NOTE

Internal Medicine

Long-term survival in a dog with primary hepatic neuroendocrine tumor treated with toceranib phosphate

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ABSTRACT. Primary hepatic neuroendocrine tumors (PHNETs) are rare in dogs, and limited information exists about the treatment of these tumors. A 12-year-old castrated male French bulldog was presented to our clinic with gastrointestinal signs. Diagnostic tests revealed increased hepatic enzyme levels, a mass in the hepatic quadrate lobe, multiple intrahepatic nodules, and enlarged hepatic hilar lymph nodes. The liver mass was diagnosed cytologically as a malignant epithelial tumor suspected to be of neuroendocrine origin. The dog was treated with single-agent toceranib phosphate (TOC) and survived 25.1 months after the initial presentation. On necropsy, a liver mass was found and was subsequently diagnosed as a PHNET on histopathology. To the best of our knowledge, this is the first report of long-term survival in a dog with PHNET treated with TOC.

KEY WORDS: carcinoid, dog, long-term survival, primary hepatic neuroendocrine tumor, toceranib

Primary hepatic neuroendocrine tumors (PHNETs) are relatively rare and account for 2−14% of all primary liver tumors in dogs [16, 17, 22]. PHNETs in dogs tend to occur at a slightly younger age than dogs with other primary hepatobiliary tumors [16, 17]. Thirty-three percent of dogs with PHNETs have nodular lesions; the remainder have diffuse lesions [16, 17]. PHNETs in dogs often form lesions in multiple hepatic lobes or have already undergone metastasis at the time of diagnosis; metastases are commonly found in regional lymph nodes, the peritoneum, and other sites, indicating high biologic behavior [16, 17]. For this reason, surgical treatment is often not indicated, and no standard treatment has been established.

Toceranib phosphate (TOC) is a multi-targeted receptor tyrosine kinase inhibitor (TKI) approved for use in dogs. TOC was originally indicated for the treatment of recurrent Patnaik grades 2 and 3 cutaneous mast cell tumors (MCTs) [11] but has also been shown to be effective against various solid tumors, including neuroendocrine tumors (NET) [1, 8, 12, 20]. This report describes an overview and the treatment outcomes in a case of canine PHNET with multiple metastases where long-term survival was achieved with TOC therapy.

A 12-year-old castrated male French bulldog weighing 13.1 kg presented to a primary veterinary clinic with the chief complaint of anorexia and acute vomiting. The dog had increased serum hepatic enzyme levels, and a liver mass was found on abdominal ultrasonography. The dog was then referred to the Japan Small Animal Cancer Center, Saitama, Japan, for further evaluation. On the initial presentation at our center, the physical examination revealed a skin mass on the left trunk (21 mm × 25 mm × 6 mm) and a subcutaneous mass on the left lateral thigh (20 mm × 18 mm × 5 mm). A complete blood count (IDEXX ProCyte Dx Hematology Analyzer; IDEXX Laboratories, Tokyo, Japan) was within the reference interval, whereas the blood chemistry panel (FUJIFILM DRI-CHEM 7000V; FUJIFILM, Tokyo, Japan) revealed increased alanine aminotransferase (ALT, 1,042 U/l; RI, 17−78 U/l), aspartate aminotransferase (AST, 62 U/l; RI, 17−44 U/l), alkaline phosphatase (ALP, 6,087 U/l; RI, 47−254 U/l), and gamma-glutamyl transferase (GGT, 40 U/l; RI, 5−14 U/l) activities, and hypercholesterolemia (T-cho, 445 mg/dl; RI, 112−312 mg/dl). Thoracic and abdominal radiography revealed no apparent abnormalities. Abdominal ultrasonography (ARIETTA 70; HITACHI Health Care, Tokyo, Japan) showed a hyperechoic mass measuring 64.9 mm at the longest diameter in the quadrate lobe

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of the liver (Fig. 1A), and an enlargement of the left hepatic lymph node to 11 mm in diameter.

