8 October 2015
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Imrestor (EMEA/V/C/002763/0000)

International non-proprietary name: pegbovigrastim

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

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Introduction

On 29 August 2014, the applicant Eli Lilly and Company Limited submitted an application for a marketing authorisation to the European Medicines Agency (The Agency) for Imrestor, through the centralised procedure falling within the Article 3(2)a of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP on 14 June 2012 as Imrestor contains a new active substance pegbovigrastim, which is not yet authorised as a veterinary medicinal product in the Community.

The rapporteur appointed is C. Friis and co-rapporteur is S. Louet.

The applicant initially applied for the following indication: To assist in the restoration of immune function by increasing the activity and number of circulating neutrophils as an aid in reduction of the incidence of disease e.g. clinical mastitis in periparturient dairy cows and heifers. The product is intended for use as a part of a health management strategy.

Imrestor is a solution for injection for subcutaneous use in cattle, containing pegbovigrastim as the active substance (pegylated recombinant bovine granulocyte colony stimulating factor, PEG-bGCSF). Bovine granulocyte colony stimulating factor is a naturally occurring protein produced by cattle, which is responsible for increasing the numbers of neutrophils produced by the bone marrow. The product is intended to restore normal neutrophil function to cows during the periparturient period, thus reducing their susceptibility to clinical mastitis infections.

It is presented in pre-filled syringes (10, 50 or 100 per pack). The proposed withdrawal period is zero days (milk, meat and offal). The product is indicated for use as an aid in the reduction of the risk of clinical mastitis in periparturient dairy cows and heifers during the 30 days following calving.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC.

On 8 October 2015, the CVMP adopted an opinion and CVMP assessment report.

On 9 December 2015, the European Commission adopted a Commission Decision granting the marketing authorisation for Imrestor.

Scientific advice

The applicant received scientific advice from the CVMP on 16 June 2010 on efficacy and on 13 December 2012 on quality, safety and efficacy.

In general the scientific advice given was followed in the dossier submitted. In the scientific advice(s) the CVMP defined the clinical signs associated with clinical mastitis.

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 (dated September 2013) 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 Union or in a third country.

Manufacturing authorisations and inspection status

The active substance is manufactured by Eli Lilly and Company Ltd. Speke Operations, Liverpool, UK.

The finished product is manufactured and packed in the European Economic Area (EEA) Batch release for the EU will be carried out by Eli Lilly and Company Ltd. Speke Operations, Liverpool, UK.

A declaration signed by the qualified person (QP) is provided for the active substance, which confirms that the active substance is manufactured in line with GMP requirements. All relevant sites have valid manufacturing authorisations or valid GMP certificates as appropriate. The inspection of two sites where an intermediate of the active substance is manufactured was recommended. The first site was inspected in March 2015 and the second site was inspected in February 2015 and both are GMP compliant.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system is considered in accordance with legal requirements.

The GMP status and manufacturing authorisation for both the active substance and the finished product manufacturing sites have been satisfactorily established and are in accordance with legal requirements.

Part 2 - Quality

Composition

Imrestor is a 15 mg solution for injection presented in a 2.7 ml single dose pre-filled syringe.

The product contains pegbovigrastim as active substance and the following excipients: citric acid monohydrate, arginine hydrochloride, arginine and water for injections.

Container

Active substance

Pegbovigrastim is filled into a 12 litre multi-layer co-extruded polymeric 2D bioprocess bag.

Final product

The final product is filled into single-use 3.0 ml polypropylene syringes with a chlorobutyl piston/stopper.

Studies on extractables and leachables and in addition information regarding sterilisation of the packaging material has been provided and deemed acceptable.

Development pharmaceutics

Active substance

Manufacturing development for the active substance is outlined in sufficient detail. A number of changes have been introduced to the manufacturing process over time, including new manufacturing sites. Analytical data support the comparability of the active substance manufactured over the time of the process development and manufacturing sites. Further, the data presented support that the active substance manufactured over time, and used in clinical trials, is comparable to that to be launched on the market. Characterisation and analytical data were also presented for field batches.

Quality by Design (QbD)

QbD tools have been used for the characterisation of the active substance manufacturing process. As recommended by the relevant guidelines addressing QbD issues (ICH Q8, ICH Q9), a Quality Target Product Profile (QTPP) was established first for the product. The QTPP gives the quality characteristics of the product, which will ensure that the desired quality of Imrestor is achieved. The next step for the QbD development was designation of Quality Attributes as Critical (CQA) or, non-Critical Quality Attributes (QA). A CQA is defined as a physical, chemical, biological or microbiological property, or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality. Quality attributes of bGCSF and PEG-bGCSF have been evaluated for their known or possible impact on safety, efficacy, PK/PD and/or immunogenicity. This approach was acceptable and in most aspects in line with the ICH recommendations. CQA designation has been discussed extensively.

After designation of quality attributes as critical or not, risk-assessment tools were used to define those process parameters, which may have an impact on the CQAs identified. Overall, acceptable and relevant critical process parameters (CPPs) have been identified by relevant and acceptable Design of Experiments (DoEs). The DoEs and the statistics applied for the DoE evaluation are acceptable. Based on the DoEs, Design Spaces have been claimed, and have been accepted, for some steps in the manufacturing process of the active substance.. The design space constitutes both critical and non-critical process parameters.

The consequences/action taken have been outlined, if the acceptable range of a CPP or non-CPP, which is part of the Design Space, is exceeded. The consequences/action taken have also been outlined for CPPs or non-CPPs not part of the Design Space.

Final product

The rationale for the choice of formulation and container closure system as well as the development of the manufacturing process is considered properly described.

No Design Space or statistically designed experiments were applied in the product manufacturing process, but QbD concepts linking the Target Product Profile to the manufacturing design, process controls, specifications and process validation were maintained.

Method of manufacture

Active substance manufacturing process

The active substance is produced in *E. coli* cells by recombinant DNA technology. The manufacturing process begins with a fermentation of the *E. coli* cells where the protein accumulates intracellularly as inclusion bodies. The inclusion bodies are separated and stored frozen until the downstream purification is initiated.

The downstream process consists of a refolding step followed by clarification, CM chromatography, and diafiltration. The protein is then pegylated and purified by cation exchange chromatography. The pegylated protein is diafiltered, passed through a bioburden reduction filter and filled into 12 L bags and stored frozen.

The active substance manufacturing process is considered properly described. Details on holding times were presented.

Active substance process validation

Two process validations were conducted. The aim of the first validation was to validate "*several process parameters at the lower end of the established "Design Space" ranges (=1.0 process validation)*. The second validation focused on the "higher end of the established Design Space").

All release specifications were met, all CPPs were within their acceptable ranges, the intermediate specification for pegbovigrastim were met and acceptable removal of host cell proteins (HCP), DNA and bioburden was demonstrated. The level of CQAs (aggregation, oxidation, carbamylation and disulfide bond scrambling, acetyl hydrazide, residual solvent and bioactivity) during the manufacturing process for the validation batches were tested and shown to be within acceptable/historical ranges. Based on this, the active substance manufacturing process is considered well validated and with demonstrated capability of robust and consistent production.

