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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

Withdrawal assessment report for Sirolimus TriviumVet (EMEA/V/C/006230/0000)

INN: Sirolimus

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

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Table of contents

Introduction

The applicant TriviumVet DAC submitted on 25 April 2023 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Sirolimus TriviumVet, through the centralised procedure under Article 42(2)(c) of Regulation (EU) 2019/6 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 10 November 2022 as Sirolimus TriviumVet contains an active substance which was not authorised as a veterinary medicinal product within the Union at the date of the submission of the application (Article 42(2)(c)).

At the time of submission, the applicant applied for the following indication:

'Management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM) in cats.'

The active substance of Sirolimus TriviumVet is sirolimus (also known as rapamycin), a macrolide compound, which inhibits mTOR complex 1 (mTORC1), a protein that is part of a signalling pathway regulating cell growth, autophagy, and cell death. According to the applicant, intermittent dosing with sirolimus specifically inhibits mTOR complex 1 (mTORC1), which results in a reduction in cardiac hypertrophy, an increase in mitophagy and autophagy and a reduction in oxidative stress. The target species is cat.

Sirolimus TriviumVet gastro-resistant tablets contain 0.4 mg, 1.2 mg, and 2.4 mg sirolimus and was presented in packs containing 12 tablets.

The applicant was 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 8 of Regulation (EU) 2019/6 – full application.

On 18 March 2025, TriviumVet DAC withdrew the application during the assessment of the responses to the list of outstanding issues at day 187 of the procedure. In its letter notifying the Agency of the withdrawal of application, the applicant stated the reason for the withdrawal: CVMP considers that the data provided does not allow the Committee to conclude on a positive benefit-risk balance.

Scientific advice

The applicant received scientific advice (EMA/CVMP/SAWP/424725/2020) from the CVMP on 5 November 2020. The scientific advice pertained to the safety section of the dossier.

The applicant followed the scientific advice provided by the CVMP in respect of the provision of proprietary developmental toxicity and carcinogenicity studies (See Part 3 of this report). At Day 180 of the procedure, the CVMP considered the scientific advice provided (concerning whether the omission of certain proprietary safety studies performed with the active substance rapamycin could be compliant with the requirements of Annex II of Regulation (EU) 2019/6) relevant to the current application.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant provided a summary of the pharmacovigilance system master file which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided the applicant has in place a pharmacovigilance system master file (PSMF), has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

Active substance

Manufacture, quality control testing and packaging of the active substance sirolimus were proposed to take place outside the EEA. A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site manufacturer of dosage form on behalf of all QPs involved. The declaration was based on an onsite audit by a third party.

Finished product

Batch release of the finished product was proposed to take place within the EEA. The site has a valid manufacturing authorisation issued by the relevant competent authority. The latest GMP certificate from the batch certification site which confirms that site is authorised for the batch certification of imported products and references an inspection was available on the EudraGMP database.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file was considered to be in line with legal requirements.

At Day 180 of the procedure, the GMP status of both the active substance and finished product manufacturing sites was satisfactorily established and in line with legal requirements.

Part 2 - Quality

Composition

The products were gastro-resistant tablets for oral administration, containing either 0.4 mg, 1.2 mg or 2.4 mg of the active substance sirolimus, together with several other ingredients as excipients, and enteric coatings. The enteric coatings include different colouring materials, thus allowing differentiation of the different tablet strengths.

The products were proposed to be packaged in a box containing a perforated, blister of 12 tablets. The blister packaging containing the tablets was child-resistant.

Containers and closure system

The primary packaging of the product was described in the dossier as 12 count, perforated child resistant blister pack. The blister specification includes tests for description, dimensions, and identification by IR. A certificate of compliance of the primary packaging with various EU packaging Regulations and Directives was provided. This includes confirmation of compliance with Commission Regulation (EU) No. 10/2011.

Product development

The product was developed as a gastro-resistant tablet of three strengths (0.4 mg, 1.2 mg and 2.4 mg), differentiated by colour and containing the active substance sirolimus. The active substance has low solubility and high permeability.

The excipients included in the formulation are commonly used for this dosage form. With the exception of the enteric coatings, the excipients were all monographed in the Ph. Eur. A compatibility study between excipients in the formulation was performed.

The dissolution test method was developed in line with Ph. Eur. 5.17.1 Recommendations on Dissolution Testing for delayed-release formulations. The discriminatory power of the dissolution method was demonstrated. At Day 180 of the procedure, the deviation from Ph. Eur. recommendations regarding the volume of buffer used in the dissolution methods still required justification.

Formulation studies were performed in order to optimise the quantitative and qualitative components of excipients within the formulation, with respect to tablet characteristics such as dissolution, disintegration, assay and related substance content. Some minor questions remained at Day 180 of the procedure.

The formulation development was evaluated by the applicant through the use of risk assessment to identify the critical quality attributes and the critical process parameters. A risk analysis was performed in order to define critical process steps and process parameters that may have an influence on the finished product quality attributes. The risk identification was based on the prior knowledge of products with similar formulations and manufacturing processes as well as on the experience from formulation development, process design and scale-up studies. The critical process parameters were adequately identified. Although all aspects of the risk assessments were not considered fully justified, questions were not raised on aspects that did not impact on the control strategy adopted for the product manufacture and testing. The control strategy was generally acceptable and specific issues relating to control during manufacture, release and shelf life are dealt with in the relevant sections of this report.

The development pharmaceutics report includes details of a number of trial batches which were manufactured in order to optimise the product formulation and manufacturing process, using results from earlier risk assessment and compatibility studies. The dossier includes a systematic and thorough description of the formulation development work. The formulation development was satisfactorily well described and the applicant’s conclusions throughout this process were supported by the data presented.

Description of the manufacturing method

The manufacturing process follows a conventional approach for gastro-resistant solid dose manufacturing. The manufacturing process consists of steps including generation of an active substance (API) dispersion, granulation, compression, coating and packaging.

The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

The dossier includes a stepwise narrative description of the manufacturing process and a manufacturing process flow chart. Factorisation for active substance assay content is applied. At Day 180 of the procedure, an update to the manufacturing description regarding weighing of the binder solution remained to be provided.

The proposed commercial batch sizes were provided.

Process validation was conducted on commercial scale batches. The parameters employed for manufacture of these batches were consistent with parameters detailed in the manufacturing process description. Major steps of the manufacturing process were validated for each tablet strength. Additional in-process control testing was performed during validation.

It was demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. A separate packaging validation was performed, using a bracketing approach with regard to the tablet strengths examined.

A process validation protocol was provided for future production batches at the largest batch size of each tablet strength.

Control of starting materials

Active substance

The active substance sirolimus (also known as rapamycin) is a macrocyclic lactone compound produced by fermentation from *Streptomyces hygroscopicus* that has been developed for use in human medicine as an immunosuppressant.

The active substance, sirolimus, is not monographed in the Ph. Eur. Data on the active substance was provided in the form of Active Substance Master File (ASMF).

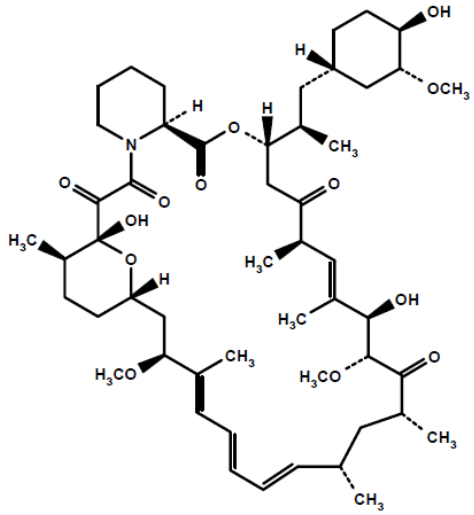
The chemical names of sirolimus are

[3S(3R*(S*(1R*,3S*,4S*)),6S*,7E,9S*,10S*,12S*,14R*,15E,17E,19E,21R*,23R*,26S*,27S*,34aR*]]
9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,27-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3Hpyrido[2,1-c][1,4]oxaazacyclohentriacontine-1,5,11,28,29(4H,6H,31H)-pentone.

Sirolimus is an off-white to yellow crystalline powder, odourless with a melting range of 182 - 185°C and is not hygroscopic in nature. It is soluble in methanol, ethanol, acetone, ethyl acetate, dichloromethane and chloroform, sparingly soluble in ethyl ether, hexane and petroleum ether, insoluble in water.

Sirolimus has 15 chiral centres and 3 reported isomer A, B and C and a specific optical rotation of -145° to -160° (in methanol).

Sirolimus has the following structure:



Molecular formula: $C_{51}H_{79}NO_{13}$

Molecular weight: 914.17

The applicant's active substance specification includes tests for description, identification, loss on drying, specific optical rotation, residue on ignition, related substances, content of isomers, assay, residual solvents and microbial quality. The specification was generally considered to be acceptable, although some questions remained to be satisfactorily resolved at Day 180 of the procedure. The test methods used for the control of the active substance, and the validation thereof, were as per those of the ASMF. Batch analysis data were provided, although some questions remained to be satisfactorily resolved too. Acceptable information was provided for the reference standard used by the applicant for the control of the active substance.

With respect to the Applicant's Part of the ASMF, sufficient information was provided on the nomenclature, structure and general properties of the active substance. The manufacturing process for sirolimus consists of fermentation using the microorganism *Streptomyces hygroscopicus,* followed by extraction and purification. Process flow diagrams were provided along with a brief description of the process.

Data were provided on the characterisation of the active substance. Information was provided for related substances, residual solvents, microbial purity, mycotoxins, residues of the producer microorganism, mutagenic impurities and elemental impurities. At Day 180 of the procedure, an open issue remained regarding residual solvents.

The active substance specification includes tests for description, identification, loss on drying, specific optical rotation, residue on ignition, related substances, content of isomers, assay, residual solvents and microbial quality. The specification was generally considered to be acceptable, although some questions remained to be satisfactorily resolved at Day 180 of the procedure. The test methods were well described and considered to be acceptable. The method validation provided is in accordance with the VICH guidelines VICH GL 1 and VICH GL2 and so was acceptable, and acceptable validation data were also provided for the test methods for microbial control in accordance with the pharmacopoeial requirements. Compliant comparative batch analysis data were provided for batches of the active substance for all tests in the proposed specification. Satisfactory justification was provided for the specifications. Satisfactory information regarding the reference standards was presented.

Acceptable specifications were provided with regards the packaging of the active substance, and declarations of the compliance of the immediate packaging contact material with the current requirements of Regulation (EU) No 10/2011, as amended.

