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Combining radiation therapy with zoledronate for the treatment of osteo-invasive feline oral squamous cell carcinoma

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Abstract

Feline oral squamous cell carcinoma (FOSCC) is the most common oral tumour diagnosed in pet cats and carries a poor prognosis with <10% one-year survival despite multi-modal therapies. Tumours of the mandible or maxilla are frequently osteo-invasive and pain can result from osteolysis. Zoledronate is a bisphosphonate that inhibits osteoclasts and reduces bone resorption. Radiation therapy (RT) is used to treat FOSCC due to anti-cancer activity and ability to improve quality of life. We hypothesized RT can be safely combined with zoledronate, and that this combinatory therapy would be efficacious, well tolerated, and result in decreased bone resorption in cats with FOSCC. SCCF1 cell line was treated with zoledronate before, concurrently, or after RT, and clonogenic assays were performed to determine if an optimal dosing schedule would be identified. Nine cats with osteoinvasive FOSCC were recruited for treatment with 4 weekly doses of 8 Gy RT combined with zoledronate administered at the first and fourth treatments. Serial CT scans were performed to assess tumour response. Safety and tolerability were monitored with hematologic and biochemical parameters, and acute radiation effects were characterized. Serum c-telopeptide (CTx) and relative bone mineral density (rBMD) by dual-energy X-ray absorptiometry (DEXA) quantified bone resorption. In vitro studies showed no clear benefit to timing of zoledronate with RT, therefore all zoledronate was administered concurrently with RT in FOSCC patients. Based on tumour volume, 4/9 (44.4%) cats achieved partial remission, 4/9 (44.4%) stable disease and 1/9 (11.1%) had progressive disease. The combinatory therapy was well-tolerated based on biochemical measurements, and all patients experienced decreased serum CTx. Combining RT with zoledronate in tumour-bearing cats is safe, well-tolerated, results in a partial remission rate of up to 44%, and decreases serum CTx, a marker of bone resorption.

KEYWORDS
bisphosphonate, neoplasia, pain relief, palliative, serum c-telopeptide

1 | INTRODUCTION

Oral tumours are commonly diagnosed in the domestic cat, comprising about 3–12% of all tumours diagnosed in this species. Of the different tumour types that can affect the oral cavity of cats, 60–75% is feline oral squamous cell carcinoma (FOSCC).[1–3] FOSCC has a low metastatic rate[4] but local invasion is the most common cause of death or euthanasia in cats, resulting in a one-year survival rate of less than 10%.[5,6] Mortality associated with this tumour is largely due to progressive local disease despite treatment, resulting in invasion of surrounding tissue and severe pain causing difficulty eating, drinking, and grooming. Cats are usually euthanized due to poor quality of life.

Treatment options include combinations of surgery, radiation therapy, chemotherapy and pain medications with very little improvement in survival times; multimodal therapy typically provides improved response to treatment when compared to any single modality.[1,6–10] Surgery is rarely an option due to the size and location of the tumour relative to the size and shape of a cat's head. FOSCC is associated with a poor prognosis, and treatment is primarily focused on palliative care. Management of feline bone pain due to cancer is challenging and includes surgical removal when possible, radiation therapy and oral analgesics. As noted, surgery is infrequently attempted due to the extent of invasion, though marginal removal may be performed to decrease the burden of disease. Administration of oral analgesics can be difficult in a patient with an oral tumour, and cats can be difficult to medicate orally because of their small size and resistance to restraint. Additionally, options for long-term oral analgesia are limited in cats due to species-specific toxicity with NSAIDs.

Bisphosphonates inhibit osteoclasts and can be given intravenously and infrequently, usually once monthly. This group of drugs can decrease cancer-related bone pain that is due to osteolysis in people and dogs, with less information available for cats.[11–14] Zoledronate reduces bone loss in mouse models using FOSCC cell lines, and this can be enhanced when combined with meloxicam.[15,16] Additionally, zoledronate decreases vascular endothelial growth factor (VEGF) production in FOSCC in vitro and may have radiosensitization effects in various other tumour histologies.[17–20] Radiation therapy, particularly hypo-fractionated and accelerated protocols, is an important treatment option for this cancer because it can slow tumour progression, improves quality of life, and is non-invasive thus overcoming limitations of surgery.[21–25]

The aim of this study was to assess a combined radiation and zoledronate protocol for the treatment of osteoinvasive FOSCC. Specific objectives were to determine optimal dosing, efficacy, toxicity and reduction of osteolysis.

