Received: 31 December 2020 | Accepted: 31 August 2021

DOI: 10.1111/jvim.16264

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Outcomes of adjunctive radiation therapy for the treatment of mast cell tumors in dogs and assessment of toxicity: A multicenter observational study of 300 dogs

Sarah L. Mason[1,2] | Charles Pittaway[2,3] | Begona Pons Gil[1] | Onne-Marju Russak[3,5] | Katie Westlake[2] | Davide Berlato[4,5] | Jérôme Benoit[1] | Joanna Morris[6] | Jane Margaret Dobson[2]

[1]Southfields Veterinary Specialists, Basildon, United Kingdom

[2]Queens Veterinary School Hospital—Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

[3]Dick White Referrals, Cambridge, United Kingdom

[4]AniCura Animal Oncology and Imaging Center, Hünenberg, Switzerland

[5]Animal Health Trust, Newmarket, United Kingdom

[6]University of Glasgow, Glasgow, United Kingdom

Correspondence
Sarah Mason, Southfields Veterinary Specialists, 1 Bramston Way, Basildon SS15 6TP, United Kingdom.
Email: sarah.mason@southfields.co.uk

Abstract

Background: Radiation therapy is commonly used as an adjunct to incomplete surgical excision in dogs with mast cell tumors (MCT), but the optimal dose and fractionation regimen have yet to be determined.

Hypothesis: We assessed outcomes (time to local recurrence, patient survival and toxicity) of a large population of dogs with MCT that received adjunctive radiation therapy.

Animals: Three hundred dogs with 302 MCT treated using adjunctive radiation therapy.

Methods: Retrospective observational study. Clinical records of 4 veterinary radiation centers were reviewed.

Results: Local recurrence rates were similar regardless of radiation protocol with 6.6% of patients developing recurrent cutaneous MCT at a median of 526 days. Local recurrence rate was similar between high and low-risk MCT. Mast cell tumor related death was reported in 19% of all dogs, with 13% of dogs with low-risk MCT dying of their disease compared to 29% of dogs with high-risk MCT. No SC MCT (SCMCT) recurred after radiation therapy and only 7% of dogs with SCMCT were reported to have died of their disease. Mild late toxicity was common in both protocols and severe late toxicity occurred in 1.9% of dogs many years after treatment.

Conclusions and Clinical Importance: Our study supports the use of adjunctive radiation for the long-term control of incompletely or narrowly excised cutaneous and SCMCT in dogs. More moderate dose and fractionation protocols may be appropriate in the adjunctive treatment of low-risk MCT in dogs. Large multicenter prospective

Abbreviations: 95CI, 95% confidence interval; BED, biologically effective dose; cMCT, cutaneous mast cell tumor; CTV, clinical target volume; Hpf, high power field; ICRU, International Commission on Radiation Units and Measurements; LN, lymph node; LTF, lost to follow-up; MCT, mast cell tumor; MCTSST, mast cell tumor specific survival time; MeV, megavoltage x-rays (electrons); MI, mitotic index; MST, median survival time; MV, megavoltage x-rays (photons); NS, not specified; OAR, organs at risk; OST, overall survival time; PTV, planned target volume; RT, radiation therapy; SCMCT, SC mast cell tumor; TKI, tyrosine kinase inhibitor; TLR, time to local recurrence; VRTOG, Veterinary Radiation Therapy Oncology Group

studies are required to establish the optimal dose and fractionation for MCT of different risk categories.

KEYWORDS
canine, oncology, radiation, radiotherapy, toxicology

1 | INTRODUCTION

Mast cell tumors (MCT) account for up to 20% of cutaneous and SC tumors in dogs and their presentation and clinical course vary among individuals. Low and intermediate Patnaik and Kiupel low grade MCT often are slow growing and relatively benign in clinical behavior, causing problems by local invasion and ulceration rather than metastatic spread.[1-3] Histopathologic grade provides information regarding potential for local recurrence or metastasis, but factors such as size, clinical appearance, location, proliferation or mutation markers, and clinical stage also affect recurrence and metastasis.[3-6] Some MCT are considered high risk including those with high expression of proliferation markers or metastatic disease, despite low or intermediate grading.[6]

Wide surgical excision is the preferred treatment for localized cutaneous or SC MCT (cMCT/SCMCT) and is curative when margins are complete. However, complete excision is not always feasible in areas in which spare skin or deep facial planes are lacking.[3,7,8] Several studies report high cure rates for low to intermediate grade MCT, even when margins are incomplete,[9-14] making the recommendation for radiation challenging because some patients will be overtreated.

Adjunctive radiation is considered standard of care for incompletely excised stage 1 and 2 cMCT when revision surgery is not possible.[15-21] Control rates after adjunctive radiation of microscopic cMCT range from 65% to 96%.[15-21] Radiation causes acute (erythema, desquamation, and ulceration) and late (leukotrichia, hyperpigmentation and cutaneous fibrosis) toxicity to normal tissues within the treated field. More serious toxicities including vascular or lymphatic damage and subsequent lymphedema, osteoradionecrosis and rarely, second tumor development may occur.[22-24]

Various adjunctive radiation protocols are reported for dogs with MCT, with total doses between 48 and 57 Gy in daily or alternating daily fractions of 3 to 4 Gy.[15-21] The optimal total radiation dose or regimen for adjuvant treatment of MCT in dogs is not yet established. No specific report of the outcomes of dogs with SCMCT treated with adjunctive radiation is available although this approach is commonly employed.[15-19] Biological effective dose (BED) is a concept that defines radiation prescriptions with regard to their acute (Gy_{10}) and late (Gy_3) effects on tumor and tissues and is more appropriately used to describe protocols than is total dose.[25] Radiosensitivity is influenced by several factors and can vary widely across tumors, even those of similar histopathogenesis. Mast cell tumor cell lines are of medium radiosensitivity, compared with a range of other tumor cell lines in dogs.[26]

We hypothesized that long-term control rates for dogs with cMCT and SCMCT treated using adjunctive radiation would be similar and independent of protocol. Objectives were to assess outcomes for high dose fractionated protocols and lower dose coarse fractionated regimens, to report tumor control in dogs with cMCT and SCMCT, and high-risk vs low-risk MCT and to assess the prevalence and severity of acute and late radiation toxicity.

2 | MATERIALS AND METHODS

2.1 | Study design

Ours was a retrospective observational multicenter study. Dogs were enrolled from 4 UK veterinary radiation centers and the study was approved by each center's ethical committee. A standard spreadsheet (MSOffice Excel v365) and information sheet quantifying methodology for data collection were provided. Patients treated from 1 January 2008 until 31 December 2016 were included. Data were collected between September 2017 and June 2018.

Case records of dogs that received adjunctive radiotherapy (RT) for cMCT/SCMCT were retrospectively reviewed. Inclusion criteria were histopathological diagnosis of cMCT/SCMCT and adjunctive RT. Primary and recurrent MCT were included, as were MCT with incomplete or narrow histopathological margins and those with reported clear histopathological margins but clinical uncertainty. Exclusion criteria were macroscopic MCT, macroscopic lymph node (LN) metastasis (palpable) or mucosal MCT.

Data collected included signalment, MCT position (SC vs cutaneous) and location (distal or proximal limbs, thoracic or abdominal wall, head, muzzle, dorsum, inguinal, perineal, other), histopathological grading, mitotic index (MI), Ki67 score when performed, clinical staging and any treatment before, during or after surgery or radiation therapy (eg, prednisolone, chemotherapy tyrosine kinase inhibitors), time from surgery to first radiation treatment (defined as first surgery in the case of recurrent tumors). Tumor size was not available in most patients as they presented to the radiation center after surgery in a local practice.

