

Multi-center, Placebo-controlled, Double-blind, Randomized Study of Oral Toceranib Phosphate (SU11654), a Receptor Tyrosine Kinase Inhibitor, for the Treatment of Dogs with Recurrent (Either Local or Distant) Mast Cell Tumor Following Surgical Excision

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Abstract Purpose: The purpose of this study was to determine the objective response rate (ORR) following treatment of canine mast cell tumors (MCT) with toceranib phosphate (Palladia, SU11654), a kinase inhibitor with both antitumor and antiangiogenic activity through inhibition of KIT, vascular endothelial growth factor receptor 2, and PDGFR β . Secondary objectives were to determine biological response rate, time to tumor progression, duration of objective response, health-related quality of life, and safety of Palladia.

Experimental Design: Dogs were randomized to receive oral Palladia 3.25 mg/kg or placebo every other day for 6 weeks in the blinded phase. Thereafter, eligible dogs received open-label Palladia.

Results: The blinded phase ORR in Palladia-treated dogs ($n = 86$) was 37.2% (7 complete response, 25 partial response) versus 7.9% (5 partial response) in placebo-treated dogs ($n = 63$; $P = 0.0004$). Of 58 dogs that received Palladia following placebo-escape, 41.4% (8 complete response, 16 partial response) experienced objective response. The ORR for all 145 dogs receiving Palladia was 42.8% (21 complete response, 41 partial response); among the 62 responders, the median duration of objective response and time to tumor progression was 12.0 weeks and 18.1 weeks, respectively. Palladia-treated responders scored higher on health-related quality of life versus Palladia-treated nonresponders ($P = 0.030$). There was no significant difference in the number of dogs with grade 3/4 (of 4) adverse events; adverse events were generally manageable with dose modification and/or supportive care.

Conclusions: Palladia has biological activity against canine MCTs and can be administered on a continuous schedule without need for routine planned treatment breaks. This clinical trial further shows that spontaneous tumors in dogs are good models to evaluate therapeutic index of targeted therapeutics in a clinical setting.

Mast cell tumors (MCT) are the second most common malignant tumors in dogs. Most originate in the skin or s.c. tissues but they can occur as primary tumors in the intestines, liver, or spleen (1). Canine MCTs possess a wide range of biological

behaviors, from benign to extremely aggressive leading to metastasis and eventual death. Several prognostic factors have been identified that help predict the biological behavior of a MCT, although histologic grade using the Patnaik system

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Translational Relevance

Small-molecule inhibitors are used to treat several human cancers. While clinical responses are observed, toxicities can be substantial and resistance develops over time. Unfortunately, murine models often fail to predict both the spectrum of toxicities and appropriate dosing regimens. The following clinical trial describes the first large-scale, registrational evaluation of a small-molecule inhibitor of a vascular endothelial growth factor receptor/PDGFR/Kit (Palladia [SU11654]) for use in dogs. A phase I study of Palladia in dogs with cancer predicted that a vascular endothelial growth factor receptor/PDGFR/Kit inhibitor would have activity in several tumor types, particularly those harboring Kit juxtamembrane domain mutations, and accurately predicted the spectrum of clinical toxicities subsequently observed in people treated with similarly targeted tyrosine kinase inhibitors. The present study expands on these previous findings, demonstrating that Palladia can be administered on an alternating day schedule, avoiding the need for long treatment breaks to address toxicity. Furthermore, these data show that malignant mast cell disease is responsive to Palladia, supporting the notion that human mast cell and eosinophil disorders responsive to Gleevec (systemic mastocytosis, hypereosinophilic syndrome) will be responsive to other similarly targeted tyrosine kinase inhibitors. Lastly, this work again supports the notion that spontaneous tumors in dogs can serve as strong predictors of both clinical toxicities and response to therapy for targeted therapeutics.

(grade 1, low; 2, intermediate; 3, high) has proven to be the most reliable (2, 3).

Treatment for dogs with MCTs consists of surgical excision followed by local radiation therapy if complete tumor removal is not possible (1). Chemotherapy (lomustine, vinblastine, cyclophosphamide, prednisone) is used when metastatic disease has been identified, or negative prognostic indicators are present, although no standard of care is currently established (4–9). Unfortunately, dogs with aggressive MCTs rarely survive beyond 6 months postdiagnosis even after treatment with surgery, chemotherapy, and/or radiation therapy (1).

The potential role of the receptor tyrosine kinase Kit in mast cell disorders was first recognized in 1994 when point mutations inducing ligand-independent activation were identified in the catalytic domain of c-Kit in malignant mast cell lines (10–12). Subsequently, point mutations in the catalytic domain of c-Kit were shown in up to 90% of human patients with aggressive systemic mastocytosis, supporting the notion that Kit dysfunction may contribute to the malignant transformation of mast cells (13–15). An investigation of Kit dysregulation in dog MCTs showed the presence of novel mutations consisting of internal tandem duplications (ITD) in the juxtamembrane domain that resulted in constitutive activation of Kit in the absence of ligand binding (16, 17). The prevalence of Kit mutations in canine MCTs is approximately 9% to 30%, with higher-grade tumors

more likely to possess a mutation (16, 18–20). Additionally, Kit ITDs are associated with increased risk of metastasis and local recurrence, a higher tumor proliferation index, and aberrant Kit localization (16, 18–20). More recently, activating point mutations in the extracellular domain of c-Kit (exons 8 and 9) have been identified in a proportion of MCTs (21). These mutations are similar to those found in acute myelogenous leukemia, suggesting that the spectrum of Kit dysregulation in dog cancer resembles that of human cancer (22–24).

Given the presence of activating Kit mutations in canine MCTs and the importance of wild-type Kit in mast cell growth and survival, it was reasoned that these tumors would likely respond to a small-molecule Kit inhibitor. Additionally, as mast cells are known to produce vascular endothelial growth factor (VEGF) and contribute to the process of angiogenesis, inhibition of VEGF receptor 2 (VEGFR2) signaling might also display biological activity against MCTs. A phase I trial was conducted to explore the biologic activity of Palladia (SU11654), a multitargeted inhibitor active against the split kinase family of receptor tyrosine kinases (Kit, VEGFR2, PDGFR β) in dogs with spontaneous tumors (25). Eleven of 22 dogs with MCTs in this study had tumors that possessed the c-Kit ITD. Response rates were approximately 90% in dogs with MCTs possessing a c-Kit ITD, and 25% in dogs without c-Kit mutations. A subsequent study showed down-regulation of Kit phosphorylation *in vivo* in previously untreated MCTs after a single dose of Palladia, thereby establishing a distinct pharmacokinetic/pharmacodynamic relationship (26). The observed responses provided evidence that a kinase inhibitor with activity against Kit, VEGFR2 and PDGFR β could exhibit biological activity against canine MCTs *in vivo*. Furthermore, adverse events observed following Palladia administration, including anorexia, diarrhea, and lethargy, were predictive of those subsequently observed in human cancer patients treated with similar multitargeted kinase inhibitors (27). Lastly, activity of Palladia against MCTs possessing Kit juxtamembrane domain mutations predicted activity of similar multitargeted agents against human gastrointestinal stromal tumors with Kit juxtamembrane domain mutations (28, 29).

