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Masitinib is Safe and Effective for the Treatment of Canine Mast Cell Tumors

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Background: Activation of the KIT receptor tyrosine kinase is associated with the development of canine mast cell tumors (MCT).

Hypothesis/Objective: To evaluate the efficacy of masitinib, a potent and selective inhibitor of KIT, in the treatment of canine MCT.

Animals: Two hundred and two client-owned dogs with nonmetastatic recurrent or nonresectable grade II or III MCT.

Methods: Double-blind, randomized, placebo-controlled phase III clinical trial. Dogs were administered masitinib (12.5 mg/kg/d PO) or a placebo. Time-to-tumor progression (TTP), overall survival, objective response at 6 months, and toxicity were assessed.

Resulsts: Masitinib increased overall TTP compared with placebo from 75 to 118 days ($P = .038$). This effect was more pronounced when masitinib was used as first-line therapy, with an increase in the median TTP from 75 to 253 days ($P = .001$) and regardless of whether the tumors expressed mutant (83 versus not reached [$P = .009$]) or wild-type KIT (66 versus 253 [$P = .008$]). Masitinib was generally well tolerated, with mild (grade I) or moderate (grade II) diarrhea or vomiting as the most common adverse events.

Conclusions and Clinical Importance: Masitinib is safe and effective at delaying tumor progression in dogs presenting with recurrent or nonresectable grade II or III nonmetastatic MCT.

Key words: Dog; KIT; Mast cell tumor.

Mast cell tumors (MCT) are the most common cutaneous tumors in dogs, accounting for 7–21% of all skin tumors. The behavior and progression of MCT are highly heterogeneous; some MCT are behaviorally benign, develop slowly, and persist for years without increasing in size, whereas others exhibit aggressive growth and progress rapidly to a fatal metastatic disease.[1,2]

The most commonly used system for grading MCT, developed by Patnaik et al[3] defines a grade I MCT as a well-differentiated tumor, grade II as a tumor with an intermediate phenotype, and grade III MCT as a poorly differentiated tumor. They reported that 4 years after surgical excision, survival for grade I MCT is 93%, but it decreases to only 44 and 6% for grades II and III, respectively. A more recent report by Murphy et al[4] reported 1-year survival rates of 100, 92, and 46% for grades I, II, and III, respectively. Because grade I and most grade II MCT do not typically metastasize, they usually can be controlled by complete surgical removal or marginal resection, followed by radiation therapy.[5–7] Chemotherapy (eg, IV vinblastine[8]or lomustine[9]), is reserved for dogs in which surgery and radiation therapy are not feasible, as an adjunct to these treatments, or for dogs presenting with grade III MCT. Thamm et al[8] reported an overall response rate to chemotherapy (vinblastine and prednisone) of 47% for grade II or III MCT, with a median response duration of 154 days. Although chemotherapy can prolong life by transiently controlling the disease, it has adverse effects, especially digestive disorders and hematologic abnormalities such as neutropenia. Therefore, existing therapeutic options are not satisfactory, and there is an unmet medical need for dogs suffering from MCT, especially those with nonresectable or recurrent grades II and III tumors.

Studies by us[10] and others[11–13] have shown that 20–30% of canine MCT express a mutated form of KIT, a receptor tyrosine kinase involved in the control of mast cell growth and differentiation. These studies have shown that the most frequent mutations in KIT lie in the juxtamembrane domain and cause kinase activation, although our study revealed that activating mutations in the 5th immunoglobulin-like domain on the extracellular portion of the receptor are also common. These mutations activate the KIT tyrosine kinase, implicating it in the pathogenesis of canine MCT. In support of this, mutations in KIT are associated with higher histologic grade

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MCT and poor prognosis.[11,12] Furthermore, a Phase I trial published in 2003 showed that SU11654 (Pfizer), an inhibitor of several receptor tyrosine kinases including KIT, causes significant shrinkage or stabilization of mast cell tumors at concentrations that inhibit KIT in vivo.[14,15] A more recent study by Kobie et al[16] also found that the tyrosine kinase inhibitor imatinib (Novartis) induces regression of xenografted canine mast cell tumors in SCID mice, and Gleixner et al[17] recently reported that a variety of tyrosine kinase inhibitors that can inhibit KIT suppress the proliferation of canine mastocytoma cell lines.

We have developed masitinib[a] as a potent and selective inhibitor of KIT. Masitinib inhibits wild-type human and murine KIT in vitro with an IC_{50} of approximately 200 nM, and administered PO masitinib blocks the growth of tumors expressing juxtamembrane-mutated KIT in mice (P. Dubreuil et al[18]). Here, we report on the results of a randomized-controlled phase III clinical trial to determine the safety and therapeutic potential of masitinib in dogs with nonresectable or recurrent grade II or III nonmetastatic MCT.

