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Response of Canine Soft Tissue Sarcoma to Stereotactic Body Radiotherapy

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Canine soft tissue sarcoma (STS) has served as a preclinical model for radiation, hyperthermia, experimental therapeutics, and tumor microenvironmental research for decades. Stereotactic body radiotherapy (SBRT) demonstrates promising results for the control of various tumors in human and veterinary medicine; however, there is limited clinical data for the management of STS with SBRT. In this retrospective study, we aimed to define overall efficacy and toxicity of SBRT for the treatment of macroscopic canine STS to establish this preclinical model for comparative oncology research. Fifty-two canine patients met inclusion criteria. Total radiation dose prescribed ranged from 20–50 Gy delivered in 1–5 fractions. Median progression-free survival time (PFST) was 173 days and overall survival time (OST) 228 days. Best overall response was evaluable in 46 patients, with 30.4% responding to treatment (complete response n = 3; partial response n = 11). For responders, OST significantly increased to 475 days vs. 201 days ($P = 0.009$). Prognostic factors identified by multivariable Cox regressions included size of tumor and metastasis at presentation. Dogs were 3× more likely to progress ($P = 0.009$) or 3.5× more likely to experience death ($P = 0.003$) at all times of follow up if they presented with metastatic disease. Similarly, every 100-cc increase in tumor volume resulted in a 5% increase in the risk of progression ($P = 0.002$) and death ($P = 0.001$) at all times of follow up. Overall, 30.8% of patients developed acute toxicities, 7.7% grade 3; 28.8% of patients developed late toxicities, 11.5% grade 3. Increased dose administered to the skin significantly affected toxicity development. SBRT serves as a viable treatment option to provide local tumor control for canine macroscopic STS, particularly those with early-stage disease and smaller tumors. The results of this study will help to define patient inclusion criteria and to set dose limits for preclinical canine STS trials involving SBRT. **© 2021 by Radiation Research Society**

INTRODUCTION

Cancer accounts for the death of 40–50% of all dogs over the age of ten and is the leading cause of death in canine patients (1, 2). Soft tissue sarcomas (STS) are a common mesenchymal cell tumor of dogs and are the fourth most commonly diagnosed tumor type in in this species (3). Alternatively, STS are rare cancers in humans, making up approximately 1% of adult malignancies (4). Despite its rarity in people, STS accounts for greater mortality than testicular cancer, Hodgkin's lymphoma, and thyroid cancer combined (5, 6). Human STS have more than 100 different histologic types, and due to the complexity and low clinical occurrence of this cancer, access is needed to representative translational models that reflect this diversity (7, 8). Overall, it is recognized that progress has been slow in finding new effective therapeutic approaches for treating STS. Through the field of comparative oncology research, studying the biological and clinical impacts of treating spontaneous, naturally occurring canine cancer may be used to translate findings and inform therapeutic approaches for correlating human cancers (9).

Canine and human soft tissue sarcomas share biological and clinical similarities. Because of this, naturally occurring canine STS has been utilized as a relevant and prevalent model of this rare yet devastating human cancer [reviewed in (10, 11)]. While there are some differences in the histopathologic classification and grading of soft tissue sarcomas between humans and dogs, a study using canine soft tissue sarcomas to compare pathologic diagnoses between veterinary and medical pathologists showed that the majority of canine tumors were given diagnoses congruent with the human counterpart (12). Molecular genetic testing improves diagnostic accuracy in human STS (13), but is not readily available nor performed for canine

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patients. Despite lack of access, gene expression in canine tumors is equivalent to relevant human subtypes (14). Canine STS is graded histologically on a scale of 1–3, with tumor aggression and metastatic propensity increasing with grade (15–17). Tumor grading has historically been the most important prognostic tool for STS with clinical outcomes and treatment protocols varying widely by tumor grade (16–19). Canine STS are locally invasive and metastatic rates range from less than 15% with grade 1–44% with grade 3 (20). The lung is the most frequent metastatic site in human patients, similar to dogs. Patients presenting with metastasis have poor long term survival, although this is improving in recent years; however the best chance of survival is a result of surgical resection of distant metastases (21, 22). While the development of metastasis is possible and devastating, more commonly it is the primary tumor that affects health and compromises quality of life (18, 23–25). Frequently, dogs present with STS that have extensive local invasion and complete surgical excision is impossible or would be associated with undesirable morbidity. Clinically, this proposes a significant problem as complete surgical excision, or incomplete excision with adjuvant radiation therapy to treat residual microscopic tumor cells, is considered the current standard of care for STS in both human and canine patients, likely to provide substantial long-term local tumor control (18, 26, 27). Similarly, in humans, soft tissue sarcoma of the extremities often requires aggressive therapy such as limb salvage or amputation. Both of which have detrimental effects to perception of quality of life (28). Current research needs for human STS are similar to those in the veterinary world, such as defining optimal radiotherapy techniques and adjuvant therapies for unresectable tumors, as well as characterization of normal tissue toxicities (29, 30).

Immunomodulation and radiation response in canine STS is similar to human cases. Dogs have been a model of STS tumor microenvironment (TME) for decades, such as investigations into effects of tumor hypoxia and hypothermia (31–33). More recently tumor specific immune responses to radiation have been analyzed in canine models, including the release of soluble factors such as cytokines and tumor associated antigens, which promote immunogenic cell death and abscopal effects (34). This has been explored in canine clinical trials showing efficacy of oncolytic viruses in treating canine sarcoma and other cancers (35, 36). Similarly, in recent years there has been an increase of human clinical trials of oncolytic viruses (37) as well as their potential use with other immunotherapeutics such as checkpoint inhibitors (38) and in combination therapies (39). Canine sarcoma cell lines have been shown to express similar checkpoint inhibitory blockades as human cancers, such as programmed cell death ligand, PDL-1 (40) which may be targeted by canine specific anti-PDL-1 antibodies (41). Radiation also induces changes in tumor endothelium, and modulation of immune cell subsets. Adoptive cellular therapy using activated canine natural killer cells has been investigated in an osteosarcoma model (42), while modulation of the canine tumor microenvironment through radiation and immunotherapy has been investigated using indoleamine deoxygenase inhibition (43) as well as nanotechnology-based immune adjuvants (44). Additionally, dogs receiving fractionated radiotherapy developed significant and sustained lymphopenia after treatment (45), which has been similarly shown in human radiation therapy to effect clinical outcomes in oropharyngeal tumors (46). Moving forward, investigations into the combined use of SBRT with immunotherapy in canine cancer patients may serve as a translational model due to the similar immunomodulatory effects as human cancers (47).

While the intrinsic radiosensitivity of a canine STS cell line was evaluated in vitro and characterized as radiosensitive (48), this does not reflect the clinical response for most dogs with macroscopic STS. Conventional radiation therapy only temporarily controls macroscopic canine STS. For example, a curative-intent megavoltage radiation protocol consisting of 10 fractions delivering a total of 45 and 50 Gy provided control rates of 48 and 67%, respectively, at one year and dropped to 33% for those receiving 50 Gy at two years (49). Coarsely, hypofractionated radiation treatment has been investigated to manage dogs with macroscopic STS. A coarsely fractionated 4×8 Gy protocol delivered once per week extended survival for 286–309 days (50), while hypofractionated radiation treatment in a 5×6 Gy protocol with fractions administered twice per week extended survival 368–658 days (51). In regard to chemotherapy providing tumor control, one study showed only 30% of dogs with high grade STS responded to doxorubicin (20). These suboptimal long-term treatment response rates underscore the need for a more aggressive treatment approach for dogs with macroscopic STS.

Stereotactic Body Radiotherapy (SBRT) is an advanced radiotherapy technique that has recently emerged in veterinary medicine. It differs from conventional radiation therapy as it delivers high-dose radiation with high precision, targeted directly to the tumor, with a steep dose drop off to spare normal tissues from significant radiation exposure (52, 53). Not only does SBRT provide tumor control with fewer treatments, which translates to fewer anesthetic episodes for veterinary patients, in human studies it has been associated with fewer side effects and improved success rates compared to conventional therapy for early-stage lung cancer, pancreatic cancer and liver tumors (54–56). It has also been shown that SBRT can serve as an alternative therapeutic option for local therapy for STS where wide surgical margins may be difficult to achieve (57). Despite these potential benefits, its clinical utility has not been greatly explored. One retrospective study of 23 human patients found SBRT of unresectable STS of the trunk with prescribed doses of 20–48 Gy in 1–5 fractions resulted in a local control rate of 52% and an OS rate of 39% at 5 years, with improved outcomes for benign vs. malignant tumors (19). In the context of human STS, SBRT

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has been mostly reserved to treat non-surgical lung metastasis (58, 59).

