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Preliminary evidence for biologic activity of toceranib phosphate (Palladia®) in solid tumours*

C. London[1], T. Mathie[1], N. Stingle[1], C. Clifford[2], S. Haney[2], M. K. Klein[3], L. Beaver[3], K. Vickery[3], D. M. Vail[4], B. Hershey[5], S. Ettinger[6], A. Vaughan[7], F. Alvarez[8], L. Hillman[9], M. Kiselow[10], D. Thamm[11], M. L. Higginbotham[12], M. Gauthier[13], E. Krick[14], B. Phillips[15], T. LaDue[16], P. Jones[17], J. Bryan[18], V. Gill[19], A. Novasad[20], L. Fulton[21], J. Carreras[22], C. McNeill[23], C. Henry[24] and S. Gillings[25]

[1]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA; [2]Red Bank Veterinary Hospital, Tinton Falls, NJ, USA; [3]Southern Arizona Veterinary Specialty and Emergency Center, Tuscon, AZ, USA; [4]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, Madison, WI, USA; [5]Integrative Veterinary Center, Phoenix, AZ, USA; [6]Animal Specialty Center, Yonkers, NY, USA; [7]Las Vegas Veterinary Referral Center, Las Vegas, NV, USA; [8]Coral Springs Animal Hospital, Coral Springs, FL, USA; [9]MedVet Memphis, Memphis, TN, USA; [10]Veterinary Medical Specialists, San Mateo, CA, USA; [11]Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA; [12]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA; [13]Mississauga Oakville Veterinary Emergency Hospital, Oakville, ON, Canada; [14]Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA; [15]Veterinary Specialty Hospital of San Diego, San Diego, CA, USA; [16]Southeast Veterinary Oncology, Orange Park, FL, USA; [17]Capital Area Veterinary Specialists, Austin, TX, USA; [18]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA; [19]Katonah-Bedford Veterinary Center, Brightheart Veterinary Centers, Bedford Hill, NY, USA; [20]Sugar Land Veterinary Specialists, Sugar Land, TX, USA; [21]MedVet Medical Center, Columbus, OH, USA; [22]Gulf Coast Veterinary Specialists, Houston, TX, USA; [23]The Hope Center for Advanced Veterinary Medicine, Vienna, VA, USA; [24]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA; [25]Animal Cancer Specialists, Seattle, WA, USA

Abstract The purpose of this study was to provide an initial assessment of the potential biologic activity of toceranib phosphate (Palladia®, Pfizer Animal Health, Madison, NJ, USA) in select solid tumours in dogs. Cases in which toceranib was used to treat dogs with apocrine gland anal sac adenocarcinoma (AGASACA), metastatic osteosarcoma (OSA), thyroid carcinoma, head and neck carcinoma and nasal carcinoma were included. Clinical benefit (CB) was observed in 63/85 (74%) dogs including 28/32 AGASACA [8 partial response (PR), 20 stable disease (SD)], 11/23 OSAs (1 PR and 10 SD), 12/15 thyroid carcinomas (4 PR and 8 SD), 7/8 head and neck carcinomas [1 complete response (CR), 5 PR and 1 SD] and 5/7 (1 CR and 4 SD) nasal carcinomas. For dogs experiencing CB, the median dose of toceranib was 2.8 mg kg^{-1}, 36/63 (58.7%) were dosed on a Monday/Wednesday/Friday basis and 47/63 (74.6%) were treated 4 months or longer. Although these data provide preliminary evidence that toceranib exhibits CB in dogs with certain solid tumours, future prospective studies are necessary to define its true activity.

Keywords
carcinoma, dog, sarcoma, toceranib, tumour

Correspondence address:
C. London
The Ohio State University
1025 Coffey Road
VMAB 454
Columbus, OH 43214, USA
e-mail: cheryl.london@cvm.osu.edu

Introduction

Toceranib phosphate (Palladia®) is a small molecule inhibitor that blocks a variety of tyrosine kinases expressed on the cell surface, these are known as receptor tyrosine kinases (RTKs).[1–3] It works as a competitive inhibitor of adenosine triphosphate, thereby preventing receptor phosphorylation and subsequent downstream signal transduction. Targets of toceranib include several members of the split-kinase family such as vascular

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endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR), Kit, colony stimulating factor-1 receptor (CSF-1R) and Flt-3.[1–3] On the basis of its structural and functional similarity to sunitinib (Sutent) toceranib likely also inhibits an additional RTK called rearranged during transfection (RET).[4,5] Toceranib was originally developed as an anti-angiogenic agent as inhibition of VEGFR and PDGFR family members limit angiogenesis in a variety of murine tumour models.[6,7] However, inhibition of other members of the split-kinase family such as Kit and possibly RET can result in direct antitumour activity as well.[8–10]

