21 June 2018
EMA/427104/2018
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Arti-Cell Forte (EMEA/V/C/004727/0000)

Common name: chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells

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

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Introduction

The applicant Global Stem cell Technology submitted on 21 June 2017 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Arti-Cell Forte, 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 19 January 2017 as Arti-Cell Forte contains a new active substance, chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells (ciMSCs), which was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

The applicant applied for the following indication: For the treatment of recurrent lameness due to non-infectious joint inflammation in horses.

The active substance of Arti-Cell Forte is chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells (ciMSCs), which aims to activate chondroprotective mechanisms, such as producing extracellular matrix, stimulating local cells by paracrine effects and/or integrating into the injured tissue. The target species are horses.

Arti-Cell Forte contains 1.4-2.5 x 10^6 chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells and is presented in single packs containing 1 vial of 1 ml stem cell suspension plus 1 vial of 1 ml plasma suspension (1 dose).

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

The rapporteur appointed is Frida Hasslung Wikström and the co-rapporteur is Wilhelm Schlumbohm.

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

On 21 June 2018, the CVMP adopted an opinion and CVMP assessment report.

Following the request from France, the written procedure for the adoption of the Commission implementing decision granting a marketing authorisation for Arti-Cell Forte was stopped and a plenary meeting of the Standing Committee on Veterinary Medicinal Products convened on 24 September 2018.

The Standing Committee noted that there were certain questions which, in its view, required further clarification by the CVMP (EXT/759852/2018).

Based on the discussion, the Standing Committee recommended that the Commission services suspend the adoption procedure for Arti-Cell Forte, in line with Art. 35(4) of Regulation (EC) No 726/2004 and that the application for Arti-Cell Forte be referred back to EMA for further consideration, and in particular in order to clarify specific questions which were raised during the meeting of the Standing Committee by the Member States (EXT/7598553/2018). Therefore, the Commission requested EMA to address the issues raised and to further consider their opinion as soon as possible.

CVMP endorsed 8 November 2018 Frida Hasslung Wikström to further act as a rapporteur and Wilhelm Schlumbohm as the co-rapporteur in preparation of the CVMP responses. CVMP further decided to establish a scientific advisory group (SAG) in line with Article 56(2) of Regulation (EC) No 726/2004. The SAG will be asked to provide input that will be used by CVMP to respond to the

questions of the Member States.

The SAG meeting was convened on 8 February 2019.

The applicant, Global Stem cell Technology NV, attended the SAG meeting for an oral explanation. The applicant gave a presentation covering the questions from the EC.

The SAG raised some questions to the applicant in regard to quality and efficacy of the product. The answers from the applicant were later on taken into account by the SAG to draw their conclusions.

The applicant, Global Stem cell Technology NV, attended the CVMP February 2019 meeting for an oral explanation. The applicant gave a presentation covering the questions from the EC.

At its February 2019 meeting the Committee endorsed the SAG report '*Responses to questions from the European Commission for further clarification of CVMP opinion*' which includes the responses to the EC questions, the scientific rationale for CVMP answers and the final CVMP answer taking SAG comments into consideration. The CVMP confirmed its previous conclusions and considered that no changes were needed to the initial opinion, assessment report and product information for Arti-Cell Forte

On 29 March 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for Arti-Cell Forte.

Scientific advice

The applicant received scientific advice from the CVMP on 7 May 2015. The scientific advice pertained to quality, safety and clinical development of the dossier. The applicant has followed the scientific advice given.

MUMS/limited market status

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

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated 2018-06-06) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place at Global Stem cell Technology, Noorwegenstraat 4, 9940 Evergem, Belgium. The site has a manufacturing authorisation issued on 17 February 2016 by Federaal Agentschap voor Geneesmiddelen en Gezondheidsproducten (AFMPS-FAGG). 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.

Secondary packaging and batch release takes place at the same site as stated above.

The active substance is manufactured at the same site as stated above. A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP).

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

Composition

The veterinary medicinal product Arti-Cell Forte is a cellular suspension for intraarticular injection, used for the treatment of recurrent lameness due to non-infectious joint inflammation in horses. The product consists of two vials of 1 ml each, both supplied frozen: one vial contains 1.4-2.5 x 10^6 chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells (ciMSCs; colourless clear suspension) as active substance, suspended in dimethyl sulfoxide (DMSO) and Dulbecco's Modified Eagle Medium Low Glucose (DMEM LG) as excipients and a second vial that contains the excipient equine allogeneic plasma (EAP), a yellow, clear suspension which improves the stem cell viability. Prior to administration, the contents of both vials are mixed and then administered intraarticularly as a 2 ml dose per animal.

Containers

The primary packaging is cyclo olefin co-polymer (COC) vials closed with sterile thermoplastic elastomer (TPE) stoppers and sealed with high density polyethylene (HDPE) caps, for both the chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells and the plasma. The peripheral blood-derived mesenchymal stem cells vial has a coloured cap and the EAP another coloured cap. The material complies with the relevant European Pharmacopoeia (Ph. Eur.) requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. One vial of chondrogenic induced mesenchymal stem cell suspension and one vial of equine allogeneic plasma suspension are packed together into a transparent polycarbonate container.

Development pharmaceutics

The active substance is the chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells (ciMSCs). A literature review on their mode of action and effectiveness in the treatment of degenerative joint disease was performed. To date, fundamental mechanisms or patterns describing the survival, distribution and homing effects in horses are scarce. However, for chondrogenic induced mesenchymal stem cells (MSCs), the mode of action is proposed to be narrowed towards chondroprotective mechanisms, such as producing extracellular matrix, stimulating local cells by paracrine effects and/or immunomodulation by reducing local inflammation. Moreover, previous studies indicate that pre-differentiating MSCs avoid possible formation of ectopic tissues and ensure the presence of the desired cell type, in this case being chondroblasts.

The proposed dose of 1.4 x 10^6 cells/ml concurs with the clinically qualified minimum and the maximum dose of 2.5 x 10^6 cells/ml with the clinically qualified maximum.

Dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle's Medium Low Glucose (DMEM-LG) and equine allogeneic plasma are used as excipients:

- DMSO is used for the cryopreservation of haematopoietic stem cells at a concentration of 10%. This has been justified by a study which showed reduced cell viability at lower amounts.
- Dulbecco's Modified Eagle's Medium Low Glucose (DMEM-LG) serves as nutrient for the cells as it contains amino acids, vitamins, minerals and carbohydrates. It is used for a wide variety of cell culture applications and is reported to be a suitable component for freezing of equine MSCs according to literature.
- Equine allogeneic plasma (EAP) is added to the stem cell suspension immediately before administration with the purpose of improving cell viability. This claim is supported in the dossier with data representative for Arti-Cell Forte. Data regarding the beneficial effect of EAP on cell viability have been provided, including specifications for the attribute platelet count, which are clinically qualified.

All studies have been conducted using material from pilot batches produced from one intermediate stock isolated from one blood sampling from a healthy horse. Pilot batches correspond to the final product in all relevant aspects and the representability of these batches for the commercial drug product and therefore for the submitted process validation study has been established. Data from two additional commercial batches, produced from additional intermediate stocks isolated from separate blood samplings from two horses are also provided in the dossier.

In the early steps of product manufacturing, antibiotics (penicillin, streptomycin and amphotericin B) are added to the culture media. According to theoretical calculations performed by the applicant, the manufacturing process should be able to wash out these antibiotics to levels close to limit of detection (LoD). The evaluation of the possible impact of these antimicrobial substances on the sterility quality controls for this product has been sufficiently addressed.

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

In the absence of a defined Master Cell Bank (MCB), the applicant has chosen to introduce an intermediate stage, intermediate cell stock (ICS), to test the characteristics of the isolated cells while

providing a sufficient number of cells for release and release testing. This approach is considered acceptable.

In order to analyse the effect of the manufacturing process on the genetic stability of the equine peripheral blood-derived MSCs, karyotyping studies were performed on blood samples from two donors, the two corresponding ICSs, all pilot batches used in the clinical studies as well as two commercial batches. The reports provided in the dossier indicate a normal karyotype without any detectable chromosomal abnormalities for all samples tested. Genetic stability is indirectly controlled at the level of ICS and DP via determination of the population doubling time. This approach is considered acceptable.

Potency assay

A suitable potency assay based on a validated surrogate marker has been established to identify subpotent batches. Within the test, an induced sample is compared to the non-induced negative control and the level of increase of the marker is analysed.

