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STANDARD ARTICLE

# Plasma cytokeratin-18 concentrations as noninvasive biomarker of early gastrointestinal toxicosis in dogs receiving toceranib

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**Background:** No biomarkers for the early detection of gastrointestinal (GI) toxicosis secondary to antineoplastic treatment are recognized in veterinary medicine. Toceranib causes GI toxicosis in dogs.

**Hypothesis/Objective:** To assess if changes in plasma cytokeratin 18 (CK18) concentration, measured in dogs being treated with toceranib phosphate, can predict the onset of GI toxicosis. We hypothesize that an increase in CK18 concentrations will be detected before the development of GI toxicosis in dogs treated with toceranib phosphate.

**Animals:** Twenty healthy client-owned dogs and 25 client-owned dogs with surgically excised mast cell tumor (MCT).

**Methods:** Prospective cohort study. Dogs were treated with toceranib (2.75 mg/kg PO q48h). Plasma was collected weekly for 4 weeks. Plasma CK18 concentration was measured on days 0, 7, 14, 21, and 28. vascular endothelial growth factor was measured on days 0 and 28.

**Results:** Mean plasma CK18 concentration on day 0 in dogs with MCT was not significantly different than healthy controls (313.5 $\pm$ 592.8 pg/mL, 119.7 $\pm$ 76.9 pg/mL, mean $\pm$ SD $P = 0.27$). Mean plasma CK18 concentration decreased by 98.69 pg/mL from day 0 to day 28 ($P < 0.001$). Plasma CK18 concentration was not a significant predictor of the development of signs of GI toxicosis.

**Conclusions and Clinical Importance:** Plasma CK18 concentration was not a clinically useful biomarker for the early detection of GI toxicosis secondary to toceranib administration in dogs with MCTs.

KEYWORDS

cancer, canine, chemotherapy, mast cell tumor, tyrosine kinase inhibitor

## 1 | INTRODUCTION

Tyrosine kinase inhibitors (TKIs) are small molecular inhibitors administered with increasing frequency in both human and veterinary oncology as antineoplastic agents. Adverse events (AEs) occur frequently with the administration of these drugs in dogs and humans. A total of 93% of dogs receiving toceranib for treatment of macroscopic mast cell tumors (MCT) developed AE, with 55% requiring a dose adjustment in dosage, frequency, or both.[1]

Gastrointestinal (GI) AE occur in approximately 40% of dogs receiving toceranib.[1–3] The majority of these are grade 1 or 2 according to the

**Abbreviations:** AE, adverse event; CK18, cytokeratin 18; ECOG, Eastern Comparative Oncology Group; EDTA, ethylenediaminetetraacetic acid; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase-1; GI, gastrointestinal; IACUC, Institutional Animal Care and Use Committee; MAPK, mitogen-activated protein kinases; MCT, mast cell tumor; MTD, maximum tolerated dose; NSAID, nonsteroidal antiinflammatory drug; PI3K, phosphatidylinositol 3-kinase; TKI, tyrosine kinase inhibitor; VCOG-CTCAE, Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v.1.1.[4] Management of AE involves administration of concomitant medications and changes to the dosage, frequency, or both factors of drug administration. In some cases, discontinuation of toceranib is required to manage AE or is requested by the owner because of impact on quality of life of the dogs.

The impact of changes to dose intensity and treatment withdrawal on outcome for dogs receiving toceranib has not been evaluated to the author's knowledge. In people with renal cell carcinoma receiving sunitinib, changes to dose, frequency, or both lead to reduced response rates, progression free survival, and overall survival.[5] Thus, the ability to identify patients at risk for the development of GI AEs could allow for early intervention of preventative treatments thereby reducing the need for dose reductions or delays.

Recently, the detection of cytokeratin 18 (CK18) in the plasma of people treated with chemotherapy for lymphoma was demonstrated as a clinically relevant biomarker for the early detection of signs of GI toxicosis.[6] Plasma CK18 concentrations are higher in patients who experience higher grade GI toxicosis than those who do not.[6] This finding suggests that plasma CK18 could represent a clinically useful biomarker to predict patient populations at risk for chemotherapy-associated toxicosis and identify patients that could benefit from more aggressive preventative treatment.

