, in order to establish relevant specifications.
- A correspondence between the established potency assay (and its specifications) to a relevant biological activity related to a clinical effect was not demonstrated in developmental studies and/or in clinical studies unequivocally supporting the efficacy of the product. The included clinical studies are not considered conclusive and efficacy of treatment has not been demonstrated.
- In the absence of an established potency indicating assay, the stability of DS and finished product cannot be considered demonstrated.

Accumulative population doublings of active substance in the finished product

The number of accumulative population doublings (PD) has been estimated and the passage has been appropriately justified.

Genetic stability

Karyotype and soft agar assay tests were carried out. No chromosomal aberrations were detected.

Bacteria, bacterial endotoxins and fungi

Tests are done according to Ph. Eur. monographs.

- Endotoxin test according to Ph. Eur. 2.6.14.
- Test for bacteria and fungi according to Ph. Eur. 5.1.6. and 2.6.27

A complete validation of the method has been provided with all the organisms listed in the Ph. Eur 2.6.27.

Mycoplasma determination

This test is performed during manufacture and it is also a test for batch release. For the fresh product, the mycoplasma test is done in the discarded culture media.

Virus contamination

A test for virus contamination is not carried out in the finished product batch. A risk assessment regarding viral contamination was provided but was not considered adequate as this did not include the contamination risk derived from the raw materials used neither a justification for the proposed strategy for viral testing during manufacturing.

Stability

Stability studies were performed on the MCB, the cryopreserved active substance and the finished

product.

MCB stability

Stability data on eleven lots of MCB were provided. Vials were thawed approximately and seeded to continue with the cell culture process. Stability controls are viability, spindle shape and adherence to plastic. Based on those controls the applicant has set an acceptance criterion and proposed a shelf life of one year for the MCB.

Active substance stability

From 5 different MCB batches, 32 different active substance batches were made. The proposed stability controls are viability, spindle shape and adherence to plastic. Based on these a shelf life of one year has been proposed.

Final product stability

Fresh product: The applicant proposed the viability, sterility, mycoplasma, endotoxins, purity and potency as the main variables measured. The only control used to set the shelf life was the cell viability. Results showed a great reduction in viability from 72 hours at 15 ºC to 25 ºC. The applicant proposed a shelf life of 72 hours for the fresh finished product.

Frozen product: The applicant provided data on the cell viability of one batch of Horse Allo 20 for up to 18 months of storage. Additionally, four-month data from another frozen finished product batch was submitted suggesting that sterility, mycoplasma, endotoxins, purity and trilineage differentiation (previously considered as potency indicator) are meeting the proposed specifications.

As stability data according to all relevant stability indicating specifications (including an adequate potency assay) have not been provided, the stability claim cannot be supported for any of the formulations proposed.

Overall conclusions on quality

The dossier was extensively based on bibliographic references and not adequately supported by real data obtained from Horse Allo 20 cells.

The application for Horse Allo 20 is mainly based on R&D batches manufactured in a GMP-like environment. Data have been submitted for R&D batches and pre-commercial batches. However, release data according to the full specification (including the proposed potency assay) on at least one relevant batch have still not been provided and therefore the exercise cannot be considered conclusive.

The identity and purity of Horse Allo 20 are determined by the same method, flow cytometry. The flow cytometry-based assay is therefore central in demonstrating the quality of Horse Allo 20. A comprehensive validation of this method as per VICH GL1/2 has not been performed and submitted.

The current data submitted for the newly proposed ELISA method cannot fully support the suitability of this test as potency indicator. Furthermore, the relevance of the potency data provided is not yet demonstrated and therefore the proposed limits cannot be supported.

Horse Allo 20 is presented in two formulations, ready to use (‘fresh’) and frozen, each of which has different excipients, and different primary packaging, storage conditions and shelf-life. Due to major differences in the composition of the ready to use and frozen presentations of Horse Allo 20 (DMSO), additional clarifications and data on important quality related issues are still missing. The relevance of

the submitted results intended to support the comparability between the two presentations is questioned as the formulation of the investigated samples is not clearly stated in the dossier. Therefore the submitted data cannot be considered conclusive.

In conclusion, Horse Allo 20 cannot be considered approvable from the quality perspective, as several major objections have not been resolved.

Part 3 – Safety

No data demonstrating equivalence between the two presentations in terms of their clinical characteristics was presented. Therefore, the proposed frozen presentation was not accepted

Safety documentation

Pharmacodynamics

No original pharmacodynamic studies have been performed. Literature data relevant to pharmacodynamic properties are discussed in Part 4.

Pharmacokinetics

See part 4.

Toxicological studies

No toxicity data for equine allogeneic mesenchymal stem cells in laboratory animal species were provided. This is considered acceptable.

As the active component in Horse Allo 20 consists of stem cells the general study data requirements for pharmaceutical products do not apply. There are no general EU veterinary guidelines for stem cells; as a result recommendations regarding development plans and evaluation requirements are generally given on a case-by-case basis for each product by the CVMP via the scientific advice procedure. The major safety concern for a product containing stem cells is considered to be related to potentially malignant transformation and tumorigenic effects. There are no adequate *in vivo* models for investigating tumorigenic potential. A well-controlled production process with adult mesenchymal stem cells that have been cultured for a limited number of passages and are controlled for identity, purity and genomic stability in terms of population doubling time (PDT) and karyotype will however contribute to a low risk for tumorigenicity.

Single dose toxicity

No single dose toxicity data relating to the active substance of the final product were provided. Three studies using cells from different species that had been prepared according to a partially similar process to that used for the final product have been presented. The relevance of this data to describe safety/toxicity of Horse Allo 20 in the target species is, however, unclear due to the differences in cell origin and manufacturing processes, animal species, administration route and disease model and a low number of treated animals.

Summary of conclusions from the three studies presented:

Rodríguez Hurtado I, Gómez Lucas R, García-Castro J, Mariñas Pardo L, Hermida Prieto M. Uso de células progenitoras mesenquimales alogénicas en patologías ortopédicas en caballos. Equinus. 2014 May; 39:48-60	A preliminary study of the use of allogeneic MSCs in different orthopaedic conditions in a total of 6 horses. Different doses were administered including the dose recommended for Horse Allo 20 (from 5 to 20 x 10^6 cells). No adverse effects were observed in the horses. Relevance for the present product is unclear.
Pérez-Merino EM, Usón-Casaús JM, Duque-Carrasco J, Zaragoza-Bayle C, Mariñas-Pardo L, Hermida-Prieto M, Vilafranca-Compte M, Barrera-Chacón R, Gualtieri M. Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Endoscopic and histological outcomes. Vet J. 2015 Dec;206(3):391	An uncontrolled study to determine the safety of adipose tissue derived MSC therapy in dogs with inflammatory bowel disease (IBD). Different species, disease condition and route of administration (i.v.). Relevance for the present product is unclear
Mariñas-Pardo L, Mirones I, Amor-Carro O, Fraga-Iriso R, Lema-Costa B, Cubillo I, Rodríguez Milla MÁ, García-Castro J, Ramos-Barbón D. Mesenchymal stem cells regulate airway contractile tissue remodeling in murine experimental asthma. Allergy. 2014 Jun;69(6):730-40	Murine adipose tissue derived MSCs expressing green fluorescent protein (GFP) administered i.v. in a murine asthma model. Migration of the cells to the area with lesions was reported. No adverse events were detected. The study investigated different species (mice), disease condition (asthma) and different route of administration (i.v.) compared to Horse Allo 20. Relevance for the present product is unclear.

In addition to the findings of the above studies 16 bibliographic references were provided. The conclusions from these studies cannot be extrapolated to Horse Allo 20 as the manipulation, animal or tissue origin and tests for identification are not the same as for the proposed product.