Stability data were provided for production-scale batches of the active substance at long-term conditions, and for batches of the active substance on accelerated conditions. All results are within specification, however updated documentation was requested at Day 180 of the procedure. Forced degradation data provided shows the stability-indicating nature of the methods. Photostability data provided demonstrates that the proposed packaging was suitable for the active substance.

At Day 180 of the procedure, a number of points remained to be addressed on the Restricted Parts of the ASMF.

Excipients

With the exception of the coating components, all excipients were well known pharmaceutical ingredients and their quality compliant with Ph. Eur. standards. There were no novel excipients used in the finished product formulation. The list of excipients was included in section 2 of the SPC.

Where functionality-related characteristic testing was specified within an individual Ph. Eur. monograph, but not included on the specifications proposed in the dossier, justification for the absence of these tests was provided. Microbial controls within certain excipients were absent and justification for the absence of controls provided to support their absence.

The non-pharmacopoeial excipients used in this product were the components of the enteric coating. The qualitative composition of the enteric coating used in the manufacture of each tablet strength was provided. Specifications were provided which include appearance, colour difference, dispersion and identification. The quantitative compositions of the coatings were provided; the colourings consist wholly of substances listed in the Ph. Eur. and food grade materials recorded in the relevant compendia (E numbers).

Representative certificates of analysis for the excipients were provided demonstrating compliance with the proposed specifications.

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

None of the starting materials used for the active substance or the finished product were risk materials as defined in the current version of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev 3). The product was therefore out of the scope of the relevant Ph. Eur. monograph and the Note for guidance.

Control tests on the finished product

The finished product release specification controls relevant parameters for a tablet dosage form, including tests for appearance of the product, identification and assay of the active substance, water content, uniformity of dosage units, dissolution, HPLC related substances and microbial quality. The specification presented was generally acceptable, however some questions remained to be satisfactorily resolved at Day 180 of the procedure.

An elemental risk assessment on the control of elemental impurities in the veterinary medicinal product in line with the requirements of the 'Reflection paper on risk management requirements for elemental impurities in veterinary medicinal products' EMA/CVMP/QWP/15364/2018 was conducted and a summary report presented in the dossier.

The analytical methods for each test are presented, validated in line with VICH GL1: *Validation of analytical procedures: definition and terminology* and VICH GL2: *Validation of analytical procedures*. Some questions remained to be satisfactorily resolved at Day 180 of the procedure.

Batch analysis data were provided for full scale batches of each tablet strength. All results were within specification confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability

Stability data of full scale batches of finished product of each tablet strength stored under long term conditions for 12 months at 25 ºC/60% RH and for up to 6 months under accelerated conditions at 40ºC/75% RH according to the VICH guideline GL3 were provided. The batches were identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

The proposed shelf-life of the veterinary medicinal product as packaged for sale was 2 years when stored below 25°C. On the basis of the data presented, no temperature storage precaution was considered warranted, and the applicant was therefore asked to amend the storage conditions to "This veterinary medicinal product does not require any special storage conditions".

At Day 180 of the procedure, the proposed shelf-life specification required amendments.

One batch of each tablet strength was exposed to light as defined in the VICH guideline GL5 on photostability testing of new veterinary drug substances and medicinal products. Results of the photostability study were within specification and no light storage precaution was therefore considered necessary.

For the long term (12 months) and accelerated stability (6 months) studies conducted, all results remained within specification. The proposed shelf life of 2 years might have been acceptable by extrapolation with no special storage conditions.

New active substance (NAS) status

The applicant requested the active substance sirolimus contained in the Sirolimus TriviumVet product to be considered a new active substance as it was not hitherto authorised in a veterinary medicinal product in the European Union at the date of submission of the application.

The applicant substantiated the NAS claim with reference to the macrolide ring being structurally conserved as the therapeutic moiety of sirolimus. This ring was not present in any other substance authorised for veterinary use in the union and therefore the active substance does not expose the animal to the same therapeutic moiety as any other approved veterinary product in the union.

Overall conclusions on quality

The products were gastro-resistant tablets for oral administration, containing either 0.4 mg, 1.2 mg or 2.4 mg of the active substance sirolimus. The primary packaging of the product was 12 count, perforated child resistant blister pack.

Information on the development, manufacture and control of the active substance and the finished product was satisfactory.

Within the product development section, the applicant provided an evaluation of the active substance and excipients, a summary of the formulation work performed and the development of the manufacturing process. Development of the dissolution test method, containing an acid and buffer stage with limits in line with Ph. Eur. for delayed-release formulations was presented.

The manufacturing process consists of the generation of intra-granular and extra-granular phases, compression into tablets and the addition of an enteric coating. The process parameters and in-process controls were acceptable. Process validation at the lower batch size for each tablet strength was provided and deemed acceptable at Day 180 of the procedure.

Information on the control of starting materials was provided. The active substances sirolimus is not monographed in a pharmacopoeia and data on the active substance were provided according to the Active Substance Master File (ASMF) procedure.

Most excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. monographs with the addition of functionality-related characteristic testing and microbial testing where relevant. The enteric coatings used are non-monographed excipients, the full quantitative and qualitative composition and confirmation of compliance of the coating colours with legislation were provided. Data were presented to give reassurance on TSE safety.

The finished product release specification controls relevant parameters for the dosage form. A summary report of the risk management to justify the absence of control strategy for elemental impurities was provided. Analytical methods and validation data were provided and deemed acceptable at Day 180 of the procedure.

Product stability data in compliance with VICH GL 3 were provided to support the proposed shelf-life of 2 years with no special storage conditions required.

At Day 180 of the procedure, there were still a number of issues to be addressed by the applicant before a conclusion regarding the quality of the product could be made.

Part 3 – Safety documentation (Safety and residues tests)

The active substance of Sirolimus TriviumVet is sirolimus, a macrolide compound which inhibits mTOR complex 1. At the time of submission, Sirolimus TriviumVet was intended for use in cats for the management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM), however the indication would have required further amendment had the procedure progressed to the granting of a Marketing Authorisation (MA). Sirolimus is a new active substance, which was not authorised for a veterinary medicinal product in the EU at the date of submission of the application.

A safety file largely in accordance with Article 8 of Regulation (EU) 2019/6 was provided and is described below. The safety file comprises both proprietary studies (repeat dose toxicity and genotoxicity studies) and reference to publicly available bibliographic data. Scientific advice was provided by the CVMP and this advice concerned the requirement for submission of proprietary developmental and carcinogenicity studies in support of safety of a rapamycin-containing veterinary medicinal product. The advice provided was followed by the applicant. At Day 180 of the procedure, the CVMP considered the scientific advice provided (concerning whether the omission of certain

proprietary safety studies performed with the active substance rapamycin could be compliant with the requirements of Annex II of Regulation (EU) 2019/6) relevant to the current application.

Safety tests

Pharmacology

Pharmacodynamics

A summary of the pharmacodynamics of the active substance sirolimus as they relate to immunosuppression (the authorised indication for the sirolimus-containing human medicinal product) was provided.

The pharmacodynamic effect of sirolimus is brought about by its binding to the cytosolic immunophilin protein FKPB-12, and the resulting complex binding to and inhibiting the cell cycle regulatory protein 'mammalian target of rapamycin' (mTOR). The mTOR inhibition results in suppression of induced cell proliferation by inhibition of progression of the cell cycle. Sirolimus inhibits T cell activation resulting in reduced lymphocyte activation (by blockade of signal transduction) and immunosuppression.

Further information on pharmacodynamics (that relates to the purported therapeutic effect in the proposed indication) is provided in Part 4. Information concerning the primary pharmacodynamic effect (immunosuppression) of the active substance sirolimus in humans was provided in this section, however, no appropriate pharmacological NOEL/LOEL could be determined.

Pharmacokinetics

A bibliographic review of scientific literature concerning absorption, distribution, metabolism and excretion (ADME) of sirolimus in humans and other non-target species was provided and is summarised as follows:

Low oral bioavailability of sirolimus is reported in humans. Variability in pharmacokinetics between individuals is also reported and is considered to be related to variations in expression of hepatic cytochrome P450 3A alleles.

Non-linear saturable absorption is reported in humans. Once absorbed, most sirolimus partitions into red-blood cells. Protein-binding (to albumin) also occurs. Sirolimus is lipophilic and distributes widely in lipid membranes. Extensive metabolism of sirolimus occurs in the liver and is reported to be cytochrome P450 3A dependent (in humans and rats). Most sirolimus metabolites are excreted in faeces and renal excretion is minimal. The half-life in adult (humans) is reported to be between 67 and 80 hours.

Toxicology

One repeat-dose toxicity study and three genotoxicity studies were provided. Bibliographic data were also provided.

Single-dose toxicity

Based on publicly available information, an oral LD50 for sirolimus in rats and mice is > 800 mg/kg and > 2,500 mg/kg respectively. Based on data generated in a genotoxicity study presented with this

dossier, the maximum tolerated dose (MTD) for sirolimus in male and female mice is determined to be 250 mg/kg bw/day and 500 mg/kg bw/day respectively.

Repeat-dose toxicity

A GLP-compliant 90-day repeated dose oral toxicity study in Wistar rats was performed in accordance with OECD TG 408. In this study sirolimus was administered orally at three doses (0.03, 0.3 and 3 mg/kg bw/day, in groups referred to as LD, MD and HD groups respectively), to rats once daily for 90 days. Increased mean organ to body weight ratio of the heart in the HD group is reported, however in the absence of histomorphological observations in cardiac tissue and no statistical difference in mean heart weights between groups, at day 180 of the procedure the CVMP accepted that this observation was unlikely to impact target animal safety when the intended posology for the veterinary medicinal product is taken into consideration.

It was concluded based on the results of this study that histopathologic changes in the liver (and related increases in circulating levels of total, HDL and LDL cholesterol) observed in male animals in the HD group are due to tissue injury caused by sirolimus. As such, the toxicological no observed adverse effect level (NOAEL) for sirolimus was determined to be 0.3 mg/kg bw/day under the conditions of this repeat dose-toxicity study in rats.

Tolerance in the target species

See Part 4.

Reproductive toxicity, including developmental toxicity.

Study of the effect on reproduction

No data were provided on the effects of sirolimus on reproduction which is in accordance with Regulation (EU) 2019/6.

Study of developmental toxicity

No proprietary developmental toxicity data were presented. Publicly available data on developmental toxicity were provided and it was concluded that the developmental toxicity potential of sirolimus is incompletely defined. Generally, it was shown that mTOR inhibitors increase foetal mortality in experimental animals, however no teratogenic effects were observed in either rats or rabbits. Furthermore, the product information for the sirolimus-containing human product indicates that sirolimus can cause embryo / foetal toxicity.

