Received: 20 January 2017 | Revised: 9 March 2017 | Accepted: 6 April 2017

DOI: 10.1111/vco.12319

WILEY Veterinary and Comparative Oncology

ORIGINAL ARTICLE

Intratumoral injection of radioactive holmium (^{166}Ho) microspheres for treatment of oral squamous cell carcinoma in cats

S. A. van Nimwegen[1] | R. C. Bakker[2,3] | J. Kirpensteijn[1] | R. J. J. van Es[4] | R. Koole[3] | M. G. E. H. Lam[2] | J. W. Hesselink[1] | J. F. W. Nijsen[2]

[1]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[2]Department of Radiology and Nuclear medicine, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Oral and Maxillofacial Surgery, University Medical Center Utrecht, Utrecht, The Netherlands

[4]Department of Head and Neck Surgical Oncology, UMC Utrecht Cancer Center, Utrecht, The Netherlands

Correspondence
S. A. van Nimwegen, DVM, PhD, Diplomate ECVS, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, PO Box 80154, 3508 TD Utrecht, The Netherlands.
Email: S.A.vanNimwegen@uu.nl

Funding Information
Dutch Cancer Society research, Grant/Award number: UU 2014 7075.

Background & Aims: A "microbrachytherapy" was developed as treatment option for inoperable tumours by direct intratumoral injection of radioactive holmium-166 (^{166}Ho) microspheres (MS). ^{166}Ho emits β-radiation which potentially enables a high, ablative, radioactive-absorbed dose on the tumour tissue while sparing surrounding tissues.

Materials & Methods: Safety and efficacy of ^{166}Ho microbrachytherapy were evaluated in a prospective cohort study of 13 cats with inoperable oral squamous cell carcinoma without evidence of distant metastasis.

Results: Local response rate was 55%, including complete response or partial response (downstaging) enabling subsequent marginal resection. Median survival time was 113 days overall, and 296 days for patients with local response. Side effects were minimal. Tumour volume was a significant predictor of response.

Discussion: Response rate may be further improved by optimizing the intratumoral spatial distribution of ^{166}Ho MS.

Conclusion: ^{166}Ho microbrachytherapy has potential as a minimally invasive, single procedure radio-ablation treatment of unresectable tumours with minimal morbidity.

KEYWORDS

head and neck, brachytherapy, holmium, tongue, feline, translational model, radionuclide, tumour

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy in cats and humans.[1,2] In humans, OSCC accounts for approximately 200 000 (2.7%) of all new cancer cases and 100 000 deaths worldwide annually.[3] The incidence of OSCC has increased with 1.8% per year between 1989 and 2011.[4] This is probably because of the increased prevalence of tobacco abuse, alcohol consumption and slaked lime or betel nut chewing, which are the major risk factors for developing OSCC in humans.[4,5] The aetiology of OSCC in felines remains largely unknown. Advanced age, indirect tobacco smoke, poor oral hygiene and papillomavirus exposure are possible risk factors.[5–7] Apart from the resemblance of risk factors, human and feline OSCC also share comparable molecular parameters (eg, overexpression of epidermal growth factor receptor, Vascular Endothelial Growth Factor and p53 mutation). Furthermore, OSCC has comparable biological and histological characteristics (eg, tumour growth and metastasis), as well as their response to therapy.[8] OSCC, is commonly located in the gingiva with invasion of underlying bone. In cats and humans, OSCC is also frequently located in the tongue; ventral in cats and lateral in humans.[9]

Feline OSCC (FOSCC) is, therefore, an optimal and most relevant translational animal model to study various new treatment modalities before their application in humans. The survival of both veterinary and human patients with OSCC mainly depends on the stage of the disease

at time of diagnosis. In humans, stage I and II carcinomas, still confined to the primary site, are treated by surgical resection and adjuvant radiotherapy on indication, with a 5-year survival rate of 80%-90%. However, only 30%-50% of the patients present with this stage. For advanced (stage III-IV) carcinomas, the 5-year survival rate drops to approximately 45%.[10] Despite medical advancements, only limited improvements in survival have been achieved for humans with OSCC over the last decades and curative-intent surgery with ≥1 cm margins remains the most effective therapy for both humans and animals with OSCC. However, morbidity of surgical treatment is high for stage II or higher disease, urging the need for new treatment modalities for OSCC.

Treatment-related loss of tongue functions severely decreases the quality of life in both humans (speech, swallowing) and felines (feeding and grooming). In cats, the diagnosis of OSCC is commonly made in an advanced stage of the disease. Consequently, surgery is considered impossible or unethical in most cases. Surgery is only considered in a very select group of cats with small, localized tumours. Effectiveness of palliative radiation protocols (consisting of 3-6 sessions of 6-10 Gy) and stereotactic radiation is low in felines with response rates of 39% or less and median overall survival (OS) times of 60 to 106 days.[11–13] More recently, several case series of accelerated hypofractionated radiotherapy protocols with or without chemotherapy and/or surgery suggest improved survival outcomes of 86 to 163 days.[14–17] However, these are intensive treatment protocols that require multiple anaesthetic interventions and are accompanied by considerable costs and therapy-induced morbidity, with 91% overall complication rate and up to 78% grade 3 oral mucositis.[12,14] Chemotherapy, tyrosine kinase inhibitors and immunotherapy alone have not achieved durable responses in macroscopic FOSCC.[18,19]

Holmium-166 (^{166}Ho) loaded microspheres (MS) are radioactive microspheres with a diameter of 10 to 30 μm that emit β-radiation ($E_{\beta,max}$ = 1.84 MeV, $t_{1/2}$ = 26.8 hours) which has a mean tissue penetration depth of 3.2 mm and a maximum tissue penetration of 8.7 mm. The combination of a localized intratumoral microsphere application with the short β-penetration depth enables a high, tumour-ablative radiation-absorbed dose in a single treatment, without causing extensive collateral damage to surrounding tissues. Furthermore, ^{166}Ho also emits a small fraction of γ radiation (6.71%, 80.6 keV) and can be visualized using nuclear Single-photon emission computed tomography (SPECT). In addition, ^{166}Ho can also be observed using Computed Tomography (CT) because of its high density, and with magnetic resonance imaging (MRI) because of its unique paramagnetic properties.[20–23] These qualities provide an excellent opportunity for in vivo multimodality imaging, enabling treatment guidance and monitoring.

In human patients, ^{166}Ho MS are currently used in an intra-arterial transcatheter radio-embolization treatment of metastatic liver cancer.[22] Radio-embolization of small head and neck tumours is technically more demanding with the risk of unintended retrograde flow of particles into the carotid artery and into the brain that may result in serious complications including hemiparesis or even death.[24,25] To overcome these obstacles, a direct intratumoral injection approach has been proposed for FOSCC. This concept of intratumoral injection of ^{166}Ho MS, also referred to as ^{166}Ho microbrachytherapy, has been investigated in laboratory animals and veterinary patients with spontaneous cancers. In these in vivo studies, renal cell carcinoma-bearing mice, Vx2 tumour-bearing rabbits and feline patients with spontaneous liver tumours were treated with ^{166}Ho microbrachytherapy. In these studies, it was shown that extremely high tumour-absorbed doses could be attained (in excess of 6 times the absorbed dose that can be achieved with external beam radiotherapy) without major associated side effects.[26] ^{166}Ho microbrachytherapy can therefore potentially be a safe and effective treatment option for both human and veterinary patients with head and neck cancer for whom no other treatment options are available.[26–28] The objective of this study was to prospectively evaluate safety and efficacy of ^{166}Ho microbrachytherapy in cats with spontaneous unresectable FOSCC. Therefore, a pilot study and subsequent dose reduction study were performed in feline patients.

