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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

Withdrawal assessment report for EQUITEND (EMEA/V/C/002774/0000)

Common name: polycarboxymethyl glucose sulfate acetate

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

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Introduction

The applicant OTR3 submitted on 27 January 2017 an application for a marketing authorisation to the European Medicines Agency (The Agency) for EQUITEND, 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 14 July 2016 as EQUITEND contains a new active substance polycarboxymethyl glucose sulfate acetate (OTR4131) 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: Aid in healing flexor tendinopathy in sport horses.

The active substance of EQUITEND is polycarboxymethyl glucose sulfate acetate (OTR4131), a dextran derivative polymer, specifically designed to replace degraded heparan sulfates in injured muscle and tendonal structures. The pharmacological mechanism of action of polycarboxymethyl glucose sulfate acetate (OTR4131) is based on the binding and protection of growth factors and structural proteins in the extracellular matrix to restore the matrix scaffold and tissue architecture, and provide a micro-environment that is recognized by the cells of origin, aiding the natural process of tissue regeneration. The target species is horse.

EQUITEND contains 1 ml of 10 µg/ml polycarboxymethyl glucose sulfate acetate solution for injection and is presented in packs containing 1 vial.

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

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

On 5 November 2018, OTR3 communicated the withdrawal of the marketing authorisation application at day 120 of the procedure. In its letter notifying the EMA of the withdrawal of the application, the applicant stated the reason for the withdrawal was based on insufficient funds to perform a new clinical field study as requested by the CVMP to support the claimed indication.

Scientific advice

The applicant received scientific advice from the CVMP on 13 June 2013. The scientific advice pertained to quality, safety and clinical development of the dossier.

From the quality point of view, critical discrepancies were detected between the different batches of finished product presented in the development pharmaceutics and adherence to the scientific advice was not generally maintained and deviations were not justified. The points concerned are addressed under the appropriate sections of this report.

Regarding safety and clinical development, the applicant followed the recommendations of the scientific advice.

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 the horse is considered a minor species

and the proposed indication in horse is considered a minor use.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant provided a detailed description of the pharmacovigilance system (version 1), which however had a number of deficiencies at the time of withdrawal of the marketing authorisation application.

Manufacturing authorisations and inspection status

The proposed manufacturer of the dosage form is outside the European Union. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture of products aseptically prepared and terminally sterilised, was provided. The site was considered appropriately certified as complying with GMP requirements.

The manufacturer proposed for batch release and EU importation has a manufacturing authorisation issued by the corresponding national competent authority. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the batch release of sterile products aseptically and terminally sterilised, was provided.

The manufacturer proposed for EU importation and storage of the veterinary medicinal product has GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the chemical/physical quality control testing.

The manufacturer proposed for storage of the active substance and storage and batch control of the finished product has GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the chemical/physical quality control testing.

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

Overall conclusions on administrative particulars

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

Additional information was requested in regard to the detailed description of the pharmacovigilance system.

Part 2 - Quality

Composition

The finished product is presented as a single-use solution for injection for horses containing 10 µg/ml of polycarboxymethyl glucose sulfate acetate (OTR4131) as active substance and three excipients.

The product is presented in colourless type I glass vial closed with a bromobutyl stopper and sealed with an aluminium cap as described in section 6.5 of the proposed SPC.

Containers

The primary packaging is described as colourless type I glass vial closed with a bromobutyl stopper.

At the time of withdrawal of the application some questions on the primary and secondary packaging remained to be addressed.

Development pharmaceutics

The product is a 10 μg/ml single-use solution for injection of polycarboxymethyl glucose sulfate acetate (OTR4131) for horses.

The active substance is a mixture of polysaccharides of the alpha 1,6 glucose type, substituted by carboxymethyl groups, sulfate groups and acetyl groups. Reference is made to ASMF's applicant's part.

This mixture of polysaccharides is mainly characterised by the degree of substitution of carboxymethyl groups, sulfate groups and acetyl groups, optical rotation and the mixture of mass fractions.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the proposed SPC.

Major discrepancies were noticed between the different batches of finished product presented in the development pharmaceutics (batches for clinical studies, batches for preclinical studies, batches of finished product for the pharmaceutical development):

- The active substance employed: although it was always named polycarboxymethyl acetyl sulfate (OTR4131), different specifications were provided for this substance regarding the molecular weight distribution, degree of substitution of sulfates and carboxymethyls and optical rotation. It was concluded that the mixtures of polysaccharides used in the clinical batch, preclinical batches and in the batches of finished product used in the pharmaceutical development are not the same and neither equivalent to that proposed within the ASMF.
- Preclinical and clinical batches were not manufactured by the finished product manufacturer proposed for commercial batches.
- The manufacturing process and formula: one of the excipients contained in the proposed final formulation was not employed in the manufacture of the clinical and the preclinical batches.
- The batches of finished product during development contained traces of one excipient and showed an unjustified increase of pH during the first six to seven months after the manufacture. There were not enough data at the time of withdrawal to demonstrate that this increase of pH in the finished product is not evidence of instability of the finished product when this excipient is used in trace amounts.

As a result of the differences in the manufacturing process/formula, there were critical differences between clinical/preclinical and finished product batches regarding the pH.

Also, at the time of withdrawal of the application detailed information about batches that have been employed for safety/efficacy studies was missing.

In view of all these facts, it was concluded that neither the formulation nor the identity of the active substance used during clinical studies are the same as those intended for marketing. Consequently,

in order to support the studies included in Part 3 and Part 4 of the dossier, the rationale for considering clinical batches, preclinical batches and the proposed formulation for commercialisation equivalent was requested.

Method of manufacture

The proposed manufacturing process consisted of four main steps: preparation of the solution, membrane filtration, aseptic filling and immediate packaging. It was indicated that the finished product is manufactured under aseptic conditions. However, no clear information was provided regarding the pre-sterilisation process of the individual components prior to the preparation of the solution or the established limit of bioburden. The process was considered to be a non-standard manufacturing process. A justification for choosing a non-terminal sterilising process was requested.

Although the submission of validation data post-authorisation could have been acceptable, in accordance with the Guideline on quality data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/QWP/128710/2004) relevant information regarding this non-standard process was absent and was requested. Information requested included: the validation scheme of the manufacturing process, the levels and specification limits for endotoxins and bioburden in components and bulk solutions, complete description of the filters, including filter area together with a description of the filter integrity testing (principle of the test and details of when the tests are performed including limits before and after filtration), specifications of the in-process controls proposed during the manufacturing process. Moreover, the description and validation of the analytical methods of aseptic filling of vials: microbiological control (bioburden), controls for sterility and endotoxins were also requested.

Control of starting materials

Active substance

The name of “polycarboxymethyl glucose sulfate acetate”, although used to designate the active substance in a general manner throughout the dossier, is a chemical name for a family of polysaccharides.

OTR4131 is the specific name used for the active substance. The active substance is defined as mixture of polysaccharides of the α-1,6-glucose type, substituted by carboxymethyl groups, sulfates groups and acetyl groups.

The active substance is a white or off-white lyophilised mass, very hygroscopic, freely soluble in water and insoluble in organic solvents.

It exhibits stereoisomerism due to the presence of multiple chiral centres.

Polymorphism was not considered a critical parameter as the active substance was intended for a solution.

The information on the active substance was provided according to the Active Substance Master File (ASMF) procedure.

There is not a monograph of polycarboxymethyl glucose sulfate acetate in the Ph. Eur. The control tests were carried out to comply with the specifications and test methods of the manufacturer of the active substance internal monograph.

The characterisation of the active substance and its impurities were in accordance with the CVMP

guideline on chemistry of new active substances, only minor concerns remained at the time of withdrawal. Potential and actual impurities were discussed with regards to their origin and were characterised but data and discussion on the purge of the impurities derived from the starting material and the risk of introducing those impurities into the active substance were requested.

Detailed information on the manufacture of the active substance, from the introduction of the starting material, was provided in the restricted part of the ASMF which was considered satisfactory, however a brief description was requested to be provided in the ASMF Applicant's Part.

Polycarboxymethyl glucose sulfate acetate (OTR4131) is synthesised in two main steps using commercially available well defined starting material with Ph. Eur. specifications but no conclusion on its acceptability was reached pending data and discussion on impurities derived from the manufacturing process of the starting material.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents were presented.

There was a major concern related to the use of a particular batch of active substance in the manufacture of a clinical batch.

