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A retrospective study of 101 dogs with oral melanoma treated with a weekly or biweekly 6 Gy × 6 radiotherapy protocol

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Abstract

One radiotherapy (RT) protocol used for canine oral melanoma (OM) gives 36 Gy total, in six weekly or biweekly fractions (6 Gy × 6). This retrospective study characterizes oncologic outcomes for a relatively large group of dogs treated with this protocol and determines whether radiation dose intensity (weekly vs. biweekly) affected either progression-free or overall survival (PFS and OS). Dogs were included if 6 Gy × 6 was used to treat grossly evident OM, or if RT was used postoperatively in the subclinical disease setting. Kaplan–Meier statistics and Cox regression modelling were used to determine the predictive or prognostic value of mitotic count, bony lysis, World Health Organization (WHO) stage (I, II, III, or IV), using systemic anticancer therapies, tumour burden at the time of RT (macroscopic vs. subclinical), radiation dose intensity (weekly vs. biweekly), and treatment planning type (manual vs. computerized). The median PFS and OS times for all dogs (n = 101) were 171 and 232 days, respectively. On univariate analysis PFS and OS were significantly longer ($p = <.05$) with subclinical tumour burden, WHO stages I or II, and weekly irradiation. On multivariable analysis, only tumour stage remained significant; therefore, cases were grouped by WHO stage (I/II vs. III/IV). With low WHO stage (I/II), PFS and OS were longer when irradiating subclinical disease (PFS: risk ratio = 0.449, $p = .032$; OS: risk ratio = 0.422, $p = .022$); this was not true for high WHO stage (III/IV). When accounting for other factors, radiation dose intensity had no measurable impact on survival in either staging group.

KEYWORDS
acute radiation toxicity, oral mucositis, oral tumours, radiodermatitis, veterinary oncology

1 | INTRODUCTION

Canine oral melanoma (OM) is characterized by locally invasive tumours and high rates of metastasis to regional lymph nodes (LN) and distant organs (e.g. lungs and other).[1–14] Measurable responses to platinum chemotherapeutics occur in about 30% of cases; however, such treatment has no demonstrable anti-metastatic or life-prolonging effect when used as adjunctive therapy.[1,10–12] The literature is also inconsistent with regard to demonstration of antineoplastic efficacy for available immunotherapies (e.g. ONCEPT; Merial,

Duluth, GA).[1–5] Surgery has long been a mainstay of therapy; with surgery alone, published median overall survival (OS) times range from 150 to 1020 days.[15–19] If surgery is not performed, radiotherapy (RT) can be considered as an alternative primary treatment. RT can also be used postoperatively with a goal of preventing or delaying locoregional recurrences.

Various RT protocols have been described, but an optimal fractionation scheme has not been defined. Current practice patterns favour use of hypofractionated RT; reported response rates in dogs with gross disease exceed 80%, with at least 44% having complete responses and published median OS times ranging from 122 to 363 days.[1,6–10,20] A commonly used but infrequently reported protocol is 36 Gy total in six fractions of 6 Gy (hereafter referred to as '6 Gy × 6'). Published data are limited to two studies. Hoopes and colleagues reported delivery of 6 Gy × 6, with three fractions per week given every-other-day along with an experimental immunotherapeutic; sample size was small (N = 5 dogs), and no firm conclusions can be drawn regarding tolerability or efficacy.[20] A prospective study by Freeman et al. investigated a once-weekly 6 Gy × 6 protocol in dogs with microscopic OM (i.e. irradiated postoperatively with no evidence of metastasis); RT was given with radiosensitizing low-dose carboplatin chemotherapy. The local recurrence rate was 15%, and 20 of the 39 dogs developed nodal or distant metastasis. The median OS time was 363 days.[10] These results suggest that with appropriate case selection and multimodal combinations of surgery and RT, outcomes can be optimized. However, important gaps in knowledge remain when considering application of the 6 Gy × 6 protocol for OM.

