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Committee for Medicinal Products for Veterinary Use

CVMP assessment report for ZACTRAN to add new target species, sheep (EMA/V/C/000129/X/0034)

International non-proprietary name: gamithromycin

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



Introduction	3
Scientific advice	4
MUMS/limited market status	4
Part 1 - Administrative particulars	4
Detailed description of the pharmacovigilance system	4
Manufacturing authorisations and inspection status	4
Overall conclusions on administrative particulars	4
Part 2 - Quality	4
Part 3 – Safety.....	5
Pharmacodynamics	5
Pharmacokinetics	5
Tolerance in the target species of animal	5
User safety	5
Environmental risk assessment.....	6
Residues documentation	6
Identification of the product concerned	6
Pharmacokinetics	6
Depletion of residues.....	6
MRL status	8
Analytical method	8
Withdrawal periods	9
Overall conclusions on the safety and residues documentation	9
Part 4 – Efficacy	9
Pharmacodynamics	9
Development of resistance	10
Pharmacokinetics	11
Target animal tolerance	12
Dose justification	12
Dose confirmation studies	14
Field trials.....	14
Overall conclusion on efficacy	16
Part 5 – Benefit-risk assessment.....	16
Introduction	16
Benefit assessment	17
Direct therapeutic benefit	17
Additional benefits	17
Risk assessment	17
Risk management or mitigation measures.....	17
Evaluation of the benefit-risk balance	18
Conclusion	18

Product profile

Invented name:	ZACTRAN
Active Substances:	Gamithromycin
Target Species:	Cattle, pigs and sheep
Pharmaceutical Form:	Solution for injection
Strength:	150 mg/ml
Therapeutic Indication:	Cattle: Treatment and metaphylaxis of bovine respiratory disease (BRD) associated with <i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> and <i>Histophilus somni</i> . The presence of the disease in the herd should be established before metaphylactic use. Pigs: Treatment of swine respiratory disease (SRD) associated with <i>Actinobacillus pleuropneumoniae</i> , <i>Pasteurella multocida</i> , and <i>Haemophilus parasuis</i> . Sheep: Treatment of foot lesions and lameness associated with <i>Dichelobacter nodosus</i> and <i>Fusobacterium necrophorum</i> .
ATCvet code	QJ01FA95
Pharmacotherapeutic group	Antiinfectives for systemic use
Applicant	MERIAL

Introduction

The applicant MERIAL submitted on 19 May 2016 an application for an extension to the marketing authorisation for ZACTRAN to the European Medicines Agency (The Agency) in accordance with Article 19 of Commission Regulation (EC) No 1234/2008 and Annex I thereof.

ZACTRAN 150 mg/ml was first authorised in the EU on 24 July 2008, and is available as a solution for injection for cattle (since 2008) and pigs (since 2016). ZACTRAN is presented in packs containing 1 vial.

This extension application is for a new target species, sheep.

The applicant applied for the following indication for sheep: "Treatment of foot lesions and lameness associated with *Dichelobacter nodosus* and *Fusobacterium necrophorum* in sheep such as foot rot." However, based on the data provided, the indication "Treatment of infectious pododermatitis (foot rot) associated with virulent *Dichelobacter nodosus* and *Fusobacterium necrophorum* requiring systemic treatment" was considered to be more appropriate.

The active substance of ZACTRAN gamithromycin is an azalide, 15-membered semisynthetic macrolide class antibiotic with uniquely positioned alkylated nitrogen at 7a-position of the lactone ring.

The proposed route of administration is subcutaneous, and the withdrawal period for sheep meat and offal is 29 days. ZACTRAN is not authorised for use in lactating animals producing milk for human consumption.

The rapporteur appointed is Ellen Magrethe Vestergaard and the co-rapporteur is Gabriel Beechinor.

The dossier has been submitted in accordance with Article 19 of Commission Regulation (EC) 1234/2008 and Annex I point 3 (change or addition of target species) thereof (extensions).

On 16 March 2017 the CVMP adopted an opinion and CVMP assessment report.

On 15 May 2017 the European Commission adopted a Commission Decision granting the extension to the marketing authorisation for ZACTRAN.

Scientific advice

The applicant received scientific advice from the CVMP on 13 February 2014. The scientific advice (EMA/CVMP/SAWP/780305/2013) concerned questions in regard to safety (the establishment of the MRL) and clinical development (dose determination, comparator in field studies). The applicant has followed the advice.

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 (MSD-014217, version 2.0, dated 19 August 2015) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place at a duly authorised site within the EEA.

Manufacture for batch release takes place at Merial SAS, Toulouse France. The site has a manufacturing authorisation issued on 10 May 2013 by French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France. Reference to the EudraGMP database has been provided for GMP certification, which confirms the date of the last inspection and shows that the site is authorised for manufacture for batch release of such veterinary dosage forms..

A GMP declaration for the active substance manufacturing site based on an on-site audit was provided.

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

For this extension for a new target species, sheep, no pharmaceutical development has been conducted as ZACTRAN is already registered for cattle and pigs with the same injectable formulation, same strength

and same presentations as will be applicable to the new target species. Therefore, no new data about the active substance or finished product are provided in this application, and cross-reference is made to the data already submitted and assessed with previous applications. This is considered acceptable.

Seven presentations of ZACTRAN have already been registered in Europe for cattle and swine: 50 ml (glass), 150 ml (glass and polypropylene), 250 ml (glass and polypropylene), 500 ml (glass and polypropylene).