The following study was a randomized, placebo-controlled, double-blind, multicenter study to evaluate the efficacy and safety of Palladia in dogs with recurrent, Patnaik grade 2 or 3 MCTs with or without lymph node involvement for the purpose of registration of Palladia as a new veterinary drug. Observed response rates were analyzed for an association with treatment (Palladia versus placebo), tumor grade, the presence or absence of regional lymph node metastasis, and the presence or absence of c-Kit mutation.

Materials and Methods

Patient selection. Dogs >1 y of age and with ≥ 5 kg body weight with pathologically confirmed recurrent MCT and at least 1 measurable MCT ≥ 20 mm in its longest diameter were included. Recurrence was defined as the postoperative occurrence of a new MCT, whether locally within/near the original surgery site or distant to the previously excised tumor; the nature of the lesion(s) was not determined by clonality. Eligibility criteria included performance status of 0 or 1 on Karnovsky's performance scale modified for dogs (30), life expectancy of >12 wk, up to 1 previous radiation therapy regimen and/or 1 systemic chemotherapy regimen completed at least 14 d prior to enrollment, and adequate hematologic, renal, and hepatic function. Dogs were excluded if they were intended for breeding or had evidence of gastrointestinal bleeding, a

serious systemic disorder incompatible with the study, evidence of systemic MCT, and/or involvement of more than one lymph node region. Baseline evaluations included medical history, physical examination, thoracic and abdominal radiographs, abdominal ultrasound, fine-needle aspiration cytology from lymph nodes, splenic or hepatic lesions suspicious for MCT, assessment of performance status, complete blood count, biochemical profile, prothrombin time, partial thromboplastin time, and urinalysis. Tumor samples were obtained prior to study entry to assess for c-Kit mutation. Dogs were excluded if they received corticosteroids within 14 d prior to enrollment due to their potential effect (e.g., anti-inflammatory) on tumor size. Dog owners were required to give written informed consent. The study was conducted in compliance with the principles of Good Clinical Practice (31).

Study design. This study was a double-blind, centrally randomized, multicenter group sequential clinical trial. Dogs were randomized to receive either Palladia or placebo in a 4:3 ratio. Dogs were stratified based on regional lymph node metastasis (yes or no) and tumor grade (2 or 3). Randomization grade was based on a histopathology report available at enrollment whereas analysis was based on the results of a central pathology review. The interim analysis assessed sample size and futility ($n = 70$).

Treatment plan. Dogs received either Palladia (3.25 mg/kg orally every other day) or an equivalent number of placebo-matched tablets. Dose reductions and dose interruptions for up to 2 wk were permitted to manage adverse events (Supplemental Data, Table 1). Dogs in both treatment groups that showed complete response, partial response, or stable disease at the end of the 6-week blinded phase and placebo-treated dogs that showed progressive disease at any time during the blinded phase were permitted to enter the open-label phase and receive Palladia. Dogs receiving Palladia during the blinded phase that had progressive disease at the final blinded phase visit were discontinued from study. Any dog experiencing a grade 4 adverse event at the end of the blinded phase was not eligible to enroll in the open-label phase. Concomitant medications, other than corticosteroids, were permitted to manage adverse events. Treatment continued until approximately 6 mo after the last dog enrolled; thereafter, eligible dogs could continue receiving Palladia under a separate continuation protocol where formal collection of efficacy and safety data was discontinued and, hence, is not included here.

Safety was assessed weekly during the blinded phase, at week 3 and week 6 of the open-label phase, and every 6 wk thereafter, using the National Cancer Institute Common Toxicity Criteria v.2.0, adapted for dogs (Supplemental Data, Table 2, Canine-Adapted Common Toxicity Criteria). If a grade 4 adverse event occurred, the dog was discontinued from study. Treatment was delayed for a maximum of 2 wk or dose was decreased for adverse safety changes as described in Supplemental Table S1. Safety assessments included adverse events, hematology, clinical chemistry profiles, prothrombin time, partial thromboplastin time, and urinalyses. A Health-Related Quality of Life (HRQL) questionnaire was completed by dog owners throughout the study (Supplemental Data, Table 3).

The primary study end point was the objective response rate at the end of the 6-week blinded phase, defined as the proportion of dogs with confirmed complete response or partial response. Clinical response was assessed according to Response Evaluation Criteria in Solid Tumors, modified for the evaluation of canine MCTs, every 6 wk. The longest diameter of each target lesion was measured by two evaluators and the mean of the sum of these measurements was used for response assessment. At baseline, tumor lesions were categorized as target or nontarget lesions. Measurable lesions up to a maximum of three were identified as target lesions and recorded and measured at baseline. All other lesions, measurable or not measurable, were recorded as nontarget lesions. The sum of the longest diameters for all target lesions was calculated and reported as the baseline sum. Complete response was defined as the disappearance of all lesions. Partial response was defined as at least a 30% decrease in the sum of the longest diameter of target lesions, nonprogression of nontarget lesions, and no new lesion(s). Progressive disease was defined as at least a 20% increase in the sum

of the longest diameter of target lesions using the smallest sum of the longest diameter recorded since treatment initiation as the reference, progression of nontarget lesions, or appearance of a new lesion(s). Stable disease was defined as neither partial response nor progressive disease. When a lymph node was included as a target lesion, the best possible response was a partial response. Criteria for complete response or partial response must have been met at week 6 of the blinded phase to be assigned as a blinded phase objective response. Dogs discontinued from the study prior to the 6-week blinded phase time point were considered to have negative objective responses.

Secondary efficacy end points were based on data from the blinded and open-label phases including biological response, response after escape, duration of response (DOR), and time to tumor progression (TTP) or death. Biological response applied to cases treated with Palladia for at least 6 wk and was defined as stable disease for at least 10 wk, or a complete response or partial response. Cases that did not have a biological response prior to progressive disease were considered failures. Placebo-escape dogs were those treated with placebo in the blinded phase subsequently treated with Palladia in the open-label phase. Palladia-treated dogs were those treated with Palladia in the blinded phase that may or may not have continued treatment in the open-label phase. Response assessments for placebo-escape dogs utilized a comparison-to-tumor size at the end of the blinded phase. DOR was defined as the time between the first documentation of an objective response to progressive disease or withdrawal due to death from any cause with dogs censored on the day of termination from study if no progression or death occurred. TTP was defined as the interval between the first dose of Palladia to progressive disease or withdrawal due to death from any cause, with censored observations handled as above.

