Veterinary and Comparative Oncology

Original Article

DOI: 10.1111/j.1476-5829.2012.00355.x

Evaluation of ifosfamide salvage therapy for metastatic canine osteosarcoma

K. Batschinski, N. G. Dervisis and B. E. Kitchell

Veterinary Medical Center, Center for Comparative Oncology, Michigan State University, East Lansing, MI, USA

Abstract

A retrospective study was performed to assess toxicity and response rate of ifosfamide salvage treatment for dogs diagnosed with metastatic osteosarcoma (OSA). Dogs diagnosed with OSA and previously treated with standard chemotherapy were included in the study. Nineteen dogs met the inclusion criteria, and 17 dogs were evaluable for response. Ifosfamide doses ranged from 375 to 425 mg m^{-2} (median dose 375 mg m^{-2}), with a median of two doses administered per dog (range 1–7 doses). The overall response to ifosfamide was 11.8% [complete response (CR) = 1/17, partial response (PR) = 1/17, stable disease (SD) = 2/17, progressive disease (PD) = 13/17]. Two dogs were hospitalized due to ifosfamide toxicosis. The median survival duration from the first dose of ifosfamide to death was 95 days. Ifosfamide was well tolerated, but minor anti-tumour activity was observed.

Keywords
canine, chemotherapy, ifosfamide, metastasis, osteosarcoma

Introduction

Osteosarcoma (OSA) is the most common primary bone tumour in dogs. It comprises 85% of all canine skeletal neoplasms and 5–6% of all canine malignancies.[1–5] OSA is a locally invasive and highly metastatic disease. Microscopic metastasis is thought to arise early in the course of the disease, with variable time to progression of micrometastatic lesions. Less than 15% of dogs have radiographically detectable pulmonary or osseous metastasis at presentation, but development of pulmonary metastasis is typically the ultimate cause of death.[6–8] It appears that the addition of certain chemotherapy agents after surgery to remove the primary tumour increases both time to onset of gross metastases and overall survival duration. Dogs treated with surgery alone have a median survival duration that ranges from 134 to 175 days,[8–11] whereas patients treated with adjuvant chemotherapy protocols have median survival durations that range from 262 to 540 days.[10–18] Grossly metastatic OSA is associated with a grave prognosis, however. In one study, dogs with OSA, metastatic to any body site at the time of diagnosis, had a median survival time of 76 days, regardless of type of treatment attempted. In this same study, dogs with metastasis to bone and dogs that were treated palliatively with radiation therapy and chemotherapy had the longest survival times.[19] In a second study, the median survival duration of a population of 10 dogs with histologic evidence of regional lymph node metastasis at the time of amputation was 59 days, despite the fact that 9/10 dogs were treated with chemotherapy in an adjuvant setting.[20] The response rates and duration of response have been disappointing for dogs with gross metastatic OSA treated with several chemotherapy drugs. Single agent therapy with doxorubicin, cisplatin or mitoxantrone was used to treat 45 dogs with measurable metastatic OSA. In this study, only one dog achieved a partial response that lasted for 21 days and the remaining dogs had progression of disease.[21] Poirier *et al.*[22] utilized paclitaxel as a salvage chemotherapy for nine dogs with metastatic OSA. Two of these dogs had partial response, two dogs had stable disease and the rest of the dogs had progressive disease.

Ifosfamide [3-(2-chloroethyl)-2-[(2-chloroethyl)-amino]tetrahydro-2 H-1,3,2-oxazaphosphor

Correspondence address:
Dr K. Batschinski
Veterinary Medical Center
Center for Comparative Oncology
Michigan State University
736 Wilson Road
East Lansing, MI 48842, USA
e-mail:
batska@cvm.msu.edu

