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

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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.


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 auto regulatory 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.

KIT0158 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...
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 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.

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 10-fold dilutions of KTN0158 (ranging from 10 nmol/L to 1 pmol/L) or isotype control antibody KTN0062C (10 nmol/L), nilotinib (1 µM/L), or imatinib (1 µM/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).

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; 2013A0000049). 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 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/VEGFR2/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.
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 study entry. Continued prednisone administration was permitted as indicated to manage adverse events.

Drug product and concomitant medications KTN0158 was produced by Kolltan Pharmaceuticals, Inc. and stored at 4°C. To maintain optimal potency, KTN0158 was secured in a refrigerated 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 and 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 (IC50) 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/y2).

Results KTN0158 is a potent inhibitor of KIT activation and signaling KTN0158 inhibited KIT phosphorylation in M-07e cells (Fig. 1A) with a mean IC50 value of 117 pmol/L (n = 3), which was similar to IC50 values observed in transfected CHO cells expressing wild-type human KIT (mean IC50 = 169 pmol/L, n = 13; data not shown). Imatinib and nilotinib (1 μmol/L) also inhibited KIT phosphorylation. Downstream signaling as...
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 day 14. These hematologic and biochemical events were subclinical, and no other adverse events were documented.
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

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Hematologic adverse events

Table 2A. 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 ≥28 days). PRs were noted in 5/11 dogs in 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).

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 MCLs 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 MCLs 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

Table 2B. Other adverse events

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
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 isoforms that can heterodimerize leading to KIT activation 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 hematoxic 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 FcεRI, resulting in the release of proinflammatory mediators and anaphylaxis in patients (39). Other mechanisms of mast cell activation include IgG receptor (FcγRI and FcγRIII) cross-linking or complement binding, inducing
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...
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

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