

## Standard Article

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## Effects of Toceranib Phosphate on the Hypothalamic-Pituitary-Thyroid Axis in Tumor-Bearing Dogs

K.R. Hume , V.L. Rizzo, J.R. Cawley, and C.E. Balkman**Background:** Thyroid dysfunction is associated with the use of tyrosine kinase inhibitors (TKI) in people.**Hypothesis/Objectives:** To determine whether dysfunction in the hypothalamic-pituitary-thyroid axis occurs in dogs receiving the TKI, toceranib phosphate.**Animals:** Forty-three client-owned dogs with cancer.**Methods:** Prospective, observational study. Concentrations of total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT3), and thyroid-stimulating hormone (TSH) were evaluated on day 0, 30, and 90. Dogs also were evaluated for the presence of thyroglobulin autoantibodies.**Results:** The proportion of dogs with low TT4, low FT4, low TT3, high TSH, or primary hypothyroidism (increased TSH and decreased TT4, FT4 or both) did not change over 90 days. Hormone concentrations remained within laboratory reference intervals, but FT4 ( $P = 0.0032$ ) and TSH ( $P < 0.0001$ ) changed over time. Mean FT4 was 1.22 ng/dL (95% confidence interval [CI], 1.10–1.34) on day 0 and 1.00 ng/dL (95% CI, 0.86–1.16) on day 90. Mean TSH was 0.17 ng/mL (95% CI, 0.13–0.23) on day 0 and 0.34 ng/mL (95% CI, 0.24–0.48) on day 90. Furthermore, TT4/TT3 ratio also changed over time ( $P = 0.0086$ ). Mean TT4/TT3 ratio was 2.57 (95% CI, 2.26–2.88) on day 0 and 2.02 on day 90 (95% CI, 1.61–2.44). Thyroglobulin autoantibodies were not detected in any dog.**Conclusions and Clinical Importance:** Toceranib phosphate can disrupt the hypothalamic-pituitary-thyroid axis in dogs. Periodic evaluation of TT4, FT4, TT3, and TSH should be carried out in dogs receiving long-term treatment with this medication.**Key words:** Cancer; Canine; Hypothalamic-pituitary-thyroid axis; Hypothyroidism; Nonthyroidal illness; Tyrosine kinase inhibitors.

Toceranib phosphate<sup>a</sup> is a tyrosine kinase inhibitor (TKI) with activity against several members of the split receptor tyrosine kinase family, including stem cell growth factor receptor (KIT), vascular endothelial growth factor receptor-2 (VEGFR-2), and platelet-derived growth factor receptor-B (PDGFR-B).<sup>1</sup> It is approved for use in dogs with Patnaik grade II or III, recurrent cutaneous mast cell tumors (MCT) with or

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## Abbreviations:

AHDC	Cornell University Animal Health Diagnostic Center
CUHA	Cornell University Hospital for Animals
FT4	free T4
GIST	gastrointestinal stromal tumor
HCC	hepatocellular carcinoma
KIT	stem cell growth factor receptor
MCT	mast cell tumor
MWF	Monday, Wednesday, and Friday
NSAID	nonsteroidal anti-inflammatory drug
NTI	nonthyroidal illness
PDGFR-B	platelet-derived growth factor receptor-B
SVRC	Summit Veterinary Referral Center
T3	triiodothyronine
T4	thyroxine
TGA	thyroglobulin autoantibodies
TKI	tyrosine kinase inhibitor
TSH	thyroid-stimulating hormone
TT3	total T3
TT4	total T4
UPC	urine protein:creatinine ratio
VEGF	vascular endothelial growth factor
VEGFR-2	vascular endothelial growth factor receptor-2

without regional lymph node involvement.<sup>2</sup> Toceranib phosphate also is used off-label to treat other tumor types in dogs.<sup>3</sup> The TKIs generally are well tolerated, but have been associated with several adverse effects. Similar toxicities have been observed in both people and dogs, including gastrointestinal toxicity, muscle or joint pain, hypertension, and neutropenia.<sup>4–9</sup>