Both Patnaik[27] and Kiupel[28] gradings were recorded if available for cMCT. Histopathology reports were reviewed by a board-certified oncologist when available. Incomplete excision was considered if it was directly reported or tumor cells were seen at the edges of excised tissue. Narrow excision was defined histologically as ≤1 mm margin, vs complete excision as >1 mm.[29] SCMCT was defined as tumor confined to the subcutis.

High-risk MCT was defined as any tumor of high Patnaik or Kiupel grade, MI ≥5/10 per high power field (hpf) for cMCT,[30] MI > 4/10 for SCMCT,[31] Ki67 above threshold,[4,32-34] pathologist

description of the MCT as high grade, and clinical stage 2 or 3, even if histopathologic description did not include MI or Ki67.

The MCT were considered low risk if defined as any of the following: low-grade Kiupel or low to intermediate Patnaik grade, MI <5 for cMCT or <4 for SCMCT, Ki67 below threshold and clinical stage was 1 or not recorded within these histopathological and immunohistochemical features.

TABLE 1 World Health Organization (WHO) staging system

Stage	Criteria
I	One tumor confined to dermis (or subcutis for SC mast cell tumor) without regional LN involvement
II	One tumor confined to dermis with regional LN involvement[a]
III	Multiple dermal tumors, large infiltrating tumors with or without regional LN involvement
IV	Any tumor with distant metastasis including blood or bone marrow

[a]Regional LN involvement can be cytological or histopathological diagnosis of metastasis.

The MCT were considered of unknown risk if the histopathology report did not provide a grade, or if grade was intermediate with no MI or Ki67 available. Tumor stage was determined by the modified World Health Organization (WHO) staging system (Table 1).

Follow-up data were collected from medical records, or by email or telephone follow-up with referring veterinarians. Follow-up data included recorded radiation toxicity, recurrence, metastasis, or new MCT and the dates observed, the date the animal was last seen alive and healthy (no MCT) or was euthanized or died, and the cause of death where known.

Dogs were treated with different protocols and linear accelerators (Table 2).

Treatments were grouped into:

1. Coarse fractionated total dose ≤40 Gy (≥5 Gy fractions))
2. Fractionated total dose >40 Gy (daily, or Monday, Wednesday, Friday ≤4 Gy fractions)

Treatment data collected included total radiation doses, fraction numbers, field sizes in cm (converted to equivalent square by the

TABLE 2 Treatment machines and treatment protocols used in each center

Treatment center	Linear accelerator	Coarse fractionation protocols	Definitive intent protocols	Standard electron prescribing	Standard photon prescribing
A (36 MCT)	Varian Clinac 23EX	7 4 × 8 Gy weekly (32 Gy) 1 5 × 6 Gy weekly (30 Gy) 2 6 × 6 Gy biweekly (36 Gy) **(BED = 57.6 Gy_{10}, 117.3 Gy_3)**	23 16 × 3 Gy (48 Gy) 1 14 × 3.5 Gy (49 Gy) 1 15 × 3.2 Gy (48 Gy) 1 15 × 2.7 Gy (40.5 Gy) **(BED = 60.1-63.4 Gy_{10}, 90-99.2 Gy_3)**	100% dose to the skin using appropriate bolus	Single field—100% dose to the skin using appropriate bolus Parallel opposed fields—100% dose to the midpoint using appropriate bolus
B (28 MCT)	Siemens Oncor Impression Plus	20 (71%) 8 × 5 Gy biweekly 5 Gy (40 Gy) **(BED = 60 Gy_{10}, 106.6 Gy_3)**	7 (25%) 12 × 4 Gy MWF (48 Gy) 1 (4%) 11 × 4 Gy (44 Gy) **(BED = 67.2 Gy_{10}, 112 Gy_3)**	95% to 100% dose to the skin using appropriate bolus	As above
C (102 MCT)	Varian Clinac DMX 6100	93 (91%) 4 × 8 Gy weekly (32 Gy) 3 (3%) 4 × 7.5 Gy weekly (30 Gy) 3 (3%) 3 × 8 Gy/1 × 7 Gy weekly (31 Gy) 3 (3%) 3 × 8 Gy/1 × 7.5 Gy (31.5 Gy) **(BED = 52.5-68.4 Gy_{10}, 105-130 Gy_3)**	N/A	95% to 110% to the skin using appropriate bolus	As above
D (136 MCT)	Varian Clinac 600C	67 (49%) 4 × 8 Gy weekly (32 Gy) weekly 12 (9%) 4 × 9 Gy weekly (36 Gy) **(BED = 57.6-68.4 Gy_{10}, 117-144 Gy_3)**	47 (34%) 16 × 3 Gy (48 Gy) 1 (0.7%) 16 × 3.1 Gy (49 Gy) 1 (0.7%) 17 × 3 Gy (51 Gy) 1 (0.7%) 18 × 3 Gy (54 Gy) 7 (5%) 15 × 3 Gy (45 Gy) **(BED = 58.5-70 Gy_{10}, 90-108 Gy_3)**	N/A	As above

Note: The range of biologically effective dose is displayed in bold.
Abbreviations: BED, biologically effective dose; MCT, mast cell tumors; N/A, not applicable.

formula $2 \times X \times Y/(X + Y)$, number and arrangement of fields, type of energy (photons vs electrons), energy used, bolus or blocks used, manual or computer plan, margins around clinical target volume (CTV) defined as surgical scar or tumor bed.

Radiation toxicity was scored retrospectively by Veterinary Radiation Therapy Oncology Group (VRTOG) criteria.[22]

2.2 | Statistical analysis

A power study estimated 90% tumor control in the fractionated group based on previous studies and 85% in the hypofractionated groups based on first principles and clinical experience. Using 80% chance of detecting a difference with 5% significance >1000 dogs were required to reach statistical power, which was higher than the number of available cases. Hence, we did not attempt statistical comparison of outcomes between treatment groups.

Kaplan-Meier product estimates were used to compare overall survival time (OST), MCT specific survival time (MCTSST) and time to local recurrence (TLR) for the group and TLR and MCSST between risk groups. Time to local recurrence was calculated from the date RT started to the date the tumor recurred. Dogs with no recurrence, that died from other causes, or those lost to follow-up (LTF) were censored at the last date seen alive and MCT free.

The MCTSST was calculated for dogs that died from confirmed or suspected local or systemic MCT. Death was considered MCT related if signs could be attributed to MCT, even if unconfirmed. Dogs alive at the end of the study period, LTF, or that died from other causes while MCT free were censored on the date last seen.

Descriptive statistics were performed across groups to assess distribution of characteristics and to compare toxicity by Chi-squared and Kruskal-Wallis tests. Significance was set at $P = .05$.

3 | RESULTS

3.1 | Study population

Three hundred dogs were included: 154 female neutered, 109 male neutered, 17 female, and 20 male intact. Median age was 8 years (range, 1-16). Breeds are summarized in the Supporting Information.

Three hundred and two MCT were treated in 300 dogs. Center A contributed 36 MCT in 35 dogs, center B 28 MCT in 28 dogs, center C 102 MCT in 101 dogs and center D 136 MCT in 136 dogs. Most MCT were appendicular (243/302, 80%) with 184 (76%) on the distal limb and 59 (24%) on the proximal limb. There were 19 (6%) on the muzzle, 15 (4.9%) on the head, 5 (1.6%) on the thoracic wall, 5 (1.6%) on the dorsum, 3 (1%) on the abdominal wall, 1 (0.3%) inguinal, and 4 (1.3%) perineal. Seven (2.3%) were classified as other (2 tail base, 1 prepuce, 1 umbilical, 3 not recorded).

