14 September 2017
EMA/641255/2017
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Masipro (RFD)

International non-proprietary name: masitinib

Procedure No. EMEA/H/C/004159/0000

Note

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

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List of abbreviations

AB1010	Masitinib mesylate
ABL-1	Abelson Murine Leukemia-1
AE	Adverse Event
AFIRMM	*Association Française pour les Initiatives dans la Recherche sur le Mastocyte et la Mastocytose*
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ASM	Aggressive Systemic Mastocytosis
(A)SMAHNMD	(Aggressive) Systemic Mastocytosis Associated clonal Hematologic Non-MC-lineage Disease
ASMF	Active Substance Master File = Drug Master File
AST	Aspartate Aminotransferase
Bid	bis in diem/twice a day
BSA	Body Surface Area
C-Findings	Clinical Findings
CDR	Central Documentation Review
CM	Cutaneous mastocytosis
CRF	Case Report/Record Form
CS&E	Clinical Safety and Epidemiology
CR	Clinical Research
CYP	Cytochrome P450
DLT	Dose Limiting Toxicity
DSC	Differential Scanning Calorimetry
EC	European Commission
ECOG	Eastern Cooperative Oncology Group
ECG	Electrocardiogram
EGFR	Epidermal Growth Factor Receptor
EU	European Union
FcR	Fragment Fc Receptor
FLT3	FMS-like tyrosine kinase 3
FGFR3	Fibroblast Growth Factor Receptor 3
FIP1L1	Factor Interacting with PAP like 1
FIS	Fatigue Impact Scale
FSH	Follicle Stimulating Hormone
GC	Gas Chromatography
GCP	Good Clinical Practice(s)
GEE	Generalized estimating equation
GIST	Gastro Intestinal Stromal Tumor
HAMD	Hamilton Rating scale for Depression
HCl	Hydrobhloric acid
HDPE	High Density Polyethylene
HGF	Hematopoietic Growth Factor
HPLC	High performance liquid chromatography
IC50	Inhibitory Concentration 50
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP	Inductively coupled plasma Optical Emission spectrometry
IEC	Independent Ethics Committee
IFN	Interferon
IGF1R	Insulin-like Growth Factor 1 Receptor
IMP	Investigational Medicinal Product
IR	Infrared
IRB	Institutional Review Board
ISM	Indolent Systemic Mastocytosis
ISMwH	Indolent Systemic Mastocytosis with Handicap
JAK2	Janus Kinase 2
JM	Juxtamembrane
KL	Kit Ligand

LH	Luteinizing Hormone
LC-MS	Liquid chromatography mass spectrometry
LDPE	Low density polyethylene
LOCF	Last Observation Carried Forward
Lyn	Lck/Yes novel tyrosine kinase
MC	Mast Cells
MCGF	Mast Cell Growth Factor
MCL	Mast Cell Leukemia
MTD	Maximum Tolerated Dose
NOAEL	No Observed Adverse Effect Level
OPA	Overall Patient Assessment
PDGF	Platelet-Derived Growth Factor
Ph. Eur.	European Pharmacopoeia
PKC	Protein Kinase C
p*h	Patient * handicap
p.o.	per os/ by mouth/orally
RTK	Receptor Tyrosine Kinase
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SCF	Stem Cell Factor
SL	Steel Factor
SM	Systemic Mastocytosis
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SRC	Steroid Receptor Coactivator
SSM	Smouldering Systemic Mastocytosis
UP	Urticaria Pigmentosa
uPLC/UV	ultra-high performance liquid chromatography with ultra-violet detector
uPLC/MS	ultra-high performance liquid chromatography with mass spectrometry detector
VEGFR	Vascular Endothelial Growth Factor Receptor
WBC	White Blood Cell Count
WHO	World Health Organization
WT	Wild-type

1. Background information on the procedure

1.1. Submission of the dossier

The applicant AB Science submitted on 27 April 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Masipro, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 February 2015.

Masipro was designated as an orphan medicinal product EU/3/04/242 on 16 November 2004 in the following condition: treatment of mastocytosis.

The applicant applied for the following indication:

- treatment of adult patients with smouldering or indolent systemic severe mastocytosis unresponsive to optimal symptomatic treatments.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that masitinib was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0093/2015 on the granting of a (product-specific) waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance masitinib mesylate contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Scientific Advice / Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 28 July 2005, 21 September 2006, 20 October 2011, 25 September 2014, 23 October 2014, 26 February 2015, 23 April 2015, 25 June 2015 and 24 September 2015. The Protocol Assistance pertained to the quality, non-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Bjorg Bolstad Co-Rapporteur: Filip Josephson

- The application was received by the EMA on 27 April 2016.
- The procedure started on 19 May 2016.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 5 August 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 8 August 2016. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 19 August 2017.
- During the meeting on 15 September 2016, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 December 2016.
- The following GCP inspection was conducted at the request of the CHMP and its outcome taken into consideration as part of the Safety/Efficacy assessment of the product:
  - A GCP inspection at 3 sites (Sponsor site in France and two clinical sites, one in France and one in the USA) between 29 September 2016 and 18 November 2016. The outcome of the inspection carried out was issued on 23 January 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 January 2017.
- During the PRAC meeting on 9 February 2017, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP.
- During the CHMP meeting on 23 February 2017, the CHMP agreed on a list of outstanding issues to be sent to the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 22 March 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 7 April 2017 and 12 April 2017.
- During the CHMP meeting on 20 April 2017, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.

- During the CHMP meeting on 18 May 2017, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion recommending not granting a marketing authorisation to Masipro on 18 May 2017.

1.3. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Paula Boudewina van Hennik Co-Rapporteur: Natalja Karpova

- The applicant submitted written notice to the EMA on 31 May 2017 to request a re-examination of Masipro CHMP opinion of 18 May 2017.
- During its meeting on 22 June 2017, the CHMP appointed Paula Boudewina van Hennik as Rapporteur and Natalja Karpova as Co-Rapporteur.
- The applicant submitted the detailed grounds for the re-examination on 17 July 2017 (Appendix 2 of Final Opinion). The re-examination procedure started on 18 July 2017.
- The rapporteur's re-examination assessment report was circulated to all CHMP members on 16 August 2017. The co-rapporteur's assessment report was circulated to all CHMP members on 18 August 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's detailed grounds for re-examination to all CHMP members on 31 August 2017.
- During a meeting of the SAG on 04 September 2017, experts were convened to consider the grounds for re-examination The CHMP considered the views of the SAG/Expert group as presented in the minutes of this meeting.
- During the CHMP meeting on 12 September 2017, the detailed grounds for re-examination were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 14 September 2017, the CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the marketing authorisation.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Systemic mastocytosis, is a myeloproliferative disease characterized by infiltration of clonally derived mast cells in different tissues, including bone marrow, skin, the gastrointestinal tract, the liver, and the spleen. (Akin C, Metcalfe DD Annual Rev. Med. 2004; 55: 419-32; Bain BJ, Br J Heamatol 1999 106(1): 9-17). Different types of mastocytosis according to WHO include cutaneous mastocytosis; Indolent systemic mastocytosis (systemic mast cell disease); Systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease and aggressive forms such as agressive systemic mastocytosis, mast cell leukemia and mast cell sarcoma (P. Valent et al; Blood 2010 116:850-851).

The initially claimed indication was as follows: Masitinib is indicated for the treatment of adult patients with smouldering or indolent systemic severe mastocytosis unresponsive to optimal symptomatic treatments.

The Applicant subsequently revised the proposed indication to: Masipro is indicated for the treatment of adult patients with smouldering or indolent systemic mastocytosis with severe mediator release-associated symptoms unresponsive to optimal symptomatic treatments.

2.1.2. Epidemiology

Indolent systemic mastocytosis (including smouldering systemic mastocytosis) has an estimated European prevalence of 3.8 people per 100,000.

2.1.3. Biologic features

Mast cells play a pivotal role in the pathogeneses of mastocytosis. Normal mast cell growth, differentiation and survival are dependent on the ligation of stem cell factor (SCF) to c-Kit [Akin. C., Metcalfe D.D. Annual Rev. Med. 2004; 55: 419-32]. c-Kit, located on the surface of mast cells, is a single-spanning transmembrane protein possessing an intracellular tyrosine kinase domain, as well as 5 extracellular Ig-like domains that regulate ligand binding and receptor dimerisation. Stem cell factor is a haematopoietic cytokine that is the principal ligand of c-Kit. SCF binding to the extracellular domain of c-Kit triggers receptor homodimerisation leading to the activation of the intracellular kinase domain [Broudy, 1997]. Activated in this manner, c-Kit undergoes a series of autophosphorylation events causing a conformational change that permits the binding of key downstream signalling molecules, which in turn regulate mast cell function [Brockow, 2010]. Several gain-of function mutations of KIT (the gene that encodes for the protein c-Kit) have been described in mastocytosis, resulting in an ‘over active’ mast cell c-Kit receptor. The most common mutation is the Asp-816-Val (D816V) mutation in the kinase (phosphotransferase) domain of c-Kit. This mutation is associated with ligand-independent constitutive activation of c-Kit/SCF signalling, leading to uncontrolled mast cell proliferation, resistance to apoptosis and mediator release. Other c-Kit mutations have been observed in mastocytosis patients; however, their occurrence is far less frequent.

2.1.4. Clinical presentation, diagnosis and stage / prognosis

Mastocytosis is a heterogeneous condition with a large disease spectrum, ranging from indolent to aggressive forms of the disease. Patients can be classified depending on the degree of mast cell infiltration, mast cell activation, and the severity of symptoms. Of the adult patients that are diagnosed with systemic mastocytosis, 33% suffer from severe symptoms, whereas 67% suffer from non-severe symptoms. These symptoms typically include pruritus, flushing, syncope, hypotensive shock, dizziness, abdominal pain, nausea, vomiting, diarrhea, fatigue, memory loss, depression, tachycardia, palpitations, breathing difficulties, fractures/osteoporosis, and pain in the muscles, joints, and bones. Indolent patients can suffer from organ damage and a very wide variety of clinical manifestations. The associated mast cell mediator release symptoms endured by patients has a highly negative effect upon their quality-of-life to the point of being disabling [Hermine 2008].

The World Health Organization (WHO) has described an official standard for the classification and diagnosis of mastocytosis. According to the WHO guidelines, indolent systemic mastocytosis is defined as the excess of mast cells or abnormal mast cells in at least two organs. Diagnosis is based on histological and cytological analysis of the bone marrow.

In indolent systemic mastocytosis, around 90% of patients carry the gain-of-function Asp-816-Val (D816V) mutation in the kinase (phosphotransferase) domain of c-KIT [Hermine 2008]. The remaining 10% of adult patients display wild type (WT) c-Kit. The oncogenic driver in these patients is unknown, however it is said to be c-Kit dependent [Hermine 2008].

Patients with smouldering or indolent systemic mastocytosis have a normal life-expectancy.

2.1.5. Management

There are no registered treatments in the EU for patients with smouldering or indolent systemic mastocytosis.

This is a life-long condition and although the symptoms can be considered as handicaps most patients experience fluctuations in their symptoms. Thus although there is no urgent need for treatments for these patients, there is an unmet medical need in patients who do not adequately respond to existing symptomatic treatments.

Indolent systemic mastocytosis (including smouldering systemic mastocytosis) has an estimated European prevalence of 3.8 people per 100,000.

About the product

Masitinib is a tyrosine kinase inhibitors (TKI) for which the foremost cellular target is the mast cell, which plays a crucial role in the pathogenesis of mastocytosis. Masitinib is proposed to be able to regulate mast cell activity mainly due to its inhibitory potential against c-Kit, Lyn and Fyn [P. Dubreuil et al, Pone 2009], all of which are suitable targets for indolent forms of mastocytosis. These kinases are highly expressed in mast cells and control many essential cell functions including mast cell growth, differentiation, survival and degranulation. Masitinib is proposed to reduce the activation of mast cells mainly via its ability to inhibit WT c-Kit. Additionally, through its inhibition of Lyn and Fyn masitinib can reduce mast cell degranulation. Lyn and Fyn are key components of the transduction pathway leading to IgE induced degranulation [Gilfillan, 2006; Gilfillan, 2009]. Masitinib has been demonstrated to inhibit, in a dose-dependent manner, the release of both histamine and TNF-α by mast cells [Dubreuil, 2009].

In addition, masitinib also inhibits mutant c-Kit receptors, including mutations in the extracellular (exon 9) and juxtamembrane (JM) (exon 11) regions.

Masitinib under the tradenames Masican and Masiviera has previously applied for MA for the treatment of GIST and pancreatic cancer, respectively. Both applications concluded negatively. Masitinib has an MA for the treatment of mast cell tumours in dogs (tradename Masivet).

2.2. Quality aspects

2.2.1. Introduction

The finished product was proposed as film-coated tablets containing 100 mg and 200 mg of masitinib (as mesylate) as active substance.

Other ingredients were: microcrystalline cellulose, povidone, crospovidone, magnesium stearate, polyvinyl alcohol, titanium dioxide, macrogol, talc and sunset yellow lake (E110).

The proposed packaging was high density polyethylene (HDPE) bottles with child resistance closures.

2.2.2. Active Substance

An ASMF for masitinib mesylate was submitted by Excella GmbH. A letter of access to the ASMF in relation to the application for the proposed 100 mg and 200 mg film-coated tablets was provided. The discussion below refers to this source alone, as it is the only proposed for marketing. Masitinib mesylate is considered by the Applicant to be a new active substance.

General information

The chemical name of masitinib mesylate is 4-[(4-methyl-piperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)-1,3-thiazol-2-yl]amino}-phenyl)benzamide, methane sulphonic acid salt and has the following structure:

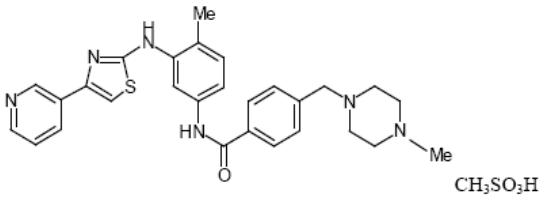


Figure 1 Structure of masitinib mesylate

The molecular structure of masitinib mesylate has been confirmed by elemental analysis, UV spectroscopy, IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR, and LC-MS using a reference batch of active substance.

Masitinib mesylate is a white to pale yellow powder, slightly hygroscopic, practically insoluble in acetone, slightly soluble in ethanol, sparingly soluble in methanol. The molecular structure does not contain asymmetric carbon atoms.

Three polymorphic forms of masitinib mesylate were identified by differential scanning calorimetry and X-ray spectrometry. It has been demonstrated that masitinib mesylate consistently manufactured by the proposed manufacturer is the polymorphic Form DRX1, anhydrous which is the most stable form. The polymorphic forms can be differentiated by melting point/range. Melting point is included in the active substance specification. Manufacture, characterisation and process controls.

The active substance is synthesised in a convergent synthesis consisting of several chemical steps, purification, salt formation followed by drying and sieving. The synthesis is comprised of 6 steps (with step 4 being divided into 3 sub-steps). Steps 1 to 4.1 are synthetic steps (bond breaking/formation), steps 4.2 to 6 comprise purification and salt formation.

The proposed starting materials comply with the general principles for selection of the starting materials as outlined in ICH Q11 and the EMA reflection paper and were found to be acceptable. The information provided on the route of synthesis of the starting materials allows for an adequate assessment of the impurities arising from their synthesis. Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Comprehensive details and discussion are provided on impurities. Potential and actual impurities were well discussed with regards to their origin. Specified impurities are controlled in compliance to the ICH Q3A guideline. Several mutagenic impurities were identified and control strategies in accordance with ICH M7 guideline have been proposed for these impurities.

The active substance is packed in double LDPE bags which comply with EC 10/2011 and with the Ph. Eur. monograph 3.1.4.

Specification

The active substance specification includes tests for appearance (colour), identity masitinib (IR, UPLC/UV) identity mesylate ion (^{1}H-NMR), melting point (DSC), water content (Ph. Eur.), sulphated ash (Ph. Eur), heavy metals (Ph. Eur.), related substances (UPLC/UV), residual solvents (GC), microbiological purity (Ph. Eur), particle size distribution (laser diffraction), assay masitinib (UPLC/UV)

and assay mesylate ion (titration). In addition, tests for the metal impurities and tests for a number of potential mutagenic impurities (GC - UPLC/MS) are included.

The analytical methods used have been adequately described and the non-compendial methods appropriately validated in accordance with the ICH guidelines.

The specifications and their limits are considered to be acceptable and have been appropriately justified. The potential formation of impurities has been investigated and appropriate specifications have been set.

Batch analysis data on 3 production scale batches of the active substance were provided. The batches were all analysed according to the analytical methods presented in the dossier. The results were within the proposed specifications and consistent from batch to batch.

Stability

Stability data on three production scale batches of the active substance from the proposed manufacturer stored for 12 months under long term conditions at 25 ºC / 60% RH and for 6 months under accelerated conditions at 40 ºC / 75% RH were provided. These batches were tested according to the specifications and with the analytical methods presented. No trend was observed and no significant differences between room temperature and accelerated conditions occurred.

A forced degradation study was conducted on one batch of masitinib mesylate in solid state (at high temperature) and in aqueous solution (at high temperature, UV, acidic, alkaline and oxidative conditions). The impurity test method used for release testing was used. In oxidative conditions masitinib showed nearly complete degradation. Some degradation was observed at aqueous high temperature (80 °C) UV and acidic conditions. There was no significant degradation in solid state at high temperature (120°C) or in aqueous alkaline conditions.

Two batches of masitinib mesylate were exposed to light for about 24 h (1.2 MioLux/h), according to ICH guideline Q1B. This photostability study revealed no significant changes due to the UV-light exposure; masitinib mesylate appears not to be photosensitive.

Additional stability data on six batches of the active substance manufactured before certain changes to the manufacturing process of the active substance were implemented were also provided. These batches were tested according to the specifications and using the analytical methods in force at the time of the studies. The results from long-term stability studies at 25 ºC / 60% RH (3 batches x 60 months and 3 batches x 48 months) as well as from the accelerated stability studies at 40 ºC / 75% RH (6 batches x 6 months) show that the active substance is stable, all parameters comply with the established specification limits; no trend is observed and no significant differences between room temperature and accelerated conditions occurred.

Overall the batch data provided justify the retest period of 48 months for the masitinib mesylate active substance.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The finished product was proposed as light-orange, capsule-shape, film-coated tablets containing 100 mg or 200 mg of masitinib (as mesylate) as active substance.

The excipients are microcrystalline cellulose (Avicel pH101 and pH200), povidone, crospovidone type A and magnesium stearate. The film coat contains polyvinyl alcohol, titanium dioxide, macrogol, talc and

sunset yellow lake (E110). The two strengths of the formulation are manufactured from one common blend.

The proposed container closure system was an HDPE bottles, 30 tablets per bottle, closed with a childproof closure system.

The pharmaceutical development for masitinib 100 mg and 200 mg film-coated tablets has been adequately described. The aim was to develop an immediate release dosage form of masitinib.

Masitinib mesylate exists in three polymorphic forms. Polymorphic form DRX 1, the most stable form, is used to manufacture the finished product. This form exhibits a pH-dependant solubility profile across the physiological pH range with highest solubility at low pH (34 g/l at pH 1.3). The particle size of the drug substance is reduced by impact milling and is controlled in the active substance specification.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The compatibility of the active substance and excipients was investigated. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

In early clinical development masitinib 50 mg capsules were used. Later on, an immediate release tablet was developed as the commercial formulation. Bioequivalence has been demonstrated between the phase 1 capsule formulation (2 x 50 mg) and the tablet formulation (1 x 100 mg). The applicant provided detailed information on the selection of dissolution method to bridge between the clinical tablet formulation and the proposed commercial tablet formulation. Since the tablets used during phase 2 and phase 3 studies have the same composition and are manufactured by a simple manufacturing process, the bridging between clinical formulations and the proposed commercial formulation, supported by relevant dissolution data, was considered acceptable.

A major objection was initially raised relating to the discriminatory power of the dissolution method proposed for quality control testing. The applicant subsequently submitted additional experimental data justifying the selection of the dissolution test conditions (i.e. paddle speed, medium, pH) and demonstrating the discriminatory power of the dissolution test and proposed specification limits (by testing batches of uncoated tablets formulated without disintegrant and testing batches manufactured with variable granulation parameters (equipment, granulation times, blade speed, water content and subsequent hardness). The major objection was considered to be resolved.

The primary packaging is HDPE bottles closed with a polypropylene child-resistant closure with an induction sealed aluminium/polyethylene liner. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. The material complies with Ph.Eur. and EC requirements. During storage the tablets are in contact with the protective polyethylene liner, however after opening, the polyethylene liner is removed and the tablets are in contact with a waxed white pulp board attached to the polypropylene closure. A specification for the waxed white pulp board liner has not been provided by the applicant at the time of opinion.

Manufacture of the product and process controls

The manufacturing process consists of 8 main steps: weighing, preparation of binder solution, granulation, drying, milling, compression, tablet coating and packaging. The manufacturing process is considered a standard manufacturing process.

Based on the submitted batch data the batch size ranges are considered approvable. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form. Process

validation for three production scale batches has been carried out on some steps only. In response to questions from CHMP, the applicant updated the process validation protocol to include all relevant steps in the manufacturing process. The updated process validation protocol is considered acceptable.

Product specification

The finished product release specification include appropriate tests for this kind of dosage form, such as appearance, identification (HPLC, UV), uniformity of dosage units by mass variation (Ph. Eur.), average weight, dissolution (Ph. Eur), moisture content (Ph Eur.), impurities (HPLC), microbiological quality (Ph. Eur.) and assay (HPLC).

The specifications have been justified in accordance with relevant guidelines and pharmacopoeial requirements. The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for three batches of 100 mg tablets and six batches of 200 mg tablets manufactured at the proposed commercial manufacturing site, at commercial scale, confirming the consistency of the manufacturing process.

Stability of the product

Stability data of two batches of the 100 mg tablets and six batches of the 200 mg tablets (all at commercial scale) stored under long term conditions at 25ºC / 60% RH for up to 48 months and for up to 6 months under accelerated conditions at 40ºC / 75% RH according to the ICH guidelines were provided. Samples were tested for appearance, moisture content, hardness, assay, impurity content, dissolution and microbial testing.

The batches of medicinal product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing. The stability data presented do not show degradation in any of the batches presented under any condition.

Force degradation was carried out under various stress conditions as part of the analytical validation. Degradation was observed under acidic, alkaline and oxidative conditions. Satisfactory mass balance data showed that the analytical procedure for impurities is stability indicating.

In addition, a photostability study as per ICH Q1B conducted on one batch of tablets from each strength showed a slight fading of the colour of film-coating; however the proposed HDPE primary packaging offers sufficient protection from light exposure. No change was observed in any of the other parameters tested.

A holding time study was conducted for each dosage strength on one batch of core tablets and one batch of film-coated tablets packaged and stored in bulk in PE bags. The holding time of 1 month before coating step and 6 months as coated tablet was confirmed.

Based on available stability data, the shelf-life of 36 months when stored in the original container to protect from light are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used. It is confirmed that the raw materials used for the production of magnesium stearate are of synthetic or plant origin.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

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

At the time of the CHMP opinion, there was a minor unresolved quality issue having no impact on the Benefit/Risk ratio of the product:

- A specification for the liner of the bottle cap consisting of waxed white pulp board should be established. The specifications should include a test for identification of the material, which is in contact with the finished product after removal of the protective liner.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. The only information missing from the quality part of the dossier is the specification for the liner of the bottle cap. This has no impact on the benefit/risk of the product.

2.2.6. Recommendation(s) for future quality development

A specification for the liner of the bottle cap consisting of waxed white pulp board should be established. The specifications should include a test for identification of the material, which is in contact with the finished product after removal of the protective liner.

2.3. Non-clinical aspects

2.3.1. Introduction

The nonclinical dossier was already been assessed in the context of previous MA applications for masitinib. It has been resubmitted with the addition of a new assessment of the primary and secondary pharmacodynamics, novel data on systemic metabolites in mouse and rat are assessed and taken into consideration for safety evaluation, novel data on repeat dose pharmacokinetics in mouse, rat and dog for the evaluation of the toxicology studies where the original toxicokinetic data were GLP non-compliant and the importance of the non-clinical safety profile in the current indication.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The proposed mode of action of Masitinib mesylate in the pathology of indulgent systemic mastocytosis is shown in the figure below:

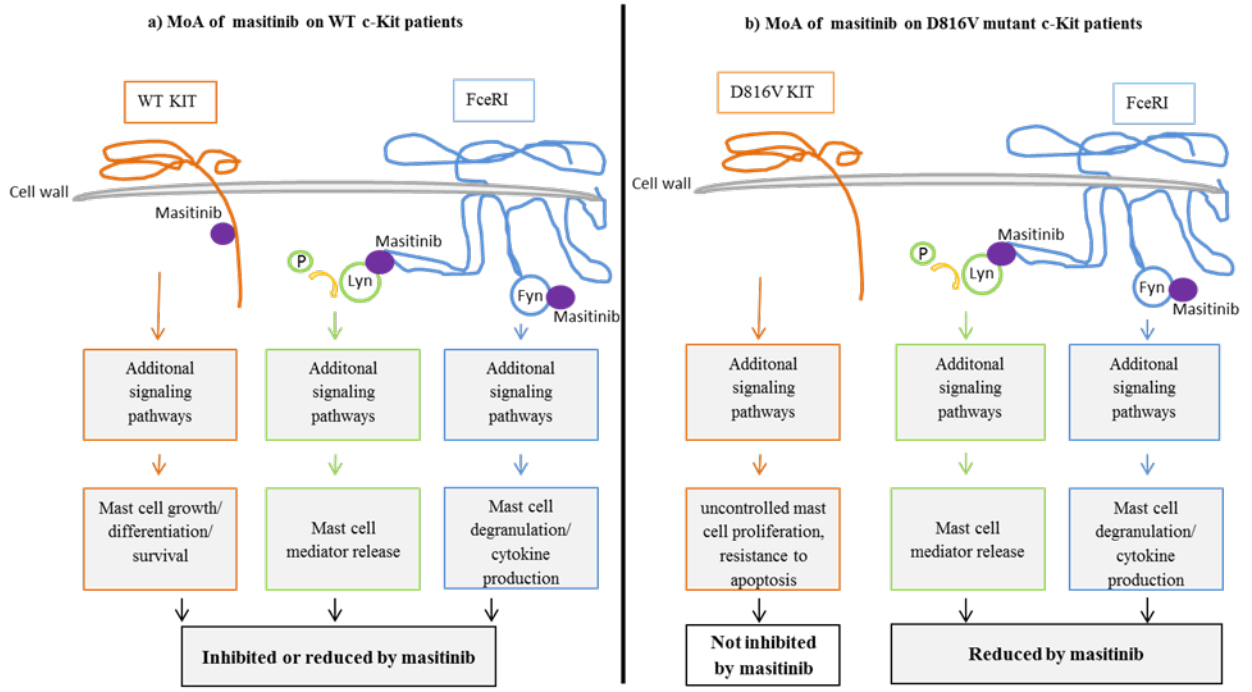


Figure 2 The c-Kit receptor is primarily responsible for mast cell growth, differentiation and survival with mast cell mediator release being initiated through the integration of downstream signaling pathways of c-Kit and FcεRI. D816V mutant c-Kit receptors result in uncontrolled mast cell proliferation and resistance to apoptosis. Masitinib inhibits WT c-Kit, Lyn and Fyn. In WT c-Kit mast cells (panel a) masitinib directly inhibits mast cell activation via inhibition of WT c-Kit, while mast cell mediator release and cytokine production is inhibited through targeting of Lyn and Fyn. In D816V mutant c-Kit mast cells (panel b) masitinib inhibits mast cell degranulation and cytokine production via Lyn and Fyn inhibition.

Primary pharmacodynamic studies conducted to demonstrate the mode of action of masitinib on the primary targets in indolent systemic mastocytosis, listed in the table below.

Table 1 Pharmacodynamic studies conducted to demonstrate mechanisms of action of masitinib in indolent systemic mastocytosis

Target	Type of study	Study title	MoA of masitinib
c-Kit D816v	Enzyme (*in vitro*)	Analysis of the kinase selectivity profile of masitinib (ABS AB1010 selectivity)	- Masitinib does not inhibit the D816V c-Kit mutation
	Cell (*in vitro*)	Inhibition of c-Kit phosphorylation by AB1010 (ABSMRS AB1010 Bioch 2)	
c-Kit WT	Enzyme (*in vitro*)	*In vitro* AB1010-mediated inhibition of c-Kit tyrosine kinase activity (Study N ABSMRS AB1010 Bioch(1))	- Masitinib selectively and potently inhibits WT c-Kit - Masitinib effectively inhibits the autophosphorylation of WT c-Kit
		In vitro selectivity of AB1010 for c-Kit (study No ABSMRS AB1010 Select)	
		Analysis of the kinase selectivity profile of masitinib (ABS AB1010 selectivity)	

	Cell (*in vitro*)	Inhibition of c-Kit phosphorylation by AB1010 (ABSMRS AB1010 Bioch 2)	- Masiinibs main active metabolite retains the activity and selectivity profile of AB1010
		Comparative study of efficacy and selectivity of AB1010 and its major metabolite AB3280 (Study No. ABSMRS AB1010 versus AB3280)	
		Comparative analysis of the kinase selectivity profile of masitinib and its competitors in clinical trials (unpublished report)	
Lyn	Enzyme (*in vitro*)	Analysis of the kinase selectivity profile of masitinib (ABS AB1010 selectivity)	- Masitinib selectively inhibits Lyn
Fyn	Enzyme (*in vitro*)	Analysis of the kinase selectivity profile of masitinib (ABS AB1010 selectivity)	- Masitinib selectively inhibits Fyn
Degranulation	Cell (*in vitro*)	Anti- degranulating activities of AB1010 on human normal mast cells (Study N ABSMRS AB1010 Degra)	- Masitinib blocks the release of histamine and TNFα by mast cells
		Masitinib (AB1010), a Potent and Selective Tyrosine Kinase Inhibitor Targeting KIT (Publication – Dubreuil 2009)	- Masitinib inhibits human mast cell degranulation, cytokine production.
Kinase selectivity	Enzyme and Cell (*in vitro*)	Study of the selectivity of AB1010, a potent inhibitor of c-Kit (ABSMRS AB1010 Select)	- Masitinib highly selective for c-Kit in comparison to other kinases

***In vitro* studies**

Analysis of the kinase selectivity profile of masitinib

Study ABS AB1010 assessed the effect of masitinib on the phospho-transfer activity of 50 different kinases.

Table 2 Masitinib IC_{50} values on a panel of 50 kinases

Kinase	IC_{50} (nM)	Kd (nM)*	Kinase	IC_{50} (nM)	Kd (nM)*
Receptor Tyrosine kinases			Src-family kinases		
ALK	>1000	na	FYN	240 ± 130	140
CSF1R	90± 35	7.6	HCK	2000 ± 200	690
FGFR1	>1000	na	LCK	300 ± 145	31
FGFR3	>1000	na	LYN	225 ± 40	61
FLT3	>1000	na	SRC	2300 ± 300	900
Kit	20 ± 2	8.1			
KIT D816H	2400	850			
KIT D816V	>1000	1300			
KIT T670I	>1000	na			
KIT V654A	75 ± 20	20			
MET	>10000	na			
PDGFRα	50 ± 17	25			
PDGFRα D842V	>10000	0			
PDGFRβ	110 ± 25	8.4			

*From Davis et al. 2011

Inhibition of c-Kit phosphorylation by AB1010 (Study No. ABSMRS AB1010 Bioch (2))

AB1010 was assayed in a variety of cellular systems to evaluate its activity in wild Type and mutant Kit. Phosphorylation of c-Kit was monitored by western blot analysis using anti-phosphospecific antibodies following cell treatment with various concentrations of AB1010.

Table 7: Inhibition of autophosphorylation of c-Kit by AB1010

Inhibition of autophosphorylation c-Kit Ba/F3	AB1010 IC_{50} [µM]
Ba/F3 m Kit Δ27	<0.1
Ba/F3 m Kit WT	<0.1
Ba/F3 m D814V	>10
Ba/F3 h Kit WT	<0.1
Ba/F3 h Kit D816V	>10

m: murine; h: human

These data demonstrate the activity against mouse and human WT c-Kit, and the murine Δ27 mutant. No activity was seen with the mutants D814V (murine) and D816V (human).

In vitro AB1010-mediated inhibition of c-Kit tyrosine kinase activity (Study No. ABSMRS AB1010 Bioch(1))

AB1010 was assayed *in vitro* for inhibition of c-Kit tyrosine kinase activity. Experiments were performed using the purified intracellular soluble domain (567_976) of c-Kit expressed in baculovirus

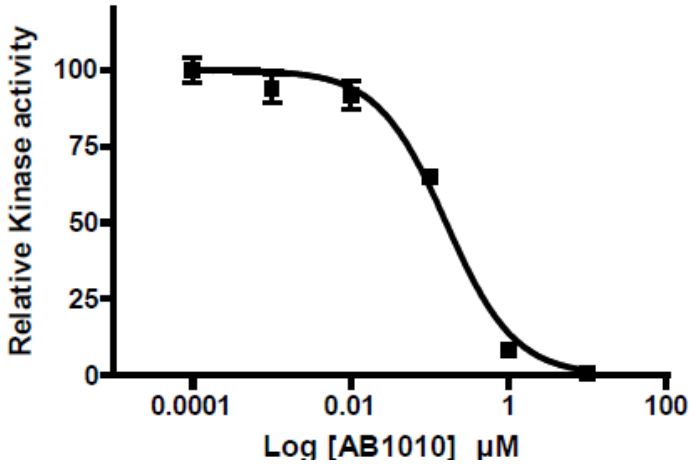


Figure 3 In vitro inhibition of the catalytic domain of c-Kit (JM and WT) tyrosine kinase by AB1010

In vitro selectivity of AB1010 for c-Kit (Study No. ABSMRS AB1010 Select)

The selectivity of AB1010 for c-Kit tyrosine kinases inhibition was investigated *in vitro* by using both an enzymatic purified kinase assay and a cell proliferation assay using Ba/F3 cell lines expressing various tyrosine kinases.

Table 3 Masitinib IC_{50} values using an enzymatic kinase assay and a cell proliferation assay

Enzyme inhibition

Enzymes	IC50 [µM]
c-Kit	0.2*
PDGF-beta	0.49
ABL1	5.7
VEGFR1	IC50 > 100
EGFR	IC50 > 100
FGFR1	IC50 > 100
FLT3	IC50 > 100
JAK2	IC50 > 100
AKT1	57
PKC-alpha	100
SRC	IC50 > 100
IGF1R	IC50 > 100
PIM1	19

Inhibition of cell proliferation

Cells lines	IC50 [µM]
Ba/F3 Kit	IC50= 0.15
Ba/F3 PDGFR	IC50<0.1
Ba/F3 p210Bcr-Abl	IC50=4
Ba/F3 EGFR	IC50≥1
Ba/F3 FGFR3	IC50≥1
Ba/F3 IL3	IC50>1
Ba/F3 FMS	IC50>1
Ba/F3 RET	IC50>1
Ba/F3 TRKB	IC50>1
Ba/F3 FGFR1	IC50>1
Ba/F3 FLT3 WT	IC50>1
Ba/F3 FLT3 ITD	IC50>1
Ba/F3 FLT3 D835V	IC50>1
Ba/F3Tel-JaK1	IC50>1
Ba/F3Tel-JaK2	IC50>1
Ba/F3Tel-JaK3	IC50>1
Ba/F3Tel-Abl	IC50>1

In the set of cell lines tested in the assay, masitinib only should submicromolar activity at c-Kit and the PDGF receptor.

Comparative study of efficacy and selectivity of AB1010 and its major metabolite AB3280 (Study No. ABSMRS AB1010 versus AB3280)

The major metabolite of masitinib formed in vivo is the N-desmethylated derivative, named AB3280. Its plasma level contributes between 6 to 30% to the total masitinib plasma level (AUC), depending on the species, and therefore its pharmacological profile is of interest. The activity of AB1010 and AB3280 on cell proliferation and cell survival in Ba/F3 cell lines expressing various receptor and non-receptor tyrosine kinases was studied.

Table 4 Masitinib and AB3280 IC_{50} values by a cell proliferation – cell survival assay

Cell line	IC_{50} [µM] AB1010	IC_{50} [µM] AB3280
Ba/F3 IL3	5	5
Ba/F3 h Kit	0.15	0.3
Ba/F3 m Kit delta27	0.005	0.015
Ba/F3 m Kit V558D	0.005	0.01
Ba/F3 h Kit D816V	5	5
Ba/F3 PDGFRβ	0.03	0.05
Ba/F3 p210Bcr-Abl	5	5
Ba/F3 p210Bcr-Abl T315I	8	10
Ba/F3 EGFR	2-5	8
Ba/F3 FGFR3	2.5-5	2.5
Ba/F3 FGFR1	2.5-5	2.5
Ba/F3 RET	7	7
Ba/F3 TRKB	5	4
Ba/F3Tel-JaK2	10	10

At the targets where masitinib showed activity (c-Kit, PDGF receptor) AB3280 showed similar activity as masitinb. AB3280 also showed a similar reactivity/non-reactivity to c-Kit mutants. No novel target was identified for AB3280.

Anti-degranulation activities of AB1010 on human normal mast cells (Study No. ABSMRS AB1010 Degra)

The activation and release of mast cell-derived mediators, including histamine and prostaglandins are said to cause many of the systemic manifestations associated with mastocytosis. This study assessed the anti-activating effect of masitinib on human normal mast cell activation. Cells were activated using anti IgE.

Table 5 Inhibition of histamine and TNF-α release (%) by human normal MC treated by AB1010

Concentration of AB1010 (µM)	1	0.1	0.01
Histamin release inhibition (%) Mean ± SD	25.0 ±12.2	7.1 ± 6.9	5.4 ± 7.3
TNF-α release inhibition (%) Mean ± SD	42.8 ± 20.1	14.1 ± 16.7	3.6 ± 8.9

Masitinib was shown to inhibit the release of main mediators of mast cell activation such as histamine and TNF-α.

Inhibition of human mast cell degranulation and cytokine production of mast cells

This study assessed masitinib's ability to inhibit the FcεRI-mediated degranulation of human cord-blood-derived mast cells. Comparative results to imatinib are presented in the figure below.

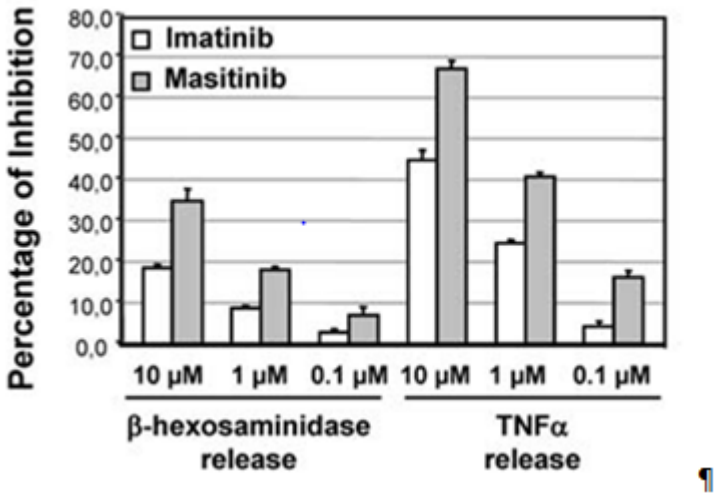


Figure 4 Inhibition of mast cell degranulation and cytokine production of mast cells

Anti-proliferative and pro-apoptosis of AB1010 on various mammalian cell lines expressing mutated and WT c-Kit (Study No. ABSMRS AB1010 Prolif)

The ability of AB1010 to inhibit cell proliferation was assessed in various mammalian cell lines, including Ba/F3, HMC1, TF1, KU812 cells and human T lymphocytes, using a thymidine incorporation assay and a colorimetric survival assay. Ba/F3 is an immortalised murine pro-B cell line. The study included a number of Ba/F3 expressing WT or mutant c-Kit where c-Kit is critical for proliferation HMC-1 is a human mast cell line, where sub-clones expressing mutated c-Kit have been isolated (see table below).

Table 6

Cell line	Inhibition of proliferation IC_{50} [µM] AB1010	Induction of apoptosis IC_{50} [µM] AB1010
Ba/F3 m/h c-Kit WT IL3	5	-
Ba/F3 m Kit Δ27 (exon 11)	0.005	<0.1
Ba/F3 m c-Kit WT	0.1	-
Ba/F3 m Kit V558D (exon 11)	<0.1	-
Ba/F3 m c-Kit D814V (exon 17)	>2.5	>5
Ba/F3 h c-Kit WT	0.15	0.2
Ba/F3 h c-Kit D816V (exon 17)	5	>5
Ba/F3 h c-Kit ins[6] 502 (exon 9)	0.1	--
Ba/F3 Bcr-Abl	4	
Ba/F3 EGF-PDGFRα	0.01	-
HMC-1 α155 (Kit V560G)	0.05	0.1
HMC-1 5C6 (Kit D816V)	5	>5
TF1	0.1	0.2
MO7e	0.15	-
BMMC (bone marrow derived mast cell line)	<0.25	-
Human T lymphocytes PHA	>5	-
Human T lymphocytes IL2	>5	-
PDGFRβ	0.00025	-
KU812	2	>5

These data demonstrate that masitinib acts on WT c-Kit and on several clinically occurring c-Kit mutations. However, no activity was seen at the c-Kit D816V mutation.

Anti-proliferative and pro-apoptosis activities of AB1010 on cell lines rendered resistant to STI571 (Study No. ABSMRS AB1010 Active on STI571 resistant cells)

This study was performed the test the ability of AB1010 to overcome resistance to STI571 (imatinib) in Ba/F3 cells expressing c-kit Δ27. Ba/F3 Δ27 were grown in sequentially increased concentrations of STI571 and a resistant cell line was obtained. The cell line retained sensitivity to apoptosis induced by AB1010 Similar findings were demonstrated using STI571 resistant HMC-1 α155 cells.

***In vivo* studies**

Antitumor activity study of AB1010 following per os administrations in Balb/c nude mice bearing subcutaneous transfected BA/F3 tumors (Study No. PRT/03104-1)

The anti-tumor activity of AB1010 was studied following oral administration in a nude mice model with a subcutaneous graft of transgenic murine hematopoietic cell line, Ba/F3, transfected with gene encoding Kit JM Δ27.

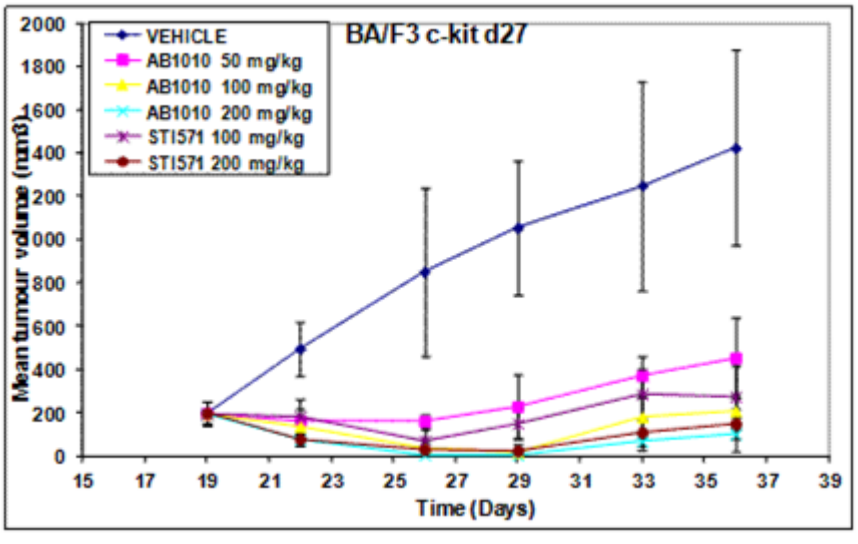


Figure 5 Anti-tumour activity in transfected Balb-c mice

Antitumor activity study of AB1010 following IP and per os administration in Balb/c nude mice, bearing subcutaneous transfected BA/F3 tumors (Study No. AB1010-vivo-1)

Irradiated mice were grafted with a subcutaneous injection in the right flank of 1.5x106 Ba/F3 c-Kit Δ27 cells. Treatment, with AB1010 at 30 mg/Kg (Group AB1010) or vehicle (Vehicle) by IP injection

twice a day every 12 hours began when tumors reached a mean volume of 400 mm3 on day 19 after graft. Mice were treated for 25 consecutive days. The treatment efficiency was assessed on day 29. The tumor growth inhibition (T/C) in the AB1010 group (30 mg/kg) was 24%. The median survival times for vehicle and AB1010 were 30.5 and 42 days, respectively.

In a further set of experiments, oral doses of AB1010 of 30 mg/kg and 45 mg/kg (twice daily) resulted in tumour growth inhibition of 24% and 21% respectively. A dose of 100 mg/kg bid resulted in completely blocked tumour growth.

The efficacy of masitinib in the treatment of canine Mast Cell Tumors (MCT)

In a phase III study in dogs with non-metastatic mast cell tumors, masitinib increased time to tumor progression.

Pharmacodynamic studies conducted to demonstrate the mode of action of masitinib on relevant targets in aggressive forms of mastocytosis, including JM c-Kit mutation, using as targets JM and EC (extracellular) mutants c-Kit, mast cell proliferation and imatinib-resistant cells.

