The Journal of Veterinary Medical Science

NOTE

Internal Medicine

Effects of toceranib phosphate (Palladia) monotherapy on multidrug resistant lymphoma in dogs

Hiroki YAMAZAKI[1,2)]*, Naoki MIURA[1)], Yu-Chang LAI[1)], Masashi TAKAHASHI[3)], Yuko GOTO-KOSHINO[4)], Momoi YASUYUKI[5)], Munekazu NAKAICHI[6)], Hajime TSUJIMOTO[4)], Asuka SETOGUCHI[7)] and Yasuyuki ENDO[3)]

[1)]Veterinary Teaching Hospital, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
[2)]Veterinary Medical Center, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano, Osaka 598-8531, Japan
[3)]Laboratory of Veterinary Internal Medicine, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
[4)]Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[5)]Laboratory of Veterinary Diagnostic Imaging, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
[6)]Laboratory of Veterinary Radiology, Department of Veterinary Medicine, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan
[7)]Bayside Animal Clinic, 22-9 Sakaecho, Yokohama-shi, Kanagawa 221-0052, Japan

J. Vet. Med. Sci.
79(7): 1225–1229, 2017
doi: 10.1292/jvms.16-0457

Received: 11 September 2016
Accepted: 18 May 2017
Published online in J-STAGE: 6 June 2017

ABSTRACT. We examined whether multidrug resistant (MDR) canine lymphoma increases gene expression for platelet-derived growth factor receptor α (*PDGFRα*), vascular endothelial growth factor receptor 2 (*VEGFR2*), and *c-KIT*, and whether toceranib phosphate (TOC) has potential as a treatment for MDR canine lymphoma. The clinical data showed that *PDGFRα* expression was higher in canine subjects with MDR T-cell lymphoma than in those with untreated T-cell lymphoma, and that *c-KIT* expression was greater in subjects with T-cell lymphoma than in those with B-cell lymphoma. TOC monotherapy was well tolerated without serious adverse effects, and two of the five subjects that received TOC exhibited partial responses. The data presented here could contribute to the assessment of TOC-based therapy for dogs with MDR or T-cell lymphoma.

KEY WORDS: dog, lymphoma, multidrug resistance, toceranib phosphate

Although multidrug therapy based on doxorubicin (DOX) is highly effective against canine lymphoma, it leads to multidrug resistant (MDR) lymphoma and, ultimately, to treatment failure [17]. It has been reported that this resistance is facilitated by the activation of P-glycoprotein (P-gp), other drug transporters, or a combination of both [18]. Angiogenesis is required for the survival of hematologic cancers [20] and the expression patterns of angiogenic growth factors, including platelet-derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR), correlate with clinical stage and historical grade in canine lymphoma [2, 15]. Additionally, overexpression of PDGFR and VEGFR promotes resistance to several anti-cancer drugs [1, 14]. Therefore, determining the relationship between multidrug resistance and the expression of PDGFR and VEGFR in canine lymphoma may provide medically valuable information for its treatment.

Toceranib phosphate (TOC) is a tyrosine kinase inhibitor (TKI) that targets PDGFR, VEGFR and KIT. It is approved for the treatment of canine mast cell tumors, and some groups have reported its efficacy in dogs with several types of carcinomas [3, 7, 13]. Other groups have demonstrated that TOC has high bioavailability and is usually well tolerated [16]. Masitinib, another TKI that targets PDGFR, VEGFR and KIT, also inhibits the growth of canine lymphoma cells *in vitro* [6, 19]. Moreover, the same groups have reported that KIT is expressed at high levels in dogs with high-grade T-cell lymphomas, and that masitinib shows antitumor effects in these dogs [5, 6]. However, whether TOC can be used to treat canine lymphoma is unknown. Evaluating its effects may ultimately improve the treatment of MDR canine lymphoma.