Repeat dose toxicity

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

A target animal safety study where treatment was repeated three times with 15-day intervals has been presented, see part 4. In summary, few conclusions can be drawn from the results due to a limited number of animals tested, the complex study design and inadequate presentation of the data. Only conclusions regarding general aspects can be made; 6 of the 8 horses showed local adverse reactions (lameness and/or local inflammatory reactions) and all of these occurred in limbs that had been treated with the stem cells and after the second or third administration of the product. No local adverse reactions were demonstrated in limbs treated with the control product (CP) placebo. No systemic adverse reactions were demonstrated.

Tolerance in the target species of animal

Tolerance in the target animal species is discussed in part 4. Data to describe safety in the target species were obtained in both a target animal safety study and the pivotal field study.

Reproductive toxicity

The product is not intended for use in pregnant females. No specific studies were provided to support the safety of EA-MSCs on reproduction or developmental toxicity. This is acceptable.

Genotoxicity

No genotoxicity tests in accordance with to the standard test battery were conducted with equine adipose mesenchymal stem cells. The tumorigenic potential of mesenchymal stem cells is best controlled by the quality of the cultured product, i.e. by specifications of identity, purity and genomic stability in terms of population doubling time (PDT) and karyotype (see part 2). Furthermore, in the CHMP Guideline on human cell based medicinal products (EMEA/CHMP/410869/2006) it is stated that genotoxicity studies are not considered necessary for human cells, unless the nature of any expressed product indicates an interaction directly with DNA or other chromosomal material.

Carcinogenicity / Tumorigenicity

No data from tumorigenicity studies were provided. The tumorigenic potential of mesenchymal stem cells is best controlled by the quality of the cultured product, i.e. by specifications of identity, purity and genomic stability in terms of population doubling time (PDT) and karyotype (see part 2).

Studies of other effects

Examination of immunological functions

No study to test for any effects of this product on the immune system has been included.

Bibliographic references have been provided in general support of an immune privileged state of MSCs. The references were noted, however, in the light of the high number and relative severity of local adverse reactions noted in the pivotal clinical trial, the concept of immune privilege was questioned for the cells in this product. Indeed, recent publications on equine allogeneic MSCs have reported a strong immunogenic potential of the cells that the authors attribute to mismatching between the donor and recipient MHCs. It was not clear from the study report or data records provided for the clinical trial which individuals received repeated administrations of the test product. It was therefore not possible to conclude on any potential correlation of repeated administration to adverse events, but from the information available it was considered that the adverse reactions could be due to immunogenic reactions initiated by the cells, and that these could be aggravated by repeated administrations of the product. The applicant has not clarified the causes of the adverse reactions and has not presented any further data with respect to immunogenic properties of the cells. *In vitro* data could help to characterise the cells further with respect to potential immunogenicity and would therefore have been of value.

Excipients

The proposed ready to use (‘fresh’) formulation of this product included Dulbecco’s Modified Eagle’s Medium (DMEM) as the only excipient. A proposed frozen formulation included dimethylsulphoxide

(DMSO) and DMEM as excipients. However, since no data demonstrating equivalence between the two presentations in terms of their clinical characteristics was presented the proposed frozen presentation was not accepted by the CVMP.

DMEM contains several proteins, vitamins and soluble factors and is not considered to pose any risk for the skin, mucosa and for the eye.

User safety

The applicant has presented a user safety risk assessment which has largely been conducted in accordance with the CVMP guideline on user safety of pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1). The most likely routes of user exposure are dermal and/or oral exposure and accidental self-injection, with the latter representing the worst case scenario. The user risk assessment has addressed the risk of possible severe immunological responses, pain and swelling at the injection site, thrombosis, possible unwanted homing, and the risk of ectopic tissue formation. As long as the product meets the quality specifications severe physiological or immunological changes at the injection site would not be expected following accidental injection. There is not considered to be a risk of unwanted homing or ectopic tissue formation by MSCs in a xenogeneic environment. Expected adverse events include pain, local inflammatory reactions and swelling at the site of injection, all of which are expected to resolve spontaneously.

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

The proposed frozen presentation of the product included the excipient DMSO. The permitted daily exposure (PDE) for DMSO has been established as 50 mg/day (ICH guideline Q3C (EMA/CHMP/ICH/82260/2006) for human medicinal products and VICH guideline GL18(R) for veterinary medicinal products (EMA/CVMP/VICH/502/99-Rev.1). The frozen presentation was, however, not accepted due to the lack of adequate clinical data.

Environmental risk assessment

According to VICH GL6-Environmental Impact Assessment (EIA) for Veterinary Medicinal Products the environmental risk assessment (ERA) can stop in Phase I and no Phase II assessment is required because the product is intended to treat only a small number of animals (e.g. not as a herd treatment) and consequently environmental exposure can be expected to be well below levels that would have an environmental impact.

Horse Allo 20 was not expected to pose a risk for the environment when used according to the proposed SPC.

Residues documentation

MRLs

The active substance contained in Horse Allo 20, equine adipose tissue derived mesenchymal stem cells, 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 annex to part 2C of the dossier contains the composition of the DMEM, including quantitative information. All components are included in Regulation (EC) No 37/2010 or the list of substances considered as not falling within the scope of Regulation (EC) No 470/2009 (EMA/CVMP/519714/2009), with the exception of ferric nitrate nonahydrate and sodium pyruvate, which are not considered to be pharmacologically active at the dose administered to the target animal and so are not considered to fall within the scope of Regulation (EC) No 470/2009 when used as in this product. In addition, a worst case consumer exposure estimate was provided along with data/argumentation to support the view that the exposure from these substances would not represent a hazard for the consumer.

Residue studies

No residue depletion studies were provided.

Withdrawal periods

Since the active substance and DMEM are considered to be out of scope of Regulation (EC) No 470/2009 a withdrawal period of zero days is considered acceptable.

Overall conclusions on the safety and residues documentation

No toxicity data, including data on repeat dose toxicity for equine allogeneic mesenchymal stem cells in laboratory animal species, were provided. This can be accepted as the target animal safety, user safety and consumer safety are addressed by means of other data.

In the pivotal clinical trial local adverse reactions to treatment were numerous and severe. The cause of the local adverse reactions has not been evaluated, and there is no data or discussion on mode of action of the treatment providing plausible explanations. It is indicated from the data presented that repeated treatment results in increased frequency and severity of reactions. Considering the known characteristics of MSCs and that the local reactions showed clear inflammatory signs it is likely that the cause is immunological/inflammatory (see Part 4).

No specific studies were provided to support the safety of equine allogeneic mesenchymal stem cells on reproduction or developmental toxicity. This is acceptable since treatment with the product is not indicated in pregnant animals.

No data from genotoxicity or tumorigenicity studies were provided. The tumorigenic potential of mesenchymal stem cells is best controlled by the quality of the cultured product, i.e. by specifications of identity, purity and genomic stability in terms of population doubling time (PDT) and karyotype (see part 2).

The use of this product is not expected to represent a user safety concern. The main potential routes of accidental contact identified for the user include those of dermal and/or oral exposure and as the worst case scenario, accidental self-injection. As long as all quality aspects in the manufacture of the product are met, no severe physiological or pathological changes, including any formation of tumour cells, are expected after accidental systemic self-administration of xenogeneic MSCs. Expected adverse events may include pain, local inflammatory reactions and swelling at the site of injection.

As the MSCs are unlikely to survive and/or differentiate in a xenogeneic environment due to the lack of necessary stimuli, is it not considered that there would be any risk for unwanted homing or ectopic tissue formation by equine allogeneic MSCs in a xenogeneic environment.

The ERA can stop in phase 1. The product is not expected to pose a risk to the environment when used according to the product information.

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

Part 4 – Efficacy

Pharmacodynamics

No proprietary studies were performed in order to assess the pharmacodynamic properties of Horse Allo 20. A summary of published information on osteoarthritis in horses and stem cells discussing various aspects and possible modes of action was presented. This information could be relevant as a basis for the design of studies to explore and describe functional properties of the cells in Horse Allo 20. There was, however, no product specific data presented, and the relevant functional characteristics of Horse Allo 20 are therefore largely unknown.