On Day 7, contrast-enhanced computed tomography (CT) was performed using an 80-MDCT scanner (Aquilion Prime TSX-303 A; Canon Medical Systems, Tochigi, Japan) under general anesthesia. CT scans were acquired at 120 kVp, 350 mA, 0.5 sec spin time, 0.5 mm slice thickness, 0.813 beam pitch, and 0.5 mm reconstruction interval using a standard algorithm. For contrast enhancement during CT, 2.5 ml/kg (750 mg/kg) of iopamidol (Oypalomin; Fuji Pharma, Tokyo, Japan) was injected into the cephalic vein over 10 sec, and scans were acquired at 20, 50, and 120 sec after injection. The acquired CT data were analyzed using image processing software (OsiriX; Pixmeo SARL, Geneva, Switzerland). CT scans revealed a multilocular mass measuring 67 mm in the longest diameter of the quadrate lobe of the liver. The mass was strongly enhanced in the arterial phase, showed residual enhancement in the portal phase, and was isointense with the surrounding liver parenchyma in the equilibrium phase (Fig. 2). In addition, nodules with similar contrast enhancement patterns were detected at two sites in the lateral left lobe (both 3 mm) and one site in the caudate process of the caudate lobe (5 mm). Nodules showing similar contrast enhancement patterns were
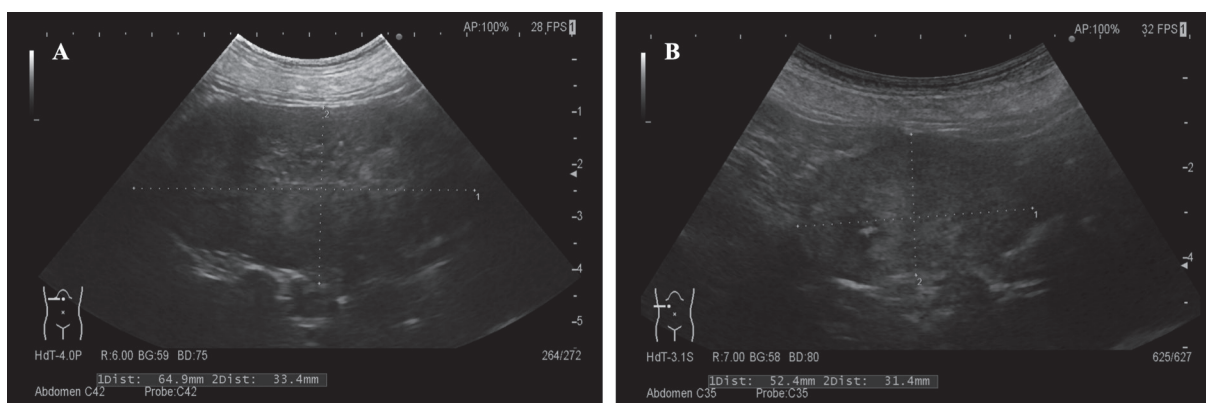
Fig. 1. Cross-sectional ultrasound images of the mass in the quadrate lobe of the liver. **(A)** On Day 1, the quadrate lobe mass measured 64.9 mm in the longest diameter. **(B)** On Day 203, the quadrate lobe mass measured 52.4 mm in the longest diameter, with an about 20% reduction in size (stable disease).
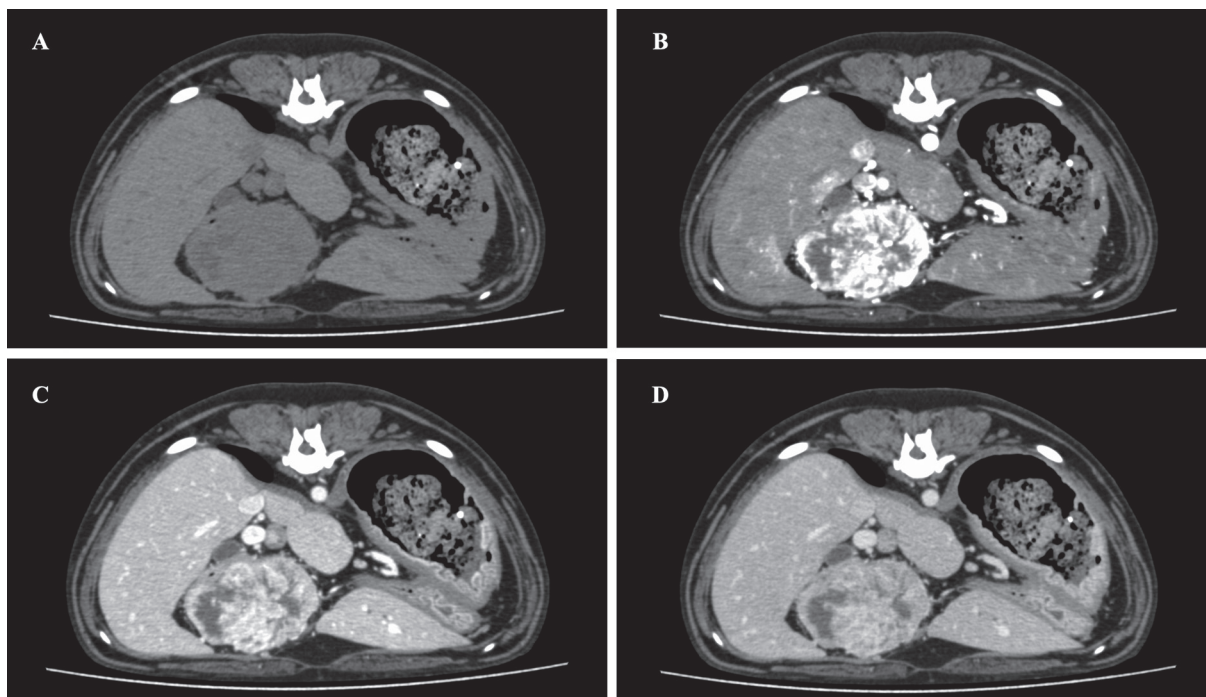
Fig. 2. Cross-sectional computed tomography scans of the mass in the quadrate lobe of the liver. A multilocular mass lesion with poorly contrast-enhanced areas inside is seen in the quadrate lobe. **(A)** A precontrast image. **(B)** In the arterial phase, the mass lesion is strongly contrast-enhanced. **(C)** In the portal phase, contrast enhancement of the mass lesion remains. **(D)** In the equilibrium phase, the mass lesion is isointense with the surrounding liver parenchyma.