Product manufacturing process

The manufacturing process is initiated with preparation of the formulation buffer solution. The active substance is thawed and added to the formulation buffer. The mixture is passed through a bioburden reduction filter, sterile dual filtered and filled into syringes.

The proposed in-process controls are considered fully justified.

Product process validation

Process validation including media fill validation has been performed successfully and is considered properly described.

Control of starting materials

Active substance

The active substance is pegbovigrastim - pegylated bovine granulocyte colony stimulating factor (PEG-bGCSF).

Results of physicochemical and biological characterisation as well as an evaluation of potential impurities are found acceptable.

The proposed active substance specification includes relevant parameters such as, identity, content, purity, and physicochemical properties in accordance with VICH GL40 (Test procedures and acceptance criteria for new biotechnological/biological veterinary medicinal products). The analytical methods have been properly described and relevant methods have been validated in accordance with VICH GL1 (Guideline on validation of analytical techniques).

Batch analysis data are provided for 14 full-scale batches tested according to the proposed commercial specification. All results are within specification.

A 33 month shelf-life for the active substance when stored at -20 °C±5 ºC was proposed which is supported by the stability data provided.

The source, history, and generation of the cell substrate have been properly described. The master cell bank and working cell bank is tested in accordance with ICH Q5D (Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products).

Excipients

All excipients are of European pharmacopoeial quality.

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

No materials on animal origin were used during the establishment of the cell banks or as components of the active substance or product manufacturing process. This medicinal product is therefore not expected to present any risk of transmitting TSE or viral contamination. The product complies with 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).

Control tests during production

Active substance and final product

Relevant in-process controls have been established and the process validation studies demonstrated compliance with the acceptance criteria.

Control tests on the finished product

The final product release and shelf-life specifications include relevant parameters such as identity, purity, content and potency. In addition, relevant parameters related to the pharmaceutical form (solution for injection), such as sterility, endotoxins, particulate matter, pH and osmolality, have been included. The specifications of the finished product are acceptable.

The potency assay is based on the proliferation of mouse-derived cells.

The analytical methods have been properly described and validated.

Batch analysis data for three primary stability batches and four developmental and clinical batches demonstrate compliance with the specification.

Stability

The proposed shelf-life for the product is 24 months when stored at 2–8 °C. This is supported by long-term data and is acceptable.

Post Approval Change Management Protocols

Three post approval change management protocols (PACMPs) have been included in the submission concerning the following changes:

- New site for the formulation and filling of the product
- Active Substance Scale Up
- Expansion of the Active Substance Manufacturing Facility

The PACMPs are acceptable.

Overall conclusions on quality

A Quality by Design approach has been applied to the quality documentation for this product. The active substance and excipients meet their current respective Ph. Eur. requirements.

The proposed finished product specifications, both at release and end of shelf life, are acceptable.

The proposed shelf life of 24 months when stored at 2–8 °C is accepted.

Three post approval change management protocols have been accepted.

Information on the development, manufacture and control of the active substance and the finished product has been presented in a satisfactory manner. The results of tests carried out indicate the 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.

Part 3 – Safety

Pegbovigrastim is a glycoprotein cytokine which stimulates the production of neutrophils in bone marrow and increases the numbers of circulating neutrophils. Secondary activities are limited due to specificity of binding sites and such effects noted in cattle are unlikely to be relevant to human safety assessment. Like many proteins such as insulin it is not active orally as it is subject to the normal digestive processes in the gastrointestinal tract. Studies in rats confirmed that it is not orally bioavailable and therefore devoid of pharmacodynamic activity after oral administration.

The safety of pegbovigrastim has been reviewed by CVMP in the context of the application regarding the establishment of MRLs and as a result, a "No MRL required" status was recommended for the substance. Pegbovigrastim was entered into Table 1 of the Annex to Regulation (EC) No 37/2010 in 2012 and a summary of the safety data provided is described in the EPMAR (EMA/CVMP/190918/2010). No further safety studies except tolerance in the target species (reported in part 4) have been provided in this application.

Pharmacodynamics

Pegbovigrastim is a modified form of the naturally occurring immunoregulatory cytokine, bovine granulocyte colony stimulating factor, which is a naturally occurring protein produced by mononuclear leukocytes, endothelial cells and fibroblasts. Colony stimulating factors regulate the production and functional activities of immune cells. The immunoregulatory activities of granulocyte colony stimulating factor concerns notably cells of the neutrophilic granulocyte lineage which bear cell surface receptors for the protein.

Well conducted pharmacological studies were presented. These studies show that the administration of pegbovigrastim to cows at a dose of 20 µg/kg bw specifically increases the absolute neutrophil counts. In addition to increasing the numbers of circulating neutrophils, pegbovigrastim activates the function of

mature neutrophils for example; activated neutrophils exhibit enhanced myeloperoxidase hydrogen peroxide halide mediated microbiocidal capabilities, but pegbovigrastim does not appear to improve other neutrophil functions as phagocytosis, neutrophil extracellular trap formation and oxidative burst.

Pegbovigrastim presents additional functions beyond its action on neutrophils and these may be direct or indirect functions on other cells/receptors and cytokine pathways.

Pharmacokinetics

In humans, recombinant human GCSF is cleared from the circulation partly by the kidneys, and partly by binding to specific receptors and is likely to follow the same pattern in cattle. Pegbovigrastim is subjected to the normal digestive processes in the gastrointestinal tract and studies in rats confirmed that it is not orally bioavailable.

Attempts to develop an antibody assay to evaluate the elimination rate of pegbovigrastim in the target species were unsuccessful. However, as pegbovigrastim was specifically designed to be as similar to the naturally-occurring cytokine as possible, the CVMP agreed that it would be difficult to design an assay intended to assess the amount of iatrogenically introduced bGCSF in cattle. Therefore no pharmacokinetic data for cattle are available, which is acceptable.

Toxicology

There are no conventional toxicology studies available with pegbovigrastim, which is acceptable in view of the poor oral bioavailability and gastrointestinal deactivation through digestion. Studies in rats dosed 10 to 2500 µg/kg bw either orally or subcutaneously, have not revealed any abnormal clinical effects.

There are no data available on genotoxicity and carcinogenicity but as a protein closely related in structure to the endogenous substance, genotoxic or carcinogenic properties would not be predicted. The product was not a skin or eye irritant when tested in rabbits.

Immunogenicity

bGCSF is an endogenous cytokine constantly produced by bovines (hence unlikely to be immunogenic), and the polyethylene glycol (PEG) polymer chains are not expected to be immunogenic either. Thus, although the immunogenic potential of pegbovigrastim cannot formally be ruled out, its occurrence (if any) appears unlikely and of negligible impact in current practice.

Target animal safety

See part 4.

