Masitinib for treatment of severely symptomatic indolent systemic mastocytosis: a randomised, placebo-controlled, phase 3 study

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Summary

Background **Indolent systemic mastocytosis, including the subvariant of smouldering systemic mastocytosis, is a lifelong condition associated with reduced quality of life. Masitinib inhibits KIT and LYN kinases that are involved in indolent systemic mastocytosis pathogenesis. We aimed to assess safety and efficacy of masitinib versus placebo in severely symptomatic patients who were unresponsive to optimal symptomatic treatments.**

Methods **In this randomised, double-blind, placebo-controlled, phase 3 study, we enrolled adults (aged 18–75 years) with indolent or smouldering systemic mastocytosis, according to WHO classification or documented mastocytosis based on histological criteria, at 50 centres in 15 countries. We excluded patients with cutaneous or non-severe systemic mastocytosis after a protocol amendment. Patients were centrally randomised (1:1) to receive either oral masitinib (6 mg/kg per day over 24 weeks with possible extension) or matched placebo with minimisation according to severe symptoms. The primary endpoint was cumulative response (≥75% improvement from baseline within weeks 8–24) in at least one severe baseline symptom from the following: pruritus score of 9 or more, eight or more flushes per week, Hamilton Rating Scale for Depression of 19 or more, or Fatigue Impact Scale of 75 or more. We assessed treatment effect using repeated measures methodology for rare diseases via the generalised estimating equation model in a modified intention-to-treat population, including all participants assigned to treatment minus those who withdrew due to a non-treatment-related cause. We assessed safety in all patients who received at least one dose of study drug. This trial is registered with ClinicalTrials.gov, number NCT00814073.**

Findings **Between Feb 19, 2009, and July 15, 2015, 135 patients were randomly assigned to masitinib (n=71) or placebo (n=64). By 24 weeks, masitinib was associated with a cumulative response of 18·7% in the primary endpoint (122·6 responses of 656·5 possible responses [weighted generalised estimating equation]) compared with 7·4% for placebo (48·9 of 656·5; difference 11·3%; odds ratio 3·6; 95% CI 1·2–10·8; p=0·0076). Frequent severe adverse events (>4% difference from placebo) were diarrhoea (eight [11%] of 70 in the masitinib group *vs* one [2%] of 63 in the placebo group), rash (four [6%] *vs* none), and asthenia (four [6%] *vs* one [2%]). The most frequent serious adverse events were diarrhoea (three patients [4%] *vs* one [2%]) and urticaria (two [3%] *vs* none), and no life-threatening toxicities occurred. One patient in the placebo group died (unrelated to study treatment).**

Interpretation **These study findings indicate that masitinib is an effective and well tolerated agent for the treatment of severely symptomatic indolent or smouldering systemic mastocytosis.**

Funding **AB Science (Paris, France).**

Published Online
January 6, 2017
http://dx.doi.org/10.1016/S0140-6736(16)31403-9

See Online/Comment
http://dx.doi.org/10.1016/S0140-6736(16)31655-5

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Introduction

Mastocytosis is a rare disease characterised by mast cell neoplasia and aberrant mast cell activation in various tissues, leading to a heterogeneous clinical presentation and wide variety of symptoms, such as pruritus, flushes, depression, and asthenia.[1,2] Although life expectancy is similar to that of the general population in the relatively indolent variants of mastocytosis—cutaneous mastocytosis and indolent systemic mastocytosis, including the subvariant of smouldering systemic mastocytosis—about a third of patients will experience severe symptoms of mast cell mediator release. A greatly increased occurrence and severity of such symptoms is reported in systemic mastocytosis when compared with cutaneous mastocytosis.[1,2] Treatment decisions are based on the presence of bone marrow mast cell infiltration and severity of symptoms, with the main objective being a sustained improvement of symptoms—ie, inhibition of mast cell mediator release.

Genetic aberrations are known to be involved in the pathogenesis of systemic mastocytosis, predominantly the KIT Asp816Val (D816V) mutation, with an emerging understanding that a wide variety of other KIT mutations and mast cell regulatory genes might also be implicated.[3,4] Indeed, type and severity of symptoms are independent of KIT Asp816Val status.[1,5,6] Masitinib is an oral tyrosine

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For the **statistical analysis plan, study protocol, and summary of protocol changes** see http://www.ab-science.com/pdf/Lortholary_et_al_Lancet_Protocol_online.pdf

See Online for appendix

Research in context

Evidence before this study

We searched PubMed without date restriction for reports pertaining to phase 2 or 3 clinical trials in indolent systemic mastocytosis using the search terms of "systemic mastocytosis" [All Fields] filtered for "Clinical Trial, Phase II" and "Clinical Trial, Phase III" [publication type]. We did not apply any language restrictions, but used search terms in English only. We identified 11 previous clinical trials matching these search criteria, describing seven potential treatments for indolent systemic mastocytosis. No trial focused solely on indolent systemic mastocytosis, with this cohort representing a subgroup of overall populations that also comprised advanced systemic mastocytosis or cutaneous mastocytosis patients. All trials were open label, non-comparative (single-arm), phase 2 studies evaluating various compounds including masitinib, dasatinib, everolimus, imatinib, interferon alfa, nilotinib, and thalidomide. Results were mixed, varying from potential therapeutic benefit in select patients to no appreciable clinical efficacy.

Added value of this study

To our knowledge, this is the first phase 3 prospective, randomised placebo-controlled study of a treatment for indolent systemic mastocytosis. We show a significant and clinically meaningful treatment benefit in this difficult-to-treat population, with a demonstrated possibility of effective long-term management.

Implications of all the available evidence

The observed positive benefit–risk ratio supports the use of masitinib for patients with severely symptomatic indolent systemic mastocytosis. Masitinib might be a new treatment option for adult patients with severely symptomatic indolent systemic mastocytosis, including those with the subvariant of smouldering systemic mastocytosis, who are unresponsive to existing symptomatic treatments. Statistical design features of the study and mechanistic implications of targeting of non-clonal mast cells or KIT Asp816Val-independent signalling pathways could influence future trial design in mastocytosis.

kinase inhibitor that targets wild-type KIT (50% inhibitory concentration [IC_{50}] 200 nM) with greater potency in vitro than KIT Asp816Val (IC_{50} 5·0 μM), as well as targeting LYN and FYN at submicromolar concentrations.[7] Wild-type KIT, LYN, and FYN have crucial roles in the survival and function of mast cells, including mediator release.[8,9] Masitinib has a higher selectivity for these targeted kinases than other KIT inhibitors, which is likely to lower the risk of off-target toxicities.[10,11] Phase 2 study results have shown its potential efficacy in patients with indolent forms of mastocytosis, regardless of their KIT Asp816Val status.[12]

We aimed to assess the safety and efficacy of masitinib for treatment of indolent forms of systemic mastocytosis in patients who were unresponsive to optimal symptomatic treatment.

Methods

Study design and participants

This multicentre, parallel-group, randomised, double-blind, placebo-controlled, phase 3 study (AB06006) was initiated in 2009 and conducted in 15 countries (Austria, Czech Republic, France, Germany, Greece, India, Italy, Latvia, Poland, Russia, Slovakia, Spain, Switzerland, UK, and USA) across 50 active centres.

The study protocol and amendment were approved by the relevant institutional review boards at individual enrolment centres or ethics committees and conducted according to the Declaration of Helsinki. All patients provided written informed consent.

Eligible patients were aged 18–75 years and had indolent or smouldering systemic mastocytosis according to the WHO classification,[13–15] or documented mastocytosis based on histological criteria of typical mast cell infiltrates in a multifocal or diffuse pattern in skin or bone marrow biopsy. The latter criterion encompasses all patients satisfying the WHO classification but also selects those patients matching inclusion criteria from the masitinib phase 2 trials and AFIRMM survey.[1,12] Consequently, these inclusion criteria are broader than the WHO classification. To ensure consistency in the investigators' application of diagnostic criteria, a blinded central document reading was used to verify patient eligibility for inclusion to the intention-to-treat (ITT) population based on a set of unifying criteria that encompassed the WHO classification (appendix p 2). We did primary analysis on the ITT population as defined via this central document review. Additional eligibility criteria included severe symptoms of mast cell mediator release at baseline—pruritus score of 9 or more determined via a patient perception questionnaire,[1] at least eight flushes per week, Hamilton Rating Scale for Depression score of 19 or more,[16] or Fatigue Impact Scale total score of 75 or more[17]—and documented failure of at least one symptomatic treatment used at optimal dose—eg, H1-antihistamines, H2-antihistamines, proton pump inhibitors, sodium cromoglicate, antidepressants, and leukotriene antagonists (appendix p 12). Patients were ineligible if presenting with one of the following variants of mastocytosis: cutaneous mastocytosis (as per the amended protocol version 6.0), undocumented indolent systemic mastocytosis or smouldering systemic mastocytosis, systemic mastocytosis with an associated clonal haematological non-mast-cell-lineage disease, mast cell leukaemia, or aggressive systemic mastocytosis; patients presenting with inadequate organ function defined via blood test levels; vulnerable populations such as patients with life expectancy of less than 6 months, known diagnosis of human

immunodeficiency virus (HIV) infection, known cardiac disorders, or Eastern Cooperative Oncology Group performance status greater than 2; previous treatment with any tyrosine kinase inhibitor or treatment with any investigational agent within 4 weeks prior to baseline; and change in the symptomatic treatment of mastocytosis or administration of any new treatment of mastocytosis within 4 weeks prior to baseline (appendix p 7).

Randomisation and masking

Patients were centrally randomised to masitinib or placebo in a 1:1 ratio using an interactive voice response system and minimisation method according to the covariates of pruritus score, number of flushes per week, depression (measured by the Hamilton Rating Scale for Depression), asthenia (measured by the Fatigue Impact Scale), and country. Masitinib and placebo capsules were identical except for the active ingredient and both produced by Excella GmbH (Feucht, Germany), with no difference in dispensing of medication. The investigators, patients, data analysts, and the trial funder were blinded to the randomisation sequence and treatment assignment.

Procedures

Masitinib was administered orally at 6 mg/kg per day in two daily doses over 24 weeks with a possibility of a double-blind extension period. Long-term analysis was done over the timeframe of weeks 8–96. Patients were assessed at weeks 8, 12, 16, 20, and 24 during the 24-week treatment period, with assessments every 12 weeks thereafter if entering the 2-year (96-week) extension period. In the event of severe toxicity related to masitinib, treatment interruption or dose reduction was permitted according to predefined criteria (appendix p 10). Protocol amendments were implemented between 3·5 years and 2 years prior to database unmasking, owing to an emergent risk of masitinib-related severe neutropenia and severe skin toxicity. Protocol amendment version 6.0 aimed to modify the protocol's benefit–risk balance to identify the patient population with greatest medical need. The amendment introduced four key changes: enrolment was restricted to patients with severe baseline symptoms of mast cell mediator release; enrolment was restricted to indolent systemic mastocytosis, including the subvariant of smouldering systemic mastocytosis, because these patients exhibit greater symptom severity than do those with cutaneous mastocytosis;[1] the threshold for positive treatment response was increased from 50% to 75%, thereby enhancing the clinical relevance of improvement; and, as recommended in the European Medicines Agency (EMA) guidelines for clinical trial design for rare diseases, the treatment effect was tested using a repeated measures methodology—namely, longitudinal analysis with respect to symptoms as opposed to patient response rate at a single point in time.[18] Patients with severely symptomatic systemic mastocytosis were defined as those with at least one severe baseline symptom of mast cell mediator release. Thus, only patients with indolent systemic mastocytosis meeting the prospectively declared inclusion criteria specified in this amendment were included for final analysis—ie, the ITT population.

Administration of concomitant optimal symptomatic treatments was allowed (appendix p 12); however, administration of any other kinase inhibitor, interferon alfa, or cladribine was not permitted during the study period.

Outcomes

The prospectively declared primary endpoint (referred to hereafter as 4R75%) was cumulative response in at least one of four severe baseline symptoms of mast cell mediator release (pruritus, flushes, depression, or asthenia). We defined response as a 75% improvement from baseline for any of these four symptoms. We defined cumulative response as the number of actual responses between weeks 8 and 24, divided by the total number of possible responses over the same treatment period (ie, with five scheduled visits, each patient had a maximum of five to 20 possible responses depending on the number of severe baseline symptoms).

