SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Masivet (as published in November 2008). For information on changes after this date please refer to module 8B.

1. Introduction

Mast cell tumours are the most common cutaneous tumours in dogs, with an incidence of 1:1000 (grade II/III about 6.5/10.000). It is assumed that the prevalence of mast cell tumours is similar across European member states. Mast cell tumours tend to occur in middle aged to elderly dogs (average of 9 years) and are classified as Grade I (well differentiated), Grade II (intermediate differentiated) or Grade III (poorly differentiated). Mast cell tumours may be (but are not always) a life-threatening condition. The primary disease can sometimes be adequately controlled by surgery and radiotherapy, resulting in less than 20% of the grade II mast cell tumours metastasising.

Although mast cell tumour is one of the major cancer diagnoses in dogs, the total incidence of canine mast cell tumours grade II or III in dogs is very low. Since this disease is a life threatening condition and the overall number of dogs suitable for treatment is expected to be low, the CVMP agreed that the CVMP guidelines on "Minor-Use-Minor-Species (MUMS) data requirements" could be applied when assessing the dossier.

Masivet is an antineoplastic agent for the treatment of mast cell tumours (Grade 2 or 3, non-resectable) in dog carrying the mutated for of the tyrosin kinas receptor. The product is presented as film-coated tablets in two different strengths (50 mg and 150 mg), to be used in different combinations together to achieve accurate body-weight dependant dosing. The active substance is masitinib (in form of masitinib mesylate), a protein-tyrosine kinase inhibitor.

In vitro masitinib selectively and effectively inhibits the c-Kit tyrosine kinase receptor (the mast cell growth factor receptor) and shows the highest affinity for mutated forms of c-Kit which are commonly found in tumours.

In vivo masivet showed a significantly longer Time-to-Tumour Progression (TTP) (median of 241 days) as compared to placebo (median of 83) in dogs with mast cell tumours (Grade II or III) with confirmed mutated c-KIT tyrosine kinase receptor. The most common side effects are gastrointestinal reactions (diarrhoea and vomiting).

The approved indication is: "Treatment of non-resectable dog mast cell tumours (Grade 2 or 3) with confirmed mutated c-KIT tyrosine kinase receptor".

2. QUALITY ASSESSMENT

Composition

Masivet is presented as a film-coated tablet available in two strengths, 50 mg and 150 mg with the active ingredient Masitinib mesylate.

Excipients used in the tablet core include microcrystalline cellulose, povidone, pig liver powder, crospovidone, magnesium stearate. Tablets coating film contains poly(vinyl alcohol), titanium dioxide (E171), polyethylene glycol, talc and FD&C yellow#6/sunset yellow FCF aluminium lake (E110)

Container

The tablets are packaged in multidose HDPE-bottles covered by a child-resistant closure cap.

Development Pharmaceutics

For safety and palatability reasons, the tablets are presented as film-coated tablets. The pig liver powder is intended to prevent the tablets from being rejected if chewing of the tablets occurs.

Method of manufacture

Tablets are manufactured according to a standard process including wet-granulation, compression and coating. Three batches of each dosage strength have been manufactured at industrial scale. All batches correspond with the finished product specification. Validation of the manufacturing process at commercial scale will be conducted prior to marketing on three batches of each strength.

CONTROL OF STARTING MATERIALS

Active substance

The specification includes limits for the main impurities observed in batches produced by the defined route of synthesis. The impurity limits are qualified with reference to toxicology studies and do not present a safety problem. The analytical methods used in routine controls are described. Method validation studies are in agreement with the current VICH-guidelines. The synthesis of Masitinib mesylate is completed in 4 main steps. A description of the manufacturing process has been provided including reaction conditions, quantities of raw materials and yields. Specifications for starting materials and intermediates have been established.

Packaging for storage and transport is described. Masitinib mesylate has no chiral centres and it is not optically active. The solubility of Masitinib mesylate in aqueous solutions depends on pH; it is very soluble in acid, but insoluble at alkaline pH. Three different crystalline forms are known.

Stress testing did not reveal any degradation pathways. Masitinib mesylate is not sensitive to light, but unstable under oxidative stress conditions. Formal stability data of 3 pilot batches performed under VICH conditions and representative of the material manufactured by the commercial process were presented. No apparent change of the drug substance quality was observed in these studies. A re-test period of 12 months was acceptable. In addition, the first next three production scale batches will be placed on long term stability studies over 3 years.

Excipients

All excipients contained in the tablet core except the flavouring agent pig liver powder are of pharmacopoeial grade quality. The magnesium stearate is of vegetal origin. Documentation on the pig liver powder has been provided. The Committee considered that a more rigorous procedure for inactivation of viruses and microorganisms should be used. The applicant committed to instantly apply the inactivation method requested by the Committee.

For preparation of the film a ready-for-use coating mixture is used. For the non-pharmacopoeial excipients, pig liver powder and coating agent, in-house specifications have been established.

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

There are no concerns in relation to TSE with any of the ingredients of the product. The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

Control tests on the finished product

A specification has been developed for release of the tablets which includes tests for identification, dissolution, content uniformity, related substances and assay by HPLC and microbiological characteristics. Control methods have been validated and the specification is considered to be relevant for a product of this type. Results of three batches per dosage strength manufactured at commercial scale have been provided. The results indicate compliance with the specification and uniformity of the finished product.

Stability

The shelf-life specification provided was acceptable. The shelf-life specification is the same as the release specification, with the exception of the additionally specified moisture content. The analytical methods applied are those described for finished product testing at release.

Three commercial scale batches per dosage strength have been placed on accelerated and long term stability studies. A proposed matrixing plan (one third reduction scheme, 36 month long term testing, 6 months accelerated testing) was accepted. In addition, one half-sized batch per dosage strength will be placed on accelerated and long-term stability studies.

A shelf-life of 12 month was accepted. The storage recommendation “Keep the bottle tightly closed” has been included in section 6.4 of the SPC.

OVERALL CONCLUSION ON QUALITY

Masivet is presented as film-coated tablets available in two strengths, 50 mg and 150 mg containing the active ingredient masitinib mesylate and various excipients.

The tablets are packaged in multidose HDPE-bottles covered by a child-resistant closure cap.

Development pharmaceutics, the steps in the manufacturing process and the specifications for the active substance, the excipients and for the finished product at release and shelf-life have been sufficiently addressed.

