# Postoperative Adjuvant Combination Therapy with Doxorubicin and Noncytotoxic Suramin in Dogs with Appendicular Osteosarcoma

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## ABSTRACT

**Although conventional treatment of dogs with osteosarcoma (OSA) by amputation and chemotherapy results in reported survival times (STs) of 262–413 days, no major improvements in STs have occurred in the past 2 decades. Suramin is a polysulfonated napthylurea, which at noncytotoxic concentrations *in vitro*, increases tumor sensitivity to chemotherapy, including doxorubicin. The study authors evaluated the combination of noncytotoxic suramin and doxorubicin after amputation in dogs with OSA. The hypothesis was that treatment of dogs with appendicular OSA with amputation, adjuvant doxorubicin, and noncytotoxic suramin would be well tolerated and result in STs at least comparable to those of doxorubicin alone. Forty-seven dogs received 6.75 mg/kg of suramin IV followed by 30 mg/m$^2$ of doxorubicin IV 4 hr later. Treatment was repeated *q* 2 wk for five doses. The median disease free time (DFI) was 203 days (range, 42–1,580+ days) and the median ST for all dogs was 369 days (range, 92–1,616+ days). There was no statistical difference in ST and DFI between greyhounds and nonngreyhounds. Adjuvant doxorubicin and noncytotoxic suramin was well tolerated in dogs with OSA following amputation. Additional studies are needed to determine if this combination treatment protocol provides additional clinical benefit compared with doxorubicin alone.** (*J Am Anim Hosp Assoc* 2014; 50:12–18. DOI 10.5326/JAAHA-MS-5958)

## Introduction

Osteosarcoma (OSA) is the most common primary bone neoplasm in dogs.[1] The treatment of choice for dogs with OSA is either amputation or limb-sparing surgery followed by either adjuvant single-agent or combination chemotherapy.[2–6] The median survival times (STs) for dogs with appendicular OSA treated by amputation alone ranges from 134 days to 175 days, whereas in dogs treated with surgery and single-agent chemotherapy, the median ST ranges from 262 days to 413 days.[2–11] Although preliminary data using combination chemotherapy after amputation were encouraging, resulting in a median ST of 471 days.[12] Subsequent studies by the same author (R. Chun) using the same protocol demonstrated that adjuvant combined doxorubicin and carboplatin therapy does not appear to offer any benefits over single-agent chemotherapy.[13]

Despite the use of adjuvant chemotherapy after either amputation or limb-sparing surgery, the STs for dogs with OSA have not improved markedly in the past 2 decades.[3–13]

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CBC *complete blood count;* DFI *disease-free interval;* FGF *fibroblast growth factor;* FS *fractional shortening;* OSA *osteosarcoma;* PD *polydipsia;* PU *polyuria;* ST *survival time*

Consequently, a novel, more effective treatment approach is needed. Resistance of tumor cells to chemotherapy appears to be a major challenge in dogs with OSA. Drug resistance is often multifactorial, and overexpression of drug efflux proteins is a common mechanism of resistance documented in preclinical studies.[14,15] However, clinical studies have been unable to corroborate that inhibition of the drug efflux proteins significantly improves the effectiveness of chemotherapy in human cancer patients, suggesting the existence of additional mechanisms of chemoresistance.

Previously, this study group demonstrated that acidic and basic fibroblast growth factor (FGF) receptors are involved in cancer chemoresistance.[16] Suramin, a polysulfonated naphthylurea, historically has been used for the treatment of certain African parasitic infections, such as Rhodesian and Gambian trypanosomiasis.[17] Suramin inhibits the binding of several polypeptide growth factors (e.g., platelet-derived growth factor, basic FGF, transforming growth factor-β, epidermal growth factor, and insulin-like growth factor 1) to their respective receptors.[18,19] The study authors also demonstrated that the chemoresistance conferred by FGFs is reversed in the presence of low and noncytotoxic concentrations of suramin (10–50 μM) *in vitro* and that the activity of doxorubicin in mouse xenograft prostate, lung, breast, and bladder tumors is enhanced by adding low doses of suramin. [20–27]

