DOI: 10.1111/vco.12519

Adjuvant anthracycline-based versus metronomic chemotherapy versus no medical treatment for dogs with metastatic splenic hemangiosarcoma: a multi-institutional retrospective study of the xxxx

Marconato, L.[1], Chalfon, C.[2], Finotello, R.[3], Polton, G.[4], Vasconi, M.E.[5], Annoni, M.[6], Stefanello, D.[7], Mesto, P.[8], Capitani, O.2, Agnoli, C.2, Amati, M.[9], Sabattini, S.[2]

[1]Centro Oncologico Veterinario, Bologna, Italy

[2]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

[3]Department of Small Animal Clinical Science, Institute of Veterinary Science, University of Liverpool, Neston, UK

[4]North Downs Specialist Referrals, Bletchingley, UK

[5]Centro Veterinario Torinese, Turin, Italy [6]Clinica Veterinaria Tibaldi, Milan, Italy

[7]Department of Veterinary Medicine, University of Milan, Milan, Italy

[8]Centro Medico Veterinario BMVet, Bari, Italy

[9]Ospedale Veterinario Città di Pavia, Pavia, Italy

Correspondence

Laura Marconato, Centro Oncologico Veterinario, via San Lorenzo 1/4, 40037 Sasso Marconi, Bologna, Italy.
Email: lauramarconato@yahoo.it

Keywords: dog, hemangiosarcoma, maximum tolerated chemotherapy, metastasis, metronomic chemotherapy, prognosis

Abstract

Treatment options for dogs with metastatic (stage III) splenic hemangiosarcoma are limited. A doxorubicin-based chemotherapy regimen is commonly administered; however, there are no published data to support this practice. The aim of this study was to investigate the impact of maximum-tolerated-dose chemotherapy (MTD), metronomic chemotherapy (MC) and no adjuvant treatment on outcome in dogs with stage III splenic hemangiosarcoma undergoing splenectomy. Medical records of dogs with stage III splenic hemangiosarcoma that underwent splenectomy followed by MTD chemotherapy, MC or no adjuvant treatment were retrieved. Time to progression (TTP), survival time (ST) and toxicity were evaluated. One hundred three dogs were identified: 23 received adjuvant MTD, 38 MC, and 42 were not medically treated. Overall median TTP and ST were 50 (95% CI, 39-61) and 55 days (95% CI, 43-66), respectively. Dogs treated with adjuvant MTD had a significantly longer TTP and ST compared with dogs receiving MC (median TTP, 134 versus 52 days, P=0.025; median ST, 140 versus 58 days, P=0.023, respectively). Dogs treated by splenectomy only had the shortest median TTP (28 days) and ST (40 days). However, treatment-related adverse events (AEs) were significantly more frequent in the MTD group (P=0.017). The

outcome for dogs with metastatic splenic hemangiosarcoma is poor. While MTD showed greater efficacy compared to MC, toxicity was higher in this group. Treatment-related AEs need to be carefully balanced against this modest survival prolongation when offering adjuvant MTD to dogs with advanced stage hemangiosarcoma.

Introduction

Canine visceral hemangiosarcoma is a highly malignant neoplasm of vascular endothelial cells or bone marrow-derived endothelial progenitors. [1,2.] The most common primary site is the spleen, however hemangiosarcoma can develop at any site having a vascular supply. [1,3-7]

Splenic hemangiosarcoma is characterized by rapid growth and early distant metastasis, with liver, lungs, peritoneum, and central nervous system sites commonly involved.[1,3,8] The mainstay of therapy comprises surgery followed by adjuvant chemotherapy.

Previous studies have shown that dogs with gross metastatic disease at the time of diagnosis (stage III) have shorter survival time (ST) compared with dogs with stage I or II disease. [9-12] Reported median ST of dogs with stage III splenic hemangiosarcoma ranges from 27 to 65 days following splenectomy only.[7,9-10.] The addition of adjuvant maximum-tolerated dose chemotherapy (MTD), including an anthracycline as single agent,[10,12,13] doxorubicin in combination with cyclophosphamide or dacarbazine,[14] or doxorubicin in combination with vincristine and cyclophosphamide,[15,16] resulted in median ST of 62 195 days. Overall, survival remains disappointing and most dogs will eventually succumb to progressive disease.