CK18 is a member of the intermediate filament family of cytoskeletal proteins expressed in epithelial cells.[7,8] It compromises approximately 5% of total protein content in epithelial tissues including liver, exocrine pancreas, and the GI tract.[9] During apoptosis, caspase-cleaved CK18 fragments are released into the extracellular space.[10] Therefore, the presence of CK18 fragments in plasma can be utilized as a biomarker for epithelial cell apoptosis.[6–8,10–12] Cellular stress, such as that caused by anticancer drugs, results in the activation of the intrinsic apoptotic pathway and eventual activation of downstream effector caspases 3 and 7.[7,13] These caspases cleave a variety of substrates including CK18.[8,9]

The primary objective of this study was to evaluate the feasibility of measuring plasma CK18 concentrations in healthy dogs and dogs being treated with toceranib for MCT, and if CK18 concentrations in dogs receiving toceranib correlated with the development of GI AE. We hypothesized that dogs receiving toceranib would have higher CK18 concentrations at the conclusion of the study period compared with the healthy controls, and CK18 concentrations would increase from baseline in dogs that developed GI AE.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

This study was designed as a prospective cohort study. Client-owned dogs were enrolled from August 2016 through December 2017 at The Ohio State University Veterinary Medical Center, Colorado State University Veterinary Teaching Hospital, New England Veterinary Oncology Group, Regional Veterinary Referral Center, and Veterinary Health Center at Kansas State University. The clinical trial was approved at each site's Institutional Animal Care and Use Committee (IACUC), Clinical Review Board or both. In order to be eligible for enrollment, dogs must have had prior surgical excision of a cutaneous or subcutaneous MCT confirmed with histopathology and had an indication for adjuvant treatment with toceranib as deemed appropriate by the attending clinician. On evaluation of the histopathology report, margins were considered incomplete if tumor cells were <5 mm of the nearest margin. Additional criteria included age ≥1-year, adequate organ function as indicated by standard laboratory tests, and performance status of 0 or 1 (according to the modified Eastern Comparative Oncology Group [ECOG] performance score).[4] Dogs were not eligible for enrollment if there was a history of signs of GI disease within 5 days of enrollment, surgery within 14 days of enrollment, any prior chemotherapy or radiation treatment for MCTs, presence of local macroscopic metastatic disease, or other serious systemic disease. A 5-day washout period was required for dogs receiving steroids or NSAIDs before being eligible for enrollment. All dogs were required to have a physical examination, complete blood count, serum biochemistry profile, urinalysis, urine protein to creatinine ratio, and an abdominal ultrasound. Aspirates of the liver and spleen and blood pressure measurement were performed at the discretion of the attending clinician.

### 2.2 | Study schedule

The day that toceranib was started was considered day 0. A physical examination, body weight, collection of 6 mL of blood for plasma CK18 concentration, and toceranib was prescribed at this visit. Rechecks were required at day 7, 14, 21, and 28. At these visits, a physical examination, body weight, and collection of blood for plasma CK18 concentration were performed. Blood was collected for plasma vascular endothelial growth factor (VEGF) concentration on day 0 and 28. Based on clinician discretion, a CBC was performed at day 14. At the end of the trial on day 28, a complete blood count, serum biochemistry, urinalysis, and urine protein to creatinine ratio were performed. Blood pressure assessment at day 28 was at the discretion of the attending clinician. The owner completed a quality of life assessment form at each visit. Concomitant medication and AEs were recorded and prospectively graded based on the Veterinary Comparative Oncology Group Common Terminology Criteria for Adverse Events v1.1.[4] Owners completed a daily diary documenting any signs potentially associated with GI toxicosis including, diarrhea, vomiting, reduced appetite, anorexia, or nausea. Owners were asked to quantify and characterize any abnormalities noted. The owner diary was collected at day 7, 14, 21, and 28.