Thyroid dysfunction has been noted in people receiving several different TKIs, including sunitinib, sorafenib, imatinib, and others.<sup>10</sup> In a prospective study of euthyroid patients being treated with sunitinib for

gastrointestinal stromal tumors (GIST), 36% (15/42) developed persistent, primary hypothyroidism, defined as persistent increase in thyroid-stimulating hormone (TSH)  $>5.0$  mU/L.<sup>11</sup> In another prospective study, 27% (16/59) of patients treated with sunitinib for metastatic renal cell carcinoma or imatinib-resistant GIST developed subclinical (TSH above the upper limit of normal with normal free thyroxine index [FTI]) or clinical (increased TSH with decreased FTI) hypothyroidism requiring treatment.<sup>12</sup> The TKIs also have been linked to recurrence of hypothyroidism in patients with well-controlled, preexisting hypothyroidism. One study of 8 patients being treated with imatinib for metastatic medullary thyroid cancer showed that all patients on previously stable thyroid hormone replacement after thyroidectomy developed significantly increased TSH and decreased serum free thyroxine concentrations (FT4).<sup>13</sup>

Several mechanisms may play a role in TKI-induced hypothyroidism including decreased thyroid gland capillary density, induction of type 3 deiodinase activity, inhibition of iodine uptake, inhibition of peroxidase activity, altered thyroid hormone transport, decreased thyroid hormone synthesis, and in some patients, thyroid autoimmunity resulting in thyroid failure.<sup>14–18</sup> Studies performed in rats exposed to sunitinib showed changes in thyroid gland capillary density, which was both structural and functional in nature. These changes were hypothesized to be caused by the antiangiogenic effects of vascular endothelial growth factor (VEGF) receptor inhibition and increased concentrations of circulating endothelin-1.<sup>14,19</sup> Decreased iodine-123 uptake occurred in patients that developed hypothyroidism while receiving sunitinib.<sup>15</sup> This finding could not be duplicated in cell culture experiments using rat thyroid cells exposed to sunitinib.<sup>20</sup> It was hypothesized that decreased iodine uptake in patients may be secondary to decreased blood flow to the gland.

To our knowledge, hypothyroidism has not been reported in dogs receiving TKIs. Hypothyroidism is known to negatively affect quality of life in both people and dogs. Signs can be vague and may include lethargy, anemia, and weakness, clinical signs that overlap with the adverse event profile of TKIs. It would be useful to know whether TKIs influence the hypothalamic-pituitary-thyroid axis in dogs, so as to inform appropriate clinical monitoring and prevent premature discontinuation of anticancer therapy for perceived drug intolerance that could be otherwise managed with hormone supplementation. We hypothesized that the TKI toceranib phosphate would affect the hypothalamic-pituitary-thyroid axis in dogs and designed a 90-day prospective study to test our hypothesis.

## Materials and Methods

Dogs were enrolled through either the Cornell University Hospital for Animals (CUHA) in Ithaca, NY, or Summit Veterinary Referral Center (SVRC) in Tacoma, Washington. Our goal was to have 20 dogs complete the study. This number would provide 80% power to detect an increase in hypothyroidism prevalence to 35%

in our study population, assuming a null proportion of 1% ( $\alpha = 0.05$ ), with diagnosis based on laboratory detection of high TSH and low total thyroxine concentrations (TT4) or low FT4.

## Inclusion and Exclusion Criteria

Any dog scheduled to receive toceranib phosphate as part of its anticancer treatment was eligible for enrollment as long as no exclusion criteria were present. Exclusion criteria were life-threatening systemic illness, expected survival  $<3$  months, thyroid carcinoma, previous neck irradiation, concurrent chemotherapy, concurrent radiation therapy, or some combination of these. Dogs receiving phenobarbital or sulfonamides also were excluded.

## Clinical Monitoring

All dogs were monitored for adverse events according to a standard protocol. Complete blood counts, serum biochemistry profiles, and urinalyses were performed 2 and 4 weeks after starting toceranib phosphate, and at monthly intervals thereafter, or with increased frequency as indicated by clinical signs or development of abnormalities. Clinical characteristics also were recorded: signalment, body weight, tumor type, tumor measurements, drug dosage, subsequent dosage modifications, response to treatment,<sup>21</sup> adverse events, concurrent medications, and patient outcome.