In this study, our aim was to determine the effect of chemoresistance on the expression patterns of *PDGFRα*, *VEGFR2* and

*Correspondence to: Yamazaki, H., Veterinary Medical Center, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano, Osaka 598-8531, Japan. e-mail: hyamazaki@vet.osakafu-u.ac.jp

Table 1. Summary of clinical information for the 16 canine subjects with lymphoma

No.	Age (year)	Sex [a]	Breed [b]	Location	Type [c]	Stage [d]	Protocol [e]	Response [f]	PFI [g] (days)	Sampling [h]
1	10	M	MD	Multicentric	B	2a	CHOP	CR	336	Twice
2	12	F	BG	Multicentric	B	3a	CHOP	CR	252	Twice
3	9	M	WC	Multicentric	T	3a	LCHOP	PR	105	Twice
4	14	M	MD	Multicentric	B	4b	LCHOP	CR	189	Once
5	7	F	MS	GI tract	T	/	LCHOP	SD	42	Once
6	10	M	GS	Multicentric	B	3a	LCHOP	CR	168	Twice
7	8	F	FB	Multicentric	B	4b	CHOP	CR	420	Twice
8	13	F	MD	Cutaneous	T	/	LCHOP	PR	126	Twice
9	9	F	LR	Multicentric	B	3b	LCNOP	CR	210	Twice
10	14	F	SS	Multicentric	B	4b	LCNOP	PR	147	Once
11	9	M	WC	Multicentric	B	3a	CHOP	CR	294	Twice
12	10	F	Pug	Multicentric	B	3b	LCHOP	CR	378	Twice
13	8	M	MD	Mediastinal	T	/	LCHOP	PD	/	Once
14	13	F	MS	Multicentric	B	2a	CHOP	CR	315	Twice
15	12	F	BH	Multicentric	B	3a	LCHOP	CR	336	Twice
16	10	M	Mix	GI tract	T	/	LCHOP	SD	63	Twice

a) F: Female, M: Male. b) BG: Beagle, BH: Basset Hound, FB: French Bulldog, GS: German Shepherd Dog, LR: Labrador Retriever, MD: Miniature Dachshund, MS: Miniature Schnauzer, SS: Shetland Sheepdog, WC: Welsh Corgi. c) B: B-cell Lymphoma, T: T-cell Lymphoma. d) Clinical staging of multicentric lymphoma at first visit (extranodal lymphoma was not shown). e) L: L-asparaginase, C: Cyclophosphamide, H: Doxorubicin, N: Mitoxantrone, O: Vincristine, P: Prednisone. f) CR: Complete Response, PR: Partial Response, SD: Stable Disease, PD: Progressive Disease. g) PFI: Progression-free interval. h) Once: Sample was collected before treatment, Twice: Samples were collected before and after treatment.

c-KIT in canine lymphoma, and how TOC affects dogs with MDR lymphoma. We hypothesized that MDR lymphoma would cause high levels of expression of *PDGFRα*, *VEGFR2* and *c-KIT*, and that TOC had potential as an effective canine anticancer therapy.

Canine lymphoma cell lines (CL-1 and GL-1) [8, 10] and DOX-resistant lymphoma cells were used in this study. The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Grand Island, NY, U.S.A.), supplemented with 10% heat-inactivated fetal bovine serum (Cosmo Bio, Tokyo, Japan) and 1% L-glutamine (BioWhittaker, Walkersville, MD, U.S.A.) with 5% CO_2 at 37°C. DOX-resistant cells were generated from the CL-1 and GL-1 lines in accordance with a previously reported procedure [18], the details of which are given in the Supplementary Methods, including Tables and Figures.

Samples were collected from 16 dogs with lymphoma during their first visits to a veterinary teaching hospital at Kagoshima University between 2012 and 2016. All subjects were treated with a standard multidrug protocol (L-asparaginase, cyclophosphamide, DOX, vincristine, and prednisone) as a first-line therapy. In 12 out of 16 subjects, samples were collected for a second time, from a relapsed tumor after the first-line therapy failed. The following were excluded from sampling: (1) subjects affected by multiple distinct malignant neoplasms or nonmalignant diseases; and (2) subjects that had undergone surgery or radiation therapy. All subjects were diagnosed with high-grade lymphoma, in accordance with the updated Kiel classification or with the World Health Organization system, on the basis of cytological or histopathological evaluation. Moreover, types of lymphoma (B-cell or T-cell origin) were classified by using polymerase chain reaction to analyze antigen receptor gene rearrangements [4], by immunostaining with antibodies against CD3 and CD79a at a commercial laboratory, or using a combination of these methods. Table 1 summarizes the clinical information collected from the 16 subjects. Popliteal lymph node tissues were sampled from a healthy 1-year-old, intact female beagle by needle biopsy, for use as control samples. All samples were used for analysis of target gene expression, as described in the next subsection.

