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Clinical Cancer Research

KTN0158, a Humanized Anti-KIT Monoclonal Antibody, Demonstrates Biologic Activity against both Normal and Malignant Canine Mast Cells

Cheryl A. London[1,2], Heather L. Gardner[2], Sarah Rippy[2], Gerald Post[3], Krista La Perle[1], Linda Crew[4], Lori Lopresti-Morrow[4], Andrew J. Garton[4], Gerald McMahon[4], Theresa M. LaVallee[4], and Richard Gedrich[4]

Abstract

Purpose: KTN0158 is a novel anti-KIT antibody that potently inhibits wild-type and mutant KIT. This study evaluated the safety, biologic activity, and pharmacokinetic/pharmacodynamics profile of KTN0158 in dogs with spontaneous mast cell tumors (MCT) as a prelude to human clinical applications.

Experimental Design: Cell proliferation, KIT phosphorylation, and mast cell degranulation were evaluated *in vitro*. KTN0158 was administered to 4 research dogs to assess clinical effects and cutaneous mast cell numbers. Thirteen dogs with spontaneous MCT were enrolled into a prospective phase I dose-escalating open-label clinical study of KTN0158 evaluating 3 dose levels and 2 schedules and with weekly assessments for response and clinical toxicities.

Results: KTN0158 was a potent inhibitor of human and dog KIT activation and blocked mast cell degranulation *in vitro*. In dogs, KTN0158 was well tolerated and reduced cutaneous mast cell numbers in a dose-dependent manner. Clinical benefit of KTN0158 administration in dogs with MCT ($n = 5$ partial response; $n = 7$ stable disease) was observed regardless of *KIT* mutation status, and decreased KIT phosphorylation was demonstrated in tumor samples. Histopathology after study completion demonstrated an absence of neoplastic cells in the primary tumors and/or metastatic lymph nodes from 4 dogs. Reversible hematologic and biochemical adverse events were observed at doses of 10 and 30 mg/kg. The MTD was established as 10 mg/kg.

Conclusions: KTN0158 inhibits KIT phosphorylation, demonstrates an acceptable safety profile in dogs, and provides objective responses in canine MCT patients with and without activating KIT mutations, supporting future clinical evaluation of KTN0158 in people. *Clin Cancer Res; 1–10. ©2016 AACR.*

Introduction

KIT is a member of the type III receptor tyrosine kinase family that includes PDGFR and VEGFR. KIT consists of five extracellular immunoglobulin-like domains, a single transmembrane domain, and an intracellular catalytic region containing kinase and autoregulatory domains (1). The second and third membrane distal domains of the extracellular domain play a role in recognition of the ligand stem cell factor (SCF), which upon binding to KIT, initiates homodimerization through the fourth Ig-like domain, autophosphorylation, and protein tyrosine kinase activity (2, 3). KIT is expressed on a variety of cells, including hematopoietic stem cells, melanocytes, various cells of neural crest origin, and mast cells. In mouse strains with *Kit* mutations, several defects are observed, such as anemia, infertility, susceptibility to infection, and pigment loss. Spontaneous mutations in *KIT* resulting in constitutive activation occur in human tumors, including gastrointestinal stromal tumors (GIST), acute myelogenous leukemia, melanoma, and systemic mastocytosis (4). Small-molecule inhibitors that target KIT have had significant clinical success, most notably in the treatment of GIST, where imatinib is now the standard of care for patients with high-risk disease (5). Despite their efficacy, the use of these inhibitors invariably results in the development of resistance, often driven by mutations occurring in the kinase domain that preclude drug binding (6). In addition, several other tumor types exhibit aberrant expression of KIT, but small-molecule KIT inhibitors do not appear to have substantial activity in these settings.

KTN0158 is a humanized anti-KIT mAb that binds the extracellular immunoglobulin-like domain 4 (D4) of KIT, which mediates homotypic interactions essential for KIT activation (7). It blocks receptor homodimerization and prevents the interaction of KIT with its ligand, SCF, blocking downstream signaling. KTN0158 binds to the extracellular domain of canine, feline, monkey, and human KIT, but not rodent KIT (7). The binding of KTN0158 to canine KIT provides a unique opportunity to evaluate activity in the setting of spontaneous canine cancer.

Mast cell tumors (MCT) are the most common cutaneous malignancy in dogs, representing between 7% and 20% of all skin tumors (8). They exhibit a wide range of biologic behaviors from benign curable disease to aggressive growth with subsequent metastasis and death (8, 9). Approximately 30% of canine MCTs are known to possess activating mutation in *KIT* consisting

[1]Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio. [2]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, Ohio. [3]The Veterinary Cancer Center, Norwalk, Connecticut. [4]Kolltan Pharmaceuticals, Inc., New Haven, Connecticut.

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Corresponding Author: Cheryl A. London, Ohio State University, 1925 Coffey Road, Columbus, OH 43210. Phone: 614-915-7409; Fax: 614-292-7599; E-mail: london.20@osu.edu

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Translational Relevance

The receptor tyrosine kinase KIT is known to be dysregulated in several cancers, including gastrointestinal stromal tumors (GIST), among others. It has been successfully targeted in the clinical setting with small-molecule inhibitors, including imatinib and sunitinib, for the treatment of GIST. Despite these successes, resistance to therapy is common, and new approaches to target KIT are needed. Canine mast cell tumors express activating *KIT* mutations similar to those found in human GIST and serve as a spontaneous large animal model of KIT dysregulation for the interrogation of novel therapies that target this receptor kinase. The current study evaluated KTN0158, a novel humanized anti-KIT IgG1 mAb, in dogs with spontaneous mast cell tumors and demonstrated both safety and efficacy, supporting the notion that KTN0158 will have activity in human patients with KIT-driven malignancies.

predominantly of internal tandem duplications (ITD) in exon 8 or 11, which are associated with an increased risk of local recurrence and metastasis (10–14). Exon 11 mutations in canine MCTs (8, 14, 15) and human GIST (16–18) promote ligand-independent activation, supporting the notion that shared molecular aberrations in driver oncogenes transcend species and histology to contribute to the malignant phenotype. Canine MCTs have been previously used as a model of KIT dysregulation for preclinical evaluation of novel therapeutics. For example, toceranib, the multi-targeted small-molecule inhibitor of KIT/VEGR2/PDGFR, was evaluated in dogs demonstrating safety, efficacy, and *in vivo* target modulation prior to initiation of human clinical trials with its closely related analogue sunitinib (19, 20).