Table 7 Masitinib's secondary mechanisms of action relevant to aggressive forms of mastocytosis (anti-proliferation and pro apoptosis of mast cells, including cells with JM and EC mutants c-Kit, WT c-Kit and imatinib-resistance)

Target	Type of study	Study title	MoA of masitinib
JM mutant c-Kit	Enzyme (*in vitro*)	*In vitro* AB1010-mediated inhibition of c-Kit tyrosine kinase activity (Study N ABSMRS AB1010 Bioch(1))	- Masitinib inhibits the autophosphorylation of JM mutant c-kit - Masitinib inhibits the proliferation of cell that express JM mutations
	Cell (*in vitro*)	Inhibition of c-Kit phosphorylation by AB1010 (ABSMRS AB1010 Bioch 2)	
		Anti-proliferative and pro-apoptosis activities of AB1010 on various mammalian cell lines expressing mutated and WT c-Kit (ABSMRS AB1010 Prolif)	
	Cell (*ex vivo*)	Anti-proliferative and pro-apoptosis activities of AB1010 on various mammalian cell lines expressing mutated and WT c-Kit (ABSMRS AB1010 Prolif)	
Proliferation	Cell (*in vitro*)	Anti-proliferative and pro-apoptosis activities of AB1010 on various mammalian cell lines expressing mutated and WT c-Kit (ABSMRS AB1010 Prolif)	- Masitinib inhibits the proliferation of cell expressing WT c-Kit
	Cell (*in vivo*)	Antitumor activity study of AB1010 following per os administrations in Balb/c nude mice bearing subcutaneous transfected BA/F3 tumors (PRT/03104.1)	- Masitinib inhibits tumor growth in murine models - Masitinib exhibited efficacy in

		Antitumor activity study of AB1010 following IP and per os administration in Balb/c nude mice bearing subcutaneous transfected BA/F3 tumors (AB1010-vivo-1)	the treatment of mast cell tumors (expressing either JM and EC mutants or WT c-Kit) in dogs
		The efficacy of masitinib in the treatment of canine Mast Cell Tumors (MCT) (AB04003; Publication - Hahn 2008)	
Imatinib-resistant cells	Cell (*in vitro*)	Anti-proliferative and pro-apoptosis activities of AB1010 on cell lines rendered resistant to STI571 (ABSMRS AB1010 Active on STI571 resistant cells)	- Masitinib inhibits proliferation and induces apoptosis in imatinib-resistant cells

The ability of AB1010 to inhibit cell proliferation was assessed in various mammalian cell lines, including Ba/F3, HMC1, TF1, KU812 cells and human T lymphocytes, using a thymidine incorporation assay and a colorimetric survival assay. Masitinib was a potent inhibitor of proliferation of cells expressing wild-type c-Kit, cKit mutated in exon 9 and 11 and an EGF-PDGFRβ construct as well as cells expressing PDGFRα.

Masitinib was less active in cells expressing c-Kit mutated in exon 17 (kinase domain) and IL-3 stimulated Ba/F3 cells expressing wild-type c-Kit. Overall, masitinib appears to be a more potent inhibitor of cells expressing wild-type as well as mutated c-Kit when compared to imatinib. Imatinib on the other hand was a more potent inhibitor of BCR-ABL stimulated cell proliferation when compared to masitinib.

Masitinib was a potent inducer of apoptosis in cells expressing wild-type c-Kit and c-Kit with mutations in the juxtamembrane domain (Ba/F3 c-Kit Δ27, HMC-1 α155 V560G) while cells expressing c-Kit mutated in the catalytic domain (Ba/F3 c-Kit D816V, HMC-1 5C6 V560G & D816V) were not affected. In addition, cells expressing the BCR-ABL protein were not affected by masitinib treatment.

AB1010 was tested on ex vivo primary murine mast cells expressing endogenous WT c-Kit. The Applicant proposes that the results indicate a complete inhibition of tyrosine phosphorylation of c-Kit in cells treated with AB1010. However, no study details are available and no dose response can be identified. Thus, the relevance of this study is questionable.

Secondary pharmacodynamics effects of masitinib include anti-tumour activity in nude mice bearing subcutaneous transfected BA/F3 tumours, leading to tumour growth inhibition and enhanced survival in this type of studies.

Experiments performed in STI571-resistant cell lines (including cell lines with JM mutant c-Kit) remain sensitive to higher concentration of AB1010 but not to STI571, while the parental cell lines are highly sensitive to both STI571 and AB1010.

No in vivo PD studies were presented to support the relevance for using masitinib in the sought indication, even though literature indicates that relevant models exist (e.g. publication by Ranieri et al., 2015 which includes description of animal models of mastocytosis (including KitD814V transgenic mice and zebrafish model)).

Secondary pharmacodynamic studies

Data referred to as secondary pharmacodynamics by the applicant are presented in the section on primary pharmacodynamics.

Safety pharmacology programme

Table 8 Results from the safety pharmacology studies conducted with Masitinib

Organ System Evaluated (Study Report No.) GLP-status	Species/ Number	Dose/ Method of Administration	Results	NOAEL
Cardiovascular system (2-24367-sac) GLP	Beagle dogs/6 females/group	10, 50, 150 mg/kg p.o. (gavage) single dose	3/6 and 6/6 animals vomited within an hour after administration of 50 and 150 mg/kg, respectively. Hence, any treatment-related effect on CV parameters could not be evaluated at 150 mg/kg. No effect on heart rate, diastolic, systolic and mean arterial blood pressure or duration of PQ, QRS, QT intervals when evaluated via implantation of telemetric devices.	**50 mg/kg p.o. (n=3)**
hERG channel (4-ps05d91) GLP	4 cells/group	0.1, 1, 10, 30 µmol/L	Masitinib inhibited the hERG tail current by 8%, 14%, 54% and 73% at 0.1, 1, 10 and 30 µM in HEK cells stably expressing the hERG potassium channel. IC_{50}: 8.3 µM Positive control: E-4031 (0.1 µmol/L)	**<0.1 µM**
Respiratory System (1-24366-sar) GLP	Conscious Sprague-Dawley rats/8 females/group	15, 50, 150 mg/kg p.o. (gavage) single dose	No effect on respiratory rate, peak inspiratory and expiratory flows, tidal volume, minute volume or enhanced pause when measured using whole body plethysmography. Positive control: carbamylcholine	**150 mg/kg p.o.**
Central nervous system (3-24368-sar) GLP	Sprague-Dawley rats/ 8 females/group	15, 50, 150 mg/kg p.o. (gavage) single dose	No effect observed in a functional observation battery. Positive control: chlorpromazine (10 mg/kg)	**150 mg/kg p.o.**

Safety pharmacology studies in rats revealed no treatment-related effect on the central nervous system (3-24368-sar) or respiratory system (study (1-24366-sar) at single oral doses up to 150 mg/kg. Toxicokinetic sampling was not performed but based on allometric scaling a dose of 150 mg/kg administered to rats corresponds roughly to twice the recommended daily human dose (12 mg/kg/day).

In Study 4-ps05d91, Masitinib induced a concentration-dependent reduction in hERG tail current over the concentration range 0.1 to 30 µM. The lowest concentration tested (0.1 µM) gave rise to a hERG current inhibition of 8% while the IC_{50} value was 8.3 µM. Considering a masitinib free fraction in human blood of 2.12%, the reported clinical plasma Cmax of 1206 ng/mL masitinib corresponds to an unbound plasma concentration of approximately 51.3 nM. Hence, only a minimal effect on the hERG channel is expected at clinical Cmax.

In study (2-24367-sac), no effect was observed on electrocardiogram parameters in telemetered dogs (n=3) receiving 50 mg/kg. This dose level roughly corresponds to the recommended daily dose for patients receiving masitinib.

Pharmacodynamic drug interactions

No non-clinical studies evaluating the potential for pharmacodynamic drug interactions have been submitted.

2.3.3. Pharmacokinetics

Absorption

In vitro studies in Caco-2 cells, indicate that masitinib may be a substrate of P-gp mediated transport at concentrations <10 µM. At higher concentrations (≥10 µM), masitinib appears to be an inhibitor of P-gp mediated transport which is likely to be due to saturation of P-gp-mediated efflux. It was not possible to establish an exact IC50, however approximated IC50 values were calculated, and in the range of 63.24 to 154.22 µM. The free fraction of masitinib in plasma is well below this range, hence inhibition of systemic P-gp is considered unlikely, whereas the concentration of masitinib in the gut is much higher than the approximated IC50 values, and inhibition of P-gp in the gut is a risk.

Table 9 Pharmacokinetic parameters based on total radioactivity plasma data derived following administration of ^{14}C-masitinib

Study ID / GLP	Species / N / Sex	Route	Dose (mg/kg)	C_{max} (ng-eq/g)	T_{max} (h)	AUC_{0-inf} (ng-eq*h/g)	$t_{½, el}$ (h)	F (%)
SR-2-24364-pac / GLP	Beagle dogs/3/males	i.v.	5	953	-	6088	24.7	-
		p.o.	10	794	2.67	10,303	10.6	84.6
	Beagle dogs/3/females	i.v.	5	980	-	6457	19.7	-
		p.o.	10	901	1.67	10,517	10.2	81.4
SR-1-24363-par / GLP	Sprague Dawley rats/3/males	i.v.	5	1683	-	4206	5.95	-
		p.o.	10	973	4	5810	4.69	69.5
	Sprague Dawley rats /3/females	i.v.	5	1784	-	5634	4.98	-
		p.o.	10	974	4	8455	4.48	74.8

Moreover, the volume of distribution (Vd) was determined to 10.2 and 6.38 L/kg for male and female Sprague-Dawley rats, respectively, while the plasma clearance for males and females was 19.8 and 14.8 mL/min/kg, respectively.

The absorption of masitinib was studied after single i.v. (5 mg/kg) and p.o (10 mg/kg) administration of 14C-masitinib to Beagle dogs and Sprague-Dawley rats. No gender differences were observed. Tmax following p.o. dosing was 2.67? h in dogs and 4 h in rats. Elimination half-life (T½) following oral administration was 4.6 h and 10.4 h in rats and dogs, respectively. The bioavailability was relatively high with a mean value of 83% in dogs and 72% in rats. Masitinib had a relatively large volume of distribution (Vd) with values of 10.2 and 6.38 L/kg for male and female Sprague-Dawley rats, respectively. The plasma clearance for male and female rats was 19.8 and 14.8 mL/min/kg, respectively.

In male Swiss mice after oral administration of AB1010 at four doses: 30, 125, 250 and 500 mg/kg, the tmax was reached 0.5 or 1 hour after oral (gavage) administration. Systemic exposure (as measured by the Cmax and AUC0-24h) increased with dose-level. Repeated dosing to rats revealed higher exposure in females. However, exposure to the major metabolite AB3280 was approximately two times higher in male rats. AB3280 Tmax was in the range from 3-4 h while the elimination half-life varied from 3.55 to 4.23 h. No gender differences were observed with respect to masitinib absorption in dogs following repeated oral administration. Results from TK analysis in rats revealed a supra-linear increase in exposure at lower dose levels (between 10 and 30 mg/kg).

Distribution

The binding of ^{14}C-Masitinib mesylate was determined on human blood cells, human plasma proteins and isolated human plasma proteins as well as on rat, mouse, dog and rabbit blood cells and plasma. The results indicate that for the ^{14}C-Masitinib mesylate concentration used, 100-3000 ng/mL the binding to human, rat, mouse, dog and rabbit plasma proteins was high 93.93%, 92.15%, 86.12%, 93.33% and 97.50%, respectively and constant within the tested concentration range.

Following intravenous injection and oral gavage, the test item distributed throughout the body. Higher distribution was observed in the adrenals, kidneys, spleen and intestines. However, rapid elimination was observed from all tissues as only trace amount was observed after 24 hours and 7 days respectively. A new *in vivo* study in rats has been performed to further investigate the passage of masitinib to the brain. It was shown that masitinib indeed crosses the blood brain barrier in rats and Cmax levels appear to be sufficiently high to inhibit c-Kit kinase directly in the brain tissues. Albeit, no treatment-related CNS adverse events were observed in the non-clinical models (*in vivo).*

Metabolism

The Phase I metabolism of masitinib was investigated in hepatic microsomes from CD-1 mice, Sprague-Dawley rats, New Zealand White rabbits, Beagle dogs, *Cynomolgus* monkeys and humans. While the identical five metabolites were detected in mice and rabbits (AB3280, MET1, MET2, MET3 and AB1187.3), four metabolites were seen in rats, monkeys and humans (AB3280, MET1, MET2, MET3). While AB3280 was the major metabolite in hepatic microsomes derived from mice, rats, monkeys and humans (≥18%), AB3280 was not formed in dog hepatocytes *in vitro*. Hence, the dog microsomes formed MET1, MET2 and MET3. No human specific metabolites were detected.

In vitro metabolites

Comparative in vitro metabolism studies of ^{14}C-masitinib with freshly isolated CD-1 mouse, Sprague-Dawley rat and human hepatocytes (SR-5-abs-05, GLP)

The metabolism of 5 µM 14C-masitinib was investigated in freshly isolated hepatocytes capable of performing both Phase I and Phase II reactions. The extent of metabolism following 4 hours incubation was 100% in mouse, 56.7% in rat and 57.7% in human hepatocytes, hence masitinib was extensively metabolised by mouse hepatocytes. An overview of the detected metabolites is given in the below table.

Table 10 Masitinib metabolites formed following 4 hours incubation of 5 µM ^{14}C-masitinib with freshly prepared hepatocytes. Values are given as percentage of total sample radioactivity

Metabolite	Mouse	Rat	Human
AB2436	47.3%	16.1%	-
AB3280	6.6%	20.8%	19.4%
MET 1/AB5235	27.9%	-	-
MET 2	<3.7%	<3.7%	<5.5%
MET 3	<3.7%	<3.7%	<5.5%
AB6465	<3.7%	11.3%	-

-, not detected

While AB3280 was the major metabolite in rat and human hepatocytes, AB2436 was the predominant metabolite in mouse hepatocytes. Moreover, the metabolites MET1 and AB6465 were only formed to a significant extent in mouse and rat hepatocytes, respectively.

Comparative *in vitro* metabolism studies of ^{14}C-masitinib with CD-1 mouse, Sprague-Dawley rat, New Zealand White rabbit, Beagle dog, *Cynomolgus monkey* and human hepatic microsomes (SR-3-xtc-03, SR-4-xtc-04 (both GLP))

The Phase I metabolism of 5 µM 14C-masitinib was studied over an incubation period of 30 min in microsomes prepared from rat, dog, rabbit, mouse, Cynomolgus monkey and human liver. Following 30 min incubation, the extent of parent 14C-masitinib metabolism by mouse, rat, dog, rabbit, monkey and human microsomes were 64%, 49%, 50%, 79%, 72% and 61%, respectively. An overview of the detected metabolites is given in the table below.

Table 11 Masitinib metabolites formed following 30 min incubation of 5 µM ^{14}C-masitinib with hepatic microsomes from various species. Values are given as percentage of total sample radioactivity

Metabolite	Mouse	Rat	Rabbit	Dog	Monkey	Human
AB3280	18.0%	17.9%	19.2%	-	34.5%	31.7%
MET 1	15.0%	5.86%	20.9%	1.00%	2.71%	1.5%
MET 2	4.25%	2.33%	5.57%	1.78%	4.66%	3.08%
MET 3	10.5%	12.5%	13.2%	27.2%	13.3%	11.0%
AB1187.3	1.62%	-	3.12%	-	-	-

-, not detected

In all species except dog, MET3 and AB3280 were major microsomal metabolites. The dog was the only species that did not produce the major human metabolite AB3280.

In a subsequent study, the identity of the major metabolites produced in hepatic microsomes was investigated. According to the results, MET1 corresponds to AB1187.3, MET2 is a carbamimidothoic acid or thiourea metabolite of masitinib, AB3280 corresponds to N-demethylated masitinib while MET3 is the N-oxide of masitinib. However, in this study AB3280 was neither detected in hepatic microsomes from mice, rats nor dogs. This discrepancy was ascribed a poor response in the mass spectrometer for AB3280.

In vivo metabolites

Plasma metabolites (SR-1-24363-par, SR-2-24364-pac (both GLP compliant))

Following administration of i.v. and p.o. ^{14}C-masitinib to Sprague-Dawley rats as well as Beagle dogs, the metabolic pattern analysis for plasma samples from both routes, only showed a single peak corresponding to parent drug, which indicates the absence of metabolites. However, in dogs the metabolic profile was only determined in plasma samples collected up to 30 min and 1 h following i.v. and p.o. dosing, respectively. Hence, it is likely that the analysis of plasma samples collected at later time point may have revealed masitinib plasma metabolites. In rats, the metabolic profile was determined in plasma samples collected up to 30 min post i.v. dosing and 4 h following p.o. dosing.

Urinary metabolites (SR-1-24363-par, SR-2-24364-pac (both GLP compliant))

Up to 24 h following i.v. and p.o. dosing, parent drug as well as three metabolites were detected in the urine from the dosed Sprague-Dawley rats and Beagle dogs (SR-1-24363-par, SR-2-24364-pac). The metabolites represented one major metabolite and two very minor metabolites. Following hydrolysis with Glucuronidase and arylsulfatase enzymes, the minor metabolite peaks disappeared and tended to disappear in rats and dogs, respectively. This finding indicates that the two minor metabolites may represent glucuro or sulfo-conjugate metabolites.

Plasma and urinary metabolites analysed with a more sensitive method (Study reports 14398-B,C,D,E)

A fully validated analytical method for analysis of metabolites in plasma and urine in mice and rats was developed. This method demonstrated that the aniline metabolite AB2436 was present in mouse plasma with an AUC 4 times higher than the AUC for AB1010. In rats the AB2436 exposure was 200 times lower than the AB1010 exposure. For comparison, in human the steady-state AUC for AB2436 is less than 3% of the AB1010 AUC.

Proposed metabolic pathway

Masitinib contains an amide functional group and when hydrolysed two hydrolysis products are formed: a carboxylic acid part containing the phenylpiperazine moiety (AB1187.3) and an aniline part containing the thiazole heterocycle (AB2436). AB2436 is subject to oxidation to a further metabolite (AB5235).

The Phase I and II metabolism of masitinib was studied in hepatocytes from CD-1 mice, Sprague-Dawley rats and humans. While AB3280 was the major metabolite in hepatocytes from rats and humans AB2436 was the major metabolite in mice hepatocytes *in vitro* (>47%). AB2436 was less abundant in rats (16%) and it was not formed in human hepatocytes. Moreover, MET1/AB5235, which is genotoxic in the presence of S9 fraction *in vitro*, was only detected in mouse hepatocytes. Again, no human specific metabolites were observed.

In initial *in vivo* i.v. and p.o. metabolite studies conducted in rats and dogs, no masitinib plasma metabolites were detected. With a more sensitive method, it was shown that the aniline metabolite AB2436 is present in plasma in mice and rats. In mice, it is present at high levels (AUC for AB2436 is

4x AUC for AB1010) while in rats the levels are low (AUC for AB2436 is 0.005x AUC for AB1010). In humans the AUC for AB2436 at steady state is <3% of the AUC for AB1010.

The major metabolite AB3280 was quantified during the course of repeat-dose studies in mice, rats and dogs. Based on the sum of *in vitro* data, plasma, urinary and faecal data, an overview of the expected metabolism of masitinib in mice, rats, dogs and humans has been gathered. N-demethylation of masitinib to AB3280 takes place in mice, rats, dogs as well as humans and AB3280 represents the major masitinib metabolite in plasma. Based on the presence of AB3280 and/or its counterpart AB1187.3 in urine, the cleavage of the amide bond leading to the formation of AB1187.3 and the aniline AB2436 occurs in all species tested. N-oxidation and hydroxylation appear to be minor metabolic pathways. N-oxides of either masitinib or AB3280 or both were found as minor metabolites in urine and faeces of rats and dogs and were not specifically searched for in plasma of any species. Hydroxylated derivatives of masitinib were identified as minor metabolites in urine and faeces of rats and dogs. To conclude, the major metabolites detected in humans are also formed in animals and as such the species used for toxicity testing are considered valid animal models.

Excretion

The excretion of ^{14}C-masitinib was evaluated in rats and dogs over a 168 hour period; no gender differences in excretion pattern were observed.

Table 12 Results on ^{14}C-masitinib excretion -as average of values obtained in males and females

Study GLP	Species N	Dose (mg/kg)	Route	Cage wash (% dose)	Urine (% dose)	Faeces (% dose)	Recovery (% dose)	Time (h)
SR-2-24364-pac GLP	Beagle dog 3/sex	5	i.v.	0.66	5.58	91.35	97.6	168
		10	p.o.	0.86	4.65	91.25	96.8	168
SR1-24363-Par GLP	Sprague-Dawley rat/3/sex	5	i.v.	1.12	7.57	91.65	100.3	168
		10	p.o.	1.96	9.82	88,45	100.2	168

Following i.v. dosing of rats, the radioactivity in the faeces and urine was eliminated fast with >81% of the total recovered dose in the faeces and urine being excreted within 24 hours following injection. Similarly, the administered radioactivity was excreted relatively rapidly following p.o. dosing with >91% of the total recovered dose in faeces being eliminated within the first 48 hours after oral gavage while >84% of the total recovered dose in urine was excreted within 24 hours.

Pharmacokinetic Drug Interactions

Non-clinical PK drug interaction studies were not submitted.

2.3.4. Toxicology

Single dose toxicity

An overview of the single-dose toxicity studies conducted for masitinib is given in the below table.

Table 13 Results from single – dose toxicity studies with masitinib

Study ID	Species / Sex / Number / Group	Dose / Route	Approx. lethal dose / observed max non-lethal dose	Major findings
SR-1-26276-tar (GLP)	Sprague-Dawley rats/5/sex/group	2000 mg/kg[A]; p.o. (gavage)	2000 mg/kg	2/5 females died within 5 days after dosing. From days 1 to 7: hypoactivity, sedation, piloerection, dyspnea. Reduced weight gain in surviving animals.
SR-3-26785-tar (GLP)	Sprague-Dawley rats/5/sex/group	2000 mg/kg[B]; p.o. (gavage)	2000 mg/kg	1/5 males found dead on day 5. From day 1 to 2: hypoactivity, piloerection, dyspnea.
SR-2-26277-tar (GLP)	Sprague-Dawley rats/5/sex/group	100 mg/kg; i.v.	>100 mg/kg	Hypoactivity following injection.

[A]masitinib batch produced under non-GMP conditions; [B]GMP-compliant batch

Repeat dose toxicity

Repeat dose toxicity studies have been conducted of 4, 13 and 26 weeks duration in the rat and 4, 13 and 39 weeks duration in the dog. Repeated dose toxicity studies were performed in the mouse up to 3 months duration. The vehicle applied in the studies was 0.9% NaCl.

The following parameters were evaluated in the pivotal repeat-dose toxicity study in rats (26-weeks): clinical signs, body weights, food consumption, ophthalmology, haematology, clinical biochemistry, urinalysis, toxicokinetics, necropsy, organ weights and histopathology. In the pivotal repeat-dose toxicity study in dogs (39-weeks) the following parameters were evaluated: clinical signs, body weights, food consumption, ophthalmology, electrocardiography (ECG), respiratory minute volumes, haematology, clinical biochemistry, urinalysis, toxicokinetics, organ weights and gross observations at necropsy, histopathology. In addition, ECG was evaluated as part of the 4-week and 13-week repeat-dose toxicity studies conducted in Beagle dogs are presented in the table below. The doses are given as glycopyrronium base.

Table 14 Results from repeat-dose toxicity studies conducted in rats and dog

Study ID GLP status Duration	Species N	Dose/ (mg/kg/day) Route	NOAEL (mg/kg/ day)	Major findings
MICE				
SR-9-29399-tcs GLP but parts of the dosage form analysis & the bio-analysis was non-GLP 13-weeks	Swiss mice 10/sex/group	p.o. (gavage) Phase I: 50, 150, 300 Phase II (Day 94-124): former 50 mg/kg/day group administered 450 mg/kg/day	<50	**≥ 50 mg/kg/day:** ↓ leucocytes **≥ 150 mg/kg/day:** ↑ liver weight ♂ **300 mg/kg/day:** Haematology: ↓ RBC, ↓ Hb, ↓ PCV ♀, ↑ MCV, ↑ mean cell Hb Organ weight: ↑ liver weight ♀ Microscopy: Urinary bladder ♂ - females not evaluated - (minimal to moderate transitional cell hyperplasia sometimes accompanied by vacuolar degeneration and/or single cell necrosis of transitional cells, mitosis in the basal layer, vacuoles in the urothelium), liver (hepatocellular hypertrophy) **450 mg/kg/day:** Haematology: ↓ RBC, ↓ Hb, ↓ PCV ♀, ↑ MCV, ↑ mean cell Hb, ↓ leucocytes Microscopy: minimal to moderate centrilobular hypertrophy
RATS				
SR-1-24370-tsr GLP 4-weeks + 2 weeks recovery	Sprague-Dawley rats 10-16/sex/group Recovery: 6/sex/group from control and high dose groups	15, 50, 150 p.o. (gavage)	<15	**≥ 15 mg/kg/day:** ↓ RBC ♂, ↓ glucose Microscopy: bone marrow ♀ (slight to moderate hypocellularity), uterus/& vagina (signs of irregular oestrous cycle) **≥ 50 mg/kg/day:** Haematology: ↓ Hb ♂, ↓ MCV ♂, ↑ MCH ♂, ↓ leucocytes ♂, ↓ lymphocytes ♂, ↓ triglycerides ♀, ↑ AST ♀ (1.3-fold), ↑ ALT ♀ (1.5-fold) Organ weight: ↓ spleen ♂ Macroscopy: blackish coloration of the ovaries in some cases associated with ovary enlargement Microscopy: bone marrow ♂ (slight to moderate hypocellularity) **150 mg/kg/day:** ↓ body weight gain ♀, ptyalism Haematology: ↓ RBC ♀, ↓ Hb ♀, ↓ PCV ♀, ↓ MCV ♀, ↑ MCH ♀, ↓ PTT ♂, ↓ APTT , ↓ fibrinogen ♀ Clinical chemistry: ↑ urea, ↑ creatinine, ↑ total protein, ↑ albumin, ↓ triglycerides ♂, ↑ ALP ♀ (1.9-fold), ↑ AST ♂ (2.1-fold), ↑ ALT ♂ (1.8-fold) Urinalysis: ↑ proteins ♀ Organ weight: ↑ adrenals, ↑ heart ♀, ↑ kidney ♀, ↑ liver ♀, ↑ ovaries ♀ (47%), ↓ thymus ♂, ↓ thyroids ♂ Microscopy: lungs (foamy alveolar macrophages), uterus/& vagina (absence of normal cyclical changes including epithelial hyperplasia and haemorrhagic follicular cysts) **Recovery**: ↓ RBC, ↑ MCV, ↑ MCH, ↓ creatinine ♂, ↓ ALP ♂, ↑ heart ♀, evidence of reversibility for foamy macrophages and bone marrow hypocellularity
SR-3-24372-tcr GLP 13 weeks + 4 weeks recovery	Sprague-Dawley rats 10-16 /sex/group Recovery: 6/sex/group from the control and	10, 30[A], 100 p.o. (gavage)	<10	**≥ 10 mg/kg/day** Haematology: ↓ leucocytes, ↓ neutrophils ♂, ↓ monocytes Clinical chemistry: ↓ triglycerides ♀ **≥ 30 mg/kg/day** Haematology: ↓ RBC ♂, ↑ MCV, ↑ MCH, ↓ lymphocytes ♂ Clinical chemistry: ↑ ALP ♀ (1.5 fold), ↑ AST ♂ (1.7 fold) IgM response: ↓ IgM level ♀ only at 30 mg/kg/day Organ weight: ↑ adrenals ♀, ↑ heart ♀, ↓ thymus

Study ID GLP status Duration	Species N	Dose/ (mg/kg/day) Route	NOAEL (mg/kg/ day)	Major findings
	high dose groups			Microscopy: thymus (lymphoid depletion), bone marrow (medullary hypocellularity) **100 mg/kg/day** Clinical signs: ptyalism Ophthalmology: pallor of the fundus ♀ Haematology: ↓ RBC ♀, ↓ Hb, ↓ PCV, ↓ APTT ♂, ↓ fibrinogen Clinical chemistry: ↑ urea, ↑ total protein ♀, ↓ triglycerides ♂, ↓ cholesterol ♀, ↑ ALT (1.4 fold) Urinalysis: ↑ urine volume ♂, ↓ mean specific gravity ♂, ↑ urine pH ♂, ↑ urinary protein ♀ Organ weight: ↑ adrenals ♂, ↑ heart ♂, ↑ liver, ↑ ovaries Microscopy: ovaries (developing follicles associated with few or no corpora lutea, haemorrhagic follicles and some foci of haemosiderosis), uterus (hypertrophy/hyperplasia), vagina (epithelial hyperplasia, hyperkeratinisation and mucinification), liver (hepatocellular hypertrophy), adrenals ♀ (cortical cell hypertrohy), lungs (foamy alveolar macrophages) **Recovery:** ↓ body weight gain, Haematology: ↓ RBC, ↑ MCV, ↑ MCH Organ weight: ↑ adrenal, ↑ liver ♀, ↓ thymus Macroscopy: grey/greenish discolouration of the lacrimal gland, foci of discolouration in the ovaries. No microscopic evaluation was performed on the recovery animals.
SR-6-26099-tcr GLP but bioanalysis was non-GLP 26-weeks		10, 30, 100 p.o. (gavage)	<10	**≥ 10 mg/kg/day** Haematology: ↓ RBC, ↓ Hb ♂, ↓ PCV ♂, ↑ MCH ♀, ↓ monocytes ♂ Clinical chemistry: ↓ triglycerides ♂ Microscopy: ovaries (follicular cysts, very few or no corpora lutea) **≥ 30 mg/kg/day** One female found dead. Clinical signs: ptyalism in 1/20 females Haematology: ↓ Hb ♀, ↑ MCV, ↑ MCH ♂, ↓ APTT ♂, ↓ fibrinogen ♂ Clinical chemistry: ↑ creatinine ♂, ↑ AST ♂ (1.7-fold) Organ weight: ↑ heart weight ♀, ↓ thymus ♀ Macroscopy: small thymus Histopathology: kidney (moderate degenerative/necrotic nephropathy characterized by tubular cell degeneration/necrosis and tubular dilatation with flattened epithelium), bone marrow (slight to marked hypocellularity), thymus (more severe lymphoid depletion than in controls), mesenteric lymph nodes (swollen histiocytes with granular cytoplasm), lungs (foamy alveolar macrophages), heart ♂ (slight myocardial degeneration and fibrosis) **100 mg/kg/day** One male found dead and one female euthanized Clinical signs: ptyalism in 11/20 females and 12/20 males, ↓ body weight gain (-11-15%) Haematology: ↓ PCV ♀ Clinical chemistry: ↓ triglycerides ♀, ↑ urea, ↑ creatinine ♀, ↑ AST ♂ (2-fold), ↑ ALT ♂ (2-fold) Urinalysis: ↑ protein content Organ weight: ↑ heart weight ♂, ↑ kidney, ↑ liver, ↑ ovaries, ↓ thymus ♂

Study ID GLP status Duration	Species N	Dose/ (mg/kg/day) Route	NOAEL (mg/kg/ day)	Major findings
				Histopathology: bone marrow (slight to moderate hyperostosis in femoral bone and sternal bone), liver (slight periportal swollen macrophages), female genital organs (findings indicative of a disturbed oestrous cycle: moderate to large number of luteal and/or follicular hematocysts, few corpora lutea, very few or few follicular developments), heart ♀ (slight myocardial degeneration and fibrosis)
BEAGLE DOG				
SR-2-24371-tsc GLP 4-weeks + 2 weeks recovery	Beagle dog 3-4/sex/group Recovery: 2/sex/group from control and high dose groups	15, 50, 150 p.o. (gavage)	15	**≥ 15 mg/kg/day** Clinical signs: vomiting, regurgitation, soft faeces, hypoactivity, abnormal vocalization, excessive salivation before or after dosing, abnormal breathing **≥ 50 mg/kg/day** Clinical signs: pallor of nose and/or oral region, reddish colour of the litter ♀ Haematology: ↓ RBC, ↓ Hb, ↓ PCV, ↓ reticulocytes, ↓ leucocytes, ↓ neutrophils, ↓ eosinophils, ↑ APTT Clinical chemistry: ↑ glucose Microscopy: acute oesophagitis ♀, liver (vacuolated Kupffer cells, brownish pigment-laden Kupffer cells, bile canalicular plugs), mesenteric lymph nodes (foamy macrophages), sternum (medullary hypocellularity) **150 mg/kg/day** One male died on Day 29. Macroscopic findings consisted of greyish/whitish foci in the lungs and lesions in the mucosa of the gastro-intestinal tract. Marked acute esophagitis, moderate alveolitis with few granulomas and amorphous substance present in alveoli were noted at the microscopic examination. Death was considered to be due to lung lesions after the frequent regurgitation of the dosage forms (vomited on 17 occasions). Clinical signs: reddish colour of the litter ♂, reddish or greenish coloured faeces, emaciated appearance, ↓ body weight, ↓ food consumption Haematology: ↑ fibrinogen Bone marrow: markedly ↑ neutrophil & neutrophil metamyelocytes, slightly ↑ neutrophils, markedly ↓ proerythroblasts, basophilic erythroblasts, polychromatophilic erythroblasts and normoblasts, slightly ↑ lymphocytes and plasma cells Clinical chemistry: ↓ calcium, ↓ total protein, ↓ albumin, ↑ chloride ♂, ↑ ALT (2.5-fold), ↑ ALT ♀ (2.4-fold), ↑ creatinine kinase ♀, ↑ ALP ♂ (1.7-fold) Urinalysis: ↓ urinary pH ♀, presence of blood and protein in the urine, ↑ bilirubin ♂ Macroscopy: liver (enlarged and pale) Microscopy: acute oesophagitis ♂, liver (vacuolated hepatocytes), mandibular lymph nodes (decrease in germinal center development), spleen (histiocytosis) **Recovery:** pallor of nose and oral cavity, ↓ RBC, ↓ haemoglobin, ↓ PCV, ↑ reticulocytes, ↑ leucocytes, ↑ neutrophils, ↑ fibrinogen, grey/green colour of the liver, enlarged spleen, bile canalicular plugs with very few brownish pigment-laden macrophages were noted with a lower incidence than following the 4-week treatment period.

Study ID GLP status Duration	Species N	Dose/ (mg/kg/day) Route	NOAEL (mg/kg/ day)	Major findings
SR-4—24373-tcc GLP 13-weeks + 4-weeks recovery	Beagle dogs 3-4/sex/group	5, 15, 15 p.o. (gavage)	15	**≥ 5 mg/kg/day** Clinical signs: excessive salivation ♂ **≥ 15 mg/kg/day** Clinical signs: vomiting ♀, regurgitation ♀, excessive salivation ♀, abnormal breathing ♀, hypoactivity ♀ Organ weights: ↑ liver **50 mg/kg/day** Clinical signs: soft or liquid faeces ♀, vomiting ♂, regurgitation ♂, pallor, cold to touch ♀ Haematology: ↓ RBC, ↓ Hb, ↓ PCV, ↓ leucocytes ♀, ↓ neutrophils ♀, ↑ APTT ♂, ↑ fibrinogen ♂ Clinical chemistry: ↓ albumin ♀, ↑ chloride, ↑ ALP ♀ (3 fold) Microscopy: liver (hepatocellular hypertrophy) **Recovery** Pallor within the first two weeks
SR-7-26100-tcc GLP but bioanalytical data was non-GLP 39-weeks	Beagle dog 4/sex/group	3, 10, 30 p.o. (gavage)	3	**≥ 3 mg/kg/day** Clinical signs: transient excessive salivation following dosing **≥ 10 mg/kg/day** Clinical signs: pallor of the oral region and/or oral mucosa ♀ Haematology:↓ RBC, ↓ Hb, ↓ PCV, ↓ neutrophils, ↓ leucocytes Microscopy: liver (pigment deposition in Kuppfer cells), spleen (pigment deposition), mesenteric lymph nodes ♂ (ceroid pigment deposition) **30 mg/kg:** 1 ♀ euthanized on Day 225 (severe anemia, leucopenia, ↑ thrombocytes & fibrinogen, ↑ blood sodium, chloride & urea levels, ↓ blood protein and albumin levels; ↑ blood creatine kinase and lactate deshydrogenase , presence of proteins & blood in the urine, ↓ urinary pH, pallor, ↓ size of spleen, thyroid glands & thymus, oedema in pericardium, pancreas, thymus, subcutaneous tissue and the iliac and portal lymph nodes of the pancreatic region, serous contents in the abdominal cavity, severe lymphoid depletion in the thymus, thickened pericardium which also had a serous content.) Clinical signs: pallor of the oral region and/or oral mucosa ♂ Clinical chemistry: ↓ protein & albumin Organ weight: ↑ liver, ↓ thymus Macroscopy: small thymus in ♀, testes (vacuolation of the epithelium in the seminiferous tubules), epididymides (oligospermia)

RBC, red blood cells; Hb, haemoglobin; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell haemoglobin; APTT, activated partial thromboplastin time

[A]microscopy was only performed on adrenals, femur, sternum, liver, ovaries, thymus, uterus and vagina from animals of the intermediate-dose group.

Genotoxicity

Table 15 Results from the genotoxicity studies

Type of	Test system	Concentration range/	Metabolising	Results

test / study ID / GLP	system		
Gene mutations in bacteria/SR-1-24351/GLP	Salmonella strains TA1535, TA1537, TA98, TA100, TA102 E. Coli WP2 uvrA	Experiments without S9 WP2 uvrA: 156.3-2500 µg/plate TA98, TA100: 19.53-312.5 µg/plate TA1535, TA1537, TA102: 39.06-625 µg/plate Experiments with S9 WP2 uvrA: 312.5-5000 µg/plate TA1537, TA100: 19.53-312.5 µg/plate TA98, TA102, TA1535: 39.06-625 µg/plate	Negative
Gene mutations in mammalian cells/SR-2-24352/GLP	Human lymphocytes	Experiments without S9 3 h treatment/20 h harvest: 2.29-20.58 µg/mL 20 h treatment/20 h harvest: 2.5-10 µg/mL 44 h treatment/44 h harvest: 30 µg/mL Experiments with S9 3 h treatment/20 h harvest: 2.29-30 µg/mL 3 h treatment/44 h harvest: 30 µg/mL	Negative
Gene mutations in mammalian cells/SR-3-24354-mly/GLP	L5178Y $TK^{+/-}$ mouse lymphoma cells	Experiments without S9 3 hours treatment: 1.3-20 µg/mL 24 h treatment: 0.16-7.5 µg/mL Experiments with S9 3 h treatment: 2.5-40 µg/mL	Negative
Chromosomal aberrations *in vivo*/SR-1-24353-mas/GLP	Mouse, micronuclei in bone marrow; 5/sex/group	437.5, 875, 1750 mg/kg/day for two days p.o. (gavage) Sacrificed 24 h after treatment	Negative

Doses up to 5000 µg/plate masitinib were tested in the Ames test (SR-1-24351). While no precipitation was observed, a moderate to strong toxicity was noted generally at dose-levels ≥ 500 µg/plate or ≥ 2500 µg/plate, towards Salmonella typhimurium strains or Escherichia coli strain, respectively.

A moderate precipitate was observed at 5000 µg/mL in the human lymphocyte study while a slight precipitate was observed in the culture medium at dose-levels ≥ 555.6 µg/mL (SR-2-24352). A more than 50% decrease in mitotic index was observed following 3, 20 and 44 hours treatment at masitinib doses ≥ 62, 10 and 20 µg/mL, respectively, at treatment conditions without S9 mix. In the experiments conducted with S9, a moderate to marked toxicity was induced at dose-levels ≥ 20 µg/mL for the 3 hour treatment/20 hour harvest time, as shown by 57-100% decrease in mitotic index. At the 44-hour harvest time, no noteworthy decrease in mitotic index was observed.

In the mouse lymphoma test, a marked toxicity was induced at dose-levels ≥ 10 µg/mL following 3-hours treatment in the absence of S9 (RTG=5% at 15 µg/mL) (SR-3-24354-mly). Following 24-hours treatment without S9 mix, a slight to marked toxicity was induced at dose-levels ≥ 1.25 µg/mL. In the presence of S9, a moderate to marked toxicity was induced at dose-levels ≥ 20 µg/mL (RTG=14% at 40 µg/mL).

Exposition of the bone marrow cells was achieved in the mouse micronucleus test, since plasma levels at the 1750 mg/kg/day dose-level were 15333 ± 2113 ng/mL in males and 28800 ± 10059 ng/mL in females 4 hours after the second administration (SR-1-24353-mas).

Carcinogenicity

Table 16 Overview of the carcinogenicity studies performed

Study ID /GLP	Dose (mg/kg/day)	Species/ No. of animals/ Route	NOAEL (mg/kg /day)	Major findings
SR-1-29400-tcs/ *In vivo* phase: GLP Dosage form analysis: partly GLP	Group 1: Vehicle (isotonic saline) Group 2: 30/20 Group 3: 150/100/40 Group 4: 500/300/80	CD-1 mice/ 50/sex/group/ p.o. (gavage)	30/20	Urinary bladder neoplasms
SR-2-29402-tcd GLP	Group 1: vehicle (isotonic saline) Group 2: 10 Group 3: 30 Group 4: 75/60	Sprague-Dawley rats /50/sex/group	10	Uterine adenocarcinomas Pulmonary cystic keratinizing epitheliomas Thyroid follicular adenomas

The vehicle consisted of a sterile, isotonic saline solution.

Long-term carcinogenicity study in CD-1 mice (SR-1-29400-tcs)

Mortality

CD-1 mice (50/sex/group) were administered masitinib via oral gavage at doses up to 300 mg/kg/day. During the course of the study 30/50 male mice died in group 1, 37/51 died in group 2, 37/52 died in group 3 and 37/52 died in group 4. With respect to the female animals, 30/50 died in group 1, 32/50 died in group 2, 33/53 died in group 3 and 43/58 died in group 4. Hence, the overall survival rates ranged from 26-38% in the treated animals versus 40% in the control group. Due to high mortality rates, the study treatment period was reduced to 80 weeks (males: groups 3 and 4), 86 weeks (females: group 4), 94 weeks (males: group 2), 101 weeks (males: group 1; females: group 2) and 103 weeks (females: groups 1 and 3). Due to the marked toxicity, the dose levels were reduced during the study. The deaths of four high-dose males were related to transitional carcinoma of the urinary bladder, and this tumour was considered to be masitinib-related. The death of one (found dead) high-dose female was caused by necrosis of the liver; this was considered to be test item treatment-related.

Neoplastic findings

After repeated administration of masitinib for 2 years, urinary bladder transitional carcinomas and papillomas were seen in five high-dose males, while transitional papillomas were observed in the intermediate dose group. Urinary bladder transitional cell hyperplasia was also seen in group 3 and 4 males and females with a greater incidence than in controls and low-dose mice. The incidence of neoplastic and pre-neoplastic findings in the urinary bladder is given in the below table.

Table 17 The vehicle consisted of a sterile, isotonic saline solution

Sex	Male			
Dose-level (mg/kg/day)	0	30/20	150/100/40	500/300/80
Number examined	50	51	51	52
Urinary bladder				
- carcinoma; transitional	-	-	-	5#
- papilloma; transitional	-	-	2#	3#
- hyperplasia; transitional	1	1	15*	19*
. grade 1	1	1	8	7
. grade 2	-	-	7	6
. grade 3	-	-	-	3
. grade 4	-	-	-	2
. grade 5	-	-	-	1

Sex	Female			
Dose-level (mg/kg/day)	0	30/20	150/100/40	500/300/80
Number examined	50	50	53	57
Urinary bladder				
- hyperplasia; transitional	-	-	7*	21*
. grade 1	-	-	6	8
. grade 2	-	-	1	13

-: not observed.
#: Statistically significant (Peto test).
*: Statistically significant (Armitage test).

Non-neoplastic findings

Increased incidences and/or severity of gall bladder hyperplasia were noted in group 3 and 4 males and females. Statistical significance was obtained in males treated at 500/300/80 mg/kg/day. This tendency was seen for terminally sacrificed males and females, but not in found dead or prematurely sacrificed mice. Since this lesion was seen in dose-related manner in group 3 and 4 males and females, it was considered to be treatment-related. Since this change could be preneoplastic, it was considered as adverse.

Some liver changes were observed with a higher incidence in treated mice when compared to controls mice, namely: hepatocellular hypertrophy, hepatocellular necrosis and hepatocellular microvacuolation/ enlargement but with a moderate incidence and severity and as the test item is metabolised in liver, these changes were considered not to be adverse with the exception of a death of one group 4 female.

Pigment deposits were observed in numerous organs with a greater incidence in treated animals than in controls. Hence, renal dark grey pigment was observed in group 4M and F while brown pigment was seen in multiple organs at all dose-levels in both sexes (kidneys, heart, brain, liver, adrenals, adipose tissue, skeletal muscle, tongue, spleen, lymph nodes, thyroid glands, etc.). The morphology and location of brown pigment were suggestive of lipofuscin and/or ceroid pigments. This overload of age-related intracellular pigment was considered not to be adverse. The dark grey pigment observed in renal tubules was not found at any other location and was more suggestive of compound deposition. It was not thought to be adverse in the absence of associated degenerative changes.

Long-term carcinogenicity study in Sprague-Dawley rats (SR-2-29402-tcr)

Mortality

No apparent treatment-effect on survival was noted at scheduled termination in Sprague-Dawley rats administered 10, 30 or 75/60 mg/kg/day masitinib via oral gavage for 104 weeks. Based on advice from the FDA, the high dose-level was reduced to 60 mg/kg/day from week 27 in order to ensure an appropriate level of survival in the high-dose group animals after 104-week treatment. In all groups, the most common causes of mortality were pituitary neoplasia, and dosing-related deaths (esophageal perforation or aspiration pneumonia), in males, and pituitary neoplasia, mammary neoplasia and dosing-related deaths in females.

Neoplastic findings

Trend test statistics, conducted according to Peto et al. (1980), revealed statistically significant increases for neoplasms in several organs at the dose level of 76/60 mg/kg/day. The data are presented in the below table.

Table 18 Incidence of selected uterine neoplastic and pre-neoplastic findings

Sex	Female			
Dose-level (mg/kg/day)	0	10	30	75/60
Number examined	50	50	50	50
Uterus				
- malignant adenocarcinoma	-	1	-	**5**
- atypical hyperplasia	-	-	2	7

-: not observed.

The incidence of uterine adenocarcinomas was considered to be related to Masipro at 75/60 mg/kg/day.

Table 19 Incidence of selected thyroid neoplastic and pre-neoplastic findings

Sex	Female			
Dose-level (mg/kg/day)	0	10	30	75/60
Number examined	50	50	50	50
Thyroid				
- carcinoma; follicular cell	-	1	1	**1**
- adenoma; follicular cell	-	-	1	**5**
- hyperplasia; focal; follicular cells	-	-	4	**3**

-: not observed.

The incidence of follicular cell adenomas (benign tumours) at 75/60 mg/kg/day was higher than that noted in female Sprague-Dawley rats in CIT control data or in the literature, and therefore the high incidence at 75/60 mg/kg was considered to be related to the test item. The incidence recorded at 30 mg/kg/day was within the range of that recorded in CIT control data or in the literature, and was not considered to be treatment-related. The single occurrence of a follicular cell carcinoma (malignant tumour) noted for each dose-levels of 10, 30 and 75/60 mg/kg/day in females was within the range of that recorded in the literature in control female Sprague-Dawley rats.

