Phase I Dose-Escalating Study of SU11654, a Small Molecule Receptor Tyrosine Kinase Inhibitor, in Dogs with Spontaneous Malignancies\textsuperscript{1,2}

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ABSTRACT

Purpose: The purpose of the following study was to investigate the safety and efficacy of the novel multitargeted indolinone receptor tyrosine kinase (RTK) inhibitor, SU11654, using a canine model of spontaneous tumors. This p.o. bioavailable compound exhibits potent inhibitory activity against members of the split kinase family of RTKs, including vascular endothelial growth factor receptor, platelet-derived growth factor receptor, Kit, and Flt-3, resulting in both direct antitumor and antiangiogenic activity.

Experimental Design: This was a Phase I trial in which successive cohorts of dogs with spontaneous tumors that had failed standard treatment regimens received escalating doses of SU11654 as oral therapy. Pharmacokinetics, toxicity, and tumor response were assessed.

Results: Fifty-seven dogs with a variety of cancers were enrolled; of these, 10 experienced progressive disease within the first 3 weeks. Measurable objective responses were observed in 16 dogs (including 6 complete responses), primarily in mast cell tumors ($n = 11$), mixed mammary carcinomas ($n = 2$), soft tissue sarcomas ($n = 2$), and multiple myeloma ($n = 1$), for an overall response rate of 28\% (16 of 57). Stable disease of sufficient duration to be considered clinically meaningful (>10 weeks) was seen in an additional 15 dogs, for a resultant overall biological activity of 54\% (31 of 57).

Conclusions: This study provides the first evidence that p.o. administered kinase inhibitors can exhibit activity against a variety of spontaneous malignancies. Given the similarities of canine and human cancers with regard to tumor biology and the presence of analogous RTK dysregulation, it is likely that such agents will demonstrate comparable antineoplastic activity in people.

INTRODUCTION

Cancer is a leading cause of death for companion dogs; >50\% of dogs that live to 10 years of age will develop at least one type of malignancy (1). Common spontaneous malignancies in dogs include lymphomas, MCTs,\textsuperscript{4} sarcomas (fibrosarcoma, osteosarcoma, and hemangiosarcoma), carcinomas (mammary carcinoma, squamous cell carcinoma, and transitional cell carcinoma of the bladder), and malignant melanoma (1, 2). In most cases, the biological behavior of these tumors mimics the biological behavior of similar tumors found in humans: the tumors arise over months to years, and microscopic metastatic disease is usually present at the time of diagnosis. Additionally, current treatment regimens for canine malignancies are based on established therapies for similar human cancers. Therefore, spontaneous malignancies in the canine population may serve as a relevant model to test the safety and efficacy of novel antineoplastic therapeutics.

The aberrant growth of solid tumors and hematologic malignancies requires multiple, complex interactions between the neoplastic cells and the surrounding normal tissue (3). Thus, tumor growth is dependent on factors derived from the tumor (autocrine stimulation) and the tumor microenvironment (paracrine stimulation). Many of the processes involved in tumor growth, progression, and metastasis are mediated by ligand-receptor interactions involving RTKs and downstream signaling molecules (3). Because of the demonstrated importance of RTKs in the control of many cellular processes (particularly proliferation; Ref. 4) and the role of aberrant RTK signaling in

\textsuperscript{1} The abbreviations used are: MCT, mast cell tumor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; ITD, internal tandem duplication; GIST, gastrointestinal stromal tumor; QD, once daily; MS/MS, tandem mass spectrometry; MS, mass spectrometry; CT, computed tomography; IL, interleukin.
Several RTKs have been found to be dysregulated in a variety of both human and canine malignancies. Mechanisms of dysregulation for human cancers include mutation leading to constitutive activation (Met in renal carcinoma and Kit in GISTs; Refs. 10–15), overexpression (Her-2/neu in breast cancer; Refs. 16, 17), and autocrine loops of activation (hepatocyte growth factor/Met in osteosarcoma, PDGFR/PDGFR in sarcomas, and VEGF/VEGFR in melanoma; Refs. 18–24). In dogs, dysregulation of RTKs in spontaneous cancers is less well characterized; however, those canine tumors that have been evaluated demonstrate similar mechanisms of RTK dysregulation when compared with their human counterparts. For example, aberrant MET oncogene expression in canine and human osteosarcomas is found at a similar incidence (19, 20, 25). Additionally, 30–50% of malignant canine MCTs possess activating mutations in c-Kit consisting of ITDs in the negative regulatory juxtamembrane domain (26–29). The constitutive phosphorylation of Kit noted in canine MCTs closely mimics the situation in human GISTs in which a large percentage of these patients also express activated Kit secondary to mutations in the juxtamembrane domain (12–15, 30). Additionally, the majority of human patients with systemic mastocytosis possess activating mutations in Kit (31–34). Therefore, the role of RTK dysregulation is likely to be similar in many human and canine cancers.

In addition to their role in stimulation of cancer cells, RTKs also participate in the process of angiogenesis, the growth of new blood vessels from existing host vasculature. Of particular interest are the receptors for VEGF, PDGF, and fibroblast growth factor, as these RTKs are now known to be critical in endothelial cell migration, proliferation, and survival (21, 35–37). Although angiogenesis is essential for sustained tumor growth, the physiological requirement for angiogenesis in a normal host is modest, suggesting that angiogenesis inhibitors might offer a significant therapeutic benefit to cancer sufferers at well-tolerated dosages (37–39).

Most RTK inhibitors function by competitive inhibition of ATP binding, thereby preventing subsequent phosphorylation of the receptor (40–43). In general, the amino acid sequence of the ATP binding pocket is well conserved among species, and a given small molecule RTK inhibitor would, therefore, be likely to work across a variety of species. Antitumor activity of an RTK inhibitor in a certain canine malignancy could theoretically predict antitumor activity in its human counterpart, making spontaneous tumors in dogs a rationally selected preclinical model for the testing of novel RTK inhibitors prior or parallel to their testing in people with advanced cancers.

SU11654 (SUGEN Inc.; Fig. 1), a small molecule with an indolone chemical structure, is a selective inhibitor of the tyrosine kinase activity of several members of the split kinase RTK family, including Flk-1/KDR, PDGFR, and Kit (see Table 1). SU11654 and structurally related compounds have been shown to compete directly with ATP at the intracellular kinase domain of the RTK, thus preventing tyrosine phosphorylation and subsequent signal transduction (44–47). In vitro, SU11654 and related compounds exert a potent antiproliferative effect on endothelial cells (48). Additionally, SU11654 treatment can induce cell cycle arrest and subsequent apoptosis in tumor cell lines expressing activating mutations in split kinase RTKs (47). In mouse xenograft models, oral administration of SU11654 and related compounds affects the growth of multiple tumor cell lines originating from a variety of tissues, leading to either tumor regression or tumor growth inhibition (48).