There were 244 (81%) cMCT, 55 (18.2%) SCMCT, and 3 (1%) not specified (NS), which were either cutaneous or SC on review of histology. Five (1.6%) MCT had clear margins of excision (4 [80%] low risk, 1 [20%] high risk), 29 (9.6%) narrow margins (7 [24%] high risk, 21 [72%] low risk, 1 [3%] unknown risk), 265 (87.7%) had incomplete margins (88 [33%] high risk, 163 [62%] low risk, 14 [5%] unknown risk), in 3 (1%) MCT margins were recorded as not known (2 [66%] unknown risk, 1 [33%] low risk).

Not all MCT had Patnaik/Kiupel grading reported. Of 238/244 cMCT (97.5%) that had a Patnaik grade, 19 (7.9%) were low grade,

TABLE 3 Mast cell tumors (MCT) results populations

	Coarse fractionated group	Fractionated group	*P* value (significance ≤.05)
Patient median age (y)	8 (range, 1-13.7)	8 (range, 2-13)	
Total number MCT	211	91	
Cutaneous	165 (78%)	79 (87%)	.57
SC	43 (20%)	12 (13.2%)	.49
Not recorded	3 (1.4%)	0	NA
Stage 1	150 (71%)	68 (75%)	.79
Stage 2	25 (12%)	17 (19%)	.17
Stage 3	4 (1.8%)	6 (6.6%)	.04
Stage not recorded	32 (15%)	0	NA
Received any chemotherapy	46 (22%)	25 (27%)	.4
Incomplete margins	186 (61.6%)	83 (27.4%)	.85
Complete margins	4 (1.3%)	1 (0.3%)	.62
Narrow margins	18 (5.9%)	7 (2.3%)	.82
Not recorded margins	3 (1%)	0	NA
Median time from surgery to radiation therapy (d)	32 (range, 4-277)	36 (range, 9-173)	.09

207 (87%) intermediate grade, and 12 (5%) high grade. Of 231/244 (95%) that had Kiupel grading, 206 (89%) were low grade, and 25 (11%) high grade. When grouped for high-risk characteristics 96 (32%) MCT (83 cMCT and 13 SCMCT) were included, 189 (63%; 149 [79%] cMCT and 40 [21%] SCMCT) MCT were included in the low-risk group and 17 (5.6%; 12 cMCT [71%], 2 [12%] SCMCT, 3 NS [17.6%]) in the unknown risk group.

Two hundred and eighteen (72%) MCT were recorded as clinical stage I, 42 (14%) stage II, 10 (3%) stage III, and stage was not available for 32 MCT (11%). One of 2 dogs with 2 MCT was considered stage III and, in the other, stage was not recorded. Stage was distributed equally across treatment groups except stage 3 where more received fractionated protocols (Table 3).

3.2 | Radiation treatment

Most primary field treatments (275/302 [91%]) were single beam, 22/302 (7.4%) were parallel opposed beams and in 5/302 (1.6%) beam arrangements were not recorded. Only 1 MCT was treated using a computer-generated 3-dimensional plan, the remainder were manually planned. Tissue equivalent bolus 5 to 10 mm (Superflab, CIVCO, Kalona, Iowa) was used in all cases. Treatment was prescribed to the 95% to 100% isodose line depending on energy used for single field electron treatments, to maximum depth (dMax) for single beam photon fields and to the midpoint of the limb for parallel opposed photon fields.

Because most patients were presented after surgery in the microscopic setting, the surgical scar was used to estimate clinical target volume (CTV). Commonly 2 to 3 cm combined CTV and planned treatment volume (PTV) margins were applied proximally, distally, and when possible, laterally to include the scar. The retrospective nature of the study did not permit more detailed assessment. Twenty-seven dogs (9%; 26 limb MCT, 1 head MCT) had the regional LN treated with radiation at the clinician's discretion, and prophylactically in all cases. All LN were irradiated in situ. Field size to the regional LN when not included in the primary field was recorded in 16 (60%) patients and the remaining 11 (40%) LN were included in the primary field. Median equivalent square field size was available for 281 (93%) treated primary fields and was 10.66 cm^2 (range, 2.66-18.75 cm^2). Median LN field size was 6.88 cm^2 (range, 4-10 cm^2).

A total of 179/302 (59%) MCT were treated with 6 MV photons, and the remainder with electrons: 99 (33%) primary fields were treated with 6 MeV electrons, 6 (2%) with 8 MeV electrons, 8 (3%) with 9 MeV electrons, 5 (2%) with 10 MeV electrons, 3 (1%) with 12 MeV electrons, 1 (1%) with 15 MeV electrons, and in 1 field electron energy was not recorded. In patients in which the energy to the node was recorded 10/27(37%) received 6 MV photons and 4/27 (15%) 6 MeV, 1/27 (3.7%) 8 MeV, and 3/27 (1.1%) 9 MeV electrons. One dog (3.7%) received primary site 6 MeV, LN 9 MeV, and 2 (7.4%) dogs received 9 MeV to the primary site and 6 MeV to the LN.

The use of blocks was recorded in 97 (32%) treatments, not reported in 63 (21%) treatments and 142 (47%) treatments were performed without blocks.

Two hundred and eleven (70%) MCT received coarse and 91 (30%) conventionally-fractionated treatments. Characteristics for each group are summarized in Table 3.

3.3 | Chemotherapy

Seventy-one dogs received chemotherapy/tyrosine kinase inhibitor (TKI) treatment (Table 4). Of dogs receiving any chemotherapy, 38 (53%) stage I, 23 (32%) were stage II, 3 (4%) stage III and in 7 (10%) stage was not recorded. Forty (56%) of the dogs that received chemotherapy were high risk, 24 (34%) low risk, and 7 (10%) unknown risk. Thirteen of 71 (18%) dogs that received combination RT and chemotherapy received RT to the local LN. Dose reductions were performed if toxicity was observed at the maximumly tolerated dose.

Median follow-up time for the entire population was 975 days (range, 20-3610 days).

TABLE 4 Chemotherapy received by dogs in each treatment group and by timing of radiation

Chemotherapy/ medical therapy	All dogs	Fractionated high-risk group	Fractionated low-risk group	Fractionated unknown risk group	Coarse fractionated high-risk group	Coarse fractionated low-risk group	Coarse fractionated unknown risk group
Before/during radiotherapy	23 dogs	1 VBL, 3 CCNU, 1 OTH	1 VBL/CCNU, 3 VBL, 1 CCNU	N/A	2 VBL	2 VBL, 2 CCNU, 1 CHL, 2 OTH	3 VBL, 1 CCNU
Before/during and after radiotherapy	28 dogs	1 VBL/MAS, 1 VBL/TOC, 1 VBL, 1TOC/ CCNU	1 VBL/MAS, 1 VBL, 1 OTH, 1 CCNU/OTH	N/A	6 VBL, 2 TOC, 2 MAS/OTH, 1 MAS, 3 CCNU, 1 CCNU/ VBL, 1 CCNU/ TOC, 1 OTH	1 VBL	2 CCNU
After radiotherapy only	20 dogs	4 VBL, 1 VBL/CCNU	1 CCNU, 1 MAS, 1 OTH	N/A	1 CHL, 2 VBL, 4 CCNU	3 VBL, 1 CCNU	1 VBL
Total	71	14	12	0	26	12	7

Abbreviations: CCNU, lomustine; MAS, masitinib; OTH, other; TOC, toceranib; VBL, vinblastine.