Given the defined role of KIT in canine MCT and the binding of KTN0158 to canine KIT, dogs represented an ideal large animal model in which to interrogate the potential activity of this antibody. Therefore, the objectives of the studies described here were to characterize the effects of KTN0158 on KIT signaling *in vitro* and to assess the safety, pharmacokinetic and pharmacodynamics parameters, and antitumor activity of KTN0158 administered to healthy research dogs and client-owned dogs with spontaneous MCT.

Materials and Methods

In vitro assays

KIT activation in human mast cells. Primary human mast cells were differentiated *in vitro* from $CD34^+$ progenitor cells isolated from human peripheral blood (obtained from the New York Blood Center, New York, NY) by standard methods (21). To assess KIT phosphorylation, human mast cells were starved overnight in cytokine-free media. Cells were plated in a 96-well culture plate and incubated with serial 3-fold dilutions of KTN0158 or the isotype control antibody KTN0062C at final concentrations ranging from 100 nmol/L to 14 pmol/L or imatinib at final concentrations ranging from 10 μmol/L to 14 nmol/L for 2 hours and then stimulated with human SCF (100 ng/mL) for 10 minutes. The cells were then washed and lysed, and phosphorylated KIT was measured using a sandwich immunosorbent assay format with electrochemiluminescent (ECL) detection (see Supplementary Methods for details).

To measure mast cell degranulation, mast cells were starved overnight in cytokine-free media and loaded overnight with biotinylated human myeloma IgE. IgE-loaded mast cells were then washed, resuspended in HEPES buffer, pH 7.4, and preincubated with serial dilutions of KTN0158, the isotype control antibody KTN0062C, or imatinib for 30 minutes. Cells were stimulated with SCF for 30 minutes, followed by cross-linking biotinylated IgE with streptavidin (2 ng/mL) for 30 minutes. The β-hexosaminidase release assay was performed as described previously (22). Additional details are provided in the Supplementary Methods.

KIT phosphorylation and proliferation in cells in vitro The human acute megakaryoblastic leukemia cell line M-07e (19) was obtained from the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and used within 6 months of receipt. To measure the effects on KIT phosphorylation, M-07e cells were serum starved overnight, either left untreated or incubated with serial 10-fold dilutions of KTN0158 (ranging from 10 nmol/L to 1 pmol/L) or isotype control antibody KTN0062C (10 nmol/L), nilotinib (1 μmol/L), or imatinib (1 μmol/L) for 2 hours. The cells were then stimulated with SCF (100 ng/mL) for 10 minutes, washed, and lysed in iced-cold lysis buffer containing sodium vanadate and protease inhibitors, and Western blotting was performed to assess KIT phosphorylation status (see Supplementary Methods for details).

To assess the effect on M-07e cell proliferation, logarithmically growing cells were resuspended in growth media without GM-CSF and incubated with serial 3-fold dilutions of the antibodies KTN0158 or KTN0062C (isotype control) at concentrations ranging from 100 nmol/L to 15 pmol/L, or imatinib or nilotinib at concentrations ranging from 10 nmol/L to 150 pmol/L for 2 hours prior to the addition of SCF. Cells were incubated for 6 days at 37°C in a CO_2 incubator, and proliferation was measured using the CellTiter-Glo assay according to the manufacturer's instructions (Promega Corp.).

Chinese hamster ovary (CHO) cells expressing wild-type human or canine KIT were generated by stably transfecting an expression vector containing the full-length human KIT cDNA or full-length dog KIT cDNA, selecting with G418 and cloning by FACS. KIT phosphorylation was measured using an ELISA) See Supplementary Methods for details on the phosphorylation assays.

Evaluation of KTN0158 in healthy dogs

The effects of KTN0158 on hematologic parameters and mast cells *in vivo* were evaluated in healthy research mongrel dogs. This study was approved by the Ohio State University (OSU; Columbus, OH) Institutional Animal Care and Use Committee (IACUC; 2013A00000049). Dogs (2 male and 2 female) were treated with a single infusion of KTN0158 at 10 or 30 mg/kg and followed for 28 days. Daily observations for evidence of clinical toxicities and a complete blood count (CBC) and biochemical profile were performed on days 0, 7, 14, 21, and 28. Skin biopsies were obtained under sedation prior to treatment, on day 7 and again on day 28 to quantitate mast cell numbers. Briefly, four 8-mm skin punch biopsies were obtained at each time point from the dorsum and then fixed in formalin and embedded in paraffin. Slides were stained with hematoxylin and eosin for standard histopathologic evaluation and Toluidine blue to identify mast cells. Mast cells

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in superficial dermal, periadnexal, and deep dermal areas were counted in three random 400× fields in each biopsy sample ($n = 4$) at each time point for each dog and reported as mean number of mast cells.

Clinical trial in spontaneous canine MCT

Thirteen dogs with measurable MCT were enrolled into an open-label clinical trial. Twelve dogs were treated at the OSU College of Veterinary Medicine, and one dog was treated at the Veterinary Cancer Center (Norwalk, CT). Data from all dogs were used to evaluate toxicity; data from 12 dogs were evaluable for efficacy (the remaining dog was removed from the study prior to the first target lesion assessment).

This study was conducted following the established VCH guidelines on Good Clinical Practice and was approved by The OSU CVM Clinical Research Committee and IACUC (2014A00000065). Prior to enrolment, all dog owners were required to give written informed consent. Dogs more than 1 year of age with a cytologic or histologic diagnosis of a new or relapsed cutaneous or subcutaneous MCT were enrolled. To be considered eligible for enrolment, dogs were required to undergo complete staging, including history and physical examination, CBC, serum biochemical profile, urinalysis, abdominal ultrasound, thoracic radiographs, and tumor measurement. Any prior treatment had to be completed 2 weeks prior to study entry. Continued prednisone administration was permitted in dogs that demonstrated progression of disease while on corticosteroids prior to study entry. Dogs with any serious systemic disorder or evidence of systemic mast cell disease involving the spleen and/or liver were excluded.

