

**Evaluation of a multi-agent chemotherapy protocol combining lomustine, procarbazine and prednisolone (LPP) for the treatment of relapsed canine non-Hodgkin high-grade lymphomas**

J.B. Tanis,<sup>1</sup> S.L. Mason,<sup>2</sup> T.W. Maddox,<sup>1</sup> L. Blackwood,<sup>1</sup> D.R. Killick,<sup>1</sup> I. Amores-Fuster,<sup>1</sup> A. Harper<sup>1</sup> and R. Finotello<sup>1</sup>

*<sup>1</sup>Department of Small Animal Clinical Science, Institute of Veterinary Science, University of Liverpool, Leahurst Campus, Neston, CH64 7TE*

*<sup>2</sup>Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES*

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Corresponding author. Dr Riccardo Finotello, Department of Small Animal Clinical Science, Institute of Veterinary Science, University of Liverpool, Leahurst Campus, Neston, CH64 7TE

*E-mail address:* [riccardofi@libero.it](mailto:riccardofi@libero.it)

1 **Abstract**

2 The standard of care treatment for canine lymphoma is multi-agent chemotherapy containing  
3 prednisolone, cyclophosphamide, vincristine and an anthracycline such as doxorubicin (CHOP)  
4 or epirubicin (CEOP). Lomustine, vincristine, procarbazine, and prednisone (LOPP) has been  
5 evaluated as a rescue, with encouraging results; however, resistance to vincristine is likely in  
6 patients relapsing on CHOP/CEOP, and this agent may enhance LOPP toxicity without  
7 improving efficacy. The aim of this study was to evaluate responses to a modified LOPP  
8 protocol that does not include vincristine (LPP) and is administered on a 21-day cycle. Medical  
9 records of dogs with high grade multicentric lymphoma from 2012 to 2017 were reviewed.  
10 Dogs with relapsed lymphoma that received LPP as a rescue protocol were enrolled. Response,  
11 time from initiation to discontinuation (TTD) and toxicity of LPP were assessed. Forty-one  
12 dogs were included. Twenty-five dogs (61%) responded to LPP including 12 complete  
13 responses (CR) and 13 partial responses (PR). Responders had a significantly longer TTD ( $p$   
14  $<0.001$ ) compared to non-responders with 84 days for CR and 58 days for PR. Neutropenia was  
15 documented in twenty dogs (57%): 12 grade I-II, 8 grade III-IV. Thrombocytopenia was  
16 infrequent (20%): 5 grade I-II, 2 grade III-IV. Twelve dogs developed gastrointestinal toxicity  
17 (30%): 10 grade I-II and 2 grade III. Nineteen dogs had elevated ALT (59%): 9 grade I-II, 10  
18 grade III-IV. Treatment was discontinued due to toxicity in 8 dogs (19%). The LPP protocol  
19 shows acceptable efficacy and toxicity-profile and minimises in-hospital procedures.

20

21 *Keywords:* Canine Lymphoma; Relapse; Procarbazine; Lomustine; Dog; Chemotherapy;

22 Rescue

## 23 **Introduction**

24 Haematopoietic neoplasia is common in dogs, with canine non-Hodgkin lymphomas (cNHLs)  
25 making up over 80% of all hematopoietic cancer.<sup>1,2</sup> Among cNHLs, high grade B-cell  
26 lymphomas prevail and first-line chemotherapy consists of maintenance free CHOP or CEOP-  
27 based protocols including prednisolone, vincristine, cyclophosphamide and an anthracycline  
28 such as doxorubicin (CHOP) or epirubicin (CEOP).<sup>2-8</sup> High grade T-cell lymphomas are less  
29 common and though response rates and duration are shorter, treatment is similar.<sup>9</sup> The overall  
30 response rate for CHOP/CEOP protocols is between 89-100% and the median first remission  
31 duration is 142 to 302 days.<sup>2-8</sup> However, median first remission duration for T-cell cNHL is  
32 shorter than 5 months, compared to 11 months for B-cell cNHL.<sup>2-9</sup>

33 Despite an initial response, most dogs relapse and rescue chemotherapy is used to achieve a  
34 second remission. Rescue strategies are usually less effective than first-line protocols, showing  
35 a wide range of response rates (18-77%) and remission durations (56-238 days).<sup>10-25</sup>

36  
37 ATP-binding cassette superfamily (ABC) proteins such as P-glycoprotein (P-gp) or multidrug  
38 resistance-associated proteins may be involved in the development of resistance to first-line  
39 chemotherapy.<sup>26-29</sup> ABC protein superfamily proteins should not mediate resistance to  
40 alkylating agents, so their use in a rescue setting is appealing.<sup>30</sup>

41  
42 In 2002, Rassnick and colleagues evaluated the efficiency and tolerability of a  
43 mechlorethamine, vincristine, procarbazine and prednisolone protocol (MOPP).<sup>24</sup> MOPP  
44 demonstrated adequate efficacy (65% response rate) and an acceptable toxicity profile;  
45 however, due to the difficulty in obtaining mechlorethamine, LeBlanc and colleagues replaced  
46 it with carmustine (BOPP) or lomustine (LOPP).<sup>19</sup> Both protocols demonstrated good remission  
47 rates (50-52%) but severe adverse effects occurred. Consequently, Fahey and colleagues

48 proposed a modified-LOPP protocol (UF-LOPP) with decreased dose intensity.<sup>15</sup> The response  
49 rate was similar to the previous study, but fewer adverse effects were recorded.

