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ORIGINAL ARTICLE

Oral melphalan for the treatment of relapsed canine lymphoma

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Oral melphalan has been included in multi-agent rescue protocols for canine lymphoma but its activity as a single-agent for this purpose has not been established. Inexpensive cost, ease of administration and tolerability make oral melphalan an attractive candidate for single-agent rescue therapy of canine lymphoma. Retrospective evaluation of 19 cases of relapsed canine lymphoma treated with oral melphalan was performed. Melphalan was primarily administered (n = 16) via a high dose protocol (HDM) with a median dosage of 19.4 mg m^{-2}. Fifteen dogs (78.9%) were treated concurrently with corticosteroids. Response evaluation was possible for all dogs with a calculated overall clinical benefit (partial response [PR] + stable disease [SD]) of 31.6% (PR 3/19; SD 3/19). Times to progression following melphalan (TTP-M) were 14, 24 and 34 days for responders and 20, 28 and 103 days for dogs experiencing SD. Twelve of 17 dogs evaluable for toxicity experienced an adverse event (AE) with only 3 dogs experiencing a grade III or higher AE. Haematologic toxicity was common (11/17) while gastrointestinal toxicity was rare (1/17). Although treatment resulted in limited clinical benefit and non-durable responses, oral melphalan was well-tolerated and may be a reasonable rescue option in cases where minimal effective agents remain.

KEYWORDS

canine, chemotherapy, lymphoma, melphalan, rescue

1 | INTRODUCTION

High-grade canine lymphoma is initially a chemoresponsive disease with remission rates to standard CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone)-based chemotherapy protocols of greater than 80% to 90%.[1–3] However, despite this high rate, the majority of dogs eventually become refractory to these frontline agents. When relapse occurs, secondary treatment (ie, "rescue") protocols can be employed, although remission rates and durations are usually considerably less compared with frontline protocols. Both multidrug and single-agent rescue protocols have been evaluated in this setting, with a wide range of reported response rates and durations.[4–14] Many protocols carry both increased cost and risk of toxicity compared with initial regimens, making them potentially undesirable to clients who have already undergone significant financial commitment and for whom quality of life is typically the utmost concern. Therefore, inexpensive and well-tolerated alternatives are needed.

Melphalan (Alkeran; Glaxo-Smith-Kline, Research Triangle Park, North Carolina), is a bifunctional alkylating agent in the nitrogen mustard subclass that enters the cell via active transport through amino acid transporters.[15,16] The drug's inexpensive cost, ease of administration and tolerability make melphalan an attractive candidate for single-agent rescue therapy of canine lymphoma. Melphalan is commercially available in both intravenous and oral formulations, with the 2-mg oral tablets being relatively inexpensive compared with other commonly used oral alkylating agents such as chlorambucil and procarbazine. Oral bioavailability in the dog is reported to be high, with rapid absorption and peak serum levels occurring approximately 30 minutes after administration.[17] Dosage and schedule of oral melphalan administration vary considerably, ranging from lower dose daily-to-every other day schedules to higher dose treatment administered every few weeks.[18–22] However, regardless of the protocol, melphalan has been reported to be reasonably well-tolerated, with minimal clinically relevant short-term side effects and the most common long-term side effects being haematologic in nature.

In both human and veterinary oncology, melphalan's main indication is in combination with prednisone for the treatment of multiple myeloma.[15,18] Other published veterinary experiences include use in combination with prednisolone for canine chronic lymphocytic leukaemia,[19] alone or in combination with other therapies for malignant melanoma[20,21] and as a single-agent following surgery for canine apocrine gland adenocarcinoma of the anal sac.[22] Of particular relevance for our study, melphalan has also been included in combination protocols for relapsed or refractory canine lymphoma.[5,23,24] However, its contribution to both response and outcome in the multi-agent setting is difficult to assess.

Although melphalan has been anecdotally used alone or in combination with corticosteroids for canine lymphoma, there are no published studies evaluating its use as the sole cytotoxic agent. A phase I study of intravenous melphalan did include 2 dogs with lymphoma but no response or outcome data was included due to the nature of that study.[25] Single-agent high-dose melphalan has been shown to have activity in relapsed Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) in people[26–28] and, thus, may also be active in the canine counterpart. Therefore, the purpose of this retrospective study was to evaluate the efficacy and adverse event (AE) profile of oral melphalan for the treatment of relapsed canine lymphoma.

