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

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Abstract

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

Keywords: Canine Lymphoma; Relapse; Procarbazine; Lomustine; Dog; Chemotherapy; Rescue

Introduction

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

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

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

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

proposed a modified-LOPP protocol (UF-LOPP) with decreased dose intensity.[15] The response rate was similar to the previous study, but fewer adverse effects were recorded.

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

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

Materials and methods

Study population

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

Medical record review

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

Diagnosis and staging

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

Treatment protocol

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

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

Response assessment

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

Assessment of toxicity

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

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

Statistical analysis

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

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

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

prednisolone and procarbazine), procarbazine dosing schedule, prednisolone use, occurrence of grade III-IV neutropenia, ALT increase and gastrointestinal toxicity. For the purpose of statistical analysis, procarbazine dosing schedule was divided as: continuous procarbazine (C-PCB: daily administration or one day off a week) and "pulsatile" procarbazine (P-PCB: every other day administration or one day off every three days). The daily average procarbazine doses (mg/m^2/day and mg/kg/day) was calculated from the total cumulative intended dose divided by the 14 intended days of administration. The influence of these variables on response (CR/PR vs SD/PD), occurrence of gastrointestinal toxicity (including anorexia, vomiting and diarrhoea), grade III-IV neutropenia and grade III-IV ALT increase was analysed. For univariable analysis, Fisher exact test, *t*-test and Wilcoxon rank sum test were used to analyse the influence of categorical, continuous normal and continuous non-normal variables on the type of response, respectively. Binomial logistical regression was used for multivariable analysis.

Log rank test was used to compare TTD according to previously mentioned categorical predictor variables and Cox proportional-hazard regression was used for continuous variables and multivariable analysis.

Variables significant at 0.2 on bivariate analyses were entered into a multivariable model followed by a backwards stepwise protocol. All analyses were two-sided, and $p<0.05$ was considered to be significant. All statistical calculations were carried out with the R statistical software version 3.2.0 using the "survival" package. [33,34]

Results

Patients

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

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

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

Previous treatments

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

At relapse, cytological confirmation of disease progression was obtained in 13 dogs (31.7%). The median number of relapses prior to LPP was 2 (range 1-3). The median number of previous chemotherapy protocols was 2 (range 1-4) and the number of previous chemotherapy agents was 5 (range 4-9). The median duration from the date of diagnosis to the date starting LPP was 202 days (range 33-397). L-asparaginase was used within 2 weeks prior to LPP in 8 cases (19.5%). None of the dog that received L-asparaginase were in CR at the initiation of the LPP

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

LPP protocol

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

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

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

2 dogs (25%) responded to LPP. The median average daily procarbazine dose for responders was 1.8 mg/kg/day whereas it was 1.47 mg/kg/day for non-responders. Eleven of twelve dogs (92%) with gastrointestinal toxicity responded to LPP whereas response was identified in 15 dogs out of 29 (48%) without gastrointestinal toxicity. Although the association between procarbazine dosing schedule and response was not statistically significant (p=024), the overall response rate for dogs in the P-PCB group was 77% whereas it was only 56% for dogs in the C-PCB group.

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

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

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

Toxicity

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

At baseline, all dogs had a CBC performed. None of the dogs were neutropenic, 3 had VCOG grade I thrombocytopenia and 6 were anaemic (4 grade I, 1 grade II). CBC was performed one 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 ($p<0.001$),

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Tables and figure captions

Table 1. Prednisone was administered orally at 30 mg/m^2/day from day 1 to day 15, then reduced to 20mg/m^2/day from day 15 to 21 and then maintained at 15mg/m^2/day for the following cycles.

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

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

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

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