Drug product and concomitant medications KTN0158 was produced by Kolltan Pharmaceuticals, Inc. and stored at 4°C in a secured location, protected from light and diluted in 0.9% NaCl prior to intravenous administration. No concomitant antineoplastic therapy (chemotherapy, radiotherapy) was permitted during this study. Concomitant medications, including anti-emetics, anti-diarrheals, anti-histamines, analgesics, and corticosteroids, were permitted as indicated to manage adverse events.

Study design This study was not randomized. Dogs were administered KTN0158 at 1, 10, or 30 mg/kg intravenously over 4 to 6 hours once or twice during 28-day or 42-day study periods, respectively (Supplementary Table S1). Tumor biopsies were obtained prior to the first KTN0158 treatment and then post-KTN0158 administration on days 1 and 7. If possible, tumor biopsies were also collected on the final scheduled study visit. Blood samples were obtained pre- and post-KTN0158 treatment on day 0 and 21 (for dogs receiving 2 doses), then again every 7 days during the study period. *KIT* mutation status was determined by the Colorado State University College of Veterinary Medicine Clinical Immunology Laboratory. Histopathologic evaluation of tumors and lymph nodes was performed by the Applied Pathology Service at the OSU CVM.

Toxicity assessment Dogs were assessed for adverse events at each study visit using the VCOG-CTCAE established guidelines (23). Adverse events were defined using specific criteria as any expected or unexpected grade 1–3 toxicity. Serious adverse events were any grade 4 or 5 toxicity. Disease progression or events definitely related to disease were not considered adverse events.

Tumor response assessment Dogs were considered evaluable for response if there was a baseline assessment and at least one follow-up lesion(s) assessment following KTN0158 treatment. Tumor measurements were performed with calipers, and response assessments were performed using the VCOG Response Evaluation Criteria for Solid Tumors in Dogs v.1.0 (24). A complete response (CR) was defined as the disappearance of all target and nontarget lesions. A partial response (PR) was defined as >30% decrease in the sum of the longest diameter of all target lesions. Progressive disease (PD) was defined as ≥20% increase in the sum of the longest diameter of all target lesions or the appearance of a new lesion. Stable disease (SD) was defined as the absence of either a response or disease progression for a duration of ≥28 days.

Pharmacokinetic analyses Serial blood samples and tumor biopsies were obtained pre- and posttreatment throughout the study period. KTN0158 in dog serum was measured using a sandwich immunosorbent assay format with ECL detection. Additional details are provided in the Supplementary Methods.

Antidrug antibody analyses Antibodies to KTN0158 in dog serum were detected by solid-phase extraction with acid dissociation following sample pretreatment with biotinylated KTN0158 and then direct detection by immunoassay (25). The anti-KTN0158 antibody KTN0209 was used as a positive control. Additional details are provided in the Supplementary Methods.

Analysis of KIT phosphorylation in tumors Tumor biopsies were obtained using a 4-mm punch instrument prior to the first KTN0158 treatment on day 1 and day 7 (and day 42 for 2-dose cohorts) following treatment. The tumor specimens were divided and flash frozen in liquid nitrogen prior to storage at −80°C. To prepare tumor lysates, samples were weighed then lysed in RIPA buffer plus protease and phosphatase inhibitor cocktails by homogenization. KIT phosphorylation was measured using a sandwich immunosorbent assay format with ECL detection similar to the method described above for human mast cells, except the capture antibody was the anti-CD117 antibody ACK45 (BD Biosciences). Because of limits in the sensitivity of the assay, samples with day 0 RLU values ≤100 were not considered evaluable. Additional details are provided in the Supplementary Methods.

Statistical analysis

Graphing and half-maximal (IC_{50}) values were generated using GraphPad Prism 6.05 (GraphPad Software, Inc.). Curve fitting was performed using a 4- or 5-parameter nonlinear regression algorithm. For serum KTN0158 concentrations, curves were fitted using a 4-parameter logistic nonlinear regression analysis with weighting ($1/y^2$).

Results

KTN0158 is a potent inhibitor of KIT activation and signaling

KTN0158 inhibited KIT phosphorylation in M-07e cells (Fig. 1A) with a mean IC_{50} value of 117 pmol/L ($n = 3$), which was similar to IC_{50} values observed in transfected CHO cells expressing wild-type human KIT (mean IC_{50} = 169 pmol/L, $n = 13$; data not shown). Imatinib and nilotinib (1 μmol/L) also inhibited KIT phosphorylation. Downstream signaling as