Recently, the authors studied the pharmacokinetics of noncytotoxic suramin in combination with doxorubicin in tumor-bearing dogs and evaluated the potential enhancement of doxorubicin activity without increasing toxicity.[28] In that same study, the authors determined that a median plasma suramin concentration of approximately 50 μM was achieved 3 hr after the end of the suramin infusion and, at the time, the doxorubicin infusion was started.[28]

Based on those previous studies, the authors hypothesized that the addition of noncytotoxic doses of suramin to adjuvant doxorubicin monotherapy would enhance DFIs and STs. As a first step to testing that hypothesis in prospective studies, the goals of this study were to evaluate the DFIs and STs after either amputation or limb-sparing surgery and adjuvant chemotherapy with noncytotoxic suramin and doxorubicin in dogs with spontaneously occurring OSA and to evaluate the toxicity of that protocol. Additionally, because The Ohio State University Veterinary Medical Center is intimately involved in the rescue of retired racing greyhounds, the authors evaluate a large number of greyhounds with OSA. Absorption and metabolism of some drugs is different in greyhounds than in nongreyhounds.[29] Consequently, the authors analyzed the greyhounds as a separate subgroup and compared differences in DFIs and STs between greyhounds and nongreyhounds.

## Materials and Methods

### Inclusion and Exclusion Criteria

Dogs with histologically diagnosed appendicular OSA and no evidence of metastases on thoracic radiographs were entered prospectively into the study. Dogs with documented decreased myocardial contractility (baseline fractional shortening [FS] $< 25\%$) were excluded from the study. For greyhounds, a FS of 22% was used as the lower limit based on the fact that greyhounds have lower FSs than other dog breeds.[30,31] Informed signed consent was obtained from the owners. This study was approved by the Veterinary Medical Center Hospital Board and by the Institutional Animal Care and Use Committee. Treatment was continued for five cycles either until the development of pulmonary metastases on thoracic radiographs or until unacceptable toxicity occurred. Dogs with unacceptable toxicity and those not completing the protocol were excluded for calculation of DFI and median ST but were included for assessment of toxicity.

### Evaluation, Treatment, and Monitoring

Samples for histopathology were obtained by either core biopsy or amputation. In patients referred with a previous histopathologic diagnosis of OSA, a board-certified veterinary pathologist at The Ohio State University College of Veterinary Medicine reviewed the slides to confirm the diagnosis. The primary tumor was surgically removed by either amputation or limb-sparing surgery and treated with adjuvant chemotherapy using a suramin/doxorubicin protocol postsurgically.

On the first treatment visit, patients were evaluated by physical examination; complete blood count (CBC); serum chemistry profile; urinalysis; thoracic radiographs, included right and left lateral views and either a ventrodorsal or dorsoventral; echocardiogram; and imaging targeted at any other specific clinical signs or physical examination findings prior to treatment. The physical examination and CBC were repeated before each treatment. A serum biochemical analysis, urinalysis, and thoracic radiographs were repeated before the third and fifth treatments, and an echocardiogram was repeated before the fifth treatment.

The treatment protocol consisted of suramin at a dose of 6.75 mg/kg (diluted to a final volume of 20 mL) given by IV infusion at a rate of 1 mL/min through a peripheral vein indwelling catheter. Four hr after completing the suramin infusion, doxorubicin was given at a dose of 30 mg/m$^2$ (diluted in saline solution (NaCl

0.9%) to a final concentration of 0.5 mg/mL) and administered as a slow, 30 min IV infusion using a 35 mL or 60 mL syringe through the same IV catheter. A total of five treatments were given at 2-wk intervals. The median time between surgery and starting chemotherapy was 14 days (range, 3–85 days).

### Long-Term Evaluation

Patients were re-evaluated at 3 mo, 6 mo, 9 mo, 12 mo, 18 mo, 21 mo, and 24 mo after last chemotherapy administration. The evaluation consisted of physical examination and thoracic radiographs, CBC, serum biochemical profiles, and urinalysis were performed when deemed necessary.