In regard to target animal safety, the omission of developmental toxicity data was considered an appropriate approach in the scientific advice provided by the CVMP, provided a suitable restriction on use in breeding and pregnant queens was stated in the SPC. Consequently, the product was not recommended for use during pregnancy or lactation, or in cats intended for breeding at Day 180 of the procedure.

Genotoxicity

The genetic toxicology potential of sirolimus was evaluated in a standard test battery in accordance with VICH guideline GL23 and with relevant OECD guidelines (that is OECD TG 471, 487 and 474).

Based on the results of the above studies, it was concluded that sirolimus does not appear to have genotoxic effects.

Carcinogenicity

No proprietary carcinogenicity data were provided, however bibliographic data concerning this topic were considered. These data indicate that while increased risk of malignancy is associated with long-term immunosuppression therapy, this risk does not appear to be associated with mTOR inhibitors specifically. Noting that there is no evidence that sirolimus has genotoxic properties, the absence of proprietary carcinogenicity data was considered justified. This approach follows the scientific advice provided by the CVMP in respect of this matter and was considered acceptable at Day 180 of the procedure.

Other requirements

Special studies

No signs of immunotoxicity and neurotoxicity were observed in a repeat dose toxicity study. Based on this information and the proposed presentation of the candidate VMP (that is as a gastro-resistant tablet) no special studies investigating potential of the formulation to cause sensitisation or irritation were provided. The approach was considered acceptable. Please refer to the user risk assessment for more detail.

Observations in humans

Published observations of the diverse range of adverse effects observed when sirolimus is used in humans were summarised, while taking into account that the human patient population being considered in the available literature are usually renal transplant recipients receiving long-term immunosuppressive therapy. As such, attribution of the observed adverse effects to sirolimus alone is not straight-forward. Data from elderly, but healthy humans receiving a daily dose of 1 mg sirolimus (0.017 mg/kg bw) for at least 8 weeks showed some adverse effects. Gastrointestinal adverse events, fever, facial rash, decreased erythrocyte parameters, increased cholesterol, and elevated plasma ALP are noted as adverse events associated with sirolimus exposure in otherwise healthy humans (in the context of the afore-mentioned clinical trial in elderly patients, accidental consumption or deliberate overdose). A case study reported that a 2.5-year-old child who ingested 3 mg presented with elevated ALP, fever and gastroenteritis, symptoms that were likely related to sirolimus.

Excipients

Excipients of the product are used in oral pharmaceutical medicines and do not raise any specific toxicological concern. Excipients were further considered in the user risk assessment.

User safety

The applicant presented a user safety risk assessment conducted broadly in accordance with CVMP guideline EMEA/CVMP/543/03/Rev.1.

Considering the toxicity of the active substance sirolimus, reference is made to the data presented in the Toxicology section, above. Based on the results of a repeat-dose oral toxicity study performed in

rats, a toxicological NOAEL of 0.3 mg/kg bw was derived. This toxicological NOAEL is above the clinical doses used in human medicinal products (according to the SPC of a human medicinal product: 6 mg initial dose and 2 mg maintenance dose, which corresponds to 0.1 mg/kg bw and 0.03 mg/kg bw). However, 0.3 mg/kg bw is the clinical dose for the target animals, and at this dose level adverse effects were observed in a target animal study. Therefore, user risk assessment should be based on an appropriate pharmacological reference value. A pharmacological reference value of 0.017 mg/kg bw based on a clinical trial performed in a small number of elderly human patients was proposed by the applicant with some justification of why this value is appropriate, namely no evidence of changes in the immune parameters tested in the study.

Considering the toxicity of the formulation, the proposed presentation of the tablets with a gastro-resistant coating (in accordance with the relevant requirements as stated in Ph. Eur. 01/2018:0478 as part of the FPS) will significantly limit exposure of a user to the final formulation during normal use / handling of the candidate VMP.

In respect of predicted exposure(s) of a user to the candidate VMP, a user is defined as a non-professional adult (including pregnant persons), and a child (in the case of accidental ingestion).

The routes of exposure considered by the applicant are: 1. frequent / probable dermal contact with the tablet coating in the course of normal expected use once weekly; 2. infrequent / low probability of dermal contact (and possible consequent hand to mouth / eye contact) with 10% (0.36 mg) of the whole formulation if the coating is compromised for example, after an episode of vomiting; and 3. infrequent / unpredictable oral exposure to the maximum dose for a large cat (3.6 mg) of the whole formulation in the event of accidental ingestion by a child.

As regards risk arising from these routes of exposure, it was accepted that the risk posed by normal handling of the candidate VMP is negligible, as this scenario will result in exposure of the user to the gastro-resistant coating only, and no exposure to the active substance is foreseen. This includes pregnant users, therefore, the risk for the woman and the unborn can be considered low.

The risk in the following worst-case scenario was quantified: accidental ingestion of a dose of 3.6 mg sirolimus by a 12.5 kg child. In such a scenario, a margin of exposure (MOE) < 100 is returned and as such, risk mitigation measures were proposed.

The risk mitigation measures proposed by the applicant include provision of appropriate storage instructions on the package leaflet, user safety warnings in the SPC and provision of the candidate VMP in a child-resistant blister pack.

Regarding the user safety warnings for inclusion in section 3.5 of the SPC, the applicant considered the quantitative risk assessment, in particular the need for a warning concerning accidental ingestion by a child.

The blister packaging containing the tablets was child-resistant.

At Day 180 of the procedure, and pending minor updates to the proposed user safety warnings, the CVMP was in a position to conclude that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the CVMP/VICH guidelines.

The environmental risk assessment can stop in Phase I and no Phase II assessment is required because the veterinary medicinal product will only be used in non-food producing species. It was

accepted that when the candidate product is used in accordance with the SPC it will have limited environmental exposure and will not pose a risk to the environment. No specific warnings or risk mitigation measures concerning the environment were proposed for the SPC at Day 180 of the procedure.

New active substance (NAS) status

Not assessed under Part 3.

Overall conclusions on the safety documentation: safety tests

Pharmacology:

The pharmacodynamic effect (that of immunosuppression) of sirolimus in humans is brought about by its inhibition of the cell cycle regulatory protein mTOR and resultant suppression of induced cell (lymphocyte) proliferation.

Toxicology:

In a repeat dose toxicity study, the oral toxicity profile consists of hypercholesterolaemia consistent with liver damage identified in rats. The NOAEL in a rat 90-day toxicity study is 0.3 mg/kg bw/day.

In the absence of studies on the effects on reproduction, a suitable restriction on use in breeding animals and pregnant and lactating queens was stated in the SPC.

Based on the standard genotoxicity test battery, sirolimus is not genotoxic. Carcinogenicity studies were not performed and were not requested.

The data presented was considered adequate to characterise the toxicity profile of the active substance.

User safety:

A user safety assessment broadly in line with the relevant guidance document was presented.

At Day 180 of the procedure, it was accepted that the product was unlikely to pose an unacceptable risk to the user, pending further minor updates to the user safety warnings in the SPC.

Environmental risk assessment:

An appropriate environmental risk assessment was provided. At Day 180 of the procedure, the product was not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Pre-clinical studies

Sirolimus TriviumVet was a range of oral gastro-resistant tablets containing 0.4 mg, 1.2 mg and 2.4 mg sirolimus. At the time of submission, Sirolimus TriviumVet was intended for use in cats for the management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM),

however the indication would have required further amendment had the procedure progressed to granting of a MA. The product is proposed for once weekly oral administration at an approximate dose rate of 0.3 mg sirolimus/kg bw.

Sirolimus is a new active substance, which has not been authorised in a veterinary medicinal product in the EU at the date of submission of the application.

Pharmacology

Pharmacodynamics

The pharmacodynamic data provided were derived from studies conducted in rodents and humans, and no cat-specific pharmacodynamic were presented. However, the applicant considered the general pharmacology of sirolimus to be common across species.

Sirolimus is an inhibitor of the mTOR protein complex, primarily causing inhibition of mTORC1 with consequent impacts on protein synthesis, cell growth and proliferation, autophagy, cell metabolism and stress responses. However, at high or chronic dosing sirolimus may also cause inhibition of mTORC2, a regulator of cell survival and polarity.

The targeted pharmacodynamic effect for the candidate product is the inhibition of cardiac hypertrophy associated with hypertrophic cardiomyopathy in cats, whilst retaining the capacity of the heart to adapt to load. The applicant suggests that with inhibition of mTORC1 and mTORC2, inhibition of hypertrophy will be observed, but so will impairment of the heart's capacity to adapt to stress. Therefore, selective inhibition of mTORC1 is preferable. Inhibition of mTORC1 stimulates autophagy, eliminating damaged and harmful cells and sustains cell survival by recycling cytosolic components and inhibiting hypertrophy. Sirolimus activates mitophagy which helps to eliminate abnormal mitochondria, improving cellular homeostasis and mitochondrial function. Inhibition of mTORC1 also reduces proliferation of vascular smooth muscle, intimal hyperplasia and inflammatory cell infiltration. It can be accepted that the bibliographic data provided by the applicant supports the above observations.

Further, attenuation of cardiac hypertrophy and improvement in cardiac function, were observed in a number of studies conducted in rodent models of cardiomyopathy following the administration of rapamycin at dosages ranging from 0.2 – 2 mg/kg bw for treatment durations ranging from 14 to 70 days.

It can be accepted that the data presented provide evidence of positive effects of sirolimus on heart muscle structure and function in rodents and humans; however, it was noted that neither sirolimus nor any other mTOR inhibitor were authorised for the management of cardiac disease/dysfunction in humans (that is, the effects observed have not translated into an authorised indication). Further, it was noted that none of the data on pharmacodynamic effects were generated in the target species, the cat.

With regards secondary pharmacodynamic effects, it is noted that in human medicine, sirolimus is authorised as an immunosuppressant for the prevention of transplant rejection. However, the exact mechanisms by which sirolimus impacts on the immune system are unclear. Although in humans receiving treatment with mTOR inhibitors, adverse effects such as fever, pneumonitis and anaemia of chronic disease have been observed, studies have also demonstrated an improvement in vaccine responsiveness with mTOR inhibition. In humans, it is recognised that long term use of sirolimus has the potential to result in immunosuppression and glucose intolerance associated with mTORC2 suppression; however, it can be accepted that the results of some studies presented do appear to indicate that the unwanted effects of rapamycin associated with mTORC2 suppression can be

reduced/eliminated with intermittent dosing or alternatively, by the use of rapamycin analogues, such as everolimus or temsirolimus. These observations will be commented on further, later in this assessment report under the dose justification, target animal safety and clinical studies.