User safety

A user risk assessment has been provided taking into account the CVMP Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1).

The users identified are the farmers and the veterinarians.

The most relevant exposure scenarios are considered to be skin and eye contact resulting from spillage during opening of the packaging and accidental self-injection.

Skin and eye irritation following spillage are not considered to represent a serious concern as the product has been tested for its skin and eye irritation properties and was concluded not to be irritant. Furthermore, the dose that might be received would be very low. However, the possibility of hypersensitivity reactions following dermal contact cannot be ruled out as no studies to test for potential hypersensitivity following dermal contact have been performed. A recommendation to wear gloves during administration is included in the product literature and this is considered appropriate.

Accidental self-injection of 1 ml would be possible. A number of adverse effects have been reported following administration of granulocyte colony stimulating factor products used in human medicine, including bone and musculoskeletal pain, as well as hypersensitivity-type reactions, including skin rash, urticaria, angioedema, dyspnoea, erythema, flushing, and hypotension. Self-administration of Imrestor in human beings may cause symptoms similar to those reported for other GCSF products and injection of 1 ml dose volume of the drug would result in a dose of 5.56 mg of pegbovigrastim or 92.7 µg/kg for a 60 kg adult. This dose is at the same level as the therapeutic dose of the human product pegfilgrastim. Studies in cattle have shown that they respond equally to human recombinant-GCSF treatment as to bGCSF and the different preparations must therefore be regarded as equipotent, at least in terms of the neutrophil and leukocyte responses. The warning included in the SPC adequately manages the risks posed by human exposure to the bGCSF molecule.

Environmental risk assessment

The Predicted Environmental Concentration for soil was calculated in accordance with VICH GL6 and the CVMP guideline on the Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1). The maximum predicted environmental concentration in soil was 0.227 µg/kg assuming total excretion of the active substance as the parent compound. This predicted level occurred following simultaneous total herd treatment, with all of the active substance being excreted during the housed period, and assuming that animals are only housed for 50% of the year. Based on the data provided the ERA can stop at Phase I. Imrestor is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

There are no data available on genotoxicity and carcinogenicity but as a protein closely related in structure to the endogenous substance, genotoxic or carcinogenic properties would not be predicted. The product was not a skin or eye irritant when tested in rabbits.

The immunogenic potential of pegbovigrastim appears unlikely and of negligible impact in current practice.

Target animal safety is addressed in part 4.

A user safety risk assessment has been provided in which self-injection is identified as the most relevant user safety concern. Self-injection of 1 ml of Imrestor will lead to a dose of pegbovigrastim at the same level as the therapeutic dose of the human product pegfilgrastim. Adverse effects have been reported following administration of granulocyte colony stimulating factor products used in human medicine, including bone and musculoskeletal pain, as well as hypersensitivity-type reactions, including skin rash, urticaria, angioedema, dyspnoea, erythema, flushing, and hypotension. Self-administration of Imrestor in human beings may cause symptoms similar to those reported for other similar type products.

When used at the recommended dose and according to the SPC Imrestor is unlikely to pose a risk to the environment.

Residues documentation

Residue studies

Pegbovigrastim is not bioavailable after oral administration and consumer exposure as a result of exposure to residues in food of animal origin is therefore not relevant. Any residues present, along with any endogenous GCSF and indeed any other proteins, will be subject to denaturation by acids and digestion by enzymes in the human gastrointestinal tract.

MRLs

The active substance in Imrestor 15 mg solution for injection for cattle is an allowed substance as described in table 1 of the annex to Commission Regulation (EU) No 37/2010:

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions	Therapeutic classification
Pegylated bovine granulocyte colony stimulating factor	NOT APPLICABLE	Bovine	No MRL required	NOT APPLICABLE	NO ENTRY	Biological/ Immunomodulator

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.

Withdrawal periods

A withdrawal period is not required to ensure consumer safety. A zero day withdrawal period is established.

Overall conclusions on the residues documentation

Pegbovigrastim is not bioavailable after oral administration. Consumer exposure as a result of exposure to residues in food of animal origin does not represent a concern. A zero day withdrawal period is established.

Part 4 – Efficacy

Pharmacodynamics

Pegbovigrastim pharmacodynamics properties have been described in part 3 safety.

GCSF factors have a number of pharmacodynamic activities the most important of which is the induction of the production of neutrophils by the bone marrow stem cells with activation of part of their functional capabilities. The functionality of neutrophils in dairy cattle, including random migration and antibody-dependent cell-mediated toxicity, has been shown to decline significantly in the periparturient period and to reach a major decline in the first week after calving. An increase in neutrophils then may play a role in defense against infection by pathogenic organisms.

Development of resistance

Not applicable.

Pharmacokinetics

See part 3.

Dose determination/justification

Dose determination

Three laboratory studies were carried out in calves and cows to find a dose and a regimen which lead to a rise in absolute neutrophil count (ANC). All studies were well conducted and GCP accredited. Based on the pharmacodynamics properties the applicant selected the absolute neutrophil counts (ANC) as the primary end-point. Single doses between 40 and 160 µg pegbovigrastim/kg bw (subcutaneously) were tested and a clear dose-effect relationship could be demonstrated.

Dose confirmation

The increased neutrophil counts alone were deemed insufficient evidence to take any one dose forward into field trials. Therefore a programme of studies was conducted to investigate the clinical effect of reduction in mastitis rates.

Two studies were conducted with *E. coli* induced infections and three further studies with naturally infected cows.

The two controlled studies were performed using *E. coli* inoculation into healthy quarters of early lactating dairy cows and ANC and any change in morbidity as a result of infection were determined as outcomes of the studies. In one of the studies cows were treated at day -7 (prior to calving) with a single dose of 4–35 µg pegbovigrastim/kg. In the second study 80 and 160 µg pegbovigrastim/kg were administered followed by a second dose at Day 3 after calving. At Day 5 after calving each cow was challenged with an intramammary infusion of 2 ml of an inoculum containing 30 cfu/ml *E. coli* into the right front quarter. Animals were monitored at morning and evening milking until Day 10 after calving. Pegbovigrastim increased ANC as expected, but there was no significant difference in morbidity of *E. coli* infection between the pegbovigrastim and control groups. From these studies it was concluded that a direct intramammary inoculation of *E. coli* was a too rigorous test for a product, which had no direct antibacterial action, but whose activity was achieved by increasing the numbers of circulating neutrophils.

Three further well-conducted GCP-compliant dose titration studies were conducted to evaluate the efficacy of pegbovigrastim against naturally occurring mastitis in periparturient dairy cows, administered either as a single dose of 20 µg/kg at 7 days prior to anticipated calving date in one of the studies or at doses of 20 or 40 µg/kg at 7 days prior to calving followed by a second dose within 24 hours of calving in the other 2 studies. Clinical mastitis morbidity rates per cow were the primary efficacy parameter evaluated. Clinical mastitis was defined as a clinical status score ≥2 ("abnormal quarter and/or questionable milk") in combination with a California Mastitis Test (CMT) score greater than trace. This resulted in identification of mastitis cases with a range of clinical severity.

