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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Stelfonta (EMEA/V/C/005018/0000)

INN: tigilanol tiglate

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

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Introduction

The applicant QBiotics Netherlands B.V. submitted on 3 October 2018 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Stelfonta, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 19 April 2018 as Stelfonta contains a new active substance (tigilanol tiglate), which is not yet authorised as a veterinary medicinal product in the Union.

The applicant applied for the following indication: "For the treatment of all non-metastatic (WHO staging) cutaneous mast cell tumours, and subcutaneous mast cell tumours located at or distal to the elbow or the hock in dogs. Tumours may be of any cytological grade and must be accessible to intratumoral injection".

The active substance of Stelfonta is tigilanol tiglate, an antineoplastic agent that activates the protein kinase C (PKC) signalling cascade; in addition, necrosis is induced in cells that are in direct contact with the active substance. Stelfonta is a solution for injection containing 1 mg/ml tigilanol tiglate and is presented in packs containing 1 vial (2 ml).

The product is intended for use in dogs for the treatment of non-resectable, non-metastatic cutaneous and subcutaneous mast cell tumours. Treatment consists of a single intratumoral injection of 0.5 ml per cm^3 of tumour volume and may be repeated once (after 4 weeks), if needed. Treatment should be administered together with corticosteroids and H1 and H2 receptor blocking agents, and analgesics, if needed.

The product has been classified as MUMS/limited market and therefore reduced data requirements apply that have been considered in the assessment.

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

The rapporteur appointed is Peter Hekman and the co-rapporteur is Gesine Hahn.

The dossier has been submitted in line with the requirements for submissions under Article 31 of Regulation (EC) No 726/2004 - for full new application.

On 7 November 2019, the CVMP adopted an opinion and CVMP assessment report.

On 15 January 2020, the European Commission adopted a Commission Decision granting the marketing authorisation for Stelfonta.

Scientific advice

The applicant received scientific advice from the CVMP on 15 June 2017. The scientific advice pertained to safety and clinical development of the dossier. The provided dossier generally was in compliance with the scientific advice.

MUMS/limited market status

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

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated 24 July 2018) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place outside the EEA. The site has a manufacturing authorisation issued by the UK competent authority. Good Manufacturing Practice (GMP) certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

Batch release in the EU takes place at Virbac S.A., Carros Cedex (France), which holds a manufacturing authorisation issued by the French authority.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by a third party which has taken into consideration the GMP certificate available for the active substance manufacturing site issued by the Australian government following inspection.

Overall conclusions on administrative particulars

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

The GMP status of both the active substance and finished product manufacturing sites has been satisfactorily established, and they are in line with legal requirements.

Part 2 - Quality

Composition

The finished product is presented as an aqueous solution for injection containing 1 mg/ml of the antineoplastic agent tigilanol tiglate as the active substance.

Other ingredients are propylene glycol, sodium acetate trihydrate, glacial acetic acid and water for injections.

The product is presented in single-dose glass injection vials containing 2 ml.

Containers

The primary packaging consists of a colourless Type I glass vial closed with a PTFE-coated grey chlorobutyl rubber stopper and an aluminium flip-off cap. The materials all comply with the relevant Ph. Eur. and/or EU requirements.

The choice of the container-closure system has been validated by stability data and is adequate for the intended use of the product.

The secondary packaging is a cardboard box, each box containing 1 vial of 2 ml. The pack size is consistent with the dosage regimen and duration of use.

Development pharmaceutics

Active substance

Tigilanol tiglate is extracted from the kernel of *Fontainea picrosperma*, *Euphorbiaceae*, commonly known as Blushwood.

Tigilanol tiglate is a white to off-white, fine to granular amorphous powder, freely soluble or even very soluble in various organic solvents including ethanol, methanol, acetone, ethyl acetate and propylene glycol. Tigilanol tiglate has one single polymorphic form.

Since tigilanol tiglate is insoluble in water, it was necessary to dissolve the active substance into a suitable organic compound such as propylene glycol (the vehicle) first, and then complete the formulation of the injectable product with a minimal amount of water.

Formulation and production

Propylene glycol and water for injections are the major excipients and form the solvent system for the product. Sodium acetate and acetic acid are used in small quantities as a buffer system.

All the excipients used are well established pharmaceutical substances and common in such solutions for injection. The choice of excipients is justified. There are no novel excipients used. The list of excipients is included in section 6.1 of the SPC.

Type I clear glass vials (of 5 ml total capacity, containing 2 ml of the product) were selected as the primary packaging for the finished product. Photostability studies on exposed product in the clear glass vials showed that degradation occurred, however, when the product was protected from light in the secondary packaging (cardboard boxes) no degradation occurred.

The 20 mm PFTE-coated (Fluorotec) grey chlorobutyl rubber stoppers were demonstrated to be compatible with the product.

Sterilisation of the product is by filtration and aseptic processing.

Dose-finding studies using three different strengths of tigilanol tiglate injection (0.2, 0.5 and 1.0 mg/ml) established the highest concentration (1 mg/ml) of tigilanol tiglate as the most suitable.

The formulation used during clinical studies is the same as that intended for marketing.

The manufacture of tigilanol tiglate solution for injection does not require any formulation or manufacturing overages.

A quality target product profile (QTPP) was defined considering the product as an intratumoral injection and on the basis of the market and patient's needs.

The initial Critical Quality Attributes (CQA) relevant to the product were identified as per the VICH GL39 guideline. Appearance, identification and assay of tigilanol tiglate, related substances, container content, pH, particulate matter, and sterility were considered as CQAs.

The manufacturing method for the bulk solution involves preparation of the acetate buffer solution, dissolution of the active substance in propylene glycol and then mixing with the acetate buffer solution,

adjustment of the pH and dilution to the required volume with the acetate buffer solution. The bulk solution is then sterile filtered through two pre-sterilised 0.22 µm filters.

The manufacturing process development involved the manufacture of laboratory scale batches, technology transfer for pilot batch manufacturing, manufacture of three pilot scale batches and process control studies. Knowledge gained regarding the Critical Process Parameters (CPP) during laboratory scale manufacture was then used to determine the process controls for the manufacture of pilot scale batches.

Similarly, knowledge gained during both pilot scale manufacture and various process control studies was utilised in a risk assessment (Failure Mode Effect Analysis (FMEA)) to support further scale-up for commercial manufacture and a process validation plan.

The information and knowledge gathered during the early formulation development studies, laboratory scale manufacture, primary batch manufacture at pilot scale and various other laboratory studies has provided scientific understanding and supports the proposed manufacturing process for commercial batches. The CPP of the manufacturing process and CQA of the final product are fully understood. Manufacturing data and release testing of the three primary (pilot scale) batches has proven the ability to deliver a consistent product quality. Furthermore, the (ongoing) stability study data for the three primary batches also shows a state of control and consistent product performance.

Method of manufacture

Manufacture of the bulk solution involves preparation of the acetate buffer solution, dissolution of the active substance in propylene glycol and mixing with the acetate buffer solution, then adjustment of the pH and dilution to the required volume with the acetate buffer solution.

The bulk solution is filtered through two pre-sterilised 0.22 µm filters, connected in series through sterilised tubing to the bulk solution tank. The bulk solution is filled aseptically into the glass vials and then closed with the rubber stoppers and sealed with the aluminium caps. A slight overfill has been justified.

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

A pre-filtration bioburden sample is taken prior to filtration of the bulk solution to ensure the bioburden is not more than 10 CFU/100 ml. The choice of sterilizing filter has been explained and sufficient validation tests have been provided.

The stoppers and the caps are pre-sterilised by autoclaving, whereas the vials are pre-sterilised by dry heat (250 °C for 1 hour) using a depyrogenation oven. The sterilisation conditions represent standard sterilisation processes.

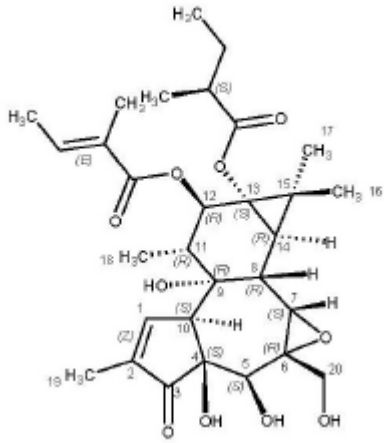
The choice of a non-terminal sterilisation process is justified according to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/850374/2015) and is acceptable.

The process may be considered a standard manufacturing process. with validation at the commercial scale is required only post-approval.

Control of starting materials

Active substance

The chemical name of tigilanol tiglate is (4S,5S,6R,7S,8R,9R,10S,11R,12R,13S,14R)-12-(2E)-2-methyl-but-2-enoatyl-13-[(2S)-2-methylbutyroyl]-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliaen-3-one and it has the following structure:



Tigilanol tiglate is a granular amorphous powder, at least freely soluble in ethanol, methanol, acetone, ethyl acetate and propylene glycol, and insoluble in water and heptane.

Tigilanol tiglate is only slightly hygroscopic.

Tigilanol tiglate has a single chiral molecular structure.

No polymorphism has been observed for this active substance.

There are no physico-chemical characteristics liable to affect its bioavailability in the product since tigilanol tiglate occurs as a stable molecule with a single polymorphic form, and particle size is not relevant for a solution dosage form.

All the information on the active substance is provided within the dossier. The active substance is manufactured by a single manufacturer.

The characterisation of the active substance is in accordance with the Guideline on the chemistry of active substances for veterinary medicinal products (EMA/CVMP/QWP/707366/2017). Defined potential and actual impurities were sufficiently discussed with regards to their origin and are characterised.

Information on the manufacture of the active substance has been provided in the dossier. The active substance is using a well-defined starting material, in line with ICH Q7.

Sufficient in-process controls are applied during the purification of the active substance. The specifications and control methods for intermediates, starting materials and reagents have been presented. Appropriate validation data have been provided for the HPLC methods used for analysis of the starting material and subsequent intermediates.

There is no monograph for tigilanol tiglate in the Ph. Eur. and an in-house monograph is defined. The active substance specification is generally acceptable and includes tests for appearance, identity, assay and impurities, water content, residual solvents, residual formic acid, residue on ignition and

microbial limits. The residual solvent limits have been justified in accordance with VICH GL18.

The analytical methods used have been sufficiently described and the in-house HPLC method for assay and related impurities, the in-house GC and HPLC methods for residual solvents are all appropriately validated in accordance with VICH GL2.