2 | MATERIALS AND METHODS

2.1 | Cell lines and reagents

A feline oral squamous cell carcinoma cell line SCCF1 (provided by Dr. Thomas J. Rosol, Ohio State University) was utilized. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (100 IU/mL each). Cells were maintained at 37°C in 5% CO_2. Zoledronic phosphonic acid monohydrate (1 mg/mL) were obtained from Novartis Pharmaceuticals Ltd.

2.2 | Cell line validation statement

Cell line validation was not performed in this study.

2.3 | Clonogenic cell survival assay

SCCF1 cells were plated at increasing densities (400, 1000, 3000, 5000, 10 000 and 15 000 cells/well) into six well plates depending on RT dose (0, 2, 4, 6, 8 and 10 Gy), and allowed to adhere no more than 15 h. Cells were treated with either radiation, zoledronate (0, 3 and 10 μM)[17] for a 2-h incubation, or a combination of the two. Radiation was delivered using a cobalt-60 machine, which has a Dmax of 5 mm and so approximately 5 mm of media was present at the time of irradiation to allow build up to the monolayer of cells at the bottom of the cell culture dishes. For combined treatments, zoledronate was administered either 2 h prior to RT, immediately preceding RT, or 2 h post-RT to determine the ideal timing of zoledronate administration. Media was replaced, and cells incubated for 10 days. Cell colonies were fixed using crystal violet and counted. Surviving fraction (SF) was calculated as follows: SF = colonies counted/cells plated, normalized SF = treated SF/control SF (plating efficiency). Surviving fraction curves were generated in logscale with commercially available software Prism 9 (GraphPad Software Inc). The experiment was performed in triplicate and values are reported as mean ± SD.

2.4 | Naturally occurring osteo-invasive feline oral squamous cell carcinoma study population

Nine patients were prospectively evaluated and enrolled into the study. All patients had a diagnosis of OSCC confirmed by cytology or histology. Pet owners were informed of all available treatment options, and study patients were treated according to the animal care guidelines of the University of Illinois Institutional Animal Care and Use Committee (IACUC # 18018). All cats that met eligibility had baseline complete blood count (CBC), serum biochemical profile and urinalysis performed. Patients underwent computed tomography (CT) scan of the head to obtain pretreatment tumour measurements and to confirm bone invasion by the tumour, and either had thoracic CT scan or thoracic radiographs for staging. Mandibular lymph node aspirates were obtained for complete staging when possible; patients were excluded if there was evidence of nodal or thoracic metastasis at the time of treatment. Patients received a hypo-fractionated RT protocol of 8 Gy once weekly for 4 weeks utilizing a Cobalt-60 radiation machine (Theratron 780). Though treatment angles were guided by CT scan images, all treatment protocols were calculated manually based on field size and depth of treated area of the tumour. No beam shaping was used. Given the relatively shallow (5 mm)