The first evaluation of toceranib in dogs with cancer was a phase I clinical trial exploring its safety and activity.[1] In 57 dogs with a variety of tumours [carcinomas, sarcomas, mast cell tumours (MCTs), melanomas and lymphomas], objective responses occurred in 16 dogs (28%) with stable disease (SD) in an additional 15 dogs for an overall biological activity of 54%. Responding tumours included sarcomas, carcinomas, melanomas, myeloma and MCTs. The highest response rate was in MCTs, with 13/22 dogs with Kit mutations exhibiting responses ($n = 11$) or SD ($n = 2$). A placebo controlled randomized field study of toceranib was then performed in dogs with nonresectable grades II and III MCTs.[2] During the blinded phase, the response rate in toceranib-treated ($n = 86$) dogs was 37.2 [7 complete response (CR) and 25 partial response (PR)] versus 7.9% (5 PR) in placebo-treated ($n = 63$) dogs. Of 58 dogs that received toceranib following placebo-escape, 41.4% (8 CR and 16 PR) experienced an objective response. The overall response rate for all 145 dogs receiving toceranib was 42.8% (21 CR and 41 PR). These two clinical trials demonstrated that toceranib has biologic activity in canine MCTs and possibly has activity in a variety of other tumour types either through inhibition of angiogenesis, direct antitumour effects or a combination of both. Toceranib was approved by the Food and Drug Administration for the treatment of canine MCTs in June 2009. Since that time, dogs with a variety of non-MCT histologies have been treated with toceranib, either alone or as part of a metronomic protocol in which low doses of chemotherapy (usually cyclophosphamide or chlorambucil) often combined with a nonsteroidal anti-inflammatory drug (NSAID) is used to inhibit neo-angiogenesis in tumours. Anecdotal evidence of potential biologic activity of toceranib has been reported in several solid tumours including apocrine gland anal sac adenocarcinomas (AGASACAs) and thyroid carcinomas.

The toceranib label indicates dosing should be initiated at 3.25 mg kg^{-1} [the maximum tolerated dose (MTD)] every other day (EOD) and dose reduced as needed based on clinical side effects.[2] Although 3.25 mg kg^{-1} has been established as the MTD, clinical evidence exists that good biologic activity occurs when doses are initiated below the 3.25 mg kg^{-1} rate and flexible dosing schedules may also be effective. For example, in the phase I study, of 16 dogs treated with toceranib at 2.5 mg kg^{-1} EOD, 6/16 (37.5%) exhibited response to therapy (4 complete and 2 partial) while an additional 5 dogs had SD for greater than 10 weeks.[1] This compares favourably with 20 dogs treated with 3.25 mg kg^{-1} EOD in which 8 (40%) had objective responses (2 complete and 6 partial) and an additional 4 dogs had SD for 10 weeks. On the basis of these data in a small number of patients, it is possible that lower doses of toceranib will be still associated with clinical activity. This is important as at 2.5–2.75 mg kg^{-1} EOD may be better tolerated than the higher dose, resulting in fewer adverse events (AEs), better owner compliance and importantly, fewer drug holidays (shorter periods of drug discontinuation).

The purpose of the following study was to survey the Oncology Listserve to gain additional information regarding tumour histologies that may be responsive to toceranib, to identify dosing regimens currently employed by those using toceranib and to provide preliminary data regarding the impact of dose rate and regimen on biologic activity of toceranib. Such information represents a critical prerequisite for the design and implementation of future prospective clinical investigations to determine the true activity of toceranib for treating additional histologies.

Materials and methods

Case selection

The American College of Veterinary Internal Medicine Oncology Listserve, an e-mail-based

forum for case discussion among Oncology Diplomates, was used to solicit for information regarding cases in which toceranib phosphate was used to treat dogs with several different solid tumours including AGASACA, metastatic osteosarcoma (OSA), thyroid carcinoma, head and neck carcinomas [squamous cell carcinoma (SCC) and ceruminous gland carcinoma] and nasal carcinoma. Cases were eligible for analysis if all of the following information was available: signalment (breed, age and gender), cytological or histological confirmation of tumour, previous treatment, documentation of gross disease before initiation of toceranib, toceranib dose and schedule, at least one documented response assessment (except for nasal tumours), duration of toceranib therapy, duration of response to therapy, concomitant medications (including NSAIDs and other metronomic drugs) and reported AEs.

Response to therapy

Response to therapy was defined as CR (resolution of all target and nontarget lesions, no new lesions), PR (at least 30% decrease in the longest diameter of target lesions, no progression of nontarget lesions and no new lesions), SD (decrease in target lesions of less than 30% or increase of target lesions less than 20%, no progression of nontarget lesions and no new lesions for at least 10 weeks) or progressive disease (PD, greater than 20% increase in target lesions, progression of nontarget lesions and new lesions). Dogs were defined as experiencing clinical benefit (CB) if they had CR, PR or SD.

Data analysis

Given the retrospective nature of this study and the informal manner in which data was collected, it was not subjected to formal statistical analysis. However, descriptive statistics were performed on the following factors: age, gender, weight, toceranib dose, toceranib schedule, toceranib regimen, response to therapy, duration of response, duration of therapy and the use of NSAIDs and cyclophosphamide.

Results

Dogs

There were 85 cases with sufficient information for inclusion into the analysis and patient demographics are provided in Table 1. The median age of all dogs was 10 years and most were either spayed females ($n = 40$) or neutered males ($n = 41$). The most common breeds represented were mixed breed ($n = 19$), Labrador retriever ($n = 16$), Golden retriever ($n = 9$), Greyhound ($n = 4$) and three each of German shepherd, Shetland sheepdog and beagle. There were 32 cases of AGASACA, 23 cases of metastatic OSA, 15 cases