Patients and Methods

Study Design

The project was a multicenter, randomized, double-blind, placebo-controlled clinical field study with client-owned dogs with measurable nonresectable or recurrent grade II or III MCT without nodal or visceral metastasis, previously treated or not. On day 0, dogs were clinically staged on the basis of the following information and according to Patnaik et al[3]: CBC, serum biochemistry, thoracic radiography (2 views), abdominal sonography, regional lymph node fine needle aspiration biopsy (if palpable), buffy coat analysis, and biopsy for histopathology and grading of the tumor (if not performed previously).

Dogs eligible for the study were randomized to receive either placebo or masitinib, respectively. After enrollment, the dogs were seen by the investigator on days 7 ± 1, 14 ± 1, 28 ± 2, 42 ± 2, 56 ± 2, 84 ± 4, 112 ± 4, 140 ± 6, and 168 ± 6. Dogs entering the compassionate program also were seen every 12 weeks. At each visit, the following were performed: physical examination, clinical assessment of the tumor(s), urinalysis, CBC, and serum biochemistry. In addition, rectal temperature, heart rate, respiratory rate, and body weight were recorded on days 112 and 168; abdominal ultrasonography and thoracic radiography were performed, allowing for the detection of visceral metastases. Before initiation of the study, the protocol was reviewed and approved by the United States Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products. Each participating veterinary hospital followed guidelines established for Good Clinical Practice, and all dogs were cared for in accordance with each institution's animal care and use protocols.

Patients

Client-owned dogs were recruited from 25 veterinary centers in the United States and France. Inclusion criteria for the study were as follows: male and female dogs of any breed, at least 6 months old, at least 3.3 kg in weight, and at least 1 histopathologically confirmed, measurable, recurrent or nonresectable, nonmetastatic (without nodal or visceral metastasis) grade II or III MCT. Nonresectable tumors were those that were either medically nonresectable (eg, because the microscopic margins were unlikely to be free of disease if surgery was performed) or nonresectable according to the pet owner (ie, a resection leading to amputation would not be acceptable to the owner). Tumors were graded by the pathologist working for each investigator according to the system of Patnaik et al.[3] An experienced pathologist (BP) performed a secondary analysis of the skin biopsies to confirm the grading. Exclusion criteria included the following: lactating or pregnant bitches, dogs used for breeding, dogs under treatment (including corticosteroids, chemotherapy, or radiation therapy or some combination of these) within 2 weeks of entry into the study, dogs having a blood urea nitrogen (BUN) or serum creatinine concentration >1.5 times the upper limit of the normal reference range, dogs experiencing gastrointestinal bleeding as assessed by clinical signs, dogs with a life expectancy of <3 months, an absolute neutrophil count $<3{,}000/\mu L$, liver enzyme activity >2.0 times the upper limit of the normal reference range, or an abnormal liver structure as assessed by ultrasonography. After confirmation that the dog fulfilled all the inclusion and exclusion criteria, the owner signed the "Owner Information/Consent" form before any study procedures were started.

Drug Product and Concomitant Medications

Masitinib was available in nondivisible coated tablets.[a] It was initially administered at a daily dosage of 12.5 mg/kg PO, based on its IC_{50} versus human and murine KIT ($\sim$200 nM; P. Dubreuil, manuscript submitted) along with our toxicity and bioavailability studies in dogs and rats (unpublished observations: Pharmacokinetic studies in beagle dogs show that at an oral dose of 10 mg/kg, the maximum concentration (C_{max}) of masitinib in serum reaches 794 ± 94 ng-Eq/g for males and 901 ± 63 ng-Eq/g for females. This is equivalent to 1.3–1.5 μM). Medications for the treatment of adverse events, such as antibiotics, antiemetics, antidiarrheals, and antihistamines were allowed. The following treatments for MCT were prohibited during the study: surgery, radiation therapy, and other chemotherapy treatments (eg, vinblastine, lomustine, and prednisone). Masitinib was initially administered with an intent-to-treat period of 6 months unless progressive disease was confirmed. Dogs with a complete, partial, or stable response after 6 months of treatment (see "Assessment of tumor response" below) were eligible to continue receiving the medication after entering a compassionate program, wherein they were seen every 12 weeks until progression of the disease.