### Toxicity

Toxicity was evaluated using standard criteria.[32] Cardiac toxicity was evaluated by sequential echocardiography. FS was compared at each time point and compared with baseline values. Treatment was discontinued if FS was either $< 25\%$ for nongreyhounds or 22% for greyhounds. Doxorubicin dose reductions of 20% were instituted in patients with grade $\geq 3$ or 4 hematologic toxicity, patients with $> 2$ grade gastrointestinal toxicity, or in patients that developed grades 2, 3, or 4 gastrointestinal or hematologic toxicity with presence of fever.

### End Points

Because only dogs free of radiographic evidence of metastatic disease were entered in the study, standard response criteria (i.e., complete response, partial response, stable disease, progressive disease) were not used. Instead, end points were DFI, ST, and toxicity. DFI was defined as the time (in days) from surgery until either tumor relapse or evidence of metastases. ST was defined as the time (in days) from the surgery until either death or euthanasia. Death and euthanasia were qualified as either tumor related or nontumor related based on the results of necropsy (when performed).

### Statistical Analysis

DFIs and STs were estimated using the Kaplan-Meier method. DFIs and STs in greyhounds were compared with those of nongreyhounds using the log-rank test and Cox proportional hazards regression model. Univariate analyses were performed to assess for prognostic value of the different covariates using Cox proportional hazards regression models. Dogs were censored for DFI if no either evidence of metastases was found at the time of evaluation or death, and dogs were censored for ST if they were still alive at the time of data evaluation, if they were lost for follow-up, or died because of an unrelated cause. Statistical significance was established as $P \leq 0.05$[a,b].

## Results

Forty-seven dogs with appendicular OSA were enrolled from June 2003 through June 2006. Of those 47 dogs, 18 were females (15 spayed) and 29 were males (26 castrated). There were 21 greyhounds and 26 nongreyhounds (6 rottweilers, 6 mixed-breed dogs, 3 golden retrievers, 2 Doberman pinschers, and 1 each of the following breeds: Australian sheepdog, Bernese mountain dog, boxer, German shepherd dog, Great Pyrenees, Great Dane, Labrador retriever, mastiff, and Staffordshire bull terrier). Mean patient age was 7 yr (range, 2–12 yr) and mean body weight was 34.2 kg (range, 15.7–86.5 kg). The anatomic location of the tumor was proximal humerus (n = 17), distal radius (n = 10), distal tibia (n = 8), distal femur (n = 8), proximal tibia (n = 3), and midulna (n = 1).

Forty-six dogs underwent a limb amputation and one dog with a distal radius lesion had a limb-sparing procedure. The median interval between surgery and initiation of chemotherapy treatment was 14 days (range, 3–85 days).

Forty-three of the 47 dogs (24 nongreyhounds and 19 greyhounds) were included for DFI and ST analysis. At the time of data analysis, the median DFI for the 43 dogs was 203 days (mean, 296 days; range, 42–1,580+ days) as shown in **Figure 1** and the median ST for all dogs was 369 days (mean, 457; range, 92–1,616+ days) as shown in **Figure 2**. When the dogs were evaluated in two separate groups, the median DFI for the nongreyhounds (n = 24) was 224 days (mean, 368 days; range, 42–1,580+ days) and the median DFI for the greyhounds (n = 19) was 201 days

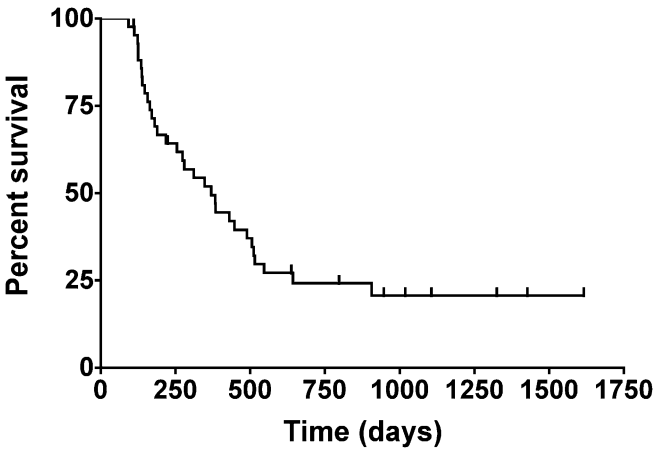


**FIGURE 1** *Disease-free interval (DFI) in 43 dogs with appendicular osteosarcoma (OSA) treated with suramin and doxorubicin after surgery (median DFI, 203 days). The small vertical lines in the curve correspond to censored patients.*

