

Alma Mater Studiorum Università di Bologna  
Archivio istituzionale della ricerca

Canine indolent and aggressive lymphoma: Clinical spectrum with histologic correlation

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Aresu L., Martini V., Rossi F., Vignoli M., Sampaolo M., Arico A., et al. (2015). Canine indolent and aggressive lymphoma: Clinical spectrum with histologic correlation. VETERINARY AND COMPARATIVE ONCOLOGY, 13(4), 348-362 [10.1111/vco.12048].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/702699> since: 2019-10-18

*Published:*

DOI: <http://doi.org/10.1111/vco.12048>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13

This is the final peer-reviewed accepted manuscript of:

Finotello R, Henriques J, Sabattini S, Stefanello D, Felisberto R, Pizzoni S, Ferrari R, Marconato L. A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma. Vet Comp Oncol. 2017 Jun;15(2):493-503. doi: 10.1111/vco.12193. Epub 2016 Jan 21. PMID: 26792231.

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

**A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma**

**Riccardo Finotello,<sup>1</sup> Joaquim Henriques,<sup>2</sup> Silvia Sabbatini,<sup>3</sup> Damiano Stefanello,<sup>4</sup> Ricardo Felisberto,<sup>2</sup> Selene Pizzoni,<sup>5</sup> Roberta Ferrari,<sup>4</sup> Laura Marconato<sup>5</sup>**

<sup>1</sup>Small Animal Teaching Hospital, School of Veterinary Sciences, University of Liverpool, Neston, UK

<sup>2</sup>Centro Veterinario Berna, Lisbon, Portugal

<sup>3</sup>Department of Veterinary Medical Sciences, University of Bologna, Italy

<sup>4</sup>Department of Veterinary Science and Public Health, University of Milan, Milan, Italy

<sup>5</sup>Centro Oncologico Veterinario, Sasso Marconi, Italy

**Short title:** chemotherapy switch in aggressive haemangiosarcoma

**Keywords:** haemangiosarcoma, metronomic, thalidomide, chemotherapy switch, dog

Findings of this study were presented in part at the European Society of Veterinary Oncology Annual Meeting, Krakow, Poland, 2015.

Corresponding author: Laura Marconato, DVM, Diplomate ECVIM-CA (Oncology), Centro Oncologico Veterinario, via San Lorenzo 1-4, I-40037 Sasso Marconi (Bologna), Italy; email: [marconato@centroncologicovet.it](mailto:marconato@centroncologicovet.it)

## Abstract

Haemangiosarcoma (HSA) has an aggressive biological behaviour and carries a poor prognosis, with less than 10% of treated dogs surviving longer than one year.

In this retrospective study a varied metronomic chemotherapy (MC) regimen preceded by ~~standard~~ adjuvant doxorubicin-based maximum-tolerated dose chemotherapy (MTDC) was compared to MTDC, in terms of efficacy (time to metastasis, TTM, and survival time, ST) and safety in dogs with biologically aggressive HSA. Dogs were eligible if they had no metastasis after MTDC and received either no further chemotherapy or MC maintenance.

~~Twenty-two dogs were enrolled: 12~~Twelve dogs received MTDC, and 10 received MC thereafter. Median TTM and ST were significantly longer for dogs receiving MTDC-MC (not reached versus 150 days, P=0.028; and not reached versus 168 days, P=0.030, respectively). Treatment was well tolerated.

MTDC followed by MC is safe and suggests improveds TTM and ST in dogs with surgically removed, biologically aggressive HSA that are treated in the microscopic setting.

## Introduction

Haemangiosarcoma (HSA) is a common mesenchymal tumour in dogs, arising in three different forms: dermal, subcutaneous/muscular and visceral, the latter mainly involving spleen, right atrium or auricle, and liver.<sup>1-3</sup> With the exception of the dermal form, which may behave in a less aggressive fashion, subcutaneous/intramuscular and visceral HSA is a highly malignant cancer, spreading rapidly to lungs, liver, peritoneum and central nervous system.<sup>4,5</sup> Unfortunately, visceral HSA has a silent evolution for a quite long time, and is accompanied by non specific clinical signs. As a consequence, when detected, it is usually in an advanced or metastatic stage, therefore precluding cure.<sup>1,2</sup>

The mainstay of treatment consists of surgery followed by adjuvant intravenous chemotherapy.<sup>6,7</sup> Doxorubicin-based chemotherapy protocols have been administered to dogs with HSA, including doxorubicin as single agent,<sup>6</sup> or combined with ifosfamide,<sup>8</sup> vincristine and cyclophosphamide,<sup>7,9-11</sup> and epirubicin as single agent.<sup>12</sup> Although a three weekly regimen is the commonest schedule administration of doxorubicin, one study attempting to increase dose intensity by more frequent administrations showed such strategy to be well tolerated; however, survival time was not improved.<sup>13</sup>

Although the combination of doxorubicin and dacarbazine has provided promising results in a recent clinical trial, it is still common knowledge that < 10% of the dogs diagnosed with HSA will survive one year after diagnosis, being attributable to the development of metastatic disease during or after completion of maximum-tolerated dose chemotherapy (MTDC).<sup>14</sup> Thus, it appears obvious that MTDC is unlikely to provide a durable response in such biologically aggressive solid tumours.