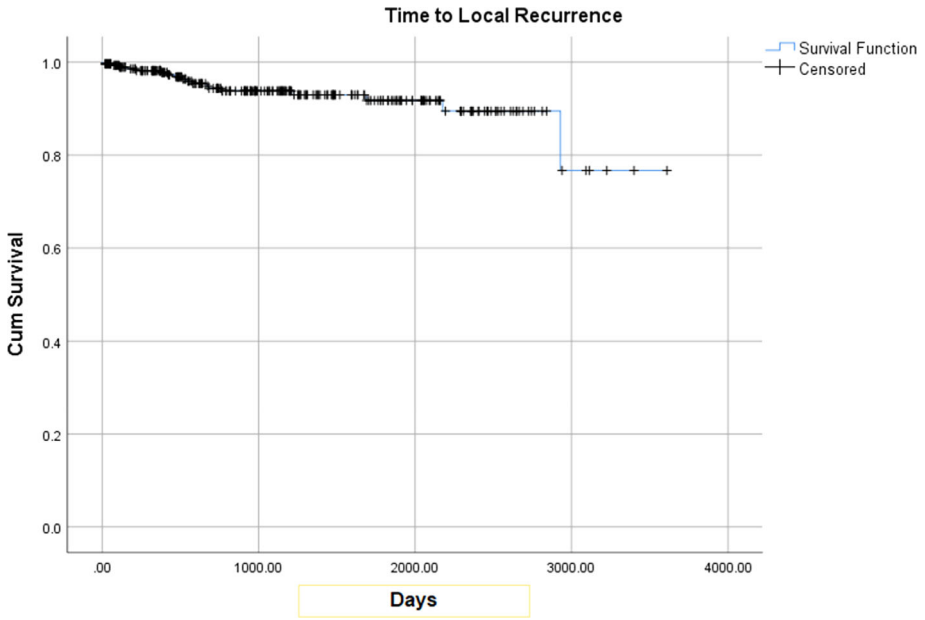

FIGURE 1 Time to local recurrence for 18 of the 20 dogs, in which the date of recurrence was recorded

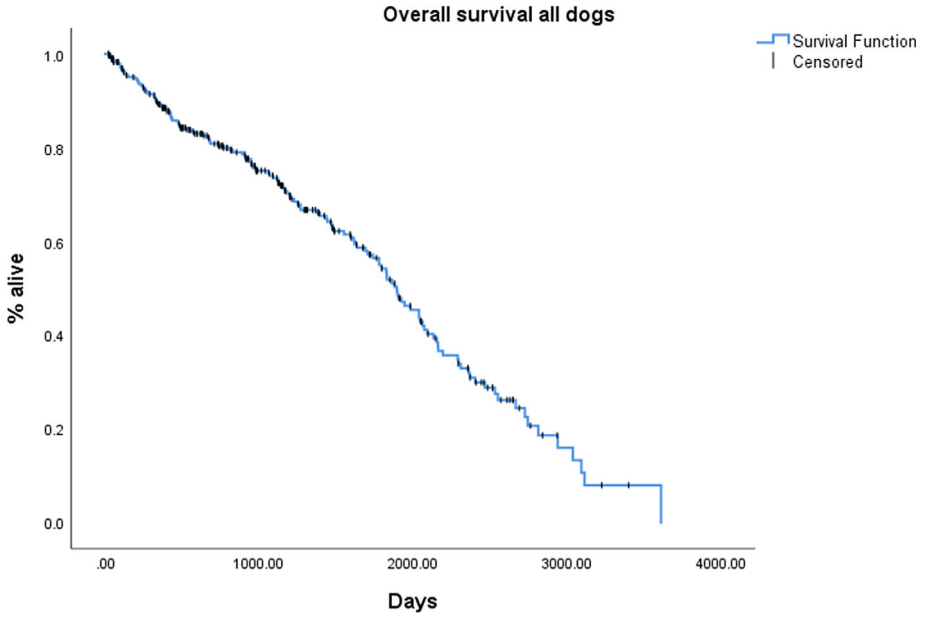

FIGURE 2 Overall survival time for the whole cohort. Censored dogs were alive or lost to follow-up

3.4 | Outcomes

Twenty of 302 (6.6%) tumors recurred locally with a median TLR of 526 days (range, 21-2929 days; Figure 1). All were cMCT; no SCMCT recurred. Six of 99 MCT treated with photons (6%) and 14/179 MCT treated with electrons recurred (8%; $P = .61$). Of 20 recurrent tumors, 6 (30%) had the local LN treated, 5/6 (83%) were high-risk MCT, 2/6 (33%) had documented LN metastasis after radiation. Thirteen tumors recurred after coarse fractionated protocols (6.2%) and 7 recurred after conventionally fractionated radiation protocols (7.7%). Dogs with tumor recurrence had a median OST of 972 days (range, 244-3037 days).

For 7/91 (7.7%) MCT recurrent after conventionally fractionated RT, 2 (29%) were high risk and 5/7 (71%) low risk. Surgery to radiation time was 173 (date of recurrence not recorded) and 31 days (recurrence, 515 days) for the high-risk recurrent tumors. The first patient received chemotherapy with lomustine and then toceranib and survived 233 days. The other survived 678 days.

The low risk recurrent MCTs had a median time from surgery to radiation of 40 days (range, 33-55 days) for the 4 patients in which this time was recorded. Median time to recurrence for low-risk dogs that received fractionated radiation was 462 days (range, 380-669 days). Five (71%), (3 [60%] systemic and 2 [40%] local