A shelf-life of 12 month was accepted.

3. SAFETY ASSESSMENT

Pharmacodynamics

The applicant provided a number of *in vitro* studies investigating the effects of masitinib as inhibitor of c-Kit tyrosine kinase activity, the anti-proliferative and pro-apoptosis activities of masitinib on various mammalian cell lines and anti-degranulating activities on normal human mast cells.

Masitinib proved to be a potent and selective c-Kit tyrosine kinase inhibitor, which is a competitive inhibitor of binding of adenosine triphosphate (ATP), a critical substrate in the tyrosine phosphorylation reaction. Alterations of the c-Kit are significantly associated with histologically higher-grade (2 or 3) tumours. It was shown that masitinib selectively and effectively inhibits the c-Kit tyrosine kinase receptor (the mast cell growth factor receptor). The affinity of masitinib was tested on kinase assay and cell proliferation assay on cell lines expressing endogenous or transfected receptors. A variety of human and murine receptor and non-receptor kinases was initially tested. In two subsequent studies, several isoforms of human, murine and canine c-Kit were also evaluated.

Masitinib shows the highest affinity for isoforms of c-Kit with mutations in the juxta-membrane domain (IC_{50} ranging from 0.005 µM to 0.1 µM) and, to a lesser extent c-Kit wild-type (WT, IC_{50} ranging from 0.1 to 0.2 µM).
In addition to these findings, the platelet-derived growth factor (PDGF) receptor is inhibited (IC_{50}<0.1µM). The selectivity of masitinib is important as it does not affect other members of the type III receptor kinase family.
Poor inhibiting activity was found against isoforms of c-Kit with mutations in the catalytic domain (IC_{50} values > 10 µM for D814V murine c-Kit and D816V human c-Kit) or different growth factor receptors, which are also tyrosine kinases (IC_{50} > 1 up to > 100 µM) and non-receptor kinases.

Studies on secondary pharmacodynamics included potential effects on cardiac function, respiratory functions and central nervous system. Whereas no adverse effects related to the central nervous system and the lungs were recorded, it was noted, that masitinib at high concentration (14 µM) is able to inhibit *in vitro* the cardiac potassium channel. However, no evidence of cardiotoxicity could be found in dogs.

In addition, two *in vivo* studies were submitted investigating the antitumor activity in Balb/c nude mice tumour models. In the SC BA/F3 c-Kit d27 tumour model, masitinib induced a significant increase of the life time compared to the reference drug STI571, but was ineffective in the subcutaneous BA/F3 p210 bcr-abl tumour model.

Pharmacokinetics

Following oral administration in dogs at a dose of 10 mg/kg bodyweight, masitinib is rapidly absorbed and the time to maximal concentration (t_{max}) is approximately 2 hours. The concentration of masitinib increased with increasing dose-levels in a nearly dose proportional manner. No evidence of saturation or a time-dependent effect of absorption was recorded. After repeated dose administrations in rats for 4, 13 and 26 weeks, t_{max} values of 2 to 7 hours were recorded. In rats, a gender-dependent effect leading to a twofold higher exposure of females was observed. This effect, even though to a much smaller extent, was also seen in dogs after single dose administration.
The mean half-life ($t_{½}$) of masitinib in rats and dogs after single administration is about 5 hours, whereas in humans after repeated dose application $t_{½}$ values of about 13 hours were recorded, which increased up to 33 hours with ascending doses. In rats, terminal half life was slightly shorter for masitinib (range 2.72 to 3.16 hours) than for AB3280, a major metabolite (range: 3.55 to 4.23 hours), and showed no variation related to gender or over time after repeated doses for 14 days.
Masitinib has a high protein binding (92% binding to dog blood cells, 93% binding to plasma proteins) approximately 93 % bound to plasma proteins.

Although masitinib was not able to induce different cytochrome P450 isoforms, it inhibits (human) CYP3A4, 2C9 and 2D6 with IC_{50} values of 14 µM, 20 µM and > 30 µM, respectively. This inhibition is partly reversible. However, this inhibition as well as the high protein binding are of concern because of the potentials for drug-drug interactions, which are common in tumour patients due to the fact that these patients received often more than one drug. The major metabolite AB3280 was in contrast to that not able to inhibit/induce these CYP isoforms.

Studies concerning the tissue distribution of masitinib (single oral administration) in rats revealed an enrichment of the test item in adrenals, kidneys, spleen and intestine (0.006 to 6.43% of the dose at 24 hours).

Masitinib is metabolised predominantly by N-dealkylation. The main metabolite in dog faeces is a N-desmethyl derivative of masitinib, which is formed by gut microflora. The second major metabolite in dog faeces corresponds to sulfate conjugate of mono-hydroxy-masitinib, whereas the major metabolite in urine is a carboxylic acid metabolite of the parent compound after hydrolysis of the central amid linkage. Apart from the parent compound up to 12 metabolites were detected in urine and 8 metabolites were identified in the faeces. Data on the pharmacological activity of the main metabolites in dogs are not available; however, mutagenic potential of the different metabolites is unlikely because a structural alert is not obvious.

In dogs, approximately 90% of masitinib is excreted with faeces, mainly as parent compound, and only 5% of the dose is found in urine with a moderately rapid elimination within 48 hours.

Toxicity

The test compound masitinib is of low acute toxicity in rats ($LD_{50\ intravenous}$ >100mg/kg bodyweight and $LD_{50\ oral}$ > 2000 mg/kg bodyweight).

A number of repeated oral dose toxicity studies in rats and beagle dogs were submitted. In rats, the toxicity of masitinib was assessed in 2-week, 4-week, 13-week and 26-week studies and at dose levels from 10 to 300 mg masitinib mesylate/kg bodyweight/day.

In beagle dogs, the toxicity of masitinib was assessed in a 4-week study (15 to 150 mg masitinib mesylate/kg/day), 13-week (15 to 50 mg masitinib mesylate/kg/day) and 39-week (3 to 30 mg masitinib mesylate/kg/day) study. Details on these studies are presented in Part 4 (tolerance).