Metronomic chemotherapy (MC) refers to the frequent administration of cytotoxic drugs at doses significantly lower than the maximum tolerated dose, with no prolonged drug-free breaks, leading to an anti-angiogenic effect and immune-modulation.<sup>15-16</sup> In veterinary oncology, MC has been mainly used in a palliative setting with good

response rates and safety profile.<sup>17-18</sup> A continuous low-dose chemotherapy strategy consisting of cyclophosphamide, etoposide, and piroxicam has been proposed as an alternative treatment for dogs with HSA, yielding comparable results to conventional MTDC, therefore suggesting a beneficial effect of this regimen in delaying disease progression in canine HSA.<sup>19</sup> A more recent study suggested that the combination of both MTDC and MC was more efficacious in dogs with splenic HSA than either type of chemotherapy alone in the early follow-up period; however, no significant prolongation of survival time was observed during the late follow-up period when compared with dogs undergoing splenectomy only.<sup>20</sup>

A “chemo-switch schedule” refers to the introduction of a new and potentially non-cross-resistant agent after completion of first-line chemotherapy, such as the administration of MC after MTDC.<sup>21</sup> In the current study, we retrospectively compared MC preceded by doxorubicin-based MTDC to MTDC treatment only, in terms of efficacy (time to metastasis, TTM, and survival time, ST) and safety in dogs with biologically aggressive HSA. It was hypothesised that chemo-switch would improve long-term tumour control.

## **Material and methods**

### ***Inclusion criteria***

The databases of the Centro Oncologico Veterinario (Bologna, Italy), Centro Veterinario Berna (Lisbon, Portugal) and University of Milan Teaching Hospital (Milan, Italy) were reviewed to identify client-owned dogs with histologically confirmed and biologically aggressive HSA (2011-2014).

109 Haemangiosarcoma was considered as “biologically aggressive” if arising from any  
110 visceral, bone and muscular location or, in case of subcutaneous tumours, if the largest  
111 diameter was > 6 cm.<sup>1-4</sup>

112 Eligible dogs for inclusion in the analysis set were those that had no evidence of  
113 macroscopic disease after completion of MTDC based on imaging and that received  
114 either no further chemotherapy or MC maintenance.

115 Pre-surgical, pre-dosing, and post-dosing investigations included physical examination,  
116 haematology, serum biochemistry, abdominal ultrasound and at least two lateral views  
117 thoracic radiographs or computed tomography (CT) if performed.

118 Dogs were monitored at least every three months after MTDC or during MC  
119 maintenance, as listed above.

120 Dogs were staged according to the World Health Organization (WHO) staging system  
121 for domestic animals.<sup>22</sup>

122

### 123 ***Treatment protocol***

124 Based on owners' and clinicians' preference, dogs received MTDC followed by MC  
125 (Group 1) or MTDC only (Group 2). MTDC consisted of a discontinued doxorubicin-  
126 based chemotherapy protocol. MC was administered orally and consisted of low-dose  
127 cyclophosphamide (Endoxan®, Baxter s.r.l., Lurago d'Erba, Como, Italy) administered  
128 q24h or q48h at 7-15 mg/m<sup>2</sup>, and the cyclooxygenase-2 (COX-2) inhibitor firocoxib  
129 (Previcox®, Merial, Lyon, France), meloxicam (Metacam®, Boehringer Ingelheim,  
130 Milan, Italy), or a non-selective COX inhibitor (Piroxicam®, Pfizer Italia s.r.l., Latina,  
131 Italy) administered daily at the standard recommended dose. The non-steroidal anti-  
132 inflammatory drug (NSAID) varied depending on clinician's preference. In case of  
133 haemorrhagic cystitis, cyclophosphamide was discontinued and dogs received oral

134 chlorambucil (Leukeran®, GlaxoSmithKline S.p.A., Verona, Italy) at the dosage of 4  
135 mg/m<sup>2</sup> q24h or q48h.<sup>23</sup>

136 Depending on availability, oral thalidomide at 2-3 mg/kg (Thalidomid, Bichsel AG,  
137 Interlaken, Switzerland) was also administered q24h or q48h depending on clinician's  
138 preference. The dose of thalidomide was arbitrarily chosen based on some of the  
139 authors' experience.<sup>24</sup> Owners intending to have thalidomide administered were  
140 informed on its known teratogenic effect.<sup>25</sup>

141

#### 142 *Assessment of toxicity*

143 Toxicity resulting from MTDC was assessed in both groups based on the dog's history,  
144 physical examination and complete blood counts (CBCs) 7-10 days after chemotherapy  
145 and before the beginning of each next cycle, as stated by the Veterinary Co-operative  
146 Oncology Group.<sup>26</sup> In Group 2, urinalysis was also carried out in the case of suspected  
147 urothelial toxicity (i.e. haematuria, stranguria, pollachiuria).