Given the demonstrated direct antitumor and antiangiogenic activity of SU11654 in vitro as well as in mouse models, it was predicted that SU11654 would have activity against spontaneous tumors in larger animal models. Therefore, the primary objective of the following Phase I study was to assess the safety, pharmacokinetics, and biological activity of SU11654 administered p.o. to dogs with advanced spontaneous malignancies.

**Table 1 Inhibitory activity of SU11654**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Biochemical $K_i$ (μM)</th>
<th>Receptor phosphorylation (μM)</th>
<th>Ligand-dependent proliferation (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flk-1/KDR</td>
<td>0.006</td>
<td>0.005–0.05$^a$</td>
<td>&lt;0.007$^b$</td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>0.005</td>
<td>0.01–0.1$^d$</td>
<td>&lt;0.07$^c$</td>
</tr>
<tr>
<td>c-Kit</td>
<td>ND$^c$</td>
<td>0.01–0.1$^f$</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$ Determined using recombinant enzyme.
$^b$ Determined using serum-starved NIH-3T3 cells expressing Flk-1, stimulated with VEGF.
$^c$ Determined using HUVEC stimulated with VEGF.
$^d$ Determined using serum-starved NIH-3T3 cells expressing PDGFR, stimulated with PDGF.
$^e$ ND, not determined.
$^f$ Determined using MO7E (human acute myeloid leukemia) cells expressing c-kit, stimulated with SCF.

**PATIENTS AND METHODS**

**Eligibility**

This study was sponsored by the Center for Companion Animal Health at the University of California-Davis School of Veterinary Medicine. SU11654 was administered to dogs with spontaneous tumors that had failed conventional therapy or for which there was no therapeutic alternative. Informed consent from the owner of each dog was requested according to federal and institutional guidelines. To be eligible for the study, the dog...
must have had a histologically confirmed, advanced spontaneous malignancy, and met all of the inclusion criteria and none of the exclusion criteria. Eligible malignancies included soft tissue sarcoma, osteosarcoma, malignant melanoma, MCT, transitional cell carcinoma of the bladder, multiple myeloma, squamous cell carcinoma, mammary carcinoma, and lymphoma. Additional eligibility criteria included: >1 year old at study entry with estimated life expectancy of at least 6 weeks; adequate organ function; at least 2 weeks since prior investigational therapy, chemotherapy, or radiation (3 weeks for a surgical procedure); and no evidence of brain metastases or any serious systemic disorder incompatible with the study at the discretion of the investigator.

**Drug Product and Concomitant Medications**

SU11654 drug product \(\{5-\text{fluoro-2-oxo-1,2-dihydroindol-}(3)-\text{ylidenemethyl}-2,4\text{-dimethyl}-1\text{H}-\text{pyrrole}-3\text{-carboxylic acid (2-pyrrolidin-1-ethyl)-amide; Fig. 1}\}\) was available in 10- and 20-mg capsules, compounded with lactose. Capsules were stored at room temperature. SU11654 was generally delivered after food late in the evening; no difference in oral bioavailability was seen in earlier pharmacokinetic studies when the drug was delivered with or without food. In the previous Beagle dog dosing studies investigating high doses of SU11654, gastrointestinal toxicities occurred at higher drug doses and after prolonged drug dosing. To prevent or treat drug-related gastrointestinal toxicities, supportive care was administered as needed to dogs enrolled in the daily dosing cohorts. This typically consisted of famotidine, metronidazole, and/or metoclopramide. For the alternate daily dosing cohorts, premedication with famotidine and metronidazole was not used unless indicated by clinical symptoms. Antihistamines were administered to dogs with MCTs, as these tumors are known to release histamine. Other supportive care administered to dogs consisted of prednisone and piroxicam to treat tumor-associated inflammation and for pain control.

**Study Design**

This study was a Phase I dose escalating, open-label assessment of the safety and pharmacokinetics of SU11654 in companion dogs with spontaneous advanced malignancies. Safety was assessed on enrollment of at least 3 evaluable animals into dose-escalating cohorts. Each dog was evaluated every 1–3 weeks for toxicity and tumor response. Dose escalation occurred in 1.25 mg/kg increments via three separate dosing schedules (daily, alternate daily therapy, and 7-day loading dose followed by alternate daily maintenance therapy), until unacceptable toxicity occurred. The definition of unacceptable toxicity was ≥2 animals in a single dose group who experienced grade 3 or higher toxicity (excluding hematologic, nausea/vomiting) refractory to standard supportive care, or grade 4 hematologic, nausea or vomiting lasting longer than 7 days. Toxicity guidelines were adapted based on those established by the National Cancer Institute Common Toxicity Criteria, version 2.0. Except where adapted for use in canines, the study has used the grading system of adverse events (1–5) and procedures for attribution of adverse events as outlined in this manual. Disease progression or signs and symptoms definitely related to disease were not considered adverse events.

The initial starting dose and dosing regimen was set at 1.25 mg/kg QD. Because the capsule formulation did not permit exact dosing, each dose was approximated to the closest possible based on the capsule size. The first dog was administered one 20 mg capsule. Because of his small size, his actual dose was 2.04 mg/kg; toxicities associated with this animal were therefore later classified in the 2.5 mg/kg QD dose level (dose group #2). Subsequent dogs were started at 1.25 mg/kg QD (actual doses 1.1–1.6 mg/kg). Dogs were treated in 3-week cycles.

**Toxicity Assessment**

Every dog underwent a baseline complete history, physical examination, and predose laboratory assessment. Dogs were evaluated for adverse events at day 7, day 21, then every 21 days, at which time complete blood count with differential and clinical chemistry were performed. Urinalyses were performed at baseline and repeated only if indicated. Stipulations regarding minimal hematological requirements to continue dosing were included in the protocol: absolute neutrophil count >2,500/mm³, hematocrit >20%, and platelets >100,000/mm³. In addition, liver transaminases were to have been ≤3.0 × upper limit of normal with a normal total bilirubin and serum creatinine <3.5 mg/dl to permit continued SU11654 therapy.