### 2.3 | Treatment

Toceranib was prescribed at 2.75 mg/kg PO q48h. If clinically substantial AE occurred, a drug holiday was prescribed until resolution. Toceranib was then restarted on a Monday, Wednesday, and Friday schedule. Dogs received omeprazole (1 mg/kg PO q24h) throughout the study.

### 2.4 | CK18 and VEGF sample processing

Six milliliters of blood was sampled from the jugular vein, placed into an EDTA tube, and placed on ice until processing. Blood samples were processed within 30 minutes of collection. Samples were centrifuged

for 15 minutes at 1000*g* (or 3000 rpm) at 2°C-8°C. Plasma was aliquoted into 3 cryovials and stored at −80°C until analysis.

### 2.5 | ELISA

Plasma CK18 fragment M30 was measured in duplicate with a commercially available quantitative ELISA kit (ABClonal, Woburn, Massachusetts) according to the manufacturer's instructions. The absorbance reading of each well at 450 nM was measured with a microplate reader and recorded. A standard curve was generated for each plate using the provided standard solutions. VEGF plasma concentrations were measured in duplicate using a commercially available quantitative ELISA Kit (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions. The absorbance of each well was measured at 540 nM and was subtracted from the absorbance reading at 450 nM.

### 2.6 | Statistical analysis

A two-sample *t* test with equal variance was used to assess the mean difference in demographic variables (age and sex) and plasma CK18 concentrations between cases and controls. A multivariable mixed logistic regression was used to assess differences in the odds of a case developing signs of GI toxicosis. The main fixed effect variable of interest was average plasma CK18 values for each case. We utilized a risk factor modeling approach and included drug dose (mg/kg), breed, categorized as small, large or mix breed, sex, and age in the model as fixed effects to control for potential confounding. Drug dose and age were continuous variables, and sex and breed were categorical variables. The individual dog was included in this model as a random effect.

Two generalized linear mixed models were used to assess the potential association of plasma CK18 and VEGF blood concentrations with visit week among cases. Normality of the outcome variables was assessed using standard graphical methods.[14] The first generalized linear mixed model utilized plasma CK18 concentrations as the primary outcome and hospital visit and mean treatment drug dose as fixed effects variables. Hospital visit was categorized by visit day (0, 7, 14, 21, and 28) and mean treatment drug dose, calculated as the average drug dose between days 7 and 28, was continuous. The second generalized linear model utilized VEGF plasma concentrations as the primary outcome variable and hospital visit as the estimator of interest. Hospital visit was included as a binary variable in this model because VEGF concentrations were measured at the initial and last hospital visit. Both models utilized an exchangeable covariance structure based on the correlation matrix of outcome residuals within each subject. The random effect variable in both models was the animal identification number. We performed all statistical tests using STATA version 15.1 software (StataCorp LLC, College Station, Texas).

## 3 | RESULTS

### 3.1 | Dogs

Twenty healthy controls and 25 dogs with MCTs were enrolled (Table 1). There were no significant differences between the dogs with MCTs and the controls in age, sex, or body weight ($P$ = 0.17, 0.099, and 0.44, respectively). Twenty-one dogs completed the study. Four dogs were withdrawn from the study for a variety of reasons. Three dogs withdrew after 1 week, whereas one dog withdrew after 1 weeks. The 1st dog withdrew after 1 week because of grade 4 myositis. The 2nd dog withdrew at the client's request because of cranial cruciate ligament rupture necessitating NSAID administration and surgery. The 3rd dog withdrew also at the client's request because of grade 2 vomiting after administration of 2 doses of toceranib. Finally, the 4th dog was withdrawn at day 14 because of disease progression. There was no significant difference in body weight between the study dogs and controls (25.5 ± 12.9 and 24.2 ± 10.6, respectively, $P$ = 0.44). Twenty-three of the 25 dogs with MCT had cutaneous tumors, and 2 had subcutaneous tumors. Tissue samples from all dogs with cutaneous tumors were submitted for histopathology and received a Patnaik grade, and 21 also had a Kiupel grade. Twelve dogs had a grade 3 tumor, 9 had a grade 2 tumor, and 2 had a grade 1 tumor based on the Patnaik grading scheme. Using the Kiupel grading scheme, 15 had high-grade tumors and 6 had low-grade tumors. Evaluation of internal tandem duplication in exon 11 and exon 8 was performed in 8 dogs. Mutations were identified in exon 11 and exon 8 in 2 and 1 dogs, respectively. The remaining 5 dogs were negative for the mutation at both sites.

