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Feasibility and safety of whole lung irradiation in the treatment of canine appendicular osteosarcoma

Amanda Brehm | Heather Wilson-Robles | Tasha Miller | Jill Jarvis | Michael Deveau

Small Animal Clinical Sciences Department, Texas A&M University, College Station, Texas, USA

Correspondence
Amanda Brehm, Small Animal Clinical Sciences Department, Texas A&M University; 660 Raymond Stotzer Pkwy, College Station, TX 77845, USA.
Email: amandabrehmdvm@gmail.com

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Abstract

Whole lung irradiation (WLI) has been used successfully in humans as an adjuvant treatment for osteosarcoma. The aim of this study is to describe the feasibility and safety of WLI in dogs with appendicular osteosarcoma. Twelve client-owned dogs with appendicular osteosarcoma that had successfully completed amputation and four doses of carboplatin without evidence of gross metastasis were enrolled in this prospective clinical trial. Ten once-daily fractions of 1.75 Gy were administered to the planning target volume encompassing the lungs. Overall, WLI was well tolerated in these patients. No dogs developed symptoms of pneumonitis or pulmonary fibrosis. Haematopoietic toxicity evaluated during radiation therapy was found to be mild. The median disease free interval for WLI treated dogs was not significantly different than the median DFI for a group of historic control dogs (376 days for WLI treated dogs versus 304.5 days for control dogs; $p = 0.5461$). Although no significant improvement in outcome was observed with this study, WLI appears to be safe in dogs and warrants further investigation to characterize the efficacy and toxicity.

KEYWORDS
bone tumour, clinical trial, lung radiation effects, pulmonary metastasis, radiation oncology

1 | INTRODUCTION

Osteosarcoma is a highly malignant tumour in dogs and people. Approximately 90% of dogs with appendicular osteosarcoma will develop metastases despite successful local tumour control. The development of pulmonary metastasis is the most common cause of death in these patients. Recent advances in multi-modal local treatment options and attempts to intensify chemotherapy regimens have been unsuccessful in dramatically increasing disease free interval (DFI) or overall survival times (ST).[1–5] In addition, chemotherapy for the treatment of gross metastatic disease in dogs has been associated with poor response rates and increased toxicity.[6–8] Canine appendicular osteosarcoma shares many clinical and biologic characteristics with osteosarcoma in humans. Attempts to translate knowledge acquired across species should be made with regards to adjuvant therapies, including whole lung irradiation (WLI).

WLI has been investigated in humans with osteogenic sarcomas as a treatment for microscopic metastatic disease. The efficacy of WLI for the treatment of osteosarcoma in a microscopic disease setting has been evaluated in humans with promising results. A randomized trial comparing the use of WLI against no adjuvant treatment reported that in patients less than 17 years of age, the addition of WLI yielded a significantly improved metastasis-free percentage at 5 years.[9] Additionally, in a randomized trial evaluating the

use of adjuvant chemotherapy with or without WLI, the 9 year survival percentage was significantly greater for patients that received treatment with WLI compared with those that did not. The incidence of lung metastases was also significantly lower in the irradiated group.[10] Several other studies have been performed in humans with inconsistent outcome results.[11–13] A study by Owen and Bostock in 1973 evaluated prophylactic lung irradiation in dogs with osteosarcoma.[14] The authors did note a difference between irradiated and unirradiated lungs in that unirradiated lungs were heavier due to disease burden; however, the majority of dogs in this study had only one lung irradiated, using fraction sizes of 3–6 Gy. Additionally, sample sizes were variable and quite small. Therefore, it is difficult to draw useful conclusions from this study. There are no other current or historical trials evaluating the use of WLI in dogs with osteosarcoma.

The safety of WLI protocols in humans is well-established. Dose limiting side effects are known to be radiation-induced pneumonitis and pulmonary fibrosis.[15] Radiation-induced pneumonitis is an early inflammatory reaction that usually occurs one to 4 months after radiation therapy.[16] Pneumonitis occurs as a result of direct DNA damage and the generation of reactive oxygen species within the pneumocytes. This causes alveolar cell loss and inflammatory cell infiltration. Persistent and severe pneumonitis can lead to pulmonary fibrosis. Pulmonary fibrosis is characterized by fibroblast proliferation and collagen deposition, and typically occurs at or after 9 months following radiation therapy.[17] Most WLI prescriptions involve 1.5–1.75 Gy daily treatments to total doses of 15–20 Gy. These doses are administered to target volumes which encompass nearly 100% of the lung volume. The incidence of symptomatic cases of pneumonitis in patients treated with these protocols ranges from 0% to 12%.[9,11,18–22] In studies where pulmonary function testing is performed, mild restrictive changes and decreased lung volumes are reported. However, the majority of patients that develop these changes in function testing remain clinically asymptomatic.[19–21] The influence of concurrent chemotherapy in combination with WLI has also been studied in humans. Although actinomycin D and doxorubicin are both known radiosensitizers, only actinomycin D has been shown to significantly increase the risk of pneumonitis when combined with WLI.[23,24] Doxorubicin is more commonly included in human osteosarcoma adjuvant chemotherapy protocols, yet has not been shown to increase incidence of pneumonitis or pulmonary fibrosis, as assessed by pulmonary function testing.[21,25]

The safety of WLI in dogs has been studied preliminarily. A dose–response study for WLI has been performed in healthy Beagle dogs.[26] In this study, the incidence of radiation induced pneumonitis was 0% (0/16) for dogs undergoing 100% volume lung irradiation with doses up to 36 Gy, when delivered in 1.5 Gy fractions over 6 weeks. Another study evaluated late term side effects secondary to WLI in 59 healthy Beagle dogs.[27] In this study, all dogs received total doses at or above 18 Gy, delivered as 1.5 Gy fractions over 6 weeks. Significant changes in pulmonary function testing were not seen below total doses of 40 Gy. One dog, however, developed restrictive lung disease and died 1 year after treatment with a total dose of 31.5 Gy. These studies support the expected tolerance of WLI in dogs using human fractionation schedules, which rarely exceed a total dose of 20 Gy.