2 | METHODS

2.1 | Patient selection

All cats with FOSCC referred to the Department of Clinical Sciences of Companion Animals, Utrecht University, The Netherlands, between July 2009 and July 2015 were considered potentially eligible to participate in a prospective cohort study on ^{166}Ho microbrachytherapy.

Inclusion criteria were: diagnosis of FOSCC confirmed by histology, a tumour not amenable for surgical therapy with curative-intent because of tumour size and location, localized tumour disease suitable for manual needle injections, no detectable metastasis (locoregional and/or distant), and no life-threatening comorbidities or diseases with a life expectancy of <3 months or a deteriorated state requiring intensive care. The owners were invited to participate in the study after having received detailed information in both oral and written form concerning the treatment protocol, costs associated with work-up and hospital admission, expected risk of complications and restrictions because of radiation safety procedures. The treatments were performed in compliance and under supervision of the radiation safety officer of the Utrecht University radiation protection unit.

2.2 | Diagnosis and staging

Baseline characteristics including breed, age, weight and tumour location were recorded. Each patient received a standardized staging procedure consisting of oral inspection, tumour inspection, palpation and manual evaluation of tumour size using 3-dimensional caliper measurements under general anaesthesia. Contrast-enhanced CT imaging of the primary tumour (head), locoregional lymph nodes (neck), thorax and abdomen was performed to determine tumour size, invasion in surrounding tissues, bone involvement and possible metastasis. The tumour volume was calculated using the 3 longest perpendicular diameters as measured on CT, assuming an ellipsoid shape; tumour volume = $\pi/6 \times$ length $\times$ width $\times$ height.

Any regional lymph node that was enlarged or abnormal on CT image was examined for metastasis by cytology of an ultrasound-guided fine needle aspiration biopsy. Tumour stage was determined according to the World Health Organization Tumor, Node, Metastasis (TNM) system for classification of oral tumours in domestic animals.[29]

In addition, a complete blood count, blood biochemistry and urine analysis were performed to screen for subclinical disorders.

2.3 | Patient preparation

A treatment date was planned within 2 weeks. In case of a large exophytic growing malignancy partial laser debulking was performed with a 1064 nm Nd:YAG laser (Medilas 40 N, MBB-Medizintechnik GmbH, München, Germany) with bare optical fibre (600 μm diameter; Ultraline, Heraeus LaserSonics, Milpitas, California) for immediate reduction of clinical signs of gross tumour. Tumours with a mainly infiltrative growth pattern were not amenable for surgical debulking. Tumour volumes and dosimetry calculations were based on residual tumour volume in case of debulking prior to ^{166}Ho microbrachytherapy.

To ensure maximal patient comfort and reduce radiation dose to nursing personnel the following provisions were made prior to the ^{166}Ho microbrachytherapy. All feline patients received an esophageal feeding tube to ensure adequate food intake in case of anorexia because of pain or discomfort after treatment. The cats received prophylactic antibiotic treatment with a single injection of the long-acting antibiotic, cefovecin sodium (Convenia Zoetis B.V., Louvain-la-Neuve, Belgium). During treatment and hospitalization, all patients received buprenorphine (15 μg/kg 4dd by intramuscular injection, Buprecare, AST Farma B.V., Oudewater, the Netherlands) and meloxicam (0.05 mg/kg by subcutaneous injection or through feeding tube, after an initial dose of 0.1 mg/kg by subcutaneous injection, Metacam, Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany) for at least 7 days after ^{166}Ho microbrachytherapy.

2.4 | Microsphere preparation

The ^{166}Ho MS were prepared as previously described.[28,30] After neutron activation, ^{166}Ho MS were suspended in a solution with Pluronic F-68 (Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) 2% wt/vol solution. The ^{166}Ho MS were suspended by gentle agitation and repeatedly drawing up and down with a syringe. Subsequently, aliquots of 0.3-0.4 mL were drawn up in 1 mL Luer taper syringes.

The amount of radioactivity present in each syringe was measured in a dose calibrator (VDC-404; Veenstra Instrumenten B.V., Joure, The Netherlands). Each syringe was placed into an acrylic glass cylinder to limit β-radiation exposure of personnel, especially to the hands, during dose preparation and administration. The treatment was conducted with a fixed quantity of 200 mg of ^{166}Ho MS. The required activity needed for a given tumour treatment was obtained by varying the neutron activation time of the MS.

2.5 | Dosimetry

After selecting the planned tumour-absorbed dose for a certain tumour ("dose cohort", see later), the accompanying ^{166}Ho activity was determined based on tumour volume according to the following equation:

$$D = A \times 15.87/W$$

In which D = tumour-absorbed dose (in Gray [joules/kilogram]); A = ^{166}Ho activity in MBq; ^{166}Ho-specific tissue dose conversion coefficient = 15.87 mJ/MBq; W = tumour weight in grams. Assumed tumour tissue density was 1.0 g/cm^3.[31] Tumour-absorbed dose is based on a homogeneous distribution of the injected ^{166}Ho MS over the tumour. Furthermore, the tumour margin and its transition to normal surrounding tissue were carefully treated with ^{166}Ho microbrachytherapy. As a result, the "treatment volume" was determined to be 1.5 × the tumour volume as measured on CT images. In addition, extra activity was ordered to account for anticipated losses of ^{166}Ho MS during suspension and syringe preparation, and during the administration procedure (residual ^{166}Ho MS remaining in the syringes after intratumoral injections and possible spillage outside of the tumour).[27]

2.6 | Pilot study

Initially, the treatment was started in a pilot setting (n = 5) using a relatively high ^{166}Ho activity, aiming for a tumour-absorbed dose of 400 to 800 Gy. This relatively high-tumour dose was chosen to overcome possible focal low-absorbed dose areas in the tumour because of inhomogeneous ^{166}Ho MS distribution and peritumoral deposition of ^{166}Ho MS. Based on previous pilot experiments,[26,27] a "loss" of >50% of ^{166}Ho MS was expected during treatment due to possible leakage of ^{166}Ho MS out of the tumour needle tracts and premature settling and accumulation of ^{166}Ho MS in the syringe and needle dead space. A substantial additional loss of ^{166}Ho MS was anticipated during the process of microsphere suspension and preparation of the syringes for treatment. As a result, a double amount of radioactivity needed for the aimed tumour-absorbed dose was ordered for the pilot treatments. Subsequent analysis of the safety and observed efficacy in this pilot indicated that the applied procedures resulted in a higher than anticipated tumour-absorbed dose of >800 Gy. Therefore, the total anticipated loss of activity was adjusted to 50% of the activity needed for the intended treatment dose for further treatments. Furthermore, the study was continued as a minimal effective dose study to comply with the "As Low As Reasonably Achievable" (ALARA) principles of radiation safety guidelines, as described in the next section.

2.7 | Dose reduction study

A dose reduction study approach was added to obtain data of possible dose-related side effects and minimal effective dose. Subsequent feline patients were randomly placed in 1 of 2 cohorts: a low dose cohort of 200 Gy (n = 4) and an intermediate dose cohort of 400 Gy (n = 4), to be compared with the initial high-dose cohort of >800 Gy of the pilot study (n = 5).

2.8 | Treatment procedure

The administration procedure was performed under general anaesthesia with endotracheal intubation in a dedicated radionuclide facility of our department. Patients were prepared by Povidone Iodine of the tumour location and oral cavity. To aid in a homogenous intratumoral-absorbed dose distribution and to prevent skipping parts

of the tumour, the ^{166}Ho MS injections were orderly divided over predetermined equal tumour segments. The number of syringes was based on tumour volume. The injected volume was 0.3-0.6 mL per 1 mL tumour.