Regarding the proposed active substance specification some questions remained to be addressed at the time of withdrawal of the application as well as some adjustments in line with the batch data provided. Satisfactory information regarding the reference standards used for assay testing were presented.

Concerning the description and validation of the control methods for the active substance major concerns remained at the time of withdrawal of the application.

Major inconsistencies were found between the proposed specification of the active substance and analytical data of the batches of active substance used in the manufacture of clinical and preclinical batches of finished product.

Regarding the container closure system of the active substance, additional information was requested.

Data for two industrial size batches of active substance were provided in accordance with the "Guideline on quality requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/ limited market". The results were within the proposed specifications.

It was accepted that the active substance would be tested to full specification immediately before manufacture of the finished product as the veterinary medicinal product was classified as MUMS by the CVMP. Therefore formal stability studies according to CVMP guidelines were not required but further stress testing was requested to investigate the degradation profile and the stability-indicating characteristics of the control method. The reference standard used in the forced degradation study provided was not considered acceptable. In addition, photostability data in accordance with VICH GL5 was requested.

Excipients

All of the proposed excipients are well known pharmaceutical ingredients and their quality was compliant with Ph. Eur. There were no novel excipients used in the finished product formulation.

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

The proposed product does not contain any materials derived from human or animal origin.

Control tests on the finished product

The proposed specifications at release and at the end of shelf-life were not appropriate to control the quality of the finished product.

Validation data for some of the the proposed analytical methods was not provided. The stability-indicating characteristics of these methods were not demonstrated and neither the batch to batch consistency.

As a result of these deficiencies, the consistency of the manufacturing process was not considered to be sufficiently controlled.

Stability

Stability data were provided for two production batches of finished product stored under VICH conditions. The data available at the time of withdrawal included: 3 months under long-term conditions (25 ºC/60% RH) and 3 months under accelerated conditions (40 ºC/75% RH). The interaction of the solution with the container was not assessed with vials stored in the inverted position. The batches of product used in the stability studies are representative of those proposed for marketing and were packed in the primary packaging proposed for marketing. Additional stability data was provided for other batches of finished product and similar products, however, these data were not taken into account.

The specifications proposed at end of shelf-life and analytical methods used were the same as those at release. However, the analytical methods proposed are not stability-indicating and the proposed specifications were not considered appropriate to control the quality of the finished product.

In addition, although the absence of photostability data could be justified in accordance with CVMP Guideline for the quality data requirements for veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/QWP/128710/2004-Rev.1), the appropriate protection and labelling of the finished product was pending at the time of withdrawal of the application.

Additional stability data for the finished product in line with acceptable updated specifications was requested. No conclusion could be drawn at the time of withdrawal of the application as regards the stability of the formulation or the acceptable shelf-life period and storage conditions of the finished product.

Overall conclusions on quality

Information on the development, manufacture, control and stability of the finished product was not presented in a satisfactory manner. The results of tests carried out do not indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product would not have a satisfactory and uniform performance in clinical use.

The quality of this product was not considered acceptable at the time of withdrawal of the application. Physicochemical aspects relevant to the performance of the product were investigated but were not

controlled in a satisfactory way. Data was presented to give reassurance on TSE safety.

Despite the manufacturing method being a non-standard process and validation data on pilot-scale batches were not provided, it was accepted that full scale validation would be performed post-authorisation, in accordance with the CVMP Guideline on the quality data requirements for veterinary medicinal products intended for minor uses or minor species (MUMS)/limited market (EMEA/CVMP/QWP/128710/2004), if the applicant had provided both a satisfactory protocol for the process validation study and a confirmation to submit the data post-authorisation and the Committee had considered them acceptable.

Part 3 – Safety

EQUITEND contains the active substance OTR4131, which is a mixture of polysaccharides of the α-1,6-glucose type, substituted by carboxymethyl groups, sulfate groups and acetyl groups. OTR4131 is a new active substance not authorised yet in the EU for veterinary or human use, but is structurally related to OTR4120, a heparan sulfate proteoglycan mimetic, already present in two products currently authorised as medical devices to treat skin and eye injuries in humans. OTR4131 is intended for the treatment of flexor tendinopathy in sports horses. A full safety file in accordance with Article 12(3)(j) of Directive 2001/82/EC was provided based on new studies performed with the active substance.

The product was proposed to be used in horses that are declared as not intended for human consumption under Commission Implementing Regulation (EU) 2015/262. As these animals are not food producing animals the safety and residues data requirements are those that apply for companion animals and therefore, no residue data were provided.

Safety documentation

A comprehensive data package with recently performed studies and data from current literature on the active substance and other ReGeneraTing Agents (RGTAs) was presented.

Pharmacodynamics

OTR4131 belongs to a family of polymers engineered to protect and stabilize heparin-binding growth factors which have been shown to promote tissue repair and regeneration. The pharmacodynamics are described in Part 4.

Pharmacokinetics

The product was intended to be locally injected under ultrasound guidance in or near the injured flexor tendon. Due to the poor vascularisation of this area, it was assumed that the compound would be trapped in the injected area where it performs its healing function, as the systemic absorption is very limited. As part of tendon injuries there can be increased vascularization, metabolic rate and thus lower retention time of molecules at the site of a tendon injury. Thus, the applicant was asked to address local pharmacokinetics based on e.g. available literature and furthermore to justify why studies were not performed on local kinetics when it seems that proper detection methods for the active substance are available. Components of EQUITEND are found in many natural molecules in all living species. The peak concentration in the blood of horses is expected to be low. No pharmacokinetic studies have been conducted in horses. Two non-GLP compliant studies were provided investigating the pharmacokinetics of OTR4131 following intravenous administration of

radio-labelled OTR4131 in rats. A group of rats received a single intravenous dose of approximately 2.5 µg OTR4131/animal. The first study showed that OTR4131 is rapidly cleared from the circulation, with 1.7% and 0.8% of injected activity observed in blood and plasma after 24 h, respectively. The second study confirmed these results, demonstrating that a significant proportion of OTR4131 is excreted in urine (42%), with a concomitant reduction in the liver (21%) and blood (0.9%) after 24 h, while levels in the bone remain relatively unchanged (9.7%).

Toxicological studies

Single dose toxicity

The applicant submitted a GLP compliant single dose toxicity study performed in rats, which showed that an intravenous dose of 5 mg of OTR4131 elicits effects on treated animals including lower body weight gain and increase of the mean activated partial thromboplastin time (APTT). Furthermore, one male rat treated with OTR4131 was found dead 3 hours post injection. No clinical signs were noted prior to its death therefore, the effect of OTR4131 could not be excluded. However, taking also into account the information on target animal safety studies (TAS) it was concluded that the potential toxicity of the active substance after a single accidental injection of the prescribed dose is low.

Repeat dose toxicity

Polycarboxymethyl glucose sulfate acetate was tested by the intravenous route in rats and rabbits at the dose level of 0.005 or 0.5 mg/animal for 13 weeks.

Clinical signs, body weight, food consumption, local reactions and ocular reactions were monitored. Morbidity and mortality were checked twice a day. Hematological and blood biochemistry investigations were performed on all animals the day of sacrifice. Complete macroscopic post-mortem examination was performed on all animals. A microscopic examination was performed on designated tissues from all control and high-dose animals and on injection sites from the low-dose animals.

No unexpected mortality was observed at any dose in either species. In rats, only transient systemic clinical signs were observed sporadically at high doses. No other parameters seemed to be affected by the treatment with the active substance. Therefore, the NOEL was established at 5 µg/animal (20 µg/kg bw). Regarding rabbits, no relevant findings were noted in any animals at either the highest or the lower dose. The NOEL was hence established at 500 µg/animal (200 µg/kg bw).

Tolerance in the target species

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

Reproductive toxicity

No reproductive or developmental toxicity studies were conducted. According to the Guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1), studies on effects on reproduction are not required for target animal safety evaluation of non-food producing species unless the product is intended for use in animals which might be used for breeding.

In the absence of studies on reproductive toxicity an appropriate warning was included in the proposed product literature: ‘The safety of the veterinary medicinal product has not been established during pregnancy/lactation. Use only according to the benefit-risk assessment by the responsible

veterinarian'.

Genotoxicity

The genetic toxicology potential of polycarboxymethyl glucose sulfate acetate was evaluated in a standard test battery of tests in accordance with VICH GL23.