2 | MATERIALS AND METHODS

A medical records search was performed for dogs with relapsed lymphoma (cytologically or histologically high grade) that were treated with melphalan at the North Carolina State University Veterinary Teaching Hospital between 2003 and 2015. Dogs were included if they had clinically detectable disease at the time of first melphalan treatment and received melphalan as the sole cytotoxic chemotherapy agent. Concurrent corticosteroids were allowed. Data collected from medical records review included: breed, sex, weight (at initial visit and at time of starting melphalan), age at diagnosis, method of diagnosis, phenotype, method of phenotyping, staging performed, initial stage and substage, initial chemotherapy protocol, date of initiating chemotherapy, whether or not complete remission was achieved on initial protocol, date of first relapse, stage at initial relapse, number and types of rescue protocols prior to melphalan, date of first melphalan administration, stage at time of first melphalan administration, melphalan dosage and schedule, use of concurrent corticosteroids and type/dose/schedule, total number of melphalan doses received, toxicity, date of progression post-melphalan, subsequent chemotherapy protocols, and date and cause of death.

Although full staging was not required, when performed stage and substage were assigned at initial diagnosis and relapse according to the modified World Health Organization (WHO) Classification for canine lymphoma. Immunophenotyping of the lymphoma was also not required, however, when performed, immunophenotype was determined as previously described.[29] All dogs had cytologic confirmation of relapse prior to initiation of rescue chemotherapy, but not all had cytologic confirmation of relapse immediately prior to starting melphalan. Melphalan dosing, as well as prescribing of concurrent corticosteroids, were at the clinician's discretion. Melphalan protocols were described as either "high dose" (HDM) if each treatment dosage was ≥10 mg m^{-2} and given weekly or biweekly, or "low dose" (LDM) if <10 mg m^{-2} was given daily or every other day. The HDM dosage and schedule were adapted from the published target dosage of 20 mg m^{-2} biweekly used in the multi-agent DMAC (dexamethasone, melphalan, actinomycin and cytosine arabinoside) rescue protocol.[5,23] The LDM dosages and schedules were adapted from similar published protocols in tumour-bearing dogs[18,19,24] adjusted to the individual within the constraints of the available 2-mg tablets.

Response and toxicity were typically evaluated 7 and 14 days posttreatment with physical examination and complete blood counts. Response was generally determined via caliper measurement of the largest diameter of target lesions or haematologic evaluation when indicated. Response was assigned retrospectively according to previously published criteria,[30] as follows: complete response (CR), 100% reduction in all measurable lesions; partial response (PR), ≥30% reduction in the size of measurable lesions; stable disease (SD), <30% reduction or <20% increase in the size of measurable lesions and no new lesions identified; and progressive disease (PD), ≥20% increase in the size of measurable lesions or development of new lesions. Toxicity was graded retrospectively according to the Veterinary and Comparative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v 1.1.[31] Because several dogs had pre-existing cytopenias presumed to be either secondary to their disease or long-term chemotherapy administration, haematologic toxicity was attributed to melphalan, and thus included in analysis, only if values were reduced further from pretreatment levels.

Time to first progression (TTP-F) was defined as the time from starting the first chemotherapy protocol to the time of first progression. Time to starting melphalan (TTM) was defined as the time from diagnosis to the time of first melphalan treatment. Time to progression post-melphalan (TTP-M) was defined as the time from first melphalan treatment to the time of progression post-melphalan. To allow comparison between HDM and LDM protocols, dose intensity (DI) was calculated by determining the total number of milligrammes of melphalan per body surface area (BSA; m^2) administered per 7-day period and was expressed as mg m^{-2} wk^{-1}.

3 | RESULTS

3.1 | Case demographics

Twenty-two dogs were identified as having received oral melphalan as the sole cytotoxic agent of a rescue protocol. Three dogs were subsequently excluded due to concurrent administration of L-asparaginase (n = 2) or euthanasia 24 hours post-melphalan administration (n = 1), because accurate assessment of both response and toxicity was not possible in these cases. Table 1 summarizes the characteristics of the remaining 19 dogs that met inclusion criteria. The median weight at initial diagnosis was 26.40 kg (mean 24.05 kg; range, 4.80-41.7 kg). The most common breeds were Boxer (n = 4) and Labrador Retriever (n = 2). Lymphoma was initially diagnosed by cytology in 9 dogs, histopathology in 8 dogs, and a combination of cytology and histopathology in 2 dogs. In all but 1 dog, the diagnosis