The expression of the target genes, ATP binding cassette B1 (*ABCB1*), ATP binding cassette G2 (*ABCG2*), *PDGFRα*, *VEGFR2* and *c-KIT*, in lymphoma cells and tissue samples, were evaluated using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) after isolation of total RNA. Details are included in the Supplementary Methods.

Client consent for the TOC-based clinical trial was obtained from client owners of five out of the 16 subjects with lymphoma as described previously. These five client-owned dogs had been treated in accordance with the University of Wisconsin Madison Protocol (UW-25) as a first-line therapy. The subjects were enrolled in the clinical trial after failure of the UW-25. Before the clinical trial, each subject was restaged and underwent a complete physical examination during which all clinical signs were documented. Staging included an abdominal ultrasound, three-view thoracic radiographs or computed tomography, complete blood count, analysis of biochemical parameters, and a urinalysis including urine protein creatinine ratio (UP/C). Clinical research was performed according to the Institutional Guidelines for Animal Experiments and in compliance with the Ethics Committee of Animal Care and Experimentation at Kagoshima University.

TOC (2.4–2.8 mg/kg) (Palladia, Zoetis, Florham Park, NJ, U.S.A.) was administered orally to the five subjects, three days per week (Monday, Wednesday and Friday). While subjects were undergoing therapy, adverse events were recorded and graded every two weeks in accordance with the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events [12]. Treatment was discontinued for subjects with a UP/C >2, creatinine or blood urea nitrogen (BUN) 1.5 times higher than the upper limit of the reference range, or albumin 0.75 times lower than the lower limit of the reference range. If subjects

developed signs of a protein-losing syndrome (UP/C >2, albumin <0.75 times the lower limit of the reference range), therapy was interrupted until values had returned within acceptable limits. If this was observed for a second time, treatment was permanently discontinued. If hemolytic anemia (hemoglobin (Hb) <10 g/μl and bilirubin >1.5 times the upper limit of normal) or anemia without regeneration (Hb <10 g/μl and reticulocytes <80,000/μl) occurred, treatment was discontinued. In cases of hepatic toxicity (alanine aminotransferase, aspartate aminotransferase, or total bile acid >3 times the upper limit of normal) or neutropenia (<2,000 cells/μl), treatment was interrupted until values normalized, then continued at the same dosage used previously. If these events occurred for a second or third time, treatment was interrupted until they were resolved, then resumed at a lower dose of 2.0–2.4 mg/kg. If severe adverse reactions occurred even at this low dose, treatment was permanently discontinued. If one of the previously mentioned adverse reactions, severe diarrhea or severe vomiting, persisted after dose reduction, treatment was permanently discontinued.

Clinical response to TOC was assessed every three weeks in accordance with Response Evaluation Criteria in Solid Tumors (RECIST) [11]: complete response (CR) (disappearance of all clinical signs of disease); partial response (PR) (>50% or <100% decrease); stable disease (SD) (≤50% decrease or <20% increase); and progressive disease (PD) (≥20% increase and/or development of new lesions or metastases). The progression-free interval (PFI) included CR, PR and SD through to at least day 21. Survival time (ST) was calculated from the date of the first treatment through to the date of death. The data for subjects without follow up data prior to death or that died of another cause, were excluded from the ST data.

Statistical analyses were performed using standard software (IBM SPSS Statistics, SPSS Inc., Chicago, IL, U.S.A.). Data were analyzed using Dunnett's test or one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test. Quantitative values of the data from three separate experiments were expressed as mean ± standard deviation (SD), and P-values less than 0.05 were considered significant.

Ninety and 180 days after culture in medium that included low doses of DOX, CL-1 and GL-1 cells demonstrated time-dependent decreases in sensitivity to DOX that were significantly greater than the decreases seen in control cells (Fig. S1). The IC_{50} values of CL-1 and GL-1 control cells were 5.8 and 10.4 nM respectively, and IC_{50} values of CL-1 and GL-1 cells 180 days after culture were 98.2 and 128.2 nM, respectively. At 180 days after culture, expression of P-gp was upregulated in the GL-1 and CL-1 cells, and the relative intensities of the immunoreactive bands in GL-1 cells were significantly greater than those of the controls (Fig. S2). The relative expression of *ABCB1* and *ABCG2* mRNA in the CL-1 and GL-1 cells increased time-dependently while the cells were being cultured, and expression levels were significantly higher in these cells than in the controls 180 days after culture (Fig. S3). The data demonstrates that the cells developed DOX-resistance because of increases in activity of drug transporters. Moreover, the relative expression of *PDGFRα*, *VEGFR2*, and *c-KIT* also increased time-dependently and simultaneously during culture. At 180 days post-culture, expression levels of these genes were significantly different to levels in the control cells. The data suggests that expression of *PDGFRα*, *VEGFR2*, and *c-KIT* increased after the cells developed DOX resistance.