This study was designed to evaluate the use of a WLI protocol for dogs with osteosarcoma. The primary objective was to evaluate the safety and feasibility of this protocol in 12 client owned dogs after standard of care treatments (amputation and chemotherapy). The secondary objective was to evaluate the effect of WLI on the DFI of the treatment group receiving WLI compared to a group of historic control dogs receiving only standard of care treatments. Our hypothesis was that WLI would be safe and well tolerated.

2 | METHODS

2.1 | Animal population

This study was a prospective, open label, single arm clinical trial. Dogs with appendicular osteosarcoma that completed surgical excision (amputation) and four doses of carboplatin without evidence of metastatic disease were eligible for enrollment. Dogs were required to be ≥1 year of age and weigh ≥15 kg. All dogs were staged at diagnosis with a complete blood count, biochemical panel, urinalysis and three view thoracic radiographs. Abdominal ultrasound was performed if clinically indicated. Thoracic radiographs were repeated prior to the third dose of carboplatin, and again 3 weeks after the 4th dose of carboplatin. Dogs with evidence of metastatic disease identified on thoracic radiographs at any point prior to administration of WLI were not eligible for enrollment. Dogs were required to have no major biochemical abnormalities (ALT and GGT ≤2 times the upper reference limit, ALP ≤4 times the upper reference limit, bilirubin ≤1.5 g/dL, BUN and creatinine ≤ the upper reference limit). No previous therapy for osteosarcoma (other than amputation and carboplatin chemotherapy) was allowed. Dogs were required to have a modified Eastern Comparative Oncology Group (ECOG) performance score of 0–2 (Supplementary Table S1). Dogs who had a known sensitivity to carboplatin, were currently pregnant or likely to become pregnant, or had a serious, uncontrolled endocrine disorder or concurrent medical condition (hepatic, cardiovascular, other malignancy) at enrollment were excluded. All dogs were recruited and treated at the author's institution (Texas A&M University College of Veterinary Medicine). Radiation treatment was initiated for all dogs between 9/25/2018 and 1/13/2020.

Experimental protocols followed the animal care guidelines of the author's institution (Texas A&M Animal Care and Use Committee). Signed written owner consent was obtained prior to screening each client owned dog.

A control population ($n = 14$) was identified from dogs treated at the author's institution between 2009 and 2019. Dogs were included in the control group if they were treated with standard of care treatments only (amputation and four doses of carboplatin) and had no evidence of metastatic disease until after completion of their

chemotherapy protocol. Dogs included in the control group were required to meet the same inclusion and exclusion criteria as the treatment group.

2.2 | Treatment protocol

WLI was initiated 21–28 days after the final carboplatin treatment. Prior to radiation therapy, a computed tomography (CT) image set of the thorax was acquired for treatment planning. The patient was scanned in a large bore (80 cm) CT scanner (Siemens Somatom Definition AS). Patients were scanned and treated in sternal recumbency with a moldable positioning cushion (Vac-Lok™, Civco Medical Solutions, Coralville, IW) and with an in-house canine-specific bite block fixation device. The image set was transferred to a VelocityAI (Varian Medical Systems Inc., Palo Alto, CA) workstation for contouring and treatment planning. The gross tumour volume (GTV) was considered to be 100% of the lung volume. No margin for clinical tumour volume (CTV) was applied. Inhalation and exhalation breath-hold CT were performed to characterize the respiratory motion envelope.[28] Ninty five percent of the planning target volume (PTV) was set to receive prescription dose. No part of the PTV was allowed to receive greater than 107% or less than 93% of prescription dose. Organs at risk were defined as external (skin), bone, heart, spinal cord, oesophagus, stomach, trachea, liver, left kidney and right kidney. Radiation was delivered using a linear accelerator (Tomotherapy, Inc., Madison, WI) using 6 MV photons. Dogs received a total of 17.5 Gy that was delivered in 10 daily fractions of 1.75 Gy. The intended protocol was to administer daily fractions Monday through Friday in two consecutive weeks (over a span of 12 days total). All dogs were placed under general anaesthesia for treatment. Megavoltage CT images (approximately 3.0 cGy per scan) of the thorax were obtained daily and used to confirm correct patient positioning.

2.3 | Toxicity assessment

Toxicity during radiation therapy was assessed through daily patient evaluations by the attending clinician and quality of life forms completed weekly by owners (Supplementary Figure S1). The quality of life form asked the owner to assess if the following symptoms occurred and/or necessitated a visit to a veterinarian: vomiting, fatigue/lethargy, diarrhoea, rash, constipation, not eating or drinking, respiratory distress, behavioural changes, urination changes and pain. The form also provided a designated area to list any additional symptoms, comments or concerns. A complete blood count at the first and sixth fractions of radiation was performed. Hematologic toxicities were graded using the Veterinary Cooperative Oncology Group Common Toxicity Criteria for Adverse Events (VCOG-CTCAE).[29] Toxicities attributed to WLI were graded using the Veterinary Radiation Therapy Oncology Group (VRTOG) acute morbidity scoring scheme.[30]

Dogs were evaluated with thoracic radiographs 6 weeks after the completion of WLI, and then every 8 weeks thereafter. At each visit a physical exam was completed by the attending clinician and a quality of life form was completed by the owner.