## Monitoring for Hormonal Alterations in the Hypothalamic-Pituitary-Thyroid Axis

Serum was collected for evaluation on days 0, 30, and 90. For dogs presenting to CUHA, whole blood was collected and submitted to the Cornell University Animal Health Diagnostic Center (AHDC), Ithaca, NY, for processing and evaluation of TT4, total triiodothyronine concentration (TT3), FT4, TSH, and thyroglobulin autoantibodies. For dogs presenting to SVRC, whole blood was collected. After clotting and centrifugation, serum was separated and stored at  $-20^{\circ}\text{C}$ . Samples then were shipped in batches to Cornell University for the same analysis at the AHDC. Procedures used in thyroid testing of dogs by the AHDC have been described previously.<sup>22</sup> Before May 1, 2014, the AHDC used a 2-step radioimmunoassay to quantify FT4. When commercial production of that kit ceased, the Immulite<sup>b</sup> assay was used. The radioimmunoassay used a specific antibody and an incubation and double wash step to remove any protein-bound T4, whereas the Immulite chemiluminescent assay does not have a separation step.

## Statistical Analysis

To determine whether the proportion of dogs with low TT4, low TT3, low FT4, or high TSH changed over time (as compared to day 0), a paired proportion comparison was performed using McNemar's test. This analysis reflects within dog effects. Low and high were defined as lower than or higher than the AHDC reference interval. The AHDC reference intervals are as follows: TT4, 1.5–3  $\mu\text{g/dL}$ ; TT3 0.6–1.4 ng/mL; FT4 0.7–2.5 ng/dL; TSH 0–0.5 ng/mL. A Bonferroni correction was used to adjust for multiple comparisons among time points for each response variable. This same paired proportion comparison method was used to determine if the proportion of dogs with hormonal changes consistent with primary hypothyroidism (ie, high TSH with low TT4 or FT4) changed over time. Association of other variables with changes in proportions ultimately was not tested because of the low overall number of events.

To determine whether the continuous variables TT4, TT3, FT4, TSH, TT4/TT3 ratio, body weight, and toceranib phosphate dosage changed over time, linear mixed model analyses were

performed. Time was used as a fixed effect in the model (days 0, 30, and 90, categorically) with individual dog as a random effect because of the repeated measures taken on each dog over time. This analysis produces subject-specific estimates because of the random dog effect. Transformations of the response variable were performed as necessary to ensure model assumptions of normality and homogeneous variance were met. Post hoc pairwise comparisons were made with Tukey's correction to adjust for multiple comparisons. When changes in hormone concentrations or ratios were detected, the following variables were incorporated into the mixed model as individual fixed effects: FT4 assay, tumor type (MCT versus other), macroscopic versus microscopic disease, prednisone/prednisolone usage (yes or no), nonsteroidal anti-inflammatory drug (NSAID) usage (yes or no), antibiotic usage (yes or no), site of enrollment, body weight, toceranib phosphate dosage, age, and sex. This was done in an effort to ensure results of the analysis did not reflect other potential influences on thyroid function or test result. For toceranib phosphate dosage, the influence of concurrent treatment with prednisone/prednisolone and NSAIDs was evaluated in the same manner.

Significance was defined as  $P < 0.05$ . All statistical analyses were performed using statistical software.<sup>c</sup>

Our study was approved by the Cornell University Institutional Animal Care and Use Committee, and informed client consent was obtained for each dog. Owners were responsible for the costs associated with routine clinical monitoring; evaluation of the hypothalamic-pituitary-thyroid axis was provided at no cost to owners. Toceranib phosphate was provided at no cost to owners of enrolled dogs.

## Results

Forty-five dogs were evaluated for study enrollment. One dog had concurrent dermatologic disease, high cholesterol, low TT4, low TT3, low FT4, and normal TSH concentrations. A second dog had concurrent dermatologic disease, low TT4, low TT3, low FT4, and increased TSH concentrations. Owners of both of these dogs elected to begin thyroid supplementation and did not wish to continue participation in the study. Forty-three dogs ultimately were enrolled. Thirty-one dogs were enrolled at CUHA from August 1, 2012, to February 1, 2016. Twelve dogs were enrolled at SVRC from December 1, 2014, to December 1, 2015. Twenty-four dogs were castrated males; 19 dogs were spayed females. Median age was 8 years (range, 3–16 years). Median body weight was 30.1 kg (range, 6.7–63.7 kg). There were 9 mixed breed dogs and 34 purebred dogs. There were 7 Golden Retrievers, 4 Labrador Retrievers, 3 Basset hounds, 3 Miniature schnauzers, 2 Boxers, and 1 each of 15 other breeds. A variety of tumor types were represented. Twenty-three dogs had MCT; 4 dogs had soft tissue sarcoma; 3 dogs had MCT plus a second tumor type (1 each of heart base, adrenal, and prostate); 2 dogs had nasal tumors (1 carcinoma and 1 unknown); 2 dogs had osteosarcoma; the remaining dogs had the following diagnoses: angiosarcoma, bronchoalveolar carcinoma, ceruminous adenoma/adenocarcinoma, hepatocellular carcinoma (HCC), HCC and anal sac apocrine gland adenocarcinoma, HCC and melanoma, urinary bladder transitional cell carcinoma, synovial sarcoma, and undifferentiated cutaneous malignancy. Twenty-nine dogs had macroscopic disease when treatment was initiated; 14 dogs had microscopic disease.