It is unclear whether outcomes are different when dogs are treated with once- or twice-weekly fractionation (which anecdotally represent the most commonly utilized treatment schedules). Both approaches are expected to produce a similar risk for late radiation-induced tissue toxicity,[1] and there may be certain clinics and/or pet owners that choose weekly or biweekly treatment schedules due to logistical considerations. From a biological perspective, some clinicians prefer biweekly scheduling because it may limit tumour cell repopulation. Theoretically, accelerated irradiation schemata increase the potential efficacy of a given dose of radiation by reducing the amount of tumour clonogen proliferation that occurs during a course of RT. That is particularly relevant to prolonged courses of RT, where there may be 'accelerated repopulation'.[1,21,22] In veterinary medicine, it is often suggested that accelerated repopulation becomes a concern after about 4 weeks of treatment. There are also reports that accelerated repopulation may become problematic as soon as 14 days into treatment of head and neck squamous cell carcinomas.[22] The kinetics of tumour cell proliferation have not been well characterized in canine OMM, and there is likely some heterogeneity between patients. Nonetheless, it does seem probable that completing treatment with a biweekly protocol (~17 days total) would have some benefit with regard to protocol efficacy as compared with a weekly protocol, which takes approximately twice as long to complete (~35 days). Still, a more succinct course of RT may also be preferred to attempt to quickly reduce tumour-associated pain in dogs with measurable OM. Other prescribers may prefer weekly protocols, as the lower dose intensity should reduce the risk for acute radiation side effects.[1] This may be an important consideration for some pet owners and clinicians, especially since the long-term prognosis for survival in canine OM is generally regarded as poor, and in this patient population, there is often a strong clinical emphasis on maximizing quality of life, and minimizing cancer treatment associated morbidity.

The goals of this multi-institutional retrospective study were to: (1) report oncologic outcomes for a large group of dogs treated with a 6 Gy × 6 protocol, delivered once or twice weekly, as either primary therapy for macroscopic tumours, or as a postoperative adjunct, in effort to control residual subclinical tumour burdens (either at the primary tumour site, the regional LN, or both); and (2) to test the hypothesis that in dogs with OM, biweekly 6 Gy fractionation is associated with longer OS as compared with a once-weekly protocol.

2 | METHODS

2.1 | Data acquisition

Electronic and paper medical records from three veterinary RT centres located within in a single state in the United States were searched for dogs whose OM was treated with RT between June 1, 2007, and September 15, 2020. Dogs prescribed 6 Gy × 6 with or without prior cytoreductive surgery were included. Dogs prescribed alternative dosing regimens were excluded. Data collected included: signalment, prior history, concurrent disease, results of diagnostic imaging tests performed for OM staging, and pathology results (histopathology and/or cytology of the primary tumour and regional LN). Descriptions of adverse effects of RT, and adjunctive chemotherapies and immunotherapies were recorded, when available. Adverse events were retrospectively classified according to the Veterinary Radiation Therapy Oncology Group (VRTOG) classification scheme.[23] Toxicities were deemed acute if first observed within 90 days of RT, and late if first noted >90 days from the time of RT.[24] Date and cause of death, and disease progression was also recorded. Radiation treatment plans were reviewed to gather pertinent details, including RT schedule and dates, total radiation dose, and RT modality, which was categorized as being either computerized (intensity modulated RT or three-dimensional conformal RT) or non-computerized (manual/clinical setup).

2.1.1 | Statistical analysis

Kaplan–Meier survival curves were generated. The OS time was defined as the number of days from initiation of RT until death due to any cause; cases were censored if alive at the time of analysis, or if lost to follow-up. Progression-free survival (PFS) time was defined as days from initiation of RT until a tumour-specific event (local progression detected during follow-up physical examinations, or/and detection of confirmed or suspected locoregional or distant metastasis via imaging or examination) occurred. Cases were censored from the PFS

analysis if they died of causes presumed to be unrelated to OM. Timing of follow-up examinations was not standardized. Dogs who did not complete the prescribed 6 Gy × 6 protocol were included in the OS and PFS analysis.

Univariable analyses were performed via Cox proportional hazard modelling to calculate risk ratios (RR) and *p*-values. Variables included potential prognosticators: World Health Organization (WHO) stage (stage I: primary tumour <2 cm, no LN involvement; stage II: primary tumour 2–4 cm, no LN involvement; stage III: primary tumour >4 cm and/or LN involvement; stage IV: distant metastasis),[25] mitotic count, presence of bony lysis (assessed via computed tomography [CT]), and primary tumour size (the longest tumour dimension of the primary tumour diameter on physical examination and/or diagnostic imaging). Potential predictive factors were also assessed: disease setting (primary RT of grossly evident macroscopic tumours vs. postoperative RT to address subclinical and/or microscopic disease at the primary site and/or in the regional LN), RT modality (computerized vs. non-computerized), RT schedule (once-weekly vs. twice-weekly), and the use of adjunctive therapies (chemotherapy or immunotherapy). WHO stages were considered individually as pairwise comparators and in groups: low WHO stage disease (I & II) versus high WHO stage disease (III & IV). Multivariable Cox proportional hazard regression models were constructed to evaluate for potential associations with survival. Variables were entered into the multivariable models if they had a *p*-value < .2 on univariable analysis or if they could be confounding (i.e. have been previously identified as potential prognostic factors). Backward selection was applied with the predictor with the largest Wald *p*-value being considered for removal at each stage. Reported results reflect *p*-values for the Wald statistic, and unit RR. Analyses were performed using commercial software (JMP Pro version 15; SAS Institute, Cary, NC and Prism version 8; GraphPad Software, San Diego, CA). Statistical significance was set at $p < .05$.

