Clinical features and outcome in a dog with pulmonary metastatic osteosarcoma treated with toceranib phosphate

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CASE REPORT A 10-year-old female spayed Jack Russell Terrier was evaluated after 4 weeks of left forelimb lameness. Results of haematology, serum biochemistry, urinalysis and thoracic and left shoulder radiography were unremarkable except for mild elevation in serum alkaline phosphatase and osteoproliferation in the left proximal humerus. Left forequarter amputation was performed and histopathology revealed osteoblastic osteosarcoma in the proximal humerus. Adjuvant chemotherapy was administered, consisting of three doses of doxorubicin and three doses of carboplatin; and subsequently, antiangiogenic therapy (piroxicam, doxycycline, and tamoxifen) for another 6 months before evidence of pulmonary metastases became radiographically detectable. Toceranib phosphate was commenced at 2.85 mg/kg PO on Monday/Wednesday/Friday. The dog achieved a partial response and was still alive more than 45 months after amputation.

CONCLUSION To the authors' knowledge, this is the first report of a dog with osteosarcoma responding to toceranib therapy for more than 34 months after detection of pulmonary metastases.

KEYWORDS appendicular osteosarcoma; bone cancer; dogs; pulmonary metastasis; tyrosine kinase inhibitor

ABBREVIATIONS ALP, alkaline phosphatase; c-Kit, stem cell factor receptor; CT, computed tomography; MST, median survival time; RECIST-VCOG v1.0, response evaluation criteria for solid tumours in dogs: a Veterinary Cooperative Oncology Group consensus document (version 1.0); VCOG-CTCAE, Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events

Osteosarcoma is the most common primary bone tumour in dogs, representing >80% of malignant bone tumours.[1,2] The reported median survival time (MST) with amputation alone is approximately 4 months,[3,4] and with amputation and adjuvant chemotherapy the MST is between 10 and 12 months.[5] Although standard-of-care treatment with amputation and adjuvant chemotherapy has greatly improved survival in dogs with osteosarcoma, 80–90% of these dogs will still die as a result of pulmonary metastases,[1,2,6] despite no clinical evidence of metastasis at the time of initial presentation.

Osteosarcoma commonly metastasises to the lung, but may also spread to regional lymph nodes, bones, internal organs (such as the liver and spleen)[3,6] and/or skin.[7] Dogs with metastatic (stage III) osteosarcoma have a grave prognosis. The MST in one study of 90 dogs with stage III osteosarcoma was 76 days (range 0–4.3 years).[8] The 1-, 2- and 3-year survival rates were 6.6%, 4.7%, and 3.5%, respectively. However, the dogs with metastasis only to bone had a significantly longer survival time, compared with those with metastasis to visceral sites and/or lymph node. The 6 dogs that survived the longest (i.e. from 11.8 months to 4.3 years) all had metastases to bone with no evidence of pulmonary metastases. The MST for 38 dogs with pulmonary metastatic osteosarcoma was 59 days.

The response to chemotherapy in dogs with measurable metastatic osteosarcoma is poor. One study[6] assessed the response of 45 dogs with metastatic osteosarcoma treated with cisplatin (31), doxorubicin (11) and/or mitoxantrone (3). Only 1 (2.2%) dog, treated with doxorubicin, experienced partial remission (defined as >50% decrease in the sum of diameters of all measurable lesions) for 21 days and an overall survival following detection of metastasis of 159 days. The MST of the other 44 dogs that did not respond to treatment (progressive disease in 39 [88.6%] dogs and stable disease in 5 [11.4%] dogs) was 61 days (range, 2 weeks to 6.3 months). In another study, 27 dogs with stage III osteosarcoma were treated with surgery and adjuvant chemotherapy (cisplatin or carboplatin, doxorubicin or a combination of doxorubicin and cisplatin or carboplatin). The MST reported was 78 days (range, 0–4.3 years); however, the responses to treatment and response duration were not recorded.