After the failure of standard multidrug therapy, the relative expression levels of *ABCB1* and *ABCG2* were significantly higher in MDR lymphomas than in untreated lymphomas (Fig. 1): this demonstrated that chemoresistance developed due to an increase in drug transporter expression. Moreover, the relative expression of *PDGFRα* was significantly higher in MDR T-cell lymphomas than in untreated T-cell lymphomas. Expression of *c-KIT* did not differ significantly between MDR and untreated lymphoma, although was significantly higher in T-cell lymphomas than in B-cell lymphomas (Fig. 1). These results suggest that the pathogenic mechanism of chemoresistance correlates with the expression of *PDGFRα* in T-cell lymphoma, and that TOC might show antitumor effects on MDR T-cell lymphoma in dogs with elevated *PDGFRα* and *c-KIT* expression.

Five subjects received TOC for MDR lymphoma (Table 2). The tumor responses in two of these subjects (Numbers 8 and 16) were classified as PRs for 105 and 84 days, respectively. The response in one subject (Number 2) was classified as a SD for 42 days, but the responses in the other subjects (Numbers 6 and 12) were classified as PDs. The two subjects with tumor responses classified as PRs had diarrhea and neutropenia one month after the administration of TOC; however, these adverse events were mild and limited, and they did not result in discontinuation. Of the five subjects that received TOC, expression levels of the targeted genes were highest in the group with responses classified as PRs: expression levels of *PDGFRα* and *c-KIT* were highest in Number 8, and the expression level of *VEGFR2* was the highest in Number 16 (Fig. 1). After TOC monotherapy failed, all subjects were retired from the clinical trial. For four of the five subjects, the owners decided to continue administering the medication beyond the end of the clinical trial. Three (Numbers 2, 6 and 8) of the four subjects were treated with lomustine (CCNU, 50–70 mg/m^2, orally, every three weeks), and another subject (Number 16) received nimustine (ACNU, 30–35 mg/m^2, intravenously, every three weeks) as a rescue therapy. Number 12 received supportive care without additional chemotherapy after the clinical trial.

In these studies, we demonstrated that expression of *PDGFRα* was significantly higher in subjects with MDR T-cell lymphomas than in those with untreated T-cell lymphomas, and *c-KIT* expression was greater in subjects with T-cell lymphoma than in those with B-cell lymphoma. Overexpression of PDGF and PDGFR is a major player in oncogenesis and drug resistance [14]. Moreover, one study suggested that *c-KIT* might be expressed on canine lymphomas, depending on their origin or histological grade, and is related to clinical outcomes [5]. Therefore, we suggested that there was a mismatch in the *c-KIT* expression patterns between *in vitro* and clinical samples due to the difference in origin, histological grade or clinical stage. On the basis of these findings, we believe that multidrug resistance and its development in T-cell lymphoma may correlate with the expression of PDGFR, resulting in poor clinical outcomes. However, elevating the expression of targeted genes is also a potential therapeutic strategy for overcoming multidrug resistance. One study reported that masitinib reverses DOX resistance in canine lymphoma cells by inhibiting P-gp activity [19]. Therefore, TOC may have the potential to reverse drug resistance in refractory or T-cell lymphoma in dogs.

The clinical data showed that the overall response rate (ORR) to TOC was 40% (2/5) in subjects with MDR lymphoma, and