**Tumor Response Assessment**

Tumor assessments were completed before study entry, at day 7, day 21, and every 3 weeks thereafter, or at the time of suspected tumor progression. Responses were assessed by the investigator according to predefined protocol criteria. The response in dogs with assessable disease was performed by tumor markers, clinical examination, radiography, ultrasonography, or spiral CT scans, as indicated by normal standards of practice in the institution. Many lesions were not amenable for quantitative radiographic imaging, but were followed either by serial clinical examination (superficial lesions or palpable lymph nodes) or by ultrasonography (bladder or liver). Thoracic lesions were assessed by both thoracic radiography and spiral computerized tomography; abdominal lesions were assessed by ultrasonography. Dogs with multiple myeloma were followed by serial immunoelectrophoresis and radiographs.

The response in dogs with measurable disease was judged by the investigator on the basis of response evaluation criteria in solid tumors criteria (49). A complete response was defined as disappearance of all of the disease on two measurements separated by a minimum period of 3 weeks. A partial response was defined as >30% reduction in the sum of the longest diameter of the target lesions documented by two assessments separated by at least 3 weeks. An increase of >20% in the size of all of the measurable tumor areas as measured by the sum of longest diameters of the target lesions taken as reference the smallest sum since initiation of therapy, or the appearance of any new lesion(s) would qualify as progressive disease. Stable disease was defined by the absence of criteria for either a response or progression. Response in multiple myeloma was adapted from that used by the Myeloma Task Force (50) used for human disease, a scale that involves serial assessment of serum and urine M-protein levels, soft tissue plasmacytomas, and energy
levels secondary to change in bone pain. Dogs who had no evidence of tumor progression and who had not experienced any unacceptable toxicity were eligible for extended treatment cycles. Dose escalation in an individual dog was permitted if the dog was tolerating therapy.

### Specimen Collection and Analysis

#### SU11654 Serum Levels.

Serum samples were collected during the first cycle on days 7 and 21 at specified intervals: before the dose, and at 30, 60, 120, 180, and 240 min. Additional collections for dogs in long-term therapy were collected sporadically thereafter. Blood samples were drawn from the jugular vein into a red-top (serum collection) vacuum glass tube. Specimens were kept at room temperature, allowed to clot, centrifuged at 1500 rpm at 4°C for 10 min, and the serum was transferred using a pipette to cryovials and frozen at −70°C pending analysis. Serum SU11654 determinations were made at University of California Davis School of Veterinary Medicine.

Blood sample analysis was performed using either MS/MS (ThermoFinnigan LCQ ion-trap MS) or single quad MS (Aglient 1100 MSD). The high-performance liquid chromatography mobile phase A contained 10% acetonitrile, 90% aqueous (10 mM NH₄Ac and 0.1% formic acid); and mobile phase B contained 90% acetonitrile, 10% aqueous (10 mM NH₄Ac and 0.1% formic acid). An isocratic elution with 65% of mobile phase A and 35% of mobile phase B at 0.8 ml/min flow rate was used. A postcolumn tee splitter was used to reduce the flow rate to 0.3 and 0.1 ml of the appropriate working solutions. The standard curve and quality control(s) samples was adjusted to pH 7.9 using 1:1 NH₄OH:water (v:v). The final serum concentrations were 5, 10, 20, 50, 100, 200, and 500 ng/ml for calibrators 1–7, and 100 ng/ml for quality control(s) (3 replicates). After vortex mixing, the pH of all of the samples was adjusted to pH 7–9 using 1:1 NH₄OH:water (v:v). The assay was validated, and found to be linear, precise, accurate, and free of interferences because of the biological matrix.

Formal pharmacokinetic analysis was not performed for this study, because standard kinetic analysis of blood samples by either compartmental or noncompartmental method was not possible. This was because of the fact that sample collection did not occur past 240 min (for practical purposes), and drug concentrations did not clearly reach a maximal concentration or enter an elimination phase in any dog tested. In samples that were declining at the last time point sampled (either 3 or 4 h after dose), there were too few points to calculate a terminal half-life or area under the curve with any confidence. As the concentration might have increased after the last time point sampled, it cannot be stated that the Cmax, and therefore Tmax, was reached for any dog. Pharmacokinetic information that could be obtained from these data included trough values on chronic dosing and an approximation of the steady state concentrations within the first 4 h after dosing.

#### Analysis of MCT for c-kit Mutation.

Biopsies from dogs with MCTs were obtained before study entry. Using a 2- or 4-mm punch biopsy instrument, samples were taken from the tumor, skin adjacent to the tumor, and normal skin at a site distant from the tumor. The samples were placed in individual microcentrifuge tubes and digested overnight at 37°C in a standard proteinase K buffer (26). The following day, the genomic DNA was precipitated out of the digest using isopropanol at a 1:1 ratio. The DNA was washed twice in 70% ethanol, then resuspended in Tris-buffered EDTA. Samples were quantitated and were then used for PCR to detect ITDs present in the juxtamembrane domain of c-kit as reported previously (26). The forward primer for this reaction (5’-cccatgtatgaagtacagtggaag-3’) was placed at the 5’ end of exon 11, and the reverse primer (5’-gttccaaagctgtgacg-3’) was placed in the 5’ end of intron 11, yielding a PCR product of ~170 bp. The PCR reaction was carried out as follows: 94°C (5 min), 35–40 cycles consisting of 94°C (1 min), 95°C (1 min), 72°C (1 min), then 72°C (10 min). PCR products were analyzed by agarose gel electrophoresis (4%); tumors possessing tandem duplications in c-kit generated both a wild-type PCR product as well as a larger product representing that containing the tandem duplication. Controls for these studies included the genomic DNA derived from the C2 canine mastocytoma cell line (containing an ITD and no wild-type Kit) and canine cerebellum (26).