Secondary endpoints were cumulative response in at least one of three severe baseline symptoms of mast cell mediator release (pruritus, flushes, or depression) with response defined as an improvement of at least 75% from baseline for any of these three symptoms (referred to hereafter as 3R75%); cumulative response in pruritus or flushes with response defined as an improvement of at least 75% from baseline for either symptom (2R75%); cumulative response in pruritus alone; improvement of urticaria pigmentosa as measured via cumulative change in affected body surface area relative to baseline; disappearance of Darier's sign; mean change of tryptase level at week 24 relative to baseline in patients with baseline tryptase level greater than 20 µg/L; cumulative response in micturition and stool frequency among patients with a baseline of eight or more per day and four or more per day, respectively; and quality-of-life measures such as the AFIRMM questionnaire (version 2). The safety profile of masitinib was compared with placebo according to occurrence and severity of adverse events, regardless of causality.

Statistical analysis

For the primary efficacy analysis, a cumulative total of 1065 possible response evaluations was required to detect a difference of 12·5% in 4R75% between treatment arms (based on the assumption of an average 1·5 severe baseline symptoms per patient, over five assessment timepoints for 142 patients, and a response of 21·0% in the masitinib arm *vs* 8·5% in the placebo arm) with a power of 80% and significance level of 0·05 (two-sided log-rank test). The hypothesised response estimates were based on empirical knowledge from phase 2 data.

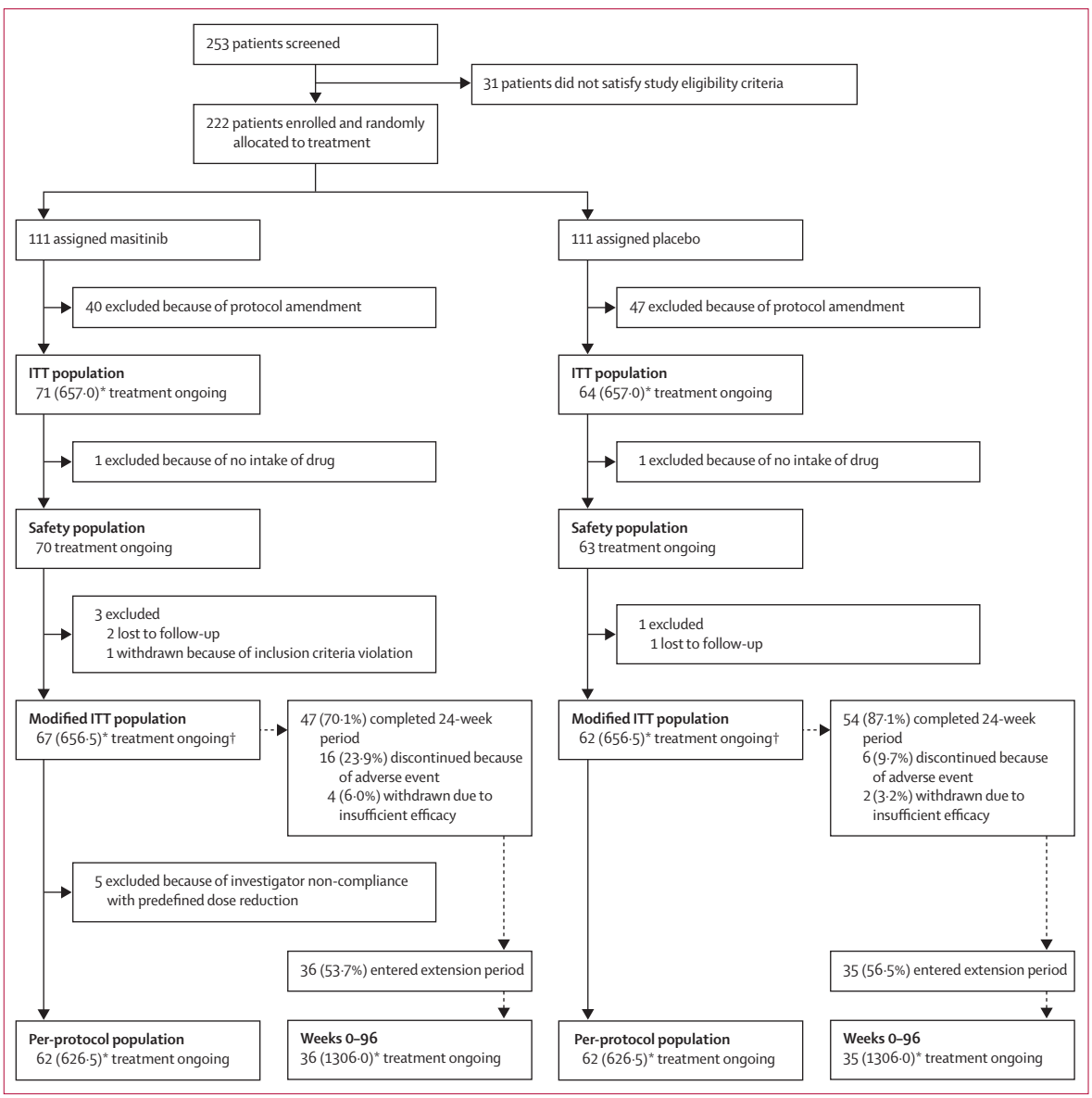

Figure: **Trial profile**

A study amendment (August, 2013, as per protocol version 6.0) restricted enrolment to patients with severe indolent and smouldering systemic mastocytosis. Consequently, 87 patients with cutaneous mastocytosis or non-severe systemic mastocytosis recruited prior to this amendment were excluded from the ITT population. A weighted GEE model was used to provide total possible cumulative responses assessable in calculation of study endpoints. ITT and per-protocol populations represent sensitivity tests of primary analysis. ITT=intention-to-treat. GEE=generalised estimating equation. *Total possible cumulative responses assessable in calculation of the 4R75% endpoint according to GEE model. †Primary endpoint corresponds to modified ITT population.

We did primary efficacy analysis according to a modified ITT population (ITT population minus those withdrawing for a well documented, non-treatment-related cause—eg, no intake of drug), with results verified via analysis on the ITT population (all eligible patients assigned to treatment, irrespective of actual treatment received), as well as other sensitivity analyses including the per-protocol population (modified ITT minus those with a major protocol deviation) and modified ITT observed cases dataset (see appendix p 2). The safety population comprised all ITT patients who received at least one dose of study medication. All main, sensitivity, and subgroup analyses reported here were prespecified in the study's statistical analysis plan prior to unblinding, and we did no interim analyses. We considered missing data as failure for primary and secondary analyses, did sensitivity analyses with the last observation carried forward or observed cases approach, and obtained the statistical test p value for the primary analysis via a re-randomisation (10 000 replicate) test.

We calculated the difference between treatment arms using the generalised estimating equation (GEE) approach (logit-link function) with treatment, symptom (pruritus, flushes, depression, and asthenia), and assessment schedule (weeks 8, 12, 16, 20, and 24) included as parameters in the model.[19] This approach simultaneously tests for effect in all four outcome measures specified in the primary endpoint, taking into account correlation across variables and across time so that valid inferences can be assured.

This trial is registered with ClinicalTrials.gov, number NCT00814073.

Role of the funding source

The funder (AB Science; Paris, France) was involved in the study design; data collection, analysis, and interpretation; and manuscript preparation and submission. OH, OL, and AM had full access to all the data in the study and final responsibility to submit for publication.

Results

Between Feb 19, 2009, and July 15, 2015, 253 patients were screened, from which 222 were randomised to masitinib (n=111) or placebo (n=111). A protocol amendment in August, 2013, restricting enrolment to severe systemic mastocytosis resulted in exclusion of 87 patients (40 from the masitinib arm, 47 from the placebo arm). As a result, the prospectively declared ITT population consisted of 135 patients (71 masitinib, 64 placebo), 108 (80%) of whom satisfied the WHO classification[13,15] for systemic mastocytosis. The safety (n=133), modified ITT (n=129), and per-protocol (n=124) populations were defined from the ITT population (figure, appendix p 2). Notably, the ITT and modified ITT populations were almost identical for efficacy assessment because no patient excluded from the modified ITT population had data beyond week 8, which was the first timepoint included for cumulative data analysis. Database lock was on Nov 24, 2015.

Baseline characteristics for the modified ITT population are provided in table 1, and were similar for the ITT, per-protocol, and safety populations (data not shown). Mean exposure to masitinib in the modified ITT population over the study duration was 18·9 (SD 22.0) months (range 0·1–74·1) versus 16·4 (19·3) months (0·7–72·3) for placebo.

268 severe symptoms (pruritus, flushes, depression, and asthenia) were recorded at baseline in the modified ITT population (136 in the masitinib group *vs* 132 in the placebo group). This number corresponds to a cumulative total of 1340 possible response evaluations for the primary analysis, indicating that the study was sufficiently powered. At baseline, severe pruritus was reported in about two thirds of patients from both treatment arms, severe flushes in about 27%, severe depression in about 39%, and severe asthenia in about 75% (table 1).

	Masitinib (n=67)	Placebo (n=62)
Demographic		
Age (years)	45·3 (11·1; 19–69)	49·2 (12·7; 27–86)
Sex		
Female	50/67 (75%)	41/62 (66%)
Male	17/67 (25%)	21/62 (34%)
c-KIT status		
Clonal (KIT Asp816Val)	63/67 (94%)	53/62 (86%)
KIT wild-type	1/67 (2%)	7/62 (11%)
Unknown	3/67 (5%)	2/62 (3%)
Disease type		
Indolent systemic mastocytosis*	54/67 (81%)	49/62 (79%)
Smouldering systemic mastocytosis*	6/67 (9%)	7/62 (11%)
Unclassified	7/67 (10%)	6/62 (10%)
Severe symptoms at baseline		
Pruritus		
Cases (%)	45/67 (67%)	42/62 (68%)
Mean score (SD)	9·0 (3·0)	9·1 (3·6)
Flushes		
Cases (%)	18/66 (27%)	17/62 (27%)
Mean (SD)	8·0 (9·6)	6·4 (7·4)
Depression (HAMD-17)		
Cases (%)	23/67 (34%)	27/62 (44%)
Mean score (SD)	16·0 (7·4)	17·3 (8·1)
Asthenia (FIS)		
Cases (%)	50/66 (76%)	46/61 (75%)
Mean score (SD)	90·2 (37·1)	89·4 (34·3)
Objective marker of mast cell activation		
Tryptase level (>20 μg/L)		
Number of cases (%)	46/60 (77%)	44/62 (80%)
Mean (SD)	75·8 (120)	72·2 (75·6)
BSA urticaria pigmentosa†	87·8 (48·0)	101·0 (46·3)
Darier's sign	21/25 (84%)	19/27 (70%)

Data are mean (SD; range) or n/number assessed (%) unless otherwise stated. HAMD-17=Hamilton Rating Scale for Depression. FIS=Fatigue Impact Scale. BSA=body surface area score corrected with Wallace formula. *Classification according to investigator. †Systemic mastocytosis patients presenting with mastocytosis in the skin, specifically urticaria pigmentosa.

Table 1: **Baseline characteristics according to the modified intention-to-treat population**

At 24 weeks of treatment, masitinib was associated with a 4R75% of 18·7% versus 7·4% for placebo (odds ratio [OR] 3·6; 95% CI 1·2–10·8, p=0·0076; table 2). This positive outcome was verified in the ITT population, as well as all predefined sensitivity analyses on the primary endpoint. Subgroup analysis in patients with KIT Asp816Val showed a significant response in favour of masitinib, with a 4R75% of 20·2% (117·6 of 581·5) for masitinib versus 7·4% (42·8 of 581·5) for placebo (4·5; 1·1–17·8, p=0·0316) (appendix p 16). Subgroup analysis for other KIT cohorts was not possible owing to the small number of these patients.