in-2-oxide] belongs to the nitrogen mustard family of cell cycle phase non-specific cytotoxic alkylating agents. Ifosfamide is considered to be one of the most active single agent chemotherapy drugs available for the treatment of OSA in humans.[23,24] Several reports have demonstrated that ifosfamide has a beneficial effect even for human patients who have failed standard OSA therapy.[25–27] The reported response rates when ifosfamide is given at standard doses ($<9\,g\,m^{-2}$ treatment^{-1}) ranges from 10 to 30%.[25] The incorporation of sodium 2-mercaptoethanesulfonate (MESNA) to protect against sterile haemorrhagic cystitis and the addition of granulocyte colony stimulating factor (G-CSF) for bone marrow support have allowed this drug to be dose escalated. However, the appearance of additional dose-limiting toxicities, including high doses of nephrotoxicity and neurotoxicity,[28] have been noted. When ifosfamide is given at high doses ($12–18\,g\,m^{-2}$ treatment^{-1}) in humans with OSA, the response rates range from 27% to 62.5% with a median duration of response of 5.5 to 15 months, suggesting a potential benefit over standard doses of ifosfamide.[24,25,27,29] In veterinary medicine, there is a published report evaluating the efficacy of ifosfamide against several canine tumour types, including OSA. Rassnick *et al*. indicated that complete responses were seen in one dog with metastatic leiomyosarcoma of the urinary bladder and in one dog with metastatic cutaneous haemangiosarcoma. The former dog died of other causes 549 days after receiving three treatments with ifosfamide at $350\,mg\,m^{-2}$ and the latter dog was still alive without recurrence of disease 445 days after receiving three treatments with ifosfamide at $375\,mg\,m^{-2}$. One dog with lymphoma had a partial response to ifosfamide given at $375\,mg\,m^{-2}$ for 112 days. No responses were noted in dogs that received ifosfamide at dosages $<350\,mg\,m^{-2}$ and Rassnick *et al.*[30] concluded that a dosage of $350–375\,mg\,m^{-2}$ of ifosfamide administered every 21 days appears to be appropriate for tumour-bearing dogs.

The purpose of this retrospective study is to evaluate the toxicity, response rate and duration of response for dogs with newly diagnosed metastatic OSA treated with ifosfamide after failure of standard chemotherapy protocols.

Materials and methods

The study was performed in compliance with the Michigan State University Institutional Animal Care and Use Committee guidelines for using medical record data of animals, and owner informed consent was obtained before treating a dog with ifosfamide. Dogs were eligible for inclusion in the study if they met the following criteria: (1) had a diagnosis of OSA at any anatomic location; (2) had newly diagnosed metastatic disease to any anatomical site and (3) had prior treatment with any standard chemotherapy protocol.

Dogs were diagnosed with primary OSA through cytologic or histologic evaluation. Metastasis was inferred by board certified radiologists' interpretation of standard diagnostic imaging tests, including thoracic and skeletal radiographs and abdominal ultrasonographic evaluation. When possible, metastasis was confirmed cytologically or histologically. Palliative and supportive treatments (radiation to the primary or metastatic bone tumour sites, non-steroidal anti-inflammatory drugs, bisphosphonates, opioids, gamma-aminobutyric acid (GABA) analogues, anti-nausea and anti-diarrhoeal medications, and antibiotics) were allowed while on ifosfamide therapy. Exclusion criteria included patients with insufficient follow-up in the medical record.

Patient data collected included: signalment; weight; affected primary site (appendicular versus axial); presence of concurrent diseases; serum alkaline phosphatase (ALP) before and after ifosfamide therapy; type (surgery versus radiation therapy) and intent of local treatment (palliative versus curative) for the primary tumour; first line chemotherapy protocol; time to progression; concurrent medications with ifosfamide therapy; location of metastasis before ifosfamide treatment; dose, frequency and number of ifosfamide cycles; and toxicity, response and response duration associated with ifosfamide treatment.

Initial diagnostic work-up and clinical staging at the time of diagnosis of OSA included complete blood count (CBC), serum biochemistry analysis, urinalysis, tumour biopsy or cytology and thoracic radiographs. Abdominal ultrasound

was also performed when deemed indicated at the clinician's discretion.

Diagnostic work-up prior to ifosfamide administration included CBC, serum biochemistry analysis, urinalysis and thoracic radiographs. Abdominal ultrasound and skeletal radiographs were performed at the clinician's discretion.