Dmax of cobalt-60, no bolus was used because the overlying skin provided build up. When needed, 5 mm of wet gauze was used for buildup (for example when a portion of the tumour was exposed without overlying skin). Zoledronate was diluted in saline and administered on days 0 and 22 at 0.2 mg/kg IV over 15 minutes based on the previously published data.[17] Supportive therapy included antibiotics, non-steroidal anti-inflammatories (NSAIDs) and other medications as indicated. On day 50, 28 days following the last radiation treatment and administration of zoledronate, a CBC, biochemistry profile and urinalysis were performed to assess for treatment-related toxicity and a CT scan of the head was performed to obtain post-treatment tumour measurements. Pre- and post-contrast CT scans were performed either under sedation, or under general anaesthesia. After the trial was complete to quantify tumour volume, CT images were transferred to radiation therapy planning software (Eclipse v15, Varian, Palo Alto, CA, USA) and the gross tumour volume was identified to provide the longest dimension as well as the tumour volume. Tumour response was determined using change in the longest dimension for Response Evaluation Criteria in Solid Tumours (RECIST) criteria, as well as World Health Organization (WHO) volumetric response.[26,27] Because oral tumours can be irregularly shaped, there was concern RECIST may not capture the response accurately; therefore we chose to look at both WHO and RECIST criteria for tumour response. Responses were classified as follows: complete response (disappearance of all measurable disease by either method), partial response (at least 30% decrease in the longest dimension by RECIST or 50% decrease in volume by WHO), stable disease (less than 30% decrease or 20% increase by RECIST, or less than 50% decrease or 25% increase by WHO), or progressive disease (more than 20% increase by RECIST or 25% increase by WHO). All images and tumour contours were reviewed by a board-certified veterinary radiologist (AB). Acute (early) radiation side effects were scored at each RT treatment using VRTOG scoring;[28] chronic (late) radiation side effects typically will not occur until at least 6 months post irradiation and were not recorded in this study.

2.5 | Determination of serum CTx concentrations

Venous blood samples were collected for assessment of serum CTx concentrations from patients prior to administration of zoledronate and/or RT on days 1, 8, 15, 22 and 50. Whole blood was centrifuged at 1500 rpm × 10 min and serum was separated and stored at −80°C until analysis. Serum CTx concentrations were measured with a commercially available assay validated in cats (Serum Crosslaps, Immunodiagnostic Systems).[29]

2.6 | Determination of tumour relative bone mineral density

In conjunction with the CT scans (Days 0 and 50), bone mineral density (BMD) of the skull was measured using dual-energy X-ray absorptiometry (DEXA) (QDR-4500 W, Hologic, Bedford, MA) following the CT scan. Scans were performed to measure BMD of the tumour and an equivalent area of normal bone on the contralateral side of the skull. Relative bone mineral density (rBMD) was calculated using the formula: rBMD = BMD tumour/BMD equivalent normal bone.

2.7 | Statistical analysis

Comparison of zoledronate treatment timing on SCCF1 cells at 8 Gy radiation was evaluated with an analysis of variance comparing each time point to baseline (no zoledronate) and to one another. Change in serum CTx concentrations from baseline were evaluated with either a paired t-test (baseline versus day 8 in individual patients) or with an analysis of variance (comparing multiple points to baseline) with post-hoc comparison made with Dunnett's multiple comparisons test in grouped patients. To assess biochemical tolerability of multiple zoledronate treatments in the study patients, serum measurements of creatinine, blood urea nitrogen (BUN), calcium and phosphorus, were evaluated with analysis of variance, and post-hoc comparisons were made with Dunnett's multiple comparisons test. For assessment of biologic effects of repeated zoledronate on rBMD, a paired t-test in individual patients was performed. Statistical analysis was performed with the commercially available software (Prism 9, GraphPad Software Inc). Significance was defined as $P < 0.05$. Prior to analysis, samples were tested for normality using the Kolmogorov–Smirnov test.

3 | RESULTS

3.1 | Effects of combinatory zoledronate and radiation therapy on OSCC cell lines