3 | RESULTS

3.1 | Demographics and staging information

One hundred and one dogs met inclusion criteria. Treatment centres included Institution 1 (Varian Novalis TX; Varian Medical Systems, Inc., Palo Alto, CA; n = 22 dogs), Institution 2 (Varian 2100C; n = 35 dogs) and Institution 3 (prior to February 2021, a Varian 2100C/D was used, and a Varian Halcyon was used thereafter; n = 44 dogs). The most common dog breeds were: mixed (n = 26), Labrador Retrievers (n = 14), Golden Retrievers (n = 6), Chow Chow (n = 5), Pug (n = 4), and Yorkshire Terrier (n = 3). Median age and body weight were 12 years (range: 6–17 years) and 23.1 kg (range: 2.7–55.2 kg), respectively. There were 52 castrated males, 45 spayed females, three sexually intact males and one sexually intact female. Sixty-seven of 94 dogs (67/94; 71%) are known to have originally presented for tumour evaluation, whereas 27 tumours (27/94; 29%) were incidentally found during oral examination. For the remaining dogs, reason for presentation was not specified. Complete patient demographics data are provided in the **Supplementary Data File**.

Ninety-six (96/101; 95%) oral tumours were diagnosed by histopathology and five (5/101; 4.9%) by cytology. A mitotic count was provided in the biopsy report for 64 of 101 dogs, and was categorized as <4 mitoses per 10 high magnification field (n = 11) or ≥4 mitoses per 10 high magnification field (n = 53).[2,14,26] Seventy-eight dogs (78/101; 77%) underwent CT imaging of the primary tumour for RT planning. The longest dimension of the primary tumour was documented in 48 dogs (48/101; 47%), thus allowing retrospective WHO tumour stage categorization as <2 cm (T1; n = 13), 2–4 cm (T2; n = 24), >4 cm (T3; n = 11).[25] In 19 cases (19/101; 18%) records indicated subclinical disease burden at the time of RT with no record of primary tumour size, preoperatively; 34 dogs (34/101; 33%) had gross disease present at the time of RT, but no documentation of tumour size.

Regional LN cytology and/or histopathology was performed in 70 of 101 dogs (69%). Twenty-one dogs (21/101; 21%) are known to have had pathologically confirmed nodal metastasis at diagnosis. Sixteen dogs had mandibular LN metastasis (ipsilateral 11/21; 52%, bilateral 4/21; 19%), one had metastasis in the ipsilateral medial retropharyngeal node, and four had metastases at both nodal sites. Thoracic imaging was performed in 98 dogs (radiography in 66 dogs and CT in 32); 4 (of 98; 4%) had evidence of pulmonary metastasis at the time of diagnosis. Abdominal imaging was performed in 39 dogs (ultrasound in 13 dogs and CT in 26 dogs). No dog had evidence of distant abdominal metastasis. Findings deemed unrelated or unlikely to be related to OM on available abdominal imaging reports included: cholecystolithiasis (n = 2), cholecystic debris (n = 3), splenic nodules/splenomegaly cytologically consistent with extramedullary haematopoiesis (n = 2), hepatic nodules/hepatomegaly cytologically consistent with benign hyperplasia (n = 4), urolithiasis (n = 1), urocystic debris (n = 2), renal cortical cyst (n = 2), mild adenomegaly (n = 2), and degenerative nephropathy (n = 4).

3.2 | Radiotherapy

Sixty-eight of 101 dogs (67%) underwent surgical excision of the primary tumour before RT; 24 dogs (of 68; 35%) underwent intensive definitive-intent procedures involving removal of tumour-adjacent bone. The remainder (44/68; 64%) underwent excisional biopsy and/or debulking to obtain a diagnosis and in some cases to provide some degree of palliation. Four dogs with cytologically confirmed metastasis and one additional dog (reason for LN removal was not recorded) had their mandibular LN extirpated during surgery, and metastasis was confirmed on histopathology in four of those five cases (i.e. in the four dogs with cytologically confirmed metastasis prior to surgery).

Only five dogs failed to complete their prescribed RT protocol, and all 101 are included in this intent-to-treat analysis. The primary tumour site was included in the radiation field for all cases. Sixty-eight dogs (of 101; 67%) had gross disease present at the start of RT and

TABLE 1 Acute radiation toxicoses in 93 dogs treated with 6 Gy × 6 radiotherapy for oral melanoma[23]

Site/Tissue	VRTOG acute toxicity (Grade)	Weekly	Biweekly
Oral/Mucous membrane	1: Injection without mucositis	n = 12 dogs	n = 14 dogs
	2: Patchy mucositis without perceived pain	n = 3	n = 3
	3. Necrosis	n = 1	n = 0
Dermatologic	1: Erythema, dry desquamation	n = 8	n = 4
	2: Patchy moist desquamation without oedema	n = 5	n = 4
Ocular	1: Mild conjunctivitis and/or sclera injection	n = 0	n = 0
	2: Keratoconjunctivitis sicca requiring therapy	n = 3	n = 0
	3. Corneal ulceration	n = 1	n = 0

Abbreviations: VRTOG, Veterinary Radiation Therapy Oncology Group (acute toxicity grading scheme; see Reference [23].