Ifosfamide was given at an initial dosage of 375 mg m^{-2} as previously described by Rassnick *et al.*[30] Dose escalations up to 425 mg m^{-2} in 25 mg m^{-2} increments were performed at the clinician's discretion. MESNA was given as an IV bolus at a dosage equal to 20% of the calculated ifosfamide dosage at time 0, 2 and 5 h after ifosfamide administration for urothelial protection.[30] An indwelling intravenous catheter was placed in all dogs. Dogs received a bolus of MESNA, followed by diuresis with 0.9% NaCl at 18.3 mL kg^{-1} h^{-1} for 30 min, and then ifosfamide was given over 30 min. Saline diuresis was continued for 5 h, with repeated doses of MESNA as described above. Treatments were continued every 21 days for as long as the dogs had a complete response, partial response or stable disease, and tolerated the chemotherapy. Toxicosis associated with ifosfamide therapy was graded according to the Veterinary Co-Operative Oncology Group Common Terminology Criteria for Adverse Events criteria.[31] CBC, serum biochemistry profiles and urinalysis were performed before each ifosfamide treatment and a CBC was done 7 days post-ifosfamide therapy either at Michigan State University or by the referring veterinarian. The extent of neoplastic disease was determined immediately before the first dose of ifosfamide, and every 3–6 weeks thereafter. Thoracic radiographs were used for determination of intra-thoracic largest tumour diameter, abdominal ultrasound for intra-abdominal largest tumour diameter and physical exam with calliper measurement for largest tumour diameter for external disease. A complete response was defined as disappearance of all measurable disease. A partial response was defined as >50%, but <100% reduction in measurable disease. Stable disease was defined as <50% reduction in measurable disease with no appearance of new lesions during that period. Progressive disease was defined as >25% increase in size of measurable metastatic lesions, or the appearance of new lesions.

First line chemotherapy protocol time to progression was defined as the duration from diagnosis to the time of documented progression of disease. Ifosfamide survival duration was defined as the time of first ifosfamide treatment to date of death. For the purposes of survival calculations, dogs that had neoplasia-related death were considered completed events, and dogs lost to follow-up were censored. Outcomes were determined by the use of the Kaplan–Meier product-limit method. Log rank test and Cox regression were used for univariate and multivariate analysis of potential risk factors, respectively. The risk factors included ALP at diagnosis, ALP at time of ifosfamide treatment, type of local treatment and anatomical site of metastasis before ifosfamide treatment. For the Cox regression, the potential risk factors were entered in the regression model in a forward fashion if their $P < 0.05$ and removed if $P > 0.1$. A P value of <0.05 was considered to be statistically significant. Commercially available software was used for all statistical calculations (MedCalc Software 10.2.0.0, Mariakerke, Belgium).

Results

Patients

A total of 19 dogs were included in this retrospective study, which was conducted between 2003 and 2008. The breeds that were represented included: mixed breed dogs (five); golden retrievers (three); greyhounds (two) and English springer spaniels (two). The remaining seven dogs consisted of one of each of the following breeds: Labrador retriever, German shepherd, Newfoundland, English mastiff, Chesapeake Bay retriever, German short hair pointer and doberman pinscher. The median age at the time of diagnosis was 8.9 years (3–12 years) and the median weight was 38.6 kg (10–79 kg). Eleven dogs were spayed females, one dog was an intact female and seven were neutered male dogs.

Staging results at initial diagnosis, primary tumour location and first line therapy

The diagnosis of OSA was confirmed by histopathology in 16 dogs and by cytology in 3 dogs. No evidence of metastatic disease was found

Table 1. First-line local therapy of dogs diagnosed with osteosarcoma at the time of initial diagnosis

	Location	No. of dogs	Surgery	Full course RT	Palliative RT
Appendicular	Proximal humerus	6	5	–	1
	Distal radius	4	2	–	2
	Proximal tibia	2	2	–	–
	Distal femur	3	3	–	–
Axial	Maxilla	1	–	1	–
	Mandible	1	1	–	–
	Zygomatic arch	1	1[a]	–	–
Extraskeletal	Mammary gland	1	1	–	–

[a]Incomplete excision.

on thoracic radiographs. Seven of the 10 dogs that had an abdominal ultrasound had abnormalities noted on the scan, which included hyperechoic liver nodules (3/7), hypoechoic splenic nodules (2/7), microhepatica (1/7) and spleen with mixed echogenicity (2/7). All of these splenic and hepatic changes were aspirated and cytology results were consistent with either lymphoid hyperplasia or extramedullary haematopoiesis, indicating no evidence of metastatic disease. All dogs that had limb amputation had regional lymph node evaluation by histopathology, of which none had metastasis at the time of OSA diagnosis.

At the time of initial diagnosis, most of the dogs had appendicular OSA at metaphyseal sites as the primary tumour location (15/19), of which the proximal humerus (6/15) and distal radius (4/15) were the most commonly affected areas. Three dogs had axial OSA (one of each at rostral maxilla, mandible and zygomatic arch) and one dog had extraskeletal OSA of the mammary gland.

Primary OSA lesions were treated with local therapy, using either surgery (amputation, mandibulectomy, partial resection of the zygomatic arch, mastectomy) or radiation therapy, followed by systemic chemotherapy prior to ifosfamide administration (Tables 1 and 2).