148

#### 149 *Statistical analysis*

150 Follow-up and survival times were calculated from the date of diagnosis to the date of  
151 last visit or death. For both groups, ST and TTM (beyond regional lymph nodes) were  
152 explored with the Kaplan-Meier product limit method followed by log-rank test. In  
153 either group, timing was considered from surgical excision. In the survival analysis,  
154 dogs were censored if they were alive at the time of data accrual closure or died of no  
155 tumour-related causes, whereas for TTM dogs were censored if, by the last examination,  
156 distant metastases had not developed.

157 Causes of death were established reviewing the individual dog clinical histories and  
158 through telephone calls to owners and referring veterinarians. Dogs were considered to  
159 have died of HSA if the clinical staging work-up was consistent with the presence of



160 metastatic disease and if symptoms could be linked to HSA progression (i.e recurrence  
161 of haemoabdomen); dogs were considered not to have died because of HSA if their last  
162 staging work-up (performed no longer than one month before death) revealed no  
163 evidence of metastatic disease and if death was determined to occur due to an unrelated  
164 cause.

165 When appropriate, data sets were tested for normality by use of the D'Agostino and  
166 Pearson omnibus normality test. Values were expressed as mean  $\pm$  standard deviation in  
167 case of normal distribution, or as median with a range in case of non-normal  
168 distribution.

169 To verify whether features of the two groups differed at admission or during MTDC, the  
170 T-test (parametric variables) or Mann Whitney U test (non-parametric variables) was  
171 used to compare age, body weight, and the time occurred from the diagnosis to the  
172 beginning of MTDC. Fisher's exact test was used to compare breed (pure- vs cross-  
173 breed), sex (male vs female), primary location of the tumour (spleen vs other sites),  
174 clinical stage, number of doxorubicin cycles (<4 vs 4-6), type of chemotherapy protocol  
175 (single agent doxorubicin vs poly-chemotherapy) and MTDC-related toxicity (present  
176 vs absent). Data were analysed by use of commercial software programs (SPSS  
177 Statistics v. 19, IBM, Somers, NY, and Prism v. 5.0, GraphPad, San Diego, CA). P  
178 values  $\leq 0.05$  were considered significant.

179

180

## 181 Results

182 Twenty-two dogs met the inclusion criteria and were ~~enrolled~~included in the analysis; 10  
183 (45.5%) of them received MTDC followed by MC (Group 1), whereas the remaining 12  
184 (54.5%) were treated with MTDC (Group 2). Dogs' characteristics are listed in Table 1. Dogs  
185 were not stratified based on prognostic risk, but there was good balance between arms

186 regarding dogs' features and possible outcome variables; however, concerning sex  
187 distribution, there was a statistically significant difference between groups, as males were  
188 more common in Group 1 and females were more common in Group 2 -(P=0.043; Table 2).  
189 For all dogs, pre-surgical, pre-dosing, and post-dosing imaging investigations were performed  
190 through thoracic radiographs and abdominal ultrasound. Two dogs (case 6 and case 22; Table  
191 1) had CT scans repeated throughout the follow-up period.

192

### 193 ***Group 1 (MTDC-MC)***

194 There were 3 mixed breed dogs, 2 German shepherds, 1 Golden retriever, 1 Labrador  
195 retriever, 1 Boxer, 1 Great Dane, and 1 Italian cane Corso. Mean age was 8.9 ( $\pm$  2.6)  
196 years and mean weight was 36.4 ( $\pm$  12.0) kg. There were 8 males (n=4 neutered) and 2  
197 spayed female dogs. HSA occurred in the spleen as primary site in 8 dogs; all dogs  
198 presented with hemoperitoneum because of splenic rupture. The remaining 2 dogs had  
199 subcutaneous (n=1) and osseous (n=1) HSA.

200 All dogs underwent surgery, consisting of splenectomy, removal of the subcutaneous  
201 tumour, or amputation according to cancer location. Histopathological evaluation  
202 revealed clean surgical margins in the subcutaneous and osseous HSA; surgical margins  
203 were deemed not assessable for dogs presenting with visceral rupture.

204 According to the WHO classification, 9 dogs had stage II disease, and 1 dog with  
205 osseous HSA had stage III disease.

206 The mean time from surgery to initial MTDC administration was 20.8 ( $\pm$  15.4) days.  
207 Eight dogs received doxorubicin as single agent, and 2 dogs received a combination of  
208 doxorubicin and dacarbazine. For all dogs, the median number of doxorubicin cycles  
209 was 5 (range, 4 to 6 cycles), and the initial dose was 30 mg/m<sup>2</sup> for all dogs.  
210 Chemotherapy dose reduction was undertaken in 3 dogs receiving single agent

211 doxorubicin; this was performed at the clinician's discretion after haematological and/or  
212 gastrointestinal toxicity developed: 2 dogs had 10% and 1 had 20% dose reduction. The  
213 median total dose of doxorubicin was 132 mg/m<sup>2</sup> (range, 120 to 180 mg/m<sup>2</sup>).  
214 The median time from completion of MTDC to start of MC was 17.5 days (range, 13 to  
215 24 days). Cyclophosphamide was administered q24h in 2 dogs and q48h in the  
216 remaining 8 dogs. The median single cyclophosphamide dose was 8.5 mg/m<sup>2</sup> (range, 7  
217 to 15 mg/m<sup>2</sup>), and the median weekly cumulative dose was 44 mg/m<sup>2</sup> (range, 28 to 105  
218 mg/m<sup>2</sup>). Concerning NSAIDs, 5 dogs received piroxicam, 4 had meloxicam and 2 dogs  
219 received firocoxib. Thalidomide was given in combination with standard MC in 7  
220 (70%) of 10 dogs: 5 dogs received 2 mg/kg q24h, whereas the remaining 2 were treated  
221 at 3 mg/kg q24h.