### Statistical Analysis

Patient disposition was summarized by dose level. Baseline characteristics, including age, gender, and cancer type, were summarized, and efficacy outcomes (objective responses and stable disease of >10 weeks) were summarized. Time to tumor progression was measured from first day of study drug administration until documented progression of disease; overall survival was measured from first day of study drug administration to death. Survival probabilities were estimated by the method of Kaplan and Meier, and the median time-to-progression was determined; comparison of survival curves was performed using the Wilcoxon test. The Pearson χ² test was used to test the association of objective response with c-Kit mutation status, lymph node involvement, multicentric disease, location (limb, head, or trunk), histological grade ≤2 or 3, and dose (either 2.5 or 3.25 mg/kg every other day).
RESULTS

Demographics

A total of 57 dogs were enrolled in the study between March 2001 and April 2002. All but 4 of the dogs were treated and followed at University of California Davis School of Veterinary Medicine; other dogs were treated by Dr. Mona Rosenberg and Sue Downing (Veterinary Cancer Referral Group, Los Angeles, CA) or Dr. Gerald Post (Veterinary Cancer Referral Group, New York City, NY). The 57 dogs were enrolled into eight different treatment groups. Data from all of the dogs are assessable for toxicity; 47 are assessable for response (remaining dogs were withdrawn before the day 21 visit). Pharmacokinetic data (both day 7 and day 21) are available for 40 dogs.

Baseline characteristics for the 57 dogs enrolled into the study are presented in Table 2. Median age on entry to the study was 10 years (range, 2–14). There were 8 intact males, 18 castrated males, 2 intact females, and 29 spayed females. The most common tumor types were MCT (n = 22), lymphoma/mycosis fungoides (n = 6), mixed mammary carcinoma (n = 5), transitional carcinoma of the bladder (n = 4), soft tissue sarcoma (n = 4; 2 dogs with undifferentiated sarcoma and 1 dog each with hemangiopericytoma or nerve sheath tumor), malignant melanoma (n = 3), osteosarcoma (n = 3), hemangiosarcoma (n = 3), oral squamous cell carcinoma (n = 2), lingual or maxillary), multiple myeloma (n = 2), primary lung tumor (n = 1), bronchogenic carcinoma), poorly differentiated carcinoma (n = 1), and sebaceous gland adenocarcinoma (n = 1). As expected for an exploratory Phase I study in dogs with advanced malignancies, most dogs had received prior therapy consisting of surgery, chemotherapy, and/or radiation therapy. Median number of prior chemotherapeutic regimens for all of the patients equaled 1 (range: 0–3+ regimens; 10 dogs were chemonaive).

Study Drug Administration and Pharmacokinetics

Enrollment and toxicity per group is presented in Table 3. Dose escalation began at 1.25 mg/kg and continued to a maximum dose of 5.0 mg/kg via intrapatient dose escalation. Dose cohorts initially consisted of 1–5 dogs; two groups were expanded to 16 (2.5 mg/kg EOD) and 20 dogs (3.25 mg/kg EOD) to better define the toxicity, activity, and potential need for premedication at this dose level. Dogs were dose escalated in increments of 10–20 mg provided the dose assigned previously was well tolerated. Of the 57 dogs, 47 dogs completed the first 3-week cycle of treatment; 10 withdrew before completion of a cycle (9 for rapidly progressive disease; 1 for adverse events, described below). Forty-three of the 47 dogs continued into a second cycle of treatment. Maximum time on study is currently 76 weeks; as of November 2002, 10 dogs continue to receive drug.

Although formal pharmacokinetic analysis was not performed for this study (because of lack of postdose sampling beyond 4 h), an analysis of trough concentrations of SU11654 was performed, comparing daily and alternate daily dosing at the various doses (Fig. 2). Predose concentrations of SU11654 were consistently lower on the alternate daily dosing compared with daily dosing regimen, but predose levels were still measurable in all of the dogs, ranging from 40–60 ng/ml (daily dosing) and 3–34 ng/ml (alternate daily dosing). On the basis of preclinical work in rodent models, the therapeutic range of SU11654 for target inhibition was considered to be 50–100 ng/ml for 12 h of a 24-h dosing period. Therefore, trough levels of SU11654 on daily dosing were either at or above threshold for target inhibition, suggesting that dogs on daily dosing were never effectively leaving the therapeutic range. This is in contrast with the alternate daily dosing, where the dogs cycled in and out of therapeutic range, and all of the dogs had trough levels below therapeutic range. It is likely that the difference in trough SU11654 levels contributed to the increased frequency and severity of toxicities noted with daily versus alternate day dosing (see below).

Toxicity

On the alternate daily dosing regimen of SU11654, drug-related toxicities were limited and in most cases readily amenable to supportive care. This is in contrast to the daily dosing regimen in which several drug-related toxicities were noted including grade 1–2 appetite loss, diarrhea, vomiting, neutropenia, and hind limb weakness; only isolated cases of grade 3 toxicity occurred. These toxicities resolved in most dogs with temporary discontinuation of therapy for 2–3 days. Antihistamines were administered to dogs with MCTs to prevent signs of anaphylaxis, as these tumors are known to release histamine. In addition, to prevent or treat drug-related gastrointestinal toxicities, supportive care was administered to many of the dogs enrolled in the later dose cohorts. Supportive care typically consisted of famotidine, metronidazole, and metoclopramide. Although premedications were used for 6 of the 36 dogs that

### Table 2 Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
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<tbody>
<tr>
<td>n</td>
<td>57</td>
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<tr>
<td>Age (yr)</td>
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<tr>
<td>Median</td>
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<tr>
<td>Range</td>
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<tr>
<td>Gender, n (%)</td>
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<tr>
<td>Male</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Castrated male</td>
<td>18 (31.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>Spayed female</td>
<td>29 (51%)</td>
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<td>Primary cancer diagnosis, n</td>
<td></td>
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<tr>
<td>Mast cell tumor</td>
<td>22</td>
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<td>Lymphoma/mycosis fungoides</td>
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<td>Mixed mammary</td>
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<tr>
<td>Hemangiosarcoma</td>
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<td>Malignant melanoma</td>
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<td>Osteosarcoma</td>
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<td>Squamous cell carcinoma (lingual/maxillary)</td>
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<td>Miscellaneous*</td>
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<tr>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>3+</td>
<td>9</td>
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</table>

* Including primary lung tumor, poorly differentiated carcinoma, and sebaceous adenocarcinoma (1 each).
began therapy at a dose of 2.5–3.25 mg/kg EOD, the current recommended dose and schedule is well tolerated. Therefore, premedication with famotidine and metronidazole on the alternate daily dosing regimen is not currently used unless clinically indicated. A description of the specific system-related toxicities is given below.