The applicant presented published data where cells produced according to the same principles as Horse Allo were tested in different species and disease conditions. The relevance of this information for the present product is, however, unclear.

Pérez-Merino EM, Usón-Casaús JM, Hermida-Prieto M, Mariñas-Pardo L. Correlation between canine IBD activity indices after stem cell therapy. Vet Rec. 2016 Nov 5;179(18):464	Different species (dogs), disease condition (IBD) and route of administration (i.v.). Relevance for the present product is unclear.
Pérez-Merino EM, Usón-Casaús JM, Zaragoza-Bayle C, Duque-Carrasco J, Mariñas-Pardo L, Hermida-Prieto M, Barrera-Chacón R, Gualtieri M. Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Clinical and laboratory outcomes. Vet J. 2015 Dec;206(3):385-90	Same study as in the reference above. Different species (dogs), disease condition (IBD) and route of administration (i.v.). Relevance for the present product is unclear
Pérez-Merino EM, Usón-Casaús JM, Duque-Carrasco J, Zaragoza-Bayle C, Mariñas-Pardo L, Hermida-Prieto M, Vilafranca-Compte M, Barrera-Chacón R, Gualtieri M. Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Endoscopic and histological outcomes. Vet J. 2015 Dec;206(3):391	Same study as in the reference above. Different species (dogs), disease condition (IBD) and route of administration (i.v.). Relevance for the present product is unclear
Mariñas-Pardo L, Mirones I, Amor-Carro O, Fraga-Iriso R, Lema-Costa B, Cubillo I, Rodríguez Milla MÁ, García-Castro J, Ramos-Barbón D. Mesenchymal	Different species (mice), disease condition (asthma) and route of administration (i.v.).

stem cells regulate airway contractile tissue remodeling in murine experimental asthma. Allergy. 2014 Jun;69(6):730-40	Relevance for the present product is unclear.
Rodríguez Hurtado I, Gómez Lucas R, García-Castro J, Mariñas Pardo L, Hermida Prieto M. Uso de células progenitoras mesenquimales alogénicas en patologías ortopédicas en caballos. Equinus. 2014 May; 39:48-60	Very low level of detail. Relevance for the present product is unclear.

The proposed potency test was not considered acceptable (see part 2).

Pharmacokinetics

No proprietary studies were performed in order to assess the pharmacokinetic properties of Horse Allo 20. Bibliographic data was submitted in order to describe the persistence, migration and biodistribution of different types of stem cells, different doses and administration routes. The data provided to describe the pharmacokinetics of stem cells is limited to information obtained from other species, localisations or types of cells, and the relevance for Horse Allo 20 is therefore unclear since they concern autologous or xenogenous cells and it is unknown how the migration of cells after their administration has been evaluated. The fact that only a fraction of the administered cells were found at the administration site shortly after administration in several studies indicates that elimination of these cells is rapid and substantial.

ChondroCelect (EMEA/724428/2009)	Persistence of autologous cells evaluated in goats, cells administered locally to cartilage defects. Degree of distribution unclear.
Becerra P, Valdés Vázquez MA, Dudhia J, Fiske-Jackson AR, Neves F, Hartman NG, Smith RK. *Distribution of injected technetium(99m)-labeled mesenchymal stem cells in horses with naturally occurring tendinopathy*. J Orthop Res. 2013 Jul;31(7):1096-102	Tendon lesion, horses, autologous cells. Only limited no. of cells remained in lesion after 24 h. i.v. infusion resulted in migration to lung tissue. Clinical relevance of this finding is unclear since no adverse reactions were recorded.
de Windt TS, Vonk LA, Slaper-Cortenbach IC, van den Broek MP, Nizak R, van Rijen MH, de Weger RA, Dhert WJ, Saris DB. *Allogeneic Mesenchymal Stem Cells Stimulate Cartilage Regeneration and Are Safe for Single-Stage Cartilage Repair in Humans upon Mixture with Recycled Autologous Chondrons*. Stem Cells. 2016 Aug. Epub ahead of print	Human, allogeneic cells mixed with autologous chondrons in fibrin glue scaffold, intraarticular administration. Cartilage repair. No allogeneic cells present in repair tissue after one year.
Toupet K, Maumus M, Peyrafitte JA, Bourin P, van Lent PL, Ferreira R, et al. *Long-term detection of human adipose-derived mesenchymal stem cells after intraarticular injection in SCID mice*. Arthritis Rheum 2013 Jul;65(7):1786-94	Human cells at different doses, i.v. injection in mice – xenogenic cells. Most cells were eliminated within 10 days Relevance for the current product is unclear (xenogenic model)

Although there is a possibility that equine MSCs may migrate to other tissues it is considered unlikely that this would result in any clinically significant effects. Becerra et al. demonstrated the substantial biodistribution of equine MSCs after i.v. infusion but with no correlated adverse effects. Local administration into a joint will reduce the number of cells distributed systemically compared to i.v. administration. It therefore considered unlikely that the intraarticular injection of Horse Allo 20 would result in clinically relevant effects due to biodistribution of MSCs.

Dose justification / Dose determination

No dose determination study was conducted with Horse Allo 20. The dose of Horse Allo 20 was selected based on bibliographic data available from different species. In a study by Toupet et al. (2013) three different doses of MSCs were evaluated in mice (1×10^6, 2×10^6 and 4×10^6). No toxicity was observed in the two lower dose groups whereas in the highest dose group 2 of the 9 mice died from embolisms. The applicant concluded that the proposed dose in the horse is 10x lower than the acceptable dose in mice and that it is therefore safe in horses. This extrapolation and calculation is, however, not accepted since the study involved a xenogenic model with human cells in mice with a therefore unknown relevance for allogeneic cells in horses. The safety of the recommended treatment dose therefore remains to be demonstrated in the target animal using the final product administered by the intended route of administration.

For a MUMS application dose determination data could be waived. This is, however, only accepted if the final product used is demonstrated as safe and efficacious in clinical studies when used at the recommended dose. Adequate data in support of efficacy has, however, not been presented and an unacceptably high number of adverse events were identified in the pivotal field trial. It is therefore not possible to confirm whether the recommended dose has been adequately justified.

Horse Allo 20 is composed of equine allogeneic MSCs. Two presentations are proposed for Horse Allo 20: ready to use (fresh) and frozen. In the ready to use presentation the excipient is Dulbecco's modified eagle's medium (DMEM). In the frozen product DMEM and dimethylsulfoxide (DMSO) are excipients. To demonstrate equivalence between the two product presentations, however, in relation to the proposed frozen presentation clinical data has not been presented in order to assess the efficacy. Therefore, the frozen presentation cannot be accepted.

Dose confirmation studies

No specific dose confirmation study has been presented but the applicant refers to the pivotal clinical trial. This could only be accepted if the recommended dose of the product was demonstrated as safe and efficacious in clinical studies. However, there was insufficient data provided to support of the efficacy of the product and since an unacceptably high number of adverse events were identified in the pivotal field trial it was therefore not possible to confirm the suitability of the recommended dose of the product.

Target animal tolerance

Target animal tolerance has been evaluated in a target animal safety study and the pivotal clinical field trial.

As supportive information the company provided bibliographic data from studies of MSCs produced in different species by a process that is analogous (or almost analogous) to the process used in horses for the production of Horse Allo 20. The relevance with respect to the target animal tolerance of Horse Allo

20 is however unclear since the species, disease models (IBD and asthma) and mode of administration (intravenous) all differ from those intended for the product.