A significant reduction in acute clinical mastitis in the first 30 days after calving could be demonstrated when administering pegbovigrastim at two doses of 10 to 40 µg/kg bw where the first dose was administered approximately 7 days before calving and the second within 24 hours after calving. However,

a significant reduction in clinical mastitis was not demonstrated where the animals received a single dose of 20 µg/kg bw within 24 hours after calving only.

On the basis of these results the dose range selected for progression to field trials was 20–40 µg/kg to be administered by single volume injection on 2 occasions, one approximately 7 days prior to the anticipated date of calving and within 24 hours after calving.

This rationale was considered acceptable by the CVMP.

Target animal tolerance

Two target animal safety (TAS) studies were provided.

The first, blinded, controlled study was conducted according to the requirements of VICH GL43 to evaluate the safety of Imrestor administered subcutaneously to periparturient Jersey cows and heifers (approximately 450 kg bw). Animals were treated with 1x, 2x, and 3x the recommended therapeutic dose (RTD) of one syringe (15 mg pegbovigrastim - 2.7 ml) at three time points to simulate an overdose (7 and 3 days prior to anticipated calving date and again within 24 hours after calving), i.e. in excess of the recommended number of treatments of two (D-7 and within 24 hours of calving). Data was collected on 8 animals for each of the 4 treatment groups (dosing was 1, 2 or 3 syringes of Imrestor at each of the time points; control animals were given three syringes of saline per time point). Animals were euthanised 4 days postpartum. Calves were evaluated for 14 days. Data were collected during a pretreatment period prior to treatment to establish baseline values, and at each of the 3 treatment time points. Physical examinations were conducted prior to each treatment time point and just prior to euthanasia. Clinical pathology parameters were measured at selected time points, as well as separate composite colostrum and milk samples collected for proteomic and genomic analyses.

During the target animal safety studies there were no adverse effects on feed intake, water consumption, and vital signs (including rectal temperature, respiratory rate, heart rate, mucous membrane colour and capillary refill times). Furthermore, pegbovigrastim had no effect on serum chemistry or urinalysis test results.

Post-mortem results indicated an increase in spleen weights and spleen to body weight ratios; which would be expected of a compound that increases neutrophil production as the spleen is a site of extramedullary granulocyte or neutrophil production as well as accumulation of aged neutrophils for removal from the blood stream. In addition, extramedullary production of granulocytes was detected in lymph nodes and the mammary gland without any apparent clinical effects on the cattle. Hematology results demonstrated a leukocytosis attributable to a relative and absolute neutrophilia, entirely consistent with the mechanism of action of pegbovigrastim.

At the doses tested, there were no detectable hematology changes. At the higher doses (2x and 3x RTD), a trend toward a greater number of animals and/or greater severity for mammary gland inflammation (mastitis), uterine inflammation (metritis), and abomasal ulcers and/or erosions was noted. Abomasal erosions and ulcer incidence was significantly increased at 2–3x RTD (60 µg/kg) administered three times (i.e. one more administration than the proposed posology). The applicant considered these findings as a result of stress associated with the design and implementation of this study such as transportation, change of diet, etc. and these factors were responsible for the abnormally increased incidence of these periparturient diseases. Hence, definitive conclusions on abomasal findings being associated with treatment with Imrestor were not possible from this study.

In order to create a less stressful environment for the animals a second TAS study was conducted at a commercial dairy facility. Pegbovigrastim was administered subcutaneously to periparturient multiparous

Holstein cattle (9 cows per treatment group) at doses of 1x, 2x, 2.5x or 3x RTD (1x = 15 mg of pegbovigrastim/cow) for a total of two dosages (i.e. as the recommended number of dosages). Animals in this study were heavier than in the first study; at enrolment the cows weighed on average 680 kg (instead of 450 kg as in the first TAS study). Hence, the doses per kg were lower in the second study than in the first study. Furthermore no heifers were included.

Test article related findings were limited to increased production of neutrophils. No test article-related gross or histopathologic findings were found. Alterations noted in the pyloric region of the abomasum, including acute erosions and inflammation, occurred with similar incidence and negligible severity across treated and control groups (i.e. minimal and mild). No abomasal ulcers were reported in any of the treatment or control groups. Alterations were noted in the descending colon (red areas), urinary bladder (haemorrhage and edema), uterus (foul smell/off odour), cervix (haemorrhage), and vagina (bruise and altered contents), but were not considered test article-related.

There was no difference in milk production, food consumption, incidence of transition disease or non pegbovigrastim-related clinical pathology variables between treated and control groups. At a dose of 2-3 x RTD (70 µg/kg), a slight decrease of the bodyweights of the treated animals was observed (non-significant). Cows were not blocked by bodyweight when assigned to treatment groups. Cows were weighed one time each, on days -21, -7, 0, 7 and 14, with the primary differences in bodyweight occurring during the postpartum period. The applicant assumes that the effects of rumen fill as well as parturition may have contributed to the observed differences, which may be plausible.

Pharmacovigilance data from the use of the product during clinical studies suggests that a non-typical anaphylactoid Type 1 or immediate type hypersensitivity-like reaction may occur in some treated cows. The incidence was less than 1%. The reaction was manifested as swelling of mucous membranes, skin reactions, hypersalivation, and increased respiratory rate. One animal became recumbent and was reported as sweating profusely. Typically, Type 1 hypersensitivity reactions involve an initial sensitising exposure and a subsequent reaction on all following exposures. However, in these instances, the reaction occurred on the first exposure suggesting that a Type 1 reaction was either not present, or the effect was a Type 1 reaction to an environmental antigen. The reactions were not seen in all geographic regions where studies were conducted and other factors may have included handling stress, dust, pollen and dietary factors.

In the TAS and clinical trials, it was noted that the subcutaneous injection may induce transient local swellings at the injection site, as well as inflammatory reactions. These reactions usually resolved within 14 days post-treatment.

Conclusion on target animal safety studies

In one safety study in Jersey cows, at overdose of 60 µg/kg, administered on three occasions (1.5x the highest recommended dose), abomasal ulcers were observed. In a second safety study in a Holstein dairy cow population test article related findings were limited to increased production of neutrophils. There were no differences in milk production, dry matter intake, or clinical pathology variables, as well as no gross or microscopic test article-related tissue alterations. No safety concerns were reported when pegbovigrastim was administered subcutaneously on day -7 relative to anticipated calving date and on day 0 within 24 hours after calving at up to 3x the recommended dose level in periparturient dairy cows.

In the efficacy studies in dairy cattle, apart from the effects normally associated with dairy cattle e.g. metritis and lameness, non-typical anaphylactoid type reactions were uncommonly (0.7% of incidence) observed. Similar reactions have been reported after use in the field, through pharmacovigilance.

Injection of Imrestor may induce transient local swellings at the injection site, as well as inflammatory reactions.

