

Concurrent use of rabacfosadine and L-asparaginase for relapsed or refractory multicentric lymphoma in dogs

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Abstract

Background: Rabacfosadine (RAB), a novel antineoplastic agent conditionally licensed for the treatment of lymphoma in dogs, is efficacious in both naïve and previously treated dogs. Its use in combination with L-asparaginase (L-ASP) has not been studied.

Hypothesis/Objectives: To evaluate the safety and efficacy of L-ASP given concurrently with RAB in dogs with relapsed multicentric lymphoma.

Animals: Fifty-two dogs with relapse of lymphoma after treatment with at least 1 doxorubicin-based chemotherapy protocol.

Methods: Open-label, multicenter, prospective single-arm clinical trial. Dogs were treated with RAB at 1.0 mg/kg IV every 21 days for up to a total of 5 doses. L-asparaginase was administered at 400 IU/kg SQ concurrently with the first 2 treatments of RAB.

Results: The overall response rate (ORR) for all dogs was 67%, with 19 dogs (41%) achieving a complete response (CR). The median progression-free survival time (MPFS) was 63 days (range 5-428 days). Dogs experiencing a CR as their best response had an MPFS of 144 days (range 44-428 days). Adverse events were similar to previous studies evaluating single agent RAB. Failure to achieve a CR and having previously received L-ASP were negative prognostic factors on multivariate analysis.

Conclusions and Clinical Importance: Concurrent RAB/L-ASP appears to be both efficacious and safe for treating relapsed multicentric lymphoma in dogs. Adverse events were most often mild and no unexpected toxicoses were observed.

KEYWORDS

asparaginase, chemotherapy, dog, GS-9219, guanine, lymphosarcoma, Tanovea

Abbreviations: AE, adverse event; ALL, acute lymphoblastic leukemia; CR, complete response; DLT, dose-limiting toxicity; DOX, doxorubicin; GI, gastrointestinal; L-ASP, L-asparaginase; MPFI, median progression-free interval; ORR, overall response rate; PD, progressive disease; PR, partial response; RAB, rabacfosadine; SD, stable disease; ULN, upper limit normal; VCOG, Veterinary Cooperative Oncology Group.

1 | INTRODUCTION

Multicentric lymphoma is highly prevalent in dogs, yet treatment has remained largely stagnant for many years. Multiagent doxorubicin

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(DOX)-based chemotherapy protocols (ie, "CHOP-based" protocols including cyclophosphamide, DOX, vincristine, and prednisone) have been the staple of lymphoma treatment in dogs for over 20 years.¹ These protocols are highly effective in inducing remission for most dogs; however, more than 95% of dogs relapse with CHOP-refractory disease. Chemotherapy-refractory disease presents the greatest challenge for clinicians treating lymphoma, and there thus remains a need for novel antineoplastic agents and protocols for the treatment of lymphoma in dogs.

Rabacfosadine (RAB, Tanovea-CA1; VetDC, Fort Collins, CO), previously known as VDC-1101 or GS-9219, is a novel nucleotide analog antineoplastic prodrug. The mechanism of action has been previously reported in depth.^{2,3} Briefly, RAB is preferentially taken up by lymphoid cells and metabolized to 9-(2-phosphonylmethoxyethyl guanine), which then inhibits DNA synthesis/repair after being doubly phosphorylated.

Rabacfosadine has efficacy for treatment of lymphoma in dogs in both naïve and relapsed cases.²⁻⁴ Rabacfosadine administration has an objective response rate of 74% when used as a single agent for dogs with relapsed multicentric B-cell lymphoma.⁴ Similar to most other cytotoxic agents, RAB seems to be more effective in treating multicentric B-cell lymphomas in comparison to T-cell lymphomas.² Rabacfosadine has conditional approval from the US Food and Drug Administration for the treatment of lymphoma in dogs. The recommended dose and schedule is 1.0 mg/kg IV every 21 days.