50 The inclusion of vincristine in rescue protocols contributes in potentiating dose intensity, but  
51 its role is debatable in patients that have previously failed CHOP-based strategies. Additionally,  
52 vincristine may be the major contributor to gastrointestinal toxicity in LOPP or MOPP.<sup>15,19,24</sup>  
53 For these reasons, a modified MOPP protocol without vincristine (MPP) was designed  
54 demonstrating moderate efficacy (34% response rate) and a low toxicity profile.<sup>22</sup>

55

56 The primary aim of this retrospective study was to evaluate efficacy and toxicity of a modified  
57 LOPP rescue protocol combining lomustine, procarbazine and prednisolone (LPP) in canine  
58 relapsed lymphoma. A secondary aim was to identify predictive factors for response and  
59 occurrence of adverse effects.

60

## 61 **Materials and methods**

### 62 *Study population*

63 The computerised clinical database of the University of Liverpool was searched for dogs treated  
64 with LPP protocol January 2012 and July 2017. To be eligible for the study, patients had to  
65 fulfil the following criteria: (1) histological or cytological diagnosis of high grade cNHL  
66 performed by a veterinary board certified veterinary pathologist or clinical pathologist; (2)  
67 multicentric disease (3) lymphoma relapse following previous administration of, at least, one  
68 first line chemotherapy protocol; (4) follow-up measurement of peripheral lymph nodes from  
69 initiation to discontinuation of LPP. Patients with exclusive gastrointestinal involvement were  
70 excluded from the study. Discontinuation of prednisolone during the LPP protocol and absence  
71 of restaging prior to LPP initiation did not result in exclusion. The study was approved by the  
72 University of Liverpool ethics committee (VREC138).

73 *Medical record review*

74 Data retrieved from the medical records consisted of sex, age, breed, body weight, lymphoma  
75 stage, sub-stage, immunophenotype, number of previous chemotherapy agents/protocols  
76 received, initial response to first line protocol, number of previous relapses, administration of  
77 L-asparaginase within 14 days prior to LPP initiation, duration from initial diagnosis to LPP  
78 initiation, lomustine/procarbazine/prednisolone dosage, procarbazine dosing schedule, number  
79 of LPP cycles received, results of complete blood count (CBC) and ALT activity, response to  
80 LPP, time from initiation to discontinuation of the LPP protocol (TTD), reason for LPP  
81 discontinuation, overall survival time and cause of death.

82

83 *Diagnosis and staging*

84 Dogs were staged at time of the initial diagnosis based on the World Health Organisation  
85 (WHO) five-stage criteria for canine lymphoma.<sup>31</sup> Staging investigations included physical  
86 examination, CBC, serum biochemical analysis, lymph node cytology/histology. The following  
87 diagnostic investigations were performed according to clinician's discretion:  
88 immunohistochemistry, flow-cytometry (lymph node, blood and/or bone marrow), PARR (PCR  
89 for Antigen Receptor Rearrangement), urinalysis, thoracic radiographs, abdominal ultrasound,  
90 splenic, hepatic and bone marrow cytology.

91

92 *Treatment protocol*

93 The LPP protocol is summarized in table 1. Doses were calculated to the nearest 5 mg for  
94 lomustine (Medac GmbH, Theaterstr. 6. 22880 Wedel, Germany) and 10 or 25 mg for  
95 procarbazine (Alliance Pharmaceuticals Limited, Chippenham, Wiltshire, SN15 2BB, UK)  
96 based on the availability of smaller capsules (reformulated by Nova Laboratories Ltd, Leicester,  
97 LE18 4YL, UK). Based on the dog's weight and the available procarbazine capsule size, several

98 dosing schedules (daily, every other day, one day off a week, or one day off every three days)  
99 were used to achieve an average target procarbazine dose of 50 mg/m<sup>2</sup>/day. All dogs received  
100 S-adenosylmethionine and silybin (Denamarin<sup>®</sup>, Nutramax Laboratories Veterinary Sciences,  
101 Inc., Lancaster, SC 29720) after each lomustine treatment and the duration of this treatment  
102 was established according to clinician discretion.

103

#### 104 *Response assessment*

105 Clinical response to treatment was assessed via calliper measurements of peripheral lymph  
106 nodes. Thoracic and/or abdominal imaging was repeated to assess response at clinician's  
107 discretion. Response to treatment was assessed as follow: a complete response (CR) was  
108 clinically defined as disappearance of all measurable disease; a partial response (PR) was  
109 defined as > 50% but < 100% reduction in measurable disease; a stable disease (SD) was  
110 defined as < 50% reduction for 21 days or < 25% increase in size of all measurable disease with  
111 no appearance of new lesions; progressive disease (PD) was defined as > 25% increase in  
112 measurable disease or appearance of new lesions.<sup>15,19,22,24</sup> Transient decreases in measurable  
113 disease that persisted for < 21 days were defined as progressive disease. Thus, dogs were also  
114 divided into responders (CR + PR) and non-responders (SD + PD). Treatment was continued  
115 in patients that had CR or PR. Treatment was discontinued when there was SD, PD, toxicity or  
116 other reasons (financial constraints, euthanasia unrelated with lymphoma).

117

#### 118 *Assessment of toxicity*

119 Evidence of toxicity was assessed by evaluation of the medical record, including medical  
120 history obtained from owners and results of physical examination and clinicopathological data.  
121 CBC was recommended prior to LPP initiation, one week after the first lomustine treatment,  
122 one week after each lomustine dose adjustment and prior to each new cycle. ALT activity was

123 measured prior to LPP initiation and prior to each new cycle. Toxicity was retrospectively  
124 graded according to criteria established by the Veterinary Co-Operative Oncology Group.<sup>32</sup>  
125 Neutropenia, thrombocytopenia, increase in ALT activity, vomiting, diarrhoea and anorexia  
126 were evaluated. If baseline ALT activity was high prior to treatment, an elevation in ALT  
127 activity was recorded only if the maximum ALT activity during the LPP protocol was > 1.5-  
128 fold ALT activity at baseline. Dose adjustments and treatment delays were made according to  
129 clinician and owner's discretion, commonly if VCOG grade > II toxicity occurred.