Analysis for c-Kit mutation. Punch biopsies were obtained from MCT and normal skin distant from the tumor/tumors (opposite side of the body) to reduce the likelihood of field contamination; normal skin was obtained prior to tumor sample to minimize the possibility of DNA contamination. c-Kit mutation status was determined at the laboratory of one of the authors (CL). Methods included preparation of genomic DNA and PCR for ITD detection in exons 11 or 12 of c-Kit as previously described (18, 32, 33). All PCR products were sequenced to confirm either wild-type or mutant status.

Statistical methods. The hypothesis that the objective response rate (complete response plus partial response) in the Palladia group would be better than that in the placebo group was tested using a logistic regression analysis at the 0.05 level of significance. This model also included lymph node metastasis (yes or no), c-Kit mutation status (positive or negative), and central pathology tumor grade (2 or 3). For randomization, tumor grade was based on histopathology from the previous or current MCT. Following randomization tumors were reviewed by a single pathologist (Paul Greenlee, PALPATH; Dallas, TX) and assigned a grade then used as a covariate in the analysis. Covariates were tested for interaction with treatment but interaction terms were not included in the final model unless significant ($\alpha = 0.05$). Graphical and statistical methods (Zelen's exact test of homogeneity of odds ratios) were used to assess the degree and form of any treatment by study site interaction. The covariates prior chemotherapy, prior radiation therapy, performance status, age, and sex were tested (Zelen's exact test of homogeneity of odds ratios) for association with treatment and, because these were unplanned comparisons, were multiplicity adjusted to control the family-wise error rate. The raw P values were adjusted using Hochberg's method as implemented in the SAS multtest procedure.

Secondary objectives were to determine biological response, response after escape, TTP/death, DOR, and safety of Palladia. Biological response and response after escape were analyzed using logistic regression as previously described. TTP/death and DOR were analyzed using the Kaplan-Meier method to estimate the survival curves and the median survival times. The covariates tumor grade, lymph node metastasis, and c-Kit mutation status were tested for association with TTP/death and DOR using the log-rank test.

Table 1. Patient characteristics

Treatment Group	Placebo	Palladia
<i>n</i>	63	86
Age (y)		
Median	9.3	8.9
Range	4.0-14.6	3.1-15.3
Gender, <i>n</i>		
Male	4 (6.3%)	1 (1.2%)
Neutered male	22 (34.9%)	35 (40.7%)
Female	1 (1.6%)	0
Neutered female	36 (57.1%)	50 (58.1%)
Body weight (kg)		
Median	32.0	29.4
Range	5.7-64.8	5.4-54.0

For the blinded phase, analysis of safety data consisted of all randomized subjects that received at least one dose of Palladia or placebo. For the open-label phase, analysis of safety data consisted of all randomized subjects that received at least one dose of Palladia during the combined blinded and open-label phase. The summary of adverse events for the blinded phase consisted of the number of dogs reported to have at least one episode of a specific adverse event and the severity of the event. For those adverse events occurring in a sufficient number of dogs (≥ 5 dogs), the Pearson's χ^2 statistic was calculated and an exact *P* value was used to test for a difference between treatment groups in the blinded phase. The proportion of dogs withdrawn due to adverse events was compared. The summary of adverse events for the combined blinded and open-label phase was the same as described above except no statistical analysis was conducted. The clinical pathology variables listed in Supplemental Table S2 were summarized as frequency of occurrence, severity and, if sufficient numbers were observed, tested for a difference between treatment groups.

Concomitant treatments and number of dose reductions were summarized. For the blinded phase this consisted of the number and percent of dogs that received at least one administration of a concomitant treatment. If enough dogs (≥ 5 dogs) received a specific treatment, the Pearson χ^2 statistic was calculated and an exact *P* value determined to test for a difference between treatment groups. Descriptive statistics were used to summarize the number of dose reductions, defined as a 15% decrease in the prescribed dose. The number of prescribed dose interruptions (defined as a prescription to skip at least one dose) was summarized.

An exploratory HRQL assessment completed by the pet owner was included in the study. Responses were collected at baseline, blinded phase week 3 and 6 (or the final blinded evaluation), and every scheduled open-label phase visit until the end of study. The primary end point was the total score at blinded phase week 6 or, for dogs that discontinued the blinded phase before week 6, the final blinded phase visit. A value of 2 was assigned to a "usually" answer, 1 to a "frequently" answer, and 0 to a "hardly ever" answer for each of the questions with a highest possible total score of 24. The primary analysis was a between-arm comparison of the summary score. The analytic measure of interest was the difference computed as baseline score minus the score at the end of the blinded phase. The change from baseline was analyzed using a mixed linear model including fixed effects of treatment, lymph node metastasis, central pathology tumor grade, and c-Kit mutation status as well as random effects of site, site by treatment interaction, and residual error. An unplanned analysis was conducted that incorporated an additional covariate based on the 6-week blinded phase, tumor response. This analysis dropped tumor burden, central pathology tumor grade, and c-Kit from the model due to insignificance.

The SAS system V.9.1 (SAS Institute, Inc.) and StatXact (Cytel Software Corporation) were used for all data analyses.

Results

Evaluable cases

A total of 153 dogs (65 placebo, 88 Palladia) from 10 veterinary oncology practices in the United States were entered into the study from February 2003 to December 2004. This study ended on August 25, 2005. One dog in each group was excluded from all analyses for concurrent lymphosarcoma diagnosed at enrollment or dosing noncompliance. In addition, one dog in each group was excluded from the blinded phase efficacy analysis for failing to meet inclusion criteria – each received a single dose of corticosteroids within 14 days prior to enrollment; these dogs were included in all remaining analyses. Hence, 149 dogs (63 placebo, 86 Palladia) were evaluable for blinded phase efficacy; 151 dogs (64 placebo, 87 Palladia) were evaluable for efficacy (combined blinded and open-label phases) and safety (blinded phase and combined blinded and open-label phases). Of the 151 dogs in the blinded phase, 111 (73.5%) entered the open-label phase. Six dogs in the placebo group did not continue to the open-label phase. Thus, 145 evaluable cases were administered at least one dose of Palladia during the combined blinded and open-label phases.

Demographics

The groups were balanced with respect to age, gender, body weight, previous treatment, c-Kit mutation status and the randomization strata, tumor grade, and lymph node metastasis (Tables 1 and 2). Purebred and mixed breed dogs contributed 74.8% ($n = 113$) and 25.2% ($n = 38$) of the study population, respectively. c-Kit mutation status was available for 150 of 153 dogs; of these, 0.0% (0/2), 16.4% (18/110), and 31.6% (12/38) of grade 1, 2, and 3 tumors, respectively, were positive for the c-Kit ITD.