Rabacfosadine is generally well tolerated but can have unique adverse events (AEs) not commonly seen with other antineoplastic agents in dogs. Dose-limiting toxicoses (DLT) have included dermatopathy and gastrointestinal (GI) toxicoses, and less commonly neutropenia. Dermatopathy typically presents as otitis externa or focal erythematous skin lesions on the dorsum and inguinal regions. Rabacfosadine causes a presumed idiosyncratic pulmonary fibrosis (PF) that can be life-threatening. This AE is rare and tends to develop late after treatment with a median time to development of PF around 4-5 months (unpublished data, VetDC, Inc, Fort Collins, Colorado).

L-asparaginase (L-ASP) is an enzyme commonly used in the treatment of lymphoma in dogs. It acts by depleting systemic asparagine/glutamine which leads to decreased capacity for protein synthesis and apoptosis of cells lacking asparagine synthetase, such as lymphocytes.^{5,6}

Adverse events reported in dogs include hypersensitivity reactions, abnormal liver function tests, altered coagulation measurements, and pancreatitis.⁷ In humans, it is predominantly used in the treatment of acute lymphoblastic leukemia (ALL). The addition of L-ASP to ALL protocols has significantly increased long-term survival for children with ALL.^{8,9} In dogs, it is used in both naïve and rescue lymphoma settings.^{7,10-13} However, despite its common use for lymphoma in dogs, no prospective studies have been conducted demonstrating a clinical benefit when adding L-ASP to combination chemotherapy protocols.^{12,14}

The objective of this study was to evaluate the safety and efficacy of RAB in combination with L-ASP for the treatment of relapsed multicentric lymphoma in dogs. This was accomplished through the conduct of an open-label, prospective, single-arm clinical trial.

2 | MATERIALS AND METHODS

This study was conducted as a multi-institutional, single-arm, open-label, clinical trial. Participating sites included Colorado State University, Hope Veterinary Specialists, BluePearl Specialty and Emergency Pet Hospital North Seattle, University of Wisconsin-Madison, VCA Katonah Bedford Veterinary Center, VCA Animal Diagnostic Clinic, and The Veterinary Cancer Center. Institutional Animal Care and Use Committee/Clinical Review Board protocol approval was obtained as needed based on study site requirements, signed informed consent was obtained from all owners, and the trial was listed on the AVMA Animal Health Studies Database under protocol number AAHSD004141. Dogs were eligible for enrollment if they were older than 1 year, had a body weight >5 kg, had a cytologic or histologic diagnosis of lymphoma, had documentation of either immunophenotype (via immunohistochemistry, immunocytochemistry, or flow cytometry) or molecular clonality (via PCR for antigen receptor rearrangement), had relapsed after treatment with at least 1 DOX containing chemotherapy protocol, and had a ≥ 7 day washout from previous chemotherapy. Although histologic/cytologic confirmation of relapse was not required, the standard operating protocol of most of the sites was to confirm relapse cytologically before considering rescue treatment. All dogs were required to have a complete blood count, diagnostic profile, and urinalysis performed within 7 days of enrollment. Adequate bone marrow and organ function, defined as absolute neutrophil count ≥ 2000 cells/ μ L, hematocrit $\geq 25\%$, platelet count $\geq 75\,000$ cells/ μ L, creatinine ≤ 2.5 mg/dL, total bilirubin \leq the upper limit of normal (ULN), alanine aminotransferase ≤ 3 times ULN or if >3 times ULN, serum bile acids \leq ULN, were required. A modified Eastern Comparative Oncology Group (ECOG) performance score of ≤ 1 was required for inclusion.¹⁵