Ifosfamide therapy

Staging results prior to ifosfamide therapy

All dogs had a CBC, serum biochemistry analysis, urinalysis and thoracic radiographs performed prior to ifosfamide administration. Abdominal ultrasound and skeletal radiographs were performed in 7/19 and 4/19 dogs, respectively.

Results of the diagnostic work-up performed after failure of standard chemotherapy protocols revealed that lungs were the most common organ affected by metastasis (15/19). Other metastatic sites prior to ifosfamide administration included ribs (2/19), liver (2/19), other appendicular sites (2/19), axial sites (2/19), subcutaneous tissues

Table 2. First-line chemotherapy protocols of dogs diagnosed with osteosarcoma (OSA) at the time of initial diagnosis

First-line chemotherapy protocol	No. of dogs	Schedule of administration
Doxorubicin + carboplatin	10	– Given IV on an alternating basis every 21 days
Doxorubicin + cisplatin	3	
Actinomycin-D + carboplatin	1	
Actinomycin-D + cisplatin	1	
Single agent carboplatin	3	– Given IV every 21 days
Doxorubicin + carboplatin + cyclophosphamide	1	– Doxorubicin and carboplatin were given IV on an alternating basis every 21 days – Cyclophosphamide was given PO on days 3, 4, 5 and 6 after each doxorubicin administration

Dogs treated with surgery for their primary tumour had chemotherapy 2 weeks post surgery, at the time of staple removal. The dog that had full course radiation therapy for a maxillary OSA and the three dogs that were treated with palliative radiation therapy for their primary tumour received chemotherapy 3 weeks and 1 week after the diagnosis, respectively.

(2/19), spleen (1/19) and lymph node (1/19). Five dogs had more than one concurrent metastatic site.

Concurrent palliative and supportive treatments with ifosfamide

Two dogs were treated with palliative radiation therapy (3 × 8 Gy) for bone metastasis lesions while on ifosfamide therapy. Both of these dogs had other non-irradiated metastatic sites that were used to evaluate response to ifosfamide.

Three dogs were treated with bisphosphonates concurrently with ifosfamide for skeletal metastasis. Pamidronate was used in two of these dogs and it was given intravenously over 2 h at 1 mg kg^{-1}. The remaining dog received alendronate orally at 1 mg kg^{-1} once a day for 14 days, every other day for 14 days and then once weekly until death. Pamidronate was administered 1 week after the administration of ifosfamide, whereas alendronate was started at the time of first ifosfamide treatment. None of the ifosfamide responders were treated with bisphosphonates.

Other palliative treatments while on ifosfamide therapy included non-steroidal anti-inflammatory drugs, pain control medications, anti-nausea and anti-diarrhoeal medications, and antibiotics (Table 3).

Ifosfamide administration

Dogs received a median of two doses (range 1–7 doses) of ifosfamide chemotherapy. Overall, 37 doses of ifosfamide were administered. All dogs received their first dose of ifosfamide at a dose of 375 mg m^{-2}. Of the 10 dogs that received more than a single dose of ifosfamide, 3 dogs had dose escalation. Of these, two dogs received their second dose at 400 mg m^{-2} and one dog received a third dose at 425 mg m^{-2}.

Ifosfamide toxicity

Nineteen dogs could be evaluated for ifosfamide toxicity. Ifosfamide therapy was generally well tolerated, although mild constitutional, haematological, gastrointestinal and renal toxicities were observed (Table 4). None of the three dogs that had ifosfamide dose escalation developed treatment-related toxicities. None of the dogs died of ifosfamide toxicity. Neutropenia was the most common toxicity noted, but only one dog developed a grade IV neutropenia with sepsis. This dog was treated with supportive care, and was discharged after 24 h of hospitalization. One dog developed grade II fever in the absence of grade III/VI neutropenia and had a soft tissue swelling on the left radius 7 days after the administration of ifosfamide. The left radius was the site of a prior fracture; the dog recovered within 24 h with intravenous fluid therapy and antibiotics. None of the dogs experienced life threatening gastrointestinal toxicities. Mild gastrointestinal toxicities noted were responsive to symptomatic therapy for vomiting and diarrhoea. Proteinuria with urine protein/creatinine ratio of 6.5 (grade III) was noted in one dog after the first dose of ifosfamide. This dog had creatinine