The use of drug regimens that have been designed to kill as many tumor cells as possible by treating with MTDs of cytotoxic agents have been challenged recently by the use of metronomic chemotherapy (MC).[17-23] The term "metronomic" refers to the scheduling, which consists of chronic,

equally spaced, and generally low doses of single or combined chemotherapeutic drugs without extended drug-free breaks. Evidence exists that MC can extend survival time of dogs with stage II splenic hemangiosarcoma.[24]

At present, only one study included dogs with stage III splenic hemangiosarcoma and described their outcome after splenectomy only and splenectomy with adjuvant MTD or MC.[9] Without adjuvant medical treatment, the reported median ST was 0.9 months. The advantage of adjuvant medical treatment was not clearly deducible from the study.[9] The background prompted us to retrospectively conduct this study with the aim to investigate the outcome and treatment-related toxicity profiles of dogs that underwent splenectomy and that received no adjunctive treatment versus MTD or MC for stage III splenic hemangiosarcoma. It was hypothesized that the use of chemotherapy would be superior to no adjunctive treatment and that MC would be similarly effective to MTD but better tolerated in this population of patients with advanced hemangiosarcoma.

Material and methods

A multi-institutional, retrospective study was carried out. Members of the xxx were asked to search their records from 2011 to 2018 to identify dogs with stage III splenic hemangiosarcoma that underwent splenectomy and for

106 which medical information was sufficient to assess the site of metastatic
107 disease, treatment, treatment response and outcome. Only dogs that were
108 alive at suture removal were included in the study.
109 Eligibility for inclusion required that dogs were presented with histologically-
110 confirmed, surgically-removed splenic hemangiosarcomas and underwent
111 baseline staging within 48 hours of surgery, consisting of physical
112 examination, haematology, serum biochemistry, 3-view thoracic
113 radiographs and abdominal ultrasound or total-body computed
114 tomography (TBCT). Only dogs with stage III (measurable metastases)
115 disease were included. Visceral metastatic disease had to be confirmed
116 cytologically or histologically. Dogs with pulmonary nodules were assumed
117 to have lung metastases based solely on imaging.
118 Follow-up evaluation by thoracic radiography and abdominal ultrasound
119 were performed based on clinician's discretion and owner's preference.
120 Lack of follow-up imaging did not represent an exclusion criterion, as serial
121 imaging could be largely affected by owners' preferences, financial
122 constraints and patients' clinical conditions.
123
124 Outcome data from dogs that were treated by MTD chemotherapy, MC or
125 no adjunctive oncologic treatment were retrieved. Maximum-tolerated dose
126 chemotherapy was defined as the administration of chemotherapy agents
127 at the maximum recommended dose with long drug-free period.
128 Metronomic chemotherapy was defined as the continuous administration of
129 chemotherapy agents at low dose (below the MTD) once daily or every

other day. Treatment duration was not considered an exclusion criterion. Treatment-related adverse events (AEs) were recorded at each patient visit according to the Veterinary Cooperative Oncology Group (VCOG) guidelines.[25]

When assessed, response was defined according to the cRECIST criteria.[26]

Additional information retrieved included signalment, duration and type of symptoms, methodology of imaging, need for blood transfusion, site of metastases, time from splenectomy to initiation of medical treatment and treatment toxicity, follow-up staging results (if any), time to progression (TTP), ST, and cause of death.

Statistical analysis

Continuous data were tested for normality with the D'Agostino and Pearson omnibus normality test. Variables were summarized as mean ± standard deviation in case of normal distribution, or as median and range in case of non-normal distribution.

To assess whether features of the three treatment groups differed at admission, the distribution of possible outcome variables between groups, including duration of symptoms (cut-off arbitrarily set at the median value), blood transfusion (yes/no) and number of metastatic sites (single or multiple) was compared with Fisher's exact test/$\chi 2$ test. The time from splenectomy to adjuvant treatment initiation was compared between MTD and MC groups with the Mann-Whitney *U* test. Fisher's exact test/$\chi 2$ test were also used to

compare the proportion of dogs with treatment-related AE and 6-month survival rates between treatment groups.

TTP was calculated as the interval between splenectomy and the date of first-documented progression; dogs with no progressive disease (PD) at data-analysis closure or death were censored. PD was defined as the appearance of one or more new lesions, at least a 20% increase in the sum of diameters of target lesions and/or clear progression of existing non-target lesions documented via imaging modalities and/or the occurrence of clinical signs identified by physical examination (e.g., hemoperitoneum). Stable disease (SD) was defined as less than 30% reduction or less than 20% increase in the sum of diameters of target lesions or reduction or stable persistence of one or more non-target lesions documented via imaging modalities for at least 4 weeks.[26] ST was calculated as the interval between splenectomy and tumor-related death. Dogs deceased for tumor-unrelated causes or alive at data-analysis closure were censored. Survival plots were generated according to the Kaplan-Meier product-limit method and compared with the log-rank test. Survival estimates were presented as medians with the corresponding 95% confidence interval (CI). The influence of potentially prognostic variables, including duration of symptoms, blood transfusion, number of metastatic sites and treatment on tumor progression and tumor-related death was investigated with univariable Cox's regression analysis. Factors with a P value < 0.1 on univariable analysis were further tested for independence in a multivariable Cox proportional hazard model.