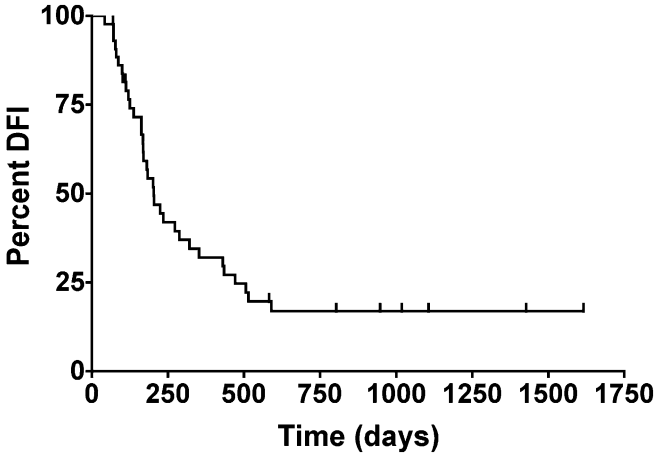


**FIGURE 2** *Survival time (ST) in 43 dogs with appendicular OSA treated with suramin and doxorubicin after surgery (median ST, 369 days). The small vertical lines in the curve correspond to censored patients.*

(mean, 337 days; range, 71–1,427+ days). There was no statistical difference in DFI between the greyhound and the nongreyhound groups (log-rank test, $P = 0.83$; **Figure 3**; Cox proportional hazards model, $P = 0.93$).

The median ST for the nongreyhounds was 383 days (mean, 451 days; range, 112–1,616+ days) and the median ST for the greyhounds was 369 days (mean, 438 days; range, 92–1,427+ days). There was no statistical difference in ST between the greyhound and the nongreyhound groups (log-rank test, $P = 0.99$; **Figure 4**, Cox proportional hazards model, $P = 0.99$). At the time of manuscript preparation, 3 of the 43 dogs were still alive at

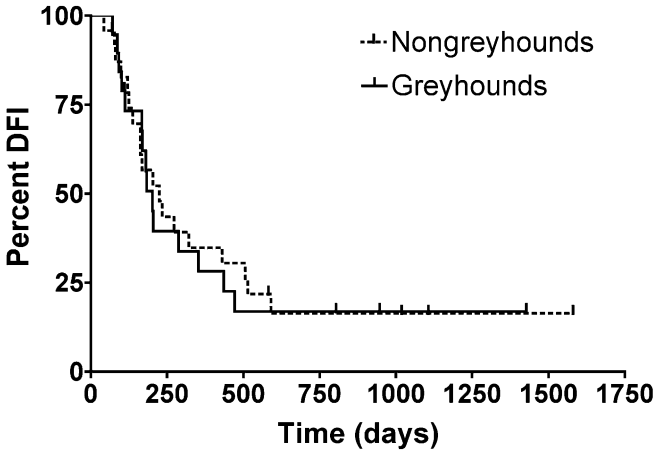


**FIGURE 3** *DFI of greyhounds (n = 19; median DFI, 201 days) and nongreyhounds with appendicular OSA (n = 24; median DFI, 224 days) treated with suramin and doxorubicin after surgery (P = 0.83). The small vertical lines in the curve correspond to censored patients.*

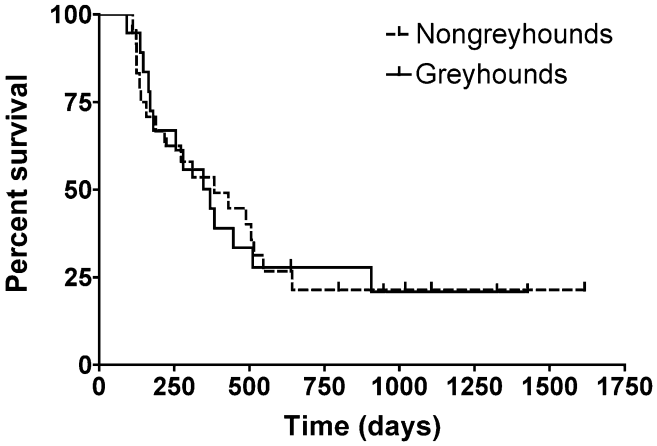


**FIGURE 4** *STs of greyhounds (n = 19; median ST, 369 days) and nongreyhounds (n = 24; median ST, 383 days) with appendicular OSA treated with suramin and doxorubicin after surgery (P = 0.99). The small vertical lines in the curve correspond to censored patients.*

