07 October 2021
EMA/576338/2021
Veterinary Medicines Division

Committee for Veterinary Medicinal Products

CVMP assessment report for Zenalpha (EMEA/V/C/005465/0000)

INN: medetomidine hydrochloride / vatinoxan hydrochloride

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Introduction

The applicant Vetcare Oy submitted on 12 May 2020 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Zenalpha, 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 10 October 2019 as Zenalpha contains a fixed combination of an existing active substance, medetomidine hydrochloride, and a new active substance, vatinoxan hydrochloride, which is not yet authorised as a veterinary medicinal product in the Union.

The applicant applied for the following indication: To provide restraint, sedation and analgesia during conduct of non-invasive, non-painful or mildly painful procedures and examinations.

The active substances of Zenalpha are medetomidine hydrochloride, a potent α2-adrenorecptor agonist authorised as a sedative, and vatinoxan hydrochloride, a selective α2-adrenoreceptor antagonist. In combination, the two active substances are proposed to prevent or attenuate the adverse cardiovascular effects of medetomidine. The target species is dogs.

Zenalpha contains 0.5 mg/ml medetomidine hydrochloride and 10 mg/ml vatinoxan hydrochloride, and is presented in packs containing 1, 5 or 10 10 ml vials.

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

The rapporteur appointed is Rory Breathnach and the co-rapporteur is Niels Christian Kyvsgaard.

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 October 2021, the CVMP adopted an opinion and CVMP assessment report.

On 15 December 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for Zenalpha.

Scientific advice

The applicant received scientific advices from the CVMP on 7 March 2013, 16 February 2017 and 11 October 2018. The scientific advices pertained to safety (data requirements) and efficacy (dose optimisation and study design). The scientific advices were broadly, but not fully, followed and any concerns relating to this are discussed throughout this report.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (Version 6, dated 19th March 2021) which is considered to fulfil the requirements of Directive 2001/82/EC.

Manufacturing authorisations and inspection status

Batch release of the dosage form takes place at Apotek Produktion & Laboratorier AB, Umeå, Sweden. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the batch release of such veterinary dosage forms, has been provided.

GMP declarations for the active substance manufacturing sites were provided from the Qualified Person (QP) at the EU batch release site. The declarations were based on on-site audits by third parties.

Overall conclusions on administrative particulars

The GMP status of the manufacturing sites of the active substances medetomidine hydrochloride and vatinoxan hydrochloride and the finished product manufacturing site have been satisfactorily established and are in line with legal requirements.

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

Part 2 - Quality

Composition

The finished product is presented as a clear, slightly yellow to yellow or brownish yellow solution for injection containing two active substances; medetomidine hydrochloride at 0.5 mg/ml and vatinoxan hydrochloride at 10 mg/ml.

The other ingredients are: mannitol, citric acid monohydrate, methyl parahydroxybenzoate, propyl parahydroxybenzoate, sodium hydroxide, hydrochloric acid and water for injections.

The product is available in clear Type 1 glass vials of 10 ml with a coated bromobutyl stopper with an aluminium seal and flip-top cap as described in section 6.5 of the SPC.

Containers

The primary packaging is Type 1 glass vials of 10 ml with a coated bromobutyl stopper with an aluminium seal and flip-top cap. The material complies with relevant European Pharmacopoeia (Ph. Eur.) chapters. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

The glass vials are packaged in outer cardboard cartons containing 1, 5 or 10 vials per carton of the solution for injection. In the 5 and 10 vial pack sizes, each individual vial is packaged in an outer cardboard carton as the formulation is sensitive to light. The pack sizes are consistent with the dosage regimen and duration of use.

Development pharmaceutics

The application for 'Zenalpha 0.5 mg/ml + 10 mg/ml solution for injection for dogs' has been submitted as a full application under paragraph 3 of Article 12 of Directive 2001/82/EC as amended.

The aim of formulation development was to produce a sterile aqueous solution for injection,

consisting of the active substances medetomidine hydrochloride and vatinoxan hydrochloride combined at a fixed ratio in a formulation with physical, chemical and microbiological characteristics appropriate for administration by intramuscular injection to dogs.

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

After investigating different combinations the optimal ratio was later determined to be 1:20 (medetomidine hydrochloride: vatinoxan hydrochloride) as it was determined that 10 mg/ml vatinoxan hydrochloride was the desired concentration of active substance which meant that the concentration of medetomidine hydrochloride was reduced to 0.5 mg/ml. In relation to the development of the formulation with respect to excipients, satisfactory information was provided on how the product formula was developed in terms of the content of mannitol and citric acid. The chosen preservative system is commonly used in veterinary medicinal products and its efficacy is supported by data from preservative efficacy studies. The use of the proposed preservative system at the specified concentrations is considered acceptable.

The manufacturing process development was carried out during formulation development. The critical process parameters (CPPs) were identified using a risk assessment approach. The CPPs related to critical quality attributes (CQAs) were studied during manufacturing process development on pilot batches. The confirmed CPPs include what could be considered standard parameters for this dosage form.

The finished product is packaged in a 10 ml clear Type 1 glass vial with a coated bromobutyl rubber stopper with an aluminium seal and flip-top cap. Both the glass vial and rubber stopper comply with relevant monographs of the Ph. Eur. Data on finished product stability presented in Part II.F confirm that the product formulation and the container closure system are compatible.

The development section discusses the microbiological control of the product and its maintenance according to manufacturing controls, ensuring container closure integrity and confirming the effectiveness of the microbial preservative system. The efficacy of the antimicrobial preservative system was confirmed at the end of shelf life in studies carried out on two pilot-scale batches as part of the stability studies.

Method of manufacture

The manufacturing process is a simple standard process with sequential addition, heating, stirring and dissolution and filling steps. The filled vials are then subject to terminal sterilisation at ≥121 °C for ≥15 minutes. The defined in-process controls are generally appropriate for this type of manufacturing process and include controls for all relevant parameters.

Process validation has been conducted on three pilot-scale batches. The critical process parameters identified during manufacturing process development were controlled in the pilot batches to support numerical limits for these parameters. The pilot batches were tested for the critical in-process controls detailed in the dossier with all results found to be within specifications. The applicant confirms that full process validation at production scale) will be conducted prior to releasing any batches to the market. A process validation scheme has been provided and refers to the validation of the process on at least three consecutive batches manufactured according to the manufacturing process described in Part 2.B The level of detail included in the process validation scheme is in accordance with that detailed in Annex I of the guideline on process validation (Guideline EMA/CHMP/CVMP/QWP/BWP/70278/2012 *Process validation for finished products – information and*

data to be provided in regulatory submissions). Overall, the approach to process validation whereby process validation on production-scale batches will be carried out post-authorisation is acceptable and in line with the aforementioned guideline for a standard process.

Control of starting materials

Active substance

Medetomidine hydrochloride

The active substance medetomidine hydrochloride is not monographed in the Ph. Eur. and data on the active substance is provided according to the Active Substance Master File (ASMF) procedure. The active substance specification includes tests for description, appearance of solution, identity (IR, chloride), assay (HPLC), related substances (HPLC), specific optical rotation, loss on drying, sulphated ash, palladium and microbial quality (Ph. Eur.). The specification is considered to be acceptable. The test methods used for the control of the active substance are as per those of the ASMF. Acceptable batch analysis data has been provided. Batch details have been provided for the reference standard used by the applicant for the control of the active substance.

Vatinoxan hydrochloride

The active substance vatinoxan hydrochloride is not monographed in the Ph. Eur. and data on the active substance is provided according to the Active Substance Master File (ASMF) procedure. The active substance specification includes tests for description, identity (IR, chloride), assay (HPLC), related substances (HPLC), enantiomeric purity (HPLC), residual solvents (GC), elemental impurities (ICP), water content, sulphated ash and microbial quality (Ph. Eur.). The specification is considered acceptable. The test methods used for the control of the active substance are as per those of the ASMF. Acceptable batch analysis data has been provided. Batch details have been provided for the reference standard used by the applicant for the control of the active substance.

Excipients

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. The excipients are also controlled for microbiological quality in line with Ph. Eur. 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.

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

Control tests on the finished product

Finished product specifications for release and shelf life have been provided in a single document. The specifications include tests for appearance of solution, appearance of packaging, degree of colouration, identity (active substances), identity (preservatives), assay (active substances), assay (preservatives), related substances of vatinoxan hydrochloride and medetomidine hydrochloride, pH, extractable volume and sterility. The finished product specifications are acceptable and include relevant test parameters for the dosage form.

The dossier describes a single HPLC method which is used for the identification and assay of the two active substances, the determination of related substances of both vatinoxan hydrochloride and medetomidine hydrochloride and the identification and assay of the preservatives methyl and propyl parahydroxybenzoate. The analytical method is well described and has been validated in accordance with VICH GL2: *Validation of analytical procedures: methodology*. The test for sterility has been validated and demonstrated to be suitable for use with this product.

Satisfactory information regarding the reference standards used for assay (active substance and preservatives) and impurities testing has been presented.

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

Stability

Stability data is presented for studies carried out on three pilot-scale batches. The batches are identical to those proposed for marketing and were manufactured using different batches of active substance and packed in the primary packaging proposed for marketing. Stability data for these batches stored under VICH long term and accelerated conditions according to VICH GL3 *Stability testing of new veterinary drug substances and medicinal products* were provided. Data is also included for batches stored under VICH intermediate conditions.

The parameters monitored on stability are appearance of solution, degree of colouration, assay, related substances (of vatinoxan hydrochloride), pH and test for sterility. The analytical procedures used are stability indicating. For all three batches in the primary stability study, results for all parameters are within specification at all VICH storage conditions however some specific trends were noted.

Long-term and accelerated stability studies on supportive stability batches at laboratory scale have been provided. The data presented for these batches indicates that the results are in-line with the data presented for the primary pilot-scale stability batches with similar trends observed.

The proposed shelf-life of the product as packaged for sale is 3 years and is considered to be supported by the data presented.

Photostability studies were conducted in accordance with VICH GL5 *Stability Testing: Photostability Testing of New Veterinary Drug Substances and Medicinal Product* using one primary stability batch. In vials exposed directly to the light source, a decrease in vatinoxan HCl assay is observed and is associated with increases in levels of related substances. Thus, the product is sensitive to light however was demonstrated to be stable when protected from light by the secondary packaging. The storage precaution 'Keep the vial in the outer carton in order to protect from light' is included on the SPC and is considered to be appropriate.

Freeze thaw studies were conducted using one laboratory scale batch. The product was analysed for visual appearance, assay (active substances and preservatives), related substances and pH. No changes were observed in any of these parameters as a result of the freeze-thaw cycling indicating no impact of freezing and thawing on the product.

The applicant is proposing a 3 month in-use shelf life and has provided data on two in-use studies carried out on two pilot scale batches which investigated shelf lives after opening of 28 days and 3 months. The 28 day study included both fresh and aged batches while the 3 month study included only aged batches. The proposed shelf-life after first opening of 3 months is considered to be supported by the data.

Overall conclusions on quality

The finished product is presented as a clear, slightly yellow to yellow or brownish yellow solution for injection containing two active substances; medetomidine hydrochloride at 0.5 mg/ml and vatinoxan hydrochloride at 10 mg/ml. The formulation is an aqueous solution for injection containing methyl parahydroxybenzoate and propyl parahydroxybenzoate as antimicrobial preservatives, mannitol as an isotonicity adjusting agent and citric acid monohydrate as a buffering agent. Hydrochloric acid and sodium hydroxide are used for pH adjustment and water for injections as the solvent.

The product is available in clear Type 1 glass vials of 10 ml with a coated bromobutyl stopper with an aluminium seal and flip-top cap.

In the development pharmaceutics section the applicant provides a summary of the development of the formulation and the development of the manufacturing process. Development of the formulation is described in a stepwise manner and satisfactory information has been provided about how the final formulation was derived in terms of the concentration of the active substances and the excipient content.

The solution for injection is manufactured in a process involving sequential mixing and dissolution of the product constituents in water for injections. The manufacturing process is a simple standard process and is adequately described in the dossier. The finished product is sterilised by terminal sterilisation. Process validation data for 3 pilot scale batches and a process validation scheme for the process validation of production scale batches post-authorisation have been provided. The process validation scheme is acceptable and is in accordance with that detailed in Annex I of the guideline on process validation. The approach to process validation can be accepted based on the standard nature of the manufacturing process.

Information on the control of starting materials has been provided. The active substances medetomidine hydrochloride and vatinoxan hydrochloride are not monographed in a pharmacopoeia and data on the active substances is provided according to the Active Substance Master File (ASMF) procedure.

All of the product excipients are supplied to Ph. Eur. grade and in addition, are controlled for microbiological quality in line with Ph. Eur. The finished product container closure system is considered to be appropriate and the required supporting information is included in the dossier.

Finished product specifications for release and shelf life have been provided in a single document. A single HPLC method is used for the identification and assay of the two active substances, the determination of related substances of vatinoxan hydrochloride and medetomidine hydrochloride and the identification and assay of the preservatives, methyl and propyl parahydroxybenzoate. The analytical method is well described and has been validated in accordance with VICH GL2: *Validation*

of analytical procedures: methodology. Satisfactory batch data for three pilot-scale batches been provided.

In terms of dosage form stability, satisfactory data is provided for three pilot scale batches stored under VICH real time, intermediate and accelerated conditions. The results of the stability studies demonstrate that the product undergoes a time related change in colour whereby the solution becomes increasingly yellow with time. Content of the active substance vatinoxan hydrochloride exhibits a slight decreasing trend over time however the decreases observed are not significant. Despite some increasing trends in levels of individual specified unidentified impurities, no significant levels of impurities are observed. All finished product batches analysed comply with the finished product specifications at shelf-life under all storage conditions and no differences were observed for data from inverted vials when compared with data for upright vials. The data indicates that the product is stable and the proposed 3 year shelf life for the finished product is supported by the available data.