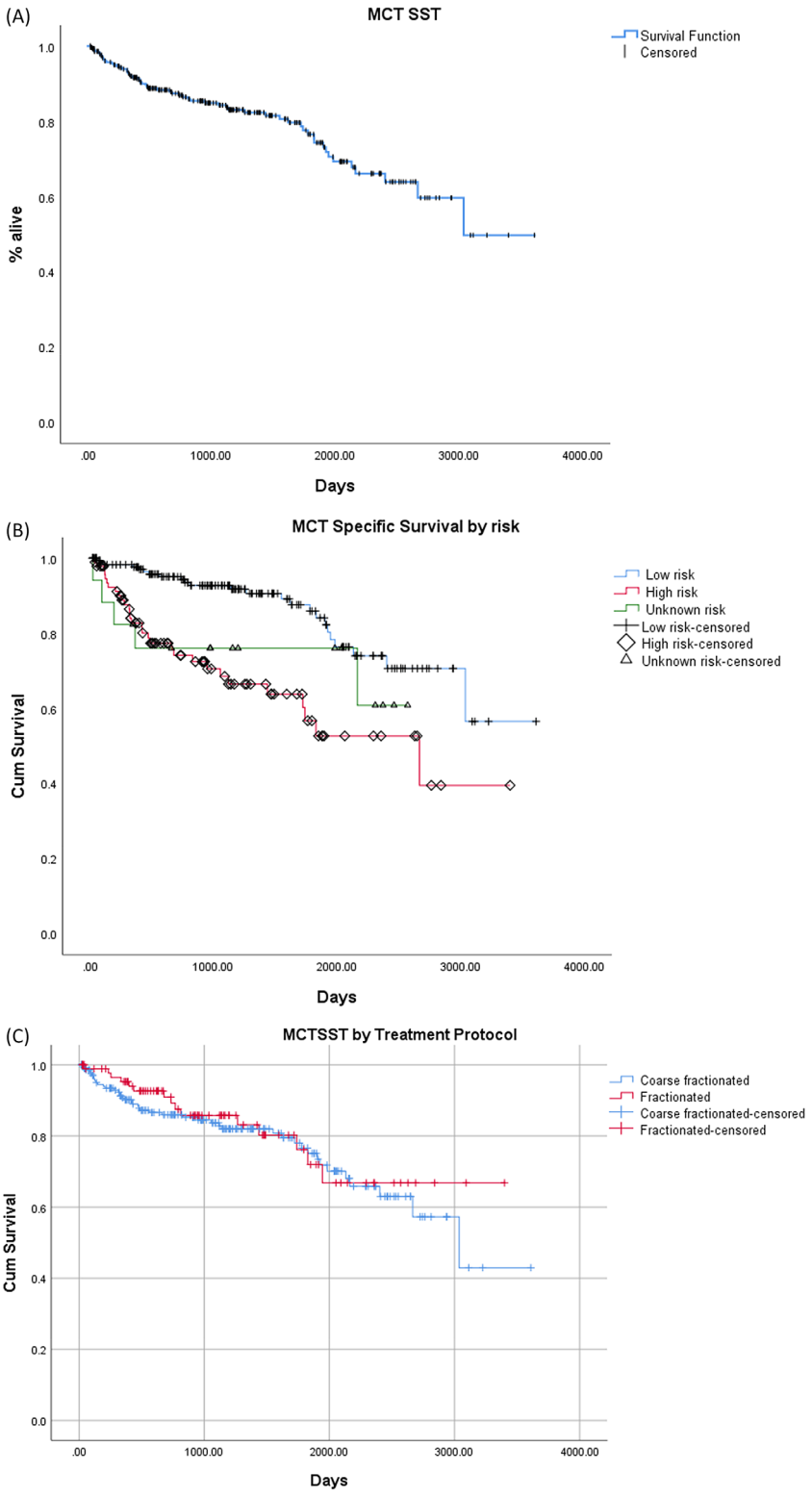

FIGURE 3 (A) Mast cell tumor specific survival time (MCT SST), censored dogs were alive, lost to follow-up or dead of non MCT related causes. (B) MCT SST by risk, censored dogs were alive, lost to follow-up or dead of non MCT related causes. (C) MCT SST by treatment protocol

disease) dogs died and 2 [29%] were censored, MCTSST was 673 days (range, 400-1122 days).

For 13/211 (6%) MCT that recurred after coarse fractionated radiation, 5 (38.4%) were high risk, 7 (54%) were low risk and 1 (7.6%) tumor was of unknown risk. One high risk, high grade and recurrent MCT which had a long time (168 days) from first surgery to second surgery and subsequent prompt radiation recurred at the last radiation fraction (21 days) and received masitinib during and after radiation. Four other high-risk tumors had a median time from surgery to RT of 21 days (range, 12-277 days), recurred at a median of 164 days (range, 110-576 days) and 2 (50%) received chemotherapy (vinblastine and other drugs). Time of recurrence was recorded for 6/7 (86%) low-risk MCT with a median time from surgery to RT of 39 days (range, 19-233 days) and median TLR was 1224 days (range, 537-2929 days). The unknown risk MCT

recurred 64 days after radiation. Three (43%) of the low-risk tumor patients also received chemotherapy. Ten (77%) of these dogs were dead at the study end, with 8/10 (80%) considered to have died of MCT (5 systemic and 3 local disease). Seven of 96 (7%) high risk, 12/189 (6%) low risk, and 1/17 (6%) unknown risk MCT recurred.

Fifty-nine of 300 (19.6%) dogs died of MCT (11 [19%] local disease, 48 [81%] systemic disease), and 77 (26%) dogs died of non-MCT related causes by the end of the study. Mean OST was 1830 days (95% confidence interval [95CI]: 1679-1982 days), and median OST was 1894 (95CI: 1716-2071 days; Figure 2).

Mean MCTSST was 2652 days (95CI: 2433-2871 days). Median MCTSST was not reached (Figure 3A). For dogs dead of MCT, MST was 486 days (range, 24-3037 days). Twenty-four of 59 (40.6%) were in the low-risk group, 30/59 (51%) in the high-risk group and 5/59 of unknown risk (8.4%). Twenty-four of 187 (13%) of low-risk MCT dogs were considered to have died of their disease compared with 30/96 (31%) high risk and 5/17 (29%) unknown risk dogs. The MCTSST by risk is shown in Figure 3B, and the protocol in Figure 3C.

Fifty-five dogs had SCMCT with median time between surgery and RT of 34 days (range, 8-152 days). Seven (12.4%) were high risk, 46/55 (84%) were low risk and 2/55 (3.6%) were of unknown risk. None had recurrent disease, and median follow-up was 987 days (range, 21-2444 days). Four of 55 patients (7.2%) were considered dead of MCT, 12/55 (22%) dead of other causes, 35/55 (64%) were alive and disease-free at the end of the study, and 3/55 (5.4%) were lost to follow-up. Eleven (20%) dogs received fractionated radiation and 44/55 (80%) received coarse fractionated radiation. Eight dogs received concurrent chemotherapy, 4/8 (50%) high risk (2/4, MI > 4, Ki67 > 1.8, 1/4, stage II), 3/8 (38%) low risk and 1/8 (12.5%) of unknown risk.

The 4 SCMCT dogs dead of MCT received coarse fractionated radiation. One had high Ki67, was treated with adjunctive vinblastine and was euthanized for MCT after 318 days. Three had low risk features. One developed presumed MCT metastasis in the axilla and was euthanized after 1121 days. The other 2 dogs were euthanized at 476 and 1054 days with no confirmed reason recorded. Thirty-five dogs (12%) developed distant de novo MCT after radiation therapy. No additional data regarding these tumors were collected.

TABLE 5 Acute radiation-related toxicity by radiotherapy protocol and VRTOG grade

	Coarse fractionated (188 dogs)	Fractionated (89 dogs)
None evident	98 (52%)	18 (20%)
VRTOG grade 1	66 (skin) (35%)	36 (skin) (40%)
	1 mucous membrane, 1 other	2 (mucous membrane)
VRTOG grade 2	16 (skin) (8%)	12 (skin) (13%)
	1 (other)	
VRTOG grade 3	5 (skin) (2.6%)	21 (skin) (23%)
Not recorded	23	2

Abbreviation: VRTOG, Veterinary Radiation Therapy Oncology Group.

3.5 | Toxicity

Toxicity assessment was not standardized and was assessed at variable times, typically at the end of and 2 weeks after completion of RT for acute toxicity. Late toxicity was assessed at the time of follow-up if the dog was not presented specifically before then. One-hundred and eighty-four of 300 (61%) dogs were reported to have had acute radiation-associated toxicity (Table 5) of higher grade in the fractionated group than in the coarse fractionated group (36% vs 10.2%, G2/G3) which was significant ($P = .02$). One hundred and eight of 300 (36%) dogs were reported to have had late radiation toxicity distributed similarly across treatment groups (Table 6). Six of 302 dogs or sites (1.9%) had grade 3 late toxicity reported after radiation treatment for MCT (Table 6).