also observed in the right (10 mm) and left hepatic (11 mm), pancreaticoduodenal, and splenic lymph nodes, all of which were suspected to be metastases from the quadrate lobe mass. After completion of CT scanning, ultrasound-guided fine-needle aspiration (FNA) biopsy was performed on the quadrate lobe mass, and FNA biopsy of the left trunk cutaneous mass and the left femoral subcutaneous mass. On cytology, the quadrate lobe mass was diagnosed as a suspected NET, while the left trunk cutaneous mass and the left femoral subcutaneous mass were both diagnosed as MCTs. A c-KIT gene mutation analysis of the MCTs showed no mutations in exons 8, 9, or 11. On the same day, treatment with diphenhydramine (Restamin; Kowa, Aichi, Japan) at 1.55 mg/kg, PO, q 8 hr and famotidine (Gaster; LTL Pharma, Tokyo, Japan) at 0.77 mg/kg, PO, q 12 hr was started to treat the MCT symptoms.

Based on these findings, the dog was strongly suspected of having PHNET in the quadrate lobe of the liver with multiple intrahepatic and intra-abdominal lymph node metastases. Two MCTs were also diagnosed on the body surface. The dog was treated with TOC (Palladia; Zoetis Japan, Tokyo, Japan), a multi-targeted TKI, at 2.56 mg/kg, PO, EOD, starting from Day 22. At the request of the owner, diphenhydramine and famotidine were discontinued on Day 42, with no obvious changes due to the suspension of these medications. After TOC therapy was initiated, two adverse events (AEs) were observed and evaluated according to the VCOG-CTCAE v1.1 [23]. The first AE, Grade 1 neutropenia (2,193 /µl; RI, 3,600−13,100 /µl), was observed on Day 49. The neutrophil count reached the nadir on the same day. The second AE, Grade 2 depigmentation of the nasal planum and metacarpal pads, was observed on Day 63. Depigmentation was not associated with pain or pruritus at any site and persisted until the dog died. After the initiation of TOC, hepatic enzymes gradually normalized, with AST and GGT reaching their reference intervals on Day 35, ALT and ALP reaching their reference intervals on Days 140 and 203, respectively. The quadrate lobe mass had shrunk to 52.1 mm by Day 203 (a 20% reduction; Fig. 1B), and the left hepatic lymph node had shrunk to 5 mm by Day 84 (a 55% reduction). These tumor shrinkages were assessed as stable disease (SD) and partial remission (PR) according to the RECIST criteria [14], respectively. Subsequently, the quadrate lobe mass remained as SD. However, the left hepatic lymph node had enlarged to 22 mm, and a new lesion measuring 43 mm in its longest diameter was identified in the left medial lobe of the liver on Day 630. Another lesion measuring 49.8 mm in the longest diameter was identified in the left lateral lobe of the liver on Day 748. Since the patient was in good physical condition and the quadrate lobe mass (the main lesion) remained as SD, TOC therapy was continued even after developing new lesions. On Day 754, TOC therapy was suspended owing to gastrointestinal signs, including decreased appetite and vomiting, and symptomatic therapy was started by the referring veterinarian. Nevertheless, the patient died at home on Day 766. The two MCTs on the body surface did not change in size throughout the TOC treatment period.

With the owner's permission, an autopsy was performed at the Japan Small Animal Cancer Center on the day of death. Macroscopic findings included masses of varying sizes in the liver, with particularly large masses in the quadrate lobe and lateral left lobe (Fig. 3). The hepatic lymph nodes were contiguous with the quadrate lobe mass, and differentiation of these structures was challenging on gross and histopathologic examinations. These liver masses consisted of atypical cell proliferation in a honeycomb pattern divided by abundant vascular connective tissue, and the tumor cells stained diffusely positive for the neuroendocrine cell marker, PGP 9.5. Both masses were histopathologically diagnosed as NETs (Fig. 4). Similar NET lesions had also formed in the heart base and pancreatic lymph nodes, which were diagnosed as PHNET metastases. Another invasive mass was also found around the gallbladder, which contained epithelial cells forming a glandular structure and negative for PGP9.5, leading to a diagnosis of bile duct carcinoma. There were interstitial pneumonia and pulmonary edema in part of the lungs.

Surgical resection is the first-line treatment for PHNETs in humans [25]. Treatment options in cases not amenable to surgical resection include transarterial chemoembolization [9], radiofrequency ablation [24], combination chemotherapy with fluorouracil, cisplatin, and other agents [15], and the somatostatin analog, octreotide [24]. In primary pancreatic NETs in people, vascular endothelial growth factor (VEGF) plays a major role in promoting angiogenesis [3, 10] and receptor tyrosine kinases (RTKs) (such as platelet-derived growth factor receptor, c-kit, and VEGF receptor) are widely expressed [4, 5, 7]. For advanced primary pancreatic NETs, the efficacy of sunitinib malate, a compound structurally and functionally similar to TOC, has been demonstrated in a multicenter, randomized, double-blind study [19]. However, it remains to be investigated whether sunitinib malate is effective against human PHNETs. In dogs, RTK expression as a target of TOC has been detected in thyroid cancer and anal sac apocrine gland carcinoma [2, 21], and TOC has been reported to be successful against these tumors [5–8]. Although no studies have examined RTK expression in canine PHNETs or other NETs, we hypothesized that the inhibitory effects of TOC on angiogenesis via RTKs might have caused prolonged suppression of PHNET progression.