In rats, identified target organs and tissues included bone marrow, heart, kidneys and liver, and in females the genital tract. Dose-dependent nephrotoxic effects were recorded at doses of 30 mg/kg bodyweight and above and included proteinuria, degenerative and necrotic lesions of the tubular epithelium. Signs of degenerative cardiomyopathy were already recorded at the lowest tested dose of 10 mg/kg/day, while myocardial degeneration / fibrosis were observed at doses of 30 mg/kg bodyweight and above. Effects on female genital tract were disturbance of the follicular development in the ovaries with secondary morphological changes of the endometrium and vagina and observed from 50 mg/kg bodyweight onwards; at dose levels of 10 mg/kg bodyweight/day and above, changes in red and white blood cell parameters (especially anaemia and lower neutrophil counts), serum chemistry, lower mean activated partial thromboplastin time and fibrinogen level.

The CVMP considered that the studies were adequate to assess the toxicity of masitinib and agreed that a dose of 10 mg/kg bodyweight/day masitinib mesylate (i.e. 8 mg masitinib free base) would represent the lowest observed adverse effect level (LOEL). Although the CVMP noted that no evidence of cardiotoxicity could be found in dogs, some concern was expressed that no sufficient safety margin exists between the lowest tested dose of 10 mg/kg/day at which signs of degenerative cardiomyopathy were already recorded and the suggested therapeutic dose of 12.5 mg/kg/day.
Reproductive toxicity including teratogenicity

The studies on reproductive toxicity included one Segment I study (male and female fertility and early embryonic development from conception to implantation) in rats, a return to fertility and embryotoxicity study in rats and two Segment II studies (embryo-foetal toxicity study from implantation to closure of the hard palate) in rats and rabbits, respectively.

In the rat segment I study, decreased fertility was manifested as an increased number of non-pregnant females, low number of corpora lutea and implantation sites, and increased pre-implantation loss in female rats exposed to 100 mg/kg/day. Cystic degeneration of corpora lutea (with accumulation of fibroblasts and a few erythrocytes) was seen at 30 mg/kg/day; however, no impact was noted on any of the fertility and pregnancy parameters.
Embryotoxicity was evidenced at 100 mg masitinib/kg bodyweight/day in rats by an increased number of early resorption and increased number of dead concepti, resulting in a low number of live concepti. There were no effects on male fertility or mating behaviour. The No Observed Adverse Effect Level (NOAEL) for parental systemic toxicity was considered to be 100 mg/kg bodyweight/day for males. In females, a NOAEL based on effects on the ovaries was established at 10 mg/kg/day. This should be considered as a NOAEL for maternal toxicity and fertility. The NOAEL for developmental toxicity was 30 mg/kg/day.
A return to fertility study was also performed in the rat and showed that adverse effects on fertility were reversible.

In the rat segment II study, signs of maternal toxicity were noted starting at doses of 10 mg/kg bodyweight/day. Reduced foetal weight and unossified or incompletely ossified bones in the skull and sternebrae were observed at 100 mg/kg bodyweight/day. No teratogenic effects were observed. The NOAEL for developmental toxicity was established at 30 mg/kg/day.
In the rabbit segment II study, there was no evidence of embryo-foetal toxicity or teratogenicity observed in the rabbit at doses up to 100 mg/kg/day. The Segment II studies were pivotal.
No study covering pre-and postnatal development and maternal function (segment III) was provided.

Based on these studies, the product is contraindicated in pregnant or lactating bitches.

Mutagenicity

A standard test battery for the assessment of genotoxicity of masitinib and its major metabolite AB3280 was conducted. Both compounds provided negative results. The CVMP concluded that masitinib, which does not contain a structural alert, was not genotoxic *in vitro* and *in vivo* as revealed in these studies.

However, considering the structure of masitinib as well as that this compound is desmethylated, a metabolism step which is also often performed by CYP 2B1, it would have been helpful to test the mutagenicity of the parent compound and AB3280 in the presence of a S9 mix prepared from Phenobarbital induced rats.

Based on the absence of any structural alerts, negative mutagenicity findings and the absence of any pre-neoplastic lesions in repeat dose toxicity studies, no carcinogenicity studies were performed. This was considered acceptable by the CVMP.

User safety

A user safety assessment was provided. Professional (veterinarians and personnel working in veterinary practice) and non-professional users (patient owners, their family etc) are considered potential user groups. Tasks and situations leading to exposure were identified and quantified, and compared with relevant NOEL values. Most likely routes of human exposure were considered accidental ingestion of tablets, inhalation and skin or eye contact (direct or via exposure to animal saliva, urine or faeces from treated dogs). In addition, the impact on female fertility was discussed.

Accidental ingestion:
Most side effects in persons treated with masitinib are mild to moderate and transitory up to doses of 12 mg/kg bodyweight/day and most commonly include oedema, nausea, vomiting, rash or diarrhoea. Doses higher than 12 mg/kg bodyweight/day may lead to gastrointestinal disorders and changes in laboratory parameters (anaemia, lymphopenia, hyperbilirubinaemia).

In adult human volunteers a single dose of 800 mg masitinib was tolerated (i.e a NOEL hazard classification dose of 11 mg/kg bodyweight) as the upper limit for a single tolerated dose. This is equivalent to more than 5 dog tablets of the highest strength; which was considered very unlikely to be taken by accident. However, the intake of an 800 mg dose by a child might lead to serum concentrations of about 50 mg/kg bodyweight and a margin of exposure (MOE) of 0.2, indicating a risk. The product is, therefore, presented with a child-resistant package and the product literature includes a special warning that children should not have close contact to treated dogs, treated dog faeces or vomit.

Inhalation:
The product is presented as a non divisible film coated tablet, a formulation which drastically reduces the risk of formation of inhalable particles. In addition, the product literature contains strong warnings that tablets should not be broken or ground. The CVMP considered, therefore, the risk of inhalation as very unlikely.

Dermal / eye contact:
In a murine Local Lymph Node Assay and eye irritation test in rabbits, masitinib proved to be a strong skin sensitiser and exhibited severe eye irritating effects. However, taking into account the formulation of the product (film-coated tablet) and the strong warnings in the product literature that tablets should not be broken or ground, the CVMP considered the risk of a direct contact between the active substance and the skin or the eyes as very unlikely.
The user may also come into dermal contact with vomit, urine or faeces of dogs under therapy. Strong warnings have therefore been included in the product literature to ensure that skin/eye contact is to be avoided and any area in contact to be rinsed immediately, protective gloves to be used when handling vomit, urine or faeces of treated dogs and that tablets should not be broken or ground:

Fertility
Reduced female fertility and embryotoxicity was observed in laboratory animals exposed to masitinib. Following repeated dermal exposure at 0.625 mg/kg/day, the margin to the female fertility NOEL (10 mg/kg/day) and the developmental toxicity NOEL (30 mg/kg/day) was less than 100 (16 and 48, respectively), indicating a risk for the user.