The activity of the prepared syringes was measured before the injection procedure. ^{166}Ho MS suspensions in the prefilled syringes for intratumoral injection were gently shaken before administration, to ensure a homogeneous suspension. The ^{166}Ho microbrachytherapy was administered through 22G 1.5″ or 22G 2.0″ needles under gradual retraction of the needle tip from the centre towards the periphery of the tumour segments. Injection positions were separated by ≤6 mm to reduce the chance of inadequate local overlap of dose distributions. After the procedure, the radioactivity of the syringes, needles, and all potentially contaminated disposable materials such as gloves and gauzes were measured in the dose calibrator. The total injected dose was calculated by subtracting the measured ^{166}Ho activity in materials from the pretreatment syringe measurements, adjusted for decay at the time of measurement.

Directly after the procedure, anterior-posterior and lateral planar scintigraphy images were obtained (Philips SKYLight, Medium-energy General-purpose Collimator) to rule out any unwanted systemic distribution (lungs or intestines) and/or contamination after ^{166}Ho microbrachytherapy.

The patients were housed in a specialized controlled radionuclide ward at the Department of Clinical Sciences of Companion Animals, Utrecht University. Patients were discharged when dose rate was below the local regulatory limit of 1 μSv/hour at 1 m, which corresponds to a maximum total accumulated dose of 40 μSv for the owner. Owners received specific follow-up radiation safety guidelines for the care of their cat for the first week.

2.9 | Follow-up

A standard follow-up protocol was used for the first 6 months after treatment, consisting of follow-up examinations at 1 week, and at 1, 2, 4 and 6 months after discharge. During these examinations, tumour response was evaluated under general anaesthesia by oral examination and taking manual caliper measurements. A physical exam was performed to evaluate general patient condition, signs of tumour progression or metastasis, and possible radiation complications such as radiation ulcers and bone necrosis. If indicated, additional laboratory investigation or imaging was performed. A CT scan was performed at 1 or 2 months if residual tumour could not be accurately measured manually using calipers.

Adverse events were scored according to the Veterinary Radiation Therapy Oncology Group VRTOG.[32] Adverse events were classified by the investigational team according to the suspected relation to the treatment with ^{166}Ho microbrachytherapy into none, unlikely, possible, likely and definitive, based on causal relationship.

In addition to the planned 6-month follow-up protocol, all patients were reviewed until death, at any time in case of suspected side effects or recurrences. Long-term follow-up data of potential side effects, disease recurrence or metastasis, survival and cause of death were recorded for all patients.

2.10 | Response

Response to therapy was categorized using the following criteria: Local Control, being complete response (CR) or partial response (PR) with sufficient tumour volume reduction enabling subsequent marginal tumour resection (ie, excision at or just beyond the visual tumour edge; Figure 3). Local Failure, in which local tumour control was not achieved, irrespective of initial volume reduction (PR).

OS was defined as the time from first ^{166}Ho microbrachytherapy treatment until subsequent death. Local disease progression free survival (LDPFS) was defined as the time from first ^{166}Ho microbrachytherapy treatment until the first clinical signs of local recurrence or progression.

2.11 | Statistical analysis

Continuous data are presented as the mean ± SD if normally distributed and as the median and interquartile range (IQR; Q1-Q3) if skewed (Shapiro-Wilk test). Categorical data are presented as numbers and proportions. Differences between the demographic and treatment characteristics of the patients with response or failure were evaluated by the χ^2 test for categorical data and by the Student's *t* test for continuous data if normally distributed. The Mann-Whitney *U* test was used if the continuous data were skewed. The 1-way analysis of variance was used to determine whether there were any statistically significant differences between the dose cohorts. The survival analysis was performed with the Kaplan-Meier method and

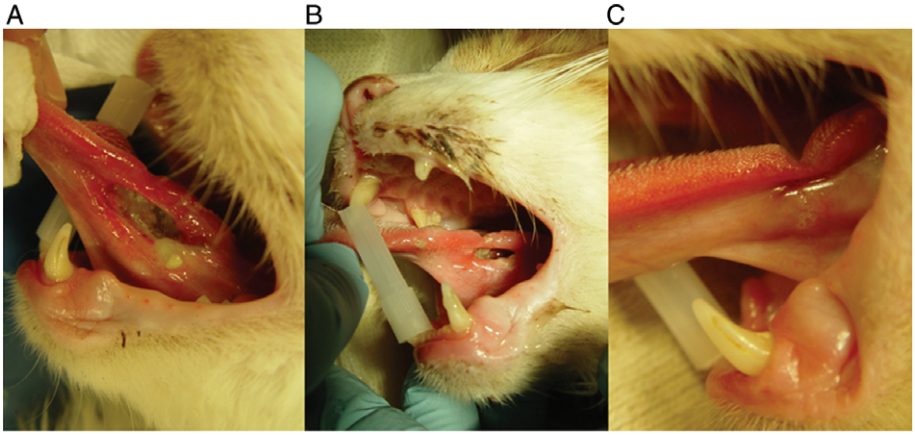

FIGURE 1 (Patient number 1) A, Tumour at presentation. B, Tumour 4 weeks after first holmium treatment. C, Result after 9 weeks (local control)

the baseline characteristic compared by the log-rank tests or Cox hazard for continuous variables. For analyses of treatment efficacy, the periprocedural deaths unrelated to the treatment were excluded. The data were registered in a dedicated database. The statistical analysis was conducted using SPSS 21. A power analysis was not performed. The significance level was set at $P \leq .05$.

3 | RESULTS

3.1 | Patients

Thirteen feline patients with FOSCC were enrolled in the ^{166}Ho microbrachytherapy study between July 2009 and July 2015 with follow-up until February 2016 (Table 1). There were 8 male and 5 female patients, with a mean age of 14.3 ± 2.0 years and a mean body weight of 4.6 ± 1.1 kg. The tumours were located ventrally in the tongue/frenulum/sublingual (n = 10), the gingiva of the mandible (n = 1) or maxilla (n = 2). Eleven patients had clinical stage T2 disease, 1 T1 and 1 T3. Bone involvement was seen in 2 patients (Patients 5 and 6) with a tumour located in the maxillary gingiva. Enlarged lymph nodes, cytologically free from tumour, were observed in 5 patients. The mean tumour volume was 3.17 ± 1.70 cm^3 with a range of 0.94 to 6.1 cm^3. Two patients (Patients 7 and 12) with a tumour of the tongue, underwent laser surgery debulking of the tumour before intratumoral ^{166}Ho microbrachytherapy. During this laser treatment, the tumour volume was decreased by approximately 80% (patient 7) and 50% (patient 12). Three patients (Patients 2, 9 and 13) received laser surgery resection after an initial PR of their lingual SCC to the ^{166}Ho microbrachytherapy treatment. Two patients (Patients 1 and 2), received a second ^{166}Ho microbrachytherapy for tumour residue/recurrence after an initial complete and PR (Table 1).

3.2 | Treatment procedure

The ^{166}Ho MS were divided over 3 to 11 aliquots depending on the tumour volume. The fraction of administered activity was 59.8% ± 17.6% of the prepared activity for injection. The administered amount of activity for the High (>800 Gy), Intermediate (400 Gy) and Low (200 Gy) dose group were 377 ± 172 MBq, 135 ± 96 MBq and 63 ± 50 MBq, respectively. The absorbed doses for the High (>800 Gy), Intermediate (400 Gy) and Low (200 Gy) dose group were 1216 Gy (925-2725), 440 ± 178 Gy and 168 ± 71 Gy, respectively. Postprocedure scintigraphy revealed distant activity of ^{166}Ho MS in the lungs of 2 patients (Patients 12 and 13). The mean hospital stay was 5.1 ± 1.6 days.

3.3 | Subsequent treatments

Additional laser ablation was performed in 5 cats: prior to ^{166}Ho microbrachytherapy in 2 (Patients 7 and 12) and after a PR in 3 cats (Patients 2, 9 and 13).