**Gastrointestinal.** On daily dosing, several dogs developed grade 1–3 diarrhea, grade 1–3 anorexia, and rarely grade 1–3 vomiting. In some cases these clinical signs continued despite supportive care consisting of metronidazole, kapectate, metoclopramide, or cyproheptadine therapy. As discussed previously, it is likely that many of these signs were secondary to persistent SU11654 blood levels above threshold for target inhibition. Whereas the exact mechanism of the gastrointestinal toxicity is not known, the gastrointestinal tract of dogs is known to be sensitive to a variety of agents with the potential to induce mucosal damage (nonsteroidal anti-inflammatory medications, prednisone, and so forth). It is believed that such mucosal damage is secondary to disruption of normal mucosal blood flow. Therefore, it is possible that sustained SU11654 blood levels caused a similar disruption of mucosal blood flow, resulting in the observed anorexia, diarrhea, and vomiting. This is supported by the fact that such side effects were much less common and resolved more rapidly with supportive care in the dogs receiving alternate day dosing of drug. Another possibility is that the function of the interstitial cells of Cajal was disrupted by SU11654 administration. As these cells are dependent on Kit signaling for survival and are responsible for regulating gastrointestinal motility, the SU11654 may have caused dysfunction of these cells leading to intestinal hypermotility and subsequent diarrhea.

**Hematologic.** In several dogs, grade 1 neutropenia was noted after 2–3 weeks of SU11654 therapy, with neutrophil counts falling between 1500–3000/μL. This did not result in any episodes of infection, and in many cases, the neutropenia appeared to resolve with continued therapy. However, some dogs had persistent neutropenia that lasted for the duration of SU11654 therapy. Interestingly, the neutropenia never worsened, and if drug was discontinued temporarily, rapidly resolved. Only 3 dogs experienced thrombocytopenia (grade 1 and 2). However, in 2 cases, this was likely because of tumor progression; both cases developed systemic mast cell disease after failing therapy, with diffuse bone marrow involvement leading to the observed decrease in platelets. The 1 dog that developed treatment-related thrombocytopenia had been dose escalated to the maximum tolerated dose (5 mg/kg QD; see below) resulting in a variety of clinical effects in addition to the decrease in platelets. Five dogs experienced grade 1–3 anemia after treatment. In all of the dogs, the anemia appeared to be secondary to tumor necrosis after the initiation of SU11654 therapy. Two of these dogs had hemangiosarcoma metastatic to the liver, and 1 had an undifferentiated sarcoma also metastatic to the liver. The remaining 2 dogs had large transitional cell carcinomas of the bladder that experienced extensive hemorrhage, although this resolved when the SU11654 therapy was discontinued.

**Neuromuscular.** Certain animals showed signs of mild-to-moderate hind limb weakness (4 of the 10 animals on daily therapy, 1 of which had pre-existing neuromotor impairment; 2 of the 6 animals administered the 7-day loading dose during the initial week of therapy). Although more pronounced with daily dosing, several dogs on the alternate daily dosing regimen also showed signs of mild hind limb weakness. The etiology of this toxicity is unclear, although a portion of this toxicity is likely to be disease or age-related in many of the dogs. It is interesting to note that in some dogs, the hind limb weakness may have been secondary to muscle pain leading to an unwillingness to walk up stairs and run/play. This is supported by the fact that in 4 dogs on the daily dosing regimen, a transient lameness (either hind limb or forelimb) was noted that resolved with rest and anti-inflammatory therapy. On physical examination no bone or joint

<table>
<thead>
<tr>
<th>Dose group</th>
<th>Regimen</th>
<th>Dose (mg/kg)</th>
<th>No. of dogs</th>
<th>Diarrhea</th>
<th>Anorexia</th>
<th>Vomiting</th>
<th>Fatigue</th>
<th>Hind limb weakness</th>
<th>Neutropenia</th>
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<tr>
<td>1</td>
<td>QD</td>
<td>1.25</td>
<td>5</td>
<td>2 (40%)</td>
<td>4 (80%)</td>
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<tr>
<td>2</td>
<td>QD</td>
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</tr>
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<td>2 (13%)</td>
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<tr>
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<td>EOD</td>
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<td>1 (33%)</td>
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</tr>
<tr>
<td>7</td>
<td>QDx7; EOD</td>
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<td>1</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>8</td>
<td>QDx7; EOD</td>
<td>3.75</td>
<td>1</td>
<td>2 (40%)</td>
<td>2 (40%)</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
<td>3 (60%)</td>
<td>1 (20%)</td>
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*Fig. 2* Trough SU11654 levels comparing daily (QD) and alternate day (EOD) dosing. The serum concentrations for dogs on SU11654 were measured at 24 h after dosing (for QD) and 48 h after dosing (for EOD) to obtain trough levels of drug. The median trough level for each dose level is shown. The --- indicates the 50 ng/ml level, a plasma concentration at which receptor phosphorylation is inhibited. Dogs on the QD dosing schedule had trough levels near or above threshold, whereas all dogs on the EOD dosing schedule had trough levels below threshold. Each data point represents the median trough level of 2–4 patients.

**Table 3** Enrollment and toxicity by dose/regimen
pain was observed but focal muscle pain was often identified, suggesting a possible myositis; however, no elevations in either creatinine phosphokinase or aspartate aminotransferase that would support evidence of muscle injury was found. Furthermore, there was no histopathologic evidence of muscle necrosis in those dogs that underwent necropsy.

**Other.** In 4 of the 47 dogs, persistent elevations in blood urea nitrogen (range, 33–102 mg/dl) and creatinine (range, 2.0–3.2 mg/dl) developed while on SU11654 therapy, indicative of chronic renal failure. However, all of these dogs had underlying conditions that could have accounted for such changes, including chemotherapy-induced chronic renal failure secondary to excess doxorubicin administration (total dose of 270 mg/m²), chronic pyelonephritis (secondary to prescrotal urethrostomy), Addison’s disease, and chronic renal insufficiency secondary to aging changes. In all of the dogs, the renal values did not worsen with continued SU11654 therapy, and the renal values did not improve with discontinuation of SU11654 therapy, additionally supporting the notion that such changes were independent of treatment. Two dogs developed transient elevations in alanine aminotransferase that were not associated with any specific clinical signs and resolved spontaneously without discontinuation of therapy. The cause for these elevations is not known but is not believed to be related to the SU11654 administration.