130

### 131 *Statistical analysis*

132 Descriptive statistics were calculated, and data were tested for normal distribution and equal  
133 variance by means of the Shapiro Wilk test and F-test, respectively.

134 The overall, CR and PR rates were defined as the number of dogs achieving CR/PR, CR and  
135 PR respectively, compared with the total number of dogs treated. The TTD was defined as the  
136 time from the initiation of the LPP protocol until discontinuation due to PD or toxicity. Overall  
137 survival time (OS) was defined as the time from diagnosis to death. Dogs that were in clinical  
138 remission at the end of the data collection or discontinued the LPP protocol because of financial  
139 constraints or lymphoma-unrelated death were censored. TTD and OS were calculated using  
140 the Kaplan-Meier method. As the timing of euthanasia is determined by a combination of  
141 clinical factors, clinician and owner judgement, OS was not an endpoint of this study.

142 The following predictor variables were used for statistical analysis : weight, age, sex, stage,  
143 sub-stage, immunophenotype, response to first line chemotherapy protocol (CR vs others),  
144 duration of first remission, time from the diagnosis to LPP initiation, number of previous  
145 protocols/chemotherapy agents and relapses, relapse during first-line protocol, use of L-  
146 asparaginase within 14 days prior to LPP initiation, lomustine/procarbazine/prednisolone dose  
147 (in mg/m<sup>2</sup> body surface area and mg/kg for lomustine; in mg/m<sup>2</sup>/day and mg/kg/day for

148 prednisolone and procarbazine), procarbazine dosing schedule, prednisolone use, occurrence of  
149 grade III-IV neutropenia, ALT increase and gastrointestinal toxicity. For the purpose of  
150 statistical analysis, procarbazine dosing schedule was divided as: continuous procarbazine (C-  
151 PCB: daily administration or one day off a week) and “pulsatile” procarbazine (P-PCB: every  
152 other day administration or one day off every three days). The daily average procarbazine doses  
153 (mg/m<sup>2</sup>/day and mg/kg/day) was calculated from the total cumulative intended dose divided by  
154 the 14 intended days of administration. The influence of these variables on response (CR/PR vs  
155 SD/PD), occurrence of gastrointestinal toxicity (including anorexia, vomiting and diarrhoea),  
156 grade III-IV neutropenia and grade III-IV ALT increase was analysed. For univariable analysis,  
157 Fisher exact test, *t*-test and Wilcoxon rank sum test were used to analyse the influence of  
158 categorical, continuous normal and continuous non-normal variables on the type of response,  
159 respectively. Binomial logistical regression was used for multivariable analysis.  
160 Log rank test was used to compare TTD according to previously mentioned categorical  
161 predictor variables and Cox proportional-hazard regression was used for continuous variables  
162 and multivariable analysis.  
163 Variables significant at 0.2 on bivariate analyses were entered into a multivariable model  
164 followed by a backwards stepwise protocol. All analyses were two-sided, and p<0.05 was  
165 considered to be significant. All statistical calculations were carried out with the R statistical  
166 software version 3.2.0 using the “survival” package.<sup>33,34</sup>

167

168

## 169 **Results**

### 170 *Patients*

171 Forty-one client-owned dogs met the inclusion criteria. The median age was 7.9 years (range  
172 2.6 –14.7 years). The median weight was 28.8 kg (range 6.5 - 76.3 kg). There were 5 intact



173 males (M), 20 neutered males (MN), 1 intact female (F) and 15 neutered females (FN). There  
174 were 8 mixed-breed dogs. Boxer (n=4), border collie (n=4), beagle (n=2), Rack Russel terrier  
175 (n=2), English springer spaniel (n=2) were the most commonly affected breeds, with 19 other  
176 breeds represented by one dog each.

177

178 High grade/large cell lymphoma was diagnosed through cytology in all patients and in 17.1%  
179 (n=7) this was also confirmed via histopathology. All dogs had at least stage III multicentric  
180 lymphoma at presentation, with 8 dogs suspected of having stage V lymphoma based on  
181 confirmed bone marrow involvement (n=1), marked CD34- lymphocytosis (n=3), bladder  
182 involvement (n=1), lymphomatous pleural effusion (n=1), suspected lung involvement (n=1)  
183 and suspected central nervous system involvement (n=1). Twenty-three (56%) and 18 (44%)  
184 were sub-stage a and b, respectively. Immunophenotype was evaluated in 25 dogs (61%). Of  
185 these, 19 (76%) had B-cell and six (24%) had T-cell lymphoma. The overall median survival  
186 time was 367 days (range 64-913 days).

187

#### 188 *Previous treatments*

189 Thirty-seven dogs (90%) had CEOP as a first line chemotherapy protocol and COP was used  
190 in 4 dogs. Thirty-three (80%) achieved complete clinical remission and 8 (20%) achieved partial  
191 remission. The median duration of first complete remission was 153 days (range 7-976).

192 At relapse, cytological confirmation of disease progression was obtained in 13 dogs (31.7%).

193 The median number of relapses prior to LPP was 2 (range 1-3). The median number of previous  
194 chemotherapy protocols was 2 (range 1-4) and the number of previous chemotherapy agents  
195 was 5 (range 4-9). The median duration from the date of diagnosis to the date starting LPP was  
196 202 days (range 33-397). L-asparaginase was used within 2 weeks prior to LPP in 8 cases  
197 (19.5%). None of the dog that received L-asparaginase were in CR at the initiation of the LPP

198 protocol. Seven dogs received lomustine prior to LPP initiation: one dog, 2 dogs and 4 dogs  
199 received 3, 2 and 1 previous lomustine administration, respectively. All dogs were receiving an  
200 ongoing chemotherapy treatment at the time of relapse and the treatment free period prior to  
201 LPP was short (median 14 days, range 5-35 days).