In total, 2 patients (Patients 1 and 2) received a second treatment with ^{166}Ho microbrachytherapy after 4.5 and 2.5 months, respectively, because of tumour recurrence after an initial complete and PR. These 2 patients had residual tumour volumes of 0.2 and 0.92 cm^3 and received a second dose of 5400 and 1088 Gy, respectively. This second treatment resulted in a CR in Patient 1. In Patient 2, the initial ^{166}Ho microbrachytherapy resulted in a 23% tumour volume reduction. The residual lesion was reduced in size by removing approximately 70% of the remaining protruding mass using laser surgery 5 weeks after the initial ^{166}Ho microbrachytherapy. However, local recurrence developed caudal to the initial lesion and a second ^{166}Ho microbrachytherapy treatment was performed 10 weeks after the first ^{166}Ho microbrachytherapy. This second ^{166}Ho microbrachytherapy did not result in a measurable response. One month later, the tumour was excised by amputation of the rostral 70% of the tongue. The cat became dependent on esophageal tube feeding and intensive grooming care by the owners. It lived free of tumour for another 144 days before the owners decided to let the cat be euthanized because it was still unable to eat or drink without assistance.

3.4 | Response and survival

Eleven of 13 patients were discharged from the veterinary hospital and followed up for tumour response. Two animals that died within 1 week of ^{166}Ho microbrachytherapy were not included in the response and survival analysis (see Section 3.5). The maximum tumour response was evaluated approximately 2 weeks after treatment. It often resulted in (temporary) improved quality of life, such as improved eating, drinking and grooming activity and a less inflamed aspect of the lesion. However, if an initial partial tumour response did not lead to a situation of local control (ie, enabled surgical resection) that case was considered a local failure. For example: Patient 4 had an initial 57% tumour volume reduction 2.5 weeks after ^{166}Ho microbrachytherapy but was still unresectable. This tumour showed a rapid tumour volume increase 2 weeks later.

Local control: CR (n = 3) and PR (n = 3) with a sufficient size reduction that enabled subsequent marginal laser excision were obtained in 6 of 11 patients (55%) after the initial ^{166}Ho microbrachytherapy. The mean tumour volume decrease was 83% ± 22% in the 6 responders. Three of 11 patients (27%) (Patients 1, 3 and 6) had a CR after a single intratumoral ^{166}Ho microbrachytherapy. One patient (Patient 1) had a small local recurrence after 3 months for which a second treatment was curative (Figure 1).

A PR was obtained in 3 patients. Subsequent marginal resection was performed in 2 patients with laser surgery and resulted in a LDPFS of 113 and 132 days. Figure 3 depicts a typical case of tumour downstaging and subsequent marginal laser excision. The third cat (Patient 2) that underwent laser debulking and a second ^{166}Ho microbrachytherapy, lived despite a near-total glossectomy for 8 months with a relatively good quality of life. Five animals had no objective response (stable disease or progressive disease)

The median OS was 113 (63-341) days. The median LDPFS was 70 (44-123) days. The median OS was better if a local control was achieved: 296 (148-676) vs 63 (52-72) days ($P < .01$) (Figure 4). In addition, the LDPFS was better if a local control was achieved 123 (87-640) vs 32 (22-57) days ($P < .01$). The cause of death after hospital discharge was OSCC-related in 81% (9/11) of the patients. One patient (Patient 3) died after 15 months because of pulmonary metastasis with no evidence of local recurrence. Eight patients had

TABLE 1 Patient characteristic, treatment details and tumour response

Patient	Breed	Age (y)	Tumour (cm^3)	Tumour stage	Tumour location	Dose cohort	Dose (Gy)	Prior laser	Tumour post ^{166}Ho (cm^3)	Response	Post-therapy	Local control	LDFS	Survival (d)	Complication	Cause of death
1	DS	13,3	3,30	T2aN0M0	Oral tongue	>800	1216		0.0	CR	19wk Second ^{166}Ho	Y	92	341		Chronic kidney failure
2	DS	16,9	1,76	T2aN1aM0	Oral Tongue	>800	939		1.36	PR	5 wk laser 10 wk second ^{166}Ho	Y	70	250		LR → tongue amputation → Inability to feed → Euthanasia
3	DS	15,9	0,94	T2aN1aM0	Mandible gingiva	>800	4162		0.0	CR		Y	466	466	Radiation lesion of adjacent tongue	Lung metastasis
4	DS	15,0	3,96	T2aN1aM0	Oral tongue	>800	912		1.69	PR		N	32	63		Local disease and LN meta's
5	MC	11,0	5,23	T3bN0M0	Maxilla/gingiva	>800	1288		33.49	PD		N	56	78		Local disease
6	DS	14,9	3,37	T2bN1aM0	Maxilla/gingiva	400	635		[a]	[a]		[a]	[a]	[a]		Kidney failure
7	DS	15,4	0,94	T1aN0M0	Oral Tongue	400	293	x	0.0	CR		Y	1304	1304[b]	Necrosis small part of the rostral tongue	Alive
8	DS	13,4	4,40	T2aN1aM0	Oral tongue	400	546		4.27	SD		N	57	66		Local disease
9	DS	10,8	3,08	T2aN0M0	Oral tongue	400	285		1.32	PR	3 wk laser	Y	132	159		Local recurrence
10	DS	14,3	6,10	T2aN0M0	Oral tongue	200	165		5.8	SD		N	30	53		Local disease
11	CB	17,0	1,38	T2aN0M0	Oral tongue	200	81		2.91	PD		N	13	50		Local disease
12	DS	15,0	4,84	T2aN0M0	Oral Tongue	200	253	x	[a]	[a]		[a]	[a]	0		Anaesthesia complication
13	DS	13,0	1,90	T2aN0M0	Oral tongue	200	174		0.92	PR	3 wk laser	Y	113	113	Necrosis small part of rostral tongue	Local recurrence

Abbreviations: CB, cross breed; CR, complete response; DS, domestic shorthair; Gy, Gray; LDFS, local disease free survival; LR, local recurrence; MC, main coon; PD, progressive disease; PR, partial response; SD, stable disease; T1, tumour <2 cm; T2, 2-4 cm; T3, >4 cm; substage Ta, without bone involvement; Tb, with bone involvement; No, no regional lymph node metastasis; N1, movable ipsilateral lymph nodes; substage Na, No evidence of lymph node metastasis; Nb, evidence of lymph node.

Tumour stage is according to WHO TNM classification for oral tumours in domestic animals.[29]

[a]Evaluation of response not available due to limited survival.

[b]Alive at data analysis.

local recurrences or progression of residual disease during follow-up. One cat (patient 1) died of progressive kidney failure after 1 year without signs of local recurrence and 1 patient (Patient 6) is still alive after 1304 days.

Tumour volume prior ^{166}Ho microbrachytherapy was significantly smaller in responders compared with non-responders, 1.9 ± 1.0 vs 4.2 ± 1.7 cm^3 (*t* test P = .028), and was a predictor of LDPFS with a hazard ratio of 1.9, 95% CI (1.0-3.3), P = .038. No relationship between the absorbed dose and survival could be demonstrated. The median survival for the 200, 400 and >800 Gy cohort was 53, 159 and 250 days, respectively (log-rank P = .21).

3.5 | Complications and adverse events

None of the patients was lost to follow-up. During the study, 3 serious adverse events occurred. One was defined as "definitely related" to the ^{166}Ho microbrachytherapy and occurred after treatment of a small superficial tumour in the mandibular gingiva. A localized radiation ulcer developed at the surface of the tongue in the area adjacent to the treated tumour. It healed within 3 weeks with minimal supportive wound care.

Two patients died during the hospitalization period. One cat (Patient 6) developed kidney failure within 1 week after the procedure. This severe adverse event was classified as unlikely related to ^{166}Ho microbrachytherapy. This complication was probably related to a combination of poor physical condition, anaesthesia and non-steroidal anti-inflammatory drugs intoxication.