**Dose-Limiting Toxicity**

The highest dose tested was in case #02. This dog was dose escalated in week 7 of therapy to 5 mg/kg QD (actual dose 4.67 mg/kg). New onset toxicities observed on escalation to this dose were similar to what had been observed in the high-dose toxicology assessment of this agent in Beagle dogs, and included grade 3–4 diarrhea (despite continued metronidazole therapy), grade 3 vomiting, grade 3 anorexia, with grade 1 thrombocytopenia, neutropenia, and gastrointestinal bleeding. The dog was hospitalized and treated with supportive care consisting of i.v. fluids, famotidine, omeprazole, sucralfate, metaclopramide, and enrofloxacain. The dog improved over the next 24 h, without vomiting or diarrhea during the hospital admission. The dog was discharged on oral famotidine, omeprazole, sucralfate, and enrofloxacain. Over the subsequent 24 h, the dog developed additional vomiting and diarrhea, and the owners elected euthanasia. Serum concentrations of SU11654 in the animal were consistently >100 ng/ml (data not shown).

**Tumor Specimens**

Tumor specimens were obtained from all of the dogs with MCTs entered into the study to determine whether an ITD was present in the juxtamembrane domain of Kit. It was predicted that dogs with MCTs possessing mutations may be more likely to respond to SU11654 therapy than those with wild-type Kit, as dysregulated Kit is clearly driving disease in these cases (26, 27). For dog #13 (8-year-old spayed Springer spaniel with a recurrent grade III MCT), biopsies of the tumor, adjacent skin, and distant apparently unaffected skin were obtained. Genomic DNA was isolated, and PCR revealed the presence of ITDs in all of the specimens evaluated (Fig. 3). Peripheral blood lymphocytes were subsequently obtained for analysis; as these did not possess an ITD, the mutation was considered to be somatic in nature (a germ-line mutation was ruled out). Repeat biopsy of the distant and adjacent skin of this animal on week 1 after initiation of SU11654 therapy was initiated demonstrated an absence of the ITD in the adjacent and distant skin based on PCR of genomic DNA. This alteration was confirmed on subsequent biopsy on week 3 (see Fig. 3). This finding is suggestive of a molecular remission of the micrometastatic disease. This dog is still alive 74+ weeks after initiation of SU11654 therapy and continues to be on drug; this compares favorably to the typical 24-week median survival time for dogs with grade III MCTs (51, 52).

**Response to Therapy**

Antitumor responses were determined on the basis of radiological assessment or tumor-marker evaluations every 3 weeks or at the time of suspected progression. Data for all of the dogs are presented in Table 4. Complete responses occurred in 6 dogs (MCTs), whereas partial responses occurred in an additional 10 dogs (MCTs, metastatic mammary carcinoma, metastatic soft tissue sarcoma, and multiple myeloma), for an overall...
Clinical Trial of a Kinase Inhibitor in Dog Tumors

The greatest number of responses were observed in dogs with MCTs, with a total response rate of 55% and an overall biological activity of 64%. Two examples are shown in Fig. 4. Patient #13, positive for a Kit ITD, experienced a durable partial response of a grade II MCT over the right dorsal aspect of the right forelimb extending to the right thoracic cavity including the 3.25 mg/kg dose, and 3 of 4 (75%) at the 3.75 mg/kg dose of 26 dogs (58%) at the 2.5 mg/kg dose, 11 of 20 dogs (55%) at 1.25 mg/kg group showing biological activity compared with 15

response rate of 28% (16 of 57). In addition to these objective responses, stable disease was noted in other tumor types, including transitional cell carcinoma of the bladder (stable disease of 11, 17, and 26 weeks duration); melanoma (2 cases of 12 weeks stable disease); 2 dogs with osteosarcoma (stable disease of 12 and 42 weeks duration); primary lung carcinoma (34+ weeks); squamous cell carcinoma (11 weeks); and T-cell lymphoma (10 weeks), for an overall biological activity of 54% (31 of 57). Biological activity in this clinical trial appeared to follow a dose-dependent pattern, with only 2 of 7 dogs (28%) in the 1.25 mg/kg group showing biological activity compared with 15 of 26 dogs (58%) at the 2.5 mg/kg dose, 11 of 20 dogs (55%) at the 3.25 mg/kg dose, and 3 of 4 (75%) at the 3.75 mg/kg dose (data not shown).

The effect of Kit ITD on time to progression and survival of dogs with MCTs was also examined. Fig. 5 shows the differences in the time to progression and overall survival of 22 dogs treated with SU11654 whose tumors either expressed (n = 11) or did not express (n = 11) this mutation. Dogs with MCTs possessing a Kit ITD survived longer than those without (36.9 versus 15.4 weeks), but this comparison did not reach statistical significance (P = 0.12), most likely because of inadequate sample sizes for such a comparison (Fig. 5b). However, the median time to progression in dogs with MCTs possessing an ITD was 21.0 weeks, compared with 3.9 weeks for those dogs without an ITD (P = 0.05; Fig. 5a). This data indicates that as predicted the effect of SU11654 therapy on tumor progression was clearly impacted by Kit mutation status.

Partial responses were also observed in pulmonary metastases of other tumor types. Of the 5 dogs enrolled with mixed mammary carcinoma, 2 dogs experienced a partial response in their pulmonary metastases of 21 weeks (patient #31) and 60+ weeks (patient #27) duration. Serial spiral CT scans are shown for patient #27, demonstrating regression of the metastatic disease (Fig. 6). Both dogs had been administered cytotoxic chemotherapy consisting of doxorubicin and cyclophosphamide after surgical resection of the primary tumor but subsequently developed pulmonary metastatic disease before beginning SU11654 therapy. Two other dogs with metastatic mammary carcinoma remained on study with stable pulmonary metastases for 27 and 38 weeks. In addition, 2 dogs with soft tissue sarcomas metastatic to the lungs had quantifiable regression of pulmonary metastases for 10 weeks (patient #40) and 47+ weeks (patient #44). One of these dogs (#44) had received adjuvant doxorubicin chemotherapy after surgical removal of the primary tumor before developing metastatic disease. Serial spiral CT scans are shown for patient #44, demonstrating regression of the metastatic disease (Fig. 6).