Assessment of Tumor Response

Tumors were measured at baseline and at each visit. All cutaneous masses with at least 1 dimension ≥ 10 mm were included in the comprehensive lesion measurement. These masses were measured in 3 dimensions, and the tumor volume in cm^3 was calculated as the product of the 3 measurements. The total tumor burden for each dog was determined as the sum of all tumor volumes (comprehensive lesion measurement). The percent of baseline tumor size was then calculated as follows: % baseline tumor size = 100% × [current comprehensive lesion measurement ÷ baseline comprehensive lesion measurement]. This value was used to grade the response to treatment according the World Health Organization guidelines[19]: 0% baseline tumor size indicated a complete response; >0 to $\leq 51\%$ with no increase in size of any previously documented area and no new lesion development (including metastases) was considered a partial response; 51–125% with no increase in size of any previously documented area and no new lesion development (including metastases) was considered stable disease; all other cases were considered progressive disease. To avoid overestimating the response rate, partial or complete responses found at 4 months of treatment were confirmed by a repeat assessment at 6 months. For the disease to be categorized as stable, the category had to be confirmed at least once with a minimum interval of 4 weeks.

Primary Endpoints

The following endpoints were assessed: time-to-tumor progression (TTP), overall survival (OS), and objective response (OR) at day 112 (confirmed at day 168). TTP was defined as the number of days from the date of 1st treatment intake to the date of tumor progression. If progression was not observed during the study, data on TTP were censored at the date of last tumor assessment without evidence of progression. OS was defined as the number of days between the date of 1st treatment intake and the date of the death. If death was not observed during the study, data on OS were censored at the last date dog was known to be alive. The OR was defined as follows: 100 × [number of responders ÷ number of enrolled dogs in the efficacy analysis]. A dog was classified as a responder if it showed a complete or partial response (see "Assessment of tumor response" above) at 4 months (day 112 ± 4) and 6 months (day 168 ± 6); all others were considered nonresponders.

Toxicity Assessment and Dose Reduction

Toxicity was graded according to a modified version of the Eastern Cooperative Oncology Group criteria[20] and the Veterinary Cooperative Oncology Group—common terminology criteria for adverse events.[21] An adverse event was defined as any unfavorable or unintended sign (including an abnormal clinicopathologic finding), clinical sign (eg, loss of mobility or gastrointestinal, genitourinary, or dermatologic signs), or disease temporally associated with the use of the treatment that may or may not be related to the treatment.[21] Accordingly, the severity of adverse events was graded as follows: 1, mild; 2, moderate; 3, severe; 4, life-threatening; 5, death related to the adverse event. After a grade III or IV adverse event, treatment was discontinued until the adverse event resolved, and the dosage then was reduced by approximately 25 or 33% (ie, from 12.5 to 9 mg/kg/d or from 9 to 6 mg/kg/d).

Determination of KIT Mutation Status

Percutaneous biopsies (~5 mm) were obtained from an enlarged mass (eg, previous tumor or cutaneous metastasis) previously confirmed to be a mast cell tumor. Biopsies were submerged immediately in RNAlater[b] to a volume of approximately 1 mL and were stored at room temperature. Total RNA was isolated with an RNeasy mini kit[c] as recommended by the manufacturer. The extracted RNA (200 ng) was reverse transcribed in a 25-μL reaction containing random hexamers[d] and the StrataScript First-strand Synthesis System.[e] A 2.5-μL sample of the resulting cDNA then was amplified by PCR with the primers covering exons 8–13 and 17–19 (Table 3). PCR was carried out for 40 cycles at 94 °C for 30 seconds, 57 °C for 30 seconds, and 72 °C for 45 seconds. Amplimers were purified with the GeneClean III kit[f] and directly sequenced with the Big Dye Terminator V 1.1 kit[g] and sequencing primers (Table 3) on an ABI Prism 3130 sequencer.[h] Observed mutations were systematically checked in a new reverse transcription reaction. This method was able to detect the mutated allele when present in 5–10% of the cells.

Statistical Analyses

Differences in dog (eg, age, sex, and breed) and tumor characteristics (eg, grade and size) were analyzed by a *t*-test for continuous variables and Pearson's χ^2 or Fisher's exact tests for categorical variables. Outcome measures (TTP, OS, OR) were computed with the product-limit method, and curves were drawn by the Kaplan-Meier method. Dogs were stratified by tumor characteristics (eg, KIT mutation, first-line treatment, tumor grade), and differences in the actuarial estimates were tested by the Log-rank method. In this study, dogs were censored in the analysis when (i) they were lost to follow-up, (ii) death was not caused by MCT or treatment, or (iii) relapse had not occurred before the end of the study period. Differences were considered significant at $P < .05$. Statistical analysis of data was performed using SAS 9.1.[i]

Results

Baseline Patient Characteristics

Between February 2005 and October 2006, 202 dogs were evaluated for response to and toxicity from treatment with masitinib. Detailed characteristics of the dogs are shown in Table 1. The most common breeds were Labrador Retrievers, Golden Retrievers, and Boxers. Dogs receiving first-line treatment with masitinib comprised 42.1% of the trial population, and the remaining 57.9% had received previous medical treatment for MCT. The majority of dogs enrolled in this study (65.3%) had nonresectable tumors, and the remainder (34.6%) had tumors that were recurrent after surgery. Diagnosis was made <12 months before initiation of treatment with masitinib in most (75.9%) of the dogs. Also, most of the dogs (85.6%) had grade II MCT, and the remainder (14.4%) had grade III tumors. The characteristics of the dogs and the MCTs were not significantly different between the masitinib and placebo arms of the study (Table 1).