Pérez-Merino EM, Usón-Casaús JM, Zaragoza-Bayle C, Duque-Carrasco J, Mariñas-Pardo L, Hermida-Prieto M, Barrera-Chacón R, Gualtieri M. *Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Clinical and laboratory outcomes*. Vet J. 2015 Dec;206(3):385-90	I.v. infusion in dogs with confirmed IBD, 2x10e5 cells/kg, 42 day follow up period. No acute or chronic adverse events detected
Pérez-Merino EM, Usón-Casaús JM, Duque-Carrasco J, Zaragoza-Bayle C, Mariñas-Pardo L, Hermida-Prieto M, Vilafranca-Compte M, Barrera-Chacón R, Gualtieri M. *Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Endoscopic and histological outcomes.* Vet J. 2015 Dec;206(3):391	Same study as in the reference above
Pérez-Merino EM, Usón-Casaús JM, Hermida-Prieto M, Mariñas-Pardo L. *Correlation between canine IBD activity indices after stem cell therapy.* Vet Rec. 2016 Nov 5;179(18):464	Same study as in the reference above
Mariñas-Pardo L, Mirones I, Amor-Carro O, Fraga-Iriso R, Lema-Costa B, Cubillo I, Rodríguez Milla MÁ, García-Castro J, Ramos-Barbón D. *Mesenchymal stem cells regulate airway contractile tissue remodeling in murine experimental asthma*. Allergy. 2014 Jun;69(6):730-40	Asthma model in mice, allogenic cells i.v. administration. 72h or 2 weeks follow up period. No MSCs detected in lung tissue
Rodríguez Hurtado I, Gómez Lucas R, García-Castro J, Mariñas Pardo L, Hermida Prieto M. *Uso de células progenitoras mesenquimales alogénicas en patologías ortopédicas en caballos*. Equinus. 2014 May; 39:48-60	Only abstract is available in English, very low level of detail. Uncontrolled study involving only 5 horses. Unclear if product used corresponds to final product.

The target animal safety study (TAS - Horse Allo 20) was placebo controlled and designed to evaluate repeated administration of Horse Allo 20 at the recommended dose in multiple joints. Eight (8) animals were enrolled, and each was administered injections in 4 joints (left and right distal interphalangeal and metatarsophalangeal joints), two joints on one side with Horse Allo 20 and the two contralateral joints with placebo. Four (4) horses were treated with the ready to use presentation of Horse Allo 20 and 4 horses with the frozen presentation. Administrations were repeated three times with intervals of 15 days between injections.

No systemic reactions were observed throughout the study period. No local adverse reactions were demonstrated after the first injection with Horse Allo 20. Local adverse reactions consisting of lameness, pain, swelling and synovitis were observed in 6/8 horses in joints treated with Horse Allo 20 after the second and third administrations of the product. No adverse reactions were demonstrated in the joints treated with placebo, indicating a treatment related inflammatory reaction. According to the applicant, synovial fluid data did not support an immunogenic reaction. However,

due to the timing of the sampling of the synovia, as well as a high proportion of missing samples it is not possible to conclude on immunological/inflammatory reactions after treatment based on this data. It cannot be excluded that inflammatory reactions to treatment are of immunogenic origin, in particular since reactions were only demonstrated after the second and third administrations.

Due to the complex study design and the low number of animals included in the study it is not possible to conclude on potential differences in the safety profile of the two presentations (that is, the ready to use presentation and the frozen presentation).

The pivotal field trial was a placebo controlled multi-centre field trial including a total of 72 horses of which 39 were treated with Horse Allo 20 (ready to use presentation) and 33 with placebo. A total of 36 adverse events (AEs) in 21 horses were reported during the study. 29 of these adverse events occurred in horses treated with Horse Allo 20 and 7 in the placebo treated horses. Of the 21 horses with AEs, 16 were administered Horse Allo 20 and 5 placebo. Of the 16 horses showing adverse events classified as “Probable” or “Possible” related to treatment by the investigator, 15 were from the group treated with Horse Allo 20 whereas one was from the placebo group indicating that adverse reactions were related to the cellular component of the product, and not associated with the intraarticular injection itself. Adverse reactions were described as local signs related to the site of administration and included joint inflammation, local pain, synovitis, signs of heat at the treated joint and increased lameness which normally occurred a few days after injection of the product. A total of 17 individual animals were treated with a non-steroidal anti-inflammatory drug (NSAID) due to adverse reactions during the study. Of these horses were 15 from the group treated with Horse Allo 20 and 2 from the placebo group. Four (4) animals required repeated treatment, all of these in the group treated with Horse Allo 20. Adverse reactions resulted in several cases of more serious conditions than the initial condition of osteoarthritis intended to be treated, such as increased lameness to grade 4 or 5, a total of 11/29 (38%) of AEs classified by the investigator as related to treatment in the treated group did not resolve during the study period, but suffered complications mentioned as “sequelae”, “remaining static” or “worsened”.

Since the information presented was not clear on the adverse events after repeated administration this issue is not considered sufficiently addressed.

The applicant presented a new analysis of adverse events in the pivotal field study after the blinding was broken. This was not considered acceptable, however, regardless of the new analysis and classification of adverse events presented by the applicant, the safety of the product is not considered acceptable due to the high number and severity of local adverse reactions demonstrated in the pivotal field trial. The adverse reactions were clearly related to treatment with the MSCs and are not considered acceptable for an intraarticular treatment of osteoarthritis in horses.

An inflammatory/immunological cause of the local adverse reactions has not been appropriately investigated. The applicant concluded that local adverse reactions were not caused by an immune reaction elicited as a result of treatment, but that swelling was a result of synovitis and increased fenestration of blood vessels. It would, however, appear that synovitis is an inflammatory reaction of immunological origin. It is furthermore not considered a plausible explanation that swelling is caused by increased vascular fenestration unrelated to an inflammatory reaction. Swelling as a result of increased vascular permeability is one of the cardinal symptoms of inflammation and it is unlikely that it should be present after treatment in a much higher proportion of test treated animals than controls if there was no treatment related local inflammatory reaction.

It is well known that MSCs can result in inflammatory reactions and local adverse reactions after intraarticular administration have been described. The absence of leucocytes in synovial samples does

not provide evidence that an inflammatory reaction did not take place. The synovial samples were collected at time points (two weeks after treatment) when it would not be expected to find clear evidence of an inflammatory reaction that resolved within a few days after treatment. There was also a high number of samples missing from the analysis. In the target animal safety study local adverse reactions were also more frequent and more severe after repeated injection of the product indicating an immunogenic component. The fact that severe local adverse reactions indicating a clear inflammatory/immunological genesis (swelling, pain, heat, timing in relation to treatment) were demonstrated in the field study and that these were predominantly seen in test product treated horses, strongly indicate adverse effects related to the immunological/inflammatory characteristics of the active substance have not been sufficiently investigated or addressed.

Clinical field trial

One Pivotal field study was presented. The study was a placebo controlled multi-centre study designed to investigate the efficacy and safety of Horse Allo 20 in the treatment of osteoarthritis in horses. Seventy-two (72) horses aged ≥2 years old were enrolled for the study divided in two groups; Horse Allo 20 (n=37) and placebo (n=33). At the time of their inclusion in the study the horses had suffered from osteoarthritis for varying periods of time; the duration of lameness varying between a few weeks to several years. Baseline data for lameness upon inclusion was collected over a period of up to two weeks before the start of the study. It is likely that lameness could have improved in some cases between the first lameness assessment and the start of the study. This is of particular concern since the primary endpoint for the evaluation of efficacy was a reduction in lameness score of only one grade, which could be subtle changes, and there were other factors that introduced variability into the study (see further discussion below). There was an imbalance between the groups with respect to lameness grade on inclusion where a higher proportion of the horses treated with Horse Allo 20 had a higher lameness grade compared to those treated with placebo.

The horses were injected (intraarticularly) with the product in the treatment group, or 2 ml of CP placebo in the control group. According to the study report horses that showed an unsatisfactory response (poor or fair responders) on day 45 after the first treatment were given an additional administration of Horse Allo 20. 29/37 (78.4%) of the horses treated with Horse Allo 20 were treated again on day 45 due to an unsatisfactory initial response.