In order to support the link between the selected potency assay and clinical efficacy of Arti-Cell Forte, the applicant presented bibliographical references, an in vitro assay as well as a clinical proof-of-concept study and a clinical field trial.

The bibliographical and experimental data presented are considered sufficient to justify the choice of the marker and its accepted limits as appropriate for identification of batches with relevant clinical effect:

- The relevance of the in vitro data presented in the supportive bibliographical study for Arti-Cell Forte cells has been clarified.
- The study designed to support the proposed mode of action (MoA) included eight Arti-Cell Forte batches obtained from five different donors, five of them with marker values above the proposed accepted limit and three of them with marker values below the proposed accepted limit. Submitted data indicated that batches with sufficient marker induction levels are able to homogenously adhere to the cartilage defects and form multiple cell layers as opposed to the batches with insufficient marker levels.
- The relevance of the percentage of non-adherent cells for the functionality of Arti-Cell Forte has been discussed: this term is not intended to correlate with the marker levels, yet indicates if an expected amount of cells did adhere to the explant in the first place. If an insufficient proportion of cells adhered to the explants, other factors (such as excessive cell death, dilution errors, seeding errors, etc.) could have contributed to non-homogenous adhesion rather than the actual functional capacity of the cells. This clarification is endorsed.
- Additionally, the term of homogenous cell adhesion has been defined and relevant illustrative pictures have been included.

In a proof of concept clinical study, further discussed in the clinical assessment, statistically significant differences were observed between the test group and the placebo group in terms of chondrocyte area (as determined by Alcian Blue/Safranin O staining) and of other immunohistochemistry markers for chondrocyte presence and function. These data indicate a biological effect of Arti-Cell Forte. Moreover, an additional batch with a marker value within proposed accepted limit has been included in clinical data which is deemed acceptable. As a result, the proposed accepted limit can be considered clinically qualified.

Method of manufacture

The manufacturing process for Arti-Cell Forte is briefly described and flow charts of the entire production are provided, including the in-process controls and final product control tests used to ensure the consistency and reproducibility of Arti-Cell Forte.

Validation of the production process

The manufacturing process was validated using three pilot batches obtained from the same ICS and therefore from blood collected from one single donor. Two of these batches are used in the clinical trials. Further validation data is available from two more recent commercial batches produced from two additional blood samplings, originated from two horses. According to the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMEA/CVMP/IWP/123243/2006-Rev 3), validation studies with pilot batches are accepted, as long as these are representative for the commercial process. Additional information about the pilot batches in relation to the commercial DP has been provided, including specific characteristics and batch size, confirming their relevance for the validation of the manufacturing process of Arti-Cell Forte.

A validation report was provided for three pilot batches. All in-process controls and a final product testing meet the acceptance criteria. The microbiological monitoring showed satisfactory results.

In line with the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/IWP/12343/2006-Rev3), stability results of one commercial batch should be provided post-authorisation.

Control of starting materials

Active substance

Additional information has been provided and the selection of donor horses, quarantine and testing are deemed acceptable. Selection and testing of reagents and their qualities are adequate. Testing of starting materials and raw materials for the possible presence of extraneous agents has been sufficiently addressed.

The different media used for culture and induction of tri-lineage cells are presented and supported by relevant references for their suitability.

Any biological starting material used in the production has been assessed for its suitability and transmissible spongiform encephalopathy (TSE) risk was found to be in line with the TSE guideline (EMEA/410/01 rev2). Additional justification for biological starting material safety has been submitted in the context of the absence of extraneous viruses.

The origin of the donor horse(s) and their appropriate control is of crucial importance with regard to the quality and safety of the product. For the selection and control, the applicant has followed the requirements for donor horses as specified in Ph. Eur. monograph 30 on Immunosera for veterinary use. The horses are tested in sufficient intervals for general health and diseases with a specific focus on relevant transmittable diseases. Testing for infectious diseases follows the Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010-Rev.1) except for testing on Hendra virus which is not performed. The omission is sufficiently justified as Hendra virus is relevant for Australia only. Additional measures have

been implemented or elaborated upon as outlined in the Questions and Answers on allogenic stem cell-based products for veterinary use: Specific questions on extraneous agents (EMA/CVMP/ADVENT/803494/2016) by the by Ad Hoc Expert Group on Veterinary Novel Therapies (ADVENT) of the CVMP. Moreover, the suitability of the respective test methods which are listed in the batch release document is considered justified.

Raw materials of animal origin are tested for the presence of extraneous agents and especially extraneous viruses in line with the CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (Annex2) (EMA/CVMP/IWP/206555/2010-Rev.1) and Questions and Answers on allogenic stem cell-based products for veterinary use: Specific questions on extraneous agents (EMA/CVMP/ADVENT/803494/2016) as well as the Ph. Eur. monograph 5.2.5. Substances of animal origin for the production of immunological veterinary medicinal products.

Excipients

The microbiological controls including sterility, mycoplasma and endotoxins are deemed acceptable and in line with current Ph. Eur. Monograph requirements. A risk analysis has been performed in line with Ph. Eur. Chapter 5.1.7. and 5.2.5. requirements.

The EAP is used as an excipient to improve the stem cell viability. The EAP is not enriched in platelets but contains a platelet number within the physiological range for horses. For the production of EAP the same criteria as for the active substance with regard to donor horses apply and are considered acceptable.

DMSO specifications are according to Ph. Eur. monograph 763 requirements and its use is considered acceptable.

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

A risk assessment for potential TSE contamination in accordance with section 4 of Ph. Eur. Chapter 5.2.8. requirements for all materials used in production has been provided.

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

In conclusion, the risk analysis and risk mitigations are in line with the Ph. Eur. Chapter 5.2.8. monograph requirements and are therefore found acceptable.

Intermediates

The final product consists of two components: the stem cell suspension and the equine allogeneic plasma (EAP). An intermediate cell stock is prepared only for the stem cell suspension.

Control tests for the intermediate cell stock have been established. Testing includes differentiation, cell morphology, total cell number, viability and proliferation identity and purity, impurity, sterility and mycoplasma. For all tests an appropriate description is provided.

The specifications for the intermediate cell stock in preparations for the stem cell suspension are in line with the specifications for the finished product. The choice of methods used to control the intermediate

cell stock is in essence found acceptable. Their validation and suitability is further addressed in part 2E.

In the context of extraneous agents, a virus risk analysis has been performed in line with Ph. Eur. Chapter 5.1.7. and 5.2.5. requirements.

Control tests during production

The final product consists of two components: the stem cell suspension and the equine allogeneic plasma. An intermediate cell stock is prepared only for the stem cell suspension.

Control tests for the intermediate cell stock have been established. Testing includes differentiation, cell morphology, total cell number, viability and proliferation, include identify and purity, impurity and sterility, including testing for mycoplasma. For all tests an appropriate description is provided.

The specifications for the intermediate cell stock in preparations for the stem cell suspension are in line with the specifications for the finished product. The choice of methods used to control the intermediate cell stock is found acceptable. Their validation and suitability is further discussed below.

Control tests on the finished product

General characteristics of the finished product

The final product consists of two components/vials that when mixed, make one 2 ml single dose: one vial containing 1 ml of stem cell suspension and one vial containing 1 ml of equine allogeneic plasma. The stem cell suspension vial and the equine allogeneic plasma vial are mixed immediately before administration to obtain a 2 ml dose per animal. The stem cell suspension was tested before and after freezing. Individual release tests and specifications apply for each component.

The quality control tests of the finished product are cell morphology and cell count, proliferation, identity, purity, impurity, potency, sterility, endotoxin and mycoplasma tests.

Identification and assay of active substance

Cell morphology

As part of the batch release of the intermediate cell stock and the final product morphology scoring is performed to determine the efficacy of the chondrogenic induction. The morphology scoring has been validated for the following parameters: repeatability, intermediate precision, linearity, range and accuracy. This is considered acceptable.

Population doubling time (PDT)

The PDT is considered to reflect the growth efficiency and proliferation rate of cells. A PDT out of the set specifications should be able to detect any potentially tumorigenic cells. The PDT is by using the pre-defined formula. The PDT should remain within specifications for the finished product A satisfactory validation report for the PDT determination has been submitted.