A few reports on the treatment and prognosis of PHNETs in dogs have been published. In a report of 10 dogs diagnosed with hepatic neuroendocrine carcinoma on histopathology, all dogs were euthanized within 8 days of diagnosis [18]. In 2019, Morgan *et al.* treated a dog with a primary hepatic neuroendocrine carcinoma with metronomic chemotherapy with cyclophosphamide combined with doxorubicin, resulting in the suppression of tumor progression for 10 months and survival for 15.5 months [13]. The efficacy of TOC has also been demonstrated in dogs with insulinoma, a functional primary pancreatic NET [1, 6]. Although the biological behavior of PHNETs in dogs is unclear, in this case, the mass shrank by up to 20% after TOC administration, and the patient survived for 25.1 months, suggesting that the PHNET might have responded to TOC and resulting in long-term survival.

The limitation of this report is that we did not perform tissue biopsies of the quadrate lobe mass in the liver before using TOC and could not confirm RTK expression and the changes in expression. In addition, although the dog survived more than 2 years, the presence of a more indolent form of this disease cannot be excluded.

In the present case, a dog with PHNET with multiple metastases was treated with TOC, resulting in tumor shrinkage and long-term survival. To further demonstrate the efficacy of TOC against canine PHNET, not only basic molecular biological and genetic studies of canine PHNET are needed, but also multicenter clinical studies of this relatively rare disease.
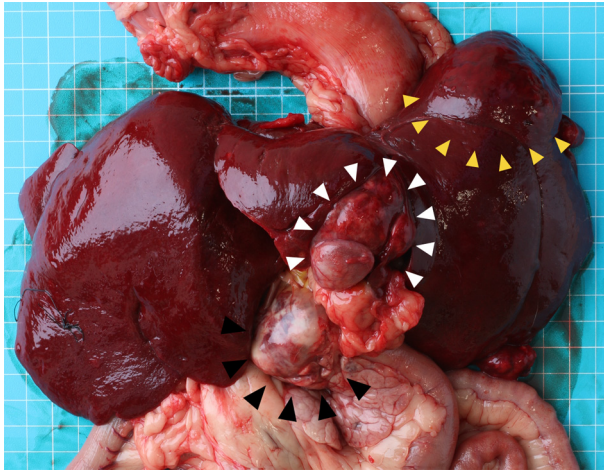
Fig. 3. The gross appearance of the liver on necropsy. A mass surrounding the gallbladder (black arrowheads), a mass in the quadrate lobe (white arrowheads), and a mass in the lateral left lobe (yellow arrowheads) are indicated.
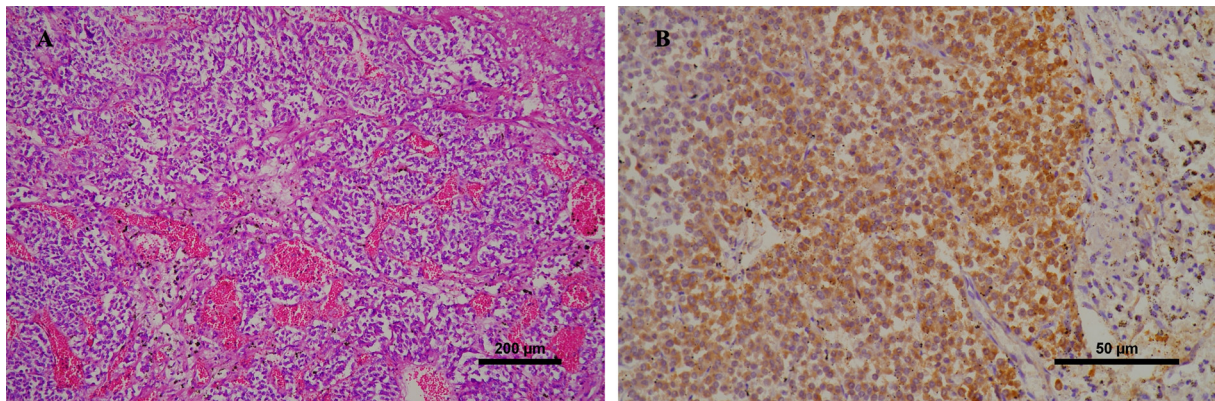
Fig. 4. Photomicrographs showing the histopathologic findings of the hepatic quadrate lobe mass. **(A)** Tumor cells have poorly demarcated weakly eosinophilic fine granular cytoplasm and atypical oval nuclei. H&E stain, bar=200 μm. **(B)** Immunostaining for PGP 9.5. Tumor cells are diffusely positive for PGP 9.5. Bar=50 μm.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