Table 20 Incidence of selected neoplastic and pre-neoplastic findings in the lungs

Sex	Male				Female			
Dose-level (mg/kg/day)	0	10	30	75/60	0	10	30	75/60
Number examined	50	50	50	50	50	50	50	50
Lungs								
- cystic keratinizing epithelioma	-	-	-	-	-	-	-	**4**
- hyperplasia, bronchoalveolar grade 4	-	-	-	-	-	-	-	**10**
- hyperplasia, bronchoalveolar grade 5	-	-	-	-	-	-	-	**1**

-: not observed.

Pulmonary cystic keratinizing epithelioma was found in 4/50 high-dose females whereas it was not recorded in the CIT control data or in the compilation of spontaneous neoplasms of control Sprague-Dawley rats and therefore was considered to be induced by masitinib. Two cases of astrocytoma and a single case of oligodendroglioma was seen in the male high-dose group comprising 50 animals while a single case of astrocytoma was noted in a female control animal. In addition, a reduced incidence of pituitary adenomas was observed in high-dose animals.

Non-neoplastic findings

Treatment with the test item induced the death of some rats secondary to renal, cardiac, pulmonary or adrenal cortical lesions. Nephropathy contributed to the death of a few males treated with the test item at all dose-levels and of one female at 75/60 mg/kg/day. Cardiac lesions (cardiomyopathy and/or atrial thrombosis) contributed to the death of three and five males treated with masitinib at 30 or 75/60 mg/kg/day, respectively, and of two females at 75/60 mg/kg/day. Foamy alveolar macrophages in the lungs contributed to the death of 4/50 males treated at 75/60 mg/kg/day. An increase of the incidence of follicular cysts was observed at the dose-levels of 30 and 75/60 mg/kg/day and an increase of the incidence and severity of ovarian atrophy at 75/60 mg/kg/day. When compared with controls, there was a higher incidence of cystic endometrial hyperplasia in females given the test item at 75/60 mg/kg/day. Squamous metaplasia of the endometrium was also observed with higher incidence in the uterus of females administered with masitinib at all doses than in controls. At the high-dose only there was a higher incidence of hyperplasia of the cervix. In a few females at 30 mg/kg/day and 75/60 mg/kg/day, squamous epithelial cysts were noted in the cervix but were not seen in any other groups.

Administration of masitinib induced in the adrenal cortex in both sexes an increase in incidence and severity of cystic degeneration from the dose-level of 30 mg/kg/day. Cystic degeneration is a term

commonly used for severe forms of vacuolation in the adrenal cortex characterised by cell loss and formation of cystic spaces which may contain blood. In severe cases the entire cortex was replaced by large cystic and/or blood-filled spaces. Mineralisation was also increased in incidence and severity in females at 30 and 75/60 mg/kg/day. It was observed in males given the test item at 30 and 75/60 mg/kg/day and not in control males. This finding was secondary to necrosis and haemorrhage accompanying the cystic degeneration (dystrophic mineralisation). When compared with controls, the incidence and severity of thymic atrophy/regression was increased in females treated with the test item from the dose-level of 30 mg/kg/day and in males at 75/60 mg/kg/day. There was an increased incidence of epithelial hyperplasia in high-dose males, and an increased incidence and severity of epithelial hyperplasia in females at 30 and 75/60 mg/kg/day. When compared with controls, there was an increased incidence of tubular cell loss/atrophy in the testes from high-dose males generally associated in the epididymides with sloughed cells in the lumens and oligospermia. These changes were accompanied by prostate and seminal vesicles atrophy in a few animals.

There was a dose-related increase in incidence and severity of cardiomyopathy in males and females treated at the dose-levels of 30 and 75/60 mg/kg/day. This lesion was characterised by a variable association of fibrosis, necrotic cardiomyocytes, and inflammatory infiltrate of macrophages and lymphocytes, located predominantly in the left ventricle, interventricular septum and papillary muscle. In addition, in 2/50 high-dose males and 2/50 females there was minimal to marked thrombosis in the atria (3/4 affected rats) or ventricles (1/4 rats).

There was an increased severity of nephropathy in males and females at the dose-level of 75/60 mg/kg/day when compared with the other groups. Nephropathy is a spontaneous chronic progressive renal disease in rats which can be exacerbated by administration of a variety of xenobiotics. Moreover, focal hyperplasia of the transitional epithelial cells was increased in incidence and severity in males and females at 75/60 mg/kg/day. This was accompanied by an increased incidence of vascular ectasia just below the hyperplastic epithelium.

There was an increased incidence of myofiber necrosis/regeneration in the skeletal muscle from 10 mg/kg/day, and in the tongue and esophagus at 75/60 mg/kg/day. In addition, vacuolation of the myofibers was observed in the skeletal muscle in both sexes given the test item at all dose-levels, and in the esophagus of a few males at 30 and 75/60 mg/kg/day. The tongue was not affected by vacuolation. These minor changes were considered to be of limited biological relevance in the context of a 2-year study.

When compared with controls, there was a dose-related increased incidence and severity of foamy alveolar macrophages in males and females administered with the test item from the dose-level of 10 mg/kg/day. Collections of foamy alveolar macrophages were accompanied by hyperplasia of the broncho-alveolar epithelium in both sexes at 30 and 75/60 mg/kg/day. In marked (grade 4) or severe (grade 5) cases, hyperplasia was accompanied by alveolar squamous metaplasia.

Administration of the test item induced hepatocellular centrilobular hypertrophy in males at 30 and 75/60 mg/kg/day and in females at 75/60 mg/kg/day only. Administration of the test item induced single cell necrosis in males and females at 30 and 75/60 mg/kg/day. Males were more affected than females. However, centrilobular necrosis was found in 5/50 and 6/50 females at 30 and 75/60 mg/kg/day respectively, and only in 1/50 control females, whereas this lesion was found with similar incidence in control and treated males. There was also an increase in the incidence and severity of bile duct hyperplasia in males and females given the test item at 75/60 mg/kg/day.

Administration of masitinib at the dose-levels of 30 and 75/60 mg/kg/day in both sexes induced slight to moderate hypocellularity affecting all lineages.

While dark grey pigment was found only in the kidneys, especially in the glomeruli, brown pigment was present in a wide variety of tissues.

Reproduction Toxicity

Table 21 An overview of the performed reproductive and developmental toxicity studies

Study type/ Study ID / GLP	Species; Number Female/ group	Route & dose	Dosing period	Major findings	NOAEL (mg/kg/day)
Male fertility/SR-1-26311-rsr/GLP but not the bioanalysis	Sprague-Dawley rat; 24 males/group	10, 30, 100 mg/kg/day p.o.	29 days prior to mating - female sacrifice	None	100
Female fertility/SR-1-26311-rsr/GLP but not the bioanalysis	Sprague-Dawley rat; 24 females/group	10, 30, 100 mg/kg/day p.o.	29 days prior to mating – day 7 *post-coitum*	↓fertility indices, ↓ corpora lutea, ↓implantation sites, ↑ pre-implantation loss	10
Female fertility/SR-2-aa19859/GLP	Sprague-Dawley rat; 25 females/group	15, 50 mg/kg/day p.o.	28 days followed by a recovery period of two weeks before mating	Acyclic oestrous cycle	15
Embryo-fœtal development/SR-1-29395-rsr/GLP but not the bioanalysis	Sprague-Dawley rat; 24 females/group	10, 30, 100 mg/kg/day	Day 6 - 17 *post-coitum*	F0: ↓ body weight gain, ↓ food consumption, macroscopic findings F1: ↓ fetal weight, skeletal variations	F0: <10 F1: 30
Embryo-fœtal development/SR-2-29398-rsl/GLP but not the bioanalysis	New Zealand White rabbit; 22 females/group	10, 30, 100 mg/kg/day	Day 6 – 18 *post-coitum*	F0: ↓ body weight F1: skeletal variations	F0: <10 F1: 100

GD, gestation day

In all studies, the vehicle control group received isotonic saline.

Male Sprague-Dawley rats were treated p.o. with 10, 30 or 100 mg/kg/day masitinib from 29 days prior to mating until sacrifice of the mated females. Pthyalism and soft faeces were observed in males administered 100 mg/kg. No treatment-effect on mating parameters, testes weight, epididymides weight, macroscopy or seminology (sperm counts, morphology or motility) was noted at any dose-level.

Female Sprague-Dawley rats were treated p.o. with 10, 30 or 100 mg/kg/day masitinib from 29 days prior to mating until day 7 *post-coitum*. The animals were sacrificed on day 15 *post-coitum* and hysterectomies were performed. In the female group given 100 mg/kg/day, the body weight gain was reduced during gestation, mainly at the end of the dosing period. The latter effect was coupled with reduced food consumption during the first week of gestation. In addition, pthyalism was observed in females administered 100 mg/kg.

The females had normal oestrous cycles. There were no effects on mating behavior, whereas the fertility of females given 100 mg/kg/day was affected, as indicated by the number of non-pregnant females (3/24, compared to 0/24 in the vehicle), the low number of *corpora lutea* and implantation sites and the high pre-implantation loss. At 100 mg/kg/day, the increased number of early resorptions in addition to the increased number of dead concepti resulted in a low number of live concepti.

At necropsy of females given 100 mg/kg/day, haemorrhagic cysts were noted in the ovaries (4/24). Brown content was observed in the uterus (4/24) and may be related to the presence of resorptions. The microscopic examination of the ovaries showed haemocysts in many *corpora lutea* (with large central blood-filled cavity) in all the females given 100 mg/kg/day. These haemocysts were accompanied by haemorrhagic foci and brown pigments. Small areas of hyalinization were observed in the ovarian parenchyma. At this dose-level, the number of *corpora lutea* was lower than in the control group and the *corpora lutea* were of small size. Cystic degeneration of *corpora lutea* (with accumulation of fibroblasts and a few erythrocytes) was seen at 100 and 30 mg/kg/day (respectively, 17/24 and 6/24 females).

Blood samples for determination of plasma levels of the test item were taken 4 hours post-dosing on study day 16. The measured concentrations were 371, 1523 and 2019 ng/mL in males administered 10, 30 and 100 mg/kg/day, respectively, and 729, 3125 and 8240 ng/mL in females administered 10, 30 and 100 mg/kg/day, respectively. Although the data are non-GLP, they may indicate that exposure levels higher than observed in patients administered the recommended daily dose were obtained in the mid- and high-dose animals. Altogether, the NOAEL for male and female fertility is considered 100 mg/kg/day and 10 mg/kg/day, respectively.

Study 2-aa19859

Six out of 25 females given 50 mg/kg/day became acyclic compared with two in the control group and one in the 15 mg/kg/day group. Apart from this finding, no treatment-related effects were noted on any of the evaluated parameters. The NOAEL for this study is considered 15 mg/kg/day.

Embryo-fetal development toxicity in rats (SR-1-29395-rsr)

Three groups of 24 mated female Sprague-Dawley rats received masitinib by oral (gavage) administration from day 6 until day 17 *post-coitum*, at dose-levels of 10, 30 or 100 mg/kg/day. One female receiving 10 mg/kg/day was found dead on gestation day (GD) 18. Females administered 100 mg/kg/day exhibited hypersalivation, reduced mean body weight gains (-18 to -41%) and reduced food consumption. Although the incidence was not dose-related in the masitinib-treated groups, the following necropsy findings were not made in the control animals: spleen (enlarged, granular surface, yellowish areas), kidney (paleness), uterus (enlarged cervix, dilatation of uterine horn, coloured contents in uterine horn), vagina (brownish contents, coloured contents), enlarged placentas.

Litter data showed a lower (-10%) mean fetal body weight in the high-dose group. While visceral or skeletal malformations were not observed, masitinib-treatment was associated with variations in the form of unossified or incompletely ossified bones of the head, sternebrae and ribs. The incomplete ossifications were observed at doses ≥ 30 mg/kg/day.

At day 17 post-coitum, masitinib $AUC_{0.5\text{-}24h}$ was 4176, 20,191 and 72,245 ng*h/mL in animals receiving 10, 30 and 100 mg/kg/day, respectively (non-GLP data). Overall, the NOAEL for maternal toxicity is considered < 10 mg/kg/day. While the skeletal variation observed (cases of unossified bone) are reversible and as such not adverse to the animal, the reduced foetal body weight seen at 100 mg/kg/day is considered adverse. Hence, the NOAEL for developmental toxicity is considered 30 mg/kg/day.

Embryo-fetal development toxicity in rabbits (SR-2-29398-rsl)

Three groups of 22 mated female New Zealand White rabbits received masitinib daily, by oral (gavage) administration, from day 6 until day 18 *post-coitum*, at dose-levels of 10, 30 or 100 mg/kg/day. The overall body weight gains (day 6 to day 19 *post-coitum*) were +35% (ns), -15% (ns) and -74% ($p<0.001$), when compared with the control value for the groups given 10, 30 or 100 mg/kg/day, respectively. All groups had a mean net body weight loss (body weight change adjusted for gravid uterus weight) from day 6 *post-coitum*, but this was markedly greater than control at 100 mg/kg/day. Food consumption was significantly reduced at 100 mg/kg/day. Morever, all females given 100 mg/kg/day had purple-colored urine.

Unossification of 1st metacarpals was observed in fetuses from the 100 mg/kg/day dose group, achieving statistical significance for the fetal incidence ($p<0.05$), however the fetal and litter incidences were within CIT Historical Control Data ranges. Increased incidences of unossified 5th and 6th sternebra were observed at 30 and 100 mg/kg/day although this finding only reached statistical significance at 30 mg/kg/day.

At day 18 post-coitum, masitinib AUC_{1-24h} was 3464, 15,018 and 110,938 ng*h/mL in animals receiving 10, 30 and 100 mg/kg/day, respectively (non-GLP data). Since the skeletal variation observed (cases of unossified bone) are reversible and as such not adverse to the animal, the NOAEL for developmental toxicity is considered 100 mg/kg/day. The NOAEL for maternal toxicity (reduced body weight) is considered < 10 mg/kg/day.

Toxicokinetic data

An overview of the toxicokinetic data obtained in the repeat-dose toxicity studies conducted with masitinib is given in the table below. Please note that the data described for the 26-week rat study as well as the 39-week study in dogs are non-GLP and as such have indicative value only. Also, the animal-human exposure margin is calculated from human data on the dose 12 mg/kg whereas the recommended dose in mastocytosis is 6 mg/kg.

Table 22 Overview of the masitinib plasma exposures (AUC) obtained in the conducted repeat-dose toxicity studies

Study	Dose (mg/kg/day)	AUC_{0-24h} (ng.h/mL)		Cmax (ng/mL)		Animal:human exposure margin AUC[b]		Animal human exposure margin Cmax	
		♂	♀	♂	♀	♂	♀	♂	♀
4-weeks	15	1871	4997	240	660	0.2	0.6	0.2	0.5
rat	50	16333	28840	1815	2300	1.9	3.5	1.5	1.9
	150	50933	65838	4025	4570	6.1	7.8	3.3	3.8
13-weeks	10	4339	11673	507	1110	0.5	1.4	0.4	0.9
rat	30	23832	44099	2375	4910	2.8	5.2	2.0	4.1
	100	50588	84365	3260	6250	6.0	10.0	2.7	5.2
26-weeks	10	4110	8346	385	675	0.5	0.9	0.3	0.6
Rat	30	19637	36293	1340	3605	2.3	4.3	1.1	3.0
Non-GLP	100	66041	110946	4885	10245	7.9	13.2	4.1	8.5
4-weeks	**15**	9106[a]	9527	968	938	1.1	1.1	0.8	0.8
dog	50	26871	28929	2417	2503	3.2	3.4	2.0	2.1
	150	47631	50051	3474	3610	5.7	5.9	2.9	3.0
13-weeks	5	1288[a]	1180	140	162	0.1	0.1	0.1	0.1
dog	**15**	6508	7358	711	942	0.8	0.8	0.6	0.8
	50	18378	32252	1782	2746	2.2	3.8	1.5	2.3
39-weeks	**3**	827	1061	111	114	0.09	0.1	0.09	0.09
dog	10	5164	4177	422	431	0.6	0.5	0.3	0.4
Non-GLP	30	14158	10576	906	857	1.7	1.3	0.7	0.7

The doses marked with bold represent the NOAEL

a, $AUC_{0-t;}$ b, human C_{max} and AUC_{0-24h} were 1206 ng/mL and 8410 ng*h/mL in a single patient exposed to 12 mg/kg

In order to provide GLP compliant exposure data from repeat dosing in mice, pharmacokinetic data were collected from dedicated studies using novel, fully validated GLP-compliant analysis methods (see table below).

Table 23 Summary of toxicokinetic results

Study	Dose (mg/kg/day)	AUC_{0-24h} (ng.h/mL)		Cmax (ng/mL)	
		♂	♀	♂	♀
13-week Mouse 36735-TCS	300	37391	ND	5781	ND
4-week Rat 36882-TSR	10 30 100	2080 11558 40604	4961 23376 73083	325 1358 4381	609 3220 5087
4-week Dog 6883-TSC	3 10 30	361 3074 14532	212 3507 11742	70.9 508 1453	46.9 526 1297

Local Tolerance

Evaluation of skin sensitization potential in mice using the local lymph node assay (LLNA) (SR 33510-tss).

Masitinib induced delayed contact hypersensitivity in the murine Local Lymph Node Assay. According to the EC3 value obtained in the experiment (0.7%), masitinib should be considered as a strong sensitiser when applied on the skin.

Acute dermal irritation in rabbits (SR 33511-tal)

Masitinib was slightly irritant when applied topically to rabbits for up to 72 hours. Hence, mean scores over 24, 48 and 72 hours were 0.3, 1.0 and 0.7 for erythema and 0.0, 0.0 and 0.0 for oedema.

Acute eye irritation in rabbits (SR 33512-tal)

Masitinib was severely irritant when administered by ocular route to rabbits.

Other toxicity studies

An immune response evaluation was performed as part of the 13-week repeat-dose toxicity study in rats (SR-3-24371). When compared to the control group, no statistical differences in the primary antibody response to a T-cell dependent antigen (KLH) were observed in the three treated groups (10, 30 and 100 mg/kg/day) one week following KLH immunisation.

A noteworthy decrease in the IgM level was observed in 3/6 females given 30 mg/kg/day. However, in absence of dose-relationship and in view of the haematological and histopathological investigations performed, this was considered to be without relationship to treatment with the test item. DMSO was applied as vehicle for the test substances.

Table 24 Results from the *in vitro* genotoxicity studies conducted with the metabolites AB3280 and AB2436

Type of test / study ID / GLP	Test system	Concentration range / Metabolising system	Results
		Metabolite AB3280	
Gene mutations in bacteria/SR-1-29421/GLP	Salmonella strains TA1535, TA1537, TA98, TA100, TA102 E. Coli WP2 uvrA	AB3280 dissolved in DMSO Without S9 TA98, TA102, TA1537: 7.81-250 µg/plate TA100, TA1535: 15.6-500 µg/plate WP2 uvrA: 62.5-2000 µg/plate With S9 TA102: 7.81-250 µg/plate TA100, TA1535: 15.6-500 µg/plate TA98, TA1537: 31.3-750 µg/plate WP2 uvrA: 62.5-2000 µg/plate	Negative
Gene mutations in mammalian cells/SR-2-30107/GLP	Human lymphocytes	AB3280 dissolved in DMSO 1.56- 37.5 µg/mL – S9 6.25-50 µg/mL + S9	Negative
		Metabolite AB2436	
Gene mutations in bacteria/SR-4-34708/GLP	Salmonella strains TA1535, TA1537, TA98, TA100, TA102	AB2436 dissolved in DMSO 156.3-2500 µg/plate +/- S9	Positive in the presence of S9 in TA98, TA100, TA102 & TA1537
Gene mutations in mammalian cells/SR-5-34709-mlh/GLP	Human lymphocytes	AB2436 dissolved in DMSO 0.078-1.25 mM – S9 0.039-0.937 mM + S9	Positive in the presence of S9

AB3280 2-week toxicity study in rats (SR-3-30428)

The toxicity of the plasma metabolite AB3280 was evaluated in Sprague-Dawley rats (n=6/sex/group) via p.o. administration of 100, 250 or 600 mg/kg/day for 14 days. The following parameters were evaluated: clinical signs, body weight, food consumption, haematology, clinical chemistry, toxicokinetics, macroscopy, organ weight (designated organs), microscopy (animals in the control and high-dose groups and on macroscopic lesions from animals in the low- and mid-dose groups).

Lower body weight gain and food consumption were noted in males given 600 mg/kg/day. Moreover, loud breathing was observed in a few animals given 600 mg/kg. Lower glucose not dose-related levels were noted in males given 100 mg/kg/day and in animals given 250 and 600 mg/kg/day (approximately -20%). Lower cholesterol levels were noted in males given 250 mg/kg/day and in animals given 600 mg/kg/day (-24 to -32%).

2.3.5. Ecotoxicity / environmental risk assessment

An ERA had been performed in the context of previous applications. The Applicant has performed a Phase 1 calculation of PEC surface water and determined LogKow for masitinib mesylate. The use of masitinib in the applied indication is not considered to be associated with an environmental risk.

2.3.6. Discussion on non-clinical aspects

The Applicant has presented several *in vitro* studies regarding the mode-of-action for masitinib as a potent inhibitor of wild-type c-KIT. In indolent systemic mastocytosis, only 10% of patients present with wild-type (WT) c-Kit receptors and 90% present with D816V mutant c-Kit receptors. *In vitro*, assays have studied inhibition of proliferation and induction of apoptosis in cell lines expressing wildtype c-Kit and c-Kit mutants. These clearly show that masitinib completely lacks activity on cells expressing c-Kit D816V (IC50 >10000 µM) and it therefore appears that alternative mechanisms of action must be implicated for the therapeutic benefits observed in indolent systemic mastocytosis patients with D816V mutant c-Kit.

Lyn is a downstream kinase that once phosphorylated, initiates mast cell mediator release. Fyn is another kinase crucial in the FcεRI-associated mast cell degranulation and cytokine production. It could be hypothesised that these kinases may be involved in the mode-of-action for masitinib in the sought indication; however, the data presented lack proof-of-concept for the effect in systemic mastocytosis.

Studies addressing mast cell activation, and of direct relevance to the clinical indication, were performed with normal human mast cells, however these data gives no information on what is the main target for the masitinib inhibition, although the inhibition of c-Kit is likely most important.

No studies in animal models of systemic mastocytosis have been performed for the application, even though several models exist (e.g. publication by Ranieri et al., 2015, including KitD814V transgenic mice and a zebrafish model). However, the Applicant has presented a comprehensive discussion regarding *in vitro* data for support of the proposed mode-of-action for masitinib in mastocytosis. This includes data that tryptase levels pre and post masitinib treatment may represent mechanistic evidence of product efficacy in KIT-816V patients by reducing mast cell activation. Clinical data from study AB06006 showed statistically significant but partial decrease in serum tryptase level in the masitinib treatment-arm, indicating a reduction of mast cell degranulation. Furthermore, masitinib demonstrated significant activity on two other objective markers of mast cell activation, namely, lesions of urticaria pigmentosa and Darier's sign.

The major human metabolite, AB3280 was present in animals to an extent allowing toxicological qualification. The mutagenic aniline metabolite AB2436 was shown to be a minor circulating metabolite in humans (<3% if the AUC for masitinib) but was present in high levels in mouse (4x AUC for AB2436). As discussed below, the genotoxic metabolite may be associated with a carcinogenic risk.

In a long-term carcinogenicity study in CD-1 mice after repeated administration of masitinib for 2 years, urinary bladder transitional carcinomas and papillomas were seen in five high-dose males, while transitional papillomas were observed in the intermediate dose group. Urinary bladder transitional cell hyperplasia was also seen in group 3 and 4 males and females with a greater incidence than in controls and low-dose mice.

In the long-term carcinogenicity study in Sprague-Dawley rats the incidence of uterine adenocarcinomas seen at the high-dose was higher than that observed in CIT control data or in the literature, therefore this neoplasm was considered to be related to the administration of the test item at 75/60 mg/kg/day. The increased incidence observed in the current study may be related to the increased incidence of ovarian follicular cysts as this lesion is reported to be associated with such cysts and is thought to be the result of prolonged oestrogen stimulation (Boorman et al., 1990). Pulmonary cystic keratinizing epithelioma was found in 4/50 high-dose females whereas it was not recorded in the CIT control data or in the compilation of spontaneous neoplasms of control Sprague-Dawley rats and therefore was considered to be induced by masitinib. The cystic keratinizing epithelioma appears to be a proliferative lesion limited to the rat and is rarely seen in other species (Boorman et al., 1996). In the current study, it was thought to be secondary to the irritation elicited in the alveoli by the presence of foamy macrophages induced by the test item administration. Bronchoalveolar hyperplasia in the lungs and squamous metaplasia in the lungs, trachea and larynx were considered to be regenerative and secondary to the injury elicited by the presence of foamy alveolar macrophages.

In an embryo-fetal development toxicity in of 24 mated female Sprague-Dawley rats litter data showed a lower (-10%) mean fetal body weight in the high-dose group. While visceral or skeletal malformations were not observed, masitinib-treatment was associated with variations in the form of unossified or incompletely ossified bones of the head, sternebrae and ribs. The incomplete ossifications were observed at doses ≥ 30 mg/kg/day.

In an embryo-fetal development toxicity in 3 groups of 22 mated female New Zealand rabbits unossification of 1st metacarpals was observed in fetuses from the 100 mg/kg/day dose group, achieving statistical significance for the fetal incidence ($p<0.05$), however the fetal and litter incidences were within CIT Historical Control Data ranges. Increased incidences of unossified 5th and 6th sternebra were observed at 30 and 100 mg/kg/day although this finding only reached statistical significance at 30 mg/kg/day.

Studies on developmental and reproductive toxicity are needed to make well-educated decisions in those rare cases where exposure occurs during early or late pregnancy. A decision to waive or postpone a study on pre- and postnatal toxicity must be justified by the fact that fertile women are rare in the patient population, or that data from the embryo-foetal toxicity study suggest such pronounced embryotoxicity that a pre- and postnatal toxicity study is unlikely to provide data of additional value. In absence of a valid justification, only in the case of a major clinical benefit it would have been acceptable for such data to be provided as a post – approval commitment (see discussion on clinical efficacy).

There were tumour findings in both the mouse and the rat carcinogenicity study. In the current application, masitinib is indicated for symptomatic treatment in patients with mastocytosis, a non-life-threatening indication. Based on the findings of the carcinogenicity study in mice, uncertainties and the lack of a full characterisation of metabolic pattern in humans, a carcinogenic risk of masitinib for patients, based on the bladder tumour findings in mice, cannot be fully excluded. However, it is anticipated that further nonclinical evaluation will not clarify this risk. Should the product be found to be of important clinical benefit in the target population, these findings would be described in the product information and proposed to be managed in the context of the risk management program (See discussion on benefit- risk).

2.3.7. Conclusion on the non-clinical aspects

Uncertainties in the non-clinical information submitted in support of the application of Masipro are related to the lack of developmental and reproductive toxicity studies and carcinogenic findings in rats. Should a significant clinical benefit be proven (see discussion on Clinical efficacy, Clinical Safety and Benefit / Risk) such uncertainties could be managed with appropriate warnings in the PI and measures in the RMP.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The applicant claimed that the clinical trials were performed in accordance with GCP.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table 25 Tabular overview of clinical studies

Study no.	Study design	Subjects	Objective	Drug, dose, adm. route
PIHV03001	Phase I, double blind, placebo-controlled, dose escalation, single dose	Healthy male subjects, N=40	Determine safety / tolerability and PK parameters of masitinib	Masitinib, powder for solution, ascending doses (40, 100, 200, 400 and 800 mg), oral
PIHV03003	Phase I, double blind, placebo-controlled, dose escalation, single dose	Healthy subjects, N=32	Determine safety / tolerability and PK parameters of masitinib	Masitinib, powder for solution, ascending doses (40, 100, 200, 400 and 800 mg), oral
AB 14004	Single-center, open-label, active-controlled, 4-sequence study of 28 days duration	Healthy subjects, N=15	Evaluate PK of masitinib and its metabolites after CYP3A4/P-gp-inhibition. Develop a popPK model Investigate QTc	Masitinib, tablet, 3 mg/kg/day at day 1, oral. Masitinib, tablet, 3 mg/kg/day from day 17 to 24, oral. moxifloxacin 400 mg at Day 1 Itraconazole 200 mg once daily (QD) from Day 9 to Day 13
AB 03002	Phase I, open	Patients with	Define maximum	Escalating doses of oral masitinib

	label, sequential cohort, dose escalation study	advanced solid tumors N=40	tolerated dose and assess PK of masitinib in patients with solid tumors	daily for up to three months or until unacceptable toxicity or documentation of disease progression or any other reason for patient's withdrawal
AB 04010	Phase II, open-label, randomised	Patients with mastocytosis (no D816V) N=25	PK/PD relationship, supportive study efficacy/safety	Masitinib, tablet 3 mg/kg or 6 mg/kg per day, oral.
AB 06013	Phase II, open-label, randomised	Patients with mastocytosis (with D816V) N=25	PK/PD relationship, supportive study efficacy/safety	Masitinib, tablet 3 mg/kg or 6 mg/kg per day, oral.
PIHV 05031	Cross-over, single dose	Healthy subjects, N=12	Evaluate food intake on PK profiles	Masitinib tablet 200 mg
PIHV 04015	Cross over, single dose	Healthy subjects, N=12	Compare relative BA of masitinib from two formulations	Masitinib tablet/ capsules 100 mg
AB06006	Phase III, prospective, multicentre, randomised, double-blind, placebo-controlled, 24 weeks + extension phase	Smouldering or indolent systemic mastocytosis N=224 (135 for efficacy analysis)	Assess safety and efficacy of masitinib	Masitinib or placebo tablet 6 mg/kg/day
AB04009	Multicenter, uncontrolled, open-label, 12 weeks + extension phase	Aggressive mastocytosis with point mutation in c-Kit (e.g. D816V) N=8	Assess safety and efficacy of masitinib	Masitinib tablet 6 mg/kg/day

Data from studies PIHV03001, PIHV03003, AB 04015, PIHV05031 and AB 03002 were used to develop a population PK model. Data from study AB 14004 were used to develop a second population PK model.

2.4.2. Pharmacokinetics

Clinical PK data are provided based on eight studies (table 26). Single oral doses of masitinib up to 800 mg has been given to healthy subjects (HV) and up to 1000 mg to patients. Table 27summarises the *in vitro* studies included in the clinical pharmacology package.

Table 28 Overview of studies included in the clinical pharmacology package of masitinib

Description	Phase	Subject	n	Dose	Reference	
Single dose escalation	1	HV (M)	40	40, 100, 200, 400, 800 mg	PIHV03001	old[a]
Multiple oral dosing	1	HV (M)	23	100, 200, 400, 800 mg	PIHV03003	old
Relative F	1	HV	12	100 mg	PIHV04015	old
Food interaction	1	HV	12	200 mg	PIHV05031	old
DDI itraconazole	1	HV	15	3 mg/kg	AB14004	new
					AB14004 PPK	new
MTD	1	Pats	40	40, 100, 150, 250, 500, 800, 1000 mg od or 9, 12 mg/kg.day	PIST03002	old
Efficacy/safety	IIa	Pats	25	3, 6 mg/kg	AB04010	new
Efficacy/safety	IIa	Pats		3, 6 mg/kg	AB06013	new
PPK[b]		HV/Pats				new

[a] old = has been submitted and assessed in earlier procedures

[b] data from studies 3001, 3003, 5031, 4015 and 3002

Table 29 Overview of *in vitro* studies included in the clinical pharmacology package of masitinib

Description	Masitinib concentration	Reference	
In vitro protein binding masitinib	100, 300, 500, 1000, 3000 ng/ml (<=> 0.2, 0.5, 0.8, 1.6, 5 µM)	PR6592-1/CC2099	old
In vitro protein binding AB3280	AB3280 4 µM	AB3280	new
In vitro metabolism in HLM	[14C]masitinib 5 µM	XTC/03	old
Met id following incubations in HLM	[14C]masitinib 5 µM	XTC/04	old
In vitro metabolism in human hepatocytes	[14C]masitinib 5 µM	ABS/05	old
CYP id	[14C]masitinib 5 µM	ABS/02	old
CYP2C8 id	5 µM	DC15041	new
CYP450 inhibition	Masitinib 0.05, 0.5, 5 µM; 5, 10, 30 µM AB3280 0.02, 0.2, 2 µM	PR6513-3/VT2081	old
CYP2C9, 2D6, 3A4/5 inhibition	Masitinib 3, 10, 30, 75, 150 µM	ABS-RF6513-2	new
CYP2C9, 2D6, 3A4/5 inhibition	Masitinib 3, 10, 30, 75, 150 µM µM	ABS-RF6513-2-Ki	new
Induction – CYP1A2, 2C9, 2C19, 3A4	Masitinib 0.2, 1, 2 µM AB3280 0.2, 1, 2 µM	PR6537-5/VT2085	old
Induction – CYP1A2, 2B6, 3A4	Masitinib 1, 3, 10, 30, 75, 150 µM	PR6537-2013	new
Pgp – substrate, inhibitor, Caco-2	Substrate – 1, 50, 500 µM Inhibitor – 1, 10, 100 µM	ABS/03	old
Pgp inhibition	25, 50, 100, 150, 200, 300 µM	AB1010/MDR1	new
BCRP inhibition	0, 0.1, 0.3, 1, 3, 10, 30, 100, 300 µM	DC14839	new
BCRP inhibition – Ki determination	0, 0.25, 0.5, 1, 2, 5, 30 µM	DC15926	new
Inhibition – OATP1B1, OATP1B3	0, 0.1, 0.3, 1, 3, 10, 30, 100, 300 µM	DC14841	new
OATP1B1, OATP1B3 - substrate	1, 100 µM	DC15904	new
Inhibition – OCT2, OAT1, OAT3		DC15040	new

Absorption

Single ascending oral dosing in healthy subjects (PIHV03001)

Single ascending oral doses were given to healthy subjects in a double blind, placebo controlled, parallel groups design. A total of 40 healthy subjects were included divided in five dose groups 40, 100, 200, 400 and 800 mg. Blood samples were taken before treatment and up to 48h *post* dose. Urine was collected in pre-defined intervals also up to 48h *post* dose. The systemic exposure, as well as the urinary excretion of masitinib and the metabolite AB3280 were determined.

Masitinib was administered as a powder for a solution to drink.

The figure below shows mean plasma concentration-time profiles of masitinib and AB3280. The basic PK are shown in the below table.

Masitinib **AB3280**

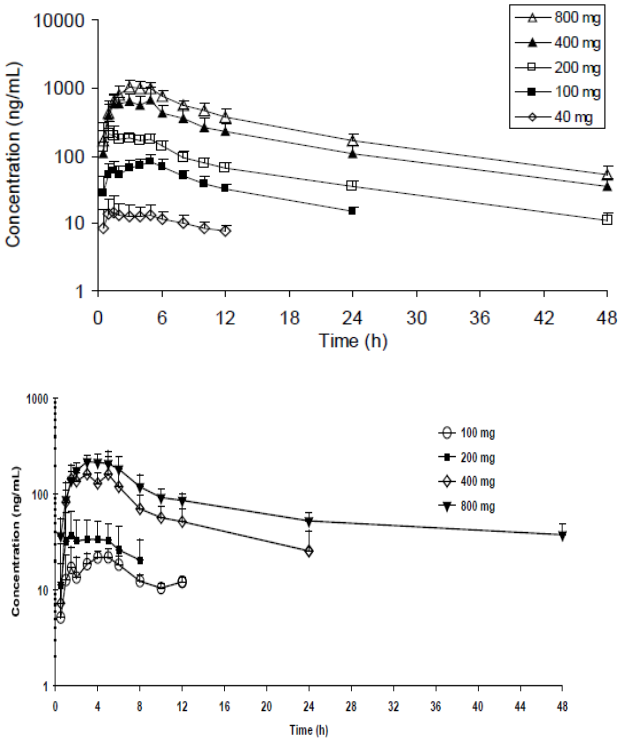


Figure 6 Mean(SD) plasma concentration of masitinib *vs* time following single oral doses of 40, 100, 200, 400 or 800 mg to healthy subjects

Table 30 Geometric mean (CV) PK of masitinib and the metabolite AB3280 following single oral doses of 40, 100, 200, 400 or 800 mg to healthy subjects

Masitinib

Dose (mg)	C_{max} (ng/ml)	t_{max}[a] (h)	AUC_{tot} (ng/ml.h)	$t_{1/2}$ (h)	$fe_{,0-48h}$ (%)	CL_R (ml/min)
40	15 (59)	2 [1 – 5]	269 (56)	12 (76)	0.8 (37)	24 (29)
100	82 (23)	5 [1.5 – 5]	1201 (19)	13 (22)	1.1 (22)	17 (30)
200	239 (32)	1.5 [1 – 5]	2823 (21)	13 (18)	1.3 (24)	17 (18)
400	711 (35)	3.5 [1.5 – 5]	8951 (30)	14 (13)	1.2 (51)	9 (35)
800	1122 (21)	3.5 [3 – 5]	14456 (22)	13 (9)	1.5 (39)	15 (29)

AB3280

Dose (mg)	C_{max} (ng/ml)	t_{max}[a] (h)	AUC_{tot} (ng/ml.h)	$t_{1/2}$ (h)	$fe_{,0-48h}$ (%)	CL_R (ml/min)
40	–	–	–	–	0.6 (37)	–
100	25 (25)	4.5 [1.5 – 5]	–	–	0.8 (21)	36 (12)
200	36 (62)	1.5 [1.5 – 5]	–	–	0.6 (39)	34 (3)
400	169 (37)	4 [1.5 – 5]	2125 (42)	15 (25)	0.6 (42)	21 (40)
800	235 (22)	4 [3 – 5]	5441 (25)	34 (35)	1.0 (17)	35 (17)

[a] median [min - max]

Bioequivalence

Relative bioavailability between capsule and tablet (PIHV4015)

In an open, randomised, two-way cross-over design, 12 healthy subjects received a single oral dose of 100 mg masitinib as two capsules one 50 mg and as one tablet 100 mg. Blood samples were taken during 36h post dose.

The concentration-time profiles of masitinib following dosing with two capsules 50 mg and as one tablet 100 mg are shown in the below figure. The exposure was comparable following dosing with the two formulations with 90% confidence intervals within the pre-defined criteria for bioequivalence 0.80-1.25 (see table below).

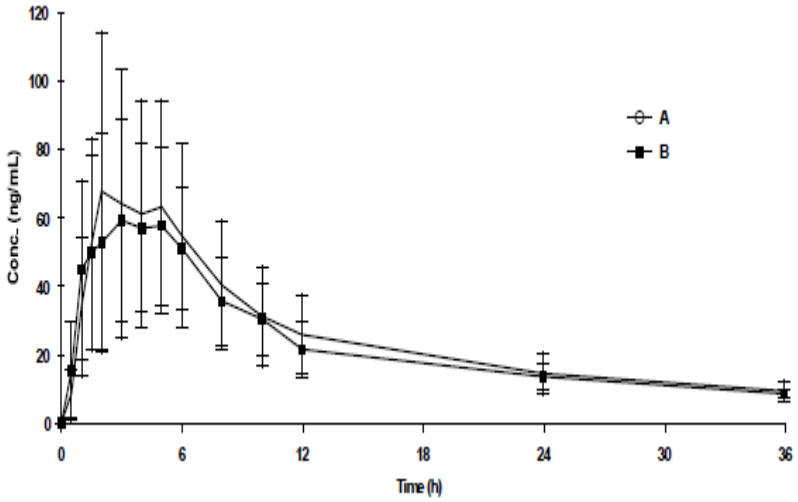


Figure 7 Mean(SD) plasma concentration of masitinib *versus* time following an oral dose of 100 mg as one tablet 100 mg (A) and 2 capsules 50 mg (B)

Table 31 Basic PK (geomean CV%) of masitinib following an oral dose of 100 mg as one tablet 100 mg and two capsules 50 mg

	C_{max} (ng/ml)	t_{max}[a] (h)	AUC_{tot} (ng/ml.h)	$t_{1/2}$ (h)
Tablet	67	3.5 [1.5- 6]	977	14
Capsule	66	4 [1.5 – 6]	977	16
Ratio Tablet / Capsule	1.02 [90%CI 0.84 -1.23]		1.00 [90%CI 0.87 – 1.15]	

[a] median [min - max]

Influence of food (PIHV0531)

Twelve healthy male subjects received a single oral dose of 200 mg masitinib in an open, randomised, two-way cross-over design during two treatments fasted and fed a high fat breakfast. Blood samples were collected pre-dose and regularly up to 144h (6 days) post dose.

Masitinib was administered as one 200 mg tablet.

The plasma concentration-time profiles of masitinib and of AB3280 are shown in the figure below. The systemic exposure increased about 20% when co-administered with food, for Cmax and AUC 19% and 23%, respectively (see table below).

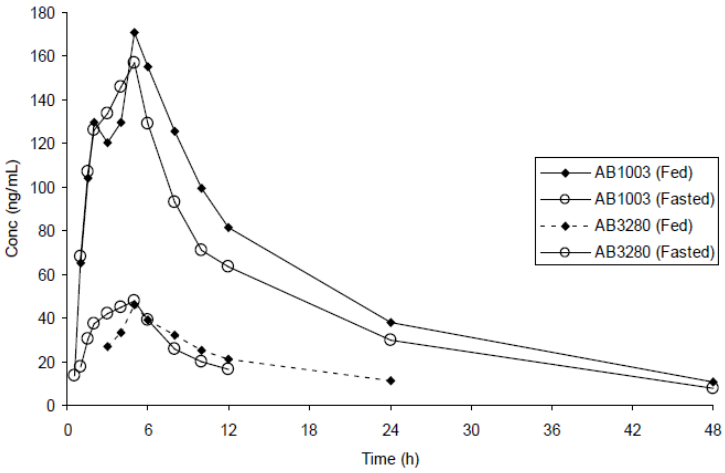


Figure 8 Mean plasma concentrations of masitinib and AB3280 *versus* time following an oral single dose of 200 mg in fasted and fed condition

Table 32 Summary of masitinib and AB3280 PK (geomean, CV%) in fasted and fed condition following an oral single dose of 200 mg

	C_{max} (ng/ml)	t_{max}[a] (h)	AUC_{tot} (ng/ml.h)	$t_{1/2}$ (h)
Masitinib				
Fed	189 (41)	5 [1.6 – 6]	2494 (36)	13 (29)
Fasted	163 (34)	4 [2 – 6]	2224 (29)	11 (14)
Ratio Fed/Fasted	1.19 [90%CI 98 - 145]		1.23 [90%CI 113 - 134]	
AB3280				
Fed	46 (34)	5 [2 – 6]	886 (42)	16 (51)
Fasted	50 (34)	4.5 [2 – 6]	673 (47)	10 (69)
Ratio Fed/Fasted	0.93 [90%CI 82 – 106]		1.33 [90%CI 109 – 162]	

[a] median [min - max]

Distribution

In vitro protein binding of masitinib (PR6592-1-CC2099)

The in vitro protein binding of [14C]masitinib was determined by equilibrium dialysis at 100-3000 ng/ml (0.2-5 µM) masitinib. The plasma protein binding was determined to 93.9% ie the unbound fraction (fu) was calculated to 6.1%.

The mean fraction [14C]masitinib bound (fb) to human serum albumin, α1-acid glycoprotein and gamma globulins was 91, 74 and 46%, respectively.

In vitro protein binding of the metabolite AB3280 (AB3280-PB)

The plasma protein binding of AB3280 4 µM was determined by the use of a blood partitioning method where the dialysis membrane is replaced by the biological membrane of the erythrocytes. The fb is reported to be 93.3% <=> with a fu of 6.7%.

Elimination

The calculated mean terminal $t_{1/2}$ for masitinib was 13-19h following both a single and repeated dosing. The mean t1/2 for AB3280 ranged between 15-38h independently of single or repeated dosing

About 2.5% of a single oral dose of masitinib was excreted in the urine, 1.5% as unchanged compound and 1% as AB3280

Metabolism

In vitro

Human microsomes (XTC/03)

The *in vitro* metabolism of 14C-masitinib 5 µM was studied following 30 min incubation with pooled human liver microsomes (HLM) .

Masitinib was extensively metabolized. A total of four radiolabelled metabolites AB3280 (N-demethylation) and MET1 to MET3 were detected using HPLC analysis.

Metabolite identification following incubation of 14C-masitinib in HLM (XTC/04)

The samples from the incubation of 14C-masitinib with HLM (XTC/03) were re-analyzed using LC-MS/MS aiming to identify MET1 to MET3.

MET2 was identified as the carbamimidothioic acid (or thiothiourea) metabolite of masitinib and MET3 as an N-oxide metabolite.

Human hepatocytes (ABS/05)

The *in vitro* metabolism of 14C-masitinib 5 µM was investigated following 4h incubation in freshly isolated human hepatocytes. Samples were analyzed by HPLC with on-line radiodetection and selected samples also by UPLC-MS/MS (ultra-performance liquid-chromatography tandem mass spectrometry).

The most common metabolic pathways detected included N-demethylation and N-oxidation; cleavage of the amide bond was also detected but to a minor route in human hepatocytes.

Identification of metabolizing enzymes involved in the metabolism of masitinib (ABS/02)

The *in vitro* metabolism of 14C-masitinib was studied in HLM to identify the cytochrome P450 (CYP450) enzymes involved in the metabolism. Reaction phenotyping was undertaken in the presence of NADPH, by incubation of 14C-masitinib with HLM, selective CYP450 inhibitors and recombinant CYP450 enzymes (CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4).

14C-masitinib was metabolised up to four metabolites by HLM in the presence of NADPH with AB3280 identified as the main metabolite formed. The below table summarises the results.

Table 33 In vitro incubation with 14C-masitinib with HLM in the presence of NADPH and chemical inhibitors

Enzyme	Inhibitor	% inhibition of 14C-masitinib metabolism
CYP 1A2	Furafyllin	<6
CYP 2C8	Quercetin	68
CYP 2C9	Sulphaphenazole	0
CYP 2B6 and 2C19	Ticlopidine	23
CYP 2D6	Quinidine	<2
CYP 2E1	Diethyldithiocarbamate	26
CYP 3A4	Ketoconazole	68

14C-masitinib was incubated with cDNA expressed CYP450 enzymes. CYP3A4 catalysed the formation of MET 1 - 4 in a profile similar to that found in HLM. CYP2C8 catalysed the formation of AB3280 as a major metabolite with lesser amounts of MET 2. CYP2D6 catalysed the formation of minor amounts of AB3280. 14C-masitinib was not metabolised by CYP1A2, CYP2B6, CYP2C9, CYP2C19 or CYP2E1.