Part 3 – Safety

Safety documentation

No new safety studies have been conducted with regards to single dose toxicity, repeat dose toxicity, reproductive toxicity, genotoxicity, carcinogenicity or special studies, and cross-reference is made to data that have already been submitted and assessed for previous application(s). This is considered acceptable.

Pharmacodynamics

See Part 4.

Pharmacokinetics

See Part 4.

Tolerance in the target species of animal

See Part 4.

User safety

No new data on user safety have become evident since the original User Safety Assessment was written to support the initial application for ZACTRAN in cattle. However, the CVMP Guideline on user safety for pharmaceutical veterinary medicinal products has been revised and the revised document became effective in March 2010 (EMA/CVMP/543/03-Rev.1). Consequently, a new user risk assessment has been written in compliance with the new guideline.

Current user safety warnings refer to the potential of gamithromycin to cause irritation to eyes and/or skin. Adequacy of user safety warnings in the product literature was confirmed at renewal of the cattle marketing authorisation for ZACTRAN in 2013, and at the time of the addition of the new target species, pigs, in 2016.

The product is intended to be used in sheep with the same route of administration as in cattle (subcutaneous), the same dosage (mg/kg) and the same treatment duration (single administration) as for the authorised target species, cattle and pig. Therefore, user exposure to the formulation when used in sheep is not expected to be different or greater than user exposure associated with use in cattle and pig. Consequently, the existing user safety statements (agreed in relation to use in cattle and pigs) are equally applicable to use in sheep. The user precautions in the proposed Summary of Product Characteristics (SPC) can be accepted and are considered adequate to ensure user safety.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the VICH guidelines. Predicted environmental values in soil for sheep on pasture are below 100 µg/kg. Consequently, no further studies are required.

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

Residues documentation

Identification of the product concerned

In the pivotal studies the final formulation of ZACTRAN has been used. ZACTRAN contains 150 mg gamithromycin/ml and is recommended for administration to sheep at a dose of 6 mg/kg bw by subcutaneous injection.

Pharmacokinetics

An *in vivo* pivotal pharmacokinetic study was conducted with the final formulation of ZACTRAN. Twenty four sheep (5 month of age, 41-52 kg) were treated with either a single intravenous dose of 6 mg/kg bw gamithromycin or with a single subcutaneous dose of 3, 6 or 12 mg/kg bw in a parallel design. Each group comprised 6 animals. Plasma samples were collected before treatment and at predetermined time points up to 12 days after treatment. The resulting plasma samples were analysed using a validated method with a limit of quantitation (LOQ) of 2 ng/ml. Pharmacokinetic parameters were determined using a non-compartmental model.

Following a single intravenous injection of 6 mg/kg bw, the mean area under the curve extrapolated to infinity (AUC_{inf}) was 10.0 ± 0.70 µg.h/ml, and the mean terminal plasma half-life ($t_{1/2}$) was 34.6 ± 6.86 hours. The volume of distribution at steady state (V_{ss}) and clearance (Cl_{obs}) were 19.0 ± 4.30 l/kg and 602 ± 42.8 ml/h/kg, respectively. For animals treated with subcutaneous injections of 6 mg/kg bw, the AUC_{inf} was 8.88 ± 2.33 µg.h/ml which is comparable to the AUC_{inf} following the same dose given intravenously, resulting in 89% absolute bioavailability. The AUC_{inf} for subcutaneous injections of 3 and 12 mg/kg bw were 4.51 ± 0.78 and 15.9 ± 4.12 µg.h/ml, respectively. The mean maximum plasma concentrations (C_{max}) were 147 ± 27.8 , 448 ± 180 , and 534 ± 302 ng/ml for subcutaneous doses of 3, 6, and 12 mg/kg bw, respectively. The time to reach the maximum plasma concentration in the three subcutaneous injection groups was 1.50 ± 0.84 , 2.30 ± 2.40 , and 4.07 ± 2.99 hours, indicating a rapid absorption. The terminal plasma half-life ($t_{1/2}$) following subcutaneous treatment was 46.8 ± 7.96 , 42.5 ± 5.25 , and 53.1 ± 8.79 hours, respectively, for doses at 3, 6, and 12 mg/kg bw, values which are comparable to the half-life following intravenous administration.

The average C_{max} values for the 3, 6 and 12 mg/kg bw doses did not increase proportionally with dose, but dose proportionality of the AUC_{inf} was established.

Depletion of residues

The depletion of residues has been examined in one pivotal study where ZACTRAN was injected subcutaneously at the intended therapeutic dose of 6 mg/kg bw once to sheep. A total of 37 healthy sheep (19 males and 18 females) were included in the study. The animals were approximately 7 months old and weighed between 51.6 and 73.2 kg (day -1). The animals were allocated into 8 groups. Group 1 consisted of one male and one female sheep that were used as untreated controls and Groups 2-8 consisted of

5 animals of both genders. Animals in Groups 2-8 were treated with gamithromycin 15% w/v by subcutaneous injection on the dorsal left neck side at 1 ml/25 kg bw (equivalent to 6 mg gamithromycin/kg bw) once on day 0. The two control animals in Group 1 were euthanised on day 1. Groups 2, 3, 4, 5, 6, 7, and 8 were euthanised at 5, 9, 14, 21, 28, 35, and 47 days post dose, respectively and samples of and plasma, liver, kidney, muscle, skin, fat and injection site (inner and outer perimeter) were collected. Urine and faeces excreted over the whole day were collected from the male animal in Group 1 on day 1, and two randomly selected male animals in Group 5 on days 1, 2, 3, 4, 5, 7, 9, and 14 post dose.