We also noted improvement in secondary endpoints with masitinib (table 2). Endpoints representative of

	Masitinib	Placebo	Difference	Odds ratio (95% CI)	p value
Primary outcome					
4R75%					
Modified ITT (MDF)	122·6/656·5 (19%)	48·9/656·5 (7%)	11·2%	3·6 (1·2–10·8)	0·0076*
ITT	122·7/657·0 (19%)	49·8/657·0 (8%)	11·1%	3·3 (1·2–9·1)	0·0102
Modified ITT (OC)	136·4/565·0 (24%)	44·4/565·0 (8%)	16·3%	4·9 (1·6–15·1)	0·0014
Per-protocol	125·8/626·5 (20%)	46·5/626·5 (7%)	12·7%	3·9 (1·3–11·5)	0·0048
Secondary outcomes					
3R75%					
Modified ITT (MDF)	104·5/423·5 (25%)	41·5/423·5 (10%)	14·9%	3·1 (1·4–6·9)	0·0071
Modified ITT (OC)	117·2/362·0 (32%)	37·5/362·0 (10%)	22·0%	4·1 (1·8–9·1)	0·0008
Per-protocol	107·0/403·5 (27%)	39·4/403·5 (10%)	16·8%	3·3 (1·5–7·5)	0·0038
2R75%					
Modified ITT (MDF)	81·9/301·5 (27%)	32·2/301·5 (11%)	16·5%	2·6 (1·1–6·6)	0·0380
Modified ITT (OC)	92·2/254·5 (36%)	28·8/254·5 (11%)	24·9%	3·8 (1·5–9·4)	0·0042
Per-protocol	83·7/284·0 (29%)	30·3/284·0 (11%)	18·8%	2·9 (1·2–7·2)	0·0220
Pruritus					
Modified ITT (MDF)	47·1/214·5 (22%)	15·6/214·5 (7%)	14·7%	3·1 (1·1–8·9)	0·0322
Modified ITT (OC)	53·0/178·5 (30%)	14·0/178·5 (8%)	21·8%	4·2 (1·5–12·0)	0·0071
Per-protocol	50·0/202·0 (25%)	14·7/202·0 (7%)	17·5%	3·7 (1·3–10·5)	0·0146
Flushes					
Modified ITT (MDF)	34·7/87·0 (40%)	16·6/87·0 (19%)	20·8%	3·0 (0·9–10·7)	0·09
Modified ITT (OC)	39·3/76·0 (52%)	14·8/76·0 (20%)	33·3%	3·8 (1·1–13·8)	0·0402
Per-protocol	33·8/82·0 (41%)	15·6/82·0 (19%)	22·2%	3·1 (0·9–10·9)	0·09
HAMD-17					
Modified ITT (MDF)	22·7/122·0 (19%)	9·3/122·0 (8%)	11·0%	2·7 (0·7–11·1)	0·17
Modified ITT (OC)	24·9/107·5 (23%)	8·7/107·5 (8·1%)	15·0%	3·3 (0·8–13·8)	0·10
Per-protocol	23·2/119·5 (19%)	9·1/119·5 (8%)	11·8%	2·9 (0·7–11·8)	0·14
FIS					
Modified ITT (MDF)	18·1/233·0 (8%)	7·4/233·0 (3%)	4·6%	4·8 (1·0–23·4)	0·0499
Modified ITT (OC)	19·3/203·0 (10%)	6·9/203·0 (3%)	6·1%	7·0 (1·1–46·3)	0·0448
Per-protocol	18·8/223·0 (8%)	7·1/223·0 (3%)	5·2%	5·5 (1·1–27·4)	0·0370

Cumulative response based on generalised estimating equation (GEE) model, with missing data considered as failure (MDF), unless stated otherwise. Response rates expressed as weighted ratio of sum of actual responses between weeks 8 and 24 divided by the total number of possible responses over the same treatment period. Primary and secondary analyses were based on the modified ITT (MDF) dataset. Sensitivity analyses were based on the ITT, modified ITT (OC), and per-protocol datasets. 4R75%=cumulative response in at least one of four severe baseline symptoms (pruritus, flushes, depression, or asthenia). ITT=intention-to-treat population. OC=observed cases dataset. 3R75%=cumulative response in at least one of three severe baseline symptoms (pruritus, flushes, or depression). 2R75%=cumulative response in at least one of two severe baseline symptoms (pruritus or flushes). HAMD-17=Hamilton Rating Scale for Depression. FIS=Fatigue Impact Scale. *Based on re-randomisation.

Table 2: **Efficacy results for primary, secondary, and sensitivity analyses, according to cumulative response analysis (weighted GEE model)**

objective markers of mast cell activation were also positive (appendix p 11). At week 24, the mean change of tryptase level from baseline in the modified ITT population was a decrease of 18·0% in the masitinib arm versus an increase of 2·2% in the placebo arm—an absolute difference of 20·2% (p<0·0001). The response of urticaria pigmentosa lesions to masitinib differed when compared with placebo (p=0·0210) as evidenced by a decrease in average body surface area of 12·3% for masitinib versus an increase of 15·9% for placebo—an absolute difference of 28·2%. The response to masitinib included one KIT Asp816Val-positive patient who had a complete response at week 24 (from baseline body surface area of 18%). This observation was supported by abolition of Darier's sign in 18·9% of patients treated with masitinib versus 2·7% treated with placebo—an absolute difference of 16·2% (p=0·0187; appendix p 11).

Among patients entering the extension period, of whom 36 received masitinib treatment and 35 received placebo, a sustained response was observed in the masitinib group when compared with placebo for primary, secondary, and sensitivity outcomes (appendix p 11).

Table 3 shows a summary of safety results during the 24-week treatment period, regardless of causality. The most frequently occurring severe adverse events were diarrhoea (eight [11%] of 70 in the masitinib group *vs* one [2%] of 63 in the placebo group), rash (four [6%] *vs* none), asthenia (four [6%] *vs* one [2%]), peripheral oedema

(two [3%] *vs* none), pruritus (three [4%] *vs* one [2%]), and neutropenia (three [4%] *vs* one [2%]; appendix p 13). The most frequent serious adverse events were diarrhoea (three patients [4%] *vs* one [2%]) and urticaria (two [3%] *vs* none; appendix p 13). No deaths were reported in the masitinib group, whereas one death, unrelated to study treatment, was reported in the placebo group. Overall, more adverse events occurred during the first 6 months in the masitinib group than in the placebo group (table 3, appendix p 13).

Long-term safety over the extension period was assessed according to incidence per patient-months of exposure; this measure is more appropriate than frequency of adverse events given that some patients had been exposed to masitinib for over 2 years. This analysis revealed a comparable incidence of severe and serious adverse events between masitinib and placebo (table 4).

Discussion

Treatment with masitinib resulted in a therapeutic benefit across a diverse range of symptoms in patients with severely symptomatic indolent systemic mastocytosis who were unresponsive to optimal symptomatic treatments. Moreover, the response criterion of greater than 75% improvement in at least one severe baseline symptom constitutes a clinically meaningful effect, as evidenced by comparison with published recommendations on response evaluation.[20] Data from the extension period showed that masitinib can maintain remission of symptoms for over 2 years; this is supported by results of the GEE model—a powerful tool for making statistical inference on longitudinal data.[21] This observation is important, given that indolent systemic mastocytosis is a chronic condition that requires lifelong management.

The primary analysis was supported by improvements seen in the predefined sensitivity analyses, notably the ITT population, and secondary analyses relating to patient-reported symptomatic endpoints, as well as objective endpoints representative of mast cell activation (tryptase, Darier's sign, and urticaria pigmentosa). Depression and asthenia are potential psychiatric manifestations of mast cell activation and can have a negative influence on the wellbeing of patients with systemic mastocytosis.[1,2,22,23] Thus, the improvement in endpoints, such as the Fatigue Impact Scale and Hamilton Rating Scale for Depression (non-significant), and their associated 4R75% and 3R75% composite endpoints is indicative that masitinib can positively affect neuropsychiatric manifestations of systemic mastocytosis. These efficacy data confirm observations from related phase 2 studies (appendix p 20).[24]

A common issue for clinical trials in orphan diseases is low sample number. In this study, we circumvented this problem by using the EMA-recommended repeated measures methodology.[18] Despite the odds ratio confidence intervals for primary and secondary endpoints being wide, a lower boundary of at least unity supports the superiority of masitinib over placebo. Use of a blinded central document reading introduced a risk of post-randomisation imbalance that was managed via weighting of each observation (appendix p 2). Sensitivity analyses that omitted the weighting function showed this process introduced negligible bias with closely matched data to the primary analysis (data not shown). Another complication for data interpretation arises because of the definition of indolent systemic mastocytosis used in this study is broader than the WHO classification. Among the 135 patients with severe systemic mastocytosis according to the blinded central document reading, 108 (80%) fulfilled the criteria for WHO classification of indolent systemic mastocytosis. Hence, 27 patients (20%) did not comply with the standard WHO classification but were still eligible for inclusion in the ITT population according to the non-standard protocol definition of indolent systemic mastocytosis based on histological criteria of typical mast cell infiltrates in a multifocal or diffuse pattern in skin or bone marrow biopsy (appendix p 21). Finally, although protocol amendments made during the study to improve the benefit–risk balance of the protocol are less than optimal, such changes did not bias the key findings (appendix p 5).

With regard to the mechanism of action, because the KIT Asp816Val mutation might not activate mast cells to release pro-inflammatory mediators—which is consistent with clinical observations that type and severity of symptoms are KIT Asp816Val-independent—the inactivity of masitinib against this target is not necessarily a limitation.[1,5,6,25] The treatment effect is hypothesised to be a result of masitinib targeting wild-type mast cells, leading to a reduction in mast cell burden (an effect seen in long-term treatment of chronic myeloid leukaemia with the wild-type KIT-inhibitor imatinib; appendix p 17),[26,27] or by reducing activation of KIT Asp816Val mast cells. The latter proposed mechanism is mediated through dual inhibition of LYN and FYN, which contribute to modulation of mast cell degranulation in a KIT Asp816Val-independent manner.[7] The decrease we noted in mean tryptase levels in patients

	Masitinib (n=70)	Placebo (n=63)	Difference
At least one adverse event	70 (100%)	63 (100%)	0·0%
Death	0	1 (2%)	−1·6%
Non-fatal serious adverse event	20 (29%)	12 (19%)	9·5%
Severe adverse event	35 (50%)	22 (35%)	15·1%
Adverse event leading to permanent discontinuation (except death)	17 (24%)	4 (6%)	17·9%
In per-protocol population	12/62 (19%)	4/62 (6%)	12·9%
Adverse event leading to study treatment dose reduction	15 (21%)	1 (2%)	19·8%

Data are number of patients (%) affected. All data refer to safety population unless otherwise stated. For the per-protocol masitinib arm, five patients were excluded owing to investigator non-compliance to predefined protocol safety rules regarding dose reduction. Adverse events reported according to any causality. Adverse event intensity count is cumulative.

Table 3: **Safety summary over 24-week treatment period**

	Weeks 0–24			Extension period			Overall study period		
	Masitinib (pm=307)	Placebo (pm=326)	Difference	Masitinib (pm=1016)	Placebo (pm=705)	Difference	Masitinib (pm=1321)	Placebo (pm=1031)	Difference
At least one adverse event	22·8	19·3	3·5	3·3	3·7	−0·4	5·3	6·1	−0·8
Death	0·0	0·3	−0·3	0·0	0·0	0·0	0·0	0·1	−0·1
Non-fatal serious adverse event	6·5	3·7	2·8	1·0	1·1	−0·1	2·1	1·8	0·3
Severe adverse event	11·4	6·7	4·7	1·7	1·4	0·3	3·2	2·9	0·3
Adverse event leading to permanent discontinuation (except death)	5·5	1·2	4·3	0·3	0·0	0·3	1·5	0·4	1·1
Adverse event leading to dose reduction	4·9	0·3	4·6	0·3	0·0	0·3	1·4	0·1	1·3

Incidence in patient-months (pm) is number of patients with at least one adverse event divided by the sum of exposure durations (in months). Frequency is calculated per 100 pm. Adverse events reported according to any causality.

Table 4: **Long-term safety according to incidence in patient-months**

in the masitinib treatment arm is consistent with either of these effects—as are the individual decreases seen in most masitinib-treated patients (34 of 40, 85%; appendix pp 15–16). However, unknown factors could also contribute to these effects, as evidenced by the non-universal patient susceptibility to masitinib, with identification of predictive markers for patient treatment selection remaining a goal for future research.