All dogs had an abdominal ultrasound performed before enrollment. Fifteen dogs had liver and splenic aspirates performed, whereas an additional 5 dogs had only splenic aspirates performed. The remaining 5 dogs had abdominal ultrasound without aspirates performed. No cytological evidence of metastatic MCT in the liver or spleen was identified in any of the dogs. Three dogs had surgical excision of their draining lymph node. Two dogs had histologic evidence of lymph node metastasis. Ten dogs had incompletely excised tumors.

The median (range) dose of toceranib administered for 28 dogs was 2.73 mg/kg PO q48h (2.39-2.95 mg/kg). Adverse events were documented in 22 of 25 dogs (88%). A total of 64 AE were reported in 25 dogs. Categories and grades of AE are displayed in Table 2. Greater than 95% of the AE were grade 1 or 2. Drug holidays occurred in 2 dogs, of which 1 dog also had a dose adjustment. One dog had a 1-week drug holiday for grade 2 diarrhea. Toceranib was restarted at the same dose and frequency without recurrence of diarrhea. The 2nd dog had a 1-week drug holiday for grade 2 diarrhea and grade 3 anorexia. The dog's dose was reduced from 2.87 mg/kg PO q48h to 2.47 mg/kg PO Monday, Wednesday, and Fridays. However, the dog's diarrhea and inappetence recurred after 2 weeks at this dose so toceranib was discontinued. The most common group of AE was GI, accounting for 53% (34 events) of all reported AE. Diarrhea (50%) was the most common GI AE, followed by vomiting (29%), nausea (12%), and anorexia (8%). The second most common group of AE was hematological, accounting for 11% (7 events). Neutropenia (5 events) was the most common followed by one episode each of lymphocytosis and anemia.

### 3.2 | CK 18

Plasma CK18 was measured in the 20 control dogs and in 25 MCT dogs at baseline (day 0). All 25 MCT dogs had plasma CK18 measured at day 7. Twenty-two had measurements recorded at day 14. Twenty-one were

**TABLE 1** Dog characteristics

| Variable | Controls (n = 20) | Study dogs (n = 25) |
|---|---|---|
| Age (years) | 5.8 ± 3.3 | 7.1 ± 3.1 |
| Sex | 11 male neutered | 9 male neutered |
| | 6 female spayed | 15 female spayed |
| | 3 intact male | 1 intact male |
| BW (kg) | 24.2 ± 10.6 | 25.5 ± 12.9 |
| Patnaik grade | | 12 grade 3<br>9 grade 2<br>2 grade 1<br>2 subcutaneous |
| Kiupel grade | | 15 high grade<br>6 low grade<br>2 subcutaneous<br>2 not reported |
| c-KIT mutation (n = 8) | | 1 exon 8 ITD |
| | | 2 exon 11 ITD |
| Breeds | Mixed (9) | Mixed (10) |
| | Rottweiler (2) | Boxer (4) |
| | German shepherd (2) | Labrador retriever (2) |
| | Golden retriever (2) | Beagle (1) |
| | Lhasa Apso (1) | Cocker spaniel (1) |
| | Staffordshire terrier (1) | English bulldog (1) |
| | English setter (1) | German shorthair pointer (1) |
| | Boxer (1) | Newfoundland (1) |
| | German wirehair pointer (1) | Staffordshire terrier (1) |
| | | Poodle (1) |
| | | Pug (1) |
| | | St. Bernard (1) |

ITD, internal tandem duplication.