Biological activity of toceranib phosphate in canine metastatic osteosarcoma has been previously reported.[9,10] In one study,[9] 3 dogs with osteosarcoma and radiographically detectable evidence of pulmonary metastases treated with toceranib experienced stable disease according to the response evaluation criteria in solid tumours (RECIST version 1.1) guidelines using unidimensional measurements;[11] however, the duration of response was not reported. Another study[10] included 23 dogs with osteosarcoma and radiographically detectable evidence of pulmonary metastases treated with toceranib. In that retrospective case series, approximately 48% of dogs showed a clinical benefit, with 1 dog (4.3%) achieving a partial response (defined as >30% decrease in target lesions) and 10 dogs (43.5%) experiencing stable disease (defined as decrease in target lesions of <30% or increase of target lesions <20%). The median duration of clinical benefit was 6 months (range, 2.3 to >9.7 months). Pulmonary metastatectomy is another treatment option for dogs with pulmonary metastatic osteosarcoma that have had primary tumour control for at least 1 year, <3 metastatic lesions, and a slow doubling time of the metastatic lesions.[12]

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In general, despite treatment, the outcome for dogs with pulmonary metastatic osteosarcoma is poor and very few dogs have been reported to have extended survival with treatment. This case report describes the clinical features and outcome in a dog with stage III pulmonary metastatic appendicular osteosarcoma successfully managed with toceranib for >34 months after detection of pulmonary metastases, alerting veterinarians to the potential for dogs with pulmonary metastatic osteosarcoma treated with toceranib to have long-term survival.

CASE REPORT

Clinical features

A 10-year-old female spayed Jack Russell Terrier weighing 8.2 kg was evaluated because of a 4-week history of progressive, intermittent lameness in the left forelimb. Physical examination revealed non-weight-bearing lameness in the left forelimb with pain localised on palpation of the proximal humerus. Haematology, serum biochemistry and urinalysis were performed and revealed no notable abnormalities except for a mild elevation in serum alkaline phosphatase (ALP) at 178 IU/L (reference range, 1–150 IU/L). The monocyte and lymphocyte counts were within reference ranges at 0.3×10^9 cells/L (reference range, $0.0–1.1 \times 10^9$ cells/L) and 2.2×10^9 cells/L (reference range, $0.9–3.5 \times 10^9$ cells/L), respectively. Left lateral and craniocaudal radiographs of the left shoulder were performed and revealed moderate periosteal proliferation and sclerosis in the left proximal humerus (Figure 1). Three-view thoracic radiographs showed no radiographically detectable evidence of pulmonary metastases.

Treatment

Based on the radiographic changes in the left proximal humerus, a diagnosis of primary bone neoplasia, most likely osteosarcoma, was suspected. Multiple 1-inch 12G Jamshidi needle bone biopsies of the left proximal humerus lesion were performed under general anaesthesia and histopathology revealed osteosarcoma. A left forequarter amputation and surgical extirpation of the normal-sized left axillary lymph node (measuring $9 \times 7 \times 5$ mm) was performed 7 days later. Histopathology confirmed a completely excised, intermediate grade (grade II)[13] osteoblastic osteosarcoma with moderate mitotic activity, necrosis and pleomorphism of osteoblasts (Figure 2). The left axillary lymph node showed sinus histiocytosis, but no histological evidence of metastatic osteoblastic osteosarcoma (Figure 3).
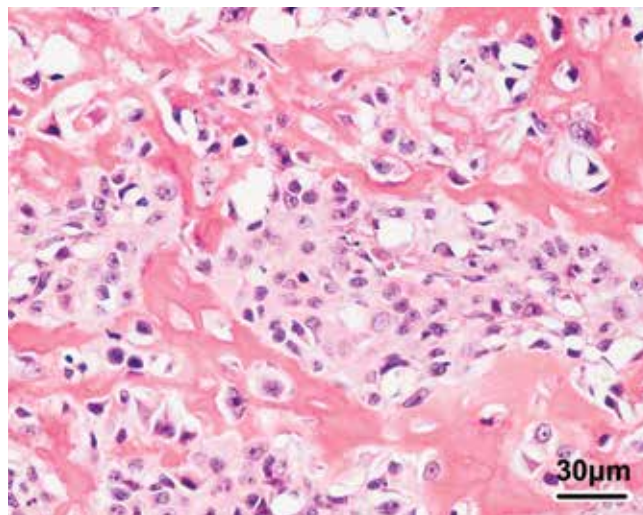

FIGURE 2. Photomicrograph of a section of the left proximal humerus of a 10-year-old female spayed Jack Russell Terrier with a 4-week history of left forelimb lameness. There is irregular extracellular brightly eosinophilic osteoid formed by large, angular, pleomorphic osteoblasts (H&E; bar = 30 μm).