In the Ames test, polycarboxymethyl glucose sulfate acetate was negative for induction of reverse mutations in the five tester strains of *Salmonella typhimurium* in the presence and absence of metabolic activation. However, additional detail on the study was requested.

In an *in vivo* micronucleus test in rats, polycarboxymethyl glucose sulfate acetate was administered orally twice at a dose level of 1000, 500 and 250 mg/kg/day (male rats) or 500 – 250 and 125 mg/kg/day (female rats). However, in the description of the method, it is stated that rats were treated intravenously. Clarification was required on the route of administration used for this study. The test substance did not induce a statistically significant increase in the frequency of micronucleated immature erythrocytes of the bone marrow of males and females separately at any dose, when compared with the concurrent negative control.

A Mutation Assay at the TK Locus in L5178Y Mouse Lymphoma Cells using OTR4131 demonstrated the absence of mutagenic potential at concentrations up to 5000 µg/ml. The active substance was shown to be non-mutagenic in this test, both in the presence and in the absence of metabolic activation, up to the highest concentration tested.

While the above studies indicated that polycarboxymethyl glucose sulfate acetate is not genotoxic some further clarifications were requested before a final conclusion could be reached.

Carcinogenicity

No carcinogenicity data was provided. This would have been considered acceptable if the lack of genotoxic potential was confirmed, as there are no structural alerts, and there were no findings relevant to neoplasic lesions in repeat dose toxicity studies.

Studies of other effects

In a guinea pig study the administration, by intradermal route, of concentrations up to 10% of OTR4131 did not show any skin sensitization potential. The substance was also shown to be non-irritant in the preliminary study.

No specific studies on the eyes irritation potential of the substance were provided.

Excipients

No toxicity data on the final formulation were provided and the toxicity of the excipients was not described. However, the excipients are well known substances widely used in the pharmaceutical industry. While one of them is a corrosive irritant to the skin, eyes, and mucous membranes at high concentrations, in this product it is only used for pH adjustments.

User safety

The user risk assessment (URA) provided was not in accordance with the structure recommended by the Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-

Rev.1).

No toxicity data on the final formulation was provided. However, based on the single dose toxicity data generated with the active substance, and the target animal safety data, it was concluded that the potential toxicity of the active substance after a single accidental injection of the prescribed dose is low.

A guinea pig sensitization study performed with OTR4131, confirmed the absence of sensitization potential for this compound. Since none of the excipients was expected to impact on the sensitization potential of the final formulation, the sensitization study conducted only with the active substance was considered acceptable. However, no information on the irritation potential of the final formulation was provided. Therefore, irritancy of the product to the skin and eyes was requested to be addressed.

No reproductive toxicity studies were performed. According to the 'Guideline on Safety and Residue data requirements for veterinary medicinal products intended for minor uses or minor species, EMEA/CVMP/SWP/66781/2005', studies on effects on reproduction are not required for non-food producing species unless the product is intended for use in animals which might be used for breeding. However, toxicological data on the safety of the product on reproduction/fertility could have been needed to address the risk for the user of the veterinary medicinal product. In this case, in view of the conditions under which the product would have been administered, the risk of significant exposure was considered to be low and the absence of reproductive toxicity data was accepted.

Exposure scenarios were properly identified. The most probable scenario would have been accidental self-injection but still very improbable considering that the product was to be administered by an experienced veterinarian under ultrasound guidance. Other exposure scenarios such as accidental ocular or skin exposure due to splashing from the syringe were considered as well even though the possibility of their occurrence is very low.

Although the hazards of the active substance were described throughout the dossier and the main exposure scenarios were identified, an adequate quantitative and qualitative risk characterisation, including an estimation of the margin of exposure was missing.

The proposed user warnings were considered appropriate and address the identified risks but an update was requested in order to reflect the conclusions of the URA.

Environmental risk assessment

A Phase I Environmental Risk Assessment (ERA) in accordance with the Guideline on environmental impact assessment for veterinary medicinal products - Phase I (CVMP/VICH/592/98-FINAL) as well as the Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL 38 (EMEA/CVMP/ERA/418282/2005-Rev.1) was submitted.

The environmental risk assessment stopped in Phase I and no Phase II assessment was required because the veterinary medicinal product was not to be used in animals intended for slaughter for human consumption.

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

The disposal advice given under section 6.6 of the proposed SPC is "Any unused veterinary medicinal product or waste materials derived from such veterinary medicinal product should be disposed of in accordance with local requirements", which is in accordance with current guidelines.

Residues documentation

Not applicable, since the product was intended for non-food horses. However, given that the target species is used for human consumption, it was requested that a warning be included in the corresponding section of the proposed SPC.

MRLs

The use of EQUITEND was to be restricted to horses not intended for slaughter for human consumption. The product fulfils the requirements of Article 6(3) of Directive 2001/82/EC and consequently maximum residue limits did not need to be established for the pharmacologically active substances contained in the product.

Horses treated with EQUITEND would need to be declared as not intended for slaughter for human consumption in the identification document (sometimes referred to as the “horse-passport”) accompanying registered equidae.

Withdrawal period(s)

As EQUITEND was intended for use in horses that will not enter the food chain a withdrawal period was not required. The following wording was included in the proposed SPC:

Not to be used in horses intended to produce meat or milk for human consumption. Treated horses may never be slaughtered for human consumption. The horses must have been declared as not intended for human consumption under Commission Implementing Regulation (EU) 2015/262.

Overall conclusions on the safety documentation

A comprehensive data package with recently performed studies and data from current literature on the active substance and other RGTAs was presented.

The pharmacological properties of OTR4131 were described based on available literature on the structurally similar OTR4120 and other RGTAs and laboratory studies performed by the applicant with the compound in question. Although the information provided was limited, it was considered acceptable and in line with the Guideline on safety and residue data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/SWP/66781/2005).

The applicant submitted a single dose toxicity study performed in rats, which showed that an intravenous dose of 5 mg of OTR4131 elicits effects on treated animals. However, taking account of the target animal safety data it was concluded that the potential toxicity of the active substance after a single accidental injection of the prescribed dose was low.

Two repeated dose toxicity studies performed with the intravenous administration of the active substance (OTR4131) in accordance with GLP and current guidelines were submitted. The NOEL was established at 5 µg/animal in rats and 500 µg/animal in rabbits.

The product was well tolerated by the target species at the dose tested (150X dose) and only minimal side effects such as mild swelling of the tendon following injection were observed.

No reproductive or developmental toxicity studies were conducted. According to the Guideline on safety and residue data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/SWP/66781/2005) studies on effects on reproduction are not required for the target animal safety evaluation of non-food producing species unless the product is intended for

use in animals which might be used for breeding.

In the absence of such studies an appropriate warning was included in the proposed product literature: 'The safety of the veterinary medicinal product has not been established during pregnancy/lactation. Use only accordingly to the benefit-risk assessment by the responsible veterinarian'.

A battery of *in vivo* and *in vitro* mutagenicity studies was performed. The results in all of the studies provided suggest that the active substance does not have mutagenic potential at the tested concentrations, however some clarification was requested before a final conclusion on the genotoxicity could be reached.

Concerning user safety, the user risk assessment provided by the applicant is not in accordance with the structure recommended by the Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1).

No toxicity data on formulation were provided. A guinea pig sensitization study performed with OTR4131, confirmed the absence of sensitization potential for this compound. Since none of the excipients was expected to impact on the sensitization potential of the final formulation, the sensitization study conducted only with the active substance was considered acceptable. However, no information on the irritation potential of the product was submitted. Therefore, irritancy of the product to the skin and eyes was requested to be addressed.

Although the hazards of the active substance were described throughout the dossier and the main exposure scenarios were identified, a quantitative and qualitative risk characterization, including an estimation of the margin of exposure was missing.

The proposed user warnings were considered appropriate but an update was requested in order to reflect the conclusions of the URA.

Regarding the toxicity for the environment, a risk for the environment was not expected when used according to the proposed SPC.

The use of EQUITEND was to be restricted to horses not intended for slaughter for human consumption. The product fulfils the requirements of Article 6(3) of Directive 2001/82/EC and consequently maximum residue limits did not need to be established for the pharmacologically active substances contained in the product. However, horses treated with EQUITEND would have needed to be declared as not intended for slaughter for human consumption.

