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ORIGINAL ARTICLE

# Survival analysis of dogs with advanced primary lung carcinoma treated by metronomic cyclophosphamide, piroxicam and thalidomide

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Unresectable or metastatic (advanced) primary pulmonary carcinoma (PPC) represents a therapeutic challenge where surgery may be contraindicated and the therapeutic role of maximum-tolerated dose (MTD) chemotherapy remains uncertain. This study was undertaken to explore the impact of metronomic chemotherapy (MC) in dogs with advanced PPC. Previously untreated dogs with advanced (T3 or N1 or M1) PPC, with complete staging work-up and follow-up data, receiving MC (comprising low-dose cyclophosphamide, piroxicam and thalidomide), surgery, MTD chemotherapy or no oncologic treatment were eligible for inclusion. For all patients, time to progression (TTP) and survival time (ST) were evaluated. Quality-of-life (QoL) was only evaluated in patients receiving MC. To assess QoL, owners of dogs receiving MC were asked to complete a questionnaire before and during treatment.

Ninety-one dogs were included: 25 received MC, 36 were treated with surgery, 11 with MTD chemotherapy and 19 received no treatment. QoL was improved in dogs receiving MC. Median TTP was significantly longer in patients receiving MC (172 days) than patients undergoing surgery (87 days), receiving MTD chemotherapy (22 days), or no oncologic treatment (20 days). Median ST was similarly longer in patients receiving MC (139 days) than those undergoing surgery (92 days), MTD chemotherapy (61 days) and no oncologic treatment (60 days). In dogs with advanced PPC, MC achieved a measurable clinical benefit without significant risk or toxicity. This makes MC a potential alternative to other recognized management approaches.

**KEYWORDS**

advanced stage, canine, dog, lung carcinoma, metronomic chemotherapy, prognosis, thalidomide

## 1 | INTRODUCTION

Primary pulmonary tumours are uncommon in dogs and typically originate from the airway epithelium or alveolar parenchyma.[1]

Several prognostic factors have been associated with tumour progression and dogs' survival. Dogs that demonstrate clinical signs at the time of diagnosis are expected to have a shorter disease-free interval (DFI) and survival time (ST) compared to those where the pulmonary neoplasia is diagnosed as an incidental finding.[2] Locoregional lymph node involvement is also a predictor of short DFI and ST.[2–5] With regards to histology features, dogs with well-differentiated tumours are likely to have longer DFI and ST than dogs with moderately or poorly differentiated tumours; moreover, dogs with squamous cell differentiation are likely to experience a shorter ST.[4,6] Other factors significantly associated with tumour remission are the degree of primary tumour extension, the lack of gross tumour residual disease after surgical intervention and the lack of pulmonary metastatic disease.[4] Tumour localization and tumour volume are also considered predictors of ST with peripheral lesions associated with better outcomes than lesions involving an entire lobe, as well as tumour volumes smaller than 100 $cm^3$.[6]

Findings of this study were presented in part at the European Society of Veterinary Oncology Meeting, Lyon, 2017.

  

The treatment strategy for canine primary pulmonary carcinoma (PPC) mainly varies according to anatomic location, stage and histologic type. Surgical resection represents the recommended treatment of primary lung tumours and it is currently the only curative-intent approach. However, surgical excision is not always feasible or indicated because of the precise intrapulmonary location, invasion of neighbouring organs, or the presence of nodal or distant metastases, the latter occurring in 13.5%-23.1% of dogs at diagnosis.[7] Therefore, there is a significant unmet medical need to control tumour growth in dogs with advanced (unresectable or metastatic) PPC.

Conventional maximum-tolerated dose (MTD) chemotherapy has been used as a treatment for unresectable PPC with variable results. In 1 study, 2 of 7 dogs with macroscopic lung tumours treated with vinorelbine experienced >50% reduction in tumour volume.[8] More recently, vinorelbine was administered as primary treatment or following other chemotherapeutic agents in 16 dogs with macroscopic disease. Three dogs experienced partial remission (PR), 7 stable disease (SD) and 6 had progressive disease (PD). Overall median time to progression (TTP) and ST of 55 and 92 days, respectively, were reported.[9] Other drugs, including doxorubicin and mitoxantrone, have been described in the treatment of PPC with some anti-tumour activity.[10,11] However, study design, low case numbers and heterogeneity of patients and tumours limit extrapolation to the population of dogs diagnosed with PPC at large.

In the landscape of anti-tumour therapy, metronomic chemotherapy (MC) has gained traction recently as an attractive treatment modality due to its favourable toxicity profile and ease of administration in comparison to traditional MTD chemotherapy.

MC refers to the practice of administering cytotoxic drugs without prolonged drug-free breaks and at doses significantly lower than MTD, with the therapeutic outcome of both anti-angiogenic and immune-modulatory effects.[12]

In veterinary oncology, MC has been mainly used in a palliative setting. There is heterogeneity among reported treatment protocols qualifying as MC in terms of the choice of cytotoxic agents and supporting therapies used.[13–20] Similarly, a range of different cancer contexts have been used as the substrate for testing these various treatment protocols, including soft tissue sarcoma, haemangiosarcoma, osteosarcoma and transitional cell carcinoma.[13,14,17,18,20] Treatment of macroscopic tumours occasionally achieves measurable improvement in tumour dimensions with 3%-11% of cases achieving PR or complete remission (CR).[15–17] However, consistent with the proposed method of action of MC, establishment of a stable or minimally progressive state may instead be achieved and is reported in 30%-67% of cases treated with macroscopic tumour.[15–17] This is further supported by evidence relating to adjuvant therapy for microscopic disease in haemangiosarcoma and incompletely excised soft tissue sarcomas.[14,20,21] No advantage was seen in cases of osteosarcoma given MC following amputation and conventional chemotherapy.[18]