Gamithromycin and declad (a metabolite formed by the loss of the dideoxy sugar moiety, cladinose) concentrations in faeces, urine and edible tissues were determined using validated LC-MS/MS methods. A semi-quantitative metabolite profile was also performed in matrices using HPLC coupled with LC-high resolution MS (LC-HRMS) and extracted ion chromatogram (XIC) were obtained.

The gamithromycin residue concentrations in both urine and faeces are much higher than its metabolite declad. The cumulative amount of gamithromycin and declad residues in excreta showed that the majority of the residues were recovered in excreta over 7 days post administration. The cumulative amount of gamithromycin and declad residue at 14 days post administration amounted to approximately 25% of the dose in faeces and approximately 18% in urine. Thus total recovery of gamithromycin and declad in excreta was 43% of the dose, which appears to be low. However, low recovery has also been observed in cattle and pigs.

Depletion of gamithromycin from tissues followed first order kinetics with liver showing the slowest depletion rate among all tissues analysed. The half-lives of gamithromycin depletion in liver, kidney, muscle, fat, injection site core, and injection site ring were calculated to be 5.48, 4.22, 2.55, 2.82, 4.43, and 2.39 days, respectively. The half-lives of declad were longer than those of gamithromycin.

The metabolite profiles obtained using LC-HRMS showed qualitatively similar metabolites in tissues and excreta. The amount of metabolites varied in different tissues and in excreta at various time points. The major biotransformation pathways included hydrolysis with a loss of the cladinose sugar moiety to form declad and N-dealkylation to form M8 (N-despropyl N-desmethyl gamithromycin) and M9 (N-despropyl gamithromycin). Gamithromycin also underwent biotransformation via a minor pathway to form the translactone derivative M5 (TDO) (ML-1,620,759) through an intra-molecular rearrangement. Other minor biotransformation pathways were mostly oxidative (+O and +O-2H).

In liver, gamithromycin and declad were the major residues present. As the %XIC, gamithromycin decreased from 83.9% on day 5 to 63.2% on day 28. Declad concentrations as %XIC increased from 12.0% on day 5 to 32.4% on day 28. Declad was the only major metabolite in liver that exceeded 10% of XIC. None of the other metabolites exceeded 3% of XIC.

A similar pattern was noted in kidney where gamithromycin and declad were the major residues. Gamithromycin declined from 89.7% on day 5 to 67.8% on day 28. As with liver, declad was the only metabolite that exceeded 10% of XIC, with none of the other metabolites exceeding 3% of XIC.

For injection site core muscle, gamithromycin and declad were the major residues. Gamithromycin declined from 91.9% on day 5 to 78.5% on day 35. Declad increased from 3.76% on day 5 to 16.9% on day 35. As with liver and kidney, declad was the only metabolite that exceeded 10% of XIC and none of the other metabolites exceeded 3% of XIC. Residues of gamithromycin at the injection site remained above the limit of quantification (25 µg/kg) until the final sampling point (47 days).

In muscle, in addition to gamithromycin and declad, metabolites M8 and M9 were the major residues. As %XIC, gamithromycin declined from 70.2% on day 5 to 8.19% on day 21 while declad increased from

8.94% on day 5 to 13.7% on day 14, and decreased to 8.57% by day 21. Concentrations of M8 and M9 increased from 6.91% and 5.89% on day 5 to 48.06% and 18.1% on day 21, respectively. However, the concentrations of gamithromycin and declad in muscle at day 21 were well below the LOQ of the assay and so the concentrations of M8 and M9 were also very low.

In fat, gamithromycin, declad and M9 were the major residues present. Gamithromycin decreased from 83.1% of XIC on day 5 to 37.9% by day 21 whereas declad and M9 increased from 7.3% and 2.79% on day 5 to 37.7% and 18.7% on day 35. No other metabolites exceeded 5% of XIC at any time point.

MRL status

On 14 July 2016 the CVMP recommended the extension of the entry for gamithromycin in table 1 of the annex to Commission Regulation (EU) No 37/2010 to all ruminant except bovine, in accordance with the table below. The Commission Implementing Regulation (EU) 2016/2045 was published in the Official Journal of the European Union on 24 November 2016.

Pharmacologically active substance	Marker residue	Animal species	MRL µg/kg	Target tissues	Other provisions	Therapeutic classification
Gamithromycin	Gamithromycin	Bovine	20	Fat	Not for use in animals from which milk is produced for human consumption	Anti-infectious agents / Antibiotics
			200	Liver		
			100	Kidney		
		All ruminants except bovine	50	Muscle		
			50	Fat		
			300	Liver		
Porcine	200	Kidney	No entry			
	100	Muscle				
	100	Skin and fat in natural proportion				
			100	Liver		
			300	Kidney		

In addition, as outlined in the EPMAR for gamithromycin, the CVMP established injection site residue reference values (ISRRV) of 1500 µg/kg for use in the derivation of withdrawal periods, as described in the CVMP draft reflection paper on injection site residues: considerations for risk assessment and residue surveillance (EMA/CVMP/520190/2007-Rev.1).

The excipients listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Analytical method

The analytical method for monitoring of residues for gamithromycin in sheep tissues has been evaluated during the MRL evaluation and reviewed by the relevant European reference laboratory. The method was considered validated and adequate for monitoring of residues.

Withdrawal periods

The withdrawal periods were calculated for edible tissues liver, kidney, muscle, fat and injection site core based on the data from the residue depletion study for gamithromycin in sheep and the established MRL, making reference to the statistical approach described in the CVMP Note for Guidance: approach towards harmonisation of withdrawal periods (EMA/CVMP/036/95-FINAL).