Masitinib was associated with increased frequency of adverse events during the first 6 months of treatment compared with placebo, although no toxicities were life-threatening, and over the long term (>1000 patient-months) the incidence of adverse events was similar between masitinib and placebo. Toxicities were predominantly gastrointestinal or skin events, consistent with the known adverse-event profile of masitinib, and which can be managed by dose reduction. Emerging evidence from the overall safety profile of masitinib shows that a substantial improvement in tolerance of masitinib occurs after the initial 12-week treatment period (unpublished data). These toxicities could be mitigated via implementation of a dose-escalation scheme—eg, initial dose of 3·0 or 4·5 mg/kg per day with increments of 1·5 mg/kg per day every 4 weeks depending on absence of toxicity until reaching the target dose of 6 mg/kg per day. Nevertheless, the safety profile of masitinib (including tolerance and toxicities) still compared favourably against that reported for interferon alfa, thalidomide, and cladribine—three drugs used in indolent mastocytosis. Treatment with interferon alfa has been associated with a variety of severe adverse events in almost every organ system, as well as with high levels of severe depression and severe cytopenia in patients with systemic mastocytosis.[28,29] Use of thalidomide in patients with systemic mastocytosis is associated with severe peripheral neuropathy and severe myelosuppression.[30] A retrospective study[31] of 68 patients showed frequent severe (grade 3–4) adverse events with use of cladribine in mastocytosis, including lymphopenia, neutropenia, and opportunistic infections, of which one was fatal.

Unlike aggressive forms of mastocytosis, indolent systemic mastocytosis—a rare condition with high, unmet medical need—has no registered or established standard treatment. Results from this study have shown a positive benefit–risk ratio for masitinib in severely symptomatic patients with indolent systemic mastocytosis, including the subvariant of smouldering systemic mastocytosis, as evidenced by a sustained response and long-term incidence of adverse events that was equivalent to placebo. Masitinib might therefore be an important new treatment option for these patients; moreover, these data suggest a possibility for effective longer-term management of this difficult-to-treat disease.

Contributors

OH, OL, AM, LBA, J-PK, and PD contributed to study design. OL, MOC, CBL, CP, GG, EJ, MN, SB, SV, CG, GD, DC, SF, LL, SGL, KH, JA, RG, and PD contributed to data collection. OH, OL, AM, and CM did the initial data interpretation. CM wrote the manuscript with major contributions from AM, OH, OL, and LBA. All authors contributed to final data interpretation and approved the final draft of the manuscript.

Declaration of interests

Masitinib is under clinical development by the study funder, AB Science. AM, CM, KH, and JA are employees and shareholders of AB Science. OH is the President of the Scientific Committee of AB Science. PD, CA, and J-PK are consultants and shareholders of AB Science. All remaining authors have no competing interests.

Acknowledgments

We thank the patients enrolled in the study and their families. We also thank all of the investigators (appendix p 23) who contributed to the study. Financial support for medical editorial assistance (CM) was provided by AB Science.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Lortholary O, Chandesris MO, Livideanu CB, et al. Masitinib for treatment of severely symptomatic indolent systemic mastocytosis: a randomised, placebo-controlled, phase 3 study. *Lancet* 2017; published online Jan 6. http://dx.doi.org/10.1016/S0140-6736(16)31403-9.

Supplement to Lortholary O, Chandesris MO, Bulai Livideanu C et al., "A randomized, placebo-controlled, phase 3 study of masitinib for treatment of severely symptomatic indolent systemic mastocytosis"

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I. SUPPLEMENTARY METHODS AND STUDY DESIGN

A. Definition of populations and datasets

Blinded central document reading definition of systemic mastocytosis

Eligible patients had: (i) ISM/SSM according to the WHO classification; or (ii) documented mastocytosis based on histological criteria of typical mast cell infiltrates in a multifocal or diffuse pattern in skin and/or bone marrow biopsy. The latter criterion encompasses all patients satisfying the WHO classification but additionally selects those patients matching inclusion criteria from the masitinib phase 2 trials and AFIRMM survey. Consequently, these inclusion criteria are slightly broader than the WHO classification.

To ensure consistency in the investigators' application of diagnostic criteria a blinded central document reading was used to verify patient eligibility for inclusion to the intent to treat (ITT) population based on a set of unifying criteria. Possible post-randomization imbalance was managed via weighting of each observation (see below).

The retained classification of systemic mastocytosis was based on an excess of mast cells or a presence of abnormal mast cells in at least two organs. All patients have excess presence of mast cells in the skin, expressing D816V mutation or not. The central document reading was therefore based on findings from the bone marrow or digestive organs.

Patient classification via the central document reading was reviewed and validated before unblinding by Olivier Hermine (Head of the Centre de Référence des Mastocytoses, CEREMAST, France) and Olivier Lortholary, international coordinator of study AB06006.

Central document reading defined systemic mastocytosis based on the following criteria, present in the records of the patients:

1) Bone marrow biopsy or aspirate associated with at least one sign of abnormality of mast cells, wherein said abnormal signs are:
   a) Abnormal aggregates of mast cells in a sample in bone marrow:
      The criterion was deemed satisfied if the aggregate was: i) quantified and strictly above 15 mast cells per aggregated (corresponding to WHO major criterion), or ii) not quantified but had been described as nodule, seat, cluster, focus, or granuloma and therefore pathological.
   b) More than 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 with microscopic testing that could 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 criterion was deemed satisfied if the infiltration was: i) quantified and is strictly above 3% in the biopsy, or ii) not quantified but is abnormal as described with infiltration, contingent of mast cells, or proliferation and therefore pathological.
2) Detection of KIT-D816V in the bone marrow without evidence of mast cells in bone marrow but with evidence of KIT-D816V in skin, justifying clonality.
3) Excess of mast cells in digestive organs.

A tryptase serum level of greater than 20 ng/mL, which is a condition of the WHO classification, was not retained in the central document reading classification because this criterion is not specific to systemic mastocytosis; for example, from the AFIRMM study in 593 patients, 32% of cutaneous mastocytosis have elevated tryptase level above 20 ng/mL [Hermine O, Lortholary O, Leventhal PS, et al. Case-control cohort study of patients' perceptions of disability in mastocytosis. PLoS One. 2008 May 28;3(5):e2266].

Patients with rare (low) and normal presence of mast cells in the bone marrow biopsy or aspirate and without signs of abnormality of mast cells were not retained in population of systemic mastocytosis as defined by the central document reading, as per recommendation of the medical experts.

80% of patients from the central document reading-based ITT population satisfied the WHO definition for indolent systemic mastocytosis (ISM) or smouldering systemic mastocytosis (SSM).

Weighting of the primary and secondary efficacy analysis

Because the aforementioned central review was performed after randomization, it was necessary to manage any post-randomization treatment-arm imbalance in the number of patients with a given severe mast cell mediator release symptom (pruritus, flushes, depression, or asthenia) via weighting of each observation.

The following weighting formula was used:

$$\text{Weight}_i = \frac{Ntot/2}{N\,trt_i}$$

Where:

- Ntot: is the total number of patients with a given symptom (either pruritus, flushes, depression, or asthenia)
- $Ntrt_i$ is the number of patients with a given symptom (either pruritus, flushes, depression, or asthenia) in the treatment group 'i' (either masitinib or placebo)

The primary efficacy analysis was performed with a weighted generalized estimating equation (GEE) model, using weights computed as defined above.

For example, if treatment group A comprises 40 patients with a given symptom and treatment group B comprises 50 patients with that symptom, for a total of 90 patients with this symptom, then the weights allocated to each observation will be equal to 45/40 = 1·125 in treatment group A and 45/50 = 0·9 in the treatment group B.

Likewise, secondary efficacy analyses were performed with similar weighted statistical models using weights computed as defined above in the appropriate number of severe mast cell mediator release symptoms.

Intention-to-treat population (ITT)

The ITT population was defined as all randomized patients presenting a documented systemic mastocytosis with severe mast cell mediator release symptoms as defined above. Patients were classified according to the treatment-arm to which they have been randomized, irrespective of the actual treatment received.

The ITT population comprised a total of 135 patients (71 and 64 patients in the masitinib and placebo arms, respectively).

Modified intention-to-treat (mITT) - primary efficacy analysis

The mITT population was prospectively defined as the population for primary efficacy analysis. The mITT population included all ITT patients with the exception of patients withdrawing prematurely from the study during the 24-week treatment period (W0–W24) for a well-documented non-treatment related cause.

Non-treatment related causes of patient withdrawal are as follows:

- No treatment intake
- Lost to follow-up
- Violation of inclusion and/or exclusion criteria
- Non-compliance with protocol for reason not related to toxicity
- Withdrawal of informed consent due to travel or move

Conversely, examples of treatment related causes of withdrawal, considered as failure, are as follows:

- Adverse events (related or not)/toxicity
- Lack of efficacy
- Withdrawal of informed consent due to study procedure
- Withdrawal of informed consent due to unknown reason

Six patients from the ITT population, two from the placebo arm and four from the masitinib arm were excluded from the mITT population based on this definition. Among these patients, two were excluded due to no treatment intake, three patients were lost to follow-up, and one patient was withdrawn by the investigator due to violation of inclusion/exclusion criteria (low absolute neutrophil at baseline). Hence, the mITT population comprised a total of 129 patients (67 and 62 patients in the masitinib and placebo arms, respectively).

Notably, of those patients excluded from the mITT population only one had data at week 8 but was lost to follow-up thereafter. Consequently, the ITT and mITT populations were almost identical for efficacy assessment because pre-defined endpoints were based on cumulative response methodology, i.e. longitudinal analysis according to symptoms

(not patients) between weeks 8 and 24 with missing data considered as failure. That is to say, the ITT and mITT populations differed by just one patient-visit for the primary efficacy endpoint.

Per protocol (PP) population

The PP population included all mITT patients except those considered as being in major protocol deviation by a blinded Data Review Committee. The criteria for a major protocol deviation are as follows:

- Investigator protocol violation involving discontinuation of a patient despite protocol safety rules stating that they should continue with or without dose reduction,
- Patients did not express willingness to discontinue study,
- Investigator recognized violation.

For the PP masitinib arm five patients were excluded due to investigator protocol violation of non-compliance to pre-defined protocol safety rules regarding dose reduction. Hence, the PP population comprised a total of 124 patients (62 and 62 patients in the masitinib and placebo arms, respectively).

Observed Cases (OC) dataset

For the observed cases dataset, no data imputation was performed for non-observed values, as opposed to replacement of incomplete data using the missing data considered as failure (MDF) approach.

Safety population (SAF) - primary safety analysis

The safety population (SAF) included all ITT patients with severe systemic mastocytosis who took at least one dose of study treatment (masitinib or placebo).

Two patients from the ITT population, one from each treatment-arm, were excluded from the SAF population due to no treatment intake. Hence, the SAF population comprised a total of 133 patients (70 and 63 patients in the masitinib and placebo arms, respectively).

B. Protocol amendment

Detailed explanation of the protocol amendment in study AB06006

Initial protocol versions, up to version 4·0, included mastocytosis patients with moderate and severe mast cell mediator release symptoms (also referred to as handicaps). During the course of study AB06006 there was a change in the safety profile of masitinib due to an emergent risk of severe neutropenia and severe skin toxicity. An amendment to the protocol of study AB06006 was therefore an unavoidable consequence of this development and was made with an objective to improve the benefit/risk balance. It took two protocol amendments (version 5·0 and version 6·0) to reach the intended benefit/risk balance for study AB06006. This safety amendment was therefore implemented between 3·5 and 2 years prior to unblinding.

- *Change in the severity of condition for eligible patients*

To increase the benefit/risk ratio of masitinib in non-oncology indications and following discussion with the authorities, the protocol for study AB06006 was amended to include only mastocytosis patients with severe handicap. EMA was consulted on this question through scientific advice in October 2011 and validated the increase of severity of handicaps. The implementation of this increase was performed in version 5·0 and version 6·0 of the protocol. Handicaps specified in inclusions criteria of study protocol v4·0 to v6·0 were strengthened:

- Pruritus score from ≥ 6 to ≥ 9
- Flushes frequency per week from ≥ 7 to ≥ 8
- Hamilton score from ≥ 10 to ≥ 19
- FIS score from ≥ 40 to ≥ 75

It took two protocol versions to reach the intended severity level of handicap because protocol version 5·0 still had some level of handicaps incompatible with severe handicap. For example:

- For depression, as measured by the Hamilton rating for depression (HAMD-17), definition of severity is a score ≥ 19. In protocol version 5·0 the severity level for inclusion was ≥ 14, a score of 14 corresponding to moderate depression.
- For frequency of flushes per week, a frequency ≥ 7 (protocol version 5·0) was interpreted as one per day, on average, whereas ≥ 8 is more than one per day.