176 Analyses were carried out using a commercial software program (SPSS
177 Statistics v19, IBM, Armonk, NY, USA); the significance level was set at 0.05.
178
179
180 **Results**
181
182 *Dogs and tumors' characteristics*
183 One hundred three dogs were included. Their signalment is summarized in
184 Table 1. At admission all dogs were symptomatic. Reported clinical signs and
185 symptoms included: lethargy/weakness (n=74; 71.8%), abdominal distension
186 (n=33; 32%), loss of appetite (n=20; 19.4%), collapse (n=16; 15.3%), pale
187 mucous membranes (n=8; 7.8%), vomiting (n=4; 3.9%), polydipsia and
188 polyuria (n=2; 1.9%), hyperthermia (n=2; 1.9%), and one (0.9%) each of the
189 following: weight loss, hypothermia, and lameness. The median duration of
190 clinical signs prior to splenectomy was 2 days (range, 1-60 days).
191 Regarding imaging, at the time of splenectomy 73 (70.9%) dogs underwent
192 thoracic radiography and abdominal ultrasonography, 29 (28.2%) had a
193 TBCT scan performed, and 1 (0.9%) dog had only three-view thoracic
194 radiography prior to exploratory laparotomy.
195 Ninety-seven (94.2%) dogs had hemoperitoneum at admission, and 35
196 (33.9%) dogs received a blood transfusion during or after splenectomy.
197 Fifty-four (52.4%) dogs underwent biopsy and 44 (42.7%) dogs underwent
198 fine-needle aspiration cytology of suspicious metastatic lesions. These
199 diagnostic tests confirmed the presence of metastatic disease in all cases.

Five (4.9%) dogs with pulmonary metastases did not undergo cytological nor histological sampling. All dogs had gross metastatic disease left after splenectomy.

Overall, 80 (77.7%) dogs had single-site metastases: hepatic (n=59; 73.7%), omental (n=13; 16.2%), pulmonary (n=5; 6.2%), muscular (n=1; 1.2%), bladder (n=1; 1.2%), and distant nodal (n=1; 1.2%).

Twenty-three (22.3%) dogs had metastases at multiple sites: liver and omentum (n=9; 39.1%); liver and lungs (n=3; 13%); liver, kidney, heart and lungs (n=1; 4.3%); liver, lungs and muscles (n=2; 8.7%); liver, lungs and omentum (n=1; 4.3%); liver, omentum and diaphragm (n=1; 4.3%); liver and skin (n=1; 4.3%); liver and adrenal gland (n=1; 4.3%); liver and heart (n=1; 4.3%); liver and kidney (n=1; 4.3%); lungs and sternal lymph node (n=1; 4.3%); omentum and abdominal wall (n=1; 4.3%).

Adjuvant treatments

Twenty-three (22.3%) dogs received MTD, 38 (36.9%) received MC, and 42 (40.8%) were not medically treated. Treatment groups were well-balanced concerning possible prognostic variables, including duration of symptoms, blood transfusion, number of metastatic sites and time from splenectomy to adjuvant treatment initiation (Table 2).

Among the cases receiving MTD, 17 (73.9%) dogs were treated with doxorubicin as a single agent, 2 (8.7%) dogs with doxorubicin and cyclophosphamide, 1 (4.3%) dog with doxorubicin and dacarbazine, and 3 (13.1%) dogs received epirubicin as single agent. Among dogs receiving

doxorubicin, 16 dogs were treated at 30 mg/m^2 every 3 weeks, while one was treated at 25 mg/m^2. Epirubicin was administered at a dose of 30 mg/m^2 every 3 weeks. Dacarbazine was administrated at 200 mg/m^2 once daily for 5 days, starting on the day of doxorubicin administration. Cyclophosphamide was administered at a dose of 200 mg/m^2 over 4 days, starting on the day of doxorubicin administration. Median time from splenectomy to MTD initiation was 15 days (range, 5 to 30 days), and the median number of treatments administered was 4 (range, 1 to 8). The median duration of MTD was 88 days (range, 21 to 168 days). Four (17.4%) dogs in this group also received MC at the end of the anthracycline-based protocol, consisting of thalidomide, cyclophosphamide and piroxicam in 3 dogs, and cyclophosphamide in one dog. Fourteen of 23 dogs underwent follow-up imaging during treatment: 12 had SD and 2 had PD.