222

#### 223 ***Group 2 (MTDC)***

224 There were 5 mixed breed dogs, 2 Labrador retriever and 1 each of Boxer, German  
225 shepherd, Pitt Bull, Rottweiler and Yorkshire terrier. Mean age was 9.8 ( $\pm$  2.2) years  
226 and mean weight was 27.2 ( $\pm$  10.4) kg. There were 8 female (n=4 spayed) and 4 males  
227 (n=1 neutered) dogs. HSA occurred in the spleen as primary site in 11 dogs; 10 of them  
228 presented with hemoperitoneum because of splenic rupture. One dog had a  
229 subcutaneous HSA.

230 All dogs underwent surgery, consisting of splenectomy and removal of the  
231 subcutaneous tumour according to cancer location. Histopathological evaluation  
232 revealed clean surgical margins in the subcutaneous HSA; surgical margins were  
233 deemed not assessable for dogs presenting with visceral rupture.

234 According to WHO, 11 dogs had stage II disease, and 1 had stage I disease. The dog  
235 with stage I disease had a splenic HSA.

236 The mean time from surgery to initial MTDC administration was 25.0 ( $\pm$  12.1) days.  
237 Nine dogs received doxorubicin as single agent and 3 dogs received a combination of  
238 doxorubicin and dacarbazine. The median number of doxorubicin cycles was 4 (range, 2  
239 to 5 cycles) and all dogs received a starting dose of doxorubicin of 30 mg/m<sup>2</sup>.  
240 Chemotherapy dose reduction was performed in 2 dogs receiving single agent  
241 doxorubicin; this was performed at the clinician's discretion due to haematological  
242 and/or gastrointestinal toxicity: one dog had 10% and one had 20% dose reduction. The  
243 median total dose of doxorubicin was 120 mg/m<sup>2</sup> (range, 60 to 180). In the three dogs  
244 receiving doxorubicin and dacarbazine, the protocol was designed as previously  
245 reported.<sup>14</sup> Cases' data are summarized in Table 1.

246

#### 247 ***Clinical outcome***

248 Three (30%) out of the 10 dogs included in Group 1 (MTDC-MC) developed metastatic  
249 disease after 119, 151 and 460 days, respectively. Metastases were found in the  
250 peritoneum (n=2) and liver and lung (n=1). The two dogs with metastases to the  
251 peritoneum developed haemoabdomen.

252

253 Nine (75%) of the 12 dogs included in Group 2 (MTDC) developed metastatic disease  
254 after a median of 134 days (range, 89 to 174 days). Metastases were found in lung  
255 (n=3), peritoneum (n=2), liver (n=2), lung and brain (n=1) and lung, stomach and liver  
256 (n=1). The two dogs with metastases to the peritoneum developed haemoabdomen.

257

258 Overall, median TTM was significantly longer for dogs receiving MTDC-MC compared  
259 to those receiving MTDC only (not reached versus 150 days, respectively; P=0.028;  
260 Figure 1).

261

262 Six (60%) out of the 10 dogs included in Group 1 (MTDC-MC) were dead at the end of  
263 the study. Three (27.2%) dogs with splenic HSA died as a result of disease progression  
264 after 152, 191 and 487 days. Three dogs with splenic HSA died of tumour-unrelated  
265 causes after 165, 292 and 730 days, respectively, with no evidence of tumour recurrence  
266 or metastasis. One dog (splenic HSA) was lost to follow-up after 680 days from the  
267 diagnosis; at the last visit this dog had no evidence of macroscopic disease.  
268 Three dogs (osseous, n=1, and splenic, n=2) were still alive with no evidence of disease  
269 after 311, 640 and 1280 days, respectively.

270  
271 Ten (83.3%) out of the 12 dogs in Group 2 (MTCD) were dead at data analysis closure:  
272 9 (75%) died as a result of HSA progression with a median survival time of 156 days  
273 (range, 97 to 341 days). Of these 9 dogs, 7 had splenic stage II HSA, 1 had splenic stage  
274 I HSA, and one had subcutaneous stage II HSA. The remaining dog (splenic stage II  
275 HSA) died 803 days after the diagnosis because of tumour-unrelated causes.  
276 Two dogs with splenic HSA were still alive with no evidence of disease at 437 and 608  
277 days, respectively.

278  
279 Overall, dogs receiving MTDC followed by MC had a significantly longer median ST  
280 than those receiving MTDC only (not reached versus 168 days, respectively; P=0.030;  
281 Figure 2).