RTKs inhibited by SU11654 (Flk1/KDR and PDGF-R) have also been implicated in the pathogenesis of multiple myeloma. One dog with advanced multiple myeloma, refractory to melphalan with prednisone and doxorubicin, showed a 50% decline in her serum IgA levels, lasting for 6 months; this level of decline in her paraprotein is equivalent to that shown with prior chemotherapy (Fig. 6). On an increase in this paraprotein, the animal was coadministered cyclophosphamide with continued SU11654 therapy, resulting in stabilization of her IgA levels; an increase in the SU11654 dose (from 2 to 4 mg/kg EOD) again led to a decline in the IgA level (Fig. 6). In total, the dog has been maintained in response or stable disease for 76+ weeks, an encouraging length of time for this typically rapidly progressive disease.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Number enrolled</th>
<th>Objective response (tumor shrinkage)</th>
<th>SD ≥ 10 weeks</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>22</td>
<td>11</td>
<td>2</td>
<td>13 (59%)</td>
</tr>
<tr>
<td>ITD positive</td>
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<td>9</td>
<td>1</td>
<td>10 (91%)</td>
</tr>
<tr>
<td>ITD negative</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Lymphoma</td>
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<td>0</td>
<td>1</td>
<td>1 (17%)</td>
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<td>2</td>
<td>2</td>
<td>5 (80%)</td>
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<td>Soft tissue sarcoma</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2 (50%)</td>
</tr>
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<td>Bladder carcinoma</td>
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<td>3</td>
<td>3 (75%)</td>
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<tr>
<td>Lung carcinoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

Table 4 Response rate by tumor type

The effect of Kit ITD on time to progression and survival of dogs with MCTs was also examined. Fig. 5 shows the differences in the time to progression and overall survival of 22 dogs treated with SU11654 whose tumors either expressed (n = 11) or did not express (n = 11) this mutation. Dogs with MCTs possessing a Kit ITD survived longer than those without (36.9 versus 15.4 weeks), but this comparison did not reach statistical significance (P = 0.12), most likely because of inadequate sample sizes for such a comparison (Fig. 5b). However, the median time to progression in dogs with MCTs possessing an ITD was 21.0 weeks, compared with 3.9 weeks for those dogs without an ITD (P = 0.05; Fig. 5a). This data indicates that as predicted the effect of SU11654 therapy on tumor progression was clearly impacted by Kit mutation status.

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DISCUSSION

Over the past few years tremendous interest has developed in the use of kinase inhibitors to treat cancer. The potential utility of such agents is supported by the significant activity of the bcr-abl/Kit inhibitor, Gleevec, against chronic myelogenous leukemia and GISTs in people (13, 53–55). Agents that target the split kinase family of RTKs, (including Flk-1/KDR, PDGFR, fibroblast growth factor receptor, Flt3, and Kit), are likely to work via a complementary mechanism of action compared with therapies that directly target the tumor cells or endothelial cells alone. Depriving tumor cells of an RTK-derived growth stimulus combined with denial of nutrients and oxygen through disruption of endothelial cell growth may force tumor cells into an apoptotic pathway, resulting in significant tumor regression. Given their lack of genomic instability, endo-

Table 5  Response rate in MCT by mutation and lymph node disease

<table>
<thead>
<tr>
<th></th>
<th>Lymph node negative</th>
<th>Lymph node positive</th>
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<tr>
<td>Kit mutation negative</td>
<td>50% (2/4)</td>
<td>0% (0/7)</td>
</tr>
<tr>
<td>Kit mutation positive</td>
<td>100% (6/6)</td>
<td>60% (3/5)</td>
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Fig. 4  Response of MCTs possessing Kit ITDs to SU11654 therapy. a, patient #13 had a recurrent grade III MCT that had failed treatment with both surgery and chemotherapy. PCR of genomic DNA revealed that the tumor possessed an ITD in Kit. This patient began SU11654 therapy, and a partial remission was achieved by week 9 of treatment. This patient remains on SU11654 therapy for 74+ weeks. b, patient #46 had a chemotherapy resistant diffuse grade II MCT. PCR of the genomic DNA demonstrated the presence of a Kit ITD. The patient began SU11654 therapy, and a partial remission was achieved by week 6 of treatment. This patient remains on SU11654 therapy for 42+ weeks.
epithelial cells would be less prone to develop resistance, additionally amplifying the antitumor response (56). Lastly, use of such agents may produce additional benefit through prevention of tumor cell escape into the circulation by maintaining the integrity of the intercellular connections between endothelial cells. Therefore, a single molecular entity that inhibits RTKs involved in both the proliferation of malignant cells and endothelial cell signaling would be predicted to have great therapeutic potential.

SU11654, a novel synthetic compound, is a potent and selective inhibitor of several members of the split-kinase domain family of RTKs, including VEGFR, PDGFR, Flt3, and Kit. These RTKs are involved in the stimulation of tumor growth through autocrine and paracrine mechanisms, as well as mutations leading to ligand-independent RTK signaling (12, 14, 15, 18, 34, 43, 57–60). Additionally, both VEGFR and PDGFR are important in tumor angiogenesis (36, 56). Previous studies of similar inhibitors in mouse models of cancer (xenografts, and so forth) have demonstrated promising activity (61–63). However, such models do not predict the actual activity against spontaneous tumors, as transplanted tumors in mice often consist of a homogeneous population of tumor cells that has developed over a relatively short period of time. Additionally, the blood vessels in such tumors are rapidly growing and homogenous, making them more amenable to antiangiogenic therapy. Therefore, we were interested in evaluating the activity of SU11654 in a tumor model that more closely represents the situation observed in human cancers. We chose to use companion dogs with spontaneous malignancies, because in most cases, the biological behavior of cancer in dogs closely mimics that observed in people. As such, the primary objective of this study was to determine the toxicities and pharmacokinetics of p.o. administered SU11654 in companion dogs with spontaneous malignancies; data on response of tumors to therapy was also collected. Rigorous collection of safety, activity, and (whenever possible) molecular data were captured for each animal.

The initial dosing schema was based on preclinical xe-
nograft models that incorporated daily dosing via oral gavage. However, toxicities observed in the dogs treated with a daily dosing regimen increased with increased time on study, and were likely because of constant inhibition of the target RTKs (Fig. 2). An alternate daily dose regimen resulted in significantly less observed toxicities and is, therefore, the preferred regimen for this agent. Serum concentrations of 50–100 ng/ml for half of the dosing interval (12 of 24 or 24 of 48 h, the proposed target concentration based on animal studies) are achieved using 2.5 and 3.25 mg/kg EOD.