Among cases receiving MC, 33 (86.8%) dogs were treated with thalidomide, cyclophosphamide and piroxicam, 2 (5.3%) with thalidomide and piroxicam, 2 (5.3%) with cyclophosphamide and meloxicam, and 1 (2.6%) with cyclophosphamide and piroxicam. Cyclophosphamide was administered at 10-15 mg/m^2 once daily or every other day, thalidomide at 2-4 mg/kg daily, while meloxicam and piroxicam were given daily at the standard dose. The median time from splenectomy to initiation of MC was 15 days (range, 1 to 45 days). The median duration of MC was 35 days (range, 5 to 421 days). Twelve of 38 (31.6%) dogs underwent follow-up imaging at some point during treatment: 6 had SD and 6 had PD.

Toxicity

Among dogs receiving MTD, 10 (43.5%) experienced AEs. The complete list of AEs recorded for each dog and number of episodes is listed in Table 3. Four dogs needed a dose decrease, causing dose delay. Five dogs (n=2 with febrile neutropenia, and n=3 with severe gastrointestinal symptoms) required hospitalization for the symptomatic treatment of AEs, including intravenous fluids, antibiotics, antiemetic and gastro protectant drugs.

Among dogs receiving MC, 6 (15.8%) experienced AEs (Table 3). None needed hospitalization, dose decrease or treatment interruption.

The number of dogs with treatment-related AEs was significantly higher in the MTD group (P=0.017). When considering only the AEs requiring hospitalization, the difference was still statistically significant (P = 0.005).

Outcome and analysis of prognostic variables

At the end of the study, 101 (98.1%) dogs had died (100 for cancer-related causes, 1 for cancer-unrelated causes) and 2 (1.9%) dogs were still alive after 26 and 81 days. Overall, median TTP and ST were 50 days (95% CI, 39-61) and 55 days (95% CI, 43-66), respectively.

Dogs receiving adjuvant medical treatment had a significantly better outcome compared with dogs treated with splenectomy alone (median TTP, 68 vs 28 days; P < 0.001; median ST, 80 vs 40 days; P < 0.001).

Regarding individual treatment groups, TTP was 134 days (95% CI, 73-194) for dogs treated with MTD and 52 days (95% CI, 32-72) for dogs treated with MC. Survival time was 140 days (95% CI, 123-157) for dogs treated with MTD and

58 days (95% CI, 33-83) for dogs treated with MC. Dogs treated with MTD had a significantly better TTP and ST compared with dogs treated with MC (TTP P = 0.025; ST P = 0.023; Figure 1).

The 6-month survival rate was 0% in the splenectomy alone group and 12% for dogs receiving adjuvant chemotherapy (P = 0.039). The 6-month survival rate was not significantly different between dogs treated with MTD (23%) or MC (6%; P = 0.09).

Beside treatment, an additional variable significantly associated with a higher risk of tumor progression and tumor-related death both in univariable and multivariable survival analysis was symptom duration (Tables 4-6). Patients with clinical signs referable to their tumour that had a duration of greater than two days had a statistically significantly higher hazard of death (HR = 1.8; 95% CI = 1.2-2.8; P = 0.004; Table 4).

Discussion

During the past decade, enormous efforts have been devoted to overcoming the lethality of canine splenic hemangiosarcoma. [10-16, 27-29] However, the effectiveness of doxorubicin-based protocols appears to have reached a plateau, with fewer than 10% of dogs diagnosed with hemangiosarcoma surviving one year after diagnosis.[1] To aggravate clinical research, once hemangiosarcoma has spread to other sites, it has to be considered incurable, whereupon the role of adjuvant treatments is

296 uncertain.[7,9-16] Thus, identifying the population of dogs that may benefit from
297 adjuvant chemotherapy is challenging.
298 Based on the results obtained here, MTD showed greater efficacy compared
299 to MC. Dogs treated with MTD had a median ST of 140 days as opposed to
300 those treated with MC (58 days). The data obtained here are in line with
301 those that have been previously published, with reported median ST ranging
302 between 62 and 195 days after MTD.[9-16]
303 Nevertheless, when considering the proportion of dogs that were alive at 6
304 months, there was no significant difference between the MTD and MC
305 groups, although the small number of cases may have influenced this
306 analysis.
307
308 It has been previously shown that MC may be an alternative treatment for
309 dogs with stage II HSA, yielding comparable results to conventional MTD,[24]
310 thereby challenging the paradigm "the higher, the better" by "the more
311 frequent, the better". MC is actually designed to administer at least the
312 same amount or, more commonly, even a greater amount of drugs, in total,
313 over time.[30,31] In the current study the MC approach did not provide a
314 survival benefit. This might be due to the fact that stage III
315 hemangiosarcoma is rapidly fatal, often not giving sufficient time for the
316 drugs to prove efficacy.[9,29] It may be speculated that in case of stage III
317 hemangiosarcoma, the tumor growth rate may be too fast to allow an
318 efficacious antitumor response to be documented. If this is true, it could be