Satisfactory information regarding the primary and secondary reference standards used for the assay testing has been presented. Furthermore, sufficient information on available reference standards for all three specified impurities has also been provided.

Batch analysis data for five development batches and five commercial scale batches of the active substance have been provided. The results are well within the specifications proposed and are consistent from batch to batch.

Stability results were provided for three production scale batches of tigilanol tiglate from the proposed manufacturer stored in the proposed container (amber Type I glass vials closed with polypropylene screw caps with silicon seals, vinyl-methyl-silicone (VMQ) coated on one side with polytetrafluoroethylene (PTFE)), for 36 months at 2—8 °C, 12 months at 25 °C/60% RH, and in addition, one month at 40 °C/75% RH.

The following parameters were tested: appearance, water content, assay and related impurities. The analytical methods used were in accordance with the active substance specifications and were stability indicating.

All tested parameters were well within specification and no real trends in water content, assay or impurity profiles were observed.

Photostability testing in line with VICH GL5 was performed on development batches.

Results from stress testing of the active substance by acid hydrolysis, base hydrolysis, thermal hydrolysis, and oxidation on development batches were also provided.

The stability results indicate that tigilanol tiglate is generally very stable but it is light sensitive, and the proposed retest period of 36 months when stored at 2—8 °C and protected from light in the proposed primary (vial) and secondary (carton) containers has been justified.

Excipients

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

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

The product does not contain any materials derived from human or animal origin. Valid TSE declarations from the manufacturers of the active substance and the finished product have been provided.

Control tests on the finished product

The specifications proposed for use at release and at the end of shelf life are appropriate to control the quality of the finished product. The finished product specification includes tests for appearance, pH, assay and related impurities, extractable volume, particulate matter and sterility.

The analytical methods used have been sufficiently described and appropriately validated in accordance with the relevant VICH guidelines.

Satisfactory information regarding the reference standards used for assay testing has been presented.

Batch analysis results are provided for three pilot scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability

Stability data on three pilot scale batches of finished product stored for 36 months at 5 °C, and 18 months at 25 °C/60% RH were provided.

The batches of finished product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Vials were stored inverted and samples were tested for appearance, pH, assay and related impurities, particulate matter and sterility. In addition tests were performed for bacterial endotoxins (informative only).

The analytical procedures used are stability indicating.

The observed physical and chemical changes were small, and not likely to have a significant effect on the efficacy and/or safety of the product when used according to the directions in the SPC.

In addition one batch was exposed to light, as defined in the VICH guideline GL5 on photostability testing of new veterinary drug substances and medicinal products. The study revealed that tigilanol tiglate is light sensitive.

Based on the available stability data, the proposed shelf-life of 42 months when stored in a refrigerator (2 °C – 8 °C) and in the original carton in order to protect from light, as stated in the SPC, is acceptable.

Overall conclusions on quality

The finished product is a colourless aqueous solution for injection (for intratumoral use) containing 1 mg tigilanol tiglate per ml as the active substance.

Other ingredients are propylene glycol, sodium acetate trihydrate, acetic acid (glacial) and water for injections.

The product is presented in clear Type I glass vials sealed with PTFE-coated grey chlorobutyl rubber stoppers and aluminium caps containing a nominal volume of 2 ml, as described in section 6.5 of the SPC. The materials comply with the relevant Ph. Eur. and/or EU requirements. The choice of the container-closure system has been validated by stability data and is adequate for the intended use of the product.

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

The choice of a non-terminal sterilisation process was justified according to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/850374/2015) as investigation of the effect of terminal sterilisation demonstrated that the product cannot be steam sterilised without substantial degradation of the active substance. The use of aseptic processing and sterilisation by filtration was therefore fully justified. The

development production and manufacturing processes have been described in detail and the in-process controls are adequate for this type of manufacturing process.

The process may be considered a standard manufacturing process. Validation of the finished product manufacturing process at the commercial scale is required only post-approval and will be performed using three commercial scale batches. A suitable process validation protocol for full commercial scale batches has been provided. As the manufacturing method is a relatively simple standard process and validation data on pilot-scale batches were provided, it was accepted that full scale validation would be performed post-authorisation, in accordance with the CVMP Guideline on the quality data requirements for veterinary medicinal products intended for Minor Uses or Minor Species (EMEA/CVMP/QWP/128710/2004). The applicant provided both a protocol for the process validation study and a commitment to submit the data post-authorisation and the Committee considered this to be acceptable

Full information on the active substance tigilanol tiglate is provided in the dossier. The active substance is using a well-defined starting material, in line with ICH Q7.

Detailed information on the manufacture of the active substance has been provided. Sufficient in-process controls are applied during the purification. The specifications and control methods for intermediates, starting materials and reagents have been presented. The specifications for the starting materials, raw materials, solvent and reagents are appropriate.

There is no monograph for tigilanol tiglate in the Ph. Eur. and a suitable in-house monograph is defined.

Batch analyses results of batches of the tigilanol tiglate demonstrate compliance with the proposed active substance specification.

The excipients are well known pharmaceutical ingredients and their quality is compliant with their respective current Ph. Eur. monographs.

The finished product specifications proposed for use at release and at the end of shelf life are acceptable. Batch analyses results of pilot scale batches demonstrate compliance with the proposed finished product specification.

Based on the available stability data, the proposed shelf life 42 months when stored in a refrigerator (2—8 °C) and the additional storage conditions 'Do not freeze.' and 'Keep the vial in the outer carton in order to protect from light.' are justified. Considering the small volume/flacon, no in-use shelf life was felt needed.

Sufficient and clear information has been provided in the dossier to support the authorisation of this medicinal product, and current regulations and guidelines have been taken into account.

In addition, the applicant is recommended to provide the following information post-authorisation:

1. Process validation studies on the first three consecutive commercial batches.
2. Hold time study data as soon as the process validation report is complete.
3. Stability commitments:
    a. To complete the long-term stability study for the three primary batches (B150455, B150561 and B150562) up to 48 months.
    b. To place the first three production batches on long term and accelerated stability studies.
    c. To place at least one production batch per year on long term stability studies (unless none is produced during that year).

Part 3 – Safety

The active substance tigilanol tiglate of Stelfonta, a protein kinase C-(PKC) activating compound, is a new active substance not authorised for a veterinary medicinal product in the EU before. A full safety file in accordance with Article 12(3)(j) has been provided.

Safety documentation

A data package with (recently performed) studies has been presented by the applicant. The pharmacological and toxicological properties of tigilanol tiglate have been characterized and described in the dossier based on available literature and laboratory studies performed by the applicant.

Pharmacodynamics

See part 4.

Tigilanol tiglate is an antineoplastic agent that activates the protein kinase C (PKC) signalling cascade; in addition, necrosis is induced in cells that are in direct contact with the active substance.

Pharmacokinetics

See also part 4.

Relevant for user safety assessment is the potential excretion via urine, faeces and saliva but also the potential residue leakage of tigilanol tiglate onto the surface of the treated tumour. In line with a scientific advice (EMA/CVMP/SAWP/178983/2017), relevant information has been provided.

A study investigating potential residue leakage of tigilanol tiglate onto the surface of the treated tumour of 6 dogs (7 treatments) indicated that leakage of tigilanol tiglate from the injection site post-treatment occurred mainly during the first 24 hours (up to 1.6 µg in most animals, with the exception of 9.6 µg in one animal receiving a double treatment), and only sporadically very small amounts of tigilanol tiglate are excreted/discharged from the tumour beyond day 2 post treatment (up to 1.199 µg) with no excretion detected at 7 and 14 days post-treatment. The maximum amount of surface residue was detected one hour after treatment, representing 1.93% of the administered dose.

In conclusion, only minimal leakage of tigilanol tiglate from the injection site post-treatment is expected to occur following intratumoral (IT) injection.

Another study focusing on the potential excretion via urine, faeces and saliva following intratumoral treatment of 11 dogs revealed that minimal tigilanol tiglate appears to be excreted in urine, faeces and saliva. Samples were taken 4-6 hours and on day 1, 2, 4 and 7 after treatment. However, measurable concentrations of tigilanol tiglate could only be detected in a total of 5 samples, in three dogs on days 4 or 7 after treatment: i.e. Dog 1 (day 4): 11.6 ng/ml (urine) and 43.8 ng/g (saliva); Dog 2 (day 7) 11.3 ng/g (faeces) and 18.5 ng/g (saliva); and Dog 3 (day 7) 10.9 ng/g (faeces).

In canine excreta (faeces/urine/saliva), residues of tigilanol tiglate and one metabolite were detected (due to insufficient extract no other metabolites were analysed). However, based on in vitro bioactivity tests with metabolites of tigilanol tiglate (see part 4) it is most likely that a significant fraction of metabolites of tigilanol tiglate have lower bioactivity and toxicity. An increased bioactivity of metabolites is unlikely. Moreover, based on a rough estimate, taking the parent to metabolite ratio obtained from an in vitro metabolism study in dog hepatocytes , the worst case assuming that 4.35% of the total residue in the excreta is tigilanol tiglate and the rest are all active metabolites, it was estimated that the user

would need to be exposed to unrealistically high amounts of faeces, urine and saliva to reach a toxicological level.

In addition, no signs of irritation have been observed at the mucous membrane, the urinary tract or the rectum of dogs. Hence, an irritating potential of the excreta is unlikely.

Toxicological studies

Single dose toxicity

Multiple single dose studies are available, in mouse, rat and dog, with administration of tigilanol tiglate via the subcutaneous, intravenous and intratumoral route. Also, a single dose study via the dermal route was available for Stelfonta. It is noted that most studies included a limited number of animals and focussed primarily on establishing the maximum tolerated dose (MTD). With respect to systemic effects, the intravenous administration route can be considered worst case.

Effects were mainly limited to local effects at the site of injection, including oedema and erythema. In some cases this developed into a wound, which took a long time to resolve.

Systemic effects included lethargy and death (killed in extremis- one mouse) at high intravenous doses in the mouse, and some changes in haematology and clinical chemistry parameters upon intratumoral administration in the rat. In dogs, following subcutaneous and intravenous administration, a reduction in body temperature, reduced food consumption and retching, (mucous) vomiting, urination, salivation, defecation, decreased activity, lateral position, panting, swaying gait, breathlessness and thirst were noted, and tachypnoea, lethargy, tachycardia, hypertension, emesis, and salivation following intratumoral administration (see also part 4, target animal safety).

However, no NOAEL or LOAEL can be determined from the single dose toxicity studies, because the study designs were not appropriate to serve that purpose (low number of animals, adverse effects in the vehicle groups or intratumoral application).