Identification of CYP2C8 involvement in the metabolism of masitinib (DC15041)

The involvement of CYP2C8 in the metabolism of masitinib was studied *in vitro* by the use of recombinant CYP2C8 as well as by simultaneous incubation of masitinib and the CYP2C8 inhibitor montelukast in pooled HLM.

10-min incubation of masitinib 5 µM together with recombinant CYP2C8 resulted in a biotransformation ratio of 19%. This is confirmed by a formation of A3280.

10-min incubation of masitinib in HLM together with montelukast 10 µM resulted in a partial inhibition of the formation of AB3280, the formation was inhibited by 52%.

In vivo

AB3280 has been monitored following oral administration. The table below shows that the exposure of AB3280 is about 24-38% compared to the total exposure of masitnib. The time for C_{max} was about 4h. No differences are observed between single and repeated dosing. The mean $t_{1/2}$ for AB3280 ranged between 15-38h independently following single or repeated dosing.

When masitinib was co-administered together with itraconazole, a strong CYP3A inhibitor, about a 50% increase was seen in the exposure of AB3280 (as well as in the exposure of masitinib).

Table 34 Systemic exposure of masitinib and AB3280 following oral single doses

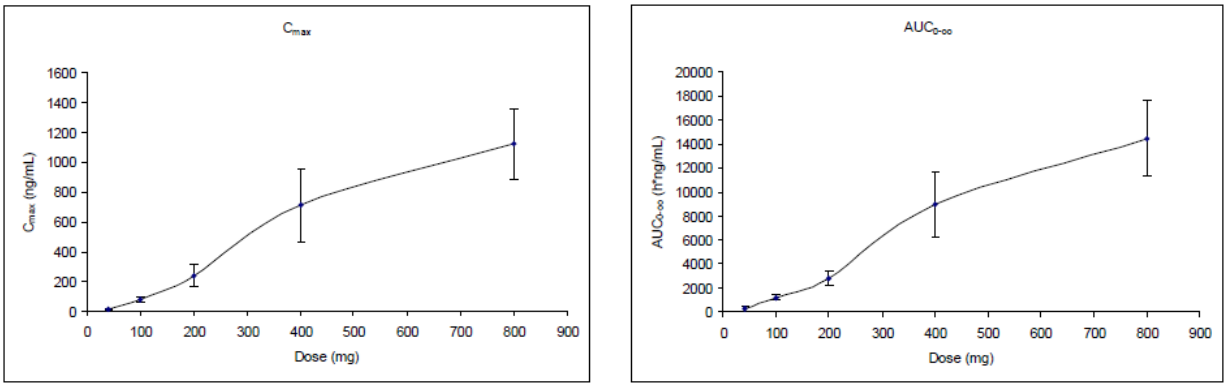
	Dose	% AB3280	Study
Masitinib	200 mg fed		PIHV05031
AB3280		36	
Masitinib	200 mg fasted		
AB3280		30	
Masitinib	400		PIHV03001
AB3280		24	
Masitinib	800		
AB3280		38	
Masitinib	3 mg/kg		AB14004
AB3280		27	
Masitinib[a]			
AB3280[a]		27	

[a] together with CYP3A inhibitor itraconazol•

Dose proportionality and time dependency

In the single ascending dose study the systemic exposure increased more than dose-proportionally (PIHV03001).

Masitinib



AB3280

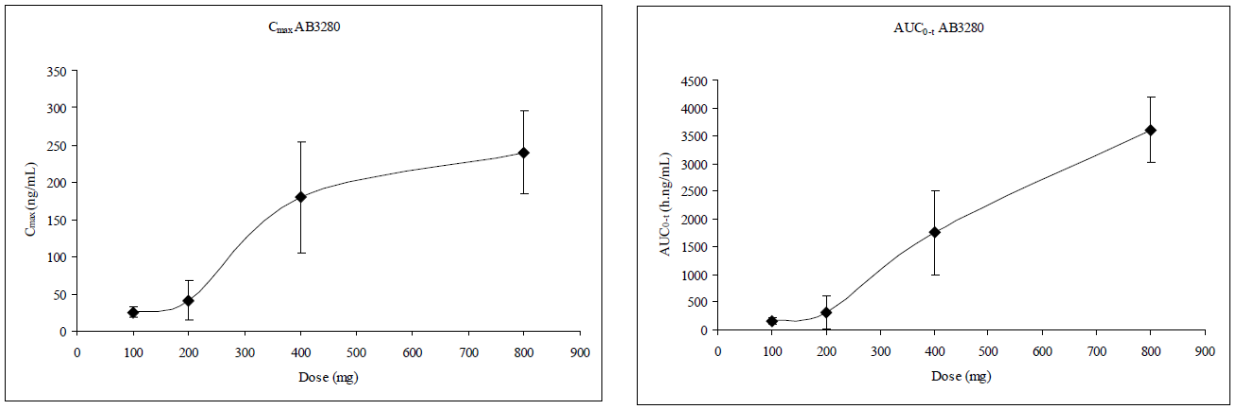


Figure 9 C_{max} and AUC of masinitib (upper panel) and AB3280 (lower panel) *versus* dose following doses of 40 – 800 mg administered as an oral solution in healthy subjects (PIHV03301)

Open dose escalating oral doses in patients with advanced solid tumours (AB03002)

The systemic exposure of masitinib and AB3280 were determined in patients with advanced solid tumours in an open, non-randomised, sequential cohorts, dose-escalation design aiming for defining the maximum tolerated dose (MTD). Patients were treated once daily up to three months. PK blood samples were taken on Day 1 and Day 14. The study was started using capsules but change to the use of tablets at two strengths 100 mg and 200 mg. At this study of the systemic exposure of masitinib after a single dose and after two weeks treatment an accumulation was seen, however, less than 2-fold when comparing the exposure after a single dose with steady state levels. The increase in exposure with dose was more than dose-proportional especially at the higher dose range.

Dose proportionality and time dependencies

Ascending multiple oral doses in healthy male subjects (PIHV03003)

Healthy male subjects were (n=30) were enrolled in the double blind, randomised, multiple ascending dose study including four dose groups 100, 200, 400 and 800 mg. Masitinib was administered once daily for one week. Doses were administered in an escalating manner.

Blood samples were taken frequently up to 12h after the first dose and up to 72h after the last dose. Pre-dose samples were taken daily as well as a sample taken at 4h *post* dose on days 2, 4 and 6. Urinary samples were collected at pre-dose and 0-24h after the first dose and at 0-24h and 24-48h after the last dose.

The systemic exposure of masitinib *versus* time on Day 1 is shown in the below figure. The below table shows the basic PK of masitinib and AB3280. The exposure of both masitinib and AB3280 was about 2-fold higher following one week treatment compared to a single dose. The time for C_{max} varied between 1-5h for masitinib independently if after a single dose or after one weeks treatment. For AB3280 t_{max} varied between 2-6h on both the first and the las day dosing.

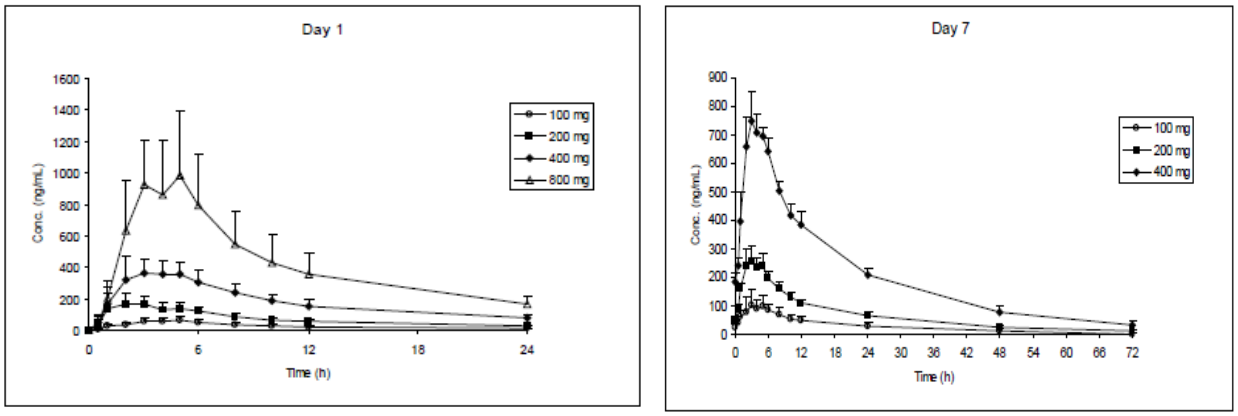


Figure 10 Mean (SD) plasma concentrations of masitinib on Day 1 and 7 following once daily dosing of 100, 200, 400 and 800 mg. *NB* different scales on the axes

Table 35 Mean (CV%) exposure of masitinib and AB3280 following once daily dosing for 1 week

Masitinib

Dose (mg)	C_{max} (ng/ml) Day 1	Day 7	AUC_{0-T} (ng/ml.h) Day 1	Day 7	C_{trough} (ng/ml) Day 1	Day 7	$t_{1/2}$ (h) Day 7
100	63 (33)	100 (43)	675 (33)	1249 (40)	12 (32)	28 (45)	16 (27)
200	176 (39)	264 (17)	1772 (23)	3179 (13)	27 (20)	67 (18)	18 (19)
400	380 (26)	764 (11)	4317 (26)	10000 (8)	81 (26)	210 (11)	19 (14)
800	1063 (38)	–	9724 (43)	–	168 (29)	–	–

AB3280

Dose (mg)	C_{max} (ng/ml) Day 1	Day 7	AUC_{0-T} (ng/ml.h) Day 1	Day 7	C_{trough} (ng/ml) Day 1	Day 7	$t_{1/2}$ (h) Day 7
100	19 (45)	29 (57)	91 (81)	562 (26)	–	15 (5)	25 (92)
200	54 (37)	73 (29)	362 (26)	851 (20)	–	21 (10)	35 (40)
400	74 (27)	139 (19)	805 (22)	1862 (16)	18 (25)	49 (17)	38 (26)
800	272 (40)	–	2283 (47)		37 (40)	–	–

Pharmacokinetics in target population

Population pharmacokinetics

The population PK model built in the context of the sought indication takes into account PK data obtained in healthy volunteers and patients with solid tumours. In total, 1419 plasma concentrations at given time-points were available for analysis from 116 subjects (98 males, 18 females). Each study is briefly discussed below.

Study AB03001

This study was a double blind, placebo-controlled, phase I study to determine the safety, tolerability and pharmacokinetic profiles of ascending, single oral doses of AB1010 in healthy volunteers. 40 subjects were enrolled in this study, receiving masitinib doses of 40, 100, 200, 400, and 800 mg, 6 subjects at each dose level and 10 patients receiving placebo. Blood and urinary samples were collected at predose then regularly up to 48 hours after dosing (sampling time-points: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 48 hours).

Study AB03003

This study was a double blind, placebo-controlled study with a parallel group ascending dose design. 30 subjects were randomized to receive ascending doses of 100mg, 200mg, 400mg, and 800mg of masitinib or matching placebo. 6 subjects in each dose group and 8 placebo patients were enrolled. Masitinib was administrated as one daily morning intake for seven consecutive days. Blood samples were collected on day 1 and day 7, at pre-dose then regularly after dosing up to 12.0 hours and 72.0 h, respectively. Sampling times were pre-dose, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00 h post-dose on Day 1 and pre-dose, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 24.00, 48.00 and 72.00 h post-dose on Day 7.

Study AB04013

This was a single centre, open, two-way cross over study. Twelve healthy male volunteers received a single oral dose of AB1010 base (100 mg) on Day 1 of each of both treatment periods. The two administrations were separated by at least a one-week interval where no AB1010 was taken in order to prevent any carry over effect.

The aim of the study was to compare the relative bioavailability of AB1010 from two different formulations (capsule or tablet) in 12 healthy male volunteers after a 100 mg AB1010 base single oral administration. Blood samples were collected at predose and then 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0 and 36.0 hours after dosing.

Study AB05031

This was a single centre, open, randomised, two-way crossover study. There were 2 study periods (i.e. Period 1 and Period 2), each with a duration of 6 days. On Day 1 of both periods, each subject received a single oral dose of 200 mg of AB1010base under fed or fasted condition. There was at least a 2-week washout period between the two study periods in order to prevent any carry over effect. The duration of the study, up to the end-of-study visit, was about 7 weeks.

The aim of the study was to evaluate the food intake influence on pharmacokinetic profiles in 12 healthy male volunteers after single oral administration of 200 mg AB1010 tablets.

Blood samples were collected at pre-dose then 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0, 72.0, 96.0 and 144.0 hours after dosing.

Study AB03002

This was a multicenter, non-randomized, open-label, sequential cohort, dose-escalation phase I study of oral masitinib in adults with advanced and/or metastatic cancer. Patients with advanced solid tumors were included in subsequent cohorts of escalating treatment dose. In total, 40 patients were included in this study, including 19 patients with GIST.

The first dose levels were administered as initially planned in the protocol. Successive cohorts of one patient each received escalating doses of oral masitinib daily for up to three months or until unacceptable toxicity or documentation of disease progression or any other reason for patient's withdrawal. Masitinib was administered at increasing doses based on the following classical design for dose escalation.

The primary aim of this study was to assess the safety and tolerability of oral masitinib administered daily as a single agent in patients with solid tumors, i.e. to define the maximum tolerated dose of the drug.

The secondary objectives of the study were to assess the pharmacokinetic profile of masitinib in human subjects with cancer and to assess the clinical activity of masitinib. Plasma samples were taken on Day 1 at pre-dose and in regular intervals thereafter until 48 hours after administration (sampling times: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 hours). Plasma samples were also taken on Day 14 at pre-dose and regular intervals thereafter (sampling times: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, and 72 hours. Sampling was only performed up to 12 hours after dosing for certain patients in cohorts #5, #7, and #8. The different cohorts are shown below.

The following cohorts were included:

#1 = patients who received masitinib at the dose of 40 mg/day (n=1)

#2 = patients who received masitinib at the dose of 100 m/day (n=3)

#3 = patients who received masitinib at the dose of 150 mg/day (n=3)

#4 = patients who received masitinib at the dose of 250 mg/day (n=4)

#5 = patients who received masitinib at the dose of 500 mg/day (n=3)

#6 = patients who received masitinib at the dose of 1 000 mg/day (n=6)

#7 = patients who received masitinib at the dose of 800 mg /day (n=6)

#8 = patients who received masitinib at the fixed dose of 9 mg/kg/day (n=7)

#9 = patients who received masitinib at the fixed dose of 12 mg/kg/day (n=7)

Data were analysed using the nonlinear mixed effect modelling software program Monolix version 3.1s (http://wfn.software.monolix.org). Parameters were estimated by computing the maximum likelihood estimator of the parameters without any approximation of the model (no linearization) using the stochastic approximation expectation maximization (SAEM) algorithm combined to a MCMC (Markov Chain Monte Carlo) procedure. The number of MCMC chains was fixed to 10 for all estimations. A constant error model was used to describe the residual variability, and the between subject variabilities (BSV, η) were described by an exponential model.

The pharmacokinetics of plasma masitinib in patients was satisfactorily described by a two-compartment open model with linear elimination. The main covariate effects were related to body weight which influenced all PK parameters and to albumin which influenced the clearance and the

central volume of distribution and decrease inter-individual variabilities. The final parameter estimates are shown in the table below.

Table 36 Parameter estimates of the final masitinib population model in 116 subjects

Parameter	Covariate effect(s)	Estimate (%rse)	BSV (%rse)
Ka (h^{-1})	NA	**0.33** (20)	0.57 (17)
CL, ($L.h^{-1}$ .70 kg^{-1})	$TVCL*(BW/70)^{0.75}*(ALB/40)^{\beta}$ $\beta ALB = 1.24$	**85.8** (9)	0.625 (8)
V1 (L 70 kg^{-1})	$TVV1*(BW/70)^{1}*(ALB/40)^{\beta}$ $\beta ALB = 0.975$	**396** (11)	0.385 (27)
Q (L/h)	$TVQ*(BW/70)^{0.75}$	**21.4** (34)	0.931(31)
V2 (L)	TVV2* (BW/70)	**416** (25)	NA
Residual variability	/	Constant error model	104 (2)

Key: %rse, percent relative standard error; BSV, between-subject variability; CL and Q, elimination and inter-compartmental clearances; V1 and V2 central and peripheral volumes of distribution; NA, Not Applicable; TV, Typical Value.

An excerpt of the individual concentration predictions are displayed below.

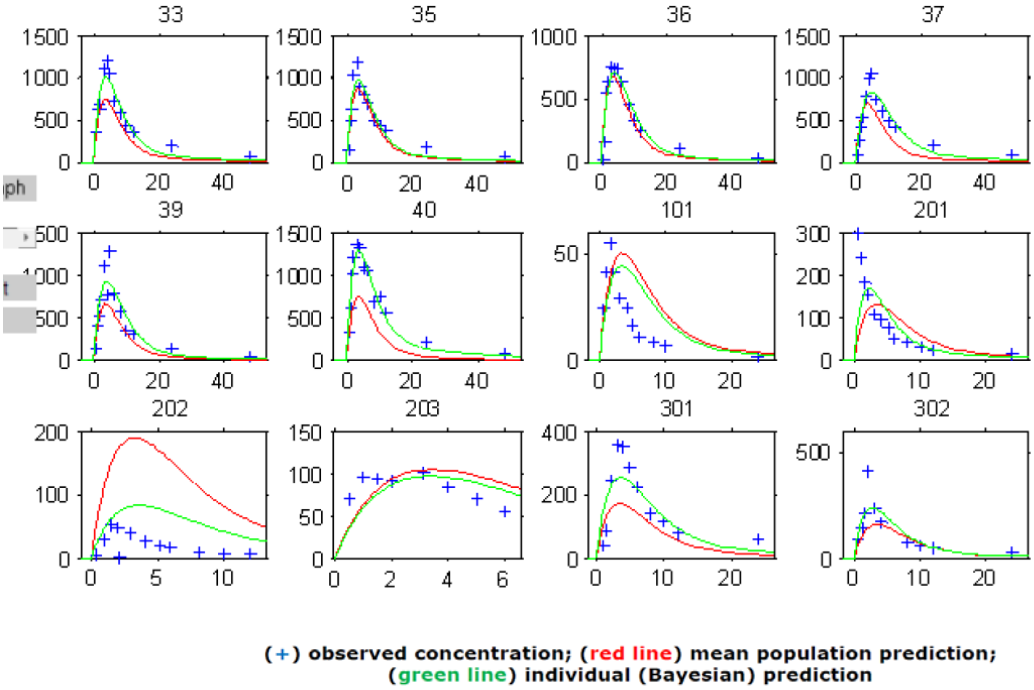


Figure 11 Observed and model-predicted individual masitinib concentration-time profiles

Special populations

Studies with Masipro in patients with renal or hepatic impairment have not been submitted.

Pharmacokinetic interaction studies

The PK interaction potential of masitinib has been evaluated in previous submissions in a number of *in vitro* studies and one *in vivo.*

Table 37 Summary of the *in vitro* results

Enzyme	Substrate	Inhibitor	IC50 (µM)	Clinical relevance	Induction Clinical relevance
CYP3A4/5	Yes	Yes	17	Yes - gut	Yes – gut[a]
CYP2C8	Yes				
CYP2D6	(Yes)	Yes	>30	No	
CYP2C9		Yes	17	No	

[a] cannot be ruled out as relevant clinical concentrations could not be studied due to downregulation/cytotoxicity

Masitinib was not a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19 or CYP2E1

Masitinib was not an inhibitor of CYP1A2, CYP2C8, CYP2C19 and CYP2E10; no data on CYP2B6 are available

Masitinib has no induction potential towards CYP1A2 and CYP2B6

AB3280 was not an inhibitor of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5

Table 38 Summary of results with transport proteins

Transporter	Substrate	Inhibitor	IC50 (µM)	Clinical relevance
Efflux transporters				
Pgp	Yes at 1 µM	Yes		Yes - gut
BCRP		Yes	0.7	Yes
Uptake transporters				
OATP1B1		Yes	>100	No
OATP1B3		Yes	130	No
OAT1		Yes	>100	No
OAT3		yes	>100	No
OCT2		Yes	17	No

Investigation of masitinib as a potential Pgp substrate and inhibitor (ABS/03)

Masitinib was incubated with Caco-2 cells to assess whether it should be classified as a Pgp substrate or inhibitor. Propranolol and mannitol, classified with high and medium/low permeability, respectively, were used as positive controls.

The apparent permeability (P_{app}) for Apical–Basolateral (A-B) and Basolateral-Apical (B-A) transport was determined at 1, 50 and 500 µM masitinib. The B-A permeability was determined in absence and presence of verapamil a known Pgp inhibitor.

The potential of masitinib (1, 10 and 100 µM) to inhibit Pgp was investigated by determining P_{app} for A-B and B-A for Pgp substrate vinblastine.

The table below shows that masitinib is a Pgp substrate at lower concentrations.

Table 39 Apparent permeability of masitinib in Caco-2 cells, in the absence and presence of verapamil, as well as apparent permeability of positive controls

Test compound	Papp (*10^{-6} cm.sec^{-1})		Pgp efflux ratio
	A-B	B-A	
Masitinib 1 µM	2.1	19	8.9
Masitinib 50 µM	5.9	14	2.4
Masitinib 500 µM	7.6	9.3	1.2
Masitinib 1 µM + verapamil 20 µM	8.3	20	2.4
Masitinib 50 µM + verapamil 20 µM	7.2	17	2.4
Masitinib 500 µM + verapamil 20 µM	10	11	1.1
Propranolol 50 µM	26	32	1.2
Mannitol 50 µM	3.5	2.2	0.6
Vinblastine 10 µM	1.6	34	21
Vinblastine + verapamil	4.4	25	5.7

Masitinib is characterised as a Pgp inhibitor at higher concentrations (see table below).

Table 40 Apparent permeability of masitinib in Caco-2 cells, in the absence and presence of verapamil, as well as apparent permeability of positive controls

Test compound	Papp (*10^{-6} cm.sec^{-1})		Pgp efflux ratio
	A-B	B-A	
Vinblastine + masitinib 1 µM	0.8	27	32
Vinblastine + masitinib 10 µM	2.2	22	10
Vinblastine + masitinib 100 µM	4.9	11	2.2
Propranolol	26	31	1.2
Mannitol	1.7	0.2	0.1
Vinblastine	2.6	29	11
Vinblastine + verapamil	3.7	16	4.3

In vitro investigation of masitinib as a potential Pgp inhibitor (AB1010/MDR1)

Masitinib is classified as a Pgp inhibitor (ABS/03). The present study aimed at determining the *in vitro* K_i value. Purified cell membrane fractions prepared from insect cells transfected by the human MDR1 gene were used. The inhibitory potential of masitinib 25-300 µM towards verapamil 5-40 µM was tested and actinomycin D 10 µM as reference compound. The effect of the test compound on ATPase activity was measured.

Masitinib showed concentration dependent Pgp inhibition (see below figure).

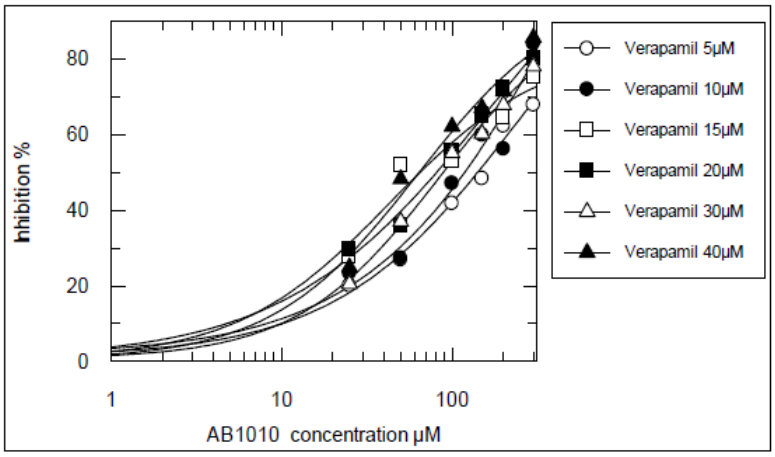


Figure 12 *In vitro* Pgp inhibition of verapamil by masitinib 25-300 µM

The Ki value was not calculated as the mechanism of inhibition by masitinib was concluded not competitive (see below figure).

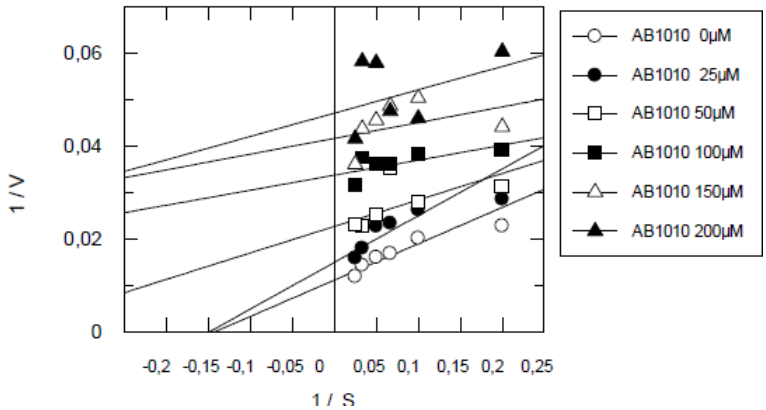


Figure 13 Lineweaver-Burk plot of inhibition of Pgp transport of verapamil by masitinib

In vitro assessment of masitnib as a potential BCRP inhibitor (DC14839)

The inhibitory potential of masitinib towards the BCRP transporter was investigated using plasma membranes vesicles prepared from cells over-expressing human BCRP protein. Masitinib in the concentration range 0-300 µM was studied, estrone-3-sulfate was used as BCRP probe substrate and sulfasalazine 5 µM as reference inhibitor.

The IC50 value was calculated to 0.7 µM.

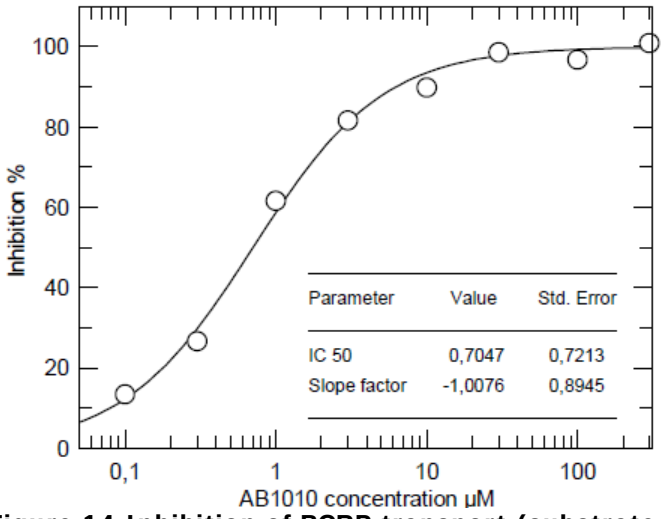


Figure 14 Inhibition of BCRP transport (substrate estrone-3-sulfate) by masitinib

Masitinib a BCRP inhibitor – Ki determination (DC15926)

Masitinib is characterised a BCRP inhibitor (DC14839). The present study aimed at determining the Ki value by studying the inhibition of the BCRP-mediated estrone-3-sulfate by masitinib 0-30 µM. The inhibition was investigated at different concentrations of estrone-3-sulfate (0.4-6 mM) to evaluate the inhibition kinetics. Sulfasalazine was used as BCRP reference inhibitor. The test system was inverted plasma membrane vesicles (inside-out membrane vesicles) prepared from insect cells transfected with human BCRP gene.

Masitinib showed a concentration-dependent inhibition of the BCRP-mediated transport of estrone-3-sulfate. The inhibition at 30 µM was >80%.

The inhibition of estrone-3-sulfate transport *via* BCRP transporter is indicated to be in a competitive manner and the Ki value is calculated to be 1.4 µM (see figure below).

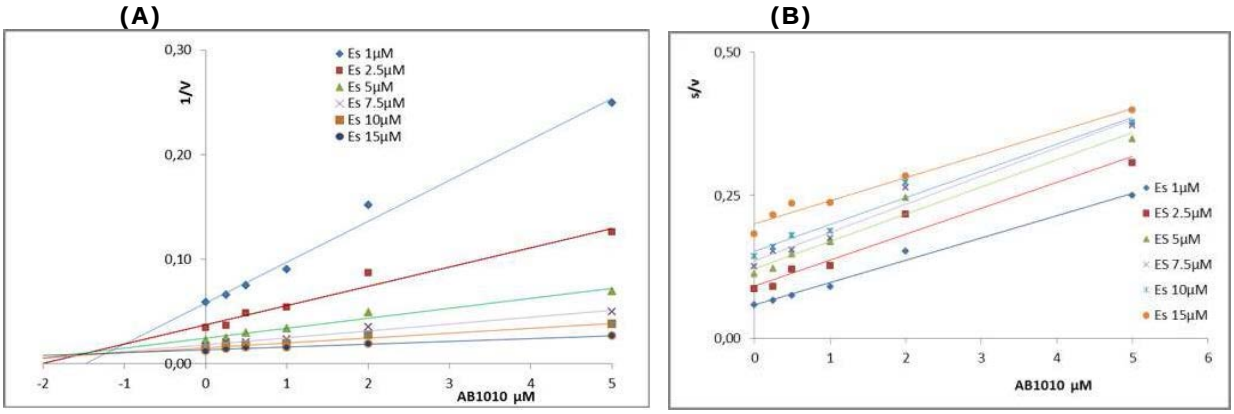


Figure 15 Dixon (A) and Cornish-Bowden (B) plot of BCRP-mediated estrone-3-sulfate transport inhibition by masitinib

Masitinib is characterised as a BCRP inhibitor *in vitro* as the transport of estrone-3-sulfate was inhibited in a concentration-dependent manner. The IC50 value was calculated to 0.7 µM, Ki to 1.4 µM.

Transport proteins for cellular uptake

In vitro inhibition of OATP1B1 and OATP1B3 (DC14841)

The potential properties of masitinib, 0.1-300 µM, to inhibit the active transport of OATP1B1 and OATP1B3 substrates, estrone-3-sulfate (0.1 µM) and Fluo-3, respectively, was investigated using mammalian CHO cells expressing human OATP1B1 or human OATP1B3. Cerivastatin and fluvastatin were used as reference inhibitors for OATP1B1 and OATP1B3, respectively.

Masitinib inhibited OATP1B1-mediated transport at 300 µM but not at lower concentrations and OATP1B3-mediated at >100 µM.

The IC50 for OATP1B3 was calculated to be 130 µM.

Determination whether masitinib is characterized as OATP1B1 and OATP1B3 substrates (DC15904)

To assess potential active uptake of masitinib by OATP1B1 and OATP1B3, masitinib was incubated using HEK-293 cells over-expressing human OATP1B1 or human OATP1B3 transporters. Transfected cells and mock cells (transfected with an empty vector) were incubated with two concentrations of masitinib, 1 and 100 µM, for three incubation times in the absence or presence of rifampicin, a known OATPs inhibitor. Estradiol-17β-glucuronide, a known OATP1B1/1B3 substrate, was run in parallel to validate the system.

Masitinib was not a OATP1B1 substrate. No differences were in masitinib levels between OATP1B1 transfected cells and mock cells independently of concentration or duration of incubations. The presence of rifampicin did not change the uptake.

Masitinib was neither characterized as an OATP1B3 substrate. Similar uptake of masitinib was seen in transfected and mock cells independently of concentrations and incubation time. The presence of rifampicin did not change the uptake.

Masitinib (1 and 100 µM) was not characterised as a substrate of OATP1B1 and OATP1B3 *in vitro* using transfected HEK-cells.

In vitro inhibition of OCT2, OAT1 and OAT3 (DC15040)

The potential of masitinib 0-300 µM to inhibit OCT2, OAT1 and OAT3 was assessed using over-expressing CHO (OCT2, OAT1) or MDCK (OAT3) cells. Metformin 10 µM, aminohippuric acid 5 µM and estrone-3-sulfate 1 µM were used as probe substrate for OCT2, OAT1 and OAT3, respectively. Verapamil, benzbromarone and probenecid were used as reference inhibitors for OCT2, OAT1 and OAT3, respectively.

OCT2-mediated transport of metformin was inhibited by masitinib. The IC50 value was calculated to 17 µM.

OAT1 p-aminohippuric acid transport was slightly inhibited by masitinib. An IC50 value was estimated to >100 µM.

OAT3-mediated estrone-3-sulfate transport was slightly inhibited by AB1010. The IC50 value was estimated to >100 µM.

Masitinib was an inhibitor OCT2-mediated metformin transport in transfected CHO cells with an IC50 value of 17 µM.

In OAT1 and OAT3 transfected CHO and MDCK cells, respectively, was masitinib characterized as a weak inhibitor with an IC50 value of >100µM for both p-aminohippuric acid (OAT1) and estrone-3-sulfate (OAT3) transport.

In vivo drug-drug-interactions

In vivo CYP3A inhibition by itraconazole (AB14004)

The PK of masitinib and AB3280 was studied in the absence and presence itraconazole, a strong CYP3A inhibitor. The potential inhibition of the masitinib metabolism was evaluated in one treatment sequence in the QT/QTc study performed.

Fifteen healthy subjects received a single dose of masitinib 3 mg/kg on Day 3 (a single dose of moxifloxacin 400 mg had been administered on Day 1). Itraconazole 200 mg (capsule 2x100 mg) was given orally once daily from Day 9 to Day 13. On Day 12 a single dose of masitinib 3 mg/kg was administered 1h after the itraconazole dose.

Frequent blood samples were collected up to 72h post dose of masitinib in the absence of itraconazole and up to 120h in the presence of itraconazole.

The total exposure and C_{max} of masitinib increased by 46% and 28%, respectively, when co-administered with itraconazole. The $t_{1/2}$ increased from 16h to 21h *ie* about 30% when inhibiting CYP3A4 with itraconazole.

C_{max} and AUC_{inf} for AB3280 increased by 11% and 52%, respectively, when masitinib was administered in the presence of itraconazole. The $t_{1/2}$ was doubled.

In line with the recommendations in the draft guideline on the investigation of drug interactions (CPMP/EWP/560/95 Rev 1), the CYP450 inhibitory properties of masitinib and AB3280 towards CYP1A2, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4/5 were investigated in human liver microsomes.

- AB1010 was a weak to moderate inhibitor of the CYP3A4/5 and CYP2C9, as well as CYP2D6 with IC50 values of 14 µM, 20 µM and > 30 µM, respectively. This inhibition is partly reversible.
- 2) AB1010 does not increase the activity of CYP3A4/5, CYP2C9 and 2C19 but slightly increase CYP1A2 activity. AB1010 does not affect the mRNA of CYPs 1A2, 2B6 and 3A4/5.
- 3) At lower concentrations (<10 µM), AB1010 appears to be a substrate of P -gp mediated transport while at higher concentrations (≥10 µM), AB1010 appears to be an inhibitor of P-gp mediated transport which is likely to be due to competitive inhibition of P-gp-mediated efflux.
- 4) AB1010 is not an inhibitor of OATP1B1 (IC50 not reached) and a weak inhibitor of OATP1B3 (IC50 of 130µM).

Pharmacokinetics using human biomaterials

N/A

2.4.3. Pharmacodynamics

Mechanism of action

No PD studies on the mechanism of action have been submitted.

Primary and Secondary pharmacology

No specific primary PD studies were submitted.

Secondary pharmacology

Study AB14004 was a single-centre, single-arm, open-label, active-controlled, 4-sequence study evaluating the potential effect of masitinib on the Fridericia-corrected QT interval (QTcF; primary objective) and other ECG parameters (i.e. heart rate (HR), PR and QRS interval; secondary objective) in healthy subjects. The study period was 28 days and each subject received in sequence the 4 following treatments:

- Treatment Sequence 1: a single dose of moxifloxacin 400 mg was administered on Day 1.
- Treatment Sequence 2: a single dose of masitinib 3 mg/kg was administered according to the subject's weight at screening in the morning of Day 3.
- Treatment Sequence 3: repeated dose of itraconazole 200 mg was administered once daily (QD) from Day 9 to 13 (5 days). In the morning of Day 12, a single dose of masitinib 3 mg/kg was administered 1 hour after the administration of itraconazole 200 mg.
- Treatment Sequence 4: repeated dose of masitinib 6 mg/kg/day BID was administered according to the subject's weight at screening from the evening of Day 17 to the morning of Day 24 (7 days).

In total, 37 subjects were screened and 15 subjects were treated and analysed (ITT/mITT). Among the 15 subjects included in the study for whom a baseline value was available, 5 were classified as non-responders to moxifloxacin. The maximum observed baseline-corrected mean change in QTcF induced by moxifloxacin occurred 3 hours after administration and was 5.97 ms (90% CI: 2.20, 9.74 ms; p=0.01). As expected, administration of moxifloxacin caused an increase in QTcF. However, the detected increase in QTc is rather small compared to the values reported in earlier studies for the QTc prolonging effect of oral moxifloxacin (normally in the range of 10 to 15 ms).

Within 12 hours after a single dose of 3 mg/kg of masitinib, the largest mean change in the QTcF interval was -7.74 ms (90% CI: -11.5, -3.97 ms; p=0.0011) observed 3-hours post dose. The QTc shortening was statistically significant ($p<0.05$) at 1, 1.5, 3, 4 and 5 hours post dosing. For two subjects (13.3%), there was a new (not present at baseline) QTcF of $>450 \leq 480$ ms observed after administration of both moxifloxacin and a single-dose of 3 mg/kg of masitinib. Furthermore, within 12 hours after a therapeutic dose of 6 mg/kg, a non-statistically significant QTc shortening with a largest mean change in the QTcF interval of -4.65 ms (90% CI: -8.73, -0.58 ms; p = 0.06) was observed 5-hours post dose. Though, a small QTcF prolongation of +4.98 ms (90% CI: 0.91, 9.06 ms; p = 0.045) was observed 12 hours after repeat-dose of masitinib. No clear effect of either single-or repeat-dose administration were observed in HR, PR and QRS width.

2.4.4. Discussion on clinical pharmacology

A bioanalytical assay for determination of total plasma concentrations of masitinib and the active metabolite AB3280 has been developed and pre-validated. PK samples have been collected in two phase II studies AB04010 and AB06013 including the target population *i.e.* patients with mastocytosis.

The pharmacokinetics of masitinib has been described for healthy subjects. Although the underlying disease of mastocytosis can affect the function of several organs including liver and GI tract, the PK in the target group has not been sufficiently investigated.

Available PK data does not give a clear picture on whether masitinib exhibits time-dependent PK or not.

Masitinib is characterised as a BCS IV compound with pH dependent solubility. Thus, there is a potential for an interaction with medicinal products that moderate pH in the gut (*i.e.* proton pump inhibitors, H2-receptor antagonists, antacids). The Applicant committed to investigate the effect of increased stomach pH on the gastrointestinal absorption of masitinib following PPI dosing, should a MA be granted.

No mass balance study has been conducted. Lack of such studies has only been accepted by the CHMP for *non-malignant* conditions provided specific minimum requirements are met, however, in this case these have not been fulfilled by the Applicant. Based on *in vitro* data, masitinib is claimed to be extensively metabolised. A number of metabolites are proposed, including AB3280 which is considered to be the primary metabolite (CYP2C8 mediated metabolism). It remains unclear to which extent metabolites have been identified in humans. Based on available data it can be concluded that about one-third of the overall elimination pathways are characterised (*i.e.* CYP3A mediated metabolism). The contribution of CYP2C8 *in vivo* is not known, and depending on the results from the mass balance study further studies may be required. Although renal elimination appears to be of minor importance, no data on absolute bioavailability has been presented and thus the contribution of renal excretion to the total elimination of masitinib is not known.

The PK of masitinib in special populations has not been satisfactorily investigated. No study with masitinib has been performed in patients with renal impairment or hepatic impairment.

The distribution of patients included in the clinical program with respect to race is unknown and there is only limited data in the elderly population (>65 years). Thus potential influence on the PK and clinical outcome are not clear.

A number of questions related to potential for DDI are raised that require further discussion and additional studies. Studies investigating masitinib as a substrate of OCT2, OAT1 and OAT3 transporters *in vitro* and BCRP *in vivo* are lacking. Depending on the results, the clinical relevance of the *in vitro* findings needs to be further investigated. Furthermore, interaction potential of the metabolite AB3280 has not been elucidated. The potential for interaction with CYP2B6 has not been investigated as recommended in the DDI guideline CPMP/EWP/560/95/Rev.1 Corr.**.

An *in vitro* study to investigate masitinib inhibition potential on CYP2B6 should be performed. The potential risk of CYP3A4 intestinal induction requires further study, and the *in vivo* potential for clinically relevant CYP3A4 inhibition by masitinib through PBPK simulation should be evaluated. Depending on the results further studies may be needed.

The popPK model analysis supporting the weight-based posology is considered a post-hoc approach, since clinical studies already were performed before the popPK model was developed. The

appropriateness of the applied tablet strengths of 100 mg and 200 mg in relation to a weight-based posology is questioned.

The pharmacodynamics of masitinib has only been superficially investigated. With the exception of the study AB14004 (masitinib effect on the QTc interval), no specific studies on primary or secondary pharmacology have been performed. Dose-response and exposure-response data exists for only a small dataset, and no formal investigation of the PK/PD relationship has been performed. A longitudinal DER model (efficacy and safety) accounting for dose adjustments and co-variates should be considered to further explore the dose-exposure-response relationship.

The Applicant conducted a phase I drug-drug interaction study to evaluate any potential effect of masitinib on QTcF. Although administration of the positive control moxifloxacin caused an increase in QTcF, the detected increase (5.97 ms; 90% CI: 2.2, 9.7 ms) is rather small compared to values reported in earlier studies for the QTc prolonging effect of oral moxifloxacin (normally in the range of 10 to 15 ms). Consequently, this result questions the validity of the study. Moreover, a small QTcF prolongation of +4.98 ms (90% CI: 0.91, 9.06 ms; p = 0.045) was observed 12 hours after repeat-dose of masitinib. Since QT-prolongation is a recognised class effect of TKIs, and the data from the non-clinical trials do not exclude an increased risk of *cardiotoxic* effects of masitinib, additional data would be required in the event of a proven clinical benefit.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology of Masipro has been insufficiently investigated.

The lack of information on the metabolism and excretion pathways in humans, the lack of *in vivo* studies investigating the potential for interactions, lack of investigation of PK in the target population and in special populations (liver/renal impairment), and a poor investigation of the PK/PD relationship represent deficiencies of the MAA submission. Further investigation of class effects of TKIs such as QT prolongation is needed.

In the absence of a mass balance study, which represents a major gap in the understanding of the elimination pathways, further studies are needed to elucidate masitinib elimination and its potential for DDI. In the event of a proven clinical benefit with Masipro, lack of information on DDI could not be managed through the RMP or PI warnings as in the context of symptomatic treatment for a condition where patients need a range of other medications and knowledge of DDI is essential.

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

No formal dose-response studies have been submitted.

2.5.2. Main study(ies)

Study AB06006

Title: A 24-week with possible extension, prospective, multicentre, randomised, double blind, placebo-controlled, 2-parallel group with a randomisation 1:1, phase 3 study to compare efficacy and safety of

masitinib at 6 mg/kg/day to placebo in treatment of patients with smouldering systemic, indolent systemic or cutaneous mastocytosis with handicap *(cutaneous mastocytosis patients are not part of main analysis and claim as per protocol amendment version 6.0)*

Methods

Study Participants

According to the WHO classification and definition of systemic mastocytosis a patient can be diagnosed with systemic mastocytosis if he/she presents with the major criterion, along with one minor criterion, or at least 3 minor criteria as outlined in the table below.

Table 41 WHO diagnostic criteria for systemic mastocytosis - Diagnosis of systemic mastocytosis: the major criterion and 1 minor criterion or at least 3 minor criteria

Major Criterion / C Findings
Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organs
Minor Criteria / B findings
a. In biopsy sections of bone marrow or other extracutaneous organs, >25% of the mast cells in the infiltrate are spindle shaped or have atypical morphology or, of all mast cells in bone marrow aspirate smears, >25% are immature or atypical.
b. Detection of an activating point mutation at codon 816 of KIT in bone marrow, blood, or other extracutaneous organ.
c. Mast cells in bone marrow, blood, or other extracutaneous organ express CD2 and/or CD25 in addition to normal mast cell markers.
d. Baseline serum tryptase levels >20 ng/mL (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid).

Table 42 Central Document Review (CDR) diagnosis criteria for systemic mastocytosis

1. Bone marrow biopsy or aspirate associated with at least a sign of abnormality of mast cells:
   Signs of abnormality of mast cells are:

   a) Abnormal aggregates of mast cells in a sample in bone marrow:
   The criterion is deemed satisfied if the aggregate is: i) quantified and strictly above 15 mast cells per aggregated (corresponding to WHO major criterion), or ii) not quantified but is described as nodule, seat, cluster, focus, or granuloma and therefore pathological

   b) >25% atypical mast cells in a sample of bone marrow (corresponding to WHO minor criterion)
   c) c-Kit point mutation at codon 816 in bone marrow (corresponding to WHO minor criterion)
   d) Abnormal mast cells in the sample of bone marrow while microscopic testing that can be described by the following words: spindled, abnormal, atypical, fusiform,

	dystrophic, pathologic, dysmorphic (corresponding to WHO minor criterion) e) Abnormal immunohistochemistry signs: mast cells in bone marrow express CD2 or/and CD25 present (corresponding to WHO minor criterion) f) Abnormal infiltration of mast cells in the bone marrow The criteria is deemed satisfied if the infiltration i) is quantified and is strictly above 3% in the biopsy, or ii) is not quantified but is abnormal as described with infiltration , contingent of mast cells, or proliferation and therefore pathological.
	2. Detection of c-Kit 816 in the bone marrow without evidence of mast cells in bone marrow but with evidence of c-Kit 816 in skin, justifying clonality
	3. Excess of mast cells in digestive organs

Inclusion criteria:

1. Patient with one of the following documented mastocytosis as per WHO classification:

- Smouldering systemic mastocytosis (SSM)
- Indolent systemic mastocytosis (ISM)

2. Patient with documented mastocytosis and evaluable disease based upon histological criteria: typical infiltrates of mast cells in a multifocal or diffuse pattern in skin and/or bone marrow biopsy.