evaluated on days 21 and 28. For the control dogs, the mean (range) plasma CK18 was 119.7 pg/mL (61.09-359.33 pg/mL). For the MCT dogs, the mean (range) CK18 concentrations at day 0 was 313.54 pg/mL (24.12-2979.32 pg/mL), at day 7 was 262.76 pg/mL (23.74-2681.26 pg/mL), at day 14 was 261.02 pg/mL (46.67-2562.58 pg/mL), at day 21 was 271.38 pg/mL (25.78-2236.26 pg/mL), and at day 28 was 244.37 pg/mL (0-2368.86 pg/mL). There was no statistically significant difference in plasma CK18 between the control dogs and the MCT dogs at day 0 ($P = 0.27$) (Figure 1) or day 28 ($P = 0.21$). Plasma CK18 concentrations at day 0 in the MCT dogs were significantly higher ($P < 0.001$) compared to concentrations at day 28 (313.54 ± 118.55 and 244.37 ± 112.63, respectively). The average plasma CK18 among cases on day 28 was 98.69 pg/mL less than the average on day 0 ($P < 0.001$). The most significant decrease in plasma CK18 concentrations occurred between day 0 and day 7 ($P < 0.003$) (Figure 2). The average plasma CK18 among cases at day 7 was 50.8 pg/mL less than the average on day 0. Plasma CK18 concentration was not a significant predictor of the development of signs of GI toxicoses.

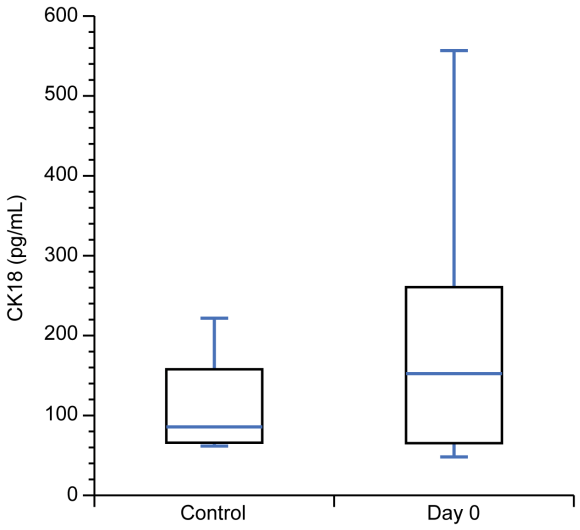


**FIGURE 1** Box and whisker plot illustrating plasma cytokeratin 18 (CK18) concentrations in healthy control dogs and dogs receiving toceranib at day 0 (baseline). The boxes represent the 25th and 75th percentiles and the central lines represent the mean values. The whiskers represent the 10th and 90th percentiles. No statistically significant difference between the groups was observed

## 3.3 | VEGF

Plasma VEGF concentration was measured in 21 MCT dogs at day 0 and day 28. The mean (range) VEGF concentration at day 0 was 16.23 pg/mL (2.745-114.94 pg/mL), and 106.39 pg/mL (7.472-929.61 pg/mL) at day 28. Mean VEGF concentration at day 28 was significantly higher than concentration at day 0 ($P = 0.022$) (Figure 3).

**TABLE 2** Adverse events in dogs receiving toceranib

| Adverse event | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Metabolic | 11% of all AEs | | | |
| Elevated ALT | 2 | | | |
| Elevated AST | 2 | | | |
| Elevated ALP | 1 | | | |
| Elevated CPK | 1 | | | |
| Elevated creatinine | 1 | | | |
| Hematologic | 11% of all AEs | | | |
| Neutropenia | 5 | | | |
| Lymphocytosis | 1 | | | |
| Anemia | | 1 | | |
| Gastrointestinal | 53% of all AEs | | | |
| Diarrhea | 12 | 4 | 1 | |
| Vomiting | 9 | 1 | | |
| Nausea/ptyalism | 4 | | | |
| Anorexia | 1 | 1 | 1 | |
| Constitutional | 6% of all AEs | | | |
| Lethargy | 2 | 1 | | |
| Fever | | 1 | | |
| Miscellaneous | 19% of all AEs | | | |
| Cutaneous masses | 3 | | | |
| Rash: acne/acneiform | 2 | | | |
| Allergy reaction/hypersensitivity | 1 | | | |
| Dyspnea | 1 | | | |
| Other (otitis) | 1 | | | |
| Pruritus | 1 | | | |
| Skin ulceration | 1 | | | |
| Lameness | | 1 | | |
| Myositis | | | | 1 |
| Grand total | 51 | 10 | 2 | 1 |

AE, adverse event; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatinine phosphokinase.