Repeat dose toxicity

It is noted that no oral and dermal repeated dose toxicity studies are presented in the dossier. The repeated dose studies are limited to the intravenous exposure route. This can however be considered worst case with respect to systemic exposure. It is noted that most studies included a limited number of animals and focussed primarily on establishing the MTD. Further, the available rat and dog repeated dose toxicity studies included a very limited number of exposures (i.e. two, three or four exposures separated by one week), with a LOAEL of 0.1 mg/kg bw/week for rats (based on three exposures, separated by one week resulting in clinical and systemic effects) and a LOAEL of 0.025 mg/kg bw/week for dogs (based on four exposures, separated by one week resulting in local and clinical effects).

The CVMP noted the limited data available, but considered that a toxicological reference value based on a repeated dose study with only 3 or 4 exposures is acceptable when evaluating user safety for the professional (the veterinarian), as good hygienic practice will be followed and appropriate risk mitigation measures have been included in the product literature. In addition, significant residues are only expected to occur within the first two days of treatment. Frequent exposure of household members can therefore be considered to be rather low and sporadic beyond 2 days post-treatment. Hence, this toxicological reference value would be acceptable when evaluating the exposure scenarios for the dog-owner.

Tolerance in the target species of animal

See part 4.

Reproductive toxicity

Study of the effect on reproduction

No studies focussing on effects on reproduction have been presented in the dossier. In line with the scientific advice provided by the CVMP (EMA/CVMP/SAWP/178983/2017), new reproductive toxicity studies were not considered necessary for this MUMS application, if the safety of professional users as well as pet owners can be ensured; in this regard, appropriate warnings for pregnant animals and animals used for breeding are included in the product information.

Study of developmental toxicity

In a preliminary rabbit prenatal developmental toxicity study, no treatment-related adverse effects on development were noticed. However, in this study tigilanol tiglate was applied during a limited period of the pregnancy, with a limited number of animals. No final conclusion on developmental toxicity could be derived based on this study, and consequently, an appropriate warning for pregnant or breastfeeding women has been added to the SPC.

Genotoxicity

A battery of genotoxicity tests has been presented, in accordance with the data requirements as described in VICH GL 23. Negative results were obtained from a bacterial reverse mutation assay, an *in vitro* chromosomal aberration assay and an *in vivo* rat micronucleus assay. Based on these data, it is concluded that tigilanol tiglate does not have genotoxic properties.

Carcinogenicity

No carcinogenicity studies have been presented in the dossier.

The association of PKC with tumour promoting effects appears to be complex, and depends on the specific isoforms involved, the timing of PKC activation, the cell lineage, the stage in the cell cycle, and the general cellular signalling environment. From several literature studies it appeared that PKC down-regulation or loss of function, and not activation was associated, with tumour developments.

Prolonged exposure to phorbol esters, which are structurally related to tigilanol tiglate, has been shown to result in dephosphorylation and down-regulation of PKC. This might therefore also be the case for tigilanol tiglate. Moreover, prolonged dermal exposure (to phorbol esters other than tigilanol tiglate) also produced a chronic local inflammation; in combination with a previous treatment with a local mutagen this resulted in the increase in dermal tumours partly developing into malignant squamous cell carcinomas. This might also be the case for tigilanol tiglate.

Therefore, tumour promoting effects cannot be fully excluded; however, these appear to be associated with prolonged exposure of the same dermal area, which is unlikely for Stelfonta.

Studies of other effects

Stelfonta is considered to be irritating or corrosive to the skin based on the results of an *in vitro* study using reconstructed human epidermis. The high incidence of local reactions at the site of injection in many studies also confirms the irritating potential.

Eye irritation would not be expected based on the outcome of an eye irritation study using the bovine corneal opacity and permeability (BCOP) assay. However, most substances that are irritating to the skin are also at least irritating to the eyes. Also, two cases of eye irritation of veterinarians during the clinical trial in dogs were reported. Therefore, it is assumed that the final formulation is at least irritating to the eye.

To date, there have been no reports of hypersensitivity responses in human studies of tigilanol tiglate following repeated intratumoral injection nor have reports of hypersensitivity responses been reported in the animal studies (field, target animal safety) supporting this application. However, hypersensitivity reactions from dermal use have been reported as an uncommon adverse reaction for a related compound (ingenol mebutate). A skin sensitisation potential of the excipient propylene glycol has been established in man. Warnings have been included in the SPC that people with known hypersensitivity to tigilanol tiglate or to propylene glycol should avoid contact with the product.

A single case of accidental injection (occurring with a veterinarian during the target animal clinical trial) revealed local effects at the site in injection (thumb) which included pain and necrosis. A wound developed at the site of injection which healed over a period of three months. Warnings have therefore been included in the SPC on accidental self-injection.

In clinical trials in humans with intratumoral injection of tigilanol tiglate mainly local effects at the site of injection occurred, such as transient tumour site swelling and associated pain, and bruising/erythema. However, one serious adverse effect (SAE) was observed following treatment of a tumour located on the side of the neck. Effects with this patient included localised swelling that compounded pre-existing lymphoedema, resulting in slight constriction of the patient's windpipe and necessitating intervention. A second SAE was observed in a patient with a tumour on the lower leg. The SAE occurred after the tumour sloughed off, leaving a wound that became infected approximately six weeks after treatment. Treatment was initiated with systemic antibiotics. A third SAE was presented in a patient with abdominal pain related to constipation, which was treated with laxatives.

Excipients

The toxicity of this product will be mainly determined by its active substance tigilanol tiglate. Excipients are of low toxicity, with the exception of propylene glycol. Sodium acetate and glacial acetic acid are included, in minimal amounts, for pH adjustment. No clear adverse effects are expected from these chemicals. Propylene glycol is commonly used as an excipient in human and veterinarian medicinal products and is authorised in food production and cosmetics. According to CHMP, the safety threshold for oral and parenteral administration is 1 mg/kg per day for children up to 4 weeks, 50 mg/kg bw for children up to 5 years as well as pregnant or breastfeeding women or persons with kidney disease, and 500 mg/kg bw for adults. Propylene glycol has been linked with hypersensitivity reactions in man and neurotoxicity, and a warning has been included in the SPC and product literature for Stelfonta.

User safety

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

The main potential routes of accidental contact with the product are considered to be dermal exposure and accidental self-injection during administration of the product. Also, after treatment, drug exuding from the tumour site may result in dermal contact. Contact with excreta could potentially result in exposure to the active substance and/or metabolites.

Based on the information available, it is concluded that dermal contact with the product may result in skin irritation. Also, hypersensitivity reactions cannot be excluded. Eye irritation would not be expected based on the outcome of the eye irritation study. However, two cases of eye irritation in humans were observed in the clinical trial in dogs, and appropriate warnings have been included in the SPC and product information to avoid skin, eye or mucosal contact.

The product is to be administered only by a veterinarian. During the handling of the product, it is anticipated that dermal exposure might amount to a single drop of the product, resulting in an exposure level of 0.83 µg tigilanol tiglate/kg bw. The lowest toxicological reference value is the LOAEL of 0.025 mg/kg bw derived from the dog study (TAS; based on four intravenous exposures, separated by one week; adverse effect were transient/mild clinical signs including salivation, retching or vomiting). Based on these inputs the MOE would be 30. Moreover, some product may leak from the tumour site during administration as was observed in some animal studies. The wearing of gloves, as is recommended in the product information, would limit exposure. Therefore, no risks are anticipated for the user in relation to dermal exposure to the product when wearing gloves. However, the site of administration should be covered for the first day after treatment in order to prevent direct contact with residual or leaking product.

The professional user may also accidentally self-inject the product, resulting in a reasonable worst-case exposure level of 8.3 µg tigilanol tiglate/kg bw. This would result in a MOE of 3 (based on a LOAEL from an intravenous application study, see also above). Systemic effects observed in this study were transient/mild clinical signs including salivation, retching or vomiting. Moreover, severe local effects, including pain, oedema, and necrosis are expected. Considering the mode of action and the fact that severe adverse effects cannot be excluded after accidental injection of the product, as a precautionary principle additional warnings were included in the SPC.

After administration, the owner or children might come in contact with the treated animal. While no data on possible residues on the body surface of the treated animals are available, they can be considered negligible due to the route of administration. Residues of tigilanol tiglate caused by leakage were however measured on top of the treated tumour site; residues of tigilanol metabolites on the tumour surface are unlikely. Dermal exposure levels of respectively 0.76, 0.096 and 0.017 µg/kg bw for 1 hour, 48 hours and 4 days after-treatment period were estimated for children. Compared to the LOAEL of 0.025 mg/kg bw (based on intravenous application), this would result in a MOE of 33 at 1h after treatment period. At 48 hours after treatment the outcome of the MOE would be 260; and 4 days after treatment the outcome of the MOE is 1471. It is noted that full absorption is assumed for the dermal exposure levels which is very worst case. Moreover, the MOE is based on a LOAEL as transient/mild clinical signs including salivation, retching and vomiting were observed after intravenous exposure to 25 µg/kg bw in dogs. These effects were not observed after subcutaneous exposure to 26 or 32 µg/kg bw in the target animal safety study. Therefore, these effects may be due to a difference in Cmax and consequently to use the LOAEL from an intravenous study may be very conservative. However, using the values derived from subcutaneous exposure as a NOAEL, the MOE would still not be acceptable when considering the 1 hour time point after treatment period. The site of administration should be covered for the first day after treatment in order to prevent direct contact with residual or leaking product. In case of severe leakage of wound debris, which may occur in the first weeks following administration of the product, the wound should be covered.

The owners, including children, might also become exposed to residues of the product via excreta (saliva, faeces, urine). However, with respect to the parent compound tigilanol tiglate, the vast majority of residues in excreta were below LOQ, except for some animals. Based on the maximum levels observed in faeces (11.3 ng/g), in urine (11.6 ng/ml) and in saliva (18.5 ng/g), the user would need to be exposed to 27655 g of faeces, to 26940 ml of urine or to 16892 g of saliva to reach the

LOAEL of 0.025 mg/kg bw (or 312.5 µg for a 12.5 kg child). No safety factors are taken into account for this calculation, however, full absorption is assumed. Therefore, it is concluded that there will be no risks even if the owners, including young children, are exposed to residues of the product in excreta, based on the parent compound.

Effects of administration of tigilanol tiglate in rabbits during the period of foetal development did not show treatment-related adverse effects on development. However, in this study tigilanol tiglate was applied during a limited period of the pregnancy with a limited number of animals, which precluded any final conclusion on developmental toxicity. Consequently, an appropriate warning for pregnant or breastfeeding women has been added to the SPC.