As the veterinary radiation oncology community has been increasingly treating canine macroscopic STS with SBRT, the purpose of this study was twofold: 1. to analyze the safety and efficacy of SBRT in treating canine macroscopic STS; and 2. identify prognostic factors associated with outcome that should be considered in the development of translational canine comparative oncology trials investigating SBRT for STS with respect to biological and/or clinical endpoints. The tumors in this population of dogs were treated with a variety of SBRT protocols across a spectrum of clinical scenarios including cases in which resection was not feasible or would be associated with undesirable morbidity, as well as owner preference for a non-surgical approach, ranging from definitive intent of achieving local control to palliation. We defined patient outcomes according to tumor progression, survival time, and incidence of acute and late radiation toxicities.

MATERIALS AND METHODS

Case Selection

Dogs with macroscopic STS treated with SBRT at Colorado State University (CSU) Flint Animal Cancer Center between February 2010 and February 2018 were included in this study. Inclusion criteria consisted of: 1. dogs with macroscopic tumors that had been definitively diagnosed with STS by biopsy or fine needle aspirate, 2. treatment with SBRT, and 3. follow-up time of at least 90 days after the start of treatment for individuals still alive at time of analysis. Patients with multiple malignant neoplastic processes at time of treatment, tumors in the oral or nasal cavity, or with primary histologic or immunohistochemical diagnosis of osteosarcoma, hemangiosarcoma, histiocytic sarcoma, and spinal nerve-root tumors were excluded from this study due to their differences in biological behavior from other subtypes of soft tissue sarcomas (23).

Data collected included: presenting clinical signs and physical examination findings, previous surgical resections and therapeutics, SBRT treatment parameters, post-SBRT surgical resections and therapeutics, complete blood cell count (CBC) and serum chemistry values pre- and post-SBRT, acute and late effects of radiation characterized according to the Veterinary Radiation Therapy Oncology Group (VRTOG) Radiation Toxicity Scoring Scheme (60), other adverse events, response to therapy, progression free survival, pattern of failure, overall survival, and cause of death. Complete blood cell count data was further analyzed using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 lymphopenia grading (61). The normal reference range for lymphocyte counts in our diagnostic laboratory is 1000–4800 cells/μl. Dogs were only included in analysis of circulating leukocyte changes if both pre- and post-SBRT CBCs were available within 3 months of the start of treatment and prior to the start of adjunct or concurrent chemotherapy.

Histologic Analysis

Biopsy samples from cases of STS available at CSU were reviewed and graded by a single pathologist (KLH) according to the Dennis *et al*. cutaneous and subcutaneous soft tissue sarcoma grading system in dogs (16). The additional histologic reports from remaining cases were also reviewed for grade information (KLH). Tumors were not assigned a grade if diagnosis was made by cytology or if insufficient cellular description was available in the report. If other tumor types could not be ruled out by Hematoxylin & Eosin staining alone, immunohistochemistry was performed. Cases concerning for histiocytic sarcoma were stained for CD204 positive macrophages (MSRA:CD204, SRA-E5 clone, TransGenic Inc.), lymphoma for CD3 positive T cells and Pax5 positive B cells (monoclonal mouse anti-human CD3, LN10 clone, Leica Biosystems Newcastle Ltd; monoclonal mouse anti-human Pax5, DAK-Pax5 clone; Dako North America Inc.). Cases were excluded if positive for CD204, CD3, or Pax5.

Radiation Treatment Planning

Imaging was performed using a Philips Gemini TF Big Bore 16-slice Computed Tomography (CT) scanner (Philips Medical Systems, Nederland, B.V.) at CSU Flint Animal Cancer Center for radiation planning. A non-contrast volumetric (helical) dataset was obtained prior to a post contrast series after IV injection (2.2 mL/kg) of omnipaque 350 contrast media (GE Healthcare, Princeton, NJ). Images were reconstructed at 2.0 mm contiguous intervals with a 512 matrix. Both the 2.0 mm precontrast and postcontrast CT scan were used for inverse treatment planning performed using a Varian Eclipse treatment planning system (Varian Medical Systems, Inc Palo Alto, CA). Organs at risk (OAR) and gross tumor volume (GTV) were identified and contoured. A 2–6 mm isotropic planned target volume (PTV) expansion encompassed the GTV to account for daily set-up positioning error (Fig. 1). No clinical target volume (CTV) was utilized for any treatment. All plans were designed using coplanar or noncoplanar, isocentrically placed 6 and/or 10 MV radiation beams or volumetric modulated arc therapy (VMAT). Radiation beams were modulated using sliding-window technique. The intent for each radiation plan was to deliver 100% of the radiation prescription to 99% of the GTV and 95% of the PTV. Quality assurance was performed by gamma analysis using the Varian portal dosimetry system on individual fields. A minimum of 95% gamma for a 3 mm distance to agreement and a 3% absolute dose difference have been institutionally defined as a passing score.

Retrospective data collection from radiation treatment plans included: Gross tumor volume (GTV), planning target volume (PTV), organs at risk (OAR), dose prescription, dose administered to 99% of GTV, dose administered to 95% of PTV, maximum tumor dose, minimum tumor dose, and mean doses to the PTV.

To compare results across various SBRT protocols, biological effective dose (BED) was calculated for 99% GTV, 95% PTV, and to evaluate dose to the skin in 1 cc, maximum, and full thickness. Biologically effective dose allows for comparison of different fractionation regimens with differing total dose using a common numerical score (62) BED was calculated as

$$\mathrm{BED} = \mathrm{D}\left(1 + \left[\mathrm{d}/\left(\frac{\mathrm{a}}{\mathrm{b}}\right)\right]\right). \qquad \text{Eq. (1)}$$

(62), where

D = total dose (number of fractions × dose per fraction) in Gy;
d = dose per fraction, in Gy;
α/β ratio = 4 Gy.

An α/β ratio of 4 Gy was used for soft tissue sarcoma is recognized as a tumor type with a relatively low (−0.5 to 5.4) α/β ratio (63–66), determined to be approximately 4 Gy in previous studies (63–66). An α/β ratio of 10 Gy was utilized to evaluate early effects to the skin and 3 Gy was utilized to evaluate late effects to the skin (67).

Radiation Treatment

Patients were anesthetized with varying protocols for radiation treatment, generally consisting of an opioid pre-medication with propofol induction and inhalant maintenance. They were positioned in customized immobilization devices from CT simulation through each fraction of SBRT. Accuracy of positioning was confirmed with online registration of the simulation CT using an on-board cone beam CT. SBRT beams were delivered with a Varian Trilogy® system linear accelerator (Varian Medical Systems, Inc. Palo Alto, CA).

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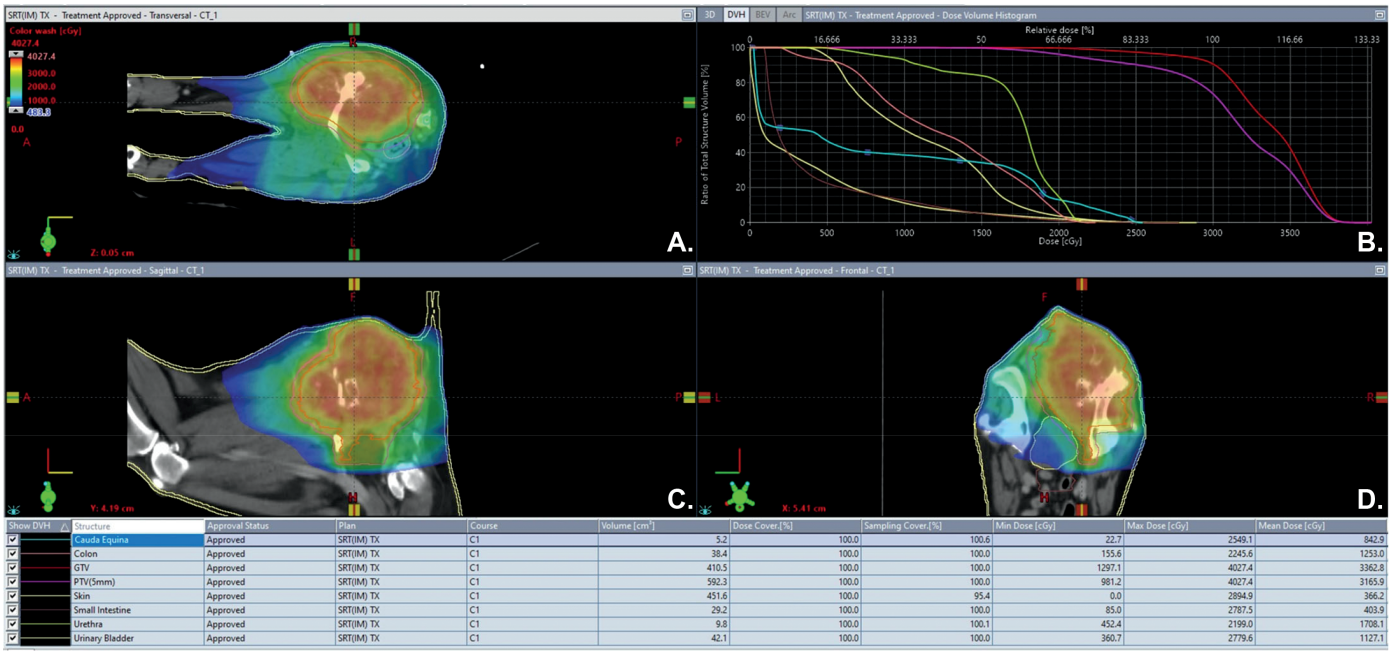