The CVMP expressed some concern about the risk of the product for female fertility, in particular with repeated dermal contact to masitinib. The Committee, therefore, suggested strengthening the user warnings in the proposed SPC and product literature to avoid any skin contact with the product.

Environmental safety

An environmental risk assessment stopped in Phase I. Since the product is intended for use in individual companion animals, no assessment of the potential impact of masitinib on the environment was performed. Masivet is intended for use in non-food producing animals only, and is not considered as a DNA-reactive compound with potential for unusual ecotoxicity; therefore, the termination of the EIA at Question 3 in Phase I was accepted by the CVMP without further exposure assessment.

OVERALL CONCLUSIONS ON SAFETY

Masitinib is a tyrosine kinase inhibitor which *in vitro* selectively inhibits the c-Kit tyrosine kinase receptor (the mast cell growth factor receptor) with a high affinity for a mutated form of c-Kit and to a lesser extent c-Kit Wildtype (WT). Studies on secondary pharmacodynamics included potential effects on cardiac function, respiratory functions and central nervous system; however, no such adverse effects could be found in dogs.

Following oral administration, masitinib is rapidly absorbed. No accumulation is expected using the treatment dose and masitinib is moderately rapidly excreted (within 48 hours), mainly as parent compound via faeces. A number of metabolites have been identified; however, no data on the pharmacological activity of these are available. Masitinib binds to a high degree to plasma proteins and inhibits different isoforms of human cytochromes P450 isoforms, indicating the possibility of drug-drug interactions.

Masitinib is not considered cytotoxic and of low acute toxicity in rats. However, following repeated oral administration in rats, some effects were already recorded at the lowest tested dose of 8 mg masitinib/kg/day (signs of degenerative cardiomyopathy, changes in haematology). CVMP expressed concern that an insufficient safety margin exists between this dose and the suggested therapeutic dose of 12.5 mg/kg bodyweight/day.

For reproductive toxicity, the maximum tolerated dose (NOAEL) in rats was considered to be 100 mg/kg/day in males and 10 mg/kg/day in females (based on effects on the ovaries). No study covering pre-and postnatal development and maternal function was provided. Based on these studies, the product is contraindicated in pregnant or lactating bitches.

A standard test battery for the assessment of genotoxicity of the parent compound and its major metabolite was conducted and both compounds provided negative results. Based on the absence of any structural alerts, negative mutagenicity findings and the absence of any pre-neoplastic lesions in repeat dose toxicity studies, carcinogenicity studies were not considered necessary.

Masitinib is a strong skin sensitiser and a severe eye irritant. Appropriate warnings have, therefore, been included in the SPC and product literature, also the advice not to ground or break the tablets; in addition, the product is presented as a film-coated tablet (no contact to the core expected). Reduced female fertility and embryotoxicity was observed in laboratory animals and the CVMP expressed some concern in regard to female fertility. However, it was concluded that the user warnings in the SPC and product literature would sufficiently address this issue. Following oral administration of masitinib, gastrointestinal reactions and changes in laboratory parameters have been reported in people with a NOEL hazard classification dose of 11 mg/kg bodyweight. Since accidental oral ingestion by a child was considered a potential risk, a child-proof container was considered necessary and particular warnings have been included in the product literature.

The product is intended for use in individual, non-food producing animals only and is not considered as a DNA-reactive compound with potential for unusual ecotoxicity. The risk for the environment was therefore considered low and no further exposure assessment was required.

4. EFFICACY ASSESSMENT

Tolerance

Information on target animal tolerance was provided in three toxicity studies and also with the results of the clinical study.

The tolerance of masitinib was investigated in three repeated oral dose toxicity studies in dogs. The studies were conducted in young beagle dogs (treatment start at 6-7 months of age) investigating the effects of different doses of masitinib mesylate administered orally (gavage) over 4 weeks (0, 15, 50, 150 mg/kg/day), 13 weeks (0, 5, 15, 50 mg/kg/d) and 39 weeks (0, 3, 10, 30 mg/kg/d). The 4-week and 13-week studies were followed by a 14 days treatment free period. All studies were well conducted and documented according to GLP. Despite some shortcomings in these studies (e.g. the use of a different pharmaceutical form), the following conclusions could be drawn:

Clinical signs affecting the gastrointestinal tract occurred in all treatment groups. While the incidence of clinical gastrointestinal effects (diarrhoea, vomiting, regurgitation) was low in animals receiving 15 mg masitinib mesylate/kg bodyweight/day (equivalent to 12.5 mg masitinib) and vomiting in dogs receiving 30 mg/kg/day, it was frequent in groups treated with at least 50 mg/kg/day.

As expected from an inhibitor of tyrosine kinase c-Kit, masitinib shows effects on the haematopoetic system. This becomes clinically apparent as pallor of the oral region, which could be observed after some weeks of treatment in almost all dogs receiving 50 mg/kg/day and all dogs receiving 150 mg/kg/day. This is correlated to laboratory signs of anaemia and hypocellularity of the bone marrow in the animals of these groups. The white blood cell line is also affected, as displayed by a slight decrease in leucocyte (mainly neutrophil) counts, which is already seen at 15 mg/kg/day (but statistically significant only from 50 mg/kg/day and more). There was also evidence for further effects on the immune system at high doses (150 mg/kg/day).

The liver was affected by high doses of masitinib. In long-term use (13 weeks, 39 weeks), a liver weight gain was noted particularly in the 30- and 50 mg groups. In the 4-week study, the administration of high doses (50 mg/kg, 150 mg/kg) led to the formation of bile canalicular plaques and the presence of vacuolated Kupffer cells, and very high doses (150 mg/kg) to visible enlargement and/or paleness of the organ. This effect was not fully reversible. Liver enzyme levels, accounting for liver cell death, were also elevated in dogs receiving 150 mg/kg/day. In the 13-week study, 50 mg/kg/day led to hepatocellular hypertrophy.