Dogs were excluded from the study if they had received chemotherapy within 1 week of enrollment, had received RAB before enrollment, had received radiation therapy within 6 weeks of enrollment, had pulmonary fibrosis or a history of chronic pulmonary disease that could predispose to fibrosis, had concurrent malignancy or significant comorbidities, had previously been treated with bleomycin, or were receiving alternative therapies within a day of enrollment (permitted supplements included chondroitin sulfate, vitamins, essential fatty acids, glucosamine). Dogs having received L-ASP previously were permitted so long as they had not received a dose within 1 week of enrollment. West Highland White Terriers were excluded because of a genetic predisposition for idiopathic pulmonary fibrosis. Staging tests such as abdominal ultrasound and bone marrow aspiration cytology were recorded if performed previously but were not required for enrollment in the study. Thoracic radiographs were highly recommended before enrollment, but not required. Recorded variables included signalment (eg, age, sex, neuter status, breed, body weight), immunophenotype, and previous treatment (corticosteroids, previous chemotherapy protocols, etc.).

Rabacfosadine was provided by VetDC, Inc. Signed informed consent was obtained from all owners before study entry. All dogs received RAB at a dose of 1.0 mg/kg. Rabacfosadine was reconstituted and diluted with sodium chloride for injection, USP to

achieve a total infusion volume of 2 mL/kg, and was administered IV as a 30-minute infusion. Rabacfosadine was administered every 3 weeks up to a total of 5 doses per the label instructions. L-asparaginase was administered SC at a dose of 400 IU/kg, concurrent with the first 2 treatments of RAB. In dogs whose calculated L-ASP dose was between 10 000 and 15 000 IU, the dose was rounded down to 10 000. Dogs whose calculated dose was <10 000 IU or >15 000 IU received exactly 400 IU/kg. Concurrent use of steroid treatment was allowed in this study and no standard dose or dosing scheme was dictated by the trial design. Prophylactic anti-nausea and anti-diarrheal medications were permitted and prescribed based on individual clinician discretion. Antihistamines were also permitted as pretreatment for L-ASP.

Response to treatment was determined by using the Veterinary Cooperative Oncology Group (VCOG) response evaluation criteria for lymphoma.¹⁶ A complete response (CR) was defined as disappearance of all evidence of disease. A partial response (PR) was defined as $\geq 30\%$ reduction in the sum of the longest diameters of peripheral lymph nodes measured as compared to baseline measurements. Stable disease (SD) was defined as <30% reduction or >20% increase in the sum of the longest diameters of peripheral lymph nodes measured as compared to baseline measurements. Progressive disease (PD) was defined as >20% increase in the sum of the longest diameters of peripheral lymph nodes measured as compared to the smallest recorded measurements. Dogs experiencing CR received a total of 5 RAB treatments; thereafter, monthly rechecks were performed until PD was noted. Dogs experiencing PR or SD after 5 treatment cycles were considered off-study upon completion of the fifth treatment cycle and censored from outcome analysis at that point. Dogs experiencing PD were removed from the study and were eligible for other treatment as deemed appropriate by the investigator.

Hematological AEs were evaluated 7 days after the first treatment. Thereafter, clinical, hematological, and biochemical AEs were assessed every 21 days based on the history provided by the owner, physical examination, and blood work (Table 1). Adverse events were graded according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v1.1.¹⁶ Dose-limiting toxicoses were defined as any grade 3 or 4 non-hematologic toxicosis, any uncomplicated (eg, no fever,

bleeding, etc.) grade 4 hematologic toxicosis, or any complicated grade 3 or 4 hematologic toxicoses. In addition, dermatological lesions deemed less than grade 3 according to VCOG-CTCAE v1.1 criteria but considered clinically substantial or extensive enough to warrant protocol alterations were considered DLTs. Exceptions that were not considered DLT included AEs not related to RAB; hyporexia, vomiting, or diarrhea remediable within 24 hours by supportive medical treatment; or elevations in liver enzymes or total bilirubin which resolved without medical intervention. Dose reductions and delays of up to 2 weeks were permissible to manage AEs. If a DLT was observed, the dose was reduced by up to 20% for future RAB administrations.