FIGURE 1. Medial to lateral radiograph of the left proximal humerus at the time of presentation of a 10-year-old female spayed Jack Russell Terrier with a 4-week history of left forelimb lameness. There is circumferential osteoproliferative new bone at the level of the proximal metaphysis of the left humerus.
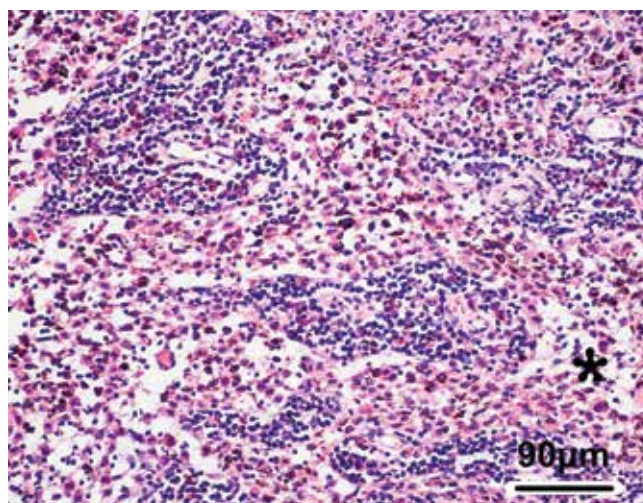
FIGURE 3. Photomicrograph of a section of the left axillary lymph node of a 10-year-old female spayed Jack Russell Terrier with a 4-week history of left forelimb lameness. Haemosiderophages filling sinuses (*) without evidence of metastatic osteosarcoma can be seen (H&E; bar = 90 μm).

At 16 days after amputation, adjuvant chemotherapy was commenced, comprising three doses of doxorubicin (1 mg/kg IV) administered 14 days apart, followed by three doses of carboplatin (250 mg/m^2 IV) administered 21 days apart,[5] with no dose reductions or dose delays. Prophylactic treatment with sulfadiazine–trimethoprim was administered at a dosage of 15 mg/kg PO twice daily for 14 days with each cycle of doxorubicin chemotherapy and after the first cycle of carboplatin only. The dog tolerated chemotherapy well, with only grade 2 gastrointestinal toxicity

(anorexia and diarrhoea) occurring 4 days after the final carboplatin treatment, according to the Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events (version 1.1) [VCOG-CTCAE v1.1].[14] The gastrointestinal toxicity resolved within 24 h following treatment with subcutaneous fluids (20 mL/kg of 0.9% sodium chloride) and maropitant (1 mg/kg SC). Three-view thoracic radiography was performed after three doses of doxorubicin (approximately halfway through the chemotherapy protocol) and again following the completion of chemotherapy, and revealed no radiographically detectable evidence of pulmonary metastases.

Following completion of adjuvant chemotherapy (4 months after amputation), the owners elected to proceed with antiangiogenic therapy consisting of piroxicam (0.3 mg/kg PO once daily), doxycycline (5 mg/kg PO twice daily) and tamoxifen (1 mg/kg PO once daily), all with food.[15,16] The dog tolerated this combination well, except for development of a moderately enlarged vulva consistent with pseudo-oestrus (16 weeks after commencement of antiangiogenic therapy), which resolved within a few days following discontinuation of tamoxifen. Three-view thoracic radiography was performed every 3 months.

At 6 months following commencement of antiangiogenic therapy (10.5 months after amputation), three-view thoracic radiographs revealed multiple (up to 12 mm diameter) pulmonary nodules, most of them consistent with pulmonary metastases from the osteosarcoma (Figure 4). At that time, haematology, serum biochemistry, urinalysis and the urine protein–creatinine ratio were unremarkable. The previously used antiangiogenic combination was discontinued and toceranib phosphate (Palladia®, Zoetis Inc., Kalamazoo, MI, USA) was commenced at 2.85 mg/kg PO three times per week (Monday/Wednesday/Friday) with food.

During toceranib therapy, monitoring consisted of repeated physical examination, Doppler systolic blood pressure measurements (Ultrasonic Doppler Blood Pressure Monitor, Model 811-B, Parks Medical Electronics Inc., Aloha, HI, USA), body weight, haematology, serum biochemistry, urinalysis, protein-creatinine ratio and three-view thoracic radiography,