It is noted that in some studies leakage of the product from the site of injection is observed directly after administration. The user and especially the professional user might be exposed in this scenario. Also, it is noted that in a few cases, leakage of tigilanol tiglate might occur due to tumour ulceration or wound debris during the first weeks after administration. In some cases tumours sloughed off. The concentration of tigilanol tiglate in the wound debris or sloughed off tumour has not been determined. An appropriate warning to the user was added to the SPC.

It was concluded that appropriate warnings are included in the SPC, in order to safeguard user.

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 animals.

Based on the data provided the ERA can stop at Phase I. Stelfonta is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

Pharmacodynamics

Tigilanol tiglate is an antineoplastic agent that activates the protein kinase C (PKC) signalling cascade; in addition, necrosis is induced in cells that are in direct contact with the active substance.

Pharmacokinetics

Following intratumoral administration, tigilanol tiglate is rapidly absorbed into the blood stream. Minimal tigilanol tiglate appears excreted in urine, faeces and saliva of dogs.

Single dose toxicity

No NOAEL or LOAEL can be determined from the single dose toxicity studies, because the study designs were not appropriate to serve that purpose (low number of animals, adverse effects in the vehicle groups or intratumoral application).

Repeat dose toxicity

The available rat and dog repeated dose toxicity studies included a very limited number of exposures (i.e. two, three or four exposures separated by one week) and number of animals, with a LOAEL of 0.1 mg/kg bw/week for rat (based on three exposures, separated by one week resulting in clinical and systemic effects) and a LOAEL of 0.025 mg/kg bw/week for dog (based on four exposures, separated by one week resulting in local and clinical effects). The adverse effects in the dog study were transient/mild clinical signs.

Reproductive toxicity

No studies focussing on effects on reproduction have been presented in the dossier. This was accepted for this MUMS application and was in line with the CVMP scientific advice (EMA/CVMP/SAWP/178983/2017).

Effects of administration of tigilanol tiglate during the period of foetal development were examined in rabbits. No treatment-related adverse effects on development were noticed. However, in this study tigilanol tiglate was applied during a limited period of the pregnancy, with a limited number of animals which precluded any final conclusion on developmental toxicity. Consequently, an appropriate warning has been added to the SPC.

Genotoxicity

Tigilanol tiglate does not have genotoxic properties.

Carcinogenicity

Carcinogenicity studies have not been performed. Based on literature data, the applicant presented a detailed discussion of the potential tumour promoting properties of tigilanol tiglate with focus on the pharmacodynamic effects of phorbol esters. The association of PKC with tumour promoting effects appears to be complex, and depends on the specific isoforms involved, the timing of PKC activation, the cell lineage, the stage in the cell cycle, and the general cellular signalling environment. Tumour promoting effects cannot be fully excluded, however, appear to be associated with prolonged exposure, which is not expected from the proposed use of the product.

Studies of other effects

Based on *in vitro* studies tigilanol tigilate was classified to be irritating or corrosive to the skin, but non-irritant to the eye. However, two cases of eye exposure with the final formulation showed transient irritating effects in people. The CVMP therefore also considered Stelfonta to be an eye irritant and recommended to avoid using the product in the proximity of sensitive tissues, in particular the eye, and added appropriate user warnings.

No studies on the skin sensitisation potential of Stelfonta have been provided. No reports of hypersensitivity responses in human or animal studies have been reported. However, hypersensitivity reactions from dermal use have been reported for a related compound (ingenol mebutate). Propylene glycol has been linked with hypersensitivity reactions in man. Warnings have been included in the SPC that people with known hypersensitivity to tigilanol tiglate or to propylene glycol should avoid contact with the product.

A single case of accidental injection (occurring with a veterinarian during the target animal clinical trial) revealed local effects at the site in injection (thumb) which included pain and necrosis, and wound development at the site of injection, which healed over a period of three months.

Excipients

Excipients are of low toxicity. Sodium acetate and glacial acetic acid are included, in minimal amounts, for pH adjustment. No clear adverse effects are expected from these substances. However, propylene glycol has been linked with hypersensitivity reactions in man.

User safety

The most relevant routes of accidental contact are self-injection as well as dermal and eye exposure. For the non-professional user, dermal exposure may occur via contact with treated animal and the excreta. Contact with the product may result in skin or eye irritation. Also, hypersensitivity reactions cannot be

excluded. Accidental self-injection would result in the worst-case exposure. Severe local effects, including pain, oedema and necrosis might occur.

Appropriate warnings are included in the SPC. The safety of the veterinary medicinal product has not been established during pregnancy or lactation. Thus, appropriate information has been included in the SPC that pregnant women or breastfeeding women should take care to avoid accidental self-injection, contact with the injection site, leaking product and tumour debris.

Environmental risk

An appropriate environmental risk assessment was provided. The product is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Pharmacodynamics

Stelfonta belongs to the group of antineoplastic agents. The active substance, tigilanol tiglate, is a novel compound in veterinary medicines, and there is little literature in the public domain concerning this molecule, owing to its novelty to both human and veterinary medicine. The product is intended for use in dogs for the treatment of non-resectable, non-metastatic cutaneous mast cell tumours and subcutaneous mast cell tumours that are located at or distal to the elbow or the hock. Tigilanol tiglate is a well characterised small diterpene ester molecule that is purified from a commercially sustainable natural source.

The applicant provided references on pharmacodynamics (PD) derived from *in vitro* studies and *in vivo* mice model studies. No PD studies were performed in dogs, nor were any PD studies performed on mast cell tumours (MCT).

Tigilanol tiglate is a diterpene ester that activates protein kinase C (PKC) and causes PKC-dependent haemorrhagic necrosis. The role of PKC in carcinogenesis has been recognized for decades. Effects start to occur within hours after treatment. Since treatment results in cell necrosis, the product possesses cytotoxic properties. The pharmacodynamic effect is not specifically confined to neoplastic cells. However, the product is not considered a 'conventional cytotoxic drug'. Though treatment results in necrosis, this effect is not caused by a genotoxic mechanism: the substance is not a known mutagenic and is not DNA-reactive. Also, the product is not intended for systemic use; therefore, the CVMP guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008) does not fully apply for this product. The local effect on neoplastic tissue is achieved by direct contact, due to the intratumoral use. In addition, an increased permeability of tumour vasculature, as well as vascular swelling and apparent disruption of vessel morphology occur. Tumour ablation is associated with local inflammation, haemorrhagic necrosis, and cellular effects consistent with PKC activation in both tumour and host cells. Mice models have demonstrated an enduring antitumour effect.

Development of resistance

Stelfonta is a new chemical entity that has not been used in veterinary medicine before. The exact risk of resistance development with regard to the use of this product is therefore currently not known. However, systemic exposure of the product is very limited, and the pharmacodynamic properties of the product are very distinct. Finally, development of *de novo* MCT in dogs is known to occur often. The applicant presented an interim report in which 34 dogs (44 tumours) received a second repeated treatment with Stelfonta for either a *de novo* (n=22) or recurrent (n=22) MCT. Nine of these dogs (18

tumours) received a third treatment, and three dogs (six tumours) received a fourth treatment. One dog received five treatments in three tumours over a 3-year period. There was no indication that the repeated treatment altered the efficacy of the product, though interpretation of these results is difficult, since no control group or blinding technique was applied.

The development of resistance following treatment with tigilanol tiglate is therefore not considered very likely.

Pharmacokinetics

In support of the pharmacokinetics of tigilanol tiglate, the applicant provided data from dogs following intratumoral treatment (field studies), as well as a number of laboratory *in vitro* and *in vivo* studies, some of which however could only be considered supportive, and are not further described here.

PK data following intratumoral *treatment (field studies):*

Pivotal pharmacokinetic data following intratumoral (IT) treatment were obtained from two field studies, a dose determining study and a dose confirmation study, that included tumour-bearing, client-owned dogs. After IT injection, the maximum concentration of tigilanol tiglate appears rapidly in the blood stream, t_{max} occurred within 5 to 30 minutes, $t_{1/2}$ ranged from 1.24 to 10.8 hours and 1.4 to 12.4 hours. Systemic exposure of tigilanol tiglate could only be measured up to 0.145 mg/kg bw.

Both studies had limitations with regard to the limited sampling points for PK data. As a result, definite C_{max} and AUC values could not be reliably determined. Measurements however indicated a mean C_{max} of 5.86 ng/ml (range: 0.36-11.1 ng/ml) and a mean AUC_{last} of 14.59 h*ng/ml (range: 1.62-28.92 h*ng/ml). However, variability in the PK data is not surprising and cannot be prevented, considering that the data is likely to depend on tumour location, dimensions, and on complexity of dosing by fanning with variable dose volumes at variable doses. Also, due to insufficient case load, the population characteristics (maximum tumour size) were limited and lower dose ranges of 0.002 - 0.145 mg/kg bw were investigated rather than the initially proposed maximum treatment dose of 0.25 mg/kg bw.

PK data following subcutaneous or intravenous treatment (laboratory studies)

PK data was also obtained following other routes of administration, species or models. Use of healthy, non-tumour bearing dogs was considered acceptable, since no laboratory model on canine MCT exists. Since IT injection is not possible in healthy laboratory animals, tigilanol tiglate was administered intravenously (IV) and/or subcutaneously (SC). None of the laboratory studies was performed to a GLP standard. However, studies did appear well designed, appropriately conducted and well documented.

Absorption (systemic exposure by bridging data IV- and IT administration):
In the pivotal target animal safety (TAS) study, plasma concentrations up to approximately 55 ng/ml following IV administration (0.075 mg/kg bw) were described, and elimination half-life did not vary over the dose levels tested. Tigilanol tiglate appears to exhibit flip-flop kinetics (sustained release rate), since a considerable shorter half-life of 0.54 hours was determined after IV infusion than after IT administration (1.24 to 12.4 hours). Tigilanol tiglate exhibited dose proportional kinetics over the range evaluated in this study (0.025 to 0.075 mg/kg bw, IV). The IV doses were lower than the recommended intratumoral dose (0.15 mg/kg bw), but the highest peak exposure following IV administration (55 ng/ml at a dose of 0.075 mg/kg bw) was higher than the highest peak exposure following IT administration (13.8 ng/ml at a dose of 0.094 mg/kg bw).