Field trials

The applicant provided two pivotal GCP-compliant European field studies, supported by four GCP-compliant field trials carried out in other regions, i.e. New Zealand, Australia and the US.

For all these trials, the same study protocol was used with some exceptions depending on local geography and policy. Good Clinical Practice training was provided to all parties participating in the trials. One study conducted in Germany in 2012 was not conducted according to GCP and with a number of deviations from the protocol. Data was not included in the analysis.

The product was used in addition to usual treatments at drying off.

The concept was to assess the efficacy and clinical safety of Imrestor, by examining the incidence of clinical mastitis in the first 30 days after calving as the primary parameter for assessing efficacy. The CVMP noted however, that the definition of clinical mastitis differed between trials, in particular considering the complementary examinations undertaken in the different regions - California mastitis test (CMT) and somatic cell counts (SCC).

In most of the field studies the definition of a clinical mastitis was "abnormal quarter (clinical signs in the quarter such as swelling, heat, pain) and/or abnormal milk (clots or flakes, watery appearance, discoloration) with or without general signs (fever, loss of appetite)". In the second European pivotal study, abnormal milk appearance was also investigated and in case of this, the case was considered as a clinical mastitis only if SCC > 200,000 cells/ml. The bacteriological testing made on all morbid quarters was not taken into account to classify a case as clinical mastitis. In 48% of quarters no major mastitis pathogen was isolated from clinical milk samples taken at the time of clinical signs of mastitis. Detection of mastitis was determined by farm and trial personnel at the time of milking.

All cows received one subcutaneous injection of Imrestor or saline control at -7 days before anticipated calving date and within 24 hours of calving. The interval between dosages was in median 8 days. The actual dose rate of Imrestor varied due to variability in size of animals in the field. The dose range was 20–40 µg pegbovigrastim/kg of body weight. To determine clinical safety, milk quality (fat/protein concentration), milk production (yield), gestation length, percentage of live birth of calves, calf health and first service pregnancy rate, data was also collected where possible (in some regions, these data were not adequately recorded on farm sites and therefore not always available). All adverse events were recorded.

First EU study (France, Spain, UK)

The first EU study was carried out on multiple sites in France, Spain and the UK and investigated the effectiveness and clinical safety of 20–40 µg pegbovigrastim/kg bw against naturally occurring clinical mastitis and subclinical mastitis in 486 periparturient cows and heifers under field conditions.

Imrestor or a control was administered by subcutaneous injection at two occasions, at the estimated day minus 7 prior to calving and a second dose on the day of calving (within 24 hours of birth). Animals included were mainly adult dairy cows (75% approx) and heifers (25% approx) both large frame, Holstein Friesian and small frame, Jersey types, weighing from 350–700 kg. Accordingly, one dose/pre-filled syringe of 2.7 ml (15 mg) equated to approximately 20–40 µg/kg bw. The control product was 0.9% NaCl - sterile saline. Animals eligible for inclusion in the study comprised periparturient cows/heifers with a normal clinical presentation at the time of the physical examination, and without other medications prior to enrolment except dry cow intramammary treatments and teat sealants. Animals which were treated with any anti-infectious or anti-inflammatory product by systemic or intramammary route in the previous

30 days were excluded. Primary endpoint was the incidence of clinical or subclinical mastitis and clinical safety during the first 30 days after calving.

Clinical mastitis was identified if one or more quarters met the morbidity criteria (abnormal milk and/ or abnormal quarter and CMT≥2) at any milking time point during Days 3–30 of lactation; subclinical mastitis was identified if one or more quarters exhibited a CMT= trace or 1, and if the same bacterial pathogen(s) was isolated at two of three days. Secondary end-points included absolute leukocyte counts microbiology on milk from mastitis cases. Clinical safety (not included in all study sites) was determined by assessment of milk composition, milk production (yield), length of gestation, percentage of live births, health observations in calves and first service/conception rates in dams.

Although the results indicated a reduction in the incidence of clinical mastitis in Imrestor-treated cows (13.2%; i.e. 32 mastitis cases out of 242 cows) compared to the control group (15.3%; i.e. 37 mastitis cases out of 242 cows) this effect was not statistically significant. The applicant considered that this statistically not significant effect was due to the overall low mastitis incidence in this trial.

Second EU study (UK, Germany, The Netherlands, Hungary)

The second EU trial was carried out as a combination of two studies conducted over 2 years, pooling the two studies in order to comprise one Final Study Report to ensure sufficient statistical power. The trial involved 35 farm sites in UK, Germany, The Netherlands and Hungary using 2465 cows across treatment and control groups. The objective of the study was to evaluate the effectiveness of pegbovigrastim against naturally occurring mastitis in periparturient cows and heifers. The study protocol was similar to the first EU study (see above). However, the primary criteria was "abnormal milk and/or abnormal quarter" with no CMT. Somatic cell counts (SSC) were obtained from cows with no signs of clinical mastits, only from the UK sites at 10 and 30 days. In Germany, Hungary and The Netherlands, SCC was only determined on the cows with questionable clinical parameters recorded after the calving date in order to support a mastitis diagnosis.

The results showed a statistically significant difference in the incidence of clinical mastitis. The original analysis including cows with questionable milk samples and negative bacteriology but SCC >200,000 cells/ml as mastitis cases resulted in a p-value of 0.0095 with 12.7% of mastitis cases in the control group and 9.4% in the Imrestor group. Classification of the cows with questionable milk samples with SCC >200,000 cells/ml and a negative bacteriology as not mastitic, resulted in a p-value of 0.0094 with 12.4% of mastitis cases in the control group (152 out of 1230 cows) and 9.1% in the Imrestor group (113 out of 1235 cows).

The results describe a relative reduction in mastitis incidence of 26% (p=0.0094). Both Gram-positive and Gram-negative bacteria isolated from positively identified mastitic milk samples were isolated. Milk samples from a total of 223 and 242 quarters, respectively, in the treated and the control group were tested. 46% (102/223) of morbid quarters/questionable milk were isolated with major pathogens in the treated group versus 60% (145/242) in the control group. Among the 247 isolated pathogens, 13% were coliforms, 44% were *streptococci* and 41% were *staphylococci*.

Due to the normal physiological changes and periparturient health conditions affecting cows through the period around calving, a number of abnormal health observations were noted in animals in both groups, including lameness, dystocia, milk fever and teat injuries, which were not considered treatment related. However, five adverse events observed 15 to 30 minutes after the first administration with Imrestor were indicative of a transient non-typical anaphylactoid type reaction and occurred at 0.4% incidence in treated cows.

Supporting (non EU) clinical trials

In addition to the European studies, the applicant provided the results of four clinical field trials, which were performed in the USA, Australia and New Zealand. While there may be differences in animal husbandry practices and clinical mastitis in these regions, there remain many similarities to the husbandry practices and mastitis conditions encountered in the EU. These data are submitted in support of the pivotal EU generated data discussed above. Study protocols similar to those employed in the EU were employed in all non-EU regions.