Flow cytometry to determine purity and impurity

Flow cytometry is used to determine cell identity (purity and impurity). MSCs are identified by positive and negative antibody markers visualised using flow cytometry. The validation report confirmed that the antibodies bind to horse cells (compared to isotype control) and the method was considered suitable.

Potency

The chosen marker expression is used as a surrogate marker for the chondrogenic induction process. The marker levels of the cells are determined by PCR and the results are depicted as “fold change” values.

Identification and assay of excipient components

Equine allogeneic plasma (EAP)

EAP is used as an excipient to improve the stem cell -viability. EAP is tested for appearance, identity and platelet count. Control tests have been established and testing on sterility, endotoxin test and testing for mycoplasma follow the respective monograph requirements of the Ph. Eur. Batch results for three batches are provided in the dossier which is considered acceptable. All batches comply with the set specifications.

Dulbecco´s Modified Eagle Medium Low glucose (DMEM LG)

Control tests are performed by the manufacturer, including bacterial, fungal, pH and osmolality testing according to Ph. Eur. requirements. Appearance is tested according to an internal company specification. Data for three batches are provided which is considered acceptable. All batches comply with the set specifications.

DMSO

DMSO is used as a cryoprotectant and inhibits aggregation, clotting and damage of MSCs. DMSO specifications are according to Ph. Eur. Monograph (01/2017:0763) current edition, requirements.

Stability

Stability studies were performed on the Intermediate Cell Stock and the finished product. Data on one ICS, three pilot batches and two commercial batches have been included.

Data on ciMSC and EAP stability at -80 °C are available for up to 24 months post-packaging, which is the proposed shelf life. The lower limit for the viability release specification was set to 90% in order to ensure 70% viability at the end of shelf life.

Arti-Cell Forte stability data are provided for three pilot batches, which is considered acceptable. In line with the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/IWP/12343/2006-Rev3), results from one industrial scale batch should be provided as a post-authorisation recommendation.

Testing includes appearance, cell count, viability, morphology, cell identity, impurity, potency, population-doubling time, mycoplasma and sterility, for ciMSC and appearance, cell count, sterility and mycoplasma, for EAP. The set specifications are met for all parameters. As in the case of batch analysis, a high variability in the potency results is observed. The acceptance limits for cell viability established for the stability exercise have been set to mirror the batch data submitted.

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

Additional testing to establish the consistent quality of the final diluted product for batches of ciMSC and EAP during and at the end of their current individual shelf lives has been performed. The results confirmed that addition of EAP does not alter the characteristics of the active substance. Additional

evidence for an improved survival/function has been submitted, further supporting the use of EAP in the final product formulation.

Overall conclusions on quality

The dossier submitted for Arti-Cell Forte is of appropriate quality and provides adequate information on the development, manufacture and control of the finished product.

Post-authorisation recommendations

i) As a post-authorisation recommendation results of stability data of one industrial batch should be provided in line with the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/IWP/12343/2006-Rev3).

ii) The applicant is also requested to provide the data set used for the calculation of the lower specification for one of the markers used based on the modified gating strategy introduced to improve the detection of the PBMC-impurities.

Part 3 – Safety

The active substance chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells (ciMSCs) of Arti-Cell Forte, is a new active substance not authorised for a veterinary medicinal product in the EU before. A safety file in accordance with Article 12(3)(j) of Directive 2001/82/EC has been provided. Adequate justifications have been provided and considered acceptable for any data not provided.

Safety documentation

Pharmacodynamics

See Part 4.

Pharmacokinetics

See Part 4.

Toxicological studies

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

As the active component in Arti-Cell Forte consists of stem cells the general study data requirements for pharmaceutical products do not apply. There are no general guidelines for stem cells; as a result recommendations regarding development plans and evaluation requirements are given case by case for each product by CVMP via the scientific advice procedure. The major safety concern for a biological product with stem cells is considered to be related to potentially malignant transformation and tumorigenic effect. 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 PDT and karyotype is considered to contribute to a low risk for tumorigenicity (see Part 2).

Single dose toxicity

No single dose toxicity data relating to the active substance were provided. This was accepted as target animal safety, user safety and consumer safety were addressed by means of other studies.

A summary of median lethal dose (LD_{50}) values of DMSO estimated in single dose studies from a set of bibliographic references was provided. The relevance of LD_{50} values of DMSO for the evaluation of target animal safety and user safety is considered to be low. The values reported for DMSO in various laboratory species (range from 2500 mg/kg following intraperitoneal administration in mouse and intravenous administration in dog to ≤40 000 and ≤50 000 mg/kg following administration to the skin of rat and mouse, respectively) do however indicate that the acute toxicity of DMSO is low.

Repeat dose toxicity

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

A summary of maximum tolerable doses (MTDs) of DMSO estimated from repeated dose toxicity studies from bibliographic references was presented. The relevance of the reported MTDs for DMSO (from 1200 mg/kg bw/day following 24 days of intravenous administration in dog to 11000 mg/kg bw/day following 10 days of oral administration in rat) for the evaluation of target animal safety and user safety of occasional exposure to DMSO is considered to be low.

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

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

Tolerance in the target animal species

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

Reproductive toxicity

Active substance:

Equine allogeneic chondrogenic induced mesenchymal stem cells

No data from reproductive toxicity studies of equine allogeneic chondrogenic induced mesenchymal stem cells in laboratory animal species or in the target animal species were provided. This is acceptable.

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

For human cell-based medicinal products there are no standard requirements for reproductive toxicity studies (Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006). As the cells are xenogeneic, the risk for pregnant women accidentally exposed to Arti-Cell Forte is considered as negligible, see User safety.

Excipients:

DMSO

For reproductive toxicity of DMSO the applicant referred to a safety data sheet from a chemical company. The safety data sheet refers to data and concludes that DMSO is not teratogenic at low levels regardless of the route of administration and that the teratogenicity of DMSO is dependent on the route of administration, the dose level and gestation stage at exposure. For example, in a mouse teratology study, a NOEL of 12 g/kg/day was reported for a 50% DMSO solution given orally. However, without access to the data the conclusion on the teratogenic potential of DMSO cannot be verified.

Nevertheless there is a permitted daily exposure (PDE) of 50 mg/day, i.e. 0.83 mg/kg/day set for exposure to DMSO via human medicinal products (ICH guideline Q3C (EMA/CHMP/ICH/82260/2006) that was also used for the user risk assessment (see repeated dose toxicity) by the applicant, and thus the lack of assessable data on reproductive toxicity of DMSO was considered acceptable, on the basis that this limit is not expected to be exceeded, therefore providing reassurance of a low risk for the potential reproductive toxicity of DMSO.

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

Other excipients:

See relevant paragraph at the end of the section.

Genotoxicity

Active substance:

Equine allogeneic chondrogenic induced mesenchymal stem cells

No data from genotoxicity studies of the allogeneic chondrogenic induced MSCs were provided. Due to the nature of the product this was considered acceptable.

According to the CVMP Scientific Advice (EMA/CVMP/SAWP/80218/2015), the tumorigenic potential of

Arti-Cell Forte is best controlled by the quality of the cultured product, i.e. by specifications of identity, purity and genomic stability in terms of PDT and karyotype (see Part 2). Furthermore, in the Guideline on human cell based medicinal products (EMEA/CHMP/410869/2006) it is stated that genotoxicity studies are not considered necessary for human cells, unless the nature of any expressed product indicates an interaction directly with DNA or other chromosomal material.

As the data are considered sufficient to allow for a well-controlled production process of the stem cells the risk for tumorigenicity of Arti-Cell Forte is considered to be low.

Excipients

DMSO

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

Other excipients:

See relevant paragraph at the end of the section.

Carcinogenicity

Active substance:

In line with the CVMP scientific advice, no data from tumorigenicity studies were provided (EMA/CVMP/SAWP/80218/2015). There are no adequate *in vivo* models for investigating tumorigenic potential of stem cells in horses. In accordance with CVMP recommendations, PDT and karyotype stability was investigated.

A karyotype analysis using the shallow whole genome sequencing method showed that up to 15 passages with chondrogenic induction did not result in any detectable chromosomal abnormalities. The PDT for chondrogenic induced MSCs fell outside the specification at a concentration of 0.1% induced pluripotent stem cells (iPS) cells or more. PDT and karyotype analyses are used to provide a well-controlled production process with respect to genomic stability, which together with controls for identity and purity are considered to contribute to a low risk for tumorigenicity.