Based on the laboratory studies, the main target organs of toxicity in dogs were identified as the gastrointestinal tract, the haematopoetic system, the kidney and the liver. Treatment related adverse effects mostly occur at doses exceeding the recommended starting dose of 12.5 mg masitinib (i.e. 15 mg masitinib mesylate) and are mainly reversible. As a result of the 4-week and 13-week studies, the No Observed Adverse Effect Level (NOAEL) or Maximum Tolerated Dose (MTD) was established at 15 mg masitinib mesylate per kg bodyweight/day (i.e. 12.5 mg/kg masitinib), as only minor clinical adverse effects, mainly vomiting on single occasions, and only a tendency for haematological changes were present at this dose level. As no treatment related effects were noted in the 10 mg masitinib mesylate/kg-group, this dose level was chosen as NOAEL in the 39-week study (i.e. 8 mg masitinib/kg bodyweight/day).

Resistance

No specific studies on resistance have been presented, however, resistance has been observed in tumours of human patients treated with tyrosine kinase inhibitor (mainly imatinib) and cell lines sensitive to tyrosine kinase inhibitors can be rendered insensitive by progressively increased doses. Since cross-resistance between chemotherapy and tyrosine kinase inhibitors cannot be ruled out, an appropriate warning was added to the SPC.

Dose justification

The proposed dose is 12.5 mg masitinib / kg bodyweight per day. Following carefully monitoring, it is suggested that this dose could be reduced to 9 mg or 6 mg/kg bodyweight/day, if considered necessary. The applicant justified the dose with the results of the findings in the tolerance studies; i.e. the 4-week and 13-week target animal toxicity studies where the MTD of 12.5 mg masitinib / kg/day was established.

The Committee agreed that it is, in principle, acceptable to choose the MTD of a tyrosine kinase inhibitor as treatment dose for an oncology product, especially if a dose reduction in case of adverse effects is part of the SPC. However, the Committee expressed concern that when using the MTD for dose determination, no safety margin exists. In addition, this recommended dose is only supported by the 4-week and 13-week studies; whereas in the third tolerance study of 39 weeks a MTD of 8 mg masitinib/kg bodyweight/day was established.

The Committee also noted that considerable pharmacokinetic variability exists and the exposure in individual animals is therefore difficult to predict. However, it is important to ensure accurate dosing in order to minimise the risk for lack of efficacy, resistance development or adverse reactions. Under field conditions, the tablet strengths are inadequate to ensure accurate dosing in small dogs, and the proposed dose reduction in case of severe adverse events, depending on the dog's bodyweight, could not be performed correctly.

Field studies

Two clinical field studies were submitted. One of them was only considered a pilot study and could therefore only be considered supportive in the assessment.

The pivotal multicentre, randomised, placebo-controlled field study was conducted in 21 centres in the USA and 2 centres in Europe in 2005-2006. It included dogs of different breeds and gender, with one or more histologically diagnosed measurable grade 2 or 3 mast cell tumour(s), and either recurrence after failure of surgery or presence of a non-resectable tumour. Dogs were either carriers of the "mutated" c-Kit or the "normal" wildtype c-Kit. Most dogs (95%) received concomitant treatment, mainly antihistamines for systemic use (71%), anaesthetics (64%) or analgesics (51%).

Dogs excluded from this study were e.g. lactating/pregnant bitches, dogs intended for breeding, animals younger than 6 months age or less than 3.3 kg; dogs that received or had received until recently various other treatments including chemotherapy, radiation therapy, or surgical removal (within 2 weeks prior to treatment initiation and/or incompletely healed surgical incisions prior to treatment initiation). Most of these groups of dogs are also contra-indicated in the proposed SPC.

The dogs received either tablets containing 25 mg, 100 mg or 150 mg of masitinib at a dose of 12.5 mg/kg bodyweight once daily for 6 months or received a placebo (i.e. untreated control group). In about 8 % treated dogs, this dose was reduced due to adverse events to 9 mg/kg/day or 6 mg/kg/day.

Efficacy:

The applicant chose as primary efficacy criterion the "Objective Response to treatment" measured at Day 112 (i.e. 4 months) and confirmed 56 days later (at 6 months), with the overall response being "complete response" (i.e. 0%) or "partial response" (i.e. at least 51%) in regards of comprehensive lesion measurement (CLM) as compared to baseline CLM (i.e. 100%) at Day 0. Secondary efficacy criteria in both groups (up to 6 months) included the time to tumour progression (TTP), the tumour progression-free survival and the overall survival; and also the response rate over time.

The analysis of the primary variable, Objective Response (OR), showed no statistical difference between masitinib and placebo treated animals. Likewise, for most secondary efficacy criteria, there were no statistically significant differences between masitinib and placebo treated animals (Complete Response Rate, Overall Response, Control Disease Rate, Best Response, Progression Free Survival and Overall Survival). The only significant improvement for the entire study population was seen in regard to Time to Tumour Progression (TTP) analysis, demonstrating that masitinib treated animals extended the median TTP by approximately 43 days as compared to placebo (p=0.038).

Since masitinib *in vitro* showed higher affinity for inhibition of the mutated form of c-Kit than to the wildtype c-Kit, the applicant performed a subgroup-analysis of the data obtained in the field study. This analysis included a comparison of treatment results regarding time to tumour progression from dogs that are carriers of a mutated c-Kit and dogs that are carriers of the wild-type c-Kit. The analysis indicated differences in treatment results of mutated c-Kit and wild-type c-Kit carriers. In the overall analysis, the prolongation of TTP was about 6 months (230 days; p=0.006) in the mutated c-Kit carriers, whereas the effect on wild-type c-Kit carriers was not different between groups (masitinib group 83 days; placebo group 98 days).

The applicant also provided further data from a subgroup predefined in the protocol (dogs with non-resectable *versus* recurrent post-surgery tumours and dogs with grade 2 *versus* grade 3 tumours) and from other subgroups, that were retrospectively defined and analysed such as dogs that had not received other chemotherapy or radiotherapy prior to masitinib treatment or "first line treatment".