REFERENCES

1. Alonso-Miguel, D., García-San José, P., González Sanz, S., Clarés Moral, I. and Pérez-Alenza, M. D. 2021. Evaluation of palliative therapy, alone or in combination with toceranib phosphate, in dogs diagnosed with metastatic or recurrent beta-cell neoplasia. *N. Z. Vet. J.* **69**: 234–239. [Medline] [CrossRef]
2. Campos, M., Kool, M. M., Daminet, S., Ducatelle, R., Rutteman, G., Kooistra, H. S., Galac, S. and Mol, J. A. 2014. Upregulation of the PI3K/Akt pathway in the tumorigenesis of canine thyroid carcinoma. *J. Vet. Intern. Med.* **28**: 1814–1823. [Medline] [CrossRef]
3. Casanovas, O., Hicklin, D. J., Bergers, G. and Hanahan, D. 2005. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* **8**: 299–309. [Medline] [CrossRef]
4. Fjällskog, M. L., Hessman, O., Eriksson, B. and Janson, E. T. 2007. Upregulated expression of PDGF receptor beta in endocrine pancreatic tumors and metastases compared to normal endocrine pancreas. *Acta Oncol.* **46**: 741–746. [Medline] [CrossRef]
5. Fjällskog, M. L., Lejonklou, M. H., Oberg, K. E., Eriksson, B. K. and Janson, E. T. 2003. Expression of molecular targets for tyrosine kinase receptor antagonists in malignant endocrine pancreatic tumors. *Clin. Cancer Res.* **9**: 1469–1473. [Medline]
6. Flesner, B. K., Fletcher, J. M., Smithee, T. and Boudreaux, B. 2019. Long-term survival and glycemic control with toceranib phosphate and prednisone for a metastatic canine insulinoma. *J. Am. Anim. Hosp. Assoc.* **55**: e55105. [Medline] [CrossRef]
7. Hansel, D. E., Rahman, A., Hermans, J., de Krijger, R. R., Ashfaq, R., Yeo, C. J., Cameron, J. L. and Maitra, A. 2003. Liver metastases arising from well-differentiated pancreatic endocrine neoplasms demonstrate increased VEGF-C expression. *Mod. Pathol.* **16**: 652–659. [Medline] [CrossRef]
8. Heaton, C. M., Fernandes, A. F. A., Jark, P. C. and Pan, X. 2020. Evaluation of toceranib for treatment of apocrine gland anal sac adenocarcinoma in dogs. *J. Vet. Intern. Med.* **34**: 873–881. [Medline] [CrossRef]
9. Huang, Y. Q., Xu, F., Yang, J. M. and Huang, B. 2010. Primary hepatic neuroendocrine carcinoma: clinical analysis of 11 cases. *Hepatobiliary Pancreat. Dis. Int.* **9**: 44–48. [Medline]
10. Inoue, M., Hager, J. H., Ferrara, N., Gerber, H. P. and Hanahan, D. 2002. VEGF-A has a critical, nonredundant role in angiogenic switching and pancreatic β cell carcinogenesis. *Cancer Cell* **1**: 193–202. [Medline] [CrossRef]

11. London, C. A., Malpas, P. B., Wood-Follis, S. L., Boucher, J. F., Rusk, A. W., Rosenberg, M. P., Henry, C. J., Mitchener, K. L., Klein, M. K., Hintermeister, J. G., Bergman, P. J., Couto, G. C., Mauldin, G. N. and Michels, G. M. 2009. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. *Clin. Cancer Res.* **15**: 3856–3865. [Medline] [CrossRef]