282  
283 **Toxicity**  
284 During MTDC, neutropenia occurred in 4 (40%) dogs in Group 1. One dog had one  
285 episode of grade 1 neutropenia, 2 dogs had one episode of grade 2 neutropenia, whereas  
286 1 dog had 2 episodes of grade 2 neutropenia. In all dogs haematological toxicities  
287 resolved without sequel.

288 In Group 2, 1 (8.3%) dog developed 2 episodes of grade 4 non-febrile neutropenia, and  
289 1 (8.3%) dog developed one episode of grade 2 anaemia.

290 Gastrointestinal toxicity was the second most common adverse event in both groups,  
291 and consisted of vomiting, diarrhoea and decreased appetite of mild to moderate  
292 severity. Gastrointestinal toxicity of grade 2 occurred in 2 (20%) dogs in Group 1.  
293 These dogs had no concurrent episodes of haematological toxicity. In Group 2, 3 (25%)  
294 dogs developed gastrointestinal toxicity: 1 dog had one episode of grade 3 anorexia and  
295 2 dogs had 1 episode of grade 2 vomiting (1 concurrently had grade 2 anaemia).  
296 The overall frequency of MTDC related side effects did not differ between groups  
297 (Table 2).

298  
299 During MC, 4 (40%) dogs developed gastrointestinal, haematological and/or urothelial  
300 adverse events. Two dogs developed grade 2 sterile haemorrhagic cystitis after 180 and  
301 470 days, respectively; in both cases cyclophosphamide was discontinued and  
302 chlorambucil was started; cystitis resolved within 4 weeks in both cases. One dog  
303 developed grade 1 diarrhoea and in one case grade 1 vomiting and diarrhoea occurred  
304 simultaneously. Gastrointestinal signs resolved with symptomatic treatment and did not  
305 recur.

306

## 307 **Discussion**

308 The treatment of HSA continues to be extremely challenging in veterinary oncology.  
309 Unfortunately, little progress has been made over the years, and prognosis for dogs with  
310 HSA is poor as a result of the aggressive nature of the disease, leading to invasion of  
311 nearby organs and vessels, early metastasis and limited treatment options providing  
312 durable disease control. Surgery is designed to remove all macroscopic tumours and  
313 prevent further risk of acute haemorrhage, but is considered purely palliative. The

addition of chemotherapy in an effort to treat microscopic disease has been documented to provide a modest improvement in outcome, with reported median survival times in the range of 6-8 months and less than 10% of dogs being alive at 12 months.<sup>1,2</sup>

The “cell kill” paradigm associated with MTDC has been successful in the treatment of human and canine haematological neoplasia, but unfortunately this has not provided long-lasting responses in the majority of advanced solid tumours.<sup>21</sup> Failure of MTDC may be multifactorial, being attributable to the heterogeneity of cancer cells, genetic make-up, and the influence of tumour microenvironment, thereby giving rise to treatment resistance.<sup>21</sup> Based on the above, the Gatenby’s hypothesis of controlling tumour growth instead of trying to eradicate it may become a more rational strategy.<sup>27</sup>

Maintenance therapy refers to a treatment that is given to avoid disease progression after the cancer has been successfully controlled with the initial therapy.<sup>21</sup>

An effective maintenance therapy should accomplish good patient tolerability, lack of cumulative toxicities, and cost-effectiveness. Maintenance therapy may consist of “continuation” therapy where one drug of the initial therapy is continued after the induction phase of the protocol, or of “switch” maintenance in which a new agent is introduced.<sup>21,28</sup>

Switch maintenance has been recently investigated in canine stage I-II splenic HSA by administering the tyrosine kinase inhibitor toceranib phosphate. Toceranib mainly targets the stem cell factor receptor KIT, platelet derived growth factor receptor and vascular endothelial growth factor receptor (VEGFR), which are typically expressed by canine HSA.<sup>29</sup>

As in our study, the switch maintenance was administered in the microscopic disease setting after completion of doxorubicin MTDC. Unfortunately, disease-free interval nor ST were improved when comparing dogs receiving or not receiving maintenance toceranib.<sup>29</sup>

340 It has become progressively clear that the endothelial cell compartment is an attractive  
341 target for anticancer therapy as a result of the evident importance of the tumour  
342 vasculature for sustaining tumour growth and metastasis. Also, the endothelial cells are  
343 sensitive to the action of conventional cytotoxic drugs, including cyclophosphamide,  
344 if the dosing regimen is altered to the so-called anti-angiogenic scheduling.<sup>15</sup>

345 In a previous study, dogs with stage II HSAAS receiving an oral adjuvant therapy  
346 consisting of alternating low-dose daily cyclophosphamide and etoposide in  
347 combination with piroxicam had comparable survival times to historical controls treated  
348 with conventional doxorubicin chemotherapy.<sup>19</sup> Starting from the promising results of  
349 the mentioned study, Based on the promising results obtained by MC in the treatment of  
350 surgically-removed canine HSA,<sup>19</sup> we hypothesised that outcome might be improved, if  
351 a MC schedule is to be administered after MTDC as a consolidation strategy. To this  
352 end, we retrospectively compared HSA dogs receiving MTDC versus MTDC followed  
353 by MC in the microscopic setting. Beside cyclophosphamide and NSAID, thalidomide  
354 was added to this combination in the majority of dogs.