To determine if there was an ideal dosing strategy of zoledronate in relation to timing of radiation to guide treatment in our patient population, we looked at whether the effect of radiation therapy could be enhanced with the addition of zoledronate (Figure 1A,B by a clonogenic survival assay. We chose the in vitro concentrations based on the previously published work from our group.[17] Because the circulating half-life of bisphosphonates is very short (<2 h) and up to 70% of bisphosphonates administered accumulate in the bone where it can reside for years,[30,31] blood levels will not necessarily correspond to pharmacodynamic effects. When SCCF1 was exposed to increasing doses of radiation and variable timing and dosing of zoledronate, there was a benefit to the addition of zoledronate when compared to no zoledronate. When looking at the cells treated at 8 Gy (how the feline patients were treated), there was a statistical difference ($p < .05$) when comparing zoledronate-treated groups to the no zoledronate-treated group. However, there was no difference based on timing of zoledronate administration relative to RT in the 3 or 10 μM group. Therefore, we treated the patient population by administering zoledronate concurrently (within 30 min prior to) with RT for convenience.
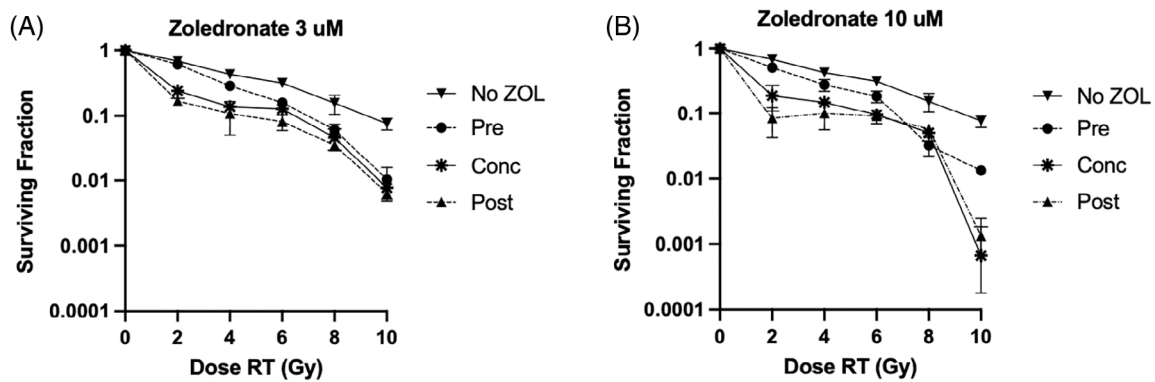
FIGURE 1 Surviving fraction of SCCF1 with 3 μM zoledronate (A) and 10 μM zoledronate (B) with increasing dosages of radiation therapy (2–10 Gy). Zoledronate was either omitted (no ZOL) or administered 2-h pre-RT (Pre), immediately preceding RT (conc), or 2-hours post-RT (post). Values plotted as mean with standard deviation

TABLE 1 Summary of tumour location, tumour response to therapy (reported in % change in the longest dimension and in tumour volume), survival time and additional anti-cancer therapies following completion of the trial

Patient	Tumour location	Change in the longest dimension (%)	RECIST response	Change in tumour volume (%)	WHO response	Survival time (days)	Additional therapies following trial?
1	L Mandible	0	SD	−18	SD	119	Yes[a]
2	L Maxilla	−13	SD	−56	PR	71	No
3	L Maxilla	−42	PR	−88	PR	160	Yes[a]
4	L Maxilla	+3	SD	N/A	SD	41	Yes[a]
5	L Maxilla	−17	SD	−62	PR	136	Yes[a]
6	R Mandible	+4	SD	+27	PD	200	Yes[a,b]
7	L Mandible	−20	SD	−7	SD	60	Yes[a,c]
8	R Maxilla	+2	SD	−3	SD	183	Yes[a]
9	L Maxilla	−74	PR	−98	PR	190	Yes[a,b,c,d]

[a]Zoledronate.
[b]Palladia (toceranib phosphate).
[c]Mitoxantrone.
[d]Cyclophosphamide.

3.2 | Anti-tumour effects of combinatory zoledronate and radiation therapy in pet cats with OSCC

The patient population consisted of four castrated males and five spayed females. Breeds represented were domestic shorthair (7), domestic longhair (1) and Bengal (1). The patient population age and weight (mean, range) were 10.36 (3.5–16) years and 4.18 (2.6–7.3) kg, respectively. Supportive care included oral and/or ocular antibiotics ($n = 8$), artificial tears ($n = 1$), ocular serum ($n = 1$), NSAIDs ($n = 2$), steroids ($n = 2$), narcotics ($n = 6$), gabapentin ($n = 3$), anti-nausea medications ($n = 1$), appetite stimulants ($n = 1$) and one patient was treated for hyperthyroidism with methimazole. No patients required the support of feeding tubes throughout the trial. The mean weight of patients at the start of the trial was 4.18 kg (ranges 2.6–7.3), and at the end of the trial was 3.93 kg (ranges 2.3–6.6 kg). The body condition score (BCS) was assessed at each visit using the nine-point scale;