The CVMP acknowledged that the modest postponement of approximately 40 days of the time until the mast cell tumour progresses (TTP) in the overall dog population might be the result of the high efficacy of treatment in dogs with mutated c-Kit; whereas no or less efficacy might occur in dogs with wildtype c-Kit. The presented results indicate that masitinib treatment of dogs with mutated c-Kit might have a prolonging effect on TTP. However, since some subgroup analyses were performed retrospectively and sub-populations were not well defined, likely highly auto-correlated and/or not pre-specified in the protocol, the CVMP did not consider the results of the studies sufficient to support a claim. A confirmation study would be necessary, specifically designed to address the different subgroups using groups of dogs with mutated and with wildtype c-Kit that are comparable to each other with respect to all other relevant parameters.

Moreover, CVMP considered that, under normal clinical conditions including veterinary oncologist practices, it would not be possibly to differentiate between the two dog subpopulations due to the lack of available diagnostic tools. It would be unacceptable to treat non-responders (unnecessarily) with masitinib. These animals would not only receive a treatment without beneficial results, but would experience side effects and would also be deprived of other treatment options (due to the veterinarian's reliance on this product).

Safety:

Results from the pivotal field study showed that treatment with masitinib was associated with a number of adverse events, mainly affecting the gastrointestinal tract (vomiting, diarrhoea); but also including neutropenia, fatigue, anorexia/decreased appetite, increase of liver enzymes, oedema and anaemia as well as a high rate of renal disorders indicated by proteinuria.

Although some adverse reactions such as gastrointestinal signs, occurred in both groups (treatment and placebo), the frequency in treated dogs was significantly higher. E.g. diarrhoea was more frequently seen in masitinib treated animals (37%) than in the placebo group (17%) and of longer duration (15 ± 44 days as compared to 4.5± 8.8 days).

The results indicate that animals suffering from grade 2 and 3 mast cell tumours suffer a wide range of disease-related clinical signs. The CVMP expressed concern that some of the clinical signs of the adverse reactions are similar to those related to the disease and would potentially worsen the clinical condition of dogs under the treatment, in particular those that are non-responders.

The use of supportive co-medication such as antibiotics, analgesics, anti-inflammatory drugs or antihistamines, was equally intense in the masitinib and the placebo group, indicating that treatment did not improved life quality. The CVMP expressed particular concern for the occurrence of treatment-related gastrointestinal signs as these may hamper adequate pain relief by use of NSAIDs, as these compounds are also known for gastrointestinal disturbances.

The increased risk for adverse events is of particular concern in view of the fact that a life time treatment strategy is proposed.

The applicant argued that most adverse events were mild to moderate, short-lasting and transient and provided a post-hoc analysis of the dogs' quality of life performed by pet owners and investigators. However, this argumentation was not accepted by the CVMP because the quality of life analysis was considered inconclusive and retrospectively. Furthermore, the data clearly indicated a higher frequency of adverse events in the treatment group than in the placebo group. In addition, the CVMP expressed concern that masitinib might only be palliative and did not improve quality of life. The applicant disagreed with the CVMP on this.

Conclusion on efficacy:

The tolerance of masitinib in dogs was investigated in three repeated oral dose toxicity studies over 4-week, 13-week and 39-weeks and in the clinical study. Based on the 4-week and 13-week studies, a MTD (maximum tolerated dose) of 12.5 mg/kg was established; however, the MTD following 39-week treatment was established at 8 mg/kg bodyweight/day. Treatment related adverse effects mostly occurred at doses exceeding the recommended starting dose and were mainly reversible. The main target organs of toxicity in the dog are the gastrointestinal tract, the haematopoietic system and the liver.

Resistance has been observed in tumours of human patients treated with tyrosine kinase inhibitor and cell lines sensitive to tyrosine kinase inhibitors can be rendered insensitive by progressively increased doses. The CVMP expressed concern that cross-resistance between chemotherapy and tyrosine kinase inhibitors could not be ruled out and an appropriate sentence is included in the SPC and product literature.

The proposed dose of 12.5 mg masitinib/kg bodyweight/day is based on the MTD established in the 4-week and 13-week toxicity studies The CVMP expressed concern about this proposed dose taking into account that when using the MTD for dose determination, no safety margin exists. Also, the recommended dose of 12.5 mg/kg is only supported by studies up to 13-weeks; whereas in a 39 week study 8 mg/kg bodyweight was established as MTD. The efficacy of lower doses and alternative dose intervals were not explored by the applicant.

The pivotal multicentre, randomised, placebo-controlled field study was conducted in USA and France in dogs suffering from mast cell tumours grade 2 or 3. Dogs were treated with an estimated dose of 12.5 mg masitinib/kg bodyweight once daily for 6 months and compared to an untreated (placebo) control group. In cases of severe adverse reactions, the dose was reduced to 9 mg/kg/day or to 6 mg/kg/day. For the entire study population, only a modest postponement of the time until the mast cell tumour progresses (TTP) of approximately 40 days could be demonstrated.

A subgroup-analysis was conducted comparing treatment results from dogs that are carriers of a mutated c-Kit and dogs that are carriers of the wild-type c-Kit. A prolongation of TTP was seen in the mutated c-Kit carrier group (approximately 25% of dogs), whereas masitinib did not show any clinically relevant effect in the overall population of dogs carrying the wildtype c-Kit, as compared to placebo.

Overall, CVMP considered that the results were not conclusive. In addition, in the absence of suitable diagnostic tools, differentiation of the subpopulations of dogs would currently not be possible for the veterinarian under practice conditions.

Results from the pivotal field study showed that treatment with masitinib is associated with adverse events of a frequency and magnitude that potentially reduce the quality of life of the animal patients. Since the clinical signs of the adverse reactions were similar to those related to the disease, the clinical condition of dogs could potentially worsen under the treatment. Furthermore, gastrointestinal disturbances caused by the treatment may hamper the possibility to provide the dog with essential analgetic/anti-inflammatory treatment. This was of serious concern, in particular when considering the proposed life-long treatment.

A proposal by the Applicant to use masitinib as a general treatment in dogs with grade 2 or 3 mast cell tumours, non resectable or recurrent after surgery, and to continue the treatment for the rest of their lives independently from the tumour response, was not accepted by the CVMP for animal welfare reasons; since a large number of animals may encounter severe adverse effects without any expected efficacy.

5. BENEFIT – RISK BALANCE

Benefits:

Based on the field study, a modest postponement of the time until the mast cell tumour progresses (TTP) of approximately 40 days could be demonstrated in the overall study population as compared to placebo. A retrospective subgroup-analysis of the field study indicated that this effect might be due to a small sub-population of dogs with mutated c-Kit.