33 (of 101; 33%) were treated in the subclinical disease setting (after cytoreductive surgery). The regional LNs were irradiated in 85 dogs, 21 of which had pathologic evidence of nodal metastasis at the time of irradiation. Thus, 64 of the 85 dogs (75%) underwent prophylactic regional LN irradiation. In all cases, prophylactic nodal RT included the bilateral mandibular and medial retropharyngeal LNs. The same RT prescription was used for primary and nodal sites in all dogs except one (whose LNs were reported as having been irradiated to a total dose of 33 Gy in 6 biweekly fractions).

Treatment planning was performed using inverse planning for intensity modulated RT in 18 cases, forward planning for three-dimensional conformal RT in 43 cases, and by clinical setup with manual dose calculation in 30 cases. For 10 dogs, no information about the RT treatment plan (beyond the RT prescription, treatment schedule, and planning modality) was available. In all other cases, RT plans were constructed with 2–11 coplanar beams (6 MV photons, n = 83 dogs; 10 MV photons, n = 2 dogs) or a single electron field (n = 6 dogs). Beam modifiers (blocks, multileaf collimators, wedges, and/or bolus materials) were used per clinician preference. Dose statistics for organs at risk were not recorded for purposes of this retrospective study. All available radiation plan, and associated dosimetry, data are summarized in the Supplementary Data File.

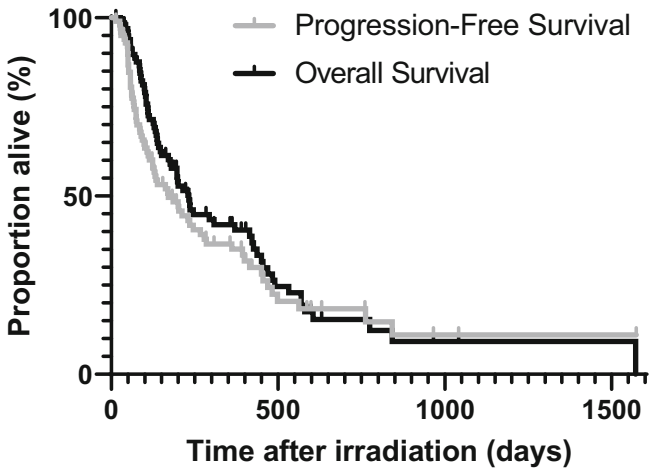

FIGURE 1 Kaplan–Meier curve depicting progression-free (grey line; 171 days, 95% confidence interval: 114–267 days) and overall survival times (black line; 232 days, 95% confidence interval: 171–415 days) for the entire cohort of 101 dogs. Tick marks represent the censored subjects

3.3 | Adjunctive medical therapies

Eighty-two of the 101 dogs (81%) received systemic therapy in the form of systemic chemotherapy, the ONCEPT melanoma vaccine, or both. Twenty-four dogs (of 101; 24%) received carboplatin chemotherapy; in 19 cases, it was given upon completion of RT (19/24; 79%); in two cases (2/24, 8.3%) it was given concurrent with RT; in 3 cases (3/24; 12%), it was given before starting RT. The median dose was 287.5 mg/m^2 (range: 225–300 mg/m^2), at a median 3-week dosing interval (range: 2–4 weeks). Dogs received 8 (n = 1), 7 (n = 1), 5 (n = 1), 4 (n = 10), 3 (n = 3), 2 (n = 4), and 1 (n = 1) doses of carboplatin. Other chemotherapy drugs that were administered included dacarbazine (n = 2 dogs), toceranib phosphate (n = 1 dog; 2.42 mg/kg, given Monday, Wednesday, Friday for 130 days), chlorambucil (n = 1 dog; 7 mg/m^2 every 48 h for 135 days) and temozolomide (n = 1 dog). Information regarding dose, duration, and frequency of administration were not frequently available for these additional chemotherapies. Seventy-seven dogs (77/101; 77%) received ONCEPT (median number of doses was 4; range: 2–7). Nineteen (19/101; 19%) dogs received both chemotherapy and ONCEPT immunotherapy.