Table 1. Summary of patient demographics, treatment and outcome

	All	CR/PR	SD	CR/PR/SD	PD
Number of dogs	85	20 (24%)	43 (51%)	63 (74%)	22 (26%)
Age	10 (3–18)	10 (7.5–13)	10 (3–18)	10 (3–18)	10 (4–13)
Gender					
FS	41	8	25	33	8
M	4	3	0	3	1
MC	40	9	18	27	13
Dose (mg kg^{-1})	2.8 (2.2–3.25)	2.8 (2.48–3.25)	2.8 (2.2–3.25)	2.8 (2.2–3.25)	2.8 (2.2–3.25)
≥3 mg kg^{-1}	25 (29%)	6 (30%)	11 (26%)	17 (27%)	8 (36%)
<3 mg kg^{-1}	60 (71%)	14 (70%)	32 (74%)	46 (73%)	14 (64%)
MWF schedule	50 (59%)	7 (35%)	30 (70%)	37 (59%)	13 (59%)
Metronomic con meds					
NSAID	17	6	5	11	6
Low-dose CTX	14	3	9	12	2
NSAID/CTX	12	3	7	10	2
Total	43 (51%)	12 (60%)	21 (49%)	33 (52%)	10 (45%)
Duration of TX (weeks)	N/A	22 (4–48+)	25 (10–50+)	23 (4–50+)	N/A

FS, spayed female; MC, castrated male; CTX, cyclophosphamide; TX, treatment

of thyroid carcinoma, 8 cases of head and neck carcinomas and 7 cases of nasal carcinoma included in this retrospective analysis.

Response to therapy

Apocrine gland anal sac adenocarcinoma

Of the 32 dogs with AGASACA treated with toceranib, there were 9 Labrador retrievers and 2 Labrador mixed breed dogs suggesting that this breed may be overrepresented. The median age of all dogs was 11 years (range 7–18 years), 13 were spayed females and 19 were neutered males. Prior therapy (surgery, chemotherapy, radiation therapy or a combination of these) had been given to 25/32 dogs and 7 were hypercalcaemic secondary to disease. Metastasis was present in most dogs ($n = 28$) including sublumbar lymph nodes ($n = 25$), lungs ($n = 9$), liver ($n = 2$) and other sites ($n = 11$). The median toceranib dose used was 2.81 mg kg^{-1} (mean 2.81 mg kg^{-1}, range 2.2–3.25 mg kg^{-1}). Most of the dogs (22/32, 68.8%) were treated on a Monday, Wednesday and Friday (MWF) schedule with the remainder receiving drug EOD ($n = 8$) or twice per week ($n = 2$). Of the 32 dogs treated, 8 (25%) experienced PR and 20 (62.5%) experienced SD for a CB rate of 87.5%. Examples of two dogs with metastatic AGASACA that experienced PR to toceranib therapy are shown in Fig. 1. The median duration for PR was 22 weeks (mean 23.1 weeks, range 10–40 weeks) and the median duration for SD was 30.5 weeks (mean 30.9 weeks, range 10–47 weeks). The median duration of treatment for all 32 dogs treated with toceranib was 25 weeks (range 0–47+ weeks).

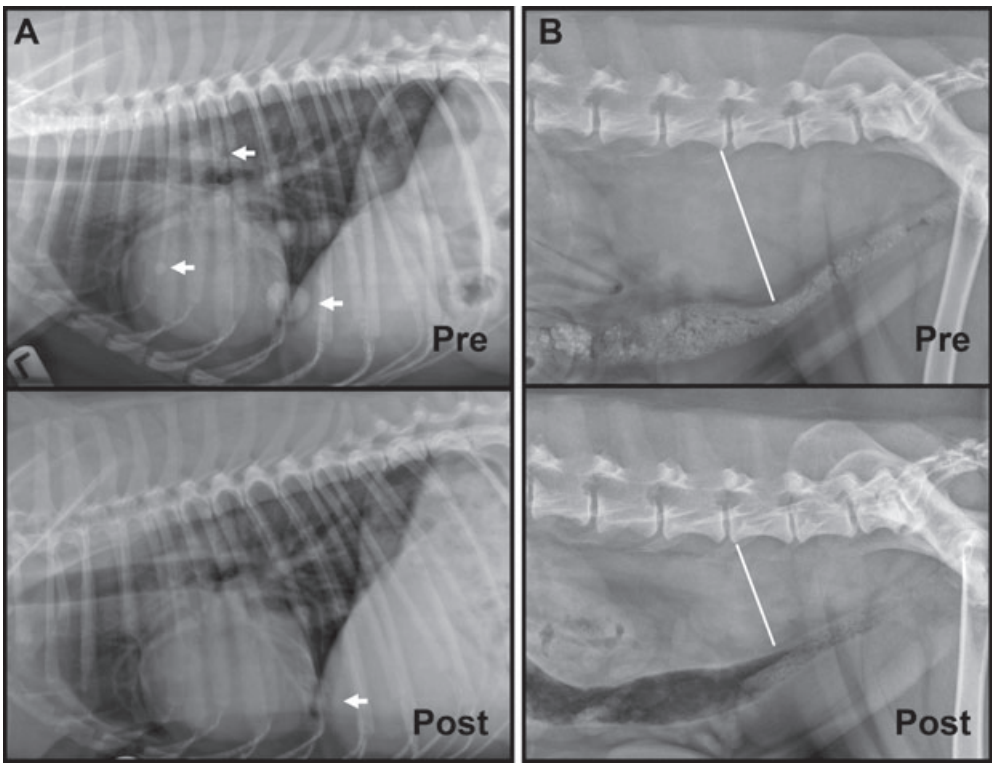

Figure 1. Response of AGASACA to toceranib. (A) An 8-year-old MC mix breed with metastatic AGASACA to the sublumbar lymph nodes and lungs was treated with toceranib after recurrence postsurgery, radiation therapy and chemotherapy. Pictured above are radiographs before the start of Palladia and again at day 46 of therapy. Regression of several pulmonary metastatic lesions is noted. (B) A 9-year-old spayed female Golden retriever with metastatic AGASACA to the iliac lymph nodes, retroperitoneal space and lungs was treated with toceranib after having failed surgery and multiple rounds of chemotherapy. Shown is a radiograph of her caudal abdomen before toceranib administration, and then again 4 weeks later demonstrating regression of her iliac lymph nodes. She also experienced regression of her pulmonary nodules (not shown).