319 suggested that MC probably does not benefit all stages of splenic
320 hemangiosarcoma.
321
322 While MTD was associated with a prolonged survival. this improved long-term
323 survival rate and prognosis still remain disappointing. It is commonly
324 expected that MTD only achieves palliation in dogs with advanced solid
325 cancer, potentially due to the fast development of resistance[32] however, in
326 this study MTD was associated with a significantly prolonged survival and this
327 data is noteworthy.
328 This improvement in overall survival in dogs treated with MTD was
329 counterbalanced by a significantly higher incidence of treatment-related
330 toxicity when compared to MC, often requiring additional supportive care,
331 including hospitalization, and treatment modifications.
332 In the current series of dogs, the difference in outcome between patients
333 receiving MTD and MC amounted to almost three months. As oncologists
334 with a keen interest in improved survival for defined patient populations, this
335 appears to indicate a meaningful improvement. However, accepting the
336 absence of validated quality of life metrics in this retrospective study, the
337 authors would also like to invite readers to consider the value of these extra
338 82 days. The incidence of AEs was significantly different between groups.
339 Whether this improvement in survival probability satisfactorily exceeds the
340 detrimental impact of increased adverse effect probability is for the
341 individual clinician and owner to decide. It is hoped that the presentation of

342 the data herein will at least enable that conversation to take place with an
343 improved foundation in evidence.
344 The Cox's proportional hazards model identified two covariates to be
345 prognostically significant, use of MC versus MTD, and the presence of clinical
346 signs referable to the underlying disease. Longer duration of clinical signs
347 prior to splenectomy was prognostically favourable. This was contrary to
348 expectation. It had been presumed that the gravity of haemorrhage would
349 be the most significant determinant of outcome in this context Severe
350 hemorrhage would have a peracute history. More limited serial hemorrhages
351 would result in a more chronic history. In view of the studied observation, the
352 authors propose that the more significant biological determinant of
353 outcome is the pathophysiological change that
354 accompanies longer-standing disease. In the context of the study cohort,
355 this chronicity enables further-advanced metastasis.
356 Cancer language classically records extent of disease with a simple clinical
357 stage notation that fails to accommodate an expression for the extent of
358 metastasis yet it is known that marked variations exist. In the context of
359 metastatic and non-metastatic cancers, this variation might appear trivial,
360 but in the context of stage 3 splenic hemangiosarcoma cases only, this
361 variable may assume greater significance. A prospective analysis of stage III
362 hemangiosarcoma cases could more accurately record measurable
363 differences in metastatic burden. Review of such a case series might reveal
364 genuine differences in suitability of patients with differing burdens of
365 metastatic disease for MTD versus MC.

This study has a few limitations, mainly due to its retrospective nature. Information on quality of life could not be accurately captured from the medical records, so only evident AEs were reported.

Also, there was some heterogeneity regarding the medical treatment protocols in both groups. Although the majority of dogs in the MC group received a combination of piroxicam, thalidomide and cyclophosphamide, 5 (13.2%) dogs received 2 drugs only. Similarly, the majority of dogs in the MTD group received doxorubicin as single agent, while a small group received doxorubicin combined with an alkylating agent or epirubicin. Four dogs in this group also received MC after completion of the dose-intense protocol.

Finally, follow-up imaging was not standardized, thereby challenging the assessment of TTP. Dogs receiving MTD chemotherapy were more likely to undergo serial follow-up imaging due to the fact that they survived longer, possibly enhancing owners' compliance. Conversely, dogs that were not medically treated and approximately two thirds of those receiving MC did not undergo serial follow-up imaging, which is not surprising, given that MC allows the administration of treatment with minimal monitoring, thereby reducing the need for hospital visits and, as a consequence, the chance to offer follow-up imaging.

Post mortem examination was not performed in any of the patients making the cause of death only presumptive; however, the authors believe that given the advanced clinical stage and the rapid clinical progression typical

of metastatic HSA, death because of tumour progression/tumour related causes was most likely in these cases.