The study data presented indicate that diabetes mellitus, hyperlipidaemia (potentially leading to cardiovascular disease), with consequent hypertriglyceridemia and vasculitis may be observed consequent to sirolimus administration. These effects may be dose-dependent and reversible with cessation of treatment. These observations were taken into consideration when evaluating the target animal safety data presented with the application. On this point, it is noted that diabetes mellitus was diagnosed in one sirolimus-treated cat in the field trial presented with this application.

The applicant described how drug interactions may be observed when sirolimus is administered concurrent with products that affect cytochrome p450 3A and P-glycoprotein, with inhibitors of these proteins resulting in an increase in the absorption of sirolimus, whilst inducers of CYP314 and P-gp may reduce absorption of sirolimus thus reducing plasma concentration.

The applicant made reference to these effects under SPC section 3.8; and included examples of other active substances used in veterinary medicine which may impact upon the bioavailability of sirolimus.

The applicant proposed text for inclusion in section 4.2 of the SPC detailing a proposed mechanism of action of sirolimus based on the data summarised above, however at Day 180 of the procedure, the applicant had been requested by CVMP to further update this text such that it can be considered to be substantively supported by data in the target species, cats. In the absence of relevant data in the target species, the applicant was also requested to clearly identify the target species in which the data being cited were generated for the end-user / reader of the SPC.

Pharmacokinetics

In order to characterise the pharmacokinetic profile of sirolimus in the target species, the applicant derived some information from published studies (not conducted in the target species), and cat-specific pharmacokinetic data were generated in two proprietary target animal safety studies. These studies were designed to investigate target animal safety and consequently are not compliant with the relevant Guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/99-FINAL). Notwithstanding this, they provide the following information.

In a pilot target animal safety study, cats were administered sirolimus orally at one of 3 dose rates, 3 times weekly for 12 weeks. The dose rates investigated were: 0.15, 0.45 and 0.75 mg/kg bw in groups T1, T3 and T5 respectively. Blood samples (for measurement of whole blood concentrations of sirolimus) were taken on study days 0 and 26, and on each occasion, sampling spanned the time from pre-administration until 72 h post-administration. The tablets used in the study were not the final formulation and were coated with gastro-resistant coating.

$AUC_{0\text{-tlast}}$ values were calculated for each animal and compared within treatment groups. At Day 0, $AUC_{0\text{-tlast}}$ values were 30.67, 74.56 and 105.30 h*ng/ml for T1, T3 and T5 respectively. At Day 26, $AUC_{0\text{-tlast}}$ values were 44.29, 122.06 and 216.16 h*ng/ml for T1, T3 and T5 respectively.

In the pivotal target animal safety study, sirolimus was administered once weekly to cats in the fed state for 24 weeks at one of the following dose rates, 1x the recommended treatment dose (RTD) (0.26 – 0.38 mg/kg bw), 3xRTD (1.0 – 1.13 mg/kg bw) or 5xRTD (1.76 – 1.88 bw). At 1xRTD, the following PK parameters (± SD) were observed on study Day 147 (after 6 months of weekly dosing): AUC_t = 514.25 (± 391.15) ng/ml*h, C_{max} = 27.54 (± 12.04) ng/ml and t_{max} = 3.5 (± 1.83) h. An elimination rate ($t_{½}$) of 91.09 ± 42.41 h in the 1XRTD group was observed (also at study day 147).

It was postulated based on the results generated in these studies that the rate of absorption of sirolimus can be saturated at higher dosing levels (higher than the RTD) in cats. It also appears that the pharmacokinetics of sirolimus in cats are non-linear; however, the extent of this non-linearity was not fully investigated. These observations are largely in keeping with observations in humans and laboratory animal species. The gastro-resistant coating may delay absorption of sirolimus in cats (as is the intention).

Despite provision of an incomplete and non-guideline compliant pharmacokinetic data set in the target animal species, it was concluded that the approach taken by the applicant characterised the pharmacokinetics of sirolimus such that very basic information concerning the behaviour of the active substance at the proposed dose in the target animal species cats was provided. Considering the proposed life-long duration of treatment intended for the IVP, it was accepted that slight accumulation of sirolimus occurs after repeated dosing; at Day 180 of the procedure, the applicant had been requested to include this information in SPC section 4.3. The general pharmacokinetic characteristics of the active substance that may be of clinical relevance to the target animal species for the treating veterinarian were adequately described in the SPC, although minor updates to the text had also been requested.

Based upon the data provided, it was concluded that following oral administration of sirolimus, absorption is rapid; however, oral bioavailability is low due to a high first pass effect in the liver and intestinal tract with a high degree of individual variability. Extensive metabolism occurs with the majority of sirolimus eliminated via metabolites in the faeces and minimal renal excretion. A precaution in the SPC informs that the VMP should not be prescribed in cats with known functional hepatic impairment to ensure safe and appropriate use.

It was noted that the species-specific information proposed for SPC section 4.3 correctly reflects the data derived from the target animal safety study in which the IVP was administered as proposed, and this was considered acceptable.

The pharmacokinetic dataset can be considered generally adequate with regard to rate and extent of exposure after administration of the proposed treatment dose, however it was noted that the available data do not allow for an evaluation of exposure versus effect (PK/PD response relationship) for the proposed indication in cats and were not adequate to make informed decisions on dose selection from an efficacy perspective.

Development of resistance and related risks in animals

Whilst sirolimus is a macrolide produced by *Streptomyces hygroscopicus*, published data supports the applicant's conclusion that it does not demonstrate antibacterial effects and therefore, the omission of resistance data can be accepted. Antiparasitic effects cannot be ruled out; however, in the context of this application, an influence on resistance development was not considered relevant.

Dose determination and confirmation

Dose justification

The applicant provided a summary of bibliographic data which was taken into consideration when determining the most appropriate dose regimen for the IVP.

From the data provided, it can be accepted that intermittent dosing regimens can serve to reduce the incidence of adverse effects associated with sirolimus (rapamycin) administration. Further, based on a

study conducted by O'Donnell et al. (2008), the applicant considered that a 10 mg everolimus weekly dosing regimen provided an optimal efficacy profile for the IVP, given that it resulted in full suppression of S6K1 (a protein kinase which induces protein synthesis and cell proliferation) for only part of the dosing period. However, the relevance of this information for establishing the appropriate dosing interval for sirolimus when used in cats for the management of HCM was unclear at Day 180 of the procedure.

With regards the actual dose rate, the applicant extrapolated data derived from use of the sirolimus (rapamycin) analogue everolimus in humans using a 1:1 conversion factor and applied allometric scaling. Whilst it can be accepted that the application of a conversion factor was evaluated (as detailed in a paper by Carvalho et al., 2011), comparability of efficacy in that study was based upon use of sirolimus and everolimus for the purpose of immunosuppression in renal transplant patients, whereas the IVP is proposed for the treatment of hypertrophic cardiomyopathy in cats. Therefore, the use of a conversion factor is not entirely applicable in this case. Limited information was provided as to the allometric scaling conducted by the applicant, however, it is noted that Nair et al. (2016), considers this approach most applicable for 'drugs with lesser hepatic metabolism, low volume of distribution, and excreted by renal route', which does not reflect the metabolism and excretion profile of the IVP. Generally, an allometric scaling approach is not suitable for a highly protein-bound active substance that undergoes extensive metabolism and active transport.

With regards the studies from which a 5 – 10 mg everolimus dose for humans was concluded by the applicant as optimal (O'Donnell et al., 2008, and Mannick et al., 2014), it was noted that these studies either evaluated use of everolimus in cancer treatment or evaluated its effect on immune function immunosenescence. Given the different active substance (everolimus vs sirolimus/rapamycin), species (humans vs cats) and indication (cancer therapy, immunosenescence vs HCM), the information from these studies is limited in relevance when establishing the appropriate dose for sirolimus when used in cats for the management of HCM.

The applicant referenced one paper in which the effects of rapamycin on age-related cardiac function was evaluated in dogs at dose rates of 0.05 mg/kg bw (n=5) or 0.1 mg/kg bw (n=10) three times weekly, with parameters evaluated including echocardiographic changes. The study authors claim that this study provides the first evidence that rapamycin may partially reverse age-related heart dysfunction in dogs by improving measures of both diastolic and systolic functions. Although the effects reported do not reach statistical significance ($p < 0.05$) for each measure (likely due to the relatively small sample size and high individual heterogeneity), all three of the outcomes (ejection fraction, fractional shortening and E/A ratio) showed trends toward improved function following rapamycin treatment. It can be accepted that this study provides some evidence that pharmacological inhibition of mTOR in older dogs may have effects on heart function that are consistent with a reversal of age-related functional changes. However, as concluded by the study authors, this would need to be confirmed in studies involving larger numbers of dogs and the clinical relevance of these effects would also need to be investigated (clinical relevance in terms of impact on survival and the onset and prevalence of various diseases that share aging as their common risk factor).

While the findings of the dog study cannot be considered definitive, they do provide some support that rapamycin has positive effects on cardiac function when administered to dogs at dose rates of 0.05 mg/kg bw or 0.1 mg/kg bw three times weekly.

Overall, the justification for the dose of sirolimus for use in cats was very weak. Although the applicant considers that these data were adequate to select doses for further investigation in the context of a pilot tolerance/dose determination study (0.15 mg, 0.45 mg and 0.75 mg rapamycin/kg bw administered three time weekly), the lack of robust justification for the proposed dose was a significant

deficiency that, at Day 180 of the procedure, was taken into account by the CVMP in the overall risk-benefit assessment for the candidate VMP.

Dose determination studies

For the purpose of dose determination, the applicant provided the results of a pilot study, which was not designed for that purpose, but rather to evaluate tolerance in cats when rapamycin is administered at 0.15 mg/kg, 0.45 mg/kg and 0.75 mg/kg three times weekly for a total of 12 doses administered over a 26 day period, and to gain information on toxicokinetics. This study is discussed in more detail below (see 'Tolerance in the target animal species'). However, it was noted that whilst in many cases adverse effects appeared to be observed across the treatment groups, for systolic blood pressure, a greater frequency of readings over baseline were observed in the middle dose and high dose treatment groups compared to the low dose group. Similarly for clinical pathology, an increased frequency of abnormalities was observed in the middle dose and high dose treatment groups over the low dose treatment group (0.15 mg/kg bw three times weekly). In light of the above findings, the applicant's selection of a comparatively low dose for further evaluation was considered reasonable (based on safety considerations only). The study does not allow for conclusions on efficacy.