Metastatic osteosarcoma

To be included in this retrospective analysis, dogs must have had radiographic evidence of pulmonary metastatic disease as it was not considered possible to accurately assess primary bone lesions for response to therapy. Of the 23 dogs with OSA treated with toceranib, there were 4 mixed breeds, 4 Golden retrievers, 3 Labrador retrievers, 3 Greyhounds, with the remainder a variety of other primarily large breed dogs. The median age of all dogs was 9 years (range 3–12 years), 13 were spayed females and 10 were neutered males. Nearly, all dogs (21/23) had undergone surgery to remove the primary tumour and received chemotherapy before the development of metastasis; one dog had received no treatment and one dog had received palliative radiation therapy only. One dog had a metastatic lesion in the triceps muscle in addition to pulmonary metastasis. The median toceranib dose used was 2.7 mg kg^{-1} (range 2.3–3.25 mg kg^{-1}). Approximately, half of the dogs (13/23, 56.5%) were treated on a MWF schedule with the remainder receiving drug EOD. Of the 23 dogs treated, 1 (4.3%) experienced PR and 10 (43.5%) experienced SD for a CB rate of 47.8%. Figure 2 shows thoracic radiographs from a dog that experienced stable pulmonary metastasis following toceranib therapy. The median duration of treatment for the 11 dogs that experienced CB was 24 weeks (range 10–42+ weeks).

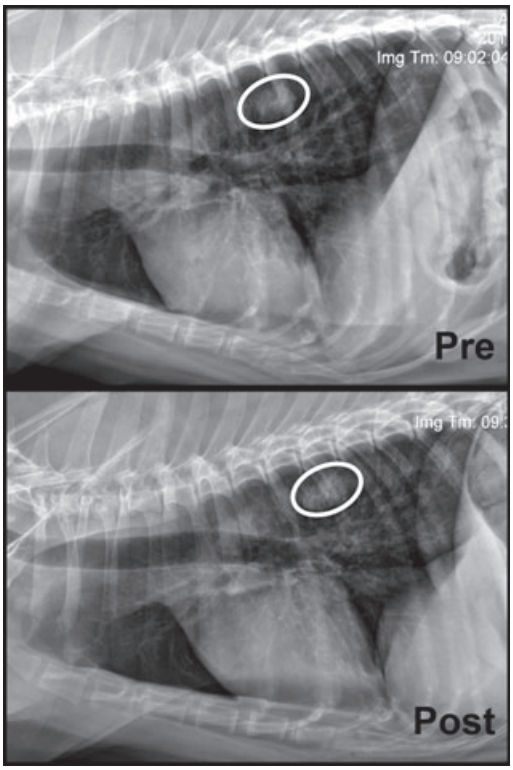

Figure 2. Stable metastatic OSA following toceranib administration. A 9-year-old spayed female mixed breed dog was placed on toceranib therapy after developing pulmonary metastasis 8 months following amputation and chemotherapy for OSA. Shown are thoracic radiographs before and 5 months after beginning toceranib; the metastatic lesion is circled. This dog did eventually go on to develop additional pulmonary lesions consistent with metastatic OSA.

Thyroid carcinoma

Of the 15 dogs with thyroid carcinoma treated with toceranib, there were 4 mixed breeds, 2 Labrador retrievers, 2 beagles, with the remainder a variety of other breeds. The median age of all dogs was 10 years (range 7.5–13 years), 9 were spayed females, 2 were intact males and 4 were neutered males. Prior therapy had been given to 10/15 dogs including surgery ($n = 4$), chemotherapy ($n = 7$) and radiation therapy ($n = 3$). None of the dogs had clinical evidence of hyperthyroidism and of T4 levels of the 6 dogs for which this was performed were normal. The primary tumour was present in 13 dogs, 9 dogs had metastatic disease to the lungs and 1 dog had metastatic disease to the liver and spleen. The median toceranib dose used was 2.75 mg kg^{-1} (range 2.47–3.25 mg kg^{-1}). More than half of the dogs (9/15, 60%) were treated on a MWF schedule with the remainder receiving drug EOD ($n = 5$) or every third day ($n = 1$). Of the 15 dogs treated, 4 (26.7%) experienced PR and 8 (53.3%) experienced SD for a CB rate of 80%. Figure 3 shows two dogs with pulmonary metastasis that experienced PR or SD following toceranib therapy. The median duration of treatment for the 12 dogs that experienced CB was 24.5 weeks (range 11–50+ weeks).