For muscle and fat, only Day 5 and 9 time points were above LOQ, which are not sufficient to have a meaningful regression analysis for these two tissues. However, this has no impact on the assessment of overall withdrawal period because the residue levels in all loin muscle and fat tissues were very low and the overall withdrawal period of gamithromycin in sheep is not driven by the withdrawal period for these two tissues.

The withdrawal periods for liver, kidney, and injection site core tissues were determined to be 26, 25 and 29 days, respectively, based on the statistical linear regression model with a 95% tolerance limit and a 95% confidence interval. Therefore, the overall withdrawal period is 29 days (based on depletion of residues at the injection site to the injection site residue reference value (ISRRV)).

Gamithromycin is not for use in animals from which milk is produced for human consumption, and ZACTRAN should not be used in pregnant animals within 2 months of expected parturition (cows, heifers) or 1 month (ewes). The limit of 1 month before parturition in ewes as proposed by the applicant was considered acceptable, taking into consideration the half-life of the substance, the time to reach virtual clearance in plasma, the time interval for residues to deplete from edible tissues, and the addition of a safety span.

Overall conclusions on the safety and residues documentation

Cross-reference has been made to toxicity, genotoxicity, carcinogenicity and special studies, which have been submitted and assessed as part of previous applications for the product, which is considered acceptable.

A user risk assessment report in line with current guidance was provided, and the extension does not pose an unacceptable risk to the user when used in accordance with the SPC.

A new Phase I environmental risk assessment (ERA) was provided and the extension for sheep is not expected to pose a risk for the environment when used according to the SPC.

An ADI value of 600 µg/person has been assigned to gamithromycin.

The applicant has presented a well conducted residue study which has been assessed in the MRL procedure. A withdrawal period of 29 days was established.

Part 4 – Efficacy

Pharmacodynamics

Gamithromycin is a semisynthetic azalide which has a 15-membered semisynthetic lactone ring with a uniquely positioned alkylated nitrogen atom at the 7a-position. Azalides, in common with other macrolides, disrupt protein synthesis by reversibly binding to 50S subunits of the ribosome, thereby inhibiting the transpeptidation and translocation process and causing premature detachment of incomplete polypeptide chains. Macrolides are generally bacteriostatic agents but they may also be bactericidal. Gamithromycin acts in a bactericidal manner.

In the original submission, minimum inhibitory concentrations (MICs) of gamithromycin were determined in 4 strains of *Dichelobacter (D) nodosus* and 18 strains of *Fusobacterium (F) necrophorum*. Data were compared with corresponding MIC values for tilmicosin. Each strain originated from foot swab samples taken from clinical cases of foot rot in sheep and the strain selection complied, as far as possible, with the requirements of the draft CVMP Guideline on the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/261180/2012). The strains were isolated between 2011 and 2015, in 3 different countries (France, UK and Germany) from 15 different farms. The test system used standardised agar dilution MIC methodology with supplemented Brucella blood agar, as described by the Clinical and Laboratory Standards Institute (CLSI) in guideline M11-A8. The tilmicosin MIC against the quality strain (*C. difficile*) fell within the prescribed range, and gamithromycin and tilmicosin MICs against the other quality control strains were reproducible, thereby validating the MIC determinations.

In response to questions, the applicant provided MIC data on seventeen new isolates of *D. nodosus* and twelve new isolates of *F. necrophorum* collected in the field or obtained from different European laboratories. When including these additional isolates with the samples already submitted in the original dossier, a total of 21 isolates of *D. nodosus* and 30 isolates of *F. necrophorum* have been considered

Minimum Inhibitory Concentrations (MIC) have been determined according to CLSI methodology; all reported data were in accordance with CLSI published QC criteria.

MIC results for the initial *D. nodosus* strains (n=4) were 0.016 µg/ml for gamithromycin and between 0.031 and 0.063 µg/ml for tilmicosin. Including the results from all isolates (n=21), 100% of the strains possesses MIC values equal to 0.008 (n=9) or 0.016 µg/ml (n=12).

Two strains of *D. nodosus* were screened by PCR and were found to be of the virulent subtype based on aprV2 and aprB2 genes. The other *D. nodosus* subtypes were not genetically characterised, but the strains have all been collected during clinical outbreaks of foot rot (including severe/virulent clinical cases), ensuring that the *D. nodosus* strains tested include subtypes of severe/virulent foot rot.

MIC results for the original 18 *F. necrophorum* strains ranged between 1 and 64 µg/ml with an MIC₅₀ of 4 µg/ml, and between 1 and 16 µg/ml for tilmicosin with a MIC₅₀ of 4 µg/ml. A single strain was resistant to tilmicosin (MIC>128 µg/ml). With the addition of another 12 strains of *F. necrophorum*, the distribution of *F. necrophorum* MIC (n=30), 27 out of 30 strains possessed MIC's in the range 1 to 32 µg/ml, with MIC₉₀ of 32 µg/ml, and MIC₅₀ of 2 µg/ml.

Development of resistance

Gamithromycin belongs to the macrolide class of antimicrobials which cover a number of molecules. All appear to act via the same mechanism and there is no reason to expect a much higher selection pressure against bacterial pathogens than already present. Development of resistance for gamithromycin has been fully assessed in the initial Marketing Authorisation (2007-2008), at renewal in 2013 and at the time of extension to include pigs as a target species.