2.4 | Statistical analysis

Statistical analyses were conducted using a commercially available statistical software program (GraphPad Prism version 8.0.0 for Mac, GraphPad Software, San Diego, CA, www.graphpad.com). DFI was defined as the time from surgery (amputation) to the time of development of any local, regional or distant metastasis. ST was defined as the time from surgery (amputation) to death. Dogs were censored from DFI or survival analysis if they were alive at the end of the study or died from diseases unrelated to osteosarcoma. DFI and ST curves were generated using the Kaplan–Meier product limit method and compared via log-rank test with a P-value of ≤0.05 considered significant.

2.5 | Cell line validation statement

No cell lines were utilized for this research and no cell line validation testing was performed.

3 | RESULTS

3.1 | Patient data

A total of 19 client-owned dogs were screened for the treatment group. Seven dogs were not enrolled in the study. Reasons for not enrolling included surgical site infection resulting in a delay of chemotherapy >21 days following amputation ($n = 1$), progression of a comorbidity (chronic renal disease, $n = 1$), and development of pulmonary or bone metastasis prior to WLI ($n = 5$). Twelve dogs received WLI and were included in the final analyses. Fourteen dogs were contemporaneously identified for the control population and consisted of patients that were treated at the author's institution with amputation and four doses of carboplatin. Animal and tumour characteristics are included in Table 1 for dogs in the treatment group and Table 2 for dogs in the control group. Dog breeds included in the treatment group were mixed breed ($n = 2$) and one of each of the following: Labrador, Rottweiler, Great Dane, Golden Retriever, Anatolian Shepherd, Boxer, Pit Bull, Mastiff, Dutch Shepherd, Standard Poodle. Dog breeds in the control group included Golden Retriever ($n = 4$), Greyhound ($n = 2$), and one of each of the following: Labrador Retriever, mixed breed, Pit Bull, Rottweiler, Mastiff, Irish Setter, Brittany Spaniel, and German Shepherd. Histopathology of the primary tumour was available for all dogs and confirmed a diagnosis of osteosarcoma. Histologic subtypes for the treatment group included chondroblastic osteosarcoma ($n = 3$), osteoblastic osteosarcoma ($n = 2$), and no subtype given ($n = 7$). Histologic subtypes for the control group were similar, including chondroblastic osteosarcoma ($n = 1$), osteoblastic osteosarcoma ($n = 3$), fibroblastic osteosarcoma ($n = 2$), telangiectactic

TABLE 1 Characteristics of dogs in WLI treatment group ($n = 12$) at time of amputation

Median age, years (range)	**8.5 (5.0–12.0)**	
Median weight, kg (range)	36.0 (17.0–62.0)	
Sex	***n***	**%**
Male, castrated	5	42
Male, intact	1	8
Female, spayed	6	50
Location of tumour	***n***	**%**
Proximal humerus	2	17
Distal radius	4	33
Distal tibia	2	17
Proximal tibia	2	17
Proximal femur	1	8
Distal femur	1	8
Serum ALP	***n***	**%**
Low (<110 U/L)	5	42
High (>110 U/L)	7	58
Monocytes in circulation	***n***	**%**
Low (<400/μL)	6	50
High (>400/μL)	6	50
Lymphocytes in circulation	***n***	**%**
Low (<1000/μL)	3	25
High (>1000/μL)	9	75

TABLE 2 Characteristics of dogs in control group ($n = 14$) at time of amputation

Median age, years (range)	**8.0 (1.0–12.0)**	
Median weight, kg (range)	32.0 (15.2–56.0)	
Sex	***n***	**%**
Male, castrated	9	64
Female, spayed	5	36
Location of tumour	***n***	**%**
Proximal humerus	6	43
Distal radius	4	29
Distal tibia	1	7
Proximal tibia	3	21
Serum ALP	***n***	**%**
Low (<110 U/L)	8	57
High (>110 U/L)	6	43
Monocytes in circulation	***n***	**%**
Low (<400/μL)	3	21
High (>400/μL)	11	79
Lymphocytes in circulation	***n***	**%**
Low (<1000/μL)	3	21
High (>1000/μL)	11	79

osteosarcoma ($n = 1$) and no subtype given ($n = 7$). In the treatment group, eight dogs had lymph nodes removed at the time of amputation; no lymph nodes were considered to be metastatic based on histopathologic evaluation. Similarly in the control group, six dogs had lymph nodes removed at the time of amputation and no lymph nodes were considered to be metastatic through histopathology.

3.2 | Chemotherapy side effects

All 12 dogs in the treatment group received four doses of carboplatin at 300 mg/m^2. No dose reductions were required. Chemotherapy was well tolerated. Three dogs developed grade 1 gastrointestinal toxicity. Four dogs developed neutropenia; the worst reported grade was grade 1 in 2 dogs and grade 2 in 2 dogs. Six dogs developed thrombocytopenia; the worst reported grade was grade 2 in 2 dogs, grade 3 in 3 dogs and grade 4 in 1 dog. Two dogs required a 1 week dose delay starting at the 2nd dose of carboplatin to allow for hematologic toxicity recovery.

In the control arm, chemotherapy was similarly well tolerated. The mean dose of carboplatin was 296 mg/m^2. Two dogs required a dose delay. One dog required a 1 week dose delay due to neutropenia. One dog received a 2 week dose delay due to neutropenia and scheduling issues. Eight dogs developed thrombocytopenia; the worst reported grade was grade 1 in 1 dog, grade 2 in 1 dog, grade 3 in 5 dogs and grade 4 in 1 dog. Four dogs developed neutropenia; the worst reported grade was grade 1 in 3 dogs and grade 3 in 1 dog. Five dogs developed gastrointestinal toxicity; the worse reported grade was grade 1 in 3 dogs and grade 2 in 2 dogs.