The primary efficacy endpoint was the comparison of the percentage of responders versus non-responders between treatments on day 45±2. A responder was defined as an animal with a reduction in 1 or more grades in lameness evaluation grade according to the 5 graded the American Association of Equine Practitioners (AAEP) scoring versus its pre-Day 0 grade. Secondary efficacy endpoints were the percentage of responders on days 15±2, 60 ±2 and 90±2, mean lameness grade at these time points, distribution of lameness grade over time and number of animals that required a second treatment. Data from the secondary endpoints is, however, of limited value to support the efficacy of treatment since these were based on the same lameness data as the primary endpoint.

The clinical relevance of improvement of 1 grade in lameness in treatment of osteoarthritis is unclear. There are uncertainties in the lameness data presented due to lack of reliable baseline lameness data and also because of substantial variation in evaluations between different investigators which introduce variability into the results.

Results for the comparison of the percentage of responders on day 45 showed no statistically significant difference between the groups. The applicant performed a post-hoc analysis to exclude horses >20 years of age after the primary analysis of the full data set was performed. Post-hoc analyses are not accepted since there is an obvious risk that decisions are driven by data if analyses

are made after breaking of the blinding. The removal of results from horses >20 years of age from the analysis is therefore inappropriate and has not been clinically justified. The treatment of osteoarthritis in horses >20 years of age is highly relevant, regardless of economic considerations, and in the absence of clear evidence that stem cell treatments are not effective in a population above a certain age there is no clinical/biological justification for exclusion based on age only.

Altered immune function at advanced age as proposed by the applicant as a reason for excluding old horses has not yet been justified and would need to be confirmed in relation to the treatment of osteoarthritis with stem cells. Furthermore, since analysing a subgroup of animals represents one type of multiplicity in a clinical trial the type-one error rate should be adjusted in order to control the overall type-one error rate. This has not been done. Since the subgroup analysed was not pre-specified but defined post-hoc based on actual results, the effect on the type-one error is difficult to estimate. What is clear, however, is that the actual risk of a type I error is larger than the observed p-value. No discussion on the level of evidence given by this post-hoc analysis has been presented.

Seventeen (17) horses were treated with an NSAID (phenylbutazone) due to adverse events. Of these, 15 horses were in the test product treated group and 2 in the control group. Phenylbutazone is authorised for `treatment of osteoarthritis, inflammation and other orthopaedic diseases´. In studies, oral treatment with NSAIDs in horses diagnosed with osteoarthritis has been demonstrated to significantly reduce the lameness grade. The concomitant treatment with phenylbutazone in this study can therefore not be excluded to have had an impact in the reduction of lameness in the horses diagnosed with osteoarthritis, regardless of the timing of its administration in relation to the efficacy evaluation. It is not accepted that a period of 10 days between treatment with phenylbutazone and efficacy evaluation would ensure that the substance did not influence the condition of the joints and thus interfere with the outcome of the efficacy evaluation of Horse Allo 20. This is of particular concern due to the fact that phenylbutazone treatment was much more prevalent in the test group than in the placebo group.

It is unclear if a more pronounced/rapid reduction in lameness score can be expected from horses presented with a higher grade of lameness at Day 0 compared to horses with a milder grade of lameness. This is indicated by a clear tendency towards a higher response rate for horses with higher baseline lameness grade observed in the study. Since there was an imbalance between the two treatment groups with respect to grade of lameness at the start of the study, it can be assumed that this has influenced the efficacy results and is therefore considered a major issue.

Inappropriate statistical methods were used in order to support homogeneity between the study groups with respect to the lameness grade at inclusion. As stated in CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (EMA/CVMP/EWP/81976/2010) it is not appropriate to conduct hypotheses tests to compare baseline data between treatment groups in a randomised study. Any imbalance in baseline data in a randomised study is by definition due to chance. However, a numerical imbalance in baseline data could still influence the outcome.

Repeated hypothesis tests designed for finding differences were conducted and from the absence of significant differences it was concluded that the groups are equivalent. This is an invalid statistical conclusion. The absence of statistically significant differences is not the same as equivalence, as stated in the CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (EMA/CVMP/EWP/81976/2010) page 14; "…the conclusion of equivalence from the non-rejection of the null hypothesis of no difference is never acceptable".

The observed (numerical) differences in baseline values in relation to outcomes were presented in one analysis. According to this, there is a clear tendency towards a higher response rate for horses

with a higher baseline lameness grade. This was dismissed by the applicant with reference to a non-significant p-value. This is an invalid statistical conclusion.

The applicant argued that one-sided tests are appropriate for the statistical analysis of efficacy data. The level of significance is normally set to 5% two-sided when conducting a confirmatory trial. Using this as a standard assures that the burden of proof is the same across studies and across products and that regulatory decisions are based on the same requirement. It could be argued that the analysis question is one-sided and that the test used therefore should be one-sided. However, this is the case in most clinical trials and not unique for this product. Therefore, the significance level should still be the same as used for all other products, i.e. 5% two-sided or, if a one-sided test is preferred, 2.5% one-sided. (Refer to CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (EMA/CVMP/EWP/81976/2010 page 13). This was, however, not done for this application as 5% one-sided tests were used, which is not acceptable.

Two horses were excluded from the intention to treat (ITT) population due to use of concomitant medication. This is a violation of the ITT principle and is not acceptable. These two individuals should have been included in the analysis counted as failures.

In conclusion, there were several serious flaws in the design and conduct of the pivotal field trial precluding conclusions to be drawn with the respect to efficacy of the product. These include inappropriate use of statistical methods and inaccurate conclusions from analyses, unclear clinical relevance of data obtained for the primary efficacy endpoint, uncertainty of the reliability of data obtained from various examining veterinarians showing variability in their evaluations and the concomitant use of NSAIDs in test treated animals. Although specific EU veterinary guidelines are lacking for stem cell products, basic principles for study design, data handling and statistical analysis are fully applicable, as for any product regardless of their MUMS status, and should be used in the case of this product.

The deficiencies of the pivotal clinical trial were of such severity that the data was not considered reliable and efficacy of treatment has not been demonstrated. Furthermore, since the product's efficacy has not been confirmed the chosen dose is not considered justified.

In addition, an unacceptably high number and severity of adverse reactions were observed in the field trial. A large proportion of the test treated horses received concomitant NSAID treatment due to severe adverse events such as grade 4 lameness. This is not considered acceptable for an intraarticular treatment of osteoarthritis in horses.

Concerns regarding tolerance in the field study also remain unresolved as major concerns.

Overall conclusion on efficacy

Pharmacodynamics:

No proprietary studies were performed in order to assess the pharmacodynamic properties of Horse Allo 20 and the relevant functional characteristics of Horse Allo 20 are therefore largely unknown.

Pharmacokinetics:

No proprietary studies were performed in order to assess the pharmacokinetic properties of Horse Allo 20. Bibliographic data only was submitted in order to describe the persistence, migration and biodistribution stem cells.

Dose determination:

No dose determination studies were performed but the recommended dose was chosen based on published data. This could be acceptable according to the CVMP Guideline on the Efficacy and Safety Data Requirements for Veterinary Medicinal Products intended for Minor Uses or Minor species (EMEA/ CVMP/EWP/ 117899/2004) provided that safety and efficacy of the chosen dose is adequately demonstrated in clinical trials.

However, since the efficacy and safety of the proposed product has not been adequately demonstrated in the clinical trials the dose is not considered to have been justified.

Target animal tolerance:

Target animal tolerance was evaluated in a target animal safety study where horses were treated with Horse Allo 20 on repeated occasions and in multiple joints. No systemic adverse reactions were reported, but local adverse reactions consisting of lameness, swelling and synovitis were seen in joints treated with the product after the second and third administration. No adverse reactions were seen in joints treated with placebo. According to the applicant synovial fluid data did not support an immunogenic reaction. However, due to the timing of the sampling of synovia it is not possible to conclude on immunological/inflammatory reactions after treatment.