Regarding the risk to public health, available data suggest that the use of gamithromycin since its first approval in 2008 for the treatment of bovine respiratory disease and its subsequent extension to use in pigs for swine respiratory disease has not compromised the use of macrolides for the treatment of infection in man. Accordingly, it is considered unlikely that the use of gamithromycin in the treatment of foot rot in sheep will prejudice the use of macrolides, in the limited cases where they are used, nor other drug classes, for the treatment of human infections.

Pharmacokinetics

In support of the application for the new target species, two GLP pivotal pharmacokinetic studies were submitted.

In the first study, twenty six (13 male and 13 female) Merino sheep aged approximately 5 months old and in the bodyweight range 41.8-52.4 kg were either untreated (n=2) or treated with either a single IV dose of 6 mg/kg gamithromycin (n=6) or with a single subcutaneous dose of 3, 6 or 12 mg/kg (n=6 per dose rate) in a randomised parallel design. Gamithromycin administered once to sheep by the subcutaneous route demonstrated rapid absorption, high bioavailability, approximate dose proportionality of AUC_{inf}, rapid and extensive distribution to tissues, and a rather slow elimination rate with a terminal half-life of 46 to 53 hours. The average C_{max} values for the 3, 6 and 12 mg/kg doses did not increase proportionally with the dose. For more details see above (Residues, pharmacokinetics).

A second study examined the distribution of gamithromycin to skin. Seventeen 5-6 month old healthy Merino sheep were included in this study. Two animals were randomly selected to form the untreated control group. The remaining 15 animals received gamithromycin once on Day 0 at 6 mg/kg bw subcutaneously. Blood samples were collected prior to treatment and at pre-determined times up to 21 days. Following euthanasia, samples of skin of the lower limb of both front legs were collected from randomly selected sheep (Day 2 = 2 control and 3 treated animals / Day 5 = 3 treated animals / Day 10 = 3 treated animals). The concentration of gamithromycin in plasma and skin was analysed using an LC-MS/MS method.

The time to reach the maximum plasma concentration ranged from 10 minutes to 6 hours indicating a rapid absorption, with a maximum plasma concentration of 573 ng/ml at mostly 15 minutes after treatment. The average concentration of gamithromycin in tissue skin was 805, 393 and 276 ng/g on Days 2, 5 and 10 respectively. No skin concentrations are available between Day 0 and 2. The estimated half-life for depletion of gamithromycin skin was 5.25 days.

The extent to which these data can be relied on for PK/PD analyses is probably limited. However, it is accepted that the data presented suggest that the concentrations of gamithromycin achieved in total skin are greater than the concentrations achieved in plasma but the relevant matrix representing the infection sites is unknown (extracellular/intracellular). The gamithromycin concentration in the skin was higher than the proposed *in vitro* MICs for *D. nodosus*. Regarding *F. necrophorum*, the *in vitro* MICs are higher than the concentration reached in the skin.

A PK/PD analysis has nevertheless been conducted to support clinical efficacy at the selected dose (in particular for *D. nodosus*). The PK/PD analysis showed that the concentration of gamithromycin in the skin rapidly exceeds the MIC₉₀ of *D. nodosus* (0.016 µg/ml) and remains high in a sustainable manner

However, it is recognised that the skin is a heterogeneous tissue made of different cellular and non-cellular components and therefore an accurate estimate of the gamithromycin concentration to which the bacteria are actually exposed is not possible to quantify.

The ratio between the gamithromycin concentration measured in the skin and the *D. nodosus* MIC₉₀ provides a crude estimate of the level to which the bacteria are exposed. It suggests that gamithromycin is present at high concentrations (above the MIC₉₀) in a sustainable manner in the target tissue. PCR and bacteria culture at the end of the PK- study (showed a significant decrease of the bacterial load, and the efficacy rate was high in the field trial, suggesting that *D. nodosus* is adequately exposed to a concentration of gamithromycin (above the MIC₉₀) as suggested in the PK/PD analysis.

As regards *F. necrophorum*, the MIC₉₀ and MIC₅₀ are higher (2 and 32 µg/ml, respectively, based on both MIC studies pooled) than the concentration of gamithromycin in the skin. However, as noticed for

Dichelobacter nodosus, the clinical studies have demonstrated efficacy of ZACTRAN in treating foot rot in animals in which the presence of *Fusobacterium necrophorum* has been confirmed.

Target animal tolerance

Target animal tolerance was evaluated in a GLP-compliant target animal safety study and in the clinical field studies.

A target animal tolerance study was conducted in accordance with VICH GL43 Target animal safety: pharmaceuticals (CVMP/VICH/393388/2006). Thirty two healthy sheep 3 to 4 months of age were allocated to 4 treatment groups and received either saline or 1X, 3X and 5X the recommended use level of 6 mg/kg bw gamithromycin on 3 occasions (Days 0, 5 and 10) representing up to 30 mg/kg of gamithromycin. The maximum volume was of 5 ml per injection site (IJS). The injection sites were observed (scored for pain, skin appearance, hardness, heat, swelling/oedema) for reactions following treatment on Days 1, 5, 6, 7, 10, 11, 12, 13 and 14. Sheep were euthanized on Days 15, 16 or 17 for a full gross necropsy examination and samples were collected for histopathology. Clinically, diffuse (>2 cm) swellings (accompanied by heat in some animals) were noted at the injection site of many animals, correlating at necropsy (day 10) with a discoloured firm nodule (+/- cavitated, +/- haemorrhagic), and correlating microscopically with a granulomatous inflammatory process. The changes in the injection sites occurred in a dose- and time-dependent manner. The injection site inflammatory processes range from minimal to mild in the 1X group, and minimal to moderate in the 3X and 5X groups. The local reaction was transient and typically resolved within a few days. An overall dose-dependent moderate increase in mean plasma fibrinogen concentrations was observed. The increase in plasma fibrinogen was considered to be in response to the injection site inflammation. All other observations were considered to be biological variation.