Blinded phase

Response to therapy. There was a statistically significant improvement in the primary end point (objective response) for Palladia treatment compared with placebo treatment ($P = 0.0004$; Table 2). The objective response rate in Palladia-treated dogs was 37.2% (32/86; 7 complete response, 25 partial response) compared with 7.9% (5/63; 5 partial response) for placebo-treated dogs. The odds of an objective response were 6.5 (95% confidence interval, 2.3-18.3) times higher in Palladia-treated dogs compared with placebo-treated dogs. Significantly more placebo-treated dogs (66.7%; 42/63) showed progressive disease during the 6-week blinded phase compared with Palladia-treated dogs (34.9%; 30/86; $P = 0.0004$). Additionally, the median TTP was significantly shorter for placebo-treated dogs (3 weeks) compared with Palladia-treated dogs during the blinded phase (>6 weeks; $P < 0.0001$; Fig. 1).

The treatment by study site interaction was significant based on Zelen's exact test of homogeneity of odds ratios ($P = 0.004$). The association was primarily the result of absence of an objective response among Palladia-treated dogs and two objective responses among eight placebo-treated dogs at one site. The mean baseline sum longest diameter for Palladia-treated versus placebo-treated dogs at this site was 114 mm versus 50 mm, respectively; between-group differences of this magnitude were not observed at other sites. The *P* value for Zelen's test without this site is 0.063. Other secondary covariates shown in Table 2 were tested for interaction

with treatment; none were associated with the effect of treatment on tumor response. As unplanned comparisons, these tests were corrected for multiple comparisons. Zelen's exact test for homogeneity of odds ratios and the Hochberg adjustment for multiplicity were used for testing.

Regardless of treatment group, dogs with tumors positive for c-Kit ITD were more likely to have an objective response compared with those negative for c-Kit ITD (44.8%, 13/29 versus 20.3%, 24/118, respectively; $P = 0.009$). Within the Palladia-treated group, dogs with c-Kit-positive tumors were more likely to respond than those with c-Kit-negative tumors (60.0%, 12/

20 versus 31.3%, 20/64, respectively; $P = 0.0099$; odds ratio, 4.41). Tumor grade and regional lymph node metastasis were not associated with objective responses ($P = 0.349$ and $P = 0.109$, respectively).

Safety. The median duration of blinded phase study treatment was 42 days for Palladia-treated and 21 days for placebo-treated dogs. The percentage of Palladia-treated dogs on blinded phase study treatment at week 6 was 70.1% (61/87) compared with 34.4% (22/64) for placebo-treated dogs. Although Palladia-treated dogs had a greater opportunity to experience adverse safety changes due to the longer time on blinded phase study

Table 2. Objective response rates in the placebo-treated versus Palladia-treated dogs at the end of the 6-wk blinded phase and in all dogs treated with Palladia in the combined blinded and open-label phases

	Objective response								
	6-wk blinded phase				P*	Combined blinded and open-label phases			
	Placebo		Palladia			Placebo-escape [†]		Palladia [‡]	
	No. in group	No. (%)	No. in group	No. (%)	No. in group	No. (%)	No. in group	No. (%)	
Total population	63	5 (7.9)	86	32 (37.2)	0.0004 [§]	58	24 (41.4)	87	38 (43.7)
Lymph node involvement					0.699				
No	38	4 (10.5)	52	22 (42.3)		38	17 (44.7)	53	27 (50.9)
Yes	25	1 (4.0)	34	10 (29.4)		20	7 (35.0)	34	11 (32.4)
Central pathology tumor grade					0.058				
I	2	0 (0.0)	0	NA		1	1 (100.0)	0	NA
II	41	5 (12.2)	69	25 (36.2)		38	14 (36.8)	70	31 (44.3)
III	20	0 (0.0)	17	7 (41.2)		19	9 (47.4)	17	7 (41.2)
Lymph node-tumor grade					Not done				
No-I	1	0 (0.0)	0	NA		1	1 (100)	0	NA
No-II	27	4 (14.8)	44	19 (43.2)		27	11 (40.7)	45	24 (53.3)
No-III	10	0 (0.0)	8	3 (37.5)		10	5 (50.0)	8	3 (37.5)
Yes-I	1	0 (0.0)	0	NA		0	NA	NA	NA
Yes-II	14	1 (7.1)	25	6 (24.0)		11	3 (27.3)	25	7 (28.0)
Yes-III	10	0 (0.0)	9	4 (44.4)		9	4 (44.4)	9	4 (44.4)
Juxtamembrane c-kit mutation					0.482				
No sample	0	NA	2	0 (0)		0	NA	2	0 (0.0)
Negative	54	4 (7.4)	64	20 (31.3)		49	17 (34.7)	65	25 (38.5)
Positive	9	1 (11.1)	20	12 (60.0)		9	7 (77.8)	20	13 (65.0)
Prior chemotherapy					1.00				
No	36	4 (11.1)	47	20 (42.6)		35	14 (40.0)	47	22 (46.8)
Yes	27	1 (3.7)	39	12 (30.8)		23	10 (43.5)	40	16 (40.0)
Prior radiation therapy					0.166				
No	59	3 (5.1)	81	31 (38.3)		54	22 (40.7)	82	37 (45.1)
Yes	4	2 (50.0)	5	1 (20.0)		4	2 (50.0)	5	1 (20.0)
Age in y					1.00				
≤9	30	3 (10.0)	49	19 (38.8)		28	13 (46.4)	50	23 (46.0)
>9	33	2 (6.1)	37	13 (35.1)		30	11 (36.7)	37	15 (40.5)
Sex					1.00				
Female	37	4 (10.8)	50	19 (38.0)		33	14 (42.4)	51	25 (49.0)
Male	26	1 (3.8)	36	13 (36.1)		25	10 (40.0)	36	13 (36.1)
Performance status					1.00				
0-Normal	62	5 (8.1)	79	30 (38.0)		57	23 (40.4)	80	36 (45.0)
1-Restricted	1	0 (0.0)	7	2 (28.6)		1	1 (100.0)	7	2 (28.6)
Median duration of therapy in d (range)	21 (6-49)		42 (7-49)			97 (2-735)		63 (7-812)	

NOTE: All objective responses in the placebo group were partial responses. Objective responses in the Palladia group included 7 (8.1%) complete responses and 25 (29.1%) partial responses.

*P value for test of the interaction of treatment by covariate. The test for chemotherapy, radiation, age, sex and performance status were adjusted for multiple comparisons using the Hochberg method (SAS, proc multtest) due to these tests being unplanned comparisons.

[†]Dogs treated with placebo in the blinded phase that were treated with Palladia in the open-label phase; includes one dog excluded from the blinded phase efficacy analysis because it received one dose of prednisone within 2 wk of enrollment.

[‡]Dogs treated with Palladia in the blinded phase; includes one dog excluded from the blinded phase efficacy analysis because it received one dose of prednisone within 2 wk of enrollment.

[§]Odds ratio, 6.46; 95% confidence interval, 2.3-18.3.