TABLE 1 Characteristics of 19 dogs with relapsed or refractory lymphoma treated with melphalan

Characteristic	Value	Percentage (%)
Age at time of melphalan (years)		
Median	10	
Mean ± SD	9.37 ± 3.27	
Range	14-15	
Weight at time of melphalan (kg)		
Mean± SD	24.36 ± 12.49	
Range	5.09-43.80	
Median	25.8	
Gender		
Female spayed	8	42.1
Male castrated	11	57.9
Breed		
Boxer	4	21.1
Labrador Retriever	2	10.5
Mixed breed	2	10.5
Other purebreed	11	57.9
Immunophenotype		
B-cell	9	47.4
T-cell	3	15.8
Not determined	7	36.8
Initial WHO stage		
III	8	42.1
IV	4	21.1
V	7	36.8
Initial WHO substage		
a	13	68.4
b	6	31.6
Melphalan schedule		
High dose	16	84.2
Low dose	3	15.8
Concurrent corticosteroids		
Prednisone	10	52.6
Dexamethasone	5	26.3
None	4	21.1

Abbreviations: SD, standard deviation; WHO, World Health Organization.

was made via sampling of an enlarged lymph node. In the remaining dog, a diagnosis was made via cytology of abdominal fluid and urine. In this case, the disease burden was isolated to the bladder at the time of initial diagnosis. Cellular morphology was described as "large" in 10 dogs, "intermediate-to-large" in 6 dogs and "intermediate" in 3 dogs. Immunophenotype was determined in 12 of 19 dogs: 9 dogs were identified as having B-cell lymphoma and 3 dogs as having T-cell lymphoma. The majority of dogs (n = 10) were immunophenotyped using flow cytometry, with the remaining determinations made by immunohistochemistry (n = 2). One additional Boxer dog had polymerase chain reaction (PCR) for antigen receptor rearrangements (PARR) performed which was consistent with T-cell clonality; however, no additional immunophenotyping diagnostics were performed to confirm T-cell immunophenotype.

Fifteen dogs were initially treated with a CHOP-based chemotherapy protocol. Of these 15 dogs, 5 received L-asparaginase as an induction agent at the start of their CHOP protocol, and 4 had mitoxantrone substituted for doxorubicin at some point during their initial CHOP protocol. The remaining 4 dogs received a variety of protocols including L-asparaginase, vincristine and doxorubicin (n = 2), L-asparaginase followed by single-agent doxorubicin (n = 1) and a multi-agent protocol consisting of vincristine, cyclophosphamide, doxorubicin, lomustine and procarbazine (n = 1). The median TTP-F was 152 days (range, 50-1195 days). None of the dogs received melphalan as their first rescue protocol. The median number of chemotherapy protocols received between first relapse and starting melphalan was 3.4 (range, 1-6). Six dogs restarted the initial CHOP protocol with mitoxantrone substituted for doxorubicin if a cumulative doxorubicin dosage of 180 mg m^{-2} had previously been reached. All dogs received lomustine as a rescue agent prior to melphalan either with (n = 8) or without (n = 11) L-asparaginase. Various other drugs were also used alone or in combination prior to melphalan.

The median TTM was 245 days (range, 119-1317 days). Most dogs did not undergo routine re-staging at the time of starting melphalan, so the majority (n = 12) were designated as "at least stage III" based on generalized peripheral lymphadenopathy. Three dogs were designated stage V by documentation of circulating lymphoblasts or other extranodal disease. All dogs received the oral formulation of melphalan. The median weight at the time of starting melphalan was 25.80 kg (mean 24.36 kg; range, 5.09-43.80 kg). The median DI for all dogs was 10.0 mg m^{-2} wk^{-1} (mean 12.7; range, 6.3-22.7). Sixteen dogs received HDM with a median dosage and dose of 19.4 mg m^{-2} (range, 17.9-29.9) and 17 mg (range, 6-38), respectively. The median DI was 10.1 mg m^{-2} wk^{-1} (mean 12.7; range, 6.3-20.2), and the median number of doses was 1 (range, 1-5). Two dogs received LDM every other day, with dosages of 2.1 and 1.7 mg m^{-2}, respectively. One dog received alternating daily dosages of 4.6 and 2.3 mg m^{-2}. The median DI for the 3 LDM dogs was 8.2 mg m^{-2} wk^{-1} (mean 12.6; range, 6.8-22.7). Fifteen dogs (78.9%) received either prednisone (n = 10) or dexamethasone (n = 5), with the majority of dogs (n = 13) administered corticosteroids daily.