Part 4 – Efficacy

Pharmacodynamics

Regenerating agents (RGTAs) are chemically synthesized dextran derivative polymers, specifically designed to replace degraded heparan sulfates in damaged tissue. To date, two RGTA-based dextrans, OTR4120 (alpha1-6 polycarboxylmethylsulfate glucose) and OTR4131 (alpha1-6 polycarboxylmethylsulfateacetate glucose), have been developed for clinical use. OTR4120 is already used in two commercially available medical devices for the treatment of skin and corneal lesions in humans. OTR4131 differs from OTR4120 only by the addition of acetate groups, leading to changes in hydrophobicity, and is hence more adapted to the treatment of tissue injuries with a hydrophobic nature such as tendon. Since the position and amount of other carboxyl and sulfate groups remains unchanged between OTR4120 and OTR4131, characteristics underlying the mode of action associated

with tissue repair, including growth factor and extra cellular matrix (ECM) protein binding and protection and resistance to enzymatic digestion are considered to be shared between these molecules.

The applicant provided different types of data including published papers, a scientific PhD-thesis, preliminary experiments and field studies and clinical studies to describe the pharmacodynamic effects of the regenerating agent OTR4131. Data from clinical studies were assessed and the results are discussed in Dose confirmation studies and Clinical field trials sections.

Considering that the horse is a minor species, that EQUITEND was classified as a MUMS product by the CVMP and that information regarding the mechanism of action of OTR4131 is limited, the CVMP accepted that available data about the mode of action of OTR4120 could be considered to support the mode of action of OTR4131 (the addition of acetate groups render OTR4131 more hydrophobic and therefore adapted to the treatment of some injured tissues with a hydrophobic nature, such as tendinous tissue) in general terms. However, some questions remained unanswered at the time of withdrawal of the application related to the specific characteristics underlying the mode of action of these molecules:

- Ability to specifically localize to a site of tissue injury: this was assessed in a rat model in which 99mTc-labelled OTR4131 was administered via intravenous injection. Only a preliminary analysis of this study was submitted, so it was not possible to assess it in detail. To support these results a published paper (Meddahi *et al*. 2002) of RGTA11 in a rat EDL muscle crush model was presented. It explains that, to date, only two RGTA (OTR4120 and OTR4131) have been developed for clinical use; thus, it is not clear if RGTA11 is one of these two or if it is another type of RGTA. The applicant was requested to clarify the molecule which corresponds to RGTA11. In addition, RGTA134 is described in a monocentre uncontrolled field study, but whether it corresponds to OTR4131 or OTR4120 was not clear either.
- Ability to bind ECM structural proteins and growth factors: the data provided was limited and part of it could not be assessed. However, from the results obtained by Thong *et al*. it could be assumed that the molecule OTR4120 has this binding property. In the additional findings obtained in the PhD-thesis, no difference in binding to Vascular Endothelial Growth Factor (VEGF) was observed between both RGTAs, so it could be assumed that OTR4131 also has the ability to bind that growth factor. On the other hand, the hydrophobicity of OTR4131 seemed to stimulate higher binding to other growth factors compared to OTR4120. These results were supported by those obtained in the study performed by Chevalier *et al*. where higher binding to the growth factors assessed was obtained compared to placebo groups. Therefore, it could be accepted that the molecule OTR4131 has this ability as part of its mode of action.
- Ability to protect matrix proteins from degradation: a thesis was submitted although published in the form of nine scientific papers. Only 4 out of the nine publications (Chevalier *et al*. 2015, D. Papy-Garcia *et al*. 2005, Tong *et al*. 2012 and J. Van-neck *et al*. 2012) were submitted and only one of them referred to OTR4131 (Chevalier *et al*. 2015). The data provided to describe the ability of OTR4131 to protect matrix proteins from degradation was limited and part of it could not be assessed. In the thesis, some information was provided which seemed to reveal the protective effect of OTR4120. However, the dose that protects most of the collagenases (1 µg/ml) is inferior to the one intended for use in EQUITEND (10 µg/ml) and required concentration increases 100 times when the protection is necessary against glycanases. On the other hand, it could be assumed from Tong *et al*. 2012 that OTR4120 improves healing quality by reducing collagen type III (amongst other factors) and that the increase of TGF-β1 and VEGF observed strengthens the concept of the protective role of OTR4120 on growth factors. Although it is

generally accepted that the mechanism of action is considered to be similar between both molecules, taking into account that they are not identical, that this study was performed with different animals and species and that there was no comparative data between both RGTAs regarding this property, it was not clear if EQUITEND would provide the same grade of protection. Therefore, the applicant was asked to further comment on this issue and on the possible extrapolation of this ability of OTR4120 to EQUITEND.

- Finally, the resistance to degradation by enzymes was supported by one published reference (Ikeda *et al*. 2011) where a mice model of skin necrosis induced was used to evaluate the wound healing properties of OTR4120. In this study, OTR4120 was resistant to degradation by all the assayed glycanases including chondroitinase ABC, dextranase, hyaluronidase, and heparitinases I, II, and II, the most common endoglycosidases known to digest natural GAGs. From these data the authors assumed that heparan mimetics' resistance to enzymatic degradation should allow these products to replace damaged natural GAGs in an injured tissue and thus protect natural signals needed for tissue regeneration. This ability for the molecule OTR4120 was accepted but further clarification was requested on whether the similarity between both RGTAs is adequate to justify the extrapolation and to award this property to the molecule of EQUITEND.

The therapeutic effect of EQUITEND was justified on the basis of several papers, a preliminary uncontrolled field study (Coudry *et al*. 2014) and a mouse necrotic ulcer wound model study (proprietary study evaluating the wound healing properties of an OTR4120-injectable formulation). This new injectable formulation was expected to reduce the time of closure or at least have the same results in terms of efficacy/time of closure, as OTR4120 as topical application. This was assessed as the improvement in wound healing in a mouse necrotic induced ulcer model and on four studies (EQUITEND dose ranging study, efficacy study of EQUITEND intra-lesional injection in superficial digital flexor tendon (SDFT) on horses, EQUITEND multicentre randomized controlled trial and EQUITEND monocentric uncontrolled field study) submitted in the clinical part of the dossier.

The dose ranging study was performed to determine the optimal dose of EQUITEND (1 ml of a 1, 10, 100 or 1000 µg/mL solution versus placebo) administered by intralesional injection in the SDFT on the healing of surgically injured tendons in horses. The study concluded that a dose of 10 µg/ml improved the majority of criteria associated with tendon healing including cross section area, tranversal lesion architecture and extent and MRI signal length.

The efficacy study of EQUITEND intra-lesional injection in SDFT on horses was conducted to evaluate the efficacy of a specific dose of EQUITEND (1 ml of a 10 µg/ml solution) administered by intralesional injection in the SDFT on the healing of surgically injured tendons in horses. An improvement of several criteria indicative of tendon healing (tendon strength, transversal lesion and longitudinal architecture, increase in endotendon neovascularization and decrease in endotendon thickness) was concluded.

The EQUITEND multicentre randomized controlled trial was conducted to evaluate the efficacy of a specific dose of EQUITEND (1 ml of a 10 µg/ml solution) administered by intralesional injection in the SDFT, on the healing of spontaneous SDFT injuries. The study concluded a positive primary outcome with a significant decrease in cross section area after one month treatment ($p=0.04$).

In the EQUITEND monocentric uncontrolled field study, where 137 horses were treated with EQUITEND (10 µg/ml or 30 µg/ml), improvement in tendon healing assessed by ultrasonography was observed in 89.9% of followed tendon injuries. The study concluded that OTR4131 is easy to use and well tolerated in horses, may decrease the duration between injury and return to race, and is associated with improvement of the tendon lesion, high rates of return to racing and a better level of earnings per start after injury.

The results obtained by Coudry *et al*. 2014 (performed with EQUITEND) were partially satisfactory given that the parameters related to the time taken to return to competition and earnings are considered as secondary efficacy parameters and are very variable dependent on the breed and type of the sport practised. On the other hand, from the Chevalier *et al*. 2015 study (performed with OTR4131) and the proprietary study (performed with OTR4120) it could be assumed that both molecules have a positive influence on accelerating wound healing. However, based on the results of these clinical studies (see comments in sections Dose confirmation studies and Clinical field trials) the therapeutic effect of OTR4131 could not be confirmed.