Show DVH	Structure	Approval Status	Plan	Course	Volume [cm³]	Dose Cover.[%]	Sampling Cover.[%]	Min Dose [cGy]	Max Dose [cGy]	Mean Dose [cGy]
☑	Cauda Equina	Approved	SRT(IM) TX	C1	5.2	100.0	100.6	22.7	2549.1	842.9
☑	Colon	Approved	SRT(IM) TX	C1	38.4	100.0	100.0	155.6	2245.6	1253.0
☑	GTV	Approved	SRT(IM) TX	C1	410.5	100.0	100.0	1297.1	4027.4	3362.8
☑	PTV(5mm)	Approved	SRT(IM) TX	C1	592.3	100.0	100.0	981.2	4027.4	3165.9
☑	Skin	Approved	SRT(IM) TX	C1	451.6	100.0	95.4	0.0	2894.9	366.2
☑	Small Intestine	Approved	SRT(IM) TX	C1	29.2	100.0	100.0	85.0	2787.5	403.9
☑	Urethra	Approved	SRT(IM) TX	C1	9.8	100.0	100.1	452.4	2199.0	1708.1
☑	Urinary Bladder	Approved	SRT(IM) TX	C1	42.1	100.0	100.0	360.7	2779.6	1127.1

FIG. 1. Representative CT-guided radiation treatment plan for a case of canine soft tissue sarcoma. Representative CT guided radiation treatment plan for a case of canine soft tissue sarcoma depicting with the distribution of the radiation via dose color wash in (panel A) transverse, (panel C) sagittal, and (panel D) frontal views with (panel B) corresponding dose volume histogram (DVH) for treated tumor volumes and organs at risk. Dose prescribed was three fractions of 10 Gy.

Follow-Up

Two- and four-week post-SBRT follow-up appointments were recommended to all patients to screen for and monitor acute radiation therapy effects. Additional recheck physical examinations and staging diagnostic tests (CBC, serum chemistry, thoracic radiographs) were recommended every 3 to 6 months for the first two years, then every 6 to 12 months after that to evaluate the irradiated tumor site and monitor for evidence of late toxicity. More frequent monitoring was recommended for patients with high grade tumors or those receiving adjunct chemotherapeutics.

Tumor Response

Tumor response was evaluated using Response Evaluation Criteria in Solid Tumor (RECIST) guidelines for target lesion and overall response (18, 68). Response criteria were defined as follows: Complete Response (CR) as the disappearance of the tumor, Partial Response (PR) at least 30% reduction in sum of longest diameter (LD) of the tumor, stable disease (SD) less than 30% reduction or 20% increase in the LD of the tumor, and progressive disease (PD) the appearance of one or more new lesions or at least a 20% increase in the LD of the tumor, taking as reference the smallest LD on study. Maintenance of the treatment response for at least 90 days was required for classification of local response. Identified tumor baseline measurements were taken by calipers with physical examination or measurements from CT imaging if caliper measurements were not available in the record. Overall response rate included both complete and partial response. Response was deemed unevaluable (NE) if no follow-up tumor measurements were taken before time of death, nor progressive changes adequately described. Those with unevaluable responses were not included in analysis. Patients with symptomatic deterioration, defined as disease progression affecting quality of life described without quantitative tumor measurements recorded from physical examination or diagnostic imaging were categorized as PD.

Statistical Analysis

Progression free survival time (PFST) was defined as the time from start of treatment until either local tumor progression, metastasis, or symptomatic deterioration. Overall survival time (OST) was defined as the time from start of treatment until death. Patients were censored if lost to follow-up, or still alive at the end of the study. For PFST, patients were also censored if there was no documented progression before time of death. Survival analysis was performed on an intent-to-treat basis; events included time to progression, time to first adverse event, and death of the patients. To assess patient outcomes, univariate time-to-event analysis was conducted by generating Kaplan-Meier curves with comparisons using the Cox proportional hazard test for OS and PFS; the variables that met the criteria for $P < 0.20$ were selected for inclusion in a multivariable model. Forward, stepwise multivariable Cox regressions were used to assess the influence of demographics, tumor, and treatment factors on OS and PFS. The favored model for each Cox regression included covariates with $P < 0.05$ in the final model and any variable whose removal causes a greater than 10% change in the hazard ratio of a variable of interest. All variables were evaluated for interaction in the favored model. For all time-to-event analyses, time was measured from the start of radiation treatment.

Association of categorical predictors and RECIST treatment response was examined by Fisher's exact test. Continuous predictors for RECIST treatment response and development of radiation induced toxicities were evaluated by the independent t-test. Pre and post leukocyte counts were compared with a paired t-test and changes in lymphopenia grade by Fisher's exact test. Shapiro-Wilk test was used to confirm normal distribution. If data were not normally distributed, the non-parametric Mann-Whitney U test was performed to compare outcomes. Survival time was interpreted as the median value with 95% confidence intervals. Descriptive data were described using means and 95% confidence intervals, or medians and range. A P value less than 0.05 was considered for statistical significance. STATA/IC 16.1 (StataCorp, College Station, TX) and GraphPad Prism version 8.00 for

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TABLE 1
Tumor Grade and Categorization of Failure

	Total		Presented with metastasis		Progressed distantly		Progressed locally		Did not progress	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Grade 3	19	36.5%	4	21.1%	11	57.9%	11	57.9%	1	5.3%
Grade 2	20	38.5%	4	20.0%	8	40.0%	13	65.0%	5	25.0%
Grade 1	9	17.3%	2	22.22%	4	44.4%	2	22.2%	4	44.4%
No grade	4	7.7%	1	25.0%	1	25.0%	0	0.0%	2	50.0%
Total	52	100.0%	11	21.2%	24	46.2%	26	50.0%	12	23.1%

Mac (GraphPad Software, La Jolla, CA) were used for all statistical analyses.

RESULTS

Patient Inclusion

Ninety-three canine patients with STS treated with SBRT were initially identified. Twenty-seven were excluded by descriptive record review: those with a tumor of the oral or nasal cavity, spinal nerve root tumors, and with a histologic or clinically presumptive diagnosis of histiocytic sarcoma, hemangiosarcoma, osteosarcoma, and chondrosarcoma. Five patients were excluded due to secondary malignant neoplastic processes identified at time of treatment. Five additional patients were excluded for insufficient follow up time (less than 90 days), and two were determined to be treated with three-dimensional conformal radiation therapy upon retrospective analysis of radiation treatment plans and were excluded.

Biopsy samples from 23 available cases of soft tissue sarcomas were reviewed and graded by a single pathologist (KLH). There were five cases in which other types of tumors could not be completely ruled-out by hematoxylin & eosin (H&E) alone. One that stained CD204 positive – diagnostic for histiocytic sarcoma – was removed from the study. Immunohistochemistry was additionally performed for one suspect lymphoma case confirmed to be CD3 and Pax5 negative (markers for B and T cells) and three high-grade soft tissue sarcomas were CD204 negative with no evidence of osteoid or chondroid matrix; these four cases were kept in the study. One additional case was removed due to description in the available histologic report being most consistent with histiocytic sarcoma.