Dose confirmation studies

For the purpose of dose confirmation, the applicant provided the results of a pilot clinical field trial designed to evaluate the safety of chronic sirolimus treatment when administered at two different dose rates, 0.3 mg/kg bw and 0.6 mg/kg bw and its effectiveness in reversing or slowing the progression of myocardial hypertrophy and consequent cardiac dysfunction. This study is discussed in more detail below (see clinical trials). However, when evaluating the study findings for the purpose of dose confirmation, it was noted that whilst an improved safety profile may have been demonstrated for the 0.3 mg/kg bw dose, upon analysis of individual safety data for study animals, there remains a concern that in both treated groups, animals experienced progression (echocardiographic and / or clinical signs) of HCM. This was considered relevant for concluding on the benefit-risk balance of the candidate product. With regards an improved efficacy profile for the 0.3 mg/kg bw dose, concern was raised regarding the *post hoc* selection of a primary efficacy parameter, particularly given that the efficacy parameters selected *a priori* failed to identify a statistically significant difference at study conclusion. It was noted that the applicant conducted an additional dose confirmation study in a feline model of HCM (investigating two doses 0.15 mg / kg and 0.3 mg / kg once weekly), although the provision of the study report was awaited before interpretation of the data arising from this study in the context of dose confirmation could be finalised. Notwithstanding this point, at Day 180 of the procedure the CVMP remained of the opinion that the data provided for the purpose of confirmation of the proposed dose regimen were weak and consequently that a major concern in respect of the overall benefit-risk balance of the candidate VMP remained outstanding.

Tolerance in the target animal species

The applicant provided the results of one pivotal and one pilot target animal tolerance study.

With regards the pivotal target animal safety study, this was a well conducted GLP compliant target animal safety study, largely in accordance with VICH GL43. A total of 32 healthy domestic short haired (DSH) cats (16 male and 16 female) were allocated to four study groups with each group comprising 4 males and 4 females, in accordance with VICH GL43. The animals were 10-11 months of age and whilst these could be considered young mature animals, adequate guidance concerning this point was provided under section 3.5 of the product SPC. Study animals ranged from 2.8 to 5.0 kg in bodyweight

(in the field trial conducted study animals ranged from 3.3 -14 kg, therefore a broad weight range would appear to have been evaluated overall). Sirolimus was administered on a weekly basis for 24 weeks, which is considered acceptable. The applicant advised that whilst the recommended dose is 0.3 mg sirolimus/kg bw, due to tablet strength constraints, the maximum dose that will be administered to some client-owned cats could be up to 0.38 mg sirolimus/kg bw. However, this dosage has been taken into account in this target animal safety study at approx. 1X, 3X and 5X of the highest recommended dose level (RTD), in accordance with VICH GL43. That said, in accordance with section 3.9 of the SPC for the product, the dose range proposed for the product is in fact 0.25 – 0.43 mg/kg bw, with animals weighing between 8.3 – 9.3 kg proposed to be administered dosages ranging from 0.39 – 0.43 mg sirolimus/kg bw. The safety of this additional margin was considered during the evaluation of any adverse effects observed at the higher dose groups for this study. The test article was administered orally with food provided both before and after administration, which can be accepted as consistent with the SPC which states that the IVP should be administered at time of feeding.

The study was both randomised and blinded, with study personnel conducting measurements while unaware of treatment allocation, in accordance with VICH GL43. Assessments included detailed physical examinations conducted by a veterinarian, daily general observations, clinical observations conducted on treatment days, diet consumption, body weight recording and blood pressure measurements, clinical pathology (haematology, clinical chemistry and urinalysis) with the inclusion of additional parameters relevant to the active substance and its recognised adverse effects (SDMA and glucose tolerance) and necropsy (pivotal study only) with both gross observations and histopathology. The parameters evaluated were considered sufficiently comprehensive both in terms of parameters evaluated and temporality.

It was noted under SPC section 3.6 for the IVP, that the applicant stated that elevated liver enzymes occur very commonly. However, a number of abnormalities were observed over the course of the study in all treatment groups, which were considered necessary for inclusion as adverse effects of the product. With regards to clinical observations, in the pivotal study, gastrointestinal signs were observed in one animal in the 1X treatment group and two animals in the 3X group during the study and it was noted that gastrointestinal signs such as diarrhoea were very common adverse effects noted when the active substance is used in humans.

Whilst reductions in bodyweight and feed intake were observed, the applicant concluded that these changes were neither statistically nor clinically significant. This conclusion was accepted by CVMP.

Systolic blood pressure was evaluated on a monthly basis over the course of the study. The conclusion that hypertension associated with sirolimus use is not a specific risk for the target animal species was accepted by CVMP.

With regards clinical pathology and focusing on haematological and coagulation parameters, the applicant concluded that although haemoglobin, MCHC and eosinophils were significantly lower and red cell distribution width (RDW), neutrophils, fibrinogen and platelet counts were significantly higher in the treatment groups compared to the control, these differences were not clinically significant and in many cases the values observed were within the reference range of the controls. However, with regards the RBC indices, it was noted that reductions in HCT were also observed in all treatment groups and whilst occasionally transient, low RBCs are a known adverse effect of the product in humans and categorised as ‘very commonly occurring’ in the SPC for the human product. Notwithstanding this point, the applicant’s conclusion that there exists no pattern to suggest that changes in red blood cell parameters in response to treatment should be captured as an adverse event in section 3.6 of the SPC was accepted by the CVMP. However, at Day 180 of the procedure, related information concerning this point was pending inclusion in section 3.10 of the SPC.

Additionally, it was noted that significant elevations in platelets and fibrinogen were observed in the treated groups for the pivotal study with concurrent reductions in prothrombin time (reductions observed in 2/8 control animals, 7/8 of the 1X RTD group, 8/8 of the 3X RTD group and 6/8 of the 5X RTD treatment group) with similar findings in the pilot TAS study. It was noted that blood clots are a common adverse effect for the human product and may also be a particular concern for this IVP given that a proportion of the target population may be pre-disposed to arterial thromboembolism. As was not conclusively demonstrated that treatment with sirolimus will *not* increase the risk of a thromboembolic event in a subpopulation known to already be at risk of coagulopathy, and the available safety data include clinical indicators that this risk may be increased (to some degree), at Day 180 of the procedure the applicant was requested to provide relevant information concerning this point in section 3.5 of the SPC, noting this point was also considered in the overall benefit-risk analysis for the candidate product.

For all other haematological parameters, the applicant concluded that no statistically significant differences were observed; however, when taking individual values into consideration, reduction in lymphocytes were observed in all treatment groups for the pivotal study with values dropping as low as 1.13 x 10^9/L (reference ranges indicating a normal range of 1.5 – 7 x 10^9/L). As discussed under pharmacodynamics, the immunosuppressant properties of the active substance are a targeted pharmacodynamic effect in human medicine and reductions in white blood cell parameters are listed as very common adverse effects for humans in the SPC for the authorised human medicinal product, along with observations of infections. The significance of this observation was further evaluated, taking into consideration the impact of an increased risk of infection on pre-existing cardiovascular disease. The CVMP considered the argumentation provided sufficient to conclude that the extent of the reduced lymphocyte counts observed in both the pilot and pivotal TAS studies, if these changes were treatment related (noting that there does not appear to be a clear dose-relationship associated with the findings), does not indicate an increased risk of infection when cats are dosed according to the proposed dosing regimen. This conclusion was supported by the literature references provided, and the observation that no signs of clinically significant immune suppression were observed in the animals treated with sirolimus in the studies provided with this application. The same conclusion was made with regard to potential impact on vaccinations or tumour promoting effects.

With regards biochemical parameters and hepatic enzymes, elevations in AST, ALT and LDH were observed in the treated groups and whilst elevations in these parameters were on occasion also observed in the control group, they were of a lesser magnitude. It is additionally noted that elevations in liver enzymes are reported as very common adverse effects in humans in the SPC for the authorised human medicinal product. 'Elevated liver enzymes' was included in the proposed SPC as a 'very common' adverse event. Elevations in cholesterol levels and triglycerides were also observed, although there was insufficient evidence that this is treatment-related to support inclusion of information in the SPC.

Phosphate levels were reduced both from baseline in all treatment groups and compared to the control group whilst potassium was reduced in the 1X and 3X RTD group compared to control and reduced in all groups from baseline. As for other parameters, low blood potassium and low blood phosphorus are recognised adverse effects of the active substance in humans as described in the SPC for the authorised human medicinal product. It was concluded based on available data, that changes in potassium and phosphorous do not merit inclusion in section 3.6 of the SPC, but that information in section 3.10 concerning reductions in blood phosphorous levels following overdose with sirolimus was justified.

With regards abnormalities observed at necropsy, it was noted that mean weights for spleen and liver and gallbladder were reduced for the treatment groups compared to the control. However, the CVMP

could accept the conclusion of the applicant that there is no clinically relevant impact of the trend of reduced splenic weights and that no causal association between splenic weight and haematological changes can be determined. A trend in reduced thyroid and parathyroid weight was observed with dose increments and considered clinically insignificant. Specifically, for the left thyroid and parathyroid gland, mean weights were 0.26 g, 0.23 g, 0.17 g and 0.20 g and for the right thyroid gland and parathyroid, weights were 0.22 g, 0.22 g, 0.178 g and 0.17 g for groups 1, 2, 3 and 4, respectively.

The applicant acknowledged changes in absolute testes weight which reached statistical significance for the 3X RTD and 5X RTD groups; however, as a statistically significant difference was not observed for relative testes weight and histopathological changes were not observed, the organ weight differences were considered incidental. However, as a dose-relationship was observed and this would appear consistent with reports in the literature of reduced testicular weight in both humans and mice administered rapamycin on a daily or intermittent basis, suitable information was included in section 3.10 of the SPC. It was noted that no NOEL could be derived under the conditions of the study.

The applicant additionally presented the results of a pilot target animal tolerance study, which was both randomised and blinded and included 32 healthy DSH cats (11 male and 21 female) ranging from 2.4 – 4.5 years of age and 3.2 – 5.8 kg bw. The animals were randomised to 4 groups with 8 animals included per group; group T0 was the control group (orally administered placebo), group T1 were administered approximately 0.15 mg sirolimus/kg bw (i.e. 0.5 x RTD), group T3 were administered approximately 0.45 mg sirolimus/kg bw (approx. 1.5 x RTD) and group T5 were administered 0.75 mg sirolimus/kg bw (2.5 x RTD). Treatments were administered three times weekly over a 4-week period, with the animals subsequently allowed a 4-week recovery period over which observations were made and clinical pathology samples were collected for analysis. It was noted that the housing / husbandry conditions (specifically cage size) in the pilot TAS study were not compliant with the requirements of Directive 2010/63.