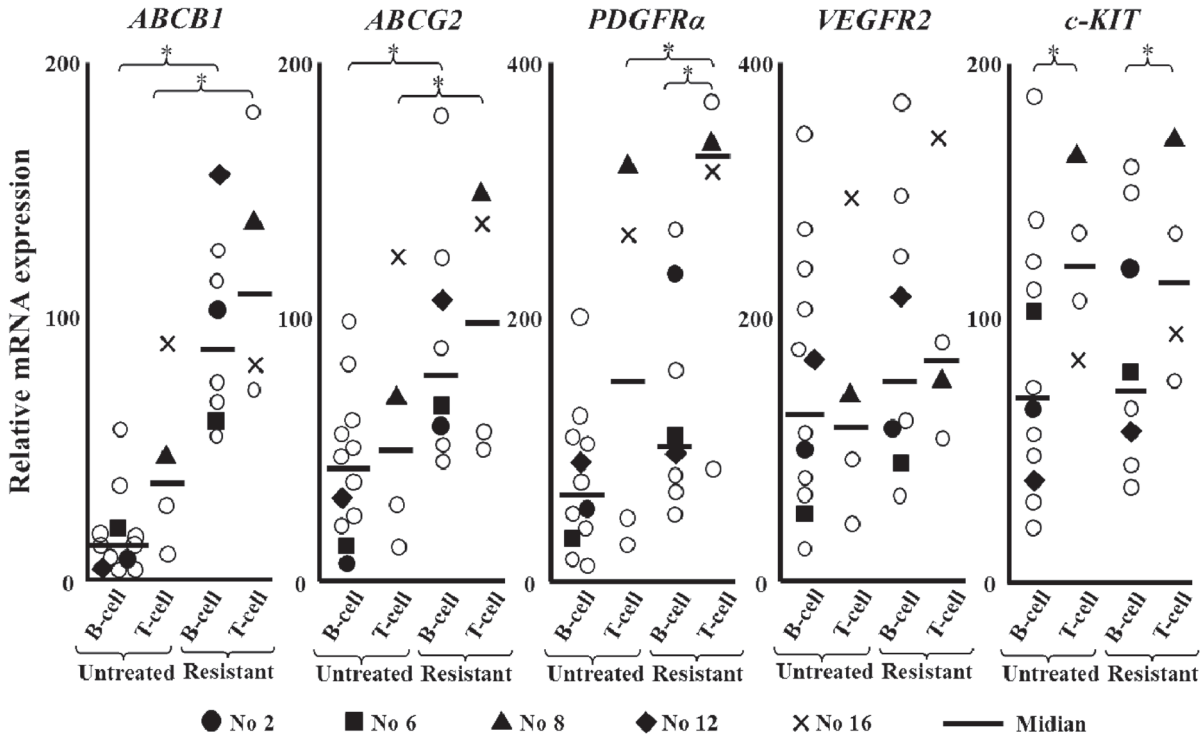

Fig. 1. Comparative analysis of relative mRNA expression of *ABCB1*, *ABCG2*, *PDGFRα*, *VEGFR2* and *c-KIT* between untreated lymphoma (n=16) and multidrug resistant lymphoma (n=12) were performed with the qRT-PCR technique. All expression levels were normalized to those of the controls (lymphocyte in normal dogs). *P<0.05 vs. untreated lymphomas (Dunnett's test).

Table 2. Outcome of the clinical trial for the five canine subjects with lymphoma

No.	TOC [a] (mg/kg)	Response [b]	PFI [c] (day)	ST [d] (day)	Adverse events [e]	Supportive care
2	2.6	SD	42	336	None	No treatment
6	2.8	PD	/	240	None	No treatment
8	2.4	PR	105	298	Diarrhea (G1)	Metronidazole (No discontinuation)
12	2.6	PD	/	412	None	No treatment
16	2.8	PR	84	218	Neutropenia (G1)	No treatment (No discontinuation)

a) TOC; Toceranib phosphate was administered orally on 3 days (Monday, Wednesday and Friday) per week. b) CR: Complete Response, PR: Partial Response, SD: Stable Disease, PD: Progressive Disease. c) PFI; Progression-free interval. d) ST; Survival time. After failure of TOC therapy, Numbers 2, 6 and 8 were treated with lomustine (CCNU), No 12 had supportive care only, and No 16 was treated with nimustine (ACNU). e) G; Grade, Adverse events were recorded and graded according to the Veterinary Co-operative Oncology Group-Common terminology criteria for adverse events (VCOG-CTCAE).

these two subjects had PFIs of 105 and 84 days without severe adverse events or discontinuation. One study reported that the ORR to masitinib was 70% in canine epitheliotropic T-cell lymphoma. In the same study, the median PFI of the subjects that exhibited CRs was 85 days, and that of the subjects that exhibited PRs was 60.5 days [6]. In this clinical trial, it was suggested that TOC might be more effective against T-cell lymphoma than B-cell lymphoma. Moreover, expression of the TOC-targeted genes was greater in the two subjects that exhibited PRs than in the other subjects. To reinforce the suggestion that expression levels of these genes may allow estimation of therapeutic effects, further study is required to demonstrate phosphorylation levels of the receptor tyrosine kinases and related signal transduction pathways. This would include the analysis of phosphoprotein expression or phosphorylation activity by kinase inhibitors.