3.3 | Radiation therapy delivery

The mean time to delivery of radiation therapy was 26.1 days after the last dose of chemotherapy (range 21–32 days) and 8.7 days after the planning CT was performed (range 4–17 days). The intended protocol was to administer daily fractions Monday through Friday in two consecutive weeks (over a span of 12 days total). Eight of 12 dogs completed the protocol with this schedule. Technical failures of the linear accelerator resulted in delays in treatment for four dogs. This resulted in one dog receiving the protocol over 14 days, two dogs received the protocol over 15 days, and one dog received Monday–Friday treatments but with a 1 week delay in between the first and second week (19 days total). No biologically effective dose corrections were made for cases that received the protocol over a longer period of time. It is possible that acute toxicity was underestimated in these patients due to prolongation of their protocol, but this was considered preferable to increasing risk for late toxicity by increasing dose per fraction.

3.4 | Radiation therapy side effects

The most common side effect observed during radiation therapy was weight loss. The mean percentage of body weight loss was 2% per

TABLE 3 Hematologic side effects during radiation therapy

Patient	Fraction 6		Fraction 10	
	Grade thrombocytopenia	Grade neutropenia	Grade thrombocytopenia	Grade neutropenia
1	–	–	1	0
2	1	0	–	–
3	0	0	–	–
4	0	0	–	–
5	1	0	–	–
6	1	0	–	–
7	0	0	–	–
8	1	0	2	1
9	0	0	–	–
10	1	0	–	–
11	0	0	–	–
12	–	–	–	–

Note: Complete blood counts were obtained for each patient during the radiation protocol to monitor for hematologic toxicity. Toxicities were graded according to VCOG-CTCAE v1.1.[26]
Note: "–" designates that a CBC was not obtained on the day of that radiation fraction.

dog (range −12% to +9% body weight). Based on owner completed quality of life surveys, 11 of 12 dogs experienced no symptoms during radiation therapy. One dog experienced a grade 1 non-productive cough on the day of the tenth radiation fraction.[30] Thoracic radiographs were taken and did not reveal any pulmonary pathology. The cough had resolved by the first 6-week recheck and was attributed to tracheal irritation from repeated endotracheal intubation during radiation therapy. No other symptoms or side effects were observed for any other patient by attending clinicians via daily patient assessment and physical exams during radiation therapy. Complete blood counts were checked on the day of the first radiation fraction, the sixth radiation fraction and the tenth radiation fraction in 11/12 dogs, 10/12 dogs and 2/12 dogs, respectively. Hematologic side effects noted during radiation therapy are summarized in Table 3. One dog began radiation therapy (fraction #1) with a grade 1 thrombocytopenia presumably secondary to carboplatin administration 3 weeks prior. Six dogs experienced a grade 1 thrombocytopenia during radiation (five occurred at fraction six, one occurred at fraction ten). One dog had a grade 2 thrombocytopenia and grade 1 neutropenia (both occurring at fraction ten of radiation). Toxicity grading was performed according to VCOG-CTCAE v1.1.[29] Grade 1 thrombocytopenia included values below the lower limit of normal to 100 000/μL. Grade 2 thrombocytopenia included values from 50 000 – 99 000/μL. Grade 1 neutropenia included values below the lower limit of normal to 1500/μL. Acute radiation therapy side effects other than hematologic toxicity were not observed.

Each dog returned every eight weeks after radiation therapy for a physical exam, quality of life survey and thoracic radiographs. No clinically significant late radiation therapy side effects were noted. Quality of life abnormalities reported after radiation therapy are summarized in Table 4. At the 8 week post radiation recheck, one dog was reported to have a lameness and one dog was reported to have lethargy and intermittent diarrhoea. Both of these dogs had resolution of their symptoms at subsequent recheck. At the 24 week post radiation recheck, one dog was reported to have lameness which resolved at subsequent recheck. One dog had a chronic lameness noted from weeks 8 to 40 post radiation. This lameness was attributed to a cruciate ligament injury by orthopaedic exam and radiographic investigation. At the 32 week recheck one dog was reported to have lethargy which resolved at subsequent recheck. No other dogs had any symptoms reported by owners prior to documented progression of disease.

TABLE 4 Quality of life abnormalities reported after radiation therapy

Patient	Quality of life Abnormality	Timing after radiation therapy
1	–	–
2	–	–
3	Lethargy	32 weeks
4	–	–
5	–	–
6	–	–
7	–	–
8	Pain (Lameness)	8 weeks
9	–	–
10	Pain (Lameness)	24 weeks
11	Pain (Lameness)	8, 16, 24, 32, 40 weeks[a]
12	Lethargy, diarrhoea	8 weeks

[a]The chronic lameness reported for patient 11 was attributed to a cranial cruciate ligament injury through orthopaedic exam and stifle radiographs. All other quality of life abnormalities depicted in this table were temporary and resolved at subsequent recheck.

TABLE 5 Patient outcomes in WLI treatment group

Patient	Reason for disease progression	DFI (days)	Overall ST (days)
1	Bone and pulmonary metastasis	228	229
2	Bone and pulmonary metastasis	219	539
3	Pulmonary metastasis	297	703
4	Pulmonary metastasis	376	672
5	Pulmonary metastasis	384	523
6	Pulmonary metastasis	250	353
7	Bone metastasis	421	421
8	Bone metastasis	218	219
9	Hemoabdomen; splenic stromal sarcoma	178	206
10	Acute onset hind limb paresis	255	255
11	Acute onset multi-organ dysfunction	269	269
12	Disease free at last follow up	516	516

No dogs had evidence of pulmonary pathology on thoracic radiographs prior to disease progression. The median follow up time for dogs after completion of WLI was 24 weeks.