4 | DISCUSSION

We confirmed that adjunctive radiation can result in good outcomes in dogs with incomplete or narrowly but completely excised MCT, and

TABLE 6 Acute radiation-related toxicity by radiotherapy protocol and VRTOG grade

	Coarse fractionated (209 dogs)[a]	Fractionated (91 dogs)[a]
None evident	88 (42%)	48 (53%)
VRTOG grade 1	66 (skin) (32%)	35 (skin) (38%)
VRTOG grade 2	1 (bone) (0.4%)	0
VRTOG grade 3	3 (bone) 2 skin (1 each skin and nail bed)) (2.3%)	1 (skin) (1%)
Total reported late toxicity	72 (34%)	36 (40%)
Not recorded	51 (24%)	7 (8%)
Follow-up time (days)	991 (21-3610)	929 (42-3399)

Abbreviation: VRTOG, Veterinary Radiation Therapy Oncology Group.
[a]Dogs may have >1 toxicity reported.

assessed a large population of patients that received relatively standardized treatment protocols with long follow-up. Seventy-five percent of recurrences occurred within 2 years of treatment in our population, but 25% of patients developed recurrence between 2 and 8 years after treatment.

Our recurrence rate of 6.6% is lower than that of many previous reports,[15-21] including the up to 36% reported for incompletely excised MCT treated with surgery alone.[9,12,35] Ours is the first report of outcomes for SCMCT treated by adjunctive radiation and suggests that the combination of surgery and RT results in excellent local control of SCMCT. Radiation toxicity was similar to that previously reported with a low rate of severe late toxicity. [36]

Mast cell tumor recurrence after incomplete surgical excision in small numbers of dogs has reported rates of 4% to 36%[9-12,29,35] Patients that had high grade MCT and MCT with high proliferation indices are more likely to experience recurence.[9,12] Local recurrence rates for SCMCT are 7% to 8% overall,[31,37] (ie, 12% in dogs with incompletely excised tumors and 2% in dogs with complete excision).[31] Our local control rates of 93.4% to 100% for cMCT and SCMCT respectively are consistent with clinical benefit for the population over surgery alone, but some individual patients, especially those with low-risk MCT, may have been overtreated given the low rate of local recurrence reported with surgery. It is also likely however that a substantial number of dogs referred for radiation have clinically or histopathologically more aggressive MCT than those having surgery alone, but no studies are available to substantiate this suspicion. Future research should focus on determining prognostic factors for MCT recurrence to help determine which cases would benefit from adjunctive radiation.

Considering high risk tumors as a separate group, local recurrence rate after adjunctive RT was similar to that of low and unknown risk tumors, although not statistically assessed because of the low number of recurrences and variation in grade, risk, and margin status. More dogs with high-risk tumors (31%) died of MCT disease, likely because of metastasis, despite chemotherapy in many cases, compared with low-risk dogs (13%). This observation was not statistically assessed because of the number of unknown risk dogs included in the study. We acknowledge that MCT specific death was not confirmed in most patients because of the retrospective nature of the study, and lack of necropsy, meaning that dogs that died of other causes may have been included. High-risk MCT in our study subjectively recurred sooner than did low-risk MCT. Low recurrence rate and different radiation protocols limited interpretation of this suspicion but time to recurrence may reflect cell proliferation and specific cellular responses to radiation.

Our good control rates may in part be related to the inclusion of 55 (18%) SCMCT in the study, which have lower recurrence rates than do cMCT.[31] However, a similar situation was likely to have occurred in historical reports on the role of adjunctive RT, most of which were undertaken before the separate categorization of SCMCT.[38] A rate of 7% SCMCT related death in our population is consistent with previous findings.[31,38,39]

We wanted to compare local control rates for MCT treated with higher dose conventional fractionated regimens with those treated with lower dose coarse fractionated regimens, but the number of cases available was not high enough to achieve statistical significance. The inclusion of tumors of mixed grade, different clinical stages and nonstandardized radiation protocols contributed further to the lack of statistical power. Recurrence rates however were subjectively similar between protocols, which suggests that moderate dose radiation protocols may be adequate for local control of most MCT in dogs.

A previous study reported 10% recurrence in 20 dogs that received 48 to 60 Gy in daily or alternate day fractions.[19] Considering that 93% were Patnaik low or intermediate grade and that other studies with definitive intent protocols reported similar recurrence rates of 7% to 16%, our results support the hypothesis that total radiation dose or fractionation may be less important than, for example, tumor-related factors in determining outcome.

Fewer than 10% of dogs in our population had the local LN irradiated despite 42 dogs being in stage 2. Interpretation of stage is limited because of the retrospective nature of the study, with some dogs having had cytological suspicion of LN metastasis and others having had the LN extirpated. Unfortunately, many clinical records were incomplete regarding LN management. Almost all of the LN in our study were treated with electrons of variable energy. Because most were limb MCT, it is likely that the LN was superficial and palpable, and that dosimetry was appropriate in these cases, but this aspect was impossible to quantify from the clinical records. Additionally, LN treated were not documented, and may not have represented the draining LN in all cases. Prophylactic LN radiation remains controversial with many historical studies reporting it as an alternative to surgical excision, but recent evidence suggests that LN micrometastasis is less prognostic than previously thought, with many patients experiencing long survivals, particularly if lymphadenectomy is included in the treatment, which is now recommended.[40,41] One study that evaluated prophylactic radiation of regional LNs in 6 dogs with MCT reported improved locoregional control[42], but evidence for surgical expiration currently is more strongly supported.[41,43]

Radiation therapy should commence as soon as possible after wound healing to allow the best chance of tumor control. Median time from surgery to radiation in our population was 32 days in the coarse fractionated group and 36 days in the fractionated group, longer than normal wound healing. This delay likely reflects the fact that radiation is often a late treatment consideration after incomplete excision is confirmed and delays in organizing referral have occurred. Some dogs did not receive radiation for a prolonged time after surgery because of owner-related factors.

The median time from surgery to radiation in patients with MCT that recurred in our study was 39.5 days, which is slightly longer than the median for the population overall, but this time was not statistically assessed because of the low numbers of recurrences. The 2 patients with rapidly recurring MCT after long periods of time from surgery to the start of RT had recurrent tumors that were debulked

again before RT. It is likely these patients would have a less favorable response to RT, given demonstratable clinically aggressive behavior of their tumors. It would have been preferable to clarify the number of patients treated with recurrent vs initial MCT, because this difference also may influence the success of RT. Unfortunately, clinical records from the referring veterinarians were often incomplete with regard to this factor. Previous studies however also included mixed populations of patients having initial and recurrent MCT irradiated, but unfortunately not specifying the numbers that were recurrent.[15-21]

The optimal margin of healthy tissue to be included in the radiation field has not been established for dogs with MCT, and although combined CTV/PTV margins of 2 to 3 cm were common it was not possible to establish whether these were from the macroscopic tumor margin or the surgical scar, because patients were commonly referred for radiation by primary care veterinarians after surgery without tumor measurements. In the latter situation topographical omission of tumor tissue is possible. Most patients referred for radiation had surgery at the local practice and in most cases tumor measurements were not available. With improved veterinarian education and more access to digital photography, all patients should have presurgical photographs and measurements recorded to assist in accurate treatment planning and to minimize risk of missing affected tissue.

Not all dogs in our study had full staging and thus tumor stage may have been underestimated, but the low number of patients that died of mast cell disease makes this possibility unlikely. It is unlikely given the poor prognosis associated with stage 4 MCT that clinicians would recommend RT for these patients.[44]

One consideration in offering adjunctive radiation is the chance of the patient developing de novo MCT in the future, estimated as up to 21%.[3] In our study, a rate of 12% was within the expected range.