Drugs that are commonly used in metronomic regimens include, among others, cyclophosphamide and piroxicam.[13,14,18] There are fewer reports of thalidomide use in dogs, either used as a single agent or as part of a metronomic regimen in combination with cyclophosphamide and piroxicam. These reports have shown a favourable toxicity profile and some anti-tumor activity.[20,22–24]

This manuscript describes a prospectively enrolled cohort of dogs treated at a single centre (Centro Oncologico Veterinario) with MC as first-line therapy for advanced PPC: outcomes evaluated included TTP, ST and health-related quality-of-life (HRQoL). In an effort to provide the reader with an approximate comparator population of patients, by which to judge the response to MC, data were also retrospectively recovered from dogs with advanced PPC treated contemporaneously with other modalities from the same and other Specialist veterinary hospital archives.

## 2 | MATERIALS AND METHODS

### 2.1 | Case selection

This study reports outcomes for canine patients with advanced PPC. All patients were initially presented between January 2011 and January 2017 to 1 of 5 institutions (Centro Oncologico Veterinario, Sasso Marconi, Italy; North Downs Specialist Referrals, Surrey, UK; Small Animal Teaching Hospital, University of Liverpool; Division of Radiation Oncology, Vetsuisse-Faculty, University of Zurich, Switzerland; Centro Veterinario Torinese, Turin, Italy). Eligibility for inclusion required that dogs were presented with previously untreated, clinically advanced, cytologically or histologically confirmed PPC that underwent baseline staging consisting of haematology, biochemistry, urinalysis and total-body computed tomography (TBCT) or 3-view thoracic radiographs. Clinical stage criteria were adapted from the WHO TNM recommendations for lung tumours (Appendix A).[25] With regards to lymph nodes, only those that were enlarged or that showed a contrast enhancement pattern on CT were sampled.

PPC was considered "advanced" if clinical stage evaluations indicated stage T3 or N1 or M1, or if imaging revealed insufficient apparently normal bronchial tissue between tumour and trachea for lobectomy to be safely performed.

Information concerning signalment, methodology of diagnosis, type of carcinoma, site of origin, longest diameter of the primary pulmonary lesion, clinical stage, site of metastasis, treatment, response to treatment, TTP, ST and cause of death were retrieved from the clinical records. TTP was calculated from the date of diagnosis to the date of first-documented progression or death if PD was not recorded. ST was defined as the time interval between diagnosis and death or last follow-up contact.

Cases were excluded if they had other known advanced cardiac, hepatic and/or renal diseases or other conditions with the potential of being life-threatening; moreover cases were excluded if follow-up information was unavailable.

### 2.2 | Metronomic chemotherapy group

MC was administered orally and consisted of low-dose cyclophosphamide (10 mg/m$^2$ q24 h or q48 h; Endoxan, Baxter s.r.l., Lurago d'Erba, Como, Italy), piroxicam (0.3 mg/kg q24 h; Piroxicam, Pfizer Italia s.r.l., Latina, Italy) and thalidomide (2 mg/kg q24 h; Talidomide, Fagron

Italia s.r.l., Quarto Inferiore, Bologna, Italy); MC drugs were compounded for individual patients. Owners administering thalidomide were comprehensively informed of its known teratogenic effect. Dogs receiving MC were considered eligible if they did not receive concurrent MTD chemotherapy.

A HRQoL questionnaire was applied during the study based on investigators' clinical expertise and this was assessed at baseline (before starting MC) and during treatment (after 1 month, 2 months and every 3 months thereafter; Appendix B).

Disease evaluation by thoracic radiography, haematology, biochemistry and urinalysis were performed every 6 to 8 weeks during the first 6 months of treatment, and every 3 months thereafter. Response was defined according to cRECIST criteria into CR, PR and SD, which, for this study, was required to last more than 4 weeks. Response rate (RR) was defined as the sum of all patients achieving CR and PR. Clinical benefit rate (CB) was defined as the sum of all patients achieving CR, PR and SD.[26]

Adverse events were recorded according to the Veterinary Cooperative Oncology Group (VCOG) guidelines.[27]

All MC cases were recruited through the Centro Oncologico Veterinario because of the difficulties in obtaining thalidomide supplies for some institutions and the restrictions in force for centres based in the United Kingdom.

### 2.3 | Other clinical groups

Data from dogs with advanced PPC that were treated at any of the participating centres by surgery, MTD chemotherapy or no oncologic treatment were retrieved for presentation as comparator populations for outcome analyses. Information concerning outcome was essential for inclusion.

For dogs receiving MTD chemotherapy, response, CB and adverse events were assessed as previously described.

### 2.4 | Statistical analysis

Descriptive statistics were used in the analysis of dogs and tumour characteristics. When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. Values were expressed as mean ± SD in case of normal distribution, or as median with a range in case of non-normal distribution. The $\chi^2$ test and Fisher exact probability text were applied to evaluate differences in stage distribution among treatment groups.

Differences in the total and partial HRQoL scores at baseline and during MC were assessed with paired Student's *t*-test.

The influence of the received treatment and of other potential prognostic variables (tumour size, location, stage, T, N, M, substage, pleural effusion and treatment) on TTP and ST was investigated with univariable and multivariable Cox regression analyses.

To be included in the analysis of prognostic factor, variables had to be recorded for 70% or more of the cases.