Continuous data were expressed as median and range, and categorical data as frequencies and percentages. The ORR and progression-free survival (PFS) were the primary efficacy endpoints. The ORR was defined as the percentage of evaluable dogs experiencing CR or PR as their best response at any time. The PFS was calculated from the date of treatment initiation to the date of PD using the Kaplan-Meier method. Dogs were censored if they had not developed PD at the time of data analysis, or if they were withdrawn or lost to follow-up before PD development. The effect of secondary variables (eg, degree of pretreatment, substage, immunophenotype) on ORR was evaluated using a 2-tailed Fisher exact test. The effect of secondary variables on PFS was evaluated using logrank and/or Cox proportional hazards analysis. Variables with a univariate *P* value of <.15 were incorporated into a forward stepwise logistic regression multivariable Cox proportional-hazards model to compare the multiple variables for effect on PFS. Variables with values of $P \leq .05$ were considered significant. All statistical analysis was performed with commercial software packages (Prism v.8, GraphPad Software, La Jolla, California; SPSS v.25, IBM, Armonk, New York).

3 | RESULTS

3.1 | Dog population

Fifty-two dogs in total were prospectively enrolled in the study. Information regarding age, weight, stage, substage, immunophenotype, and

TABLE 1 Study schedule

Day	RAB treatment	L-asparaginase treatment	Lymph node evaluation	CBC	Serum chemistry	UA	Thoracic radiographs
Pre-enrolment (day -7 to -1)			X	X	X	X	+/-
0	X	X	X	X			
7				X			
21	X	X	X	X			
42	X		X	X	X	X	
63	X		X	X			
84	X		X	X	X	X	+/-
Monthly rechecks			X				Recommended every other month

TABLE 2 Characteristics of study population

Age	Median (range)	8 (2-15)
Weight	Median (range)	30.9 (5.5-52.1)
Original approximate stage	2	1 (2%)
	3	29 (56%)
	4	15 (29%)
	5	5 (10%)
	NR	2 (4%)
Original substage	A	29 (56%)
	B	19 (37%)
	NR	4 (8%)
Immunophenotype	B	38 (73%)
	T	12 (23%)
	Mixed T/B	1 (2%)
	NR	1 (2%)
Lines of previous treatment	1	25 (48%)
	2	18 (35%)
	>2	9 (17%)
Previous asparaginase treatment	No	26 (50%)
	Yes	26 (50%)

previous treatment before RAB/L-ASP is given in Table 2. A total of 9 of the 52 dogs (17%) did not receive concurrent steroid treatment. Treatment was variable for all dogs receiving a concurrent steroid (prednisolone, prednisone, dexamethasone), including use of every other day dosing or a tapering course. Thirteen of the 52 dogs (25%) received an antihistamine before treatment for at least 1 of the L-ASP treatments.

3.2 | Adverse events

Fifty-two dogs were evaluable for assessment of AEs. Forty-three dogs (81%) had at least 1 AE reported during their treatment protocol. Frequency of the most commonly reported AEs are summarized in Table 3. The most common AEs were GI in origin, with grade 1 GI AEs being more common than grade 2 or 3 GI AEs. Two dogs experienced a grade 4 AE, and no dogs had a grade 5 AE. One dog with a grade 4 AE was thought to have developed Evan's syndrome. The most common hematologic AE reported was anemia; hematologic AEs were predominantly low grade. Fifteen dogs developed dermatopathy during treatment, which was first noted a median of 42 days after treatment initiation (range 7-114 days). One dog developed radiographic changes that could have been consistent with pulmonary fibrosis 167 days post starting treatment. One dog was suspected of having a hypersensitivity reaction after L-ASP administration. This dog had received L-ASP in a previous protocol and was on prednisone but did not receive an antihistamine before L-ASP.