These changes to the inclusion criteria effectively restricted the study's target population by excluding a certain group of patients that were initially eligible. Consequently, those patients that became ineligible were no longer included as part of the intention-to-treat (ITT) population, or any other dataset for efficacy analysis; however, data already collected would still be applicable for supportive safety analysis.

- *Change in patient population*

In an effort to further improve the benefit/risk balance, protocol v6·0 restricted the targeted population to severely symptomatic indolent systemic mastocytosis patients (including the subvariant of smouldering systemic mastocytosis). This decision was based on design considerations to target a more homogeneous population and also evidence from the scientific literature identifying indolent systemic mastocytosis as representing a more severe variant of the disease when compared against cutaneous mastocytosis; the exclusion of patients with cutaneous mastocytosis was therefore a consequence of this change. Regarding this latter point, the AFIRMM pathophysiological study showed that systemic mastocytosis patients experienced significantly more severe handicap than patients with cutaneous mastocytosis [Hermine et al., PLoS One. 2008 May 28;3(5):e2266]. For example, according to the overall patient assessment (OPA) results, 28% (23/82) of systemic mastocytosis patients reported severe to intolerable handicap versus 15% (5/33) of the cutaneous mastocytosis patients (P=0·0386). According to the AFIRMM questionnaire score results, systemic mastocytosis patients experienced more severe handicap than cutaneous mastocytosis patients with median AFIRMM scores of 124 and 84, respectively (P=0·0225). Overall, OPA and AFIRMM score data show that systemic mastocytosis patients experienced more severe handicap than patients with cutaneous mastocytosis. Again, this change to the exclusion criteria effectively restricts the study's target population. Patients no longer meeting this revised criteria, i.e. patients with cutaneous mastocytosis, were excluded from the final ITT population for efficacy analysis of study AB06006; although, data already collected could still be applicable for supportive safety analysis.

- *Use of repeated measures in the statistical analysis*

In the protocols up to version 5·0, the main statistical analysis was the response on at least one handicap at week 24. To counteract rarity of the restricted target population, in what was already a very limited population due to mastocytosis being an orphan disease, the main statistical analysis was amended to be performed on repeated measurements over time and over handicaps. This approach is endorsed in the EMA guideline on clinical trials in small populations

(CHMP/EWP/83561/2005). In this guideline EMA recommends to "minimize bio-noise" in the context of a small population study, by using repeated measures analysis. In the guideline it is mentioned that "Repeated measurements over time – or in different body locations – may also improve the efficiency of an analysis".

The implementation of the repeated measures analysis was performed in version 6·0 of the protocol, with main statistical analysis being the analysis of response repeated over handicap and over time from week 8 to week 24. Thus, cumulative response by patient*handicap was the primary variable/endpoint for the analysis. 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 (from among pruritus, flushes, depression and asthenia).

- *Increase in the cut-off point for response up to 75% improvement of the baseline handicap*

To further improve the benefit/risk balance following discussion with authorities for indications in non-oncology and 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.

EMA was consulted on this question through scientific advice in October 2011 and EMA validated the increase in the cut-off point for response to at least a 75% improvement of the baseline handicap. EMA commented 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). As previously mentioned, it took two protocol versions to reach the intended cut-off point for response; protocol version 5·0 still had some cut-off points for response below 75%. For instance, in protocol version 5·0, the response for Hamilton score was stated as an improvement of two severity categories (HAMD-17 categories: 0-7 normal, 8-13 mild depression, 14-18 moderate depression, 19-22 severe depression, ≥ 23 very severe depression), which corresponds for a baseline level of 14 as an improvement of 50%, and for a baseline level of 19 as an improvement of 30%, even lower that the initial cut-off point for response of 50%. This category-based approach was therefore inappropriate for achieving the intended effect of enhanced clinical relevance; consequently the Hamilton response was define using a cut-off point equal to 75% improvement, as for the other primary analysis handicaps.

C. Patient eligibility for study AB06006

Full patient eligibility criteria for study AB06006 (as per the amended protocol version 6·0) are presented below.

Inclusion criteria

1. Patient with one of the following documented mastocytosis as per WHO classification:
- Smouldering Systemic Mastocytosis
- Indolent Systemic Mastocytosis

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 optimized dose:
- Anti H1
- Anti H2
- Proton pump inhibitor
- Osteoclast inhibitor
- Cromoglycate Sodium
- Antileukotriene

4. Handicapped status defined as at least two of the following handicaps, including at least one among pruritus, flushes, depression and 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 ≤ 2.
7. Patient with adequate organ function:
- Absolute neutrophils count (ANC) ≥ 2·0 x 10^9/L
- Haemoglobin ≥ 10 g/dL
- Platelets (PTL) ≥ 100 x 10^9/L
- AST/ALT ≤ 3 x ULN (≤ 5 x ULN in case of liver mast cell involvement)
- Bilirubin ≤ 1·5 x ULN
- Creatinine clearance >60 mL/min (Cockcroft and Gault formula)
- Albumin >1 x LLN
- Urea ≤ 1·5 x ULN
- Proteinuria < 30 mg/dL on the dipstick; in case of proteinuria ≥ 1+ on dipstick, 24 hours proteinuria should be <1·5 g/24 hours

8. Male or female patient aged 18 to 75 years, weight > 50 kg, 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. Acceptable forms of contraception include:
- A documented placement of an intrauterine device (IUD) or intrauterine system (IUS) and the use of a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).
- Documented tubal ligation (female sterilization). In addition, a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository) should also be used.
- Double barrier method: Condom and occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.

- Any other contraceptive method with a documented failure rate of <1% per year.
- Abstinence when this is in line with the preferred and usual lifestyle of the patient.

10. Male patients must use medically acceptable methods of contraception if your female partner is pregnant, from the time of the first administration of the study drug until three months following administration of the last dose of study drug. Acceptable methods include:

- Condom
- If you have undergone surgical sterilization (vasectomy with documentation of azoospermia) a condom should also be used

Male patients must 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. The acceptable methods of contraception are as follows:

- Condom and occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository
- Surgical sterilization (vasectomy with documentation of azoospermia) and a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository)
- Your female partner uses oral contraceptives (combination oestrogen/progesterone pills), injectable progesterone or subdermal implants and a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository)
- Medically prescribed topically-applied transdermal contraceptive patch and a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository)
- Your female partner has undergone documented tubal ligation (female sterilization). In addition, a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository) should also be used
- Your female partner has undergone documented placement of an intrauterine device (IUD) or intrauterine system (IUS) and the use of a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository)
- Abstinence when this is in line with the preferred and usual lifestyle of the patient

11. Male or female patients of child bearing potential must agree to use two methods (one for the patient and one for the partner) of medically acceptable forms of contraception during the study and for three months after the last treatment intake. Female patients must have a negative result in the pregnancy tests at screening and baseline.
12. Patient must be able and willing to comply with study visits and procedures per protocol.
13. Patient must understand, sign, and date the written voluntary informed consent form at the screening visit prior to any protocol-specific procedures performed.
14. 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.
15. 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 leukaemia (MCL)
- Aggressive systemic mastocytosis (ASM)

2. Previous treatment with any tyrosine kinase inhibitor.
3. Patient presenting with cardiac disorders defined by at least one of the following conditions:

- Patient with recent cardiac history (within 6 months) of:
  - Acute coronary syndrome
  - Acute heart failure (class III or IV of the NYHA classification)

- 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.

<u>Previous treatment</u>

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.

D. Dose adjustment procedures for study AB06006

Subjects enrolled received a total daily dose of 6 mg/kg masitinib or a matching placebo, to be taken during meals. In the event of severe toxicity related to masitinib, treatment interruption or dose reduction was permitted according to predefined criteria.

If a dose reduction is necessary, the patient will receive 4·5 mg/kg/day. The dose of study treatment to be administered according to patient's weight, after a dose reduction to 4·5 mg/kg/day (randomization dose: 6 mg/kg/day), is shown below.

		4·5 mg/kg/day		
Patient's weight in kg		**Daily dose (mg)**	**Morning* (mg)**	**Evening** (mg)**
	≤41·6		STOP	
> 41·6	58·3	200	100	100
> 58·3	77·7	300	100	200
> 77·7	99·9	400	200	200
>99·9		500	200	200+100

**am: the tablets should be taken during breakfast; pm: the tablets should be taken during the dinner*

Described below is the general dose reduction risk management plan for an adverse event suspected to be related to study treatment. Study treatment refers to masitinib or its matching placebo.

- At the first occurrence of moderate adverse event, study treatment will be interrupted until said adverse event has returned to baseline value or mild intensity and then resumed at the same dose level.
- If the same moderate adverse event re-occurs, study treatment will be interrupted until said adverse event has returned to baseline or mild intensity and then resumed with a dose reduction (4·5 mg/kg/day).
- If the same moderate adverse event re-occurs after dose reduction, study treatment must be definitely discontinued
- In case of severe adverse event, study treatment will be interrupted until said adverse event has returned to baseline level or mild intensity and then resumed with a dose reduction (4·5 mg/kg/day).
- In case of severe adverse event re-occurs, discontinue definitely study treatment.
- In case of life-threatening or disabling adverse event, study treatment must be definitely discontinued.

No dose escalation is authorized for patients who have had a dose reduction for safety reasons.

A detailed description of safety rules for specific safety events or risk (regardless of the causal relationship to study treatment) including neutropenia, renal disorders, hypoalbuminemia, liver disorders, cardiac disorders, reproductive system disorders and pregnancy, skin toxicity, oedema, nausea or vomiting, diarrhoea, dehydration, pulmonary disorders, ocular disorders, and carcinogenicity, can be found in the online study protocol at www.ab-science.com/pdf/Lortholary_et_al_Lancet_Protocol_online.pdf.

II. SUPPLEMENTARY TABLES

Table S1. Additional efficacy results: Long-term (2-year [W8-W96]) follow-up for primary endpoint; secondary analyses representative of mast cell activity or burden (24-week treatment period, mITT dataset); other secondary analyses (24-week treatment period, mITT dataset)

	Masitinib	Placebo	Delta[a]	*P* value	Odds Ratio (CI95)
Long-term follow-up					
4R75% [W8-W96][b]	16·8%	6·8%	10·0%	0·0156	3·5 (1·3-9·7)
Secondary analyses representative of MC activity or burden					
Tryptase level (relative) [W24][c]	-18·0% (21·4)	2·2% (26·9)	20·2%	<0·0001	N/A
Tryptase level (µg/L) [W24][d]	-10 (46·9)	2·7 (20·0)	12·7 µg/L	0·0267	N/A
BSA UP [W8-W24][e]	-12·3% (26·4)	15·9% (59·8)	28·2%	0·0210	N/A
Darier's sign [W8-W24][f]	18·9%	2·7%	16·2%	0·0187	6·6 (1·0-41·5)
Other secondary analyses					
Micturition frequency [W8-W24][g]	1·6%	0·0%	1·6%	0·12	2·7 (0·4-18 2)
Stool frequency [W8-W24][h]	2·5%	12·0%	-9·5%	0·0353	0·3 (0·07-1·34)
OPA [W8-W24][i]	3·7%	0·6%	3·1%	0·06	6·33 (0·7-56·1)
AFIRMM [W8-W24][j]					
Item #1 (Itching)	22·7%	11·0%	11·7%	0·0005	2·39 (1·45-3·93)
Item #2 (Erythemateous crisis)	24·0%	11·9%	12·1%	0·0004	2·33 (1·45-3·74)
Item #12 (Stools)	11·4%	25·1%	-13·7%	0·0002	0·38 (0·22-0·64)
Item #13 (Pseudo occlusive syndrome)	33·9%	20·6%	13·3%	0·0102	1·99 (1·18-3·36)
Item #17 (Rheumatology/Mobility)	31·9%	19·9%	12·0%	0·0059	1·90 (1·20-2·99)
Item #24 (Cephalgias)	30·2%	15·3%	14·9%	0·0002	2·40 (1·51-3·82)
Item #25 (Vertigo)	34·7%	22·6%	12·1%	0·0118	2·40 (1·51-3·82)
Item #28 (Irritability)	19·0%	8·9%	10·1%	0·0015	2·39 (1·39-4·12)
Item #30 (Paranoia, hallucination)	59·6%	37·0%	22·6%	0·0315	2·56 (1·10-5·97)
Item #38 (Incontinence)	45·5%	23·9%	21·6%	0·0087	2·64 (1·27-5·47)
Item #42 (Tinnitus)	35·2%	22·0%	13·2%	0·0160	1·92 (1·13-3·27)
Item #44 (Stomatitis)	42·7%	25·0%	17·7%	0·0068	2·26 (1·25-4·07)
Item #46 (Libido)	17·7%	26·6%	-8·9%	0·0470	0·60 (0·36-0·99)
QLQ-C30 global (absolute) [W24][k]	13·0 (24·6)	12·2 (16·5)	0·8	0·72	N/A