Hazard ratios for potential risk factors were calculated by forward stepwise Cox regression model, with time-independent variables. Confidence intervals (95%) were calculated for hazard ratios. The variables with values of $P < .05$ in univariate analysis were selected for the multivariable model. Survival curves were generated according to the Kaplan-Meier product-limit method. Survival estimates are presented as medians with the corresponding 95% confidence intervals (95% CI).

For survival analysis, dogs that died of tumour-related or -unrelated causes (ST) or with documented PD (TTP) were considered as events. Dogs were censored if they were alive (ST) or had no documented PD (TTP) at the end of the study period.

Statistical analysis was performed with SPSS Statistics v.19 (IBM, New York, United States). Significance was set at $P < .05$.

## 3 | RESULTS

### 3.1 | Dogs and tumour characteristics

Ninety-one dogs matched the inclusion criteria and were included: 35 dogs from the Centro Oncologico Veterinario, 26 from North Downs Specialist Referrals, 17 from the Small Animal Teaching Hospital of Liverpool University, 10 from the Division of Radiation Oncology of the Vetsuisse-Faculty of Zurich University and 3 from the Centro Veterinario Torinese. Mixed-breed dogs predominated (*n* = 35; 38.4%), followed by Dobermann Pinscher (*n* = 7; 7.7%), Boxer (*n* = 6; 6.6%), English Springer Spaniel (*n* = 4; 4.4%), Border Collie (*n* = 3; 3.3%), Labrador (*n* = 3; 3.3%), Staffordshire bull terrier (*n* = 3; 3.3%), Bernese mountain dogs (*n* = 2; 2.2%), Cocker Spaniel (*n* = 2; 2.2%), Jack Russell Terrier (*n* = 2; 2.2%) and 1 each of Airedale Terrier, Beagle, Belgian Shepherd, Bull Mastiff, Cavalier King Charles Spaniel, Chihuahua, Dogo Argentino, English Setter, Fox Terrier, German Shepherd, German Shorthaired Pointer, Golden Retriever, Lurcher, Miniature Poodle, Miniature Schnauzer, Old English Sheepdog, Parson Russell Terrier, Pug, Segugio Italiano, Spitz, Standard Poodle, Weimaraner, West Highland White Terrier and Yorkshire Terrier. There were 55 (60.4%) females (24 spayed) and 36 (39.6%) males (19 neutered). Median age was 11 years (range, 4-16 years), and median weight was 23.4 kg (range, 3.7-50.4 kg).

Seventy-one (78%) dogs were symptomatic at presentation; coughing was the most common clinical sign (95.8% of cases), followed by haemoptysis (7%), panting (4.2%), exercise intolerance (2.8%) and weight loss (2.8%). For those dogs for which the information was reported, duration of clinical signs ranged from 2 weeks to 6 months, and the severity ranged from mild to severe. In five (5.5%) dogs PPC was an incidental finding. For the remaining 15 (16.5%) dogs, presenting clinical signs were unavailable.

Sixty-seven (84.6%) dogs underwent TBCT, 5 (5.5%) underwent thoracic CT and abdominal ultrasound, whereas in 19 (20.9%) thoracic radiography and abdominal ultrasound were performed.

Carcinomas arose in the lung periphery in 54 (59.3%) dogs (right caudal lobe, *n* = 18; left cranial lobe, *n* = 11; left caudal lobe, *n* = 7; accessory lobe, *n* = 7; right cranial lobe, *n* = 3; not specified, *n* = 8) and near the hilus in 19 (20.9%) dogs. For 18 (19.8%) dogs data concerning tumour location were unavailable.

Forty-four (48.4%) dogs had cytological or histopathological evidence of tracheobronchial lymph node metastases (N1); 46 (50.5%)

dogs had pulmonary metastases indicated by the presence of smaller pulmonary nodules in addition to the primary mass (M1). Twelve (13.2%) dogs had pleural effusion and 2 (2.2%) dogs had pericardial effusion (T3). The TNM staging for all dogs grouped according to treatment is summarized in Table 1.

Forty-eight (52.7%) dogs had a cytological diagnosis, whereas 43 (47.3%) dogs had a histopathological diagnosis ($n$ = 19 papillary carcinoma; $n$ = 13 adenocarcinoma; $n$ = 6 broncho-alveolar carcinoma; $n$ = 2 adenosquamous carcinoma; $n$ = 2 squamous cell carcinoma; $n$ = 1 poorly differentiated carcinoma). All dogs underwent US-guided or CT-guided sampling of the PPC during the initial staging work-up.

## 3.2 | Metronomic chemotherapy group

Twenty-five dogs received MC. Overall, treatment was well tolerated: 8 (32%) dogs developed grade 1 ($n$ = 3) or 2 ($n$ = 5) gastrointestinal toxicity consisting of decreased appetite and/or vomiting, and 1 (4%) dog developed grade 1 lethargy. Notably, 1 dog (4%) had treatment discontinued due to grade 3 renal toxicity, occurring 1009 days after starting MC. This dog eventually died with his PPC still considered stable after 1088 days. Autopsy was not permitted.

According to the owners, QoL was improved in 21 of the 23 symptomatic dogs (91.3%). Based on the questionnaire results, overall HRQoL score was significantly improved in dogs undergoing MC compared with baseline assessment ($P$ < .001). A statistically significant improvement was also recorded for each of the 5 individual parameters of the questionnaire (behaviour, activity appetite and pain, $P$ = .001; Table 2).