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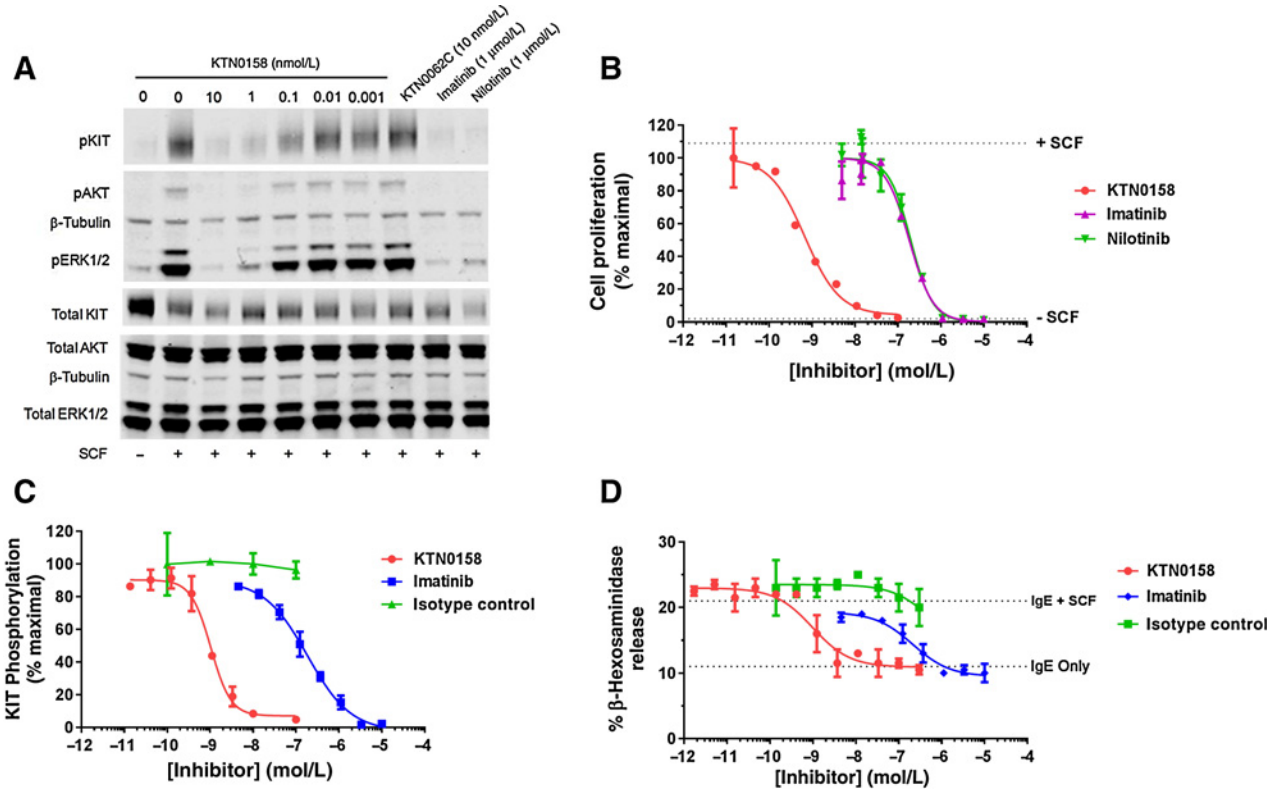

Figure 1.

KTN0158 potently inhibits KIT activity and function in M-07e cells and primary human mast cells *in vitro*. **A,** KTN0158 inhibited KIT activation and downstream signaling in M-07e cells. Dose-dependent inhibition of KIT, ERK1/2, and AKT activation were observed following KTN0158 treatment. KTN0062C is an isotype control antibody. **B,** KTN0158 was a more potent inhibitor of SCF-dependent proliferation in M-07e cells compared with imatinib and nilotinib. Dashed lines, proliferation observed in controls grown in the presence (+SCF) or absence (−SCF) of SCF. Representative data are shown for six independent experiments. Analyses were performed in duplicate (KTN0158) or triplicate (all others), and data are plotted as means ± SEM. **C,** KTN0158 was a more potent inhibitor of SCF-induced KIT phosphorylation in primary human mast cells *in vitro* than imatinib. Representative data are shown for four independent mast cell preparations and runs. Analyses were performed in duplicate, and data are plotted as means ± SEM. **D,** KTN0158 was a potent inhibitor of SCF enhancement of mast cell degranulation. **C** and **D**, The isotype control is KTN0062C and analyses were performed in duplicate with data plotted as means ± SEM.

measured by AKT and ERK phosphorylation were also inhibited by KTN0158, imatinib, and nilotinib. No effects on KIT, AKT, and ERK1/ERK2 phosphorylation were observed following treatment with an isotype control antibody (KTN0062C). No change in total KIT protein levels was observed.

Treatment of M-07e cells with KTN0158, imatinib, and nilotinib resulted in dose-dependent inhibition of SCF-dependent proliferation, with mean IC_{50} values of 1.75, 291, and 301 nmol/L ($n = 6$), respectively (Fig. 1B). Thus, although all the KIT inhibitors tested achieved complete inhibition of SCF-dependent M-07e cell growth, KTN0158 was >150-fold more potent than either imatinib or nilotinib.

KTN0158 blocks KIT activity in primary human mast cells

The effects of KTN0158 on KIT activation in primary human mast cells *in vitro* were examined. KTN0158 inhibited KIT phosphorylation following SCF treatment (Fig. 1C), with a mean IC_{50} of 680 pmol/L ($n = 4$). Imatinib also inhibited KIT activation in mast cells with a mean IC_{50} of 195 nmol/L ($n = 4$). Activation of KIT by SCF has been shown to enhance mast cell degranulation induced by IgE cross-linking (26). Both KTN0158 and imatinib also inhibited the enhancement of degranulation by SCF (Fig. 1D). Treatment with an isotype control antibody had no effect on either KIT phosphorylation or enhancement of degranulation by SCF in human mast cells.

Evaluation of KTN0158 safety and pharmacodynamics in healthy dogs

KTN0158 binds to human, monkey, canine, and feline KIT but not to mouse or rat KIT (7). To assess the effects of KTN0158 *in vivo*, studies were undertaken to characterize effects in dogs. KTN0158 was a potent inhibitor of canine KIT activation in stably transfected CHO cells *in vitro* (Fig. 2A; mean $IC_{50} = 1$ nmol/L, $n = 6$). In healthy research dogs, KTN0158 was well tolerated when administered at the 10 and 30 mg/kg dose levels. Mild infusion-related nausea was observed in some animals that resolved with modulation of infusion rate. No loss of appetite, weight loss, or other indications of KTN0158-related toxicity were observed over the 28-day study period. Grade 1 neutropenia occurred at day 7 in 3 dogs postinfusion and resolved in all by day 21. In addition, one dog experienced a grade 1 anemia that resolved by day 14. Grade 2 ALT elevations were noted in 2 dogs at day 7 after infusion and resolved by day14. These hematologic and biochemical events were subclinical, and no other adverse events were documented.