3.4 | Radiotherapy-associated toxicities

Ninety-three of 101 dogs were assessed by a veterinarian for RT-associated toxicosis after completing RT. Because of the retrospective nature of the study, the timing and frequency of follow-up examinations varied. Among the 93 dogs that were assessed, 48 (48/93; 52%) were noted to have developed acute radiation toxicosis which were expected and tolerable (Table 1). Information about late radiation toxicosis was available in 90 dogs. Late toxicosis was documented in six dogs (6.6%); two dogs had VRTOG grade 1 alopecia (alopecia), three dogs had evidence of osteonecrosis (two of the maxilla and one of the

TABLE 2 Univariable analysis of factors assessed for an association with OST and PFS for 101 dogs treated with 6 Gy × 6 radiotherapy for oral melanoma

	Overall survival		Progression-free survival	
	Risk ratio (95% CI)	*p*-value	Risk ratio (95% CI)	*p*-value
Mitotic count (# mitoses per 10 high magnification fields)				
<4 vs. ≥4 per 10 hpf	1.731 (0.761–4.648)	.2251	1.885 (0.790–5.571)	.1939
Bony lysis				
No vs. Yes	1.150 (0.544–2.204)	.6916	1.386 (0.691–2.581)	.3273
WHO stage				
I vs. II	1.393 (0.592–3.277)	.4470	1.195 (0.512–2.785)	.6805
I vs. III	0.437 (0.218–0.876	.0197*	0.400 (0.195–0.823)	.0128*
I vs. IV	0.227 (0.075–0.684)	.0084*	0.300 (0.101–0.895)	.0309*
II vs. III	0.313 (0.142–0.691)	.0040*	0.335 (0.156–0.719)	.0050*
II vs. IV	0.163 (0.051–0.519)	.0021*	0.251 (0.082–0.766)	.0151*
III vs. IV	0.520 (0.188–1.437)	.2076	0.749 (0.278–2.017)	.5678
Tumour burden				
Macroscopic vs. Subclinical	1.913 (1.129–3.242)	.0160*	2.000 (1.171–3.419)	.0112*
Planning modality				
Computerized vs. Manual	1.052 (0.618–1.791)	.8528	1.125 (0.657–1.927)	.6682
Radiotherapy schedule				
Once vs. Twice Weekly	0.610 (0.375–0.992)	.0464*	0.568 (0.351–0.919)	.0213*
Anti-neoplastic chemotherapy or immunotherapy (e.g. cancer vaccines)				
No vs. Yes	0.852 (0.479–1.634)	.6067	0.902 (0.514–1.692)	.5140

*Threshold for statistical significance set to $p < 0.05$.

mandible) which developed at 279, 109, and 96 days, respectively, one of which had concurrent oronasal fistula formation, and one dog had severe xerostomia.

3.5 | Oncologic outcomes

Five dogs did not complete the prescribed RT protocol. In three cases treated on a weekly basis, premature RT completion was attributed to severe acute radiation side effects, including oronasal fistula formation in a dog who received four fractions, and ocular effects (corneal ulceration, and moist desquamation in dogs who received three fractions; n = 1 dog each; OS times were 109, 496, and 183 days, respectively). Another dog, in the weekly treatment group, developed pulmonary metastasis after receiving three fractions; it survived 43 days. The final dog, in the weekly treatment group, survived 103 days after having developed aspiration pneumonia in association with anaesthesia for RT fraction #4.

The median PFS was 171 days (95% confidence interval [CI]: 114–267) (Figure 1). Thirty-two dogs were censored from the PFS analysis (16 were lost to follow-up, 12 were alive with no evidence of tumour recurrence and four died of unrelated reasons; one each of lymphoma, hepatobiliary dysfunction, pathologic tibial fracture secondary osteosarcoma, and haemoabdomen). Of the 69 dogs, progressive disease was determined based on follow-up thoracic radiographs in 22 dogs, based on physical examination in 31 dogs, and based on clinical signs in 16 dogs. Dogs with unconfirmed (no imaging or cytology) but likely progressive disease based on clinical presentation were considered as events.

The median OS time for all 101 dogs was 232 days (95% CI: 171–415 days) (Figure 1). Thirty-two dogs were censored from the survival analysis; 16 were lost to follow-up and 16 were alive at the time of data analysis. The median follow-up time for the 32 censored cases was 295 days (range: 14–1041 days). Of the 69 dogs that died and were considered an event, 42 dogs belonged to the weekly cohort and 27 to the biweekly cohort. Thirty-one dogs died or were euthanized because of documented locally progressive disease, 22 from the weekly treatment cohort and 11 from the biweekly. Sixteen dogs died or were euthanized due to documented metastatic disease, eight from the weekly cohort and eight from the biweekly cohort. Two dogs had both documented local recurrence and metastatic disease, one dog each from the weekly and biweekly treatment groups. The cause of death in 20 dogs was unknown or suspected to be unrelated to OM.