3.5 | Outcomes

Patient outcomes are summarized in Table 5. Eight dogs developed progressive disease suspected to be related to osteosarcoma. Of these cases, four developed soft tissue pulmonary nodules on thoracic radiographs suspected to represent metastases. Two dogs developed bone and pulmonary metastasis concurrently. Two dogs developed bone metastasis without evidence of pulmonary metastasis (confirmed at necropsy). Three dogs developed new diseases not suspected to be related to osteosarcoma. One of these dogs developed a hemoabdomen due to a splenic mass; histopathology confirmed the mass to be a grade III splenic stromal sarcoma. The second dog developed acute onset hind limb paresis and was euthanized. The third dog developed acute onset multi-organ dysfunction and was euthanized. One dog remained disease free with last documented follow up at 385 days post amputation. Dogs considered to have progressive osteosarcoma were removed from study follow-up and were allowed further treatments based on clinician and client preferences.

In the treated population, four dogs were censored from DFI analysis. Dogs were censored for progression of disease unrelated to osteosarcoma ($n = 3$) and for being still alive and disease free at paper submission ($n = 1$). Seven dogs in the treated population were censored from survival analysis. Dogs were censored from survival analysis for death unrelated to osteosarcoma ($n = 4$), and for being still alive at paper submission ($n = 3$). Median follow up for censored dogs in the treated population was 516 days. In the control population, four dogs were censored from DFI analysis. Dogs were censored for being still alive and disease free at paper submission ($n = 3$) or progression of disease unrelated to osteosarcoma ($n = 1$). Four dogs in the control population were censored from survival analysis. Dogs were censored from survival analysis for death unrelated to osteosarcoma ($n = 1$) and for being still alive at paper submission ($n = 3$). Median follow up for censored dogs in the control population was 514 days. The median DFI for dogs that received WLI was 376 days (range 178–516 days). The median DFI for dogs in the control population that did not receive WLI was 304.5 days (range 98–1408 days). The DFIs were not significantly different between groups (p = 0.5461) (Figure 1). The median overall survival for dogs that received WLI was 523 days (range 206–703 days). The median overall survival for the control group was 379 days (range 127–1524 days). The overall ST was not significantly different between groups ($p = 0.4681$) (Figure 2).

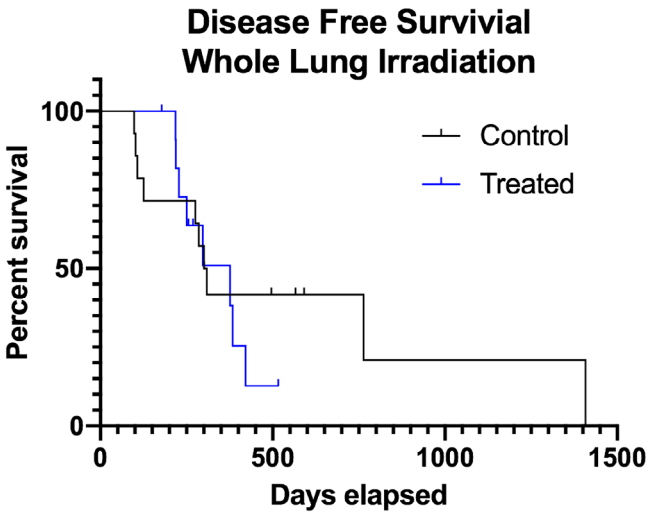

FIGURE 1 Disease free survival for WLI treated group compared to control group. Kaplan–Meier curve depicting DFI in dogs treated with standard of care plus WLI compared to control dogs treated with standard of care treatments alone. The median DFI for treated dogs was 376 days versus 304.5 days for the control group. Log-rank analysis was not statistically significant ($p = 0.5461$). Tick marks indicate censored cases

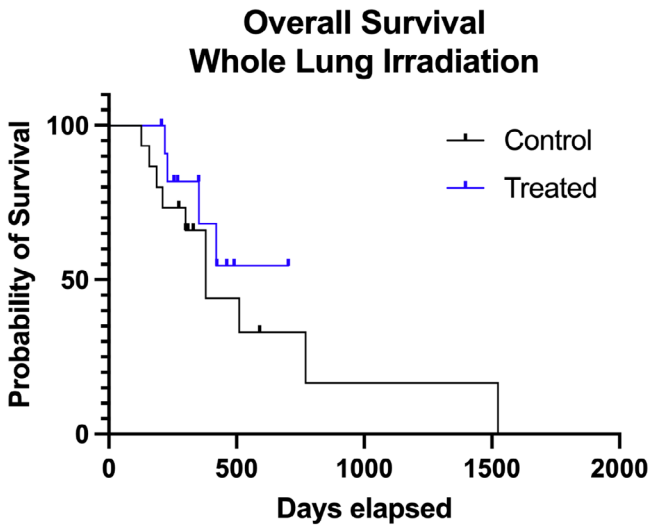

FIGURE 2 Overall survival for WLI treated group compared to control group. Kaplan–Meier curve depicting overall survival in dogs treated with standard of care plus WLI compared to control dogs treated with standard of care treatments alone. The median overall ST for treated dogs was 523 versus 379 days for the control group. Log-rank analysis was not statistically significant ($p = 0.4681$). Tick marks indicated censored cases

Three dogs had necropsies performed. One dog had confirmation of metastatic osteosarcoma to the lung and a rib, with no evidence of pulmonary fibrosis. Two dogs had evidence of mild pulmonary fibrosis (either peribronchiolar or subpleural) and confirmation of metastatic osteosarcoma to bone.