The 57 dogs enrolled in this clinical trial were representative of the population of companion dogs with spontaneous malignancies in terms of their age, gender, prior chemotherapy regimens, and mix of different tumor types. The drug was safe and well tolerated at doses up to and including 3.25 mg/kg given on alternate daily dosing. At this dose and schedule, most dogs experienced toxicities that were generally mild in severity and amenable to minimal supportive care including the administration of antacids (famotidine), antidiarrheals (metronidazole), and anti-inflammatorics (prednisone and piroxicam). Clinical laboratory tests were monitored for evidence of effects on liver function and renal function, but elevations in either liver transaminases or creatinine were only occasionally without any obvious dose-dependency. In addition, comorbid disease existed in these animals that could reasonably result in the observed toxicity. Sporadic hind limb weakness was observed in 18 dogs (more severe on the daily administration schedule). The mechanism behind this toxicity is unknown, but this resolved with short-term (48–72 h) discontinuation of therapy. Some portion of this toxicity may be related to underlying disease. Interestingly, several dogs developed grade 1 neutropenia that persisted for long periods of time during therapy. This may be secondary to inhibition of Kit signaling leading to disruption of normal granulopoiesis. Granulocytes have the shortest half-life of all of the hematopoietic cells and are, thus, the cell type most likely to be affected by disruption of normal signaling pathways in the bone marrow (64). None of the dogs experienced neutrophil counts <1500/µl, and no dogs developed any signs of infection secondary to the neutropenia. Additionally, the degree of neutropenia did not worsen with continued therapy; indeed, in many dogs it was transient, resolving over 3–4 weeks. It is possible that elevated levels of granulocyte colony-stimulating factor

Fig. 6 Serial spiral CT scans demonstrating regression of pulmonary nodules after SU11654 therapy. Spiral CT was performed on a patient with metastatic mammary carcinoma (#27) and a patient with metastatic undifferentiated sarcoma (#44) before beginning SU11654 therapy, 6 weeks after treatment initiation, and again at 18 weeks after treatment initiation. Both of these patients experienced significant regression of multiple pulmonary nodules. The course of regression for each dog is demonstrated by the identical CT image presented before treatment, at 6 weeks, and at 18 weeks. The pulmonary nodule of interest for each dog is indicated by the arrow. Both patients remain on SU11654 therapy 60+ and 47+ weeks, respectively.
Fig. 7 Reduction in serum IgA levels in a patient with multiple myeloma in response to SU11654. Patient #8 was treated for multiple myeloma (IgA monoclonal gammopathy) with melphalan and prednisone resulting in a partial response to therapy for ~6 months. After failing this standard treatment, the patient was given a single dose of doxorubicin with no response. SU11654 therapy was subsequently initiated at 2 mg/kg EOD, and the serum IgA level slowly decreased over a period of ~6 months to that achieved previously with chemotherapy. A rise in the serum IgA was then noted, and pulse dose cyclophosphamide was administered q 3 weeks, whereas the patient continued on SU11654 resulting in stabilization of the IgA level. When the SU11654 dose was increased to 4 mg/kg EOD, another decline in the IgA level occurred. This patient remains on therapy 76+ weeks.

produced in response to the neutropenia resulted in recovery with continued dosing.

In the present study, SU11654 given p.o. produced objective clinical responses in 16 dogs with advanced malignancy and stable disease for ~10 weeks in 15 additional dogs. Most encouraging antitumor data were observed in dogs with MCTs, mammary carcinoma, soft tissue sarcoma, and multiple myeloma. As is the case for similar spontaneous tumors in people, there is a molecular basis to explain the response of many of these tumor types in dogs. Approximately 30–50% of canine MCTs are known to possess activating mutations consisting of ITDs in the juxtamembrane domain of Kit; these ITDs are believed to be the primary stimulus for uncontrolled growth in these cases (26). This type of Kit mutation is very similar to that observed in GISTs in people where small deletions are found in the juxtamembrane domain of Kit, also resulting in constitutive receptor phosphorylation (14, 65). In this study, dogs with MCTs possessing Kit mutations were much more likely to respond to therapy than those tumors with wild-type Kit. These data support the notion that targeted therapies with small molecule kinase inhibitors are more likely to provide a clinical benefit if the tumor cell exhibits aberrant function of the expressed RTK through mutation, autocrine loop stimulation, or gene duplication (overexpression). It is possible that those MCTs that did not respond to SU11654 expressed other mutations independent of Kit (or other split kinases such as VEGFR and PDGFR) that promoted cell survival and proliferation, thereby circumventing kinase inhibition.

With regard to the clinical response observed in the patient with multiple myeloma, it is known (at least in the human disease) that malignant plasma cells express Kit (60, 66). It is believed that Kit signaling promotes the production of IL-6, resulting in growth stimulation of these cells. As stem cell factor is abundant in the bone marrow, inhibition of Kit signaling may suppress this stimulus. Alternatively, recent evidence suggests that VEGF produced by myeloma cells stimulates IL-6 production by stromal cells, thereby supporting myeloma cell survival and growth (67–70). Furthermore, IL-6 stimulates myeloma cells to produce more VEGF, resulting in a two-way paracrine loop. Some investigators have found that degree of neovascularization found in the bone marrow of myeloma patients may be predictive of clinical response (59, 71). Therefore, inhibition of VEGFR signaling may also contribute to regression of myeloma.

The mechanisms of SU11654-induced tumor regression in the mammary tumors and soft tissue sarcomas in this study are not known. However, in women with breast cancer, aberrant expression of VEGFR, PDGFR, and Kit has been described (58, 72, 73). High levels of Kit expression have also been noted in canine mammary carcinomas (74). It is possible that inhibition of any combination of these RTKs may have contributed to the observed regression. In soft tissue sarcomas, expression of PDGF/PDGFR and VEGF/VEGFR with resultant autocrine loops of growth factor stimulation has been documented (21, 23, 75–78). Inhibition of such autocrine loops by SU11654 may have resulted in regression of the metastatic disease in the affected dogs.

In summary, this Phase I study of an oral kinase inhibitor that targets several members of the split kinase domain RTK family represents the first evidence that such inhibitors have potent biological activity in the treatment of a variety of spontaneous malignancies. In this instance, the inhibitor was likely working through two separate yet complementary mechanisms: direct inhibition of cell signaling pathways necessary for sustained tumor cell growth and survival, and inhibition of the growth and survival of neovessels critical for sustaining the tumor. The spectrum of tumors that responded to SU11654 therapy included hematopoietic neoplasms, carcinomas, and sarcomas, demonstrating the broad activity of this drug. Given the parallel nature of canine and human cancers with regard to tumor biology and the presence of analogous RTK dysregulation, it is likely that agents similar to SU11654 will elicit comparable biological responses in human cancers.

REFERENCES


Phase I Dose-Escalating Study of SU11654, a Small Molecule Receptor Tyrosine Kinase Inhibitor, in Dogs with Spontaneous Malignancies,