In the pivotal field trial a significantly higher number of horses showing local adverse reactions were reported in the group treated with Horse Allo 20 than in the control group (15 compared to 1). Adverse reactions were described as local signs related to the site of administration and included joint inflammation, local pain, synovitis, signs of heat at the treated joint and increased lameness which normally occurred a few days after administration of the product. Adverse reactions in several cases resulted in more serious conditions than the initial condition of osteoarthritis intended to be treated. A total of 17 individual animals were treated with an NSAID (phenylbutazone) due to adverse reactions during the study. Of these horses, 15 were from the group treated with Horse Allo 20 and 2 from the placebo group. The exact cause of the adverse reactions has not been evaluated, and there is no data or discussion on the mode of action of the treatment providing plausible explanations. The adverse events reported in the pivotal field trial are considered inacceptable for an intraarticular injection for the treatment of osteoarthritis in horses.

Efficacy:

Efficacy was evaluated in the pivotal field trial. The study included horses with different grades of lameness but the duration of the disease and reliable baseline data regarding the lameness grade was missing. Efficacy was not demonstrated for the primary efficacy endpoint comparison of the percentage of responders versus non- responders between treatments on Day 45±2. A post-hoc analysis was performed after the breaking the blinding for the analysis of the primary efficacy endpoint where horses >20 years of age were excluded. This analysis was not acceptable. Concomitant treatment with NSAIDs due to adverse reactions was common in the group treated with Horse Allo 20, and this may have influenced the lameness evaluation. Furthermore, the secondary endpoints could not contribute to the demonstration of efficacy since these were based on the same lameness data as the primary endpoint. Inappropriate statistical analyses were used and incorrect conclusions were made from statistical tests. It can therefore be concluded that due to the major flaws and shortcomings in the study design, inappropriate use of statistical methods and concomitant medication, that the results from the pivotal clinical field trial efficacy of Horse Allo 20 for the treatment osteoarthritis in horses has not been demonstrated.

The ready to use presentation was used as final formulation in the pivotal field study. However, two presentations (ready to use and frozen) are proposed but since insufficient clinical data including no efficacy data has been presented for the frozen presentation and it is not accepted.

Part 5 – Benefit-risk assessment

Introduction

Horse Allo 20 is an allogeneic equine adipose derived mesenchymal stem cell (EA-MSC) suspension for intraarticular injection intended for use in horses for the following indication: For treatment of osteoarthritis in adult horses.

Horse Allo 20 is presented as a single dose injection containing allogeneic equine adipose derived mesenchymal stem cells per container. Two presentations were proposed: a ready to use suspension containing Dulbecco's modified eagle's medium (DMEM) as the excipient, and a frozen suspension with DMEM plus dimethylsulfoxide (DMSO) as excipients. The product is presented in single dose packs containing 1 syringe of 2 ml (ready to use presentation) or 1 vial of 2 ml (frozen suspension). The proposed withdrawal period is 0 days.

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 and have been considered in the assessment.

Benefit assessment

Direct therapeutic benefit

Insufficient data have been presented to demonstrate efficacy for the proposed indications.

Sufficient documentation to support the suitability of the potency method has not been provided. The proposed assay cannot be considered sufficiently robust to ensure the identification of batches with sufficient biological activity to promote a positive clinical effect.

The laboratory and field studies performed did not provide sufficient evidence of clinically relevant efficacy of treatment correlating with the proposed indications when the product was administered as intended.

Insufficient clinical data, including the absence of any efficacy data, has been presented for the proposed frozen presentation which is therefore not accepted by the CVMP.

The CVMP, therefore, considers that the data provided are inadequate to provide acceptable evidence of efficacy for the proposed indications, and that the benefit of the product has therefore not been demonstrated.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and finished product has not been presented in a satisfactory manner. Quality data provided remain incomplete. Major outstanding issues remain regarding the manufacturing process and its validation as well as the control

strategy, including methods and their validation. Therefore, the quality of the product remains unsubstantiated and unjustified.

Safety

Risks for the target animal:

Published data has been presented addressing aspects of biodistribution and persistence of MSCs in horses. Due to the high number and severity of adverse reactions in the pivotal field trial and the probable immunogenic cause the safety profile of the product remain incompletely addressed.

Risk for the user:

The use of this product is not expected to represent a user safety concern. A user risk assessment has been provided addressing the risk of accidental self-injection by the user associated with the risk for a possible severe immunological response, with pain and swelling at the injection site.

Risk for the consumer:

The active substance 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, and consequently its MRL status does not need to be further addressed. The excipients, including all components of Dulbecco's Modified Eagle Medium (DMEM) have been satisfactorily addressed and are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product. A zero day withdrawal period is considered acceptable.

Risk for the environment:

Horse Allo 20 would not be expected to pose a risk for the environment when used according to the proposed SPC recommendations.

Risk management or mitigation measures

User safety:

User safety risks have been identified, mainly the risks associated with accidental injections. Adequate warnings have been proposed in section 4.5 of the SPC.

Evaluation of the benefit-risk balance

The development, manufacture and control of the active substance and finished product of Horse Allo 20 is partly/not presented in a satisfactory manner. Assessment of the quality of the product remains incomplete as the batch potency testing has not been satisfactorily described.

The laboratory and field studies submitted failed to provide reliable results to support the proposed indication in the intended target population. There were a high number of local adverse reactions in the pivotal field study that have not been appropriately investigated or addressed. The benefit of Horse Allo 20 therefore has not been demonstrated as the safety and efficacy has not been sufficiently 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 Horse Allo 20 is not approvable since the data on quality, target animal safety and efficacy remain inconclusive. Therefore, the data do not satisfy the requirements for the granting of a marketing authorisation as set out in Regulation (EC) No 726/2004 and Directive 2001/82/EC.

The CVMP therefore considers by majority decision that the overall benefit-risk balance is negative and, therefore, recommends the refusal of the granting of the marketing authorisation for the above mentioned veterinary medicinal product.

Grounds for refusal

On the basis of all the available data, the CVMP considers that specific documentation provided by the applicant in order to demonstrate the quality, efficacy and target animal tolerance of the veterinary medicinal product Horse Allo 20 is not reliable.

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

  Potency: The applicant has proposed a potency test (ELISA). While the current scientific knowledge supports a role in the pathology of osteoarthritis (OA), it is not clearly stated nor demonstrated how this attribute can identify batches with sufficient biological activity to promote a positive clinical effect. The data submitted for the proposed ELISA method cannot fully support the suitability of this test as potency indicator since:

  - The ELISA method has not been fully validated using product specific matrix as required in VICH guideline GL1.
  - The relevance of the batch data provided for this attribute is not established since no finished product batches used in the clinical trials have been tested using this potency assay. Active substance batches used in previous studies have been used to produce new finished product. However, no information regarding the period of storage for these three different active substance batches have been described and their stability in terms of potency cannot be determined as they have not been tested with this method at time 0. Therefore their relevance as supportive data for the finished product batches used in the clinical studies is not demonstrated.
  - It is not clear how the specification limits were established. The applicant states that three batches have been tested repeatedly and the limits were established based on the minimum and maximum values obtained. The approach is not justified and the raw data on which these limits were established are not provided. Additionally, no data has been submitted to demonstrate that the proposed potency test can identify sub-potent batches, in order to establish relevant specifications.
  - A correlation between the potency assay (and its specifications) with a relevant biological activity related to clinical effect was not demonstrated in the developmental studies and/or in the clinical studies supporting the efficacy of the product. The clinical studies are not considered conclusive and the efficacy of the product has not been demonstrated.
  - In the absence of an established potency indicating assay, the stability of active substance and finished product cannot be considered demonstrated.

Relevance of the research and development (R&D) batches and GMP-like batches for the validation of the commercial process: Although the differences between the R&D batches, pre-commercial batches and commercial batches have been clarified, release data according to the full specification (including the proposed potency assay) on at least one relevant batch have not been provided. Moreover, a comparison between the R&D batches used in clinical studies and the commercial batches, including at a minimum a full set of specifications, has not been presented. Therefore the validation of the manufacturing process is not accepted.