Excipients:

DMSO

The lack of information on carcinogenic potential of DMSO was considered acceptable based on the fact that DMSO is regarded as a solvent with low toxic potential and of low risk for human health and

for which a PDE via pharmaceutical products has been set e.g. based on negative genotoxicity (ICH guideline Q3C on residual solvents in human medicinal products [EMA/CHMP/ICH/82260/2006] and VICH guideline 18(R) for veterinary medicinal products [EMA/CVMP/VICH/502/99-Rev.1]). Furthermore, the target animal will be treated with a single dose and users are expected to be exposed on single occasions only.

Other excipients:

See relevant paragraph at the end of the section.

Studies of other effects

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

Other excipients

In addition to DMSO, Dulbecco's Modified Eagle Medium Low Glucose (DMEM LG) and equine allogeneic plasma (EAP) are also excipients in Arti-Cell Forte. DMEM LG consists of inorganic salts, amino acids and vitamins, which were concluded not to cause any concern for the safety of the target animal or the user.

EAP contains thrombocytes within the physiological range of horses and is a normal constituent of horse meat. Plasma is an endogenous material. The contribution of exogenous physiological EAP to the overall level of plasma to which the horse is exposed will be negligible and is not expected to have a pharmacological effect. It can be concluded that EAP does not cause any concern for the safety of the target animal or the user.

User safety

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

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

Provided there is adequate control of cell identity, control of cell duplication, control of exponential growth and karyotype controls (See Part 2a), tumorigenic potential of the ciMSCs is considered to be low. In addition, due to multiple xenogeneic cell-surface antigens present on the equine ciMSCs, or secreted cellular components, which will be perceived as foreign to the human immune system an efficient graft-rejection is expected to occur in exposed users. The main potential routes of accidental contact with the product have been considered. It was concluded that the most likely are those of dermal and/or oral exposure and accidental self-injection of which the latter was considered to be the worst case scenario. The major risk in relation to accidental self-injection consists of local immune reactions against the foreign cells and plasma at the injection site. As long as all quality aspects in the manufacture of the product are fulfilled, no severe physiologic or pathologic changes, including formation of tumour cells, are expected after potential systemic self-administration. Expected adverse events may include pain, local inflammatory reactions and swelling at the site of injection and possibly fever, which the applicant claims will resolve after a few days, although no data have been presented

to support this. The potential for local effects after accidental injection is reflected in the risk communication.

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

The content of DMSO was not considered to represent a significant risk for the user. The permitted daily exposure (PDE) of residual levels of DMSO via pharmaceuticals has been established as 50 mg/day. Accidental injection of half the total product volume of 2 ml, that is 1 ml 5% DMSO, corresponds to the PDE for DMSO via pharmaceuticals for humans. This was considered to provide an acceptable margin of exposure, particularly as the PDE value has been established for long term exposure and users of this product would only be exposed on a single occasion.

Regarding user risk communication information on expected adverse effects in relation to accidental self-injection, i.e. pain, local inflammatory reactions and swelling at the site of injection which may persist for several weeks and possibly fever which will resolve after a few days has been included in SPC section 4.5ii.

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

> Liquid nitrogen containers should be handled by properly trained personnel only. The handling of liquid nitrogen should take place in a well-ventilated area. Before withdrawing the vials from the liquid nitrogen canister, protective equipment consisting of gloves, long sleeves and a facemask or goggles should be worn.
>
> In case of accidental self-injection this product can cause pain, local inflammatory reactions and swelling at the site of injection which may persist for several weeks and possibly cause fever; seek medical advice immediately and show the package leaflet or the label to the physician.
>
> Based on the above risk assessment the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the provisions of the SPC.

Environmental risk assessment

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

Arti-Cell Forte is not expected to pose a risk for the environment when used according to the SPC.

Residues documentation

MRLs

The active substance contained in Arti-Cell Forte, chondrogenic induced equine allogeneic peripheral blood-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.38).

According to Commission Regulation 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuff of animal origin, no MRL is required for the excipient DMSO.

Plasma is an endogenous material. The contribution of exogenous physiological EAP to the overall level of plasma to which the horse is exposed will be negligible and is not expected to have a pharmacological effect. Furthermore, the contribution of exogenous physiological EAP to the level of endogenous plasma in food from a horse injected with EAP is expected to be negligible and not to be of any safety concern for the consumer. In addition, the absorption of the constituents in plasma, e.g. proteins, is generally expected to be negligible e.g. due to proteolytic degradation in the gastro-intestinal tract. Overall, when used as in the product physiological EAP is not considered to be pharmacologically active and so is not considered to fall within the scope of Regulation (EC) 470/2009.

Dulbecco's Modified Eagle Medium Low Glucose (DMEM LG) contains amino acids, vitamins, salts and carbohydrates which are relevant nutrients to cells in culture. All components except ferric nitrate nonahydrate and sodium pyruvate are covered by Regulation (EC) 37/2010 (i.e. all vitamins are covered and remaining salts are covered by the entry for food additives with valid E-numbers) or the Out of Scope list (D-glucose). Ferric nitrate nonahydrate and sodium pyruvate are not considered as pharmacologically active at the dose administered to the target animal and so are not considered to fall within the scope of Regulation (EC) 470/2009 when used as in this product. A worst case consumer exposure estimate supports the view that this exposure would not represent a hazard for the consumer.

Residue studies

Pharmacokinetics

See part 4.

Depletion of residues

Not applicable.

Withdrawal periods

Since the active substance, EAP and DMEM LG are considered to be out of the scope of Regulation (EC) 470/2009 and no MRL is required for the excipient DMSO, a withdrawal period of zero days was proposed and considered justified for Arti-Cell Forte.

Overall conclusions on the safety and residues documentation

Arti-Cell Forte is composed of equine ciMSCs as the active substance. Excipients include EAP (horse plasma), DMEM and DMSO. As the active component in Arti-Cell Forte consists of stem cells the general study data requirements for pharmaceutical products do not apply. The safety data package consists, with the exception of a combined Target Animal Safety/distribution study and quality data of the horse MSCs, of bibliographic data. This is in line with the CVMP scientific advice (EMA/CVMP/SAWP/80218/2015).

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

General toxicity

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

Reproductive toxicity

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

Genotoxicity and cancer

As the data are considered sufficient to allow for a well-controlled production process of the stem cells the risk for tumorigenicity of Arti-Cell Forte was considered to be low.

The genotoxicity and carcinogenicity of DMSO has been adequately addressed.

User risk assessment

The main potential routes of accidental contact identified for the user include those of dermal and/or oral exposure and as the worst case scenario, accidental self-injection. As long as all quality aspects in the manufacture of the product are met, no severe physiologic or pathologic changes, including formation of tumour cells, are expected after potential systemic self-administration of xenogeneic MSCs. Expected adverse events may include pain, local inflammatory reactions and swelling at the site of injection which can persist for several weeks and possibly fever, and are reflected in the risk communication.

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

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

Environmental risk assessment

The environmental risk assessment stopped in Phase 1. Arti-Cell Forte is not expected to pose a risk for the environment when used according to the provisions of the SPC.

MRLs

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

Part 4 – Efficacy

Pharmacodynamics

Published references were provided to describe pharmacodynamic properties of mesenchymal stem cells. Effects of MSCs are thought to result from multiple mechanisms that include anti-inflammatory, angiogeneic, homing capacities and/or immunomodulatory effects. Anti-inflammatory effects of MSCs include suppression of both innate and adaptive immune cells such as macrophages, NK-cells and dendritic B-cells, CD8+ T-cells and CD4+ T-cells.

Immunomodulatory effects that have been described for stimulated equine MSCs include decreased lymphocyte proliferation, increased prostaglandin E2 (PGE2) and interleukin-2 (IL-2) secretion and decreased secretion of tumour necrosis factor -α (TNF-α) and interferon gamma (IFN-γ). Although MSCs have generally been attributed to be immunomodulatory in the sense that they can reduce immunological activity, allogeneic MSCs in pigs and horses have been demonstrated to elicit immune responses *in vivo* despite a low immunogenic profile *in vitro*. This is thought to be caused by MHC mismatch between donor and recipient. In an *in vitro* assay to evaluate the immunogenic potential of the MSCs (mixed leukocyte reaction) it was demonstrated that the cells of Arti-Cell Forte had very low immunogenic properties.