However, extrapolation of the observed, roughly linear relationship between dose and C_{max} following IT administration beyond the highest dose administered (0.145 mg/kg bw) was not considered appropriate, since the assumptions of dose proportionality and homoscedasticity cannot be verified for the extrapolated dose range. It is therefore also not possible to estimate a reliable numerical margin of

safety for a dose above the maximum dose used in the field trial. The maximum treatment dose was therefore set to 0.15 mg tigilanol tiglate/kg bw.

Metabolism
The applicant presented three exploratory laboratory studies investigating the metabolism of tigilanol tiglate:

- An *in vitro* study using canine hepatocytes, in which thirteen metabolites were identified. Structural elucidation has been provided for the five most prevalent metabolites: M4, M5, M6, M9 and M13.
- An *ex-vivo* pilot study performed in mouse cells on the metabolism of tigilanol tiglate in human blood and in mouse tissue homogenates. Human blood and tissue homogenates from mice were treated with tigilanol tiglate. The metabolites were extracted and analysed by LC-MS/MS. Metabolites were demonstrated to have a 60- to >2000-fold reduced bioactivity. The overall conclusion was that a significant fraction of tigilanol tiglate metabolites are considered essentially inactive; both pharmacologically as well as toxicologically.
- A screening pharmacokinetic study performed in healthy dogs conducted to determine the pharmacokinetic profile of tigilanol tiglate after a single SC and IV dose. Due to the explorative character of this study, no firm conclusions on the pharmacokinetics following IV or SC administration of the product could be drawn.

Excretion
The route of excretion of tigilanol tiglate or its metabolites has not been determined, and currently, the mechanism of toxicity is not fully clear and target organs could not be identified. However, in line with the scientific advice (EMA/CVMP/SAWP/178983/2017), in order to assess possible exposure of people, information on the occurrence of tigilanol tiglate and possible metabolites in faeces, urine and saliva including leakage from the tumour site has been provided (see part 3).

Dose justification / dose finding

The dose to be administered intratumorally is proportional to the size of the tumour and is limited by the body weight of the dog and the size of the tumour (up to 8 cm^3). The dose is calculated as 50% of the volume of the target tumour i.e. 0.5 ml [0.5 mg] per cm^3 of tumour size, up to a maximum of 0.15 mg/kg bw or 4 mg (equivalent to 4 ml) per animal. Independent of the number of tumours that are treated at one time, the maximum dose of 0.15 mg tigilanol tiglate/kg bw must not be exceeded, as the safety of higher doses has not been demonstrated. The proposed minimum volume of Stelfonta to be administered is 0.1 ml/tumour. The product is delivered by IT injection inside the target tumour using a fanning injection technique.

The applicant provided different studies, investigating the effect of different strengths and treatment volumes on the tumour, as well as repeated administrations in cases where no complete response was achieved upon initial treatment.

Concentration / strength

The applicant provided one non-pivotal GCP-compliant dose determination 'like' study, performed in Australia in dogs with MCT. The study was a multi-site, unmasked, uncontrolled non-randomized field study investigating different strengths of the formulation (see part 2 – development pharmaceutics). The study design was unconventional, as de-escalation was used instead of escalation of the concentration. Twenty-seven dogs were divided in three groups, and dosed at a strength of 0.2, 0.5 or 1.0 mg/ml. In all animals, treatment volume was 0.5 ml per cm^3 tumour delivery, which was the highest deliverable volume possible to maximise spread of the product throughout the tumour mass without

leakage of the product. The final formulation of Stelfonta was not used: each of the 3 concentrations was diluted in a 30% propylene glycol solution. It is however unlikely that the difference in propylene glycol concentration would have resulted in a significantly different outcome since the excipient is not known to have an anti-tumour effect. The mean dose volume tested was 0.35 ml (range 0.08-2.35 ml) and the mean treatment dose investigated was 0.025 mg/kg bw (range 0.0005 - 0.135 mg/kg bw).

Of the 10 animals treated with 1.0 mg/ml, nine experienced complete remission (CR) and one experienced Stable Disease (SD). Results demonstrated that efficacy (in terms of CR at D21) in animals treated with 1.0 mg/ml was significantly better ($p<0.05$) than in animals treated with the lower strengths. Also, only limited systemic adverse events were observed in animals treated with this concentration. Therefore, the 1.0 mg/ml concentration was considered appropriate.

The CVMP agrees that results of this study support the 1.0 mg/ml concentration to be the most appropriate strength for further studies.

Treatment volume

A dose of 0.5 ml per cm^3 tumour was the highest deliverable volume to maximise spread of the product within the tumour without leakage of the product. When the dosing volume was decreased to 0.4 ml per cm^3 tumour, this resulted in reduced efficacy. Minimum treatment volume was 0.1 ml since in tumours treated with volumes of less than 0.1 ml, the risk of ineffective treatment increased.

The effect of the proposed dose of 0.5 ml [0.5 mg] per cm^3 of tumour size was further confirmed in a dose confirmation (field) study, and in the (pivotal) field studies (see below). Dose restrictions to 5 mg/animal were introduced due to adverse events observed in a preliminary study in which treatment of soft tissue sarcomas was investigated. For the treatment of mast cell tumours, the maximum dose is 4 mg/animal since efficacy in tumours larger than 8 cm^3 was not sufficiently demonstrated.

It was noted that the CVMP guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008) was not applied: dose de-escalation was used instead of dose escalation, and the recommended strength of 1 mg/ml was not the central concentration tested. However, as stated earlier, this guideline does not fully apply for this product.

Simultaneous treatment of multiple tumours

The simultaneous treatment of multiple (up to three) distinct MCT was evaluated in one small, supportive, non-GCP study. This study included 12 dogs with multiple (up to three) tumours (26 cutaneous tumours and 1 SC MCT). At D28, 24/27 tumours achieved CR, and three achieved PR. Safety was acceptable. The safety of simultaneous treatment of more than three tumours was not evaluated.

Repeated treatment

According to the product information, treatment with Stelfonta may be repeated after 4 weeks in cases where complete response (CR) is not achieved, and where the surface of the residual mass is intact. Significant data on progression of a potential residual tumour beyond D28 are not available. However, it is considered appropriate to provide further treatment in case CR is not achieved at that point.

In the pivotal field trial, CR was obtained in 8 dogs (out of 18) that received a second dose after 28 days. A significant difference in the anticipated animal safety is not expected; the incidence of adverse events was similar in dogs re-treated in the pivotal field trial.

Conclusions

Overall, the CVMP concluded that the dose (treatment volume and concentration) and dosing scheme (single-use, potential for a second dose in case of residual tumour at D28) are adequately justified for tumours up to 8 cm^3.

Target animal tolerance

On target animal tolerance, one pivotal GLP-compliant TAS study and three supportive laboratory studies were provided. Laboratory studies primarily provided information on systemic target animal safety of the product. Study design in all of the laboratory studies, including the pivotal target animal safety study, was not fully in line with the VICH guideline GL 43 (target animal safety), since the dose applied and the method of administration were not in line with the proposed SPC. This is however considered justified since healthy, non-tumour bearing animals were used in these studies.

In addition to the laboratory studies, both systemic safety as well as local tolerance was evaluated during the clinical trials, primarily the pivotal field study.

Systemic toxicity (intravenous use; laboratory studies)

Systemic toxicity of the product in dogs was mainly assessed in laboratory studies, following intravenous (IV) infusion over 15-30 minutes.

One laboratory study investigated the maximum tolerated dose (MTD) by intravenous administration in healthy beagle dogs (n=14). The IVP (not the final formulation) was administered in 15 minutes at a concentration of 1.0 mg/ml and doses of 0.05, 0.1, 1.5, or 0.225 mg/ kg bw. The MTD in healthy male young dogs was 0.150 mg/kg bw IV. At a higher dose of 0.225 mg/kg bw IV, one dog died following infusion over 15 min, and another dog showed severe transient reactions following IV infusion of the same dose over 32 minutes. At other doses, clinical signs were transient and disappeared within 4 hours. The MTD at the repeat IV dose phase was in the range of 0.075 mg/kg bw and <0.100 mg/kg bw in female and male dogs, respectively.

In another laboratory study and the pivotal TAS study, tigilanol tiglate administered in doses up to 0.075 mg/kg bw IV over 15-minutes (i.e. within the range of the recommended IT treatment dose) was generally well tolerated, and clinical signs were mostly categorised as mild and transient. Clinical signs relating to systemic toxicity were primarily retching, emesis, salivation, tremor, lethargy (decreased activity), tachycardia, restlessness, and occurred during or shortly following (within the first 1 to 4 hours post dosing) IV administration. In the pivotal TAS study, which was a blinded 4-week repeat-dose IV infusion toxicity study followed by a 14-day recovery period in beagle dogs, there were no remaining clinical observations or local injection site reactions noted during the 14-day recovery phase.

However, neither a margin of safety nor a maximum tolerated dose with regard to the proposed conditions of use can be derived based on the data presented. The mechanism of systemic toxicity of tigilanol tiglate is unclear, and no target organs could be identified.

For the pivotal TAS study, no indications for systemic adverse events could be identified from the clinical pathology parameters (urinalysis, haematology/ biochemistry).

Systemic safety (intratumoral use; field studies)

The safety of Stelfonta when used as recommended was evaluated in a number of field studies, demonstrating that local and systemic safety was acceptable, in particular with regard to the lower part of the proposed dose range.

In all field studies, safety evaluation consisted of clinical parameters (body weight and vital signs temperature, respiratory rate and heart rate, general demeanour and body function), and observation for any adverse events (AE). All AEs were categorised and graded according to the Veterinary Cooperative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE) (v1), which is a descriptive severity scale (grade system; Grade 1-5). This is considered appropriate, and in

line with the recommendations of the CVMP guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008).

Two field studies evaluated serum chemistry, haematology and urinalysis (one study only). Overall, serum chemistry and haematology results indicated that there was no significant detectable systemic toxicity due to treatment. Urinalysis, serum biochemistry and haematological readings were mostly unremarkable, and any significant changes are likely to be of minimal clinical relevance. The observations are appropriately described in current SPC.

Clinical observations were largely unremarkable throughout the field studies. Mild fluctuations in bodyweight were however observed, and these concerned both bodyweight gain and loss. Clinical relevance of the fluctuations in bodyweight is therefore considered to be minimal.

However, deaths potentially related to treatment occurred in two dogs out of a total of 238 included in the field studies. Death in one case was presumed to be caused by degranulation of mast cells and poor owner compliance to supportive treatment, whilst in the second case it was the result of a necrotizing and suppurative panniculitis and cellulitis following treatment.