202

### 203 *LPP protocol*

204 The median starting doses of lomustine, procarbazine and prednisolone were 58 mg/m<sup>2</sup> (range  
205 47-68 mg/m<sup>2</sup>), 48 mg/m<sup>2</sup>/day (range 34-66 mg/m<sup>2</sup>/day) and 28 mg/m<sup>2</sup>/day (range 0-33  
206 mg/m<sup>2</sup>/day), respectively. In mg/kg, the median starting dose of lomustine, procarbazine and  
207 prednisolone was 1.98 mg/kg (range 1.34-2.99 mg/kg), 1.75mg/kg/day (range 0.92-2.55  
208 mg/kg/day) and 0.86 mg/kg/day (range 0-1.5 mg/kg/day), respectively. Prednisolone was not  
209 administered in 7 cases (17.1%). Thirty-two dogs (78%) received a continuous procarbazine  
210 dosing schedule (C-PCB group): 28 dogs had daily procarbazine and 4 had daily procarbazine  
211 with one day off per week. Nine dogs (22%) received a “pulsatile” procarbazine dosing  
212 schedule (P-PCB): 7 had every other day administration and 2 had one day off treatment every  
213 three days. The average daily procarbazine dose in mg/m<sup>2</sup>/day was not statistically different  
214 between the two procarbazine dosing schedule groups (p=0.26) whereas in mg/kg/day, dogs in  
215 the P-PCB group (median 2.08 mg/kg/day, range 1.55-2.48 mg/kg/day) received a significantly  
216 (p=0.03) higher dose compared to C-PCB (1.54 mg/kg/day, range 0.92-2.55 mg/kg/day).  
217 Similarly, the lomustine dose in mg/m<sup>2</sup> was not significantly different between dogs in P-PCB  
218 and C-PCB whereas when expressed in mg/kg, dogs in P-PCB received a significantly higher  
219 lomustine dose (p<0.001, P-PCB: median 2.5 mg/kg; range 2.13-2.85 mg/kg vs C-PCB median  
220 1.8 mg/kg; range 1.34-2.99). The body weight of dogs in the P-PCB group was significantly  
221 (p<0.001) lower (median=11 kg, range: 6.5-17.7 kg) than dogs receiving C-PCB (median: 29.7  
222 kg, range 8.3-76.3 kg).

223

224 Of the 41 dogs treated, 12 dogs (29%) achieved CR, 13 (32%) achieved PR, and 16 (39%) had  
225 SD or PD, for an overall response rate of 61%. CR was achieved following the first cycle in 11  
226 dogs and one dog was in PR following the first cycle and achieved CR only following the  
227 second cycle. Two dogs had repeated imaging to assess response during the protocol. One dog  
228 had peripheral lymphadenopathy and restaging prior to LPP initiation revealed a bladder mass  
229 with cytology consistent with large cell lymphoma. Therefore, focal bladder ultrasound was  
230 performed prior to each new LPP cycle. A significant reduction (>50%) of the bladder mass  
231 size was initially documented following LPP initiation although a mild thickening of the  
232 bladder wall persisted. This dog was thus considered a partial responder. LPP was discontinued  
233 following grade IV ALT increase though neither peripheral lymphadenopathy nor bladder mass  
234 enlargement was documented. Another dog had mediastinal lymph nodes involvement and  
235 thoracic ultrasound was repeated prior to each new LPP cycle. Partial response was documented  
236 following the first LPP cycle but the size of the mediastinal lymph nodes increased by more  
237 than 50% (consistent with PD) after the second cycle and LPP was discontinued.

238

239 On univariable analysis, CR on first-line protocol ( $p=0.02$ ) and gastrointestinal toxicity  
240 ( $p<0.01$ ) were significantly associated with overall response (CR/PR vs SD/PD). Other factors  
241 were not significant in univariable analysis (data not shown). Average daily procarbazine dose  
242 in mg/kg/day ( $p=0.06$ ), lomustine dose in mg/kg ( $p=0.17$ ), weight ( $p=0.07$ ), VCOG grade III-  
243 IV ALT increase ( $p=0.15$ ) were also included in multivariable analysis. On multivariable  
244 analysis, CR on first remission ( $p=0.01$ , OR 28.53 95% CI 2.14-379) and average daily  
245 procarbazine dose in mg/kg/day ( $p=0.01$ , OR 10.0 95% CI 1.18-85) were significantly associated  
246 with response (CR/PR vs SD/PD). Among the 33 dogs who achieved CR on first line protocol,  
247 23 (70%) responded to LPP. For the 8 dogs that did not achieve CR on first line protocol, only

248 2 dogs (25%) responded to LPP. The median average daily procarbazine dose for responders  
249 was 1.8 mg/kg/day whereas it was 1.47 mg/kg/day for non-responders. Eleven of twelve dogs  
250 (92%) with gastrointestinal toxicity responded to LPP whereas response was identified in 15  
251 dogs out of 29 (48%) without gastrointestinal toxicity. Although the association between  
252 procarbazine dosing schedule and response was not statistically significant ( $p=0.24$ ), the overall  
253 response rate for dogs in the P-PCB group was 77% whereas it was only 56% for dogs in the  
254 C-PCB group.