Control of active substance/finished product:

- *Specifications:*
  - The established limits for the potency assay are not considered justified
  - A population doubling test has not been included in the specifications of the ready to use formulation
  - Accepted limits for cell count have not been defined in the specification list.
- *Flow cytometry:* The flow cytometry-based assay is central in demonstrating the quality of Horse Allo 20. A comprehensive validation of this method as per VICH guidelines GL1 and GL2 has not been presented.

Ready to use /frozen presentations: Due to major differences in the composition of the ready to use and frozen presentations of Horse Allo 20 (DMSO), additional clarifications and data on quality are still missing. The relevance of the submitted results intended to support the comparability between the two presentations is questioned and the submitted data is not conclusive. Moreover, the clinical efficacy data using the frozen formulation has not been presented.

Microbiological control: adequate microbiological control during the manufacturing process has not been demonstrated.

- A root cause analysis was requested for the three batches found positive in the clinical trials. A summary of the performed analysis, although with no clear identified root cause, has been submitted only for one batch. Similar exercises were not provided for all positive batches.
- An adequate risk assessment to include the contamination risk derived from the raw materials used as well as a justification for the proposed strategy for viral testing during manufacturing has not been provided.

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

Pivotal clinical study: Due to major flaws in the design, conduct and evaluation of the pivotal clinical study, efficacy has not been demonstrated for the claimed indication. The following deficiencies of the study were identified:

- The primary efficacy parameter was not met in the test group (significant difference in the primary efficacy parameter was only demonstrated after exclusion of older horses in a post-hoc analysis).
- The more frequent use of a NSAID in the test group (15 animals in the test group vs 2 animals in the control group) and the positive impact this may have had on the lameness grade.

- Issues concerning the validity of the statistical analyses:
    - an unscheduled post hoc analysis was performed after the breaking of the blinding where horses older than 20 years old were removed from the efficacy analysis.
    - inappropriate statistical tests for basal homogeneity between groups.
    - incorrect conclusions on similarities between groups based on the absence of statistically significant differences in superiority tests.
    - the level of significance was incorrectly set to 5% in one-sided tests.
    - horses were excluded from the analysed population which is a violation of the ITT principles.
- There were differences in baseline data between study groups with respect to lameness grade. Baseline data was collected at different time points and did not reliably represent the lameness status on the day of study start.
- Clinical relevance of an improvement in lameness of 1 grade in treatment of osteoarthritis has not been justified. The involvement of several investigators that were not harmonised in their assessments in addition to uncertainties in baseline lameness data introduced variability in the efficacy evaluation. It is therefore not clear that the level of improvement as used for the primary efficacy endpoint (1 lameness grade) is a robust and clinically relevant result.

Dose determination and treatment regimen: in the absence of clinical data showing reliable efficacy of treatment but where severe adverse reactions were demonstrated the dose is not considered justified. It is furthermore not possible to conclude on whether repeated administration should be recommended and in which cases it would be expected to be beneficial. Due to insufficient data it is furthermore not clear whether simultaneous administration in more than one joint in the same horse is acceptable.

- **Ground for refusal 3 (target animal tolerance):**

Local adverse reactions: In the pivotal field trial there were concerns regarding the high number and severity of local adverse reactions to treatment associated with the product. The degree of increased lameness, swelling, heat and pain demonstrated after treatment was not acceptable for an intraarticular treatment of osteoarthritis in horses. The cause has not been appropriately investigated although the type of reactions seen indicates a strong inflammatory/immunological response to the product.

On the basis of the above, the CVMP remains concerned about major outstanding issues in regard to the quality, efficacy and target animal tolerance 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, efficacy and target animal tolerance of the veterinary medicinal product and therefore considers the benefit-risk balance to be negative.

Therefore, the CVMP recommends by a majority the refusal of the granting of the marketing authorisation for Horse Allo 20, in accordance with Article 37(1)(a) of Regulation (EC) No 726/2004.

Divergent position on a CVMP opinion on the granting of a marketing authorisation for Horse Allo 20

(EMEA/V/C/004328/0000)

Based on an unfavourable benefit-risk balance, the CVMP has adopted a negative opinion on the granting of a marketing authorisation for Horse Allo 20.

The proposed indication is “For treatment of lameness associated to osteoarthritis in adult horses”. Horse Allo 20 is a biological product; it is a stem cell suspension for intra-articular injection in horse. The recommended posology is to be administered as a single dose, if an improvement in the lameness grade is not observed on day 45, a second dose may be administered. A 2 ml dose containing $20x10^6$ mesenchymal stem cells.

The concern that has led to enunciate **a divergent opinion** is in relation to the following points:

About non-GMP compliance: We consider that it could be acceptable taking into account that the provisions on the *Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products*, particularly its point 13.12 would allow for flexibility regarding the role of ISC III, as an exception to the general rule : *“Exceptionally, when the outsourced activity is a highly specialised test (e.g. karyotype test), it is acceptable that the contract acceptor is not GMP-certified, provided that it complies with suitable quality standards relevant to the outsourced activity (e.g. ISO) and that this is duly justified.* The contract company now performs flow cytometry to test identity and purity; and new potency test (TGF-β) is now performed by Centauri with a commitment to transfer this test to the contract company.

Furthermore in the Questions and answers about stem cell products published on EMA web site *EMA/CVMP/ADVENT/751229/2016 Questions and Answers on allogenic stem cell-based products for veterinary use: specific questions on sterility it is indicated and EMA/CVMP/ADVENT/803494/2016 : Specific questions on extraneous agents* the following is indicated in the background: *The EU Guide to Good Manufacturing Practice (GMP) covers in Part I basic GMP principles for the manufacture of human and veterinary medicinal products. Annex 2 to this guide covers the manufacture of human biological products including Advanced Therapy Medicinal Products (ATMP). The principle provisions laid down in that Annex are considered to be applicable also to stem cell–based products for veterinary use.*

This interpretation is also shared and was the one included in our assessment.

Potency

- For the ELISA methodology the matrix (DMEM+FBS) was used in the validation, see annex to the response of the LoQ (*annex D180_Annex Q1_V-014_TGF-b ELISA)*.
- The DP was obtained from the DS (stored frozen) used for the pivotal efficacy study. Potency was shown to behave similarly independent of the donor horse or the time stored frozen, as it has included results of batches stored frozen from the initial pilot batches and new produced batches. All the release characteristics are tested for the stability and the potency results can be assumed not to change from the obtained results. No statistically differences can be observed between the different batches tested.
- Specification limits of the potency are established from the results obtained with the batch used for the pivotal efficacy study and from the batch used for the TAS trial. The upper limit is

established from the batch used for the safety study (TAS) and the lower limit is established from the DP batch derived from the DS batch used for the pivotal efficacy study.

- The relevance of the potency assay relies on the potency results of the batch used in the efficacy pivotal study. No mode of action is required to be described.

Relevance of R&D batches and GMP-like batches for the validation of the commercial process:

- A comparative exercise between R&D batches used in clinical trials and the commercial batches has been presented showing no differences.

Control of DS/DP:

- Specifications: As indicated for Potency, the established limits of the potency are justified.
- A population double test is implemented for the frozen presentation, it is not included in the DP fresh presentation as it is only one more passage from the DS produced in a continuous process and it has been validated as the test has been performed in passages superior to the DP passage. It is still performed for the frozen presentation.
- Cell limits are defined. The applicant has stated the product has a fixed amount of cells and has calculated the error in the counting in order to know the real content. No limits are needed if it is a fixed number and the margin of error has been calculated.
- Flow cytometry: only one outstanding issue that can be solved post-authorisation is pending. The matrix used for the product has not been tested for the identity markers but the several flow cytometry tests done showed a very constant numbers for the markers and no interferences were observed for the purity markers. In the case an identity marker were also cross-reacting with the matrix some differences should have been observed. A post-authorisation recommendation could serve to solve the issue.