Minimal, if any present, secondary pharmacodynamic effects were expected based on justifications from two publications focused on OTR4120 (Ikeda *et al.*, 2011 and Frescaline *et al.*, 2013). Ikeda *et al.* produced different methods to depolymerize Heparan Mimetics (HMs) in order to produce a library of related oligosaccharides and study their biological activities. Depolymerization was achieved by chemical methods. These oligosaccharides showed to be active *in vitro* and *in vivo* and to present specificity concerning biological functions as activation of FGF-2 mitogenicity and antithrombotic effect. The *in vivo* wound healing assay showed a higher activity for the polymeric HM which might be associated to a higher capacity of the polymeric product to form scaffolds in the extracellular matrix during tissue regeneration. Frescaline *et al.* developed a strategy associating OTR4120 with bone substitutes to optimise stem cell-based therapeutic products and showed that OTR4120 was able to potentiate proliferation, migration, and osteogenic differentiation of human mesenchymal stem cells (hMSC) *in vitro*. These findings support the ability of GAG mimetics to organize the local ECM to coordinate the host response toward the implanted biomaterial, and to inhibit the abnormal bone formation process on a subcutaneous ectopic site. However, given that it is unclear that the similarity between both RGTAs justifies the resistance to enzymatic degradation of OTR4131, a final conclusion could not be drawn regarding this issue.

The dose selected was based on the dose response curves from the mouse necrotic ulcer wound model study, the results obtained by Chevalier *et al*. 2015 and the EQUITEND dose ranging study.

The proposed SPC stated that concomitant administration of aminoglycosides could inhibit the efficacy of the product. No data was provided in support of the interaction described with aminoglycosides and was requested.

Overall, the data provided to describe the pharmacodynamics of OTR4131 was limited. The information could be partly derived (there were pending clarifications) from the experimental evidence obtained for OTR4120 but did not explicitly explain the pharmacodynamic properties of OTR4131 when administered as intralesional injection to horses with flexor tendinopathy. No information was provided to explain why the molecule OTR4131 was chosen instead of OTR4120 from which there seems to be more information available and better characterised (note that the majority of the literature references are centered in the molecule OTR4120). Additionally, no explanation was provided about the selected treatment regimen (single injection).

In conclusion, general pharmacodynamic properties of OTR4131 were not specifically characterised but could have been extrapolated from the evidence obtained for OTR4120 if clarifications on the issues explained above had been provided.

Pharmacokinetics

The applicant did not consider it necessary to perform pharmacokinetic studies on the grounds that OTR4131 is administered to the horse at 10 µg, and assuming that if the entire amount were to enter the blood stream, the maximum concentration estimated at the peak would be 0.3 µg/l (i.e. very low).

Taking into account that the product was intended to be locally injected under ultrasound guidance in the injured flexor tendon and that this area is poorly vascularized, it is plausible to assume that the molecule will be trapped in the injection zone (being able to perform its function on tendon healing).Thus, systemic absorption is expected to be very limited and in such case it is very likely that the amount entering the blood stream is lower than the one theoretically estimated (0.3 µg/l). Moreover, the intended recommended dose to use in horses (10 µg/ml) is significantly lower than the NOEL calculated in the toxicological studies performed in rats and rabbits (See Part 3 - Safety).

However, it was stated that OTR4131 is resistant to degradation by enzymes produced following tissue damage and no information was provided regarding how long OTR4131 would be present in the target tissue, how long it would exert its therapeutic effect at the site of the lesion (and if it is long enough to improve wound healing), how the molecule is eliminated from the injected area and whether more than one injection would be necessary. As part of tendon injuries there can be increased vascularization, metabolic rate and thus lower retention time of molecules at the site of a tendon injury. Thus, the applicant was asked to address local pharmacokinetics based on e.g. available literature and furthermore justify why studies on local kinetics were not performed when it seems that proper detection methods for the active substance are available.

Although no pharmacokinetic studies (ADME studies) were performed in horses, two biodistribution studies in rats with 99mTc labelled OTR4131, OTR4120 and dextran control were presented. The results of the first study (Report on radiolabelling, blood clearance assessment and biodistribution of the polysaccharides OTR4131, OTR4120 and Dextran T40) showed that OTR4120 presents the faster renal elimination (due to a greater hydrophilicity and the lowest concentration in blood at the earliest time point) and the lowest uptake, and that OTR4131 biodistribution is intermediate between dextran and OTR4120 with a lower uptake than dextran and a renal elimination slower than OTR4120. Irrespectively, it could be assumed that OTR4131 is rapidly cleared from the circulation. Also, the biodistribution analysis revealed a greater accumulation of OTR4131 in the liver and spleen compared to bladder, kidneys and total blood. Some issues remained to be clarified at the time of withdrawal of the application.

The results of the second study (Report on tissue biodistribution of the polysaccharides OTR4131, OTR4120 and Dextran T40) indicated that, overall, the amount of compound cleared via urine 24 hours after intravenous injection showed no significant difference between OTR4120 and OTR4131. Also, the profile of tissue uptake and retention was broadly similar between the 2 RGTAs.

In the pharmacodynamics part a preliminary analysis study where 99mTc labelled OTR4131 was presented. Thus, taking into account that three studies with 99mTc labelled OTR4131 were performed the CVMP questioned whether this approach (Technetium-99m labelling) or also the one employed in the Meddahi *et al*. 2002 study (H^3-labelling) (also mentioned above in the pharmacodynamics part) could not have been applied to a horse study in order to obtain more accurately the medicinal product pharmacokinetic characteristics in the target species.

Given that none of this is mentioned in the actual dossier submitted, the applicant was asked to explain if it was possible to investigate the pharmacokinetic properties of OTR4131 in the horse by labelling the molecule with Technetium-99m or H^3 and, if so, clarify why it was not performed for the present application.

Despite the question raised above, considering the characteristics of the active substance, the assumed low risk of systemic absorption and the expected concentration in the horses' blood and tissues the approach to not perform pharmacokinetics studies was accepted. Considering that the horse is a minor species and EQUITEND has been classified as a MUMS product by the CVMP, the data

requirements for MUMS products were taken into account and the absence of pharmacokinetic studies was considered justified. The information included in section 5.2 of the proposed SPC is considered adequate. Nonetheless, some issues remained to be clarified at the time of withdrawal of the application.

Target animal tolerance

No proprietary target animal safety (TAS) study has been performed which was accepted by CVMP taking into account the information presented. The safety data presented consisted of one previously performed safety study in horses (EQUITEND tolerance study) and safety data generated from clinical studies presented in the dossier.

In the EQUITEND tolerance study (non-GCP) the safety of an intra-tendinous injection of EQUITEND in the superficial digital flexor tendon (SDFT) of the forelimb in horses was studied. The OTR4131 intra-tendinous injection was compared to an intra-tendinous injection of placebo at the same volume in the homologous contralateral tendon.

It was not clear if the investigational formulation corresponds to the final formulation intended to be marketed given that the volume and the concentration of the treatment injections differed (1.5 ml and 1 mg/ml) from those present in EQUITEND (1 ml and 10 µg/ml). This and other issues remained to be clarified at the time of withdrawal of the application. To assess the margin of safety the VICH Guideline on target animal safety for veterinary pharmaceutical products (VICH GL43) recommends to include a negative control (0X), the highest proposed dose level (1X) and two multiples of this dose (3X and 5X) for a period of time in excess of the recommended maximum duration of use. Regarding the overdose levels, this study did not comply with the VICH GL43 requirements but the CVMP considered the VICH approach to be impractical when dealing with intralesional injections in tendons and, thus, systemic tolerance testing was not considered necessary for this product. However, a repeat dose study would have been beneficial, since it cannot be ruled out that a horse might receive another intralesional injection even though a single administration was recommended in the proposed SPC. Therefore, a local safety study with repeated administration of the maximum recommended treatment dose was requested. Additionally, the report did not mention whether concurrent treatment with ketoprofen (2 mg/kg) that the investigators were authorised to administer in case of severe inflammation post injection was finally given to any of the horses of the study. This could indicate a clear sign of intolerance regarding the product injected. This issue remained to be clarified at the time of withdrawal of the application.

A very thorough medical and orthopaedic examination was performed. Systemic tolerance analysis was not performed, but the exclusion was accepted due to the assumed small level of active substance that enters the systemic circulation. However, there were two minor discrepancies that needed clarification. One was regarding the time points when musculoeskeletical examination was performed and the other one was in relation to the dynamic orthopaedic examination. Clarification was requested on whether assessment of possible lameness was only performed at walk or also at trot, which is more correct. Additionally, certain evaluations included non-integer scoring, which does not correspond to the American Association of Equine Practitioners (AAEP) lameness grading system. Thus, horses with non-integer scoring had to be excluded from the analysis and the applicant was asked to recalculate the data.