With respect to the in-use stability study, data is presented to support the proposed in-use shelf life of 3 months including satisfactory data for the preservative efficacy repeat challenge study. Based on the data provided, the proposed 3 month in-use shelf life is considered acceptable for this product.

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical aspects relevant to the performance of the product have been investigated and are controlled in a satisfactory way.

Part 3 – Safety

Zenalpha is a fixed combination veterinary medicine combining two active substances, medetomidine hydrochloride and vatinoxan hydrochloride. Medetomidine hydrochloride is an alpha-2 adrenergic receptor agonist and is a widely used active substance in the EU that has been authorised in several single-component products for many years both as a human and veterinary medicinal product. Vatinoxan hydrochloride is a new alpha-2 adrenergic receptor antagonist, and a new active substance in a veterinary medicinal product.

The product is administered via the intramuscular (i.m.) route and is indicated to provide restraint, sedation and analgesia during conduct of non-invasive, non-painful or mildly painful procedures and examinations in dogs intended to last no more than 30 minutes. It is proposed that the product is available in 10 ml multidose vials containing 0.5 mg medetomidine and 10 mg vatinoxan per ml. The product is a solution for injection for dogs and the dose is to be calculated according to body surface area (BSA), to provide 1 mg medetomidine hydrochloride and 20 mg vatinoxan hydrochloride per square meter (m^2) BSA.

Safety documentation

The applicant has provided pharmacodynamic and pharmacokinetic data on medetomidine and vatinoxan using both published literature and proprietary study data.

A full safety data package for the novel active substance vatinoxan has been provided.

Reference to published literature and proprietary data for both medetomidine and dexmedetomidine is made in the dossier on the grounds that the pharmacodynamic effects of medetomidine are almost identical to those of dexmedetomidine, with studies cited highlighting that levomedetomidine

(inactive enantiomer) has no apparent sedative, analgesic or cardiorespiratory effects in dogs. While the L-enantiomer is devoid of pharmacodynamic activity, the pure dextro-enantiomer of medetomidine, dexmedetomidine (hydrochloride), is twice as potent as the racemate medetomidine (consequently, requiring half the dose of the latter). The majority of supporting studies were conducted using dexmedetomidine-containing products, which was highlighted and accepted in previous requests for scientific advice, notwithstanding any differences in pharmacokinetic profiles between dexmedetomidine and medetomidine.

Pharmacodynamics

The pharmacodynamics of medetomidine and vatinoxan when administered alone and in combination (including possible interactions) have been considered.

Medetomidine hydrochloride

(Dex)medetomidine has been widely used in veterinary medicine for an extensive period of time and its pharmacodynamic effects are widely recognised. It is a racemic mixture, which has both an inactive and an active enantiomer, levomedetomidine and dexmedetomidine, respectively. As an alpha-2 adrenergic agonist, medetomidine exhibits activity at both central and peripheral alpha-2 adrenoceptors, by which it exerts sedative, analgesic and cardiorespiratory effects. Following administration of medetomidine, vasoconstriction is initially observed, with increased blood pressure, a baro-reflex-mediated reduced heart rate and reduced cardiac output. Additionally, reduced respiratory rate and body temperature is observed. The sedative effects of medetomidine are mediated through central alpha-2 adrenoreceptors, whilst analgesic effects appear to be mediated through both supraspinal and spinal sites. The proposed dose of medetomidine is 1 mg/m^2, which is in line with the existing dose approved in already authorised veterinary medicinal products containing only this active substance.

In a GLP-compliant proprietary dose-determination study that included 8 Beagle dogs, administration of medetomidine (alone or in combination with vatinoxan) by intramuscular injection at the proposed dose of 1 mg/m^2 was associated with sedation, reduced heart rate, elevated blood pressure and reduced respiratory rate.

In another GLP-compliant proprietary study in dogs, the administration of medetomidine by intramuscular injection at the proposed dose of 1 mg/m^2 was associated with sedation and reduced heart rate. In a third GLP-compliant proprietary study, the intramuscular administration of 1 mg/m^2 medetomidine or a medetomidine/vatinoxan combination to 8 Beagle dogs was associated with adverse cardiovascular effects including reduced heart rate, reduced cardiac output, reduced cardiac index, increased blood pressure, arrhythmias and reduced respiratory rate in both groups. However, following administration of the combination treatment, the reductions in heart rate, cardiac output, cardiac index and the increase in blood pressure, were to a lesser degree than those observed following administration of medetomidine alone.

Reduced respiratory rate is a known effect of medetomidine and has been demonstrated in the above-mentioned proprietary studies conducted by the applicant in the target species. Sedative effects have been demonstrated as being dose-dependent for depth and duration.

Alpha-2 agonists are recognised as antinociceptive agents, an effect considered to be mediated by the involvement of both supraspinal and spinal sites, and studies have indicated that both the intensity and duration of analgesia induced by alpha-2 agonists are dose-dependent.

Vatinoxan

Data derived from one non-GLP-compliant study designed to investigate the binding affinity constants of the RR and RS-diastereomers of vatinoxan and their efficacy identified the RS-diastereomer of vatinoxan as the most potent isomer in terms of its antagonistic effect on human alpha-2 adrenoreceptors. Given that only the pure RS diastereomer is included in the candidate formulation, no further consideration of the potential impact of chirality with respect to safety and efficacy is needed.

In one GLP-compliant study, the intravenous (i.v.) and i.m. administration of vatinoxan to dogs at 22.5 or 30 mg/m^2, respectively (both higher than the proposed recommended treatment dose of 20 mg/m^2 i.m.) resulted in an elevated heart rate, salivation and injected sclera.

Proprietary and bibliographic studies suggest that vatinoxan does not directly elicit respiratory effects and it is not considered to exhibit either analgesic or sedative activity.

Data derived from one non-GLP-compliant study in which 64 mice were administered either saline solution, glibenclamide, vatinoxan or vatinoxan combined with glibenclamide demonstrated that vatinoxan reduces blood glucose levels. Given these findings, the applicant has included a contraindication in the proposed SPC that the product should not be used in dogs that have hypoglycemia or are at risk of developing hypoglycaemia.

According to the applicant, vatinoxan is predominantly active in peripheral tissues due to low penetration into the central nervous system. This has been investigated in a study in which vatinoxan was administered to rats and marmosets at varying dosages and by different routes of administration, with subsequent analysis of tissues demonstrating brain:plasma vatinoxan concentration ratios of 0.05:1 to 0.09:1 in rats and 0.06:1 in marmosets, respectively.

In another study, vatinoxan was tested on human P-gp-transfected cells, from which it cannot be concluded definitively whether it is suitable for use in dogs that carry the inactivating P-glycoprotein mutation, since it is known that species differences exist between canine and human P-gp function. There is, however, no indication from the field study that vatinoxan is a P-gp substrate.

Combination of medetomidine plus vatinoxan and potential pharmacodynamic interactions

In one non-GLP-compliant cross-over proprietary study, 8 Beagle dogs were administered 20 µg medetomidine/kg bw, 20 µg medetomidine plus 200 µg vatinoxan per kg bw, 20 µg medetomidine plus 400 µg vatinoxan per kg bw and 20 µg medetomidine plus 600 µg vatinoxan per kg bw intramuscularly. Following administration, the haemodynamic effects associated with medetomidine use appeared attenuated by vatinoxan in a dose-dependent manner. Cardiac output was decreased in all groups, although, at higher doses of vatinoxan, it returned to baseline by 45–60 minutes post administration. Similarly, regarding the heart rate, whilst reduced with all treatments, higher doses of vatinoxan led to an earlier return to baseline values. Clinical sedation was observed at all doses, although the duration of sedation was reduced by the combination.

In a proprietary GLP-compliant dose-determination study, 8 Beagle dogs were intramuscularly administered either medetomidine alone (1 mg/m^2) or medetomidine (1 mg/m^2) combined with 15, 30 or 50 mg/m^2 vatinoxan, with vatinoxan observed to attenuate the cardiovascular effects associated with medetomidine in both a time and dose-dependent manner for blood pressure and heart rate. Administration of vatinoxan in 4 out of 8 animals resulted in development of ventricular escape complexes (VECs), which were not observed following administration of medetomidine alone. Whilst the clinical significance of this finding is unclear, it was noted that they were associated with AV block, and, as considered appropriate, both VECs and AV block have been included with the cardiovascular effects under section 4.5 of the SPC. With regards to sedation, a depth similar to that

observed for medetomidine alone was reported even though it was of a shorter duration and, similarly, co-administration of vatinoxan resulted in a generally dose-dependent earlier loss of analgesia. The significance of this shortening in duration of sedation and analgesia has been considered within the overall benefit/risk balance of the product.

In another GLP-compliant study, 6 Beagle dogs administered 1 mg/m^2 medetomidine alone or 1 mg/m^2 medetomidine and 30 mg/m^2 vatinoxan i.m., or 0.75 mg/m^2 medetomidine and 22.5 mg/m^2 i.v., attenuation of medetomidine-associated bradycardia was observed, although increases in heart rate above baseline were observed shortly following administration of the combination treatments and again between 90–240 minutes. Gradual return to baseline was observed thereafter. Sedation scores were comparable across groups, while those administered the combination treatment exhibited a shorter duration of sedation. A slight delay in the onset of sedation following intramuscular administration of vatinoxan and medetomidine was observed in comparison to that observed following administration of medetomidine alone by intramuscular injection, which was considered likely due to individual variability in absorption from the sites of administration.

In another proprietary GLP-compliant study, in which 8 Beagle dogs were administered a combination of medetomidine and vatinoxan at the proposed dose, heart rate decreased to 50% of baseline by 5 minutes, after which it gradually increased up until the 30-minute timepoint, at which it stabilised at 11% below baseline. Mean arterial pressure also decreased, with the lowest value recorded at 50 minutes post administration.

In another proprietary GCP-compliant study, 8 Beagle dogs were intramuscularly administered either medetomidine alone (1 mg/m^2) or a medetomidine (1 mg/m^2)/vatinoxan (20 mg/m^2) combination. Following administration of the combination of medetomidine and vatinoxan, a reduction in cardiac output and heart rate, as well as cardiac index, stroke volume and stroke volume index was observed. Although none of these values returned to baseline within the 120-minute evaluation time, the values were significantly improved over those observed following administration of medetomidine alone. Although blood pressure measurements were elevated following administration of the combination, they were improved over those observed following administration of medetomidine alone. There was an initial increase in systemic vascular resistance, although of a much lesser magnitude than that observed after medetomidine administration alone, and values were observed to reduce from 10 minutes post administration onwards. Respiratory rate was slightly less reduced than when medetomidine was administered alone.

In one study designed to evaluate the effects of 10 µg medetomidine/kg bw alone or in conjunction with 250, 500 or 750 µg vatinoxan/kg bw on the bi-spectral index and clinical sedation in dexmedetomidine-sedated Beagles, the results indicated that, whilst peak sedation level attained following dexmedetomidine administration was slightly reduced by the administration of vatinoxan, the sedation level differences over the 60-minute observation period were considered minor and not clinically relevant.

In a proprietary study designed to evaluate the pharmacological effects of combinations of vatinoxan with medetomidine alone, sedation was observed more quickly than following administration of medetomidine alone. Additionally, maximal sedation lasted only 30 minutes following treatment with the combination, whilst, for medetomidine alone, deep sedation was observed for 90 minutes. In another proprietary study, following administration of the final formulation in accordance with the SPC, sedation was observed at 5 minutes, with a maximum level attained between 20–30 minutes and a gradual decline until the final observation timepoint (120 minutes), at which mild sedation was still observed.

In one non-proprietary study, 8 Beagle dogs were administered either medetomidine alone (10 µg/kg bw) or in combination with vatinoxan (250 µg/kg), and analgesic efficacy was determined. A statistically significant difference in limb withdrawal time between groups was observed at 10, 20 and 30 minutes post administration, with limb withdrawal times significantly longer following administration of medetomidine alone compared to when administered in combination with vatinoxan. Another proprietary GLP-compliant study compared toe-pinch scores of animals administered either medetomidine alone (1 mg/m^2 BSA) or medetomidine plus vatinoxan (15 or 30 mg/m^2 BSA). Comparable depth of nociception was observed, although the duration was reduced in a dose-dependent manner for the combination in both doses.

In conclusion, the peripheral effects associated with medetomidine use, particularly the adverse cardiovascular effects, appear attenuated by the combined administration of vatinoxan and medetomidine. Although the level of sedation associated with medetomidine administration does not appear to be negatively impacted (reduced) by the concomitant administration of vatinoxan, it is unclear whether the level of analgesia is negatively impacted. Vatinoxan does however appear to reduce the duration of sedation and analgesia associated with medetomidine administration. The significance of this shortening in duration of sedation and analgesia has been considered within the overall benefit/risk balance for the product.

Pharmacokinetics

A full data package on the pharmacokinetic data for the novel active substance vatinoxan has been provided.

The pharmacokinetics of medetomidine/dexmedetomidine used as a sole substance in products is considered well known and has not been reviewed in detail. Instead, a brief summary of pharmacokinetic data from published literature has been presented and reference has been made to information contained in the EPARs of two centrally authorised products. However, EPARs cannot be considered to supply sufficient information to meet the requirements of Annex I of Directive 2001/82/EC. Consequently, the information included in those EPARs is not considered adequate to support the pharmacokinetics of medetomidine in the present fixed combination product.

That said, proprietary pharmacokinetic information on the active substance medetomidine has been provided in two studies, a pivotal PK/PD study and a pilot dose-determination study. In these studies, medetomidine was administered alone and in combination with vatinoxan.