Assessments included veterinary examinations, daily clinical observations, checks for vomit on treatment days, dietary consumption, body weight recording and blood pressure measurements; the parameters evaluated, and the timing of measurements were considered sufficiently comprehensive. Additionally, the parameters evaluated for haematology, clinical chemistry and urinalysis were considered sufficiently comprehensive for the purpose of assessing tolerance to the dose range and included additional parameters relevant to the active substance and its recognised adverse effects (SDMA, fructosamine and glucose tolerance).

With regards findings considered of importance, suitable information was included in the SPC or requested of the applicant.

Based on the findings of the target animal safety studies, there appear to be no adverse effects of treatment on measures of glucose metabolism. However, available data in humans indicates that insulin resistance and diabetes mellitus may be observed consequent to sirolimus treatment. On this point, it was noted that diabetes mellitus (DM) was diagnosed in one sirolimus-treated cat in the field trial presented with this application. Notwithstanding the findings of the target animal safety studies (conducted in a small number of cats), the occurrence of DM in one treated cat in the clinical field trial is concerning and was addressed by the inclusion of a contraindication for treatment of cats with DM, and suitable advice concerning monitoring in the relevant sections of the SPC. This point was also considered in the overall benefit-risk analysis for the candidate product.

Sirolimus is a substrate of P-glycoprotein; it is therefore possible that this could lead to adverse effects other than those noted in the studies provided. However, it was concluded that no substantial impact on the benefit-risk balance for MDR-1 -/- cats could be identified compared to wildtype or +/- cats. Information that sirolimus is a substrate of P-glycoprotein was provided in section 3.8 of the SPC.

Overall, the pivotal and pilot TAS studies were considered to have been well-designed and conducted. However, a number of abnormalities common to both studies were observed, consistent with known adverse effects for the active substance in humans and rodents, along with observations from the clinical field trial. Consequently, at Day 180 of the procedure concerns remained outstanding on target animal safety, which would have required satisfactory resolution before a final conclusion on target animal tolerance and overall benefit-risk of the candidate product could be made.

The product was not recommended for use in pregnant and lactating animals, or in breeding animals. This recommendation is adequate in the view of the data available.

Clinical trial(s)

Sirolimus TriviumVet was proposed for the treatment of left ventricular hypertrophy associated with subclinical hypertrophic cardiomyopathy (HCM) in cats and was to be presented in gastro-resistant tablets containing 0.4 mg, 1.2 mg and 2.4 mg sirolimus. Although the three different strengths of tablets are not quantitatively proportional in their composition of ingredients, bioequivalence was largely justified, although at Day 180 of the procedure further information concerning the related dissolution studies had been requested.

The IVP was proposed for life-long treatment and was to be orally administered once weekly at an approximate dose rate of 0.3 mg sirolimus/kg bw. The tablets should be administered whole at time of feeding.

In support of the efficacy of the IVP, the applicant provided the results of one pilot clinical trial and referenced a number of bibliographic publications.

Clinical trial

With regards the clinical field trial conducted, it was a blinded, randomised, placebo-controlled study. Whilst GCP-compliant, it was notable that the study was planned as a pilot study and some aspects of how the study was conducted deviated from the study protocol.

Although the field study was conducted outside the EU (US), given the nature of the condition for which the product is proposed to treat (HCM in cats), the geographical location of the study was not considered important. That is, it can be accepted that the findings of this study could be extrapolated to the EU situation. It was confirmed that the formulation of the test article used for this study was the final formulation, however relevant certificates of analysis for each tablet strength used had been requested at Day 180 of the procedure.

Study group sizes were justified based upon a power analysis intended to identify clinically relevant differences from baseline and post-treatment echocardiographic assessment of left ventricular (LV) wall thickness. For this analysis, the applicant considered that a detectable difference of 0.5 mm within LV wall thickness would be clinically relevant. At Day 180 of the procedure, the basis for this assumption remained unclear.

A total of 43 client owned cats (37 males and 6 females) of different breeds and encompassing a broad age and weight range were enrolled in the study. Although some pedigree breeds of cat are considered to be at an increased risk of HCM and two mutations have been identified in the myosin binding protein C gene in cats with HCM, no preselection for cats with the mutations in question took place when selecting cats for enrolment in the trial, and no subgroup analyses were performed.

It is noted that the candidate product was initially proposed for the management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy in cats; however, the animals enrolled in the study were subclinical cases of HCM with no evidence of congestive heart failure and were otherwise

healthy. The study population was not staged further to cats at low or high risk to develop CHF, although, according to the ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats: subclinical cats can be further staged as B1 or B2 according to their low or higher risk. It is unclear if study groups were balanced with equally affected cats in placebo, low dose and high dose group, especially when it is taken into account that the mean baseline feline NTproBNP values (a peptide released in response to changes in pressure within the heart) which may correlate with the severity of the disease, differed between groups (859.43 pmol/L (placebo), 452.47 pmol/L (low dose IVP) and 649.73 pmol/L (high dose IVP)). Given that no safety or efficacy data was provided for the IVP when used in clinical cases of HCM, the most appropriate sub-group of feline HCM cases was reconsidered for the product indication and at Day 180 of the procedure it was agreed that the product should be indicated for use in cats with subclinical HCM only.

The animals were randomised to three groups, Group 1 was administered placebo, Group 2 was administered sirolimus at the recommended dose rate of approximately 0.3 mg/kg bw and Group 3 was administered a higher dose rate of approximately 0.6 mg sirolimus/kg bw.

In accordance with the SPC, animals were orally administered the test article in the fed state once weekly for 180 days. However, it was noted that for animals requiring two tablets at each dose, the dose could be divided with administration over a 12-hour timeframe, which was not consistent with the guidance provided in the SPC. The applicant provided further reassurance that this approach should have a minimal impact on bioavailability*;* however it could not be ruled out that dosing with an interval of up to 12 hours may have influenced exposure to sirolimus and hence the AUC when compared to dosing on a single occasion. At Day 180 of the procedure, this possible deviation from the guidance given in SPC section 3.9 was accounted for in the outstanding major concern addressing the overall benefit/risk assessment.

The study was blinded with both owners and clinic personnel involved in assessments. Whilst it could be accepted that the assessments conducted were largely appropriate for the evaluation of safety, with regards the primary efficacy parameters, concerns were raised by CVMP regarding their clinical relevance in the proposed population intended for treatment with the candidate VMP, and these concerns remained unresolved at Day 180 of the procedure.

This study was originally intended as a pilot study and the intended approach to statistical analysis was not fully documented in advance of study conduct, reducing confidence in the results derived from the study. As a consequence, a number of statistical questions were raised at Day 180 of the procedure. That said, it was noted that the parameters stated to be of primary interest in the study protocol (interventricular septum thickness at end-diastole (IVSd), left ventricular posterior wall at end-diastole (LVPWd) and maximum IVSd and LVPWd), included those later evaluated, with the same conclusion that a difference ≥0.5mm between measurements obtained could be considered clinically significant.

There was no precise definition of the *post hoc* defined primary parameter and of the secondary parameters. Moreover, their use was not consistent throughout the study. The primary efficacy parameter chosen by the applicant *a posteriori* was maximum wall thickness (MWT) of the left ventricle, with values obtained for end-diastolic MWT of the interventricular septum and left ventricle posterior wall (values for this parameter had not been provided by Day 180 of the procedure). In case of the primary efficacy parameter, it was stated by the applicant that 'the appropriate frame for obtaining a maximum wall thickness measurement is the frame which occurs at the onset of the QRS complex if ECG is used. This is also the frame immediately following mitral valve closure which can be observed ultrasonographically. The maximum wall thickness value recorded is the greatest measured value of either IVSd or LVPWd obtainable on this frame.'

Although M-mode and 2D-mode were allowed for evaluation of the inclusion criterion for the study, and it is known from human literature that 2D-mode echocardiography is more repeatable than M-

mode, it was accepted that use of one or other mode at study enrolment was not likely to have affected study results. It was noted that while no reference interval for maximal end-diastolic LV wall thickness is universally accepted, for the majority of normally-sized cats, an end-diastolic LV wall thickness <5 mm is considered normal, and ≥6 mm is indicative of hypertrophy (ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats). That said, the consensus statement goes on to add that in the context of screening animals for HCM "…. *It is overly simplistic to expect a single cut-off value for wall thickness to differentiate a normal ventricle from a hypertrophied ventricle*", which in the opinion of the CVMP would call into question the reliability of using MWT as the primary efficacy parameter. The extent to which LV MWT correlates with progression of HCM remained unclear (noting that many cats diagnosed with subclinical HCM will not progress to clinical disease and live normal, healthy lives, and noting in particular that in the placebo group two cats experienced a spontaneous mean reduction in MWT). In light of these and similar arguments suggesting a high variability of diagnostic criteria of HCM, at Day 180 of the procedure the applicant had been requested to further justify the choice of LV MWT as the primary efficacy parameter since suitable justification had not been provided up to that point.

Echocardiograms were conducted at baseline (Day 0, no significant differences between study groups observed), Day 60 and 180. At study day 180, a significant difference in maximum left ventricular wall thickness was detected for the 0.3 mg/kg bw group (p=0.013) and the pooled IVP group (p=0.0538), albeit, not for the 0.6 mg/kg bw group (p=0.3602). Based on this finding, the applicant concludes that sirolimus, when administered at a dose of 0.3 mg/kg bw to HCM cats, causes a reduction or stabilisation of left ventricular hypertrophy. However, when all data are considered, the findings supporting efficacy were not convincing for the following reasons:

- The appropriateness of the chosen primary efficacy endpoint, in terms of clinical relevance, remained unclear (see comment above).
- The chosen primary endpoint was not specified *a priori.*
- Tests for differences between groups were performed with a significance level of alpha=0.1 which contradicts the specification of the type I error rate in the applicable guideline (5% in case of two-sided tests, 2.5% for one-sided tests). For the chosen endpoint, a statistically significant effect (p<0.05) was claimed at Day 180 for the 0.3 mg/kg bw group only. A statistically significant effect was not detected for the higher dose group. Upon questioning, it was reported that the Bonferroni test-adjusted alpha for MWT (m = 2 contrasts) in the low-dose group versus the placebo group at Study Day 180 remains significant at p = 0.0264. However, in view of the large number of statistical tests (four *a priori* parameters of primary interest, one post-hoc primary parameter, all tested for two treatment groups at two different time points, partly also for treatment groups or time points pooled), this adjustment was considered not sufficient and the claimed significance considered not valid.
- For the parameters IVSd and LVPWd, which were specified *a priori* as parameters of primary interest, statistical significance was not observed for either treatment group at Day 60 or 180. Indeed, when focusing on the descriptive statistical analysis, it was noted that for some parameters, improvements were seen at Day 60 but not Day 180 (for the 0.3 mg/kg bw group: success for maximum IVSD at Day 60 was 86.7% but it was 71.4% at Day 180; at Day 60 success for Maximum LVPWD was 73.3% and at day 180 it was 57.1%; at day 60 maximum wall thickness at any location was 86.7%, and at day 180 it was 78.6%).
- An effect on atrial size was not detected, and although this parameter seems to be a prognostic indicator for progression of HCM insufficiency, it was noted that in a subclinical population of cats

(as were selected for the trial in question), substantial LA enlargement was not evident at the beginning of the study, and as such, no improvement in this parameter would have been expected.