[a] Delta = difference between masitinib and placebo arms (masitinib minus placebo). [b] 4R75% = cumulative response [timeframe week 8 to week 96] in at least one of four severe baseline symptoms (pruritus or flushes or depression or asthenia). Cumulative response based on GEE model, missing data considered as failure (MDF) (see text for explanation); response rates expressed as ratio of sum of actual responses over timeframe divided by the total number of possible responses over the same period with response defined as an improvement of ≥75% from baseline. [c] Mean change (±SD) of tryptase level at W24 relative to baseline in patients with baseline tryptase level >20 µg/L. [d] Mean absolute (µg/L) change (±SD) of tryptase level at W24 in patients with baseline tryptase level >20 µg/L. [e] Mean change (±SD) in body surface area affected by urticaria pigmentosa relative to baseline (GEE model, timeframe week 8 to 24). [f]Disappearance of Darier's sign (GEE model, timeframe week 8 to 24). [g] Cumulative response in micturition frequency among patients with a baseline of ≥8 per day (GEE model, model, timeframe week 8 to 24). [h] Cumulative response in stool frequency among patients with a baseline of ≥4 per day (GEE model, timeframe week 8 to 24). [i] Cumulative response in OPA score among patients with a baseline of ≥3 (GEE model, timeframe week 8 to 24). [j] Cumulative response in AFIRMMv2 item scores among patients with a baseline score of ≥3 on said item (GEE model, timeframe week 8 to 24, observed cases dataset) (only items having significant difference between treatment-arms are presented, with no difference seen for all other items). [k] Mean absolute change (±SD) of QLQ-C30 global health status at W24 according to observed cases dataset. GEE = generalized estimating equation. MC = mast cell. UP =

urticaria pigmentosa. mITT = modified intention-to-treat population. CI = confidence interval. N/A = not applicable. BSA = Body surface area corrected with Wallace formula. OPA = overall patient assessment score. Database lock 24 November 2015.

Long-term analysis was performed over the timeframe week 8 to week 96. Success in the primary endpoint was sustained over the long-term, masitinib being associated with a cumulative response of 16·8% (220·0 responses of 1306·0 possible responses) compared with 6·8% for placebo (89·3 of 1306·0; difference 10·0%; odds ratio 3·5; 95% CI 1·3–9·7; p=0·0156).

Success in the primary endpoint was also corroborated by objective endpoints representative of mast cell activity or burden (e.g. serum tryptase, Darier's sign and urticaria pigmentosa).

Other secondary analyses showed no improvement for masitinib when compared with placebo, including an inferior cumulative response rate for masitinib on the symptomatic endpoint of stool frequency (2·5% vs. 12·0%, respectively). This outcome is attributed to diarrhea being an adverse event commonly associated with masitinib treatment during the initial 6 months (50·0% vs. 20·6% in the masitinib and placebo treatment-arms, respectively; see Table S4) and therefore a confounding influence; although as seen in Tables 4 and S3 this adverse event is transitory in nature with the incidence (per 100 patient-months) improving over the long term (i.e. 96 weeks) to approach that of placebo. Likewise, secondary endpoints based on global quality of life measures showed no statistical difference between treatment-arms, an observation that could be attributed to none of the instruments used (i.e. OPA, AFIRMMv2, QLQ-C30) being validated for mastocytosis. However, significant differences in cumulative response rate are seen between treatment-arms when detailed scale items, e.g. the 52 items that comprise the AFIRMMv2 questionnaire, were individually analyzed. Presented in Table S1 are those items for which masitinib was statistically superior or inferior to placebo (P<0·05), with no difference seen between treatment-arms for all other items.

Table S2. Failure of optimal symptomatic treatment with respect to patient eligibility. The criterion of optimal symptomatic treatment failure is met for any given patient following documented failure of at least one of the listed treatments used at an optimized dose for at least one of the listed baseline severe mast cell mediator release symptoms (i.e. pruritus, flushes and depression).

	Dose	Duration
H1 Antihistamine	RUD RUD	1M† 3M‡
H2 Antihistamine	RUD	6W
Proton pump inhibitors	RUD	6W
Sodium cromoglicate	RUD	6W
LTRA	RUD	6W
Local corticosteroid		6W
Anti depressive drug		3M

†For treatment of flushes or depression. ‡ For treatment of pruritus. Severe baseline symptoms defined as: pruritus score ≥9, number of flushes per week ≥8, Hamilton rating scale for depression score ≥19. LTRA = leukotriene antagonists. RUD = Recommended usual dose. M = month, W = week.

Treatment failures included: H1- and H2-antihistamines, proton pump inhibitors (PPI), sodium cromoglicate, antidepressants, leukotriene antagonists, interferon-alpha, 2-CdA, and corticosteroids.

Failure to the cytoreductive therapies of interferon-alpha or 2-CdA was 11·9% versus 16·1% of patients from the masitinib and placebo treatment-arms, respectively. Patients were refractory to standard treatments intended to relieve the most recurrent symptoms of mastocytosis; notably pruritus, excessive gastric acid and inflammation.

- Failure to systemic or local antihistamines was 97·0% versus 96·8%, respectively.
- Failure to sodium cromoglicate was 40·3% in both arms.
- Failure to proton pump inhibitors was 23·9% versus 29·0%, respectively.
- Failure to leukotriene antagonists was 19·4% versus 12·9%, respectively.

Table S3. Most frequent (≥ 2 occurrences) non-fatal serious AEs during 24-week treatment period and overall study duration (according to incidence in patient-months) (SAF population, regardless of causality)

	N(%) of patients [W0-W24]		Incidence (Overall study period)	
	Masitinib (N=70)	Placebo (N=63)	Masitinib (pm=1321)	Placebo pm=1031)
Diarrhea	3 (4·3%)	1 (1·6%)	0·2	0·1
Urticaria	2 (2·9%)	0 (0·0%)	0·2	0·0

All data refers to safety (SAF) population. AE = adverse event. Data presented as number of patients (%) with at least one AE. AE reported according to any causality. Database lock 24 November 2015.

Table S4. Most frequent (>2·5% difference between treatment-arms) severe AEs and overall AEs during 24-week treatment period (SAF population, regardless of causality)

	Severe AE			All AE		
	Masitinib (N=70)	Placebo (N=63)	Delta[†]	Masitinib (N=70)	Placebo (N=63)	Delta[†]
Diarrhea	8 (11·4%)	1 (1·6%)	9·8%	35 (50·0%)	13 (20·6%)	29·4%
Rash	4 (5·7%)	0 (0·0%)	5·7%	11 (15·7%)	3 (4·8%)	10·9%
Asthenia[‡]	4 (5·7%)	1 (1·6%)	4·1%	18 (25·7%)	11 (17·5%)	8·2%
Peripheral edema	2 (2·9%)	0 (0·0%)	2·9%	15 (21·4%)	5 (7·9%)	13·5%
Pruritus	3 (4·3%)	1 (1·6%)	2·7%	16 (22·9%)	9 (14·3%)	8·6%
Neutropenia[*]	3 (4·3%)	1 (1·6%)	2·7%	9 (12·9%)	7 (11·1%)	1·8%

All data refers to safety (SAF) population. AE = adverse event. Data presented as number of patients (%) with at least one AE. AE reported according to any causality. [‡]asthenia = loss of energy. [*]Severe neutropenia is defined as an absolute neutrophil count <1·0 x 10^9/L. [†]Delta = difference between masitinib and placebo arms (masitinib minus placebo). Database lock 24 November 2015.

Table S5. All severe AEs during 24-week treatment period with at least one event in the masitinib treatment-arm (SAF population, regardless of causality)

System Organ Class/Preferred Term	Masitinib (N=70)	Placebo (N=63)
Blood and lymphatic system disorders	5 (7·1%)	5 (7·9%)
Neutropenia	3 (4·3%)	1 (1·6%)
Febrile neutropenia	1 (1·4%)	0 (0·0%)
Leukocytosis	1 (1·4%)	2 (3·2%)
Lymphadenopathy	1 (1·4%)	0 (0·0%)
Cardiac disorders	1 (1·4%)	0 (0·0%)
Palpitations	1 (1·4%)	0 (0·0%)
Eye disorders	1 (1·4%)	0 (0·0%)
Eyelid oedema	1 (1·4%)	0 (0·0%)
Gastrointestinal disorders	12 (17·1%)	3 (4·8%)
Diarrhoea	8 (11·4%)	1 (1·6%)
Nausea	2 (2·9%)	1 (1·6%)
Aphthous stomatitis	1 (1·4%)	0 (0·0%)
Glossitis	1 (1·4%)	0 (0·0%)
Haemorrhoids	1 (1·4%)	0 (0·0%)
Irritable bowel syndrome	1 (1·4%)	0 (0·0%)
Rectal spasm	1 (1·4%)	0 (0·0%)
General disorders and administration site conditions	6 (8·6%)	2 (3·2%)
Asthenia	4 (5·7%)	1 (1·6%)

System Organ Class/Preferred Term	Masitinib (N=70)	Placebo (N=63)
Pyrexia	2 (2·9%)	0 (0·0%)
Chills	1 (1·4%)	0 (0·0%)
Face oedema	1 (1·4%)	0 (0·0%)
Localised oedema	1 (1·4%)	0 (0·0%)
Oedema peripheral	1 (1·4%)	0 (0·0%)
Hepatobiliary disorders	1 (1·4%)	0 (0·0%)
Cholestasis	1 (1·4%)	0 (0·0%)
Immune system disorders	1 (1·4%)	1 (1·6%)
Allergic oedema	1 (1·4%)	0 (0·0%)
Infections and infestations	3 (4·3%)	1 (1·6%)
Hand-foot-and-mouth disease	1 (1·4%)	0 (0·0%)
Pharyngitis	1 (1·4%)	0 (0·0%)
Viral infection	1 (1·4%)	0 (0·0%)
Investigations	7 (10·0%)	8 (12·7%)
Neutrophil count decreased	2 (2·9%)	1 (1·6%)
Alanine aminotransferase increased	1 (1·4%)	0 (0·0%)
Aspartate aminotransferase increased	1 (1·4%)	0 (0·0%)
Blood alkaline phosphatase increased	1 (1·4%)	0 (0·0%)
Blood phosphorus decreased	1 (1·4%)	0 (0·0%)
Gamma-glutamyltransferase increased	1 (1·4%)	0 (0·0%)
Investigation	1 (1·4%)	0 (0·0%)
Lymphocyte count increased	1 (1·4%)	0 (0·0%)
White blood cell count increased	1 (1·4%)	0 (0·0%)
Musculoskeletal and connective tissue disorders	4 (5·7%)	2 (3·2%)
Intervertebral disc protrusion	1 (1·4%)	0 (0·0%)
Muscle spasms	1 (1·4%)	0 (0·0%)
Osteoarthritis	1 (1·4%)	0 (0·0%)
Pain in extremity	1 (1·4%)	0 (0·0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (1·4%)	1 (1·6%)
Bladder cancer	1 (1·4%)	0 (0·0%)
Nervous system disorders	3 (4·3%)	3 (4·8%)
Headache	3 (4·3%)	3 (4·8%)
Psychiatric disorders	1 (1·4%)	0 (0·0%)
Depression	1 (1·4%)	0 (0·0%)
Reproductive system and breast disorders	1 (1·4%)	0 (0·0%)
Genital lesion	1 (1·4%)	0 (0·0%)
Respiratory, thoracic and mediastinal disorders	2 (2·9%)	1 (1·6%)
Dyspnoea	1 (1·4%)	0 (0·0%)
Pneumothorax	1 (1·4%)	0 (0·0%)
Skin and subcutaneous tissue disorders	11 (15·7%)	2 (3·2%)
Rash	4 (5·7%)	0 (0·0%)
Pruritus	3 (4·3%)	1 (1·6%)
Drug eruption	1 (1·4%)	0 (0·0%)
Erythema multiforme	1 (1·4%)	0 (0·0%)
Palmar-plantar erythrodysaesthesia syndrome	1 (1·4%)	0 (0·0%)
Urticaria	1 (1·4%)	0 (0·0%)
Vascular disorders	1 (1·4%)	0 (0·0%)
Flushing	1 (1·4%)	0 (0·0%)