Fresh/frozen presentations:

- The only difference in the composition of the fresh presentation compared to the frozen presentation is the presence of DMSO in the frozen presentation (same active principle and same excipient; DMSO is added as "small amounts of preservative"*)*. According to the CVMP *(EMA/CVMP/ADVENT/791465/2016), "Pharmaceutical formulation of biologicals is usually minimal and cells would be expected to be suspended in the buffer in which they are cultured or thawed. In general cell products after thawing are quite vulnerable and re-formulation (e.g. to remove the DMSO used for cryopreservation) may do more harm than good". To ensure viability of the cells during the process, a small amount of cryoprotectant (excipient, DMSO) is added.*

  Comparability exercise of all release specifications has been performed and no differences were observed.

  A safety study (TAS) used DMSO in the batches and found no differences in the adverse reactions compared with the adverse reactions observed for the fresh presentation.

Microbiological control: adequate microbiological control during the manufacturing process has been demonstrated.

- Manufacture is performed in a GMP certified facility.
- A root cause analysis was provided pointing out that the manufacture process was not the cause

of the contamination even though it was not possible to identify the contamination root cause.

– Although the applicant has not clearly indicated the risk of contamination for the raw materials in a separate document, for each one of the used components a sterility certificate was provided which can assure the absence of contamination.

Primary efficacy endpoint (Pivotal field study):

Due to the design, conduct and evaluation of the pivotal clinical study, CVMP concluded that the efficacy was not demonstrated for the claimed indication because the primary efficacy parameter **established on day 45** was not met in the test group for all animals. The efficacy (the percentage of responders) was assessed at different days: 15 ±2, 45 ±2, 60 ±2, and 90 ±2 and statistical analysis showed that the **percentage of responders in the IVP group was significantly greater** on **Days 15, 60 and 90** compared with animals treated with Placebo. **On day 45**, the **percentage of responders** in the IVP group was **higher** compared with the Placebo group (51.4% vs 36.4%, respectively), although the difference was not statistically significant (P=0.1007). However, **statistical significance** was demonstrated for **animals younger than 20 years on day 45.**

Taking into account all data, efficacy has been demonstrated in terms of reduction or improvement of lameness on days **15, 45*, 60 and 90.** Although statistical significance was not demonstrated for all animals on day 45, it was achieved on previous and subsequent efficacy parameters assessments days.

Considering this product is a novel therapy based on stem cells where to date the mechanism of action has not been completely characterized, the limited current scientific knowledge might have not allowed to determine a precise primary endpoint. Appropriate efficacy was demonstrated in terms **of reduction or improvement of lameness on days 15, 45*, 60 and 90**, thus, the **efficacy of the product can be accepted whether the results and conditions of the clinical field study are appropriately reflected** in the SPC.

Concomitant use of a NSAID in the test group (15 animals in the test group vs 2 animals in the control group)

The undersigned noted that the applicant justified the use of NSAID concomitant medication (phenylbutazone) in the pivotal field study for welfare reasons in order to avoid any unnecessary pain and acute inflammatory of the treated joint. This concomitant medication was used according to the protocol. In addition, according to the pharmacokinetic of the phenylbutazone is unlikely to have affected to each specific efficacy assessment days (elimination half-life (t1/2 beta) of 5.46 h) considering 10 day margin since the last administration of phenylbutazone acceptable. This concomitant medication should be reflected in the SPC not being an issue to invalidate the global results of this study.

Issues concerning the validity of the statistical analyses

In the absence of specific guidelines for this type of products, the applicant has followed the guidance on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010).

Statistical significance was demonstrated for efficacy on days 15, 60 and 90. Furthermore, efficacy was demonstrated for **animals younger than 20 years** on day 45 which was the selected day for the primary efficacy parameter. Therefore, although statistical significance was not achieved on day 45 for all animals, there was a higher tendency to the improvement in lameness in the treated group versus placebo and efficacy was achieved on previous and subsequent efficacy parameters assessments days

(day 15, 60 and 90). However, as statistical significance on day 45 was only demonstrated for animals younger than 20 years, this information should be reflected in the SPC as a restriction.

Regarding the **basal homogeneity**, all variables were balanced and homogeneous prior to treatment administration. The basal homogeneity analysis showed that no major imbalances between treatment groups were present prior to treatment administration and that both groups were therefore comparable and no need to adjustments in the analyses were required. This approach is acceptable and reflects the real situation on the field since it is not possible to have identical populations.

Moreover, **one-sided tests** are appropriate for statistical analysis of efficacy data but 5% one-sided tests were used. Although ideally a one-sided test at 2.5% level should have been performed, this approach was justified based on the references provided by the applicant (Koch, 1991) since alternatively for certain situations for which no beneficial therapy is currently available (no authorized products based on stem cells), other tests with less significance level could be accepted.

As a conclusion, **the general approach in relation to the validity of the statistical analysis could be considered acceptable** taking into account that this is a MUMS application.

Dose determination and treatment regimen was assessed in a pivotal field study and in TAS study. The **repeated use** was also assessed in a TAS study being the observed local adverse events similar as in a single administration. The safety of the product in a single and repeated administration is not a major issue since expected local effects were observed. According to the pivotal field study, the product **was used** as a single dose, if an improvement in the lameness grade was not observed on day 45, a second dose was administered. Restrictions to the specific joints and conditions used in the pivotal field study should be reflected in the SPC. This approach is accepted.

Baseline data

The results of basal homogeneity for lameness grade showed **no statistically significant differences** between treatments. However, the lameness grade on baseline was not performed on day 0. A lameness evaluation was performed in a **mean time of 8.69 days before the day** 0 and three animals were revaluated on day 0. However, it can be assumed, not to have lameness changes for a chronic lameness at that period of time. Therefore, this approach is accepted.

Target animal safety

The undersigned remains of the opinion that **the safety of the product** is a no major issue.

Local tolerance was adequately addressed in a TAS and a pivotal field studies. Local reactions to the treatment were observed in a majority of the treated joints compared to placebo. These local reactions were related to the site of administration and included joint inflammation, local pain, synovitis, signs of heat at the treated joint and increased lameness. The administration of EA-MSC did not produce any systemic adverse events, but caused significantly more local reactions in the treated joints compared to placebo administration. In a new controlled target safety study (Protocol Code: TAS-Horse Allo 20) was assessed the tolerance after repeated administration of treatment. Detailed local and systemic tolerance data were provided as well as repeated use in multiple joints were administered and evaluated. No systemic effects were found. No local reactions were characterized after the first injection with Horse Allo 20. Some local reactions were observed after the second and third administration of the product related to potential signs of inflammation (pain, deformation and synovitis) that were resolved without further intervention. In addition, the synovial fluid data did not support an immunogenic reaction. After the repeated administration injection with Horse Allo 20, it was showed signs of potential inflammation with or without lameness. All of them were resolved in

48-96 hours without further intervention. Thus, as a conclusion, no immunogenic cause could be associated with the study. Data from a general study of the biochemistry and haematological parameters performed at day 0 and day 90 (end of follow up) showed that all of the parameters were inside the normal ranges in both treated and placebo groups including acute phase proteins like C-Reactive Protein. There was only an increase on eosinophils that can be linked to the onset of an inflammatory response considering the whole picture.

As different interpretations of adverse events were performed between investigators in the pivotal field study, the most severe classification of adverse events observed in the safety and clinical studies should be reflected in the SPC. This approach is accepted.

The safety of the product is not an issue for the target animals. Only local effects have been observed and they are considered within the expected effects for this type of products. The **benefits outweigh the risks of the product** and overall the **benefit-risk of the product is considered positive** if appropriate information and restrictions are reflected in the SPC.

In conclusion, the undersigned is of the opinion that based on the original and complementary data presented on quality, safety and efficacy for Horse Allo 20 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).

References:

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Cristina Muñoz Madero