One study conducted in New Zealand comprised 526 cows from three farm sites. Treatment with Imrestor resulted in a numerical reduction of 23% in clinical mastitis in periparturient cows; no statistically significant differences in clinical mastitis incidence could be demonstrated between the Imrestor group (11.5% i.e. 30 out of 261) and the control group (15.4%, i.e. 39 out of 254). The applicant considered this was related to the overall low number of mastitis incidence in this study.

A second New Zealand study enrolled 1094 control and 1088 Imrestor treated animals over 6 sites. Treatment with Imrestor resulted in a significant reduction in clinical mastitis (21.6%) with 14.4% (152 cases out of 1052 cows) in the Imrestor group versus 18.3% (194 out of 1062 cows) in the control group. Eight (0.7%) cows showed hypersensitivity type reactions following the first administration of Imrestor.

In an Australian study, 144 control and 152 Imrestor treated animals were enrolled, all from a single farm. No significant differences could be seen between treatment and control group. The applicant considered this as a result of the overall low number of mastitis incidence in this study (approximately 16% in both groups).

Finally a study was carried out in four sites in the United States, enrolling 320 (80 per site) control and 320 (80 per site) Imrestor treated cows. Treatment with Imrestor resulted in a statistically significant reduction (18%, i.e. 48 out of 267) in the incidence of new clinical cases of mastitis relative to the control product (26.8%, i.e. 72 out of 269). The average percentage decrease was 33% (range 23–50% across the sites). No significant treatment effect was detected on subclinical mastitis. No significant effects on any safety parameters were detected. A statistically significant increase in absolute mature neutrophil counts in the peripheral blood relative to the controls was associated with the expected biological activity of Imrestor.

Conclusions:

Imrestor is a preventive product, with a mode of action which affects the innate immune system by increasing the number and myeloperoxidase hydrogen peroxide halide mediated microbiocidal capabilities of circulating neutrophils. All studies that were adequately powered to detect a difference between treatment and untreated control (EU (2014), also US, NZ (2013)) showed a statistically significant effect of treatment on reduction in incidence of clinical mastitis. At farm level there is no relationship between the rate of clinical mastitis in the control group and the level of efficacy of Imrestor demonstrated at that site. Based on all field studies, the proportion of mastitis prevented due to herd treatment with Imrestor (Prevented Fraction) is 0.25 (with 95% confidence interval 0.14–0.35).

The reduction in mastitis was variable across the field studies conducted, however an average reduction of 25% was seen across different geographical areas, breeds and farming sytems studied.

Overall conclusion on efficacy

Pharmacodynamics: Pegbovigrastim is a pegylated recombinant bovine granulocyte colony stimulating factor that acts by increasing the number of circulating neutrophils. In addition, it activates some functions of mature neutrophils (enhanced myeloperoxidase mediated microbiocidal capabilities).

Dose determination: Preclinical studies in calves showed a dose dependant effect of pegbovigrastim. Increased absolute neutrophil counts (ANC) lasted longer with increasing doses (5–6 days for 20 µg/kg and 10–12 days for 40 and 80 µg/kg). In other studies in adult dairy cattle there was a significant decrease in milk yield at 80 µg/kg bw; therefore a dose of 20–40 µg/kg was considered suitable.

Dose confirmation: Further studies confirmed that significant reduction in clinical mastitis could not be demonstrated where the animals received a single dose of 20 µg/kg bw only. On the basis of a number of dose confirmation studies, a dose range was established of 20–40 µg/kg bw to be administered by single volume injection on two occasions, one approximately 7 days prior to the anticipated date of calving and one on the day of calving.

Target animal tolerance: Two target animal safety studies were provided. Data showed that Imrestor at the recommended dose was generally well tolerated and there were no differences in milk production, dry matter intake, or clinical pathology variables, as well as no gross or microscopic test article-related tissue alterations. No safety concerns were reported when pegbovigrastim was administered subcutaneously on day -7 relative to anticipated calving date and on day 0 within 24 hours after calving at up to 3x the recommended dose level in periparturient dairy cows. However, overdosing should be avoided.

In the clinical field studies, non-typical anaphylactoid reactions were uncommonly observed. The cows presented with swelling of mucous membranes (notably vulva and eyelid), skin reactions, increased respiration rate and salivation. In rare cases, the animal may collapse. These clinical signs typically appear between 30 minutes and 2 hours after the first dose and resolve within 2 hours.

Field trials: Two European field studies were provided.

Imrestor at a subcutaneous dose of 20–40 µg pegbovigrastim/kg (i.e. one pre-filled syringe of 15 ml) at two occasions, i.e. 7 days prior to the estimated day of calving and a second dose on the day of calving (within 24 hours of birth) resulted in a reduction of clinical mastitis. In the second European field trial, the incidence of clinical mastitis observed in the treated group was 9.1% (113/1235) and in the control group 12.4% (152/1230), showing a relative reduction in mastitis incidence of 25.8% (p=0.0094). The first European field trial was not adequately powered and showed no significant difference in mastitis incidence between treated (13.2%) and control animals (15.3%). Based on all field studies, the proportion of mastitis prevented due to herd treatment with Imrestor (Prevented Fraction) is 0.25 (with 95% confidence interval 0.14–0.35).

The data support the use of the product as an aid in a herd management programme, to reduce the risk of clinical mastitis in periparturient dairy cows and heifers during the 30 days following calving.

The benefit of the product depending on the parity of the cow was not calculated.

Part 5 – Benefit-risk assessment

Introduction

Imrestor (active substance: pegylated recombinant bovine granulocyte colony stimulating factor, bG-CSF, pegbovigrastim) is a solution for injection for subcutaneous injection in cattle. It is presented in

pre -filled syringes (10, 50 or 100 per pack) and intended to be used in dairy cows as an aid to reduce the incidence of clinical mastitis in cattle during the periparturient period.

Pegbovigrastim is a novel active substance, increases the number of circulating neutrophils. It has also been shown that it enhances myeloperoxidase hydrogen peroxide halide mediated microbiocidal capabilities of neutrophils. However, the treatment does not does not appear to improve other neutrophil functions as phagocytosis, neutrophil extracellular trap formation and oxidative burst.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

A total of 6 field trials (including pooled studies) were conducted to test the efficacy of the monodose syringe containing 15 mg of pegbovigrastim for reduction of clinical mastitis incidence. Two field trials were run in EU and 4 supportive field studies in New Zealand, Australia and USA.

Amongst the supportive studies (NZ, USA, Australia), the clinical difference was not significant in the first NZ study and the Australian study. The results were significant in the second NZ study (18.3% vs 14.4%). In the US study, the clinical difference was significant (26.8% vs 18%).

The first European field trial (FR, ES, UK) was conducted in 486 cows and, due to the low incidence of mastitis during this study, was underpowered to demonstrate the presence of a statistically significant difference in % of mastitis (15.3% vs 13.2% - control vs treated).