3. Patient with documented treatment failure of his/her handicap(s) with at least one of the following therapy used at optimised dose:

- Anti H1
- Anti H2
- Proton pump inhibitor
- Osteoclast inhibitor
- Cromoglycate Sodium
- Antileukotriene

4. Patients had to have severe symptoms defined as at least two of the following handicaps, including at least one among pruritus, flushes, depression and asthenia (fatigue):

- Pruritus score ≥ 9
- Number of flushes per week ≥ 8
- Hamilton rating scale for depression (HAMD-17) score ≥ 19
- Number of stools per day ≥ 4
- Number of micturition per day ≥ 8
- Fatigue Impact Scale total score (asthenia) ≥ 75

5. Patients with OPA > 2 (moderate to intolerable general handicap)

6. ECOG (Eastern Cooperative Oncology Group Performance Status) ≤ 2

7. Patient with adequate organ function

8. Male or female patient aged 18 to 75 years, weight > 50 kg, body mass index (BMI) between 18 and 35 kg/m²

9. Female patient of childbearing potential (entering the study after a menstrual period and who have a negative pregnancy test), who agrees to use two highly effective methods (one for the patient and one for the partner) of medically acceptable forms of contraception during the study and for 3 months after the last treatment intake.

10. Male patients must use medically acceptable methods of contraception if female partner is pregnant, from the time of the first administration of the study drug until 3 months following administration of the last dose of study drug. Male patients must use two highly effective methods of medically acceptable forms of contraception during the study and for 3 months after the last treatment intake. Patient must be able and willing to comply with study visits and procedures per protocol.

11. Patient must understand, sign, and date the written voluntary informed consent form at the screening visit prior to any protocol-specific procedures performed.

12. Patient must understand the patient card and follow the patient card procedures in case of signs or symptoms of severe neutropenia or severe cutaneous toxicity during the first 2 months of treatment.

13. Patient affiliated to a social security regimen.

Exclusion criteria:

1. Patient with one of the following mastocytosis:

- Cutaneous mastocytosis
- Not documented smouldering systemic mastocytosis or indolent systemic mastocytosis
- Systemic mastocytosis with an associated clonal hematologic non mast cell lineage disease (SM-AHNMD)
- Mast cell leukemia (MCL)
- Aggressive systemic mastocytosis (ASM)

2. Previous treatment with any tyrosine kinase inhibitor (TKI).

3. Patient presenting with cardiac disorders defined by at least one of the following conditions:

- Patient with recent cardiac history (within 6 months) of:
    - o Acute coronary syndrome
    - o Acute heart failure (class III or IV of the NYHA classification)
    - o Significant ventricular arrhythmia (persistent ventricular tachycardia, ventricular fibrillation, resuscitated sudden death)
- Patient with cardiac failure class III or IV of the NYHA classification
- Patient with severe conduction disorders which are not prevented by permanent pacing (atrio-ventricular block 2 and 3, sino-atrial block)
- Syncope without known etiology within 3 months

- Uncontrolled severe hypertension, according to the judgment of the investigator, or symptomatic hypertension.

4. Patient who had major surgery within 2 weeks prior to screening visit.

5. Vulnerable population defined as:

- Life expectancy < 6 months

- Patient with < 5 years free of malignancy, except treated basal cell skin cancer or cervical carcinoma in situ

- Patient with any severe and/or uncontrolled medical condition

- Patient with known diagnosis of human immunodeficiency virus (HIV) infection

6. Patient with history of poor compliance or history of drug/alcohol abuse, or excessive alcohol beverage consumption that would interfere with the ability to comply with the study protocol, or current or past psychiatric disease that might interfere with the ability to comply with the study protocol or give informed consent, or institutionalized by court decision.

7. Patient with any condition that the physician judges could be detrimental to subjects participating in this study; including any clinically important deviations from normal clinical laboratory values or concurrent medical events.

8. Change in the symptomatic treatment of mastocytosis or administration of any new treatment of mastocytosis within 4 weeks prior to baseline.

9. Treatment with any investigational agent within 4 weeks prior to baseline.

Treatments

Patients were randomly allocated to one of the following groups:

- Group 1: masitinib (6 mg/kg/day)

- Group 2: matching placebo

The study treatments were supplied as 100 mg and 200 mg tablets of masitinib or a matching placebo. Masitinib or placebo had to be taken in a sitting position with a large glass of water (250 ml) and during a meal.

Masitinib or placebo was administered orally in two daily doses over 24 weeks with a possibility of a double-blind extension period.

In the event of severe toxicity related to masitinib, treatment interruption or dose reduction was permitted according to pre-defined criteria.

According to the initial dose 6 mg/kg/day of masitinib or matching placebo, the steps for the dose reduction are as follows:

Starting dose	1st dose reduction	2nd dose reduction	3rd dose reduction
6 mg/kg/day	4.5 mg/kg/day	3 mg/kg/day	STOP

Concomitant treatments

Concomitant symptomatic treatments that were allowed according to the protocol:

- Anti H1
- Anti H2
- Proton pump inhibitor
- Osteoclast inhibitor (biphosphonates)
- Cromoglycate Sodium
- Antileukotriene
- Adrenaline in case of anaphylactic shocks
- Other therapies used for the symptomatic care

They should be maintained at the same dose during the study. No change in the symptomatic treatment of mastocytosis or administration of any new treatment of mastocytosis should occur.

According to the applicant, all medications being taken by the patients at study start and all medication given in addition to the IMP during the study were regarded as concomitant medications. All concomitant treatments were recorded on the patient's CRF, including name of the drug, total daily dose, route of administration, start and stop dates, and the reason for administration.

Mandatory concomitant medication

An oral antihistamine (cetirizine 10 mg/day) had to be combined systematically with the study drug for 60 days. Cetirizine was initiated at the same time as study treatment.

Prohibited concomitant treatments

- Anticancer agent (including chemotherapy, high dose of corticosteroids, biologic agents)
- 2CDA
- Interferon
- Any investigational treatment related or not related to mastocytosis

Objectives

The objective of this study is to compare the efficacy and safety of masitinib at 6 mg/kg/day to placebo in the treatment of patients with mastocytosis with handicap based on treatment effect on the pruritus score, the number of flushes per week, the Hamilton score, and the Fatigue Impact scale.

Outcomes/endpoints

Primary endpoint

Response on 4 handicaps (among pruritus, flush, depression and asthenia): Cumulative response by patient*handicap (4H75%)

- Response on a handicap was defined as an improvement with respect to the baseline values of ≥ 75% for pruritus, or flushes, or depression (as measured by the Hamilton rating for depression, HAMD-17) or asthenia (as measured by the Fatigue Impact Scale, FIS).
- Handicaps at baseline were defined as: pruritus score ≥ 9; number of flushes per week ≥ 8; HAMD-17 score ≥ 19; FIS ≥ 75.
- For every patient the response at each study visit (5 visits from week 8 to week 24) was calculated on each handicap present at baseline (among pruritus, flushes, depression and asthenia) as defined above. Thus, from 5 to 20 responses were calculated per patient: 5 if the patient presented only 1 handicap at baseline (corresponding to the 5 visits) and 20 if the patient presented all 4 handicaps at baseline (corresponding to the 4 handicaps * the 5 visits).

Secondary endpoints

Symptom related endpoints

- Response on pruritus: Cumulative response on pruritus among patients with this handicap at baseline
- Response on 3 handicaps (pruritus or flush or depression): 3H75%.
  The analysis of response on 3 handicaps was done on the patients with handicap on pruritus and/or flushes and/or depression at baseline. (*This analysis was omitted in the SAP v1.0 and was added in the Addendum to Analysis Plan post unblinding.)*
- Response on 2 handicaps (pruritus or flush): 2H75%
  Cumulative response by patient*handicap on pruritus or flushes, among patients with either of these handicaps at baseline
- Extension period analyses:
  - Response on 4 handicaps (4H75%) up to week 96 (2 years)
  - Response on pruritus up to week 96 (2 years)
  - Response on 3 handicaps (3H75%) up to week 96 (2 years)
  - Response on 2 handicaps (2H75%) up to week 96 (2 years)

Objective endpoints: Short-term measure of mast cell burden related to disease activity

- Change from baseline in serum tryptase level (in patients with tryptase higher than 20 µg/L at baseline)

Main exploratory analyses

Symptom related endpoints

- Response on flush: Cumulative response on flushes among patients with this handicap at baseline
- Response on depression: Cumulative response on HAMD-17 among patients with this handicap at baseline
- Response on asthenia: Cumulative response on FIS among patients with the handicap at baseline
- Response on miction: Cumulative response on mictions among patients with this handicap at baseline
- Response on stools: Cumulative response on stools among patients with this handicap at baseline

The response at each study visit (5 visits from week 8 to week 24) was measured. Thus, 5 responses were calculated per patient. Response was defined as an improvement with respect to the baseline value of ≥ 75% at the visit.

Objective endpoint: Long-term measures of mast cell activity nullification

- Response on urticaria pigmentosa (UP):

  - Change from baseline of body surface area (BSA) covered by UP

  - Disappearance of 'Darier's sign'

Quality of life

- Cumulative response on overall patient assessment (OPA) score among patients with "severe" or "intolerable" handicap at baseline. For patients presenting this handicap at baseline (i.e. OPA "severe" or "intolerable"), the response at each study visit (5 visits from week 8 to week 24) was calculated.
- Quality of Life : QLQ-C30 (v3) global score, functional scores and symptom scores at each visit

  QLQ-C30 score at time point, absolute and relative change from baseline for each scale was calculated according to the "EORTC QLQ-C30 Scoring Manual". Three main scores (Global Health Status score, Functional Score and Symptom score) and 14 sub-scores (Physical Functioning, Role Functioning, Emotional Functioning, Cognitive Functioning, Social Functioning, Fatigue, Nausea and Vomiting, Pain, Dyspnoea, Insomnia, Appetite Loss, Constipation, Diarrhoea, Financial Difficulties) were calculated.
- AFIRMM questionnaire:

  The AFIRMM Score V2 is a composite score built on the following items:

  52 symptoms classified in 15 categories (skin, allergy, anaphylactic shock, flushes, gastrointestinal track, rheumatology, constitutional, cardiology, neurology/psychiatry, respiratory, urology, infection/ignition, libido, endocrinology, and social life). For each of the 52 items, cumulative response among patients with "severe" or "intolerable" handicap at baseline was given.

  For the patients presenting the handicap at baseline (i.e. answer "severe" or "intolerable"), the response at each study visit (5 visits from week 8 to week 24) was calculated. Response was defined as an answer "normal" or "light" at the visit.

- Safety of masitinib

Sample size

A total of 142 patients (71 in the masitinib group and 71 in the placebo group) presenting a documented smouldering or indolent systemic mastocytosis with severe handicap as defined by protocol v6.0 were planned to be enrolled to provide a 80% power with a two-sided 5% alpha in order to compare masitinib to placebo.

The actual number of severe systemic mastocytosis patients in the ITT population was 135 patients.

Randomisation

Eligible patients were randomised by means of a computerised central randomisation system (IVRS).

Randomisation procedures included a minimisation process aimed at reducing any difference in the distribution of the handicaps/scores at baseline and country. The minimisation was performed according to the following covariates: pruritus score, number of flushes per week, Hamilton rating scale for depression, Fatigue Impact Scale score, and country. Number of patients with handicaps and the mean were balanced for each of these covariates.

Blinding (masking)

This was a double-blind study.

Statistical methods

The difference between treatment groups (masitinib versus placebo) was tested using a Generalised Estimating Equations (GEE) model using Logit as the link function. This statistical model included all the responses (yes/no) on handicaps observed from week 8 to week 24 (5 visits); thus, from 5 to 20 responses per patient depending on the number of handicaps at baseline. A difference between masitinib and placebo was concluded if the p-value associated to treatment was $\leq$ 5%. Use of repeated measures in the statistical analysis was introduced with protocol version 6.0 to counteract the rarity of the population.

To account for a possible imbalance between treatment groups in the number of patients with a given handicap (from among pruritus, flushes, depression or asthenia), each observation was weighted.

The primary efficacy analysis was to be conducted in a mITT population re-defined as all ITT patients excluding patients withdrawing prematurely from the study during the protocol part (Week0-Week24) for a well-documented non–failure cause. Non-failures consist of: Lost to follow-up; Violation of inclusion and/or exclusion criteria; Withdrawal of informed consent due to travel or move; No treatment intake

Other reasons considered as failure causes, “such as”: Adverse events related to treatment; Adverse events not related to treatment; Lack of efficacy; Non-compliance with protocol; Withdrawal of informed consent due to study procedure; Withdrawal of informed consent for reason not specified.

For the primary analysis in the mITT population with Missing Data equal to Failure (MDF) as method for replacement of missing values, the p-value of the statistical test was obtained with a re-randomization

test. This test was only performed for the primary analysis, along with a test without re-randomization as sensitivity analysis.

No interim analyses were performed during the study. Two futility analyses were done, without consumption on alpha risk function. No statistical inferences and no unblinding information were provided to the Sponsor in order to preserve the type I error.

Results

Participant flow

Due to late redefinition of the target population during the study (protocol amendments 5 and 6), 87 patients in total were excluded from the ITT population, 40 patients in the masitinib arm and 47 patients in the placebo arm (cutaneous or non-severe mastocytosis; see figure below).

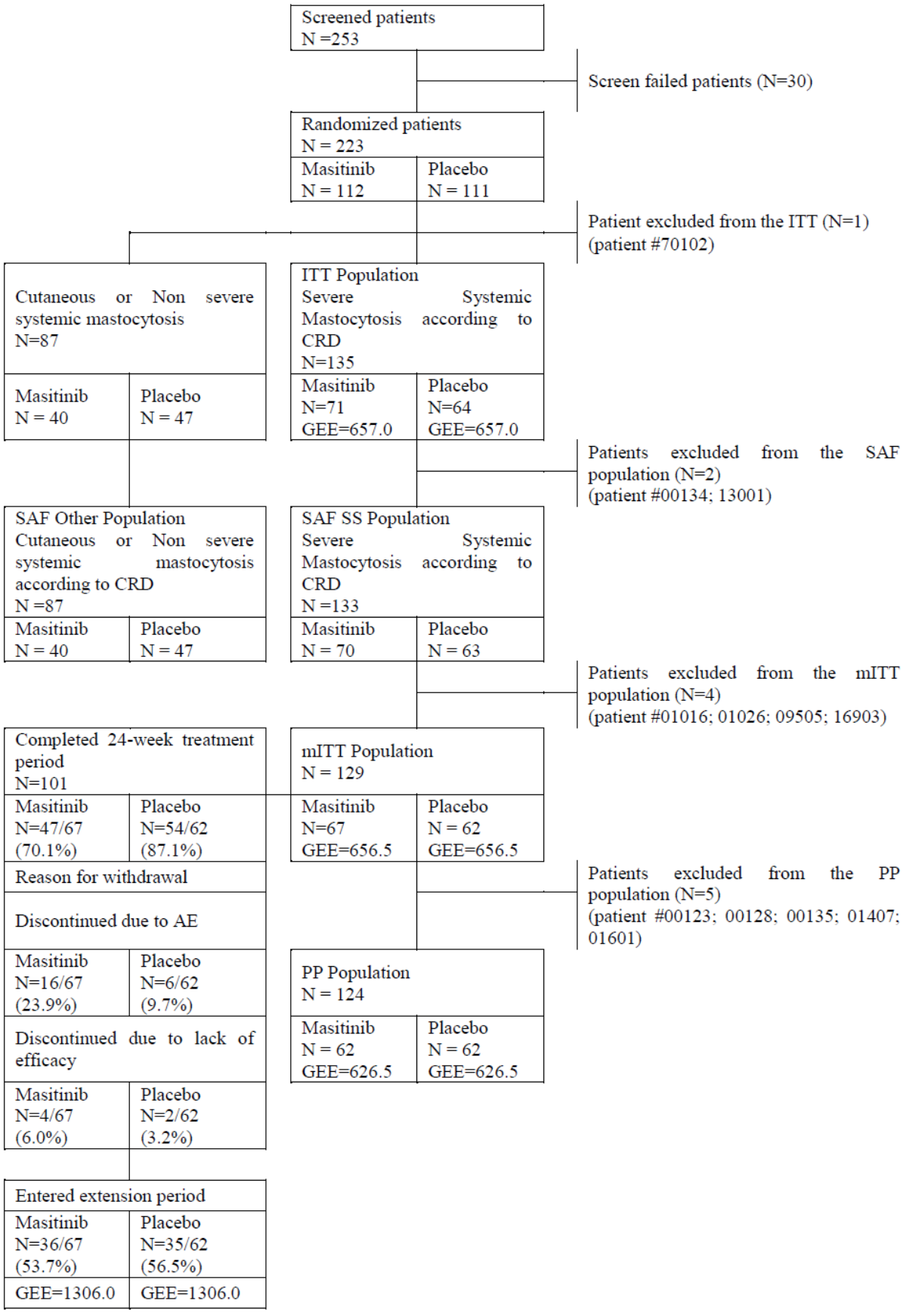


Figure 16 Participant flow

Recruitment

Clinical study centres: 51 sites randomised at least 1 patient in 15 countries. The majority of patients were enrolled in France.

The first patient was enrolled 19 February 2009, and the last patient was enrolled 15 July 2015.

Conduct of the study

Protocol deviations

Five major protocol deviations were reported as a result of poor compliance by the principal investigator having discontinued patients from the study without proposing a dose reduction.

Symptomatic treatments have been introduced or increased in a number of patients. It was not authorised per protocol but was not considered a major protocol deviation by the Blinded Review Committee. Given the severity of handicaps, such management of worsening of symptoms was considered necessary for ethical reasons by the treating physicians.

Protocol amendments

Increase of the baseline handicap severity

The initial protocol versions up to version 4.0 included mastocytosis patients with moderate and severe handicaps.

During the course of study AB06006 a change in the masitinib benefit/risk balance occurred in the non-oncology program due to severe neutropenia and severe skin toxicity episodes. The amendment of study AB06006 was considered necessary by the applicant in order to improve the benefit/risk balance.

AB Science engaged through a Scientific Advice with the EMA in October 2011 (EMA/CHMP/H/SA/573/2/FU/2/2011/PA/SME/II) as an attempt to validate the anticipated changes aimed at improving the study benefit/risk balance. SAWP then mentioned that “the increase of the baseline severity of population is in general desirable”. To increase the benefit/risk balance the protocol was amended to include only mastocytosis patients with severe handicaps. It took two protocol versions to reach the intended severity level of handicap; protocol version 5.0 still had some level of handicaps incompatible with severe handicaps. In an effort to further improve the benefit/risk balance, protocol v6.0 restricted the inclusion to patients with documented smouldering or indolent systemic mastocytosis. A significantly higher occurrence and severity of symptoms has been reported in systemic mastocytosis when compared with cutaneous mastocytosis. Study protocol version 5.0 was implemented on June 13, 2012 while version 6.0 became effective on August 9, 2013. According to the applicant, the study remained blinded at the time of these amendments and the Sponsor did not have access to any data.

Analysis on the W8-W24 time window

In protocol version 6.0, the primary analysis was planned to be performed from W8 to W24, to assess efficacy of treatment after the initial 2 months of treatment.

The non-inclusion of early efficacy measurement (W4) in the analysis was addressed in EMA scientific advice in June 2006 (EMEA/CHMP/H/SA/573/2/FU/1/2006/PA/II). The SAWP advised AB Science as follows: “The company proposes not to include the results from the first 8 weeks because there are known side effects of AB1010 in the early part of treatment. …Patients who drop out of the study

during the first 8 weeks should still be included in the primary analysis." As advised by the SAWP, missing data equal to failure method was applied to the early discontinued patients.

Increase of the cut-off for response

To enhance the clinical relevance of the response the protocol was amended to increase the cut-off point for response to at least a 75% improvement of the baseline handicap. SAWP was consulted on this question through scientific advice in October 2011 (EMA/CHMP/H/SA/573/2/FU/2/2011/PA/SME/II). SAWP mentioned that "the proposed increase in the cut-off point for response criteria would lead to more strict definition of product efficacy and, to this respect is regarded, a priori, as conservative, more clinically relevant and thus in principle desirable". The implementation of this increase was done in version 5.0 and version 6.0 for the four handicaps pruritus, flushes, depression and asthenia.

GCP inspections

The following inspection findings were identified during the two clinical site inspections: Missing adverse events from the study report, mistakes in the reporting of abnormal laboratory results as adverse events, failures in the handling of serious adverse events, lack of a database for SAEs.

In addition to the missing or duplicated adverse events linked to abnormal laboratory values previously mentioned, inconsistencies and mistakes were found in the line listings of adverse events, showing deficiencies in data management and in the review of the cases.

The Head of Pharmacovigilance is on leave since February 2016 and there is no documentation of any delegation during the absence. Training and qualification of personnel in charge of event coding prior to April 2015 has not been documented. Personnel are not periodically re-trained. In any case the identity of the person handling cases and entering information is not documented.

There is no process for medical review of non-serious adverse events and no signal detection. Deficiencies in coding were identified during the inspection. Considering the lack of medical review and of signal detection, the fact that only one case was submitted to an independent dermatology expert, though other AEs and SAEs could have indicated events of special medical interest, is also of concern.

Reporting of serious adverse events to regulatory authorities, ethics committees and investigators was not logged and it is not possible to ensure that all relevant cases have indeed been transmitted. There was no process in place to ensure a timely reporting.

Multiple patients enrolled at the two clinical sites inspected had a symptomatic treatment of mastocytosis initiated or modified during the trial or less than 4 weeks prior to their enrolment, though this was not allowed by the protocol. Some of these prohibited concomitant medications or changes were not reported to the sponsor by the investigators.

Deficiencies were identified in the monitoring process and in the overview of the trial by the sponsor, including missing site initiation visit and monitoring reports, lack of or very delayed documented review of the monitoring reports by the project manager, non-monitored data, and previously mentioned consequences of the late re-monitoring of the trial.

The list of protocol deviations in one site was incomplete: only 7 protocol deviations are listed, 2 of which are graded as minor and are not described. For instance prohibited concomitant medications (symptomatic treatments of mastocytosis) were not reported and discussed in the CSR. Protocol deviations are currently tracked by data management using an Excel spreadsheet, which was found to be missing a critical deviation known to ANSM in another study, though it occurred at the beginning of 2016. There is therefore no complete overview, evaluation and reporting of protocol deviations.

Most of the findings described above are not site-specific but are general and relevant for the whole trial.

Baseline data

Handicaps at baseline

Handicap status was defined as at least two of the following handicaps, including at least one among pruritus, flushes, depression and asthenia. Baseline data are presented in the tables below.

Table 43 Handicaps at baseline - population: mITT

	Masitinib (N=67) (P*h=136)	Placebo (N=62) (P*h=132)	p-value*
Pruritus			
N available	67	62	
Mean±SD	9.0 ± 3.0	9.1 ± 3.6	
Pruritus score ≥ 9, n (%)	**45 (67.2%)**	**42 (67.7%)**	**0.9442**
Flushes (per week)			
N available	66	62	
Mean±SD	8.0 ± 9.6	6.4 ± 7.4	
Flushes ≥ 8 per week, n (%)	**18 (27.3%)**	**17 (27.4%)**	**0.9852**
Depression (HAMD-17)			
N available	67	62	
Mean±SD	16.0 ± 7.4	17.3 ± 8.1	
Hamilton score ≥ 19, n (%)	**23 (34.3%)**	**27 (43.5%)**	**0.2829**
Asthenia (FIS score)			
N available	66	61	
Mean±SD	90.2 ± 37.1	89.4 ± 34.3	
FIS score ≥ 75, n (%)	**50 (75.8%)**	**46 (75.4%)**	**0.9636**
Stools (per day)			
N available	66	62	
Mean±SD	2.4 ± 1.6	2.2 ± 1.5	
Stools ≥ 4 per day, n (%)	**17 (25.8%)**	**10 (16.1%)**	**0.1821**
Mictions (per day)			
N available	66	62	
Mean±SD	9.1 ± 6.7	7.0 ± 2.8	
Mictions ≥ 8 per day, n (%)	**38 (57.6%)**	**26 (41.9%)**	**0.0770**

Table 44 Demographics of the patients eligible for primary analysis (mITT population)

	Masitinib (N=67) (P*h=136)	Placebo (N=62) (P*h=132)
Age (years)		
Mean ± SD	45.3 ± 11.1	49.2 ± 12.7
Min; Max	18.8 ; 68.6	27.4 ; 86.2
Median	45.1	49.0
Sex		
Male	17 (25.4%)	21 (33.9%)
Female	50 (74.6%)	41 (66.1%)
Country		
France	36 (53.7%)	33 (53.2%)
Poland	3 (4.5%)	8 (12.9%)
Germany	4 (6.0%)	6 (9.7%)
Russia	2 (3.0%)	4 (6.5%)
United Kingdom	4 (6.0%)	2 (3.2%)
Czech Republic	3 (4.5%)	2 (3.2%)
USA	3 (4.5%)	2 (3.2%)
Austria	3 (4.5%)	1 (1.6%)
Slovakia	2 (3.0%)	2 (3.2%)
Italy	1 (1.5%)	2 (3.2%)
Switzerland	3 (4.5%)	0 (0.0%)
Spain	2 (3.0%)	0 (0.0%)
Greece	1 (1.5%)	0 (0.0%)
Ethnicity		
White or North African	55 (82.1%)	54 (87.1%)
Other	8 (11.9%)	4 (6.5%)
Hispanic or Latino	4 (6.0%)	4 (6.5%)
Reproductive status		
NA (male, post-menopausal or sterile female)	36 (53.7%)	36 (58.1%)
Hormonal contraceptive	14 (20.9%)	11 (17.7%)
Non Hormonal contraceptive	16 (23.9%)	8 (12.9%)
Contraception used but type unknown	1 (1.5%)	6 (9.7%)

Table 45 c-Kit mutation status - (mITT population)

c-Kit based on central analysis (AFIRMM) and eCRF when missing		Masitinib (N=67)	Placebo (N=62)	Total (N=129)
Clonal	D816V pure	53 (79.1%)	47 (75.8%)	100 (77.5%)
	D816V chimeric	10 (14.9%)	6 (9.7%)	16 (12.4%)
Not Clonal	WT	1 (1.5%)	7 (11.3%)	8 (6.2%)
Unknown	Unknown status	3 (4.5%)	2 (3.2%)	5 (3.9%)

Clonal patients are defined as patients bearing the D816V c-Kit mutation in at least one organ in which c-Kit sequencing was performed. Several sequencing procedures could be performed for a given patient and for the same organ.

- If the D816V c-Kit mutation has been found in all the organs in which sequencing was performed the patient was classified as D816V “pure”.
- On the other hand, if the D816V variant is found in one organ and the WT allele in a second organ the patient was classified as D816V “chimeric”.

Wild-type (WT) patients are patients for whom the detection of the D816V c-Kit mutation was negative k in the organ(s) in which c-Kit sequencing was performed.

Considering that the large majority of the patients were classified as mutated, the current study cannot confirm that masitinib is effective, regardless of mutational status.

Table 46 Previous treatments for mastocytosis: Failure with specific symptomatic treatments - mITT population

AT LEAST ONE FAILURE; N (%)	Masitinib (N=67) (P*h=136)	Placebo (N=62) (P*h=132)
Any failed previous treatments	66 (98.5)	61 (98.4)
Systemic local antihistamines	65 (97.0)	60 (96.8)
Cromoglicate	27 (40.3)	25 (40.3)
Treatment with vitamin D and analogues	17 (25.4)	15 (24.2)
Proton pomp inhibitors	16 (23.9)	18 (29.0)
Antileukotriene	13 (19.4)	8 (12.9)
Neurologic treatments containing an antidepressant molecule	11 (16.4)	1 (1.6)
Calcium as supplement	11 (16.4)	10 (16.1)
Osteoclast inhibitor	10 (14.9)	10 (16.1)
Cladribine/interferon/2CDA	8 (11.9)	10 (16.1)
Systemic treatments containing corticosteroid	7 (10.4)	9 (14.5)
Dermatological corticosteroids	2 (3.0)	2 (3.2)
Treatment for functional GI disorders excl propulsives	1 (1.5)	4 (6.5)

*P*h= patients per handicaps*

Table 47 AFIRMM, QLQ-C30 and tryptase level at baseline - population: mITT

	Masitinib (N=67) (P*h=136)	Placebo (N=62) (P*h=132)	p-value
AFIRMM Score			
N available	66	62	
Mean ± SD	238.9 ± 111.7	263.9 ± 111.3	0.2075 (A)
OPA Score			
N available	66	58	
(0,1) n(%)	5 (7.6%)	1 (1.7%)	0.1297 (C)
(2,3,4) n(%)	61 (92.4%)	57 (98.3%)	
QLQ30 - Global health score			
N available	67	62	
Mean ± SD	41.8 ± 20.5	38.7 ± 16.5	0.3507 (A)
QLQ30 - Functional score			
N available	67	62	
Mean ± SD	52.9 ± 22.5	52.9 ± 19.5	0.9836 (A)
QLQ30 - Symptom score			
N available	67	62	
Mean ± SD	41.0 ± 16.5	43.2 ± 18.0	0.4860 (A)
Tryptase level			
N available	60	55	
Mean ± SD	75.8 ± 120.4	72.2 ± 75.6	0.8476 (A)
Tryptase level ≥ 20 micro g/L n (%)	46 (76.7%)	44 (80.0%)	0.6651 (C)

*(A) Analysis of variance (C) Chi-square test P*h= patients per handicaps*

Numbers analysed

Table 48 Overall patient disposition by treatment-arm

Population	Masitinib	Placebo	Total
ITT	71	64	135
mITT	67	62	129
PP	62	62	124
>3 Month	54	57	111
SAF	70	63	133

The primary efficacy analysis was performed on the modified intent-to-treat (mITT) population.

Outcomes and estimation

The cut-off date for efficacy analysis was November 23, 2015.

Primary endpoint

Cumulative response was calculated as the number of actual responses between weeks 8 and 24, divided by the total number of possible responses over the same treatment period (i.e. with 5 scheduled visits each patient had a maximum of 5 to 20 possible responses depending on the number of handicaps at baseline. Missing data was considered as failure (MDF) and the statistical test p-value was obtained via a re-randomisation (10,000 replicate) test.

Table 49 4H75% response (pruritus, flush, depression and asthenia) from W8 to W24

Population/ Missing data method	N	Masitinib	Placebo	Re-randomization p-value*	Odds ratio
mITT/MDF	129	18.7%	7.4%	0.0076	3.63
PP/MDF	124	20.1%	7.4%	0.0048	3.88
ITT/MDF	135	18.7%	7.6%	0.0102	3.28
mITT/OC	129	24.1%	7.9%	0.0014	4.90

Secondary endpoints

Table 50 3H75% response (pruritus, flushes and depression) from W8 to W24

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	Odds ratio
mITT/MDF	129	24.7%	9.8%	0.0071	3.06
PP/MDF	124	26.5%	9.8%	0.0038	3.33
mITT/OC	129	32.4%	10.4%	0.0008	4.05

Table 51 2H75% response (pruritus and flushes) from W8 to W24

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	Odds ratio
mITT/MDF	129	27.2%	10.7%	0.038	2.63
PP/MDF	124	29.5%	10.7%	0.022	2.89

Table 52 Pruritus score – Response on pruritus from W8 to W24

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	Odds ratio
mITT/MDF	129	22.0%	7.3%	0.0322	3.13
PP/MDF	124	24.7%	7.3%	0.0146	3.69
mITT/OC	129	29.7%	7.8%	0.0071	4.21

Long-term efficacy

Table 53Cumulative response rates (4H75%, 3H75% and pruritus) from W8 to W96 in the mITT population

4H75%

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	OR
mITT / MDF	129	16.8%	6.8%	0.0156	3.51
PP / MDF	124	18.4%	6.8%	0.0098	3.82
ITT/MDF	135	16.8%	6.9%	0.1840	3.21
mITT / OC	129	27.1%	10.2%	0.0031	3.66

3H75%

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	OR
mITT / MDF	129	21.8%	8.3%	0.0031	3.17
PP / MDF	124	23.8%	8.3%	0.0013	3.51
mITT / OC	129	35.1%	12.1%	0.0003	3.87

Pruritus

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	OR
mITT / MDF	129	19.2%	6.2%	0.0338	3.06
PP / MDF	124	21.6%	6.2%	0.0147	3.65
mITT / OC	129	31.9%	9.3%	0.0047	4.33

Table 54 Change from baseline in tryptase level

Short-term	Masitinib	Placebo	p-value
N of patients with tryptase level ≥20 µg/L at baseline	**46**	**44**	**.**
N with evaluation at W24 or final visit	40	42	.
Absolute change from baseline			
Mean±SD	-10 ± 46.9	2.7 ± 20.0	0.0267
Relative change from baseline			
Mean±SD	-18 ± 21.4	2.2 ± 26.9	0.0001
Response at 25%			
n(%)	14 (35.0%)	5 (11.9%)	0.0172
Response at 25% (MDF)			
n(%)	14 (30.4%)	5 (11.4%)	0.0322

Exploratory endpoints

Table 55 Cumulative response from W8 to W24 on flush, depression and asthenia

Population/ Missing data method	N	Masitinib	Placebo	Observed p-value	Odds ratio
Flush 75%					
mITT/MDF	129	39.9%	19.0%	NS	3.03
PP/MDF	124	41.2%	19.0%	NS	3.06
mITT/OC	129	51.7%	19.5%	NS	3.83
Depression (HAMD-17 75%)					
mITT/MDF	129	18.6%	7.6%	NS	2.71
PP/MDF	124	19.4%	7.6%	NS	2.87
mITT/OC	129	23.1%	8.1%	NS	3.34
Asthenia (FIS 75%)					
mITT/MDF	129	7.7%	3.2%	0.0499	4.84
PP/MDF	124	8.4%	3.2%	0.037	5.51
mITT/OC	129	9.5%	3.4%	0.0458	6.96

Table 56 Urticaria Pigmentosa and Darier's sign

Objective endpoint	Population / Missing data method	Masitinib	Placebo	p-value
N of patients **Relative change from baseline in the BSA covered by UP**	mITT / LOCF	-12.34 ± 26.41%	15.91 ± 59.79%	0.0210
N of patients **Darier's sign disappearance (Yes/ No)**	mITT / MDF	7/37 18.92%	1/37 2.70%	0.0187

Quality of life

There was no improvement in the quality of life measured by QLQ-C30 global score, AFIRMM questionnaire, and OPA score. However, exploratory analyses performed on the 75% reduction in the score of the detailed items of FIS, HAMD-17, and AFIRMM score showed that masitinib generated a benefit on a greater number of quality of life related items as compared with placebo.

Ancillary analyses

To further assess the implications of the protocol changes, the applicant was requested to provide information regarding the number of patients enrolled before and after protocol amendment 5, and also after protocol amendment 6. In addition, information regarding handicap level (and other relevant information) for patients who were no longer considered eligible after amendment 5 and 6 has been provided.

Most patients in the study were randomised before version 5 of the protocol. The restriction of the inclusion criteria with the protocol amendments clearly made it more difficult to find eligible patients within the timeframe of the study. Only 135 patients were finally considered to have documented severely symptomatic smouldering or indolent systemic mastocytosis.

DESCRIPTION	<V5	V5-V6	<V6	>V6
Screened Subjects (N)	160	52	212	38
Screen Failure (N(%))	16 (10%)	5 (10%)	21 (10%)	9 (24%)
Randomised Subjects (N(%))	144 (90%)	47 (90%)	191 (90%)	29 (76%)

Summary of main study(ies)

Summary of main efficacy results

The following table summarises the efficacy results from the main study supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 57 Summary of efficacy for trial AB06006

Title: "A 24-week with possible extension, prospective, multicenter, randomised double blind, placebo-controlled, 2-parallel group with a randomisation 1:1, phase 3 study to compare efficacy and safety of masitinib at 6 mg/kg/day to placebo in treatment of patients with Smouldering Systemic or Indolent Systemic Mastocytosis with handicap"		
Study identifier	AB06006	
Design	AB06006 was a prospective, randomised, placebo-controlled, parallel-group, phase 3 study, conducted in 15 countries (Austria, Czech Republic, France, Germany, Greece, India, Italy, Latvia, UK, USA, Poland, Russia, Slovakia, Spain, Switzerland), evaluating the efficacy and safety of masitinib in adult patients with indolent or smouldering severe systemic mastocytosis unresponsive to optimal symptomatic treatments. Patients were randomly allocated to one of the two following groups: – Group 1: masitinib (6 mg/kg/day) – Group 2: matching placebo Masitinib or placebo was administered orally in two daily doses over 24-weeks, with a possibility of double-blind extension period. To be eligible, patients had to have severe symptoms defined as at least **two** of the following handicaps, including at least **one** among pruritus, flush, depression and asthenia (fatigue): – Pruritus score ≥ 9 – Number of flushes per week ≥ 8 – Hamilton rating scale for depression (HAMD-17) score ≥ 19 – Fatigue Impact Scale total score (asthenia) ≥ 75 – Number of stools per day ≥ 4 – Number of micturition per day ≥ 8	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	24 weeks <not applicable> Up to 96 weeks (2 years)
Hypothesis	Superiority: assess efficacy and safety of masitinib versus placebo	
Treatments groups	Group 1	Masitinib 6 mg/kg/day, 24 weeks, 112 patients randomized
	Group 2	Placebo, 24 weeks, 111 patients randomized
Database lock	24 November 2015	
Results and Analysis		
Analysis description	**Primary Analysis**	

Analysis population and time point description	Primary efficacy analysis, the 4H75% response endpoint, was performed according to the mITT population, with results verified via analysis on the ITT population, as well as other sensitivity analyses including the PP population and mITT observed cases (OC). Missing data was considered as failure (MDF) and the statistical test P-value was obtained via a re-randomization (10,000 replicate) test (for the primary endpoint). Response was defined as a 75% improvement from baseline for any one of handicaps pruritus, flushes, depression, asthenia. Analysis time points were Week 8 to Week 24 (listed below) and Week 8 to Week 96.		
Results	4H75% (mITT/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	18.7% vs. 7.4%
		Odds Ratio	3.63
		CI95	1.21-10.83
		P-value	0.0076
	4H75% (PP/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	20.1% vs. 7.4%
		Odds Ratio	3.88
		CI95	1.31-11.50
		P-value	0.0048
	4H75% (ITT/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	18.7% vs. 7.6%
		Odds Ratio	3.28
		CI95	1.18-9.12
		P-value	0.0102
	4H75% (mITT/OC)	Comparison groups	Masitinib vs Placebo
		Response rate	24.1% vs. 7.9%
		Odds Ratio	4.90
		CI95	1.59-15.14
		P-value	0.0014
Analysis description	**Secondary analysis: 3H75% (pruritus, flushes, depression), 2H75% (pruritus, flushes) and Pruritus 75%**		
Analysis population and time point description	Secondary efficacy analysis, the 3H75% and 2H75% response endpoints, were performed according to the mITT population, with results verified via analysis on the PP population, as well as other sensitivity analyses including the mITT observed cases (OC). Missing data was considered as failure (MDF). Response was defined as a 75% improvement from baseline for any one of the aforementioned handicaps. Analysis time points were Week 8 to Week 24 (listed below) and Week 8 to Week 96 (available in Section 2.7.3.2).		
Results	3H75% (mITT/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	24.7% vs. 9.8%
		Odds Ratio	3.06
		CI95	1.36-6.92
		P-value	0.0071
	3H75% (PP/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	26.5% vs. 9.8%
		Odds Ratio	3.33

		CI95	1.48-7.53
		P-value	0.0038
	3H75% (mITT/OC)	Comparison groups	Masitinib vs Placebo
		Response rate	32.4% vs. 10.4%
		Odds Ratio	4.05
		CI95	1.79-9.06
		P-value	0.0008
	2H75% (mITT/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	27.2% vs. 10.7%
		Odds Ratio	2.63
		CI95	1.06-6.55
		P-value	0.038
	2H75% (PP/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	29.5% vs. 10.7%
		Odds Ratio	2.89
		CI95	1.17-7.17
		P-value	0.0222
	2H75% (mITT/OC)	Comparison groups	Masitinib vs Placebo
		Response rate	36.3% vs. 11.3%
		Odds Ratio	3.79
		CI95	1.52-9.44
		P-value	0.0042
	Pruritus 75% (mITT/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	22.0% vs. 7.3%
		Odds Ratio	3.13
		CI95	1.10-8.88
		P-value	0.0322
	Pruritus 75% (PP/MDF)	Comparison groups	Masitinib vs Placebo
		Response rate	24.7% vs. 7.3%
		Odds Ratio	3.69
		CI95	1.29-10.53
		P-value	0.0146
	Pruritus 75% (mITT/OC)	Comparison groups	Masitinib vs Placebo
		Response rate	29.7% vs. 7.8%
		Odds Ratio	4.21
		CI95	1.48-11.98
		P-value	0.0071

Analysis performed across trials (pooled analyses and meta-analysis)

N/A

Clinical studies in special populations

Studies in special populations were not submitted.

Supportive study(ies)

The applicant has provided two uncontrolled phase 2 studies, AB04010 and AB06013. In both studies, a very limited number of patients were included that had mastocytosis with severe handicaps, 15 and 12 evaluable patients respectively. Study AB04010 was supposed to give support for the efficacy of masitinib in mastocytosis also in patients not bearing activating point mutations in c-Kit. However, only 8 patients may be considered true WT in this study. It is unclear how many of these had severe handicaps. Both studies differ in many aspects from the pivotal study. Considering the low number of patients with the relevant level of handicaps, low response rate on individual handicaps, and the different methodology applied no direct support for the pivotal study can be concluded.

2.5.3. Discussion on clinical efficacy

One pivotal phase 3 trial (AB06006) and two phase 2 trials (AB04010 and AB06013) have been submitted with the MAA for masitinib. The applied indication for Masipro is as follows: Masipro is indicated for the treatment of adult patients with smouldering or indolent systemic severe mastocytosis unresponsive to optimal symptomatic treatments.

Design and conduct of clinical studies

The WHO has defined specific criteria for diagnosing systemic mastocytosis (currently under revision that has no consequence for this assessment). The applicant did not strictly adhere to these criteria, i.e. the applicant allowed a wider definition. Of the total study population of 135 patients, 108 were found to fulfil the WHO criteria. Post-hoc efficacy analyses have been performed in patients fulfilling the WHO criteria.

The patients eligible for this study had to have severe symptoms defined as at least two of the following handicaps, including at least one among pruritus, flushes, depression and asthenia (fatigue): Pruritus score ≥ 9, Number of flushes per week ≥ 8, Hamilton rating scale for depression (HAMD-17) score ≥ 19, Number of stools per day ≥ 4, Number of micturition per day ≥ 8, Fatigue Impact Scale total score (asthenia) ≥ 75. These are typical symptoms of mastocytosis, and the severity of these symptoms for eligibility into the study is considered debilitating, especially flushes, pruritus, depression and fatigue. However, there are issues with the use of the HAMD-17 in this study. The GCP inspection found that investigators were not properly trained and actually patients themselves filled in the questionnaires. In addition, incorrect versions of the FIS questionnaire were used during the first few months at some sites. Therefore, the cut-off for inclusion and grading of severity is not considered reliable.

Both patients with and without c-kit D816V mutation were eligible for participation in the pivotal trial. The vast majority of the patients harboured the c-kit D816V mutation. The applicant considers masitinib to be effective independently of mutational status and pre-clinical data seems to corroborate this. The phase 2 trial that included wild-type (WT) patients (AB1010) is supposed to support that masitinib works independently of mutational status. However, very few of these patients were pure WT and no conclusions can be drawn on such a small data base. Thus, the clinical data is too limited to

confirm that patients not harbouring the c-kit D816V mutation derive a benefit of masitinib treatment similar to the c-kit D816V mutation positive patients.

Placebo is considered an acceptable comparator for patients with severe symptoms of mastocytosis not responding to symptomatic treatment.

On request, the applicant has provided data on the use of baseline and concomitant symptomatic treatment. The GCP inspection requested by the CHMP of two sites of the pivotal study found that despite protocol recommendations, changes in background symptomatic medications were frequently undertaken during the study and use of new concomitant medications was not systematically reported to the sponsor. As symptom control was the primary aim of the study, the quality of the study is questionable and the consequences as to outcome measures are hard to foresee, as the imposed changes could seriously impact outcomes.

An oral antihistamine (cetirizine 10 mg/day) was mandatorily combined with the study drug for 60 days. Cetirizine was initiated at the same time as study treatment. The recommended daily dose of masitinib for treatment of severe systemic mastocytosis is 6 mg/kg divided on two doses, together with a meal. Based on the presented documentation it has not been convincingly demonstrated that this is the optimal dose. No formal dose-finding study has been performed.

The protocol has been changed several times during the conduct of the study to e.g. increase the severity of the handicaps at baseline and to increase the cut-off point for response (amendments 5 and 6). In addition, cumulative response as primary endpoint was introduced and the statistical methods for analysing efficacy were changed. There is a concern that these changes could have been informed by data obtained during the study. According to the applicant, these changes were undertaken before unblinding. However, due to major differences in reported AEs it is not possible to exclude unblinding of study subjects as experienced investigators might draw conclusions as regards to pattern of activity in different populations. In addition, the GCP inspection has found that the blinding was compromised during the trial. It is not possible to exclude that the protocol changes were partly driven by the applicant's impression of patient response during the conduct of the study.

Insufficient information is provided for the 85 patients randomised but retrospectively excluded from the analysis. It is therefore impossible to evaluate the impact of those patients on the presented results; nonetheless it cannot be excluded that such an effect could be profound.

The applicant has discussed several issues and changes to the protocol in CHMP scientific advice during the course of the study. However, the primary endpoint that the applicant finally chose was not discussed. Originally, the primary endpoint was the change from baseline to week 24 analysed using Chi-square test. To further investigate the implications of the changes to the protocol, the applicant was asked to provide efficacy analyses as initially planned. These analyses are further discussed in the *Efficacy* section below. In scientific advice the applicant was warned that the study integrity might be compromised by the extensive changes to the study protocol.

Cumulative response by patient*handicap (4H75%) was the primary endpoint. Response on a handicap was defined as an improvement with respect to the baseline values of ≥ 75% for pruritus, or flushes, or depression, or asthenia. Cumulative response was calculated as the number of actual responses between weeks 8 and 24, divided by the total number of possible responses over the same treatment period (i.e. with 5 scheduled visits each patient had a maximum of 5 to 20 possible responses depending on the number of handicaps at baseline). Missing data was considered as failure (MDF).