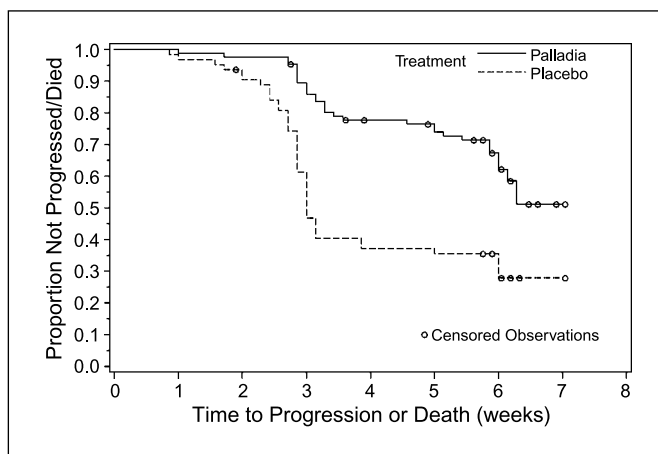


Fig. 1. Time to tumor progression in placebo-treated and Palladia-treated dogs. The median TTP in the blinded phase was 3 wk compared with >6 wk in placebo-treated and Palladia-treated dogs, respectively ($P < 0.0001$).

treatment compared with placebo-treated dogs, no adjustments were made in the statistical comparisons for this disparity.

Table 3 compares the incidence of common (>10% of Palladia-treated dogs) adverse events and laboratory abnormalities for dogs in the blinded phase, most of which were grade 1 or 2 in severity. Diarrhea, blood in stool (includes gastrointestinal bleed and hemorrhagic diarrhea), neutropenia, and weight loss were significantly more common in dogs receiving Palladia compared with placebo. Grade 3 or 4 adverse events were reported in 20.7% of Palladia-treated versus 15.6% of placebo-treated dogs ($P = 0.527$).

Combined blinded and open-label phases

Response to treatment. The objective response rate among Palladia-treated and placebo-escape dogs was 42.8% (62/145; 21 complete response and 41 partial response; Table 2). The observed biological response rate was 59.5% (78/131) and included 16 (12.2%) dogs with stable disease. The presence of a c-Kit ITD and the absence of regional lymph node metastasis were significantly associated with objective response ($P = 0.0008$ and $P = 0.037$, respectively). Tumor grade was not significantly associated with objective response ($P = 0.916$). Six (7.0%; 6/86) Palladia-treated dogs with stable disease at blinded phase week 6 had an objective response (4 complete response, 2 partial response) in the open-label phase (median time to objective response, 84 days; range, 83-181 days). The blinded phase week 6 lesion assessment for these dogs indicated the target lesion(s) were smaller (range, -8.5% to -29.3% versus baseline) but did not meet criteria for partial response.

Among dogs with an objective response ($n = 62$), the median TTP was 18.1 weeks. Dogs with grade 2 tumors had a longer TTP compared with those with grade 3 tumors ($P = 0.008$). Dogs without regional lymph node metastasis had a longer TTP than those with lymph node involvement but this was not significant ($P = 0.056$). c-Kit mutation status was not significantly associated with TTP ($P = 0.152$).

The median DOR in the 62 dogs was 12 weeks. Dogs with grade 2 tumors had a longer DOR than those with grade 3 tumors ($P = 0.019$). Regional lymph node metastasis and c-Kit mutation were not significantly associated with DOR ($P = 0.090$ and $P = 0.239$, respectively).

Safety. Of 87 dogs treated with Palladia and 64 dogs treated with placebo in the blinded phase, 53 (60.9%) and 58 (90.6%), respectively, entered the open-label phase. The median duration of Palladia treatment for the 145 dogs that received at least one dose of Palladia in the combined blinded and open-label phases was 68 days (mean, 144 days; range, 2-812 days). Palladia treatment continued in 24.8% (36/145) of dogs for over 6 months. The most common (>10% of Palladia-treated dogs) adverse events in these 145 dogs are summarized in Table 4. Among the 21 dogs with complete response, 2 had grade 4 adverse events that were possibly drug-related. One dog died with acute pancreatitis 56 days after initiating Palladia, 37 days after achieving complete response. The second dog died with gastric perforation 221 days after initiation of Palladia, 99 days after achieving complete response. There was no evidence of MCT at necropsy in either case.

Concomitant treatments and dose modifications. Significantly more Palladia-treated dogs received metronidazole (36.8%) compared with placebo-treated dogs (14.1%; $P = 0.003$). There were no other significant between-group differences in the frequencies of specific concomitant treatments. During the blinded phase, dose reductions (or drug holidays) were made for 6.3% (11%) of placebo-treated dogs and 19.5% (48.3%) of Palladia-treated dogs.

Exploratory health-related quality of life. Initial results suggest that there was no measurable decrease in the HRQL for the overall treated versus non-treated populations. The change from baseline in HRQL between the placebo-treated and Palladia-treated dogs was not statistically different at the end of the blinded phase ($P = 0.770$). Additional exploratory analysis indicated a significant difference in the total HRQL score between responders (complete response or partial response) compared with non-responders (stable disease or progressive disease) among Palladia-treated dogs at the end of the blinded phase ($P = 0.030$); dogs with an objective response had a positive change (+2) in HRQL score compared with a negative change (-7) for non-responders. Further psychometric analyses will be conducted to validate the instrument for future use in clinical studies.

Discussion

The current placebo-controlled trial of Palladia in dogs with grade 2 or 3 MCTs was conducted as a registrational study to assess the objective response rate and safety profile. Our data show that Palladia treatment resulted in a statistically significant increase in the 6-week objective response rate compared with placebo treatment (37.2% versus 7.9%). Following placebo escape, 41.4% of dogs responded to Palladia, and the overall objective response rate for all dogs that received at least one dose of Palladia in this study was 42.8%. These results confirm that Palladia has clinical activity in canine MCTs.