FIGURE 4. Ventrodorsal thoracic radiograph performed 10.5 months after forequarter amputation in a 10-year-old female spayed Jack Russell Terrier. There are multiple well-defined, variably sized, round soft tissue nodules within the pulmonary parenchyma (arrows).
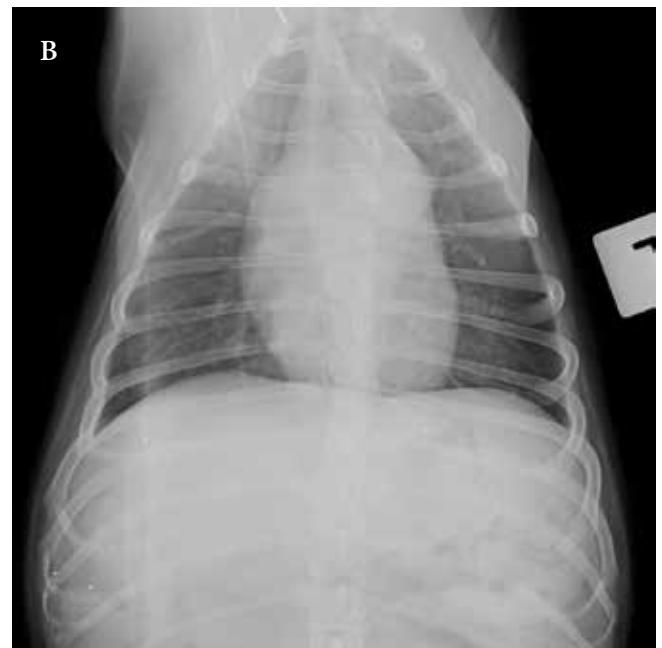

FIGURE 5. Ventrodorsal (A) and left lateral thoracic radiographs (B) at 9 months after commencement of toceranib (20 months post-amputation) in a 10-year-old female spayed Jack Russell Terrier. There is resolution of some of the previously noted pulmonary nodules (A, B), but there is still evidence of small pulmonary nodules (visible on high-resolution monitors), consistent with a partial response.

performed every 2–3 months. The dog experienced VCOG-CTCAE grade 1 gastrointestinal toxicity (nausea and vomiting) 5 days after commencement of toceranib, which resolved following administration of maropitant (2 mg/kg PO), metoclopramide (0.7 mg/kg PO) and ondansetron (0.5 mg/kg PO), 30–60 min before each dose of toceranib (still ongoing). The dog also experienced VCOG-CTCAE grade 1 elevation in blood urea nitrogen 30 days after commencement of toceranib, which improved and remained stable following ongoing administration of omeprazole at 1.4 mg/kg PO once daily and changing to a low-protein diet. No other adverse effects were noted and there were no dose delays. At 9 months after commencement of toceranib, the dose was reduced to 2.3 mg/kg (1 × 15-mg toceranib phosphate tablet) PO three times weekly because of the unavailability of 10-mg toceranib phosphate tablets; the owners elected to continue at this dose.

At 4 months after commencement of toceranib, three-view thoracic radiographs showed <30% reduction in the sum of diameters of target lesions (stable disease according to the Response Evaluation Criteria for Solid Tumours in Dogs (Version 1.0) [RECIST-VGOC v1.0[17]]). At 9 months after beginning toceranib, three-view thoracic radiographs showed >30% reduction in the sum of diameters of target lesions, consistent with a partial response according to the RECIST-VCOG v1.0 (Figure 5). At 15 months after commencement of toceranib, two new pulmonary nodules were seen on three-view thoracic radiographs, consistent with progressive disease compared with 6 months previously, according to the RECIST-VGOC v1.0, although the original nodules were smaller or absent (Figure 6). Toceranib therapy was continued at the owners' request and follow-up three-view thoracic radiography performed 1 month later (16 months after commencement of toceranib) and further follow-up thoracic radiography performed every 2–3 months thereafter showed that the new nodules had stabilised and no further lesions have arisen. At the time of last follow-up examination, (34 months after commencement of toceranib; 45 months after amputation),

the dog was clinically well with no evidence of further toxicity from toceranib and three-view thoracic radiographs showed stable disease compared with 19 months previously; the overall disease burden remains >30% reduced compared with 34 months previously when toceranib was started (Figure 7).

DISCUSSION

To the author's knowledge, this is the first report of a dog with pulmonary metastatic osteosarcoma surviving > 34 months after radiographically detectable evidence of pulmonary metastases.