Taking into account that the active substance is a new molecule, the small number of animals used and the general lack of other safety information in the target species, histological examination of the tendons would have been necessary according to VICH GL43. However, given that the same 6 horses were also enrolled 3 weeks later in the dose ranging study where the animals were euthanized to

examine the tendons (histology and biochemistry), the omission of histological study of the tendons in this safety study was considered acceptable.

Two out of the five protocol deviations registered were considered relevant for the integrity of the study.

In summary, mild reactions were detected in this study during three days following injection in both EQUITEND and placebo treated groups suggesting that these were not test item specific and that EQUITEND is well tolerated in healthy horses when administered at 150-fold the therapeutic dose. However, due to the study flaws and protocol deviations the results were difficult to interpret and could only be considered supportive data.

Additionally, the applicant was requested to discuss, based on available literature, the potential for adverse effects related to the intratendinous injection technic (e.g. biomechanical detrimental effects on the tendon, pressure-induced tissue necrosis, focal tendon hypoxia and the risk of focal infections) and the potential influence of these side effects on treatment outcome.

Considering that the horse is a minor species and that EQUITEND was classified as MUMS product by the CVMP, the data requirements for MUMS products were taken into account. According to the Guideline on the efficacy and target animal safety data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/EWP/117899/2004), a basic controlled study demonstrating the safety of the (near) final formulation in the target species at the recommended therapeutic dosage and duration of therapy is needed in addition to field safety data. However, it also states that if systemic exposure is assumed to be negligible, and there are no safety concerns, no specific tolerance study is needed, and tolerance can be demonstrated based on the field study or from published literature data.

In conclusion, adequate information on the target animal safety of the product could therefore be generated as part of the clinical efficacy studies. Overall, from the target animal tolerance studies presented in the dossier, it can be deduced that the product would be well tolerated and minimal side effects would occur. However, it was noted that the clinical studies submitted did not provide sufficient evidence for the efficacy of EQUITEND at the recommended treatment dose and that in the majority of these studies no individual data were provided to enable the CVMP to properly evaluate the safety of the treatment.

Dose determination / finding studies

A non-GCP placebo controlled dose ranging study assessing different concentrations 1, 10, 100 or 1000 µg/ml for the determination of the optimal dose of EQUITEND when administered by intralesional injection was submitted (EQUITEND dose ranging study).

The number of animals recruited was low (10) as was the number of horses included in each group (2 horses for each dose level including placebo). Moreover, 6 out of the 8 horses from the treated group were also those included in the tolerance study described earlier and several of these horses presented a discrete lesion at the site of injection from the previous study. It was argued that this is not expected to affect the study conclusions as no clinically meaningful differences in the average grades for echogenicity, transversal length or transversal architecture were observed between or within either the group of previously used (n=6) or non-used (n=4) horses at D0. The CVMP did not agree with this argumentation given that the low number of animals enrolled and per treatment group make compilation of statistics on numbers difficult and do not allow a robust statistical analysis including baseline data to be performed.

Additionally, concurrent treatment with ketoprofen was authorised in case of severe inflammation post injection but it was unknown if this was used in any of the horses. Further information and clarification was requested on this issue.

A complete medical and orthopaedic examination was performed. However, the timepoints when medical examination was performed need to be clarified as well as whether the dynamic examination (degree of lameness) was performed only at walk or also at trot.

The study report did not specify if any adverse events, amendments and/or protocol deviations occurred. A clarification was requested.

Another limitation identified in the study was that the individual variations related to the intrinsic capacity of each horse to heal more or less quickly were not taken into account and also that the spontaneous healing of each horse could interfere with the effect of the test product.

With respect to the more subjective parameters monitored, deformation profile of the palmar region and evaluation of the sensitivity-reactivity, it seemed that there was no difference between placebo and a 10 µg/ml treatment dose and between placebo and a 100 µg/ml treatment dose, respectively. Regarding the rest of the parameters evaluated, the placebo dose generated the best improvement on echogenicity and longitudinal architecture whereas the dose 10 µg/ml generated the best improvement on cross sectional area of the tendon, transversal lesion architecture, transversal lesion extent, MRI signal length. Also the doses of 1 µg/ml and 100 µg/ml provided results close to those obtained with 10 µg/ml, but the tendon healing was less satisfactory with the dose of 1000 µg/ml. Further discussion of these results was requested.

Further analysis of both the histology and biochemistry remained to be provided at the time of withdrawal of the application along with corresponding statistical analyses.

In conclusion, due to the major flaws of the study it was not possible to reliably determine an adequate dose, since statistical significances could not be obtained.

Dose confirmation studies

A second placebo controlled study (Efficacy study of EQUITEND intra-lesional injection in SDFT on horses) was conducted to evaluate the efficacy of a single dose of EQUITEND (1 ml of a 10 µg/ml solution) administered by intralesional injection in the SDFT of surgically injured tendons in horses. This study was not performed under GCP conditions, which was not considered appropriate.

The number of animals recruited was low (12 + 1 control). However, as there were animals from both sexes (6 females and 6 males) and they were representative of the class (sport horses) and age (2 to 4 years) in which the veterinary medicinal product would have been used, this was accepted. It remained unclear why the horses of this study received treatment 21 days after surgical induction of lesions as compared to 8 days in the dose ranging study. The applicant was asked to discuss this approach.

The surgical dilacerations of fibres model applied was considered adequate. The data obtained at baseline revealed no statistical significant difference for the majority of ultra-sonographic parameters between treatment groups, with the exception that EQUITEND treated horses exhibited a significant increase in transversal lesion extent at Day 0. Moreover, at Day 30, the treatment group also experienced a statistically significant higher grade of this parameter. Although the reproducibility of the surgical induction model employed in this study and the homogeneity of the lesions induced could be accepted, the applicant was asked to comment on the clinical significance of the differences of this transversal lesion extent at Days 0 and 30.

Scoring of certain clinical or histological parameters was not defined clearly (e.g., palmar deformation, semi-quantitative ultrasound parameters). The same applied to histological parameters (e.g., neovascularization, morphology of nucleus). The majority of tables were lacking proper explanations and in certain tables, the numbers did not add up. The applicant was asked to provide additional information regarding these issues.

In the statistical analysis, age was introduced as a fixed effect covariate since EQUITEND treated animals were on average older than their saline treated counterparts and since previous epidemiological studies have shown that increasing age is associated with an increased risk of tendon injury (Kasashima *et al*. 2004), which may reflect age-related degenerative changes to the matrix. A negative correlation between horse age and tendon maximal force, true stress and elastic modulus tests were observed in the horses participating in this study. However, the presentation of the results indicated that statistical analyses were based on forelimbs of horses, i.e. group sizes of twelve. However, the individual horse was randomized to a treatment group and not the forelimb. Thus, the horse should have been the statistical unit. The applicant was asked to reanalyze the data.

With respect to the clinical examination, both the physical deformation profile of the palmar region and lameness did not show differences among the studied groups. Thus, it can be deduced that this criterion (assessment of lameness) is not sufficiently sensitive for assessing a difference in healing process regarding tendinopathy (not a reliable measure of tendon healing).

Although echogenicity results on average showed faster healing in horses treated with EQUITEND, this difference was not statistically significant. Moreover, using the criteria echogenicity and longitudinal architecture, a healing index was calculated and a statistical analysis performed. No statistically significant difference in the healing index was observed between both groups. However, a treatment tendency with a p-value of 0.08 was obtained after one month. This, in addition with the significant increase in transversal lesion extent of the treated group at Day30 commented above, suggests a global positive evolution for the treatment group regarding ultrasonography criteria.