The mutational status of KIT in the tumor biopsies was determined by reverse transcription-PCR for 191 of the 202 dogs and is described in detail elsewhere.[10] Of the masitinib-treated dogs, 26.7% had a mutation in KIT, and KIT mutations were found in 25.6% of the dogs treated with placebo. Of the 50 dogs with mutations, most (n = 32) had mutations in the juxtamembrane domain (exon 11). Mutations also were frequently found in exons 8 (n = 9) and 9 (n = 8) (extracellular immunoglobulin-like domains 4 and 5), and a single dog had a mutation in exon 17 (kinase domain). These mutations all appear to cause constitutive activation of the kinase domain.[10] The fraction of dogs with mutant forms of KIT was not significantly different between the masitinib and placebo arms of the study (Table 1).

Efficacy of Masitinib in Canine MCT

Treatment with masitinib significantly prolonged TTP in all dogs compared with placebo (75 versus 118 days; $P = .038$; Fig 1A and Table 2). This effect was even more pronounced when dogs received masitinib as first-line treatment (75 versus 178 days; $P = .001$; Fig. 1B and Table 2), and first-line treatment with masitinib significantly increased the TTP regardless of whether the tumors expressed a mutant (83 versus not reached [$P = .009$]) or wild-type form of KIT (66 versus 253 [$P = .008$]) (Fig 1C). Also, dogs with a mutant form of KIT receiving masitinib as second-line or later treatment had a longer TTP compared with placebo (97 versus 202 days; Fig 1D and Table 2), but the difference was not statistically significant due to the small number of dogs in this subgroup (n = 25).

Table 1. Patient characteristics.

Parameter	Masitinib (n = 161)	Placebo (n = 41)	*P*-Value	Total (n = 202)
Age (years), mean ± SD	8.5 ± 3.0	8.7 ± 2.4	.740[a]	8.5 ± 2.8
Sex				
Male, n (%)	68 (42.2%)	18 (43.9%)	.847[b]	86 (42.6%)
Female, n (%)	93 (57.8%)	23 (56.1%)		116 (57.4%)
Time to treatment (months)				
Mean ± SD	10.7 ± 19.3	12.0. ± 16.9	.835[a]	11.0 ± 18.8
Range	0.0–120.9	0.0–67.7		0.0–120.9
≤12 (months), n (%)	124 (78.5%)	27 (65.9%)	.090[b]	151 (75.9%)
>12 (months), n (%)	34 (21.5%)	14 (34.1%)		48 (24.1%)
Previous chemotherapy or radiotherapy				
Without, n (%)	122 (75.8%)	30 (73.2%)	.730[a]	152 (75.2%)
With, n (%)	39 (24.2%)	11 (26.8%)		50 (24.8%)
Surgical status				
Nonresectable	106 (65.8%)	26 (63.4%)	.771[b]	132 (65.3%)
Recurrent after surgery	55 (34.2%)	15 (36.6%)		70 (34.7%)
Line of treatment				
First-line	67 (41.6%)	18 (43.9%)	.791[a]	85 (42.1%)
Second-line or beyond	94 (58.4%)	23 (56.1%)		117 (57.9%)
KIT mutation status				
Mutated	40 (26.7%)	10 (25.6%)	.862[b]	50 (26.5%)
Wild type	110 (73.3%)	29 (74.4%)		139 (73.5%)
Tumor grade				
II	138 (85.7%)	35 (85.4%)	.955[b]	173 (85.6%)
III	23 (14.3%)	6 (14.6%)		29 (14.4%)
Breed				
Labrador	26 (16.1%)	4 (9.8%)	.728[b]	30 (14.9%)
Golden Retriever	12 (7.5%)	6 (14.6%)		18 (8.9%)
Boxer	12 (7.5%)	2 (4.9%)		14 (6.9%)
Rhodesian ridgeback	6 (3.7%)	2 (4.9%)		8 (4.0%)
Weimaraner	6 (3.7%)	2 (4.9%)		8 (4.0%)
Mixed	40 (24.8%)	11 (26.8%)		51 (25.2%)
Other	59 (36.6%)	14 (34.1%)		73 (36.1%)
Previous treatment for MCT	58 (36.0%)	13 (31.7%)	.605[b]	71 (35.1%)
Corticosteroid for systemic use	41 (25.5%)	8 (19.5%)	.427[b]	49 (24.3%)
Antineoplastic agents	39 (24.2%)	8 (19.5%)	.524[b]	47 (23.3%)
Antihistamines for systemic use	11 (6.8%)	7 (17.1%)	.060[c]	18 (8.9%)
Drugs for acid-related disorders	10 (6.2%)	4 (9.8%)	.489[c]	14 (6.9%)
Corticosteroids, dermatological preparations	3 (1.9%)	0 (0.0%)	1.000[c]	3 (1.5%)
Antidiarrheals, intestinal anti-inflammatory, and anti-infective agents	3 (1.9%)	0 (0.0%)	1.000[c]	3 (1.5%)
Vitamins	2 (1.2%)	1 (2.4%)	.496[c]	3 (1.5%)
Surgical dressings	2 (1.2%)	0 (0.0%)	1.000[c]	2 (1.0%)
Blood substitutes and perfusion solutions	1 (0.6%)	0 (0.0%)	1.000[c]	1 (0.5%)
Therapeutic radiopharmaceuticals	8 (5.0%)	5 (12.2%)	.144[c]	13 (6.4%)