Also, a number of adverse events were observed following treatment of subcutaneous tumours located on the trunk. In consequence, the indication for subcutaneous MCT is restricted to locations at or distal to the elbow or hock. Once concomitant treatment (consisting of H1 and H2 receptor blocking agents as well as prednisone) was optimised and prescribed as mandatory, and inclusion criteria were narrowed to only include treatment of subcutaneous tumours located at or distal to the elbow or hock, no further deaths were observed that were presumably caused by treatment (such as degranulation of mast cells), and fewer degranulation events were observed that could be related to treatment.

Only a small number of the animals treated in the field studies, including the pivotal field study, received doses above the maximum dose. It appears that the incidence rate of severe AEs was higher in dogs treated with doses >0.15 mg/kg bw compared to lower doses (adverse event grading for: doses >0.15 mg/kg: Grade 3 (per patient): 0.375; Grade 4 (per patient): 0.125; all doses combined: Grade 3 (per patient): 0.2; Grade 4 (per patient): 0.3).

In an additional, supportive field study using tigilanol tiglate IT injection for a different indication (soft tissue sarcomas), three dogs experienced severe adverse reactions, including death of one dog after administration of 9.25 mg (0.25 mg/kg bw) of tigilanol tiglate following cardiopulmonary arrest. Post-mortem examination of this dog showed various sites of haemorrhage (abdomen, lungs, brain). Another dog was euthanised due to the development of a large area of necrosis after having received 8.9 mg (0.25 mg/kg bw) tigilanol tiglate. A third dog was euthanised due to seizures that commenced 2 days after treatment with 2.4 mg (0.24 mg/kg bw) of tigilanol tiglate. It cannot be excluded that the deaths observed in this study were treatment related.

It should be noted that, further to the restriction of the tumour volume to a maximum of 8 cm^3, a maximum dose of 4 mg/animal is now possible.

Local tolerance (field and laboratory studies)

Local reactions following intratumoral injection were noted in all the field studies. The local adverse events observed in the field studies were very common and mainly mild (Grade 1-2) and included wound formation and pain at the treatment site.

In the pivotal field study, wounds secondary to tumour destruction occurred in 94% of the naïve patients treated with tigilanol tiglate.

None of the cases that did *not* experience wound formation developed CR. These wounds are therefore considered directly related to the mode of action of tigilanol tiglate in tumour destruction. In most of

these cases, maximum wound size was recorded at 7 days after treatment, although in a small number of cases (n = 14) wound size increased between 7 and 14 days post treatment. More than 50% of the wounds had a maximum surface area of less than 4 cm^2. The average wound size at D7 for dogs treated with Stelfonta was 3.3 cm x 2.4 cm (average tumour size before treatment: 1.9 x 1.6 x 0.9 cm). Wound size had reduced by almost 50% at D28 to approximately 2.0 x 1.3 cm.

However, in some cases, larger wounds developed that required additional measures. In the pivotal field study, while less than 20% of wounds exceeded 16 cm^2, there were 9 cases that formed very large wounds (>32 cm^2). However, all but one of these was fully healed by day 84. Some wounds appeared to have been extensive, even in smaller tumours – with a maximum single wound size of 15.5 x 5.1 cm recorded in an animal treated for a tumour volume of 3.5 cm^3. In 20% of the animals that had developed wounds, antimicrobials were required. Wound management (such as bandaging and wound dressing and sedation) was only required in four patients. Formation of large wounds occurred more often in larger tumours. Wound size is however not reliably predictable from tumour volume, neither could wound formation reliably be predicted from tumour location and draining lymph node reactivity at screening. Therefore, there remains a non-negligible risk of the development of unpredictably large wounds following treatment. Surgery is therefore considered the gold standard for mast cell tumours, whenever possible, irrespective of their cytological grade. This is appropriately addressed in the SPC.

In case of significant wound formation, a substantial time to heal (>28 days) was often necessary. Larger wounds and wounds on lower limbs resolved more slowly.

Even in case local toxicity was graded as serious or severe, results of the pivotal field trial demonstrated that the adverse events could be managed through the course of treatment and a clinical benefit (CR) was achieved in the majority of these cases. Safety warnings included in the SPC and product literature are considered appropriate.

Additionally, one laboratory study was conducted to investigate the maximum tolerated dose by subcutaneous administration in beagle dogs. The results showed that the maximum tolerated SC dose was between 0.026 to 0.032 mg/kg bw (total dose was administered in four SC injections, each containing 5-8 µg/kg bw, respectively). At these dose levels, local effects occurred (oedema, erythema, wound development).

Conclusions

Tigilanol tiglate is a substance of considerable systemic toxic potential, although the mechanism of toxicity and target organs of toxicity have not yet been characterised. Available information does not allow for a reliable extrapolation of the systemic exposure when the product is dosed IV compared to IT beyond doses of 0.145 mg/kg bw.

Whilst the recommended treatment dose was generally systemically well-tolerated when administered as recommended (intratumorally), unintentional IV administration, especially at the maximum recommended dose, should be avoided at all times, since this is expected to cause severe systemic effects. Clinical signs relating to systemic toxicity were primarily retching, emesis, salivation, tremor, lethargy (decreased activity), tachycardia, restlessness, and occurred during or shortly following (within the first 1 to 4 hours post dosing) IV administration. This is appropriately addressed in the SPC.

Following the recommended route of administration (intratumoral injection), mild to moderate local adverse events and pain are likely to occur in all animals that are treated, which however can lead to the development of substantial wounds that could require additional treatment. Surgery therefore is considered the gold standard for mast cell tumours. Adequate risk mitigation measures regarding wound management are included in the SPC.

Overall, it is concluded that local and systemic tolerance is acceptable when the product is administered according to SPC recommendations.

Clinical field trials

The applicant provided a dose determination study (described under *Dose justification / dose finding* section above), a dose confirmation study and one pivotal clinical study, conducted under field conditions. All field studies included client-owned animals at veterinary clinics in the USA and Australia. Considering that the standard of care in USA and Australia is comparable to Europe, and canine MCT are not impacted by geographic factors, this is not considered to have an impact on the outcome of the studies, and it is expected that Stelfonta should perform similarly in the EU as in the USA and Australia.

In all field studies, determination of efficacy was based on objective tumour measurements made according to the Response Evaluation Criteria in Solid Tumours (RECIST) v.1.1 guideline (Eisenhauer *et al.,* 2009). Response to therapy was defined as complete response (CR, complete resolution of the target lesion), partial response (PR, at least 30% decrease in the longest diameter of target lesion), stable disease (SD, decrease in the target lesion of less than 30% or increase of the target lesion less than 20%) or progressive disease (PD, greater than 20% increase in the target lesion).

Tumour volume was assessed as follows:

Tumour Volume (cm^3) = ½ (length (cm) x width (cm) x height (cm))

The assessment of the primary outcome parameter (response) is adequately described, and clinical assessment of response (i.e. complete response and partial response) is clearly defined in the study protocol. Primary outcome is considered in line with the CVMP guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008).

Dose confirmation

The GCP-compliant dose confirmation study was a non-randomized, non-masked, single-arm field study. Though, according to the CVMP guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008), a randomized placebo/BSC (best supportive care) controlled design is preferred, the applicant justified the need for a one-armed design. The study was performed in the USA and included eleven MCT (six cutaneous MCT and five subcutaneous MCT in ten animals). The animals were treated with 0.5 ml tigilanol tiglate/cm^3 of tumour volume with the final formulation (concentration of 1 mg/ml). The maximum dose of Stelfonta was set to 0.25 ml/kg body weight, with no more than 5 mg to be administered per dog. The primary efficacy criterion was complete response (CR) at D14, defined as disappearance of the target lesion. At evaluation on D14, 10 MCT experienced CR (91%), whilst 1 tumour experienced PD. At D28 (end of the study), one dog experienced relapse, and efficacy was therefore considered to be 82% (9/11).

In the context of a dose confirmation study and taking into consideration the MUMS status of this product, the range of tumour volumes enrolled is adequate to support the efficacy of the proposed dose (0.5 ml tigilanol tigilate/cm^3 of tumour volume). Nevertheless, neither the maximum recommended dose volume of 5 ml, nor the maximum planned treatment dose of 0.25 mg/kg bw was investigated in this study. Thus, with regard to dose confirmation, no conclusions can be drawn for doses above those tested in this study (0.002 mg/kg - 0.145 mg/kg bw).

Clinical studies

One pivotal, multicentre, randomised, fully blinded, negative controlled clinical field study was conducted to evaluate the effectiveness and field safety of Stelfonta. The study was conducted in 11

veterinary clinics in the USA and did adhere to GCP. In addition, several supportive studies were provided to investigate the efficacy and clinical safety of the product for the proposed indication.

The pivotal efficacy study was considered appropriate and well designed, GCP-compliant, randomised, negative controlled, and blinded. The objective of this study was to evaluate the effectiveness and field safety of tigilanol tiglate (final formulation) when administered via intratumoral injection into cutaneous and lower limb subcutaneous mast cell tumours.

In this study, 123 (121 in the PP population) dogs of various breeds, sex, age (3.5-16 years old) and weights (3.2 to 64.0 kg) were included. Eighty-one of the 123 animals were randomly allocated to the IVP group, whilst 42 were allocated to the negative control group (intent-to-treat population) (2:1 ratio). For blinding purposes, the tumour area was prepared for treatment in both groups. The number of dogs is considered appropriate, and the dogs included in the study were considered to reflect the patient population accurately.

Eligibility criteria included cytological diagnosis of a subcutaneous (SC)/cutaneous MCT; in case of SC MCT, the tumour was required to be located at or distal to the elbow or hock. Only stage Ia and IIIa tumours were included. In all of these animals, presence of a MCT was confirmed via fine needle aspiration (FNA). Confirmation of a MCT by means of cytology is considered appropriate. However, accurate grading (Kiupel system) is not always possible by means of cytology, and in 46 patients accurate cytological grading was not possible. Since the results indicated that at least 9% of all of the included cases were high grade MCT, the clinical trial population is considered to sufficiently reflect the general patient population.

Only a limited number of cytologically confirmed high-grade MCT were included in this pivotal field study [(suspected) high grade: n=13, of which n=10 were ultimately treated]. Five of these animals achieved a complete response after 1 or 2 treatments, four of which were still tumour free after 84 days from their final treatment. This aspect is appropriately addressed in the SPC.