The radiation prescription also should consider organs at risk (OAR), total dose required for tumor control and dose fractionation.[25] The biologically equivalent dose should be calculated and applied to the individual situation and OAR.[25] In our study, most tumors were appendicular, where the OAR consists of skin, bone, and vasculature.

Acute toxicity was more severe in the fractionated group than in the coarse fractionated group, with 36% of patients in the former group developing VRTOG grade 2/3 dermatitis or desquamation or both. This adverse effect takes a median of 25 days to resolve,[45] often requires analgesia or other topical treatment and can be distressing for the dogs and their owners. Additionally, severe acute toxicity can lead to consequential late toxicity, none of which was seen in our population although the follow-up may have been too short. A high percentage of dogs in our study were reported as having no toxicity compared to the patients in a previous study.[36] This difference may reflect less robust reporting, less frequent assessment of toxicity, or genuine differences in population. In centers C and D, it is uncommon for radiation patients to receive concurrent prednisolone, compared to the group reported previously that all received adjunctive glucocorticoids.[36]

Late toxicity in this population was similar between the standard and coarse fractionated groups, mainly mild and comparable to that previously reported[15-21] consisting mainly of alopecia, leukotrichia and cutaneous fibrosis. Substantial late toxicity (considered life changing) was reported in 1.9% dogs and occurred 910 to 2600 days after radiation. All of the critical late effects were associated with coarse fractionated protocols with high $BEDGy_3$ values, some of which are no longer used in the United Kingdom. Although 3 dogs that received 32 Gy in 4 weekly fractions developed severe complications, these all occurred >1800 days after RT. Because it was the most common protocol used in the study (63% of dogs) and dogs that received this protocol often were more commonly treated in the earlier years of the study, it is unclear whether these complications were a direct consequence of coarse fractionation, or if they reflect longer follow-up. The development of second tumors is consistent with previous reports of outcomes 2 to 6 years postradiation,[23,24] with osteoradionecrosis occurring in 2 dogs within the reported timeframe of 1.2 to 8.7 years.

The observed low rate of 1.9% for severe late complications may reflect the high percentage of dogs treated using electrons in our population (all affected dogs received photons). Additional studies and longer follow-up are required to assess the incidence of late complications. Consideration should be given to the age of the patient at the time of radiation, and the lifetime risk compared with the benefit of tumor control.

Our study had some limitations associated with its retrospective design. Because patients were commonly presented to the radiation center having already had surgery, tumor size, an important prognostic indicator, was not available. Considering radiation field size and mainly appendicular location, however, our population is comparable with those of previous studies and reflects clinical practice.[15-21] Clinical records and staging were not always complete and histopathology specimens were not reassessed. Given the large number of patients and low number of recurrences however these limitations are unlikely to have had substantial impact on the results. Recurrence of MCT, new MCT occurrence and MCT-related death may be under reported and MCT related death may be over-, or under-estimated because no dogs were known to have had necroscopy performed. Some patients received adjunctive, nonstandardized chemotherapy, which may have contributed to improved systemic control. Toxicity also may have been underreported. Given the retrospective nature of the study and format of the clinical records, it was impossible to apply International Commission on Radiation Units and Measurements (ICRU) reporting guidelines.[46]

We have shown that local control of MCT in dogs is very good after combined surgical excision and radiation therapy, even for dogs with high-risk MCT. Dogs with SCMCT achieved excellent local control using this regimen. Mast cell tumor related death did occur, and was attributed more to confirmed or suspected metastatic disease rather than local failure and more commonly in high-risk MCT cases, but most patients still experienced good survival times after radiation. Late radiation toxicity was common and similar between protocols. Clinically relevant late toxicity was uncommon.

We propose, based on these results, that more moderate dose and fractionation protocols may be appropriate in the adjunctive treatment of MCT in dogs. Radiation oncologists should work

together to prospectively evaluate an appropriate regimen, based on current evidence and biologically equivalent dose calculations.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Sarah L. Mason https://orcid.org/0000-0003-4922-1675
Onne-Marju Russak https://orcid.org/0000-0002-2626-5504
Davide Berlato https://orcid.org/0000-0001-7951-428X