The other cat (Patient 12) died while under anaesthesia for the treatment procedure. This severe adverse event was classified as possibly related to ^{166}Ho microbrachytherapy. Post-mortem SPECT imaging of the thorax of this cat revealed the presence of ^{166}Ho activity in the lung region, and at the autopsy, MS were detected in the capillaries of the lung. Radiation damage was considered an unlikely cause of morbidity because of the short time frame between the treatment and death. No MS were detected in the trachea or bronchi, which argue against inhalation. Acute pulmonary arterial embolization after accidental intravenous injection of ^{166}Ho MS could be a possible explanation for this complication. However, the calculated 99 mg ^{166}Ho MS that was injected during the ^{166}Ho microbrachytherapy procedure in this patient was divided over 5 syringes that were each used to inject 3 to 4 ^{166}Ho MS depots. Therefore, accidental intravenous injection of large amounts of MS was not likely. Furthermore, ^{166}Ho activity was also found in the lung region after ^{166}Ho microbrachytherapy in one other patient in this study (Patient 13) without clinical signs during the follow-up period. Quantitative scintigraphy imaging, using SPECT-CT to enable correction of the scintigraphic images for local radiation attenuation from surrounding tissues, was not performed in this study. However, the fraction of activity in the lungs can be roughly estimated from the available scans using the recorded counts in the lung region and tumour region. This resulted in an estimated deposition of 30% of administered ^{166}Ho MS in the lung region of Patient 13 without complications after treatment. In comparison, an estimated 21% of administered ^{166}Ho MS was detected in the lung region of the cat (Patient 12) that died during the procedure. A more probable cause for the death of this cat was an anaesthetic complication. The cat underwent multiple consecutive procedures in different locations (esophageal feeding tube placement in anaesthesia induction room, laser debulking in OR and ^{166}Ho microbrachytherapy in the radionuclide facility) during a single anaesthetic period under propofol maintenance and spontaneous breathing. Especially during the ^{166}Ho microbrachytherapy anaesthetic monitoring might have been insufficient, resulting in hypoxia from apnoea that was not recognized on time.

A laser surgery-related complication occurred in 2 patients: a small unilateral rostral portion of the tongue, several centimetres rostral of the mid-ventral tumour location, became necrotic and was lost (Figure 2). This complication developed shortly after laser surgery and was seemingly independent of ^{166}Ho microbrachytherapy in both patients. Loss of a small portion of the tongue was discovered 11 days after laser debulking, prior to ^{166}Ho microbrachytherapy in Patient 6. Acute necrosis with distinct demarcation margin was detected 4 days after laser surgery of a residual tumour mass in Patient 11. This tumour had decreased 57% in volume after ^{166}Ho microbrachytherapy 16 days earlier without noticeable changes to the tip of the tongue at the time of laser surgery. Therefore, this complication was most likely because of ipsilateral vascular (ie, lingual artery) damage during Nd:YAG laser surgery. The tongue healed within 2 to 3 weeks without apparent functional consequences during follow-up in both cats.

4 | DISCUSSION

To our knowledge, this is the first study on intratumoral treatment with radioactive microparticles in feline patients with OSCC. This study shows that cats with small unresectable OSCC can be curatively treated with a high dose of ^{166}Ho microbrachytherapy. Complete response was obtained in 27%, partial response in 36% and stable disease in 18% of the cats. Treatment-related side effects were mild in most animals; however, some serious adverse events occurred. A possible relation to ^{166}Ho microbrachytherapy will be investigated in further research and potential preventive measures proposed.

The outcome in this study compares favourably with currently available treatments for FOSCC with or without bone involvement in respect of clinical efficacy and complications: Several external beam palliative radiation schemes have been used in cats with 3 to 6 fractions of 6 to 10 Gy with only limited effect.[11–13] Radiation side effects were severe, resulting in a significantly diminished quality of life and early treatment termination: 43% of the patients did not complete the radiation protocol in 1 study.[11] In a large study of 54 animals, only 3 (6%) animals had a complete response, 30 (61%) had a partial response and 16 (33%) had stable disease at completion of treatment. The majority (49/54 animals, 91%) experienced complications compared with only 1 of 11 (9%) in the present ^{166}Ho microbrachytherapy study.[12] The median OS in these studies was 60, 92 and 111.5 days with a median local progression free survival of 43 days, respectively.[11–13]

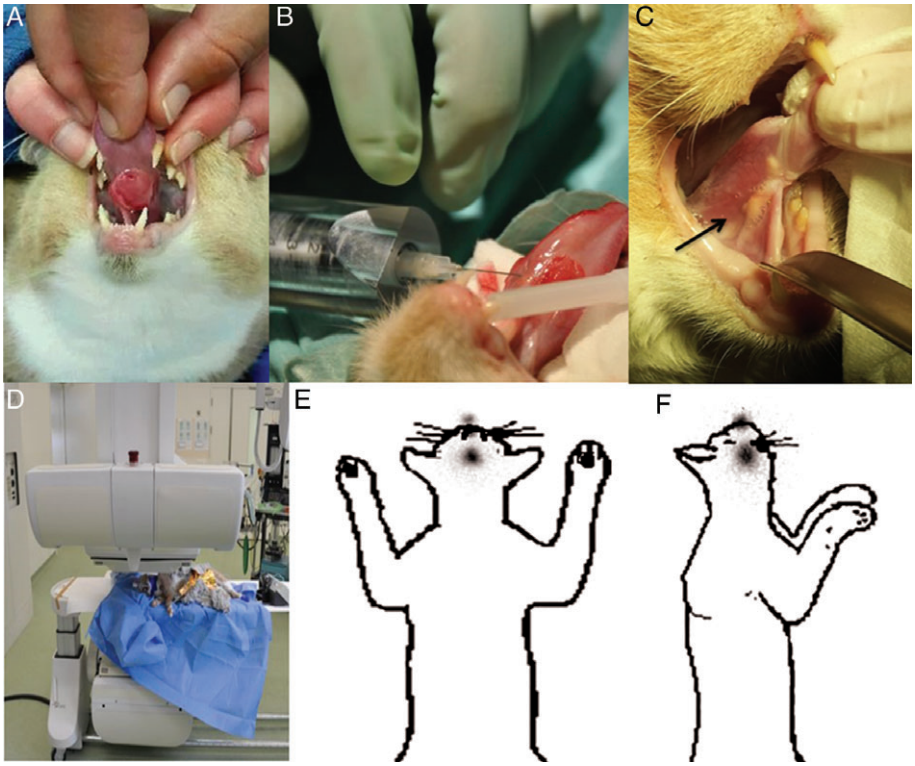

FIGURE 2 Example of a complete tumour respons to ^{166}Ho microbrachytherapy and a typical example for the complication of laser surgery, where the rostral part of the tongue became necrotic after debulking (Patient number 7). A, The tumour at first visit. B, Two weeks after the laser surgery a unilateral rim of the tip of the tongue became necrotic. This was identified prior to the ^{166}holmium treatment. C, Follow-up visit 2 months after the ^{166}holmium treatment showing a complete tumour response and loss of a right rostral rim of the tongue edge; holmium depositions are visible (black arrow). D, Set-up of scintigraphy. E and F, Scintigraphy with ^{99m}Tc marker positioned at the nose (anteroposterior and lateral view). This animal with a complete response is still under follow-up after 1304 days