Head and neck carcinoma

There were 8 dogs with head and neck carcinomas including 3 SCCs of the nasal planum, 4 SCCs of the oral cavity (mandible, maxilla and lingual) and

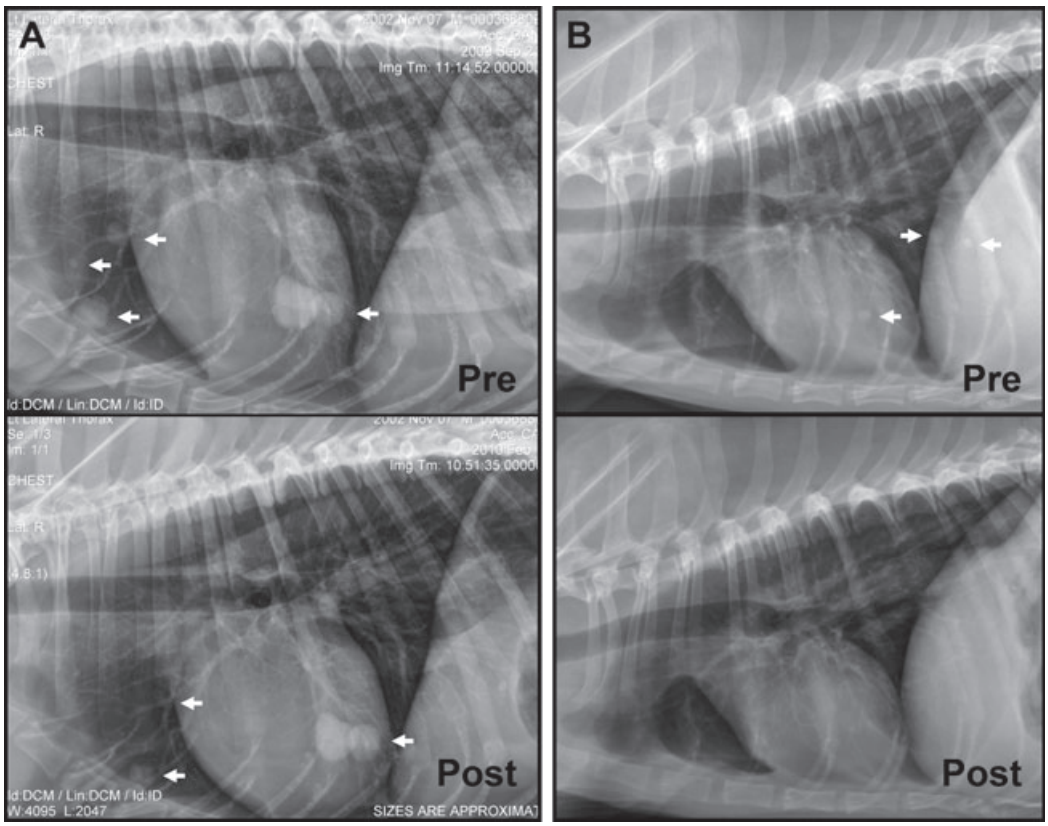

Figure 3. Response of metastatic thyroid carcinoma to toceranib. (A) A 15-year-old intact male Shih Tzu presented with a primary thyroid carcinoma and pulmonary metastasis. Toceranib therapy was initiated resulting in partial regression of the primary tumour (not shown) and complete regression of the pulmonary metastases. Shown are thoracic radiographs before and 4 weeks after beginning toceranib. (B) A 9-year-old male neutered Labrador retriever presented with metastatic thyroid carcinoma in May 2009. He underwent surgical excision of the primary mass and five cycles of chemotherapy with a doxorubicin/cyclophosphamide/5-fluoruracil regimen. He experienced progression of his pulmonary metastases and was placed on toceranib. Shown are his thoracic radiographs before initiating toceranib in September 2009 and 5 months later in February 2010.

1 dog with a ceruminous gland adenocarcinoma. There were 3 mixed breeds, 2 Labrador retrievers, 2 Golden retrievers and 1 Boxer. The median age was 11.5 years (range 10–13 years), and there were 3 spayed females, 2 intact males and 3 neutered males. The majority ($n = 5$) had received no prior treatment, while 2 had undergone surgery and 1 had completed radiation therapy. Metastasis to the mandibular nodes was present in 2 dogs and to the lungs in 1 dog. The median toceranib dose used was 2.87 mg kg^{-1} (range 2.5–3.2 mg kg^{-1}). Half of the dogs were treated on a MWF schedule and half received drug EOD ($n = 5$). Of the 8 dogs treated, 1 (12.5%) experienced CR, 5 experience PR (62.5%), 1 experienced SD although this was not of sufficient duration to meet criteria for inclusion and 1 experienced PD for a CB of 75%. Figure 4 shows a dog with an oral SCC that responded to toceranib therapy. The median duration of treatment for the 6 dogs that experienced CB was 19.5 weeks (range 4–48+ weeks).

Nasal carcinoma

There were 7 dogs with nasal carcinoma eligible for inclusion in the analysis. The median age was 10 years (range 8–13 years), and there were 3 spayed females and 4 neutered males. The majority ($n = 4$) had received prior radiation therapy, and metastasis to the lymph nodes was present in 2 dogs and to the lungs in 1 dog. The median toceranib dose used was 2.67 mg kg^{-1} (range 2.4–3.2 mg kg^{-1}). Six of the