The second EU field trial (UK, DE, NE, HU) was conducted in 2467 cows across 35 farm sites and demonstrated the presence of a statistically significant difference in the incidence of mastitis (12.4% vs 9.1% - control vs treated). The study concluded that herd treatment with Imrestor resulted in a relative reduction in the incidence of clinical mastitis during the peri-parturient period of 26% (95% CI: 7–41%). Placed into context with other contemporary herd management strategies aimed at reducing the incidence of clinical mastitis in dairy cows, the size of this therapeutic benefit (relative risk reduction of 26%) can be considered clinically relevant.

Based on all field studies, the proportion of mastitis prevented due to herd treatment with Imrestor (Prevented Fraction) is 0.25 (with 95% confidence interval 0.14–0.35)

The reduction in mastitis was variable across the field studies conducted, however an average reduction of 25% was seen across different geographical areas, breeds and farming sytems studied.

Based on the data provided, the benefits of Imrestor are its use as an aid in a herd management programme, to reduce the risk of clinical mastitis in periparturient dairy cows and heifers during the 30 days following calving.

Additional benefits

Where Imrestor reduces the incidence of clinical mastitis it can be expected that the product may reduce the need for antimicrobial treatment.

Risk assessment

Quality

The formulation and manufacturing process of pegbovigrastim active substance and Imrestor product is well described and controlled. QbD principles have been used in the development of the product and its manufacturing process. Further, as part of the QbD development an appropriate control strategy has been established. Two Design Spaces have been applied for; one for the fermentation process and one for the solubilisation, refolding and purification steps. Specifications set will ensure that a product of consistent quality will be produced.

Target animal safety

In the first target animal safety study in Jersey cows (430 kg in average) abomasal erosions and ulcer incidence was significantly increased at a dose of 60 µg/kg (1.5x the highest recommended dose) repeated three times (instead of two as recommended in the SPC).

In the second target animal safety study performed in Holstein cows (680 kg in average), test article related findings were limited to increased production of neutrophils. There were no differences in milk production, dry matter intake, or clinical pathology variables, as well as no gross or microscopic test article-related tissue alterations. At a dose of 2–3 x RTD (70 µg/kg), a slight non-significant decrease of the bodyweights of the treated animals was observed.

According to the target animal safety studies, pegbovigrastim may be well tolerated up to twice the recommended dose. In the clinical studies, non-typical anaphylactoid reactions were uncommonly observed. The cows presented with swelling of mucous membranes (notably vulva and eyelid), skin reactions, increased respiration rate and salivation. In rare cases, the animal may collapse. These clinical signs typically appear between 30 minutes and 2 hours after the first dose and resolve within 2 hours.

Subcutaneous administration of the product may induce transient local swelling at the injection site as well as inflammatory reactions which resolve within 14 days post treatment.

User safety

Adverse effects have been reported following administration of human granulocyte colony stimulating factor products used in human medicine, including bone and musculoskeletal pain, as well as hypersensitivity-type reactions, including skin rash, urticaria, angioedema, dyspnoea, erythema, flushing, and hypotension. Self-administration of Imrestor in human beings may cause symptoms similar to those reported for other similar products. The user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

Environmental safety

Imrestor does not pose a risk for the environment when used according to the proposed SPC.

Consumer safety

Imrestor does not pose a risk to the consumer when used according to the proposed SPC.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall. The product has been shown to be efficacious for the indication as an aid in a herd management programme, to reduce the risk of clinical mastitis in periparturient dairy cows and heifers during the 30 days following calving.

The formulation and manufacture of Imrestor is well described and specifications set will ensure that product of consistent quality will be produced.

It is well tolerated at the recommended dose by the target animals and presents an acceptable risk for users, consumers and the environment.

A zero day withdrawal period is established.

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 Imrestor is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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

Divergent position on a CVMP opinion on the granting of a marketing authorisation for Imrestor (EMEA/V/C/002763/0000)

Imrestor is a new product which contains pegbovigrastim as the active ingredient. The claim of the product is "as an aid in a herd management programme, to reduce the risk of clinical mastitis in periparturient dairy cows and heifers during the 30 days following calving."

The benefit:risk ratio of the above product is considered negative for the following reasons.

CLINICAL BENEFIT

Major issues

The clinical benefit of Imrestor is mainly based on one pivotal European study (Eur 2013). The study involved 35 farm sites in UK, Germany, the Netherlands and Hungary testing 2467 cows and heifers divided between treatment and control groups. Imrestor was used in this study following the usual drying off treatments (e.g. antimicrobials).

The results of the study showed a statistically significant difference in the incidence of clinical mastitis: 113 clinical mastitis cases out of 1235 cows and heifers treated (9.1%) in the Imrestor group versus 152 out of 1230 (12.4%) in the control group. Imrestor decreased the incidence of clinical mastitis by only 3%. Therefore, the clinical effect in this study can be qualified as very small. Also, a small number of dairy herds (7/39 herds) experienced an increase in the incidence of clinical mastitis after Imrestor.

The comparability between the treated and the control groups before treatment was not demonstrated for the criteria that are known to impact the incidence of mastitis, such as drying off treatment, duration of drying off and milk somatic cell counts in the previous lactation. In the absence of well-defined study group for comparability, it cannot be fully concluded that the small observed clinical effect is truly related to Imrestor treatment, or not by chance alone.

There was no information on the severity of the clinical mastitis cases identified in this study. On this point, the clinical benefit of Imrestor is inadequately documented. Also, in the pivotal European study (Eur 2013), where significance was shown, the abnormal milks samples were not confirmed with a California Mastitis Test (CMT). As soon as the milk was visually considered abnormal, the cows were classified as having clinical mastitis. The clinical aspect of the quarter (normal quarter or abnormal quarter with signs of swelling, pain and/or heat) was not recorded in the study, nor was the presence of general signs (such as fever, loss of appetite).

Performing a CMT is a standard objective measure used worldwide in the dairy industry to quantify the degree of abnormality of the milk and would have provided a robust study parameter. Abnormal milk samples were tested bacteriologically in the study, but, surprisingly, the result was not used in the definition of the clinical mastitis. Only 46% (102/223) of the abnormal milk samples were identified as isolating major pathogens in the treated group and 60% (145/242) in the control group. In 35% of abnormal milk samples, no bacterial growth was observed.

In total, 56% (151/272 cases) of clinical mastitis cases were identified during the first four days post calving that is to say during the colostral period where milk can typically appear abnormal, even in the absence of mastitis.

The applicant has presented five other clinical trials: a 2nd pivotal study in Europe (Eur 2012) and four supportive studies performed out of Europe (two in New Zealand, one in Australia and one in USA). The 2nd European pivotal study (Eur 2012) as well as two of the supportive studies (AU 2012, NZ 2011) failed to show any significant effect of the product. One supportive study (NZ 2013) did show a significant but small effect as the main pivotal European study (Eur 2013) discussed above with a decrease of 4% of the clinical mastitis incidence. The only study that showed a higher benefit was the supportive study performed in USA (2011) with a decrease of 8.2% of the incidence of clinical mastitis.