Absorption

In a pivotal PK/PD interaction study, bioavailability of vatinoxan alone after i.m. administration (dose 30 mg/m^2) was 100% and slightly lower at 91.7% when administered in combination with medetomidine. The bioavailability for medetomidine (dexmedetomidine enantiomer) when administered alone via the i.m. route (dose 1.0 mg/m^2) was 76.5% and 95% when administered in combination with vatinoxan. However, it is noted that absolute bioavailability has been calculated using different doses of medetomidine and vatinoxan administered via the i.v. and i.m. routes, respectively, and, as such, these values should be considered as estimates only.

The overall mean exposure (as measured by $AUC_{0-12\,h}$) for dexmedetomidine appeared to be much higher when the substance was administered alone (either i.m. or i.v.) than when in combination with vatinoxan (28.6 h*ng/ml alone vs 13.5 h*ng/ml in combination by i.m. and 29.4 h*ng/ml alone vs 10.8 h*ng/ml in combination by i.v., respectively). Time to individual peak plasma concentrations of dexmedetomidine ranged from 0.167 to 0.75 h when administered alone and from 0.083 to

0.333 h when administered with vatinoxan. It can be accepted that, when administered in combination by the i.m. route (at a dose ratio of 1:30), vatinoxan shortened the T_{max}, reduced the plasma concentration ($AUC_{0-12\ h}$) and increased the clearance of medetomidine.

In a non-GLP-compliant pilot dose-determination study, medetomidine and vatinoxan were co-administered at differing dose ratios (1:10, 1:20 and 1:30) and maximum plasma concentration (C_{max}) as well as time to reach maximum plasma concentration (T_{max}) were calculated. The absorption of medetomidine was increased when administered in combination with vatinoxan at the proposed ratio of 1:20 (C_{max} was 3.8 µg/l for medetomidine alone vs 5.5 µg/l when administered with 400 µg of vatinoxan). Time to maximum concentration for medetomidine absorption was significantly decreased when administered in combination with vatinoxan at the proposed ratio of 1:20 (T_{max} was 42.5 minutes for medetomidine alone vs 16.9 minutes when administered with 400 µg of vatinoxan). This study demonstrated that absorption of medetomidine was accelerated when administered in combination with vatinoxan.

Plasma/tissue distribution

The distribution of vatinoxan was investigated in two pre-clinical studies investigating the plasma protein binding of vatinoxan in dogs.

The first study (non-GLP-compliant) investigated the level of plasma protein binding of vatinoxan in dogs. Mean observed plasma bound (F_b) and unbound fractions (F_{ub}) were calculated at concentrations of 1 and 10 µM vatinoxan, with 78% and 62% binding reported, respectively. It was noted that there was less binding at the higher concentration, which the applicant suggests may be indicative of saturation of binding. Given the limited data available, no firm conclusions can be drawn from this study other than that approximately two thirds of the administered dose of vatinoxan appears to be bound to plasma protein.

In another non-GLP-compliant study, lower concentrations of vatinoxan (0.1, 1 and 10 µM) were used to determine the binding specificity to plasma proteins, human albumin serum (HAS) and alpha 1 acid glycoprotein (AGP) in dog plasma. In this study, identical binding was observed for vatinoxan at 0.1 and 1 µM (73%) in dog plasma, HSA and AGP, whilst saturation of binding was observed at the 10 µM concentration (57%). When combined with medetomidine (medetomidine to vatinoxan ratio: 1:20), little or no effect on protein binding by vatinoxan in dog plasma was observed (73% bound at 1 µm vs 62% bound at 10 µm).

The distribution of vatinoxan was further investigated *in vivo* during the toxicokinetic analysis of the pivotal target animal safety study using the final formulation where concentrations of vatinoxan and dexmedetomidine were analysed in plasma and cerebrospinal fluid (CSF) samples from dogs at necropsy. The dogs had been administered the fixed combination product at 1X 3X and 5X the proposed dose (medetomidine to vatinoxan ratio: 1:20) by i.v. administration for 4 consecutive days. Mean vatinoxan CSF concentrations were approximately 125 times lower than mean plasma concentrations and mean dexmedetomidine CSF concentrations were approximately 10 times lower than mean plasma concentrations. It can be accepted that vatinoxan does not appear to distribute to the CSF to a similar extent as dexmedetomidine.

Metabolism

Two studies identifying the metabolite profile of vatinoxan were performed.

In the first non-GLP-compliant study, 10 µM of vatinoxan in DMSO were incubated with dog and rat

cryopreserved hepatocytes for 180 minutes to obtain the metabolic profile of vatinoxan. Nine metabolites were observed in rat hepatocytes (M1, M3, M4, M5, M6, M7, M8, M9 and M10), while only four were observed in dog hepatocytes (M1, M2, M3 and M4). The same metabolites M3 and M4 were the most abundant in both dogs and rats, with M3 at 1.3 and 3.3% in dogs and rats, respectively, and M4 at 0.6 and 2.8% in dogs and rats, respectively. Overall, the levels of metabolites formed was low in both species and after 180 minutes the main component observed was the parent product, which accounted for 97.8% and 92.5% of the relative LC/MS peak area in dogs and rats, respectively.

In the second non-GLP-compliant study, samples were collected from 8 dogs, 30 or 60 minutes after they had received 200, 400 or 600 µg/kg vatinoxan together with 20 µg/kg medetomidine via the i.m. route. The samples were assayed for concentrations of known metabolites (M3, M4, M11, M12, M13 and M14). Metabolites M11, M12, M13 and M14 were not observed in the previous study. The maximum level of metabolite detected in this study was 37 ng/ml (M3, vatinoxan administered at 600 µg/kg), which suggests that only a small proportion of the drug is metabolised. It can be accepted that the metabolism of vatinoxan has been suitably investigated in these studies and that the metabolite M3 appears to be present at the highest concentration in both *in vitro* and *in vivo* models, but represents no more than 5.3% of the administered dose.

The inhibition potential of vatinoxan towards metabolising cytochrome P450 (CYP) enzymes in dogs was investigated in one non-GLP-compliant study. Vatinoxan demonstrated low or very low inhibition potential toward each studied enzyme. The highest inhibition potential was observed towards CYP3A12 (lowest IC_{50}: 56 µM). IC_{50} values for all other studied CYPs were above 100 µM.

In another non-GLP-compliant study, the induction potency of vatinoxan towards canine CYP enzymes was investigated. No induction with respect to any CYP activity was observed. The results from this study appear to confirm the results from the previous study and therefore it can be accepted that vatinoxan is unlikely (at concentrations of 1 to 100 µM) to cause a significant induction of CYP activity (therefore unlikely to cause drug interactions) in the target species dogs.

Excretion

In the pivotal PK/PD study, where dogs were administered medetomidine and vatinoxan i.v. (ratio of 0.75:22.5) and via the i.m. routes (ratio of 1:30), the mean clearance of medetomidine was almost twice as fast when administered in combination with vatinoxan either by the i.v. route (25,600 ml/h/m^2 alone vs 71,000 ml/h/m^2 in combination) or by the i.m. route (35,500 ml/h/m^2 alone vs 76,200 ml/h/m^2 in combination), whereas the mean clearance of vatinoxan was similar when administered alone or in combination by either route (2,160 ml/h/m^2 alone vs 2,050 ml/h/m^2 in combination by i.v. and 2,170 ml/h/m^2 alone vs 2230 ml/h/m^2 in combination by i.m.). Furthermore, it is noted that the overall mean exposure ($AUC_{0-12\ h}$) for dexmedetomidine appears to be much higher when the substance was administered alone (by either the i.m. or i.v. route) than when administered in combination with vatinoxan (28.6 h*ng/ml alone vs 13.5 h*ng/ml in combination by i.m. and 29.4 h*ng/ml alone vs 10.8 h*ng/ml in combination by i.v.).

These results are considered to clearly indicate an interaction between vatinoxan and medetomidine with a reduction in the $t_{1/2}$ for medetomidine when administered in combination with vatinoxan (i.m. route: $t_{1/2}$ 1.27 h when administered alone vs 0.679 h when administered in combination; i.v. route: $t_{1/2}$ 1.49 h when administered alone vs 0.603 h when administered in combination).

In the target animal safety study (see part IV for details), urinary excretion of vatinoxan was investigated. Only a very small amount of vatinoxan was detected (2–5%) as the parent compound in the urine (collected 16–24 h post administration), which suggests that urine is not the major

route of excretion for vatinoxan. Therefore, it can be accepted that the most likely route of excretion is in the faeces via the hepatobiliary route, although no data are available to confirm this.

Toxicological studies

Proprietary studies to investigate single dose toxicity and repeat dose toxicity of vatinoxan were provided.

No proprietary acute dose toxicity or repeat dose toxicity studies for medetomidine have been provided. Instead, toxicological information obtained from the EPARs from two centrally authorised products and one published study has been included.

The information included in those EPARs is not considered adequate to support the acute toxicity of medetomidine in the fixed combination product. That said, it is noted that medetomidine has been widely used in veterinary medicine for an extensive period of time with a known toxicological profile, and it is acknowledged that relevant and appropriate information is available in the MRL summary report for the related compound detomidine. Given that the proposed dose of medetomidine is in line with that of already authorised veterinary medicinal products that include medetomidine as sole active substance and an attenuation of toxico-pharmacological effect appears to arise when medetomidine is administered with vatinoxan, it can be accepted that further toxicity data for medetomidine are not required.

Single dose toxicity

To characterise the toxicological effects of vatinoxan, three single dose toxicity studies were performed, although, due to problems encountered with the test article formulation, only two of the studies are considered of use.

A preliminary non-GLP-compliant study in SPF mice was performed broadly in accordance with OECD TG 420. Only a single dose was investigated following intravenous administration injected into the tail vein of 10 mice (100 mg/kg), which was selected based on the results from a sighting study. No abnormal severe clinical signs were observed following administration of either vatinoxan stereoisomer. All animals survived until they were euthanised. As only a single dose (100 mg/kg) was administered and no cases of lethality were recorded, it can be accepted that the minimum lethal dose exceeds 100 mg/kg. The applicant considers the maximum tolerated dose (MTD) from this study to be at least 100 mg/kg, which appears to be a reasonable conclusion.

No maximum tolerated dose (MTD) could be concluded from a second study using intravenous administration due to injection site issues. This non-GLP-compliant study was conducted in 50 Wistar rats that were administered doses of 100 mg/kg and 200 mg/kg by either a single i.v. injection or by i.v. infusion over 5 minutes to 4 treatment groups. Two different batches of test item vatinoxan were administered. One batch caused skin ulcers at the site of administration (tail) in 40–60% of rats and the other batch caused tail lesions in 10% of rats. No other gross pathologic lesions were found in any animals. It is noted that a single i.v. administration of both batches of vatinoxan, either by bolus or infusion, caused dose-dependent changes in behaviour, somatomotor activity, gait and posture in all administered animals. Nonetheless, an MTD could not be determined in this study.

In a third GLP-compliant study, a range of doses was investigated (100, 200, 400, 800 and 2000 mg/kg) when administered orally to rats. The applicant concluded that the MTD of vatinoxan hydrochloride after oral administration was 800 mg/kg, which can be accepted given that lethality

was observed at the next highest dose investigated (2000 mg/kg).

It can be accepted that, due to injection site reaction issues, the product was administered orally in the final study. While the gastrointestinal reactions observed in this study may have been due to the oral route of administration, given that no adverse effects appear to have been observed in the group receiving the lowest dose but were seen in all other dose groups, it is reasonable to conclude that they are likely to be test article-related. Of interest is the finding of sedation in rats caused by the two highest doses of vatinoxan (800 and 2000 mg/kg), with the sedative effect increasing with dose.

Repeat dose toxicity

According to Annex I to Directive 2001/82/EC, "[i]n the case of pharmacologically active substances or veterinary medicinal products intended solely for use in non-food producing animals, a repeat-dose toxicity study in one species of experimental animal shall normally be sufficient".

A repeat-dose toxicity study to investigate the subacute toxicity potential of vatinoxan has been conducted in rats. In this GLP-compliant study, vatinoxan was administered orally at three doses (20, 60 and 200 mg/kg) to Wistar rats once daily for 28 days. A 14-day recovery period was observed for the highest dose group. Doses were selected based on the MTD (800 mg/kg) determined in the above-mentioned single dose toxicity study.

The main clinical signs observed were temporary soft consistency of faeces during the administration period. No treatment-related effects were found regarding bodyweight, food consumption, haematology or gross pathology. Some statistically significant differences between groups and within groups for cholesterol and protein levels in plasma and specific gravity, blood and ketone presence in urine were observed. Thyroid and prostate weights were altered in the highest dose group. Whilst these changes could be related to treatment, they were generally mild and of questionable clinical significance (and in most cases reversed in high dose animals during the recovery phase).

The NOAEL was determined to be 20 mg/kg bodyweight for oral administration of vatinoxan to rats for 28 days and, considering that no clinical signs of toxicity were observed in this group throughout the study, this can be accepted.

The repeat dose toxicity of the combination product has been investigated further in the pivotal target animal safety study.

Tolerance in the target species of animal

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

Reproductive toxicity

Study of the effect on reproduction

No studies on the effects on reproduction have been conducted for vatinoxan or the combination product. In the absence of studies specifically investigating reproductive toxicity, the applicant has proposed that the product is contraindicated for use in animals intended for breeding. This is considered appropriate in the absence of reproductive toxicity data.

Study of developmental toxicity

No studies on the effects on embryo-foetal development have been conducted with vatinoxan or the combination product. In the absence of studies specifically investigating developmental toxicity, the applicant has proposed that the product is contraindicated for use in animals intended for breeding.

According to Annex I of Directive 2001/82/EC, a study of developmental toxicity shall be performed if the product is intended for use in female animals which may be used for breeding or where use of the veterinary medicinal product would result in significant exposure to users. Given that the product is not to be indicated for use in female animals for breeding and extensive user exposure is not anticipated, the proposed warning is considered appropriate in the absence of developmental toxicity data.