Concerning anti-tumour response, 4 dogs (16%) obtained PR, 19 dogs (76%) had SD (median response duration: 99 days; range: 28-1088) and 2 dogs (8%) progressed. RR was 16%; CB rate was 92%. Median TTP was 172 days (95% CI, 44-300).

Twenty dogs died due to their cancer ($n$ = 18; 88%), its treatment ($n$ = 1; 4%) or cancer-unrelated causes ($n$ = 1; 4%) during the study period, whereas 5 (20%) were alive at data analysis closure with a median follow-up of 351 days (range, 62-1088 days). Overall median ST was 139 days (95% CI, 0-282.6).

**TABLE 2** Results of a health-related quality-of-life questionnaire in 25 dogs with advanced primary pulmonary carcinoma at baseline and during metronomic chemotherapy

| Parameters | Baseline score (mean ± SD) | Score during MC (mean ± SD) | $P$ |
|---|---|---|---|
| Behaviour | 2.3 ± 0.6 | 1.4 ± 0.6 | <.001 |
| Activity | 2.5 ± 0.5 | 1.5 ± 0.6 | <.001 |
| Appetite | 2.1 ± 0.5 | 1.4 ± 0.6 | <.001 |
| Pain | 2.6 ± 0.5 | 1.4 ± 0.6 | <.001 |
| Total | 19.4 ± 2.3 | 11.7 ± 3.6 | <.001 |

Abbreviation: MC, metronomic chemotherapy.

## 3.3 | Surgery group

Thirty-six dogs underwent surgical excision of the PPC as their sole treatment. Six dogs were euthanised under anaesthesia during surgery, 1 dog died 1 day post-operatively, another dog was euthanised after 10 days. Reasons for euthanasia included transpleural infiltration (3), thoracic carcinomatosis (1), carcinoma progression within bronchus beyond carina (1) and not recorded (1).

Median TTP was 87 days (95% CI, 48.4-125.6).

All dogs were dead at data analysis closure (34 for tumour-related and 2 for tumour-unrelated causes). Median ST was 92 days (95% CI, 61.1-122.9).

The median TTP and ST of the 28 dogs that survived surgery and the immediate post-operative period were 110 and 111 days, respectively.

## 3.4 | MTD chemotherapy group

Eleven dogs received MTD chemotherapy as sole treatment. Eight dogs received vinorelbine (15-18 mg/m$^2$ once weekly), 2 received carboplatin (300 mg/m$^2$ once three-weekly), and 1 received gemcitabine (800 mg/m$^2$ once weekly). One dog treated with vinorelbine

**TABLE 1** TNM staging for 91 dogs with primary pulmonary carcinoma stratified by treatment groups

| | Metronomic chemotherapy ($n$ = 25) | Surgery ($n$ = 36) | MTD chemotherapy ($n$ = 11) | No oncologic treatment ($n$ = 19) | $P$ |
|---|---|---|---|---|---|
| Stage | | | | | <.001 |
| 1 | 0 | 0 | 8 | 1 | |
| 2 | 2 | 8 | 2 | 1 | |
| 3 | 23 | 28 | 1 | 17 | |
| T | | | | | .732 |
| T1 | 6 | 6 | 3 | 3 | |
| T2 | 7 | 7 | 2 | 7 | |
| T3 | 12 | 23 | 6 | 9 | |
| N | | | | | .202 |
| N0 | 16 | 18 | 6 | 6 | |
| N1 | 9 | 18 | 5 | 13 | |
| M | | | | | <.001 |
| M0 | 7 | 29 | 5 | 5 | |
| M1 | 18 | 7 | 6 | 14 | |

Abbreviations: M, metastasis; MTD, maximum-tolerated dose; N, node; T, tumour.

obtained PR; this dog, with 13 doses, was the only patient who received more than 4 chemotherapy treatments. Three dogs remained stable, and 7 experienced PD. RR was 9%; CB rate was 36.4%. Two dogs experienced haematological toxicity (1 grade 1 neutropenia and 1 grade 4 neutropenia), 1 experienced grade 2 gastrointestinal toxicity (decreased appetite and vomiting) and 3 experienced grade 2 lethargy.

All dogs died due to their cancer. Median TTP and ST were 22 (95% CI, 0-52) and 61 days (95% CI, 0-124), respectively.

### 3.5 | No oncologic treatment group

Nineteen dogs received no oncologic treatment. Dogs were treated palliatively with prednisone ($n$ = 12), meloxicam ($n$ = 6) or piroxicam ($n$ = 1). According to the owners, 6 dogs experienced a slight symptom improvement, 5 of which received prednisone ($n$ = 4) or meloxicam ($n$ = 2), while the others progressed. Median TTP and ST were 20 (95% CI, 10.0-30.0) and 60 days (95% CI, 45.9-74.1), respectively.

### 3.6 | Outcome comparisons

There were no significant differences in T-stage and presence of lymph node metastasis among treatment groups ($\chi^2$, $P$ = 0.732 and 0.202, respectively). However, the frequency of distant metastases was higher in the metronomic and no treatment groups, and stage 3 tumours were less numerous in the MTD chemotherapy group ($P$ < .001; Table 1).

Both TTP and ST were significantly different between the MC group and each of the other 3 groups ($P$ < .001; Figures 1 and 2). In particular, dogs not receiving MC showed a 2.6 increased risk of tumour progression (95% CI, 1.5-4.5; $P$ < .001) and a 2.7 increased risk of death (95% CI, 1.6-4.7; $P$ < .001) (Tables 3 and 4).