Patient Demographics

Fifty-two canine patients met inclusion criteria for this study. The median age of patients at time of treatment was 10 years (range, 3–15 years). Patient population included 29 spayed females (55.8%), 18 castrated males (34.6%), and 5 intact males (9.6%). Genetic background of patients varied, with the most common being those of mixed breeds (n = 14; 26.9%), Labrador Retrievers (n = 12; 23.1%), Golden Retrievers (n = 6; 11.5%), and German Shepherds (n = 4; 7.7%). Other breeds included Brittany Spaniels (n = 2; 3.8%), Miniature Schnauzers (n = 2; 3.8%), Portuguese Water Dogs (n = 2; 3.8%), and one each (1.9%) of the following breeds: Beagle, Cairn Terrier, Corgi, German Shorthaired Pointer, Goldendoodle, Great Dane, Greyhound, Skye Terrier, Standard Poodle, and Weimaraner. Median patient weight was 31.5 kg (range, 3.4–68.1 kg).

Tumor Population

With respect to anatomic location, 24 tumors were truncal (46.2%), 11 were appendicular (21.2%), and 3 were in the head and neck region (5.8%). Tumors ranged in size from 4.7 cm^3 to 10778.3 cm^3 with a median gross tumor volume of 345.5 cm^3. For outcome analysis, patients were divided into groups based on tumor volume, with a small tumor (n = 11; 21.2%) being less than 80 cm^3, a medium sized tumor (n = 17; 32.7%) falling in the range of 80–400 cm^3, and a large tumor (n = 24; 46.2%) being greater than 400 cm^3.

Twenty-three tumors were recurrent at presentation (44.2%); eight tumors (15.4%) were excised two or more times prior to SBRT treatment. Eleven tumors (21.1%) had metastasized to either the lungs (n = 5, 9.6%), regional lymph nodes (n = 2, 3.8%), or both (n = 4, 7.7%) at the time of treatment. Six additional patients (11.5%) presented with lymphadenopathy of the draining lymph node at the start of treatment that were not cytologically confirmed to be metastatic STS. Grading and tumor type were confirmed for the patients with biopsy samples available for review at CSU (n = 22; 42.3%). Most tumors were high grade on presentation, 19 were a histologic grade 3 (36.5%), 20 grade 2 (38.5%), nine grade 1 (17.3%); the remaining four were not assigned a grade (7.7%) due to cytology diagnosis (n = 3) or incisional biopsy (n = 1) with insufficient cellular description. Of the 19 patients with grade 3 tumors, four presented with metastasis (21.1%); of the 20 grade 2 tumors, four patients presented with metastasis (20.0%); and of the 9 patients with grade 1 tumors, two presented with metastasis (22.2%) (Table 1). Size of tumor did not differ by grade.

Treatment Protocols

Several SBRT protocols were utilized in this study with total dose prescribed ranging from 20 to 50 Gy with a median of 30 Gy in 1–5 consecutive or alternating daily treatments, delivered over a Monday–Friday schedule. The median overall dose administered to patients in 99% of the GTV was 22.3 Gy (range, 4.6–50.4 Gy) and in 95% of the

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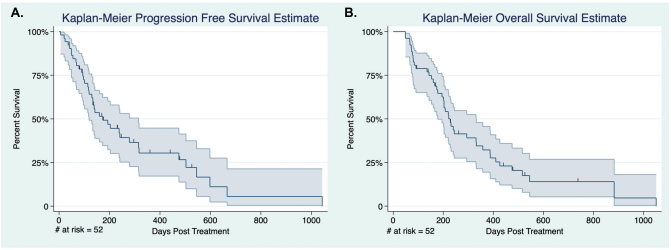

FIG. 2. Progression free and overall survival time. Kaplan-Meier curves of PFS and OST. Panel A: Median progression free survival was 173 days (95% CI = 119–315). Panel B: Median overall survival time for the entire cohort was 228 days (95% CI = 178–332) (Fig. 2). Tick marks indicate time of censoring, faded areas indicate 95% confidence intervals.

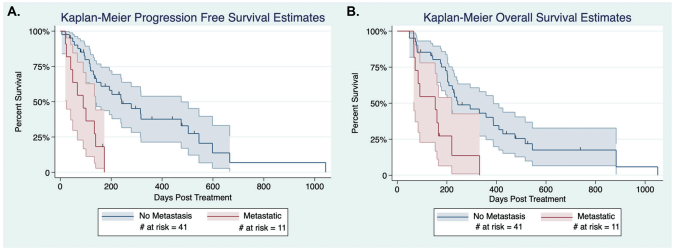

FIG. 3. Patients with metastatic disease treated with SBRT have worse survival outcomes. Kaplan-Meier curves of PFS and OST by metastasis at presentation. Panel A: Median PFST of 90 (95% CI = 24–139) vs. 241 days (95% CI = 134–475 days) for those with metastatic disease ($P = 0.009$). Panel B: Median OST of 153 days (95% CI = 67–220) vs. 244 days (95% CI = 203–338) ($P = 0.003$). Tick marks indicate time of censoring, faded regions indicate 95% confidence intervals.

PTV was 21.9 Gy (range, 7.8–46.8 Gy). The median administered relative dose expressed as a percent of the prescribed 99% of the GTV and 95% of the PTV dose was 72.7% and 74.7%, respectively.

Secondary sites, loci of lung and lymph node metastasis, were irradiated at time of initial treatment for two patients. A single patient's mass changed significantly in size requiring a repeat CT and radiation planning for the final, third treatment. One patient received a second SBRT treatment one year after initial therapy due to development of progressive disease.

Twenty-five patients (48.1%) were treated with adjunct chemotherapy. The most common type being doxorubicin (n = 15, 28.8%). Other chemotherapeutics prescribed included cyclophosphamide (n = 13, 25.0%), toceranib (n = 8, 15.4%), lomustine (n = 2, 3.8%), mitoxantrone (n = 2, 3.8%), chlorambucil (n = 1, 1.75%), epirubicin (n = 1; 1.9%), and electrochemotherapy with cisplatin (n = 1; 1.9%). Many of these adjunct therapies were utilized in combination or in series, often with the addition of a non-steroidal anti-inflammatory (NSAID) (n = 38, 73.1%). Specific NSAIDs prescribed included carprofen (n = 30, 57.7%), meloxicam (n = 3, 5.8%), deracoxib (n = 2, 3.8%), piroxicam (n = 2, 3.8%), firocoxib (n = 1, 1.9%). Chemotherapeutics were commonly administered to patients with grade 3 tumors (68.4%) while 35.0% with grade 2 and 44.4% with grade 1 tumors received adjunct chemotherapy.

Finally, 10 dogs (19.2%) underwent surgical excision of their tumor post-SBRT. The median time to surgery after SBRT was 113 days (range, 23–317 days). Of these patients that underwent post-SBRT surgery, three dogs (5.8%) also were treated with adjuvant doxorubicin chemotherapy.

Response to Treatment

Median follow up time was 214 days (95% CI = 168–253). Best overall response (Fig. 4) was calculated for 46 patients (88.5%). Response rate (CR+PR) was 30.4% (CR n = 3, 6.5%; PR n = 11, 23.9%). Nineteen dogs maintained stable disease (41.3%) after SBRT and 13 dogs had progressive disease (28.3%). These patients (SD + PD) were classified as non-responders. Tumor response in the other six patients was inevaluable due to insufficient follow up data. Median duration of best overall response was 212 days (95% CI = 155–364). Median time to response for those that were classified as responders (CR + PR) was 95 days (95% CI = 47–194). Median duration of stable disease was 174 days (95% CI = 114–239). Of those classified as responders, nine (64.3%) went on to progress before death. Despite ultimate progression, only five of these responders (35.7%) died due to their soft tissue sarcoma. Twenty-one patients (65.6%) classified as non-responders (SD + PD) succumbed due to disease, with 11 patients (57.9%) classified as a best overall response of stable disease continuing to progress prior to death. Best overall response was not a statistically significant predictor for cause of death ($P = 0.746$). Patients that were non-metastatic at presentation were more likely to respond to treatment. None of the animals with available response data that presented with metastasis responded (n = 9), while 35% of animals (n = 13) without metastasis at presentation responded to treatment ($P = 0.044$).

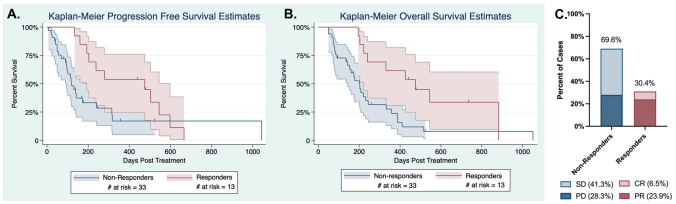

FIG. 4. Patients with local tumor response to SBRT have improved survival outcomes. Kaplan-Meier curves of PFS and OST by response. Panel A: Median PFST was 475 days (95% CI = 193–598) vs. 119 days (95% CI = 90–241) ($P = 0.0547$). Panel B: Median OST was 475 days (95% CI = 222–714) vs. 201 (95% CI = 143–244) for non-responders ($P = 0.0093$). Tick marks indicate time of censoring. Panel C: Stacked bar chart showing breakdown of best overall response by those classified as CR (6.5%), PR (23.9%), SD (41.3%), and PD (28.3%). Tick marks indicate time of censoring, faded regions indicate 95% confidence intervals.