255

256 The protocol was discontinued because of disease progression or toxicity in 30 (73%) and 8  
257 dogs (19.5%) respectively. In 1 dog, LPP was discontinued because of financial constraints and  
258 1 dog was euthanised due to deterioration of osteoarthritis. One dog was still in CR at the time  
259 of data analysis.

260

261 The median TTD was 34 days (range 12-167 days) and the median number of LPP cycle was  
262 one (range 1-7). On univariable analysis, neutered female ( $p=0.042$ ), dogs without recent L-  
263 asparaginase ( $p<0.01$ ), complete and partial responders ( $p<0.001$ ), dogs receiving P-PCB  
264 ( $p=0.05$ ) and dogs with grade III-IV ALT increase ( $p=0.05$ ) had significantly increased TTD  
265 (Table 2). Other factors were not significant in univariable analysis (data not shown). On  
266 multivariable analysis, only the achievement of partial ( $p<0.001$ , HR 0.1, 95% CI 0.03-0.34 for  
267 TTD) or complete response ( $p<0.001$ , HR 0.02, 95% CI 0.006-0.11 for TTD) was significantly  
268 associated with TTD. The median TTD for complete responders was 84 days (range 23-167  
269 days), 58 days (range 19-75 days) for partial responders and 21 days (range 19-22 days) for  
270 SD/PD (Figure 1).

271

272 After failing LPP, 28 dogs (68%) went on to receive additional rescue protocols, with DMAC  
273 (dexamethasone, melphalan, actinomycin D, cytosine arabinoside) and LMP (chlorambucil,  
274 methotrexate and prednisolone) being the most common. The median survival time following  
275 LPP discontinuation was 25 days (range 0-676).

276

277

### 278 *Toxicity*

279 Gastrointestinal toxicity occurred after at least one treatment in 12 dogs (29 %). Among them,  
280 7 (58%), 3 (25%), and 2 (17%) dogs had VCOG grade I, II and III gastrointestinal toxicity  
281 respectively (Table 3). Diarrhoea was the most common gastrointestinal adverse effect (n=10).  
282 Six dogs (15%) were hospitalised due to gastrointestinal toxicity and recovered rapidly with  
283 supportive management. Several factors were significantly associated with the occurrence of  
284 gastrointestinal toxicity on univariable analysis: weight (p=0.04), CR on first-line protocol  
285 (0.04), response to LPP (<0.01) and the average daily procarbazine dose in mg/kg/day  
286 (p<0.001). On multivariable analysis, only the average daily procarbazine dose in mg/kg/day  
287 was significant (p<0.01). The median dose for dogs with or without gastrointestinal toxicity  
288 was 2.14 mg/kg/day (range 1.34-2.55 mg/kg/day) and 1.57 (range 0.92-2.48 mg/kg/day),  
289 respectively. The weight for dogs with gastrointestinal toxicity (median 19.45 kg; range 9.75-  
290 41.7 kg) was significantly lower than those without toxicity (median 29.4 kg, range 6.5-76.3  
291 kg). Interestingly, 50% of the dogs weighing less than 14kg had gastrointestinal toxicity  
292 whereas only 23% of the dogs weighing more than 14kg experienced gastrointestinal toxicity.

293

294 At baseline, all dogs had a CBC performed. None of the dogs were neutropenic, 3 had VCOG  
295 grade I thrombocytopenia and 6 were anaemic (4 grade I, 1 grade II). CBC was performed one  
296 week following the first lomustine dose for 35 dogs (85%) and before every new cycle for all

297 dogs. Neutropenia was documented in 20 dogs (57%) 7-10 days following lomustine  
298 administration. Thrombocytopenia was documented in 7 dogs (20%). Eight dogs (23%) and 2  
299 dogs (5%) had VCOG grade III-IV neutropenia or thrombocytopenia, respectively (Table 2).  
300 None of the dogs with grade III-IV neutropenia were hospitalised. On univariable analysis,  
301 weight ( $p=0.03$ ) and procarbazine dosing schedule ( $p=0.03$ ) were associated with the occurrence  
302 of grade III-IV neutropenia. Due to the low number of grade III-IV neutropenia, multivariable  
303 analysis was not performed. The weight of dogs with high-grade neutropenia was significantly  
304 lower (median 17 kg, range 9.75-28.9 kg) than for those without high-grade neutropenia  
305 (median 29.4 kg, range 6.5-76.3 kg). Four of 9 dogs (44%) receiving P-PCB had high-grade  
306 neutropenia whereas only 12% of dogs receiving C-PCB had high-grade neutropenia.

307

308 Serum ALT activity was available in 39 dogs at baseline and among them 14 dogs (36%) had  
309 ALT increase prior to LPP initiation. All dogs received at least 7 days of Denamarin®. ALT  
310 activity was monitored in 32 dogs during the LPP protocol. Twenty-eight dogs (87.5 %) had an  
311 ALT increase during the protocol but among these, only 19 dogs (59%) had an ALT increased  
312  $>1.5$  x baseline. VCOG grade III-IV ALT increase was documented in 10 dogs (31%). None of  
313 them had clinical signs of liver dysfunction. Among the 10 dogs with grade III-IV ALT  
314 increase, one had progressive disease and the other one received 2 lomustine administrations  
315 prior to LPP initiation. The median number of LPP cycles prior to grade III-IV ALT increase  
316 was 2.5 (range 1-4 cycles). None of the analysed factors was significantly associated with grade  
317 III-IV ALT increase.