Median toceranib phosphate dosage was 2.55 mg/kg (range, 1.49–2.89 mg/kg) PO on Mondays, Wednesdays, and Fridays (MWF). Day 0 thyroid hormone data were missing from 1 dog. Thirty-six dogs remained in study at day 30. Seven dogs were withdrawn between day 0 and 30 because of progressive disease ( $n = 1$ ), potential treatment-related adverse events ( $n = 5$ ), and protocol noncompliance ( $n = 1$ ). Seventeen dogs remained in study at day 90. Nineteen dogs were withdrawn between day 30 and 90 because of progressive disease ( $n = 11$ ), potential treatment-related adverse events ( $n = 4$ ), concurrent progressive disease and potential treatment-related adverse events ( $n = 2$ ), and protocol noncompliance ( $n = 2$ ). The potential treatment-related adverse events included gastrointestinal toxicity ( $n = 7$ ), proteinuria ( $n = 1$ ), hindlimb weakness ( $n = 1$ ), weakness and tremors ( $n = 1$ ), and a vestibular event ( $n = 1$ ). Ultimately, we had > 95% power to detect an increase in prevalence to 35% at day 30, and we had 70% power to detect such an increase at day 90. Tumor responses, all complete responses,<sup>21</sup> were documented in 4 of 12 dogs with measurable MCT.

Prednisone/prednisolone use occurred in 40, 42, and 41% of dogs at day 0, 30, and 90, respectively. Median prednisone/prednisolone dosage was 0.67 mg/kg (range, 0.26–1.3). Use of NSAID occurred in 16, 14, and 12% of dogs at day 0, 30, and 90, respectively. Prescribed NSAIDs included carprofen and piroxicam. Dogs that received glucocorticoids or NSAIDs generally received these medications on non-toceranib phosphate days. Antibiotic use occurred in 19, 17, and 6% of dogs at day 0, 30, and 90, respectively. Prescribed antibiotics included amoxicillin/clavulanic acid, cephalexin, enrofloxacin, metronidazole, and tylosin.

The proportion of dogs with low TT4, low TT3, low FT4, or high TSH did not change during the 90-day study period (Table 1). Although 8 dogs had hormonal changes consistent with primary hypothyroidism on at least 1 serum evaluation, no significant change was observed in the proportion of affected dogs during the 90-day study period (5%,  $n = 2$  on day 0; 11%,  $n = 4$  on day 30,  $P = 0.82$ ; 24%,  $n = 4$  on day 90,  $P = 0.31$ ). Of these 8 dogs, 4 were normal at day 0 and hypothyroid at day 30. Three of these 4 dogs were off study by day 90; the 4th dog's TSH normalized at day 90. Two dogs were normal at day 0 and 30 and hypothyroid at day 90. Two dogs were hypothyroid at day 0 and 90 but not day 30; at day 30, TSH remained increased but TT4 was within the reference interval, whereas it was below the reference interval on day 0 and 90. For these 2 dogs, FT4 was within the reference interval at all 3 time points, although concentrations decreased over time. Clinical information was available on 7 of these dogs. Minor increases in body weight were noted in 4 dogs at the time of hypothyroidism, with an increase of 1–4% from baseline. One dog had mild nonregenerative anemia (PCV, 39%) at the time of hypothyroidism. One dog had an increased cholesterol concentration at the time of hypothyroidism (360 mg/dL; range, 138–332 mg/dL). For descriptive purposes, other patterns of hormonal changes were considered. At day 0, 3 of 42

**Table 1.** Hormonal patterns in dogs receiving toceranib phosphate.

Day (n)	Low TT4		Low TT3		Low FT4		High TSH		Hypothyroid <sup>a</sup>	
	% (n)	<i>P</i> Value	% (n)	<i>P</i> Value	% (n)	<i>P</i> Value	% (n)	<i>P</i> Value	% (n)	<i>P</i> Value
0 (42)	40 (17)		29 (12)		7 (3)		12 (5)		5 (2)	
30 (36)	53 (19)	0.58	25 (9)	>0.99	14 (5)	0.82	28 (10)	0.19	11 (4)	0.82
90 (17)	59 (10)	0.64	18 (3)	0.16	12 (2)	0.64	35 (6)	0.09	24 (4)	0.31

Summary statistics indicating the proportion of affected dogs at each time point. *P*-values associated with paired proportion comparisons to day 0 are also provided.