[a] *P*-value calculated by Wilcoxon's test.
[b] *P*-value calculated by χ^2 test.
[c] *P*-value calculated by Fisher's exact test.

Masitinib appeared to increase the OS compared with placebo, although the difference was significant only when considering only those dogs with tumors expressing a mutant form of KIT (417 versus 182 days; $P = .015$). The OR assessed 4 months (day 112 ± 4) after initiation of treatment and confirmed at 6 months (day 168 ± 6) was not significantly increased by masitinib. In addition, between dogs receiving masitinib and placebo, there were no significant differences in the proportion showing a complete response (11.2 versus 4.9%; $P = .378$ by Fisher's exact test) or a partial response (4.6 versus 9.8%; $P = .305$ by Fisher's exact test) on day 168.

Safety and Tolerability of Masitinib

The median duration of treatment was 83 days (range = 2–598 days). Of the 161 dogs receiving masitinib, 58 (36%) entered the compassionate program upon study completion and were followed up to an additional 18 months.

There were no significant differences in the incidence of severe adverse events (Table 3) or death between the 2 treatment groups. Thirty-one deaths occurred in the course of the study. Of these, 24 (14.9%) were in the masitinib arm and 7 (17.1%) were in the placebo arm ($P = .731$). Of the 31 deaths, 20 occurred after development of progressive disease, 14 of which (8.7%) were in

the masitinib arm and 6 (14.6%) were in the placebo arm ($P = .251$). There were 8 deaths unrelated to progressive disease in the masitinib arm, 4 due to euthanasia (owner dissatisfaction with quality of life), 3 after serious adverse events, and 1 for an unknown reason (patient was found dead by the owner). The percentage of deaths after a treatment-related serious adverse event and the percentage of dogs with an adverse event leading to permanent discontinuation of treatment were not significantly different between the 2 treatment groups.

The 2 most common treatment-related adverse events and the only ones significantly more frequently in masitinib-treated dogs were diarrhea and vomiting (Table 3). In these cases, 96.2% were of mild (grade 1) or moderate (grade 2) intensity. In addition, in 92% of these dogs, the events were transient (mean duration 15 days) and without sequelae. The incidence of diarrhea or vomiting was 56.5% during the 1st 3 months of treatment, but the incidence after 3 months of treatment (33.3%) was no different than observed in dogs receiving placebo (36.6%). The serious adverse events (grade 3 and 4) leading to discontinuation of masitinib were diarrhea or vomiting, renal insufficiency (increased BUN or creatinine concentration), and edema.

Neutropenia was observed in 10 dogs treated with masitinib (6.2%) and in none of the dogs receiving placebo ($P = .218$). According to the Veterinary Co-operative Oncology Group[20] scale for neutropenia as an adverse event, 4 dogs had grade 1 neutropenia ($<1,500/\mu L$), 5 had grade 2 (1,000–1,499/μL), and 1 had grade 4 ($<500/\mu L$). Because none of the dogs with neutropenia demonstrated clinical signs, these were not considered severe adverse events. Furthermore, most of the dogs with neutropenia (7 of 10 dogs) had low neutrophil counts at baseline (ie, $<2,000/\mu L$).

Renal disorders occurred in 12 dogs (7.5%) in the masitinib arm and 2 dogs in the placebo arm (4.9%) ($P = .562$). Those dogs having renal dysfunction (increased BUN or creatinine concentrations) were diagnosed with glomerulonephritis, renal failure, nephrotic syndrome, or proteinuria. Increases in BUN or creatinine concentrations were noted in all dogs that had results 1.0–1.5 times the upper limit of the normal reference range at baseline. Of the 12 masitinib-treated dogs developing renal dysfunction, 6 recovered without sequelae, 3 were unchanged or had ongoing abnormalities, and 3 were euthanized.