Gamithromycin has been administered at the recommended therapeutic dose and recommended route of administration in 229 animals. At this dosage, the product was very well tolerated without any treatment-related findings except for the frequent reporting of transient mild to moderate and diffuse reactions at the injection site observed in 19/200 animals in the clinical trials that generally fully resolved within 4 days, and generally consisting of diffuse oedema/swelling and characterised microscopically by a minimal to moderate subacute/chronic granulomatous inflammation of the subcutaneous tissues.

Overall, the studies showed that gamithromycin was well tolerated in sheep, without any systemic signs and only mild tissue reactions at the injection site, which resolve within a number of days. However, it is noted that the safety of the product was not investigated in sheep intended for breeding or in pregnant or lactating animals. As a consequence, it is proposed that the text for section 4.7 of the SPC, originally agreed for cattle, be retained: "*Based on laboratory animal data, gamithromycin has not produced any evidence of selective developmental or reproductive effects. The safety of gamithromycin during pregnancy and lactation has not been evaluated in target species. Use only according to the risk/benefit assessment by the responsible veterinarian.*" This can be accepted.

Dose justification

One GCP compliant study was performed to provide data on dose determination to evaluate and compare the effectiveness of a single subcutaneous injection of gamithromycin at 3 and 6 mg/kg bw for the treatment of foot rot in naturally infected sheep with typical foot rot lesions and/or lameness.

Forty-six female sheep of various breeds, aged 4.5 to 12.7 years old on Day 0 and in the bodyweight range 36.8 to 63.0 kg bodyweight on Day-1, were studied in this controlled and blinded, single site clinical efficacy study. The animals were ranked by ascending lameness and ascending average foot rot lesion

scores and were randomly allocated to one of the three treatment groups: 1) saline treated control group (10 animals), 2) group treated with gamithromycin at 3.0 mg/kg (18 animals), 3) group treated with gamithromycin at 6.0 mg/kg (18 animals).

Treatment doses were administered once on Day 0 by subcutaneous injection anterior to the right shoulder. After the treatment, the animals were observed once daily throughout the study and lameness and foot rot lesions scores were recorded on Days 1, 2, 5, 7, 9, 14 and 21. Swabs for PCR and bacteriology were collected on Day-6 and Day 21.

Lameness was scored according to a 4-score system:

- 0 = no obvious lameness, bears weight evenly on all feet;
- 1 = uneven posture, short stride, slight nodding;
- 2 = distinct nodding, not weight bearing on affected limb when standing;
- 3 = not weight bearing on affected limb, kneeling, reluctant to move.

Foot rot lesion was scored according to a 6-score system:

- 0 = normal, dry interdigital skin, hair
- 1 = hoof temperature high, inflammation of interdigital skin, interdigital hair loss
- 2 = necrotising interdigital dermatitis, characteristic smell
- 3 = under-running of the medial wall and soft horn of the heel
- 4 = under-running of sole, extending to the outer edge
- 5 = necrotising inflammation extending to the tip, separation of the hoof

The two treated groups were compared with the control as well as with one another. Primary efficacy variables were lameness and foot rot lesion scores. Primary efficacy comparison was the Day 21 changes from baseline (scores on Day -6). Secondary efficacy comparisons were the Day 7 and Day 14 changes from baseline.

No adverse events related to treatment were observed during the study.

During the study, the mean lameness score slightly increased in the control group from 1.9 on Day -6 to 2.5 on Day 21 whereas it decreased in both treated groups. By study Day 21, 10%, 77.8% and 88.9% of animals were reported to have an improvement in lameness scores in group 1 (control), group 2 (3 mg/kg) and group 3 (6 mg/kg), respectively. Lameness score change from baseline on Day 7, Day 14 and Day 21 was significantly different in Group 2 ($p \leq 0.002$) and Group 3 ($p \leq 0.001$) when comparing to Group 1 (saline). No significant difference was observed between the 2 treated groups.

Regarding the foot rot lesion score, the change from baseline on Day 7, Day 14 and Day 21 was also statistically different in both treated groups ($p < 0.001$) when comparing to Group 1 (saline). On Day 21, 10% of the sheep treated with saline had improvement in lesion scores compared to 88.9% and 94.4% of the sheep treated at 3 mg/kg and 6 mg/kg respectively. No statistically significant difference was observed between the two treated groups.

Bacteriological data showed that at Day -6, all animals were tested "positive" to "very strongly positive" regarding PCR against virulent strains of *D. nodosus*. On Day 21, animals from the control group remained "very strongly" or "strongly" positive. In the two treated groups, most of the animals were "positive" or "weakly positive", and 2 of them being "negative" in the group treated at 6 mg/kg.

This was a well conducted study showing that gamithromycin is efficient in reducing lameness score and lesion score in foot rot infected sheep. Based on the results of this study, it can be accepted that gamithromycin administered at 6 mg/kg bw resulted in a statistically significant reduction in lameness and lesion scores measured 7, 14 and 21 days after single treatment administration compared to a

negative control group. There were no statistical differences in either lameness score or lesion score between the two tested doses of 3 and 6 mg/kg at Day 21. However the CVMP accepts that a higher proportion of animals administered 6 mg gamithromycin/kg were observed to have a reduction in lameness and lesion scores (compared to animals administered 3 mg gamithromycin/kg) and considered that there is therefore sufficient evidence to support carrying forward the higher dose rate of 6 mg/kg for further investigation of effectiveness under field conditions of use.