The efficacy of the test product was also assessed at a functional level (*in vivo* and post-mortem biomechanical tests). The *in vivo* biomechanical test was based on the measure of axial speed of sound (SOS) at days 0, 30, 60 and 90. The measurements were performed using an ultrasonic probe applied over the average SDFT metacarpal region. This probe comprised a transmitter and 2 receivers. The SOS measures show the propagation velocity between the two receivers of the first wave propagated along the major axis of the fibers of the tendon. The *ex vivo* biomechanical study (tensile tests) was performed 1-7 hours after death at a constant speed of 60 mm/min in the testing machine. True strain of each tendon's region of interest (approximately 7 cm length), tendons' cross-sectional area and true stress were analyzed. These tests reflected the tendon's capacity to recover to the adequate level required for sports performance. The analysis performed in this study revealed a significant increase in the SDFT maximal force compared to placebo and a trend towards increased stress at maximal force in EQUITEND treated animals compared to placebo, when age was considered. Therefore, it was suggested that tendon strength was increased following treatment with EQUITEND.

Finally, histological analyses of isolated tendon tissue revealed a significant decrease in endotendon thickness and an increased endotendon neovascularization in EQUITEND treated tendons after 3 months. Biochemistry analysis revealed no significant effect of EQUITEND treatment.

In conclusion, due to the study flaws and shortcomings such as lack of predefined primary endpoint and deficiencies in the statistical analysis, this study could only be considered explorative.

Clinical field trials

To evaluate the efficacy of EQUITEND a pivotal efficacy trial (EQUITEND multicentre randomized controlled trial) was provided. This study was not performed under GCP conditions which raised a major concern. It was placebo controlled and was conducted to evaluate the efficacy of EQUITEND (1 ml of a 10 µg/ml solution) when administered via an intralesional tendon injection in horses suffering from SDFT tendinopathy.

In the study design, 75 horses were supposed to take part in the trial. Initially, 31 horses were included, but eight horses were excluded afterwards. One horse died during ultrasound examination, but no explanation was given. The total number of animals recruited was initially acceptable, i.e. 30 horses. However, due to the low numbers of Thoroughbred and Eventers included in this study, and since the rehabilitation program and specific prerequisites were different for each breed, the statistical analysis and results presented were focused only on French Standardbred Trotters (22), aged between 4 and 7 years, presenting acute or subacute SDFT tendinopathy on one forelimb. Thus, the real number of animals to assess in this study was 22 horses (11 geldings, 8 males and 3 females) 14 of them being assigned to the EQUITEND treatment group and 8 to the placebo group (the ratio 1/3 for placebo horses and 2/3 for EQUITEND treated horses mentioned in the study protocol was almost respected). The study protocol should have been decided *a priori* and, if it differed between the different types of horses such that the outcomes had to be analysed separately, then the treatment claim may have needed to be restricted to the specific study population investigated and for which effectiveness was demonstrated. One of the inclusion criteria was “horses with recent tendinopathy of one forelimb in which the SDFT is involved” but the term “recent” was not defined (in days/weeks/months). One of the non-inclusion criteria was horses with SDFT tendinopathy older than 4 weeks, so it was assumed that “recent” refers to tendinopathy not present for more than 4 weeks. It was mentioned that these 22 horses presented acute or subacute SDFT tendinopathy. The study report indicated that there were 3 horses (number 9, 14 and 17) with unknown duration of SDFT tendinopathy, 1 horse (number 7) with duration of 6 months and the rest of the horses with duration ranged between 0 and 23 days. According to the non-inclusion criteria, horse number 7 should have been excluded from the efficacy analysis. In view of these data, the inclusion criteria applied to the selection of the study animals appeared to be unachieved and the non-inclusion criteria not fully respected. Therefore, the applicant was asked to comment on this issue and to clarify the duration of the SDFT tendinopathy in horses 9, 14 and 17.

Additionally, the applicant reported that horses included in the study presented acute or subacute SDFT tendinopathy, which refered to duration of 3-14 or 2-4 weeks of onset, respectively (e.g., *Wissdorf et al*. 2002). Except for 3 horses with unknown duration of SDFT tendinopathy and 1 horse with duration of 6 months, which did not meet the inclusion criteria, no chronic cases were examined.

Regarding the test item, 4 different batch numbers were cited and 3 certificates of analysis of the batches used in the clinical trial were provided. Moreover, neither the study report nor the certificates submitted gave any information with respect to the assignment of each batch to the individual horses in the study. These data were requested.

Certain endpoints, e.g. lameness, were reported as non-integer scores even though this does not correspond to the AAEP lameness grading system or had not been defined previously.

The primary efficacy endpoint of the study was defined as the improvement of tendon healing, evaluated by ultrasound examination one month (4 weeks) after EQUITEND administration. The ultrasound examination performed was composed of:

- One quantitative variable: cross section area (CSA) of the tendon, and

- Five semi-quantitative variables: echogenicity, tissue architecture (longitudinal and transversal section) and extent of possible ultrasonographic lesion induced by the injection (transversal and longitudinal extend).

Ultrasound examination was not defined with a numeric value to clearly distinguish what can be considered improvement and what not (the sum of the scores of all the variables included within the parameter ultrasound examination). Although it was not mentioned, a healing index was determined for all the horses included initially in the study with the sum of the semi-quantitative variables scores (difference between day 0 and month 4). The statistical analysis concluded that no significant difference was observed between both groups. Given that one variable (cross section area) was not included in this healing index and that a value to establish what is considered ultrasound improvement was not defined, the primary efficacy endpoint was not well defined and, thus, this study could not be qualified as a pivotal field trial. This study was considered an explorative study and, therefore, it only provided supportive data.

Additionally, in accordance with the CVMP guideline on statistical principles (EMA/CVMP/EWP/81976/2010) the primary outcome should provide a reliable measure of an important clinical benefit. Ultrasound measurements are regarded as surrogate variables and therefore the applicant should have justified that they are a true predictor of clinical outcome (ICH E9). Three secondary efficacy endpoints were defined. The study monitoring schedule of two of them (improvement of performance criteria and the recurrence of tendinopathy), needed clarification.

In summary, the statistical analysis of the primary and secondary efficacy endpoints could not be assessed reliably since there were important flaws which made it difficult to reach any firm conclusions regarding the efficacy of the product. The significant decrease in tendon CSA after one month of treatment obtained together with the outcomes of secondary endpoints did not demonstrate the efficacy of EQUITEND. Additionally, excluding horses in knowledge of the data might have been introduced a bias and was, therefore, not acceptable. Details on the statistical analysis (e.g. inclusion of covariates) were only given in the study report but not in the study plan.

Finally, with regard to the clinical safety of EQUITEND it was stated that intralesional injections were well tolerated by all horses and no adverse effects were reported post-treatment but no individual data were provided to enable the evaluation of the safety of the treatment. Additionally, no detailed information on the approach taken by the applicant to sufficiently secure that potential adverse event were detected during the study was provided.

The efficacy and safety of EQUITEND as an aid in healing flexor tendinopathy in horses could not be derived from this pivotal study.

In addition to this pivotal efficacy study, a scientific thesis (EQUITEND monocentric uncontrolled field study) that was conducted to evaluate the ultrasonographic evolution and outcome of tendon injuries treated by intralesional injection of ReGeneraTing Agents (RGTA) at 10 and 30 µg/ml which was not performed under GCP conditions was submitted. This study was performed before the dose ranging study and the dose confirmation study.

The following main observationswere found:

- Another RGTA, i.e. RGTA 4134, was used in the study but whether it corresponds to OTR4131 or OTR4120 is not clear.
- One of the inclusion criteria was “horses presented with acute, subacute and chronic injuries of the SDFT and SL injuries”. However, as in the pilot study, the terms “acute” and “subacute” were not defined (in days/weeks/months).

- The final number of animals that could be considered for this study was 67 horses (SDFT lesions).
- The inclusion of horses with bilateral and concomitant injuries and the presence of small groups limited the statistical analysis.
- A control group was not included and non-inclusion criteria, exclusion criteria and primary and secondary efficacy endpoints were not defined.
- The batch, expiry date and analysis certificate of the test item have not been provided.
- In 8 SDFT cases a second intralesional injection was performed. Additionally, one out of these 8 cases was treated again at month 2.

In summary, the interpretation of the results from this study was limited by the study design and the statistically significant effect of EQUITEND was difficult to assess. The results obtained in this thesis could only be taken into account as supportive data prior to commencing an experimental trial. It was considered as a preliminary clinical study.

Finally, with regard to the clinical safety of EQUITEND no individual data obtained from physical examination of the horses were provided to enable the CVMP to evaluate safety of the treatment

In conclusion, due to the flaws in the study design and the results obtained, the efficacy of EQUITEND as an aid in healing flexor tendinopathy in horses could not be concluded from these studies. Therefore, additional data was requested in order to sufficiently demonstrate the efficacy of EQUITEND for the claimed indication.