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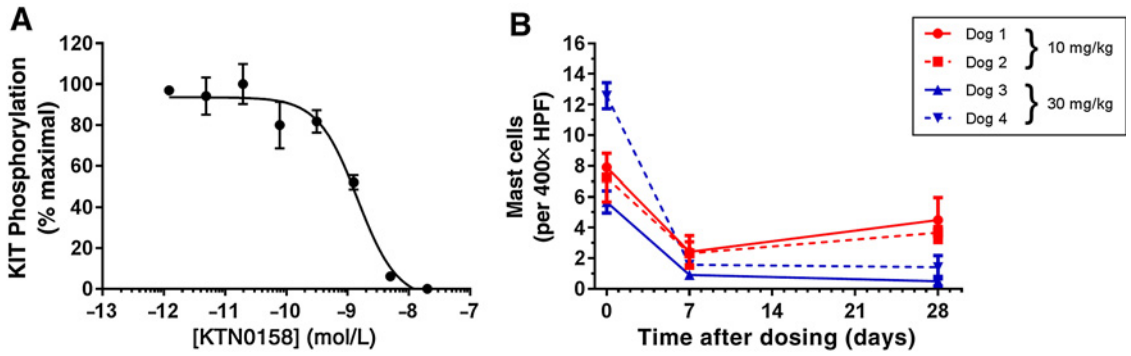

Figure 2.

KTN0158 is a potent inhibitor of canine KIT and modulates canine mast cells *in vivo*. **A,** KTN0158 inhibited SCF-induced KIT activation in CHO cells stably expressing wild-type canine KIT. **B,** KTN0158 decreased the number of mast cells in the skin of healthy dogs within 7 days following drug administration. HPF, high-power field. Data are plotted as means ± SEM. This effect was dose dependent, with 30 mg/kg demonstrating continued suppression of mast cells at day 28 posttreatment.

Pharmacodynamic effects of KTN0158 on mast cells in serial skin biopsies were also examined (Fig. 2B). No overt histopathologic lesions were noted; however, mast cell numbers in the skin were markedly decreased at day 7. In the dogs that received 30 mg/kg of KTN0158, skin mast cell counts on day 28 were similar to those observed on day 7, whereas counts in the 10 mg/kg cohort showed evidence of recovery, suggesting a dose-related effect, although numbers were still lower than those present at baseline. In addition, mast cells that were present in the skin of treated dogs at days 7 and 28 were often shrunken, pyknotic, karyorrhectic, or possessed karyolytic nuclei often with leakage of granules, features of apoptosis, and necrosis.

Clinical trial of KTN0158 in dogs with spontaneous MCT

Demographics and study drug administration. Thirteen dogs with measurable MCT were enrolled into an open-label clinical trial. Data from all dogs were used to evaluate toxicity; data from 12 dogs were evaluable for efficacy (the remaining dog was removed from the study prior to the first target lesion assessment). Baseline patient demographic information is provided in Table 1. Of the 13 dogs enrolled, 3 had tumors with exon 11 *KIT* ITDs, and 6 had evidence of local lymph node metastasis.

Cohort 1 received a single dose of 10 mg/kg administered, and cohort 2 received a single dose of 30 mg/kg; both cohorts were monitored for 28 days. Additional dogs were enrolled using a modified dosing regimen of 1 or 10 mg/kg (cohorts 3 and 4) administered twice (day 0 and day 21) and followed out to day 42 to evaluate the safety and efficacy of administration of two doses of KTN0158. KTN0158 was administered 21 days apart to allow for recovery from treatment-related adverse events. Two dogs in cohort 3 were withdrawn from the study due to PD, and 2 dogs in cohort 4 were withdrawn due to adverse events.

KTN0158 pharmacokinetics and antidrug antibody evaluation Dose-related increases in serum KTN0158 concentrations were observed on day 0 in all cohorts and on day 21 in the 2-dose cohorts (Supplementary Fig. S1). As expected, the maximum concentration was observed immediately after dosing on day 0 in all dogs. For the single-dose cohorts, the maximum concentration on day 0 following dosing with 10 and 30 mg/kg of KTN0158 was 471.16 mcg/mL and 3,620.87 mcg/mL, respectively. On day 0, the mean maximum concentration was 118.59 and 461.98 mcg/mL after 1 and 10 mg/kg doses, respectively. Serum concentrations slowly decreased in both 10 and 30 mg/kg cohorts

Table 1. Baseline patient characteristics

		Cohort 1 ($n = 3$)	Cohort 2 ($n = 3$)	Cohort 3 ($n = 3$)	Cohort 4 ($n = 4$)
Age (years)	Median	10	10	9	8.5
	Range	4–13	8–11	8–11	7–10
Breed	Pure breed	1	3	3	2
	Mixed breed	2	0	0	2
Gender	Male castrated	1	1	2	1
	Male intact	0	0	0	0
	Female spayed	2	2	1	3
	Female intact	0	0	0	0
Weight (kg)	Median	28.8	33.4	15.1	24.8
	Range	19.2–41.6	13.8–38.6	10.16–15.1	7.6–45
Lymph node status	Metastasis	3	1	0	3
	No metastasis	0	2	3	1
Prior chemotherapeutics	Yes	0	2	1	0
	No	3	1	2	4
Tumor status	Naïve	2	0	1	4
	Recurrent	1	3	2	0
KIT mutation status	Exon 11 mutation	0	2	0	1
	Exon 8 mutation	0	0	0	1
	No mutation	3	1	3	2

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between day 0 and day 7, followed by high interanimal variability and more rapid decreases in concentration on days 14 and 21. In both of the 2-dose cohorts, serum KTN0158 concentrations rapidly increased on day 21 after the second dose and were below the limit of detection at day 28, likely due to the presence of neutralizing antibodies. Consistent with these results, antidrug antibodies to KTN0158 were detected in 8 dogs by day 7, in 10 dogs by day 14, and in 11 of 12 dogs at study completion (Supplementary Table S2).

Safety assessment. The most common adverse events associated with KTN0158 administration were reversible dose-dependent hematologic changes, including, anemia, neutropenia, and thrombocytopenia (Table 2). Hematologic changes occurred between 7 and 14 days post-KTN0158 administration consistent with the transient hematologic effects noted in healthy dogs. Liver transaminase elevations were observed in a subset of dogs, but these were transient and independent of KTN0158 dose.

Two dogs ($n = 1$ each cohort 3 and 4) developed hypersensitivity reactions during the second administration of KTN0158, consistent with the development of anti-KTN0158 antibodies. Drug administration was discontinued and both dogs recovered fully. Dose limiting toxicities (DLT) were seen at 30 mg/kg, including erythema/edema, grade 4 neutropenia, and grade 3 thrombocytopenia. The primary DLT in dogs that received two KTN0158 infusions was an acute hypersensitivity reaction. Therefore, the MTD of KTN0158 in dogs was determined to be 10 mg/kg administered as a single dose.