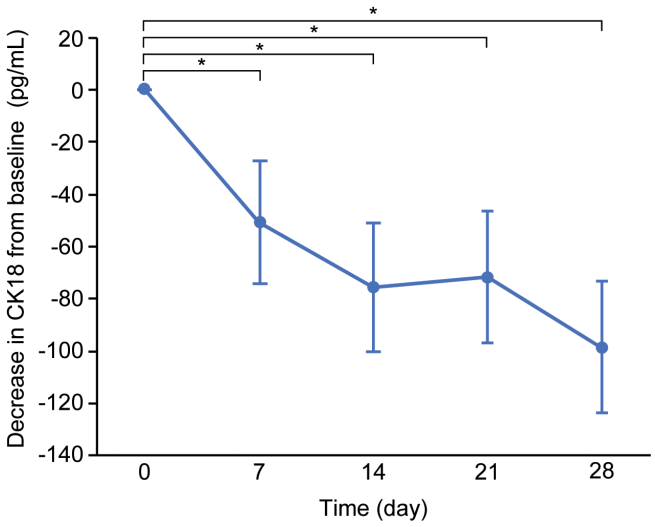


**FIGURE 2** Line graph representing mean plasma CK18 concentrations in dogs receiving toceranib over 4 weeks. Error bars represent standard error. Asterisks indicate a statistically significant difference between day 0 (baseline) and each time point

## 4 | DISCUSSION

Our study sought to assess the feasibility of evaluating plasma CK18 in both healthy dogs and dogs receiving toceranib as adjuvant treatment for MCTs following surgical excision. There was no difference between plasma CK18 concentrations in the healthy controls and the dogs with MCT at day 0. However, plasma CK18 concentrations in the dogs with MCT declined over the study period and did not predict the development of signs of GI toxicosis in dogs receiving toceranib. These findings could have resulted from several factors. In all, 88% of study dogs experienced AE and over half of AE were GI. However, only 2 were grade 3 AE and no grade 4 GI AE occurred. This low incidence of higher grade GI AE could have limited the ability to detect a difference between dogs experiencing signs of GI disease and those that did not. Furthermore, the mechanism of toceranib-induced GI AE is not well understood and might not correlate with plasma CK18 concentrations.

We chose to evaluate plasma CK18 in dogs receiving toceranib, because of the high reported incidence of GI toxicosis including vomiting, diarrhea, and anorexia. A total of 20.7% of dogs receiving

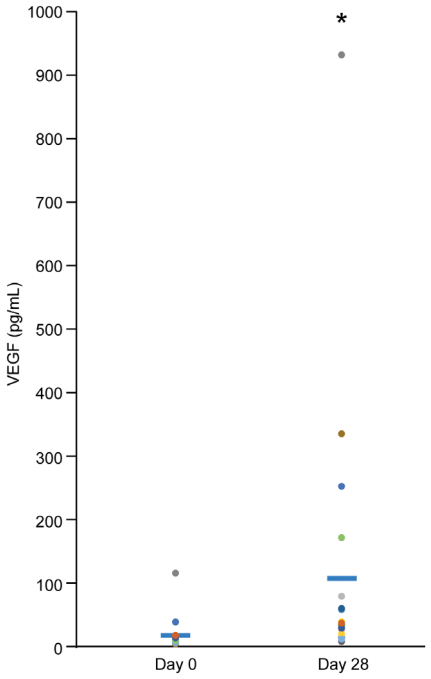


**FIGURE 3** Plasma vascular endothelial growth factor (VEGF) concentrations at day 0 and day 28 in dogs receiving toceranib. Bars represent mean plasma VEGF concentrations. A significant increase was observed from day 0 to day 28

toceranib at the maximally tolerated dose (MTD) for MCTs experienced grade 3 or 4 toxicoses.[3] At doses below the MTD, approximately 50% of dogs developed either diarrhea or colitis.[2]