Last, the relatively small sample size may have led to a type II error.

Nevertheless, the novelty of the study is that it is an endeavor to address the issues of skepticism surrounding adjuvant treatment for this population of dogs. We believe the findings of the current study would add significant values to clinical decision-making.

In conclusion, MTD had AEs for limited benefit, raising the question of whether it can be recommended. When efficacious treatments are no longer options for dogs with terminal cancer, it is the authors' view that the focus should shift from prolonging life to maintaining quality-of-life by sparing unnecessary toxic effects.

References

1. Thamm DH. Hemangiosarcoma. In: *Withrow & MacEwen's Small Animal Clinical Oncology*. Withrow SJ, Vali DM, eds. 5th ed. St Louis, Saunders Elsevier, 2013: 679–688.
2. Lamerato-Kozicki AR, Helm KM, Jubala CM, Cutter GC, Modiano JF. Canine hemangiosarcoma originates from hematopoietic precursors with potential for endothelial differentiation. *Experimental Hematology* 2006; 34:870–878.

3. Brown NO, Patnaik AK, MacEwen EG. Canine hemangiosarcoma: retrospective analysis of 104 cases. *J Am Vet Med Assoc* 1985; 186:56–58.
4. Hammond TN, Pesillo-Crosby SA. Prevalence of hemangiosarcoma in anemic dogs with a splenic mass and hemoperitoneum requiring a transfusion: 71 cases (2003-2005). *J Am Vet Med Assoc* 2008; 232:553-558.
5. Schultheiss PC. A retrospective study of visceral and nonvisceral hemangiosarcoma and hemangiomas in domestic animals. *J Vet Diagn Invest* 2004; 16:522-526.
6. Pintar J, Breitschwerdt EB, Hardie EM, Spaulding KA. Acute nontraumatic hemoabdomen in the dog: a retrospective analysis of 39 cases (1987-2001). *J Am Anim Hosp Assoc* 2003; 39:518-522
7. Johnson KA, Powers BE, Withrow SJ, Sheetz MJ, Curtis CR, Wrigley RH. Splenectomy in dogs. Predictors of neoplasia and survival after splenectomy. *J Vet Intern Med* 1989; 3:160-166.
8. Waters DJ, Hayden DW, Walter PA. Intracranial lesions in dogs with hemangiosarcoma. *J Vet Intern Med* 1989; 3:222-230.
9. Wendelburg KM, Price LL, Burgess KE, Lyons JA, Lew FH, Berg J. Survival time of dogs with splenic hemangiosarcoma treated by splenectomy with or without adjuvant chemotherapy: 208 cases (2001-2012). *J Am Vet Med Assoc* 2015; 247:393-403

436 10. Kim SE, Liptac JM, Gall TT, Monteith GJ, Woods JP. Epirubicin in the
437 adjuvant treatment of splenic hemangiosarcoma in dogs: 59 cases
438 (1997-2004). *J Am Vet Med Assoc* 2007; 231:1550-1557.
439 11. Sorenmo KU, Baez JL, Clifford CA, et al. Efficacy and toxicity of a
440 dose-intensified doxorubicin protocol in canine hemangiosarcoma. *J*
441 *Vet Intern Med* 2004; 18:209-223.
442 12. Matsuyama A, Poirier VJ, Mantovani F, Foster RA, Mutsaers AJ.
443 Adjuvant doxorubicin with or without Metronomic Cyclophosphamide
444 for canine splenic Hemangiosarcoma. *J Am Anim Hosp Assoc* 2017;
445 53:304-312.
446 13. Sorenmo K, Samluk M, Clifford C, et al. Clinical and pharmacokinetic
447 characteristics of intracavitary administration of pegylated liposomal
448 encapsulated doxorubicin in dogs with splenic hemangiosarcoma. *J*
449 *Vet Intern Med* 2007; 21:1347-1354.
450 14. Finotello R, Stefanello D, Zini E, Marconato L. Comparison of
451 doxorubicin with doxorubicin-dacarbazine for the adjuvant treatment
452 of canine hemangiosarcoma. *Vet Comp Oncol* 2017; 15:25-35.
453 15. Hammer AS, Couto CG, Filppi J, Getzy D, Shank K. Efficacy and
454 toxicity of VAC chemotherapy (vincristine, doxorubicine, and
455 cyclophosphamide) in dogs with hemangiosarcoma. *J Vet Intern Med*
456 1991; 5:160-166.
457 16. Alvarez FJ, Hosoya K, Lara-Garcia A, Kisseberth W, Couto G. VAC
458 protocol for treatment of dogs with stage III hemangiosarcoma. *J Am*
459 *Anim Hosp Assoc* 2013; 49:370-377.