Fundamental mechanisms or patterns describing the survival, distribution and homing effects of MSCs in horses are lacking in literature, but from studies done using equine MSCs it is indicated that these correspond largely to MSCs from other species in terms of characteristics and mechanism of action. It has been suggested that by the chondrogenic induction of MSCs, the mode of action is narrowed towards chondroprotective mechanisms such as production of extracellular matrix, stimulation of local cells by paracrine effects and/or immunomodulation by reducing local inflammation. Studies of equine peripheral blood MSCs similar to the cells of Arti-Cell Forte *in vitro* have been published. Chondrogenic induction of the MSCs increased adhesion and incorporation into equine cartilage and homogenous layers of chondrogenic induced MSCs were demonstrated over cartilage explant surfaces. MSCs adhered to the surface of explants and penetrated into lesions in unloaded samples, but physiological

mechanical loading significantly reduced surface adherence and lesion filling. It was therefore considered unlikely that adherence/penetration of the cells into the cartilage will occur *in vivo*.

Gene expression of a marker representative of cartilage turnover is used to determine potency of the product. Cartilage turnover is indicated as the major function of the chondrogenic induced MSCs in treatment of OA in horses, however, based on the results from the clinical studies where effects are indicated on local reactions such as joint swelling that is assumed to be correlated to inflammation, it is likely that paracrine and immunomodulatory effects of the cells could also play a role in the process.

In the proof of concept study (GST-P1), effect of treatment with Arti-Cell Forte on markers of cartilage turnover and inflammation was evaluated in a model of osteoarthrosis in horses. An increase in the area of the respective marker and collagen type II was demonstrated by immunohistochemistry in the treated group compared to placebo, and similarly, a larger area of Alcian Blue/Safranin O staining for chondroitin and dermatan sulphate was demonstrated in cartilage from horses of the test group. On macroscopic joint assessment a lower degree of wear lines and synovial hyperaemia was demonstrated in the test group and viscosity of synovial fluid was higher in the test group at late time points of the study. These results indicated that treatment had an effect on cartilage turnover and the inflammatory process in the treated joint.

The final product contains, apart from the ciMSCs, 5% DMSO and 50% equine allogeneic plasma (EAP). DMSO is included as a cryoprotectant excipient at a low concentration that is not expected to have any pharmacological activity. EAP is added to the cells prior to administration into the joint with the purpose of increasing cell viability after thawing. Products based on plasma such as platelet rich plasma (PRP) and autologous protein solution (APS) have been suggested to be efficacious in treatment of osteoarthritis in horses. There is, however, no clear indication or evidence to support that the EAP included in Arti-Cell Forte should have clinically relevant effects on its own in the treatment of osteoarthritis in horses.

Pharmacokinetics

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

Biodistribution was evaluated by immunohistochemistry in the pivotal target animal safety (TAS) study. Tissue samples from joint capsule, synovial membrane and cubital lymph node were collected upon termination of the study at day 42±1 from animals treated with ciMSCs. The presence of ciMSCs was determined by a specific marker immunohistochemistry. No evidence of biodistribution of ciMSCs to the tissues surrounding the joint was demonstrated in any of the samples tested indicating that ciMSCs did not migrate to any considerable extent and persist in these tissues. Although there is a possibility that cells may have migrated to other tissues that were not tested and the period of persistence is unknown, it is considered unlikely that this would result in any clinically significant effects. No adverse events attributed to the cell treatment were reported during the study and there was no evidence of ectopic tissue formation.

Dose justification

No specific dose determination studies are presented, but the selection of dose was based on published data from preliminary and pilot studies of treatment of degenerative joint disease in horses using allogeneic peripheral blood-derived equine MSCs. Acceptable tolerance and clinical improvement was reported in the studies indicating that the dose is suitable.

A clear dose response relationship is not to be expected for this type of cell based therapy, but batches of acceptable efficacy should be identified by a relevant potency assay that correlates to the function of the cells. As a potency assay the respective marker expression is used as a surrogate marker for the chondrogenic induction process. The potency limit was justified by the use of batches of this potency in clinical studies that showed acceptable clinical improvement.

Considering that the product is classified as MUMS it is acceptable to waive specific dose justification studies provided that the selected dose is used in clinical trials and demonstrated to be safe and efficacious. In the clinical trials provided using the final formulation of the product treatment was administered in fetlock joints, at doses within the range of 1.0-2.5 x 10^6 cells per joint.

Dose confirmation studies

A dose of 2 x 10^6 ciMSCs per joint was used for treatment in a Good Clinical Practice (GCP)-compliant proof-of-concept study to assess efficacy and safety after intraarticular injection in inflamed fetlock joints using an experimentally induced osteoarthritis fetlock model. In the pivotal clinical field trial a dose of 1-2 x 10^6 cells per joint was used for treatment of recurrent lameness of the fetlock joint, and in the pivotal target animal safety study a dose of 2 x 10^6 cells per joint was used. Batches of the test product used correspond to the final product.

The proof-of concept study was performed using a model of surgically induced OA of the right fetlock joint in 12 horses where 6 horses were treated once with the test product and 6 horses received placebo (NaCl solution) on day 35 after surgery. Efficacy of treatment was evaluated for 77±1 days based on pro-and anti-inflammatory protein expression in synovial fluid, joint assessment based on histological examination of the cartilage and (immuno-) histochemical examination of the joint cartilage, capsule and synovial membrane and lameness assessment using objective and subjective methods.

Results showed a statistically significant difference between groups regarding protein expression/viscosity of synovial fluid at 63 and 78 days after treatment in favour of the test group. No difference between groups was demonstrated for a number of parameters tested in synovia (total white blood cell count, lymphocytes, monocytes, granulocytes, total protein, IL-10, PGE2, transforming growth factor beta-3 (TGFb3), hyaluronic acid (HA), insulin responsive aminopeptidase (IRAP), IL-6, IFN-γ, TNF-α, serum amyloid A (SAA), matrix metalloproteinase-13 (MMP-13), GAG). No difference between groups was demonstrated for any of the parameters tested at histological examination of synovial membrane (cellular infiltration, vascularity, intimal hyperplasia, subintimal oedema, subintimal fibrosis or changes in cell morphology).

Histological examination of the joint cartilage from the first phalanx (PI) and the third metacarpal bone (MCIII) was performed by Alcian blue/Safranin O staining to establish the uptake of the product in the cartilage. In the test group a larger mean area (%) of Alcian blue/Safranin O staining was demonstrated in the cartilage of the PI when compared to the control group. This difference was statistically significant. No statistically significant difference was seen for MCIII. Immunohistochemistry of injured cartilage demonstrated a statistically significant difference between groups for the marker

representative for cartilage turnover in the PI area in favour of the test group (67.5% of the area in the test group, 21.1% in the control group). The latter marker in the MCIII was not statistically different between the two treatment groups. The area of collagen type II staining of the cartilage (% of the area of the cartilage structure) showed statistically significant differences between the groups for both PI (78.7% of the area in the test group, 52.5% in the control group) and MCIII (68.5% of the area in the test group, 50.0% in the control group) in favour of the test group. No area of Ki67 protein (a cellular marker for proliferation) or cysteine-aspartic acid protease 3 (Caspase3) was demonstrated in any of the animals.

The joint assessment at macroscopic level showed a statistically significant difference between groups on day 78 for wear lines and synovial hyperaemia in favour of the treated group. No difference between groups was demonstrated for erosions, extent of erosions, palmar arthrosis, covering of subchondral bone with fibrocartilage, synovial petechiae or increase in villi density/thickness.

The results on histological, immunohistochemistry and macroscopic evaluations indicated an effect on cartilage turnover and the inflammatory process in the joint after treatment with Arti-Cell Forte.

Lameness was evaluated by scoring using the AAEP scale (0-5) where 0 is sound and 5 describes the non-weight bearing of the limb. The evaluation was made in trot at a straight line or at lunging. All control horses were lame from day 7 and throughout the study period whereas horses in the treatment group improved slowly after treatment on day 35 and none of the test treated horses were lame on day 77±1.

Lameness was also assessed by using two objective methods; pressure plate and lameness locator. Loading of each stride was evaluated by using the pressure plate but no statistically significant difference between the two treatment groups was obtained. The lameness locator utilises accelerometers and a gyroscope (motion sensors) to objectively quantify how a horse moves through space and bears weight. Different parameters of vector sum (VS) were then used to identify lameness in a limb. In this study the lameness locator was used on horses trotting at a straight line before and after flexion and on treadmill. Results obtained by objective and subjective methods of lameness evaluation were, however, not in full agreement and objective tools were not used in the field study.