355 The results obtained in the current study ~~document suggest~~ an advantage of the addition  
356 of maintenance MC over MTDC alone in terms of metastatic control and survival.  
357 Indeed, dogs undergoing chemo-switch after dose-intense chemotherapy had a  
358 significantly longer TTM and ST compared to dogs receiving MTDC, suggesting that  
359 chemo-switch improves long-term tumour control in biologically aggressive canine  
360 HSA. These results ~~can may~~ be explained by the following considerations.

361 The use of continuous, low-dose cyclophosphamide exerts potent anti-angiogenic  
362 properties through the inhibition of proliferation and/or induction of apoptosis of  
363 activated endothelial cells, selective inhibition of migration of endothelial cell, increase  
364 in the expression of thrombospondin-1, and sustained decrease in levels and viability of  
365 bone marrow-derived endothelial progenitor cells.<sup>15</sup> Moreover, it has been shown that



metronomic cyclophosphamide can also target the immune system by activating or restoring its antitumor properties, particularly through the inhibition of T regulatory lymphocytes and enhance the cytotoxic T lymphocytes response.<sup>30,31</sup>

Non-selective NSAIDs and COX-2 selective inhibitors such as piroxicam and meloxicam are effective in counteracting tumour angiogenesis, by boosting the effect of cyclophosphamide.<sup>32-34</sup>

Alongside its teratogenic effect, thalidomide is a potent inhibitor of angiogenesis through inhibition of VEGF, basic fibroblastic growth factor, and tumour necrosis factor alpha, and may play a role in anti-angiogenic strategies.<sup>35</sup>

A recent study has suggested that the combination of MTDC and MC may be superior to MTDC alone in the treatment of canine splenic HSA in the early follow-up period,<sup>20</sup> however survival times were modest compared to the group receiving MTDC alone and, importantly, these did not differ substantially from the published literature.<sup>1,2</sup> In the aforementioned study, 13 dogs with splenic HSA received doxorubicin and MC either sequentially (chemo-switch; n=6) or concurrently (n=7). Median survival time for these dogs was 4.3 months, and median duration of treatment was 56 days; it was hypothesised that metastatic disease rapidly progressed after chemotherapy was interrupted for whichever reason.<sup>20</sup> In the current study group, the use of MC significantly improved outcome, and it may be hypothesised that the difference between our study and Wendelburg's study may be due to the use of the potent antiangiogenic drug thalidomide or to the continuous use of MC.

While MTDC can serve to de-bulk HSA by directly targeting the cancer cells, maintenance MC may disrupt crucial angiogenic pathways, impeding the inevitable rebound and regrowth, ultimately translating into significant therapeutic benefits.

In agreement with previous studies, MC was well tolerated, and side effects were mainly gastro-intestinal and of mild severity.<sup>17,18</sup> Haemorrhagic cystitis occurred in 2

392 dogs, most likely as a consequence of prolonged treatment with cyclophosphamide;  
393 however gastrointestinal and haematological adverse event could have also been due to  
394 transient and undiagnosed comorbidities and not related to MC.

395 Limitations of this study include its retrospective nature, the low number of cases, the  
396 different tumour site origin, the variability of chemotherapy protocols used in the  
397 MTDC phase and the lack of necropsies. Five dogs received a combination of  
398 doxorubicin and dacarbazine, which has recently demonstrated encouraging results  
399 providing an increase in the chances of survival for biologically aggressive canine  
400 HSA.<sup>14,36</sup> Nevertheless, in the present series dogs receiving doxorubicin and  
401 dacarbazine were equally distributed among groups, thereby rendering unlikely the  
402 chance of having improved outcome in one group only. Dogs' features and possible  
403 outcome variables were homogeneously distributed between groups with the exception  
404 of sex: male dogs were more common in Group 1 whereas females were more common  
405 in Group 2. Although this finding is likely to be a bias due to the small sample size, we  
406 cannot exclude that the small number of dogs included in this study may have  
407 contributed to reach significance for the other variables analysed.

408 ~~This finding, although statistically significant, is likely to be a bias due to the small~~  
409 ~~sample size, and any other explanation might only be speculative. <sup>‡</sup>~~

410 Finally, it must be acknowledged that 3 dogs treated with MTDC were censored  
411 belatedly (after 437, 608 and 803 days), compared to 6 dogs treated with MTDC and  
412 MC, and among them 3 were censored early (after 165, 292 and 311 days). While this  
413 may reflect a better outcome, as fewer dogs died due to HSA in Group 1 compared to  
414 Group 2, it also could have biased the results, as early deaths due to tumour-unrelated  
415 causes may strongly influence statistics.