17. Leach TN, Childress MO, Greene SN, et al. Prospective trial of metronomic chlorambucil chemotherapy in dogs with naturally occurring cancer. *Vet Comp Oncol* 2011; 10:102-112.
18. Schrempp DR, Childress MO, Stewart JC, et al. Metronomic administration of chlorambucil for treatment of digs with urinary bladder transitional call carcinoma. *J Am Vet Med Assoc* 2013; 242:1534-1538.
19. Elmslie RE, Glawe P, Dow SW. Metronomic Therapy with Cyclophosphamide and Piroxicam Effectively Delays Tumor Recurrence in Dogs with Incompletely Resected Soft Tissue Sarcomas. *J Vet Intern Med* 2008; 22:1373-1379.
20. Tripp CD, Fidel J, Anderson CL, et al. Tolerability of Metronomic Administration of Lomustine in Dogs with Cancer. *J Vet Intern Med* 2011; 25:278-284.
21. Maiti R. Metronomic chemotherapy. *Journal of Pharmacology and* Pharmacotherapeutics 2014; 5:186–192.
22. Madondo M, Quinn M, Plebanski M. Low dose cyclophosphamide: mechanisms of T cell modulation. *Cancer Treat Rev* 2017; 42:3-9.
23. Gaspar TB, Henriques J, Marconato L, Queiroga FL. The use of low-dose metronomic chemotherapy in dogs-insight into a modern cancer field. *Vet Comp Oncol* 2018; 16:2-11.
24. Lana S, U'ren L, Plaza S, et al. Continuous low-dose oral chemotherapy for adjuvant therapy of splenic hemangiosarcoma in dogs. *J Vet Intern Med* 2007; 21:764-769.

25. Veterinary Co-operative Oncology Group. Veterinary Co-operative oncology group-common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy for biological antineoplastic therapy in dogs and cats. *Vet Comp Oncol* 2004; 2:195-231.
26. Nguyen SM, Thamm DH, Vail DM, London CA. Response evaluation criteria for solid tumours in dogs (v1.0): a Veterinary Cooperative Oncology Group (VCOG) consensus document. *Vet Comp Oncol* 2013; 13:176-83.
27. Finotello R, Henriques J, Sabattini S, et al. A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine hemangiosarcoma. *Vet Comp Oncol* 2017; 15:493-503.
28. Alexander CK, Cronin KL, Silver M, Gardner HL, London C. The addition of metronomic chemotherapy does not improve outcome for canine splenic hemangiosarcoma. *J Small Anim Parct* 2018; 32-37.
29. Bray JP, Orbell G, Cave N, Munday JS. Does thalidomide prolong survival in dogs with splenic haemangiosarcoma? *J Small Anim Pract* 2018; 59:85-91.
30. Piccart-Gebhart, M. J. Mathematics and oncology: a match for life? *J. Clin. Oncol* 2003; 21:1425–1428.
31. Kerbek RS, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer* 2004; 4:426-436.
32. Bertolini F, Paul S, Mancuso P, et al. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the

508 mobilization and viability of circulating endothelial progenitor cells.
509 *Cancer Res* 2003; 63:4342-4346.

510
511
512 **Table 1**

513 Signalment of 103 dogs presenting with metastatic splenic
514 hemangiosarcoma.

Breed	Crossbred: n = 34 (32%) German shepherd: n = 20 (19.4%) Labrador retriever: n = 14 (13.6%) Golden retriever: n = 5 (4.9%) Beagle: n = 4 (3.9%) Border collie, English setter: n = 3 each (2.9%) Czechoslovakian wolf dog, Boxer, Cane Corso, Jack Russell: n = 2 each (1.9%) White German shepherd, Airedale terrier, Dachshund, Epagneul Breton, American cocker, Bull mastiff, Irish setter, Pitt bull, Poodle, Rottweiler, Newfoundland, English springer spaniel: n = 1 each (1%)
Sex	Intact male: n = 40 (38.8%) Castrated male: n = 19 (18.4%) Intact female: n = 9 (8.7%) Spayed female: n = 35 (34%)
Age	Median: 10 years Range: 6-15 years
Weight	Median: 30 kg

	Range: 5.7-52.8 kg

Table 2

Baseline characteristics of 61 dogs with metastatic splenic hemangiosarcoma treated with splenectomy plus maximum-tolerated dose chemotherapy or metronomic chemotherapy.