<sup>a</sup>See Materials and Methods text for details regarding determination of hypothyroidism.

dogs (7%) had increases in TSH without decreases in TT4, TT3, or FT4 below the reference intervals. At day 30 and 90, 6 of 26 dogs (17%) and 2 of 17 dogs (12%), respectively, had increases in TSH but did not have decreases in the other hormones below the reference intervals. At day 0, 19 of 42 dogs (45%) had decreases in TT4, TT3, FT4, or some combination of these without an increase in TSH above the reference interval. At day 30 and 90, 17 of 36 dogs (47%) and 6 of 17 dogs (35%), respectively, had decreases in TT4, TT3, FT4, or some combination of these without an increase in TSH above the reference interval. At day 0, 18 of 42 dogs (43%) had all hormone concentrations within reference intervals. At days 30 and 90, 9 of 36 dogs (25%) and 5 of 17 dogs (29%) had all hormone concentrations within reference intervals.

Changes in specific hormone concentrations occurred over the 90-day study period. In our mixed model analyses, FT4 (*P* = 0.0032) and TSH (*P* < 0.0001) changed over time, whereas TT4 (*P* = 0.25) and TT3 (*P* = 0.39) did not (Table 2). Thyroglobulin autoantibodies were not detected in any dog. For both FT4 and TSH, results on days 30 and 90 differed from day 0. At day 0, mean FT4 was 1.22 ng/dL (95% CI, 1.10–1.34), compared with 1.04 ng/dL (95% CI, 0.93–1.16) on day 30 and 1.00 ng/dL (95% CI, 0.86–1.16) on day 90 (*P* = 0.0032). At day 0, mean TSH was 0.17 ng/mL (95% CI, 0.13–0.23), compared with 0.28 ng/mL (95% CI, 0.21–0.38) on day 30 and 0.34 ng/mL (95% CI, 0.24–0.48) on day 90 (*P* < 0.0001). None of the covariables evaluated showed statistically significant associations with FT4 or TSH. For FT4, type of test showed marginal significance (*P* = 0.05). Mean FT4 using the Immulite test was 1.17 ng/dL (95% CI, 1.04–1.30), whereas mean FT4 using the 2-step method was 0.98 ng/dL (95% CI,

0.85–1.13). For TSH, glucocorticoid usage did not reach statistical significance (*P* = 0.069). Mean TSH in dogs receiving glucocorticoids was 0.20 ng/mL (95% CI, 0.14–0.29) versus 0.29 ng/mL (95% CI, 0.22–0.40) in dogs not receiving glucocorticoids. The type of FT4 test or glucocorticoid usage did not show a significant interaction with time, nor did it affect the significance of time. For descriptive purposes, we determined the proportion of dogs that had both increased TSH and decreased FT4 compared to baseline concentrations. At day 30, 17 of 35 dogs (49%) had both changes; at day 90, 9 of 16 dogs (56%) had both changes. Some dogs had increased TSH without decreased FT4 (day 30, 11 of 35 dogs [31%]; day 90, 4 of 16 dogs [25%]). Other dogs had decreased FT4 without increased TSH (day 30, 6 of 35 dogs [17%]; day 90, 2 of 16 dogs [13%]).

We also identified an association of TT4/TT3 ratio and time, with differences between day 0 and 30 or day 90 (*P* = 0.0086). Mean TT4/TT3 ratio was 2.57 (95% CI, 2.26–2.88) on day 0, 2.19 on day 30 (95% CI, 1.86–2.51), and 2.02 on day 90 (95% CI, 1.61–2.44). The only covariable found to have an association with TT4/TT3 ratio was enrollment site (*P* = 0.030), with dogs enrolled through SVRC having higher ratios than dogs enrolled at CUHA (SVRC mean ratio, 2.76; 95% CI, 2.24–3.29; CUHA mean ratio, 2.09; 95% CI, 1.77–2.40). This association did not affect the significance of time, and the trend was the same between sites. Mean ratio at each site was lower on day 30 and 90 than on day 0 (data not shown).