**Formattato:** Giustificato, Destro 1 cm, SpazioDopo: 0 pt, Interlinea: doppia

**Formattato:** Tipo di carattere: (Predefinito) Times New Roman, 12 pt, Inglese (Regno Unito)

**Formattato:** Tipo di carattere: Times New Roman, 12 pt, Inglese (Regno Unito)

**Formattato:** Tipo di carattere: (Predefinito) Times New Roman, 12 pt

**Formattato:** Tipo di carattere: (Predefinito) Times New Roman, 12 pt

**Formattato:** Tipo di carattere: (Predefinito) Times New Roman, 12 pt

To conclude, maintenance MC is well tolerated and may prolong TTM and survival time in dogs with biologically aggressive HSA with negative staging after completion of MTDC. Although the role of thalidomide in the treatment of HSA needs further studies, it is possible that this drug used in combination with standard MC plays an important role in controlling the metastatic process of biologically aggressive canine HSA. Prospective studies with larger number of patients are required to confirm these findings.

## References

1. Thamm DH. Hemangiosarcoma. In: *Withrow & MacEwen's Small Animal Clinical Oncology*, 5<sup>th</sup> ed., SJ Withrow, DM Vail and RL Page eds., St Louis, Saunders Elsevier, 2013: 679-688
2. Smith AN. Hemangiosarcoma in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice* 2003; **33**: 533-552
3. Schultheiss PC. A retrospective study of visceral and nonvisceral hemangiosarcoma and hemangiomas in domestic animals. *Journal of Veterinary Diagnostic Investigation* 2004; **16**: 522-526
4. Shiu KB, Flory AB, Anderson CL, Wypij J, Saba C, Wilson H, Kurzman I and Chun R. Predictors of outcome in dogs with subcutaneous or intramuscular hemangiosarcoma. *Journal of the American Veterinary Medical Association* 2011; **238**: 472-479
5. Ward H, Fox LE, Calderwood-Mays MB, Hammer AS and Couto CG. Cutaneous hemangiosarcoma in 25 dogs: a retrospective study. *Journal of Veterinary Internal Medicine* 1994; **8**: 345-348

6. Ogilvie GK, Powers BE, Mallinckrodt CH and Withrow SJ. Surgery and doxorubicin in dogs with hemangiosarcoma. *Journal of Veterinary Internal Medicine* 1996; **10**: 379-384
7. Wiley JL, Rook KA, Clifford CA, Gregor TP and Sorenmo KU. Efficacy of doxorubicin-based chemotherapy for non-resectable canine subcutaneous haemangiosarcoma. *Veterinary and Comparative Oncology* 2010; **8**: 221-233
8. Payne SE, Rassnick KM, Northrup NC, Kristal O, Chretien JD, Cotter SM, Kintzer P, Frimberger AE, Morrison-Collister KE, Wood CA and Moore AS. Treatment of vascular and soft-tissue sarcomas in dogs using an alternating protocol of ifosfamide and doxorubicin. *Veterinary and Comparative Oncology* 2003; **1**: 171-179
9. Hammer AS, Couto CG, Filppi J, Getzy D and Shank K. Efficacy and toxicity of VAC chemotherapy (vincristine, doxorubicin, and cyclophosphamide) in dogs with hemangiosarcoma. *Journal of Veterinary Internal Medicine* 1991; **5**: 160-166
10. Sorenmo KU, Jeglum KA and Helfand SC. Chemotherapy of canine hemangiosarcoma with doxorubicin and cyclophosphamide. *Journal of Veterinary Internal Medicine* 1993; **7**: 370-376
11. Bulakowski EJ, Philibert JC, Siegel S, Clifford CA, Risbon R, Zivin K and Cronin KL: Evaluation of outcome associated with subcutaneous and intramuscular hemangiosarcoma treated with adjuvant doxorubicin in dogs: 21 cases (2001-2006). *Journal of the American Veterinary Medical Association* 2008; **233**: 122-128
12. Kim SE, Liptak JM, Gall TT, Monteith GJ and Woods JP. Epirubicin in the adjuvant treatment of splenic hemangiosarcoma in dogs: 59 cases (1997-2004). *Journal of the American Veterinary Medical Association* 2007; **231**: 1550-1557

13. Sorenmo KU, Baez JL, Clifford CA, Mauldin E, Overley B, Skorupski K, Bachman R, Samluk M and Shofer F. Efficacy and toxicity of a dose-intensified doxorubicin protocol in canine hemangiosarcoma. *Journal of Veterinary Internal Medicine* 2004; **18**: 209-213
14. Finotello R, Stefanello D, Zini E and Marconato L. Comparison of doxorubicin-cyclophosphamide with doxorubicin-dacarbazine for the adjuvant treatment of canine hemangiosarcoma. *Veterinary and Comparative Oncology* 2015: 1-11, doi: 10.1111/vco.12139
15. Maiti R. Metronomic chemotherapy. *Journal of Pharmacology and Pharmacotherapeutics* 2014; **5**: 186-192
16. Kareva I, Waxman DJ and Klement GL. Metronomic chemotherapy: An attractive alternative to maximum tolerated dose therapy that can activate anti-tumor immunity and minimize therapeutic resistance. *Cancer Letters* 2015; **358**: 100-106
17. Biller B. Metronomic chemotherapy in veterinary patients with cancer: rethinking the targets and strategies of chemotherapy. *The Veterinary Clinics of North America. Small Animal Practice* 2014; **44**: 817-829
18. Marchetti V, Giorgi M, Fioravanti A, Finotello R, Citi S, Canu B et al. First-line metronomic chemotherapy in a metastatic model of spontaneous canine tumours: a pilot study. *Investigational New Drugs* 2012; **30**: 1725-1730
19. Lana S, U'ren L, Plaza S, Elmslie R, Gustafson D, Morley P et al. Continuous low-dose oral chemotherapy for adjuvant therapy of splenic hemangiosarcoma in dogs. *Journal of Veterinary Internal Medicine* 2007; **21**: 764-769
20. Wendelburg KM, Price LL, Burgess KE, Lyons JA, Lew FH and Berg J. Survival time of dogs with splenic hemangiosarcoma treated by splenectomy