There are no generally agreed response criteria for systemic mastocytosis (SSM and ISM). Pruritus, flushes, asthenia and psychological impact are frequently encountered in these patients and therefore it seems reasonable to include these in the primary endpoint. The applicant was requested to provide analyses that would further substantiate the clinical relevance of masitinib treatment, e.g. an analysis showing how many of the patients were responders. The primary endpoint does not take into account handicaps that might have occurred after the baseline assessment, or handicaps that were not considered severe at baseline, but which deteriorated during the course of the study. The applicant has therefore provided post-hoc analyses that take into account new baseline handicaps and deterioration of others.

Long-term efficacy was explored in an extension period lasting up to 2 years.

Additional endpoints were cumulative response on pruritus, cumulative response on 3 handicaps (pruritus, or flushes or depression), and cumulative response on 2 handicaps (pruritus or flushes). In addition, exploratory endpoints investigated the cumulative response on each of these handicaps. The most objective endpoint was the change in tryptase level as an indication of reduced disease activity in patients with tryptase higher than 20 µg/L at baseline. According to the findings of the GCP inspection, more than one third of the tryptase samples were analysed at local laboratories rather than at the central laboratory as specified in the protocol. The implications for the reliability of the reported changes in tryptase levels are unknown.

Exploratory endpoints included change from baseline of body surface area covered by urticaria pigmentosa and disappearance of Darier's sign.

Quality of life was also an exploratory endpoint, measured by: Cumulative response on overall patient assessment (OPA) score among patients with "severe" or "intolerable" handicap at baseline; AFIRMM questionnaire (for each of the 52 items, cumulative response among patients with "severe" or "intolerable" handicap at baseline was given); QLQ-C30 (v3) global score, functional scores and symptom scores at each visit. Only the latter is a validated instrument.

According to the findings of GCP inspection requested by the CHMP, an incorrect version of the AFIRMM V2 questionnaire was used during the first months of the study, and occasionally thereafter, however the data obtained were pooled with the other data, confusing their interpretation. Consequently, the AFIRMM V2 data should be regarded with caution.

Fewer patients have been included than required to achieve an 80% power. Originally 200 patients were required to achieve 80% power at a 1% alpha level. Subsequently the alpha level was raised first to 2% (with no new sample size calculation) and then, upon failure to achieve enough conditional power at the futility IA, to 5%. In addition the primary analysis was changed and as a result 142 patients were required to achieve 80% power. Yet, only 135 patients have been recruited. The applicant explains this with the difficulty to find patients with severe enough symptoms once protocol version 6.0 was implemented. It should be noted though, that already in Q3 of 2012, 120 patients must have been enrolled (as this triggered the futility IA). Protocol version 6.0 was implemented in Q3 2013, leaving more than a year to recruit patients not affected by that change. In the responses to the LoQ adopted by CHMP, the applicant confirmed that 250 subjects were screened, of which 30 were considered failures at screening, which would leave 220 randomised subjects of which only 135 are included in the primary analysis. Before the two most influential amendments leading to protocol version 5.0 and 6.0 in fact already 160 patients had been screened and 144 randomised. Due to the changes in the study inclusion/exclusion criteria, it cannot be established whether or not those patients are still representative to allow generalisation of the results to the proposed intended population.

Major changes to the statistical analysis plan have been introduced during the study period. In the context of a request for a scientific advice, the applicant was alerted by the CHMP in 2011 that '*there is always a risk when modifying key aspects of the study design; this might potentially impact on study robustness*'. The applicant was also made aware of '*the unavoidable impact on the study credibility posed by simultaneously changing the primary endpoint, the inclusion criteria (which questions whether the different 'stages' of the trial can be sensibly combined) and introducing an interim analysis in an ongoing study*'. Even though the applicant changed the planned efficacy IA to a pure futility analysis, additional major changes on top of those already considered critical in 2011 were made. The timing of changes that resulted in protocol version 6.0 is considered questionable and raises concerns that the trial integrity might not have been sustained.

Both phase II studies were uncontrolled and included patients with systemic mastocytosis. Study AB06013 also included patients with cutaneous mastocytosis. Both studies divided the study population into two different handicap populations: moderate and severe. In both studies, the patients' population with severe handicaps is very limited; i.e. 15 evaluable patients in study AB04010 and 12 evaluable patients in study AB06013. Both studies used two dose levels; 3 mg/kg or 6 mg/kg daily. Dose adjustments up and down were allowed. The primary efficacy analysis of masitinib in both studies was based on the percentage change from baseline to week 12. However, while the analysis of study AB04010 was based on 3 handicaps (i.e. Pruritus score, Number of flushes per day and Hamilton Rating Scale for depression) study AB06013 also included asthenia (Fatigue Impact scale). The Wilcoxon Rank signed test was used to test the significance of change from baseline. Cumulative response (W8-W24) were secondary analyses.

Efficacy data and additional analyses

The most frequently reported handicap at baseline was asthenia, followed by pruritus, micturition, depression, flushes, and stools. There was no significant difference between the treatment-arms in terms of handicaps at baseline. The applicant managed to recruit >86 patients with the handicap pruritus at baseline as outlined in the sample size assumptions. However, the number of patients for the remaining individual handicaps is rather small, which makes interpretation of the results for these particular handicaps challenging.

There are inconsistencies in the information regarding the number of patients that had failed prior therapy for depression and the number of patients defined as having depression as a handicap at baseline. The GCP inspection requested by the CHMP identified 20 patients in the mITT population (9 in the masitinib group and 11 in the placebo group) with only 1 severity criterion instead of at least 2. This may be a result of inadequate definition of eligible patients in the study protocol.

The primary efficacy analysis was performed on the modified intent-to-treat (mITT) population which comprised 129 patients. Masitinib showed a statistically significant improvement over placebo for the primary endpoint (4H75%), with a response of 18.7 vs 7.4% (p-value 0.0076). All pre-planned sensitivity analyses confirmed this positive outcome for masitinib when compared with placebo. Responses were fairly consistent across visits with the lowest response at the 8 weeks visit and the highest response after 16 weeks. With reference to the GCP inspection outcome, the rating of depression and asthenia based on the HAMD-17 and FIS questionnaires are considered unreliable. This seriously hampers the credibility of the results of both primary and secondary endpoints which incorporates these handicaps. Also, as previously discussed, it cannot be excluded that use of concomitant symptomatic treatments have influenced and biased the results of the study. Altogether, this questions the robustness of the 11% difference in cumulative response for the primary endpoint shown in favour of masitinib compared with placebo.

The applicant has provided analyses to support the primary outcome. The majority of these analyses are uninterpretable due to insufficient events analysed. In addition, due to the composite nature of this endpoint it is difficult to assess its clinical relevance. Considering that patients who contributed with a response may have responded on just one of the four handicaps included in the primary endpoint and few patients had more than one or two handicaps relevant for the primary endpoint, the generalisability and clinical relevance for a patient population which often presents with a number of handicaps is questioned. Further, it remains unclear if these handicaps are completely independent of each other. Interaction and dependencies are not explored in any way. As a matter of fact, the very nature of the endpoint obscures the assessment of clinical relevance per se. Further, improvements in handicaps not considered severe at baseline are not included in the effect measure, but can nonetheless lead to indirect changes in other handicaps.

A statistically significant difference was claimed between masitinib and placebo treatment arm for the analyses of 3H75% endpoint (depression, pruritus and flush), 2H75% endpoint (pruritus and flush) and for analysis of pruritus alone, all in favour of masitinib. When excluding asthenia from the analysis the response rate tended to improve compared with the result for the primary endpoint. Thus, the most frequent handicap experienced in this study seems to respond to a lesser degree to treatment with masitinib. A further improvement was seen when excluding depression from the analysis. Thus, the best response claimed is seen when analysing patients with pruritus and flushes at baseline.

Approximately 55% of the patients continued into the extension phase. However, it is unclear how many patients were treated for how long. A tendency for lower response rates was observed for all endpoints analysed (3H75%, 2H75% and pruritus alone) for the W8 to W96 analyses compared to the W8 to W12 analyses, but still statistically significantly in favour of masitinib. No information is given on the baseline handicaps for the extension population. Very similar number/percentage of patients in the two study arms entered the extension phase and terminated participation during the extension phase. Also duration of therapy is rather similar 108 vs. 97 weeks. Interpretation of the results of the extension phase are complicated by the mentioned shortcomings, as a result the data do not support a favourable B/R of masitinib over placebo.

There was a claimed reduction of the tryptase level from baseline to last visit ([W0-W24 period]) in the masitinib arm, while there was a slight increase in the placebo arm; mean relative change was -18% in the masitinib treatment-arm versus +2.2% in the placebo arm (p=0.0001). A reduction of 25% from baseline at last visit (W0-W24), was 35% in the masitinib treatment arm versus 11.9% in the placebo arm (p=0.0172). Thus, a spontaneous reduction in tryptase level also occurred in some patients in the placebo arm. The comparative results on the reduction in tryptase level gives support for activity of masitinib on mast cells in the study population. However, it is not clear, for example, how many of the patients had a normalisation of tryptase level and if it resulted in improvement of symptoms of mastocytosis.

The result of the pre-planned exploratory analyses in patients with micturition and stools as handicap showed no effect of masitinib on micturition. On the 75% response rate on stools in the W0-W24 period, the response rate was inferior in masitinib-treated patients with a 2.5% response rate in masitinib treated patient compared to 12.0% in placebo treated patient. This is explained by diarrhoea being an adverse event associated with masitinib use (see discussion on clinical safety).

Body surface area covered by urticaria pigmentosa (UP) was claimed to be decreased in the masitinib arm (-12.3%) while it increased in the placebo arm (+15.9%) (p=0.0210) from baseline to Week 24. Disappearance of Darier's sign from baseline to Week 24 for patients with "Darier's sign" at baseline was 18.9% in the masitinib treatment-arm versus 2.7% in the placebo arm (p=0.0187, odds ratio=6.58). The improvement in UP as well as the disappearance of Darier's sign can be considered as indicators of the activity of masitinib. The baseline values UP or Darier's sign, as well as how many of the patients had UP and Darier's sign at baseline, could not easily be found in the dossier.

There was no improvement in the quality of life measured by QLQ-C30 global score, AFIRMM questionnaire, and OPA score. Exploratory analyses performed on the 75% reduction in the score of the detailed items of FIS, HAMD-17, and AFIRMM score indicated that masitinib generated a benefit on a greater number of quality of life related items compared with placebo. See previous comments on FIS, HAMD-17, and AFIRMM score. Among the 14 sub-scores of the QLQ-C30 score, 75% improvement was in favour of masitinib for Emotional Functioning, Social Functioning and Constipation. 75% improvement was in favour of placebo for Dyspnoea, Appetite loss and Diarrhoea.

In study AB04010 in patients with severe handicaps, at Week 12, a statistically significant improvement in the flush score compared to baseline was observed (-74.7 ± 27.8%) with 7/11 patients responding with a 75% improvement. The improvement was sustained up to Week 24 (83.1 ± 30.8%) with 6/11 responding with a 75% improvement. No significant improvement was observed for pruritus score and depression and only a very limited number of patients had these handicaps at baseline. This study was supposed to give support for the efficacy of masitinib in mastocytosis also in patients not bearing activating point mutations in c-Kit. However, only 8 patients may be considered true WT in this study. It is unclear how many of these had severe handicaps. No conclusion can be drawn regarding the efficacy of masitinib in WT patients based on the clinical documentation.

In Study AB06013 at Week 12**,** a significant improvement in pruritus score compared to baseline was observed (-39.9 ± 47.6%) with 4/12 patients responding with a 75% improvement. This was somewhat reduced at week 24 but still significant. No significant improvement was observed for patients with severe flushes or severe depression at baseline and only a very limited number of patients had these handicaps at baseline.

In these Phase 2 studies masitinib had some activity on flushes and pruritus. However, the trials are hampered by the very limited number of patients and the lack of a control group and may be considered hypothesis generating. Moreover, these studies cannot be considered as supportive to the pivotal trial due to differences in methodology, sample size and patient populations.

2.5.4. Conclusions on the clinical efficacy

The clinical relevance of the 11% increase in cumulative symptomatic response for masitinib, as defined, compared to placebo, is questioned due to the composite nature of the endpoint; moreover this benefit can be considered as overestimated following the findings of an GCP inspection of 2 study sites as well as the sponsor site. The accumulation of critical and major inspection findings, affecting all aspects of the trial, seriously question the reliability of the trial data.

Therefore, the benefit of treatment with Masipro for patients with smouldering or indolent systemic mastocytosis with severe mediator release-associated symptoms unresponsive to optimal symptomatic treatments is not considered adequately demonstrated.

2.6. Clinical safety

To support the MAA in the above claimed population, AB Science conducted one pivotal phase 3 study (**AB06006**) and two supportive phase 2 studies (**AB04010** and **AB06013**).

Table 58 An overview of the clinical studies for support of the present MAA

Study number	Type of study	Type of study/design features	Dosage, regimen
AB06006	Phase 3 (pivotal)	A 24-week with possible extension, prospective, multicentre, randomized, double blind, placebo-controlled, 2-parallel group with a randomization 1:1, Phase 3 study to compare efficacy and safety of masitinib at 6 mg/kg/day to placebo in treatment of patients with Smouldering Systemic, Indolent Systemic or Cutaneous Mastocytosis with handicap	222 patients were recruited. Among them: -135 patients with severe systemic mastocytosis: Population for primary efficacy and safety analysis -87 patients with cutaneous mastocytosis and non-severe systemic mastocytosis. Patients were randomized in 2 groups: Group 1: patients received masitinib at 6 mg/kg/day Group 2: patients received placebo
AB04010	Phase 2	Phase 2a, open-label, randomized study of oral AB 1010 in patients with systemic indolent mastocytosis with handicap and not bearing activating point mutations in the phosphotransferase domain of c-Kit such as the main mutation Asp-816-Val (D816V)	25 subjects have been randomized into two group (among them: 16 patients with severe systemic mastocytosis) Group 1, have orally received AB 1010 at a total daily dose of 3 mg/ kg, divided by 2 administrations. Group 2, have orally received AB 1010 at the dose of 6 mg/ kg, divided by 2 administrations.
AB06013	Phase 2	A 12-week with possible extension, prospective, multicenter, randomized, open-label, 2-parallel group, Phase 2a study to compare efficacy and safety of AB 1010 at 3 or 6 mg/kg/day in treatment of patients with mastocytosis with handicap and bearing activating point mutations in the phosphotransferase domain of c-Kit such as the main mutation Asp-816-Val (D816V).	22 subjects have been randomized into two groups (among them: 12 patients with severe systemic mastocytosis) - group 1: patients receiving daily 3 mg/kg AB 1010 (12 patients) - group 2: patients receiving daily 6 mg/kg AB 1010 (10 patients)

Patient exposure

Table 59 Population of safety analysis

Pathologies	Masitinib	Placebo	Blinded studies (masitinib/placebo)	All
Mastocytosis	156	110		266
Phase 3 (AB06006)				
-Systemic severe (SS)	70	63		133
-SS and other forms	110	110		220
Phase 2 (pooled patients from AB04010 and AB06013 studies)				
-Systemic severe (SS)	28			28
-SS and other forms	46*			46
Non oncology studies (including mastocytosis)	399	160	858	1417
Healthy volunteers	92	18		110
Total Non-oncology + Healthy volunteers[2]	481	178	858	1527

(1) 8 patients from AB04009 study conducted in aggressive mastocytosis were not included in this total number of mastocytosis patients. Aggressive mastocytosis comprised mast cell leukemia which belongs to oncology rather than non-oncology studies.
(2) The total is the sum of non-oncology patients (which include mastocytosis patients) and healthy volunteers
(Cut-off: 24 November 2015 for mastocytosis and 31 January 2015 for non-oncology)

Apart from mastocytosis, the non-oncology studies encompassed studies conducted in patients (n=243) with ALS, MS, Alzheimer, MB Crohn, severe asthma, rheumatoid arthritis, etc.

Oncology studies were not included as masitinib was combined with chemotherapy in some studies and as a higher dose (6-12 mg/kg/day) was used.

The exposure by dose and duration for masitinib in different study populations is displayed.

Table 60 Exposure of patients to different doses of masitinib

		Number of patients exposed for at least (months)		
Therapeutic Area	**Last daily dose**	**]0-6]**	**]6-12]**	**> 12**
Mastocytosis studies	6 mg/kg/day	94	39	31
	All	156	80	58
Non Oncology studies + HV (OLU+BLE)	6 mg/kg/day	381	169	77
	All	1062	578	301

(Cut-off: 24 November 2015 for mastocytosis and 31 January 2015 for non-oncology)

Globally, 94 patients with mastocytosis and 381 non-oncology patients + Healthy volunteers (including blinded studies) were treated with masitinib at 6 mg/kg/day.

31 mastocytosis patients and 77 non-oncology patients + healthy volunteers received masitinib at 6 mg/kg/day for more than a year.

For the pivotal study AB06006, the table below presents the duration of patient exposure in the safety population (SAF), which includes all patients with severe systemic mastocytosis who took at least one dose of study medication (masitinib or placebo).

Table 61 Exposure to study drug - Safety population in pivotal study AB06006

Treatment exposure (months)	Masitinib (N=70)	Placebo (N=63)
N	70	63
Mean ± SD	18.9±22.0	16.4±19.3
Median	6.3	6.1
Range	0.1-74.1	0.7-72.3
Exposure more than 6 months	36 (51.4%)	32 (50.8%)
Exposure more than 12 months	28 (40.0%)	19 (30.2%)
Exposure more than 18 months	23 (32.9%)	15 (23.8%)
Exposure more than 24 months	21 (30.0%)	13 (20.6%)
Exposure in patient-months	1,321	1,031

For the pivotal phase 3 study, patients were given either masitinib at 6 mg/kg/day or matching placebo. The patients had the possibility to reduce the dose in one or more steps as needed, in increments of 1.5 mg/kg/day. Administration of concomitant optimal symptomatic treatments was allowed.

For the phase 2 studies (AB04010 and AB06013) the intended study duration was 12 weeks, with an extension period up to 24 weeks. Masitinib was initially dosed at 3 or 6 mg/kg/day (two cohorts) with possible dose justifications (increase or reduction) if needed. Administration of concomitant optimal symptomatic treatments was allowed.

Adverse events

The summary of AE reported in AB06006 [both for the severe systemic (SS) mastocytosis and SS mastocytosis+Other data sets] and in the pooled phase 2 data were presented in the below table.

Table 62 Summary of AE with masitinib in mastocytosis during the protocol period

	Mastocytosis					
	Phase 3				*Phases 2*	
	SS (CR)		*SS+Other*		*SS*	*SS+other*
Number (%) of patients with at least one	*Masitinib (N=70)*	*Placebo (N=63)*	*Masitinib (N=110)*	*Placebo (N=110)*	*Masitinib (N=28)*	*Masitinib (N=46)*
AE	70 (100.0%)	63 (100.0%)	109 (99.1%)	109 (99.1%)	27 (96.4%)	43 (93.5%)
SAE (non fatal)	20 (28.6%)	12 (19.0%)	32 (29.1%)	15 (13.6%)	4 (14.3%)	5 (10.9%)
Death	0 (0.0%)	1 (1.6%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	0 (0.0%)
AE leading to permanent discontinuation during protocol period	16 (22.9%)	5 (7.9%)	26 (23.6%)	6 (5.5%)	6 (21.4%)	8 (17.4%)
- Per protocol	11 (15.7%)	5 (7.9%)	21 (19.1%)	5 (4.5%)	0 (0.0%)	0 (0.0%)
- As per safety rules	7 (10.0%)	4 (6.3%)	12 (10.9%)	4 (3.6%)	0 (0.0%)	0 (0.0%)
Severe AE	35 (50.0%)	22 (34.9%)	55 (50.0%)	33 (30.0%)	16 (57.1%)	21 (45.7%)
AE leading to dose reduction	15 (21.4%)	1 (1.6%)	24 (21.8%)	2 (1.8%)	6 (21.4%)	8 (17.4%)

Cut-off: 24 November 2015

Supportive Phase II studies (AB04010 and AB06013):

An overview of observed AEs at different dose levels in the phase II studies are presented in the below table.

Table 63 Frequency of adverse events (week 0-24) in AB04010 and AB06013 studies

	3.0 mg/kg/day (N=27)	4.5 mg/kg/day (N=25)	6 mg/kg/day (N=29)
All AEs	21 (77.8%)	19 (76.0%)	21 (72.4%)
Severe AEs	7 (25.9%)	4 (16.0%)	10 (34.5%)
Serious AEs (non-fatal)	3 (11.1%)	2 (8.0%)	2 (6.9%)
Discontinuation (excl deaths)	4 (14.8%)	3 (12.0%)	3 (10.3%)

Table 64 Summary of AEs – Safety population in AB06006

	N(%) of patients [W0-W24]			Incidence (patients-months) Overall study period		
	Masitinib (N=70)	Placebo (N=63)	Delta (M-P)	Masitinib (pm=1321)	Placebo (pm=1031)	Delta (M-P)
At least one AE	70 (100.0%)	63 (100.0%)	0%	5.3	6.1	-0.8
Death	0 (0.0%)	1 (1.6%)	-1.6%	0.0	0.1	-0.1
Non-fatal SAE	20 (28.6%)	12 (19.0%)	9.6%	2.1	1.8	0.3
Severe AE	35 (50.0%)	22 (34.9%)	15.1%	3.2	2.9	0.3
AE leading to study treatment permanent discontinuation (except death)	16 (22.9%)	5 (7.9%)	+14.9%	1.5	0.5	1.0
- As per protocol	11 (15.7%)	5 (7.9%)	7.8%	1.1	0.5	0.6
- As per safety rules	7 (10.0%)	4 (6.3%)	3.7%	0.8	0.4	0.4
At least one AE leading to study treatment dose reduction	15 (21.4%)	1 (1.6%)	19.8%	1.4	0.1	1.3

Incidence in patient-month: numerator is number of patients with at least one AE, denominator is the sum of exposure durations (in months). Frequency is calculated per 100 patients-months.

Whether "incidence per patient month" is a proper summary of risk obviously depends on the event pattern. For many common events, the first month is most informative. Time to SAE and time to severe (Grade 3/4) event data are likely to be more informative.

Very common adverse events

The most frequently reported adverse events in the AB06006 study (occurring in ≥30% of masitinib treated patients) were diarrhoea (50%), nausea (48.6%), haemoglobin decreased (34.3%) and eyelid oedema (30%). Serious adverse events occurred more frequently in the masitinib group compared to the placebo group (~30% vs ~20%). There were no fatal cases in the mastocytosis phase II and III studies in patients on masitinib. There was one fatal case in the placebo arm (subdural intracerebral hematoma).

The frequency of the most common adverse events (≥10%) during the initial protocol period and the incidence of the corresponding adverse events during the overall study period is presented below.

Table 65 Most common (≥10%) AEs with masitinib – Safety population

	N(%) of patients [W0-W24]			Incidence of AEs (Overall study period)		
Preferred Term	M (N=70)	P (N=63)	Delta (M-P)	M (pm=1321)	P (pm=1031)	Delta (M-P)
DIARRHOEA	35 (50.0%)	13 (20.6%)	29.4%	2.8	1.6	1.2
NAUSEA	34 (48.6%)	13 (20.6%)	27.9%	2.6	1.4	1.2
HAEMOGLOBIN DECREASED	24 (34.3%)	9 (14.3%)	20.0%	2.0	1.0	1
EYELID OEDEMA	21 (30.0%)	2 (3.2%)	26.8%	1.7	0.2	1.5
MUSCLE SPASMS	20 (28.6%)	5 (7.9%)	20.6%	1.7	1.0	0.7
BLOOD GLUCOSE INCREASED	19 (27.1%)	15 (23.8%)	3.3%	1.9	1.9	0
ASTHENIA	18 (25.7%)	11 (17.5%)	8.3%	1.8	1.3	0.5
HEADACHE	16 (22.9%)	17 (27.0%)	-4.1%	1.7	2.2	-0.5
PRURITUS	16 (22.9%)	9 (14.3%)	8.6%	1.4	1.1	0.3
ANAEMIA	16 (22.9%)	8 (12.7%)	10.2%	1.4	0.8	0.6
VOMITING	15 (21.4%)	7 (11.1%)	10.3%	1.3	0.8	0.5
ALANINE AMINOTRANSFERASE INCREASED	15 (21.4%)	2 (3.2%)	18.3%	1.3	0.4	0.9
OEDEMA PERIPHERAL	15 (21.4%)	5 (7.9%)	13.5%	1.4	0.5	0.9
BLOOD TRIGLYCERIDES INCREASED	14 (20.0%)	17 (27.0%)	-7.0%	1.4	2.1	-0.7
ABDOMINAL PAIN	14 (20.0%)	9 (14.3%)	5.7%	1.4	1.2	0.2
BLOOD PHOSPHORUS DECREASED	13 (18.6%)	5 (7.9%)	10.6%	1.1	0.9	0.2
ASPARTATE AMINOTRANSFERASE INCREASED	13 (18.6%)	3 (4.8%)	13.8%	1.4	0.5	0.9
HYPERGLYCAEMIA	13 (18.6%)	10 (15.9%)	2.7%	1.5	1.4	0.1
WHITE BLOOD CELL COUNT DECREASED	13 (18.6%)	6 (9.5%)	9.0%	1.3	0.6	0.7
BLOOD ALKALINE PHOSPHATASE INCREASED	11 (15.7%)	10 (15.9%)	-0.2%	0.9	1.1	-0.2
RASH	11 (15.7%)	3 (4.8%)	11.0%	0.8	0.3	0.5
GAMMA-GLUTAMYLTRANSFERASE INCREASED	10 (14.3%)	10 (15.9%)	-1.6%	1.1	1.1	0
NEUTROPHIL COUNT DECREASED	9 (12.9%)	4 (6.3%)	6.5%	0.8	0.7	0.1
LYMPHOPENIA	9 (12.9%)	6 (9.5%)	3.3%	0.7	0.7	0
ARTHRALGIA	9 (12.9%)	10 (15.9%)	-3.0%	1.0	1.3	-0.3
NEUTROPENIA	9 (12.9%)	7 (11.1%)	1.7%	0.8	1.1	-0.3
WEIGHT DECREASED	9 (12.9%)	1 (1.6%)	11.3%	1.1	0.1	1
FLUSHING	9 (12.9%)	1 (1.6%)	11.3%	0.8	0.5	0.3
HYPERTRIGLYCERIDAEMIA	8 (11.4%)	11 (17.5%)	-6.0%	0.9	1.4	-0.5
NASOPHARYNGITIS	8 (11.4%)	5 (7.9%)	3.5%	0.8	0.6	0.2
FATIGUE	7 (10.0%)	11 (17.5%)	-7.5%	0.6	1.2	-0.6
FACE OEDEMA	7 (10.0%)	1 (1.6%)	8.4%	0.5	0.1	0.4
BLOOD BILIRUBIN INCREASED	7 (10.0%)	4 (6.3%)	3.7%	0.6	0.7	-0.1
DEPRESSION	7 (10.0%)	5 (7.9%)	2.1%	0.8	0.7	0.1
ANOREXIA	7 (10.0%)	2 (3.2%)	6.8%	0.7	0.2	0.5
INSOMNIA	7 (10.0%)	7 (11.1%)	-1.1%	0.8	1.0	-0.2
PYREXIA	7 (10.0%)	1 (1.6%)	8.4%	0.7	0.2	0.5
BACK PAIN	7 (10.0%)	6 (9.5%)	0.5%	0.8	0.7	0.1

For several event types, the frequency is at least 10% higher in the masitinib group compared with placebo add-on including GI-events, oedema of different locations, anaemia, rash, flushing, AST/ALT increase and weight decrease.

Pruritus/rash/flush and diarrhoea were identified as very common AE in AB06006 study, but they were also amid mastocytosis symptoms reduced by masitinib. Two different groups of population might be present in AB06006: one group showing a positive effect of masitinib on the reduction of pruritus/rash/flush and diarrhoea as mastocytosis symptoms and another one experiencing them as AE. It is possible that pruritus/rash/flush and diarrhoea also had a transient increase in some patients.

Common adverse events

Adverse reactions reported in study AB06006 in <10% of patients and with a higher frequency in the masitinib group than the placebo group (M-P>4%), are displayed below:

Table 66 Main common AE (frequency >=1% and <10%) with masitinib in SS and SS+other patients in AB06006 (0-24weeks, frequency)

	SS (CR)			*SS+other*		
Number (%) of patients with at least one	*Masitinib (N=70)*	*Placebo (N=63)*	*M-P*	*Masitinib (N=110)*	*Placebo (N=110)*	*M-P*
Lymphocyte Count Decreased	6 (8.6%)	2 (3.2%)	5.4	13 (11.8%)	7 (6.4%)	5.5
Dry Skin	5 (7.1%)	-	7.1	10 (9.1%)	1 (0.9%)	8.2
Urticaria	5 (7.1%)	-	7.1	6 (5.5%)	2 (1.8%)	3.6
Blood Potassium Decreased	5 (7.1%)	-	7.1	5 (4.5%)	-	4.5
Hypophosphataemia	5 (7.1%)	1 (1.6%)	5.6	9 (8.2%)	2 (1.8%)	6.4
Dyspepsia	4 (5.7%)	1 (1.6%)	4.1	5 (4.5%)	3 (2.7%)	1.8
Swelling Face	4 (5.7%)	-	5.7	4 (3.6%)	1 (0.9%)	2.7
Eczema	3 (4.3%)	-	4.3	6 (5.5%)	2 (1.8%)	3.6
Gastroenteritis	3 (4.3%)	-	4.3	5 (4.5%)	2 (1.8%)	2.7
Viral Infection	3 (4.3%)	-	4.3	5 (4.5%)	3 (2.7%)	1.8
Chest Pain	3 (4.3%)	-	4.3	4 (3.6%)	1 (0.9%)	2.7
Cytolytic Hepatitis	3 (4.3%)	-	4.3	4 (3.6%)	-	3.6
Erythema	3 (4.3%)	-	4.3	4 (3.6%)	1 (0.9%)	2.7
Eye Pruritus	3 (4.3%)	-	4.3	3 (2.7%)	1 (0.9%)	1.8
Weight Increased	3 (4.3%)	-	4.3	3 (2.7%)	-	2.7

M-P: difference of occurrence between Masitinib and Placebo
Cut-off: 24 November 2015

The majority of the relevant common AE observed with masitinib in the pivotal AB06006 study were already identified as common AE in supportive phase 2 studies.

TKI use is associated with hypophosphatemia, and changes in bone and mineral metabolism use. The reported frequency of hypophosphatemia in masitinib treated patients was 7.1% % in the phase 3 study.

Severity

The frequencies of severe AE (ie. of Grade 3 and 4) in study AB06006 were: diarrhoea 11% vs. 2%, asthenia 6% vs. 2% and pruritus 4% vs. 2%, rash 3% vs 0%, pyrexia (3% vs 0%) and neutropenia 4% vs. 2%.

The following events occurred in more than one patient and at a higher frequency than placebo: neutropenia [(3/70 (severe systemic) and 5/110 (mastocytosis all) vs. 1/110], diarrhoea (8/70 and 10/110 vs. 1/110), asthenia (4/70 and 5/110 vs. 2/110), pyrexia (2/70 and 4/110 vs. 0/110) and rash (4/70 vs. 0/63).

An overview of actions taken for severe AEs are presented.

Table 67 Actions taken for Severe AEs - [W0-W24] - Safety population

	Masitinib (N=70)	Placebo (N=63)
Severe AEs	35 (50.0%)	22 (34.9%)
- Leading to permanent discontinuation	14 (20.0%)	3 (4.8%)
- Not leading to permanent discontinuation	21 (30.0%)	19 (30.2%)
- Not leading to permanent discontinuation nor dose reduction	12 (17.1%)	19 (30.2%)
- Worst action per patient:		
o None (No temporary interruption nor dose reduction nor discontinuation)	0 (0.0%)	1 (1.6%)
o Temporary interruption (without dose reduction nor discontinuation)	12 (17.1%)	18 (28.6%)
o Dose reduction (without discontinuation)	9 (12.9%)	0 (0.0%)
o Discontinuation	14 (20.0%)	3 (4.8%)
Skin And Subcutaneous Tissue Disorders	11 (15.7%)	2 (3.2%)
- Leading to permanent discontinuation	4 (5.7%)	1 (1.6%)
- Not leading to permanent discontinuation	7 (10%)	-
- Not leading to permanent discontinuation nor dose reduction	6 (8.6%)	1 (1.6%)
Gastrointestinal Disorders	12 (17.1%)	3 (4.8%)
- Leading to permanent discontinuation	3 (4.3%)	1 (1.6%)
- Not leading to permanent discontinuation	9 (12.8%)	2 (3.2%)
- Not leading to permanent discontinuation nor dose reduction	4 (5.7%)	2 (3.2%)

Adverse events of interest (AEOI)

The following AEs were among the most severe AEs occurring during the clinical development of masitinib: severe neutropenia, severe skin toxicities, Steven-Johnson Syndrome (SJS) and Drug-Rash with Eosinophilia and Systemic symptoms (DRESS), and also risk of carcinogenicity, see below. Other AEs of special interest are diarrhea, rash, edemas, nausea, vomiting, asthenia and fatigue, hepatotoxicity, renal toxicity, cardiotoxicity, and reproductive toxicity. See Clinical AR for further details of the latter.

Severe neutropenia

The table below presents the frequency, action taken, severity, time of occurrence of AEs related to severe neutropenia.

Table 68 Severe neutropenia - action taken, severity, time of occurrence and duration - Population: Safety – Masitinib – Safety population

	[W0-W24]		Overall Study Period	
	Masitinib (N=70)	Placebo (N=63)	Masitinib (pm=1321)	Placebo (pm=1031)
Severe neutropenia[1] - Number of patients with at least one AE	4 (5.7%)	1 (1.6%)	0.4	0.1
Grade 3	2 (2.9%)	0 (0.0%)		
Grade 4	2 (2.9%)	1 (1.6%)		
Time of occurrence (days) – Median	38	113		

[1] The MedDRA terms (PTs): agranulocytosis, bone marrow failure, febrile neutropenia, neutropenia, neutrophil count decreased.

The risk of severe neutropenia is higher in masitinib treated patients compared to untreated patients in all non-oncology studies, and according to an incidence calculation in patient-months (taking into account the difference of study duration in non-oncology studies), the risk of grade 3 and grade 4 neutropenia cases is similar in severe systemic mastocytosis patients compared with other non-oncology patients. Neutropenia increases the risk for infections (see below). Regular monitoring would thus be considered important.

Infections

The table below presents the frequency, action taken, severity, time of occurrence of AEs related to infections.

Table 69 Infections - action taken, severity, time of occurrence and duration - Population: Safety – Masitinib – Safety population

	[W0-W24]		Overall Study Period	
	Masitinib (N=70)	Placebo (N=63)	Masitinib (pm=1321)	Placebo (pm=1031)
Infections[1] - Number of patients with at least one AE	31 (44.3%)	28 (44.4%)	2.6	3.2
Worst action per patient				
Patients with reported action taken	31	28	2.6	3.2
AE leading to no action	28 (40.0%)	26 (41.3%)	2.3	3.0
AE leading to study treatment temporary interruption	1 (1.4%)	2 (3.2%)	0.2	0.2
AE leading to dose reduction	0 (0.0%)	0 (0.0%)	0.0	0.0
AE leading to permanent discontinuation	2 (2.9%)	0 (0.0%)	0.2	0.0
Number of patients by severity				
Mild severity	22 (31.4%)	13 (20.6%)	2.0	1.7
Moderate severity	9 (12.9%)	15 (23.8%)	1.3	2.0
Severe severity	3 (4.3%)	1 (1.6%)	0.5	0.1
Time of occurrence (days) – Median	49	57		

[1] The MedDRA terms (PTs): all PTs included in the SOC 'Infections ans infestations'.

With regards to Infections and infestations, 44% of subjects in both arms (31/70 vs. 28/63) reported infections during the 24w period, i.e. overall there is no increase in infectious events, but neutropenic fever constitutes a signal. However, there was an imbalance in infections in the extension period (50% vs. 28%), but, the number of patients in this phase of the study was small, precluding any firm conclusions.

Table 70 Oedema

	[W0-W24]	
	Masitinib (N=70)	**Placebo (N=63)**
Edema[1] - Number of patients with at least one AE	37 (52.9%)	9 (14.3%)
Worst action per patient		
Patients with reported action taken	37	9
AE leading to no action	33 (47.1%)	8 (12.7%)
AE leading to study treatment temporary interruption	3 (4.3%)	1 (1.6%)
AE leading to dose reduction	0 (0.0%)	0 (0.0%)
AE leading to permanent discontinuation	1 (1.4%)	0 (0.0%)
Number of patients by severity		
Mild severity	29 (41.4%)	6 (9.5%)
Moderate severity	14 (20.0%)	4 (6.3%)
Severe severity	2 (2.9%)	0 (0.0%)
Time of occurrence (days) – Median	23	39

1 The MedDRA terms (PTs) for the analyssi of edema: eye oedema, eye swelling, eyelid oedema, orbital oedema, periorbital oedema, gastrointestinal oedema, gingival disorder, gingival oedema, gingival swelling, oedema mouth, swollen tongue, tongue oedema, face oedema, generalised oedema, local swelling, localised oedema, oedema, oedema peripheral, swelling, allergic oedema, fluid overload, fluid retention, breast oedema, oedema genital, scrotal oedema, testicular swelling, laryngeal oedema, nasal oedema, oropharyngeal pain, pharyngeal oedema, periorbital oedema, swelling face

Oedema occurs early, is mainly of mild severity, but led to temporary interruption in 3 individuals and discontinuation in one individual.

Table 71 Vomiting

	[W0-W24]	
	Masitinib (N=70)	**Placebo (N=63)**
Vomiting[1] - Number of patients with at least one AE	15 (21.4%)	7 (11.1%)
Worst action per patient		
Patients with reported action taken	15	7
AE leading to no action	11 (15.7%)	6 (9.5%)
AE leading to study treatment temporary interruption	3 (4.3%)	1 (1.6%)
AE leading to dose reduction	1 (1.4%)	0 (0.0%)
AE leading to permanent discontinuation	0 (0.0%)	0 (0.0%)
Number of patients by severity		
Mild severity	10 (14.3%)	4 (6.3%)
Moderate severity	5 (7.1%)	3 (4.8%)
Severe severity	0 (0.0%)	0 (0.0%)
Time of occurrence (days) – Median	6	76

[1] The MedDRA terms (PTs): regurgitation of food, vomiting.

Note the very early occurrence of vomiting.

Table 72 Diarrhoea

	[W0-W24]	
	Masitinib (N=70)	Placebo (N=63)
Diarrhea[1] - Number of patients with at least one AE	36 (51.4%)	13 (20.6%)
Worst action per patient		
Patients with reported action taken	36	13
AE leading to no action	26 (37.1%)	10 (15.9%)
AE leading to study treatment temporary interruption	6 (8.6%)	2 (3.2%)
AE leading to dose reduction	0 (0.0%)	0 (0.0%)
AE leading to permanent discontinuation	4 (5.7%)	1 (1.6%)
Number of patients by severity		
Mild severity	13 (18.6%)	8 (12.7%)
Moderate severity	22 (31.4%)	5 (7.9%)
Severe severity	8 (11.4%)	1 (1.6%)
Time of occurrence (days) – Median	39	35

[1] The MedDRA terms (PTs): abnormal faeces, diarrhoea, diarrhoea haemorrhagic, frequent bowel movements

Note the shift towards higher severity grade.

Table 73 Asthenia and Fatigue

	[W0-W24]	
	Masitinib (N=70)	Placebo (N=63)
Asthenia and Fatigue[1] - Number of patients with at least one AE	25 (35.7%)	20 (31.7%)
Worst action per patient		
Patients with reported action taken	25	20
AE leading to no action	21 (30.0%)	19 (30.2%)
AE leading to study treatment temporary interruption	1 (1.4%)	1 (1.6%)
AE leading to dose reduction	1 (1.4%)	0 (0.0%)
AE leading to permanent discontinuation	2 (2.9%)	0 (0.0%)
Number of patients by severity		
Mild severity	10 (14.3%)	9 (14.3%)
Moderate severity	14 (20.0%)	11 (17.5%)
Severe severity	4 (5.7%)	2 (3.2%)
Time of occurrence (days) – Median	29	52

[1] The MedDRA terms (PTs): asthenia, fatigue

For the combined terms asthenia and fatigue there is only a minor difference between study arms. Asthenia, however, was more commonly reported in the masitinib arm with the reverse for fatigue (see table above).

Table 74 Hepatic events

Safety - Masitinib - Safety population

	[W0-W24]	
	Masitinib (N=70)	Placebo (N=63)
Hepatic disorders[1] - Number of patients with at least one AE	43 (61.4%)	24 (38.1%)
Worst action per patient		
Patients with reported action taken	33	19
AE leading to no action	29 (41.4%)	19 (30.2%)
AE leading to study treatment temporary interruption	1 (1.4%)	0 (0.0%)
AE leading to dose reduction	0 (0.0%)	0 (0.0%)
AE leading to permanent discontinuation	3 (4.3%)	0 (0.0%)
Number of patients by severity		
Mild severity	41 (58.6%)	24 (38.1%)
Moderate severity	8 (11.4%)	1 (1.6%)
Severe severity	3 (4.3%)	0 (0.0%)
Time of occurrence (days) – Median	43	64

[1] The MedDRA terms (PTs): ocular icterus, ascites, bile duct obstruction, biliary colic, biliary dilation, biliary tract disorder, cholangitis, cholangitis acute, cholecystitis, cholecystitis acute, cholelithiasis, cholestasis, cytolytic hepatitis, gallbladder polyp, haemorrhagic ascites, hepatic cirrhosis, hepatic cyst, hepatic failure, hepatic haemorrhage, hepatic pain, hepatisis, hepatitis toxic, hepatocellular injury, hepatomegaly, hepatosplenomegaly, hepatotoxicity, hyperbilirubinaema, jaundice, jaundice cholestatic, liver disorder, portal hypertension, portal veinocclusion, portal veinthrombosis, alanine aminotransferase, alanine aminotransferase abnormal, alanine aminotransferase decreased, alanine aminotransferase increased, aspartate aminotranferase, aspartate aminotranferase decreased, aspartate aminotranferase increased, bilirubin conjugated increased, bilirubin urine, blood albumin abnormal, blood albumin decreased, blood alkaline phosphatase abnormal, blood alkaline phosphatase decreased, blood alkaline phosphatase increased, blood bilirubin, blood bilirubin decreased, blood bilirubin increased, blood bilirubin unconjugated increased, blood lactate dehydrogenase increased, gamma-glutamyl transferase abnormal, gamma-glutamyl transferase increased, hepatic enzyme abnormal, hepatic enzyme increased, liver function test abnormal, protein total decreased, transaminases increased, urobilin urine present, bilirubinuria, yellow skin, cholecystectomy

There is a clear increase in frequency and severity of the events and permanent discontinuation was reported in 3/70 individuals.

Renal events:

There was no difference in renal events.

Cardiac toxicity

Overall the frequencies were similar, 26 vs. 24%, but treatment was discontinued in 3/70 vs. 0/63 individuals and a single case of severe toxicity was reported in the masitinib arm.

Steven Johnson syndrome (SJS) and Drug Rash with Eosinophilia and Systemic Symptoms (DRESS)

The potential cases of SJS/DRESS reported in non-oncology studies (4 potential cases of SJS and 2 potential cases of DRESS, none observed in mastocytosis studies) were all assessed by two independent experts who considered them as unlikely to be SJS and DRESS.

In oncology studies, 3 potential cases of SJS and 1 potential case of DRESS were reported of which 2 cases were diagnosed as possible/probable SJS and 1 case diagnosed as possible DRESS by the experts.

Serious adverse event / deaths / other significant events

Deaths

In masitinib-treated patients, no fatal case was reported in AB06006 phase 3 study. In the placebo cohort of AB06006 study, one fatal case was reported: the patient was diagnosed with a subdural intracerebral hematoma.

In non-oncology studies (cut-off date: 31 January 2015), 16 fatal cases were reported including the placebo-treated patient in the phase 3 study AB06006. Those cases were reported as: 9 patients in AB10015 study for Amyotrophic Lateral Sclerosis (ALS), 2 patients in AB09004 study for Alzheimer's disease, 2 patients in AB07002 for progressive multiple sclerosis, 1 patient in AB06010 study for rheumatoid arthritis, 1 patient in AB06006 study for mastocytosis, and 1 patient in AB07015 study for severe asthma

The 3 suspected cases were:

- A case (AB06010-203) was reported in a patient treated with masitinib 6 mg/kg/day for rheumatoid arthritis. The case was unblinded after the event. The patient had a medical history of cardiac disorders (congenital valvulopathy, hypertensive cardiomyopathy, dyslipidemia, toxic cardiomyopathy, total left bundle branch block and coronary arteries stenosis) and had a poor compliance to cardiac medications. The patient took masitinib for 26 days. The patient developed sudden death 4 days after stopping the treatment.
- A case (AB07002-034-010-02) was reported in a patient treated with masitinib 4.5 mg/kg/day for multiple sclerosis. The case was unblinded after the event. The patient had many cardiac risk factors including hypertension, dyslipidemia, and heavy tobacco use. Three days after starting the treatment, the patient developed acute myocardial infarction and later died after the placement of 3 stents. Coronary arteriography revealed circumflex artery occlusion.
- A case (AB07002-034-001-05) was reported in a patient receiving placebo 5 mg/kg/day for multiple sclerosis. The case was unblinded after the event. After nine months of treatment, the patient had sudden death at home. The autopsy revealed that sudden death was probably due to acute myocardial infarction. The patient had no medical history of cardiac disorders.

The not assessable case is:

- A case (AB07015-420-00801) was reported in a patient treated with masitinib or placebo 6.3 mg/kg/day for severe asthma. The patient had multiple cardiac risk factors including obesity, hypertension, and hypercholesterolemia. After 9 months of treatment, the patient died from massive pulmonary embolism and deep venous thrombosis of right lower limb.

All cases of death were not un-blinded. Thus a patient with Alzheimer was found dead in bed after 2-3 days of nausea and vomiting. No autopsy. The investigator concluded that "sudden death" was not caused by masitinib or placebo and the Sponsor agreed. Unblinding appears warranted.

It is not surprising that patients with ALS die of respiratory failure on study, but it appears hard to exclude an interaction between a poly-targeting medication and underlying disease.

These fatalities illustrate the problem with causality assessment. For example, the polykinase inhibitor ponatinib (Iclusig) indicated for the treatment of CML is associated with an increased risk for arterial thrombosis, including myocardial infarction. This risk is likely to be increased in patients with

arteriosclerosis. As myocardial infarction is a common event, number of patients exposed in well-controlled studies is crucial in the assessment of risk.