A variety of chemotherapeutics have been used to treat canine MCTs, although there is currently no formal standard of care and there are no drugs approved for the treatment of cancer of any type in veterinary medicine. Similar to the case in human systemic mastocytosis, few drugs exhibit clinical efficacy. Although robust, randomized, controlled prospective studies of chemotherapeutics commonly used to treat canine MCTs are lacking, reported response rates to various single-agent

Table 3. Summary of the most common adverse events ($\geq 10\%$ of Palladia-treated dogs) during blinded phase

Adverse event	Placebo (n = 64)		Palladia (n = 87)		P*	
	Any grade, n (%)	Grade 3 or 4, n (%)	Any grade, n (%)	Grade 3 or 4, n (%)	Any grade, n (%)	Grade 3 or 4, n (%)
Any		10 (15.6)		18 (20.7)		0.527
Gastrointestinal						
Diarrhea	17 (26.6)	2 (3.1)	40 (46.0)	6 (6.9)	0.018	0.468
Emesis	21 (32.8)	4 (6.3)	28 (32.2)	8 (9.2)	1.000	0.560
Blood in stool, gastrointestinal bleed, hemorrhagic diarrhea	2 (3.1)	0 (0.0)	11 (12.6)	2 (2.3)	0.044	—
Systemic disorder						
Anorexia, including decreased appetite	20 (31.3)	4 (6.3)	34 (39.1)	6 (6.9)	0.391	1.000
Lethargy	19 (29.7)	2 (3.1)	31 (35.6)	4 (4.6)	0.487	0.703
Weight loss	2 (3.1)	0 (0.0)	13 (14.9)	1 (1.1)	0.025	—
Musculoskeletal						
Lameness	6 (9.4)	0 (0.0)	15 (17.2)	0 (0.0)	0.234	—
Musculoskeletal disorder	4 (6.3)	0 (0.0)	10 (11.5)	1 (1.1)	0.396	—
not otherwise specified						
Laboratory abnormality						
Hematology						
Neutrophils	4 (6.3)	0 (0.0)	40 (46.0)	0 (0.0)	<0.0001	—
Platelets	13 (20.3)	0 (0.0)	21 (24.1)	0 (0.0)	0.694	—
Serum Chemistry						
ALT	14 (21.9)	3 (4.7)	21 (24.1)	1 (1.1)	0.846	—
Albumin	5 (7.8)	0 (0.0)	11 (12.6)	0 (0.0)	0.428	—

NOTE: Canine-Adapted Common Toxicity Criteria; see Supplemental Table S2. Adverse events not listed in Supplemental Table S2 were assigned grades of 1, 2, 3, or 4 for mild, moderate, severe, or life-threatening, respectively.

Adverse events reported by investigators were coded using terms provided by the Committee for Medicinal Products for Veterinary Use Veterinary Dictionary for Drug Regulatory Activities (CVMP VEDDRA Version 2.0; 18OCT04) in order to facilitate summarization of data.

Abbreviation: ALT, alanine aminotransferase.

*P value (Pearson χ^2) was calculated when at least 5 adverse events occurred. Grade 4 adverse events in dogs on placebo included emesis (3.1%), anorexia (1.6%), and ALT (1.6%). Grade 4 adverse events in dogs on Palladia included diarrhea (3.4%), emesis (2.3%), anorexia (1.1%), lethargy (1.1%), and ALT (1.1%).

therapies in several small clinical studies vary from 42% (lomustine; ref. 6), 7% to 30% (vincristine, vinblastine, or vinorelbine; refs. 34–37), and 20% to 70% (prednisone; refs. 5, 7). In most instances, the responses were of fairly short duration (2–3 months). Objective response rates ranging from 27% to 64% have been reported when vinblastine is combined with prednisone \pm cyclophosphamide (8, 9, 38). The use of corticosteroids in the current Palladia study was not permitted due to the potential effect on tumor size. The response rate to single-agent Palladia in this study (42.8%) seems to compare favorably with that observed with other single-agent and combination protocols for canine MCTs. Given the response of MCTs to prednisone and the improved response rate of vinblastine-prednisone combinations versus vinblastine alone, Palladia-prednisone combinations may yield higher response rates versus Palladia alone.

The 6-week objective response rate of 7.9% in the placebo group, comprising entirely partial responses, was not necessarily unexpected. MCTs release large quantities of inflammatory cytokines and other chemical mediators (histamine, leukotrienes, etc) that can directly influence tumor size (i.e., induce local tissue swelling; refs. 39–43), inducing waxing and waning of tumor size over time. Spontaneous complete responses have been reported in dogs with MCTs although these are more likely to occur in dogs with less-advanced disease than those enrolled in the current study.

During the combined blinded and open-label phase, the objective response rate in tumors that did not possess a c-Kit ITD was 36.8% (42/114) compared with 69% (20/29) in tumors with the mutation but there was no significant association between c-Kit mutation status and TTP or DOR. It has recently been shown that a small number of canine MCTs possess mutations in the extracellular domain of Kit, similar to those found in human acute myelogenous leukemia, which could account for a portion of the non-ITD responders (21–24). Additionally, Palladia inhibits VEGFR2 and PDGFR β and MCTs express both of these receptors. Although their contribution to tumor cell growth and survival is unknown (44),¹² inhibition of these receptors may have resulted in a direct antiangiogenic effect on the MCTs, thereby inducing tumor regression. Lastly, in a previous study investigating the effect of a single dose of Palladia on Kit in MCTs, several tumors without evidence of c-Kit ITD showed high basal levels of Kit phosphorylation that were down-regulated 8 hours following treatment (26). It is possible that some tumors exhibit significant Kit overexpression thereby resulting in spontaneous receptor dimerization and constitutive activation.

Although Kit mutation was associated with objective response, it was not associated with TTP, indicating that regardless of the

¹²C.L. unpublished.

Table 4. Summary of the most common ($\geq 10\%$ of dogs) adverse events during the study (combined blinded and open-label phases)

Adverse Event	Palladia-treated + Placebo-escape (n = 145)*	
	Any grade, n (%)	Grade 3 or 4, n (%)
Gastrointestinal		
Diarrhea	85 (58.6)	12 (8.3)
Emesis	69 (47.6)	14 (9.7)
Blood in stool, gastrointestinal bleed, hemorrhagic diarrhea	27 (18.6)	4 (2.8)
Systemic disorder		
Anorexia, including decreased appetite	72 (49.7)	12 (8.3)
Lethargy	57 (39.3)	6 (4.1)
Weight loss	31 (21.4)	4 (2.8)
Dehydration	22 (15.2)	3 (2.1)
Skin disorder		
Pruritus	18 (12.4)	0 (0.0)
Pigmentation disorder	17 (11.7)	0 (0.0)
Dermatitis	16 (11.0)	0 (0.0)
Musculoskeletal		
Musculoskeletal disorder not otherwise specified	16 (11.0)	0 (0.0)
Lameness	33 (22.8)	0 (0.0)
Laboratory abnormalities		
Hematology		
Neutrophils	65 (44.8)	2 (1.4)
Platelets	41 (28.3)	3 (2.1)
Hematocrit	16 (11.0)	4 (2.8)
Serum Chemistry		
Albumin	41 (28.3)	2 (1.4)
ALT	40 (27.6)	6 (4.1)
Creatinine	20 (13.8)	2 (1.4)

NOTE: Canine-Adapted Common Toxicity Criteria adapted for dogs; see Supplemental Table S2. Adverse events not listed in Supplemental Table S2 were assigned grades of 1, 2, 3 or 4 for mild, moderate, severe or life-threatening, respectively.

Adverse events reported by investigators were coded using terms provided by the Committee for Medicinal Products for Veterinary Use Veterinary Dictionary for Drug Regulatory Activities (CVMP VEDDRA Version 2.0; 18OCT04) in order to facilitate summarization of data.