After failure of standard multidrug therapy, a rescue protocol based on CCNU is generally beneficial to dogs with MDR lymphoma, as CCNU is a DNA-targeted alkylating agent that does not affect the activity of P-gp [9]. One study has reported that the response of canine subjects with MDR lymphoma to CCNU lasted for a median of 86 days [9]. CCNU caused chronic neutropenia and cumulative hepatotoxicity in dogs with cancer [9], and TOC induced dose-dependent acute gastrointestinal toxicity, mild neutropenia and a rare protein-losing syndrome [13]. However, when TOC was administered (2.75 mg/kg, orally, every second day) in combination with CCNU (50 mg/m^2, orally, every 3 weeks) in phase I of the study, it was well tolerated, and two out of three subjects exhibited a CR or PR [13]. Hence, a combination of TOC and CCNU may improve clinical outcomes in

MDR canine lymphoma.

The clinical data suggested that expression of *PDGFRα* was greater in subjects with MDR T-cell lymphoma than in those with untreated T-cell lymphoma, and that *c-KIT* expression was greater in subjects with T-cell lymphoma than in those with B-cell lymphoma. Our findings demonstrated that TOC monotherapy was well tolerated without severe adverse events, and two of the five subjects with MDR lymphoma exhibited PRs. The data presented here could contribute to the assessment of TOC-based therapy for canine lymphoma. To improve clinical outcomes, further research is required on enhancing the effect of TOC by combining it with conventional anticancer drugs.

ACKNOWLEDGMENT. The authors declare that they have no conflicts of interest. This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (grant numbers 22780283, 25292180, and 15K14872) and a grant from the Veterinary Teaching Hospital for Joint Faculty of Veterinary Medicine, Kagoshima University.