4 | DISCUSSION

WLI was successfully administered to 12 dogs with appendicular osteosarcoma. All dogs that started WLI completed the protocol. WLI was well tolerated, with no dogs experiencing symptoms attributable to radiation toxicity. The dose limiting side effects for WLI in humans are pneumonitis and pulmonary fibrosis. Fortunately no dogs in our treatment group developed any symptoms of pneumonitis. In addition, no radiographic evidence of pneumonitis was observed on radiographs taken at the 6 week recheck after completion of radiation therapy, nor on any subsequent recheck. CT has been shown to be most sensitive in detecting density changes within the lung parenchyma that reflect the development of pneumonitis or fibrosis.[16] Future studies could consider using this modality to monitor patients during the follow up period. However, the detection of subclinical radiographic evidence of pneumonitis or pulmonary fibrosis is of questionable significance in our canine population. Two patients were found to have mild pulmonary fibrosis at necropsy. These patients were not experiencing symptoms of pulmonary disease prior to death. They were euthanized at 4 months and 10 months post radiation therapy. It is possible that if these patients had experienced a longer ST, they may have developed symptoms related to reduced pulmonary compliance or diffusion impairment.

Pulmonary function testing is routinely performed in humans to evaluate for development of pulmonary fibrosis. When such testing is performed in patients having received WLI, restrictive lung disorders and changes in dynamic compliance are expected, although the majority of patients with abnormal function tests still remain clinically unaffected.[22] Pulmonary function testing was not performed in dogs in this study, mainly because this form of testing is not readily available in clinical veterinary medicine. The fact that function testing does not correlate with development of symptoms in people supports the use of objective quality of life measurements as an acceptable alternative. Quality of life assessment via owner survey during and after radiation therapy in this study suggested a good quality of life in our treated patients. One dog was reported to be experiencing lethargy at the 32-week follow-up; however this had resolved by the 40-week follow up date. No other changes in respiratory patterns, exercise tolerance or energy level were reported.

During radiation therapy the most common hematologic toxicity was a mild thrombocytopenia. This finding is thought to mainly be related to a consumptive process secondary to radiation therapy. After ionizing radiation is delivered to the lung parenchyma, the formation of reactive oxygen species and DNA strand breaks results in inflammatory cytokine release and inflammatory cell infiltration.[16] This inflammatory response, along with direct endothelial cell loss, leads to increased vascular permeability throughout the lungs.[31] These changes result in platelet activation and adhesion as they traverse the pulmonary vasculature, and platelets are taken out of circulation. Direct cytotoxicity to platelet precursors within the bone marrow may play a minor role in the thrombocytopenias observed in our patients. One patient in this study developed a grade I neutropenia, which was likely the result of toxicity to granulocyte precursors within the bone marrow of the ribs.

The DFI was not significantly increased in the WLI treated population compared to the control group. The median DFI of 376 days for the treatment group is longer than some historically reported medians for dogs undergoing amputation and various chemotherapy protocols, however, this study selected for patients that completed standard of care treatments.[32–36] Thus, there is a bias towards a longer survival in our patient population compared to studies where the intent-to-treat mark was amputation or the first dose of chemotherapy. Several factors could explain the lack of statistically significant improvement in ST following WLI compared to this study's control population. Most importantly, the study groups were underpowered and a much larger patient population would be required to determine if WLI would improve disease free or overall survival. A post hoc power analysis was performed using the data generated in this study where the outcome was set as the time to failure. For this parallel trial analysis the significant level was set at 0.05 and the power at 0.8.[37] This analysis determined that a total of 117 dogs would be needed to complete a parallel group clinical trial where enrolled dogs would be randomly assigned to either a standard of care carboplatin arm or a standard of care plus WLI arm.

In addition to a lack of statistical power to detect a difference in outcome between treated and control populations, it is important to consider other factors which could influence the efficacy of WLI as an adjuvant therapy for canine osteosarcoma. It is possible that the total dose of irradiation delivered in this protocol is not enough to result in meaningful cytotoxicity to pulmonary micro-metastatic lesions. A previous study in humans evaluated patients with osteosarcoma that have pulmonary metastatic lesions just invisible on radiographs (less than 5 mm). It was determined that in one of four patients, those <5 mm pulmonary nodules will contain ≤100 000 tumour cells.[38] Based on the osteosarcoma D10-value (decimal reduction value) of 4 Gy and a tumour volume of 100 000 cells, a total dose of five times the value of D10 (20 Gy, 2 Gy/fx) should be sufficient to reduce the tumour population to less than one cell.[9] However the actual cell survival curve for dogs may differ from this human model and does not account for inherent radiation resistance in some tumour cell subpopulations. The total dose administered in this study was chosen based on published efficacy in humans and the preliminary safety studies in dogs. Due to the potentially life threatening and late onset side effects of WLI, a dose escalation trial in our patient population was considered unethical.

A second factor that could have contributed to lack of improvement in ST for the treatment group may be related to the timing of administration of WLI. This study chose to administer WLI after the completion of chemotherapy, as to not delay or withhold standard of care therapy for client owned animals. However, it is possible that by waiting until after completion of chemotherapy, we have allowed for microscopic metastatic lesions to develop patterns of chemoresistance that translate to radiation resistance. The timing of WLI in combination with chemotherapy in human trials for osteosarcoma is variable. One trial administered WLI after the induction phase (9 weeks) of adjuvant multi-agent chemotherapy. Another study allowed some patients to receive WLI immediately after local therapy, while others received one dose of doxorubicin prior to WLI, and then completed the remainder of their doxorubicin protocol.[13,20] WLI may provide more therapeutic benefit if administered earlier in the disease process, such as immediately after amputation or in combination with adjuvant chemotherapy.