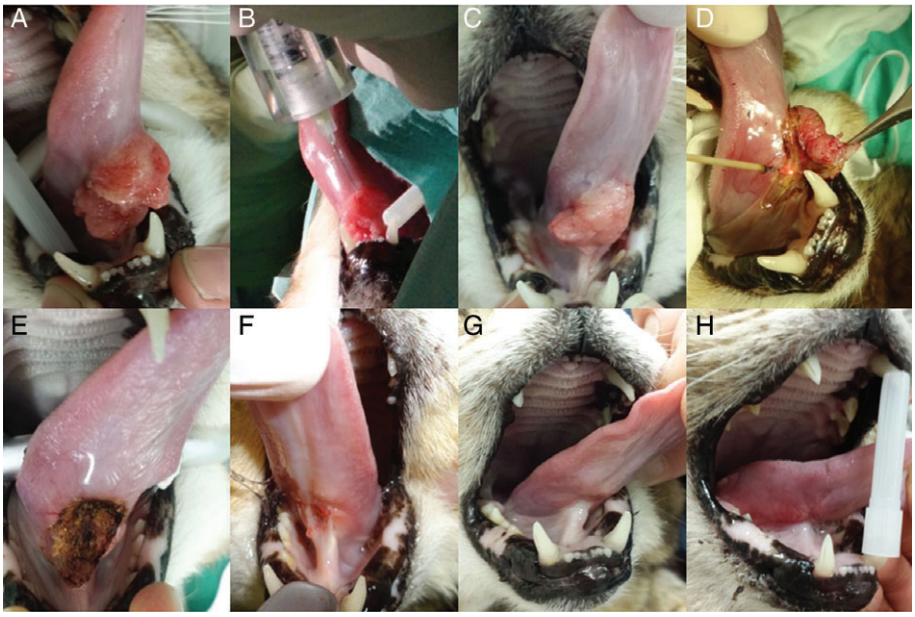

FIGURE 3 Typical presentation of downstaging and subsequent resection showing local tumour control after a partial ^{166}Ho microbrachytherapy treatment response and subsequent laser surgery (Patient number 9). A, Tumour at presentation with a volume of 3.1 cm^3. B, Intratumoral injections of ^{166}Ho-microspheres. An acrylic glass cover over the syringe blocks β-radiation to protect the hand of the operator. C, The result 12 days after ^{166}Ho microbrachytherapy with a 58% tumour volume reduction to 1.3 cm^3. D, Subsequent marginal laser resection of the remaining tumour. E, Result after tumour resection. F, Healing of the tongue at 1 month. G, Final result at 2 months. H, Local recurrence, more diffuse and caudal in the tongue at 4.5 months after initial treatment

Accelerated hypofractionated protocols might be more effective with a median OS of 86 to 174 days with a LDPFS of 91 days in 12 animals.[14,15] However, complications were still present in this twice-a-day radiation scheme resulting in grade 3 mucositis in 7 of 9 (78%) patients compared with 1 of 11 (9%) local radiation ulcer in the present ^{166}Ho microbrachytherapy study.[14] In the study by Fidel et al, 3 patients experienced a complete response 30 days after completion of the radiation, which resulted in a median OS of 298 days compared with 60 days for the partial responders. This median OS of the partial responders in the study of Fidel et al is similar to the OS of patients classified as non-responders in the present study. Furthermore, 10 of 11 (91%) of the patients in the present study did not experience any side effects.

Adding a systemic chemotherapeutic/radiosensitizer such as carboplatin or gemcitabine has been described to improve response rates, with 16 of 31 (52%) complete responders with an OS of 163 days.[14] Promising results have also been reported in 6 cats using an intensive multimodal treatment approach consisting of chemotherapy, accelerated hypofractionated radiation and surgery. This resulted in a complete response in 5 of 6 cats, and 3 cats alive with a follow-up of ≥1 year.[17]

The OS of feline patients with OSCC treated with ^{166}Ho microbrachytherapy is similar to the OS reported in the studies with the

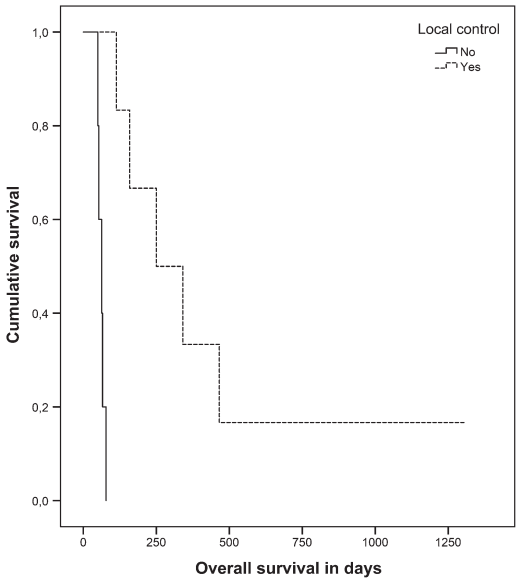

FIGURE 4 Overall survival. Kaplan-Meier analysis of the overall survival of cats in which local control was obtained (median survival 296 interquartile range [IQR] 148-676 days) vs cats where no local control was obtained (median survival 63 IQR 52-72 days; $P < .01$)

best clinical efficacy so far, using hypofractionated radiation protocols alone or in a multimodal approach, as described above (LDPFS of 70 vs 91 days, OS of 113 days vs 86-174 days).[14,15] The major advantages of this novel ^{166}Ho microbrachytherapy over other therapies are the single session approach and minimally invasive nature, without the severe side effects that are often seen with external beam radiation and chemotherapy. Especially the local toxic side effects such as mucositis, which are often observed after external beam radiation, are not seen after ^{166}Ho microbrachytherapy.

FOSCC is a locally aggressive malignancy, and most untreated patients die of local disease. In this study, only local treatments were applied: ^{166}Ho microbrachytherapy with or without laser debulking of macroscopic tumours. Despite the 55% local control rate without macroscopic tumour, most patients eventually suffered from a local recurrence after several months. Only 1 patient died of pulmonary metastasis. Recurrent disease often appeared in a more caudal location than the primary tumour site of the tongue (Figure 3). This may be related to an insufficient administration of ^{166}Ho MS in the caudal margin of the tumour. However, it could also be related to posterior perineural or lymphovascular invasion not detectable on the initial staging CT scans. These features are associated with a poor prognosis in humans and might implicate the need of a more accurate injection treatment of the caudal peritumoral region. In studies of external beam radiation therapy for OSCC, larger peritumoral treatment margins were used, regional lymph nodes were included in the radiation field, and combinations with multiple chemotherapeutical treatments were often performed. Multimodality treatment with additional systemic or regional treatment for microscopic disease might therefore also further improve the survival time after ^{166}Ho microbrachytherapy.

A local side effect seen in this study was unilateral partial necrosis of the tip of the tongue (Figure 2). This complication occurred after laser surgery in 2 cats. Considering the unilateral tongue tip location and a clear demarcation region in the acute phase, it seemed to be the result of vascular compromise during laser surgery, causing ischemic necrosis distal to the tumour site. The fact that this complication occurred before ^{166}Ho microbrachytherapy in 1 patient and 3 weeks after ^{166}Ho microbrachytherapy in the other patient indicates that this was unlikely a complication of the ^{166}Ho microbrachytherapy itself. Absence of a vascular arcade or extensive collateral arterial blood supply of the tongue in cats is the most likely explanation for this complication. In contrast, such unilateral ischemic events are not common after lingual surgery in humans, who have an extensive collateral blood supply of the tongue.

In this study, 3 serious adverse events occurred. One local radiation ulcer and 2 patients died during or shortly after the ^{166}Ho microbrachytherapy treatment procedure. Both deaths were most likely unrelated to the ^{166}Ho microbrachytherapy itself. The local radiation ulcer could be expected if healthy adjacent tissue receives a high-absorbed dose. This risk of side effects should be balanced against the chances of an incomplete response or increased risk of local recurrences. More accurate administration, for example with additional imaging, such as ultrasound needle guidance, could aid in preventing this complication.