Beside treatment, the presence of nodal metastases was the only parameter significantly associated with both TTP (HR = 1.8; 95% CI 1.1-2.8; $P$ = .011) and ST (HR = 1.9; 95% CI = 1.2-3.0; $P$ = .006) at univariable analysis (Tables 3 and 4). At multivariable survival analysis, only treatment retained prognostic significance, with the dogs not receiving MC showing a 1.7 increased risk of tumour progression (95% CI, 1.3-2.1; $P$ < .001) and a 1.5 increased risk of death (95% CI, 1.2-1.9; $P$ < .001) (Tables 5 and 6).

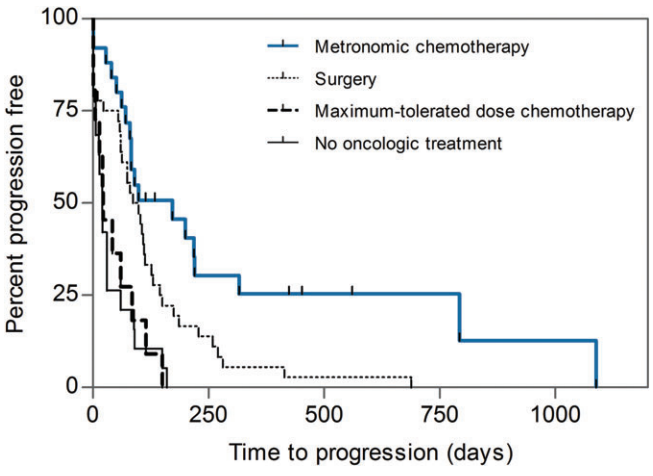


**FIGURE 1** Time to progression for 91 dogs with advanced primary lung carcinoma stratified by the received treatment. There are significant differences between the metronomic chemotherapy group and each of the other 3 groups (surgery: $P$ = .0383; maximum-tolerated dose chemotherapy: $P$ = .0009; no oncologic treatment: $P$ < .0001). Small vertical tick-marks indicate individual dogs with no disease progression at the end of the study period

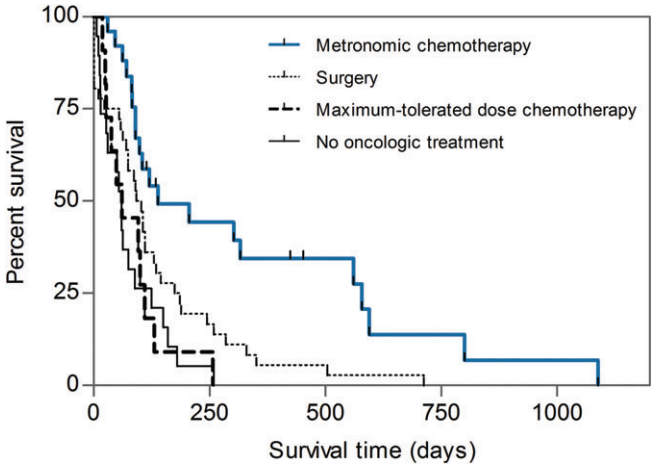


**FIGURE 2** Survival time for 91 dogs with advanced primary lung carcinoma stratified by the received treatment. There are significant differences between the metronomic chemotherapy group and each of the other 3 groups (surgery: $P$ = .0072; maximum-tolerated dose chemotherapy: $P$ = .0029; no oncologic treatment: $P$ < .0001). Small vertical tick-marks indicate individual dogs that were still alive at the end of the study period

## 4 | DISCUSSION

Clinically advanced PPC continues to be an incurable disease with a poor prognosis. This study evaluated the application of MC in this challenging clinical context. Although the value of surgery in cases of solitary PPC is undoubted, ST following surgery in cases with advanced disease has been consistently poor with reported median STs ranging from 26 to 60 days.[2,4,25] A comparable ST following surgery was even reported in cases without metastasis but classified as T3, with a median ST of 23 days according to 1 study.[25] Median ST of 92 days for the 36 dogs treated by surgery in the current study was broadly comparable with these prior data. A number of cytotoxic agents, including vinorelbine, doxorubicin and mitoxantrone, have been shown to exert some anti-tumour activity in advanced PPC and reported outcomes are similar to those following surgery.[8–11] The largest cohort reported in a single study described PR in 3 of 16 cases receiving vinorelbine for measurable disease, with a median TTP of 55 days and ST of 92 days for the whole group.[9] These results compare favourably with our findings though the margins of difference are small.

MC has shown favourable tumour responses in many tumours.[13,14,16,17,20,24] MC is not expected to cause potent cytotoxic activity against tumour cells. Rather, therapeutic advantage is thought to be gained by anti-angiogenic effects. By targeting this non-neoplastic, genomically stable cell target, positive responses can theoretically be sustained over a long period.[28,29]