The main weakness of this report is that the diagnosis of pulmonary osteosarcoma metastasis was made on evaluation of three-view thoracic radiographs, rather than histological or cytological analysis of pulmonary nodules. Although assessment of three-view thoracic radiographs is standard practice in veterinary medicine for evaluating the pulmonary parenchyma for evidence of metastatic disease, there is a possibility of misdiagnosis with this technique. A further weakness is that computed tomography (CT) imaging was not performed at baseline; thus the dog may have had CT-detectable evidence of pulmonary metastases at diagnosis. Although CT has been shown to be superior to thoracic radiography in the screening and detection of pulmonary nodules in canine osteosarcoma,[18,19] the clinical effect on survival of dogs with CT detected metastasis is currently unknown.[19]

Toceranib inhibits several tyrosine kinase receptors, including stem cell factor receptor (c-Kit), vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor and Fms-like tyrosine kinase 3, thus conferring efficacy via both direct anti-tumour effects and effects on the tumour microenvironment.[20,21] Toceranib's efficacy in canine mast cell tumours is mediated largely through c-Kit. However, activity against a wide variety of tumours, such as osteosarcoma, anal sac gland adenocarcinoma, thyroid carcinoma, nasal carcinoma, and head and neck carcinoma,[10]

FIGURE 6. Ventrodorsal thoracic radiograph at 15 months after commencement of toceranib (26 months post-amputation) in a 10-year-old female spayed Jack Russell Terrier. One soft-tissue-opacity pulmonary nodule is shown in the left cranial lung lobe (arrow), consistent with progressive disease compared with 6 months previously (see Figure 5).

FIGURE 7. Ventrodorsal thoracic radiograph at 34 months after commencement of toceranib (45 months post-amputation) in a 10-year-old female spayed Jack Russell Terrier. The soft-tissue-opacity pulmonary nodule in the left cranial lung lobe (arrowhead) remains unchanged from 19 months previously (see Figure 6), consistent with stable disease.

has been demonstrated in dogs, possibly via inhibition of other receptor tyrosine kinases.[20] Individual protein kinases, including c-Kit and platelet-derived growth factor receptor, have been found to be expressed in canine osteosarcoma cell lines.[22] Furthermore, toceranib has been shown to downregulate circulating levels of T regulatory cells in tumour-bearing dogs, suggesting some potential anti-tumour effects through immunomodulation.[10,23]

The addition of toceranib to metronomic piroxicam and cyclophosphamide therapy following amputation and carboplatin (i.e. microscopic setting) has not improved the median disease-free-interval or survival time in dogs with osteosarcoma, nor has it improved these endpoints over carboplatin alone.[20] Similarly, a clinical trial evaluating the effect of toceranib in dogs (also in the microscopic setting) with splenic haemangiosarcoma, following splenectomy and doxorubicin chemotherapy, found no survival benefit.[24] However, biological activity of toceranib in metastatic canine osteosarcoma (and other macroscopic solid tumours) has been reported.[10] It has been speculated that, although effects on tumour growth are observed in dogs with macroscopic metastatic pulmonary disease following toceranib therapy, microscopic lesions may become resistant to therapy within a short period of time, thereby negating any potential therapeutic value in this setting.[20]

Potential adverse effects of toceranib include lethargy, gastrointestinal signs, neutropenia, neuromuscular signs (lameness, weakness), hepatopathy, proteinuria, hypertension and pancreatitis.[9,21,25] In a study of canine mast cell tumours treated with toceranib at the maximum tolerated dose of 3.25 mg/kg PO every second day,[25] approximately 50% of dogs required a dose reduction and/or 'drug holiday' because of adverse effects. In contrast, in dogs with solid tumours treated with toceranib between 2.4 and 2.9 mg/kg PO every second day,[9] there were fewer VCOG-CTCAE grade 3 and 4 adverse effects (no VCOG-CTCAE grade 3 or 4 gastrointestinal events), with approximately 20% of dogs requiring dose reduction and/or drug holiday because of adverse effects. In addition, administration of toceranib at 2.4–2.75 mg/kg PO every second day has demonstrated target inhibition by showing statistically significant increases in plasma vascular endothelial growth factor, a surrogate marker of vascular endothelial growth factor receptor 2 inhibition.[9] Other studies have supported these findings by showing biological activity of toceranib at doses below label recommendations and at a less frequent dosing schedule (i.e. Monday/Wednesday/Friday).[10,21,23] For these reasons, in treating the dog described in this case report, we aimed for a toceranib dose and regimen of between 2.4 and 2.9 mg/kg PO on Monday/Wednesday/Friday rather than the label dose recommendation of 3.25 mg/kg PO every second day.