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TABLE 2
Radiation-Induced Toxicities

	None		Grade 1		Grade 2		Grade 3	
	N	(%)	N	(%)	N	(%)	N	(%)
Acute toxicities (<3 months)	36	69.2	10	19.2	2	3.8	4[a]	7.7
Late toxicities (>3 months)	37	71.2	7	13.5	2	3.8	6	11.5

[a] All four grade 3 acute skin toxicities developed either grade 2 or 3 consequential late effects.

Survival

Median progression free survival time for the entire cohort was 173 days (95% CI = 119–315) (Fig. 2). Three subjects were alive at time of data collection without clinical evidence of progression which were censored at 167, 438, and 476 days after SBRT. Six dogs were censored for PFST as they did not show clinical evidence of STS progression at time of death 73, 174, 228, 244, 294, and 358 days after SBRT. Three subjects were lost to follow-up prior to onset of clinical signs of progression and censored on the last day of communication at 92, 229, and 524 days after SBRT.

Median overall survival time for the entire cohort was 228 days (95% CI = 178-332) (Figure 2), similar to disease specific overall survival time of 203 days (95% CI = 153-244). Five subjects were alive at time of data collection and were censored at 137, 167, 438, 476, and 736 days after SBRT. Four were lost to follow-up with no documented time of death and censored on the last day of communication at 92, 229, 259, and 524 after SBRT, respectively. Cause of death was obtained for 37 of the 43 patients known to be deceased (86.1%). Death or euthanasia was tumor-associated in 28 patients (65.1%) related to primary tumor progression (n = 10; 23.2%), metastasis (n = 5; 11.6%), a combination of local and distant failure (n = 10; 23.2%), or other sequelae such as tumor-associated infection and ulceration (n = 1; 2.3%), paraneoplastic thrombocytopenia (n = 1, 2.3%), and a cardiovascular event (n = 1; 2.3%) which could not be determined to be secondary to tumor progression or radiation toxicity. For those that did not die due to local progression, the tumor was classified as CR (n = 2), PR (n = 4), or SD (n = 2) at time of death. Five animals (11.6%) died after developing additional malignancies, including two with confirmed hemangiosarcoma, two with multicentric lymphoma, and one with a thymic neuroendocrine carcinoma. Other causes of death occurred in four patients: a description of ''old age changes'' (i.e., slowing down due to arthritis) (n = 2; 4.6%), megaesophagus and subsequent aspiration pneumonia (n = 1; 2.3%), and seizure (n = 1; 2.3%).

Primary tumor status varied at time of death for those with non-STS related death (n = 15; 34.9%). One tumor had no appreciable disease at time of death (CR); this patient died due to other neoplasia. Tumors in four patients maintained a PR at time of death – three died due to other causes and cause of death was unknown for the remaining patient. The tumor was considered to be stable in two patients at time of death – one died of other neoplasia and cause of death for the other was unknown. Four tumors progressed at time of death – two of which in patients that died of other neoplasia, one of non-neoplastic causes, and one with an unknown cause of death. Local tumor response was NE for the remaining four deceased patients.

Prognostic Factors

Both metastasis and tumor volume were found to be predictive of failure for both progression free survival (Table 3) and overall survival (Table 4). Patients that present with metastasis were 3 times more likely to experience progression at all times of follow up than those without metastasis when controlling for size of tumor (HR = 3.05, 95% CI = 1.33–7.03; $P = 0.009$). Median progression free survival time for those presenting with metastasis was 90 days (95% CI = 24–139) vs. 241 days (95% CI = 134–475) for those without metastasis (Fig. 3A). Associated with each 1 unit (1 cc) of tumor volume is an increased risk of failure at all times of follow-up (HR = 1.0006, 95% CI = 1.0002–1.0009; $P = 0.002$). This means that for every 100 cc of tumor volume at the time of treatment, the risk of progression increases by 5% at all times of follow up.

Similarly, patients that present with metastasis are 3.5 times more likely to experience death at all times of follow up than those without metastasis when controlling for size of tumor and the biologically effective dose administered to 95% of the PTV in Gy_4 (HR 3.57, 95% CI = 1.4277–6.9756; $P = 0.003$). Median overall survival time for those presenting with metastasis was 153 days (95% CI = 67–220) vs. 244 days (95% CI = 203–338) for those without metastasis (Fig. 3B). Associated with each 1 cc of tumor

TABLE 3
Favored Multivariable Cox Proportional Hazards Model for Progression Free Survival Time

	HR	95% CI	*P* value
Clinical factors at presentation (n = 52)			0.0001
Metastasis	3.05 (4.08)	1.33–7.03 (1.81–9.19)	0.009 (0.0016)
Gross tumor volume (cc)	1.0006 (1.0006)	1.0002–1.0009 (1.0002–1.0011)	0.002 (0.0004)

Notes. HR, 95% CI, and *P* values reported as adjusted (crude). Alpha for inclusion in multivariable model was set at $P < 0.20$.

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TABLE 4
Favored Multivariable Cox Proportional Hazards Model for Overall Survival Time

	HR	95% CI	*P* value
Clinical factors at presentation (n = 47)			
Metastasis	3.57 (3.69)	1.53–8.35 (1.69–8.04)	0.003 (0.0026)
Gross tumor volume (cc)	1.0005 (1.0005)	1.0002–1.0007 (1.0002–1.0008)	0.001 (0.0002)
BED to 95% PTV in Gy 4[a]	0.99 (0.99)	0.98–1.00 (0.98–1.00)	0.170 (0.1728)

Notes. HR, 95% CI, and *P* values reported as adjusted (crude). Alpha for inclusion in multivariable model was set at $P < 0.20$.
[a] Confounding variable (when dropped from model results in 15% change in HR for the primary exposure metastasis.

volume is an increased risk of failure at all times of follow-up controlling for metastasis and biologically effective dose administered to 95% of the PTV in Gy_4 (HR = 1.0005, 95% CI = 1.0002–1.0007; $P = 0.001$). The risk of death increases by 0.05% for every 1 cc in tumor volume. This means that for every 100 cc of tumor volume at the time of treatment, the risk of progression increases by 5% at all times of follow up.

The following were analyzed by univariate analysis prior to inclusion in the multivariable model and did not significantly affect outcomes (Table 5): tumor grade, tumor recurrence prior to SBRT, number of surgical excisions prior to SBRT, use of adjunct chemotherapeutics, surgical excision after treatment, number of fractionations, overall prescribed dose (Gy), 99% GTV dose (Gy), 95% PTV dose (Gy), and BED as 99% GTV dose in Gy_4. Only BED as 95% PTV dose in Gy_4 was included in the final model for OS as it was a confounding variable for metastasis (resulting in a 15% change to the HR).

Survival analysis for response was not included in the multivariable model as response is dependent on treatment. Survival varied based on RECIST criteria for best overall responders (CR + PR) and non-responders (SD + PD). Responders were 50% less likely to progress at all points of follow up. This was not statistically significant (HR: 0.49, 95% CI = 0.23–1.04, $P = 0.055$). Time to progression for responders was 475 days (95% CI = 193–598) vs. 119 days (95% CI = 90–241) for non-responders (Fig. 4A). Risk of death decreased by 60% for responders vs. non-responders at all times of follow up (HR: 0.39, 95% CI = 0.18–0.82, $P = 0.009$). Overall survival for responders was 475 days (95% CI = 222–714) vs. 201 (95% CI = 143–244) days for non-responders (Fig. 4B).

Radiation-Induced Toxicities

Acute radiation toxicities (Table 2) were defined according to VRTOG toxicity criteria, for any affected organ or tissue in the treatment field occurring within three months of treatment. Overall, 16 patients (30.8%) developed acute toxicities. Ten patients developed acute toxicities with a VRTOG score of 1 (19.2%), two a VRTOG score of 2 (3.8%), and four a VRTOG score of 3 (7.7%). The most common organ or tissue affected was skin and hair in 15 patients, making up 93.8% of reported acute toxicities. Ten of the 15 skin and hair acute toxicities were grade 1 consisting of self-limiting alopecia, erythema, and pruritus of the irradiated site. One was a skin and hair grade 2 acute toxicity with two small patches of moist desquamation over the irradiated site. Four dogs developed grade 3 acute toxicity of the skin and hair with confluent erythema and moist desquamation and ulceration. The other acute toxicity was a grade 2 gastrointestinal (GI) toxicity resulting in diarrhea, which was responsive to oral medications consisting of antibiotics and pain control. The colon was a confirmed OAR.