Whilst bacteriology results indicated that 8/10 animals in the untreated control group showed evidence of infection with *D. nodosus* on study Day -6, only 1 out of 10 animals was subsequently shown to have evidence of *D. nodosus* infection on study Day 21. The reason for this finding is unclear, however, contrary to the bacteriology findings, PCR results did confirm the presence of *D. nodosus* on study Day 21.

Dose confirmation studies

Not applicable.

Field trials

The clinical efficacy and safety of gamithromycin (ZACTRAN) for the treatment of foot rot (infectious pododermatitis) in sheep associated with *D. nodosus* and/or *F. necrophorum* were compared to a positive control, containing tilmicosin, in a positive-controlled European multi-centre field study.

From a total of 6 sites located in France, Germany and UK, 364 sheep (26 males and 338 females from different breeds or cross-breeds) were enrolled from October 2014 to January 2015. Sheep were 4 months to 10 years old and weighed 16 to 113 kg. Animals displaying clinical signs of virulent foot rot (Lameness score ≥ 1 and lesion score ≥ 1 on at least 1 foot, see below) were enrolled. Site (farm) eligibility was confirmed by history of foot rot, lameness in the flock and by the isolation of at least one target pathogen per site from swabs collected prior to treatment from enrolled animals. Presence of virulent *D. nodosus* was identified by PCR (no information about the method used though) and *F. necrophorum* by culture. At each site, the sheep were randomly assigned to be treated with gamithromycin at 6 mg/kg bodyweight or tilmicosin at 10 mg/kg bodyweight subcutaneously once on Day 0. Clinical efficacy was evaluated by lameness scores as the primary end point at Day 0, Day 5 and Day 21. Foot rot lesion scores were considered as a secondary endpoint (scored according to a 6-score system). The sum and mean of lesion scores on all feet between prior to or on Day 0 and Day 21, and the mean score of affected feet (=sum of score/number of affected feet) between prior to or on Day 0 and Day 21 were reported.

The comparison of proportions of treatment success between treatment groups utilised a non-inferiority hypothesis test. Out of the 364 enrolled animals, 310 were positive for *D. nodosus* (PCR) and 120 for *F. necrophorum* (culture), 113 being positive for both isolates. A total of 359 animals out of 364 were included in the efficacy analysis.

On Day 21, the proportions of treatment success based on the lameness score were 97.8% and 93.3% in the gamithromycin and tilmicosin groups, respectively. Gamithromycin was demonstrated to be superior to tilmicosin ($p < 0.05$). For each site, the proportion of successes in the gamithromycin group was numerically equal to or higher than in the tilmicosin group (84% at one site, 100% at 5 other sites for gamithromycins versus 78-100% for tilmicosin).

For foot rot, the mean of three types of scores were similar in the two groups.

The study was considered to be well conducted with a sufficient number of animals to compare the two treatment groups, gamithromycin and tilmicosin. Suitable animals were included in the study given that the animals had to be naturally infected, sourced from a flock with a confirmed history of foot rot lesions and showed evidence of typical foot rot lesions (score ≥ 1) and lameness (score ≥ 1). Correct diagnosis was further supported by means of demonstrating presence of target pathogens (*D. nodosus* by PCR and *F. necrophorum* by culture).

The lameness and lesion scoring systems are reported in published literature and can be accepted as being sufficiently sensitive to permit clinical differentiation between animals in terms of severity of lameness and lesions for the purpose of assessing efficacy in this study.

Treatment success was high in both treatment groups with a small favour to gamithromycin. The distribution of lameness and lesions scores between the two treatment groups at Day 0 was found to be similar, ensuring that the conclusion of the study is not biased by a confounding effect arising from heterogeneous distribution of lameness or lesion scores at inclusion.

Despite the fact that the inclusion criteria required animals to have scores of ≥ 1 for both lameness and foot rot lesions, the CVMP noted that the primary efficacy outcome parameter was the proportion of success determined solely on lameness scores.

The applicant justified their choice as lameness score is a good clinical criterion for clinical trials, which can be easily and objectively measured. In order to address the concerns raised in respect of the omission of lesion scores as a pivotal efficacy parameter in the study, the applicant also performed a *post-hoc* statistical analysis of the data for lesion scores (originally only descriptive statistics were presented in the study).

The analysis showed that at Day 21, the proportion of treatment success based on lesion scores were 97.8% and 96.0% in the gamithromycin and tilmicosin group, respectively. However, despite the parameter being analysed using the same binomial variable (success/failure) as lameness scores, it is noted that the analysis of lesion scores was presented differently to that for lameness scores, which prevents direct comparison of the analyses for each of the efficacy parameters.

Nevertheless, the CVMP accepted that some evidence has been provided to suggest that the lameness scores are indicative of lesion scores and that lameness appears to be the more useful parameter for both diagnosis and assessing effectiveness under field conditions.

However, the Committee agreed that the indications should not refer to the foot lesions as initially proposed by the applicant ("Treatment of foot lesions and lameness associated with *D. nodosus* and *F. necrophorum* in sheep requiring systemic antimicrobial treatment such as severe foot rot"), but that the indications should be modified to reflect the data provided, and agreed on the following indication: "Treatment of infectious pododermatitis (foot rot) associated with virulent *Dichelobacter nodosus* and *Fusobacterium necrophorum* requiring systemic treatment."