- Although a large number of statistical tests was performed, the applicant did not correct for multiple tests until prompted to do so for the primary efficacy variable MWT (and this was not done sufficiently – see above).

At Day 180 of the procedure, the CVMP had an outstanding major concern that the available efficacy data do not robustly support the proposed (amended) indication.

Regardless of the above and as earlier noted, a further limitation of this study was the small number of animals enrolled (n=43); efficacy was based on the per-protocol population, however, withdrawal of animals due to SAEs and deviations from the protocol led to only 36 animals being included in the final analysis at Day 180 (Group 1 = 12 animals, Group 2 = 14 animals, Group 3 = 10 animals). This potentially reduces the relevance of any observations of significance at Day 180 and extrapolation to the general cat population.

Separately, it was noted that the applicant concluded that a difference of ≥0.5mm between measurements obtained would be considered clinically significant. Aside from indicating that this measurement would extend beyond biological variability, no justification as to the clinical relevance of this measurement was provided by Day 180 of the procedure. Furthermore, the pilot study was conducted in subclinical HCM cats and from the published literature provided, it would appear that many of these cats would be expected to live a normal or near normal life (Kittleson et al., 2021). Generally, no treatment was recommended in the relevant literature to date, taking into account that survival time is highly variable and not conclusively correlated with ventricular wall thickness. These cats should be closely monitored as the benefits of a therapy have not yet been proven and lifelong therapy can itself cause significant stress. In addition, Riya et al., 2018 concluded that diastolic dysfunction in HCM may not entirely be related to wall thickening.

Parameters that indicate progression, prognosis and response to therapy of the disease such as measures of diastolic function were insufficiently considered. These indicators are subject to change early in the course of HCM before the chamber is remodelled and may provide information for a meaningful benefit-risk balance. The applicant contends that taking into account 'the subclinical stage of this cat population, employing diastolic function as a primary endpoint would have yielded little value over the six-month assessment period. Such an endpoint would not have been sensitive enough to capture functional changes between these study populations.'

Reviewing the safety and efficacy data derived from this study, it was noted that the quality of life assessment failed to identify a difference between treatment groups at any point in the study, whilst a higher incidence of new cardiovascular system abnormalities were observed for the treated groups over the controls and, at conclusion of the study, three serious adverse events associated with the cardiovascular system occurred in the 0.6 mg/kg bw group (two of which culminated in death – one associated with CHF, another after a seizure which was preceded by syncopal episodes 2-3 months prior), whereas no serious adverse cardiovascular effects were observed in the control group. A treatment-related association could not be excluded in these three cases. Furthermore, upon receipt of more detail concerning non-serious adverse events in the study animals, clinically relevant changes in cardiac parameters were observed in a number of animals (particularly in the high dose group) during the course of the study – adverse events that were not categorised as serious. The CVMP considers this information highly relevant to the benefit-risk assessment of the candidate VMP, and notes that although LA enlargement in one control animal, and some murmurs and arrhythmias were reported in the control group, that the same pattern of progressive HCM does not appear to have been observed in this group of untreated animals. The CVMP considers that these data lend further support to the

concerns that 1) evaluation of efficacy based on the primary efficacy parameter (maximum ventricular wall thickness) may not be predictive of negative clinical outcomes, and 2) use of the candidate VMP in cats with subclinical HCM may in fact be associated with acceleration or decompensation of cardiac-related clinical signs. In addition, DM was diagnosed in one treated cat in the field study. This observation is concerning noting that available data in humans indicates that insulin resistance and diabetes mellitus may be observed consequent to sirolimus treatment and a suitable contra-indication for use in cats with DM has been included in the SPC. Overall, and in view of the serious adverse events reported, at Day 180 of the procedure it was unclear to CVMP how the findings of this study could be considered to support a positive benefit-risk balance when administered to HCM cats with subclinical disease.

With regards the secondary efficacy parameters, although based on the mean data provided it would appear that an increase in heart rate, potentially dose proportional, was observed for the treated groups but not the controls, it was concluded that this difference was not clinically significant.

The applicant reported that no significant differences were observed between groups at any timepoint for any of the clinical pathology parameters evaluated. However, given the small sample size, a non-significant result would not necessarily preclude a safety concern. Given the adverse effects known for the active substance in other species, the clinical pathology findings derived from the proprietary target animal safety studies and the differences in mean values observed for cholesterol, blood glucose, platelets and white blood cells, the provision of individual clinical pathology data for all animals included in the clinical study was requested and provided by the applicant.

The applicant evaluated NTproBNP at baseline and Day 180 and whilst it was determined that significant differences were not observed between treatment groups, the applicant concluded that higher baseline values positively correlated with increased baseline MWT, and more pronounced progression at baseline levels above the laboratory range was observed for the placebo cats over those administered the IVP. However, it was noted from the data provided that increases were observed in the IVP groups (mean values for the 0.6 mg/kg bw group from baseline to Day 180: 649–73 - 730.62-741–60 - 841.55; values for the 0.3 mg/kg bw group from baseline to Day 180: 452–47 - 466–60 - 471–33 - 535.57), whereas a reduction was observed in the control group (values for the control group: 859–43 - 790–00 - 714–23 - 774.50). It was noted that this enzyme can be indicative of CHF, a condition that developed in two animals in the high dose group. The applicant further considered this observation in order to conclude on whether it signals an additional risk of cardiovascular disease/disease progression. The CVMP accepted that the trends for NTproBNP are somewhat difficult to interpret and as such, it was also considered appropriate that NTproBNP was not used as an efficacy endpoint in the clinical efficacy trial. Cardiac Troponin I was also evaluated and whilst it is acknowledged that at Day 180, values in the placebo group were higher than values in the IVP groups, this was also the case at baseline; whilst a reduction in values was observed in all groups from baseline to Day 180, it was noted that a greater percentage reduction was observed in the treated groups compared to the control.

Tolerance:

With regards serious adverse events, cardiovascular effects (syncope and congestive heart failure) were observed in the 0.6 mg/kg bw dose group with none in the controls. One serious adverse event did occur in the 0.3 mg/kg bw group with consequent cardiac arrest; however, this was due to the development of diabetic ketoacidosis in a cat which had been showing signs of diabetes mellitus over the course of the study. A contraindication was included in the proposed SPC that the product is not to be used in cats with diabetes mellitus along with suitable information concerning monitoring. It is also noted that digestive tract disorders, endocrine system disorders, renal and urinary disorders and systemic disorders were reported for the IVP, some of which may have been consistent with the

adverse effects known for the active substance in other species and observed in the other proprietary studies presented. Further detail on the non-serious adverse events reported was provided by the applicant, as well as information on those adverse events which were concluded as probably or possibly product related. However further critical evaluation of these events was not provided by the applicant, and at Day 180 of the procedure, the CVMP had requested the applicant take these data into account in the overall benefit-risk assessment.

Ultimately, a number of major concerns in respect of the design, conduct and findings from this study remained, and as such final conclusions on field safety and efficacy of the product could not be made by CVMP at Day 180 of the procedure.

In further support of clinical efficacy, the applicant provided bibliographic data describing studies conducted in human and feline cases of hypertrophic cardiomyopathy. Whilst the data derived could be considered to support the applicants argument that measurement of maximal end-diastolic wall thickness may be an appropriate diagnostic tool for the diagnosis of HCM in cats (Dohy *et al*., 2021, Elliott *et al*., 2001, Payne *et al*., 2013) and links increasing ventricular wall thickness in HCM with an increased risk of mortality (Jackson *et al*., 2014), it also indicates that diastolic dysfunction in HCM may not be entirely related to wall thickening (Riya *et al*., 2018). Furthermore, in the study described by Payne *et al*., 2013, median survival time of asymptomatic cats that were not administered cardiac medications was 2171 days (range 8-2755), whereas in the pilot field trial which was conducted over the shorter duration of 6 months, no deaths were observed in the placebo group but one potentially cardiac-associated death (history of syncope) and 2 incidences of congestive heart failure were observed in the high dose group, one of which culminated in death. The study described by Fox *et al*., 1995, allows comparison of survival rates for subclinical and clinical HCM for up to 49 months, with a 76% survival rate reported for HCM cats that were asymptomatic at enrolment and were not lost to follow-up, compared to a 23% survival rate for HCM cats that were symptomatic at enrolment. When comparing the study findings derived from the asymptomatic cats included in this study with the findings derived from the clinical field trial which included only subclinical cats, it was noted that in this study 13 out of 17 cats asymptomatic at enrolment survived up until 49 months (it was unclear whether the deaths observed were associated with cardiovascular disease), whereas in the pilot field trial which was conducted over the shorter duration of 6 months, no deaths were observed in the placebo group but one potentially cardiac-associated death (history of syncope) and 1 CHF-associated death occurred in the high dose group. Also, in the high dose group, another animal was withdrawn from the study due to CHF. In addition, 19 adverse cardiovascular events were reported in the treatment groups and 9 in the negative control group. The differing incidences of deaths and cardiovascular adverse events observed between the subclinical/asymptomatic cats administered sirolimus or left untreated, were considered in the benefit-risk balance analysis for the candidate product.

In conclusion, Day 180 of the procedure the data package provided in support of safety and efficacy was considered inadequate and a major concern had been raised in relation to the overall benefit-risk balance of the candidate VMP, along with a related major concern regarding the (updated) indication as proposed.

New active substance (NAS) status

Not assessed under Part 4.

Overall conclusions on efficacy

Pharmacology

Pharmacodynamics

Sirolimus TriviumVet contains sirolimus as the active substance: an inhibitor of the mTOR protein complex, primarily causing inhibition of mTORC1 with consequent impacts on protein synthesis, cell growth and proliferation, autophagy, cell metabolism and stress responses. At high or chronic dosing, sirolimus may cause inhibition of mTORC2, a regulator of cell survival and polarity. The targeted pharmacodynamic effect for the candidate product is the inhibition of cardiac hypertrophy associated with hypertrophic cardiomyopathy in cats, whilst retaining the capacity of the heart to adapt to load. With inhibition of mTORC1 and mTORC2, inhibition of hypertrophy will be observed, but so will impairment of the heart's capacity to adapt to stress. Therefore, selective inhibition of mTORC1 is preferable.