Results significantly associated with improved PF and/or OS times on univariable analysis included low WHO stage (PFS: I vs. III RR = 0.400, $p = .0128$; I vs. IV RR = 0.300, $p = .0309$; II vs. III RR = 0.335, $p = .0050$; II vs. IV RR = 0.251, $p = .0151$: OS I vs. III RR = 0.437, $p = .0197$; I vs. IV RR = 0.227, $p = .0084$; II vs. III RR = 0.313, $p = .0040$; II vs. IV RR = 0.163, $p = .0021$)subclinical tumour burden at the time of RT (PFS 393 days, RR = 2.000, $p = .0112$; OS 437 days, RR = 9.193, $p = .0160$), and once-weekly treatment schedules (PFS RR = 0.568, $p = .0213$; OS RR = 0.610, $p = .0464$; Table 2). Tumour size (T-stage) was omitted from the analysis because of the large number of missing data points. The initial multivariable analysis

	Overall survival		Progression-free survival	
	Risk ratio (95% CI)	*p*-value	Risk ratio (95% CI)	*p*-value
Mitotic count (# mitoses per 10 high magnification fields)				
<4 vs. ≥4 per 10 hpf	1.033 (0.348–3.603)	.9554	1.235 (0.397–4.770)	.7333
Bony lysis				
No vs. Yes	1.202 (0.220–5.500)	.8174	1.135 (0.214–4.909)	.8703
WHO stage				
I vs. II	1.738 (0.441–6.857)	.4299	0.841 (0.210–3.360)	.8061
I vs. III	0.336 (0.091–1.241)	.1017	0.221 (0.057–0.856)	.0288*
I vs. IV	0.124 (0.016–0.946)	.0441*	0.233 (0.034–1.588)	.1368
II vs. III	0.193 (0.049–0.766)	.0194*	0.263 (0.066–1.050)	.0587*
II vs. IV	0.071 (0.009–0.561)	.0121*	0.277 (0.044–1.729)	.1694
III vs. IV	0.368 (0.0421–3.235)	.3675	1.053 (0.147–7.565)	.9589
Tumour burden				
Macroscopic vs. Subclinical	1.456 (0.437–4.847)	.5406	1.427 (0.441–4.616)	.5527
Planning modality				
Computerized vs. Manual	0.901 (0.302–2.687)	.8518	2.000 (0.626–6.389)	.2422
Radiotherapy schedule				
Once vs. Twice Weekly	0.215 (0.036–1.289)	.0926	1.579 (0.183–13.649	.6778
Anti-neoplastic chemotherapy or immunotherapy (e.g. cancer vaccines)				
No vs. Yes	3.693 (0.573–34.284)	.1975	2.933 (0.488–25.523)	.2704

*Threshold for statistical significance set to $p < .05$.

TABLE 3 Multivariable Cox proportional hazards regression analysis of potential prognostic factors for 101 dogs treated with 6 Gy × 6 radiotherapy for oral melanoma

	Overall survival		Progression-free survival	
	Risk ratio (95% CI)	*p*-value	Risk ratio (95% CI)	*p*-value
WHO Stage I/II				
Tumour burden				
Subclinical vs. Macroscopic	0.422 (0.194–0.862)	.022*	0.449 (0.207–0.193)	.032*
Planning modality				
Computerized vs. Manual	1.111 (0.537–2.195)	.767	1.004 (0.481–1.981)	.989
Radiotherapy schedule				
Once vs. Twice Weekly	1.177 (0.467–2.094)	.962	1.419 (0.697–2.800)	.319
Anti-neoplastic chemotherapy or immunotherapy (e.g. cancer vaccines)				
No vs. Yes	1.051 (0.449–2.878)	.915	1.238 (0.538–3.357)	.641
WHO Stage III/IV				
Tumour burden				
Subclinical vs. Macroscopic	1.117 (0.365–3.02)	.835	0.687 (0.232–1.771)	.462
Planning modality				
Computerized vs. Manual	0.537 (0.194–1.341)	.200	0.408 (0.140–1.053)	.077
Radiotherapy schedule				
Once vs. Twice Weekly	2.118 (0.728–5.96)	.159	2.059 (0.742–5.597)	.156
Anti-neoplastic chemotherapy or immunotherapy (e.g. cancer vaccines)				
No vs. Yes	0.689 (0.232–2.327)	.523	0.820 (0.314–2.638)	.696

*Threshold for statistical significance set to $p < .05$.