Risks:

Treatment is connected with a significant risk for occurrence of adverse events of a similar character as disease symptoms. In addition, CVMP considered that treatment did not reduce the need for supportive co-medication and that the ability to provide necessary analgetic/anti-inflammatory treatment may be jeopardised due to occurrence of treatment related gastrointestinal events. Thus, treatment is likely to be associated with a reduction rather than a maintenance or improvement of the dogs´ life quality.

There is limited information available of the metabolic pathway, which is of concern because of the potential for drug-drug interactions, which are common in tumour patients that often receive concomitant treatment with other medicines.

In humans, masitinib is a strong skin sensitiser and a severe eye irritant. Reduced female fertility and embryotoxicity was observed in laboratory animals and there are concerns in regard to female fertility. Following oral administration of masitinib, gastrointestinal reactions and changes in laboratory parameters have been reported in people. Accidental oral ingestion by a child is considered a potential risk because of the low margin of safety; however, attention was given in the product development to user safety by providing the product as film-coated tablets and with a child-proof container.

Benefit-Risk-Balance:

A number of risks highlighted above might be addressed / managed by appropriate warnings in the product literature (e.g. user safety).
However, satisfactory benefits of treatment for the entire proposed population have not been demonstrated and significant risks for the treated animals, constituted by potentially long lasting adverse events, are expected and this is not acceptable in view of an intended life-long treatment.
Thus the benefit-risk-balance was considered negative.

6 RECOMMENDATIONS

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded in May 2008, that the quality of Masivet was considered to be in accordance with the requirements of Directive 2001/82/EC of the European Parliament and of the Council, as amended.

However, the Committee considered that the benefits of the product in terms of a limited delay in the time-to-tumour-progression (TTP) in the proposed dog population were outweighed by the risks in terms of the frequent occurrence of adverse reactions that were considered likely to decrease the quality of life of treated dogs. The overall benefit-risk balance was, therefore, considered negative and, in line with Directive 2001/82/EC of the European Parliament and of the Council, as amended, the CVMP recommended at their meeting in May 2008 the refusal of the granting of the Marketing Authorisation for Masivet.

7. RE-EXAMINATION OF THE CVMP OPINION OF 15 MAY 2008

Following the CVMP conclusion that the benefit-risk balance of masitinib in the treatment of dog mast cell tumours, Grade 2 or 3, non resectable or recurrent after surgery was unfavourable, due to concerns about target animal tolerance and a lack of efficacy. The applicant requested a re-examination and provided detailed grounds for their request for re-examination.

During the re-examination, the CVMP consulted an Ad-Hoc Expert Group (AHEG), specially convened for this re-examination. The group concluded that the data available would not be suitable to support all the indications proposed by the applicant but that there was some evidence to support the use of masitinib in a small, well-defined subgroup of dogs, for which the tolerance data would also be acceptable.

Ground 1 for refusal: Target Animal Tolerance

The applicant acknowledged that dogs treated with masitinib had a higher occurrence of side effects than the placebo group. However, gastrointestinal events (vomiting, diarrhoea) were mainly mild to moderate in intensity and transient. Treatment discontinuations and dose reductions due to adverse events were only slightly more frequent under masitinib treatment than under placebo, suggesting that the product was well tolerated. Although gastrointestinal adverse reactions were frequent, they rarely led to treatment discontinuations or dose reduction. The CVMP noted that animals used in clinical studies did not receive additional (antiemetic) treatment which is, however, expected to be administered under practical conditions and also that most of the gastrointestinal reactions were mild to moderate. The Committee agreed that in view of the severity of the underlying disease, such adverse events could be considered acceptable in cases where treatment would be effective.

In relation to the concern of the impact of gastrointestinal events on the possibility to receive concomitant treatment, the applicant highlighted that such medicines e.g. NSAIDs, were similar in dogs receiving placebo or masitinib. The CVMP agreed with this argument and concluded that Masivet treatment did not appear to significantly adversely impair the use of NSAIDs or analgesics and also noted from that gastrointestinal adverse events did not usually result in treatment discontinuation or dose reduction.

During the pivotal field study, other adverse reactions were identified such as protein-loss syndrome, haemolytic anaemia and neutropenia. The Applicant, therefore, suggested a number of specific safety warnings, monitoring recommendations and contraindications to be included in the SPC and product literature. The CVMP considered that the (revised) management proposals included in the SPC and product literature may be considered acceptable to address these concerns.

The applicant stated the perception of pet owners that quality of life would not deteriorate under treatment with masitinib. The CVMP, however, considered that only 18.4% of the masitinib treated animals had an "improved quality of life" (versus 36.8% described as "worse" or "unacceptable") and concluded that such data could only be considered as supportive.

The applicant also argued that the safety profile of Masivet would be equal to or better than other conventionally used medication. However, the CVMP did not support this argument as the products are all non-licensed for the treatment of mast cell tumours in dogs.

The CVMP expressed concern that an accurate dosing with the proposed recommended dose of12.5 mg/kg bodyweight (and reduced doses) would not be possible in all dogs of lower bodyweight). The proposed use of the RTD in dogs of less than 15 kg bodyweight would result in a dose range of 7.5 to 15 mg/kg bodyweight. In the absence of a lower strength tablet formulation, the CVMP, therefore, added information on an acceptable dose range for the three different dose regimens and also included a warning in relation to dogs with a body weight of less than 15 kg. In addition, the applicant provided a commitment to introduce a lower strength dosage form.

Having taken account of the above concerns and the recommendations reached by the AHEG, the CVMP makes the following recommendations in relation to tolerance and safety:

- Life-long treatment with masitinib is only justified in dogs in which treatment is deemed a success (i.e. the disease remains static or goes into remission). Based on the data currently available, the tolerance profile for life-long administration of masitinib could only be considered satisfactory in dogs expressing the mutated Kit receptor.
- Although occurring at a high incidence, gastrointestinal adverse reactions may be considered acceptable in the majority of cases in terms of severity and duration. In particular, prolonged diarrhoea may not cause owners to cease treatment in the majority of cases treated. An analysis of the data suggests that most adverse gastrointestinal events occur between days 30-90, when histamine and other mediators may be released from mast cells undergoing apoptosis. The incidence and severity of such reactions appeared to decline significantly after day 90. In addition, masitinib therapy is unlikely to interfere with the administration of concomitant medicines based on the data submitted, including possible anti-emetic therapy. The SPC has been amended to address the risks of co-administration with other drugs with high protein binding capacity.