^{166}Ho activity was detected in the lungs on scintigraphy after the ^{166}Ho microbrachytherapy in 2 patients. In the 1 patient (nr. 13) with follow-up, an estimated 30% of injected ^{166}Ho MS was detected in the lung region on scintigraphy images without clinical signs during the follow-up period. The other patient (nr. 12) died during the ^{166}Ho microbrachytherapy treatment procedure. Although the cause of death was likely related to the anaesthetic procedure with inadequate monitoring, acute pulmonary artery embolization after accidental intravenous injection of ^{166}Ho MS cannot be completely ruled out as cause of death. However, the estimated 21% of administered ^{166}Ho MS that was detected in the lung region of patient 12 on scintigraphy images was less than the 30% detected in patient 13 that did not lead to any side effects. As a comparison, in the arterial ^{166}Ho MS radioembolization procedure that is commonly performed in humans with liver malignancies, a predetermined safety limit of "shunt-fraction" of MS (maximum of 20%) that may pass the liver and lodge in pulmonary vasculature is practiced. Activity in the lungs has been encountered during radio-embolization in humans without clinical signs.[22] Nevertheless, this unwanted systemic distribution of ^{166}Ho MS should be avoided if possible and monitored. Therefore, during the^{166}Ho microbrachytherapy treatment procedure, the total amount of ^{166}Ho MS is injected in multiple depots. The number of MS per injected location is therefore relatively low. The chance that such a fraction of the total dose of^{166}Ho MS, when inadvertently injected intravenously, will lead to acute life-threatening pulmonary arteriolar embolization is presumably very small.

One patient died 6 days after ^{166}Ho microbrachytherapy because of kidney failure. Leakage of free holmium from MS could, in theory, have caused nephrotoxicity as is described for gadolinium.[33] This is, however, unlikely for ^{166}Ho MS since previous research has shown a high in vivo stability of ^{166}Ho MS for more than 30 days.[28] This patient already had abnormal findings during clinical work-up (cardiac murmur, hyperglycemia and low hematocrit) and was unstable during the anaesthetic procedure for ^{166}Ho microbrachytherapy, showing ventricular premature complexes. In retrospect, this patient would

probably have had benefit of intensive postoperative care and monitoring which was, however, not possible in the current treatment setting in the radionuclide facility and according to radiation safety guidelines. The other patient died during the ^{166}Ho microbrachytherapy procedure, most probably due to anaesthetic complications. Especially, when considering that these cats are old, often have low body condition scores because of longer periods of reduced ability to eat and/or drink, periprocedural and anaesthetic care and monitoring should be optimally arranged. Considering the experimental nature of the ^{166}Ho microbrachytherapy and the radioactivity involved, anaesthesia and post-procedural care are challenging and more complicated compared with high-risk patients undergoing more common procedures. A well-trained team and constant awareness during the whole procedure are essential.

With a median-absorbed dose of 546 Gy, side effects were minimal. This is probably because of the short tissue penetration depth of the ^{166}Ho β-radiation with a mean of 3.2 mm and a maximum less than 9.0 mm.[34] The present data did not reveal a significant relation between absorbed dose and tumour response or a dose-dependent toxicity. This could be related to the small number of patients, or to the fact that all patients received a relatively high-absorbed dose compared with the 50 to 70 Gy of external beam radiation therapy. However, the spatial distribution of ^{166}Ho microbrachytherapy over and around the tumour and therefore the distribution of the radiation-absorbed dose over the entire tumour is probably more important than the calculated total absorbed dose to a tumour. A suboptimal distribution of radioactive dose is more likely to occur in larger tumours and could, therefore, explain the significantly decreased local response rate in patients with larger tumours. This effect is probably larger than a possible dose-response relation, especially with the relatively low number of patients involved in the current study. Because of the limited tissue range of ^{166}Ho β-radiation, increasing the ^{166}Ho MS specific activity only has a limited effect on the dose distribution in case of a suboptimal ^{166}Ho MS distribution, stressing the importance of the ^{166}Ho microbrachytherapy strategy. An important limitation of the ^{166}Ho microbrachytherapy procedure in the present study is the inability to monitor the intratumoral-absorbed dose distribution, especially in large tumours. The method of ^{166}Ho microbrachytherapy administration in predetermined tumour segments was aimed to create a proper intratumoral dose distribution. The resulting absorbed dose coverage of the entire tumour is, however, not known. The unique imaging properties of ^{166}Ho MS have recently been used to develop an MRI-based dosimetry method that is currently used to monitor dose distribution in humans after radio-embolization of the liver.[23] These developments are promising. A real-time imaging-guided dosimetry technique could enable optimization of intratumoral radioactive dose distribution during ^{166}Ho microbrachytherapy, which may result in improved local response rates.

5 | CONCLUSION

The application of ^{166}Ho microbrachytherapy led to promising responses in feline patients with unresectable OSCC. The intratumoral microbrachytherapy as conducted in this cohort is an effective, minimally invasive treatment with minimal morbidity and limited side effects. Tumour volume was a significant predictor of response. Optimization of the injection procedure may improve spatial distribution of ^{166}Ho MS inside the tumour after injection, especially in large tumours, and result in an improved tumour response rate in future cases. Advantages of ^{166}Ho microbrachytherapy over current treatments using external beam radiation therapy in Feline OSCC patients warrant further investigation.

ACKNOWLEDGEMENTS

R.C.B. is funded by the Dutch Cancer Society research grant: UU 2014 7075. We thank the owners and their animals that we were able to treat. Further, we would like to thank Maarten Vente, Remmert de Roos, Nikkie de Wit, Chantal Dekker, Joost Holthof, Jeannette Wolfswinkel, Kees Vos and Angelique Barten for the assistance during the holmium treatments.

Conflict of interest

J.F.W.N. is a co-founder and scientific director of Quirem Medical, and has a minority share in the company Quirem Medical. Furthermore, J.F.W.N. is an inventor on the patents related to the ^{166}Ho-PLLA-microspheres which are assigned to University Medical Center Utrecht Holding BV (patent numbers: WO2012060707 A1 and US 2005/0201940 A1). The department of Radiology and Nuclear Medicine of the UMC Utrecht receives royalties from Quirem Medical BV. M.G.E.H.L. is consultant for Sirtex, BTG, Mirada and Bayer Healthcare.

REFERENCES

1. Withrow SJ, MacEwan EG. *Small Animal Clinical Oncology*; St. Louis, Mo. : Elsevier/Saunders 2001.
2. Howlader N, Noone A, Krapcho M, et al. *SEER Cancer Statistics Review*. Bethesda, MD: National Cancer; 1975-2013.
3. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359-E386. https://doi.org/10.1002/ijc.29210.
4. Braakhuis BJM, Visser O, Leemans RC. Oral and oropharyngeal cancer in The Netherlands between 1989 and 2006: Increasing incidence, but not in young adults. *Oral Oncol*. 2009;45(9):e85-e89. https://doi.org/10.1016/j.oraloncology.2009.03.010.
5. Wypij JM. A naturally occurring feline model of head and neck squamous cell carcinoma. *Patholog Res Int*. 2013;2013:1-7. https://doi.org/10.1155/2013/502197.
6. Munday JSS, Howe L, French A, Squires RAA, Sugiarto H. Detection of papillomaviral DNA sequences in a feline oral squamous cell carcinoma. *Res Vet Sci*. 2009;86(2):359-361. https://doi.org/10.1016/j.rvsc.2008.07.005.
7. Supsavhad W, Dirksen WP, Martin CK, Rosol TJ. Animal models of head and neck squamous cell carcinoma. *Vet J*. 2015;210:7-16. https://doi.org/10.1016/j.tvjl.2015.11.006.
8. Yoshikawa H, Ehrhart EJ, Charles JB, Custis JT, Larue SM. Assessment of predictive molecular variables in feline oral squamous cell carcinoma treated with stereotactic radiation therapy. *Vet Comp Oncol*. 2016;14(1):39-57. https://doi.org/10.1111/vco.12050.
9. Snyder L. Oral squamous cell carcinoma: like owner, like cat. *Vet J*. 2012;193(1):6-7. https://doi.org/10.1016/j.tvjl.2012.02.023.