A flexion test was performed as a part of the lameness evaluation and the reaction of the flexion was assessed using a scale from 0-3. From day 49 and throughout the study period all six horses in the test group were determined as score 0 (no reaction) whereas five horses from the placebo group were determined as score 1 (mild reaction) and one horse had a severe reaction (score 3).

After surgical induction of OA, horses in both groups had mild to moderate increase in joint effusion that gradually decreased. On day 56 to 77 (21 to 42 days after treatment) lower joint effusion scores were demonstrated in the test treatment group. This difference was statistically significant. An increase in joint circumference of 0.5 to 1.5 cm was demonstrated in all horses in both treatment groups. All horses in the test group had returned to baseline values on day 56 whereas all horses in the control group had remaining increased joint circumference throughout the study period. One horse in each treatment group showed mild and transient increased heat and pain at palpation of the injection site. Evaluation of safety parameters revealed no treatment related adverse reactions.

Target animal tolerance

One pivotal GCP-compliant, randomised and blinded study was provided to investigate the target animal safety of the product. In addition, safety data was obtained from the proof-of concept study, the pivotal field trial and supportive retrospective clinical data. Batches of the test product used are

stated to correspond to the final product. Treatment was administered according to the intended route of administration (intraarticular) and within the range of the intended treatment dose (1-2.5x10^6 cells in 1 ml DMEM with 10% DMSO mixed with 2 ml EAP before administration) in all studies.

Full study title	**Results**
A GCP-compliant, randomised and blinded target animal safety study to evaluate the safety of a single intra-articular application of equine allogeneic chondrogenic induced mesenchymal stem cells (EAIMSC) in horses.	No treatment related adverse reactions. Mild lameness in both treatment groups. Mild to moderate local adverse reactions (joint effusion, increased joint circumference, increased heat on palpation, increased pain on palpation) in both groups. Normal cell morphology in cartilage of treated horses. No evidence of biodistribution of MSCs to tissues surrounding the joint. No treatment related changes in haematology/blood biochemistry or gross pathology.
A GCP-compliant proof of concept study to show the efficacy and safety of 'Equine Allogeneic Chondrogenic Induced Mesenchymal Stem Cells' (EaiMSC) on inflammation in an experimentally induced osteoarthritis fetlock model in horses.	Safety evaluation: no treatment related adverse reactions. Mild to moderate local adverse reactions (joint effusion, increased joint circumference, increased heat on palpation, increased pain on palpation) in both groups.
A multicentre, randomized, blinded and blocked clinical field study to show the efficacy and safety of Arti-Cell Forte in the treatment of recurrent lameness due to non-infectious joint inflammation in horses compared to a negative control (saline).	Safety evaluation: no treatment related adverse reactions. Increase in joint swelling on the day after treatment in one animal in each group. Increase in heat and pain at the injection site in one animal in the test group.
Supportive clinical data, retrospective analysis of horses treated with Arti-Cell Forte.	Safety evaluation: No abnormal clinical observations were made and no local adverse reactions were demonstrated

In the pivotal target animal safety study 16 healthy horses were administered a single dose of the test product (n=8) or control product (NaCl solution; n=8) and followed for 42±1 days by clinical

assessment, joint and lameness assessment, haematology/blood biochemistry and pathology/histopathology. After treatment, horses in both groups showed mild lameness and local adverse reactions (joint effusion, increased joint circumference and increased heat on palpation) to a comparable extent and severity. Histopathological examination of cartilage from treated horses demonstrated normal cell morphology, and there was no evidence of biodistribution to tissues surrounding the joint or ectopic tissue formation in samples tested. No treatment related effect on haematology/blood biochemistry or gross pathology was demonstrated. It was concluded that systemic and local tolerance of treatment was acceptable as evaluated in the study. There was no evidence of biodistribution or ectopic tissue formation in any of the samples collected during the study.

The proof-of-concept study was a GCP-compliant randomised blinded negatively-controlled laboratory study using a model of surgically induced osteoarthritis of the fetlock joint. 12 horses with induced OA were administered the test product (n=6) or control product (NaCl; n=6) and evaluated with regard to inflammation of the joint, lameness, macroscopic changes of the joint and cartilage, histology of joint cartilage, markers for cartilage remodelling, adverse events and local reactions. The observation period ended 42±1 days post treatment. Evaluation of safety parameters revealed no treatment related adverse reactions.

The pivotal field trial was a GCP-compliant, randomised, blinded, placebo controlled multicentre study including 75 horses with recurrent lameness due to osteoarthritis of the fetlock joint. Fifty (50) horses received test treatment and 25 negative control (NaCl solution) with an evaluation period of 126±7 days. All horses received an intravenous non-steroidal anti-inflammatory drug (NSAID) at the time for treatment which is likely to have reduced any inflammatory reaction. Safety evaluation consisted of clinical assessments and evaluation of local tolerance and recording of adverse events. No treatment related adverse reactions were demonstrated. An increase in the swelling of the treated joint on the day after treatment was also observed in one animal in each group. Increase in heat and pain at the injection site was observed in one animal in the test group. The frequency of adverse events was very low and comparable between groups (2 horses (4%) in the test group and 1 horse (4%) in the control group. None of the adverse events were considered related to treatment (nasal discharge in all cases).

Supportive retrospectively compiled clinical data where horses were treated on repeated occasions or with ciMSC originating from a different donor of either ciMSCs or EAP has been presented. The results indicate that safety of treatment does not vary depending on the donor. Repeated administration with an interval of 2-4 months does not result in local or systemic adverse reactions related to treatment. Results from an *in vitro* assay (mixed leucocyte reaction) were presented and describe the immunogenic profile of the ciMSCs. Although the results indicate a low level of immunogenicity of the ciMSCs in the *in vitro* test after isolation from both singly and repeatedly treated animals, the relevance of the data for the intended treatment *in vivo* is not clear.

According to the study report, horses were kept in boxes of 3 m x 3.5 m throughout the study. This approximately meets the minimum requirements for box sizes for warm-blooded horses. However, it has to be noted that additional possibilities for activity, e.g. use of a paddock, are required from an animal welfare perspective[1].

Although an impact on the study results is unlikely, the CVMP wishes to note that considerations on the improvement of the well-being of study animals should be of high priority.

[1] German Federal Ministry of Food and Agriculture (2009): Leitlinien zur Beurteilung von Pferdehaltungen unter Tierschutzgesichtspunkten.

Clinical field trial

One pivotal randomised, blinded, placebo-controlled field study was conducted to evaluate the efficacy and safety of Arti-Cell Forte in the treatment of recurrent lameness due to non-infectious joint inflammation in horses by intraarticular administration of 1-2 x 10^6 cells. The study was conducted in Belgium and did adhere to GCP. 75 client-owned horses of various breeds were included (50 in the test treatment group and 25 in the placebo group). The horses had a history of lameness from one fetlock joint of 2-6 months duration, presenting with the American Association of Equine Practitioners (AAEP) score 2 or 3 on inclusion, and had not been treated with NSAIDs within the last two weeks or corticosteroids or hyaluronic acid within the last four weeks. The horses included in the placebo group were administered saline. All horses received one concomitant treatment with NSAID intravenously. Lameness, graded according to the AAEP scale was assessed on days 21±3 and 42±5. After the study end lameness was evaluated on days 84±7 and 126±7. Retrospective information regarding the work status of the horses was also collected on D365 where the horses were classified as failure to work, rehabilitation, working at training level or return to previous work.

The primary efficacy endpoint was "relevant clinical improvement (RCI)" defined as a change in AAEP score from 2 or 3 at baseline (day 0) to 0 or 1 on day 42±5. At lameness evaluation on day 42±5 26 (52%) of the 50 test treated horses were assessed as not lame (score 0) and 12 (24%) were identified with mild lameness (score 1). None of the 25 control horses were identified with score 0 and 6 (24%) of them showed mild lameness (score 1). This difference between the groups was statistically significant. Moreover, 68% of the animals in the test treated group showed a score reduction by 2 or 3 lameness grades, compared to none of the animals in the control group ($P < 0.001$). It can therefore be concluded that the results for the pivotal efficacy data are a clinically relevant decrease in lameness grade and a statistically significant difference between the test treated group and the negative control group.