318

319 Fourteen dogs (30%) had at a least one treatment delay due to neutropenia ( $n=9$ ),  
320 gastrointestinal toxicity (3), fever/lethargy (1) and ALT increase (1). On univariable analysis,  
321 treatment delay was associated with weight ( $p<0.01$ ), grade III-IV neutropenia ( $p<0.001$ ),

322 lomustine dose in mg/kg ( $p=0.02$ ), average daily procarbazine dose in mg/kg/day ( $p<0.1$ ) and  
323 gastrointestinal toxicity ( $p<0.001$ ). In multivariable analysis, only grade III-IV neutropenia  
324 ( $p<0.01$ , OR= 48.0, 95% CI 46.7-49.3) and gastrointestinal toxicity ( $p<0.01$ , OR = 14.43, 95%  
325 CI 13.45 – 15.41) were associated with treatment delay. Treatment delays consisted of  
326 discontinuation of procarbazine for several days until documentation of a normal neutrophil  
327 count or resolution of the clinical signs. In one dog, lomustine administration was delayed by  
328 one week following grade III ALT increase. ALT activity subsequently decreased and a new  
329 LPP cycle was initiated. Eight dogs (20%) had dose reduction following the identification of a  
330 grade IV neutropenia ( $n=7$ ) or grade II gastrointestinal toxicity and lethargy ( $n=1$ ). Dose  
331 reduction was performed if more than one LPP cycle was administered and consisted of a 10-  
332 20% lomustine dose reduction according to clinician discretion and available tablet size.

333

### 334 **Discussion**

335 The primary goal of the study was to evaluate the use of LPP chemotherapy in dogs with  
336 relapsed multicentric lymphoma. A secondary goal was to identify predictive factors associated  
337 with response and toxicity.

338

339 LPP was an effective rescue protocol with an overall response rate of 61% and a median TTD  
340 of 84 days for complete responders and 58 days for partial responders. This is consistent with  
341 previous studies using alkylating agent-based rescue protocols, which reported overall response  
342 rates ranging from 34% to 65% and duration of response for responders from 63 days to 238  
343 days.<sup>10-25</sup> However, due to likely considerable differences in clinical management, geographic  
344 location and inclusion criteria, direct comparison with historical controls is not valid and only  
345 a large randomized prospective clinical trial could allow direct adequate comparison between  
346 various rescue protocols.<sup>35</sup>

347

348 When used as first line treatment for dogs with T-cell lymphoma, alkylating agent-based  
349 protocols such as LOPP and L-MOPP are associated with a high response rate (97-98%) and  
350 adequate progression-free survival (176 – 189 days).<sup>36,37</sup> However, it is expected that response  
351 rate and duration decrease in a rescue setting. Therefore, it could be postulated that the number  
352 of previous relapses could be associated with response and TTD as was also proposed by Gillem  
353 and colleagues.<sup>17</sup> In the present study, the number of previous relapses ( $p=0.98$ ), protocols  
354 ( $p=0.82$ ) and chemotherapy agents ( $p=0.92$ ) were not associated with response nor with TTD.  
355 This may be due to small sample size and bias towards dogs with more resistant lymphomas.  
356 Indeed, for dogs treated with CHOP protocol as a first line treatment, Flory and colleagues  
357 demonstrated that the duration of time off chemotherapy prior to relapse was significantly  
358 associated with the likelihood of response to a second CHOP protocol.<sup>16</sup> This suggested that  
359 most lymphomas in dogs that relapse shortly after protocol discontinuation or during  
360 chemotherapy are drug resistant and thus, may be less likely to respond to rescue protocols. All  
361 the dogs in the present study relapsed during a chemotherapy protocol and the time off  
362 chemotherapy was short (median = 14 days), supporting the hypothesis of a multi drug resistant  
363 phenotype.

364

365 For TTD, the only significant factor on multivariable analysis was response and, not  
366 surprisingly, dogs with CR had a significantly longer TTD than dogs with PR, SD or PD.  
367 Interestingly, in univariable analysis, neutered females had a significantly ( $p=0.04$ ) longer TTD  
368 compared to other sexes (42 days versus 19-23 days). Such finding was also reported by Fahey  
369 and colleagues; however, the lack of significance in multivariable analysis would suggest that  
370 “sex” may be a type I error. The use of L-asparaginase was also significantly associated with  
371 decreased TTD on univariable analysis ( $p=0.04$ ). At our institution, L-asparaginase is mainly



372 administered to dogs with sub-stage b lymphoma, bone marrow and/or central nervous system  
373 involvement. Therefore, we could argue that those dogs that received L-asparaginase had a  
374 lymphoma with more aggressive biological behaviour and, possibly, less responsive to  
375 chemotherapy. Grade III/IV ALT increase was also associated with TTD on univariable  
376 analysis. In the present study, ALT increase occurred after a median of 2.5 cycles. Therefore,  
377 as suggested by Kristal and colleagues, ALT increase is likely to be due to lomustine cumulative  
378 toxicity.<sup>38</sup> Consequently, the statistical association between TTD and VCOG grade III-IV ALT  
379 increase may be explained by cumulative lomustine toxicity and was not clinically relevant in  
380 this cohort.

381

382 Although time to progression is recommended for the assessment of treatment response  
383 duration,<sup>39</sup> TTD was considered a more objective index for this type of study. In a rescue setting,  
384 toxicity may be as important as tumour progression to justify protocol discontinuation.  
385 Additionally, progression was only assessed before starting a new cycle (every 21 days) and  
386 the time to progression could have been overestimated. The association between TTD and  
387 procarbazine dosing schedule ( $p=0.05$ ) was unexpected. This may be a confounding effect as  
388 dogs receiving P-PCB had lower body weight compared to the C-PCB and received a higher  
389 average daily procarbazine (mg/kg/day) and lomustine dose (in mg/kg). Nonetheless, neither  
390 weight ( $p=0.1$ ), procarbazine (in mg/kg/day,  $p=0.54$ ) nor lomustine dose (in mg/kg,  $p=0.31$ )  
391 were associated with TTD on univariable analysis. This result could therefore suggest that, in  
392 a rescue setting, lymphoma could respond better to “pulsatile” procarbazine administration than  
393 to continuous administration. Further studies would be warranted to investigate the effect of  
394 this procarbazine dosing schedule on large breed dogs.