Lymphopenia scoring occurring within three months of treatment was assessed according to the CTCAE v5.0. Twelve patients had relevant CBC data that could be analyzed for radiation-induced modulation of circulating lymphocytes. On average, the absolute lymphocyte count dropped from 1413 cells/μL (95% CI, 979–1846 cells/μL) to 692 (95% CI, 408–975 cells/μL, $P = 0.012$) for patients with available CBC data. Similarly, the neutrophil–lymphocyte ratio increased from 5 (95% CI, 4–7) to 17 (95% CI, 8–26) ($P = 0.025$). There was no difference in the total white blood cell count, absolute neutrophil count and absolute monocyte count (Fig. 5). Prior to treatment, nine of the 12 dogs (75%) had an absolute lymphocyte within normal range and three patients with mild-moderate lymphopenia (25%), either grade 1 or 2. After treatment, lymphopenia grade worsened for 66.7% of dogs (n = 8): five developed lymphopenia and the three with pre-existing lymphopenia worsened in severity. Six of these dogs developed severe grade 3 or 4 lymphopenia. The change in number of dogs with lymphopenia grade >1 pre- and post-SBRT was not statistically significant ($P = 0.100$)

Late toxicities (Table 2) were defined according to the VRTOG scoring scheme and occurring more than 3 months after treatment. Late toxicities were reported in 28.8% of patients (n = 15). Seven patients had a highest VRTOG score of 1 (13.5%), two a highest VRTOG score of 2 (3.8%), and six a highest VRTOG score of 3 (11.5%). Again, skin and hair were the most commonly affected tissues (n = 13). Seven of the 13 skin and hair late toxicities were grade 1 consisting of chronic alopecia, leukotrichia, and hyperpigmentation. One was a grade 2 toxicity with scarring and fibrosis of the irradiated site. Five of the dogs with late skin toxicity were grade 3 consisting of progressive ulceration and necrosis of the irradiated site and in one case suspect radiation-induced lymphedema. Bone was the only other tissue affected by late radiation toxicity (n = 3). Two grade 2 bone late toxicities were

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TABLE 5
Univariate Cox Proportional Hazards Analysis for time to event analysis

	Median progression free survival			Median overall survival		
Clinical factors at presentation	Time (days)	HR (95% CI)	*P* value	Time (days)	HR (95% CI)	*P* value
Sex						
Male (n = 23)	173	0.86 (0.44–1.67)	0.6516	220	1.12 (0.61–2.07)	0.7217
Female (n = 29)	142	Reference		240	Reference	
Sex status						
Intact (n = 5)	97	1.31 (0.40–4.32)	0.6667	203	1.97 (0.69–5.64)	0.2427
Spayed/neutered (n = 47)	193	Reference		228	Reference	
Age	-	1.02 (0.92–1.13)	0.7677	-	0.99 (0.90–1.09)	0.8617
Metastasis						
Metastatic (n = 11)	90	4.08 (1.81–9.19)	0.0016	153	3.69 (1.69–8.04)	0.0026
Non-metastatic (n = 41)	241	Reference		244	Reference	
Histologic grade						
Grade 3 (n = 19)	142	1.41 (0.55–3.63)	0.4760	231	0.88 (0.38–2.05)	0.7620
Grade 2 (n = 20)	160	0.93 (0.35–2.51)	0.8900	203	0.61 (0.25–1.48)	0.2740
Grade 1 (n = 9)	238	Reference		228	Reference	
Location						
Truncal (n = 17)	142	1.94 (0.91–4.14)	0.0850	208	1.6 (0.79–3.21)	0.1910
Head and neck (n = 5)	160	1.42 (0.38–5.29)	0.6000	244	1.41 (0.45–4.49)	0.5570
Appendicular (n = 30)	503	Reference		294	Reference	
Tumor status						
Local recurrence (n = 22)	142	1.29 (0.65–2.59)	0.4699	208	1.48 (0.78–2.79)	0.2312
Primary (n = 30)	193	Reference	-	222	Reference	
After SBRT surgery						
Resected (n = 10)	160	1.41 (0.66–3.02)	0.3942	331	1.27 (0.57–2.81)	0.5201
Not resected (n = 42)	203	Reference		222	Reference	
Gross tumor volume (cc)	-	1.0006 (1.0002–1.0011)	0.0004	-	1.0005 (1.0002–1.0008)	0.0002
Adjunct chemotherapy						
Chemotherapy (n = 26)	134	1.74 (0.89–3.39)	0.1000	231	0.98 (0.53–1.80)	0.9444
No chemotherapy (n = 26)	238	Reference		220	Reference	
99% GTV dose	-	0.99 (0.95–1.02)	0.4518	-	1.00 (0.97–1.04)	0.8178
95% PTV dose	-	0.98 (0.93–1.02)	0.3145	-	0.99 (0.95–1.03)	0.6130
BED to 99% GTV in Gy4	-	0.99 (0.99–1.00)	0.1926	-	1.00 (0.99–1.01)	0.4881
BED to 95% PTV in Gy4	-	0.99 (0.98–1.00)	0.1203	-	0.99 (0.98–1.00)	0.1728

Notes. Total number of subjects = 52; 4 values were excluded from histologic grade due to cytologic diagnosis; 5 values were excluded from biologically effective dose due to inability to calculate this value for animals receiving different Gy/fraction. Alpha for inclusion in multivariable model was set at $P < 0.20$.

characterized as radiographic radiolucencies. One grade 3 bone late toxicity resulted in a large lytic lesion and eventual pathologic fracture of the irradiated site. One patient developed both skin and bone late toxicity. Six patients (11.5%) developed both acute and late toxicities with all four of the grade 3 acute skin toxicities consequently developing grade 2 (n = 1) or 3 (n = 3) late skin toxicity, this included the patient with two organs affected by late toxicity. One patient with grade 1 acute skin toxicity developed grade 1 late toxicity and one patient with grade 2 GI acute toxicity subsequently developed a grade 1 late skin toxicity.

Skin was the most common organ at risk in this study and reported as an OAR for each patient. The dose to one cm^3 of skin, maximum skin dose, and full thickness dose to the skin was evaluated in Gy, Gy_{10} and Gy_3. There was a significant correlation between the maximum skin dose in Gy_{10} and development of acute toxicity ($P < 0.0001$). Mean maximum dose for those developing acute toxicities was 54.89 Gy_{10} (95% CI = 46.47–63.31) vs. 29.24 Gy_{10} (95% CI = 26.84–31.64) for those that did not. The one cm^3 and maximum doses (Gy) to the skin correlated with development of late toxicities ($P = 0.039$; $P = 0.010$). Mean 1 cm^3 dose for those dogs developing late toxicities was 25.95 Gy (95% CI = 23.16–28.73) vs. 22.02 Gy (95% CI = 19.87–24.16). Mean maximum skin dose for those developing late toxicities was 32.69 Gy (95% CI = 28.87–36.52) vs. 27.09 Gy (95% CI = 24.85–29.34) (Fig. 6). There was insufficient power to analyze dose to skin by grade of radiation side effect.