Toceranib phosphate dosage changed over time (*P* < 0.0001), with dosage on day 90 (mean, 2.28 mg/kg; 95% CI, 2.18–2.38) being different from both day 0 (mean, 2.57 mg/kg; 95% CI, 2.50–2.63) and day 30 (mean, 2.49 mg/kg; 95% CI, 2.42–2.56). There was no

**Table 2.** Hormone concentrations in dogs receiving toceranib phosphate.

Day (n)	TT4 (μg/dL) <sup>a</sup>	TT3 (ng/mL) <sup>a</sup>	FT4 (ng/dL) <sup>b</sup>	TSH (ng/mL) <sup>c</sup>	TT4/TT3 Ratio <sup>d</sup>
0 (42)	1.64 (1.42–1.87)	0.69 (0.63–0.75)	1.22 (1.10–1.34)	0.17 (0.13–0.23)	2.57 (2.26–2.88)
30 (36)	1.51 (1.29–1.75)	0.72 (0.66–0.79)	1.02 (0.93–1.16)	0.28 (0.21–0.38)	2.19 (1.86–2.51)
90 (17)	1.45 (1.17–1.76)	0.74 (0.66–0.83)	1.00 (0.86–1.16)	0.34 (0.24–0.48)	2.02 (1.61–2.44)

TT4, TT3, FT4, TSH, and TT4/TT3 ratio at day 0, 30, and 90 in dogs receiving toceranib phosphate. Means and 95% confidence intervals from mixed model analysis are provided.

<sup>a</sup>Differences not statistically significant.

<sup>b</sup>Results on day 30 and 90 differ from day 0 (*P* = 0.0032).

<sup>c</sup>Results on day 30 and 90 differ from day 0 (*P* < 0.0001).

<sup>d</sup>Results on day 30 and 90 differ from day 0 (*P* = 0.0086).

association between glucocorticoid ( $P = 0.67$ ) or NSAID ( $P = 0.37$ ) usage and toceranib phosphate dosage. Similarly, there was no association between toceranib phosphate dosage and FT4 ( $P = 0.10$ ) or TSH ( $P = 0.13$ ). Body weight did not change over time ( $P = 0.66$ ).

## Discussion

We detected hormonal changes consistent with TKI-mediated effects on the hypothalamic-pituitary-thyroid axis<sup>10,13</sup> in dogs receiving toceranib phosphate. After 30 days of treatment, FT4 decreased, TSH increased, and TT4/TT3 ratio decreased (Table 2). Because these changes are consistent with primary hypothyroidism,<sup>23,24</sup> it is possible that with longer duration of toceranib phosphate treatment, some dogs in our study may have eventually developed sufficiently severe enough hormonal abnormalities to cause clinical disease. Thyroid hormone abnormalities can be progressive in people with TKI-induced hypothyroidism.<sup>15</sup> In addition, some dogs in our study experienced increased TSH without decreased T4, as can occur with subclinical hypothyroidism. Although subclinical hypothyroidism has not been well described in dogs, evidence in people suggests it can progress to overt hypothyroidism.<sup>25</sup> To ascertain whether the findings we observed are transient or progressive over time, or ultimately are associated with adverse clinical consequences, a sufficiently powered study with long-term follow-up is needed.

The decrease in TT4/TT3 ratio we detected after initiation of toceranib phosphate may reflect an increase in fractional conversion of T4 to metabolically active T3 as can be seen with iodine deficiency or primary hypothyroidism caused by destruction of the thyroid gland.<sup>26</sup> Blockage of radioiodine uptake previously has been detected in a subset of patients receiving the TKI, sunitinib.<sup>15</sup> Further research is necessary to determine whether this occurs in dogs receiving toceranib phosphate.