Tumor responses, histopathology, and KIT inhibition. Response data for evaluable dogs ($n = 12/13$ enrolled) are presented in Table 3 and Fig. 3A; one dog (cohort 4) was withdrawn from the study prior to the first follow-up lesion assessment and was not eligible for the efficacy analysis due to complications resulting from the initial biopsy procedure, including swelling, edema, and necrosis, necessitating immediate tumor removal. All evaluable dogs experienced clinical benefit (CR, PR, SD $\geq$28 days). PRs were noted in 5 dogs, with dramatic tumor shrinkage in some patients. One dog with a large tumor over the dorsum that had failed surgery, radiotherapy, cytotoxic chemotherapy, and toceranib therapy experienced substantial reduction in disease within 7 days of KTN0158 administration and 46.1% reduction in tumor size by day 14 (Fig. 3B). Four of the dogs with SD experienced greater than 20% tumor shrinkage, but this did not meet the RECIST criteria for PR (Fig. 3A). Importantly, responses to KTN0158 were noted in dogs both with and without exon 11 KIT mutations.

To examine KIT target modulation, serial tumor biopsies were collected and analyzed for KIT phosphorylation. In samples with evaluable KIT phosphorylation ($n = 9$), a decrease in KIT phosphorylation was observed 24 hours after KTN0158 administration in 6 dogs (Fig. 3C; Table 3). All 3 tumors with exon 11 KIT mutations exhibited profound decreases in KIT phosphorylation. No correlation between clinical response and the magnitude of the decrease in KIT phosphorylation was observed (Table 3).

Upon completion of the clinical trial, 11 dogs underwent surgical excision of their tumors/lymph nodes. Importantly, 2 of 11 dogs with pre- and posttreatment tumor samples ($n = 1$ each in cohorts 1 and 2) had no histologic evidence of remaining MCT at the primary tumor site, despite the continued presence of a palpable mass throughout the study period. In addition, 3 of 4 dogs ($n = 2$ cohort 1; $n = 2$ cohort 4) with confirmed lymph node metastases at the time of study entry had no evidence of MCT upon examination of posttreatment lymph nodes. The lack of neoplastic cells after KTN0158 treatment was observed in dogs with and without activation mutations in KIT and was more common at higher doses (10 or 30 mg/kg).

Discussion

Small-molecule inhibitors of KIT have revolutionized the treatment of human cancers possessing *KIT* mutations, providing significant improvements in the outcome for affected patients. This has been most evident in the setting of GIST, where chemotherapy response rates are often below 5% and historical outcomes were typically dismal (27). The development of small-molecule tyrosine kinase inhibitors (TKI), most notably imatinib and sunitinib, has resulted in substantial improvement in patient survival. Spontaneous canine MCTs have been used to study KIT dysregulation and KIT-targeted therapies as a high percentage express activating mutations that recapitulate those found in human tumors, particularly GIST (8, 14, 27). Indeed, canine MCTs were used to demonstrate the safety, efficacy, and target modulation of a multi-targeted kinase inhibitor (toceranib) prior to the initiation of human studies with the sister drug sunitinib (19, 20). Following the approval of sunitinib for the

Table 2A. Hematologic adverse events

	Toxicities (number of events by grade)											
	Anemia				Thrombocytopenia				Neutropenia			
Dose group	1	2	3	4	1	2	3	4	1	2	3	4
10 mg/kg once	1	1				1	1		2		1	
30 mg/kg once	2	1			1	1	2				1	2
10 mg/kg twice	2					2			1	1		
1 mg/kg twice	1					1						

Table 2B. Other adverse events

	Toxicities (number of events by grade)																			
	ALP elevation				AST elevation				ALT elevation				Hypersensitivity reaction				Edema/erythema			
Dose group	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
10 mg/kg once							1		1	1										
30 mg/kg once	2	2							2								1	1	1	
10 mg/kg twice	2	1					1	1	2		1					1				
1 mg/kg twice	1									1				1						

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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Table 3. Response rates by dose regimen and *KIT* mutation status

Dose group	Patient nr.	*KIT* mutation	Best response (% change)	Primary tumor posttreatment histopathology	Lymph node pretreatment cytology	Lymph node posttreatment histopathology	KIT phosphorylation day 1 (% day 0)
10 mg/kg (day 0)	1	Negative	−23.3	MCT	Not done	Not done	109.5
	2	Negative	−29.2	NED	Metastatic	NED	30.2
	3	Negative	−34.3	MCT	Metastatic	NED	104
30 mg/kg (day 0)	4	Exon 11	−11.3	NED	Not done	Not done	9.7
	5	Negative	1.3	MCT	Not metastatic	Not done	52.9
	7	Exon 11	−43.2	Not done	Metastatic	Not done	38.2
10 mg/kg (day 0 and 21)	6	Negative	−46.1	MCT	Not done	MCT	Not evaluable
	8	Negative	−56.8	MCT	Not done	Not done	39.2
	9	Negative	−27.3	MCT	Not metastatic	Not done	165
1 mg/kg (day 0 and 21)	11	Negative	−27.2	MCT	Not done	Not done	Not evaluable
	12	Negative	−35.7	MCT	Metastatic	NED	Not evaluable
	13	Exon 11	−4.0	MCT	Metastatic	MCT	33.5

Abbreviation: NED, no evidence of disease.

treatment imatinib-resistant GIST, toceranib was approved by the FDA for the treatment of canine MCT.