Adverse Events Associated with Treatment

Adverse events affecting quality of life were noted in five animals (9.6%), with a median time to first event of 107 days (95% CI = 14–130). Adverse events associated with treatment that were not classified as an acute or late toxicity included: rapid progression and subsequent ulceration and necrosis (n = 1), sudden onset edema with fluid pockets, rupture, and drainage (n = 1), recurrent skin infection (n = 1), wound dehiscence, ulceration, and infection after

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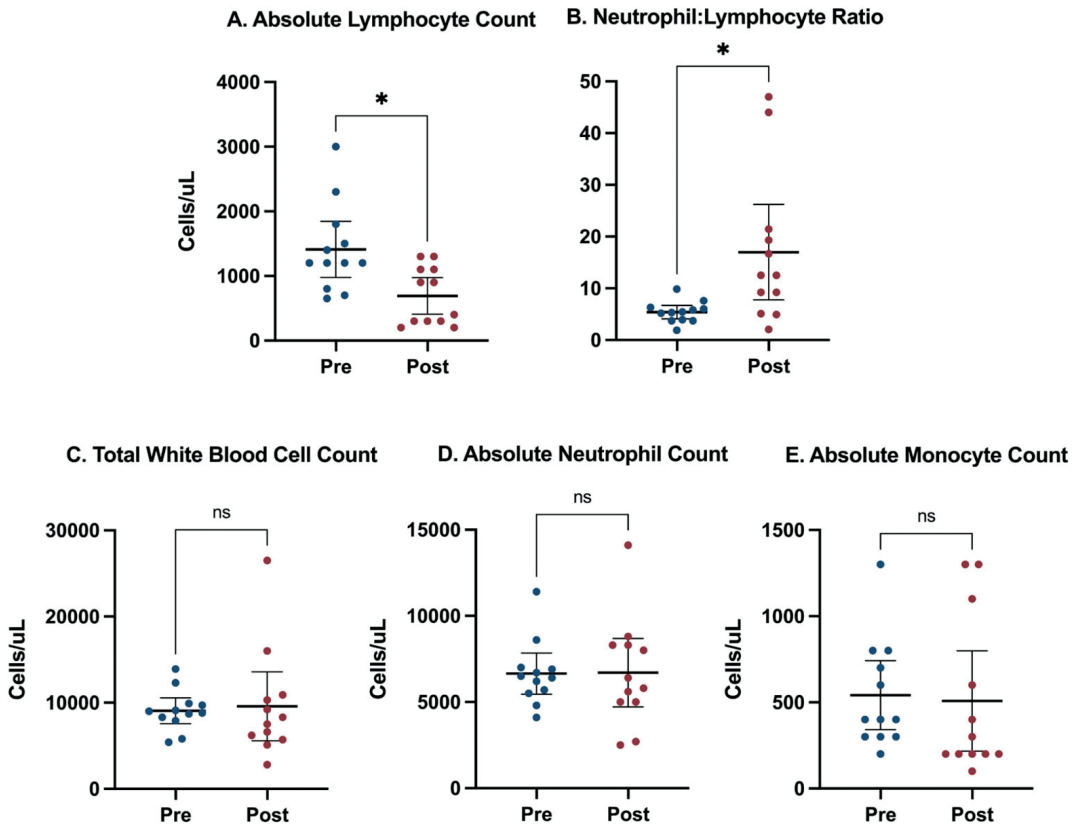

FIG. 5. Immunomodulatory effects of SBRT result in changes to circulating lymphocytes. Scatter plots with means and 95% CI of complete blood cell count differential data taken pre and post SBRT. Panel A: On average, the absolute lymphocyte count dropped from 1413 (95% CI = 979–1846) to 692 (95% CI = 408–975) (P = 0.0124, paired t-test). Panel B: The neutrophil-lymphocyte ratio increased from 5 (95% CI = 4–7) to 17 (95% CI = 8–26) (P = 0.0252, paired t-test). There was no difference in the (panel C) total white blood cell count (9067, 95% CI = 7568–10565 vs. 9592, 95% CI = 4486–13598, P = 0.7636), (panel D) absolute neutrophil count (6650, 95% CI = 5454–7846 vs. 6708, 95% CI = 4722–8695, P = 0.9431) and (panel E) absolute monocyte count (542, 95% CI = 342–742 vs. 508 95% CI = 217–799).

palliative resection (n = 1), recurrent multi-bacterial skin infection (n = 1), and progression and tumor breaking through skin leading to infection and ulceration (n = 1).

Postmortem Findings

Six patients (14.3%) underwent full necropsy and histopathologic analysis at CSU after death or euthanasia. Of note, necropsy confirmed STS metastasis to the pericardium, adrenal glands, and brain in a patient with grade 3 STS of the left hip and previous distant lung metastasis requiring partial lung lobectomy post SBRT. The cerebrum has been rarely reported as a primary site of STS in canine patients (69). Metastasis of STS to the brain in humans is rare, reported in 1–10% of cases (70, 71). Another patient with grade 2 STS of the left flank and grade 3 STS of the left stifle developed disseminated anaplastic sarcoma of the lungs, kidneys, diaphragm, myocardium, skeletal muscle, pancreas, small intestine, rectum, adrenal gland, and lymph nodes. Histiocytic and neuroendocrine origin of tumors in both patients was ruled out by IHC. Despite the absence of skin presentation of acute or late radiation effects, the tissue underlying the radiation site in both cases developed extensive necrosis. One patient with grade 3 STS of the left wing of the ilium developed bone invasion by the tumor and osteoradionecrosis of the pelvis suspected to be induced by radiation therapy, inapparent on follow-up CT. Two cases developed histologically confirmed hemangiosarcoma within the radiation field after SBRT and subsequent cytoreductive therapy or amputation, in which the primary lesion did not recur by time of death. The last report confirmed complete response of the primary grade 1 tumor, but the development of a new grade 1 STS at a distant site.

DISCUSSION

This study evaluated a large population of dogs with macroscopic soft tissue sarcoma treated with stereotactic body radiation therapy. Median progression free survival (173 days) and overall survival (228 days) time were comparable to those of other previously published studies of hypofractionated radiation, 155 and 309 days (50) and 419 and 513 days (51), respectively. Prognostic factors

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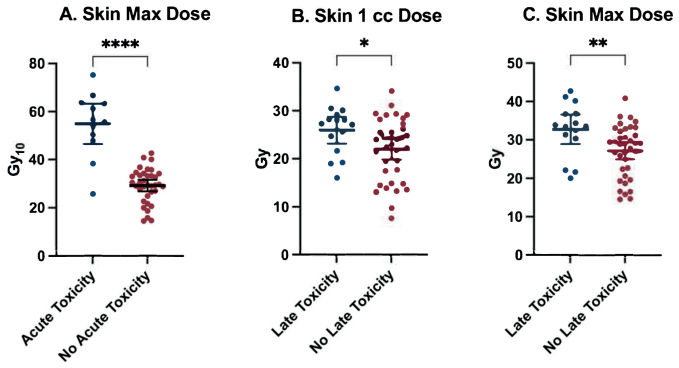

FIG. 6. Radiation dose to the skin affects incidence of acute and late radiation toxicity. Scatter Plots with means and 95% CI of dose to skin for patients developing acute or late toxicities. Panel A: The mean maximum biologically effective dose to the skin resulting in acute toxicity was 54.89 Gy_{10} (95% CI, 46.47–63.31) the mean dose for those with no acute toxicity was 29.24 (95% CI = 26.84–31.64) ($P < 0.0001$, unpaired t-test). Panel B: The mean 1 cc dose to the skin resulting in late toxicity was 25.94 Gy (95% CI = 23.16–28.73) the mean dose for those with no late toxicity was 22.02 Gy (95% CI =19.87–24.16) ($P = 0.0394$, unpaired t-test). Panel C: The mean maximum dose to the skin resulting in late toxicity was 32.69 Gy (95% CI = 28.87–36.52) the mean dose for those with no late toxicity was 27.09 Gy (24.85–29.34) ($P = 0.0095$, unpaired t-test).

identified in our study included metastatic disease at the time of treatment and size of tumor. Patients either responded to treatment (30.4%) or maintained stable disease (41.3%) for a minimum duration of 90 days. Dogs categorized as responders had significantly longer overall survival time versus non-responders. Sixty-five percent of deceased patients succumbed to disease associated events; however, disease specific survival did not differ from overall survival.

Patient demographics and tumor populations varied greatly amongst these previous studies compared to ours. The population treated with a 5×6 Gy hypofractionated RT protocol was largely made up of grade 1 tumors (52%) with only 3 patients having been diagnosed with grade 3 STS (51). In even closer comparison, a recently published study specifically reviewing the use of SBRT for treatment of STS in 35 dogs, revealed a longer PFST of 531 days and OST of 713 days (72). This study was also largely composed of grade 1 tumors (60%), and no tumors had evidence of metastasis at presentation, which differed from the study population at CSU. It is possible that our study had shorter progression free and overall survival times compared to the Gagnon and Cancedda studies due to the majority of the tumor population being high grade (grade 2: 36.85%, grade 3: 36.5%; although not a prognostic factor in this study), and 21.2% of all tumors having radiographically and/or cytologically confirmed metastasis at presentation, which significantly impacted survival. Additionally, our study population had a high percentage of recurrent tumors (44.2%) which have previously been implicated to have shorter progression free intervals (51), although again not a significant prognostic factor in our study.