TABLE 4 Multivariable Cox proportional hazards regression analysis of predictive factors, with cases categorized into one of two staging groups: low (WHO stages I and II) versus high (WHO stages III and IV) PFS for 101 dogs treated with 6 Gy × 6 radiotherapy for oral melanoma

assessing potential prognostic factors indicated that when accounting for all other variables together, only WHO stage remained significant (PFS I vs. III RR = 0.221, $p = .0288$; OS I vs. IV, RR = 0.124, $p = .0441$; II vs. III RR = 0.193, $p = .0194$; II vs. IV RR = 0.071, $p = .0121$; Table 3). This prompted this subset analysis, whereby disease burden at the time of RT was found to significantly influence survival (PFS and OS) in dogs with

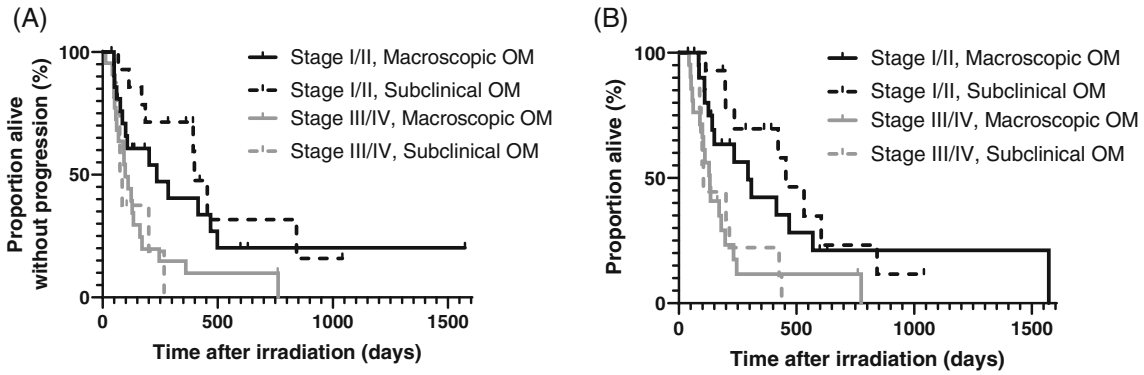

FIGURE 2 Kaplan–Meier curves depicting differences in oncologic outcome, as influenced by local tumour burden (subclinical vs. macroscopic) at the time of irradiation in dogs with either low (I/II) or high (III/IV) WHO stage disease OM. Dogs with WHO stage I or II disease and macroscopic tumour burden have shorter PF and OS as compared with dogs that have the same stage of disease but subclinical tumour burden at the time of RT (risk ratio [RR] for PFS: 0.449, $p = .032$; RR for OS: 0.422, $p = .022$). Tumour burden does not significantly impact either PF or OS times for dogs with stage III or IV disease. Tick marks represent the censored subjects

low WHO stage I and II disease (PFS 393 days, RR = 0.449, $p = .032$; OS 469 days RR = 0.422, $p = .022$; Table 4 **and** Figure 2).

4 | DISCUSSION

In this tri-institutional study of 101 dogs treated with 6 Gy × 6 for OM, the median OS time (232 days) was similar to earlier studies that reported survival times of 150–1020 days when treated with surgery, hypofractionated RT, or a combination thereof.[1–19] The OS is shorter than what has been reported in some of the more recent surgical literature, and this may be due to differences in how OS was calculated; in other words, regardless of whether RT was primary or postoperative, we calculated survival as a time that began with RT.[2–4] The new knowledge generated by this study is that in dogs with WHO stage I and II OM, the combination of surgery and RT is associated with longer PFS and OS as compared with RT alone. This is in contrast to dogs with higher stage disease (WHO stages III and IV); in that population of dogs, for whom there may be a (selection) bias with regard to surgical candidacy, we were unable to detect a difference in either PFS or OS for dogs treated with postoperative RT versus primary RT. One logical explanation for this difference could be that for dogs with early stage disease, prognosis for survival is influenced by quality of locoregional disease control, whereas dogs presenting for oncology consultation with advanced stage disease have a prognosis for survival that is more likely to be driven by factors related to both perceived quality of life, and metastasis.

One major driving force that compelled this research was a desire to know if twice-weekly irradiation leads to better disease control and longer survival than once-weekly irradiation. The univariate analysis performed herein suggested that in fact, the opposite might be true, but this observation did not hold up in multivariable modelling, and thus we conclude that on the basis of this research, at a population level, there is no evidence to support the idea that radiation dose intensity impacts survival in dogs undergoing 6 Gy × 6 either once or twice weekly for OM.