In both people and dogs with KIT-driven malignancies, responses to TKI treatment are often dramatic, but in many cases, clinical benefit is not sustained due to the emergence of drug resistance (28). For example, up to 15% of patients with advanced GIST have intrinsic resistance to first-line therapy with imatinib, and 50% develop resistance to imatinib during the course of therapy (28–30). TKIs also exhibit variable efficacy depending on the specific KIT mutation expressed in a given tumor. For example, the presence of exon 9–activating mutations in *KIT* is a strong adverse prognostic factor for response to imatinib in GIST patients, and these patients require treatment with higher doses of imatinib than those used for patients with exon 11 mutations (31). In addition, very high doses of toceranib are required to inhibit the D814V KIT mutant in a murine mastocytoma cell line in comparison with that needed to inhibit KIT with a juxtamembrane ITD mutation (32). Finally, it is important to note that GIST and other malignancies of either human or canine origin are heterogeneous and may harbor both mutant and wild-type KIT isoforms that can heterodimerize leading to KIT activation. Therefore, although KTN0158 was not evaluated in this capacity, the availability of a drug that targets both wild-type and oncogenic forms of KIT could help circumvent the development of resistance and broaden the therapeutic utility of treatment.

KTN0158 is a humanized anti-KIT mAb that binds the extracellular immunoglobulin-like domain 4 (D4) of KIT, which mediates homotypic interactions essential for KIT activation (7). It binds with high affinity to both human and canine KIT. Canine MCT represents an ideal spontaneous cancer to study this drug candidate given its known dependence on KIT signaling, the high prevalence of activating *KIT* mutations, and its prior use as a model of KIT dysregulation for the development of novel TKIs. The opportunity to evaluate both safety and pharmacodynamics in both healthy research dogs and tumor-bearing dogs provides a unique approach to generate preclinical data in support of future human studies. Furthermore, as canine and human mast cells exhibit substantial functional and molecular similarities (33, 34), the evaluation of KTN0158 in dogs provides a unique setting in which to test the effects of KTN0158 on mast cell function.

KTN0158 was safely administered to healthy dogs and dogs with spontaneous MCT. Reversible hematopoietic and biochemical effects were observed in all dose cohorts. The hematologic effects of KTN0158 may not be surprising given that KIT is expressed in bone marrow stem cells. However, in studies with cynomolgus monkeys, no hematologic effects were observed following administration of multiple doses of KTN0158 at doses up to 75 mg/kg (not shown), suggesting that there may be different effects of KTN0158 in the two species. Liver transaminase elevations were observed in some dogs. Hepatitis is an observed toxicity of some mAbs, with viral or immune-mediated causes often suspected (35). Although the mechanism of liver transaminase elevations in this study is unknown, all events were sporadic and subclinical, without obvious dose dependency and resolved in the majority of dogs upon study completion. It is possible that the sporadic use of corticosteroids or other comorbidities unrelated to KTN0158 could have contributed to these elevations.

Immunogenicity of human-derived protein sequences in humanized mAbs used in dogs is expected, ultimately limiting the use of these products in immunocompetent dogs. No complications were noted in any dogs during the first infusion of KTN0158; however, hypersensitivity reactions occurred in 2 dogs during the second treatment. This was most likely due to the presence of anti-KTN0158 antibodies, as these developed in 11 of 12 evaluable dogs within 3 weeks after the first treatment, prior to the scheduled second treatment. Furthermore, the presence of anti-KTN0158 antibodies was likely responsible for the sharp decline in serum KTN0158 concentration following the second treatment. Altered KTN0158 pharmacokinetics due to immunogenicity of the antibody likely decreased the clinical activity of the second dose of KTN0158. Furthermore, it is possible that the administration of additional doses of KTN0158 would have been associated with even greater clinical activity in the absence of neutralizing antibodies. It is important to note that the presence of anti-KTN0158 antibodies was an expected sequela following the administration of a humanized mAb to dogs. It should be recognized that immunogenicity of KTN0158 in people is also possible due to the development of anti-idiotypic antibodies and the diversity of IgG allotypes within the human population (36, 37), although such a sequela is not typical for most of the currently approved humanized mAbs.

Previous studies showed that antibodies targeting KIT demonstrated the potential for agonist activity (38). Mast cell activation occurs through multiple mechanisms, primarily through a high-affinity IgE receptor FcεR1, resulting in the release of proinflammatory mediators and anaphylaxis in patients (39). Other mechanisms of mast cell activation include IgG receptor (FcγRI and RcγRIII) cross-linking or complement binding, inducing

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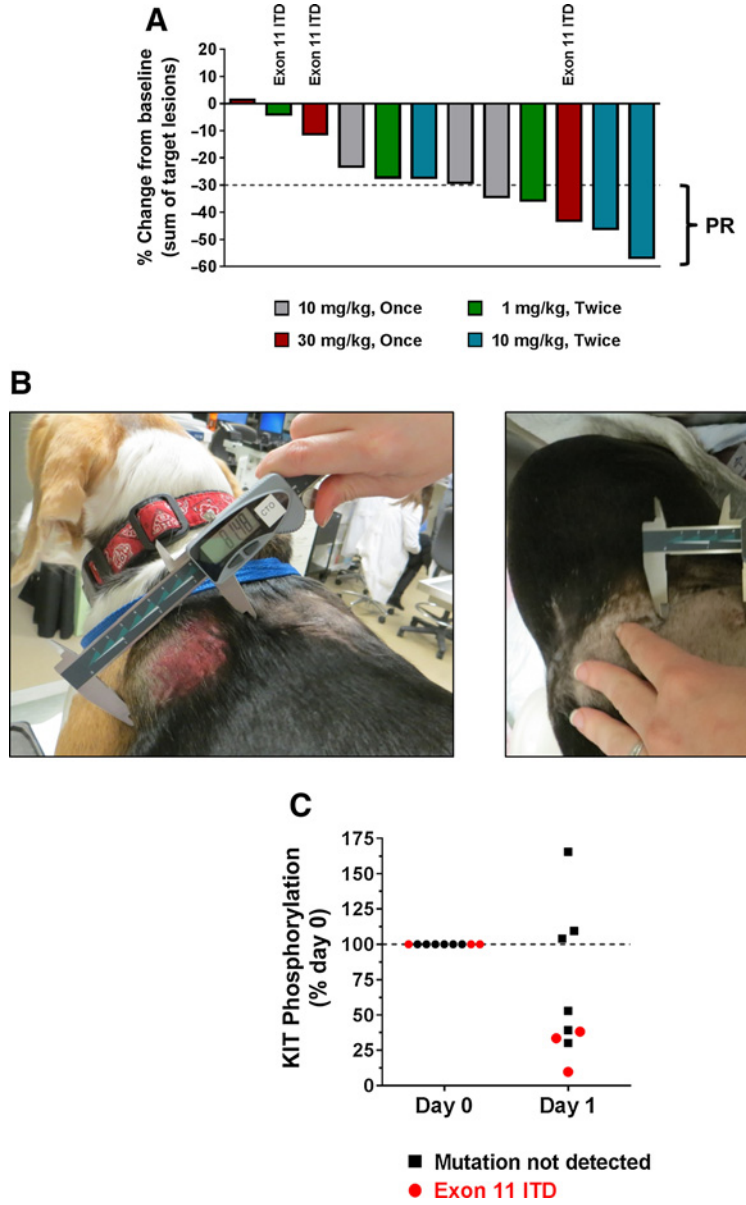