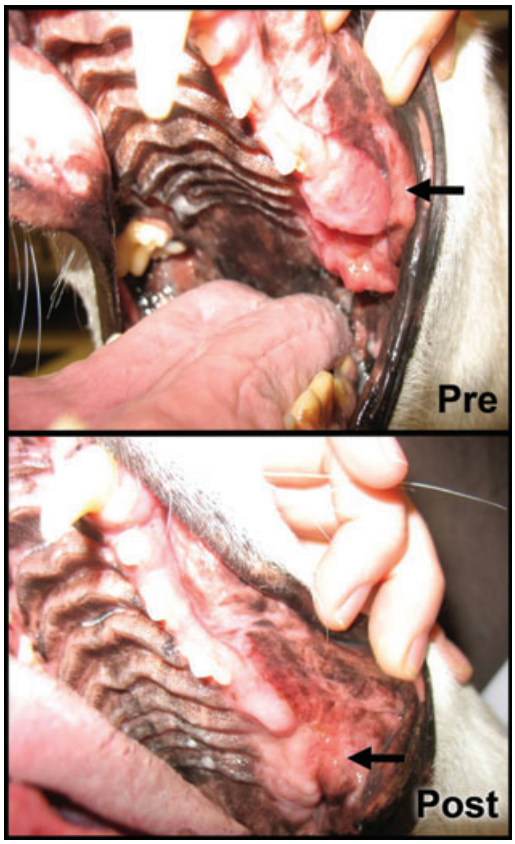

Figure 4. Regression of oral SCC after toceranib therapy. A 13-year-old spayed female Labrador retriever mix presented with a large oral SCC involving the caudal left maxilla. The owner elected to pursue toceranib therapy alone. Shown are pictures of the mass before treatment and again after near CR to therapy.

dogs were treated on a MWF schedule and only one received drug EOD. Responses in the nasal tumours are difficult to assess without serial computerized tomography (CT) and only 2/7 dogs had imaging performed following initiation of toceranib. Therefore, for those dogs with no repeat CT, evidence of biologic activity was considered positive if clinical signs (epistaxis and nasal obstruction) improved and these dogs were considered to have experienced CB. Of the two dogs that were re-imaged, 1 had a CR and 1 had SD and an additional 3 dogs experienced CB for an overall CB rate of 71.4%. Figure 5 shows serial CT scans from a dog with nasal carcinoma that experienced a CR to toceranib therapy. The median duration of treatment for the 5 dogs that experienced CB was 18 weeks (range 12–20+ weeks).

Toceranib dose and regimen

Data regarding toceranib dose and regiment for all 85 dogs are summarized in Table 1. CB secondary to toceranib therapy was noted in 63 (74%) cases (2CR, 18 PR and 43 SD) and the median dose for all 85 dogs was 2.8 mg kg^{-1} (range 2.2–3.25 mg kg^{-1}). Within the 63 dogs that experienced CB, 37 (59%) were dosed on a MWF basis, 46 (73%) received less than 3 mg kg^{-1} and 47 (75%) were on therapy for 4 months or longer. The median dose was 2.8 mg kg^{-1} for dogs that experienced SD and the median dose was 2.8 mg kg^{-1} for dogs with PR/CR. These data did not differ from the 20 dogs that experienced PD: the median dose of toceranib was 2.8 mg kg^{-1}, 13 (59%) were dosed on a MWF basis and 14 (64%) received less than 3 mg kg^{-1}.

Influence of metronomic treatments

Metronomic therapy is now being used in canine cancer therapy as part of a variety of therapeutic regimens. As previously mentioned, this generally involves the use of an NSAID and a chemotherapeutic given at a low dose continuously. The chemotherapeutic agent typically used is cyclophosphamide at 10–12 mg m^{-1}.[2] In this study, approximately half ($n = 43$, 51%) of the dogs received either an NSAID alone ($n = 17$), cyclophosphamide alone ($n = 14$) or both ($n = 12$). Within the dogs that experienced CB, 33 (52%) received an NSAID ($n = 11$), cyclophosphamide ($n = 12$) or both ($n = 10$). Although formal statistical analysis was not performed, this did not differ substantially from dogs that experienced PD; 10 (45%) received an NSAID ($n = 6$), cyclophosphamide ($n = 2$) or both ($n = 2$).

Clinical toxicities

Clinical toxicities associated with toceranib administration are primarily gastrointestinal in nature but also include muscle pain/weakness and neutropenia. While side effects occurred in 66 (77.6%) of dogs in this retrospective analysis, it was sometimes difficult to directly attribute these toxicities to toceranib administration given the advanced disease state of many dogs. Nevertheless, in order of their frequency the reported toxicities were