**TABLE 3** Univariate cox regression analysis of variables potentially associated with increased risk of tumour progression in 91 dogs with advanced primary lung carcinoma

| Variable | No. of dogs | TTP (95% ci) | HR (95% ci) | *P* |
|---|---|---|---|---|
| Tumour size[a] | | | 1.6 (0.9-2.6) | .061 |
| ≤6.8 cm | 37 | 90 (50-130) | | |
| >6.8 cm | 36 | 60 (48-72) | | |
| Tumour location | | | 1.1 (0.6-1.8) | .779 |
| Peripheral | 54 | 60 (27-93) | | |
| Hilar | 19 | 80 (67-93) | | |
| Stage | | | 1.2 (0.7-2.1) | .526 |
| 1-2 | 15 | 80 (46-114) | | |
| 3 | 76 | 71 (50-92) | | |
| T | | | 1.3 (0.8-1.9) | .291 |
| T1-T2 | 41 | 63 (34-92) | | |
| T3 | 50 | 83 (34-132) | | |
| N | | | 1.8 (1.1-2.8) | .011* |
| N0 | 46 | 87 (51-122) | | |
| N1 | 45 | 60 (35-84) | | |
| M | | | 1.2 (0.8-1.8) | .489 |
| M0 | 46 | 87 (59-115) | | |
| M1 | 45 | 60 (18-102) | | |
| Substage | | | 2.0 (0.7-5.5) | .184 |
| a | 5 | 219 (90-348) | | |
| b | 71 | 74 (53-95) | | |
| Pleural effusion | | | 1.4 (0.8-2.7) | .283 |
| No | 60 | 74 (51-97) | | |
| Yes | 13 | 60 (0-125) | | |
| Treatment | | | 2.6 (1.5-4.5)[b] | <.001* |
| Metronomic chemotherapy | 25 | 172 (44–300) | | |
| Surgery | 36 | 87 (44-130) | | |
| MTD chemotherapy | 11 | 22 (0-52) | | |
| No oncologic treatment | 19 | 20 (10-30) | | |

Abbreviations: a, not symptomatic; b, symptomatic; CI, confidence interval; HR, hazard ratio; M metastasis; TTP, time to progression; MTD, maximum-tolerated dose.

[a] Median value used as cut-off.

[b] Hazard ratio for dogs not receiving metronomic chemotherapy compared with dogs receiving metronomic chemotherapy.

*Significant.

**TABLE 4** Univariable cox regression analysis of variables potentially associated with increased risk of tumour-related death in 91 dogs with advanced primary lung carcinoma

| Variable | No. dogs | MST (95% ci) | HR (95% ci) | *P* |
|---|---|---|---|---|
| Tumour size[a] | | | 1.5 (0.9-2.5) | .088 |
| ≤6.8 cm | 37 | 139 (85-193) | | |
| >6.8 cm | 36 | 75 (47-103) | | |
| Tumour location | | | 1.2 (0.7-2.1) | .472 |
| Peripheral | 54 | 90 (61-119) | | |
| Hilar | 19 | 105 (74-130) | | |
| Stage | | | 1.2 (0.7-2.2) | .482 |
| 1-2 | 15 | 92 (46-137) | | |
| 3 | 76 | 90 (71-109) | | |
| T | | | 1.2 (0.8-1.9) | .337 |
| T1-T2 | 41 | 82 (58-106) | | |
| T3 | 50 | 105 (85-125) | | |
| N | | | 1.9 (1.2-3.0) | .006* |
| N0 | 46 | 105 (78-132) | | |
| N1 | 45 | 83 (60-106) | | |
| M | | | 1.2 (0.8–1.8) | .485 |
| M0 | 46 | 100 (77-123) | | |
| M1 | 45 | 89 (70-108) | | |
| Substage | | | 2.5 (0.9-7.0) | .083 |
| a | 5 | 595 (154-1036) | | |
| b | 71 | 96 (80-112) | | |
| Pleural effusion | | | 1.5 (0.8-2.9) | .186 |
| No | 60 | 99 (77-121) | | |
| Yes | 13 | 89 (24-154) | | |
| Treatment | | | 2.7 (1.5-4.7)[b] | <.001* |
| Metronomic chemotherapy | 25 | 139 (0–283) | | |
| Surgery | 36 | 92 (70-114) | | |
| MTD chemotherapy | 11 | 61 (0-124) | | |
| No oncologic treatment | 19 | 60 (46-74) | | |

Abbreviations: a, not symptomatic; b, symptomatic; CI, confidence interval; HR, hazard ratio; M, metastasis; MST, median survival time; MTD, maximum-tolerated dose.

[a] Median value used as cut-off.

[b] Hazard ratio for dogs not receiving metronomic chemotherapy compared with dogs receiving metronomic chemotherapy.

*significant.

Also, MC selectively depletes T regulatory lymphocyte (T-regs) number and impairs their function, thereby exerting an immune-modulating effect.[12]

Several chemotherapeutics have been used in veterinary MC trials, including cyclophosphamide, chlorambucil and lomustine; however, cyclophosphamide was preferred primarily due to the more robust literature in support of this compound.[13–17,20,24,28] The dose of cyclophosphamide used in this study (10 mg/m$^2$) was consistent with those previously reported in other MC clinical articles; however, it was lower than that recommended by the preliminary results of Burton and colleagues.[14,20,28] In their study, in fact, doses lower than 15 mg/m$^2$ were less likely to exert an effect on the T-regs component and, possibly, on microvessel density (MVD). Therefore, the 15 mg/m$^2$ dose was recommended for future MC studies.[28] However, just a small number of tissue samples were examined and the time lapse between treatment initiation and tumour sampling was not reported.[28] Moreover the assumption that lower doses of cyclophosphamide lack an anti-angiogenic effect should not be based on MVD, as this is well known to be an inaccurate means of assessing response to anti-angiogenic therapy.[30] Although a higher dose of cyclophosphamide could have been chosen for the purpose of this study, there are currently no veterinary studies that can suggest the minimally effective biological dose of cyclophosphamide able to inhibit angiogenesis in a specific tumour subtype.