The CVMP, therefore, concluded that the tolerance profile for masitinib could be considered acceptable subject to a restriction of the indications (use in dogs with the mutated Kit receptor only), a positive response to response to treatment within 4-6 weeks after treatment begin (i.e. static disease or partial/complete resolution), and clear warnings / monitoring recommendations in the SPC.

Ground 2 for refusal: Lack of Efficacy

The applicant highlighted that for the entire study population masitinib showed significant superiority over placebo on time to progression (TTP) with a median TTP of 118 days under masitinib versus 75 days under placebo (p=0.038). The CVMP agreed with the applicant that masitinib was superior to placebo on TTP; however, differences remained in terms of the significance of these benefits.

The Committee re-iterated its previous position that this modest postponement of approximately 40 days of TTP would not be acceptable (for the entire study population). However, results from subgroup analysis indicated that the overall moderate treatment effect seemed to be dependent on a small proportion of dogs.

The applicant proposed a reduced claim for Masivet, i.e. to (grade 2 or 3) non-resectable mast cell tumours independent of the presence of a mutated c-Kit on the basis of the outcome of TTP assessment for this population. However, the CVMP considered the benefit-risk balance for this proposed target population could not be regarded positive. However, a positive benefit-risk balance was accepted for those dogs with non-resectable mast cell tumours that also express a mutated tyrosine kinase c-kit receptor, since in these dogs a clear positive effect of treatment was demonstrated (median TTP of 241 days for Masivet treated dogs as compared to 83 days for placebo; p=0.003).

The applicant also presented an overview of some additional long-term efficacy data (tumour response/progression and survival over 12 and 24 month). However, the new data had not been assessed and were not considered subject of a re-examination procedure.

The issue of restricting the use of the product to a sub-group of dogs formed the basis of questions to both the applicant and the AHEG. The Committee expressed concerns regarding the possibility of identification of dogs with mutated c-Kit tumours and the definition of non-resectable tumours under practical conditions.

The AHEG agreed that for any mast cell tumour treatable by surgery, surgery should be the first choice of treatment. This would also apply for tumours recurrent after surgery and masitinib treatment should be restricted to non-resectable tumours. However, the group also concluded that only in the subgroup of dogs expressing the mutated c-Kit tyrosine kinase receptor treatment might be effective, i.e. either in the control of a tumour (no tumour progression) or treatment (partial/complete response).

A pivotal concern raised by the CVMP related to the determination of the genotype of the receptor under practical conditions, since such tests were currently not available in Europe. However, following consultation with the AHEG it appeared likely that such a test-kit for the c-Kit analysis (PCR) might be available in near future and that most laboratories should be able to offer this service. In addition, the applicant provided a commitment to facilitate the development of a commercially available test kit to identify dogs with c-Kit mutations and also recent literature reference of such a test.

Having taken account of the recommendations reached by the AHEG and the responses from the Applicant, the CVMP agreed that:

- The proposed initial indications i.e. the treatment of mast cell tumours, Grade 2 or 3, non-resectable or recurrent after surgery (“full claim”) or the proposal by the applicant for a reduced claim (treatment of mast cell tumours, Grade 2 or 3, non-resectable) are not acceptable based on the data currently available.
- The data, however, indicate a positive effect for masitinib therapy in the treatment of mast cell tumours when restricted to dogs expressing the mutated c-Kit receptor.
- The presence of a mutated tyrosine kinase Kit receptor should be confirmed prior to treatment.
- The continued, potentially life-long, use of masitinib can only be recommended in those dogs in which a positive effect of treatment is observed i.e. dogs with mutated Kit receptors that exhibit evidence of partial/complete resolution or static disease.
- A commercial test for the presence of the mutated Kit receptor is currently not available in Europe, but expected to become commercially available in the near future.

Overall conclusion on grounds for re-examination

The CVMP considered the recommendations reached by the AHEG and the responses from the applicant, and came to the following conclusions. Since the disease is a life threatening condition and the overall number of dogs suitable for treatment is expected to be low, the CVMP applied the CVMP guidelines on “Minor-Use-Minor-Species (MUMS) data requirements”.

Side effects / tolerance following treatment with Masivet were considered acceptable for dogs that are likely to exhibit a positive response to treatment expressed as a postponement of disease progression.. Such positive response is only considered likely in a small subgroup of dogs with "non-resectable grade 2 or 3 mast cell tumours with confirmed mutated c-kit tyrosine kinase receptor". The presence of a mutated tyrosine kinase Kit receptor should be confirmed prior to treatment and treatment results should be reviewed after 4 to 6 weeks in order to assess the initial response. A commercially available test Kit is expected to become available in the near future. In addition, clear warnings and monitoring recommendations have been included in the SPC and package leaflet.

Some concern remained in relation to the recommended dose. In view of a missing safety margin, and difficulties in the accurate dosing of dogs with less that 15 kg bodyweight, additional warnings were included to the product literature and an acceptable dose range (rather than a fixed dose) was mentioned. In addition, the applicant committed to provide a lower strength dosage form.

Duration of treatment depends on the response to treatment. Treatment should be maintained until disease progression is noted, provided that the product is sufficiently well tolerated. In case of tumour progression, treatment is unlikely to be successful and the treatment should be reviewed.

5. CVMP's CONCLUSION ON BENEFIT/RISK AND RECOMMENDATION:

The CVMP considered the available data submitted in the marketing authorisation application, the CVMP guidelines on "Minor-Use-Minor-Species (MUMS) data requirements", the applicant's detailed grounds for the re-examination and responses to the CVMP's List of Questions, the report from the AHEG meeting, and the oral explanations provided by the applicant.

The Committee's conclusions by majority decision at the end of the re-examination procedure were that the benefit-risk for Masivet was considered acceptable in a restricted indication for dogs in a sub-group that exhibit a positive response to treatment (i.e. treatment of non-resectable dog mast cell tumours (Grade 2 or 3) with confirmed mutated Kit tyrosine kinase receptor) and, therefore, recommended the granting of a marketing authorisation for Masivet.