Signs of GI toxicoses because of the administration of antineoplastic drugs can result in treatment delays, dose reductions, and negatively impact quality of life. Although there is no published literature regarding the impact of toceranib treatment delays or dose reductions on outcome in dogs, humans treated with sunitinib for metastatic renal cell carcinoma requiring a change to the intended treatment plan experience a negative impact on outcome, with reductions in response, progression free survival, and overall survival.[5]

As opposed to other studies evaluating toceranib in MCTs, dogs could not be enrolled into our study if they had macroscopic primary or metastatic disease. This was done to avoid the confounding impact of development of signs of GI disease secondary to mast cell disease on the assessment of plasma CK18 and toceranib-related toxicity. Dogs with macroscopic or metastatic disease are much more likely to develop clinical signs such as vomiting and diarrhea secondary to the release of mast cell mediators.[15] The incidence of GI ulceration is 35%-83% of dogs with MCTs examined at necropsy.[16,17] Although the cause of ulceration in these dogs is likely multifactorial, the release of histamine from the tumor cells and the subsequent increase in production of hydrochloric acid from the parietal cells in the stomach is the primary cause. Dogs with macroscopic MCTs have higher plasma histamine concentrations than dogs with only microscopic disease, contributing to the higher incidence of related clinical signs.[18]

We did not find any correlation between plasma CK18 concentrations and the development of signs of GI toxicosis. Analysis of correlation between grade of toxicosis and plasma CK18 was not performed because of low incidence of high grade GI toxicosis. The lack of correlation between CK18 concentrations and signs of GI toxicosis in our study might have been because of the following factors; the low number of dogs experiencing grade 3 and 4 toxicoses, the mechanism of toceranib-induced GI toxicosis, and the potential impact of toceranib on signaling mechanisms that influence intracellular concentrations of CK18.

Human studies utilizing plasma CK18 concentrations as a predictor of signs of GI toxicosis saw a significant difference when comparing patients with a grade 3 or higher toxicosis to those who experienced no or lower grade toxicoses.[6,19] In these studies, plasma CK18 concentrations peaked 3 days after administration of traditional cytotoxic chemotherapeutic agents. Plasma CK18 concentrations at day 3 also gave the greatest power to identify patients experiencing the worst toxicoses.[6] Furthermore, patients with more severe toxicoses experienced higher and more durable peaks in plasma CK18 concentrations.[6] In contrast to these studies, few high-grade toxicoses developed in our study. Only 3 dogs developed a grade 3 or higher toxicosis, and of these, only 1 was considered GI in nature. This precluded analysis based on grade of toxicosis, potentially impacting the ability to identify a relationship between plasma CK18 and signs of GI toxicosis.

In the human studies assessing plasma CK18 as a biomarker for signs of GI toxicosis, subjects received traditional cytotoxic chemotherapeutic agents.[6,19] These included doxorubicin, bleomycin, vinblastine, dacarbazine, cyclophosphamide, and vincristine. The administration of cytotoxic chemotherapy induces apoptosis of the intestinal crypts and can eventually result in crypt hypoplasia.[20,21] These changes lead to the development of mucositis and diarrhea in patients receiving chemotherapy. During apoptosis, these cells release caspase-cleaved CK18 into the extracellular space, which is measurable in plasma, thus making plasma CK18 an effective biomarker for the identification of signs of GI toxicosis secondary to cytotoxic chemotherapy administration.