The general reasons for electing for melphalan were able to be discerned from the medical record in all but 2 of the dogs. These reasons were typically multi-factorial and included tolerability (n = 9), cost (n = 8), clinician-perceived multi-drug resistance to other available agents (n = 8) and convenience/ease of administration (n = 1). The reasons behind the choice of LDM over HDM in those 3 dogs related to the clients' wishes for a less intense protocol that could be administered orally at home.

3.2 | Response evaluation

All dogs were evaluable for response. No CRs were observed. Six dogs (31.6%) experienced clinical benefit (PR or SD) consisting of 3 dogs who obtained a PR (15.8%) and 3 dogs who experienced SD (15.8%). Table 2 summarizes the characteristics of these dogs. The remaining 13 dogs experienced PD with a median TTP-M of 8 days (range, 1-14 days). Eleven received HDM and 10 received concurrent corticosteroids. No obvious factors differentiated the dogs who experienced clinical benefit from those who did not. The median DIs for the clinical benefit and PD groups were 10.0 mg m^{-2} wk^{-1}

TABLE 2 Characteristics of 6 dogs that experienced clinical benefit

Breed	Gender	Phenotype	Weight (kg)	TTM (days)	Number of previous rescue protocols	Individual melphalan dosage (mg m^{-2})	Schedule	DI[a] (mg m^{-2} wk^{-1})	Corticosteroid	Response	TTP-M (days)
Boxer	FS	ND	31.7	186	4	19.6	HDM, q1w	9.8	Prednisone	PR	34
Labrador Retriever	MC	ND	25.8	684	2	20.2	HDM, q2w	10.1	Prednisone	PR	24
Mixed	FS	ND	9.16	168	5	18.0	HDM, q1w	18.0	Dexamethasone	PR	14
Shih Tzu	FS	ND	5.09	519	1	20.0	HDM, q2w	10.0	None	SD	103
Greyhound	MC	B-cell	29.4	550	3	2.1	LDM, q48h	8.2	Prednisone/dexamethasone	SD	28
Labrador Retriever	FS	B-cell	40.0	119	1	20.1	HDM, q2w	10.0	Prednisone	SD	20

Abbreviations: DI, dose intensity; FS, female spayed; m^2, body surface area; MC, male castrated; ND, not determined; PR, partial response; q1w, weekly; q2w, biweekly; q48h, every other day; SD, stable disease; TTM, time to first melphalan administration; TTP-M, time to progression post-melphalan.

[a] Median DI for dogs with clinical benefit = 10.0; overall median DI = 10.0; median DI for non-responders = 10.8.

TABLE 3 Toxicities observed in 17 evaluable dogs[a] treated with melphalan

Toxicity	Number	Percentage (%)	Protocol (*n*)
Thrombocytopenia			
Grade 1	4	21.1	HDM (4)
Grade 2	2	10.5	HDM (1), LDM (1)
Grade 3	2	11.8	HDM (2)
Anaemia			
Grade 1	4	21.1	HDM (3), LDM (1)
Grade 2	2	10.5	HDM (2)
Neutropenia			
Grade 1	1	5.3	LDM (1)
Grade 4	1	5.3	HDM (1)
Diarrhoea			
Grade 1	1	5.3	HDM (1)

[a] Median weight (kg) of dogs with toxicity = 25.5; without toxicity = 21.1; all dogs = 26.2.

(mean 11.0; range, 8.2-18.0) and 10.8 mg m^{-2} wk^{-1} (mean 13.5; range, 6.3-22.7), respectively.