Some of the dogs treated with masitinib (2.5%) and none of the dogs treated with placebo suffered from hemolytic anemia ($P = .584$). These cases occurred after a mean of 83 days of masitinib exposure, and 3 of the 4 dogs recovered with appropriate medical management

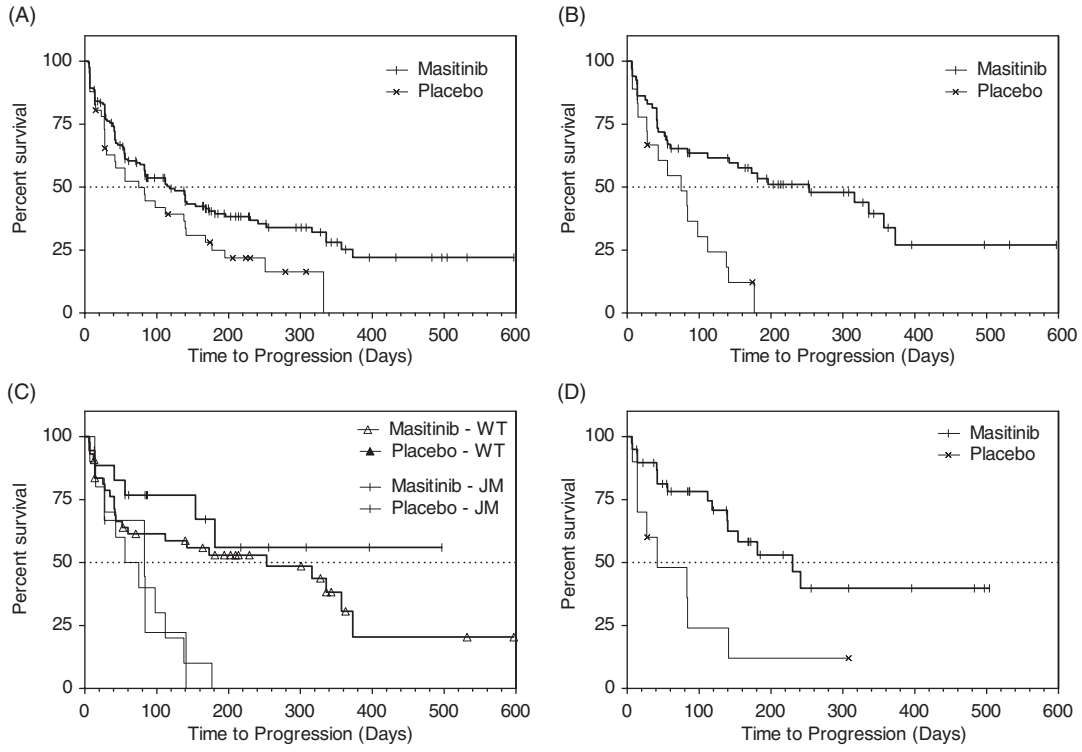

Fig 1. Time-to-tumor progression (TTP) in dogs treated with masitinib and placebo. TTP was computed by the product-limit method, and curves were drawn by the Kaplan-Meier method. (**A**) Median TTP for all dogs in the study receiving masitinib (solid line) or placebo (dotted line). (**B**) Median TTP for dogs receiving first-line therapy with masitinib (solid line) or placebo (dotted line). (**C**) Median TTP for dogs receiving first-line treatment according to the absence (dotted lines) or presence (solid lines) of mutated KIT. Thick lines indicate dogs receiving masitinib, and thin lines indicate dogs receiving placebo. (**D**) Median TTP for dogs receiving second-line (and beyond) treatment according to the absence (dotted lines) or presence (solid lines) of mutated KIT.

Table 2. Summary of efficacy.

		Median TTP (Days)			Median OS (Days)			OR at 6 (Months)		
Category	n	Mb	Plc	*P*-Value	Mb	Plc	*P*-Value	Mb	Plc	*P*-Value
All dogs	202	118	75	.038	491	340	.320	16.1	14.6	1.00
Mutated KIT[a]	50	230	42	.006	417	182	.015	20.0	10.0	.25
Wild-type KIT	139	83	98	.302	NR	NR	.944	11.8	13.8	.75
First-line										
All	85	253	75	.001	NR	340	.096	23.9	5.6	.11
Mutated KIT[a]	25	NR	83	.009	417	242	.050	36.8	0.0	.14
Wild-type KIT	53	253	66	.008	NR	NR	.722	20.9	0.0	18
Second-line and beyond										
All	117	84	140	.915	380	361	.892	10.6	21.8	.17
Mutated KIT[a]	25	230	28	.274	396	113	.078	23.8	25.0	1.00
Wild-type KIT	86	72	140	.411	434	361	.786	6.0	21.1	0.07

[a]Includes all mutations. Mutations were found in exons 8, 9, 11, 17. All of the mutations appear to cause constitutive activation of KIT.[10]
Mb, masitinib; Plc, placebo; NR, not reached; OR, objective response; OS, overall survival.

within 14 days. Analysis of 1 of these dogs did not identify the presence of autoantibodies, suggesting that masitinib does not induce hemolytic anemia by causing an autoimmune disease (data not shown).