In this pivotal field study, animals with confirmed metastatic disease were excluded, which is considered to be appropriate, since this is a localised treatment. Staging in this pivotal field study was, however, limited to palpation of regional lymph nodes and aspiration of any palpable sentinel lymph node. Staging thus did not include evaluation of lymph nodes that could not be reached by palpation, or assessments of distant metastatic disease. Therefore, results of this study are considered a worst-case scenario.

Tumour sizes included in this study are considered to represent the situation in the field. Tumours were relatively small, with a mean size of 1.7 cm^3 (range 0.1-9.8 cm^3). Only four tumours had a tumour volume over 8 cm^3. In the group of animals (re)treated at D28 (n=18 + n=33), tumour volumes ranged from 0.1 to 10.5 cm^3 (mean 1.36 cm^3), with a corresponding treatment volume of 0.1 to 4.6 ml Stelfonta (mean 0.7 ml).

At D28, effect of treatment in terms of complete response (CR) (= primary efficacy criterion) was evaluated by two masked investigators. In case a palpable lesion was still present, CR was based on FNA results. The assessment of CR is considered appropriate and in line with the CVMP anticancer guideline.

Secondary efficacy criteria were the number of dogs with objective response to treatment, defined as CR + PR; disease-free interval (DFI) on Days 42 and 84, owner assessment of QoL (Quality of Life), and wound healing assessment. The proposed secondary endpoints are considered acceptable, and criteria correspond to current acceptable RECIST Guidelines for solid tumour response assessment.

Statistically, the treatment effect on effectiveness was evaluated using a two-sided test at alpha = 0.05. Treatment effectiveness was concluded if there was a significant difference in the success rates between

the treated and untreated control groups, and if the percent success was higher in the treated group compared to the untreated control group.

Results demonstrated that 60/80 (75%) of dogs in the Stelfonta group had CR of the target lesion (primary efficacy parameter) at 28 days after one injection (compared to 5.3% in the negative control group), and 5% had a partial response, giving an objective tumour response of 80%. At Day 28, progressive disease was noted in 7.5% and 21.1% of the initially treated- and negative control group, respectively. As for DFI, by Day 84, in 96.5% of the dogs that showed initially CR, the target lesion had not recurred.

In case an animal was 'not CR' at D28, the dog received a second treatment (n=18). This allowed the evaluation of repeated (two-dose) treatment. Also, at that point, the negative control group was offered optional treatment with Stelfonta (n=33). Eight out of the 18 dogs that had received a second treatment were in CR at Day 58 (thus, the total number of CR after one-or two treatments was 68/78 (87.2%)). In seven of these eight dogs, no local occurrence was observed for up to 12 weeks after the second treatment. In the negative control group, 20/33 dogs (62.5%) had CR following initial treatment at Day 30, and 19 of these 20 dogs were still disease-free 12 weeks after treatment. Response rate in the group treated later in time was therefore somewhat lower than the group that was treated at D0 (80% versus 62.5%).

Results obtained from four other, long-term follow-up studies (that evaluated local efficacy of the product for up to 12 to 48 months) support that if the treatment resulted in CR, recurrent disease is unlikely. However, since a long-term follow up was not foreseen according to the study protocol of the individual studies, results have to be considered with caution. It was however clear that, as expected, treatment did not prevent the occurrence of *de novo* MCT at other locations.

Concomitant treatment

Stelfonta intratumoral injection leads to the *in-situ* destruction of the neoplastic mast cells (and also other cells), which may induce a severe subsequent mast cell degranulation. Concomitant treatment (with corticosteroids and H1/H2 receptor blockers) is therefore applied in order to reduce the occurrence of local and systemic adverse events related to mast cell degranulation and histamine release. It is expected that this mandatory concomitant treatment will reduce adverse reactions due to mast cell degranulation and thus improve safety. The use of H1 and H2 antagonists in animals suffering from MCT is well-known and accepted globally, and often considered necessary in animals suffering from MCT, independent of any treatment (Blackwood et al. (2012) - European consensus document on mast tumours in dogs and cats, Veterinary and Comparative Oncology).

Concomitant treatment was administered in most studies. In the pivotal field study, concomitant treatment was administered to all animals including the negative control group and consisted of H1 and H2 receptor blocking agents (e.g. diphenhydramine or chlorpheniramine and famotidine) administered between D0-D7, and prednisone (or prednisolone), administered between D2-D8. It cannot be excluded that concomitant use of corticosteroids (and H1 and H2 blocking agents) has participated in the effect, since corticosteroids are known to induce neoplastic mast cell apoptosis. However, a highly significant difference in outcome between the treated group and the negative control-group was observed. It is therefore agreed that solely the use of the concomitant medications cannot explain the results of this study. It is however appropriate that concomitant use of these products is also included in the product information in order to assure similar efficacy (and safety) when used in the field situation. The relevance and use of the mandatory concomitant treatment is adequately addressed in the SPC.

Quality of life

In line with the CVMP guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008), the pivotal field study also evaluated (owners opinion of) the Quality of Life (QoL) of the dog during the study period, by means of a questionnaire. Owner assessment of QoL suggest that quality of life during the study period, when evaluated by several categories (happiness, mental status, pain, hydration, mobility, appetite, hygiene), was not significantly negatively influenced by the treatment when compared to untreated dogs. There was no evidence of significant or debilitating pain at time points associated with maximum wound sizes following tumour destruction (Day 7/Day 14). Consistent with this low pain measure, patient mobility and engagement was high, with above average playfulness ratings recorded at these times.

Conclusions

The results of the field studies show that Stelfonta is effective in the treatment of cutaneous and subcutaneous MCT, administered IT at a dose of 0.5 ml per cm^3 of tumour volume, in tumours up to 8 cm^3. The pivotal field study demonstrated that in case of incomplete response at D28, a single repeated treatment can further increase efficacy of treatment. Treatment effect was primarily demonstrated in lower grade MCTs.

Overall conclusion on efficacy

Pharmacodynamics:

Stelfonta contains tigilanol tiglate, an antineoplastic agent intended for use in dogs for the treatment of non-resectable, non-metastatic cutaneous and subcutaneous mast cell tumours. In canine MCTs, the intended effect of tigilanol tiglate is the induction of PKC-dependent necrosis of tumour cells as well as non-neoplastic components (such as blood vessels, fibrous and connective tissues) in solid tumours. This desired effect is supposed to be achieved by causing a so called "massive necrosis", meaning that the pharmacodynamic effect is not specifically confined to neoplastic cells. The local effect on neoplastic tissue is only achieved due to the intratumoral administration.

Resistance:

Tigilanol tiglate is a new chemical entity, which has not been used in veterinary medicines before. The exact risk of resistance development with regard to the use of this product is therefore currently not known. However, the development of resistance following treatment with tigilanol tiglate is considered to be unlikely.

Pharmacokinetics:

Systemic bioavailability after intratumoral doses ranging between 0.0005 - 0.145 mg/kg bw could be directly measured. For the dose ranges tested, a dose-dependent effect is noted for C_{max} and AUC_{last}. Extrapolation beyond that dose range is not verified by PK data.

After IT injection, the maximum concentration of tigilanol tiglate appears rapidly in the blood stream, t_{max} occurred within 5 to 30 minutes, and $t_{1/2}$ ranged from 1.24 to 12.4 hours. Tigilanol tiglate appears to exhibit flip-flop kinetics (sustained release), since a considerable shorter half-life of 0.54 hours was determined after IV infusion. Measurable plasma concentrations were most frequently observed within 1 hour after treatment. Plasma levels quickly declined, and only low levels of tigilanol tiglate (<1 ng/ml) were detectable in plasma by 24 hours. Following treatment, only in isolated cases a measurable concentration of tigilanol tiglate could be observed in excretions (saliva, urine and faeces).

Dose determination:

The proposed dose for Stelfonta is 0.5 ml/cm^3 of tumour volume, with a maximum dose of 0.15 mg/kg bw, for treatment of tumours with a maximum tumour volume of 8 cm^3 (i.e. 4 mg per dog). Independent of the number of tumours that are treated at one time, the maximum dose of 0.15 mg tigilanol tiglate/kg bw must not be exceeded. The quantitative composition (strength) of 1 mg/ml was established based on a dose determination study using different strengths in a de-escalation way (1.0 mg/ml; 0.5 mg/ml; 0.2 mg/ml), with a mean injection volume of 0.35 ml (range 0.08-2.35 ml), and mean treatment dose of 0.025 mg/kg bw (range 0.0005 - 0.135 mg/kg bw). The data were supported by a dose confirmation study performed under field conditions. In addition, efficacy was confirmed in (supportive) field studies.

Tolerance:

Neither a margin of safety nor a maximum tolerated dose with regard to the proposed conditions of use can be derived based on the data presented.

When administered intravenously by infusion, the maximum tolerated dose for healthy male young dogs was observed at 0.150 mg/kg bw; at a higher dose of 0.225 mg/kg bw one dog died following infusion over 15 min, and another dog showed severe transient reactions following IV infusion of the same dose over 32 minutes. Systemic adverse reactions at 0.075 mg/kg bw over 15 minutes infusion (i.e. within the range of the recommended IT treatment dose) were considered mild and transient, and included vomiting, restlessness, lethargy, salivation, retching and tachycardia.

Following intratumoral injection as recommended in the SPC, mild to moderate local adverse events are expected to occur in over 90% of the animals. Wounds resulting from the treatment with tigilanol tiglate are considered directly related to the mode of action of the drug in tumour tissue destruction. Wound management is required in a number of cases.

Based on the data provided, the maximum treatment dose is set at 0.15 mg tigilanol tiglate/kg bw.

The field studies showed that local and systemic safety was acceptable, up to 0.15 mg/kg bw. Adverse reactions are correctly reflected in the SPC.

In patients treated intratumorally for MCT, deaths, possibly due to treatment, occurred in two animals. However, it was noticed that once concomitant treatment (consisting of H1 and H2 receptor blocking agents as well as prednisone) was optimized, and inclusion criteria were narrowed to only include treatment of subcutaneous tumours located at or distal to the elbow or hock, no further deaths were observed that were presumably caused by degranulation of mast cells, and fewer degranulation events were observed that could be related to treatment.

The mitigating measures included in the SPC in order to ensure responsible use of the product are considered appropriate.

Efficacy:

The effect of treatment administered IT at the proposed dose of 0.5 ml per cm^3 of tumour volume was demonstrated in low grade and smaller MCT (up to 8 cm^3).

The pivotal field study demonstrated that in case of incomplete responses at D28, repeated treatment can further increase efficacy of treatment.