Tumor size was also a significant prognostic factor in this study: with every 100 cc of tumor size resulting in a 5% increased chance of progression and death at all points of follow up. Overall, this study consisted of tumors of considerable size with a median tumor volume of 345.5 cm^3, mean tumor volume of 766.3cm^3, with 46.2% of the tumors being greater than 400 cm^3, up to 10778.3 cm^3. Previously reported tumor populations were significantly smaller with a median tumor volume of 81 cm^3 (50) and mean tumor volume of 118.3 cm^3 (51). Measurements for tumor volume in the study by Gagnon *et al.* were not provided, making tumor volumes between study populations difficult to compare; however, they recommended SBRT for dogs with STS too large for surgical removal (72). In contrast, our results from our study indicate the importance of tumor size in the utility of SBRT as a treatment for STS, considering large tumors are a poor predictive factor for STS (26) (73). The lower OST for our entire study cohort could have been influenced by the size of the tumors in this study population. Additionally, the size of tumors in our study may have negatively influenced owner perception of disease and led to earlier decisions for euthanasia.

Metastasis at presentation also significantly affected outcome. Patients presenting with metastasis had an increased risk of progression and death at all times of follow up compared to those without metastasis. Progression and survival in our study population was not affected by grade. The metastatic rate of STS has not been well defined, but it has been shown to vary by grade. Reported rates for grade 1 tumors range from 7–13%, from 7–33% for grade 2, and from 22–44% with grade 3 tumors (23, 27, 74). In our study, 22.2% of grade 1 tumors (2 of 9) presented with metastasis, 20.0% of grade 2 tumors (4 of 20) presented with metastasis, and 21.1% of grade 3 tumors presented with metastasis. Forty-four percent of grade 1 (4 of 9), 40% of grade 2 (8 of 20), and 57.9% of grade 3 (11 of 19) tumors developed metastatic STS after irradiation resulting in distant failure (Table 1). One former retrospective study found similar, higher metastatic rates with 29.5% in low-grade and 34.6% in high-grade tumors (75). The high percentage of metastasis and reported aggressive nature in low-grade neoplasms may have affected outcome of our study by uncharacteristically reducing survival time for low-grade tumors.

Despite an inherent risk for increased radiation toxicity, treatment dose did not influence overall survival when evaluated according to total dose prescribed, administered, or by fractionation protocol. The biologically effective dose delivered to 95% PTV in Gy_4 was included in the multivariable OS model as a confounding variable, however it was not a statistically significant predictor of failure. The relative lack of effect of radiation dose supports the known radioresistant nature of macroscopic STS (76). It is possible that the radiation threshold needed to achieve desirable treatment effects for the tumor was not reached. The

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radioresistant nature and lack of literature regarding the treatment of solid sarcomas with SBRT likely influenced the heterogenous spread of treatments; however, dose range and fractionation is similar to human studies of SBRT for both gross and metastatic tumors (19, 58).

Previous, concurrent, or adjuvant therapies, including chemotherapy and/or surgical excision of the irradiated tumor, did not affect outcome in this study; however, the low number of patients receiving each of these therapies and varied types may have resulted in low power for outcome analysis. While some patients (n = 10) went on to have surgical excision, outcome did not vary relative to others (OST ranged from 143–524). All but two patients who underwent surgical excision after SRT had not responded to treatment.

The extent of radiation induced toxicity in this study, with respect to both acute and late toxicity data or description, was available for all patients. Acute toxicities were reported in 30.8% of patients (n = 16) and late toxicities reported in 28.8% of patients (n = 15). Grade 3 acute toxicities reported in 7.7% of patients and grade 3 late toxicities reported in 11.5% of patients. In contrast, the Gagnon *et al*. study (72) evaluated only acute toxicity in 57% of the population. For those cases with toxicity data, 75% developed acute toxicities, all affecting the skin and hair, with 15% being grade 3 acute toxicity. Case evaluation for toxicity scoring in our study were reviewed by both RKT and MKB. Dose administered in the study from Gagnon *et al*. ranged from 27–48 Gy given in 2–3 fractions, which is similar to the dose limits in our study. Median maximum point dose in 2 mm thickness in the Gagnon *et al*. study was 28.6 Gy (range 24.1–49.4 Gy) for dogs receiving 3 fractions, similar to the 29.3 Gy median dose (range 14.7–36 Gy) for the 28 patients receiving 3 fractions in our study. Normal tissue constraints have not been formally set for canine patients. It remains paramount to document radiation-induced side effects and correlate with dose administered. In this study, we identified that both acute and late toxicity was associated with various dose parameters to the skin. Our study also highlighted incidence of consequential late effects to the skin as a sequalae of SBRT treatment for STS, as all grade 3 acute skin toxicities developed either grade 2 or 3 late skin toxicities. Consequential late effects occur when acute effects impair barrier function of the epithelium, which allows for continual tissue damage. This propagates a late response that also maintains acute characteristics such as ulceration and erythema along with late characteristics like fibrosis (77, 78).

In addition to physical radiation toxicity, the immunomodulatory effects of radiation could be assessed for 12 patients which met inclusion criteria. We showed significant differences in absolute lymphocyte count and neutrophil-lymphocyte ratio within 90 days of treatment with worsened lymphopenia grade in 66.7% of these patients. This is consistent with the literature (45, 79). Unfortunately, the sample size of available patients was too small for further analysis of the effect of post-SBRT lymphopenia on survival outcome and what demographic or tumor factors may influence these changes. Further research looking at how the immunomodulatory effects of SBRT on canine sarcoma may affect treatment outcome is warranted.

Five patients (9.6%) developed adverse events with a median time to first event of 107 days (95% CI = 14–130). Adverse events included tumor necrosis and infection leading to formation of fluid pockets, ulceration, rupture, drainage tracts, after SBRT surgical dehiscence and non-healing wounds. Three of these patients developed chronic Methicillin Resistant *Staphylococcus pseudintermedius* infections. These complications significantly affected patient quality of life and played a role in owner decision for euthanasia. All of these patients had one or more surgical resection prior to therapy possibly disrupting tissue architecture and increasing risk of infection. One of these patients developed severe infection and ulcerative wounds after the second SBRT treatment administered one year after the first due to progressive disease.

Of the 43 patients with a known time of death, 28 (65.1%) died or were euthanized due to symptoms related to their soft tissue sarcoma. Of the nine who died of other known causes (20.9%), five patients died due to an additional malignancy. Additionally, two patients who died due to primary disease progression had also developed additional malignancies, including hemangiosarcoma and thyroid carcinoma in one patient and multicentric lymphoma in the other. Two of the patients that died due to additional malignancies developed hemangiosarcoma within the radiation field, one of which was postulated to be a sequela of SBRT by the pathologist at time of necropsy.

Limitations of this study lie with its retrospective nature. Patients were lost to follow up, limiting the scope in which we could assess tumor and patient response to SBRT. When patient data was not available through medical record review, data gathered by follow-up client phone calls may have been skewed to owner perception with dates approximated. The process in which each patient was evaluated for progressive disease and the cause of death was detailed and dependent on the combination of reported veterinary clinical findings, necropsy reports, and owner description. Interpretation of the histologic grading of canine STS may have varied between pathologists and skewed results of grade of the tumors at presentation in our cohort. We attempted to correct for this by retrospectively reviewing the grading designation for the tumors in this study according to available reports and pathological review of available tissue sections at CSU.

Overall, this is the largest study to provide therapeutic outcomes for dogs with soft tissue sarcoma treated with stereotactic body radiotherapy. It also highlighted areas to which future research efforts should be focused when considering SBRT as a therapy for macroscopic STS, including approaches to extend local tumor control, abrogation or inhibition of metastasis, and means to widen

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the therapeutic index. Of note, for STS and SBRT research involving evaluation of biological or clinical factors at early timepoints, even dogs undergoing SBRT affected with negative prognostic factors (metastasis, large tumor volume) may still provide valuable information if the trial is designed within the limits of their anticipated progression and overall survival times. Finally, the outcome data provided in this study will be useful in designing future canine STS comparative oncology research to investigate experimental therapeutic approaches that may be used in conjunction with SBRT to treat macroscopic STS.

CONCLUSIONS

Stereotactic body radiation therapy serves as a viable treatment option for providing local tumor control for canine macroscopic STS. Dogs with small tumors, no evidence of metastatic disease at the time of treatment, and those with tumors that achieved a complete or partial response after SBRT experienced longer progression free survival and overall survival time. With respect to establishing canine STS as a preclinical comparative oncology model for translational research, we present SBRT outcome data regarding tumor response rates, duration of control times, and normal tissue tolerance so that prospective canine clinical trials can be developed according to the biological and clinical endpoints of interest.

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