Among “suspected cases” underlying conditions might explain the events, but the narrative cannot reasonably exclude a causal relation/interaction between disease and treatment with masitinib, again emphasising the need for large numbers (of unblinded cases) to enable a proper assessment of non-common events.

Serious adverse events

Selected (by the assessor) SAEs up to w. 24

Febrile neutropenia	1/70	vs. 0/63
Gastrointestinal disorder:	6/70	vs. 1/63
Oedema	1/70	vs. 0/63
Infections	3/70	vs. 2/70 (excl. febrile neutropenia)
Skin and subcutaneous tissue	7/70	vs. 1/63

One patient in the non-severe mastocytosis population experienced a case of ALT/AST grade 4 event that led to discontinuation and recovery of the event.

Other SAEs were also reported in masitinib-treated patients but in single case. An overview of actions taken for non-fatal SAEs are presented in the below table.

Table 75 Actions taken for Non-fatal SAE - [W0-W24] - Safety population

	Masitinib (N=70)	Placebo (N=63)
Non-fatal SAE	20 (28.6%)	12 (19.0%)
- Leading to permanent discontinuation	10 (14.3%)	3 (4.8%)
- Not leading to permanent discontinuation	10 (14.3%)	9 (14.3%)
- Not leading to permanent discontinuation nor dose reduction	6 (8.6%)	8 (12.7%)
- Worst action per patient:		
o None (No temporary interruption nor dose reduction nor discontinuation)	0 (0.0%)	1 (1.6%)
o Temporary interruption (without dose reduction nor discontinuation)	6 (8.6%)	7 (11.1%)
o Dose reduction (without discontinuation)	4 (5.7%)	1 (1.6%)
o Discontinuation	10 (14.3%)	3 (4.8%)
Skin And Subcutaneous Tissue Disorders	7 (10.0%)	1 (1.6%)
- Leading to permanent discontinuation	3 (4.3%)	1 (1.6%)
- Not leading to permanent discontinuation	4 (5.7%)	-
- Not leading to permanent discontinuation nor dose reduction	4 (5.7%)	-
Gastrointestinal Disorders	6 (8.6%)	1 (1.6%)
- Leading to permanent discontinuation	2 (2.9%)	1 (1.6%)
- Not leading to permanent discontinuation	4 (5.8%)	-
- Not leading to permanent discontinuation nor dose reduction	2 (2.9%)	-

Laboratory findings

The abnormal biochemistry values which were severe (grade>=3) in the target population included:

- Low blood phosphate level (Grade 3): 1.6% in masitinib-arm vs 0% with placebo during the 24-week-period,

- High triglycerides level (Grade 3): 2.0% in masitinib-treated SS patients vs 0% with placebo.

In the mastocytosis population the same severe abnormalities described above was observed. In addition, they had the following severe abnormalities:

- High Alanine aminotransferase (Grade 3): 1% in masitinib-treated patients vs 0% with placebo during the 24-week-period,

- High Aspartate aminotransferase (Grade 3): 1% in masitinib-treated patients vs 0% with placebo.

Regarding laboratory abnormalities, which occurred frequently, most of the observed abnormal biochemistry values and abnormal blood cell counts were of mild or moderate severity (grades 1 and 2), However, these findings underline the need for regular monitoring.

Masitinib has apparently no clinically significant effect on the QTcF interval, though the validity of the study evaluating the potential effect of masitinib on ventricular repolarisation is currently questioned (for more details, please refer to section 4.3.2 *Pharmacodynamics* of this Overview AR). No clinically significant effect were observed on the measured or derived echocardiographic parameters, mainly LVEF.

With regard to vital signs, no relevant change of blood pressure or heart rate was observed in masitinib-treated SS patients.

Safety in special populations

Safety in special populations

Age

The number of elderly (>65 years) exposed to masitinib was limited in mastocytosis studies (5 patients in phase 3 including 1 patient with SS mastocytosis, and 4 patients in phase 2 including 1 patient with SS mastocytosis). The analysis of masitinib safety profile according to age was carried out in non-oncology patients. In non-oncology patients, it was observed that:

- more SAEs were reported with masitinib in elderly (29.3% with masitinib versus 18.5% with placebo) in comparison to non-elderly (24.9% with masitinib versus 19.2% with placebo) during the whole study period.
- by contrast, elderly patients seemed to have a lower frequency of severe AE than non-elderly patients: the delta was even negative in elderly (-4%, with a frequency of 29.3% with masitinib and 33.3% with placebo), while it was 7.4% (frequency of 37.2% with masitinib versus 29.8% with placebo) in non-elderly patients.

Gender

In mastocytosis patients, the safety profile of masitinib can be considered comparable between males and females. For severe AEs, delta in male patients was equivalent to that of female patients in the phase 3 study: +10.1% (frequency of 55.6% with masitinib and 45.5% with placebo) versus 12.7% (frequency of 61.5% with masitinib versus 48.8% with placebo) in females during the whole study period.

Exposure to pregnant and breast-feeding women

Neither pregnant nor breastfeeding women were enrolled and exposed to masitinib in the whole clinical development of masitinib including mastocytosis and non-oncology studies.

Exposure to renal impaired patients

No study was conducted specifically in patients with renal disorders with masitinib. Patients were considered renal impaired if baseline blood creatinine was > 120 µmol/L. One SS mastocytosis patient and 1 non-oncology patient with renal impairment were exposed to masitinib.

Exposure to hepatic impaired patients

No study was conducted specifically in patients with hepatic disorders with masitinib. Patients were considered hepatic impaired if baseline blood aspartate aminotransferase (AST) was >126 IU/L, or alanine aminotransferase (ALT) >180 IU/L or Bilirubin >25.65 µmol/L or gamma-glutamyl transferase (GGT) >150 IU/L. Four mastocytosis (SS + other) and 8 non-oncology patients were hepatic impaired and received masitinib.

Safety related to drug-drug interactions and other interactions

No case of drug-drug interaction was reported by the investigators in mastocytosis and non-oncology studies.

Discontinuation due to adverse events

In AB06006 study, the most frequent adverse events leading to dose reduction in the masitinib treatment-arm versus the placebo arm during the initial protocol period were: nausea (5 pts versus 0), diarrhoea (5 pts versus 0), vomiting (3 pts versus 0) and fatigue (2 pts versus 0). Otherwise, there were various AEs appearing in single patients that led to dose reduction.

During the 24-week period of AB06006 where most of discontinuation due to AE occurred, masitinib was discontinued due to AE by 22.9% of SS patients treated vs 7.9% with placebo (M-P: +14.9%).

Overall, patients discontinued mainly due to the following AEs: diarrhea (5.7%), nausea (2.9%), asthenia (2.9%), headache (2.9%), rash (2.9%), neutropenia (2.9%), dyspepsia (2.9%), transaminases increased (2.9%).

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

The Applicant provided an update of the safety analyses during the assessment procedure. After the initial submission (0000), the Applicant performed additional re-monitoring of the investigator sites that were involved the phase 3 and phase 2 mastocytosis studies. The AEs collected during this re-monitoring were then added to the safety database. The applicant claims that the inclusion of these additional AEs has not altered the safety profile of masitinib in mastocytosis patients nor non-oncology patients. Unfortunately, no discussion or written summary/overview is provided along with the tabular formats (hundreds of pages), and this is not an adequate format to assess the safety properly, so the new numbers of these analyses is not included.

Further, and crucially, in the GCP inspection reports, it is concluded that there are serious deficiencies affecting the quality and reliability of the safety data reported.

To support the MAA in the above claimed population, AB Science conducted one pivotal phase 3 study (AB06006, including 70 patients with smouldering or indolent systemic severe mastocytosis) and two supportive phase 2 studies (AB04010 and AB06013, including 28 patients with smouldering or indolent systemic severe mastocytosis).

Supportive safety data are available from non-oncology studies in a wide spectrum of disorders altogether about 1400 patients, but thereof 858 in ongoing blinded studies. Placebo data are available in 270 individuals (mastocytosis + non-oncology) enabling a fair assessment of tolerability.

In total 220 patients were treated in the Pivotal study AB06006; 110 patients received masitinib at 6 mg/kg/day and 110 patients received placebo. In the population of the intended indication 70 patients received masitinib and 63 patients received placebo. Concomitant symptomatic treatments were allowed.

The common AEs (≥10%) were mostly reported with masitinib in SS mastocytosis patients in the pivotal AB06006 study during the 24-week-period.

The main very common AEs (frequency>10% and where a difference (>10%) of frequency between masitinib and placebo was detected) reported in masitinib-treated patients belonged to: *Gastro-intestinal disorders*: diarrhoea (50% with masitinib), nausea (48.6%) and vomiting (21.4%); *Fluid retention*: eyelid oedema (30%) and oedema peripheral (21.4%); *Hepatotoxicity* (liver enzymes increased): ALAT increased (21.4%) and ASAT increased (18.6%); *Hematotoxicity*: haemoglobin decreased (34.3%), anaemia (22.9%); *Investigations*: blood phosphorus decreased (18.6%); *Musculo-skeletal and connective tissue disorders*: muscle spasms (28.6%); *General disorders*: weight decreased (12.9%); *Vascular disorders*: flushing (12.9%); *Skin disorders* (15.7%).

The main common AE (frequency >1%, <10% and with the difference in frequency between masitinib and placebo arm, M-P≥4%) observed in SS mastocytosis patients during the 24-week-period of the pivotal AB06006 study occurred within areas of: *Skin disorders* (urticaria, dry skin, swelling face, eczema, erythema, eye pruritus), *Investigations* (lymphocyte count decreased, blood potassium decreased, weight increased), *Gastro-intestinal disorders* (dyspepsia, gastroenteritis), *Metabolism and nutrition disorders* (hypophosphatemia), *Hepatic disorders* (cytolytic hepatitis), viral infection and chest pain.

The following events were among the most severe AEs occurring during the clinical development of masitinib: severe neutropenia, severe skin toxicities, Steven-Johnson Syndrome (SJS) and Drug-Rash with Eosinophilia and Systemic symptoms (DRESS). Other AE of special interest are diarrhoea, rash, oedemas, nausea, vomiting, asthenia and fatigue, hepatotoxicity, renal toxicity, cardiotoxicity, reproductive toxicity and risk of carcinogenicity. TKI use is associated with hypophosphatemia, and changes in bone and mineral metabolism. The reported frequency of hypophosphatemia in masitinib treated patients was 7.1% % in the phase 3 study.

In the phase 3 study, the frequency of severe neutropenia in mastocytosis patients was higher in masitinib treated patients (4 pts) compared to the placebo (1 patient), both regards of grade 3 and 4 severe neutropenia (2 pts of each grade) compared to the placebo (0 and 1 patient, respectively). The median time of occurrence of severe neutropenia was 38 days in the masitinib treatment-arm, versus 113 days in the placebo arm. Neutropenia increases the risk for infections. However, the frequency of infections was balanced: 44% in both arms (31/70 vs. 28/63) during the 24 week period. There was an imbalance in infections in the extension period (50% vs. 28%). Notably, the number of patients in this phase of the study was small, and the residual uncertainties are important.

The potential cases of SJS/DRESS reported in non-oncology studies were considered as unlikely to be SJS and DRESS. In oncology studies, there were 2 cases diagnosed as possible/probable SJS and 1 case diagnosed as possible DRESS by the experts. No cases were observed in the submitted mastocytosis studies.

The potential risk of carcinogenicity was identified from preclinical studies. The following malignancies were identified: urinary bladder and uterine tumours, and (benign) thyroid tumours. In the phase 3 studies (SS mastocytosis+other patients), there were 11 cases (9.1%) of neoplasm reported in masitinib treated patients versus 7 cases (6.4%) in placebo treated patients. Based on these numbers the Applicant states that the incidence of neoplasm was comparable with masitinib treatment (0.2) versus placebo (0.3). Any firm conclusions on the absence of a carcinogenicity risk would, however, require a great number of patients exposed to masitinib for a sufficient duration of time. In the non-oncology studies, only 301 patients have received masitinib treatment for more than 12 months in different doses and across various indications. To confirm or refute the risk of secondary cancers in masitinib treated patients, additional data would need to be collected within ongoing and possible future trials and post-marketing surveillance.

During this first 24 weeks in study AB06006, SAEs were experienced by 28.6% of masitinib-treated patients vs 19.0% with placebo. The SAEs mainly concerned *skin disorders* (masitinib: 7 pts vs placebo: 1) and *gastro-intestinal disorders* (6 pts vs 1). The most frequent SAEs were diarrhoea (3 pts masitinib, vs 0 on placebo) and urticaria (2 pts vs. 0 pts). Otherwise, no individual SAE occurred in more than 1 patient. For the non-oncology population, the SAEs detected had a comparable incidence as in the pivotal study.

Non-fatal SAEs in the pivotal study were increased in the masitinib arm (about 30 vs. 20%) and severe events showed a similar pattern (50 vs. 35%). Severe skin reactions were reported in 11/70 vs. 2/63 patients. There were also signals as regards SJS (n=2) and DRESS (n=1) in the full safety database, but the diagnoses were questioned by independent experts, but still constitutes a concern. In addition a case of severe hepatic reaction and an individual case of neutropenic fever were reported.

There was one death in the mastocytosis studies, in the placebo group of study AB06006 and 16 fatal cases reported in the non-oncology studies. The causes of deaths in the non-oncology studies were: respiratory failure/ insufficiency /arrest (6 pts), sudden death (4 pts), cardio-respiratory arrest, ALS & dyspnoea, intracranial subdural hematoma, myocardial infarct, acute massive pulmonary embolism, and transaminitis (1 patient each). In all these cases where there is doubt around the possible relationship with the study drug, one cannot definitely exclude, even if most of the patients had comorbidities or pre-existing risk factors. For 12 of 16 fatal cases it is not known whether the patient received masitinib or placebo.

The most frequent adverse events leading to dose reduction in the masitinib treatment-arm versus the placebo arm in study AB06006 during the initial protocol period were: nausea (5 pts versus 0), diarrhoea (5 pts versus 0), vomiting (3 pts versus 0) and fatigue (2 pts versus 0). Otherwise, there were various AEs in single patients that led to a dose reduction. However, further important information regarding dose reductions, i.e. when patient required such reductions, and whether patients were able to resume to the same or a different dose after interruption, has not been submitted.

In the pivotal study AB06006 masitinib was discontinued due to AEs by 16 masitinib-treated SS patients (22.9%) versus 5 patients (7.9%) with placebo during the 24-week period. The target SS mastocytosis patients discontinued masitinib mainly due to the following AEs: diarrhoea (4 pts), nausea, asthenia, headache, rash, neutropenia, dyspepsia, transaminases increased (2 pts each). The main AEs leading to permanent discontinuation belonged to the SOCs Gastrointestinal disorders, and Skin and subcutaneous disorders.

The all cause discontinuation rate in the pivotal study was about 20+% with a difference vs. placebo of 15%. The main reasons for discontinuation were gastrointestinal and skin reactions. These are also the dominating adverse reactions irrespective of grade.

Masitinib has apparently no clinically significant effect on the QTcF interval, though the validity of the study evaluating the potential effect of masitinib on ventricular repolarization cannot be established. No clinically significant effect was observed on the measured or derived echocardiographic parameters, mainly LVEF.

The safety database in the target population is limited (133), and given the uncertainties due to the nature of the side effect profile of masitinib, seen in relation to the symptomatic aims of therapy, this represents a major weakness of the submission. Considering that only 31 mastocytosis patients (and 77 non-oncology patients + healthy volunteers) received masitinib at the target dose of 6 mg/kg/day for more than one year, long-term safety data are scarce. This is of concern for the potential late-appearing adverse events, e.g. the carcinogenicity potential seen in animal studies. Therefore, the toxicity profile of masitinib, especially in the longer term, can currently not be considered as sufficiently characterised.

2.6.2. Conclusions on the clinical safety

The safety database is considered limited for a possible life long, symptomatic treatment and the risk for severe, potentially irreversible adverse reactions cannot be estimated with reasonable precision. There are obvious, common and severe adverse reactions reported in patients treated with masitinib (neutropenia, skin and hepatic toxicity). Potential risks - including cardiovascular events, such as myocardial infarctions- where causality cannot be properly assessed due to the small sample size, have been identified. A potential risk of carcinogenicity was identified from non-clinical studies.

Serious deficiencies have been identified, affecting the quality and reliability of the safety data reported in the pivotal clinical study. Major inspection findings concerning inadequate safety reporting and lack of PhV system with rigorous system for reporting and collection of AEs - create substantial uncertainties concerning the safety profile of masitinib.

2.7. Risk Management Plan

Safety concerns

Table 76 Summary of the Safety Concerns as proposed by the Applicant

Summary of safety concerns	
Important identified risks	• Severe Neutropenia • Severe skin toxicity (including a potential risk of SJS and DRESS, see important potential risks) • Liver toxicity-liver transaminases and bilirubin increase • Renal toxicity – Creatinine and proteinuria increase
Important potential risks	• SJS and DRESS • Cardiac toxicity • Reproductive toxicity including embryo-toxicity/teratogenicity • Hypophosphatemia and risk of osteoporosis • Carcinogenicity (bladder, uterine and thyroid carcinomas) • Off label use • Drug-Drug interactions (DDIs)
Missing information	• Efficacy and safety in geriatric patients • Use in patients with hepatic impairment • Use in patients with renal impairment • Less common adverse effects • Long-term efficacy and safety of masitinib at 6 mg/kg/day

Pharmacovigilance plan

Table 77 Pharmacovigilance Plan.

Study / activity **Type, title and category (1-3)**	**Objectives**	**Safety concerns addressed**	**Status** (planned, started)	**Date for submission of interim or final reports** (planned or actual)
Specific severe skin toxicity follow-up questionnaire and request of biopsy and pictures Category 3	To get additional data to characterise this safety concern, mainly regarding the frequency, severity and chronology of masitinib-induced severe skin toxicity	Severe skin toxicity	Ongoing in clinical trials with masitinib Planned for Post-Authorization phase	NA
Specific severe skin toxicity follow-up questionnaire and request of biopsy and pictures Category 3	To get additional data to characterise this safety concern, mainly regarding the frequency, severity and chronology of masitinib-induced severe skin toxicity	SJS and DRESS	Ongoing in clinical trials with masitinib Planned for Post-Authorization phase	NA
Systematic hormonal work-up in ongoing non-oncology clinical trials. Category 3	To identify any hormonal imbalance in masitinib-treated women, which could increase the risk of breast or uterine carcinoma. Also, to identify other possible reproductive toxicities not observed during clinical trials. To get additional data to confirm or infirm the potential risk of reproductive disorders in masitinib-treated patients within the ongoing clinical trials.	Reproductive toxicity including embryotoxicity and teratotoxicity	Ongoing in clinical trials with masitinib	NA
Specific follow up of the mother and the infant in case of	Specific pregnancy form to collect specific information about the	Reproductive toxicity including embryotoxicity and teratotoxicity	Ongoing in clinical trials with masitinib	

cases of pregnancy Category 3	health status of the mother and the baby during the pregnancy after the delivery birth.			
Non-interventional PASS Category 3	To assess the long term safety of masitinib and less common adverse effects	Long term safety	Planned for Post-Authorization phase	
Non-interventional PASS Category 3	To assess the long term safety of masitinib and less common adverse effects	Less common adverse effects	Planned for Post-Authorization phase	
Pharmacokinetic study: specific drug-drug interactions study testing the pharmacokinetics of masitinib with inducer of CYP3A4 Category 3	To evaluate pharmacokinetic interaction between rifampicine, an inducer of CYP 3A4, and masitinib	Important potential risk Drug Drug interations with inducers of CYP3A4	Planned for Post-Authorization phase	To be advised

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

Risk minimisation measures

The applicant does not propose any additional risk minimisation measures for Masipro.

Conclusion

The CHMP and PRAC, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

N/A

2.9. New Active Substance

The applicant compared the structure of masitinib mesylate with active substances contained in authorised medicinal products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

The CHMP, based on the available data, considers masitinib mesylate to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union. However, in light of the negative recommendation, new active substance status is not applicable at this stage.

2.10. Product information

In light of the negative recommendation a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet does not yet meet the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.* In addition, also in light of the negative recommendation a satisfactory package leaflet cannot be agreed at this stage.

2.10.2. Additional monitoring

Not applicable.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Indolent systemic mastocytosis (including smouldering systemic mastocytosis) is characterised by excess of mast cells or abnormal mast cells that leads to a wide variety of signs and symptoms, including pruritus, flushing, syncope, hypotensive shock, dizziness, abdominal pain, nausea, vomiting, diarrhoea, fatigue, memory loss, depression, tachycardia, palpitations, breathing difficulties, fractures/osteoporosis, and pain in the muscles, joints, and bones. Urticaria pigmentosa is the most common sign of mastocytosis, in both cutaneous and systemic forms of the disease.

3.1.2. Available therapies and unmet medical need

There are no medicinal products approved in the EU specific for patients with smouldering or indolent systemic mastocytosis. However, symptomatic treatment options of mastocytosis include H1/H2 antihistamines, osteoclast inhibitors, anti-leukotrienes, or proton pump inhibitors. The use of interferon-alpha, thalidomide, cladribine and imatinib has been reported but is limited in indolent mastocytosis.

Smouldering or indolent systemic mastocytosis is a life-long condition, patients have a normal life-expectancy and the symptoms are considered as handicaps. Most often patients experience fluctuations in their symptoms. An unmet medical need is identified in patients who do not adequately respond to existing symptomatic treatments.

3.1.3. Main clinical studies

The MAA for masitinib is based on one pivotal study, AB06006, an international, randomized (1:1), double-blind, placebo-controlled phase 3 study including 132 patients (ITT) with smouldering or indolent systemic mastocytosis with symptoms considered as severe handicaps. The main efficacy analyses were performed on the modified intent-to-treat (mITT) population which comprised 129 patients.

In this study, the primary efficacy endpoint was cumulative response resulting from five visits from Week 8 to Week 24 on one or more of 4 predefined handicaps registered at baseline (i.e. pruritus score ≥9, flushes per week ≥8, Hamilton rating scale for depression (HAMD-17) score ≥ 19, Fatigue Impact Scale total score (asthenia) ≥ 75). A response was defined as a 75% reduction in these four handicap scores (4H75%). Cumulative response was calculated as the number of actual responses between weeks 8 and 24, divided by the total number of possible responses over the same treatment period (i.e. with 5 scheduled visits each patient had a maximum of 5 to 20 possible responses depending on the number of handicaps at baseline). Secondary and exploratory endpoints included e.g. cumulative response on individual handicaps, changes in tryptase level, body surface area covered by urticaria pigmentosa, disappearance of Darier's sign and QoL.

3.2. Favourable effects

In the main study AB06006, masitinib was associated with a statistically significant difference over placebo for the cumulative response rate 4H75% from W8 to W24, with a response of 18.7 vs 7.4% (p-value 0.0076).
Statistically significant differences between masitinib and placebo were observed for the cumulative response rates 3H75% (depression, pruritus and flush), 2H75% (pruritus and flush) and for analysis of pruritus alone from W8 to W24, with observed p-value in the range 0.01-0.03 with an overall absolute difference in response about 15% irrespective of endpoint. Cumulative response rates (4H75%, 3H75% and pruritus) from W8 to W96 also showed statistically significant difference between masitinib and placebo.

There was a reduction of the tryptase level from baseline to last visit (W0-W24) in the masitinib arm, while there was a slight increase in the placebo arm; mean relative change was -18% in the masitinib treatment-arm versus +2.2% in the placebo arm (p=0.0001). Response rate in tryptase level, defined as a reduction of 25% from baseline at last visit (W0-W24), was 35% in the masitinib treatment arm versus 11.9% in the placebo arm (p=0.0172).

Body surface area covered by urticaria pigmentosa was decreased in the masitinib arm (-12.3%), while it increased in the placebo arm (+15.9%) from baseline to Week 24 (p=0.0210).

Disappearance of Darier's sign from baseline to Week 24 was 18.9% in the masitinib treatment arm versus 2.7% in the placebo arm (p=0.0187, odds ratio=6.58).

3.3. Uncertainties and limitations about favourable effects

Due to the composite nature of the primary endpoint, it is difficult to interpret the clinical relevance of the magnitude of the effect of the improvement of 11% compared to placebo. Considering that patients who contributed with a response may have responded on just one of the four handicaps included in the primary endpoint and few patients had more than one or two handicaps relevant for the primary endpoint, the generalisability and clinical relevance for a patient population which often presents with a number of handicaps is also unclear. In addition it remains unclear if these handicaps are completely independent of each other. Interaction and dependencies of various handicaps have not been explored in any way. Further, the endpoint hampers the distinction between safety and efficacy aspects as handicaps contribute to both measures, yet in different degrees. Improvements in handicaps not considered severe at baseline are not included in the effect measure, but can nonetheless lead to indirect changes in other handicaps. Notably, no positive impact of masitinib on quality of life was documented.

A number of changes to the protocol have been implemented during the conduct of the study in order to capture patients with more severe handicaps, increase in the cut-off for response, introduce cumulative response as primary endpoint, exclude previously included patients and change statistical methods, having consequences on the blinding of the study, with impact on the randomisation and data integrity. Following the conclusion of the GCP inspection of the pivotal clinical study AB0600 the CHMP considered that the accumulation of critical and major inspection findings, affecting all aspects of the trial, seriously question the validity of the trial data. Critical and major findings include the following: blinding was compromised during the trial; serious deficiencies affect the reliability of components of the inclusion criteria, of the disease severity criterion and of the primary and secondary evaluation criteria (HAMD-17 and FIS); GCP deficiencies in the monitoring process and in the overview of the trial by the sponsor; and violation of inclusion criteria.

Although exploratory analyses performed on the 75% reduction in the score of the detailed items of FIS, HAMD-17, and AFIRMM score indicated that masitinib generated a benefit on a greater number of quality of life related items compared with placebo, there was overall no improvement in the quality of life measured by QLQ-C30 global score, AFIRMM questionnaire, and OPA score. As revealed by the GCP inspections, an incorrect version of the AFIRMM V2 questionnaire was used during the first months of the study, and occasionally thereafter, however the data obtained were pooled with the other data, which hampered their interpretation.

3.4. Unfavourable effects

Several very common AEs (≥10%) were reported with masitinib in patients in the pivotal AB06006 study during the 24-week-period; for a number of events the frequency was at least 10% higher in the masitinib group vs placebo including GI-events, oedema of different locations, anaemia, rash, flushing*, AST/ALT increase and weight decrease (also an efficacy measure).

The main common AEs (frequency >1%, <10% and with the difference in frequency between masitinib and placebo arm ≥4%) observed during the 24-week-period of the pivotal AB06006 study were within the areas of: *Skin disorders* (urticaria, dry skin, swelling face, eczema, erythema, eye pruritus), *Investigations* (lymphocyte count decreased, blood potassium decreased, weight increased), *Gastro-intestinal disorders* (dyspepsia, gastroenteritis), *Metabolism and nutrition disorders* (hypophosphatemia) and *Hepatic disorders* (cytolytic hepatitis), viral infections and chest pain.

During the first 24 weeks in study AB06006, SAEs were experienced by 28.6% of masitinib-treated patients vs 19.0% with placebo and mainly concerned *Skin disorders* (masitinib: 7 pts vs placebo: 1) and *Gastro-intestinal disorders* (6 pts vs 1). The most frequent SAE being diarrhoea (3 pts masitinib, vs 0 on placebo) and urticaria (2 pts vs. 0 pts). Severe AEs (AEs of grade 3 and 4) were reported in 50% (35/70) in the masitinib arm vs. 35% (22/63) in the placebo arm, these events lead to permanent discontinuation in 14/35 (masitinib) vs. 3/22 (placebo). The frequencies of severe AE were: diarrhoea 11% vs. 2%, asthenia 6% vs. 2%* and pruritus 4% vs. 2% (also an efficacy measure), and also rash 3% vs 0%, pyrexia 3% vs 0% and neutropenia 4% vs. 2% in the masitinib and placebo arm, respectively.

The following events occurred in more than one patient and at a higher frequency than placebo: neutropenia [(3/70 (severe systemic) and 5/110 (mastocytosis all) vs. 1/110], diarrhoea (8/70 and 10/110 vs. 1/110), asthenia (4/70 and 5/110 vs. 2/110), pyrexia (2/70 and 4/110 vs. 0/110) and rash (4/70 vs. 0/63). Skin reactions were 11/70 vs. 2/63 and 4/11 vs. 1/2, masitinib and placebo, respectively. Gastrointestinal events were 12/79 vs. 3/63 and 3/12 vs. 1/3, masitinib and placebo, respectively. Oedema (37/70 vs. 8/63) occurs early (median 23 vs. 39 days), is mainly of mild severity, but led to temporary interruption in 3 individuals and discontinuation in one individual.

If asthenia and fatigue is combined the incidences were similar (25/70 vs. 20/63) and of similar severity. Asthenia, however, was more commonly reported in the masitinib arm (26% vs 11%) with the reverse for fatigue (10% vs. 18%).

In the phase 3 study, the frequency of severe neutropenia in mastocytosis patients was higher in masitinib treated patients (4 pts) compared to placebo (1 patient) The frequency of infections was, however, balanced between the masitinib and placebo arms (31/70 vs. 28/63) during the 24 week period. There was, however, one case of neutropenic fever in the masitinib arm**.**

No potential cases of Steven-Johnson Syndrome (SJS) and Drug-Rash with Eosinophilia and Systemic symptoms (DRESS) were reported in the mastocytosis studies. In the oncology studies, there were 2 cases diagnosed as possible/probable SJS and 1 case diagnosed as possible DRESS.

In the completed phase 3 studies (including mastocytosis patients and patients with other diseases), there were 11 cases (9.1%) of neoplasm reported in masitinib treated patients versus 7 cases (6.4%) in placebo treated patients. Based on these numbers the applicant states that the incidence of neoplasms was comparable with masitinib treatment (0.2) versus placebo (0.3).

In the pivotal study AB06006, no deaths were reported in masitinib-treated population, but one death (subdural intracerebral hematoma) was reported in the placebo arm. In addition, there were 15 fatal cases reported in the non-oncology studies, (9 in ALS, 2 in Alzheimer's disease, 2 in multiple sclerosis, 1 in rheumatoid arthritis, 1 in severe asthma).

The most frequent adverse events leading to dose reduction in the masitinib treatment-arm during the initial treatment period were: nausea (5 pts), diarrhoea (5 pts), vomiting (3 pts) and fatigue (2 pts).

In the pivotal study AB06006 (during the initial 24-week period) masitinib was discontinued due to AEs by 16 patients (22.9%); 5 patients (7.9%) discontinued in the placebo arm, mainly due to GI events and skin toxicities.

3.5. Uncertainties and limitations about unfavourable effects

The general quality of the conduct and monitoring of the trial is questioned, as the GCP inspection of the sponsor site and 2 study sites revealed very serious issues including an unreliable assessment and collection of safety data and potential premature unblinding of the pivotal study. These critical issues hamper the safety assessment, causing a considerable residual uncertainty.

Among the 16 fatal cases reported in the non-oncology studies, the role of masitinib was suspected in 3 cases (2 cases of sudden death, one of myocardial infarction) and 1 case (massive pulmonary embolism and deep venous thrombosis of right lower limb) which was not assessable. In all of these cases one cannot definitely exclude the role of masitinib, even though most of the patients had comorbidities or pre-existing risk factors. However, only for 4 of the 16 fatal cases it is known which treatment was given; for the 12 other deaths, it is not known from the narratives if the patient received masitinib or placebo.

The potential risk of carcinogenicity was identified from preclinical studies. From these studies, the following malignancies were identified: urinary bladder and uterine tumours, and (benign) thyroid tumours. According to the applicant, there have been no suspected (or not assessable) cases of bladder cancer reported. Detecting or stating the absence of a carcinogenicity risk would, however, require a great number of patients exposed to masitinib for a sufficient length of time. Carcinogenicity is considered as a potential important risk of masitinib and should be further investigated.

The reported frequency of hypophosphatemia in masitinib treated patients was 7.1% in the phase 3 study. In light of the intended chronic use of masitinib in this mastocytosis population with a normal life expectancy, osteoporosis is a regarded an important concern and uncertainty, as TKI use is associated with hypophosphatemia and changes in bone and mineral metabolism.

Some of the known class effects of TKIs have been observed in the pivotal study, such as neutropenia, gastrointestinal toxicities, skin toxicities, and hepatotoxicity. The true frequency, severity and impact of these at short or long term are based on too few patients and are thus currently not known. Other TKI class effects (e.g. hypothyroidism or cardiotoxicity) have not been observed, but can still be considered an uncertainty. Masitinib has apparently no clinically significant effect on the QTcF interval, though the validity of the study evaluating the potential effect of masitinib on ventricular repolarisation is currently questioned.

The knowledge of concentration-response is very limited. Thus the clinical consequences of unexpected increases/decreases in exposure, due to potential DDI with concomitant medications, cannot be foreseen as the major part (*ca* 70%) of the elimination pathways is unknown.

Masitinib was not studied in mastocytosis patients with renal, hepatic or cardiac impairment. The clinical significance of renal, reproductive organ- and cardiovascular toxicities observed in animal studies is unclear.

Experience from long-term exposure is very limited. Therefore, the toxicity profile can currently not be considered as sufficiently characterized, especially with regard to (potential) late-appearing effects. This safety database for the relevant indication is therefore still considered as limited and the risk for severe, potentially irreversible adverse reactions, including deaths, cannot be estimated with

reasonable precision. Tumour findings were observed in both mice and rats. At current, the relevance to human safety is not sufficiently well understood and a carcinogenic risk cannot be excluded.

3.6. Effects Table

Table 78 Effects Table for Masipro

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Duration of study	Intended 24 weeks + possible extension period up to 96 weeks				70% of the patients in the masitinib arm completed 24 weeks; 87% completed 24 weeks in the placebo arm. 55% of the enrolled patients continued into the extension phase. 25 patients completed 96 weeks of masitinib treatment.	AB06006

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
		%	Masitinib	Placebo		
Primary: **4H75%** (pruritus, flush, depression, fatigue)	Cumulative response rate (75% reduction in handicap score) as measured per visit and handicap at baseline (5 visits in the period W8-W24).		18.7%	7.4%	Changes to the protocol performed during the conduct of the study might have compromised the trial integrity: changes in the inclusion criteria, increase in the cut-off for response, introduction of cumulative response as primary endpoint, and change in statistical method. Cannot exclude that these changes were data driven. The GCP inspectors found that blinding was compromised. GCP inspectors found irregularities in the handling of HAMD-17 (depression) and FIS (asthenia) questionnaires that leaves the rating of depression and asthenia unreliable. Changes in background symptomatic medications were frequently undertaken. Use of new concomitant medications was not systematically reported to the sponsor. The consequences as to outcome measures are hard to foresee as changes per se might have at least “placebo effects”.	
Secondary: **3H75%** (pruritus, flush, depression)			24.7%	9.8%		
2H75% (pruritus, flush)			27.2%	10.7%		
Pruritus			22.0%	7.3%		
Up to 2 years of treatment:	As above but last visit at W96.					
4H75% (pruritus, flush, depression, fatigue)			16.8%	6.8%		
3H75% (pruritus, flush, depression)			21.8%	8.3%		
Pruritus			19.2	6.2%		

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Unfavourable Effects*						
Pivotal study AB06006	Safety parameters in AB06006 were compared for the 24-week, double blind phase.		Masitinib N=70	Placebo N=63		
Discontinued			22.9%	7.9%	discontinuation of 5 patients as mistakenly withdrawn in the masitinib arm (protocol deviations)	
AEs	Febrile neutropenia 1/70vs. 0/63 Gastrointestinal 6/70 vs. 1/63 Oedema 1/70 vs. 0/63 Infection 3/70vs. 2/70 (excl. febrile neutropenia) Skin and subcutaneous tissue 7/70vs. 1/63		100%	100%	Most common (>25%): diarrhoea, nausea, haemoglobin decreased, eyelid oedema, muscle spasms, blood glucose increased, asthenia	
Suspected/ not assessable AEs			97.1%	87.3%	New analyses with regard to treatment-relation have been performed and lots of tables given, but no discussion is provided along with the tabular formats.	
SAEs			29%	20%	The SAEs are mainly skin and gastro-intestinal disorders	
Suspected/ not assessable SAEs			21.4%	9.5%	New analyses are performed; the numbers of suspected/not assessable SAEs are given here.	

* The GCP inspection report of the sponsor site and 2 study sites describes very serious issues including an unreliable assessment and collection of safety data and potential premature unblinding of the pivotal study, thus resulting in a large uncertainty.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Due to substantial changes of the protocol during study conduct, the trial integrity is questioned. It cannot be excluded that the changes were data driven. The critical and major findings reported in the GCP inspection regarding the conduct of the study further questions the reliability of the outcome.

A small, but statistically significant treatment effect of masitinib has been claimed with the methods applied after large amendments to the study protocol, i.e. an improvement of 11% compared to placebo. The clinical relevance is difficult to assess due to the composite nature of this endpoint and the methodological deficiencies.

The rating of depression and asthenia based on the HAMD-17 and FIS questionnaires are considered unreliable. This seriously hampers the reliability of the results of both primary and secondary endpoints which incorporates these handicaps. Also, it cannot be excluded that use of concomitant symptomatic treatments have influenced and biased the results of the study.

Common and severe adverse reactions were reported in patients treated with masitinib such as neutropenia, skin and hepatic toxicity and oedema, which are unacceptable for long term use in the context of a condition where symptomatic response at best has been documented. To this may be added potential risks, including cardiovascular events, such as myocardial infarctions, where causality cannot be properly assessed due to the small sample size. The potential risk of carcinogenicity was identified from non-clinical studies.

Further, due to GCP inspection findings, there is a general uncertainty concerning the reliability of data and prevent any valid conclusions on efficacy and safety.

3.7.2. Balance of benefits and risks

The target population is patients with systemic mastocytosis with symptoms not controlled by available therapies. This means that masitinib has to be used as add-on to a wide variety of compounds encompassing different classes of drug. As the drug interaction potential is largely unknown this constitutes a major obstacle for its clinical utility, especially with regard to other compounds putative effects on masitinib exposure, but also on the effects of masitinib on the exposure of other drugs.

A small, statistically significant treatment effect (cumulative response rate 4H75% from W8 to W24 was 18.7 vs 7.4% in the masitinib arm vs placebo arm, respectively; p-value 0.0076) has been shown with the methods applied after multiple major amendments to the study protocol. There was, however, overall no improvement in the quality of life measured by QLQ-C30 global score, AFIRMM questionnaire and OPA score. Considering that patients who contributed with a response may have responded on just one of the four handicaps included in the primary endpoint and few patients had more than one or two handicaps relevant for the primary endpoint, the generalizability and clinical relevance for a patient population which often presents with a number of handicaps is questioned.

The consequences of applying more restricted inclusion criteria in retrospect and excluding a substantial number of already randomized patients in the statistical analyses, and subsequent inferences on efficacy, are not possible to assess. Furthermore, increasing the baseline criteria required to contribute as response to the revised primary endpoint (4H) from 50% to 75% during the study is considered problematic. Applying the original 50% criterion resulted in statistically non-significant effects for all outcome measures, underlining the critical nature of this change to the protocol. Similarly, the analysis representing the originally proposed primary analysis in the less severe study population failed to support a significant difference. In light of the findings outlined in the integrated GCP inspection report, it is also not possible to reasonably ascertain that the integrity of blinding and trial conduct was maintained throughout this extensive sequence of changes.

The safety database is considered limited for a possible life long, symptomatic treatment and the risk for severe, potentially irreversible adverse reactions, including deaths, cannot be estimated with reasonable precision.

The GCP inspection conducted at the request of the CHMP concluded that there are serious deficiencies affecting the quality and reliability of the safety data reported. The safety reporting was inadequate and in general pharmacovigilance handling (reporting and collection of AEs) in the pivotal trial was poor. The applicant had not established a pharmacovigilance system with rigorous system for adverse events reporting and signal detection.

3.7.3. Additional considerations on the benefit-risk balance

N/A

3.8. Conclusions

In light of all the above the overall Benefit/Risk of Masipro for the treatment of adult patients with smouldering or indolent systemic mastocytosis with severe mediator release-associated symptoms unresponsive to optimal symptomatic treatments - is considered negative.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy for Masipro in the treatment of adult patients with smouldering or indolent systemic severe mastocytosis with severe mediator release-associated symptoms unresponsive to optimal symptomatic treatments, the CHMP considers by consensus that the efficacy and safety of the above mentioned medicinal product is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the Marketing Authorisation for the above mentioned medicinal product. The CHMP considers that:

Grounds for refusal

- **Study conduct and GCP inspection**

  The GCP inspection of the pivotal study AB06006, reports an accumulation of critical and major inspection findings, affecting all aspects of the trial, which seriously impact the reliability of the trial data. A pharmacovigilance system had not been established and safety reporting was

inadequate. These issues cannot be resolved by performing re-monitoring and retrospective analyses at the study sites.

Major changes were implemented to the study protocol during study performance, including changes in the inclusion criteria late in the study, the exclusion of previously included patients, changes in the definition of response and of the primary endpoint, and changes of the statistical method. It cannot be ascertained that these comprehensive amendments were made in ignorance of the accumulating trial data. The magnitude of bias and inflation of the false-positive error rate that has been introduced cannot be quantified. The estimated effects cannot be considered reliable for assessment.

- **Benefit / risk**

The extent of symptomatic benefit reported in the single pivotal study of Masipro cannot be considered to outweigh the uncertainties that are introduced by the fundamental concerns about the conduct of the single pivotal trial, and the observed toxicity profile.

Therefore, the overall Benefit /Risk of Masipro in the treatment of adult patients with smouldering or indolent systemic severe mastocytosis with severe mediator release-associated symptoms unresponsive to optimal symptomatic treatments, is negative.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, risk management plan and follow-up measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

5. Re-examination of the CHMP opinion of 18 May 2017

Following the CHMP conclusion that Masipro was not approvable (see section 4), the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

Detailed grounds for re-examination submitted by the applicant

Following a request from the applicant at the time of the re-examination, the CHMP convened a Scientific Advisory Group (SAG) inviting the experts to provide their views on the CHMP grounds for refusal, taking into account the applicant's response.

The applicant presented in writing and at an oral explanation the following grounds for re-examination:

Ground 1: Study conduct and GCP inspection

Related grounds for refusal adopted by the CHMP in the initial opinion:

a) The GCP inspection of the pivotal study AB06006, reports an accumulation of critical and major inspection findings, affecting all aspects of the trial, which seriously impact the reliability of the trial data. A pharmacovigilance system had not been established and safety reporting was inadequate. These issues cannot be resolved by performing re-monitoring and retrospective analyses at the study sites.

b) Major changes were implemented to the study protocol during study performance, including changes in the inclusion criteria late in the study, the exclusion of previously included patients, changes in the definition of response and of the primary endpoint, and changes of the

statistical method. It cannot be ascertained that these comprehensive amendments were made in ignorance of the accumulating trial data. The magnitude of bias and inflation of the false-positive error rate that has been introduced cannot be quantified. The estimated effects cannot be considered reliable for assessment.

Summary of the Applicant's grounds for re-examination:

GCP deficiencies

The Applicant acknowledged the GCP findings and presented in the grounds for re-examination the implemented corrective and preventive action.

The Applicant claims that all major and critical findings observed during the EMA GCP inspection have been corrected and prevented, and that the data from the mastocytosis study have been adequately reassessed retrospectively making the data now interpretable and reliable and that therefore the GCP findings should not be an obstacle in assessing the B/R of masitinib. The Applicant raises several points to support this claim: The root cause analysis of the inspection findings was performed and led to the implementation of a new monitoring system in mid-2015, re-monitoring of the data up until mid-2015, correction of the deficiencies observed in the pharmacovigilance (PhV) system following the EMA inspection, implementation of an upgraded Quality Management System (QMS), and independent external audits to confirm that the corrective and preventive actions have been made. The applicant concluded that as a consequence of these corrective actions, the updated safety data supplied can presently be considered as reliable.

Uncertainties regarding study conduct

The amendment leading to protocol version 6 contained three main modifications.

- Restriction of inclusion criteria to limit masitinib to the most severe patients
- Increase in the cut-off point for response from 50% to 75% improvement of the baseline handicap
- Change in statistical methodology from a Pearson Chi-square test based on patient response at week 24 to a GEE model overall response based on patient x handicap.

The applicant claims that the necessity of the first two modifications was duly justified and that these were completely independent from the conduct of AB06006 study. As a matter of fact, the EMA scientific advice from 2011 validated the restriction of the claim to the more severe patient population and the increase in threshold of response from 50% to 75% improvement of the baseline handicap. The Applicant argues that the necessity of the two modifications was justified and that the changes were completely independent from the conduct of study AB06006. The changes were triggered by emerging safety findings required to restrict the claim to the most severe patients and by the need to increase the response threshold from 50% to 75% in order to increase the clinical relevance of the study, based on feedback received from the peer review of the manuscript of phase 2 study AB06013.
The applicant also claims that the change in statistical methodology from a Pearson Chi-square test based on patient response at week 24 with 200 patients to a GEE model overall response based on patient*handicap with 129 patients; not formally validated through scientific advice was also necessary:

- to take into account small sample size following restriction in inclusion criteria, in application of EMA guideline on clinical trials in small populations (CHMP/EWP/83561/2005) and,
- to have a more clinically meaningful endpoint that integrates the handicap burden over the treatment period rather than at a single time point since indolent systemic mastocytosis is a fluctuating disease.

When assessing the impact of the change in statistical methodology that was not validated through scientific advice, the original analysis remained positive, considering the treatment effect observed at week 24 and the original sample size. Therefore, the applicant concluded that the protocol changes could be in principle methodologically acceptable.

CHMP assessment

GCP deficiencies

Following the EMA inspection during the initial MAA for Masipro, a large number of critical findings was recorded, in particular at the Sponsor site, and it was concluded that the identified deficiencies directly affected the quality and reliability of the efficacy and safety data and that the trial was conducted at an insufficient level of compliance with GCP.

The organisational changes in the conduct of clinical trials at the sponsor's site are acknowledged and these changes confirm in a way the severe deficiencies that were present at the time of the inspection, i.e. by the end of the pivotal clinical study supporting this application. However, as the changes were implemented only at the end of the pivotal study, this study did not benefit from most of these changes. Although the remonitoring may have improved the data, it is considered that in view of the large number and variety of critical deficiencies recorded during the inspection, and the fact that the whole trial was affected in a systematic way as a result of the deficiencies being mainly related to the sponsor, there still remain substantial doubts about the data quality which cannot fully be resolved by performing re-monitoring and retrospective analyses at the study sites.