**TABLE 5** Multivariable cox regression analysis of variables potentially associated with increased risk of tumour progression in 91 dogs with advanced primary lung carcinoma

| Variable | HR (95% ci) | *P* |
|---|---|---|
| Tumour size > 6.8 cm[a] | 1.4 (0.8-2.4) | .199 |
| Nodal metastases | 1.2 (0.7-2.2) | .467 |
| Lack of metronomic chemotherapy | 1.7 (1.3-2.1) | <.001* |

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a] Median value used as cut-off.

*Significant.

**TABLE 6** Multivariable cox regression analysis of variables potentially associated with increased risk of tumour-related death in 91 dogs with advanced primary lung carcinoma

| Variable | HR (95% ci) | *P* |
|---|---|---|
| Tumour size > 6.8 cm[a] | 1.3 (0.8-2.2) | .335 |
| Substage b | 2.0 (0.7-5.7) | .208 |
| Nodal metastases | 1.5 (0.8-2.6) | .184 |
| Lack of metronomic chemotherapy | 1.5 (1.2–1.9) | <.001* |

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a] Median value used as cut-off.

*Significant.

Thalidomide has been recently gaining interest in veterinary oncology taking part into MC strategies at doses between 1-3 mg/kg.[20,22–24] Thalidomide's anti-angiogenic effect is believed to occur through the inhibition of vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF) and tumour necrosis factor alpha (TNF-a). Moreover, thalidomide has also the potential of inducing an immunomodulatory effect inhibiting T-regs, enhancing cytotoxic T-cell and natural killer lymphocyte responses and inhibiting interleukin 12.[31] However, such mechanisms have still to be proven in small animal patients and, as a consequence, the optimal metronomic dose of thalidomide remains unknown. Overall, the limited volume of available literature, the eclectic nature of the tumours treated and the heterogeneity of patients and cancer stages make it impossible to draw meaningful conclusions from a literature review, except to say that there is certainly a foundation for further investigation in a broader multiagent MC treatment approach.

Our results suggest that MC, consisting of low-dose cyclophosphamide, piroxicam and thalidomide may be an attractive option in patients with advanced PPC because of its potential in achieving a clinically equivalent, and even superior outcome when compared to surgery, MTD chemotherapy or no oncologic therapy at all.

In the surgery and no oncologic therapy groups, there was no sustained treatment to limit further disease progression so it is easy to accept that a continuing effective therapy would be likely to achieve a superior outcome.

In the MC group dogs achieved a significantly longer ST and TTP than in the MTD chemotherapy group. For most dogs, chemotherapy was continued until death or euthanasia and response rates, according to cRECIST, were comparable among groups. However, CB was not sustained with MTD chemotherapy. This may reflect the difference between the cellular targets of these 2 treatments. As previously mentioned, anti-angiogenic therapy is considered to target the genomically stable endothelial progenitor cells while MTD chemotherapy targets the genomically unstable tumour cells, considered as a more eclectic entity with greater genetic and epigenetic plasticity and a greater readiness to acquire resistance to cytotoxic treatments.[28,29]

In the MC group, there was a disparity between RR (16%) and the proportion of dogs experiencing CB (92%). This is not the first study to report outcomes in response to "cytostatic" as opposed to "cytotoxic" therapy. But this observation certainly merits discussion because it introduces an old paradigm in cancer therapy, which is rarely reported in the scientific literature. The keystone of veterinary cancer therapy is improvement in QoL. This is increasingly pertinent in cases with incurable disease, as exemplified by the advanced PPC reported herein. Importantly, HRQoL was reported to have improved in 21 of the 23 symptomatic dogs receiving MC. However, we cannot entirely exclude that such results may be in part due to the owners' "wishful thinking."

Unfortunately, due to the retrospective data collection, it was not possible to obtain information concerning owners' perception of HRQoL in the dogs receiving MTD chemotherapy, so comparisons cannot be made.

In human oncology, the treatment approach for patients with advanced/metastatic non-small-cell-lung-carcinoma (NSCLC) is individualized and mainly dependent upon specific tumour histological/genetic subtypes.[32] Moreover, the treatment approach may even vary with time, depending on disease progression and further biopsy results. The treatment strategy takes also into account the age of the patient, performance status, presence of co-morbidities and the patient's preferences.[32] There is also a strong emotional component affecting the decision of patients in undergoing or not a proposed treatment; for example, patients who have smoked may feel guilty after diagnosis and more pessimistic about their illness and likely outcomes, all of which may have adverse implications for HRQoL. Overall, management of a patient with advanced/metastatic NSCLC may vary from a multimodal approach including palliative systemic chemotherapy, tyrosine kinase inhibitors or monoclonal antibodies, surgery and radiation to palliative single agent oral MC.[32] Oral vinorelbine represents the first MC choice for patients with NSCLC. This has shown to be a safe and effective treatment regimen for elderly patients with advanced NSCLC who are not candidates for systemic intravenous chemotherapy or multimodal approaches. However, the mechanism of action of vinorelbine in a MC setting is much less clear than for cyclophosphamide and much less predictable than for thalidomide. Concerning metronomic cyclophosphamide, this has not been widely used in advanced NSCLC; however, a recent study comparing patients with advanced NSCLC receiving radiation and MC cyclophosphamide vs radiation only has shown a small but significant survival advantage if MC cyclophosphamide was added.[33] Conversely, it should be mentioned that several randomized controlled clinical trials comparing MTD chemotherapy/chemoradiotherapy with or without thalidomide did not produce consistent results, questioning the role of thalidomide in the management of NSCLC.[34] However, we are not aware of a study combining MC cyclophosphamide and thalidomide in humans.