1,616+ days, 1,427+ days, and 947+ days and 5 dogs were lost to follow-up at 92 days, 110 days, 443 days, 638 days, and 1,106 days with no evidence of metastases. Four dogs died of unrelated causes, three without evidence of metastases (at 311 days, 638 days, and 1,019 days, respectively) and one dog with unknown status of metastases at 797 days. Twenty-one of 43 dogs (49%) achieved a ST of > 12 mo, 11 were nongreyhounds and 9 were greyhounds. Eight of the 43 dogs (19%) achieved a ST of > 24 mo, 4 were nongreyhounds and 4 were greyhounds. There was no statistical difference in ST between dogs treated within the first 21 days and dogs treated after 21 days after surgery ($P = 0.51$). The ST for the dog treated at 85 days after surgery was 1,019 days.

Eleven of 47 dogs developed hematologic toxicity (2 dogs developed grade 1, 1 dog developed grade 2, 6 dogs developed grade 3, and 2 dogs developed grade 4 hematologic toxicity) and 21 dogs developed gastrointestinal toxicity (14 dogs developed grade 1, 6 dogs developed grade 2, and 1 dog developed grade 3 toxicity). Of the 11 dogs that developed hematologic toxicity, 10 were greyhounds. Only four of the greyhounds developed grade 3 or 4 hematologic toxicity (three dogs developed grade 3 and one dog developed grade 4 neutropenia) and one nongreyhound developed grade 4 neutropenia. Grade 4 thrombocytopenia occurred only in two dogs with concurrent grade 4 neutropenia (one greyhound and one nongreyhound). No other dog developed thrombocytopenia.

Four dogs exited the study. Two dogs left due to grade 4 hematologic toxicity (grade 4 neutropenia and grade 4 thrombocytopenia). One of those dogs left after the first treatment of

suramin/doxorubicin and the other after the second treatment. Two other dogs left due to developed decreased ventricular contractility after the fourth treatment. One of those dogs was a greyhound and the other a nongreyhound. Those four dogs were subsequently treated with carboplatin and were excluded from the study for remission and survival analysis.

Fourteen dogs developed polyuria (PU) and polydipsia (PD) while on the suramin/doxorubicin protocol. The urine of those dogs was hyposthenuric (specific gravity, 1.001–1.008) and there were no concurrent serum biochemical abnormalities. Ten dogs were nongreyhounds and four were greyhounds. Six of 14 dogs had *Escherichia coli* urinary tract infections. In those dogs, the PU and PD resolved after appropriate antibiotic therapy. The PU and PD resolved after the last treatment in six additional dogs. Two dogs had PU and PD that persisted after the treatment ended. In both of those dogs, an in-house water deprivation test resulted in resolution of the hyposthenuria and PU and PD, suggesting psychogenic PD as the most likely mechanism.

## Discussion

The population characteristics in this study with respect to age and weight were similar to those in previous studies, and males appeared to be overrepresented.[2–13] The greyhound breed was overrepresented; however, The Ohio State University Veterinary Medical Center has significant involvement in the rescue of retired racing greyhounds and a large number of greyhounds with OSA is seen at the author's institution. In this study, the most common site for OSA was the proximal humerus in contrast to the distal radius and distal femur, the two most common anatomic locations in most previous studies.[2–13] There was no statistical difference for location between greyhounds and nongreyhounds.