In contrast, the mechanism of GI toxicosis secondary to administration of TKIs such as toceranib is not well understood. In humans receiving TKIs, diarrhea is a commonly encountered side effect impacting treatment outcome as well as patient quality of life. Phase III studies of sunitinib for the treatment of metastatic renal cell carcinoma and GI stromal tumors have found that 29%-53% of patients experience diarrhea, with most being grade 1 or 2 in severity.[22] Other signs of GI disease reported less frequently include, vomiting, nausea, and constipation.[23,24]

Antiangiogenic TKIs such as those inhibiting vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) appear to have a higher reported incidence of diarrhea in the human literature compared with nonangiogenic TKIs.[25] This might suggest a possible relationship between angiogenic signaling inhibition and the development of diarrhea.[26] VEGF and EGFR are important for maintaining mucosal homeostasis and epithelialization after mucosal damage. Thus, it has been hypothesized that VEGFR and EGFR inhibition might lead to disruption in GI mucosa homeostasis and healing capabilities resulting in signs of GI toxicosis, particularly diarrhea.[27]

To evaluate this, studies have been conducted in mouse and rat models of TKI-induced diarrhea. A rat model evaluating lapatinib-induced diarrhea found 81% of the rats developed diarrhea, with 67%

developing moderately severe diarrhea. At necropsy, no significant lesions were detected within the GI tract.[28] In a mouse model receiving gefitinib, after 10 days of administration every 12 hours, the mice receiving genitinib had a significant decrease in the weight of the small and large intestines, and in the absorptive surface area of the intestines because of pronounced villi atrophy compared to the control population.[29] Both of these studies suggest that the development of diarrhea in patients receiving antiangiogenic TKIs is likely not because of direct mucosal damage through apoptosis as seen with the administration of cytotoxic chemotherapy drugs.

Other potential mechanisms of GI toxicosis caused by toceranib might be inhibition of c-KIT on interstitial cells of Cajal, the pacemaker cells of the intestine.[30] Alterations to these cells and their function might impact intestinal contractions leading to altered intestinal motility and the development of diarrhea. As we did not see any correlation between CK18 concentrations and signs of GI toxicity in our study, we suggest that toceranib induced diarrhea is likely not occurring because of apoptosis of the intestinal epithelial cells as described with cytotoxic chemotherapy drug administration.

Interestingly, we observed a decline in plasma CK18 concentrations over the study period with concentrations significantly lower in dogs at day 28 compared with day 0. The greatest decrease occurred between days 0 and 7. There is evidence that the role of CK18 within the body is more complex than solely in structural support.[31,32] Relationships among CK18 and the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), Wnt, and mitogen activated protein 3 kinase (MAP3K) signaling pathways have been found.[31,32] Thus, the decrease observed in CK18 over time might be related to downstream target inhibition by toceranib, the mechanism for which is not yet understood. These pathways play an important function in normal cell function, and in tumor cell survival, proliferation, and migration. In epithelial cancer cell lines, overexpression of AKT upregulated CK18 expression.[33] Additionally, blocking the ERK MAPK signaling pathway in Sertoli cells leads to inhibition of the CK18 expression.[34] However, studies have not assessed how TKIs, such as toceranib, impact these signaling processes in normal epithelial cells. Because of the complexity and interrelationship of these signaling pathways, the downstream effects of target inhibition by toceranib might impact CK18 concentrations within the cell, leading to an overall reduction of CK18 expression in epithelial cells. In turn, if intracellular concentrations of CK18 were reduced, these changes could potentially lead to a decrease in the CK18 released by cells undergoing apoptosis, which would be reflected in a decline in plasma CK18 concentrations. Therefore, this offers one potential cause for the decline appreciated in the dogs receiving toceranib in this study.

We demonstrated that the evaluation of CK18 in the plasma of dogs was possible. We did not detect a relationship between CK18 concentrations and the development of signs of GI toxicosis in dogs receiving toceranib as adjuvant treatment for surgically excised MCTs. This finding might suggest that intestinal epithelial cell apoptosis is not the cause of toceranib-induced GI toxicosis in dogs, and further investigation is required to determine the mechanism responsible. Additionally, in order to determine if plasma CK18 concentrations could be an effective biomarker for the early detection of antineoplastic drug-induced GI toxicosis, studies are ongoing evaluating its use in dogs receiving cytotoxic chemotherapeutic drugs.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST DECLARATION

Zoetis provided the Palladia used in this study. Dr. London is a paid consultant for Zoetis and has received payment for development of educational materials and speaker honoraria from Zoetis.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

All institutions involved had IACUC or similar approval.

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