Study data presented with the application provided evidence of positive effects of sirolimus on heart muscle structure and function in rodents and humans: however, it was noted that neither sirolimus nor any other mTOR inhibitor are authorised for the management of cardiac disease/disfunction in humans (that is, the effects observed have not translated into an authorised indication). Further, it was noted that none of the data on pharmacodynamic effects were generated in the target species, the cat.

With regards secondary pharmacodynamic effects, it was noted that in human medicine, sirolimus is administered as an immunosuppressant for the prevention of transplant rejection.

The study data presented indicates that diabetes mellitus, hyperlipidaemia (with consequent hypertriglyceridemia) and vasculitis may be observed consequent to sirolimus administration. These effects may be dose-dependent and reversible with cessation of treatment.

At Day 180 of the procedure, updates to the text proposed for inclusion in section 4.2 of the SPC had been requested such that the text reflects the mechanism of action in the target species, or, in the absence of relevant data in cats, makes clear reference to the species in which the pharmacodynamic data were generated.

Pharmacokinetics

Based upon the pharmacokinetic data provided it could be accepted that following oral administration of sirolimus, absorption is rapid, however, oral bioavailability is low due to a high first pass effect in the liver and intestinal tract with a high degree of individual variability. Extensive metabolism occurs with the majority of sirolimus eliminated via metabolites in the faeces and minimal renal excretion.

Concerning pharmacokinetics in the target animal species cats, based on the results of limited pharmacokinetic data provided, it was postulated that the rate of absorption of sirolimus can be saturated at dosing levels higher than the RTD. It also appeared that the pharmacokinetics of sirolimus in cats are non-linear, although the extent of this non-linearity was not fully investigated. These observations are largely in keeping with observations in humans and laboratory species.

While the pharmacokinetic dataset could be considered generally adequate, it was noted that the available data did not allow for an evaluation of exposure versus effect (PK/PD response relationship) for the proposed indication in cats and were not adequate to make informed decisions on dose selection from an efficacy perspective.

Development of resistance and related risks to animals

Whilst sirolimus is a macrolide produced by *Streptomyces hygroscopicus*, published data concludes that it does not demonstrate antibacterial effects and therefore, the omission of resistance data was acceptable.

Dose determination and confirmation

Dose justification was based on bibliographic data, a pilot tolerance study and a pilot field trial.

Based upon the results derived from the pilot tolerance study in which rapamycin was administered at 0.15 mg/kg, 0.45 mg/kg and 0.75 mg/kg three times weekly for a total of 12 doses administered over a 26-day period, the applicant's selection of a weekly 0.3 mg/kg dose for further evaluation, was considered reasonable (based on target animal safety considerations).

The pilot field trial evaluated the safety of chronic sirolimus treatment when administered at two different dose rates, 0.3 mg/kg and 0.6 mg/kg bw and its effectiveness in reversing or slowing the progression of myocardial hypertrophy and consequent cardiac dysfunction. Whilst an improved safety profile appeared to have been demonstrated for the 0.3 mg/kg dose, no NOEL could be set and the provision of safety parameter data for individual animals appear to indicate a narrow margin of safety for the VMP in cats with subclinical HCM. With regards an improved efficacy profile for the 0.3 mg/kg dose, at Day 180 of the procedure concerns remained regarding the primary efficacy parameter selected and its clinical relevance, the statistical methods used, the reliability of the data derived from the study, and it is also noted (and now included in section 3.2 of the SPC) that the sub-group of HCM cats enrolled in the study were subclinical only. These concerns remained outstanding and precluded a final conclusion on the most appropriate dose for the product.

Tolerance in the target animal species

In the pivotal TAS study, some adverse effects were seen at all dose rates and were consistent with those observed in the pilot TAS study, the clinical field trial and adverse effects known for the active substance in other species. The applicant concluded that for cats, elevated liver enzymes is an adverse event for the product, however, given the type and incidences of adverse events observed, particularly in cats with subclinical HCM, at Day 180 of the procedure the applicant had been requested to provide further evaluation of the safety data from the clinical field trial in order to justify the omission of further data on adverse events from SPC section 3.6; in addition, the benefit-risk balance remained under consideration, inclusive of the risk of arterial thromboembolism, and treatment of cats with or at risk of diabetes mellitus. A major concern regarding the benefit-risk balance had been raised at Day 180 of the procedure.

Clinical trials

The applicant provided the results of a pilot clinical field trial in support of candidate product efficacy for the originally proposed indication, that is, the management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM) in cats. However, a number of major concerns remained at Day 180 of the procedure regarding the adequacy of the data provided in support of the proposed indication in the target population.

These include the appropriateness of the chosen primary efficacy parameter (maximum wall thickness of the left ventricle) including its clinical relevance; the representativeness of the test population (cats with subclinical HCM) for the potential target population (based on the proposed indication) and safety of long-term treatment in this subpopulation of cats (who are apparently clinically healthy at presentation).

In addition, the adequacy of the data package provided is questionable given that only a pilot field trial was presented in support of efficacy, the study comprised a small sample size with complete statistical analysis and primary efficacy variable not defined *a-priori,* and no substantive further data had been provided to conclude that study was demonstrative of safety in cats with subclinical HCM, and clinically meaningful efficacy. Another major deficiency identified with this application is that the product is proposed for the management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM) in cats, although the only proprietary efficacy data provided specific to HCM cats relates to subclinical disease, which given the absence of clinical signs is unlikely to be diagnosed during routine veterinary practice. The proposed product indication now restricts use to subclinical cases of HCM. This raises a concern about potential off label use in cats with clinical HCM given that no other product is authorised for the management of ventricular wall hypertrophy in HCM cats, with safety and efficacy of the IVP not having been evaluated in cases of clinical disease, and in the face of some indicators that treatment with the candidate product may cause or precipitate decompensation in cats with subclinical HCM, and onset of CHF.

In conclusion, given the significant short-comings of the efficacy data package presented in support of this application, and the fact that there remained significant concerns in relation to target animal safety, at Day 180 of the procedure the CVMP was unable to conclude on a positive benefit-risk balance for the candidate VMP. The applicant was at that time requested to provide a comprehensive benefit-risk assessment addressing the main outstanding concerns, as detailed above, and convincingly argue that administration of the product once weekly at a dose of 0.3 mg/kg to cats presenting with subclinical HCM will be successful at delaying progression to/onset of clinical disease and does not pose an unacceptable risk to treated animals. Furthermore, the applicant was requested to reconsider the wording of the indication to better reflect expected clinical outcome in the intended target population.

Part 5 – Benefit-risk assessment

Sirolimus TriviumVet was a gastro-resistant capsule containing 0.4 mg, 1.2 mg, and 2.4 mg of sirolimus. The active substance is innovative as it had not been authorised as a veterinary medicinal product within the Union at the date of the submission of the application.

The active substance, sirolimus, is a macrolide compound which inhibits mTOR complex 1 (mTORC1), a protein that is part of a signalling pathway regulating cell growth, autophagy, and cell death. According to the applicant, intermittent dosing with sirolimus specifically inhibits mTOR complex 1 (mTORC1), which results in a reduction in cardiac hypertrophy, an increase in mitophagy and autophagy and a reduction in oxidative stress. At the time of submission, the product was intended for use in cats for the management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM).

The application was submitted in accordance with Article 8 of Regulation (EU) 2019/6 - full application.

Benefit assessment

Direct benefit

Sirolimus was claimed as a new active substance for veterinary medicine, proposed for the management of hypertrophic cardiomyopathy in cats. No other product was authorised for this

condition in cats within the EU at the time of withdrawal of the application. However, in the presence of major and other concerns the direct therapeutic benefit could not be accepted.

Additional benefits

Sirolimus was proposed for once weekly administration, which would have facilitated good administration compliance.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and finished product was generally presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. However, at the time of withdrawal of the application there remained a number of questions regarding product development, the manufacturing process, the control of the starting materials and the control of the dosage form.

Deficiencies also arose from concerns over the (confidential) Restricted part of the ASMF. These concerns were conveyed in confidence to the ASMF holder.

Safety

Risks for the target animal

The risk of using sirolimus for hypertrophic cardiomyopathy in cats, include that its safety was not evaluated in clinical cases of the disease. Furthermore, cases of congestive heart failure and diabetes mellitus were observed following use of the product in subclinical cases of hypertrophic cardiomyopathy and adverse effects, not listed in the product literature but known for the active substance when used in other species, were observed in the target animal safety and field studies presented (and were requested to be reflected in the product information). In the presence of major and other concerns, at the time of withdrawal of the application no conclusions could be taken on the actual risk for the target animal.

Risk for the user

The most severe risk is accidental ingestion by a child. The product was intended to be marketed in child-resistant packages. Pending minor amendments to the product information, the CVMP concluded that user safety for this product could be acceptable when used according to the SPC recommendations.

Risk for the environment

Sirolimus TriviumVet would not be expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal was included in the SPC.

Risk management or mitigation measures

At the time of withdrawal of the application, risk management or mitigation measures were considered pending additional information from the applicant.

User safety

User safety risks have been identified, mainly the risks associated with exposure in children. These risks were mitigated by the presentation of the product in a child-resistant packaging. Warnings were included in the SPC, although these would have required minor amendment.

Environmental safety

No risks to the environment were identified under the conditions of normal use.

Conditions or restrictions as regards the supply or safe and effective use of the VMP concerned, including the classification (prescription status)

The veterinary medicinal product would have been subject to a veterinary prescription.

Pharmacovigilance

The MAH was required to record in the pharmacovigilance database all results and outcomes of the signal management process, including a conclusion on the benefit-risk balance. At the time of withdrawal of the application it was decided that the frequency would be determined once a conclusion on safety in the target animal species had been reached.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication: ”Management of left ventricular hypertrophy associated with hypertrophic cardiomyopathy (HCM) in cats.” In the presence of major and other concerns, no conclusions could be taken on the benefit-risk balance of the application at the time of withdrawal.

The product information had been reviewed and changes were considered necessary.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Veterinary Medicinal Products (CVMP) considered that the application for Sirolimus TriviumVet was not approvable at the time of withdrawal of the application since "major objections" and “other concerns” were identified which precluded a recommendation for marketing authorisation. At the time of withdrawal of the application, no conclusions could be taken on the benefit-risk balance of the application.