Secondary efficacy endpoints included improvement of lameness on day 21±3, where 15 (30%) of the test treated horses were assessed as not lame (score 0) and 20 (40%) were identified with mild lameness (score 1). None of the control horses was classified as grade 0 or 1. This difference was statistically significant.

Flexion tests performed on day 42±5 resulted in no reaction in 42 (84%) of the test treated horses and in 4 (16%) of the horses included in the control group. Mild reactions to flexion test (grade 1) was observed in 8 (16%) of the test treated horses and in 16 (64%) of the control horses. Moderate response (grade 2) was observed in 5 (20%) of the control horses.

None of the animals were prematurely withdrawn from the study due to treatment failure (AAEP score 4 or 5). Concomitant medication for the treatment of lameness with Interleukin-1 Receptor Antagonist Protein (IRAP) or corticosteroids after study completion (day 42±5) was administered to 2 animals (4%) in the test group and 9 animals (36%) in the control group.

The study was well designed and conducted and indicated that Arti-Cell Forte is effective in the treatment of OA in horses. The results demonstrated a significantly superior improvement in lameness in the test treatment group compared to the placebo group demonstrated by a higher number of test treated horses assessed as not lame (score 0) at both days 21±3 and 42±5. Retrospective data in terms of work status of the included horses indicates that the effect of treatment was sustained for up to one year. None of the placebo treated horses went back to the previous level of work at any of the time points evaluated (D48, D126 and D365) and 8% performed at training level at D365 whereas in the test treated group 47% of the horses performed at previous level and 37% at training level one year after treatment. It was therefore considered that a positive

effect of treatment was sustained for a significant proportion of the treated horses for a year.

In this study NSAID (ketoprofen) was administered intravenously to all horses concomitantly with the intraarticular administration of test product/placebo. Information relating to concomitant use of NSAIDs is included in the SPC.

Supportive retrospectively compiled clinical data where horses were treated either on repeated occasions with the product or with ciMSC originating from a different donor of either ciMSCs or EAP were presented. These horses had received a single oral administration of an NSAID (meloxicam) on treatment day. According to these supportive clinical data, there was no clear difference in the reduction of lameness as compared to the pivotal field study.

Overall conclusion on efficacy

Pharmacodynamics

Literature references describing properties of MSCs were provided in support of pharmacodynamics of Arti-Cell Forte. Effects of MSCs are thought to result from multiple mechanisms that include anti-inflammatory, angiogeneic, homing capacities and/or immunomodulatory effects. Although specific information on pharmacodynamic properties of Arti-cell Forte is lacking in the scientific literature, data from studies done using equine MSCs indicate that these correspond largely to MSCs from other species in terms of characteristics and mechanism of action. The cells of Arti-Cell Forte are chondrogenic induced with the purpose of narrowing the mode of action towards chondroprotective mechanisms such as production of extracellular matrix, stimulation of local cells by paracrine effects and/or immunomodulation by reducing local inflammation. A representative marker for cartilage turnover was used. Results from the proof-of-concept study indicate that Arti-Cell Forte had an effect on cartilage turnover.

Pharmacokinetics (biodistribution)

Biodistribution of Arti-Cell Forte has been satisfactorily evaluated in horses. No evidence of migration to tissues surrounding the joint was demonstrated in the target animal safety study.

Dose determination

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

Tolerance

In the Target Animal Safety (TAS) study, Arti-Cell Forte was well tolerated at single administration of the recommended treatment dose. Mild and transient local reactions were seen in both treatment groups and no treatment related effect on local or systemic tolerance was demonstrated. This was confirmed by safety data from the proof of concept study, the pivotal field trial and supportive retrospective clinical data.

Efficacy

Efficacy has been evaluated in one proof of concept study and in one pivotal field trial, both including one treatment group and one placebo group. Efficacy was demonstrated in the pivotal field trial as a statistically-significant improvement in lameness score in the test treated group compared to placebo six weeks after treatment. A positive effect of treatment was sustained over a period of one year.

Part 5 – Benefit-risk assessment

Introduction

Arti-Cell Forte is a chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cell suspension for intraarticular administration intended for use in horses for the treatment of recurrent lameness due to non-infectious joint inflammation.

One dose of Arti-Cell Forte contains 1.4-2.5 x 10^6 chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells and is presented in single dose packs containing 1 vial of 1 ml stem cell suspension and 1 vial of 1 ml plasma suspension. The two vials are mixed immediately prior to injection.

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, which has been considered in the assessment.

Benefit assessment

Direct therapeutic benefit

The benefit of Arti-Cell Forte is its efficacy in treatment of recurrent lameness due to non-infectious joint inflammation in horses, which was investigated in well-designed laboratory and field studies conducted to an acceptable standard.

Additional benefits

Arti-Cell Forte increases the range of available treatment possibilities for treatment of osteoarthritis in horses and provides a new treatment possibility for a minor species.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics.

Safety

Measures to manage the risks identified below are included in the risk management section.

Risks for the target animal:

Administration of Arti-Cell Forte in accordance with SPC recommendations is generally well tolerated. The safety of chondrogenic induced equine allogeneic peripheral blood-derived mesenchymal stem cells in horses was confirmed in a target animal safety study. Local reactions such as joint effusion and swelling were observed in some animals which were administered one dose of 2x10^6 ciMSCs that is the recommended treatment dose. However, the effects were mild and transient and could be

associated with the intraarticular injection.

Risk for the user:

Arti-Cell Forte is not expected to pose a risk for the user when used according to the recommendations in the SPC.

Risk for the environment:

Arti-Cell Forte is not expected to pose a risk for the environment when used according to the recommendations in the SPC.

Risk for the consumer:

Arti-Cell Forte is not expected to pose a risk for the consumer when used according to the SPC recommendations.

Risk management or mitigation measures

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

User safety

User safety risks in case of accidental self-injection (pain, local inflammatory reactions and swelling at the site of injection and possibly fever) have been identified. The risks will be addressed by the safety warnings in the SPC.

Consumer safety

The withdrawal period is set at 0 days.

Target animal safety

Appropriate warnings and information have been included in the product information.

Evaluation of the benefit-risk balance

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

The product has been shown to be efficacious for reduction of mild to moderate recurrent lameness due to non-septic joint inflammation in horses.

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

Conclusion

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

Cell Forte is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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

Divergent position on a CVMP opinion on the granting of a marketing authorisation for Arti-Cell Forte (EMEA/V/C/004727/0000)

We, the undersigned, have a divergent position to the final outcome for the veterinary medicinal product, Arti-Cell Forte.

The finished product consists of one vial containing 1 ml of a suspension of chondrogenic-induced Mesenchymal Stem Cells (ciMSCs) and one vial containing 1 ml of equine allogeneic plasma suspension. The analysis of the data provided in the file for granting a marketing authorisation leads to following observations:

Equine Allogenic Plasma (EAP)

It is justified by the company as an excipient, as it has been shown in vitro to contribute to stem cell (active substance) viability, and it is mixtured with the active substance just prior administration to horses. But in our opinion it has not been sufficiently characterised and the mechanism of action is unknown.

The quality characterisation performed is a basic one, including sterility/purity, identity and platelet count. In the publications not only platelet number is important for the function of the plasma (PrP, EAP or other types) on treated patients and animals, also release factors included in the plasma could contribute to the efficacy in osteoarthritis and other tendon or ligament treatments, as those factors promotes angiogenesis and tissue repair (Rozman & Bolta, 2007).

Also in a publication of the same company (Broeckx et al, 2014) an equine plasma obtained in a similar way and with platelet number close to the one used in Articell Plus (*200 x 10^6/ml in the publication and 80-175 x 10^6 in final product*) it have been shown to have clinical effect in osteoartrithis in horses when adminsitered alone.

Because of these reasons we consider that this component of the final product is not well characterised and could have a direct effect in the efficacy of the product that has been not studied.

Concomitant use NSAIDs IV in all horses in the IVP and placebo groups could affect on the efficacy and the safety of the product. This issue has not been adequate assessed.

4 - CONCLUSION

Therefore, the quality, safety and efficacy of Arti-Cell Forte cannot be demonstrated at the level mandated by the current EU legislation. This legislative standard is what all European veterinary medicinal products are held to, providing product users with guarantees of consistent quality, safety and efficacy. The undersigned have major concerns that Arti-Cell Forte is below this legislative standard.