In all studies, the comparability between the treated group and the control group at baseline was not demonstrated based upon criteria that are known to impact the incidence of clinical mastitis (e.g. drying off treatment, duration of drying off and milk somatic cell counts in the previous lactation). There was no California Mastitis Test (CMT) performed to confirm objectively the abnormality of the milk except in the first European study (Eur 2012) and in the US study (2011). However, in the first European study (Eur 2012), where CMT on milk samples was performed, there was no significance found between the two groups.

It is concluded that the assessment of the overall data package showed major methodological insufficiencies and identified a very small effect of Imrestor in the claimed indication.

A summary of the results of all the clinical studies is presented in the table below:

Study location	**Clinical mastitis definition**	**% clinical mastitis in placebo group**	**% clinical mastitis in Imrestor group**	**P value**
EU Pivotal Studies				
Eur 2013 (UK, NL, HU, DE)	At least normal quarter / abnormal milk (no CMT confirmation)	12.4% (152/1230)	9.1% (113/1235)	0.01 (*)
Eur 2012 (FR, ES and UK)	At least normal quarter/abnormal milk and CMT ≥ 2	15.3% (37/242)	13.2% (32/242)	0.63 (NS)
Non-EU (Supportive Studies)				
NZ 2011 (New Zealand)	At least normal quarter/abnormal milk (no CMT confirmation)	15.4% (39/254)	11.5% (30/261)	0.19 (NS)
NZ 2013 (New Zealand)		18.3% (194/1062)	14.4% (152/1052)	0.02 (*)
AU 2012 (Australia)		15.7% (30/191)	16% (31/194)	0.94 (NS)
USA 2011	At least normal quarter/abnormal milk and CMT ≥ 2	26.8% (72/269)	18.0% (48/267)	0.01 (*)

(*) Significant / (NS) Non significant

Additional comment

The data available were poorly investigated: for instance, no separate results were given for heifers and cows.

There was also no information on the impact of Imrestor regarding the total use of antimicrobial treatments for clinical mastitis. For example, the number of clinical mastitis treated in each group during the period of observation of the clinical study (30 days post calving) was not provided. Thus, it is unknown as to whether Imrestor had an impact on the number of antimicrobial treatments used to treat clinical mastitis during the 30 days post calving (e.g. reduced the number of antimicrobial treatments needed). Thus, it has not been demonstrated that the use of the product will lower the use of antimicrobials to control mastitis. It is also important to underline that during the field study the typical drying-off treatments were maintained and thus the product cannot be considered as an alternative to antimicrobial treatments or reducing the total antimicrobials used on the farm.

ANIMAL SAFETY

Two target animal safety (TAS) studies were provided.

In the first TAS study, Jersey cows were treated with 1x, 2x, and 3x the recommended dose of one syringe at three time points (7 and 3 days prior to the anticipated calving date and again within 24 h after calving), *i.e.* in excess of the recommended number of treatments of two (D-7 and within 24 hours of calving).

In this study, abomasal lesions were found in treated animals. The ulcers were more frequent in the animals in the 2 x and 3 x Imrestor treated groups. Two animals from the 1 x and 3 x Imrestor treated groups had perforated ulcers. The animal from the 3x treated group had to be euthanized.

The incidences of the ulcers were as follows:

Parity	Treatment Group	Incidence of abomasal erosion or ulcer / number of animals tested
Cows	Control	0/4
	Imrestor X1	1/4
	Imrestor X2*	3/4
	Imrestor X3	1/4
Heifers	Control	1/4
	Imrestor X1	0/4
	Imrestor X2	2/4
	Imrestor X3	3/4

*p=0.0576 for abomasal pylorus erosions

It is concluded from this study that the incidence of abomasal lesions is increased at doses as low as two times the dose repeated three times. Thus, the safety margin of the product is considered to be narrow.

It is possible that the stressful experimental conditions had worsened the ulcer incidence. However, ulcers were more frequent in the treated groups than the placebo group.

In the second TAS study, the product was administered to multiparous Holstein cattle at doses of 1x, 2x, 2.5x or 3x the recommended dose for a total of two dosages (*i.e.* as the recommended number of dosages). No heifers were included. The second TAS study was performed with a significantly lower level

of overdosing (the treatment was administered at three time points in the first study instead of two in the second study and the animals of the second study were significantly heavier). Consequently, the second study cannot fully overrule the first one.

In this study, the effects of Imrestor were reduced at necropsy. However, it was noticed that, in several tissues, the frequency of anomalies were more frequent in treated groups compared to control for doses as low as two times the recommended dose (mild erosion, mild inflammation, minimal congestion in abomasum, pyloric region, proximal duodenum and the descending colon, haemorrhages and oedema in the urinary bladder). No clinical signs related to these lesions were detected.

The two TAS studies showed that, starting from twice the recommended dose, Imrestor impacts the physiology of the treated animal. The observations made at necropsy in these studies may be related to the mode of action of Imrestor on the cow's immunity. It is possible that the increased number of circulating granulocytes in these animals caused an "over-shooting" response of granulocytes, resulting in an uncontrolled recruitment as well as the production of ROS (reactive oxygen species) in many tissues.

Based on the two TAS studies, it is concluded that the safety margin of the product is narrow.

In the field studies, no adverse effects were observed at the recommended dose with the product except for cases of non-typical anaphylactoid reactions. These reactions were uncommon (25 cases out of 3635 cows). However, in one farm, their prevalence reached 7% of the treated animals. The reactions were mild and transient. A treatment was implemented in half of the cases. These anaphylactic reactions, expected at the recommended doses, represent an identified risk of safety for the target animal species.

CONCLUSION

The overall data package showed major methodological insufficiencies and identified a very small effect of Imrestor in the claimed indication. The pivotal European study (Eur 2013) indicates that at least 1000 cows need to be treated to prevent 33 cases of clinical mastitis of potentially low severity (the severity of the clinical mastitis in the clinical studies was not documented).

The first TAS study with a proper overdosing (the dosages were repeated at three time points) gave alarming results with regard to formation of ulcers/erosions. The negative outcome of the second study with regard to this issue did not dispel these concerns because it was performed at lower dosages (administration of the product at two time points only and animals of higher bodyweights) and because at necropsy it was seen that the product did impact the tissues, with small effects and no clinical consequences.

It is concluded that the TAS studies showed that the safety margin of this product is narrow. Moreover, the field data indicated that non typical anaphylactoid reactions are expected at the recommended dose.

It has not been demonstrated that the use of the product will lower the use of antimicrobials to control mastitis.

This product will be used for prevention purposes, meaning that numerous cows will need to be treated and exposed to the potential adverse effects for a small benefit of prevention of only a few clinical mastitis cases.

The risk for the target animal species is considered to outweigh the clinical benefit provided by Imrestor.

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