Discussion

This study showed that masitinib is safe and effective for the treatment of canine MCT. One-quarter of the dogs in this study had mutations in KIT, including not only the juxtamembrane domain (exon 11) but also extracellular immunogluobulin-like domains 4 and 5 (exons 8 and 9) and the kinase domain (exon 17). All of these mutations appear to cause constitutive activation of the kinase domain and ligand-independent cell growth.[10] In dogs that had not received prior treatment, masitinib showed substantial efficacy regardless of KIT mutational status. Masitinib was generally well tolerated, with most adverse events transient, of mild (grade 1) to moderate (grade 2) severity, and medically manageable.

When used as a first-line therapy, masitinib significantly increased TTP compared with placebo. This effect was found in dogs with both mutant and wild-type forms of KIT. These results suggest that the possibility that masitinib inhibits the progression of MCT by another mechanism in addition to blocking KIT, for example by inhibiting other protein kinases. Indeed, masitinib also potently inhibits the platelet-derived growth factor (PDGF) receptor (P. Dubreuil, manuscript submitted). In addition, these results raise the possibility that wild-type KIT is indirectly involved in the progression or survival of MCT.

Many (57.9%) of the dogs in this study had received prior treatment with chemotherapy or radiotherapy. Within this group, the median TTP was increased by masitinib only when the MCT expressed a mutant form of KIT. Thus, prior treatments appear to limit the efficacy of masitinib. This may be due to the development of drug resistance after chemotherapy or radiotherapy.[21] This also suggests that masitinib is most effective when the tumor expresses mutant KIT, regardless of whether they develop general resistance mechanisms.

The ability of masitinib to improve TTP indicated that it can inhibit tumor progression. In addition, masitinib tended to increase OS, although the difference was significant only for dogs with mutant KIT receiving masitinib as a first-line therapy. Lack of a significant difference in the OS, for example for all dogs receiving first-line treatment, appeared to be due to a combination of insufficient study duration, loss of some dogs to euthanasia (6 dogs), and the possibility that some dogs began to receive alternative treatments, resulting in their exclusion from OS analysis.

In this study, masitinib did not significantly enhance OR. This appeared to be due to a high spontaneous rate of response; the ''rate of best response,'' defined as a compete response or as complete or partial response at any time, was 21 and 36%, respectively, in dogs receiving placebo (versus 26 and 55% in dogs receiving masitinib, data not shown). This high spontaneous response rate in placebo-treated dogs was unexpected, and it is similar to the response rates that have been reported in previous open-label studies of chemotherapy agents.[8,9] This brings into question the interpretation of efficacy data from open-label trials of chemotherapy agents for the treatment of MCT and further emphasizes the importance of using a randomized, placebo-controlled design to assess the efficacy of MCT treatments.

Masitinib had an acceptable safety profile. The more common adverse effects were diarrhea, vomiting, edema, and neutropenia, and the only ones significantly different between the masitinib and placebo arms were diarrhea and vomiting. These adverse effects generally were mild (grade 1) to moderate (grade 2) in intensity, transient, and medically manageable. Furthermore, these adverse effects appear to be common for tyrosine kinase inhibitors targeting KIT[22,23] and, therefore, may be related to KIT inhibition. The occurrence of diarrhea could be related to the inhibition of KIT in Cajal cells in the gastrointestinal tract.[24,25] In addition, inhibitors of KIT can cause mast cell apoptosis,[26] which may lead to the release of mediators that cause systemic effects such as diarrhea and vomiting.[27] If these symptoms are due to mast cell degranulation, their occurrence or severity

Table 3. Treatment-related adverse events.