10. Sant M, Aareleid T, Berrino F, et al. EUROCARE-3: survival of cancer patients diagnosed 1990-94 – results and commentary. *Ann Oncol*. 2003;14(suppl 5):v61-v118. http://www.ncbi.nlm.nih.gov/pubmed/14684501. Accessed September 1, 2016..
11. Bregazzi VS, LaRue SM, Powers BE, Fettman MJ, Ogilvie GK, Withrow SJ. Response of feline oral squamous cell carcinoma to palliative radiation therapy. *Vet Radiol Ultrasound*. 2001;42(1):77-79. https://doi.org/10.1111/j.1740-8261.2001.tb00907.x.
12. Sabhlok A, Ayl R. Palliative radiation therapy outcomes for cats with oral squamous cell carcinoma (1999–2005). *Vet Radiol Ultrasound*. 2014;55(5):565-570. https://doi.org/10.1111/vru.12157.
13. Jones PD, de Lorimier LP, Kitchell BE, Losonsky JM. Gemcitabine as a radiosensitizer for nonresectable feline oral squamous cell carcinoma. *J Am Anim Hosp Assoc*. 2003;39(5):463-467. https://doi.org/10.5326/0390463.
14. Fidel JL, Sellon RK, Houston RK, Wheeler BA. A nine-day accelerated radiation protocol for feline squamous cell carcinoma. *Vet Radiol Ultrasound*. 2007;48(5):482-485. https://doi.org/10.1111/j.1740-8261.2007.00283.x.
15. Poirier VJ, Kaser-Hotz B, Vail DM, Straw RC. Efficacy and toxicity of an accelerated hypofractionated radiation therapy protocol in cats with oral squamous cell carcinoma. *Vet Radiol Ultrasound*. 2013;54(1):81-88. https://doi.org/10.1111/j.1740-8261.2012.01970.x.
16. Fidel J, Lyons J, Tripp C, Houston R, Wheeler B, Ruiz A. Treatment of oral squamous cell carcinoma with accelerated radiation therapy and concomitant carboplatin in cats. *J Vet Intern Med*. 2011;25(3):504-510. https://doi.org/10.1111/j.1939-1676.2011.0721.x.
17. Marconato L, Buchholz J, Keller M, Bettini G, Valenti P, Kaser-Hotz B. Multimodal therapeutic approach and interdisciplinary challenge for the treatment of unresectable head and neck squamous cell carcinoma in six cats: a pilot study. *Vet Comp Oncol*. 2013;11(2):101-112. https://doi.org/10.1111/j.1476-5829.2011.00304.x.
18. Wiles V, Hohenhaus A, Lamb K, Zaidi B, Camps-Palau M, Leibman N. Retrospective evaluation of toceranib phosphate (Palladia) in cats with oral squamous cell carcinoma. *J Feline Med Surg*. 2016;19(2):185-193. https://doi.org/10.1177/1098612X15622237.
19. Fox LE, Rosenthal RC, King RR, et al. Use of cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexane platinum (II), a liposomal cisplatin analogue, in cats with oral squamous cell carcinoma. *Am J Vet Res*. 2000;61(7):791-795. https://doi.org/10.2460/ajvr.2000.61.791.
20. Seevinck PR, Seppenwoolde JH, de Wit TC, et al. Factors affecting the sensitivity and detection limits of MRI, CT, and SPECT for multimodal diagnostic and therapeutic agents. *Anticancer Agents Med Chem*. 2007;7:317-334. https://doi.org/10.1118/1.4823788.
21. Elschot M, Smits MLJ, Nijsen JFW, et al. Quantitative Monte Carlo-based holmium-166 SPECT reconstruction. *Med Phys*. 2013;40(11):112502. https://doi.org/10.2967/jnumed.112.119131.
22. Smits MLJ, Nijsen JFW, van den Bosch MAAJ, et al. Holmium-166 radioembolisation in patients with unresectable, chemorefractory liver metastases (HEPAR trial): a phase 1, dose-escalation study. *Lancet Oncol*. 2012;13(10):1025-1034. https://doi.org/10.1016/S1470-2045(12)70334-0.
23. Smits MLJ, Elschot M, van den Bosch MAAJ, et al. In vivo dosimetry based on SPECT and MR imaging of 166Ho-microspheres for treatment of liver malignancies. *J Nucl Med*. 2013;54(12):2093-2100. https://doi.org/10.2967/jnumed.113.119768.
24. Blanchard RJ, Lafave JW, Kim YS, Frye CS, Ritchie WP, Perry JF. Treatment of patients with advanced cancer utilizing Y90 microspheres. *Cancer*. 1965;18(3):375-380. https://doi.org/10.1002/1097-0142(196503)18:3<375::AID-CNCR2820180314>3.0.CO;2-U.
25. van Es RJ, Nijsen JF, van het Schip AD, Dullens HF, Slootweg PJ, Koole R. Intra-arterial embolization of head-and-neck cancer with radioactive holmium-166 poly(L-lactic acid) microspheres: an experimental study in rabbits. *Int J Oral Maxillofac Surg*. 2001;30(5):407-413. https://doi.org/10.1054/ijom.2001.0129.
26. Bult W, Vente MADD, Vandermeulen E, et al. Microbrachytherapy using holmium-166 acetylacetonate microspheres: a pilot study in a spontaneous cancer animal model. *Brachytherapy*. 2013;12(2):171-177. https://doi.org/10.1016/j.brachy.2012.08.001.
27. Bult W, Kroeze SGC, Elschot M, et al. Intratumoral administration of holmium-166 acetylacetonate microspheres: antitumor efficacy and feasibility of multimodality imaging in renal cancer. *PLoS One*. 2013;8(1):e52178. https://doi.org/10.1371/journal.pone.0052178.
28. Bult W, De Leeuw H, Steinebach OM, et al. Radioactive holmium acetylacetonate microspheres for interstitial microbrachytherapy: an in vitro and in vivo stability study. *Pharm Res*. 2012;29(3):827-836. https://doi.org/10.1007/s11095-011-0610-7.
29. Owen L. *TNM Classification of Tumours in Domestic Animals*. World Health Organization; Geneva 1980:46-47.
30. Zielhuis SW, Nijsen JFW, De Roos R, et al. Production of GMP-grade radioactive holmium loaded poly(L-lactic acid) microspheres for clinical application. *Int J Pharm*. 2006;311(1–2):69-74. https://doi.org/10.1016/j.ijpharm.2005.12.034.
31. Vente MAD, Nijsen JFW, de Wit TC, et al. Clinical effects of transcatheter hepatic arterial embolization with holmium-166 poly(L-lactic acid) microspheres in healthy pigs. *Eur J Nucl Med Mol Imaging*. 2008;35(7):1259-1271. https://doi.org/10.1007/s00259-008-0747-8.
32. Ladue T, Klein MK. Toxicity criteria of the veterinary radiation therapy oncology group. *Vet Radiol Ultrasound*. 2001;42(5):475-476. https://doi.org/10.1111/j.1740-8261.2001.tb00973.x.
33. Rose TA, Choi JW. Intravenous imaging contrast media complications: the basics that every clinician needs to know. *Am J Med*. 2015;128(9):943-949. https://doi.org/10.1016/j.amjmed.2015.02.018.
34. Nijsen JFW, Krijger GC, van Het Schip AD. The bright future of radionuclides for cancer therapy. *Anticancer Agents Med Chem*. 2007;7(3):271-290. https://doi.org/10.2174/187152007780618207.

How to cite this article: van Nimwegen SA, Bakker RC, Kirpensteijn J, et al. Intratumoral injection of radioactive holmium (^{166}Ho) microspheres for treatment of oral squamous cell carcinoma in cats. *Vet Comp Oncol*. 2018;16:114–124. https://doi.org/10.1111/vco.12319