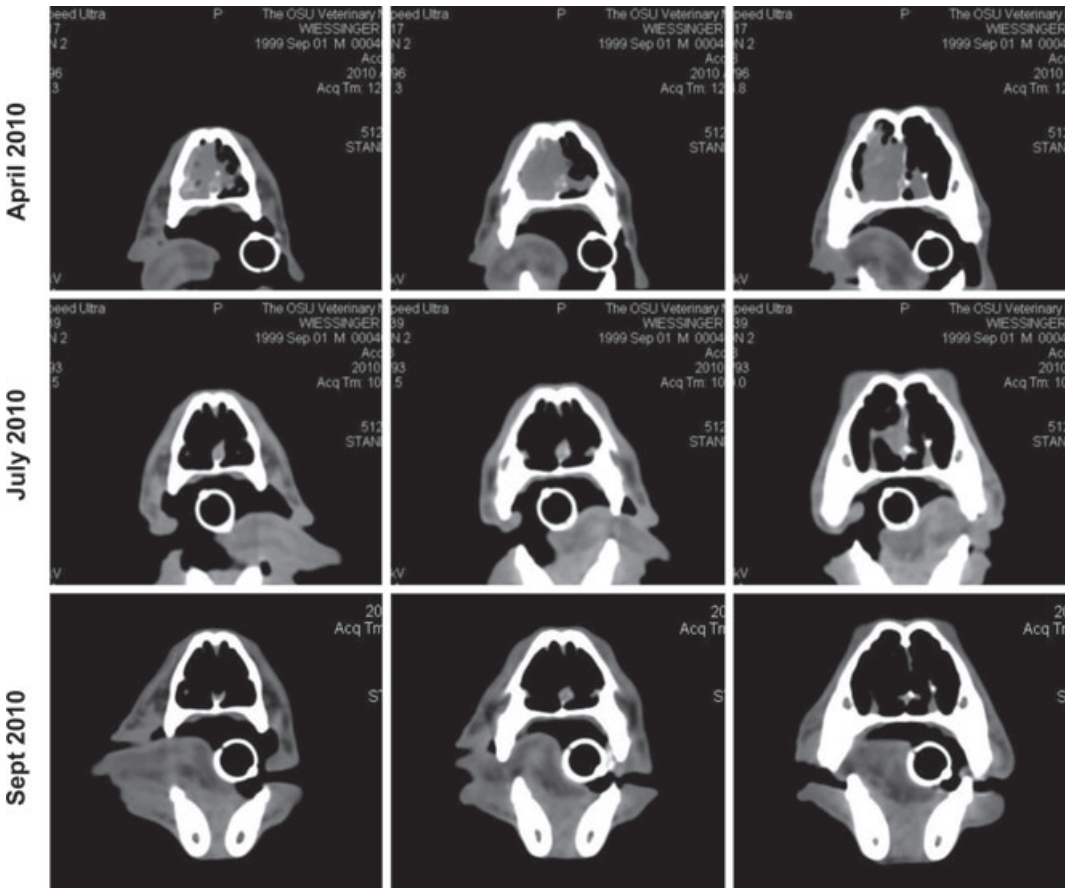

Figure 5. Serial CT demonstrating response of nasal carcinoma to toceranib. An 11-year-old male neutered Shetland sheepdog was treated with toceranib following recurrence of a nasal carcinoma after initially responding to radiation therapy in January 2010. Shown are serial matched images of the nasal cavity 3 (July 2010) and 5 (September 2010) months after beginning toceranib therapy in April 2010.

as follows: diarrhoea ($n = 44$, 51.8%), anorexia ($n = 30$, 35.3%), vomiting ($n = 16$, 18.8%), musculoskeletal pain and/or weakness ($n = 16$, 18.8%), weight loss ($n = 15$, 17.6%), positive hemoccult ($n = 13$, 15.3%), lethargy ($n = 11$, 12.9%), neutropenia ($n = 9$, 10.6%) and skin disorder (depigmentation and pyoderma, $n = 6$, 7.1%).

Discussion

Toceranib phosphate (Palladia®) is an orally bioavailable multitargeted RTK inhibitor active against several members of the split-kinase family including VEGFR, PDGFR and Kit.[1,2] Toceranib is closely related to the small molecule inhibitor sunitinib (Sutent) that is active against VEGFRs 1, 2 and 3, PGFDRα and β, Flt-3, Kit, CSF-1R and RET.[4,9] Sunitinib and toceranib were developed as anti-angiogenic agents based on their inhibition of VEGFR and PDGFR, two receptor families known to play an important role in tumour-driven angiogenesis. Given that toceranib was developed as an anti-angiogenic agent, biologic activity in a broad range of tumours without obvious driver mutations might be expected. In support of this, sunitinib has demonstrated single agent activity in gastrointestinal stromal tumours, renal cell carcinoma, thyroid carcinoma, insulinoma, angiosarcoma and hepatocellular carcinoma.[5,8,9] Furthermore, in the phase I study of toceranib, PRs and SD were noted in a variety of cancers including metastatic carcinoma, transitional cell carcinoma, multiple myeloma, melanoma and metastatic sarcoma.[2] These data suggest that toceranib may

have antitumour activity beyond primarily Kit driven MCTs. The purpose of this retrospective analysis was to provide preliminary information regarding the potential biologic activity of toceranib in several solid canine tumours and as a secondary endpoint to survey the toceranib dose and dosing regimen used in the treatment of dogs with these tumours.

Biological activity in the 85 dogs eligible for inclusion in this study was reported as 74%, with the majority of dogs experiencing SD ($n = 43$, 68.3%). On the basis of the mechanism of action of toceranib and the clinical activity of other anti-angiogenic therapies in the human arena, it is not surprising that most dogs experienced SD or slowed disease progression rather than disease regression. Interestingly, the median duration of therapy for dogs with SD was 24.5 weeks (range 10–50+ weeks) indicating that many dogs benefited from drug for approximately 6 months or longer.

The overall objective response rate to toceranib was 31.7% ($n = 20$) and most of these occurred in dogs with AGASACA (8 PR), thyroid carcinoma (4 PR) and head and neck carcinomas (1 CR and 5 PR). These objective responses suggest that specific RTKs targeted by toceranib may be dysregulated in these tumour types. In human thyroid carcinomas, objective responses to several small molecule inhibitors have been documented.[11–13] It is now evident that human papillary and follicular thyroid carcinomas possess a number of somatic mutations including those involving Ras, the cytoplasmic kinase BRAF and the RTK RET.[12] In clinical trials of the multitargeted (VEGFR, PDGFR and BRAF) small molecule inhibitor sorafenib, objective response rates ranged from 10 to 25% and approximately 50% of patients experienced SD for 6 months or longer.[14–16] Recently, sunitinib has demonstrated single agent activity against human differentiated thyroid carcinomas; phase II clinical trials of this drug are currently ongoing.[11,17] In contrast to the human disease, the molecular biology of canine thyroid carcinomas has not been investigated and as such, the basis for response to toceranib is not known.