Rabacfosadine dose reductions (from 5 to 20%) were performed in 10 dogs, and dose delays were performed in 2 dogs. Of the dogs

TABLE 3 Most common adverse events (AEs) and associated Veterinary Cooperative Oncology Group (VCOG) grades encountered in study population. Multiple events are reported for each dog if encountered during the study

Gastrointestinal					
Grade	1	2	3	4	5
Hyporexia	10	8	5 ^b		
Diarrhea	14	11 ^c	3 ^c		
Hematochezia/ melena	3	1			
Vomiting	10	5	1 ^c		
Weight loss	6	4	7 ^{b,c}		
Hematologic					
Anemia	11	1 ^c	1 ^a		
Neutropenia	4	1	1	1 ^c	
Thrombocytopenia	6	3 ^c	2 ^c	1 ^a	
Cutaneous/pulmonary					
Dermatopathy	9 ^c	5	1 ^b		
Pulmonary fibrosis	1				
Pneumonia		1			

^aEvan's syndrome suspected to be unrelated to rabacfosadine/L-asparaginase.

^bAdverse events resulting in withdrawal from study in select cases.

^cAdverse events resulting in dose reduction or dose delay in select cases.

receiving a dose reduction, 1 had grade 4 neutropenia, 1 had grade 3 thrombocytopenia, 3 dogs had grade 1 dermatitis/otitis, and 5 dogs had grade 2-3 GI AEs (hyporexia/diarrhea/weight loss). Two out of these 10 dogs had continued AEs after dose reduction and were subsequently withdrawn from study. One dog had a dose delay out of owner convenience, the other was delayed because of GI AEs. Two dogs died because of unknown causes 16 and 17 days after treatment initiation with unknown remission status. Postmortem examinations were not performed in either dog, and their deaths were treated as events for statistical purposes. Five dogs were withdrawn from study for AEs or owner-perceived reduced quality of life. The most common AE resulting in withdrawal from study was hyporexia/weight loss with 3 dogs being withdrawn for this reason. Another dog was withdrawn because of developing possible Evan's syndrome noted 1 week after the first treatment of RAB/L-ASP. The last dog was withdrawn because of grade 3 dermatopathy.

3.3 | Dog outcomes

Ten dogs were censored from PFS analysis. This was because of AEs or diminished quality of life considered unacceptable to the owner (5 cases), development of unrelated disease (2 cases), loss to follow-up while in CR (2 cases), and poor owner compliance (1 case). The median follow-up time in censored dogs was 53.5 days (range 18-102 days).

Forty-six dogs were evaluable for response assessment. The overall response rate (ORR) was 69% with 19 dogs (41%) experiencing a

CR and 13 dogs (28%) with PR as their best response. An additional 6 dogs (13%) had SD. The median time to first response and median time to maximal response were both 21 days (range 7-42 days). The median PFS (MPFS) for all dogs was 63 days (range 5-428 days). Dogs experiencing a CR as their best response had an MPFS of 144 days (range 44-428 days), and the MPFS for dogs experiencing a PR was 59 days (range 14-126 days). Dogs experiencing SD had an MPFS of 41 days (range 31-51 days).

Degree of pretreatment and before L-ASP treatment was found to significantly affect PFS (Figures 1 and 2). Dogs that only had 1 line of previous treatment had an MPFS of 86 days, dogs with 2 lines of treatment had an MPFS of 42 days, and dogs with ≥3 lines of treatment had an MPFS of 31.5 days (logrank test for trend $P = .0004$). Dogs having been treated with only 1 previous therapeutic protocol were significantly more likely to achieve a CR (61%, ORR = 74%). For dogs treated with 2 previous protocols the ORR was 73%, with only 27% achieving a CR (Figure 3). No dogs having been treated with 3 or more previous protocols achieved a CR, and only 50% of these dogs had a response to treatment. Previous treatment with L-ASP significantly impacted CR rates, with dogs not having previously been treated with L-ASP significantly more likely to achieve a CR (Figure 4; $P = .001$). Dogs not previously treated with L-ASP had an MPFS of

86 days compared to 38 days for those previously treated with L-ASP before enrollment (Figure 2; logrank $P = .0007$).