III. SUPPLEMENTARY DATA AND DISCUSSION

A. Extended discussion on masitinib effect on serum tryptase level

Success in the primary analysis was corroborated by positive results in secondary endpoints such as a significant change in tryptase level between treatment-arms (-18·0% versus +2·2%, respectively, P<0·001). Tryptase is the most abundant mediator stored in mast cell granules and the release of tryptase by mast cell granules is a characteristic feature of mast cell degranulation and mast cell burden. For this reason the change in serum tryptase level is considered to represent an objective marker for decrease in mast cell activation and/or burden. Moreover, since one of the primary mechanisms of action of masitinib is a reduction of mast cell degranulation, via Lyn and Fyn inhibition, then tryptase levels pre and post treatment may represent mechanistic evidence of masitinib treatment by reducing mast cell activation.

Additionally, because tryptase level is an established biomarker for bone marrow involvement in indolent systemic mastocytosis, the observed decrease in mean tryptase level may be indicative of a treatment related impact on mast cell infiltration in bone marrow [Donker ML, et al. Haematologica. 2008 Jan;93(1):120-3]. However, given that masitinib is a weak inhibitor of KIT-D816V any such conclusion would need to be supported by more direct evidence, such as measurement of KIT-D816V allele burden before and after treatment; a test that was not performed for this study. It is not ruled-out, however, that normal KIT-WT mast cells may have decreased.

Analysis of change in baseline tryptase level was exceptionally based on a single post-baseline timepoint at W24, rather than the repeated measures analysis used for other endpoints. It was of interest therefore to plot the relative change in baseline serum tryptase level for individual patients following 24 weeks of masitinib treatment among patients presenting with tryptase ≥20 ng/µL at baseline. It can be seen that the majority of patients tested experienced a reduction in their serum tryptase level with respect to baseline and probably therefore a reduction in mast cell activity or burden. Notably, 95% of assessable masitinib patients were positive for KIT-D816V (the remainder had unknown status) showing that this treatment impact on mast cell activity is not restricted to KIT-WT (i.e. KIT-D816V negative) patients.

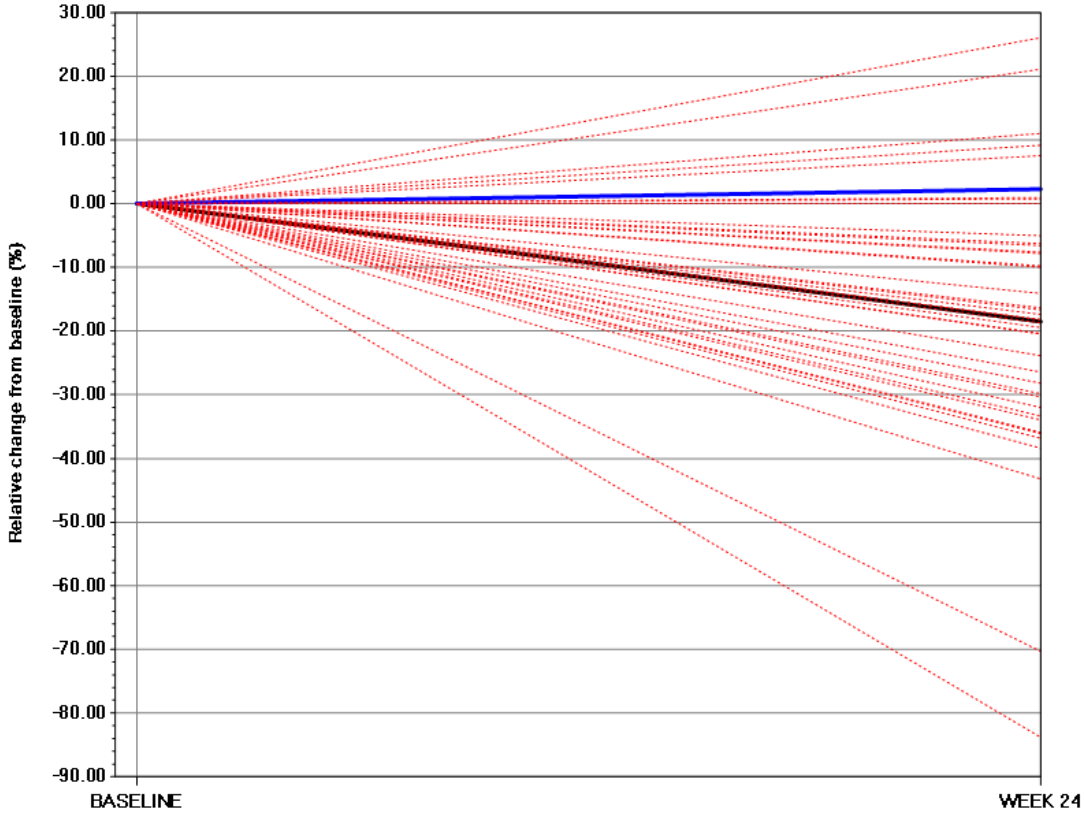

Figure S1: Relative change in baseline serum tryptase level for individual patients following 24 weeks of masitinib treatment among patients presenting with tryptase ≥20 ng/µL at baseline (mITT population). Black bold line shows the average relative change in serum tryptase for the masitinib treatment-arm (n=40). Blue bold line shows the average relative change in serum tryptase for the placebo arm (n=42).

B. Extended discussion on masitinib efficacy in KIT-816V patients

Masitinib is an oral tyrosine kinase inhibitor that targets wild-type (WT) KIT (IC_{50} 200 nM) with greater potency than KIT-D816V (IC_{50} 5·0 µM), and additionally targets LYN and FYN at submicromolar concentrations. WT-KIT, LYN and FYN play critical roles in the survival and function of mast cells including mediator release.

The KIT-D816V (KIT Asp816Val) mutation has been identified as a predominant pathogenic feature in systemic mastocytosis. Imatinib, a WT-KIT inhibitor similar to masitinib, is registered for the treatment of aggressive forms of systemic mastocytosis such as mast cell leukemia and aggressive systemic mastocytosis in which no KIT-D816V is detectable. In general, imatinib is not recommended for patients with KIT-D816V-positive systemic mastocytosis [Valent P, et al. Am J Blood Res. 2014 Dec 15;4(2):93-100]

These aspects (i.e. masitinib's inactivity against KIT-D816V and the historic use of imatinib for aggressive forms of systemic mastocytosis in WT-KIT patients only) raise a question of whether the therapeutic benefits observed for masitinib-treated patients in study AB06006 are influenced by the patient's KIT-D816V status. More specifically, can we be certain that masitinib is effective in treating severely symptomatic indolent systemic mastocytosis in which KIT-D816V is detectable (i.e. KIT-D816V positive patients).

Regarding masitinib's mechanism of action, because the KIT-D816V mutation may not activate mast cells to release proinflammatory mediators, which is consistent with clinical observations that type and severity of symptoms are KIT-D816V-independent, the inactivity of masitinib against this target is not an obstacle (see discussion below on mechanism of action, section III-C).

This reasoning is upheld by subgroup analysis in KIT-D816V-positive patients showing a significant response in favor of masitinib, with a 4R75% of 20·2% versus 7·4%, odds ratio of 4·5 (95%CI 1·1-17·8, P=0·03) (see Table S6). Secondary endpoint analyses corroborate masitinib's efficacy in patients with KIT-D816V-positive status. Furthermore, of those KIT-D816V-positive masitinib patients assessable for change in serum tryptase level following 24 weeks (n=40), the majority (85%) experienced a reduction in their serum tryptase level with respect to baseline (Figure S1). Taken together, there appears little doubt that masitinib is capable of impacting on the mast cell activity of patients with KIT-D816V-positive status leading to reduction of mast cell mediator release symptoms that is not restricted to KIT-WT (i.e. KIT-D816V negative) patients.

Subgroup analysis for other KIT cohorts was not possible due to the small number of these patients, for example, there was only one WT-KIT patient (KIT-D816V-negative status) in the masitinib treatment-arm. However, masitinib has previously demonstrated activity in KIT-WT indolent systemic mastocytosis patients with mast cell mediator release symptoms in a phase 2 study setting, as reported in the article by Paul and colleagues [Paul C, et al. Am J Hematol. 2010; 85:921-5].

Table S6. Summary of subgroup analysis for patients with positive KIT-D816V status - cumulative response analyses [week 8 - week 24] for modified intention-to-treat population (weighted GEE model[‡])

	Masitinib	Placebo	Delta[†]	*P* value	Odds Ratio (CI95)
4R75%	20·2% (117·6/581·5)	7·4% (42·8/581·5)	12·8%	0·0345[*]	4·45 (1·11-17·77)
3R75%	26·6% (100·3/377·0)	9·9% (37·3/377·0)	16·7%	0·0051	3·36 (1·44 - 7·85)
2R75%	28·8% (76·8/266·5)	10·6% (28·2/266·5)	18·2%	0·0342	2·80 (1·08 - 7·27)

[‡]Cumulative response based on GEE model, missing data considered as failure. Response rates expressed as weighted ratio of sum of actual responses between weeks 8 and 24 divided by the total number of possible responses over the same treatment period. GEE = generalized estimating equation. 4R75% = cumulative response in at least one of four severe baseline symptoms (pruritus or flushes or depression or asthenia). [*]Based on re-randomization. [†]Delta = difference between masitinib and placebo arms (masitinib minus placebo). 3R75% = cumulative response in at least one of three severe baseline symptoms (pruritus or flushes or depression). 2R75% = cumulative response in at least one of two severe baseline symptoms (pruritus or flushes). CI = confidence interval. Database lock 24 November 2015.

Finally, any preconception that masitinib's activity should be restricted to KIT-WT (i.e. KIT-D816V negative) patients from comparisons draw to imatinib's use in aggressive forms of systemic mastocytosis is erroneous because indolent systemic mastocytosis is a very different disease variant from the aggressive forms of mastocytosis, in terms of pathology, prognosis and treatment options. Moreover, imatinib is not approved for patients with indolent systemic mastocytosis, the population of relevance here; indeed, there is currently no registered or established standard treatment for indolent systemic mastocytosis.

C. Extended discussion on masitinib mechanism of action in indolent systemic mastocytosis

Mutation of the KIT gene is common in mastocytosis. In indolent systemic mastocytosis, 90% of patients display D816V mutant c-Kit receptors. The D816V mutation leads to gain-of-function and ligand-independent constitutive activity of the c-Kit receptor and consequently mast cell accumulation, degranulation, and resistance to apoptosis. The remaining 10% of patients display WT c-Kit receptors and thus mast cell infiltration and activation may be linked to another mechanism; however, in such cases mast cells may still rely on c-Kit for their survival.

Masitinib belongs to a class of compounds named tyrosine kinase inhibitors (TKI). Its foremost cellular target is the mast cell, which plays a crucial role in the pathogenesis of mastocytosis. Masitinib is thought to regulate mast cell activity mainly due to its inhibitory potential against c-Kit, Lyn and Fyn [Dubreuil, 2009]. These kinases are highly expressed in mast cells and control many essential cell functions including mast cell growth, differentiation, survival and degranulation. Masitinib reduces the activation of mast cells mainly via its ability to inhibit WT c-Kit. Masitinib also effectively reduces mast cell degranulation, as evidenced by its ability to inhibit the release of both β-hexosaminidase and TNF-α by mast cells in a dose-dependent manner, through its inhibitory action against Lyn and Fyn [Dubreuil, 2009]. Lyn and Fyn are key components of the transduction pathway leading to IgE induced degranulation [Gilfillan, 2006; Gilfillan, 2009].