Failure to identify a difference in oncologic outcomes when comparing a weekly versus biweekly fractionation schedule could represent type II statistical error (i.e. a false negative); however, for several reasons, we do not believe that to be the case. First, sample size (N = 69 events) was relatively large, and few data points regarding survival (PFS or OS) were missing. Second, we believe that in this population of dogs, the likelihood for selection bias was low since the treatment schedule was based on prescriber preference rather than perceived risk for the individual patient (i.e. at two treatment centres, all irradiations were given once-weekly, and at the third treatment centre, all irradiations were given twice-weekly). Third, data were handled in such a way that allowed us to test the hypothesis in two refined groups of dogs, with either early (WHO stages I and II) or advanced (WHO stages III and IV) OM, and in neither population was there a detectable difference in outcome that was attributable to RT schedule. From a biological perspective, it appears that a biweekly protocol does not have a clinically meaningful effect on limiting accelerated repopulation, and this may reflect disease biology or a relatively high total biologically effective dose.[1,23] Regardless, based on current knowledge, there is no reason to believe that difference in radiation dose intensity affects outcome when considering weekly versus biweekly irradiation schedules for the 6 Gy × 6 fraction protocol.

In the univariable analysis, irradiation of gross disease was associated with short PFS and OS times. However, when disease was categorized as low stage (I and II) versus advanced stage (III and IV), the multivariable analyses, indicated that treatment in the gross disease setting lost its significance for advanced stage disease. These findings suggest that in the setting of low stage disease, whenever feasible, surgical cytoreduction should be pursued before commencing RT. In advanced stage disease, the benefit of surgery is less clear; 13 of the 23 dogs treated with gross disease at the start of RT ultimately died or were euthanized due to progressive local disease. It is also possible

that for an individual patient, palliative debulking procedures could be of benefit as gross bulky tumours can directly affect quality of life by causing problems such as pain, bleeding, and unwillingness to eat.

A potential shortcoming of this study is that dogs were included regardless of mitotic count. Previous publications have indicated that OM with a mitotic count <4 per 10 high magnification fields is associated with less aggressive behaviour.[14,19,26] However, some dogs with low mitotic counts still suffer from biologically aggressive disease. Assessing a tumour's proliferative activity yields improved prognostic value when other markers are included; unfortunately, in this study we were unable to collect meaningful data regarding nuclear atypia, Ki67 or AgNORs, because such data were frequently unavailable in the medical records, and we did not have routine access to the paraffin tissue blocks that could have otherwise facilitated additional pathologic interrogation.[14,27] Thus, in the absence of a more comprehensive assessment of proliferative activity/capacity, we opted to include the few cases with low mitotic activity. Looking back, that decision seems reasonable given that in the univariable analysis, there was no statistical association between mitotic count and survival for the dogs included in this study.

Previous reports have also indicated that the presence of bony lysis at the primary tumour site is a negative prognosticator for OM; no such association was identified in the study population reported herein.[1–2,6–7] Here, only a small number of dogs had evidence of bony lysis; however, this number may be artificially low since 23 dogs did not undergo any form of oral cavity imaging, and since imaging or imaging reports were not available for review in all cases.

This study was performed as a multi-institutional, retrospective data analysis. There were several challenges. First, due to unintentional data loss at one of the treatment centres, RT plan information was incomplete for 44 of 101 cases. Second, some variables (e.g. measurement of gross tumour volume) were subjective — measurement methods were not standardized. Third, the clinical approach to tumour staging, follow-up, and delivery of adjunctive therapies was not standardized. Quality of data related to treatment-induced toxicities was limited due in part to the retrospective nature of this study, and that makes it difficult to offer firm conclusions regarding the tolerability of 6 Gy × 6 RT when delivered once or twice-weekly. Finally, the recruitment period was long, allowing for evolution of both the surgical and RT treatment techniques used. These issues could be addressed through further prospective research to verify and build upon the new knowledge generated in this study. External validation of our findings could also be pursued by re-testing the hypotheses in an entirely different set of patients with the same disease and treatment approach.

Despite these limitations, this study provides evidence that 6 Gy × 6 RT for canine OM is generally well-tolerated and can facilitate prolonged survival. Certainly, pet owners willing to pursue RT seem likely to have an increased tolerance for tumour-associated clinical signs (halitosis, bleeding, and so on). However, when left untreated, the median OS time for OM is approximately 60 days, and in comparison, when dogs in this study were treated with gross or advanced stage disease, the median OS was (subjectively) substantially longer (171 and 132 days, respectively).[28] Outcomes were even better when dogs were irradiated postoperatively, or in the setting of low stage disease; in these cases, the median OS times were 437 and 469 days, respectively. In this population of dogs, we found no evidence indicating that long-term prognosis might be improved by switching from a once- to twice-weekly protocol. Results presented herein should facilitate evidence-based decision-making for treatment of OM. But it is notable and unsurprising that a major challenge in this disease remains its high propensity for metastasizing; in this study, 23% of dogs ultimately succumbed to distant metastasis. The veterinary oncology community currently lacks drugs and immunotherapeutics with proven anti-metastatic benefit. Similarly, it remains unclear whether locoregional tumour control achieved via surgery and/or RT helps to prevent or delay distant disease dissemination.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

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

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