REFERENCES

1. Bostock DE. Neoplasms of the skin and subcutaneous tissues in dogs and cats. *Br Vet J*. 1986;142:1-19.
2. Dorn CR, Taylor DO, Schneider R, Hibbard HH, Klauber MR. Survey of animal neoplasms in alameda and contra costa counties, California, II, cancer morbidity in dogs and cats from Alameda County. *J Natl Cancer Inst*. 1968;40:307-318.
3. Blackwood L, Murphy S, Buracco P, et al. European consensus document on mast cell tumours in dogs and cats. *Vet Comp Oncol*. 2012; 10:e1-e29.
4. Abadie JJ, Amardeilh MA, Delverdier ME. Immunohistochemical detection of proliferating cell nuclear antigen and Ki-67 in mast cell tumors from dogs. *J Am Vet Med Assoc*. 1999;215(11):1629-1634.
5. Moore AS, Frimberger AE, Taylor D, Sullivan N. Retrospective outcome evaluation for dogs with surgically excised, solitary Kiupel high-grade, cutaneous mast cell tumors. *Vet Comp Oncol*. 2020;18(3):402-407. https://doi.org/10.1111/vco.12565
6. Miller RL, Van Lelyveld S, Warland J, et al. A retrospective review of treatment and response of high-risk mast cell tumours in dogs. *Vet Comp Oncol*. 2016;14:361-370.
7. O' Keefe DA. Canine mast cell tumors. *Vet Clin North Am Small Anim Pract*. 1990;20:1105-1115.
8. Govier SM. Principles of treatment for mast cell tumours. *Clin Tech Small Anim Pract*. 2003;18:103-106.
9. Donnelly L, Mullin C, Balko J, et al. Evaluation of histological grade and histologically tumour-free margins as predictors of local recurrence in completely excised canine mast cell tumours. *Vet Comp Oncol*. 2015;13(1):70-76.
10. Itoh T, Kojimoto A, Uchida K, Chambers J, Shii H. Long-term postsurgical outcomes of mast cell tumors resected with a margin proportional to the tumor diameter in 23 dogs. *J Vet Med Sci*. 2021;83(2): 230-233.
11. Milovancev M, Townsend KL, Tuohy JL, et al. Long-term outcomes of dogs undergoing surgical resection of mast cell tumors and soft tissue sarcomas: a prospective 2-year-long study. *Vet Surg*. 2020;49:96-105.
12. Seguin B, Besancon MF, McCallan JL, et al. Recurrence rate, clinical outcome, and cellular proliferation: indices as prognostic indicators after incomplete surgical excision of cutaneous grade II mast cell tumors: 28 dogs (1994-2002). *J Vet Intern Med*. 2006;20:933-940.
13. Selmic LE, Ruple A. A systematic review of surgical margins utilized for removal of cutaneous mast cell tumors in dogs. *BMC Vet Res*. 2020;16:5. https://doi.org/10.1186/s12917-019-2227-8
14. Smith J, Kiupel M, Farrelly J, et al. Recurrence rates and clinical outcome for dogs with grade II mast cell tumours with a low AgNOR count and Ki67 index treated with surgery alone. *Vet Comp Oncol*. 2017;15(1):36-45. https://doi.org/10.1111/vco.12140
15. al-Sarraf R, Mauldin GN, Patnaik AK, Meleo KA. A prospective study of radiation therapy for the treatment of grade 2 mast cell tumors in 32 dogs. *J Vet Intern Med*. 1996;10:376.
16. Frimberger AE, Moore AS, Larue SM, Gliatto JM, Bengtson AE. Radiotherapy of incompletely resected, moderately differentiated mast cell tumors in the dog: 37 cases (1989-1993). *J Am Anim Hosp Assoc*. 1997;33:320-324.
17. Chaffin K, Thrall D. Results of radiation therapy in 19 dogs with cutaneous mast cell tumours and regional lymph node metastasis. *Vet Radiol Ultrasound*. 2002;43(4):392-395.
18. Poirier VJ, Adams WM, Forrest LJ, et al. Radiation therapy for incompletely excised grade II canine mast cell tumours. *J Am Anim Hosp Assoc*. 2006;42:430-434.
19. Kry K, Boston S. Additional local therapy with primary re-excision or radiation therapy improves survival and local control after incomplete or close surgical excision of mast cell tumors in dogs. *Vet Surg*. 2014; 43:182-189.
20. Ladue T, Price GS, Dodge R, Page RI, Thrall DE. Radiation therapy for incompletely resected canine mast cell tumours. *Vet Radiol Ultrasound*. 1998;39:57-62.
21. Hahn KA, King GK, Carreras JK. Efficacy of radiation therapy for incompletely resected grade-III mast cell tumors in dogs: 31 cases (1987-1998). *J Am Vet Med Assoc*. 2004;224:79-82.
22. Laude T, Klein MK. Toxicity criteria of the veterinary radiation therapy oncology group. *Vet Radiol Ultrasound*. 2001;42:475-476.
23. Dickinson PJ, McEntee MC, Lipsitz D. Radiation induced vertebral osteosarcoma following treatment of an intradural extramedullary spinal cord tumor in a dog. *Vet Radiol Ultrasound*. 2001;42:463-470.
24. Mellanby RJ, Herrtage ME, Chantry J, Dobson JM. Sarcoma development after radiotherapy in two dogs. *Vet Comp Oncol*. 2003;1:113-119.
25. Fowler JF. The linear quadratic formula and progress in fractionated radiotherapy. *Br J Radiol*. 1989;62(740):679-694.
26. Maeda J, Froning CE, Brents CA, Rose BJ, Thamm DH, Kato TA. Intrinsic radiosensitivity and cellular characterization of 27 canine cancer cell lines. *PLoS One*. 2016;11:e0156689.
27. Patnaik AK, Ehler WJ, Macewen EG. Canine cutaneous mast cell tumour: morphologic grading and survival time in 83 dogs. *Vet Pathol*. 1984;21:469-474.
28. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol*. 2011;48:147-155.
29. Liptak JM. Histologic margins and the residual tumour classification scheme: is it time to use a validated scheme in human oncology to standardise margin assessment in veterinary oncology? *Vet Comp Oncol*. 2020;18:25-30.
30. Romansik EM, Reilly CM, Kass PH, Moore PF, London CA. Mitotic index is predictive for survival for canine cutaneous mast cell tumors. *Vet Pathol*. 2007;44:335-341.
31. Thompson JJ, Pearl DL, Yager JA, Best SJ, Coomber BL, Foster RA. Canine subcutaneous mast cell tumor: characterization and prognostic indices. *Vet Pathol*. 2011;48:156-168.
32. Scase TJ, Edwards D, Miller J, et al. Canine mast cell tumors: correlation of apoptosis and proliferation markers with prognosis. *J Vet Intern Med*. 2006;20(1):151-158.

33. Webster JD, Yuzbasiyan-Gurkan V, Miller RA, Kaneene JB, Kiupel M. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Vet Pathol*. 2007;44:298-308.
34. Maglennon GA, Murphy S, Adams V, et al. Association of Ki67 index with prognosis for intermediate-grade canine cutaneous mast cell tumours. *Vet Comp Oncol*. 2008;6:268-274.
35. Michels GM, Knapp DW, DeNicola DB, et al. Prognosis following surgical excision of canine cutaneous mast cell tumors with histopathologically tumor-free versus non-tumor free margins: a retrospective study of 31 cases. *J Am Anim Hosp Assoc*. 2002;38:458-466.
36. Blackwood L, Tanis JB, Harper A, Amores-Fuster I, Killick DR, Finotello R. Acute radiotherapy toxicity in 57 dogs with gross and microscopic mast cell tumours. *Vet Comp Oncol*. 2018;16:431-440.
37. Gill V, Leibman N, Monette S, Craft DM, Bergman PJ. Prognostic indicators and clinical outcome in dogs with subcutaneous mast cell tumors treated with surgery alone: 43 cases. *J Am Anim Hosp Assoc*. 2020;56(4):215-225. https://doi.org/10.5326/JAAHA-MS-6960
38. Newman SJ, Mrkonjich L, Walker KK, Rohrbach BW. Canine subcutaneous mast cell tumour: diagnosis and prognosis. *J Comp Pathol*. 2007;136:231-239.
39. Thompson JJ, Yager JA, Best SJ, et al. Canine subcutaneous mast cell tumors: cellular proliferation and KIT expression as prognostic indices. *Vet Pathol*. 2011;48:169-181.
40. Ferrari R, Marconato L, Buracco P, et al. The impact of extirpation of non-palpable/normal-sized regional lymph nodes on staging of canine cutaneous mast cell tumours: a multicentric retrospective study. *Vet Comp Oncol*. 2018;16:505-510.
41. Marconato L, Polton G, Stefanello D, et al. Therapeutic impact of regional lymphadenectomy in canine stage II cutaneous mast cell tumours. *Vet Comp Oncol*. 2018;16:580-589.
42. Mendez SE, Drobatz KJ, Duda LE, White P, Kubicek L, Sorenmo KU. Treating the locoregional lymph nodes with radiation and/or surgery significantly improves outcome in dogs with high-grade mast cell tumours. *Vet Comp Oncol*. 2019;18(2):239-247. https://doi.org/10.1111/vco.1254
43. Marconato L, Stefanello D, Kiupel M, et al. Adjuvant medical therapy provides no therapeutic benefit in the treatment of dogs with low-grade mast cell tumours and early nodal metastasis undergoing surgery. *Vet Comp Oncol*. 2020;18:409-415. https://doi.org/10.1111/vco.12566
44. Moirano S, Lima S, Hume K, Brodsky E. Association of prognostic features and treatment on survival time of dogs with systemic mastocytosis: a retrospective analysis of 40 dogs. *Vet Comp Oncol*. 2018;16:E194-E201. https://doi.org/10.1111/vco.12373
45. Keyerleber MA, Ferrer L. Effect of prophylactic cefalexin treatment on the development of bacterial infection in acute radiation-induced dermatitis in dogs: a blinded randomized controlled prospective clinical trial. *Vet Dermatol*. 2018;29:37-e18.
46. Keyerleber MA, McEntee MC, Farrelly J, Podgorsak M. Completeness of reporting of radiation therapy planning, dose, and delivery in veterinary radiation oncology manuscripts from 2005 to 2010. *Vet Radiol Ultrasound*. 2012;53:221-230.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Mason SL, Pittaway C, Gil BP, et al. Outcomes of adjunctive radiation therapy for the treatment of mast cell tumors in dogs and assessment of toxicity: A multicenter observational study of 300 dogs. *J Vet Intern Med*. 2021;35(6):2853-2864. doi:10.1111/jvim.16264