The MPFS was 63 days (range 14-428 days) for B-cell lymphoma and 43 days (5-86 days) for dogs with T-cell lymphoma (Figure 4). The ORR for dogs with B-cell lymphoma was 73.3% with 50% achieving a CR (Figure 5). Dogs with T-cell lymphoma had an ORR of 62.5% with 12.5% achieving a CR ($P > .05$).

Factors evaluated for independent prognostic importance based on multivariate analysis included best response (CR versus not), immunophenotype, previous L-ASP treatment and lines of previous treatment (1 versus >1). Only previous L-ASP treatment ($P = .03$; hazard ratio 2.365; 95% confidence interval between 1.097 and 5.099) and best response ($P < .001$; hazard ratio 8.878; 95% confidence

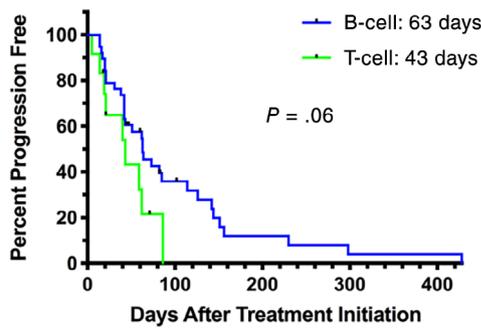


FIGURE 1 Kaplan-Meier curve depicting the effects of immunophenotype on progression free interval. P values indicate univariate log-rank values ($n = 42$). Tick marks indicate censored patients

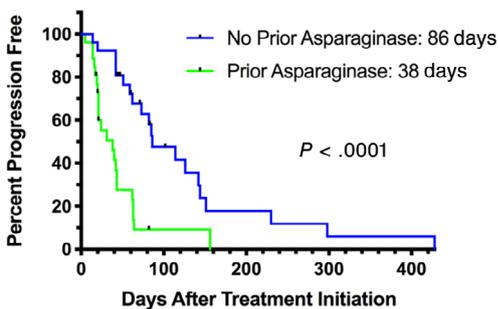


FIGURE 2 Kaplan-Meier curve depicting the effects of previous L-ASP treatment on progression-free interval. P values indicate univariate log-rank values ($n = 42$). Tick marks indicate censored dogs

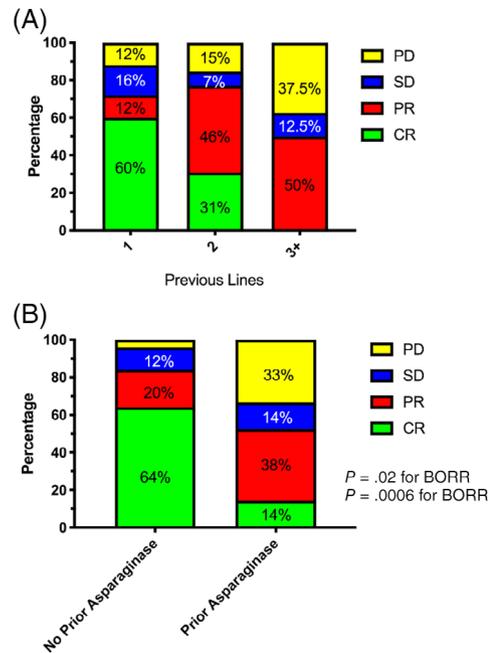


FIGURE 3 Response rates for dogs treated with RAB/L-ASP based on (A) number of previous lines of treatment before treatment with RAB/L-ASP, (B) previous treatment with L-ASP ($n = 46$)