Besides NSCLC, in human oncology, MC regimes are showing therapeutic equivalence or even superiority in progression-free

survival over MTD chemotherapy strategies.[35] MC is even recommended as a first-line therapy in metastatic cancer, geriatric patients or other significant co-morbidities, supporting the choice of exploring the role of MC in the management of advanced canine PCC.[36]

In the context of advanced cancer, when durable response to therapy is unlikely, safety issues may assume greater importance. Perioperative morbidity and mortality were a significant factor in the poor outcome for dogs undergoing surgery. Notably, 8 (22.9%) of the 36 dogs undergoing surgery died or were euthanised in the postoperative period (within 10 days).

Morbidity was less significant in the MTD chemotherapy group, with only 1 dog out of 11 experiencing severe toxicity (grade 4 neutropenia).

Among dogs receiving MC there was a comparable incidence of severe toxicity: grade 3 renal toxicity developed in 1 dog, eventually leading to euthanasia after 1088 days. It has been previously documented that piroxicam may be nephrotoxic.[37] Nevertheless, in contrast to MTD chemotherapy recipients, significant toxicity developed after a prolonged period of therapy. However, other causing factors than piroxicam could not be excluded.

In human oncology, the addition of thalidomide to MTD chemotherapy protocols has resulted in a higher incidence of severe nonhematologic toxicities (ie, dizziness, constipation, rash and venous thromboembolic events), raising significant concerns on the safety profile of such strategies. Differently, the results of this study and of previous veterinary studies would suggest that thalidomide has a safe toxicity profile even if combined with MC strategies.[34,38] Except for mild transient sedation, which is usually dammed administering the drug in the evening, side effects are usually not reported regardless of the dose or regimen.[20,22–24,39–41] Nevertheless, data on the combination of thalidomide and MTD chemotherapy are lacking and caution should be observed based on human data. There is a responsibility incumbent on the practitioner using thalidomide, to ensure that the caregivers involved in treating their pet are aware of the potential risks of treatment handling and administration. Thalidomide is a powerful human teratogen causing severe and life-threatening birth defects.[42] In many countries, thalidomide is not available to veterinary practitioners according to the rules governing drug distribution.

There were several weaknesses in the present study, inherent to its retrospective nature. Stage distribution was not perfectly balanced among treatment groups; this undoubtedly reflects a degree of selection bias. It is easy to comprehend how patients might be differentially directed towards a surgical or a medical treatment pathway when affected by different disease burdens. Surgery is less likely to be recommended for patients with nodal or distant metastasis. This was demonstrated by statistical analysis and is evident on cursory examination of Table 1. However, that bias would be expected to reduce the apparent impact of MC because the MC group had the highest rate of stage 3 cases. Indeed, the positive impact of MC in this patient group may have been underestimated.

Parameters used to measure tumour response were not available for all dogs in the control groups, and there was variability in the diagnostic and follow-up protocols. The comparator populations comprise a historic control group, use of which is less desirable than the randomized prospective allocation of dogs to treatment and control groups. These weaknesses were balanced to some degree by the use of multiple comparator populations receiving different management strategies.

Last, while combination therapy may increase efficacy, it is currently unknown whether the observed therapeutic benefit in the MC group is attributable to an additive or synergistic effect of the 3 drugs given together or solely to the thalidomide. Despite the inconsistencies of data retrieval, multivariable survival analysis demonstrated that treatment was the strongest independent prognostic factor for both tumour progression and survival.

In conclusion, our results suggest that MC it is likely to achieve a high rate of CB in dogs with advanced PCC. This together with the safe toxicity profile, the ease of oral administration and the observed amelioration of QoL may render MC an attractive treatment option for dogs with advanced PPC.

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## APPENDIX A

TNM clinical staging for primary lung tumors (Owen, 1980).

- T1: Solitary tumour surrounded by lung or visceral pleura.
- T2: Multiple tumours of any size.
- T3: Tumour invading neighbouring tissues.
- N0: No evidence of lymph node involvement.
- N1: Neoplastic lymph node enlargement.
- M0: No evidence of metastases.
- M1: Metastases present.

## APPENDIX B

Questionnaire for evaluating health-related quality-of-life in 25 dogs with primary pulmonary carcinoma receiving metronomic chemotherapy. Parameters included behaviour (questions 1, 4 and 7), activity (questions 2 and 8), appetite (question 5) and pain (questions 3 and 6). The

questionnaire was scored at baseline and after treatment, and differences were statistically assessed.

1. How much attention is your dog giving to the family?
   a. Totally indifferent (3 points)
   b. Decreased attention (2 points)
   c. Attention has not changed (1 point)

2. Is your pet still active?
   a. My pet lays in one place all day long (3 points)
   b. Occasionally (2 points)
   c. My pet moves and plays in a normal way (1 point)

3. How is your dog sleeping?
   a. Very badly/not sleeping at all (3 points)
   b. Intermittently (2 points)
   c. Normally (1 point)

4. Does your dog keep its hygienic habits (ie, does your dog clean itself)?
   a. No (3 points)
   b. Less than before (2 points)
   c. Yes (1 point)

5. Does your dog have an appetite?
   a. No (3 points)
   b. Little, it needs to be forced (2 points)
   c. Normal (1 point)

6. How is your dog's breathing?
   a. As before the start of treatment/worse (3 points)
   b. Improved (2 points)
   c. Normal (1 point)

7. How is your dog's mood?
   a. Totally altered (3 points)
   b. A bit depressed (2 points)
   c. Normal (1 point)

8. Does your dog get tired easily?
   a. Yes, always (3 points)
   b. Frequently (2 points)
   c. No (1 point)