Genotoxicity

No genotoxicity data on medetomidine has been provided. With reference to the MRL summary report for the related compound detomidine, it is noted that detomidine was not mutagenic in the Ames test in the presence and absence of metabolic activation (S9). Very high concentrations (0.3 mg/ml) produced chromosomal aberrations in an *in vitro* CHO assay in the absence of S9, but this effect may be attributed to non-specific pH effects. Lower concentrations (0.2 mg/ml) or the addition of S9 with the high concentration were not clastogenic. Detomidine at up to 20 mg/kg was not mutagenic in an *in vivo* mouse micronucleus test. Bone marrow toxicity was not demonstrated, but it is unlikely that detomidine was unable to reach these cells.

Given that the proposed dose of medetomidine is in line with that of already authorised veterinary medicinal products that include medetomidine as sole active substance, that an attenuation of toxico-pharmacological effects appears to arise when medetomidine is administered with vatinoxan and that the genotoxic potential of vatinoxan has been investigated, it can be accepted that further genotoxicity data for medetomidine are not required.

Three studies were performed to evaluate the genotoxic potential of vatinoxan in line with OECD guidelines.

A GLP-compliant bacterial reverse mutation test was conducted with vatinoxan in accordance OECD TG 471. The results of the study indicate that vatinoxan does not induce bacterial mutations in either *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 or *E. coli* strain WP2 uvrA with or without metabolic activation. It can be accepted from the results of this study that vatinoxan is non-mutagenic in this test.

A GLP-compliant chromosomal aberration test *in vitro* was conducted with vatinoxan in accordance with OECD TG 473 in cultured mammalian cells. Under the experimental conditions of the study, vatinoxan did not induce an increase in the numerical and structural chromosome aberrations in cultured human peripheral blood lymphocytes. It can be accepted that the concentrations investigated have been appropriately justified with reference to cytotoxicity findings and that the test article did not induce chromosomal aberrations under the conditions of this study in the presence and absence of metabolic activation.

A GLP-compliant *in vitro* micronucleus test was conducted in accordance with OECD TG 487 to assess the potential of vatinoxan to cause gene mutations and to identify any structural chromosomal damage (as measured by the percentage of binucleate cells with micronuclei). No significant increase in the percentage of binucleate cells with micronuclei was observed in any of the tested doses compared to the negative control. Under the experimental conditions of the study, vatinoxan did not exhibit clastogenic or aneugenic activity in cultured human peripheral blood

lymphocytes. It can be accepted that the concentrations investigated have been appropriately justified with reference to cytotoxicity findings. Results of this study indicate that, under the conditions of this study, vatinoxan does not induce structural chromosomal aberrations in cultured mammalian somatic cells.

The applicant has provided results from a further *in vivo* genotoxicity study (mouse micronucleus test) in accordance with OECD TG 474, investigating the ability of vatinoxan hydrochloride to induce the formation of micro-nucleated polychromatic erythrocytes in mice bone marrow. From this study, it can be accepted that vatinoxan hydrochloride is negative for the induction of micronuclei in polychromatic erythrocytes of mouse bone marrow at a dose of up to 500 mg/kg when administered orally.

Taking into consideration the results from this latter new study and the results from the three *in vitro* tests provided previously by the applicant, it can be accepted that no indication of genotoxicity has been observed for the active substance vatinoxan hydrochloride.

Carcinogenicity

No studies investigating carcinogenicity have been performed with medetomidine, vatinoxan or the combination drug product.

Whilst it is acknowledged that no data on carcinogenicity have been provided for vatinoxan from chronic repeated dose toxicity studies (the maximum duration of studies provided was 28 days), given the absence of any indication of mutagenicity or genotoxicity from the battery of tests conducted, it can be accepted that there appears to be no indication or findings from the repeated dose toxicity studies that might signal a potential for carcinogenicity of vatinoxan. Furthermore, the combination product is intended for single dose administration. Consequently, the absence of carcinogenicity data for vatinoxan can be accepted.

No proprietary information on the carcinogenic potential of medetomidine has been provided. However, with reference to the MRL summary report for the related compound detomidine, in which it is noted that no carcinogenicity studies were performed in the absence of signals from genotoxicity studies, it can be accepted that data on the carcinogenicity of medetomidine are not required.

Given that the proposed dose of medetomidine is in line with that of already authorised veterinary medicinal products that include medetomidine as sole active substance, that an attenuation of toxico-pharmacological effects appears to arise when medetomidine is administered with vatinoxan and that the genotoxic potential of vatinoxan has been investigated, the omission of studies investigating carcinogenicity can be accepted.

Studies of other effects

Three GLP-compliant studies using a close-to-final formulation (0.5 mg/ml medetomidine and 15 mg/ml vatinoxan; solution for injection) were performed in line with OECD test guidelines to investigate the potential for dermal irritation/corrosion, eye irritation/corrosion and skin sensitisation.

A study was conducted in accordance with OECD TG 404 to investigate the potential for acute dermal irritation/corrosion. Very slight oedema and erythema was observed in one animal but had abated by day 8 of the study. The test item was not identical to the final formulation, having a ratio of 1:30 medetomidine to vatinoxan instead of a 1:20 ratio and a minor difference in a pH adjuster.

That said, given that the amount of vatinoxan was higher in the test formulation than in the final formulation and that the pH is the same, the findings can be accepted as representative for the final formulation. Furthermore, it can be accepted that the product was shown not to be a skin irritant.

A study was conducted in accordance with OECD TG 405 to investigate the potential for acute ocular irritation/corrosion. The test item was not identical to the final formulation, with the test product having a ratio of 1:30 medetomidine to vatinoxan instead of a 1:20 ratio and a minor difference in a pH adjuster. That said, given that the amount of vatinoxan was higher in the test formulation than in the final formulation and that the pH is the same, the findings can be accepted as representative for the final formulation. Furthermore, it can be accepted that the product was shown not to be an ocular irritant.

A study was conducted in accordance with OECD TG 406 to investigate the potential for skin sensitisation. The test item in this study was not identical to the final formulation, with the test product having a ratio of 1:30 medetomidine to vatinoxan instead of a 1:20 ratio and a minor difference in a pH adjuster. That said, given that the amount of vatinoxan was higher in the test formulation than in the final formulation and that the pH is the same, the findings can be accepted as representative for the final formulation. Furthermore, it can be accepted that the product was shown not to be a skin sensitiser.

Excipients

In addition to the active substances, the solution contains the excipients methyl parahydroxybenzoate (E218), propyl parahydroxybenzoate, mannitol, citric acid monohydrate, sodium hydroxide, hydrochloric acid and water for injections.

Mannitol is a naturally occurring polyol used intravenously, orally and via inhalation to treat a range of conditions in human medicine. It is used as an excipient in pharmaceutical formulations, as a food additive and is generally recognised as safe (GRAS) by the FDA. Citric acid is widely used in pharmaceutical preparations and as a food additive. Methylparaben is an antimicrobial preservative used in injectable formulations. Hydroxybenzoates are commonly used at concentrations up to 0.25% in pharmaceutical formulations. A number are included in Table 1 of the Annex to Regulation (EU) No 37/2010 with a "no MRL-required status". Rarely, hypersensitivity reactions have been reported for injectable preparations containing hydroxybenzoates. Propylparaben, an antimicrobial preservative, is used in injectable formulations.

Whilst no information has been provided on the toxicity of either sodium hydroxide (E524) or hydrochloric acid (E507), it can be accepted that both are used as pH adjusters and are commonly included in veterinary medicinal products at similar inclusion rates as proposed.

User safety

The applicant has provided a user risk assessment, largely in accordance with the "CVMP guideline on user safety for pharmaceutical medicinal products" (EMA/CVMP/543/03-Rev.1). As the product is a prescription-only medicine to be administered either by a veterinarian or by veterinary staff under the supervision of a veterinarian, the user of this product will be the professional user in the veterinary clinic setting.

The applicant considers that the risk of dermal or ocular exposure is low. Studies conducted by the applicant have demonstrated that the product does neither demonstrate ocular or dermal irritancy/corrosive properties nor is it considered a skin sensitiser.

Whilst no reproductive toxicity data has been provided with this application, it is understood that uterine contractions, decreased foetal blood pressure and foetal deaths have been associated with medetomidine use, and that foetal toxicity, embryocidal and delayed motor development have been associated with dexmedetomidine administration to pregnant rats. On account of this identified risk, the applicant has proposed the inclusion of a warning aimed at reducing this risk, namely "pregnant women should exercise special caution to avoid exposure. Uterine contractions and decreased foetal blood pressure may occur after accidental systemic exposure". This approach is considered appropriate.

In estimating exposure, the applicant has used a worst-case exposure scenario of accidental dermal or parenteral exposure to a 0.4 ml dose by an adult, with 100% bioavailability. This equates to a potential exposure of 4 µg medetomidine per kg bw and 0.07 mg vatinoxan per kg bw. This can be accepted as representing a worst-case scenario.

With reference to the MRL summary report for the related compound detomidine, for medetomidine, a NOEL of 0.125 µg/kg has been derived from human studies involving administration of medetomidine by i.v. injection. Therefore, the potential exposure to medetomidine is well above the NOEL and as such, the risk for the user is considered unacceptably high.

With regards to vatinoxan, a NOAEL of 1.5 mg (0.025 mg/kg) has been derived from an acute intravenous study conducted in humans and, therefore, the potential exposure to vatinoxan is above the NOAEL. However, whilst this constitutes a risk, given that exposure is to the combination product, the risk posed by vatinoxan is not considered to be any greater than that posed by medetomidine.

The user safety warnings proposed by the applicant are consistent with those agreed following a previous referral procedure according to Article 78 of Directive 2001/82/EC for veterinary medicinal products containing alpha-2 adrenoreceptor agonists (EMEA/681319/2008), which is considered appropriate with regards medetomidine.

As a result, the following advice to users/warnings for the user proposed by the applicant are considered appropriate:

"Accidental exposure may cause sedation and changes in blood pressure. Caution is required during treatment administration to avoid accidental self-injection, or skin, eye or mucosal contact. Adequate restraint of the animal is recommended, as a small number of animals may react to the injection (e.g., defence reaction).

Pregnant women should administer the veterinary medicinal product with special caution to avoid self-injection since uterine contractions and decreased foetal blood pressure may occur after accidental systemic exposure.

People with known hypersensitivity to the active substance or any of the excipients should administer the veterinary medicinal product with caution.

In case of accidental self-injection or ingestion, seek medical advice immediately and show the package leaflet to the physician but DO NOT DRIVE.

In case of skin or mucosal contact, wash the exposed skin immediately after exposure with large amounts of water and remove contaminated clothes that are in direct contact with skin. In case of eye contact, rinse abundantly with fresh water. If symptoms occur, seek the advice of a physician.

To the physician: The veterinary medicinal product contains medetomidine, an alpha-2 adrenoceptor agonist, in combination with vatinoxan, a peripherally selective alpha-2 adrenoceptor antagonist. Symptoms after absorption may involve clinical effects including dose-dependent sedation,

respiratory depression, bradycardia, hypotension, a dry mouth, and hyperglycaemia. Ventricular arrhythmias have also been reported. Respiratory and haemodynamic symptoms should be treated symptomatically".

Environmental risk assessment

A phase I environmental risk assessment (ERA) was provided according to relevant CVMP/VICH guidelines (VICH GL 6). Zenalpha is intended for use in dogs, which are a non-food-producing species.

Phase I:

The ERA can stop in phase I and no phase II assessment is required because the veterinary medicinal product will only be used in non-food-producing animals.

Conclusions on the environmental risk assessment

An ERA was provided according to relevant CVMP/VICH guidelines (VICH GL 6). Based on the data provided, the ERA can stop at phase I, as none of the phase II criteria are met.

It can be concluded that the product will not present an unacceptable risk for the environment when handled, administered, stored and disposed of in accordance with the recommendations proposed for inclusion in the SPC.

Overall conclusions on the safety documentation

The applicant has provided data from the public domain and the results of proprietary studies to characterise the pharmacodynamic properties of the two active substances, medetomidine and vatinoxan, in addition to studying the interactions between the two active substances.

Concerning the combination of medetomidine and vatinoxan, whilst the central effects of medetomidine appear largely unaffected, with sedative and analgesic activity continuing to be observed albeit for a shorter duration, the peripheral affects, particularly the adverse cardiovascular affects appear attenuated by the concomitant administration of vatinoxan. Studies conducted in the target species have demonstrated dose-dependent attenuation of the reduced heart rate, reduced cardiac output and reduced cardiac index as well as the increased blood pressure and systemic vascular resistance associated with the administration of medetomidine. However, whilst administration of vatinoxan was associated with reduced incidence of rhythm variants (compared to sole administration of medetomidine), it is noted that, in one study, 50% (4 out of 8) of animals developed ventricular escape complexes, which were not observed following administration of medetomidine alone.

With regards to sedation, although the level of sedation associated with medetomidine administration does not appear to be negatively impacted (reduced) by the concomitant administration of vatinoxan, the substance does however appear to reduce the duration of sedation and analgesia associated with medetomidine administration. The significance of this shortening in duration of sedation and analgesia has been considered within the overall benefit/risk balance for the product.

The absorption of medetomidine was increased when administered in combination with vatinoxan at the proposed ratio of 1:20, while the time to maximum concentration for medetomidine absorption

was significantly decreased when administered in combination with vatinoxan at the proposed ratio of 1:20.

Regarding distribution, approximately two thirds of the administered dose of vatinoxan appears to be bound to plasma protein. When combined with medetomidine (medetomidine to vatinoxan ratio: 1:20), little or no effect on protein binding by vatinoxan in dog plasma was observed.