Table 3. Supportive medications while on ifosfamide therapy

Supportive treatment	Medications	No. of dogs
Non-steroidal anti-inflammatory drugs	Deracoxib	4
	Carprofen	2
	Firocoxib	1
	Piroxicam	1
Antibiotics	Amoxicillin Clavulanate	1
	Enrofloxacin	1
Pain-control medications	Tramadol	5
	Fentanyl Patch	2
	Gabapentin	3
Anti-nausea medications	Metoclopramide	12
	Maropitant Citrate	1
Anti-diarrhoeal medications	Metronidazole	2

Table 4. Toxicosis associated with ifosfamide therapy for dogs with metastatic osteosarcoma according to the VCOG-CTCAE criteria

Grade of Adverse Event		I	II	III	IV	V
Bone marrow	Anaemia	1	–	–	–	–
	Neutropenia	1	3	–	1	–
	Thrombocytopenia	–	1	–	–	–
Gastro intestinal	Diarrhoea	1	–	–	–	–
	Vomiting	1	–	–	–	–
	Anorexia	–	2	–	–	–
Constitutional signs	Fever	–	1	–	–	–
Renal	Creatinine	–	1	–	–	–
	Blood urea nitrogen	1	–	–	–	–
	Proteinuria	–	–	1	–	–

and blood urea nitrogen within the reference range prior to ifosfamide administration and no specific treatment was initiated. This dog received a total of three doses of ifosfamide until progression of the metastatic OSA was noted. After the third ifosfamide dose, the dog was noted to be azotemic. Blood urea nitrogen and creatinine displayed grade I and grade II toxicity, respectively. The dog died 2 weeks after progression of OSA, and post-mortem evaluation was not performed. Thus, the definitive cause of azotemia could not be elucidated.

None of the dogs developed clinical signs or any evidence of microscopic haematuria in the urine sediment that would lead to the suspicion of sterile haemorrhagic cystitis. Neurological signs were not observed in this group of patients.

Ifosfamide overall response rate

Seventeen of the 19 dogs could be evaluated for response to ifosfamide treatment. Two dogs were censored from response as being lost to follow-up, since they were never restaged after the administration of ifosfamide. One dog was lost to ifosfamide time to progression due to the addition of alternating doxorubicin with dexrazoxane after the dog attained a complete response on ifosfamide therapy. Overall response rate was 11.8% (2/17). One dog had a complete response, 1 dog had a partial response, 2 dogs had stable disease and 13 dogs had progressive disease while on ifosfamide therapy. None of the responders had ifosfamide dose escalation. The duration of response for the dogs that achieved a partial or complete response was 137 days and censored at 21 days, respectively. The duration of the stable disease was 158 and 45 days in two dogs. No risk factors for response to treatment were identified in our study population.

Ifosfamide survival duration

At the time of data analysis, all 19 dogs were dead (16 were euthanized and 3 died). Three dogs had post-mortem examination to confirm that they died of metastatic OSA, whereas in the remaining dogs the cause of death related to metastatic OSA was presumptive on the basis of physical examination findings, thoracic radiographs and/or abdominal ultrasounds.

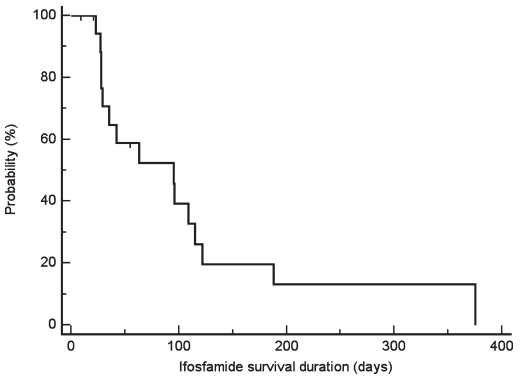

Figure 1. Median ifosfamide survival duration was 95 days, $N = 19$.

All 19 dogs were included in the ifosfamide survival duration analysis, with 3 dogs censored. Two of these three dogs were censored because of being lost to follow-up. The other remaining dog was censored for survival duration due to the addition of alternating doxorubicin with dexrazoxane after the dog attained a complete response on ifosfamide therapy. Thus, this dog's response duration of 332 days could not be fully attributed to ifosfamide therapy and the dog was censored at 21 days for evaluation of ifosfamide single agent efficacy for purposes of this report. The median survival duration for ifosfamide treated dogs was 95 days (range, 9–375) (Fig. 1). No risk factors for ifosfamide survival duration were identified in our study population.

Discussion

Metastasis is the leading cause of death for dogs with OSA. Studies regarding the effectiveness of second-line chemotherapy for canine metastatic OSA are currently limited in veterinary literature. Here, we show that ifosfamide therapy was well tolerated, but had only minor anti-tumour effect when used in this setting.