For example a deficiency that cannot be resolved by re-monitoring is that the investigators were found not to be trained in the use of the Hamilton depression rating scale (HAMD-17), which was a key inclusion criterion as well as part of the primary efficacy evaluation. At one site, the patients themselves and not the investigator or a member of staff completed the scale, and at 3 other sites at least the scale was completed by the investigator with direct contribution from patients rather than having the form completed by a trained practitioner after a structured interview with the patient (critical finding 7 from the inspection). Importantly, the forms were found not to have the name and/or signature of the staff members who interviewed and rated the subjects, and it was therefore not possible to ensure that person was qualified. Part of the forms, questionnaires and scales on which the inclusion criteria, study population and efficacy criteria were based were not available at one inspected site for 3/8 patients enrolled (38%). It is therefore impossible to check the data described in the study report for these patients. Therefore, the quality of this critical part of the data from the pivotal study, which comprises a key inclusion criterion as well as part of the primary efficacy evaluation, cannot be considered reliable.

Also with regard to the quality of the safety data, there still remain important uncertainties about the reliability of the data. With re-monitoring of data, 5 SAEs were added to the mastocytosis study – one in the masitinib group and 4 in the placebo group; 7 new SAEs were added after the medical assessment, of them 5 in masitinib group. The medical assessment of AEs led to the addition of 1 new SAE, 1 in a masitinib-treated patient in Alzheimer's disease. These additional observations need assessment and discussion. Unfortunately, the Applicant identified all these inaccuracies as not important, claiming the results of the AB06006 study are reliable. As the pharmacovigilance system at the time of conduct of the study had many severe flaws, e.g. as a result of critical deficiencies in data management and in the review of the cases and due to the use of a non-validated, non-GCP compliant, AE database (Excel sheet), it cannot be guaranteed that all AEs that occurred during the study were adequately captured and reviewed. Therefore, re-monitoring of the safety data by retrospectively assessing the medical dossiers of the patients from patients treated as early as 2009, cannot lead to a reliable safety database meeting the standards required for a pivotal study that supports a marketing authorisation, especially in the current context of one (small-sized) pivotal study.

Study conduct

Major changes to the study protocol that were implemented during study performance, including changes in the inclusion criteria late in the study, the exclusion of randomised patients from the ITT analysis, changes in the definition of response and of the primary endpoint, and changes of the statistical analysis method.

The amendments leading to protocol version 6 contained two main modifications: restriction of inclusion criteria to limit masitinib to the most severe patients and the increase in the cut-off point for response from 50% to 75% improvement of the baseline handicap.

The CHMP considered that the applied changes would not have been unacceptable per se, if data for efficacy and safety would have been compelling and robust. The fact that the observed treatment effect is highly dependent on the applied changes in the inclusion/exclusion of patients and the response criteria, serves to illustrate the lack of robustness of the efficacy result.

Regarding the change of the inclusion criteria to include patients with more severe mastocytosis in the ITT analysis, it has been reported by the Applicant that when the earlier inclusion criteria of protocol v5.0 were applied, the difference in response rate at week 24 is between the masitinib or placebo group was of a smaller magnitude (response rate 36.8% for masitinib vs. 28.4% for placebo). This indicates that the treatment effect is not robust and further undermines the external validity of the main result, as slight differences among the patients treated seem to affect the observed treatment effect. As a result of this change, 85 randomised patients were retrospectively excluded from the analysis. The lack of sufficient information about these patients has an impact on the reliability of the study results. The majority of patients were enrolled in the same country, thus raising concerns of possible selection bias that cannot be resolved. Consequently, the randomisation of other participants is considered unreliable as a result of the fact that confounders could now be unequally distributed between treatment arms, increasing the possibility of obtaining a confounded result. In this respect, the 11% difference in treatment effect could potentially be obtained by chance.

Additional changes in statistical analysis (including the change in statistical methodology from a Pearson Chi-square test based on patient response at week 24 with 200 patients to a GEE model overall response based on patient * handicap with 129 patients) were performed. Although performing statistical modelling is preferred over the Pearson Chi-square test in terms of power of the study, it is the different statistical manipulations on the same dataset itself, combined with changing of the

severity of the target population and the primary endpoint, which questions the reliability of the results.

Regarding the change of increasing the response threshold from 50% to 75%, it cannot be verified whether or not the changes were data-driven (in view of potential unblinding of the study). Even if the changes were not data-driven, it is still problematic considering that, when the original analysis with the response threshold of 50% is applied, no relevant treatment effect was observed and the results were non-significant (response rate patient*4 handicaps for masitinib 29.2% vs. 22.2% for placebo, p=0.2854). This confirms the earlier conclusion on lack of robustness of the primary efficacy result in the single pivotal study submitted. Although many attempts have been made to correct data by either re-monitoring or exclusion of wrong data, the lack of robustness persists. This is considered a key issue in the context of a single pivotal study, especially in the light of major concerns about data quality (see above), about the integrity of the trial upon the multiple and extensive protocol amendments (as cautioned for through scientific advice) and serious doubts about the benefit/risk of the product (refer to assessment of response to ground for refusal 2, below).

In conclusion, although it is agreed with the Applicant that the re-monitoring efforts may have improved the reliability of the data in part, it is considered that there remain considerable residual uncertainties regarding the data quality, which is not acceptable in the current context of a single, small-sized, pivotal study. The critical changes made to the study protocol (including changes in the inclusion criteria late in the study, the exclusion of previously included patients, changes in the definition of response and of the primary endpoint, and changes of the statistical method) are not considered unacceptable per se. However, the results of the many sensitivity analyses performed in relation to the changes made in inclusion criteria and the threshold for response, clearly illustrate that the efficacy results are not robust and that the external validity is questionable. In this context, the unknown effect on the results of the loss of randomisation upon exclusion of previously included patients is an important additional aspect, which also hampers interpretation of the results.

Considering all the above, the remaining uncertainties on the quality of the data from the single pivotal study, this first ground for refusal is not considered solved despite the additional arguments presented by the applicant in this re-examination procedure.

Ground 2: Benefit/risk

Related grounds for refusal adopted by the CHMP in the initial opinion:

a) The extent of symptomatic benefit reported in the single pivotal study of Masipro cannot be considered to outweigh the uncertainties that are introduced by the fundamental concerns about the conduct of the single pivotal trial.

b) The extent of symptomatic benefit reported in the single pivotal study of Masipro cannot be considered to outweigh the uncertainties that are introduced by the observed toxicity profile.

c) Therefore, the overall Benefit /Risk of Masipro in the treatment of adult patients with smouldering or indolent systemic severe mastocytosis with severe mediator release- associated symptoms unresponsive to optimal symptomatic treatments, is negative.

Summary of the Applicant's grounds for re-examination:

The claimed indication accounts for an orphan disease as indolent systemic mastocytosis (including smouldering systemic mastocytosis) has an estimated European prevalence of 3.8 people per 100,000 with a high unmet medical need. Of the adult patients that are diagnosed with systemic mastocytosis, 33% suffer from severe symptoms. Consequently, the targeted population in the claim is around 5,000 adult patients in the EU. Patients in the targeted population suffer from persistent symptoms due to permanent mast cell activation, which can lead to irreversible organ damages and have profound impact on patient condition and day-to day functioning [Paul, 2010]. In addition, patients are at a higher risk of occasional death (depression-related suicide, fatal anaphylaxis, progression to more aggressive disease forms).

Masitinib represents the first endeavour to bring a new treatment for those patients affected by this rare condition and there are no generally agreed response criteria for indolent systemic mastocytosis. In that respect, the fact that masitinib was assessed as a purely symptomatic drug despite evidence of activity on objective markers of mast-cell activation, and the expectation for more pharmacological data based on models that are not relevant to indolent systemic mastocytosis, reveal that the nature and causality of the disease may have been well understood.

The Applicant claims that the primary analysis was appropriate in the context of indolent systemic mastocytosis, and 11% difference in cumulative response for the primary endpoint is clinically relevant. The primary analysis was supported by the secondary analysis, in particular cumulative response on Pruritus and Flush (2H75%), and endpoint that was validated through scientific advice to demonstrate the clinical benefit in the targeted indication, and also which is not comprised by the GCP finding on Hamilton scale and fatigue impact scale and the endpoint of mast cell activation, serum tryptase level, darrier sign, and urticaria pigmentosa. In addition, the applicant claimed that most sensitivity analyses requested by the CHMP confirmed the clinical benefit associated with masitinib; odds ratio being rather similar to what is shown in the primary and secondary analyses and is stable in the sensitivity analyses.

The safety database is comprised of 266 patients in the targeted indication, including 220 patients from controlled studies at the therapeutic dose, and 1976 patients in all non-oncology indications, including 1788 from controlled studies at any dose. This is a large safety database considering that the prevalence of the disease is around 5000 patients. The size of this safety database was validated by the CHMP through scientific advice. The identified risks of severe neutropenia, severe skin toxicity, rash, diarrhoea, vomiting, and oedema are well manageable by dose reduction or discontinuation. The potential risk of cardiotoxicity and carcinogenicity is sufficiently characterized and has not been identified in humans according to date. Therefore the applicant concluded that the safety profile appears acceptable in the context of the severity of the disease and knowing that the drug will be used by haematologists who are trained to use TKI. The Applicant also concluded that the safety of masitinib "is acceptable in the claimed indication, is sufficiently characterized and important identified and potential risks have been minimized through risk management activities".

CHMP assessment

The MAA for masitinib is based on one pivotal study, AB06006, an international, randomized (1:1), double-blind, placebo-controlled phase 3 study including 132 patients (ITT) with smouldering or indolent systemic mastocytosis with symptoms considered as severe handicaps.

Efficacy

In this study, the primary efficacy endpoint was cumulative response resulting from five visits from Week 8 to Week 24 on one or more of 4 predefined handicaps registered at baseline (i.e. pruritus score ≥9, flushes per week ≥8, Hamilton rating scale for depression (HAMD-17) score ≥ 19, Fatigue Impact Scale total score (asthenia) ≥ 75). A response was defined as a 75% reduction in either one of these four handicap scores (4H75%). Cumulative response rate (75% reduction in handicap score) as measured per visit and handicap at baseline (5 visits in the period W8-W24).

In this study, masitinib was associated with a statistically significant difference over placebo for the cumulative response rate 4H75% from W8 to W24, with a response of 18.7 vs 7.4%, respectively (p-value 0.0076). Thus, there was an 11% difference in response rate (4H75%).

Statistically significant differences between masitinib and placebo were observed for the cumulative response rates 3H75% (depression, pruritus and flush), 2H75% (pruritus and flush) and for analysis of pruritus alone from Week 8 to Week 24, with observed p-values in the range of 0.01-0.03 with an overall absolute difference in response of about 15% irrespective of endpoint. Cumulative response rates (4H75%, 3H75% and pruritus) from W8 to W96 also showed a statistically significant difference between masitinib and placebo.

For patients experiencing a response, i.e. a 75% improvement on one or more of the 4 handicaps, there was a benefit of 11% for masitinib compared to placebo. However, it is unclear on how many items there was a response and how clinically relevant this is for individual patients experiencing fluctuating and different symptoms. It is also unclear whether the individual handicaps are independent of each other and whether response to one handicap could change other symptoms. Improvement of quality of life is one of the general needs of patients as defined by WHO, thus the unequivocal results in this part of the study should be a criterion for considering the benefit of masitinib. Unfortunately, there is no possibility to make a valid conclusion on the results related to the topic in the pivotal study. In the pivotal study quality of life was assessed in two different ways: overall patient assessment (OPA) score and the AFIRMM questionnaire. Additionally, the QLQ-C30 was used for the assessment of quality of life, which was the only validated instrument for this assessment. According to this instrument, there was no improvement of quality of life in patients treated with masitinib. Quality of life assessed by OPA score also failed to show differences in masitinib-treated patients compared to placebo.

The absolute 11% benefit in symptomatic score in favour of masitinib is considered a small difference and there is a concern that it may be overestimated following the findings of GCP inspections, even after re-monitoring of the data. The accumulation of critical and major inspection findings, affecting all aspects of the trial, seriously question the reliability of the trial data in general and the absolute benefit of masitinib for the proposed indication in particular.

In any case, even if hypothetically there were no GCP findings, the clinical relevance of the 11% increase in cumulative symptomatic response for masitinib, as defined, compared to placebo, is questioned due to the complexity of the endpoint.

Safety

Common and severe adverse reactions were reported in patients treated with masitinib such as neutropenia, skin toxicity (Steven-Johnson Syndrome (SJS) and Drug-Rash with Eosinophilia and Systemic symptoms -DRESS) and hepatic toxicity and oedema, which are unacceptable for long term use in the context of a condition where symptomatic response at best has been documented. To this may be added potential risks, including cardiovascular events, such as myocardial infarctions, where

causality cannot be properly assessed due to the small sample size and the uncertainties in adverse event reporting/collection. The potential risk of carcinogenicity was identified from non-clinical studies, which further adds to the uncertainties regarding the observed safety profile.

The safety database is considered very limited for a possible life-long, symptomatic treatment and the risk for severe, potentially irreversible adverse reactions cannot be estimated with reasonable precision.
Serious deficiencies have been identified by the inspectors in the pivotal clinical study, affecting the quality and reliability of the safety data reported. Major inspection findings concerning inadequate safety reporting and lack of an adequate pharmacovigilance system with a rigorous system for reporting and collection of AEs – create substantial uncertainties concerning the safety profile of masitinib. A total of 345 previously unreported AEs were identified in the re-assessed population. In total 5 SAEs were added to the mastocytosis study – one in the masitinib group and 4 in the placebo group; 7 new SAEs were added after the medical assessment, of them 5 in masitinib group; 16 SAEs were additionally reported for non-oncological studies, at least one of them in the masitinib group after the medical assessment, and two were reported by investigator as suspected to the study treatment. Although the quality of monitoring safety data has been improved following GCP inspection by the implementation of a renewed pharmacovigilance system, it cannot “repair” collected data. Therefore, the uncertainties regarding the quality of safety data remain.

Benefit / Risk

There are no medicinal products approved in the EU specific for patients with smouldering or indolent systemic mastocytosis. However, symptomatic treatment options of mastocytosis include H1/H2 antihistamines, osteoclast inhibitors, anti-leukotrienes, and proton pump inhibitors. The use of interferon-alpha, thalidomide, cladribine and imatinib has been reported but is limited in indolent mastocytosis.

Smouldering or indolent systemic mastocytosis is a life-long condition, patients have a normal life-expectancy and the symptoms are considered as handicaps. Most often patients experience fluctuations in their symptoms, even when treated with the available symptomatic treatment options. It is therefore endorsed that there is an unmet medical need in patients who do not adequately respond to existing symptomatic treatments.

The clinical benefit of masitinib in patients with severe symptomatic mastocytosis is not clear due to the composite nature of the primary endpoint, the clinical relevance of the effect as measured by an improvement in response of 11% compared to placebo is difficult to interpret. No positive impact of masitinib on quality of life was documented.

The safety database is considered very limited for a possible life-long, symptomatic treatment and the risk for severe, potentially irreversible adverse reactions cannot be estimated with reasonable precision, even with the updated safety database. From a data quality point of view, the safety reporting was inadequate and in general pharmacovigilance handling (reporting and collection of AEs) in the pivotal trial was poor, as described above. As a consequence it cannot be ascertained whether the described safety profile is representative for that to be observed in case the product would be used in clinical practice.

In light of all the above, although the unmet medical need for new drugs for the treatment of mastocytosis is endorsed, the uncertainties in efficacy and safety data do not justify a marketing authorisation for masitinib for the proposed indication.

Additional expert consultation – Report from the SAG-O meeting held on 4 September 2017

Following a request from the Applicant at the time of the re-examination, the CHMP convened a SAG meeting inviting experts, including patient representatives, to provide their views on the questions posed by the CHMP, taking into account the Applicant's response to the grounds for refusal. The questions raised by the CHMP and the views of the SAG-O are presented below.

1. **The adequacy of the endpoints and the clinical relevance of the efficacy results for the target population of adult patients with smouldering or indolent systemic mastocytosis with severe mediator release-associated symptoms unresponsive to optimal symptomatic treatments – taking into account the unmet medical need in these patients?**

The efficacy evaluation of masitinib in adult patients with smouldering or indolent systemic mastocytosis is hampered by a number of deficiencies in the design, conduct, and analysis of the pivotal clinical trial, resulting in uncertainties about the size and relevance of the observed effect, as well as of the safety profile.

Concerning the design, the patient characteristics are unclear with respect to optimal symptomatic treatments since a run-in period with stable medication was lacking before randomisation. It is unclear to what extent patients were unresponsive to optimal therapy (how long were patients observed before being considered "unresponsive"). Thus, there are uncertainties about the population recruited and the external validity of the results when applied to the target indication. The change in eligibility criteria and focus on a subgroup of the originally intended population raises doubts in terms of trial integrity and should be verified in view of the lack of strong rationale for introducing such changes.

Concerning the choice of endpoints, measuring flushing, pruritus, fatigue are in principle adequate, although diarrhoea should have been included as well. However, the chosen composite algorithm appeared exceedingly complex and the defined response based on a composite of the 4 dimensions is of unclear clinical significance as patients had variable numbers of symptoms at entry. The endpoint is also biased in favour of the experimental drug by excluding diarrhoea as a component that would otherwise be expected in this disease. A longitudinal analysis on the effect of masitinib based on each individual component, and based on the "all randomized" population as initially defined in the protocol, was lacking. The reasons and impact of patient exclusions from analysis should be carefully scrutinised. Depression and fatigue, might be relevant endpoints to measure directly and convincingly, the effect of masitinib; but the chosen tools are unfamiliar to dermatologists as evidenced by the HAMD-17 tool being administered to patients directly, instead of by trained clinicians as it was in some centres. This raises concern about the reliability of the data. Furthermore, the data generated in some of the former centres were later excluded from the analysis. In addition, an incorrect version of the Fatigue Impact Scale (FIS) was used during the first few months of the trial. There are also concerns about the use of a number of concomitant medications which could possibly confound the results. Lastly, a relevant endpoint would have been HRQoL, in particular an evaluation of the impact of masitinib on global quality of life using a validated instrument and assessed longitudinally, together with an evaluation of performance status; such assessments were not available.

In terms of analysis, the GEE method is acceptable in principle although the conclusions would be stronger if supported by the original method of analysis (chi-square) and the method may suffer in case of statistical model misspecification (the GEE model is not an obvious choice with high number of patients meeting the 75% threshold at baseline only for one or few symptoms). At least the method should have been accompanied by less assumption-dependent aggregate measures of success, less at risk of overstating the amount of available data/patients for analysis. Another major uncertainty results from the numerous patient exclusions compared to the initial population and the very small numbers in the main analyses which raises issues of external validity, robustness of the findings and potential bias.

More importantly, there are concerns with the amendments of the protocol endpoints (e.g., the change from 50% to 75% improvement to adjudicate response) and analyses (chi-square to GEE) unless it can be ensured that all changes occurred strictly under blinded conditions and for well-justified reasons. In this respect, the GCP inspection conclusion about "potential premature unblinding of the pivotal study" which is considered unresolved in the CHMP's opinion, raises serious concerns.

The primary endpoint showed an 11% improvement in cumulative response in the masitinib group (18.7%) compared to the placebo group (7.4%) which was statistically significant using the chosen method of analysis. The small difference in response rate, as defined (an improvement with respect to the baseline values ≥ 75% for pruritus, flushes, Hamilton and FIS calculated on each handicap present at baseline), is of doubtful clinical significance given the choice of these endpoints and the definition of response based on improvement on one or more individual dimensions without taking into account possible deterioration in other symptoms. Moreover, the robustness of the estimation is compromised by doubts about the integrity of the analysis. Such unclear effects and uncertain benefits are difficult to balance against the observed toxicity, including serious adverse events and lack of data on long-term toxicity in the context of a chronic disease.

2. **The acceptability of the safety database – taking into account the identified GCP deficiencies on safety reporting and the observed safety profile in the target population?**

The acceptability of the safety database is difficult to confirm as there are doubts about the completeness of the source data, which is difficult to correct post-hoc. Nevertheless, and despite the deficiencies noted in the GCP report and considered unresolved by CHMP, the safety profile of masitinib is relatively well-known so that overall, the safety database could in principle be acceptable to make a benefit-risk assessment. However, long-term safety data are missing, and this is an important deficiency given the potentially long-term treatment.

3. **The impact of the GCP findings on the ability of the data from the single pivotal study to support the applied indication? In addition, please comment on:**

    a. **the acceptability of the retrospective corrective actions performed by the applicant in response to the identified GCP deficiencies;**

    b. **the possible impact on the reliability of the resulting efficacy and safety data; and, as a consequence,**

    c. **the supportive capacity of the corrected data for the applied indication**

The SAG assessment of the impact of the GCP findings is based on the CHMP conclusions about "unreliable assessment and collection of safety data" and "potential premature unblinding of the pivotal study". The SAG is not aware of any convincing evidence to the contrary. While deficiencies in the safety reporting might be addressed using pharmacovigilance, the impact of potential unblinding of the pivotal study and the possibility of introducing bias through data-driven changes in key protocol aspects, such as the definition of response and analysis, cannot be overemphasized.

Overall, the SAG seriously questioned the ability of the data from the pivotal study to support the applied indication.

Additional information provided by the Applicant at an Oral explanation

During the Oral Explanation on 12 September 2017, the Applicant presented on the following points:

- The possible impact of the GCP findings on the reliability of the resulting efficacy and safety data (separately) from the single pivotal study to support the applied indication.
- The clinical relevance of the primary endpoint and of the observed treatment effect, taking into account the target population of patients with smouldering or indolent systemic mastocytosis with severe mediator release-associated symptoms.
- The reliability of the safety database, taking into account the identified GCP deficiencies on safety reporting and the limited size of the safety dataset in particular with regard to long-term treatment.
- The acceptability of the safety profile of masitinib in the target population, discussing 1) the acceptability of the acute toxicity profile, and 2) the impact of the identified potential risks associated with masitinib treatment (including but not limited to carcinogenicity).

Overall conclusion on the grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant and considered the views of the Scientific Advisory Group.

The CHMP acknowledged the efforts of the Applicant and organisational changes in the conduct of clinical trials at AB however, due to the late implementation of corrective measures and due to the fact that the whole trial was affected in a systematic way as a result of the deficiencies, the uncertainties on the data quality of the trial AB06006 still remain which question the reliability of the safety database and the robustness of the trial.

Further to the uncertainties derived from a number of deficiencies in the design, conduct and analysis of the pivotal clinical trial, the efficacy evaluation of masitinib in adult patients with smouldering or indolent systemic mastocytosis is hampered and no firm conclusions can be drawn on the size and relevance of the observed effect, as well as the safety profile of Masipro in the treatment of smouldering or indolent systemic mastocytosis with severe mediator release- associated symptoms unresponsive to optimal symptomatic treatments.

6. Benefit-risk balance following re-examination

6.1. Therapeutic Context

6.1.1. Disease or condition

Indolent and smouldering systemic mastocytosis are characterised by excess of mast cells or abnormal mast cells that leads to a wide variety of signs and symptoms, including pruritus, flushing, syncope, hypotensive shock, dizziness, abdominal pain, nausea, vomiting, diarrhoea, fatigue, memory loss, depression, tachycardia, palpitations, breathing difficulties, fractures/osteoporosis, and pain in the muscles, joints, and bones. Urticaria pigmentosa is the most common sign of mastocytosis, in both cutaneous and systemic forms of the disease.

6.1.2. Available therapies and unmet medical need

There are no medicinal products approved in the EU specific for patients with smouldering or indolent systemic mastocytosis. However, symptomatic treatment options of mastocytosis include H1/H2 antihistamines, osteoclast inhibitors, anti-leukotrienes, or proton pump inhibitors. The use of interferon-alpha, thalidomide, cladribine and imatinib has been reported, but is limited in indolent mastocytosis.

Smouldering or indolent systemic mastocytosis is a life-long condition, patients have a normal life-expectancy and the symptoms are considered as handicaps. Most often patients experience fluctuations in their symptoms. An unmet medical need is identified in patients who do not adequately respond to existing symptomatic treatments.

6.1.3. Main clinical studies

The MAA for masitinib is based on one pivotal study, AB06006, an international, randomized (1:1), double-blind, placebo-controlled phase 3 study including 132 patients (ITT) with smouldering or indolent systemic mastocytosis with symptoms considered as severe handicaps. The main efficacy analyses were performed on the modified intent-to-treat (mITT) population which comprised 129 patients.

In this study, the primary efficacy endpoint was cumulative response resulting from five visits from Week 8 to Week 24 on one or more of 4 predefined handicaps registered at baseline (i.e. pruritus score ≥9, flushes per week ≥8, Hamilton rating scale for depression (HAMD-17) score ≥ 19, Fatigue Impact Scale total score (asthenia) ≥ 75). A response was defined as a 75% reduction in these four handicap scores (4H75%). Cumulative response was calculated as the number of actual responses between weeks 8 and 24, divided by the total number of possible responses over the same treatment period (i.e. with 5 scheduled visits each patient had a maximum of 5 to 20 possible responses depending on the number of handicaps at baseline). Secondary and exploratory endpoints included e.g. cumulative response on individual handicaps, changes in tryptase level, body surface area covered by urticaria pigmentosa, disappearance of Darier's sign and QoL.

6.2. Favourable effects

In the main study AB06006, masitinib was associated with a statistically significant difference over placebo for the cumulative response rate 4H75% from W8 to W24, with a response of 18.7 vs 7.4% (p-value 0.0076).
Statistically significant differences between masitinib and placebo were observed for the cumulative response rates 3H75% (depression, pruritus and flush), 2H75% (pruritus and flush) and for analysis of pruritus alone from W8 to W24, with observed p-value in the range 0.01-0.03 with an overall absolute difference in response about 15% irrespective of endpoint. Cumulative response rates (4H75%, 3H75% and pruritus) from W8 to W96 also showed statistically significant difference between masitinib and placebo.

There was a reduction of the tryptase level from baseline to last visit (W0-W24) in the masitinib arm, while there was a slight increase in the placebo arm; mean relative change was -18% in the masitinib treatment-arm versus +2.2% in the placebo arm (p=0.0001). Response rate in tryptase level, defined as a reduction of 25% from baseline at last visit (W0-W24), was 35% in the masitinib treatment arm versus 11.9% in the placebo arm (p=0.0172).

Body surface area covered by urticaria pigmentosa was decreased in the masitinib arm (-12.3%), while it increased in the placebo arm (+15.9%) from baseline to Week 24 (p=0.0210).

Disappearance of Darier's sign from baseline to Week 24 was 18.9% in the masitinib treatment arm versus 2.7% in the placebo arm (p=0.0187, odds ratio=6.58).

6.3. Uncertainties and limitations about favourable effects

Due to the composite nature of the primary endpoint, it is difficult to interpret the clinical relevance of the magnitude of the effect of the improvement of 11% compared to placebo. Considering that patients who contributed with a response may have responded on just one of the four handicaps included in the primary endpoint and few patients had more than one or two handicaps relevant for the primary endpoint, the generalisability and clinical relevance for a patient population which often presents with a number of handicaps is also unclear. In addition it remains unclear if these handicaps are completely independent of each other. Interaction and dependencies of various handicaps have not been explored in any way. Further, the endpoint hampers the distinction between safety and efficacy aspects as handicaps contribute to both measures, yet in different degrees. Improvements in handicaps not considered severe at baseline are not included in the effect measure, but can nonetheless lead to indirect changes in other handicaps. Notably, no positive impact of masitinib on quality of life was documented.

A number of changes to the protocol have been implemented during the conduct of the study in order to capture patients with more severe handicaps, increase in the cut-off for response, introduce cumulative response as primary endpoint, exclude previously included patients and change statistical methods, having consequences on the blinding of the study, with impact on the randomisation and data integrity. Following the conclusion of the GCP inspection of the pivotal clinical study AB0600 the CHMP considered that the accumulation of critical and major inspection findings, affecting all aspects of the trial, seriously question the validity of the trial data. Critical and major findings include the following: blinding was compromised during the trial; serious deficiencies affect the reliability of components of the inclusion criteria, of the disease severity criterion and of the primary and secondary evaluation criteria (HAMD-17 and FIS); GCP deficiencies in the monitoring process and in the overview of the trial by the sponsor; and violation of inclusion criteria.

Although exploratory analyses performed on the 75% reduction in the score of the detailed items of FIS, HAMD-17, and AFIRMM score indicated that masitinib generated a benefit on a greater number of quality of life related items compared with placebo, there was overall no improvement in the quality of life measured by QLQ-C30 global score, AFIRMM questionnaire, and OPA score. As revealed by the GCP inspections, an incorrect version of the AFIRMM V2 questionnaire was used during the first months of the study, and occasionally thereafter, however the data obtained were pooled with the other data, which hampered their interpretation.

It is considered that although re-monitoring of the data may have improved the data, there remain important doubts about the data quality which cannot be (fully) resolved by performing re-monitoring and retrospective analyses at the study sites. This due to the large number and variety of critical deficiencies recorded during the inspection, and the fact that the whole trial was affected in a systematic way as a result of the deficiencies being mainly related to the sponsor**.**

6.4. Unfavourable effects

Several very common AEs (≥10%) were reported with masitinib in patients in the pivotal AB06006 study during the 24-week-period; for a number of events the frequency was at least 10% higher in the masitinib group vs placebo including GI-events, oedema of different locations, anaemia, rash, flushing*, AST/ALT increase and weight decrease (also an efficacy measure).

The main common AEs (frequency >1%, <10% and with the difference in frequency between masitinib and placebo arm ≥4%) observed during the 24-week-period of the pivotal AB06006 study were within the areas of: *Skin disorders* (urticaria, dry skin, swelling face, eczema, erythema, eye pruritus), *Investigations* (lymphocyte count decreased, blood potassium decreased, weight increased), *Gastro-intestinal disorders* (dyspepsia, gastroenteritis), *Metabolism and nutrition disorders* (hypophosphatemia) and *Hepatic disorders* (cytolytic hepatitis), viral infections and chest pain.

During the first 24 weeks in study AB06006, SAEs were experienced by 28.6% of masitinib-treated patients vs 19.0% with placebo and mainly concerned *Skin disorders* (masitinib: 7 pts vs placebo: 1) and *Gastro-intestinal disorders* (6 pts vs 1). The most frequent SAE being diarrhoea (3 pts masitinib, vs 0 on placebo) and urticaria (2 pts vs. 0 pts). Severe AEs (AEs of grade 3 and 4) were reported in 50% (35/70) in the masitinib arm vs. 35% (22/63) in the placebo arm, these events lead to permanent discontinuation in 14/35 (masitinib) vs. 3/22 (placebo). The frequencies of severe AE were: diarrhoea 11% vs. 2%, asthenia 6% vs. 2%* and pruritus 4% vs. 2% (also an efficacy measure), and also rash 3% vs 0%, pyrexia 3% vs 0% and neutropenia 4% vs. 2% in the masitinib and placebo arm, respectively.

The following events occurred in more than one patient and at a higher frequency than placebo: neutropenia [(3/70 (severe systemic) and 5/110 (mastocytosis all) vs. 1/110], diarrhoea (8/70 and 10/110 vs. 1/110), asthenia (4/70 and 5/110 vs. 2/110), pyrexia (2/70 and 4/110 vs. 0/110) and rash (4/70 vs. 0/63). Skin reactions were 11/70 vs. 2/63 and 4/11 vs. 1/2, masitinib and placebo, respectively. Gastrointestinal events were 12/79 vs. 3/63 and 3/12 vs. 1/3, masitinib and placebo, respectively. Oedema (37/70 vs. 8/63) occurs early (median 23 vs. 39 days), is mainly of mild severity, but led to temporary interruption in 3 individuals and discontinuation in one individual.

If asthenia and fatigue is combined the incidences were similar (25/70 vs. 20/63) and of similar severity. Asthenia, however, was more commonly reported in the masitinib arm (26% vs 11%) with the reverse for fatigue (10% vs. 18%).

In the phase 3 study, the frequency of severe neutropenia in mastocytosis patients was higher in masitinib treated patients (4 pts) compared to placebo (1 patient) The frequency of infections was, however, balanced between the masitinib and placebo arms (31/70 vs. 28/63) during the 24 week period. There was, however, one case of neutropenic fever in the masitinib arm.

No potential cases of Steven-Johnson Syndrome (SJS) and Drug-Rash with Eosinophilia and Systemic symptoms (DRESS) were reported in the mastocytosis studies. In the oncology studies, there were 2 cases diagnosed as possible/probable SJS and 1 case diagnosed as possible DRESS.

In the completed phase 3 studies (including mastocytosis patients and patients with other diseases), there were 11 cases (9.1%) of neoplasm reported in masitinib treated patients versus 7 cases (6.4%) in placebo treated patients. Based on these numbers the applicant states that the incidence of neoplasms was comparable with masitinib treatment (0.2) versus placebo (0.3).

In the pivotal study AB06006, no deaths were reported in masitinib-treated population, but one death (subdural intracerebral hematoma) was reported in the placebo arm. In addition, there were 15 fatal cases reported in the non-oncology studies, (9 in ALS, 2 in Alzheimer's disease, 2 in multiple sclerosis, 1 in rheumatoid arthritis, 1 in severe asthma).

The most frequent adverse events leading to dose reduction in the masitinib treatment-arm during the initial treatment period were: nausea (5 pts), diarrhoea (5 pts), vomiting (3 pts) and fatigue (2 pts).

In the pivotal study AB06006 (during the initial 24-week period) masitinib was discontinued due to AEs by 16 patients (22.9%); 5 patients (7.9%) discontinued in the placebo arm, mainly due to GI events and skin toxicities.

In total 31 mastocytosis patients (and 77 non-oncology patients + healthy volunteers) received masitinib at the target dose of 6 mg/kg/day for more than one year. For the target indication there are still only safety data from 58 patients, treated at various doses, and exposed for over a year. For study AB06006, the applicant confirms that only 25 patients completed 96 weeks of masitinib treatment.

Further, a total of 345 previously unreported adverse events were identified during re-monitoring. There were 5 severe adverse events reported, 1 in a masitinib-treated patient and 4 in placebo-treated patients. Other adverse events were mild and moderated adverse events.

In the masitinib treatment-arm, one case of severe peripheral oedema was identified. In the placebo treatment-arm, one case of severe asthenia, one case of severe dysmenorrhea, and two cases of severe hypertension were identified.

396 duplicated adverse events were removed from the safety database for the mastocytosis study, including 15 severe AEs, 8 in the masitinib treatment-arm, and 7 in the placebo treatment-arm. The Applicant claimed that these severe AEs which were removed do not modify the percentage of patients with AEs and that the masitinib safety profile has not been modified.

6.5. Uncertainties and limitations about unfavourable effects

The general quality of the conduct and monitoring of the trial is severely questioned, as the GCP inspection of the sponsor site and 2 study sites revealed very serious issues including an unreliable assessment and collection of safety data and potential premature unblinding of the pivotal study. These critical issues hamper the safety assessment, causing a considerable residual uncertainty.

Although the quality of monitoring safety data has been improved following GCP inspection by the implementation of a renewed pharmacovigilance system, it will not “repair” collected data from the pivotal study. Therefore, the uncertainties regarding the quality of safety data remain.

Among the 16 fatal cases reported in the non-oncology studies, the role of masitinib was suspected in 3 cases (2 cases of sudden death, one of myocardial infarction) and 1 case (massive pulmonary embolism and deep venous thrombosis of right lower limb) which was not assessable. In all of these cases one cannot definitely exclude the role of masitinib, even though most of the patients had comorbidities or pre-existing risk factors. However, only for 4 of the 16 fatal cases it is known which treatment was given; for the 12 other deaths, it is not known from the narratives if the patient received masitinib or placebo.

The potential risk of carcinogenicity was identified from preclinical studies. From these studies, the following malignancies were identified: urinary bladder and uterine tumours, and (benign) thyroid tumours. According to the applicant, there have been no suspected (or not assessable) cases of bladder cancer reported. Detecting or stating the absence of a carcinogenicity risk would, however, require a great number of patients exposed to masitinib for a sufficient length of time. Carcinogenicity is considered as a potential important risk of masitinib and should be further investigated.

The reported frequency of hypophosphatemia in masitinib treated patients was 7.1% in the phase 3 study. In light of the intended chronic use of masitinib in this mastocytosis population with a normal life expectancy, osteoporosis is a regarded an important concern and uncertainty, as TKI use is associated with hypophosphatemia and changes in bone and mineral metabolism.

Some of the known class effects of TKIs have been observed in the pivotal study, such as neutropenia, gastrointestinal toxicities, skin toxicities, and hepatotoxicity. The true frequency, severity and impact of these at short or long term are based on too few patients and are thus currently not known. Other TKI class effects (e.g. hypothyroidism or cardiotoxicity) have not been observed, but can still be considered an uncertainty. Masitinib has apparently no clinically significant effect on the QTcF interval, though the validity of the study evaluating the potential effect of masitinib on ventricular repolarisation is currently questioned.

The knowledge of concentration-response is very limited. Thus the clinical consequences of unexpected increases/decreases in exposure, due to potential DDI with concomitant medications, cannot be foreseen as the major part (ca 70%) of the elimination pathways is unknown.

Masitinib was not studied in mastocytosis patients with renal, hepatic or cardiac impairment. The clinical significance of renal, reproductive organ- and cardiovascular toxicities observed in animal studies is unclear.

Experience from long-term exposure is very limited. Therefore, the toxicity profile can currently not be considered as sufficiently characterized, especially with regard to (potential) late-appearing effects. This safety database for the relevant indication is therefore still considered as limited and the risk for severe, potentially irreversible adverse reactions, including deaths, cannot be estimated with reasonable precision. Tumour findings were observed in both mice and rats. At current, the relevance to human safety is not sufficiently well understood and a carcinogenic risk cannot be excluded.

The clinical significance of renal, reproductive organ- and cardiovascular toxicities observed in animal studies is unclear.

6.6. Effects Table

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Duration of study	Intended 24 weeks + possible extension period up to 96 weeks				70% of the patients in the masitinib arm completed 24 weeks; 87% completed 24 weeks in the placebo arm. 55% of the enrolled patients continued into the extension phase. 25 patients completed 96 weeks of masitinib treatment.	AB06006

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
		%	Masitinib	Placebo		
Primary: **4H75%** (pruritus, flush, depression, fatigue)	Cumulative response rate (75% reduction in handicap score) as measured per visit and handicap at baseline (5 visits in the period W8-W24).		18.7%	7.4%	Changes to the protocol performed during the conduct of the study might have compromised the trial integrity: changes in the inclusion criteria, increase in the cut-off for response, introduction of cumulative response as primary endpoint, and change in statistical method. Cannot exclude that these changes were data driven. The GCP inspectors found that blinding was compromised. GCP inspectors found irregularities in the handling of HAMD-17 (depression) and FIS (asthenia) questionnaires that leaves the rating of depression and asthenia unreliable. Changes in background symptomatic medications were frequently undertaken. Use of new concomitant medications was not systematically reported to the sponsor. The consequences as to outcome measures are hard to foresee as changes per se might have at least “placebo effects”.	
Secondary: **3H75%** (pruritus, flush, depression)			24.7%	9.8%		
2H75% (pruritus, flush)			27.2%	10.7%		
Pruritus			22.0%	7.3%		
Up to 2 years of treatment:	As above but last visit at W96.					
4H75% (pruritus, flush, depression, fatigue)			16.8%	6.8%		
3H75% (pruritus, flush, depression)			21.8%	8.3%		
Pruritus			19.2	6.2%		

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Unfavourable Effects*						
Pivotal study AB06006	Safety parameters in AB06006 were compared for the 24-week, double blind phase.		Masitinib N=70	Placebo N=63		
Deaths			No deaths	1 case of death (subdural intracerebral haematoma)	In other, non-oncology studies, missing mortality data for 12 of 16 fatal cases with regard to the patients actually received masitinib or placebo.	
Discontinued			22.9%	7.9%	discontinuation of 5 patients as mistakenly withdrawn in the masitinib arm (protocol deviations)	
AEs	Febrile neutropenia 1/70vs. 0/63 Gastrointestinal 6/70 vs. 1/63 Oedema 1/70 vs. 0/63 Infection 3/70vs. 2/70 (excl. febrile neutropenia) Skin and subcutaneous tissue 7/70vs. 1/63		100%	100%	Most common (>25%): diarrhoea, nausea, haemoglobin decreased, eyelid oedema, muscle spasms, blood glucose increased, asthenia	
Suspected/ not assessable AEs			97.1%	87.3%	New analyses with regard to treatment-relation have been performed and lots of tables given, but no discussion is provided along with the tabular formats.	
SAEs			29%	20%	The SAEs are mainly skin and gastro-intestinal disorders	
Suspected/ not assessable SAEs			21.4%	9.5%	New analyses are performed; the numbers of suspected/not assessable SAEs are given here.	

6.7. Benefit-risk assessment and discussion

6.7.1. Importance of favourable and unfavourable effects

Due to substantial changes of the protocol during study conduct, the trial integrity is questioned. It cannot be excluded that the changes were data driven. The critical and major findings reported in the GCP inspection regarding the conduct of the study further undermine the reliability of the outcome.

A small, but statistically significant treatment effect of masitinib has been claimed with the methods applied after large amendments to the study protocol, i.e. an improvement of 11% compared to placebo. The clinical relevance is difficult to assess due to the composite nature of this endpoint and the methodological issues identified.

The rating of depression and asthenia based on the HAMD-17 and FIS questionnaires are considered unreliable. This seriously hampers the reliability of the results of both primary and secondary endpoints which incorporate these handicaps. Also, it cannot be excluded that (changes in) use of concomitant symptomatic treatments have influenced and biased the results of the study.

Common and severe adverse reactions were reported in patients treated with masitinib such as neutropenia, skin and hepatic toxicity and oedema, which are unacceptable for long term use in the context of a condition where at best a symptomatic response has been documented. To this may be added as potential risks, amongst others, cardiovascular events, such as myocardial infarctions, where causality cannot be properly assessed due to the small sample size. A potential risk of carcinogenicity was identified from non-clinical studies.

Further, due to GCP inspection findings, there is a general uncertainty concerning the reliability of data and prevent any valid conclusions on efficacy and safety. Re-monitoring of data cannot overcome these issues.

6.7.2. Balance of benefits and risks

The target population is patients with systemic mastocytosis with symptoms not controlled by available therapies.

A small, statistically significant treatment effect (cumulative response rate 4H75% from W8 to W24 was 18.7 vs 7.4% in the masitinib arm vs placebo arm, respectively; p-value 0.0076) has been shown with the methods applied after multiple major amendments to the study protocol. There was, however, overall no improvement in the quality of life measured by QLQ-C30 global score, AFIRMM questionnaire and OPA score. Considering that patients who contributed with a response may have responded on just one of the four handicaps included in the primary endpoint and few patients had more than one or two handicaps relevant for the primary endpoint, the generalizability and clinical relevance of the results for a patient population which often presents with a number of handicaps is questioned.

The consequences of applying more restricted inclusion criteria in retrospect and excluding a substantial number of already randomized patients in the statistical analyses, and subsequent inferences on efficacy, are not possible to assess. Furthermore, increasing the baseline criteria required to contribute as response to the revised primary endpoint (4H) from 50% to 75% during the study is considered problematic. Applying the original 50% criterion resulted in statistically non-significant effects for all outcome measures, underlining the critical nature of this change to the protocol. Similarly, the analysis representing the originally proposed primary analysis in the less severe study population failed to support a significant difference. In light of the findings outlined in the integrated GCP inspection report, it is also not possible to reasonably ascertain that the integrity of blinding and trial conduct was maintained throughout this extensive sequence of changes.

Masitinib was intended to be used as add-on to a wide variety of compounds encompassing different classes of drug. As the drug interaction potential is largely unknown this constitutes an important obstacle for its clinical utility, especially with regard to other compounds, putative effects on masitinib exposure, but also on the effects of masitinib on the exposure of other drugs.

The safety database is considered limited for a possible life long, symptomatic treatment and the risk for severe, potentially irreversible adverse reactions, including deaths, cannot be estimated with reasonable precision.

The GCP inspection conducted at the request of the CHMP concluded that there are serious deficiencies affecting the quality and reliability of the safety data reported. The safety reporting was inadequate and in general pharmacovigilance handling (reporting and collection of AEs) in the pivotal trial was poor. The applicant had not established a pharmacovigilance system with rigorous system for adverse events reporting and signal detection.

Although the quality of monitoring safety data has been improved following GCP inspection by the implementation of a renewed pharmacovigilance system, it will not “repair” collected data. Therefore, the uncertainties regarding the quality of safety data remain.

6.7.3. Additional considerations on the benefit-risk balance

N/A

6.8. Conclusions

Whereas

- **Study conduct and GCP inspection**

  The GCP inspection of the pivotal study AB06006, reports an accumulation of critical and major inspection findings, affecting all aspects of the trial, which seriously impact the reliability of the trial data. A pharmacovigilance system had not been established and safety reporting was inadequate. These issues cannot be resolved by performing re-monitoring and retrospective analyses at the study sites.

  The organisational changes in the conduct of clinical trials at the sponsor’s site are acknowledged however, due to the late implementation of corrective measures and due to the fact that the whole trial was affected in a systematic way as a result of the deficiencies, the uncertainties on the data quality of the trial AB06006 still remain.

  Major changes were implemented to the study protocol during study performance, including

changes in the inclusion criteria late in the study, the exclusion of previously included patients, changes in the definition of response and of the primary endpoint, and changes of the statistical method. It cannot be ascertained that these comprehensive amendments were made in ignorance of the accumulating trial data. The magnitude of bias and inflation of the false-positive error rate that has been introduced cannot be quantified. The estimated effects cannot be considered reliable for assessment.

- **Benefit / risk**

The extent of symptomatic benefit reported in the single pivotal study of Masipro cannot be considered to outweigh the uncertainties that are introduced by the fundamental concerns about the conduct of the single pivotal trial, and the observed toxicity profile.

In view of the lack of robustness of the trial and reliability of the safety database, the overall Benefit /Risk of Masipro in the treatment of adult patients with smouldering or indolent systemic severe mastocytosis with severe mediator release- associated symptoms unresponsive to optimal symptomatic treatments, cannot be considered favourable.

7. Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data, the CHMP re-examined its initial opinion and in its final opinion concluded by consensus that the efficacy and safety of Masipro in the treatment of adult patients with smouldering or indolent systemic severe mastocytosis with severe mediator release- associated symptoms unresponsive to optimal symptomatic treatments are not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product.