In human oncology, anti-angiogenic agents such as bevacizumab have shown promise for the treatment of head and neck carcinomas, particularly when used in combination with conventional chemotherapy and radiation therapy.[18] These tumours are known to produce VEGF and express VEGFRs indicating that an autocrine loop of stimulation may exist.[19,20] However, both sorafenib and sunitinib have demonstrated little to no single agent activity suggesting that the tumour cells are not dependent on VEGF/VEGFR signalling for survival.[18,21,22] The molecular biology of canine head and neck carcinomas has not been evaluated so the underlying mechanisms responsible for the observed objective responses to toceranib are not known. It is interesting, however, that the biologic activity of toceranib in canine head and neck carcinoma appears to be much higher than that of sunitinib in the human disease. Tobacco exposure (smoking and chewing) is an established risk factor for the development of human head and neck carcinomas and not an obvious cause of the canine disease so it is possible that the molecular drivers responsible for the canine tumours are unique. As AGASACA is not a tumour that occurs in humans, no parallels can be drawn. Although the molecular mechanisms that underlie AGASACA in dogs are not known, research in the laboratory of the corresponding author (C. L.) indicates that both RET and VEGFR2 are expressed in these tumours.

One of the goals of this study was to survey the toceranib dose and dosing regimen used to treat dogs with solid tumours. Interestingly, the median dose used in dogs that experienced PD was 2.7 mg kg^{-1} compared with 2.8 mg kg^{-1} for dogs that experienced CB suggesting that doses below the MTD of 3.25 mg kg^{-1} are not necessarily associated with a lower biologic activity. These findings are also consistent with data from the phase I study in which no difference in biologic activity was noted between 2.5 and 3.25 mg kg^{-1} dosing cohorts.[1] A clinical trial is currently underway to evaluate the pharmacokinetics of lower doses of toceranib (2.5 and 2.75 mg kg^{-1}) in dogs with cancer and compare this with the known drug exposure obtained with the label dose of 3.25 mg kg^{-1}.

In addition to a lower dose, over half of the dogs were treated on a MWF basis as opposed to the label recommendation of EOD. There was no difference in the proportion of dogs treated

MWF that experienced PD (59%) versus CB (59%). It is interesting to note that while the median dose of toceranib used in dogs that experienced CR/PR was 2.8 mg kg^{-1}, only 35% received drug MWF suggesting that more frequent dosing may be associated with an increase in objective response to therapy. Future studies would be necessary to evaluate the true impact of alternative dosing regimens on toceranib's activity.

The clinical toxicities observed in this study were similar to those previously reported including primarily diarrhoea, anorexia, vomiting and musculoskeletal pain/weakness. Interestingly, although the incidence of diarrhoea was comparable with that found in the field study of toceranib in MCTs, the rates of anorexia, vomiting, lethargy and neutropenia were substantially lower.[2] There are several possible explanations for this observation, including a lack of standardized reporting for AEs resulting in underreporting, the use of altered dose/dosing regiment that may be associated with better tolerability, and the absence of malignant mast cell disease that could have been a contributing factor to at least some of the gastrointestinal toxicity observed in the field study.

Recently clinical trials in human oncology have sought to combine inhibitors of the VEGF/VEGFR axis with low-dose chemotherapy to more effectively target tumour angiogenesis. The majority of these studies have combined bevacizumab with low-dose cyclophosphamide, and other chemotherapeutics including capecitabine, 5-fluoruracil and methotrexate.[23] While not typical, a COX-2 inhibitor is sometimes included in the protocol. The success of such regimens in human cancer patients is highly variable. In advanced drug-resistant breast cancer, the combination of bevacizumab with low-dose cyclophosphamide and capecitabine or cyclophosphamide and methotrexate resulted in objective response rates of 32–48% with SD in an additional 32–41% of patients.[24,25] In contrast, the use of combined celecoxib and cyclophosphamide in metastatic renal cell carcinoma and refractory paediatric tumours of various types resulted in a CB rate of only 12.5 and 6%, respectively.[23,26,27] In this study, while a significant number of dogs received an NSAID, low-dose cyclophosphamide or both, there was wide variation in the type of NSAID used, dosing protocol for cyclophosphamide and duration of use, precluding any firm conclusions. However, there was no obvious evidence that the administration of an NSAID, low dose continuous cyclophosphamide or both substantially influenced the objective or biologic response to toceranib, as their was similar among dogs experiencing CR/PR (60%), CB (52%) and dogs experiencing PD (45%).

There are several limitations inherent in the approach used to gather information in this retrospective study including no standard procedures for confirming a histopathologic or cytologic diagnosis, for staging/restaging, for recheck exams/bloodwork schedule and for documentation of responses. In addition, there were multiple clinicians involved at multiple sites and therefore no standard procedures for assessment of clinical toxicities and dose modifications. Lastly, there was no limit to the number and type of concomitant medications used or the type/duration of metronomic therapy used. As such, the data generated from this analysis would be classified as no higher than type IV clinical evidence based on the concept of evidence-based medicine.[28] Therefore, this study is most valuable in generating rather than testing clinical hypotheses, serving as a platform for the design of future prospective clinical trials to evaluate the true biologic activity of toceranib in canine solid tumours.

Conclusions

The data reported in this retrospective analysis suggests that toceranib may have biologic activity in a variety of solid tumours in dogs. In addition, these data indicate that the administration of toceranib at doses lower than the MTD may still be associated with CB and may therefore be conducive to long-term drug administration. Future prospective studies are necessary to determine the true utility of toceranib in the treatment of canine solid tumours as well as to identify the role of additional metronomic therapeutics in toceranib-based protocols.

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