Overall conclusion on efficacy

Pharmacodynamics:

Although the general pharmacodynamic properties of OTR4131 were not specifically characterised, considering the horse is a minor species and considering the MUMS status of the product, the mode of action of OTR4131 could be extrapolated from the evidence obtained from OTR4120 as long as satisfactory clarifications on the issues raised could be provided.

Pharmacokinetics:

According to the characteristics of the active substance, the assumed low risk of systemic absorption and the expected concentration in the horses' blood and tissues the approach of not performing pharmacokinetics studies was accepted. Considering that the horse is a minor species and EQUITEND was classified as a MUMS product by the CVMP, the data requirements for MUMS products were taken into account and the absence of pharmacokinetic studies was considered justified. The information included in Section 5.2 of the proposed SPC was considered adequate. However, the applicant was asked to address local pharmacokinetics, based e.g. on available literature and furthermore justify why studies on local kinetics were not performed when it seems that proper detection methods for the active substance are available.

Tolerance:

The safety of EQUITEND was studied in a non-GCP tolerance study in which mild reactions were detected during three days following injection in both EQUITEND and placebo treated groups suggesting that the reactions were not test item specific and that EQUITEND was well tolerated in healthy horses when administered at 150-fold the therapeutic dose. However, due to the study flaws and protocol deviations the results were difficult to interpret and could only be considered as supportive data.

According to the Guideline on the efficacy and target animal safety data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/EWP/117899/2004), given that systemic exposure was accepted to be negligible, the safety of the product could have been generated as part of the clinical efficacy studies. Overall, based on the data from the target animal tolerance studies presented, it can be assumed that the product would be well tolerated and that minimal side effects would occur. However, the clinical studies submitted did not provide sufficient evidence of efficacy for EQUITEND at the recommended treatment dose and in the majority of studies no individual data were provided to enable a proper evaluation of the safety of the treatment. Additionally, a justification that local tolerance after administration of treatment is acceptable was requested.

Dose determination:

To determine the optimal dose of EQUITEND when administered by intralesional injection a non-GCP placebo controlled dose ranging study assessing different concentrations (1, 10, 100 or 1000 µg/ml) was submitted. Due to major flaws in the study it was not possible to reliably determine an adequate dose, since statistical significance could not be obtained.

Dose confirmation:

A placebo controlled study was conducted to evaluate the efficacy of EQUITEND (1 ml of a 10 µg/ml solution) administered by intralesional injection in the SDFT on the healing of surgically injured tendons in horses. Due to flaws and shortcomings in the study such as lack of predefined primary endpoint and deficiencies in the statistical analysis, this study could only be considered as explorative.

Efficacy:

To evaluate the efficacy of EQUITEND a pivotal efficacy trial was provided. The efficacy and safety of EQUITEND as an aid in healing flexor tendinopathy in horses could not be derived from this pivotal study.

Horses included in the study presented acute or subacute SDFT tendinopathy, which refers to a duration of 3-14 or 2-4 weeks of onset, respectively (e.g., Wissdorf *et al*. 2002). Except for 3 horses with unknown duration of SDFT tendinopathy and 1 horse with a duration of 6 months, which did not meet the inclusion criteria, no chronic cases were examined.

The primary efficacy endpoint was not well defined and thus this study could not be qualified as a pivotal field trial. This study was considered an explorative study and, therefore, it only provided supportive data. Moreover, the statistical analysis of the primary and secondary efficacy endpoints could not be assessed reliably since there were significant flaws which made it difficult to set any firm conclusions regarding the efficacy of the product. The significant decrease in tendon CSA after one month of treatment obtained together with the outcomes found regarding secondary endpoints did not demonstrate the efficacy of EQUITEND. Excluding horses in the knowledge of the data might have introduced a bias and was, therefore, not accepted. Details on the statistical analysis (e.g. inclusion of covariates) were only given in the study report but not in the study plan.

A scientific thesis was submitted presenting a study not performed under GCP conditions. These data were obtained before the dose ranging study and the dose confirmation study.

The interpretation of the results from this study was limited due to the study design and the statistical significance of the effect of EQUITEND was difficult to assess. The results obtained in this thesis could only be considered as supportive data prior to commencing an experimental trial. This was considered a preliminary study.

In conclusion, due to the flaws in study designs and the results obtained, the efficacy of EQUITEND as an aid in healing flexor tendinopathy in horses could not be derived from these studies. Therefore, additional data was requested to sufficiently demonstrate the efficacy of EQUITEND for the claimed indication.

Part 5 – Benefit-risk assessment

Introduction

EQUITEND is a solution for injection containing polycarboxymethyl glucose sulfate acetate (OTR4131). The active substance is innovative.

The active substance, polycarboxymethyl glucose sulfate acetate (OTR4131), is a dextran derivative polymer, specifically designed to replace degraded heparan sulfates in injured muscle and tendonal structures.

The pharmacological mechanism of action of polycarboxymethyl glucose sulfate acetate (OTR4131) is based on the binding and protection of growth factors and structural proteins in the extracellular matrix to restore the matrix scaffold and tissue architecture, and provide a micro-environment that is recognised by the cells of origin, aiding the natural process of tissue regeneration. The product was intended to aid in healing flexor tendinopathy in sport horses. The proposed dose of 10 µg (1 ml of a 10 µg/ml solution) for intralesional administration as a single dose remained to be confirmed at the time of withdrawal of the application.

The application was submitted in accordance with Article 12(3) of Directive 2001/82/EC of Directive 2001/82/EC (full application).

The product was classified as MUMS/limited market and therefore reduced data requirements apply and were considered in the assessment.

Benefit assessment

Direct therapeutic benefit

The proposed direct benefit of EQUITEND would have been its efficacy in aiding healing of tendinopathy in horses, which was investigated in one dose determination study, one dose confirmation study, one pivotal clinical trial and one uncontrolled field trial not conducted to an acceptable standard. Therefore, due to major shortcomings in the design, conduct and reporting of these studies, these data were not considered sufficient to support the proposed indication.

Additional benefits

If the product had been efficacious it would provide a new treatment possibility in a minor species and would increase the range of available treatment possibilities for flexor tendinopathies in horses.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product was not presented in a satisfactory manner. The results of tests carried out did not indicate consistency and uniformity of important product quality characteristics, and these in turn led to the

conclusion that the product would not have a satisfactory and uniform performance in clinical use.

Major deficiencies also arise from concerns over the restricted part of the ASMF. These concerns were conveyed in confidence to the ASMF holder.

Safety:

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

Risks for the target animal:

Given that systemic exposure could be accepted to be negligible, there were only a few safety concerns regarding this substance. Administration of EQUITEND in accordance with the proposed SPC recommendations was generally well tolerated; the main reported adverse reactions included mild local reactions during three days following injection when administered at 150-fold the therapeutic dose. Moreover, it was assumed from the clinical studies that the product was well tolerated and that minimal side effects occurred. However, no individual data were provided to enable the CVMP to properly evaluate safety of the treatment.

Risk for the user:

The user risk assessment provided is not in accordance with the structure recommended by the Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1). However, the available data on toxicity showed that the systemic toxicity of the product is low and that it can be considered a non-sensitizer. No information on the irritation potential of OTR4131 was provided.

An adequate quantitative and qualitative risk characterization, including an estimation of the margin of exposure, was requested.

The proposed user warnings were considered appropriate but some updates were necessary in order to reflect the conclusions of the user risk assessment.

Risk for the environment:

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

Risk for the consumer:

EQUITEND was not intended for use in horses intended for slaughter for human consumption. Horses treated with EQUITEND would have had to be declared as not intended for slaughter for human consumption.

Risk management or mitigation measures

Risk management or mitigation measures would have been considered pending additional information from the applicant.

Evaluation of the benefit-risk balance

In the presence of (major/other) concerns, no conclusions could be taken on the benefit-risk balance of the application.

Conclusion

Based on the original data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) considered that the application for EQUITEND is not approvable at the present time since "major objections" were identified which preclude a recommendation for marketing authorisation. The details of these major objections were provided in the list of questions. Major objections are critical points requiring resolution before recommendation for a marketing authorisation. Failure to resolve these major objections render the application not approvable. No conclusions could be reached on the benefit-risk balance of the application.

In addition, satisfactory answers were required to the "other concerns", which were detailed in the list of questions.