at the start of the trial the mean BCS was 4.44 (ranges 3–6) and at the completion of the trial the mean BCS was 4.33 (ranges 3–5). Eight patients finished the prescribed radiation protocol; one patient (patient number 2 in Table 1) was removed from the trial at day 22 due to heart failure; however, a CT and DEXA scan were performed at the time of study exit. Tumour response following the three radiation treatments and one zoledronate for this patient was included in the analysis, as well as serum CTx levels at days 0 and 8, and the survival time of this patient. There were a few discrepancies between RECIST and WHO methods of determining treatment response. Using RECIST criteria, 78% (7/9) patients achieved stable disease (SD) and 22% (2/9) achieved a partial remission (PR) for an overall biologic response of 100%. Using tumour volume, 44.4% (4/9) had a PR and 44.4% (4/9) had SD, with PD in one cat (27% increases). One of the four cats with stable disease had images that could not be imported into the planning software but was considered to have stable disease as the dimensions in three planes on imaging
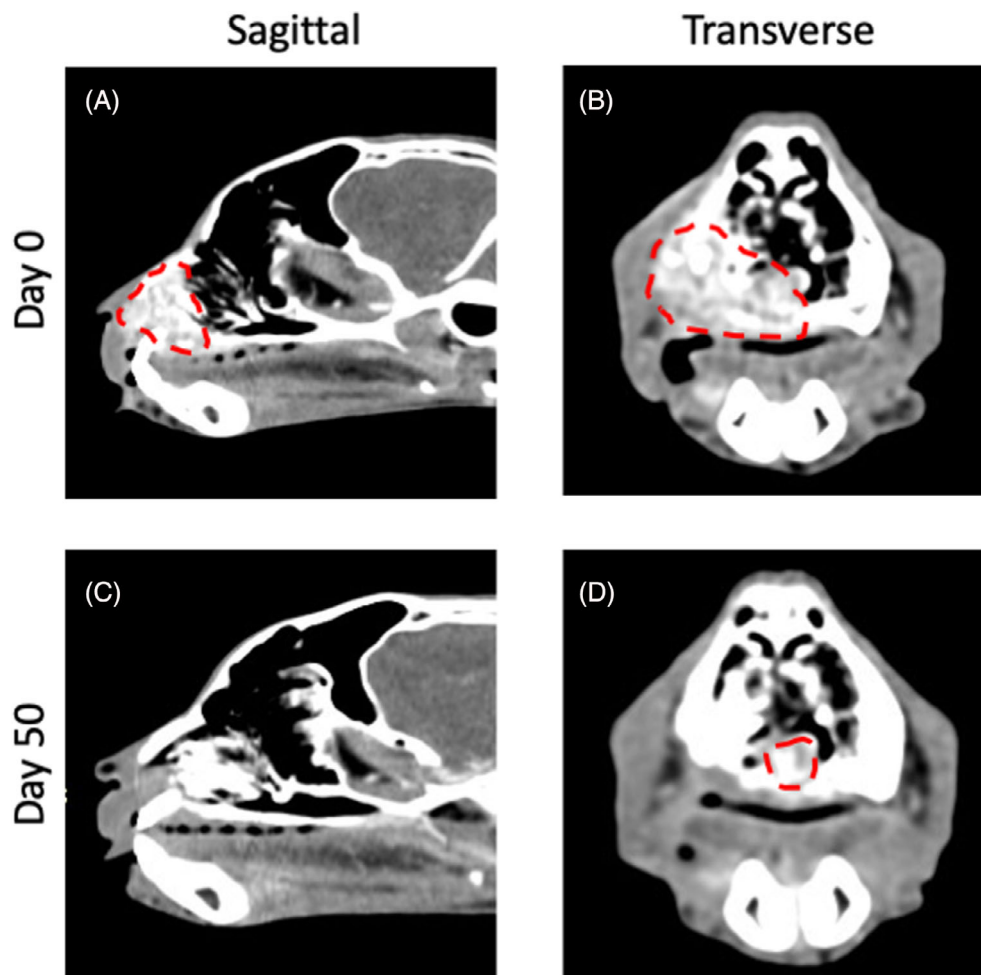
FIGURE 2 CT images of patient #9 response to therapy. Tumour is outlined in red dashed lines on sagittal (A) and transverse (B) images on Day 0 prior to starting treatment. On Day 50 following four weekly treatments of RT with two doses of zoledronate, the tumour is outlined in red dashed lines in the sagittal (C) and transverse (D) views. Tumour response for this patient using RECIST criteria is 74% reduction (partial remission) and 98% reduction (partial remission) using the WHO criteria