Variable	**MTD** (n = 23)	**MC** (n = 38)	**P**
Duration of symptoms*			0.138
≤2 days	16	18	
>2 days	7	18	
Blood transfusion			0.386
yes	10	13	
no	13	25	
Metastasis			0.111
single site	21	28	
multiple sites	2	10	
Time from splenectomy to adjuvant treatment initiation*			>0.999
≤15 days	13	21	
>15 days	10	17	

521 Abbreviations: MTD, maximum-tolerated dose; MC, metronomic chemotherapy.

522 *Median set as cut-off value.

523

524 **Table 3**

525 Adverse events recorded in 61 dogs presenting with metastatic splenic
526 hemangiosarcoma treated with splenectomy plus metronomic
527 chemotherapy or maximum-tolerated dose chemotherapy.

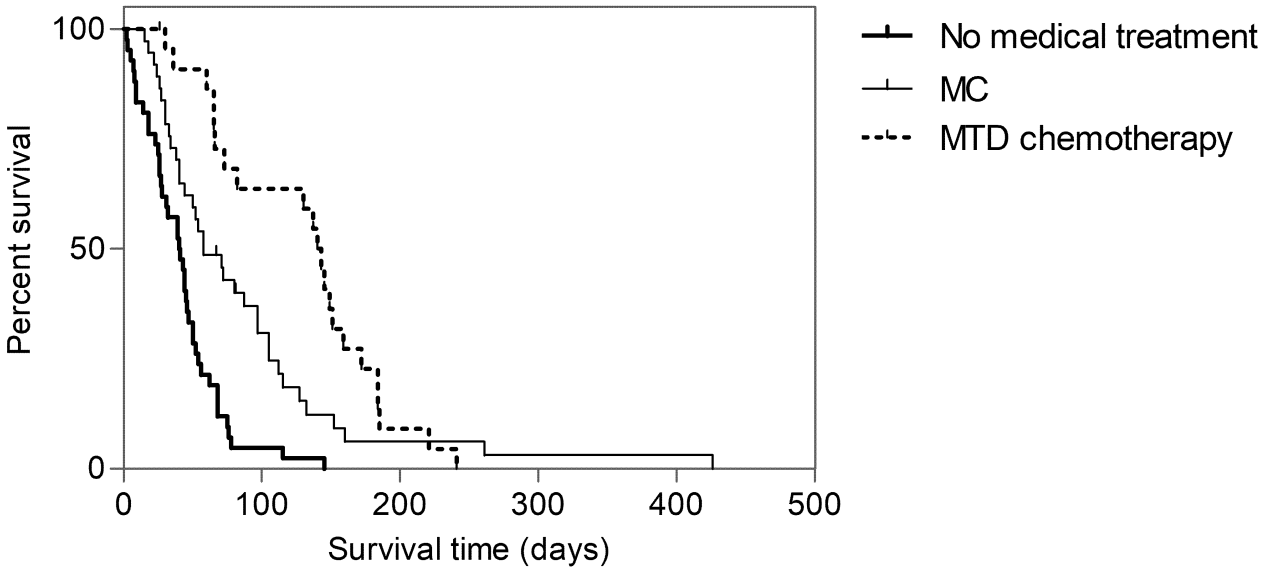
	Metronomic chemotherapy (*n* = 38)	**Maximum-tolerated dose chemotherapy** (*n* = 23)
Number of dogs with adverse events	6 (15.8%)	10 (43.5%)
Adverse events recorded for each dog (number of episodes)	Hematuria grade 1 (1) GI grade 1 (1) Hematuria grade 1 (1) GI grade 1 (1) GI grade 1 (1) GI grade 2 (1)	BM grade 2 (2), GI grade 2 (3) BM grade 2 (2) BM grade 1 (1) BM grade 1 (1) BM grade 4 (1) GI grade 3 (1) BM grade 2 (1) GI grade 1 (1) BM grade 3 (1), BM grade 2 (1) GI grade 3 (2), lethargy grade 1 (1)
Dose decrease	0 (0%)	4 (17.4%)
Hospitalization	0 (0%)	5 (21.7%)

528 **Abbreviations: GI, gastrointestinal; BM, bone marrow.**

529 **Figure legend**

530

531 Figure 1. Kaplan-Meier survival plots for dogs with stage III
532 hemangiosarcoma treated with splenectomy alone (bold line, n = 42),
533 splenectomy plus metronomic chemotherapy (MC, thin line, n = 38) and
534 splenectomy plus maximum tolerated dose chemotherapy (MTD
535 chemotherapy, dots; n = 23). Survival time was significantly longer for
536 dogs treated with MTD than dogs treated with MC (P = 0.023) or
537 splenectomy alone (P < 0.001).



538