Formattato: Italiano (Italia)

- with or without adjuvant chemotherapy: 208 cases (2001-2012). *Journal of the American Veterinary Medical Association* 2015; **247**: 393-403.
21. Malik PS, Raina V and André N. Metronomics as maintenance treatment in oncology: time for chemo-switch. *Frontiers in Oncology* 2014; **4**: 76
22. Owen LN, ed. TNM classification of tumours in domestic animals. Geneva (Switzerland): World Health Organization; 1980
23. Leach TN, Childress MO, Greene SN, Mohamed AS, Moore GE, Schrempp DR, et al. Prospective trial of metronomic chlorambucil chemotherapy in dogs with naturally occurring cancer. *Veterinary and Comparative Oncology* 2012; **10**: 102-112
24. Marconato L, Buchholz J, Keller M, Bettini G, Valenti P and 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. *Veterinary and Comparative Oncology* 2013; **11**: 101-112
25. Vargesson N. Thalidomide-Induced Teratogenesis: History and Mechanisms. *Birth Defects Research. Part C, Embryo Today: Reviews* 2015; **105**: 140-156
26. Veterinary Co-operative Oncology Group. Veterinary Co-operative oncology group- common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. *Veterinary and Comparative Oncology* 2004; **2**: 194-213
27. Gatenby RA, Silva AS, Gillies RJ and Frieden BR. Adaptive therapy. *Cancer Research* 2009; **69**: 4894-4903
28. Gerber DE and Schiller JH. Maintenance chemotherapy for advanced non-small-cell lung cancer: new life for an old idea. *Journal of Clinical Oncology* 2013; **31**: 1009-1020

29. Gardner HL, London CA, Portela RA, Nguyen S, Rosenberg MP, Klein MK, et al. Maintenance therapy with toceranib following doxorubicin-based chemotherapy for canine splenic hemangiosarcoma. *BMC Veterinary Research* 2015; **11**: 131
30. Pasquier E, Kavallaris M and André N. Metronomic chemotherapy: new rationale for new directions. *Nature Reviews. Clinical Oncology* 2010; **7**: 455-465
31. Burton JH, Mitchell L, Thamm DH, Dow SW and Biller BJ. Low-dose cyclophosphamide selectively decreases regulatory T cells and inhibits angiogenesis in dogs with soft tissue sarcoma. *Journal of Veterinary Internal Medicine* 2011; **25**: 920-926
32. Fischer SM, Hawk ET and Lubet RA. Coxibs and other nonsteroidal anti-inflammatory drugs in animal models of cancer chemoprevention. *Cancer Prevention Research (Philadelphia, Pa.)* 2011; **4**: 1728-1735
33. Iwase N, Higuchi T, Gonda T, Kobayashi H, Uetake H, Enomoto M et al. The effect of meloxicam, a selective COX-2 inhibitor, on the microvasculature of small metastatic liver tumors in rats. *Japanese Journal of Clinical Oncology* 2007; **37**: 673-678
34. Mohammed SI, Craig BA, Mutsaers AJ, Glickman NW, Snyder PW, de Gortari AE et al. Effects of the cyclooxygenase inhibitor, piroxicam, in combination with chemotherapy on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. *Molecular Cancer Therapeutics* 2003; **2**: 183-188
35. Rebuck JA and Fish DN. Thalidomide revisited. *The AIDS Reader* 1998; **8**: 7-9

36. Dervisis NG, Dominguez PA, Newman RG, Cadile CD and Kitchell BE.  
Treatment with DAV for advanced-stage hemangiosarcoma in dogs. *Journal of  
the American Animal Hospital Association* 2011; **47**: 170-178



550 **Captions to figures:**

551

552 **Figure 1:** Time to metastases for dogs treated with MTDC-MC (dots) and MTDC (line).

553 In the MTDC-MC group, dogs had a longer time to metastases (not reached versus 150  
554 days, respectively;  $P=0.028$ ).

555

556 **Figure 2:** Survival time for dogs treated with MTDC-MC (dots) and MTDC (line). In

557 the MTDC-MC group, dogs had a longer survival time (not reached versus 168 days,  
558 respectively;  $P=0.030$ ).

559