In this study, the combination protocol of noncytotoxic suramin/doxorubicin resulted in a median DFI and ST that was generally similar to other reported adjuvant chemotherapy clinical trials reported in dogs with OSA.[3–13] However, when compared with a similar previous study where dogs with OSA were treated after amputation with doxorubicin alone, the MST was significantly longer in this study (369 days versus 250 days; log-rank test, $P = 0.044$, Cox proportional hazards model, $P = 0.036$; data generously provided by the authors).[9] Admittedly, the comparison with a historical control may be confounded due to any number of variables that may exist in between studies and cannot be controlled for; however, that finding may warrant further investigation of suramin as a chemosensitizer in a larger study group with an appropriate control arm. The median ST of 369 days and 1 yr and 2 yr STs of 49% and 19%, respectively, appear to be similar to those reported in previous studies of dogs with OSA treated with amputation and adjuvant doxorubicin.[3,9] The 1 yr survival rates in those other studies were 50.5% and 37% and the 2 yr survival rates were 9.7% and 17%. The results in the current study are comparable to those with conventional chemotherapy using doxorubicin as a single agent and the combination protocol used in this study does not appear to increase toxicity. The absorption and metabolism of some drugs is different in greyhounds than in nongreyhounds.[29] In this study, the authors analyzed the greyhounds as a separate group; however, no statistical differences in DFI and median ST were found between greyhounds and nongreyhounds.

Of the 11 dogs that developed hematologic toxicity, 10 were greyhounds and 60% developed grade 1 or 2 neutropenia; however, greyhounds had lower reference ranges for neutrophil counts than other breeds; therefore, the authors propose that the prevalence of hematologic toxicity 2 wk posttreatment was overestimated due to the high proportion of greyhounds in this study.[33–35] However, importantly, as no CBCs were performed between treatments, the true hematologic toxicity of the combination of doxorubicin and suramin may have been underestimated, as nadir data were not likely recorded.

Two of the 47 dogs (4%) developed evidence of cardiotoxicity and exited the study, likely related to doxorubicin cardiotoxicity. Even though it was a small percentage of dogs, doxorubicin should be used with caution in dogs with OSA, particularly in breeds with a higher risk for cardiomyopathy. PU and PD were unexpected adverse events associated with the combination of doxorubicin and noncytotoxic suramin. In humans, mineralocorticoid insufficiency has been reported after suramin therapy.[36,37] It is unknown if a similar mechanism affecting either the aldosterone function or concentration, as the cause of PU and PD, is involved in dogs; however, the investigation of the cause of those clinical signs is beyond the purpose of this paper and warrants further investigation.

The prevalence and severity of toxicity were comparable to those in previous studies using doxorubicin as a single agent; therefore, noncytotoxic suramin can be safely administrated in combination with doxorubicin in dogs with OSA.[9] Those findings support that the use of noncytotoxic suramin with doxorubicin is safe in dogs with naturally occurring tumors, as reported in a previous study.[28] Hence, suramin can be considered as a potential chemosensitizer without increasing either bone marrow or gastrointestinal toxicity. In comparison, other chemotherapy sensitizers, such as P-glycoprotein blockers, significantly increase toxicity of chemotherapy.[38–42] Similarly, minimal toxicity was observed in human patients treated with noncytotoxic doses of

suramin in the adjuvant chemotherapy setting.[43–46] Of the 139 patients (with nonsmall cell lung, metastatic breast, and kidney cancers) that participated in phase 1/2 trials of combinations of noncytotoxic suramin with standard chemotherapies, there was only one potential drug-related hypersensitivity that was resolved with standard supportive care.[43–46] Based on the encouraging early clinical data suggesting disease control and survival benefits, noncytotoxic suramin is being evaluated as a chemosensitizer in additional randomized trials in human patients with nonsmall cell lung cancer.[43–46]

## Conclusion

Noncytotoxic suramin/doxorubicin is safe in dogs. Additional studies using a randomized, prospective clinical trial protocol and larger sample size are needed to determine whether the survival benefits in a subset of dogs with OSA are clinically meaningful.

The authors would like to thank Dr. Antony Moore for providing the ST data for dogs treated with single-agent doxorubicin. This study was supported by Morris Animal Foundation grant DO4CA-092.

### FOOTNOTES

[a] GraphPad Prism 4.0; GraphPad Software Inc., San Diego, CA

[b] StatPlus 5.8.4; Analystsoft Inc., Vancouver, BC, Canada

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