Finally, our inability to observe a significant improvement in DFI in this patient population does not necessarily indicate that WLI would not be beneficial in any patient. It is possible that WLI may be of benefit to specific patients and tumour types, such as those with a poor response to chemotherapy. Previous studies have shown that percent necrosis of the primary tumour after neoadjuvant chemotherapy with doxorubicin is able to predict survival, and that different gene expression patterns can predict chemotherapy response and clinical outcome.[3,39] Therefore it may be more prudent in future studies to consider the use of WLI in specific patient populations that are predicted to be poor responders to chemotherapy.

Limitations of this study include a small population size and a lack of randomization between treatment and control groups. In addition to a lack of statistical power to be able to detect a difference in outcome, the treatment group size of 12 may not have allowed for observation of an idiosyncratic or potentially rare adverse event. The average follow-up time for dogs in this study was 6 months after radiation therapy was completed. Pulmonary fibrosis is known to develop 9–12 months after radiation therapy.[22] Therefore, the incidence of this late term side effect may be lower than would be expected if follow-up time was longer. However it is worth noting that most dogs ($n = 8$) experienced disease progression from osteosarcoma during the follow-up period, and therefore a larger study may still not result in longer follow up times. Dogs were not randomized into treatment versus control groups. Care was taken to match the control group to the treatment group as much as possible in terms of disease status and standard of care treatment schedule. Many dogs in the control group were a part of a standard of care arm in contemporaneous clinical trials at the author's institution, which allowed for thorough staging and follow-up in that group. However, the control population did not have a uniformly standard follow-up schedule, which could have influenced the DFI and ST calculations.

In conclusion, this study supports the safety and feasibility of WLI in dogs. It emphasizes the need for further studies to confirm the expected acute and late term side effect profile. Adjustments in the timing of WLI compared to standard of care local and systemic treatments should be investigated with a larger, randomized clinical trial. In addition, delineation of osteosarcoma patient populations that display early chemoresistance may allow for the benefit of WLI to be further elucidated.

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DISCLAIMER

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CONFLICTS OF INTEREST

No conflicts of interest are disclosed.

ORCID

Amanda Brehm https://orcid.org/0000-0002-0895-3688
Heather Wilson-Robles https://orcid.org/0000-0002-6489-8468
Michael Deveau https://orcid.org/0000-0001-5334-4702

REFERENCES

1. Szewczyk M, Lechowski R, Zabielska K. What do we know about canine osteosarcoma treatment? Review. *Vet Res Commun*. 2015;39(1):61-67.
2. Frimberger AE, Chan CM, Moore AS. Canine osteosarcoma treated by post-amputation sequential accelerated doxorubicin and carboplatin chemotherapy: 38 cases. *J Am Amin Hosp Assoc*. 2016;52(3):149-156.
3. Berg J, Weinstein MJ, Springfield DS, Rand WM. Results of surgery and doxorubicin chemotherapy in dogs with osteosarcoma. *J Am Vet Med Assoc*. 1995;206(10):1555-1560.