Although documented with several TKIs, the TKI most frequently associated with hypothyroidism in human medicine is sunitinib,<sup>10</sup> which has similarities in both structure and kinase inhibition to toceranib phosphate.<sup>1,27</sup> The reported incidence of hypothyroidism associated with sunitinib use has varied from 27 to 85%.<sup>10–12,28</sup> By the end of our 90-day study, 24% of dogs had hormonal changes consistent with hypothyroidism (Table 1). Ultimately, our study was underpowered to determine whether this proportion of affected dogs varied significantly from our baseline finding of 5% affected dogs. It is clear, however, that toceranib phosphate can alter the hypothalamic-pituitary-thyroid axis, given that we did detect changes in FT4, TSH, and TT4/TT3 ratio (Table 2). We chose to conduct a 90-day study because the onset of hormonal abnormalities in people receiving TKIs is generally within 1–3 months of beginning treatment.<sup>12,28</sup> However, in 1 study in people, the mean time to development of hypothyroidism was 50 weeks, with 90% of patients affected if treatment duration was >96 weeks, and only 18% were affected at 36 weeks.<sup>11</sup> Therefore, a study

with longer follow-up may have detected a larger proportion of affected dogs. Finally, although treatment in our study was initiated with a relatively low toceranib phosphate dosage (mean, 2.57 mg/kg PO MWF), the dosage did decrease over time (mean, 2.28 mg/kg PO MWF, day 90). Our results thus may underrepresent hormonal changes in dogs maintained on a higher dosage of toceranib phosphate for an extended period of time.

Nonthyroidal illness (NTI) is known to affect thyroid hormone metabolism. A previous study in dogs found that serum TT4, FT4, and TT3 may be low and within the hypothyroid range in dogs with NTI including cancer, whereas TSH was more likely to be within the reference interval in sick dogs.<sup>29</sup> Although all of the dogs in our study had a cancer diagnosis and thus some degree of NTI, we did not detect any association of disease severity (microscopic versus macroscopic tumor burden) with the hormonal changes we detected in FT4, TSH, and TT4/TT3 ratio. Additionally, when evaluating patterns descriptively, the proportion of dogs with changes consistent with NTI (ie, low TT4, TT3, or FT4 without increased TSH) did not increase over time.

Several factors are known to influence T4 concentration in dogs, including age, body weight, and use of medications such as glucocorticoids, NSAIDs, and sulfonamides.<sup>30,31</sup> We did not detect associations with these factors in our study, likely for a variety of reasons, foremost of which is the fact that our study was not designed or powered to evaluate these factors. Although challenging to prove in dogs, in people glucocorticoids can directly suppress pituitary secretion of TSH.<sup>32–34</sup> In our study, dogs receiving glucocorticoids did not have TSH concentrations that differed from dogs not receiving glucocorticoids ( $P = 0.069$ ). However, glucocorticoid usage was infrequent and dosages were in the anti-inflammatory range in our study, whereas effects on thyroid function usually are seen with immunosuppressive dosages of glucocorticoids.<sup>35,36</sup> Importantly, glucocorticoid use did not affect the statistical significance of the association we detected between TSH concentration and time. The influence of NSAIDs varies according to the drug used and may be related to displacement of thyroid hormones from serum binding sites.<sup>37–39</sup> One report in people documented decreased T4 only in association with salsalate, but not with ibuprofen or piroxicam.<sup>39</sup> In a 60-day study of 28 dogs with osteoarthritis, TT4, FT4, and TSH did not change in dogs receiving either meloxicam or carprofen.<sup>40</sup> Less than 20% of dogs in our study received either carprofen or piroxicam, and administration only occurred on non-toceranib phosphate treatment days, and thus, it is not surprising that we did not detect any associations. By design, no dogs in our study received sulfonamides.

As a consequence of the changes we detected in the hypothalamic-pituitary-thyroid axis in dogs receiving toceranib phosphate, clinicians should periodically monitor thyroid function in dogs receiving this medication. Clinicians also should be aware that TKI-induced alterations in hormone concentrations may be transient. Therefore, until more is understood about the

consequences of TKI-induced hypothyroidism in dogs, the decision of whether to pursue T4 supplementation in affected dogs must be made on a case-by-case basis, with necessary consideration given to clinical signs and hematological and biochemical abnormalities that may be related to thyroid dysfunction.

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## Footnotes

<sup>a</sup> Palladia; Zoetis Inc., Florham Park, NJ

<sup>b</sup> Immulite 2000 Free T4; Siemens Healthcare Diagnostics, Tarrytown, NY

<sup>c</sup> JMP, Version <JMP Pro 11>; SAS Institute Inc., Cary, NC, 1989–2007

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*Off-label Antimicrobial Declaration:* Although not a component of our research, dogs in our study received a variety of antibiotics for both related and unrelated conditions, some of which were prescribed in an off-label manner.

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