In the dog reported here, adverse effects were closely monitored, as previously described, every 2–3 months. Although the dog tolerated this medication well (with only temporary VCOG-CTCAE grade 1 gastrointestinal and VCOG-CTCAE grade 1 elevation in blood urea nitrogen), careful monitoring is required in all patients receiving toceranib and any adverse effects should be treated with supportive care, drug holidays and/or dose reductions, and in some circumstances drug discontinuation.

In the case presented, the dose of toceranib was reduced to 2.3 mg/kg PO on Monday/Wednesday/Friday at 9 months after beginning toceranib, because of the unavailability of the 10-mg toceranib phosphate tablets. When the original tablets became available, the owners chose to continue at the lower dose and the dog continues to receive this dose (for another 25 months at the time of writing). The lowest dose recommended for biological activity of toceranib in one study was 2.4 mg/kg.[9] In that study, dogs (n = 3) that were dosed at between 2.30 and 2.39 mg/kg appeared to have lower concentrations of drug at all time points; however, the mean maximum concentration of drug by 30 days was > 40 ng/mL

(the concentration of toceranib predicted to be associated with effective target inhibition). Thus for the dog presented here, although pharmacokinetic data were not obtained, we hypothesise that the toceranib dose in this patient achieved effective target inhibition because the dog attained stable disease according to the RECIST-VCOG v1.0 criteria and is still alive 34 months after commencement of toceranib.

According to the RECIST-VCOG v1.0, the best response the dog achieved was a partial response for 6 months (between 9 and 15 months after commencement of toceranib). At 15 months after beginning toceranib, according to these criteria, the dog had evidence of progressive disease, because of the presence of two new pulmonary nodules seen on thoracic radiographs. Nevertheless, the owners elected to continue toceranib therapy and thoracic radiography repeated 1 month later, and every 2–3 months thereafter, showed stable disease was again achieved, with the overall tumour burden still < 30% reduced compared with 34 months previously when toceranib was started. The mechanism for this apparent change in the responsiveness of the disease from progressive to stable is unknown. In addition, although RECIST is a valuable standardised protocol for assessing response to therapy in solid tumours in veterinary cancer patients, challenges may be encountered when these guidelines are applied to therapeutics such as small molecule inhibitors, monoclonal antibodies and other immunotherapies that have different mechanisms of action from traditional cytotoxic agents.[26]

A number of poor prognostic factors have been identified for dogs with appendicular osteosarcoma, including elevated serum total or bone ALP at diagnosis,[13,27–31] histologically high-grade tumours (i.e. grade III tumours),[13] presence of regional lymph node[32] and/or distant[6,8] metastasis, higher numbers of circulating monocytes (> 0.4×10^3 cells/µL) and lymphocytes (> 1.0×10^3 cells/µL) before treatment[30] and high body weight.[31] Age at the time of diagnosis has also been shown to affect prognosis, with survival times greatest for dogs aged 7–10 years and shorter for both younger and older dogs.[3,30] There is some conflicting evidence in the literature with regard to the prognostic significance of osteosarcoma arising from the proximal humerus. Some investigators have reported decreased survival times and/disease-free intervals with osteosarcomas noted in this location,[29–31,33,34] but more recent studies did not show a statistically significant difference compared with osteosarcoma in other locations.[20,35–37] However, it can be speculated that osteosarcomas located in the proximal humerus may be associated with a worse prognosis, because this site may allow for more advanced local growth before diagnosis.[33,38–40]

The dog reported here had some poor prognostic factors identified at the time of presentation, including an elevated serum ALP, circulating lymphocytes > 1.0×10^3 cells/µL and primary tumour location in the proximal humerus. In addition, the dog developed pulmonary metastases 10.5 months after amputation. Good prognostic factors recognised in this dog included intermediate grade (grade II) of osteosarcoma, circulating monocytes < 0.4×10^3 cells/µL, lower body weight and older age at the time of diagnosis. Despite the poor prognostic factors, the dog had long-term survival after treatment with toceranib and was still alive at the time of writing, 34 months after detection of pulmonary metastases. This suggests that not all canine osteosarcomas cases with poor prognostic factors, and subsequently developing pulmonary metastases, will have a poor outcome.

CONCLUSION

Despite various available treatment options, metastatic osteosarcoma is generally associated with a grave prognosis. The present case report demonstrates that long-term survival in a dog with pulmonary metastatic osteosarcoma treated with toceranib, and that toceranib can be well tolerated long term. Further studies

on the use of toceranib in the treatment of macroscopic pulmonary metastasis in canine osteosarcoma would be of interest.

ACKNOWLEDGMENT

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