12. 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]

13. Morgan, E., O'Connell, K., Thomson, M., Boyd, S. and Sandy, J. 2019. Primary hepatic neuroendocrine carcinoma treated with doxorubicin and cyclophosphamide in a dog. *J. Am. Anim. Hosp. Assoc.* **55**: e55305. [Medline] [CrossRef]

14. 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]

15. Park, C. H., Chung, J. W., Jang, S. J., Chung, M. J., Bang, S., Park, S. W., Song, S. Y., Chung, J. B. and Park, J. Y. 2012. Clinical features and outcomes of primary hepatic neuroendocrine carcinomas. *J. Gastroenterol. Hepatol.* **27**: 1306–1311. [Medline] [CrossRef]

16. Patnaik, A. K., Hurvitz, A. I. and Lieberman, P. H. 1980. Canine hepatic neoplasms: a clinicopathologic study. *Vet. Pathol.* **17**: 553–564. [Medline] [CrossRef]

17. Patnaik, A. K., Lieberman, P. H., Hurvitz, A. I. and Johnson, G. F. 1981. Canine hepatic carcinoids. *Vet. Pathol.* **18**: 445–453. [Medline] [CrossRef]

18. Patnaik, A. K., Newman, S. J., Scase, T., Erlandson, R. A., Antonescu, C., Craft, D. and Bergman, P. J. 2005. Canine hepatic neuroendocrine carcinoma: an immunohistochemical and electron microscopic study. *Vet. Pathol.* **42**: 140–146. [Medline] [CrossRef]

19. Raymond, E., Dahan, L., Raoul, J. L., Bang, Y. J., Borbath, I., Lombard-Bohas, C., Valle, J., Metrakos, P., Smith, D., Vinik, A., Chen, J. S., Hörsch, D., Hammel, P., Wiedenmann, B., Van Cutsem, E., Patyna, S., Lu, D. R., Blanckmeister, C., Chao, R. and Ruszniewski, P. 2011. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *N. Engl. J. Med.* **364**: 501–513. [Medline] [CrossRef]

20. Sheppard-Olivares, S., Bello, N. M., Wood, E., Szivek, A., Biller, B., Hocker, S. and Wouda, R. M. 2020. Toceranib phosphate in the treatment of canine thyroid carcinoma: 42 cases (2009–2018). *Vet. Comp. Oncol.* **18**: 519–527. [Medline] [CrossRef]

21. Urie, B. K., Russell, D. S., Kisseberth, W. C. and London, C. A. 2012. Evaluation of expression and function of vascular endothelial growth factor receptor 2, platelet derived growth factor receptors-alpha and -beta, KIT, and RET in canine apocrine gland anal sac adenocarcinoma and thyroid carcinoma. *BMC Vet. Res.* **8**: 67. [Medline] [CrossRef]

22. van Sprundel, R. G., van den Ingh, T. S., Guscetti, F., Kershaw, O., Kanemoto, H., van Gils, H. M., Rothuizen, J., Roskams, T. and Spee, B. 2013. Classification of primary hepatic tumours in the dog. *Vet. J.* **197**: 596–606. [Medline] [CrossRef]

23. 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]

24. Wang, H. H., Liu, Z. C., Zhang, G., Li, L. H., Li, L., Meng, Q. B., Wang, P. J., Shen, D. Q. and Dang, X. W. 2020. Clinical characteristics and outcome of primary hepatic neuroendocrine tumors after comprehensive therapy. *World J. Gastrointest. Oncol.* **12**: 1031–1043. [Medline] [CrossRef]

25. Zhang, A., Xiang, J., Zhang, M. and Zheng, S. 2008. Primary hepatic carcinoid tumours: clinical features with an emphasis on carcinoid syndrome and recurrence. *J. Int. Med. Res.* **36**: 848–859. [Medline] [CrossRef]