Our study demonstrates that ifosfamide therapy is generally well tolerated, although toxicities were observed. Two dogs required hospitalization as a consequence of possible ifosfamide toxicosis. One dog developed proteinuria after the first treatment of ifosfamide. Unfortunately, kidney biopsy was not allowed by the dog's owner, therefore, the cause of proteinuria could not be identified. Ifosfamide treatment in humans is associated with an incidence

of renal toxicity ranging from 5 to 30% and single high doses can result in renal failure within a few days of administration.[28–32] Ifosfamide has mainly been associated with proximal renal tubular dysfunction resembling Fanconi-like syndrome. Decreased glomerular filtration rate and distal tubular damage have also been reported in people. The manifestations of ifosfamide nephrotoxicity, include phosphaturia, hypophosphatemia, glycosuria, aminoaciduria, elevation of serum alkaline phosphatase, hypokalemia, renal tubular acidosis, defective concentrating capacity and decreased glomerular filtration rate.[33,34] Another important ifosfamide-specific toxicity in humans is sterile haemorrhagic cystitis, which is typically manifested by gross or microscopic haematuria.[35] Clinical signs and urine abnormalities consistent with sterile haemorrhagic cystitis were not seen in this population of dogs, and this complication may have been prevented by the use of MESNA and diuresis. Owing to the potential for nephrotoxicity and sterile haemorrhagic cystitis, monitoring of renal function by performing chemistry profile and urinalysis prior to each ifosfamide treatment should be recommended for dogs being treated with this chemotherapeutic drug. Ifosfamide-induced neurotoxicity (encephalopathy) has been reported in the human oncology literature to affect 15–30% of people receiving this chemotherapy drug. Hallucinations, mental confusion, seizures and coma are possible encephalopathy manifestations in humans.[36] Although neurotoxicity was not noted in this study, dogs should be monitored closely for any abnormal neurological clinical sign.

The overall response rate to ifosfamide in this study was 11.8%. The minor activity of ifosfamide observed in our patients may be partially explained by the fact that the dose of ifosfamide per treatment was suboptimal, being approximately 10 times lower than what is used in human OSA therapy. The ifosfamide dose used in humans is $<9\,g\,m^{-2}$ treatment^{-1} course if the drug is administered without bone marrow G-CSF support.[25] This, along with the minimal toxicities observed in our population, suggests the need to perform a phase I trial to determine the maximum-tolerated dose (MTD) of ifosfamide in dogs and to better describe toxicities associated with this treatment. Once the MTD of ifosfamide is established, a second phase I trial to determine the MTD of ifosfamide given concurrently with G-CSF might be conducted. Eventually, a two-arm phase II trial could be completed to observe if the combination of ifosfamide and G-CSF is superior to single agent ifosfamide in dogs with metastatic OSA.

Most of the dogs in our study had large metastatic tumour burdens at study entry, which may have contributed to the observed low rate of treatment response. More frequent patient re-evaluation and staging, such as monthly physical exams and thoracic radiographs, and quarterly abdominal ultrasounds, should be utilized in dogs with OSA so that less advanced metastatic disease can be detected and second-line chemotherapy started in a setting of minimal disease burden.

Our report suffers from limitations that are inherent in retrospective studies, including the small sample size, diagnoses of OSA established by cytology only in some patients, and the heterogeneous nature of the OSA lesions seen in these patients. These issues may limit the interpretation of the impact of ifosfamide on this patient cohort.

In conclusion, this study demonstrates that ifosfamide provides minor tumour response without causing significant toxicity in what is currently the uniformly bleak setting of metastatic OSA in the dog. Future studies should utilize higher doses, possibly in combination with haematopoietic growth factor support, in a dose intense schedule to determine the true efficacy potential of this chemotherapy drug against metastatic OSA.

References

1. Ling GV, Morgan JP and Pool RR. Primary bone tumors in the dog: a combined clinical, radiological, and histologic approach to early diagnosis. *Journal of the American Veterinary Medical Association* 1974; **165**: 55–67.
2. Lui SK, Dorfman HD and Hurvitz AI. Primary and secondary bone tumors in the dog. *Journal of the American Veterinary Medical Association* 1977; **18**: 313–326.
3. Brodey RS, Sauer RM and Medway W. Canine bone neoplasms. *Journal of the American Veterinary Medical Association* 1963; **143**: 471–495.
4. Brodey RS, McGrath JT and Reynolds H. A clinical and radiological study of canine bone neoplasms:

Part I. *Journal of the American Veterinary Medical Association* 1959; **134**: 53–71.
5. Brodey RS and Riser WH. Canine osteosarcoma: a clinicopathological study of 194 cases. *Clinical Orthopaedics and Related Research* 1969; **62**: 54–64.
6. Chun R and deLorimier L-P. Update on the biology and management of canine osteosarcoma. *The Veterinary Clinics* 2003; **33**: 491–516.
7. Bacon NJ, Ehrhart NP, Dernell WS, Lafferty M and Withrow SJ. Use of alternating administration of carboplatin and doxorubicin in dogs with micrometastases after amputation for appendicular osteosarcoma: 50 cases (1999–2006). *Journal of the American Veterinary Medical Association* 2008; **232**: 1504–1510.
8. Spodnick GJ, Berg RJ, Rand WM, Schelling SH, Couto G, Harvey HJ, Henderson RA, MacEwen G, Mauldin N, McCaw DL, Moore AS, Morrison W, Norris AM, O'Obradovich J, O'Keefe DA, Page R, Ruslander D, Klausner J, Straw RC, Thompson JP and Withrow SJ. Prognosis for dogs with appendicular osteosarcoma treated by amputation alone: 162 cases (1978–1988). *Journal of the American Veterinary Medical Association* 1992; **200**: 995–999.
9. Mauldin GN, Matus RE, Withrow SJ and Patnaik AK. Canine osteosarcoma: treatment by amputation versus amputation and adjuvant chemotherapy using doxorubicin and cisplatin. *Journal of Veterinary Internal Medicine* 1988; **2**: 177–180.
10. Thompson JP and Fugent MJ. Evaluation of survival times after limb amputation with and without subsequent administration of cisplatin, for treatment of appendicular osteosarcoma in dogs: 30 cases (1979–1990). *Journal of the American Veterinary Medical Association* 1992; **200**: 531–533.
11. Shapiro W, Fossum TW, Kitchell BE, Couto CG and Theilen GH. Use of cisplatin for treatment of appendicular osteosarcoma in dogs. *Journal of the American Veterinary Medical Association* 1988; **192**: 507–511.
12. Chun R, Kurzman ID, Couto CG, Klausner J, Henry C and MacEwen EG. Cisplatin and doxorubicin combination chemotherapy for the treatment of canine osteosarcoma: a pilot study. *Journal of Veterinary Internal Medicine* 2000; **14**: 495–498.
13. Berg J, Weinstein MJ, Scheling SH and Rand WM. Treatment of dogs with osteosarcoma by administration of cisplatin after amputation or limb-sparing surgery: 22 cases (1987–1990). *Journal of the American Veterinary Medical Association* 1992; **200**: 2005–2008.
14. Morello E, Buracco P, Martano M, Peirone B, Capurro C, Valazza A, Cotto D, Ferracini R and Sora M. Bone allografts and adjuvant cisplatin for the treatment of canine appendicular osteosarcoma in 18 dogs. *The Journal of Small Animal Practice* 2001; **42**: 61–66.
15. Kraegel SA, Madewell BR, Simonson E and Gregory CR. Osteogenic sarcoma and cisplatin chemotherapy in dogs: 16 cases (1986–1989). *Journal of the American Veterinary Medical Association* 1991; **199**: 1057–1059.
16. Straw RC, Withrow SJ, Richter SL, Powers BE, Klein MK, Postorino NC, LaRue SM, Ogilvie GK, Vail DM, Morrison WB, McGee M and Dickinson K. Amputation and cisplatin for treatment of canine osteosarcoma. *Journal of Veterinary Internal Medicine* 1992; **5**: 205–210.
17. Bergman PJ, MacEwen EG, Kurzman ID, Henry CJ, Hammer AS, Knapp DW, Hale A, Kruth SA, Klein MK, Klausner J, Norris AM, McCaw D, Straw RC and Withrow SJ. Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991–1993). *Journal of Veterinary Internal Medicine* 1996; **10**: 76–81.
18. Berg J, Weinstein MF, Sprinfield DS and Rand WM. Results of surgery and doxorubicin chemotherapy in dogs with osteosarcoma. *Journal of the American Veterinary Medical Association* 1995; **206**: 1555–1560.
19. Boston SE, Ehrhart NP, Dernell WS, Lafferty M and Withrow SJ. Evaluation of survival time in dogs with stage III osteosarcoma that undergo treatment: 90 cases (1985–2004). *Journal of the American Veterinary Medical Association* 2006; **228**: 1905–1908.
20. Hillers KR, Dernell WS, Lafferty MH, Withrow SJ and Lana SE. Incidence and prognostic importance of lymph node metastasis in dogs with appendicular osteosarcoma: 228 cases (1986–2003). *Journal of the American Veterinary Medical Association* 2005; **226**: 1364–1367.
21. Ogilvie GK, Straw RC, Jamerson VJ, Walters LM, Lafferty MH, Powers BE and Withrow SJ. Evaluation of single agent chemotherapy for the treatment of clinically evident osteosarcoma metastasis in dogs: (1987–1991). *Journal of the American Veterinary Medical Association* 1993; **202**: 304–306.
22. Poirier VJ, Hershey AE, Burgess KE, Phillips B, Turek MM, Forrest LJ, Beaver L and Vail DM. Efficacy and toxicity of paclitaxel (Taxol) for the treatment of canine malignant tumors. *Journal of Veterinary Internal Medicine* 2004; **18**: 219–222.
23. Harris MB, Geiser P, Goorin AM, Ayala A, Shochat SJ, Ferguson WS, Holbrook T and Link MP. Treatment of metastatic osteosarcoma at diagnosis:

a Pediatric Oncology Group study. *Journal of Clinical Oncology* 1998; **16**: 3641–3648.
24. Harris MB, Cantor AB, Goorin AM, Shochat SJ, Ayala AG, Ferguson WS, Holbrook T and Link MP. Treatment of osteosarcoma with ifosfamide: comparison of response in pediatric patients with recurrent disease versus patients previously untreated: a Pediatric Oncology Group study. *Medical and Pediatric Oncology* 1995; **24**: 87–92.
25. Berrak SG, Pearson M, Berberoglu S, Ilhan IE and Jaffe N. High-dose ifosfamide in relapsed pediatric osteosarcoma: therapeutic effects and renal toxicity. *Pediatric Blood & Cancer* 2005; **44**: 215–219.
26. Chou AJ, Merola PR, Wexler LH, Gorlick RG, Vyas YM, Healey JH, LaQuaglia MP, Huvos AG and Meyers PA. Treatment of osteosarcoma at first recurrence after contemporary therapy. *Cancer* 2005; **104**: 2214–2221.
27. Marti C, Kroner T, Remagen W, Berchtold W, Cserhati M and Varini M. High-dose ifosfamide in advanced osteosarcoma. *Cancer Treatment Reports* 1985; **69**: 115–117.
28. Ho PT, Zimmerman K, Wexler LH, Blaney S, Jarosinski P, Weaver-McClure L, Izraeli S and Balis FM. A prospective evaluation of ifosfamide-related nephrotoxicity on children and young adults. *Cancer* 1995; **76**: 2557–2564.
29. Cardoso AA, Ferreira CG and Mendes GQ. Pharmacoeconomic analysis on high dose ifosfamide for metastatic osteosarcoma. *Journal of Clinical Oncology ASCO Annual Meeting Proceedings* 2004; **22**: 9043.
30. Rassnick KM, Frimberger AE, Wood CA, Williams LE, Cotter SM and Moore AS. Evaluation of ifosfamide for treatment of various canine neoplasms. *Journal of Veterinary Internal Medicine* 2000; **14**: 271–276.
31. Veterinary Co-operative Oncology Group. Veterinary Co-Operative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. *Veterinary Comparative Oncology* 2004; **2**: 194–213.
32. Rossi R. Nephrotoxicity of ifosfamide – moving towards understanding the molecular mechanisms. *Nephrology, Dialysis, Transplantation* 1997; **12**: 1091–1092.
33. Loebstein R and Koren G. Ifosfamide-induced nephrotoxicity in children: critical review of predictive risk factors. *Pediatrics* 1998; **101**: 1–4.
34. Stohr W, Paulides M, Bielack S, Jurgens H, Treuner J, Rossi R, Langer T and Beck JD. Ifosfamide-induced nephrotoxicity in 593 patients: a report from the late effects surveillance system. *Pediatric Blood & Cancer* 2007; **48**: 447–452.
35. Schoenike SE and Dana WJ. Ifosfamide and mesna. *Clinical Pharmacokinetics* 1990; **9**: 179–191.
36. Brunello A, Basso U, Rossi E, Stefani M, Ghiotto C, Marino D, Crivellari G and Monfardini S. Ifosfamide-related encephalopathy in elderly patients: report of five cases and review of the literature. *Drugs & Aging* 2007; **24**: 967–973.