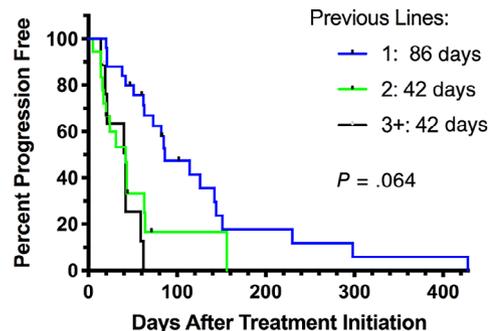


FIGURE 4 Kaplan-Meier curves depicting the effects of number of previous lines of treatment before RAB/L-ASP on progression-free interval. P values indicate univariate log-rank values ($n = 42$). Tick marks indicate censored dogs

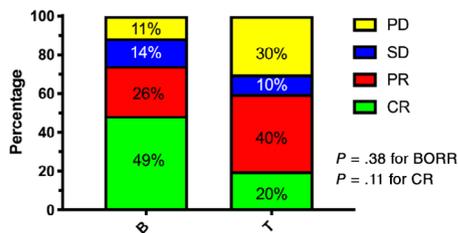


FIGURE 5 Response rates based on immunophenotype for dogs treated with RAB/L-ASP (n = 46)

interval between 3.297 and 23.384) were found to be prognostic for PFS.

4 | DISCUSSION

The results of this study suggest that the combination of RAB and L-ASP is both safe and efficacious for dogs with relapsed multicentric lymphoma. The ORR for all dogs treated in this study was 70%, with 41% experiencing a CR. The MPFS for all dogs was 63 days, and 144 days for dogs achieving a CR. The majority of AEs seen in this study population were self-limiting, and similar in frequency/severity to previously reported studies evaluating RAB.^{4,17} Five dogs were withdrawn from the study because of AEs, and the majority of these were caused by grade 3 hyporexia/weight loss. Of these 5 dogs, 2 of them had a 20% dose reduction of RAB but were later withdrawn because of continued AEs. The other 8 dogs who had RAB dose reduced were able to continue receiving RAB at a reduced dose or were withdrawn shortly after because of PD. One dog developed radiographic signs suspicious for pulmonary fibrosis 167 days after treatment initiation but had no clinical signs. A total of 15 dogs developed a dermatologic AE, the majority of which were grade I/II and did not require dose alterations. No dogs experienced a grade 5 AE.

Dogs that were more heavily pretreated before initiating RAB/L-ASP had a significantly shorter MPFS (Figure 1). This could suggest that RAB shares resistance mechanisms to other agents used commonly in the treatment of multicentric lymphoma in dogs. Alternatively, previous chemotherapy might lead to accelerated tumor cell repopulation without necessarily being refractory to chemotherapy. Of note, previous treatment with L-ASP remained statistically significant on multivariate analysis for PFS, whereas number of previous treatments did not. Five of the 21 dogs treated with L-ASP before enrollment in this study had received a variation of L-CHOP and no other protocols; therefore, not every dog that had previous L-ASP was heavily pretreated. It is unclear why dogs receiving previous L-ASP had a worse PFS when treated with RAB/L-ASP. Mechanisms of L-ASP resistance, such as development of neutralizing antibodies, would not explain resistance to RAB. Instead, it is possible that L-ASP might induce metabolic alterations or instill other molecular changes that induce resistance to this protocol. Resistance to L-ASP in human ALL can be induced by loss of huntingtin-associated protein 1.¹⁸ Huntingtin-associated protein 1 interacts with other proteins to form a complex that mediates Ca^{2+} release from the endoplasmic reticulum and through other

mechanisms can decrease entry of external Ca^{2+} into the cell. This disruption of calcium metabolism reduces activation of the Ca^{2+} -dependent calpain-1, Bid, caspase 3/12 apoptotic pathway which could potentially promote resistance to other antineoplastic drugs, such as RAB. Furthermore, the amino acid response (AAR) pathway is a well-established regulator of gene transcription. In short, depletion of essential amino acids can lead to upregulation of key proteins, such as general control nonderepressible 2 (GCN2) and activating transcription factor 4 (ATF4), which might activate pro-survival pathways.^{19,20}

In this study, as is consistent with previous RAB studies,¹⁷ dogs with T-cell lymphoma had a shorter median progression-free interval (MPFI) than dogs with B-cell lymphoma (63 days for B-cells, 43 days for T-cells). The ORR for dogs with T-cell lymphoma was 62.5%, with 12.5% achieving a CR. Reported response rates for naïve and refractory T-cell lymphoma treated with RAB range from 25 to 56%.^{2,17} The progression-free interval in this study for T-cell lymphomas was similar to slightly improved from what has been reported previously; however, any conclusions regarding superiority of RAB combined with L-ASP cannot be drawn from this study.