395

396 The achievement of CR on first-line chemotherapy protocol was significantly associated with  
397 response in univariable analysis. Similar findings were suggested by Fahey and colleagues,  
398 where dogs with relapsed lymphoma who responded to first-line protocol had a significantly  
399 longer TTD following UF-LOPP.<sup>15</sup> Therefore, dogs that do not respond to first-line protocols  
400 may be inherently more resistant to chemotherapy through overexpression of P-gp, O<sup>6</sup>-  
401 methylguanine-DNA methyltransferase (MGMT) or other mechanisms. Indeed, MGMT  
402 expression is associated with lomustine resistance in canine lymphoma cells and is also  
403 theoretically able to mediate procarbazine resistance.<sup>40-42</sup> Studies investigating the relationship  
404 between MGMT activity status in canine lymphoma and response to LPP may be warranted. In  
405 humans with malignant astrocytoma, MGMT-methylation may be associated with increased  
406 response to PAV (procarbazine, nimustine, vincristine).<sup>43</sup>

407 Interestingly, the average daily procarbazine dose, when expressed mg/kg/day, was  
408 significantly associated with response: dogs receiving higher procarbazine doses were more  
409 likely to achieve CR/PR. Dogs that had higher procarbazine doses had also more  
410 gastrointestinal adverse events and this may explain the significant association between  
411 gastrointestinal toxicity and response on univariable analysis. Not surprisingly, given the  
412 available procarbazine capsule size, smaller dogs received higher average daily procarbazine  
413 doses and were more likely to be included in the P-PCB dosing schedule. In addition, P-PCB  
414 regimen was significantly associated with TTD suggesting that pulsatile procarbazine dosing  
415 schedule may positively influence response. The higher response rate (77%) in the P-PCB group  
416 compared to C-PCB group (56%) may corroborate that.

417

418 Several studies suggested that vincristine could be the major contributor of gastrointestinal  
419 toxicity observed with LOPP or MOPP protocol.<sup>15,19,24</sup> As vincristine was not included in the  
420 LPP protocol, gastrointestinal toxicity would have been expected to be a less common finding;

421 however, 29% of dogs had gastrointestinal toxicity and among them, 58% had grade I toxicity.  
422 This was similar to the toxicity reported for UF-LOPP (36%) or LOPP (>38.6%) but higher  
423 than for the combination of lomustine-dacarbazine (22%) or L-asparaginase, lomustine and  
424 prednisolone (21% vomiting, 10% diarrhoea) for dogs with relapsed lymphoma.<sup>10,15,19,25</sup> This  
425 would suggest that vincristine may not be the main determinant for gastrointestinal toxicity.  
426 Other contributing factors or a more thorough owners' report might also explain this result.  
427 Interestingly, the addition of vincristine to lomustine and procarbazine did not significantly  
428 increase the occurrence of gastrointestinal toxicity in a human study on oligodendroglial brain  
429 tumours.<sup>44</sup> This may further support the minimal contribution of vincristine to the occurrence  
430 gastrointestinal adverse events when combined with procarbazine. In the present study, the  
431 average daily procarbazine dose (in mg/kg/day) was significantly associated with the  
432 occurrence of gastrointestinal toxicity and the weight of dogs with toxicity was significantly  
433 lower than those without gastrointestinal toxicity on univariable analysis ( $p=0.04$ , 19.45 kg vs  
434 29.4 kg). In addition, the weight was also associated with the occurrence of high-grade  
435 neutropenia. Taken together, these results highly suggested that smaller dogs might be relatively  
436 overdosed when using body surface area. Such a finding has been already established for  
437 doxorubicin, melphalan, and the combination of lomustine-cyclophosphamide.<sup>45-47</sup> Therefore,  
438 as with doxorubicin<sup>45</sup> or as proposed by Saba and colleagues for lomustine, a different  
439 procarbazine dose for smaller dogs could be beneficial to decrease toxicity.<sup>25</sup> However, this  
440 may affect response as higher procarbazine dose was associated with response to LPP in the  
441 present study.

442

443 Similarly to other lomustine-based rescue protocols such as LOPP or lomustine single agent,  
444 neutropenia was relatively common with LPP (57%) and this reflects assessment of CBC at the  
445 time of the expected lomustine nadir and clinical relevance is unlikely.<sup>19,20</sup> Indeed, only 33%

446 of dogs were reported to experience neutropenia (15% with high grade neutropenia) with the  
447 UF-LOPP protocol,<sup>15</sup> but CBC was not consistently performed at the suspected lomustine nadir  
448 and the occurrence of neutropenia was likely underestimated.<sup>15</sup> In our study, in univariable  
449 analysis, dogs with pulsatile procarbazine dosing schedule were more likely to develop high-  
450 grade neutropenia, suggesting pulse procarbazine was associated with increased  
451 myelosuppression. Pulsatile dosing was also associated with longer TTD and as high-grade  
452 neutropenia was asymptomatic in our population, the use of P-PCB may be more appropriate.  
453 This possible association between neutropenia and response duration has been previously  
454 shown for dogs with lymphoma treated with CHOP-based protocol.<sup>48</sup>

455

456 The occurrence of thrombocytopenia was low (20%) in the present study. However, as it was  
457 documented in humans<sup>30</sup> and suggested in dogs<sup>49</sup>, the long platelet lifespan may be responsible  
458 for a delayed thrombocytopenia occurrence that may occur weeks following the discontinuation  
459 of LPP protocol. Therefore, its incidence may have been underestimated.