Mean vatinoxan CSF concentrations were approximately 125 times lower than mean plasma concentrations and mean dexmedetomidine CSF concentrations were approximately 10 times lower then mean plasma concentrations. It can be concluded that vatinoxan does not appear to distribute to the CSF to a similar extent as dexmedetomidine.

It can be accepted that the metabolism of vatinoxan has been suitably investigated and that metabolite M3 appears to be present at the highest concentration in both *in vitro* and *in vivo* models, but that it represents no more than 5.3% of the administered dose.

It can also be accepted that vatinoxan is unlikely to cause a significant induction of CYP activity (therefore unlikely to cause drug interactions) in the target species dogs.

Mean clearance of medetomidine was almost twice as fast when administered in combination with vatinoxan either by the i.v. or i.m. routes, indicating an interaction between vatinoxan and medetomidine with a reduction in the $t_{1/2}$ for medetomidine when administered in combination with vatinoxan.

Only a very small amount of vatinoxan was detected (2–5%) as the parent compound in the urine, which would suggest that urine is not the major route of excretion for vatinoxan. Therefore, it can be accepted that the most likely route of excretion is in the faeces via the hepatobiliary route, although no data are available to confirm this.

Medetomidine has been widely used in veterinary medicine for an extensive period of time with a well-known toxicological profile and, given the information included in the MRL summary report for detomidine, with the proposed dose of medetomidine being in line with that of already authorised veterinary medicinal products that include medetomidine as sole active substance, further toxicity data for medetomidine are not considered necessary. Studies to investigate single dose toxicity of vatinoxan were performed. Based on the data provided, it can be accepted that the minimum lethal dose exceeds 100 mg/kg and that a maximum tolerated dose of 800 mg/kg was determined. In a repeat dose toxicity study in rats, a NOAEL of 20 mg/kg bodyweight after oral administration of vatinoxan was determined.

No studies to investigate reproductive toxicity have been conducted with vatinoxan or the combination product, as it is only intended for use in a non-food-producing target species. The proposed product information makes clear that safety has not been established in pregnant or lactating animals or in animals intended for breeding. In light of the proposed warning, the omission of reproductive toxicity data can be accepted.

No studies on the effects on developmental toxicity have been conducted with vatinoxan or the combination product, as the product is only intended for use in a non-food-producing target species. The proposed product information makes clear that safety has not been established in female animals intended for breeding. Given that the product is not to be indicated for use in female animals for breeding and extensive user exposure is not anticipated, the proposed warning is considered appropriate and the omission of reproductive toxicity data can be accepted.

No investigation of the genotoxic potential of medetomidine was conducted. With reference to the MRL summary report for the related compound detomidine, it is noted that the latter was not

mutagenic in the Ames test in the presence and absence of metabolic activation (S9), that 0.2 mg/ml or the addition of S9 with the high concentration (0.3 mg/ml) were not clastogenic and that doses of up to 20 mg/kg were not mutagenic in an *in vivo* mouse micronucleus test. Bone marrow toxicity was not demonstrated, but it is likely that detomidine was unable to reach these cells. Given that the proposed dose of medetomidine is in line with that of already authorised veterinary medicinal products that include medetomidine as sole active substance, that an attenuation of toxico-pharmacological effects appears to arise when medetomidine is administered with vatinoxan and that the genotoxic potential of vatinoxan has been investigated, further genotoxicity data for medetomidine is not required.

Results of three *in vitro* as well as an *in vivo* test generally conducted in accordance with the relevant OECD guidelines investigating genotoxicity of vatinoxan were provided, including a bacterial reverse mutation test, a chromosomal aberration test and an *in vitro* and *in vivo* (mouse) micronucleus test. Taking into consideration the results from these studies, it can be accepted that no indication of genotoxicity has been observed for the active substance vatinoxan hydrochloride.

No studies investigating carcinogenicity have been performed with medetomidine, vatinoxan or the combination drug product. Based on the well-established use of medetomidine, the information in the MRL summary report for detomidine and the findings from the genotoxicity studies conducted using vatinoxan, the omission of studies investigating carcinogenicity can be accepted.

Three GLP-compliant studies using a close-to-final combination formulation were performed in line with OECD test guidelines to investigate the potential for dermal and ocular irritation/corrosion and skin sensitisation. It can be accepted that the product was shown not to be a dermal or ocular corrosive/irritant or a skin sensitiser.

A user risk assessment largely in accordance with the "CVMP guideline on user safety for pharmaceutical medicinal products" (EMA/CVMP/543/03-Rev.1) was provided. The risk for the user is considered unacceptably high for both medetomidine and vatinoxan. However, it is concluded that the medetomidine component of the fixed combination product poses the greatest risk to the user. The user safety warnings proposed by the applicant are consistent with those agreed following a previous referral procedure according to Article 78 of Directive 2001/82/EC for veterinary medicinal products containing alpha-2 adrenoreceptor agonists, which is considered appropriate.

A phase I environmental risk assessment (ERA) was provided according to the CVMP/VICH guidelines. Given that the product is intended for use in dogs, a non-food-producing animal, the ERA can stop at phase I and a phase II assessment is not required. It can be concluded that the product will not present an unacceptable risk for the environment when handled, administered, stored and disposed of in accordance with the recommendations proposed for inclusion in the SPC.

Part 4 – Efficacy

Pharmacodynamics

Please refer to Part 3.

Development of resistance

Given the nature of the product, development of resistance is not considered relevant and therefore no assessment is required.

Pharmacokinetics

Please refer to Part 3.

Justification of fixed combination

The rationale for the fixed combination of medetomidine and vatinoxan is to improve the safety margin of the alpha-2-agonist by means of minimising adverse peripheral haemodynamic side effects, whilst maintaining the desired sedative and analgesic effects mediated in the central nervous system (CNS).

The combination of medetomidine and vatinoxan is proposed in order to prevent or attenuate the adverse cardiovascular effects of medetomidine. However, it is highlighted that the maximal sedative and analgesic effects of the combination product should not be inferior to medetomidine, although the duration of sedation and analgesia may be reduced for the fixed combination product when compared to administration of medetomidine alone.

According to the CVMP 'Guideline on pharmaceutical fixed combination products' (EMEA/CVMP/83804/2005), fixed combination products will be only considered acceptable if the proposed combination is based on valid therapeutic principles; and any fixed combination product can only be justified, if such a combination offers an advantage over their active substances, when used as single substance products.

Consequently, in order for the proposed fixed combination product to be acceptable, it is expected that the combination of vatinoxan and medetomidine offers an advantage over administration of medetomidine alone.

According to section 4.3.1 (Improvement of activity) of the aforementioned guideline, a possible advantage includes the improvement of tolerance by the addition of a substance which has been demonstrated to counteract the adverse effects produced by another substance. However, this is only justified if the adverse effect is a serious or commonly occurring one.

In light of the above, it is considered that the proposed combination satisfies the requirements of the aforementioned guideline given that the proposed combination is based on valid therapeutic principles with vatinoxan being included to counteract potentially serious cardiovascular effects of medetomidine that occur commonly.

Dose justification/determination

Medetomidine

Based on existing authorisations of medetomidine-only products, and clinical familiarity with these products, the applicant considered the recommended dose for medetomidine-only products for the same indication (1 mg/m^2 of body surface area (BSA), intramuscularly (IM)) as being appropriate for the medetomidine dose within the fixed combination product.

Vatinoxan

In order to determine the appropriate dose of vatinoxan in the fixed combination, the applicant provided a pivotal dose determination study and a pilot dose determination study both with a fixed medetomidine dose and varying vatinoxan concentrations, PK/PD modelling, and information from published literature.

Pilot dose determination study

In a pilot non-GLP dose determination study, eight healthy Beagle dogs were administered each of the following intramuscular treatments: 20 µg/kg bw medetomidine alone or mixed in the same syringe with vatinoxan at 200 µg/kg bw (1:10 ratio), 400 µg/kg bw (1:20 ratio) or 600 µg/kg bw (1:30 ratio). Heart and respiratory rates, central venous pressure (CVP), arterial mean, diastolic, and systolic blood pressures (MAP, DAP, SAP, respectively) were measured. Cardiac output (CO) was measured at 5, 15, 30 and 60 minutes after treatment administration and corrected a posterior with arterial blood gas samples (haemoglobin and sodium). Rectal temperature and continuous lead II ECG were also recorded. A composite sedation score was recorded as the sum of five parameters: resistance to positioning in lateral recumbency, palpebral reflex, position of the eye, jaw and tongue tone and general appearance at baseline and 5, 15, 20, 30, 45 and 60 minutes after administration. Maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were calculated with non-compartmental analysis.

Heart rate (HR) and cardiac output significantly decreased from baseline with all treatments by 5 minutes; no recovery towards baseline was seen over the observation period with medetomidine alone, whereas recovery occurred with all vatinoxan combinations in a dose dependent manner (HR with vatinoxan combinations at dose 600 µg/kg bw returned to baseline value by 20 mins, whereas at dose 200 µg/kg bw was still statistically lower than baseline over the whole 60 mins). MAP transiently increased with all doses (reaching significance for the combination dose of 200 µg/kg bw vatinoxan at 5 and 10 minutes) and thereafter significantly decreased from baseline with vatinoxan combination doses of 400 µg/kg bw and 600 µg/kg bw.

All dogs were considered clinically sedated during the observation period. Sedation scores increased and decreased earlier when medetomidine was administered in combination with vatinoxan than with medetomidine alone, although peak levels of sedation were the same with all treatments. With medetomidine alone, sedation scores remained high at the end of the 60-minute follow-up, whereas they had started to decrease by 45 minutes with vatinoxan combinations.

Maximum plasma concentration of medetomidine was more rapidly achieved and higher with vatinoxan combinations. Seven out of 8 dogs required reversal of medetomidine with atipamezole, as they were still heavily sedated two hours after administration of medetomidine alone, whereas only 2 out of 8 dogs in the vatinoxan 400 µg/kg bw group (1:20 ratio) were administered atipamezole.

Absorption of medetomidine was increased when administered in combination with vatinoxan at the proposed ratio of 1:20 (C_{max} was 3.8 µg/l for medetomidine alone vs 5.5 µg/l when administered with 400 µg of vatinoxan). Time to maximum concentration for medetomidine absorption was significantly decreased when administered in combination with vatinoxan at the proposed ratio of 1:20 (T_{max} was 42.5 minutes for medetomidine alone vs 16.9 minutes when administered with 400 µg of vatinoxan).

It can be accepted that the findings from this study provide supportive information on the proposed 1:20 dose ratio of medetomidine and vatinoxan for use in combination. Whilst cardiovascular benefits of concomitant administration of vatinoxan with medetomidine have been evidenced in this study, the shortening in duration of sedation of medetomidine (compared to when administered alone) represents a risk, however, this has been considered within the overall benefit/risk assessment for the product.

Pivotal dose determination study

In the GLP-compliant pivotal dose determination study the final formulation was not used. The test items were produced at the proposed concentration of medetomidine HCl in the final commercial formulation (0.5 mg/ml) and differed only in the concentration of vatinoxan (and related tonicity

and pH adjustment). Medetomidine and vatinoxan dose ratios of 1:15, 1:30 and 1:50 were administered intramuscularly to 8 dogs with a seven day washout period between treatments with medetomidine administered at a dose rate of 1.0 mg/m^2 BSA. A control group was administered 1.0 mg/m^2 BSA of medetomidine.

Cumulative analgesia scores were lowest (mean of 6) after 30 minutes for the combination, containing the highest dose of vatinoxan (1:50), whereas the score was the highest (mean of 10) for the medium combination dose (1:30). At all combination doses a reduction in the mean composite sedation scores is evident at between 30 and 45 minutes.

Mean heart rate in male dogs 3 hours after administration of medetomidine alone was well above the mean baseline heart rate (30 minutes before administration) of approximately 110 beats per minute (bpm) for dogs administered the combination drug (at 124.8, 170.8 and 186.0 bpm at dose ratios of 1:15, 1:30 and 1:50 doses, respectively). A similar result was seen in female dogs at 3 hours (baseline of approximately 75 bpm; and 132.6, 172.5 and 159.0 bpm at dose ratios of 1:15, 1:30 and 1:50, respectively). In addition, the highest individual recorded heart rates for male dogs administered combination doses were 164 bpm three hours after administration of 1:15 dose, 220 bpm three hours after administration of 1:30 dose and 224 bpm three hours after administration of 1:50 dose. As the observed tachycardia at 3 hours post administration of the combination product is concerning, the applicant has included information in the SPC advising of the potential of tachycardia to occur following administration of the product. Of further concern is the observation of ventricular escape complexes (VEC) after administration of the combination product within the first 3 – 4 hours whilst no VECs were observed when medetomidine was administered alone. Four out of eight animals demonstrated variable incidences of VECs. In dogs administered the combination drug at a ratio of 1:15, a total of 25 VECs were recorded in three dogs (with one dog alone experiencing 18 VECs), at ratio of 1:30 a total of 6 VECs were recorded and at a ratio of 1:50, a total of 7 VECs were recorded. Information about the potential occurrence of VECs as a cardiovascular effect is included in the SPC.

Eye redness or injection of the sclera was seen in dogs administered the combination drug with ratios of 1:30 and 1:50. Although this was considered by the applicant to be a minor finding as it resolved spontaneously, it is noted that this adverse event was also observed in the local tolerance study and in a PK/PD study where vatinoxan was administered alone. To ensure all relevant side effects that are possibly treatment related that have been observed are accurately reflected in the SPC, information has been included in section 4.6 of the proposed SPC.

With regards to sedation, a depth similar to that observed for medetomidine alone was reported; however, it was of a shorter duration. In fact, the time to standing (recovery from sedation) was reported to be 48.3% (i.e. less than half) of that following medetomidine administration alone when vatinoxan was administered at 15 mg/m^2 BSA which is lower than the proposed dose rate of 20 mg/m^2 BSA. The significance of this shortening in duration of sedation has however been considered within the overall benefit/risk balance for the product.