	All Adverse Events[a], n (%)			Severe Adverse Events[b], n (%)			Severe Adverse Events Leading to Discontinuation, n (%)		
	Masitinib	Placebo	*P*-Value	Masitinib	Placebo	*P*-Value	Masitinib	Placebo	*P*-Value
Diarrhea	59 (36.6%)	7 (17.1%)	.017[c]	3 (1.9%)	0 (0.0%)	1.000[d]	4 (2.5%)	0 (0.0%)	0.584[d]
Vomiting	74 (36.0%)	11 (26.8%)	.027[c]	7 (4.3%)	0 (0.0%)	.349[d]	5 (3.1%)	0 (0.0%)	0.585[d]
Alopecia	26 (16.1%)	2 (4.9%)	.062[c]	0 (0.0%)	0 (0.0%)	NC	0 (0.0%)	0 (0.0%)	NC
Decreased appetite	10 (6.2%)	0 (0.0%)	.218[d]	0 (0.0%)	0 (0.0%)	NC	0 (0.0%)	0 (0.0%)	NC
Lipoma	10 (6.2%)	0 (0.0%)	.218[d]	0 (0.0%)	0 (0.0%)	NC	0 (0.0%)	0 (0.0%)	NC
Neutropenia/decreased neutrophil count	10 (6.2%)	0 (0.0%)	.218[d]	0 (0.0%)	0 (0.0%)	NC	0 (0.0%)	0 (0.0%)	NC
Asthenia	7 (4.3%)	0 (0.0%)	.349[d]	0 (0.0%)	0 (0.0%)	NC	0 (0.0%)	0 (0.0%)	NC
Peripheral edema	9 (5.6%)	1 (2.4%)	.091[d]	2 (1.2%)	0 (0.0%)	1.000[d]	3 (1.9%)	0 (0.0%)	1.000[d]
Anemia	5 (3.1%)	0 (0.0%)	.585[d]	3 (1.9%)	0 (0.0%)	1.000[d]	1 (0.6%)	0 (0.0%)	1.000[d]
Blood urea increased	4 (2.5%)	0 (0.0%)	.584[d]	3 (1.9%)	0 (0.0%)	1.000[d]	3 (1.9%)	0 (0.0%)	1.000[d]
Haemolytic anaemia	4 (2.5%)	0 (0.0%)	.584[d]	4 (2.5%)	0 (0.0%)	.584[d]	3 (1.9%)	0 (0.0%)	1.000[d]
All Diarrhea/vomiting[e]	91 (56.5%)	15 (36.6%)	.023[c]	8 (5.0%)	0 (0.0%)	.363[d]	6 (3.7%)	0 (0.0%)	0.351[d]
All Edema[f]	15 (9.3%)	2 (4.9%)	.361[c]	4 (2.5%)	1 (2.4%)	1.000[d]	5 (3.1%)	0 (0.0%)	0.585[d]
All renal disorders[g]	12 (7.5%)	2 (4.9%)	.562[c]	8 (5.0%)	0 (0.0%)	.363[d]	7 (4.3%)	0 (0.0%)	0.349[d]

[a]Grades 1–4.

[b]Grade 3 or 4.

[c]*P*-value calculated by χ^2 test.

[d]*P*-value calculated by Fisher's exact test.

[e]Vomiting, diarrhea, hemorrhagic diarrhea.

[f]Peripheral edema, periorbital edema, pitting edema.

[g]Increased blood creatinine, increased blood urea, presence of urine in blood, increased protein-to-creatinine ratio, proteinuria, hematuria, nephropathy, azotaemia, bladder calculus, glomerulonephritis, nephrotic syndrome, renal polyuria, renal disorder, polyuria.

NC, not calculable.

could be reduced by antihistamines; however, concomitant antihistamines were not used in this study. The mechanism of the observed neutropenia is unclear, but it appears to be a common effect of tyrosine kinase inhibitors targeting KIT and the PDGF receptor.[22,23] We also observed increases in BUN and creatinine concentrations after masitinib treatment in those dogs with preexisting renal abnormalities. Although kidney biopsies showed no infiltration by inflammatory cells or anatomical damage (data not shown), care should be taken if masitinib is administered to dogs with impaired renal function. The impairment of renal function by masitinib could be related to a direct effect on renal tubules, which have been shown to express KIT,[27] or on glomerular cells that express PDGF receptors.[28,29] Finally, 4 dogs receiving masitinib developed hemolytic anemia that was generally medically manageable.

In conclusion, on the basis of its safety profile and efficacy, oral masitinib (12.5 mg/kg/d) appears to be safe and effective at delaying tumor progression in dogs with recurrent or nonresectable grade II or grade III nonmetastatic MCT. This effect was more pronounced when masitinib was used as first-line therapy, regardless of whether the tumors expressed mutant or wild-type KIT.

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Footnotes

[a] Manufactured by Cardinal Health, 14 Schoolhouse Road, Somerset, NJ. Available as nondivisible tablets containing 25, 100, or 150 mg masitinib. Excipients: microcrystalline cellulose, povidone, magnesium stearate, aroma, and coating agent

[b] RNAlater RNA Stabilization Reagent, Qiagen, Courtaboeuf, France

[c] RNeasy Mini-Kit, Qiagen

[d] Stratagene, Amsterdam, the Netherlands

[e] StrataScript, Stratagene

[f] GeneClean III, Qbiogene, Illkirch, France

[g] Big Dye Terminator V 1.1 Cycle Sequencing Kit, Applied Biosystems, Foster City, CA

[h] ABI Prism 3130xl DNA Sequencer, Applied Biosystems

[i] SAS Institute, Cary, NC

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