No adverse experiences occurred during the study that was considered to be related to treatment. It is noted that a number of animals that were administered with gamithromycin were reported to have had pain at the injection site. Further, injection site reactions were reported to have lasted for up to 4 days in this study. These findings are reflected in section 4.6 of the SPC.

Overall conclusion on efficacy

Pharmacodynamics:

Gamithromycin showed higher activity *in vitro* to *D. nodosus* than *F. necrophorum* with MIC values of gamithromycin equal to 0.008 or 0.016 µg/ml. For *F. necrophorum*, 27 out of 30 strains had MIC values of 1 to 32 µg/ml, with MIC₉₀ of 32 µg/ml and MIC₅₀ of 2 µg/ml.

Resistance development:

The product is not expected to pose a public or animal health risk in regard to resistance development, when used as proposed in the SPC and other product literature.

Pharmacokinetics:

Gamithromycin is rapidly absorbed and distributed to the skin after subcutaneous administration of 6 mg/kg bw to sheep. It is eliminated slowly with a plasma half-life of approximately 46 hours.

Target animal tolerance:

Gamithromycin is well tolerated in sheep although subcutaneous administration causes mild tissue reactions resolving within a number of days. Tolerance has been tested at up to 5 times the recommended dose without adverse reactions, apart from local tissue reaction at the injection site.

Dose determination/justification:

The applicant has presented one dose-titration study in naturally infected sheep. The dose titration study showed that gamithromycin is effective in reducing lameness score and foot rot lesion score compared to a negative control group (saline). Gamithromycin administered at 3 and 6 mg/kg bw resulted in a statistically significant reduction in lameness and lesion scores measured 21 days after single treatment administration compared to a negative control group, but there were no statistical difference between the doses. However, a higher proportion of animals administered 6 mg gamithromycin/kg were observed to have a reduction in lameness and lesion scores providing sufficient evidence to support carrying forward the higher dose rate of 6 mg/kg for further investigation of effectiveness under field conditions of use.

Clinical study:

The applicant has presented one multicentre field trial, where the efficacy of ZACTRAN at the recommended dose was compared with tilmicosin. Treatment success was high in both treatment groups and gamithromycin was shown to be non-inferior to tilmicosin for the primary outcome parameter treatment success (based on reduction in lameness scores).

Part 5 – Benefit-risk assessment

Introduction

ZACTRAN (active substance gamithromycin) 150 mg/ml solution for injection is already authorised in cattle (since 2008) and pigs (since 2016) to treat respiratory diseases. This extension application is to add a new target species (sheep) to the existing product. The agreed indication in sheep is "treatment of infectious pododermatitis (foot rot) associated with virulent *Dichelobacter nodosus* and *Fusobacterium necrophorum* requiring systemic treatment". The route of administration of subcutaneous use for a single dose of 6 mg/kg bw. The withdrawal period for sheep meat and offal is 29 days. ZACTRAN is not authorised for use in lactating animals producing milk for human consumption.

The dossier has been submitted in accordance with Article 19 of Commission Regulation (EC) 1234/2008 and Annex I point 3, change or addition of target species, thereof (extensions).

Benefit assessment

Direct therapeutic benefit

The benefit of ZACTRAN in sheep is its efficacy in the treatment of infectious pododermatitis (foot rot) associated with virulent *Dichelobacter nodosus* and *Fusobacterium necrophorum* requiring systemic treatment, which was investigated in one GCP-compliant multicentre controlled clinical field study. This disease is of serious animal welfare concern. Efficacy of a single subcutaneous injection of 6 mg/kg bw was demonstrated in a multicentre European field study.

Additional benefits

ZACTRAN would increase the range of available treatment possibilities for foot rot in sheep.

Risk assessment

Quality:

The strength and pharmaceutical form remain as authorised, and cross-reference is made to data that have already been submitted and assessed as satisfactory for the product.

Safety:

Risks for the target animal:

ZACTRAN is well tolerated in sheep. Local signs of discomfort were observed following subcutaneous injection, which were mild and transient in nature.

Risk for the user:

User safety risks have been identified, mainly concerning the risks associated with skin and eye irritation. These risks have been appropriately addressed by safety warnings in the SPC.

Risk for the environment:

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

Risk for the consumer:

A withdrawal period of 29 days for sheep meat and offal was established. ZACTRAN is not authorised for use in animals producing milk for human consumption.

Emergence of antimicrobial resistance:

The product is not expected to pose a public or animal health risk in regard to resistance development, when used as proposed in the SPC and other product literature.

Risk management or mitigation measures

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks. The re-start of the PSUR cycle is considered

appropriate to ensure more frequent pharmacovigilance monitoring since a new target species is added. The data lock point (DLP) for the first 6-monthly PSUR of the re-started cycle would be 31 July 2017.

Evaluation of the benefit-risk balance

The product has been shown to be efficacious in the proposed indication in the new target species, sheep ("Treatment of infectious pododermatitis (foot rot) associated with virulent *Dichelobacter nodosus* and *Fusobacterium necrophorum* requiring systemic treatment").

The pharmaceutical form is already in use for other species. ZACTRAN is well tolerated in the new target species and presents an acceptable risk for users and the environment and consumers, when used as recommended. Appropriate precautionary measures, including withdrawal period, have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for ZACTRAN 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 extension of the marketing authorisation for the new target species, sheep.