TABLE 2 Biochemical tolerability in OSCC cats treated with zoledronate

| Parameter	Day 0 Mean ± SD	Day 22 Mean ± SD (p-value)	Day 50 Mean ± SD (p-value)
Creatinine (0.4–1.6 mg/dL)	1.49 ± 0.68	1.16 ± 0.49 (0.454)	1.36 ± 0.45 (0.831)
BUN (18–38 mg/dL)	25.56 ± 12.45	20.88 ± 5.99 (0.393)	23.38 ± 5.24 (0.857)
Calcium (8.8–10.2 mg/dL)	9.41 ± 0.42	8.18 ± 1.36 *(0.029)	9.55 ± 1.01 (0.95)
Phosphorus (3.2–5.3 mg/dL)	4.38 ± 0.53	3.49 ± 0.81 *(0.015)	3.91 ± 0.62 (0.224)

Note: Assessment of biochemical toxicosis following two doses of zoledronate combined with four weekly RT treatments. Normal reference intervals included for parameters evaluated. Values from Day 22 and Day 50 are compared to baseline (Day 0). Values are reported as mean ± SD and significance is defined as *$P < 0.05$.

software (Vue PACS, Carestream Health, Rochester, NY) were not different comparing pre- to post-treatment. Patient information, tumours responses and survival times are summarized in Table 1. Most patients received one or more of the following additional anti-cancer therapies at completion of the trial: zoledronate ($n = 8$), toceranib phosphate ($n = 2$), mitoxantrone ($n = 2$) and cyclophosphamide ($n = 1$). The median survival time was 136 days (ranges 41–200 days). The most profound response was a 74% decrease in tumour size in one patient. CT images of this patient are shown in Figure 2.

3.3 | Safety and tolerability of combinatory zoledronate and radiation therapy in pet cats with OSCC

Biochemical toxicity from combining radiation therapy with two doses of IV zoledronate at 0.2 mg/kg IV was evaluated in the nine patients enrolled. Due to potential for renal toxicity and calcium dysregulation, serum creatinine, BUN, calcium and phosphorus were evaluated. There were no significant changes to renal values (creatinine and BUN) at days 22 and 50 when compared to baseline. Calcium and
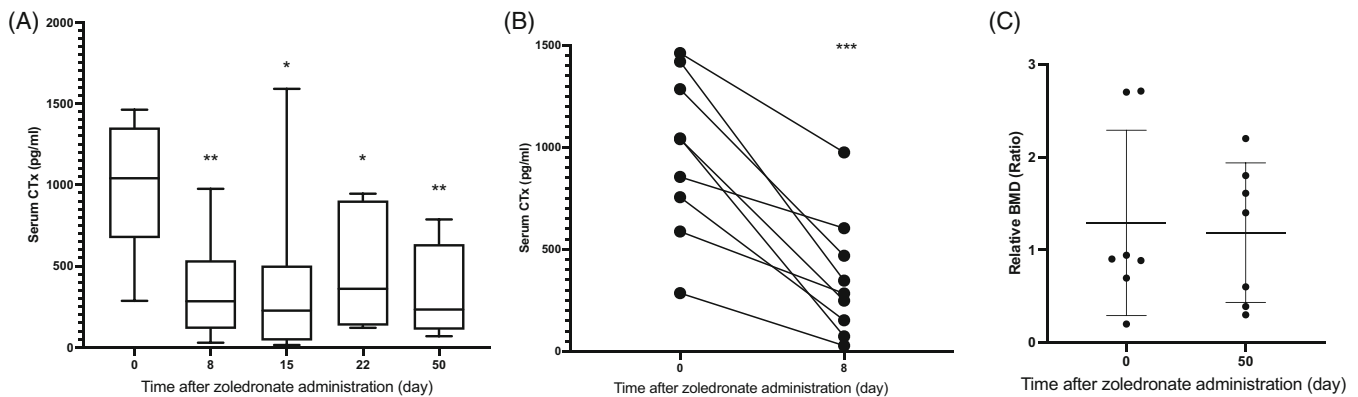
FIGURE 3   Changes in serum c-telopeptide (CTx) concentrations and relative bone mineral density (rBMD) in patients with FOSCC (n = 9). Grouped (A) or individualized (B) serum CTx concentrations after zoledronate administrations; zoledronate was administered on days 1, 22 and 50. rBMD after two zoledronate administrations (C), individual values shown with bars for mean and standard deviation. Significant decreases from baseline are indicated by significance is defined as *$P < 0.05$, **$P < 0.01$ and **$P < 0.001$