460

461 It is well recognized that lomustine can cause hepatocellular damage, resulting in serum ALT  
462 elevation in up to 86% of cancer-bearing dogs receiving therapeutic chemotherapy doses.<sup>38,50,51</sup>  
463 As Denamarin® was suggested to reduce ALT elevation in dogs treated with lomustine, all dogs  
464 in the present study received Denamarin® and ALT elevation was documented in 59% of the  
465 cases.<sup>52</sup> Despite the use of Denamarin®, the occurrence of ALT elevation was consistent with  
466 previous studies investigating lomustine-based chemotherapy protocol with or without  
467 hepatoprotectant.<sup>15,19,50-52</sup> However, as the studies are not directly comparable, the role of  
468 Denamarin® in the reduction of ALT elevation could not be assessed. There is no clear  
469 consensus to describe chemotherapy-induced ALT increase, and other factors (lymphoma  
470 infiltration, prednisolone treatment, underlying hepatopathy or procarbazine administration)

471 could participate to ALT serum activity.<sup>30,38</sup> In the present study, grade III-IV ALT increase  
472 was responsible for LPP discontinuation in 4 dogs. Among them, 3 were in CR and one was in  
473 PR at the time of LPP discontinuation. Given the absence of associated clinical signs in these  
474 dogs and the possible reversible lomustine-induced ALT elevation, it is unknown whether  
475 chemotherapy delay could have been sufficient to reduce ALT while maintaining adequate  
476 response.<sup>50</sup>

477

478 Although hospitalisation rate was consistent with previous studies, a 15% hospitalisation rate  
479 in a rescue setting can be considered relatively high. However, the recommendation for  
480 hospitalisation was at the clinician's discretion, and may reflect owners' concerns rather than  
481 the severity of clinical signs.<sup>15,19,24,37,48</sup> According to the VCOG recommendations,  
482 hospitalisation is required only in case of grade III or higher adverse events. Therefore,  
483 hospitalisation would have been required only in two dogs (5%) with VCOG grade III  
484 gastrointestinal toxicity.<sup>32</sup> The other four dogs presented VCOG grade I-II gastrointestinal  
485 toxicity and VCOG grade II lethargy.

486 Limitations of this study are its retrospective nature and the small sample size. In addition, dogs  
487 were not consistently re-staged prior to LPP initiation and during the follow-up period, making  
488 the assessment of CR challenging: CR could have been overestimated as visceral non-target  
489 lesions were not consistently assessed in all patients.<sup>39</sup> However, repeating complete clinical  
490 staging work-ups is rarely an option in dogs with relapsed lymphomas where owners face  
491 financial constraints and may try to minimise intervention due to the poor long-term prognosis.  
492 Classification of gastrointestinal toxicity was performed according to clinical records and the  
493 occurrence of gastrointestinal toxicity (especially VCOG grade 1) could have been  
494 underestimated; however, its clinical significance remains questionable. Although T-cell  
495 lymphoma has been suggested to be more responsive to alkylating-agent based chemotherapy

496 protocols, association with immunophenotype could not be analysed in our population, given  
497 the small number of dogs with T-cell lymphoma (n=6).<sup>36,37</sup> Several studies showed that  
498 different lymphoma histological types had different prognosis and response to rescue protocol  
499 may follow a similar pattern.<sup>53-56</sup> In the present study, histological classification was not  
500 consistently performed and the effect specific lymphoma histotype on response rate or TTD  
501 could not be investigated.

502

503 In conclusion, these data support the use of LPP as a rescue protocol for dogs with lymphoma:  
504 the response rate was 61%, TTD for complete responders was 84 days and toxicity was  
505 acceptable. Our results also suggested that a more pulsatile procarbazine dosing schedule may  
506 improve treatment response. In addition, the use of oral chemotherapy alone should decrease  
507 hospital visit time, which may improve owner compliance and satisfaction in a rescue setting.

508

509

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## 685 **Tables and figure captions**

686

687 **Table 1.** Prednisone was administered orally at 30 mg/m<sup>2</sup>/day from day 1 to day 15, then  
688 reduced to 20mg/m<sup>2</sup>/day from day 15 to 21 and then maintained at 15mg/m<sup>2</sup>/day for the  
689 following cycles.

690

691 **Table 2.** The use of L-asparaginase was considered only if performed within 14 days prior to  
692 LPP initiation. The C-PCB schedule includes dogs with daily procarbazine administration or  
693 with one day off a week and P-PCB dosing schedule includes dogs with every other day  
694 procarbazine administration or one day off every three days. The dog for which protocol was  
695 discontinued because of financial constraints or euthanasia due to non-lymphoma related causes

696 were censored from the TTD analysis. Only dogs for which the protocol was discontinued  
697 because of progressive disease was included in the analysis for TTP.

698

699 **Table 3.** The highest toxicity grade is reported for each patient. For gastrointestinal events,  
700 the highest VCOG grade among vomiting, diarrhoea or anorexia was considered.

701

702 **Figure 1.** Time from initiation to discontinuation for dogs who achieved CR (84 days range:  
703 23-167 days), PR (58 days, range: 19-75 days) and SD/PD (21 days, range: 19-22 days)  
704 treated with LPP ( $p < 0.001$ ). Time to progression for dogs achieving CR, PR and SD/PD was  
705 113 days (range 23-167 days), 58 days (range 19-75 days) and 21 days (range 19-22 days)  
706 respectively.