REFERENCES

1. Akiyama, K., Ohga, N., Hida, Y., Kawamoto, T., Sadamoto, Y., Ishikawa, S., Maishi, N., Akino, T., Kondoh, M., Matsuda, A., Inoue, N., Shindoh, M. and Hida, K. 2012. Tumor endothelial cells acquire drug resistance by MDR1 up-regulation via VEGF signaling in tumor microenvironment. *Am. J. Pathol.* **180**: 1283–1293. [Medline] [CrossRef]
2. Aricò, A., Guadagnin, E., Ferraresso, S., Gelain, M. E., Iussich, S., Rütgen, B. C., Mazzariol, S., Marconato, L. and Aresu, L. 2014. Platelet-derived growth factors and receptors in Canine Lymphoma. *J. Comp. Pathol.* **151**: 322–328. [Medline] [CrossRef]
3. Bernabe, L. F., Portela, R., Nguyen, S., Kisseberth, W. C., Pennell, M., Yancey, M. F. and London, C. A. 2013. Evaluation of the adverse event profile and pharmacodynamics of toceranib phosphate administered to dogs with solid tumors at doses below the maximum tolerated dose. *BMC Vet. Res.* **9**: 190. [Medline] [CrossRef]
4. Gentilini, F., Calzolari, C., Turba, M. E., Bettini, G. and Famigli-Bergamini, P. 2009. GeneScanning analysis of Ig/TCR gene rearrangements to detect clonality in canine lymphomas. *Vet. Immunol. Immunopathol.* **127**: 47–56. [Medline] [CrossRef]
5. Giantin, M., Aresu, L., Aricò, A., Gelain, M. E., Riondato, F., Comazzi, S. and Dacasto, M. 2013. Evaluation of tyrosine-kinase receptor c-kit mutations, mRNA and protein expression in canine lymphoma: might c-kit represent a therapeutic target? *Vet. Immunol. Immunopathol.* **154**: 153–159. [Medline] [CrossRef]
6. Holtermann, N., Kiupel, M., Kessler, M., Teske, E., Betz, D. and Hirschberger, J. 2016. Masitinib monotherapy in canine epitheliotropic lymphoma. *Vet. Comp. Oncol.* **14** (Supple 1): 127–135. [Medline]
7. London, C., Mathie, T., Stingle, N., Clifford, C., Haney, S., Klein, M. K., Beaver, L., Vickery, K., Vail, D. M., Hershey, B., Ettinger, S., Vaughan, A., Alvarez, F., Hillman, L., Kiselow, M., Thamm, D., Higginbotham, M. L., Gauthier, M., Krick, E., Phillips, B., Ladue, T., Jones, P., Bryan, J., Gill, V., Novasad, A., Fulton, L., Carreras, J., McNeill, C., Henry, C. and Gillings, S. 2012. Preliminary evidence for biologic activity of toceranib phosphate (Palladia®) in solid tumours. *Vet. Comp. Oncol.* **10**: 194–205. [Medline] [CrossRef]
8. Momoi, Y., Okai, Y., Watari, T., Goitsuka, R., Tsujimoto, H. and Hasegawa, A. 1997. Establishment and characterization of a canine T-lymphoblastoid cell line derived from malignant lymphoma. *Vet. Immunol. Immunopathol.* **59**: 11–20. [Medline] [CrossRef]
9. Moore, A. S., London, C. A., Wood, C. A., Williams, L. E., Cotter, S. M., L'Heureux, D. A. and Frimberger, A. E. 1999. Lomustine (CCNU) for the treatment of resistant lymphoma in dogs. *J. Vet. Intern. Med.* **13**: 395–398. [Medline] [CrossRef]
10. Nakaichi, M., Taura, Y., Kanki, M., Mamba, K., Momoi, Y., Tsujimoto, H. and Nakama, S. 1996. Establishment and characterization of a new canine B-cell leukemia cell line. *J. Vet. Med. Sci.* **58**: 469–471. [Medline] [CrossRef]
11. Nguyen, S. M., Thamm, D. H., Vail, D. M. and London, C. A. 2015. Response evaluation criteria for solid tumours in dogs (v1.0): a Veterinary Cooperative Oncology Group (VCOG) consensus document. *Vet. Comp. Oncol.* **13**: 176–183. [Medline] [CrossRef]
12. Veterinary Co-operative Oncology Group. 2016. Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Vet. Comp. Oncol.* **14**: 417–446. [Medline] [CrossRef]
13. Pan, X., Tsimbas, K., Kurzman, I. D. and Vail, D. M. 2016. Safety evaluation of combination CCNU and continuous toceranib phosphate (Palladia®) in tumour-bearing dogs: a phase I dose-finding study. *Vet. Comp. Oncol.* **14**: 202–209. [Medline] [CrossRef]
14. Wang, Y., Appiah-Kubi, K., Wu, M., Yao, X., Qian, H., Wu, Y. and Chen, Y. 2016. The platelet-derived growth factors (PDGFs) and their receptors (PDGFRs) are major players in oncogenesis, drug resistance, and attractive oncologic targets in cancer. *Growth Factors* **34**: 64–71. [Medline] [CrossRef]
15. Wolfesberger, B., Guija de Arespacohaga, A., Willmann, M., Gerner, W., Miller, I., Schwendenwein, I., Kleiter, M., Egerbacher, M., Thalhammer, J. G., Muellauer, L., Skalicky, M. and Walter, I. 2007. Expression of vascular endothelial growth factor and its receptors in canine lymphoma. *J. Comp. Pathol.* **137**: 30–40. [Medline] [CrossRef]
16. Yancey, M. F., Merritt, D. A., White, J. A., Marsh, S. A. and Locuson, C. W. 2010. Distribution, metabolism, and excretion of toceranib phosphate (Palladia, SU11654), a novel tyrosine kinase inhibitor, in dogs. *J. Vet. Pharmacol. Ther.* **33**: 154–161. [Medline] [CrossRef]
17. Zandvliet, M. 2016. Canine lymphoma: a review. *Vet. Q.* **36**: 76–104. [Medline] [CrossRef]
18. Zandvliet, M., Teske, E. and Schrickx, J. A. 2014. Multi-drug resistance in a canine lymphoid cell line due to increased P-glycoprotein expression, a potential model for drug-resistant canine lymphoma. *Toxicol. In Vitro* **28**: 1498–1506. [Medline] [CrossRef]
19. Zandvliet, M., Teske, E., Chapuis, T., Fink-Gremmels, J. and Schrickx, J. A. 2013. Masitinib reverses doxorubicin resistance in canine lymphoid cells by inhibiting the function of P-glycoprotein. *J. Vet. Pharmacol. Ther.* **36**: 583–587. [Medline] [CrossRef]
20. Zhao, Y. and Adjei, A. A. 2015. Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor. *Oncologist* **20**: 660–673. [Medline] [CrossRef]