3.3 | Toxicity

Seventeen dogs were evaluable for toxicity, with 2 dogs excluded due to insufficient follow-up. Five dogs experienced no AEs. Overall, toxicity was mild in the remaining 12 dogs (Table 3). Eleven (64.7%) experienced haematologic toxicity. Thrombocytopenia was the most common haematologic AE (*n* = 8; 47.1%) followed by anaemia (*n* = 6; 31.6%) and neutropenia (*n* = 2; 10.5%). The dog that developed grade 4 neutropenia did so after the initial dose and subsequently went on to receive melphalan at a reduced dosage (approximately 15 mg m^{-2}) for 4 additional treatments, obtaining SD for a period of 103 days. Of the 5 dogs who received >1 HDM treatment, this was the only dog who received a dose reduction. One other dog received a dose escalation to approximately 25 mg m^{-2} for its second and final treatment. Gastrointestinal toxicity was only noted in 1 dog that had received biweekly HDM, consisting of grade 1 diarrhoea that resolved with supportive care.

4 | DISCUSSION

The goal of this retrospective study was to evaluate the efficacy and tolerability of oral melphalan for the treatment of relapsed canine lymphoma. Although well-tolerated, the response rate was low (15.8%), with no dog achieving a CR, and response durations were short. However, an additional 15.8% of dogs achieved SD, resulting in clinical benefit in 31.6% of cases. Clinical benefit is an increasingly accepted measure of treatment outcome, particularly in settings where the main goal is palliation.

In addition to tolerability, oral melphalan has several other appealing qualities as a rescue therapy. Oral administration is often seen as an advantage to many clients as well as clinicians due to its convenience and reduced stress on the patient, especially when compared with intravenously administered drugs that may require

infusion over several hours of hospitalization.[5,6,11,12,23] Furthermore, melphalan's affordability may lead clients to consider the drug for additional rescue therapy if they were otherwise contemplating discontinuing treatment. At the authors' institution at the time of publication, considering only the cost of the drugs themselves for a 30 kg dog, oral melphalan at a dosage of 20 mg m^{-2} cost approximately 20% to 95% less than most other single-agent rescue protocols,[9–11,14] and 25% to 96% less than multi-agent protocols.[5–8,10,12,23] Although its response rate was found to be inferior compared with most of these protocols, this cost comparison combined with its tolerability may make oral melphalan an attractive option for clients who are financially limited and, like most clients, value their pet's quality of life.

There are multiple possibilities for the low response rate observed in this study. First, melphalan requires active transport into the cells of interest and as such it is possible that the neoplastic lymphocytes may have downregulated these transporters leading to inadequate drug uptake.[16] Additionally, tumour cells can upregulate multiple DNA repair mechanisms and anti-apoptotic/pro-survival pathways that can lead to generalized chemotherapy resistance, particularly when relapse occurs following significant pretreatment owing to selection of resistant clones.[15] The dogs in our study likely had highly chemoresistant disease at the time of starting melphalan due to pretreatment with other chemotherapy agents. In fact, 11 of 13 dogs who developed PD to melphalan had received ≥3 rescue protocols prior. This is in contrast to only 3 of 6 dogs who experienced clinical benefit that had received lomustine (+/− L-asparaginase) as their sole rescue protocol prior to melphalan.

Another potential explanation for the low overall response rate is that dogs may have received an inadequate dosage of melphalan, as the ideal dosage and schedule in the dog is unknown. The majority of dogs in this study received HDM and it is possible that when used as the sole cytotoxic agent, the dosage of melphalan may be able to be increased. In human oncology, the melphalan-containing BEAM (carmustine, etoposide, cytosine arabinoside and melphalan) protocol is a commonly used bone marrow conditioning regimen prior to stem cell transplant in patients with HL or NHL, with melphalan typically given at a dosage of 140 mg m^{-2}.[15,32,33] However, when used as a single-agent for the same purpose the melphalan dosage can be increased by 42% to 71%.[26–28] Potential dose escalation is further supported by the fact that the majority of dogs in this study experienced low grade or no AEs from treatment with only 1 dog experiencing a grade 4 AE (neutropenia). That dog had also experienced significant neutropenia during its initial CHOP protocol as well as its subsequent treatment with lomustine prior to melphalan. Furthermore, this dog was a small breed dog which weighed 5.09 kg at the time of melphalan initiation. In a phase I study of intravenous melphalan, Page et al proposed that melphalan should be dosed based on weight rather than BSA as they found that 88% of dogs weighing <14 kg experienced severe myelosuppression as compared with only 23% of dogs >14 kg.[25] In our study, only 5 dogs weighed <14 kg and 4 of these 5 dogs received HDM. Therefore, it is possible that our data underestimates the possibility of severe haematologic toxicity due to the limited number of small breed dogs included. Interestingly, in further contrast to our findings, in the Page et al paper all 4 dogs who received melphalan at a dosage of 20 mg m^{-2} experienced severe myelosuppression 1-week posttherapy regardless of body weight.