London, 21 June 2018

Cristina Muñoz Madero

Anna Wachnik-Swiecicka

Divergent position on a CVMP opinion on the granting of a marketing authorisation for Arti-Cell Forte (EMEA/V/C/004727/0000)

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1 – STEM CELLS

1.1 - Origin of Mesenchymal Stem Cells (MSCs)

Each Master Cell Bank (called 'Intermediate Cell Stock' (ICS) by the applicant) of stem cells for the veterinary medicinal product, Arti-Cell Forte, consists of MSCs isolated from equine blood. Each blood sampling results in one ICS. Several batches can be produced from each ICS, nevertheless the batch production will be limited over time, so long as the initial ICS lasts; afterwards, new blood samplings will need to be done for the establishment of new ICSs. Thus, the fundamental properties of each batch of stem cells are donor-dependent (three donor-horses are already used), since the standard isolation procedures do not lead to uniform cell populations. This results in a dilemma that equine stem cells cannot be sufficiently characterized (see below), such that consistency between batches of finished product cannot be guaranteed.

1.2 - Characterization and functional properties of MSCs

To ensure consistent quality from batch-to-batch, it is essential that equine MSCs are appropriately characterized and their functional properties verified throughout the entire production process, from the harvest to the finished product and up to the end of the shelf-life. This is the minimum standard for all veterinary medicinal products. To achieve this, the following parameters (amongst others) should be systematically verified for each batch of finished product:

1° the equine MSCs should be characterized by either the presence (identity) or the absence (purity) of a range of appropriate phenotypic surface markers (markers of a stem cell that distinguish it from ordinary cells, called 'markers of stemness' throughout the rest of this document).

2° No chromosomal alterations should be detectable in the equine MSCs.

1.2.1 - Markers of stemness

- *Of peripheral blood-derived MSCs*

The company selected surface markers for the equine peripheral blood-derived MSCs.

It is acknowledged that the company put considerable efforts into demonstrating the relevance of the selected markers, but important aspects of these stem cells remain elusive, for example:

- there is a distinct lack of scientific consensus concerning appropriate phenotypic surface markers characterizing equine MSCs,

- the same markers can be expressed in various equine cell types; moreover, equine MSCs show diverse marker expression patterns depending on the source of isolation (bone marrow, adipose tissue, tendon tissue, umbilical cord blood, umbilical cord tissue), indicating that, although they are all called 'stem cells', they are fundamentally different with diverse functional properties; hence, as the CDs are identified one by one by the fluorescence-activated cell sorting (FACS) technique (and not all together), it cannot be guaranteed that the appropriate CD phenotype is unequivocally shared by all MSCs on one hand, and that non-MSCs are unequivocally discarded on the other hand,
- the surface markers expressed by equine MSCs may also vary depending on the tissue source and cell culture procedures,
- the immune-phenotype of equine MSCs seems to be donor-dependent,
- the unequivocal immune-phenotyping of equine MSCs is hampered by the lack of specific equine markers and the limited commercial availability of monoclonal anti-horse antibodies, necessary to identify the surface markers.

- *Of chondrogenic-induced MSCs (ciMSCs)*

The company selected predefined surface markers for the equine ciMSCs; however, the same markers were already selected for the equine peripheral blood-derived MSCs, with exactly the same thresholds (which in addition are for both MSCs and ciMSCs). Nevertheless, ciMSCs represent pre-differentiated stem cells and therefore functionally different from the peripheral blood-derived MSCs. Moreover, no specific marker(s) for the equine chondrogenic phenotype are proposed, hence not allowing the differentiation between MSCs and ciMSCs.

Thus, the equine MSCs, whether blood-derived or already chondrogenic-induced, cannot be fully and unequivocally characterized; hence, consistency in quality of the batches cannot be guaranteed.

1.2.2 – Chromosome analysis

Chromosomal stability appears to be donor-dependent.

However, this assay is not included as part of the release criteria for batches of finished product. In practical terms, this means that the genetic stability of forthcoming batches cannot be guaranteed.

3 – POTENCY TEST

The test proposed by the company is based on the quantification by RT-qPCR of the mRNA encoding for a specific gene, which is presented as a surrogate marker for the chondrogenic induction process. Results are provided for various samples from different donors. From these results, the following observations are noted:

- Statistically significant differences in gene expression were shown between the ciMSCs from the different donor-horses. Thus, gene expression is donor-dependent (and probably even ciMSC-dependent, even if coming from a single donor).
- The stability studies done on frozen batches of finished product showed that the potency test produced inconsistent results over time. This casts doubt on the reliability of the potency test, as implemented by the company.
- The company demonstrated that an increase in gene expression is paramount rather than the base level of expression: the methodology measures a difference between two amounts (gene

expression from the ciMSC samples compared to gene expression of a non-induced external negative control sample). From the data available, the following observations are noted:

- the negative control sample should be accurately standardized since it becomes the reference: this is not the case, since the stem cells used as controls present the same limitations in this respect as those detailed in this document.
- the same reference sample should be used for every potency run, throughout the whole lifetime of Arti-Cell Forte. This is not the case. Hence, as the reference sample changes, the potency values of the batches of finished product cannot be interpreted with regard to efficacy/consistency of production through time.
- Since the results are expressed as relative values, the threshold retained by the applicant covers a variety of different realities with regard to efficacy/consistency of production.

Thus, this test is unable to be interpreted from the perspective of a potency test, in terms of safety (uncontrolled cartilage growth), efficacy or consistency of production of batches of finished product, even for batches fulfilling the currently accepted increase threshold retained by the applicant.

2 – EQUINE ALLOGENEIC PLASMA

An equine allogeneic plasma suspension is used to increase the viability of ciMSCs when administered to horses. Since the equine allogeneic plasma is a complex blood component which cannot be standardized, especially when coming from different donors, then consistency in quality of the plasma batches cannot be guaranteed.

3 – SAFETY AND EFFICACY PROFILES

Given the analytical deficiencies mentioned above, the safety and efficacy studies provided in the file can only be considered as proofs of concept: stem cell therapies in horses may be safe and efficacious in theory, but the circumstances under which they fulfill the safety and efficacy criteria are so miscellaneous and so largely unknown that a concrete proof of safety and efficacy is currently out of reach. Moreover, bioequivalence of MSCs/ciMSCs cannot be demonstrated because of the fundamental lack of scientific knowledge and appropriate biochemical and immunological tools for sound qualification and characterization of equine MSCs/ciMSCs. Also, since consistency in quality of plasma batches cannot be guaranteed either, then evidence of safety and efficacy will be lacking for future batches of finished product.

In comparison with the requirements for vaccines, where submission of additional safety and efficacy data are strongly recommended when a Master Cell Seed is replaced by another one of a different cell line (“Reflection paper on the replacement of cell lines used for the production of immunological veterinary medicinal products” – EMA/CVMP/IWP/37620/2014), nothing like this is requested for Arti-Cell Forte, although the degree of homogeneity between MSCs/ciMSCs (not homogenous) and cell lines (highly homogenous) are in no way comparable.

4 - CONCLUSION

It is acknowledged that the company made major efforts to demonstrate the quality, safety and efficacy for Arti-Cell Forte, in compliance with the current state-of-art knowledge regarding veterinary stem cells and according to advices and requests from the CVMP. However, the current state-of-art with regard to veterinary stem cells is largely insufficient:

- Reliable basic scientific knowledge on MSCs/ciMSCs is lacking, which makes it impossible to qualify and characterize them appropriately, as detailed above,
- Since the quality of the equine allogeneic plasma cannot be standardized, then consistency in quality of plasma batches cannot be guaranteed,
- As a direct consequence, safety and efficacy profiles will be batch-specific, and not product-specific (inconsistent safety and efficacy profiles), which is not the standard for an European veterinary medicinal product,
- Moreover, safety and efficacy data are available with regard to some of the currently available batches; however, these data do not reflect the safety and efficacy profiles of future ciMSC and plasma batches, for which no additional data will be made available,
- Finally, the general mechanism(s) of action of MSCs/ciMSCs as part of a medicinal product is/are largely unknown, and in particular when related to the treatment of equine joint diseases.

Therefore, the quality, safety and efficacy of Arti-Cell Forte cannot be demonstrated at the level mandated by the current EU legislation. This legislative standard is what all European veterinary medicinal products are held to, providing product users with guarantees of consistent quality, safety and efficacy. The undersigned have major concerns that Arti-Cell Forte is below this legislative standard.

London, 21 June 2018

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