Dubreuil and colleagues have previously published preclinical data comparing the activity of masitinib against the benchmark TKI imatinib [Dubreuil, 2009]. *In vitro*, masitinib had greater activity and selectivity against KIT than imatinib, inhibiting recombinant human wild-type KIT with an half inhibitory concentration (IC_{50}) of 200±40 nM and blocking stem cell factor-induced proliferation and KIT tyrosine phosphorylation with an IC_{50} of 150±80 nM in Ba/F3 cells expressing human or mouse wild-type KIT. Molecular modelling and kinetic analysis suggested a different mode of binding than imatinib, with masitinib more strongly inhibiting degranulation, cytokine production, and bone marrow mast cell migration when compared with imatinib.

A theoretical model for the mechanism of action of masitinib in indolent systemic mastocytosis is illustrated in Figure S2. Considering those patients with WT c-Kit mast cells (i.e. a negative D816V c-Kit mutation status), masitinib can effectively inhibit mast cell activity and survival via its strong inhibitory effect on WT c-Kit receptors [Dubreuil, 2009]. Through its inhibition of c-Kit/SCF signaling, masitinib is therefore an effective anti-mast cell agent in c-Kit WT mast cells, exerting a direct anti-activation effect.

Considering those patients bearing D816V mutant c-Kit mast cells (i.e. a KIT-D816V-positive status), *in vitro* studies show that masitinib does not effectively inhibit the D816V mutant c-Kit receptor [Dubreuil, 2009]. Thus, alternative mechanisms of action must be implicated for the therapeutic benefits observed in indolent systemic mastocytosis patients with D816V mutant c-Kit. Indeed, although the D816V c-Kit mutation has been established as a predominant genetic aberration and driver of systemic mastocytosis pathogenesis, there is an emerging understanding that a wide variety of other KIT mutations and mast cell regulatory genes are also implicated [Gleixner, 2011; Molderings, 2015]. Additionally, it has been reported that the D816V c-Kit mutation does not activate mast cells to release proinflammatory mediators, consistent with clinical observations that type and severity of symptoms might be D816V c-Kit-independent [Hermine, 2008; Broesby-Olsen, 2013; Hoermann, 2014; Saleh, 2014]. Consequently, masitinib's inactivity again the D816V mutant c-Kit would not appear to be an obstacle in its ability to reduce symptoms via inhibition of mast cells mediator release in KIT-D816V-positive patients.

Masitinib's mechanism of action in D816V mutant c-Kit mast cells is possibly realized through its inhibition of Lyn and Fyn. These represent c-Kit-independent downstream kinases that affect mast cell functionality through their involvement in the intracellular signaling cascade of a communicating receptor, FcεRI. 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 [Metcalfe, 2008]. Because the mechanism of action of masitinib via Lyn and Fyn inhibition is independent from the c-Kit signaling pathway or survival of mast cells, it will therefore affect mast cells with both WT c-Kit and mutated D816V c-Kit.

Another consequence of inhibiting mast cell degranulation could be a reduced or retarded rate of mast cell recruitment and accumulation. SCF is a chemotactic factor for mast cells with the activating D816V c-Kit mutation showing enhanced cell migration towards the SCF source [Taylor, 2001]. Furthermore, mast cells themselves possess the capacity to synthesize, store and release SCF. Together this could create a positive feedback loop for constitutively activated D816V mutant c-Kit mast cells, culminating in mast cell accumulation and organ infiltration. Preclinical data establish proof-of-concept that masitinib inhibits mast cell degranulation, thus it is a logical assumption that there will be a concomitant reduction in release of various cell migration-related chemoattractants. Such a treatment effect could lessen the rate of recruitment for both KIT-D816V mutant and KIT-WT mast cells, thereby inhibiting mast cell accumulation, cross-talk, and mediator release symptoms.

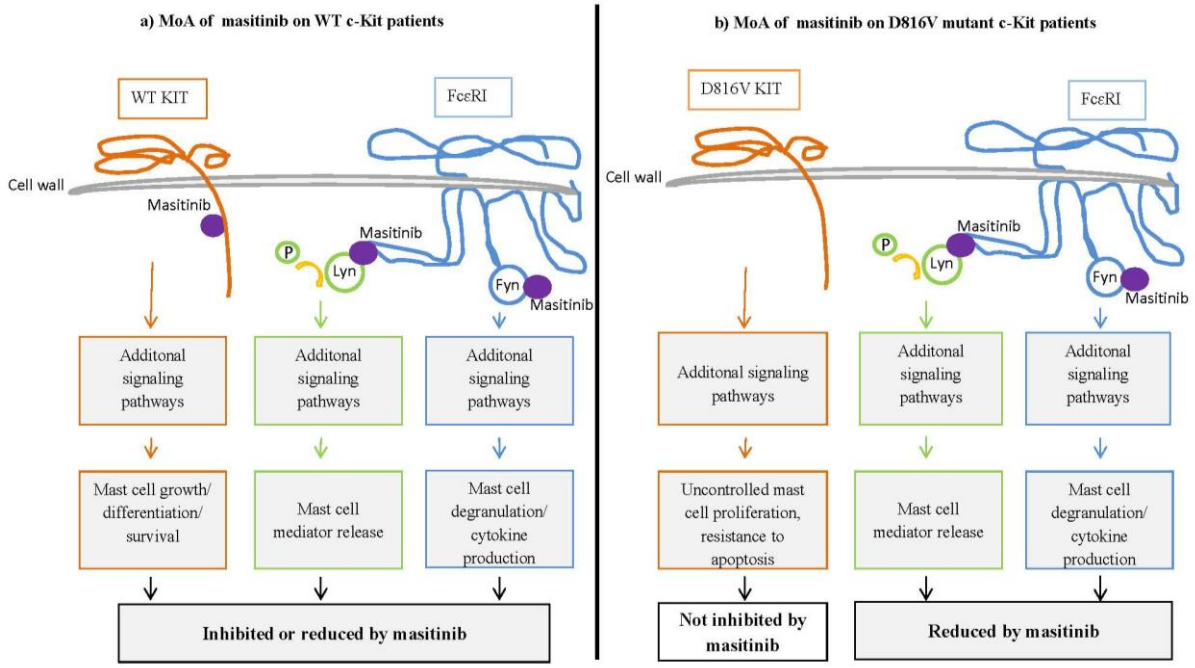

Figure S2: Theoretical model for the mechanism of action (MoA) of masitinib in indolent systemic mastocytosis. The c-Kit receptor is primarily responsible for mast cell growth, differentiation and survival with mast cell mediator release being initiated through the integration of downstream signaling pathways of c-Kit and FcεRI. D816V mutant c-Kit receptors result in uncontrolled mast cell proliferation and resistance to apoptosis. Masitinib blocks 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.

Overall, masitinib's anti-mast cell properties appear particularly well adapted to the treatment of indolent systemic mastocytosis with severe mast cell mediator release symptoms; a reduction of mast cell activity being generated via its inhibitory action on c-Kit, Lyn and Fyn tyrosine kinase activity. It is through this multifaceted mechanism of action, a feature not seen in similar c-Kit inhibitors such as imatinib, that masitinib can elicit a response in patients of both positive and negative D816V c-Kit mutation status, hence, the population of study AB06006.

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D. Extended discussion on post-hoc analysis from mastocytosis phase 2 studies

Findings from study AB06006 confirm observations from related phase 2 studies.

Two phase 2 studies were carried out:

- Study AB04010, enrolling 21 patients with indolent forms of mastocytosis without the D816V KIT mutation in at least one organ [Paul, 2010], including 16 patients with severe systemic mastocytosis.
- Study AB06013, enrolling 25 patients with indolent forms of mastocytosis with the D816V KIT mutation, including 12 patients with severe systemic mastocytosis.

Altogether, the pooled phase 2 cohort comprised 28 patients with severe systemic mastocytosis.

In a pooled phase 2 study post-hoc simulation of the phase 3 AB06006 population (n=28) the 4R75%, 3R75% and 2R75% response criteria, calculated from W8 to W24, were 28·6%, 33·6% and 39·7%, respectively [Hermine, 2015]. Likewise, the long-term 4R75%, 3R75% and 2R75% response criteria calculated from W8 to W96 were 18·0%, 21·2% and 25·2%, respectively.

Among these pooled patients, 24% received masitinib treatment for over 6 years with no signs of late toxicity.

References (section III-D):

- Hermine O, Moussy A, Mansfield C, Dubreuil P. Masitinib for the treatment of severe systemic mastocytosis: Pooled phase 2 study simulation of phase 3 population and response criteria. 2015. Proceedings of the EMBRN meeting (21-23 October 2015, Marseille, France). http://www.ab-science.com/pdf/Hermine_2015_EMBRN_Proceedings.pdf (accessed 08 November 2016).
- Paul C, Sans B, Suarez F, et al. Masitinib for the treatment of systemic and cutaneous mastocytosis with handicap: a phase 2a study. Am J Hematol. 2010; 85:921-5.

E. Extended discussion on identification of the intention-to-treat (ITT) population

Eligible patients had: (i) indolent systemic mastocytosis (ISM/SSM) according to the WHO classification; or (ii) documented mastocytosis based on histological criteria of typical mast cell infiltrates in a multifocal or diffuse pattern in skin and/or bone marrow biopsy. The latter criterion encompasses all patients satisfying the WHO classification but additionally selects those patients matching inclusion criteria from the masitinib phase 2 trials and AFIRMM survey. Consequently, these inclusion criteria are slightly broader than the WHO classification. To ensure consistency in the investigators' application of diagnostic criteria a blinded central document reading was used to verify patient eligibility for inclusion to the ITT population based on a set of unifying criteria (see section I-A). Hence, primary analysis was performed on the ITT population as defined via this central document review, which is not strictly the WHO classification.

This blinded central document reading was conducted following the end of patient recruitment and before data unblinding. Patient classification via the central document reading was reviewed and validated before unblinding by Olivier Hermine (Head of the Centre de Référence des Mastocytoses, CEREMAST, France) and Olivier Lortholary, international coordinator of study AB06006.

Among the 135 patients with severe systemic mastocytosis according the blinded central document reading, 108 (80%) fulfilled the criteria for WHO classification of systemic mastocytosis. Hence, 27 patients did not fulfil the WHO classification but were still eligible for inclusion to the ITT population according to the study's slightly broader definition of this population.

Among these 27 patients:

- 4 patients presented with an excess of mast cells in digestive organs (in addition to excess of mast cells in the skin).
- 6 patients presented with detection of c-Kit 816 in the bone marrow without evidence of mast cell infiltration in bone marrow at the histological level but with evidence of c-Kit 816 in skin, justifying clonality.
- 17 patients presented with bone marrow biopsy or aspirate associated with at least one sign of mast cell abnormality. Among these 17 patients:
    - 1 patient had 3 criteria of abnormality
        - Abnormal infiltration of mast cells in the bone marrow (>3%)
        - 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), and
        - Abnormal immunohistochemistry signs: mast cells in bone marrow express CD2 or/and CD25 present (corresponding to WHO minor criterion)
    - 7 patients had 2 criteria of abnormality, of which:
        - 4 patients with:
            - Abnormal infiltration of mast cells in the bone marrow (>3%)
            - 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)
        - 1 patient presented with:
            - Abnormal infiltration of mast cells in the bone marrow (>3%)
            - c-Kit point mutation at codon 816 in bone marrow
        - 2 patients presented with:
            - 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), and
            - c-Kit point mutation at codon 816 in bone marrow
    - 9 patients had 1 criteria of abnormality

- 1 patient presented with:
  - Abnormal immunohistochemistry signs: mast cells in bone marrow express CD2
- 5 patients presented with:
  - Abnormal infiltration of mast cells in the bone marrow (>3%)
- 2 patients presented with:
  - 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), and
- 1 patient presented with:
  - c-Kit point mutation at codon 816 in bone marrow

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