*All dogs received at least one dose of Palladia.

molecular basis of response, mechanisms of resistance to Palladia may be similar among all tumors. The potential reasons for the development of this resistance were not investigated in the present study. However, in clinical trials of other small-molecule Kit inhibitors such as Gleevec (imatinib mesylate), drug resistance is often secondary to the development of new mutations in Kit that diminish drug binding, significant over-expression of Kit overwhelming the drug effects, and/or activation of other signaling pathways that circumvent the need for Kit phosphorylation (45).

In the present study, dogs with grade 2 tumors experienced a longer DOR than those with grade 3 tumors ($P = 0.019$). Grade 3 tumors are known to exhibit a more aggressive biological behavior and historically have median survival times that do not exceed 6 months. Interestingly, the DOR was similar in dogs regardless of tumor c-Kit ITD status. This is consistent with previous work showing that although c-Kit ITDs in canine MCTs were associated with an increased risk of metastasis and local recurrence, they were not associated with an overall worse prognosis (18).

Although there are no validated HRQL surveys in veterinary medicine, an attempt was made in this study to measure changes in HRQL in dogs treated with Palladia compared with placebo. This is important because long-term administration of targeted therapeutics, particularly those with multiple targets, can result in adverse events that necessitate significant treatment breaks (27). In this study, no difference was observed in

the total HRQL score between treatment groups at the end of the blinded phase, indicating that side effects associated with Palladia treatment did not significantly impact HRQL. Importantly, within the Palladia-treated group, those dogs that responded to treatment had a significantly higher HRQL score compared with those that did not.

The range of adverse events reported in this study were largely expected based on the previous phase I study of Palladia in dogs with various malignancies (25). Neutropenia was generally limited to \leq grade 2 which compares favorably with the use of chemotherapeutics for MCT in which grade 3/4 neutropenia may occur in up to 38% of dogs (6, 34). Grade 3/4 adverse events occurred in 15.6% of placebo-treated dogs and 20.7% of Palladia-treated dogs in the blinded phase. These were primarily gastrointestinal in nature and, as they were not significantly different between placebo- and Palladia-treated dogs, it is likely that the underlying disease process (MCT) contributed in part to the adverse events. Dogs with MCTs are known to have high circulating levels of histamine which induces increased gastric acid secretion, resulting in clinical or subclinical gastrointestinal ulceration (46–48). Grade 3/4 adverse events occurred in 34.5% of all dogs treated with at least one dose of Palladia in the combined blinded and open-label phases of the study. Again, these were mainly related to gastrointestinal signs and nearly all occurred in dogs with gross disease. Therefore, without a contemporaneous control group, adverse events in the open-label phase of this study were

confounded by the presence of mast cell disease and it is difficult to attribute particular adverse events directly to Palladia treatment.

The current study provides new evidence that small-molecule inhibitors targeting the split kinase family can be used on a continuous schedule without the need for routine planned treatment breaks. Every other day treatment continued in 24.8% (36/145) of dogs for over 6 months. For many of the split kinase inhibitors, schedules include 1 to 2 weeks off treatment to ameliorate adverse events associated with drug administration (27). This could potentially result in tumor regrowth during "off" periods, thereby reducing the effectiveness of therapy. The data presented in this study show that dogs can be used to model alternative treatment schedules of targeted therapies that may result in improved tolerability.

In summary, Palladia exhibits significant biological activity against MCTs in dogs. These data provide further evidence that spontaneous tumors in dogs represent a valid model in which to test the safety and efficacy of targeted therapeutics. The use of Palladia in dogs with cancer also provides a unique opportunity to evaluate multitargeted kinase inhibitors in the microscopic disease setting where clinical efficacy may be significantly im-

proved, and to establish safe protocols for combining Palladia with other therapeutic modalities such as radiation and chemotherapy. Given the effect of Palladia on VEGFR2 and PDGFR β in addition to Kit, it is reasonable to expect that, similar to the case of multi-targeted therapeutics in human cancers, the spectrum of activity of Palladia will extend beyond MCTs to several other tumor types.