phosphorus both were significantly decreased at day 22 when compared to baseline, however this was transient and there was no statistical difference at day 50 when compared to baseline (Table 2). These transient changes to calcium and phosphorus were not appreciated clinically. Radiation toxicosis was also assessed at each visit using VRTOG criteria. Acute radiation effects were mild and all Grade 1 according to the VRTOG scoring. Cutaneous effects were the most common occurring in seven patients. Ocular side effects were noted in three patients, and oral mucosal side effects in three patients.

3.4 | Effects of repeated zoledronate on serum CTx and relative bone mineral density

A decrease in serum CTx from baseline was significant ($P < 0.05$) at all-time points when compared to baseline as shown in Figure 3A. Concentrations dropped at day 8, and remained stable at days 15, 22 and 50. When comparing serum CTx between baseline and day 8, all patients experienced a decrease in CTx levels, and this was highly significant (p < 0.001) as shown in Figure 3B. There was no significant change in rBMD in patients at Day 50 when compared to baseline (Figure 3C).

4 | DISCUSSION

The results of this study support treatment with combined radiation therapy and zoledronate in cats diagnosed with FOSCC that have an osteo-invasive component. This was an exploratory clinical trial with one treatment arm designed to test feasibility. Bone involvement of these tumours is driven by several osteolytic factors that contribute to cancer growth and bone loss[15] characterized by enhanced osteoclastic activity. Bisphosphonates are a class of drugs that inhibit osteoclastic activity, thereby decreasing

osteolysis and associated pain. In people, these are used for conditions such as osteoporosis, hypercalcemia, and primary and metastatic bone tumours.[14,32,33] In veterinary medicine, several different bisphosphonates have been identified for use in treating various conditions in dogs and cats. Zoledronate and pamidronate are aminobisphosphonates that inhibit the mevalonate pathway in osteoclasts, and have been used to treat osteolysis associated with canine and feline primary and metastatic bone lesions.[11,34] Zoledronate is more potent and is administered with a shorter duration of infusion compared to pamidronate (15 min vs. 2 h, respectively). Measuring serum CTx is the standard method of assessing the effects of anti-resorptive agents in people, and specifically measures the degradation product of the carboxyterminal telopeptide region of collagen type I.[35,36] Following a single dose of zoledronate in people treated for non-cancer diseases, levels of serum CTx remained below baseline for an average of 18 months.[37] In patients with tumour-induced osteolysis, the dosing is repeated more frequently due to continual osteoclastic activity. The cats in our study experienced the expected drop in serum CTx with an initial, and significant, decrease at one-week post administration. This response remained unchanged during the month until the second dose. This is a similar finding to reported responses in dogs receiving bisphosphonate therapy for primary appendicular bone tumours.[11]

Side effects of this protocol were mild or clinically irrelevant, and all cats experienced some clinical benefit, with some tumours regressing. Though the trial endpoint was only 50 days after starting treatment, the median survival time for cats with FOSCC is approximately three months with most reported therapies.[38] Though not a primary objective of our study, the median survival time of 136 days (4.5 months) in this study is comparable to other reports. Since no patient achieved a CR, additional chemotherapy treatments were offered to all patients at the end of the trial and three patients received additional therapies. Our study population was small, and with one third of cats receiving additional therapies, it is feasible that

the median survival time may be impacted by this. However, the achievement of stable disease or better for the 2 months' duration of the trial is clinically relevant to the behaviour of the disease when compared to a median survival time of approximately 3 months across all reported therapies.