PK-PD modelling

Based on the knowledge from published literature and the pilot study dose determination study, a PK-PD analysis and modelling of the data from the pilot dose determination study and the GLP PK/PD interaction study was conducted to help understand the PK interaction and to refine and support the final ratio of medetomidine to vatinoxan to be used in pivotal studies. In vivo data from the GLP dose determination study was then compared with the prediction of the model.

However, the final formulation was not used in either of the studies included in the PK-PD modelling. Although PK-PD information from the pivotal GLP PK/PD study was included in the

modelling, in this study the test item was administered by the intravenous (IV) route at a ratio of 0.75:22.5 medetomidine to vatinoxan and at a ratio of 1:30 for intramuscular (IM) administration route. Given the differences in pharmacokinetics when the product was administered by the IV route versus the IM route, and as the proposed administration of the final formulation is by IM, it is unclear why the IV route was included in the modelling. Furthermore, the sample pool for modelling appears to be rather limited as only six dogs in the non-GLP study, and information from only eight dogs from the pivotal dose determination study were included.

It is noted that no pharmacodynamic information from the pivotal dose determination study was included in the modelling as no pharmacodynamic information had been determined from this study.

The approach taken by the applicant appears to have been to select an appropriate ratio of medetomidine to vatinoxan in the final formulation based on only two pharmacodynamic endpoints – heart rate and sedation. Analgesia was not considered as a variable in the PK-PD modelling. The applicant has selected a heart rate of 70 beats per minute (bpm) as the number of bpm that the average heart rate prediction should reach over 120 minutes; however, no justification for the selection of 70 bpm has been provided. In the PK/PD modelling, a dose ratio was considered suitable where on average, it was not inferior to medetomidine (when administered alone) by more than 0.5 sedation units for up to 41 minutes and by more than 1 sedation unit for up to 52 minutes. However, the clinical relevance of these differences is unclear and ultimately, the suitability of the selected combination ratio had to be demonstrated under field conditions of use i.e. dose confirmation/field efficacy study.

The applicant has provided the results of further PK/PD modelling using the pharmacokinetic data derived from the target animal safety study where the final formulation was administered IV at 1, 3 and 5 x the recommended dose. This data was compared with the results from the original PK/PD modelling. It was found that by including data from the target animal safety study, the model overestimated the clearance of medetomidine at higher doses. However, it was noted that the PK/PD model could predict the exposure of dexmetedomidine and vatinoxan at the 1 x recommended dose administered (IV) in the target animal safety study.

Consequently, whilst the conclusions from this study are noted and can be used to further support the proposed dose ratio of 1:20 (medetomidine:vatinoxan) in clinical studies, ultimately, the suitability of the proposed dose of 1 mg medetomidine and 20 mg vatinoxan per square metre of body surface area is confirmed by results from the field and target animal safety studies.

Target animal tolerance

The applicant has provided the results of two target animal tolerance studies.

Pivotal target animal safety study

The first target animal safety study was GLP compliant and largely conducted in accordance with VICH GL43 on target animal safety for veterinary pharmaceuticals (EMEA/CVMP/VICH/393388/2006) and previous scientific advice.

This study was both randomised and blinded and 32 healthy Beagle dogs were enrolled in four groups. Each group comprised 8 animals, 4 of which were males and 4 females with a weight ranging from 5.4 to 9.0 kg. The animals were 4-4.5 months of age and given that the product SPC states that 'administration of the product to puppies younger than 4.5 months has not been studied', this is considered acceptable.

The test article was the same as the final formulation intended to be marketed. The control product used was 0.9% sodium chloride which is acceptable and in accordance with VICH GL43. However, in the CVMP Guideline on Pharmaceutical Fixed Combination Products (EMEA/CVMP/83804/2005), it is recommended that a reference treatment be used (mono-active product) when the rationale for the fixed combination is to increase tolerance. Whilst the recommendation in the CVMP fixed combination guideline is noted, given that the inclusion of vatinoxan is primarily aimed at minimising unwanted pharmacological effects of medetomidine and a comparison of any reduction in those effects when medetomidine is administered with vatinoxan has been investigated in pre-clinical pharmacodynamic studies and in the pivotal field safety and efficacy study, the omission of a third group administered medetomidine-only from the target animal tolerance study can be accepted.

The test and control articles were administered by intravenous injection which is not the intended route of administration (intramuscular injection). However, scientific advice was previously sought on this aspect and it was accepted that the IV route of administration represents a worst-case scenario for systemic effects. Nevertheless, it was also recommended in the scientific advice that a separate study investigating local tolerance when the product is administered intramuscularly be conducted (which has been done, see below).

The doses administered represent 1xRTD (RTD/recommended treatment dose = 1 mg medetomidine and 20 mg vatinoxan per square metre of body surface area), 3xRTD and 5xRTD or placebo; and animals were administered the test and control articles on 4 consecutive days. Detailed physical examinations were conducted by a veterinarian pre-test, at 6 time points (pre and post-administration) on each study day (Days 1 to 3) and prior to necropsy on Day 4. The parameters evaluated and the timing of measurements were largely in accordance with those specified in VICH GL43 and are considered sufficiently comprehensive for the purpose of assessing target animal tolerance.

Veterinary observations identified adverse events such as diarrhoea (observed in 1 animal in the 1xRTD group, 2 animals in the 3xRTD and 4 animals in the 5xRTD group), involuntary defection (observed in 4 animals in the 1xRTD group (5 incidences), 5 animals in the 3xRTD group (13 incidences) and 4 animals in the 5xRTD group (9 incidences)) and tremors (observed in 4 animals in the 1xRTD group, 2 animals in the 3xRTD group and 2 animals in the 5xRTD group). These adverse events were mainly consistent with those expected of sedation, and reference has been made to these in the proposed SPC with a common frequency denoted.

A reduction in body temperature was observed in all animals post-administration of the test article which was considered statistically significant. Given the nature of the product, this adverse effect is expected and is mentioned under sections 4.6 and 4.10 of the proposed SPC. Similarly, statistically significant reductions in respiratory rate were recorded, however as these remained within reference range and are an expected effect of sedation, these findings are not considered adverse reactions per se.

With regards sedation, maximum VAS (Visual Analogue Scale) scores were attained at 15 minutes post-administration of the test article with recovery within 2-4 hours. Duration of sedation increased with dosage, which is expected and has been reflected in section 4.10 of the proposed SPC. This is acceptable.

ECG results identified post-administration sinus bradycardia and sinus tachycardia which was considered likely treatment related, with a dose-dependent effect. This correlated with findings derived from heart rate measurements. Some instances of bradycardia were considered treatment related and as such, the applicant has included this finding in the proposed SPC. Considering the nature of vatinoxan, sinus tachycardia is not an unexpected finding, however, given the frequency at

which it occurred in animals administered 1 x RTD (7 incidences) with heart rates up to 228 bpm recorded at the 1, 1.5 and 2 hour time points, the applicant has included this finding in the SPC. Furthermore, given the magnitude by which the frequency of occurrence increased with dosing increments (7 occurrences at 1 x RTD, 10 occurrences in 3 x RTD and 16 occurrences in 5 x RTD), wording on the increased incidence of sinus tachycardia has been included in the overdosing section of the SPC.

Second degree atrioventricular (AV) block was identified in two animals post-administration and 3 incidences were recorded in the same animal. The applicant has proposed wording to reflect possible cardiovascular effects (e.g. bradycardia, cardiac arrhythmias) in section 4.5 of the SPC. Blood pressure values reduced significantly following administration of the test article in all groups and a dose-dependent effect was observed. The lowest values recorded were at 15 minutes with a return to baseline within 4 hours of administration. Given the nature of the product this effect is not unexpected and reference to such an effect under sections 4.5 and 4.10 of the SPC is considered appropriate. The clinical pathology results did not reveal any abnormalities that could be considered test-article related. The only abnormalities observed at necropsy, which were considered treatment-related were red discolorations at the injection sites and microscopic evidence of injection site haemorrhage. As these abnormalities were exhibited by animals in all groups, including the control group, they were considered procedure-related rather than test-article related.

Overall, the findings from this study are considered to be supportive of an acceptable level of tolerance in the target species when administered at up to 5 times the recommended dose and for a duration four times in excess of that proposed. Information relating to unwanted cardiovascular effects (i.e. bradycardia, tachycardia, EVCs, AV block) has been included in the SPC.

Local tolerance study

The second target animal tolerance study was a non-GLP-compliant local tolerance study to support local tolerance following use of the proposed intramuscular (IM) route of administration on account of the pivotal target animal safety study being conducted with a different, i.e. intravenous (IV) route of administration.

The study included 6 healthy Beagle dogs, 3 male and 3 female, aged 10 months to 2 years and 3 months, and 7.75 to 12.2 kg in body weight. Whilst the number of animals included is not in accordance with VICH GL43, given that this study is merely supportive of injection site safety and the main safety data has been derived from the pivotal target animal safety study, this is considered acceptable.

This study was randomised but was not blinded and did not include a control group. VICH GL43 recommends that for injection site safety studies, treatments administered should include a saline control and the final formulation of the test article should be used at 1xRTD. Whilst the omission of a control group is considered a deficiency in terms of permitting assessment of local tolerance to the candidate formulation, given the purpose of the study (investigation of local tolerance to supplement the pivotal target animal safety study) and the fact that the pharmacological effects following administration of the candidate product would result in unblinding, the design used is considered acceptable.

The test article used in this study included a different (higher) concentration of one of the active substances (0.5 mg medetomidine and 15 mg vatinoxan per ml) than that of the final formulation (0.5 mg medetomidine and 10 mg vatinoxan per ml). The applicant considers the difference in formulation acceptable on account of the total amount of citric acid and pH of the formulation remaining unchanged. Whilst it is expected that the final formulation would be used, given that tolerance following administration of medetomidine is well-established and the concentration of

vatinoxan included in the test article was 50% higher than in the final formulation, it can be accepted that the formulation used in this study represents a 'worst case' exposure.

Based on the data from the study, the test article was well tolerated. Although one animal exhibited signs of pain at administration, these were mild; and it can be accepted that this observation was more likely associated with the actual procedure than the product itself. Other clinical signs observed following administration of the product are recognised adverse effects of medetomidine or sedation (skin cold to touch, tremors, salivation), and relevant information has been included in section 4.5 of the proposed SPC for the product. However with regards the observation of injected sclera, although it is accepted that this may be secondary to cardiovascular changes associated with product use, given that it was observed in 5 out of 6 animals in this study and this finding was also reported in animals administered vatinoxan only in one of the PK/PD studies, the applicant has included this effect in section 4.6 the SPC.

Overall, it can be accepted that at the dose administered, the formulation used for the purposes of this study appears to be well tolerated at the site of intramuscular injection.

Clinical field trials

Dose confirmation

The applicant has not conducted a dose confirmation study and has instead proceeded directly to a clinical field trial supported by an additional study to justify the use of heart rate as a surrogate marker for cardiac output. Therefore, the efficacy of the proposed dose and ratio of 1 part medetomidine:20 parts vatinoxan had to be confirmed in the clinical field efficacy study.

Clinical studies

The pivotal GCP-compliant field study was conducted to confirm the effectiveness and safety of the fixed medetomidine/vatinoxan combination under field conditions. The study was conducted largely in accordance with the Guideline on statistical principles for veterinary clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010) and VICH GL9 on Good Clinical Practice (EMA/CVMP/EWP/81976/2010).

This field trial was conducted outside the EU. However, given the nature and intended use of the product, this is acceptable.

The study was conducted across 6 different sites with animals randomly allocated in a 1:1 ratio to medetomidine plus vatinoxan or dexmedetomidine alone. Dexmedetomidine was administered in accordance with the conditions under which a comparable product is currently authorised in the European Union, with regards dose administered, indication for use and posology. The study was designed to demonstrate non-inferiority between the Zenalpha and a veterinary medicinal product authorised for the same indication with regards to efficacy and superiority of the IVP with regards safety. Two primary efficacy parameters were specified - "ability to complete the procedure" (success) and "heart rate". The applicant assumed the proportion of successes would be 85% in the Zenalpha group and 95% in the control group and estimated a minimum sample size of 48 animals per group to demonstrate non-inferiority using a one-sided significance level of 0.025% with a power of 80% and a non-inferiority margin of 25%. However, a substantially larger sample size was enrolled (over 100 animals per group).

An interim analysis was conducted to determine whether the sample size was sufficient once data had been obtained from 103 animals. Such an adaptive approach to sample size confirmation or to check for futility is considered acceptable particularly given that it was pre-specified and well-described in the study protocol with the primary parameter "ability to complete the procedure" used to assess adequacy of sample size. Furthermore, the interim analysis was conducted by an independent statistician not involved in the final analysis and the only information provided to the sponsor was whether the sample size proposed was sufficient or the number of additional cases needed, if any.

It is noted that the control product includes dexmedetomidine whereas Zenalpha includes medetomidine, the pharmacological effects of medetomidine are however considered to be very similar to those of dexmedetomidine. Dexmedetomidine hydrochloride is the pure dextro-enantiomer of medetomidine and, as the levo-enantiomer is devoid of pharmacodynamic activity, dexmedetomidine is considered twice more potent than the racemate medetomidine (consequently, requiring half the dose of the latter). Furthermore, bibliographic data, comparing the sedative properties of the two actives, concluded comparable sedative efficacy up until 45 minutes post-administration.

A total of 226 client owned dogs (109 females and 117 males) from 6 veterinary practices in the USA were screened for this pivotal field study. The dogs ranged from 2.3 to 69.9 kg in body weight and 5 months to 14 years and 9 months in age. 223 dogs were subsequently enrolled on the study comprising 134 purebred dogs (47 different breeds) and 92 mixed breeds. The study animals included a broad range of ages, weights and breeds and are considered to be representative of the target species. The lowest weight of animal enrolled in the study was 2.3 kg and given that the product is proposed for use in animals weighing at least 2 kg, this is considered acceptable.