Disclosure of Potential Conflicts of Interest

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References

- London CA, Seguin B. Mast cell tumors in the dog. *Vet Clin North Am Small Anim Pract* 2003; 33:473-89.
- Patnaik AK, Ehler WJ, MacEwen EG. Canine cutaneous mast cell tumors: morphologic grading and survival time in 83 dogs. *Vet Pathol* 1984;21: 469-74.
- Scase TJ, Edwards D, Miller J, et al. Canine mast cell tumors: correlation of apoptosis and proliferation markers with prognosis. *J Vet Intern Med* 2006;20:151-8.
- Davies DR, Wyatt KM, Jardine JE, Robertson ID, Irwin PJ. Vinblastine and prednisolone as adjunctive therapy for canine cutaneous mast cell tumors. *J Am Anim Hosp Assoc* 2004;40:124-30.
- McCaw DL, Miller MA, Ogilvie GK, et al. Response of canine mast cell tumors to treatment with oral prednisone. *J Vet Intern Med* 1994;8: 406-8.
- Rassnick KM, Moore AS, Williams LE, et al. Treatment of canine mast cell tumors with CCNU (lomustine). *J Vet Intern Med* 1999;13: 601-5.
- Stancliff RM, Gilson SD. Evaluation of neoadjuvant prednisone administration and surgical excision in treatment of cutaneous mast cell tumors in dogs. *J Am Vet Med Assoc* 2008;232: 53-62.
- Thamm DH, Mauldin EA, Vail DM. Prednisone and vinblastine chemotherapy for canine mast cell tumor-41 cases (1992-1997). *J Vet Intern Med* 1999;13:491-7.
- Thamm DH, Turek MM, Vail DM. Outcome and prognostic factors following adjuvant prednisone/vinblastine chemotherapy for high-risk canine mast cell tumour: 61 cases. *J Vet Med Sci* 2006;68:581-7.
- Furitsu T, Tsujimura T, Tono T, et al. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand independent activation of c-kit product. *J Clin Invest* 1993; 92:1736-44.
- Tsujimura T, Furitsu T, Morimoto M, et al. Ligand-independent activation of c-kit receptor tyrosine kinase in a murine mastocytoma cell line P815 generated by a point mutation. *Blood* 1994;83:2619-26.
- Tsujimura T, Furitsu T, Morimoto M, et al. Substitution of an aspartic acid results in constitutive activation of c-kit receptor tyrosine kinase in a rat tumor mast cell line RBL-2H3. *Int Arch Allergy Immunol* 1995;106:377-85.
- Barbie DA, Deangelo DJ. Systemic mastocytosis: current classification and novel therapeutic options. *Clin Adv Hematol Oncol* 2006;4: 768-75.
- Orfao A, Garcia-Montero AC, Sanchez L, Escribano L. Recent advances in the understanding of mastocytosis: the role of KIT mutations. *Br J Haematol* 2007;138:12-30.
- Quintas-Cardama A, Aribi A, Cortes J, Giles FJ, Kantarjian H, Verstovsek S. Novel approaches in the treatment of systemic mastocytosis. *Cancer* 2006;107:1429-39.
- London CA, Galli SJ, Yuuki T, Hu Z-Q, Helfand SC, Geissler EN. Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. *Exp Hematol* 1999; 27:689-97.
- Ma Y, Longley BJ, Wang X, Blount JL, Langley K, Caughey GH. Clustering of activating mutations in c-KIT's juxtamembrane coding region of canine mast cell neoplasms. *J Invest Dermatol* 1999;112: 165-70.
- Downing S, Chien MB, Kass PH, Moore PE, London CA. Prevalence and importance of internal tandem duplications in exons 11 and 12 of c-kit in mast cell tumors of dogs. *Am J Vet Res* 2002;63:1718-23.
- Webster JD, Yuzbasiyan-Gurkan V, Kaneene JB, Miller R, Resau JH, Kiupel M. The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. *Neoplasia* 2006;8: 104-11.
- Webster JD, Yuzbasiyan-Gurkan V, Miller RA, Kaneene JB, Kiupel M. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Vet Pathol* 2007;44:298-308.
- Letard S, Yang Y, Hanssens K, et al. Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. *Mol Cancer Res* 2008;6:1137-45.
- Gari M, Goodeve A, Wilson G, et al. c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. *Br J Haematol* 1999;105:894-900.
- Schnittger S, Kohl TM, Haferlach T, et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood* 2006;107:1791-9.
- Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol* 2006;24:3904-11.
- London CA, Hannah AL, Zadovskaya R, et al. Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies. *Clin Cancer Res* 2003;9:2755-68.
- Pryer NK, Lee LB, Zadovskaya R, et al. Proof of target for SU11654: inhibition of KIT phosphorylation in canine mast cell tumors. *Clin Cancer Res* 2003;9:5729-34.
- Porta C, Paglino C, Imarisio I, Bonomi L. Uncovering Pandora's vase: the growing problem of new toxicities from novel anticancer agents. The case of sorafenib and sunitinib. *Clin Exp Med* 2007;7:127-34.
- Braconi C, Bracci R, Cellerino R. Molecular targets in Gastrointestinal Stromal Tumors (GIST) therapy. *Curr Cancer Drug Targets* 2008;8:359-66.
- Judson I, Demetri G. Advances in the treatment of gastrointestinal stromal tumours. *Ann Oncol* 2007;18 Suppl 10:20-4.
- Couto GC. Principles of cancer treatment. In: Nelson RW, Couto CG, editors. *Essentials of Small Animal Internal Medicine*. 2nd ed. St. Louis (MO): Mosby; 1998. p. 1110.
- CVM Guidelines and Guidance Documents: Good Clinical Practice. In: US Department of Health and Human Services FDA, Center of Veterinary Medicine, 2001.
- Jones CL, Grahn RA, Chien MB, Lyons LA, London CA. Detection of c-kit mutations in

- canine mast cell tumors using fluorescent polyacrylamide gel electrophoresis. *J Vet Diagn Invest* 2004;16:95–100.
33. Zavodovskaya R, Liao AT, Jones CL, et al. Evaluation of dysregulation of the receptor tyrosine kinases Kit, Flt3, and Met in histiocytic sarcomas of dogs. *Am J Vet Res* 2006;67:633–41.
 34. Grant IA, Rodriguez CO, Kent MS, Lord L, London CA. A Phase II clinical trial of vinorelbine in dogs with cutaneous mast cell tumors. *J Vet Intern Med* 2008;22:388–93.
 35. Henry CJ, Downing S, Rosenthal RC, et al. Evaluation of a novel immunomodulator composed of human chorionic gonadotropin and bacillus Calmette-Guerin for treatment of canine mast cell tumors in clinically affected dogs. *Am J Vet Res* 2007;68:1246–51.
 36. McCaw DL, Miller MA, Bergman PJ, et al. Vincristine therapy for mast cell tumors in dogs. *J Vet Intern Med* 1997;11:375–8.
 37. Rassnick KM, Bailey DM, Flory AB, et al. Efficacy of vinblastine for the treatment of canine mast cell tumors. *J Vet Intern Med*. In press 2008.
 38. Camps-Palau MA, Leibman NF, Elmslie R, et al. Treatment of canine mast cell tumours with vinblastine, cyclophosphamide and prednisone: 35 cases (1997–2004). *Vet Comp Onc* 2007;5:156–67.
 39. Amagai Y, Tanaka A, Ohmori K, Matsuda H. Establishment of a novel high-affinity IgE receptor-positive canine mast cell line with wild-type c-kit receptors. *Biochem Biophys Res Commun* 2008;366:857–61.
 40. DeVinney R, Gold WM. Establishment of two dog mastocytoma cell lines in continuous culture. *Am J Respir Cell Mol Biol* 1990;3:413–20.
 41. Goetzl EJ, Phillips MJ, Gold WM. Stimulus specificity of the generation of leukotrienes by dog mastocytoma cells. *J Exp Med* 1983;158:731–7.
 42. Lin TY, Rush LJ, London CA. Generation and characterization of bone marrow-derived cultured canine mast cells. *Vet Immunol Immunopathol* 2006;113:37–52.
 43. Takahashi T, Kitani S, Nagase M, et al. IgG-mediated histamine release from canine mastocytoma-derived cells. *Int Arch Allergy Immunol* 2001;125:228–35.
 44. Rebuzzi L, Willmann M, Sonneck K, et al. Detection of vascular endothelial growth factor (VEGF) and VEGF receptors Flt-1 and KDR in canine mastocytoma cells. *Vet Immunol Immunopathol* 2007;115:320–33.
 45. Sleijfer S, Wiemer E, Seynaeve C, Verweij J. Improved insight into resistance mechanisms to imatinib in gastrointestinal stromal tumors: a basis for novel approaches and individualization of treatment. *Oncologist* 2007;12:719–26.
 46. Fox LE, Rosenthal RC, Twedt DC, Dubielzig RR, MacEwen EG, Grauer GF. Plasma histamine and gastrin concentrations in 17 dogs with mast cell tumors. *J Vet Intern Med* 1990;4:242–6.
 47. Howard EB, Sawa TR, Nielsen SW, Kenyon AJ. Mastocytoma and gastroduodenal ulceration. Gastric and duodenal ulcers in dogs with mastocytoma. *Pathol Vet* 1969;6:146–58.
 48. Ishiguro T, Kadosawa T, Takagi S, et al. Relationship of disease progression and plasma histamine concentrations in 11 dogs with mast cell tumors. *J Vet Intern Med* 2003;17:194–8.