Our population of patients experienced a statistically significant decrease in total calcium and phosphorus at day 22, though all patients were asymptomatic and at day 50 there was no statistical change from baseline. In cancer patients receiving bisphosphonate therapy, hypocalcemia following zoledronate therapy for metastatic bone lesions is common and is often mild and asymptomatic;[39,40] those who do experience symptomatic hypocalcemia following zoledronate infusion typically have risk factors predisposing them to this including vitamin D deficiency, renal insufficiency and hypomagnesemia.[41,42] Bisphosphonate-induced hypocalcemia is infrequently documented in the veterinary literature, but has been reported to occur when using bisphosphonate therapy for the treatment of hypercalcemia.[43]

In studies evaluating people receiving anti-resorptive agents, gradual changes in rBMD often correlate with serum CTx concentrations.[35] In our study population, we did not appreciate a change in rBMD in patients treated with zoledronate therapy over 50 days. Reassessment at extended time points might show more noticeable change and is warranted in future studies. In one study of dogs treated with bisphosphonate therapy for malignant osteolysis, there was no statistical increase in rBMD at day 56 following treatment, but there was at days 84 and 112.[11] It is likely that using rBMD by DEXA scan is not optimal for assessing response to therapy for patients whose expected survival is short as we see with FOSCC patients. Furthermore, measuring a skull tumour where normal anatomical structures have a significant amount of overlay makes this a challenging diagnostic method. Primary bone tumours of dogs that underwent DEXA were all appendicular in origin, making assessment of rBMD more straightforward.[11]

At completion of our study, 6/8 (75%) patients experienced mild acute radiation toxicity. All side effects were confined to the skin, mucous membranes and eyes, as expected, and all were a grade 1 toxicity using the VRTOG scale.[28] This is similar to other reports of patients treated with palliative or hypo-fractionated protocols.[21,44] This study was not designed to compare the combination of zoledronate and RT to either treatment alone. We cannot confirm the degree of decrease in osteolysis or the response to therapy that was due to zoledronate versus RT. It is possible that either treatment, when used alone, could yield similar results; survival times were similar to those reported in the literature. The results reported here provide a basis for future studies. Our primary endpoints were tumour response, rBMD and serum CTx at day 50 as well as reporting any toxicity.

Limitations to this study include the size of our patient population and the lack of control groups including those treating with radiation alone, and zoledronate alone. The intentions of this study were to primarily determine the ability to combine radiation with zoledronate, and to assess for toxicities that may arise from the combination of the two. The tolerability of bisphosphonates alone

has been established,[17,34] and several studies have evaluated radiation therapy as a single agent;[10,25,44,45] however the combination of the two in a population of older, tumour-bearing cats had not been evaluated. This small study can be used as a basis for larger studies with control groups. Many of our patients were treated with additional therapies, including the use of NSAIDs which may have anti-cancer effects. Withholding these medications would have been unacceptable given the pain associated with the osteoinvasive nature of these tumours. In addition to concurrent medications during therapy, nine patients received a third dose of zoledronate on day 50 of the trial, and three received additional anti-cancer therapies that may affect the overall survival time of our patient population.

5 | CONCLUSIONS

When zoledronate is administered concurrently with hypo-fractionated radiation in tumour-bearing cats, it is safe and well-tolerated with no clinically relevant biochemical toxicosis. The combination of therapy resulted in 89–100% overall biologic response, with 22–44% of patients achieving a partial remission, depending on the method of measuring, and assigning response. Serum CTx decreased in all patients, though relative bone mineral density did not change in the time period of the study. Zoledronate therapy combined with radiation therapy should be considered for use in feline patients with osteo-invasive oral squamous cell carcinoma.

CONFLICT OF INTEREST
The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

DATA AVAILABILITY STATEMENT
Data is not available.

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