Zenalpha was administered as proposed in the SPC (by intramuscular injection at 1 mg medetomidine and 20 mg vatinoxan hydrochloride per m^2 body surface area) and the final formulation intended for marketing was used.

Certificates of analysis for the test article has been provided. Whilst certificates of analysis for the control articles have not been provided, this is considered acceptable, given that they were sourced commercially.

One of the inclusion criteria was that animals required non-invasive, potentially mildly painful procedures and/or examinations which required restraint and sedation. It can be accepted that the study animals included a sufficiently representative and broad range of procedures, such as blood sampling, dental procedures, radiography, biopsies and joint injections. Furthermore, 137 of the procedures were considered to be non-painful while 85 were considered to involve some degree of pain, although it is considered that the determination of whether a procedure is painful or not is largely subjective. That said, such determination is unlikely to have differed between treatment groups and therefore bias is not considered to be significant.

Results from analysis of the primary efficacy parameter 'the ability to complete the procedure' indicate 93.1% efficacy for Zenalpha compared to 87.4% efficacy for the control product. The 95% confidence interval for the difference in least square mean estimate was -23.6% to 12.2% and as the upper limit is less than the pre-specified non-inferiority margin, non-inferiority of Zenalpha compared to the control product is claimed. Whilst some of the lightest animals included in the study could not be adequately sedated, it can be accepted that individual responses to alpha- 2-agonists may vary.

The findings from the secondary efficacy parameters are broadly in agreement with the observation from pre-clinical pharmacodynamic and pharmacokinetic studies, demonstrating that vatinoxan

shortens the duration of sedation and analgesia associated with medetomidine when administered in combination.

The applicant conducted an additional study with the objective of confirming good correlation between heart rate and cardiac output. Calculations based on measured parameters and subsequent linear regression suggest a good correlation between heart rate and cardiac output. The applicant provided a supplementary statistical analysis that indicates linear correlation between cardiac output and heart rate over a range of values using repeated measures. As such, the selection of the co-primary endpoint "heart rate" as a marker of overall cardiovascular effects appears to be reasonable and can be accepted.

Results from this study demonstrated that although heart rate was reduced following administration of both Zenalpha and the control product, it was significantly more reduced following administration of the control product at all time points from 15 minutes to 180 minutes post-administration.

One of the inclusion criteria was that planned procedures should last no longer than 45 minutes and were not expected to require more than mild analgesia. Results indicate that duration of sedation, ranged from 8 minutes to 1 hour 44 minutes following administration of Zenalpha (mean duration of 43 minutes), which was significantly shorter than sedation following administration of the dexmedetomidine , which ranged from 5 minutes to 5 hours 43 minutes (mean duration of 1 hour 41 minutes) ($p<0.0001$).

It is accepted that as 94.5% and 90.9% of animals in the Zenalpha and in the control group, respectively, completed the procedure, the duration and types of procedures reflected in the study appear to be sufficiently representative of the intended use of the product. The findings from this and other studies provided with this application indicate that vatinoxan reduces the duration of sedation and analgesia associated with medetomidine (and also dexmedetomidine), therefore, in order to ensure that the product is not used for procedures longer than those investigated in this study, thereby potentially exposing animals to inadequate duration of sedation and/or analgesia, SPC section 4.2 (indications) specifies that the product should be used to provide restraint, sedation and analgesia during conduct of non-invasive, non-painful or mildly painful procedures and examinations intended to last no more than 30 minutes. This is considered appropriate given that comparable analgesia between Zenalpha and the control product (dexmedetomidine) could only be confirmed for up to 30 minutes post-administration in this study, with a significantly lower level of analgesia identified for Zenalpha at the next time point (60 minutes).

Analgesia was assessed by means of a mechanical nociceptive threshold (MNT) device. Nociceptive Threshold (NT) testing is a method of detecting responses to noxious stimuli, such as pressure or force, with the intensity of the stimulus necessary to elicit the response, indicating the threshold. The provision of analgesia can increase this threshold as a higher intensity stimulus is required to elicit a response. NT is widely used in laboratory and clinical settings and is considered suitably validated in terms of being able to detect clinically important differences between treatment groups in order for the findings from the study to be relied upon. The results of this study suggest that analgesia was initially comparable between the Zenalpha and the control product with values not significantly different at the 15 and 30 minute time points post-administration. However thereafter, the pressure required to elicit a pain response was significantly lower for the Zenalpha group, which is consistent with the pre-clinical findings of a reduction in duration of analgesia associated with medetomidine when vatinoxan is co-administered.

Rectal temperatures decreased rapidly following administration of Zenalpha, dropping below reference range. It is acknowledged that hypothermia is a known effect of sedative agents and is specified as a very common adverse effect in the proposed SPC.

It is noted that one animal in the Zenalpha group was observed to have a short period (10 minutes) of mild tachycardia. Furthermore, it is noted that at 180, 240, 300 and 360 minutes the maximum heart rates recorded for the Zenalpha group were 240, 230, 240, and 200 bpm, respectively. For the same time periods in the control group, however, the maximum heart rates recorded were 108, 134, 160 and 180 bpm. Given that tachycardia was also observed in the pivotal target animal safety study and in pre-clinical studies, the applicant has included information in the SPC advising of the potential of tachycardia to occur following administration of the product.

With regards injection site reactions, it is noted that 43.6% of animals administered Zenalpha exhibited responses at the time of injection, typified as transient pain; 42% of these responses were considered mild, 27% were considered moderate and 31% were considered severe. In light of this information, SPC section 4.5 includes a warning that adequate restraint of the animal is recommended, as some animals may react to the injection (e.g., defence reaction).

With regards other adverse effects, those most commonly identified following use of Zenalpha were diarrhoea, vomiting/nausea or retching and hypothermia. These signs have been proposed for inclusion in section 4.6 of the SPC and this is considered acceptable.

Overall and based upon the data derived from this field study, it can be accepted that the candidate product formulation, when administered in accordance with the SPC, is expected to be efficacious for the proposed indication, 'To provide restraint, sedation and analgesia during conduct of non-invasive, non-painful or mildly painful procedures and examinations intended to last no more than 30 minutes'.

Other studies

Correlation between heart rate and cardiac output

A GCP compliant study was conducted to demonstrate correlation between heart rate and cardiac output (CO) to support use of heart rate (as a surrogate measure of cardiac output) as a co-primary efficacy parameter in the pivotal field study. An additional objective was to compare the efficacy of Zenalpha with a medetomidine control product. The applicant aimed to demonstrate that Zenalpha would demonstrate less adverse cardiovascular effects than the control product with a reduction of at least 20% (control compared to Zenalpha) considered to be clinically relevant.

This was a randomised, blinded, positively controlled, two-period cross-over study conducted in 8 Beagle dogs. The investigative veterinary product (IVP) Zenalpha was administered at the proposed dose rate of 1 mg medetomidine/m^2 body surface area and 20 mg vatinoxan/m^2 body surface area by intramuscular injection. The control product (CP) was administered at a rate of 1 mg medetomidine/m^2 body surface area, again by intramuscular injection. Although the primary parameters measured were cardiac output and heart rate, a range of secondary parameters were also evaluated, and this is considered appropriate as it serves as assurance of the cardiovascular safety of the product.

Linear regression was used to investigate the correlation between cardiac output (CO) and a significant R^2 value of 0.7876 was derived from regression analysis. Variance of the CO values used for calculation of mean cardiac output ranged from 0% to 33%. The majority of CO values used for calculation of mean were within the 10% variance specified in the protocol (133 of the 176 calculations used for statistical analyses). Due to the arrhythmias induced by Zenalpha and the control product, heart rate and cardiac output varied rapidly during the 120 minute observation period of the study. This meant that it was not always possible to obtain 3 CO measurements within a variance of 10% as specified by the protocol. However, as these arrythmias were due to

administration of IVP/CP it is agreed that mean CO measurements with variance greater than 10% should be included in the statistical analysis of study variables.

Based upon the fit plot, the R^2 value, the significant p-value and the consistency in findings with respect of the other cardiac output parameters measured and the findings from a supplementary statistical analysis, it can be accepted that the findings from this study support a clinically relevant correlation between heart rate and cardiac output and consequently, the use of heart rate as a surrogate measure for cardiac output in the pivotal field study appears to have been adequately justified. Furthermore, given that at all time points cardiac output and heart rate were significantly higher following administration of Zenalpha compared to the control product, this is considered indicative of an improved safety profile of Zenalpha. Also, other adverse pharmacological effects of medetomidine administration such as increased blood pressure and systemic vascular resistance were similarly attenuated.

Given that arrhythmias and atrio-ventricular block were observed in both groups at a similar incidence, and such findings are identified in the SPCs of authorised medetomidine-containing veterinary medicinal products, the applicant has made reference to these under section 4.5 of the SPC and a contraindication for the use of Zenalpha in animals with cardiovascular disease has been specified under section 4.3 of the SPC.

Combination vatinoxan medetomidine-butorphanol

Another study cited by the applicant was designed to investigate the clinical usefulness of vatinoxan in dogs sedated for diagnostic imaging with medetomidine-butorphanol; however, only a copy of the published study has been provided. This was a randomised study conducted in the target species and whilst the dose of medetomidine (0.5 mg/m^2 body surface area) and vatinoxan (10 mg/m^2 body surface area) administered was not in accordance with that proposed for Zenalpha (1 mg/m^2 body surface area and 20 mg/m^2 body surface area, respectively), useful information may be derived from the study nonetheless.

The animals included are considered suitably representative of the target species and the procedure for which they were enrolled in the study (radiography) is consistent with the intended use of the product. The results indicate that at the dose administered, vatinoxan appeared to attenuate the reduction in heart rate associated with the medetomidine/butorphanol combination.

However, of note is the finding that a greater number of animals administered vatinoxan required a top-up dose of medetomidine after 30 minutes, in order to complete the procedure. Although a lower dose was administered in this study due to the concomitant administration of butorphanol, this is considered supportive of the concern raised in respect of the pivotal field study, i.e. concerns that the product should not be used for procedures longer than those investigated in the field study to avoid potentially exposing animals to inadequate duration of sedation and/or analgesia. Whilst the duration of study for inclusion in the field study was no longer than 45 minutes, it is noted from this study that some animals administered vatinoxan required a top-up dose of medetomidine already after 30 minutes. On account of this, itis specified under section 4.2 of the SPC that Zenalpha should not be used for procedures intended to last more than 30 minutes and it is specified under section 4.9 of the SPC that as re-administration during the same procedure has not been evaluated, Zenalpha should not be re-administered during the same procedure.

Use of atipamezole

Atipamezole is a selective and specific antagonist of both central and peripheral α_2-adrenoceptors, which was developed to reverse the effects of medetomidine. Vatinoxan is also an antagonist of α_2-adrenoceptors; however, its activity is predominantly at peripheral α_2-adrenoceptors. Although the

duration of sedation is considerably shorter when the combination product is used as opposed to when medetomidine is used alone, in cases where antagonism of the central effects are required, the applicant has proposed the inclusion of a recommendation that atipamezole may be administered 30 minutes after the candidate product. In support of the inclusion of this recommendation, the applicant has provided findings from a number of studies conducted to investigate the antagonism of a medetomidine/vatinoxan combination with atipamezole.

One non-GLP compliant study was conducted to investigate the influence of vatinoxan on dexmedetomidine induced sedation and reduced pulse rate. Study animals were administered 50 µg atipamezole/kg bw by intramuscular injection 40 minutes after administration of the sedative combination. Following administration of atipamezole, recovery was considered rapid and calm and no adverse effects were reported. Whilst this study can be considered generally supportive of the compatibility of using atipamezole following vatinoxan/medetomidine, it is noted that the dose rates and inclusion ratio of medetomidine and vatinoxan differ to that proposed, and that atipamezole was administered after 40 minutes and not 30 minutes as proposed in the SPC. It is therefore considered that inadequate information is available from this study to confirm safety of administration of atipamezole following administration of the candidate formulation. However, given that the heart rate was observed for up to 210 minutes after atipamezole administration and that tachycardia was not observed in either group up until this time point, the study can be considered to be generally supportive of the safe use of atipamezole with the fixed combination product.

Another published non-GLP compliant study was conducted to investigate the cardiovascular and sedative reversal effects of atipamezole in animals administered either medetomidine or medetomidine and vatinoxan. Although the dose rates of medetomidine/vatinoxan were not in accordance with those proposed for the product, the ratio was consistent with the final formulation (1:20). Results suggest that following atipamezole administration to the group administered medetomidine and vatinoxan, cardiovascular function appears to have been improved to a greater degree than that observed following administration to the medetomidine only group. With regards levels of sedation, recovery appeared steady in the combination group whilst in the medetomidine group recovery from sedation was interrupted, with a relapse into sedation 30 minutes post-administration. However, given that the dose of medetomidine and vatinoxan administered in this study differ to that proposed, it is considered that inadequate information is available from this study to confirm safety of administration of atipamezole following administration of the candidate formulation.

A proprietary GLP-complaint study was conducted to investigate the effects of a medetomidine/vatinoxan combination treatment when used concurrently with other treatments, including atipamezole. The dose of medetomidine/vatinoxan combination administered was consistent with that proposed for the product, the dose of atipamezole was consistent with that of atipamezole products authorised in the European Union and was administered 30 minutes following the combination product (as per the proposed SPC). Parameters were measured for up to 120 minutes post-atipamezole administration. The results of the study suggest that following administration of atipamezole to dogs sedated with medetomidine/vatinoxan combination, recovery from sedation is smooth with blood pressure, heart rate and sedation scores returning to baseline values soon after atipamezole administration. However, it is noted that 50% of animals administered medetomidine and vatinoxan with atipamezole administered 30 minutes thereafter, were reported to have tachycardia. Given the demonstrated pharmacological profile of the fixed combination and that in one study an increase in the speed of elimination of dexmedetomidine in the plasma was observed after atipamezole administration, and considering that observations were made only for 90 minutes after atipamezole administration in the proprietary study, in the opinion of the CVMP, whilst these

findings are indeed a pharmacological effect, they are considered to directly arise as a result of atipamezole administration following administration of the medetomidine/vatinoxan combination.