4. Moore AS, Dernell WS, Ogilvie GK, et al. Doxorubicin and BAY 12-9566 for the treatment of osteosarcoma in dogs: a randomized, double-blind, placebo-controlled study. *J Vet Intern Med*. 2007;21(4):783-790.
5. Bailey D, Erb H, Williams L, Ruslander D, Hauck M. Carboplatin and doxorubicin combination chemotherapy for the treatment of appendicular osteosarcoma in the dog. *J Vet Intern Med*. 2003;17(2):199-205.
6. Ogilvie GK, Straw RC, Jameson VJ, et al. Evaluation of single-agent chemotherapy for treatment of clinically evident osteosarcoma metastases in dogs: 45 cases (1987-1991). *J Am Vet Med Assoc*. 1993;202(2):304-306.
7. Batschinski K, Dervisis NG, Kitchell BE. Evaluation of ifosfamide salvage therapy for metastatic canine osteosarcoma. *Vet Comp Oncol*. 2014;12(4):249-257.
8. Laver T, London CA, Vail DM, Biller BJ, Coy J, Thamm DH. Prospective evaluation of toceranib phosphate in metastatic canine osteosarcoma. *Vet Comp Oncol*. 2018;16(1):E23-E29.
9. Breur K, Cohen P, Schweisguth O, Hart AM. Irradiation of the lungs as an adjuvant therapy in the treatment of osteosarcoma of the limbs. An E.O.R.T.C randomized study. *Eur J Cancer*. 1978;14(5):461-471.
10. Pochanugool L, Subhadharaphandou T, Dhanachai M, et al. Prognostic factors among 130 patients with osteosarcoma. *Clin Orthop Relat Res*. 1997;345:206-214.
11. Casey DL, Alektiar KM, Gerber NK, Wolden SL. Whole lung irradiation for adults with pulmonary metastases from Ewing sarcoma. *Int J Radiat Oncol Biol Phys*. 2014;89(5):1069-1075.
12. Whelan JS, Burcombe RJ, Janinis J, Baldelli AM, Cassoni AM. A systematic review of the role of pulmonary irradiation in the management of primary bone tumours. *Ann Oncol*. 2002;13(1):23-30.
13. Burgers JM, Glabbeke M, Busson A, et al. Osteosarcoma of the limbs. Report of the EORTC-SIOP 03 trial 20781 investigating the value of adjuvant treatment with chemotherapy and/or prophylactic lung irradiation. *Cancer*. 1988;61(5):1024-1031.
14. Owen LN, Bostock DE. Prophylactic X-irradiation of the lung in canine tumours with particular reference to osteosarcoma. *Eur J Cancer*. 1973;9(10):747-752.
15. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys*. 1991;21(1):109-122.
16. Giuranno L, Lent J, De Ruysscher DD, Vooijs MA. Radiation-induced lung injury (RILI). *Front Oncol*. 2019;9(877). https://doi.org/10.3389/fonc.2019.00877.
17. Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr Med Chem*. 2009;16(2):130-143.
18. Rab GT, Ivins JC, Childs DS, Cupps RE, Pritchard DJ. Elective whole lung irradiation in the treatment of osteogenic sarcoma. *Cancer*. 1976;38(2):939-942.
19. Jasmin C. A strategic adjuvant therapy of osteosarcoma. *Recent Results Cancer Res*. 1982;80:108-114.
20. Zaharia M, Caceres E, Valdivia S, Moran M, Tejada F. Postoperative whole lung irradiation with or without adriamycin in osteogenic sarcoma. *Int J Radiat Oncol Biol Phys*. 1986;12(6):907-910.
21. Ellis ER, Marcus RB, Cicale MJ, et al. Pulmonary function tests after whole-lung irradiation and doxorubicin in patients with osteogenic sarcoma. *J Clin Oncol*. 1992;10(3):459-463.
22. Ronchi L, Buwenge M, Cortesi A, et al. Whole lung irradiation in patients with osteosarcoma and Ewing sarcoma. *Anticancer Res*. 2018;38(9):4977-4985.
23. Cohen IJ, Loven D, Schoenfeld T, et al. Dactinomycin potentiation of radiation pneumonitis: a forgotten interaction. *Pediatr Hematol Oncol*. 1991;8(2):187-192. https://doi.org/10.3109/08880019109033447.
24. Margolis LW, Phillips TL. Whole-lung irradiation for metastatic tumor. *Radiology*. 1969;93(5):1173-1179. https://doi.org/10.1148/93.5.1173.
25. Carcelen A, Zaharia M, Caceres E, Moran M, Tejada F. Pulmonary function tests during adjuvant lung irradiation for osteogenic sarcoma. *Cancer Treat Rep*. 1980;64(4–5):701-703.
26. Poulson JM, Vujaskovic Z, Gillette SM, Chaney EL, Gillette EL. Volume and dose-response effects for severe symptomatic pneumonitis after fractionated irradiation of canine lung. *Int J Radiat Biol*. 2000;76(4):463-468.
27. Gillette SM, Dawson CA, Rickaby DA, Johnston MR, Gillette EL. Late response to whole-lung irradiation alone and with whole-body hyperthermia in dogs. *Radiat Res*. 1997;147(2):257-262.
28. Keall PJ, Mageras GS, Balter JM, et al. The management of respiratory motion in radiation oncology report of AAPM task group 76. *Med Phys*. 2006;33(10):3874-3900.
29. VCOG-CTCAE v1.1. Veterinary cooperative oncology group – common terminology criteria for adverse events following chemotherapy or biological anti-neoplastic therapy in dogs and cats. *Vet Comp Oncol*. 2011;14:417-446.
30. LaDue T, Klein MK. Toxicity criteria of the veterinary radiation therapy oncology group. *Vet Radiol Ultrasound*. 2001;42(5):475-476.
31. Trott KR, Herrmann T, Kasper M. Target cells in radiation pneumonopathy. *Int J Radiat Oncol Biol Phys*. 2004;58(2):463-469.
32. Bergman PJ, MacEwen EG, Kurzman ID, et al. Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991 to 1993). *J Vet Intern Med*. 1996;10(2):76-81.
33. Phillips B, Powers BE, Dernell WS, et al. Use of single-agent carboplatin as adjuvant or neoadjuvant therapy in conjunction with amputation for appendicular osteosarcoma in dogs. *J Am Anim Hosp Assoc*. 2009;45(1):33-38.
34. Saam DE, Liptak JM, Stalker MJ, Chun R. Predictors of outcome in dogs treated with adjuvant carboplatin for appendicular osteosarcoma: 65 cases (1996-2006). *J Am Vet Med Assoc*. 2011;238(2):195-206.
35. Kent MS, Strom A, London CA, Seguin B. Alternating carboplatin and doxorubicin as adjunctive chemotherapy to amputation or limb-sparing surgery in the treatment of appendicular osteosarcoma in dogs. *J Vet Intern Med*. 2004;18(4):540-544.
36. Selmic LE, Burton JH, Thamm DH, Withrow SJ, Lana SE. Comparison of carboplatin and doxorubicin-based chemotherapy protocols in 470 dogs after amputation for treatment of appendicular osteosarcoma. *J Vet Intern Med*. 2014;28(2):554-563.
37. Schoenfeld, DA. Find statistical considerations for a study where the outcome is a time to failure. Updated 3 May 2001. http://hedwig.mgh.harvard.edu/sample_size/time_to_event/para_time.html.
38. Abbatucci JS, Fourre D, Quint R, Roussel A, Urbajtel M, Brune D. Possibilities of radiotherapy in pulmonary metastases. Apropos of 150 cases. *Ann Radiol*. 1973;16(5):385-392.
39. Fowles JS, Brown KC, Hess AM, Duval DL, Gustafson DL. Intra- and interspecies gene expression models for predicting drug response in canine osteosarcoma. *BMC Bioinformatics*. 2016;17:93. https://doi.org/10.1186/s12859-016-0942-8.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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