Poor oral bioavailability may offer further explanation for both the low response rate and minimal toxicity observed in this study. As noted previously, melphalan is reported to have high oral bioavailability in the dog.[17] However, this previous research was performed in healthy female beagle dogs. Thus, it is possible that these results do not reflect the pharmacokinetics of oral melphalan in a heterogeneous population of tumour-bearing dogs. In human cancer patients, numerous pharmacokinetic studies have demonstrated that oral dosing of melphalan can result in incomplete and highly variable absorption.[34–37] These differences in bioavailability may account for some of the discordance in toxicity between our study and the Page et al study.[25] Furthermore, it has been suggested that reduced oral bioavailability may be 1 reason for poor treatment outcomes in some human patients.[35] One factor that has been shown to result in decreased oral absorption is concurrent administration of food[38,39] with a proposed mechanism being competitive inhibition by food-derived amino acids at the level of small intestinal amino acid carriers.[16,40] Consequently, it is recommended that human patients not receive oral melphalan with food.[38] As our data was acquired retrospectively, whether or not melphalan was administered with food was unknown as this was not routinely recorded within the medical record. Nevertheless, it is likely that at least some dogs, especially those who received the LDM protocol administered by their owners, received the drug with food or in close proximity to a meal. Consequently, absorption may have been decreased which could have negatively affected our results.

One possible hindrance to substantial dose escalation and a potential argument against poor oral bioavailability is the finding that nearly half (47.1%) of the treated dogs in our study experienced thrombocytopenia. However, only a third of these were categorized as grade 3 and none as grade 4. Furthermore, it is important to note that 42% of dogs evaluable for toxicity had pre-existing thrombocytopenia at initiation of melphalan suggesting that their bone marrow may have suffered previous insults from prior chemotherapy and/or their disease. Our study population had received a median of 3.5 previous rescue protocols and chronic thrombocytopenia is not uncommon in such pretreated patients. Of particular note is that all dogs had previously received lomustine, as cumulative thrombocytopenia is a known adverse effect of that drug. In their evaluation of the DMAC protocol for relapsed canine lymphoma, Alvarez et al found that previous treatment with lomustine resulted in a significantly higher likelihood of developing thrombocytopenia which occurred in 91% of such dogs.[5] In contrast, Parsons-Doherty et al did not find the same correlation in their evaluation of the DMAC protocol, though they had a lower percentage of lomustine-pretreated dogs.[23] As all dogs in the current study had previously received lomustine, it is impossible to know if the incidence of melphalan-induced thrombocytopenia would have been less in a lomustine-naïve population.

Being retrospective in nature, this study has multiple inherent limitations. First, there was no standardization of case enrollment or management, and dogs received melphalan at varying dosages and schedules. Furthermore, as the commercially available oral 2-mg formulation was used in all dogs, doses were rounded, usually down, to

the nearest whole tablet in most cases. The majority of dogs also received corticosteroids of different types and at different dosages and schedules which may have affected both response and toxicity evaluation. Although a combined (either synergistic or antagonistic) effect on response and toxicity may be possible, this seems unlikely as all dogs had been similarly treated in prior protocols and had relapsed during corticosteroid therapy. Ideally, melphalan would be evaluated prospectively as a single-agent in canine lymphoma to accurately determine activity. Such a study would be performed in a larger number of treatment-naive or minimally pretreated dogs, which illustrates 2 other limitations of this study: the small sample size and variation in the number of previous chemotherapy protocols received.

In summary, oral melphalan had limited clinical benefit in the dogs evaluated in this study. The response rate and duration presented here are inferior to a number of previously published rescue protocols, including melphalan-containing multi-agent protocols. However, in a small subset of dogs, clinical benefit was noted and for clients who wish to continue treatment for dogs that are refractory to other protocols, melphalan could provide an affordable, well-tolerated option. Due to the low incidence of higher grade AEs in the dogs treated with HDM in this study, we propose that dose escalation may be possible in minimally pretreated cases. Therefore, future prospective studies of single-agent oral melphalan, with potential dose escalation and pharmacokinetic analysis in a larger number of dogs with high-grade lymphoma, should be considered.

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