Having said that, it is noted that the results of the studies suggest that following administration of atipamezole to dogs sedated with the fixed combination product, recovery from sedation was observed as smooth with cardiovascular parameters returning to baseline values soon after atipamezole administration. Therefore, it can be accepted that the overall benefit of improved cardiovascular parameters with use of the product in combination with atipamezole (where needed) would appear to outweigh the risk of tachycardia.

Given that some (albeit limited) information has been provided in support of the safe use of atipamezole 30 minutes after administering the candidate formulation, inclusion of reference to use of atipamezole in section 4.8 of the SPC can be accepted, primarily to highlight the fact that due to the inclusion of vatinoxan in the candidate formulation, routine use of atipamezole is not warranted and that if used, tachycardia may result and also to avoid the situation whereby no information is provided and veterinarians decide to routinely administer atipamezole (unnecessarily).

Consequently, information on the administration of atipamezole following use of the product is included in section 4.8 of the SPC.

Overall conclusion on efficacy

Justification of the fixed combination

The rationale behind combining medetomidine with vatinoxan is to improve the safety margin of the alpha-2-agonist by means of minimising adverse peripheral hemodynamic side effects, whilst maintaining the desired sedative and analgesic effects mediated in the CNS. The combination of medetomine and vatinoxan is proposed in order to prevent or attenuate the detrimental cardiovascular effects of medetomidine.

Resistance

Given the nature of the product (fixed combination of an α2-adrenergic agonist and α2-adrenergic antagonist), development of resistance is not considered relevant and therefore no assessment is required.

Dose determination

The proposed dose of medetomidine (1 mg/m^2 of body surface area (BSA), intramuscularly (IM)) is based on the dose of existing authorisations of medetomidine-only products with the same indication.

To demonstrate the dose of vatinoxan in the fixed combination (20 mg/m^2 BSA), information from published literature, one pilot dose determination study, and one GLP dose determination study has been provided.

The applicant has proposed a ratio of 1 part medetomidine to 20 parts vatinoxan in the final formulation.

In a pilot non-GLP dose determination study the product was administered IM at the propose dose ratio (1:20) in addition to dose ratios of 1:10 and 1:30, however, the dose rate was not as proposed.

In this study, the absorption of medetomidine was increased when administered in combination with vatinoxan at the proposed ratio of 1:20. The time to maximum concentration for medetomidine absorption was significantly decreased when administered in combination with vatinoxan at the proposed ratio of 1:20. Although the final formulation was not used and the dosing was not

calculated using the proposed 1 mg/m^2 body surface dose (resulting in possibly lower doses being administered than that proposed), it can be accepted that overall this study does provide supportive information on the proposed 1:20 ratio of medetomidine and vatinoxan for use in combination.

In the GLP compliant pivotal dose determination study the final formulation was not used but the test items included 0.5 mg medetomidine/ml and differed only in the concentration of vatinoxan and related tonicity and pH adjustment. Cumulative analgesia scores were lowest after 30 minutes for the highest combination dose (1:50), whereas the score was the highest (mean of 10) for the medium combination dose (1:30). At all combination doses, a reduction in the mean composite sedation scores is evident at between 30 and 45 minutes. In this study, tachycardia was observed at 3 hours post administration of the combination product and as such the applicant has included information in the SPC advising of the potential of tachycardia to occur following administration of the product. Of further concern are the ventricular escape complexes (VEC) that were observed information about the potential occurrence of VECs as a cardiovascular effect has been included in the SPC.

Eye redness or injection of the sclera were seen in dogs administered the combination drug with ratios of 1:30 and 1:50. To ensure all relevant side effects that are possibly treatment related that have been observed are accurately reflected in the SPC, the applicant will be requested to include this information in section 4.6 of the proposed SPC.

With regards sedation, a depth similar to that observed for medetomidine alone was reported; it was, however, of a shorter duration. In fact, the time to standing (recovery from sedation) was reported to be 48.3% (i.e. less than half) of that following medetomidine administration alone when vatinoxan was administered at 15 mg/m^2 BSA which is lower than the proposed dose rate of 20 mg/m^2 BSA. The significance of this shortening in duration of sedation and analgesia has however been considered within the overall benefit/risk balance for the product.

The applicant also included information for dose determination from the pivotal PK/PD study; however, the dose administered was not at the final proposed ratio of 1:20 and the final formulation was not used. Whilst vatinoxan appeared to attenuate the bradycardia associated with medetomidine, increases in heart rate above baseline were observed shortly following administration of the combination of vatinoxan and medetomidine and again between 90-240 minutes.

A PK-PD analysis and modelling was developed to refine and support the final ratio of medetomidine to vatinoxan to be used in pivotal studies. However, the final formulation was not used in either of the studies included in the PK-PD modelling. Furthermore, the sample pool for modelling appears to be rather limited and pharmacodynamic information from the pivotal dose determination study was not included in the modelling as no pharmacokinetic information had been determined from this study.

Based upon the predicted modelling, the applicant has concluded that a dose ratio of 1:20 medetomidine to vatinoxan is a suitable ratio to take forward into the field efficacy study. However, very little PK/PD information has been utilised in the modelling performed and only limited pre-clinical study data is available using this ratio, with the pilot dose determination study being the only proprietary pre-clinical study to include this dose. The applicant has provided the results of further PK/PD modelling using the pharmacokinetic data derived from the target animal safety study where the final formulation was administered IV at 1, 3 and 5 x the recommended dose. This data was compared with the results from the original PK/PD modelling. It was found that by including data from the target animal safety study, the model overestimated the clearance of medetomidine at higher doses. However, it was noted that the PK/PD model could predict the exposure of dexmedetomidine and vatinoxan at the 1 x recommended dose administered (IV) in the target animal safety study.

Consequently, whilst the conclusions from this study are noted and can be used to further support the proposed dose ratio of 1:20 (medetomidine:vatinoxan) in clinical studies, ultimately, the suitability of the proposed dose ratio is confirmed by results from the field and target animal safety studies.

The applicant has not conducted a dose confirmation study and has instead proceeded directly to a clinical field trial supported by an additional study to justify the use of heart rate as a surrogate marker for cardiac output. Therefore, the efficacy of the proposed dose and ratio of 1 part medetomidine:20 parts vatinoxan will be determined the clinical field efficacy study.

Target animal safety

The applicant has provided two target animal tolerance studies; one GLP compliant pivotal study in which dogs were administered Zenalpha by the intravenous route, and a second, non-GLP compliant study investigating the local tolerance of intramuscular administration, which is the proposed route of administration.

The pivotal GLP compliant study was largely conducted in accordance with VICH GL43 and previously provided scientific advice.

Bradycardia, tachycardia and second-degree AV block were observed, and information relating to unwanted cardiovascular effects (i.e. bradycardia, tachycardia, EVCs, AV block) has been included in the SPC. Low blood pressure values were recorded in both the control and IVP groups, given the nature of the product, this effect can be expected, and the applicant has made reference to it in the proposed SPC.

With regards the local tolerance study, 5 out of 6 animals included in the study developed injected sclera following administration of the product. This finding has been also reported in other studies provided with this application. Consequently, to ensure all relevant side effects, that have been observed that are possibly treatment related, are accurately reflected in the SPC, the applicant has included this information in section 4.6 of the proposed SPC.

Field study

The applicant has provided data from a GCP-compliant clinical field trial conducted largely in accordance with VICH GL9 and the Guideline on statistical principles for veterinary clinical trials for veterinary medicinal products. It can be accepted that the study animals included a sufficiently representative and broad range of procedures including those considered to be mildly painful.

Results from analysis of the primary efficacy parameter 'the ability to complete the procedure' indicate 93.1% efficacy for Zenalpha compared to 87.4% efficacy for the control product. The 95% confidence interval for the difference in least square mean estimate was -23.6% to 12.2% and as the upper limit is less than the pre-specified non-inferiority margin, non-inferiority of Zenalpha compared to the control product is claimed. The findings from the secondary efficacy parameters are broadly in agreement with the observation from pre-clinical studies that vatinoxan shortens the duration of sedation and analgesia associated with medetomidine when administered in combination.

However, given the findings from this and other studies provided with this application that indicate that vatinoxan reduces the duration of sedation and analgesia associated with medetomidine, in order to ensure that the product is not used for procedures longer than those investigated in this study thereby potentially exposing animals to inadequate duration of sedation and/or analgesia, it is considered appropriate that the SPC includes information on the nature and duration of procedures for which the product may be used.

Analgesia was assessed by means of a mechanical nociceptive threshold (MNT) device with the results of this study suggesting that analgesia was initially comparable between the Zenalpha and the control product with values not significantly different at the 15 and 30 minutes time points post-administration. However thereafter, the pressure required to elicit a pain response was significantly lower for the Zenalpha group, which is consistent with the pre-clinical findings of a reduction in duration of analgesia associated with medetomidine when vatinoxan is co-administered.

A proprietary GCP-compliant study was conducted with the purpose of demonstrating correlation between cardiac output and heart rate to support use of heart rate (as a surrogate measure of cardiac output) as a co-primary efficacy parameter in the pivotal field study. Cardiac output was measured by intermittent bolus and linear regression was used to investigate the correlation between cardiac output and heart rate. It would appear that the findings from this study support a clinically relevant correlation between heart rate and cardiac output and therefore that heart rate may serve as a suitable surrogate measure for cardiac output. The applicant has cited an additional published study investigating the administration of vatinoxan and medetomidine in combination with butorphanol, the results of which indicated that whilst vatinoxan appeared to attenuate the reduction in heart rate associated with the medetomidine/butorphanol combination, a greater number of animals administered vatinoxan required a top-up dose of medetomidine after 30 minutes, in order to complete the procedure. Given that the product is indicated for procedures lasting no more than 30 minutes, this is considered acceptable.

The applicant has proposed the inclusion of information on administration of atipamezole in the proposed SPC. In support of its inclusion, the applicant has provided findings from a number of studies conducted to investigate the antagonism of a medetomidine/vatinoxan combination with atipamezole. It is noted that the results of the studies suggest that following administration of atipamezole to dogs sedated with the fixed combination product, recovery from sedation was observed as smooth with cardiovascular parameters returning to baseline values soon after atipamezole administration. Therefore, it can be accepted that the overall benefit of improved cardiovascular parameters with use of the product in combination with atipamezole (where needed) would appear to outweigh the risk of tachycardia.

Given that some (albeit limited) information has been provided in support of the safe use of atipamezole 30 minutes after administering the candidate formulation, inclusion of reference to use of atipamezole in section 4.8 of the SPC can be accepted, primarily to highlight the fact that due to the inclusion of vatinoxan in the candidate formulation, routine use of atipamezole is not warranted and that if used, tachycardia may result and also to avoid the situation whereby no information is provided and veterinarians decide to routinely administer atipamezole (unnecessarily).

Consequently, information on the administration of atipamezole following use of the product is included in section 4.8 of the SPC.

Part 5 – Benefit-risk assessment

Introduction

Zenalpha is a sterile multidose solution for injection containing two active ingredients, medetomidine hydrochloride (0.5 mg/ml) and vatinoxan hydrochloride (10 mg/ml). The active substance medetomidine hydrochloride is well known, however, vatinoxan hydrochloride is innovative and is considered a new active substance.

The active substance medetomidine hydrochloride, a potent α2-adrenorecptor agonist already authorised as a sedative, and vatinoxan hydrochloride, a new active substance is a selective α2-adrenoreceptor antagonist. The product is intended for use in dogs in non-invasive, mildly painful procedures and examinations that require restraint and sedation. In combination, the two actives are proposed to prevent or attenuate the detrimental cardiovascular effects of medetomidine. The dose is administrated intramuscularly and is dependent on the bodyweight of the dog.

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.

The application has been submitted in accordance with Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

Benefit assessment

Direct therapeutic benefit

The proposed benefit of Zenalpha is its efficacy in the provision of sedation and analgesia during the conduct of non-invasive, non-painful or mildly painful procedures and examinations up to 30 minutes.

The proposed indication was investigated in laboratory study models and a pivotal field trial.

Well-designed clinical trials conducted in accordance with GCP demonstrated that the product is efficacious in the proposed indication.

Additional benefits

The fixed combination is justified by the applicant to improve the tolerance of the alpha-2-agonist medetomidine by adding vatinoxan to minimise adverse peripheral haemodynamic side effects, whilst maintaining the desired sedative and analgesic effects mediated in the CNS. However, based upon the data provided, it is evident that the sedative and analgesic effects of medetomidine are also shortened significantly by the inclusion of vatinoxan in the combination product.

Risk assessment

Quality:

Information on development, manufacture and control of the active substances and finished product has been presented. 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:

Risks for the target animal:

Concerns have been raised for tolerance in the target animal species. Observations of sinus tachycardia, ventricular escape complexes and significant reductions in blood pressure were reported following administration of Zenalpha. Also, in the pivotal field trial conducted, a high incidence of injection site reactions was observed following administration of Zenalpha. The SPC is considered to adequately reflect all tolerance findings.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations.

Risk for the environment:

The product is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk management or mitigation measures

Appropriate risk management or mitigation measures have been included in the SPC and package leaflet.

Evaluation of the benefit-risk balance

The product has been shown to be efficacious in the provision of sedation and analgesia during the conduct of non-invasive, non-painful or mildly painful procedures and examinations up to 30 minutes.

Conclusion

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