Figure 3.
KTN0158 treatment results in objective responses in canine MCT with and without exon 11 mutations. **A,** Best responses after KTN0158 administration are shown and demonstrate that all dogs experienced clinical benefit. Dose and cohort are indicated. Exon 11 ITD denotes dogs with exon 11 mutations. **B,** Tumor measurements demonstrating a PR to KTN0158 treatment in one dog that occurred between day 0 (left) and day 14 (right). This dog had failed multiple treatment modalities prior to receiving KTN0158. **C,** Target modulation was observed in MCTs 24 hours after KTN0158 administration. Decreases in KIT phosphorylation were noted in most tumors with evaluable signals prior to dosing ($n = 8$), including evaluable tumors with exon 11 mutations.

potent mast cell activation (40–42). The safety data generated in both the healthy research dogs and dogs with spontaneous MCT suggest KTN0158 does not activate mast cells in dogs, which is particularly relevant given the known sensitivity of canine mast cells to degranulation in response to several agents.

One potential confounding factor in this clinical study was the use of prednisone therapy in some dogs. Prednisone was administered for a short duration to treat mast cell degranulation associated with serial tumor biopsy that occurred in a few of the dogs. Mast cell tumors are prone to bleeding and swelling post-biopsy due to the release of vasoactive and proinflammatory substances, and thus, these complications are anticipated and typically treated with a short duration of steroid administration. Steroids were also used to treat hypersensitivity reactions associated with the second KTN0158 infusion that occurred in 2 dogs. However, in these cases, the administration of steroids was limited to resolution of the observed adverse event (a few days), supporting the notion that the observed objective responses were likely to be associated with KTN0158 treatment.

Data generated from the canine MCT clinical trial described herein demonstrate that KTN0158 modulates KIT-related activities in a spontaneous model of cancer at all dosing levels regardless of *KIT* mutation status. In serial tumor biopsies, KIT phosphorylation was decreased within 24 hours of KTN0158 administration in 6 of 9 tumors with and without *KIT* mutations. However, MCT with *KIT* exon 11 mutations appeared to be particularly sensitive to KTN0158 (Fig. 3C; Table 3). Importantly, exon 11 *KIT* mutations drive ligand-independent receptor dimerization and are associated with metastasis and poor prognosis in human GIST (43–45) and canine MCT (8, 14, 15). As such, the antitumor effects of KTN0158 in dogs with MCTs containing exon 11 KIT mutations suggest that KTN0158 may benefit patients with GISTs harboring similar mutations. Furthermore, previous studies have shown that the murine precursor to KTN0158 is active

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against exon 9–mutant *KIT* (7), indicating that KTN0158 may provide therapeutic benefit to a broader population of GIST patients.

Significant biologic activity of KTN0158 was demonstrated in canine MCT patients, with 5 dogs experiencing a PR to therapy. It is interesting to note that histopathologic examination of primary tumors ($n = 2$) and lymph nodes ($n = 3$) removed following study completion showed an absence of neoplastic mast cells, transitioning 2 dogs from a clinical PR to a histopathologic CR. Three of these dogs had tumors that expressed wild-type KIT, providing further evidence that KTN0158 has activity in the setting of both wild-type and mutant KIT. It is possible that more dogs would have experienced PRs and CRs to therapy if multiple doses of KTN0158 could have been administered in the absence of neutralizing antibodies.

In summary, KTN0158 was found to be a potent inhibitor of SCF-induced KIT activation *in vitro* with evidence of pathophysiologically relevant target modulation, an acceptable safety profile and clinical benefit in canine MCT patients independent of *KIT* mutational status. These results support the notion that wild-type and oncogenic forms of KIT are relevant therapeutic targets, providing the impetus for future evaluation of KTN0158 in the treatment of KIT-driven malignancies. The fundamental molecular similarities between canine and human KIT-driven malignancies support the preclinical assessment of KTN0158 in canine tumor models as a mechanism to optimize the design and implementation of subsequent KTN0158 clinical studies in people.

Disclosure of Potential Conflicts of Interest

C. London reports receiving speakers bureau honoraria from Kolltan. T.M. LaVallee holds ownership interest (including patents) in Kolltan. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Advancing Translational Sciences, NCI or the NIH, or Kolltan Pharmaceuticals.

Authors' Contributions

Conception and design: C.A. London, T.M. LaVallee, R. Gedrich
Development of methodology: C.A. London, L. Lopresti-Morrow, T.M. LaVallee, R. Gedrich
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.A. London, S. Rippy, G. Post, K. La Perle, L. Crew, L. Lopresti-Morrow, T.M. LaVallee, R. Gedrich
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.A. London, K. La Perle, L. Crew, L. Lopresti-Morrow, A.J. Garton, T.M. LaVallee, R. Gedrich
Writing, review, and/or revision of the manuscript: C.A. London, H.L. Gardner, L. Crew, L. Lopresti-Morrow, A.J. Garton, G. McMahon, T.M. LaVallee, R. Gedrich
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.A. London, R. Gedrich
Study supervision: C.A. London, G. Post, A.J. Garton, R. Gedrich
Other (quantification of mast cells in H&E and T-blue–stained slides and interpretation of data): K. La Perle

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Cheryl A. London, Heather L. Gardner, Sarah Rippy, et al.

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