Part 5 – Benefit-risk assessment

Introduction

Stelfonta is a solution for intratumoral injection for dogs containing 1 mg/ml tigilanol tiglate as the active substance and is presented in packs containing 1 vial (2 ml). Tigilanol tiglate is an antineoplastic agent that causes necrosis of cells in direct contact with tigilanol tiglate, activates protein kinase C, and increases the permeability of the vasculature.

The product is intended for use in dogs for the treatment of non-resectable, non-metastatic cutaneous and subcutaneous mast cell tumours. Treatment consists of a single intratumoral injection of 0.5 ml per cm^3 of tumour volume, and may be repeated once (after 4 weeks), if needed. Treatment must be administered together with corticosteroids and H1 and H2 receptor blocking agents. If needed, analgesics and wound management measures should be administered.

The dossier has been submitted in line with the requirements for submissions under Article 31 of Regulation (EC) No 726/2004 of 31 March 2004; and in accordance with Article 3(2)a, as the product contains a new active substance, which was not authorised in the Community on the date of entry into force of the Regulation.

The application concerns a new active substance; however, the product has been classified as MUMS/limited market and therefore reduced data requirements have been considered in the assessment.

Benefit assessment

Direct therapeutic benefit

The benefit of Stelfonta is its efficacy in the treatment of non-resectable, non-metastatic (WHO staging) subcutaneous mast cell tumours located at or distal to the elbow or the hock, and non-resectable, non-metastatic cutaneous mast cell tumours in dogs. Tumours must be less than or equal to 8 cm^3 in volume and must be accessible to intratumoral injection.

Efficacy was investigated in well-designed field studies conducted to an acceptable standard.

The results of the field studies show that the product is effective in the treatment of non-metastatic cutaneous and subcutaneous mast cell tumours, administered intratumorally at a dose of 0.5 ml per cm^3 of tumour volume. In case of incomplete responses at D28, a second dose can further increase efficacy of treatment.

Additional benefits

The product increases the range of available treatment possibilities against non-resectable, non-metastatic cutaneous and subcutaneous mast cell tumours.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

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

Risks for the target animal:

Administration of Stelfonta in accordance with SPC recommendations is generally well-tolerated systemically, when administered intratumorally at the recommended dose (up to 0.15 mg tigilanol tiglate/kg bw). Following intratumoral injection, local reactions (including wound formation and pain at the treatment site) were noted in all the field studies and were mainly mild. However, in some cases, larger wounds developed that required additional measures. Wound size is not reliably predictable.

Risk for the user:

The most relevant routes of accidental contact are self-injection as well as dermal and eye exposure. For the non-professional user, dermal exposure may occur via contact with treated animal and the excreta. Contact with the product may result in skin or eye irritation. Also, hypersensitivity reactions cannot be excluded. Accidental self-injection would result in the worst-case exposure. Severe local effects, including pain, oedema and necrosis might occur. The safety of the veterinary medicinal product has not been established during pregnancy or lactation. Therefore, pregnant women or breastfeeding women should take care to avoid accidental self-injection, contact with the injection site, leaking product and tumour debris.

Risk for the environment:

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

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

The applicant applied for the following indication: "For the treatment of all non-metastatic (WHO staging) cutaneous mast cell tumours, and subcutaneous mast cell tumours located at or distal to the elbow or the hock in dogs. Tumours may be of any cytological grade and must be accessible to intratumoral injection".

The product has been shown to be efficacious for (subcutaneous) mast cell tumours of up to 8 cm^3 in volume, and the CVMP agreed to the following indication: "For the treatment of non-resectable, non-metastatic (WHO staging) subcutaneous mast cell tumours located at or distal to the elbow or the hock, and non-resectable, non-metastatic cutaneous mast cell tumours in dogs. Tumours must be less than or equal to 8 cm^3 in volume, and must be accessible to intratumoral injection".

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

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

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for Stelfonta is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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

Divergent position to the CVMP opinion on the granting of a marketing authorisation for Stelfonta (EMEA/V/C/005018/0000)

Stelfonta is a veterinary medicinal product intended for use in dogs for the treatment of non-metastatic cutaneous and subcutaneous mast cell tumours by intratumoral injection. The active substance tigilanol tiglate is not authorised in human or veterinary medicinal products, and it is claimed to be an antineoplastic agent acting on the cell cycle regulation, the vasculature and inflammatory response, thereby inducing necrosis and cell death.

The application for marketing authorisation is, however not considered acceptable for the following reasons:

Safety / Toxicological studies

Tigilanol tiglate is a substance of considerable acute systemic toxicity. Clinical signs of severe systemic toxicity such as retching, vomiting, urination, salivation, defecation, decreased activity, lateral position, panting, swaying gait and breathlessness were noted in young healthy beagle dogs receiving a single iv dose of 0,025-0,150 mg/kg. A dose of 0.225 mg/kg bw by intravenous infusion, i.e. 1.5 times the maximum proposed intratumoral dose (0.150 mg/kg), was fatal in a male young healthy dog. The ultimate justification for the presented single-dose toxicity studies remains unclear since no threshold limit values of toxicity could be determined. The mechanism of toxicity is unclear and target organs could not be identified.

Stelfonta was considered to be irritating or corrosive to the skin in an *in vitro* study. Tigilanol tiglate injection was tested for skin irritation using OECD 439 *in vitro* reconstructed human epidermis (EpiSkin^{tm}) test. The OECD test 439 does not discriminate between skin irritants and corrosives. In target animal safety studies, a low concentration of 0.065-0.080 mg/ml, corresponding to a dose of 0.0065-0.008 mg/kg per injection site (0.026-0.032 mg/kg total dose per animal) administered subcutaneously to healthy beagle dogs resulted in localized pain, erythema, edema and open wounds at the injection site. Although the used formulation was not the final formulation, no local dermal signs were noted with vehicle alone. The concentrations and doses used were exceedingly small compared to the final formulation and the proposed therapeutic dose.

These findings, together with mode of action of the product, lead to a concern that tigilanol tiglate is corrosive, which cannot be considered acceptable for a medicinal product (intended for veterinary use).

No carcinogenicity studies regarding the active substance were presented in the dossier. The applicant presented evidence to support a non-carcinogenic status of structurally related phorbol 12-myristate 13-acetate (PMA) or phorbol 12,13-dibutyrate (PDBU); however, there remains concern regarding tumor promoting activity of tigilanol tiglate due to the lack of specific studies.

In conclusion, the active substance tigilanol tiglate is considered very toxic and corrosive.

Safety / User safety

The user risk has been correctly addressed by the applicant and the CVMP, however, the risk pertaining to accidental self-injection by the veterinarian (or other persons helping in restraining the dog), no matter how rare an event, is deemed considerable. This risk became apparent in clinical studies, where an accidental injection in a thumb (of the treating veterinarian) resulted in necrosis and a slowly healing

wound, which healed fully only after three months. Similar serious adverse events after accidental self-injection have been reported for other veterinary medicinal products (vaccines containing mineral oil as an adjuvant), however, for those products the proper treatment of the adverse reaction is advised in the product literature.

The applicant has provided no information on how accidental injections with Stelfonta should be treated (and consequently no advice is provided in the product literature).

Efficacy / Pharmacodynamics

The active substance is a PKC activator. Pharmacodynamic characterisation of tigilanol tiglate is insufficient and no studies as to the specific pd action in mast cell tumors are presented. The mode of action is demonstrated only via intratumoral administration, and other administration routes are not possible due to severe toxicity.

The pharmacodynamic action is largely unclear. The overwhelming characteristic mode of action seems to be chemically corrosive, which does not distinguish between tumor tissue and healthy tissue to a sufficient degree but masks any possible antineoplastic action that tigilanol tiglate might have. It is considered that tigilanol tiglate is not acceptable as an active substance in a medicinal product.

Clinical efficacy

Canine mast cell tumors are biologically heterogenous and the treatment is sometimes demanding. Diagnosis is easy, however, a treatment plan requires careful consideration of the biological behaviour of the tumor and its location. Characterisation of a study population of dogs with MCT is of utmost importance to be able to conclude on the treatment safety and efficacy. Tumor characterisation through cytological grading only is not according to current standard of care in either Europe or in the US considering that grading was uncertain (low or high grade suspected) or not confirmed at screening in a significant section of the dogs (31 of the 81 IVP treated dogs corresponding to 38 %). It is also considered that patient staging was insufficient especially with regard to the too short follow up period (12 weeks) in the only pivotal field study provided.

The indication proposed (non-resectable MCT) does not reflect the inclusion criteria of the pivotal field trial. A non-resectable tumor is a relative term, as a lower-limb MCT deemed as non-resectable by a primary care veterinarian may be cured with surgery by a veterinarian trained in orthopaedic surgery or surgical oncology. It is to note that the healing period for a surgical wound managed as "open wound" in the rare cases where skin flaps or similar solutions are not possible, may be shorter than the healing periods reported for some of the patients receiving Stelfonta in the pivotal field trial.

Based on the above, only limited conclusions can be drawn as to the efficacy of the product.

Target animal safety

Due to the nature of the product traditional TAS studies are not presented (nor requested). The studies presented, and safety data from the clinical studies, show that the product causes pain upon injection and pain that decreases when the sterile abscess is drained. In almost all cases a slowly second intent healing wound of variable size develops. In the pivotal field trial 40 % of IVP treated dogs had an open wound on day 28 and 27 % on day 42. In some cases broad spectrum antibiotics were used and a majority of cases received opioids for pain (as NSAIDs were contraindicated). The numbers regarding healing time, frequency of the need for broad spectrum antibiotics and analgesics, are considered severe as 80 % of the MCTs treated were small in size (<2 cm^3). It is also noted that the applicant specifically mentions that Stelfonta is a valuable option for dogs where anaesthesia is a particular risk due to concomitant disease, however, a majority of dogs have been anesthetised for treatment administration, which is understandable considering the pain on injection and the resulting risk of accidental self-injection.

Benefit/Risk assessment

It is considered that the evidence provided points to distinct corrosive properties of the active substance. An intra-tumoral injection of Stelfonta causes a sterile abscess, pain and suffering in patient dogs, and a considerable risk for the treating veterinarian (or restraining personnel) in case of accidental self-injection. A product that by default causes a wound that may not heal in several months is not considered safe. Causing a sterile abscess is not considered an acceptable mode of action for a (veterinary) medicinal product, regardless of some specific antineoplastic activity.

The benefits of use of the product are outweighed by the negative effects seen in practically every patient, especially considering that most of the canine MCTs are cured by simple surgery.

Amsterdam, November 7th 2019

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