Recently, a study evaluated RAB alone in dogs with relapsed multicentric B-cell lymphoma having failed only 1 DOX-based protocol.⁴ In this setting, an ORR was 74% with an MPFI of 108 days. No direct conclusions can be drawn by comparing this current study to the previous study given the differences in dog populations; however, in the 23 dogs in the current study with B-cell lymphoma having failed 1 line of previous treatment, the ORR was 70% and the MPFI was 114 days, suggesting relatively similar efficacy. Taken together, these studies demonstrate that RAB has a role as a lymphoma rescue agent. Further studies are necessary to reveal whether RAB/L-ASP offers any clinical benefit to RAB alone as a rescue protocol.

The schedule for L-ASP administration in this current study was based on protocols combining L-ASP with CCNU. Two such protocols have been evaluated in the literature for relapsed lymphoma in dogs.^{7,13} The first study evaluated L-ASP concurrent with the first 2 treatments of CCNU and resulted in an ORR of 87%, with 52% achieving a CR. Median time to progression was 63 days.⁷ The second study evaluated the continuous use of L-ASP with each treatment of CCNU and resulted in an ORR of 77%, with 65% of dogs achieving a CR.¹³ Median time to progression for this study was 70 days. No obvious benefit is noted when comparing these studies, which led us to model our trial design after the first. This could be a limitation of our study as the clinical use of L-ASP has not been optimized for dogs with lymphoma. To this note, the efficacy of L-ASP is unquestioned for treating lymphoma in dogs; however, no prospective studies have been conducted with the goal of demonstrating an optimal dosing scheme for L-ASP as has been done for human leukemia. For this reason, it is unclear whether using infrequent L-ASP at the beginning of treatment, as was done in this study, is the best way to include L-ASP in a combination protocol. Further studies are required to evaluate the dosing schedule/intensity of L-ASP administration in dogs.

As stated previously, L-ASP is used most commonly in human medicine for the treatment of pediatric ALL, where it has been shown to improve 5-year survival when used as part of multi-agent

protocols.^{8,9} Furthermore, therapeutic drug monitoring via nadir serum asparaginase activity levels have been shown to improve outcomes.^{8,9,21} Nadir serum asparaginase activity can be used to detect dogs who potentially have developed neutralizing antibodies, who then can be treated with *Erwinia* derived L-ASP or pegylated forms.^{9,22} Similarly, for people developing hypersensitivity reactions, other forms of L-ASP can be safely administered. The use of L-ASP in adult onset ALL is not as well understood because of increased risk of AEs, although this might be modified with appropriate therapeutic drug monitoring.^{21,23} The understanding of how L-ASP is used and monitored in people could be the basis of further studies which might lead to improved outcomes for lymphoma in dogs.

A major limitation of this study is the lack of a control arm. Because of this study design, we cannot conclude on the superiority/inferiority of this treatment protocol in comparison to single agent RAB for relapsed lymphoma in dogs. Furthermore, this study did not further the understanding of best treatment for relapsed lymphoma in dogs. Another limitation of this study is the variable steroid usage. Steroids are commonly used in lymphoma treatment protocols and could have masked poor responses and even AEs to RAB/L-ASP in certain dogs. Furthermore, dexamethasone and prednisone might have differing benefits in lymphoma and relapsed lymphoma, although the efficacy of one over the other is not well understood. Lastly, we cannot critically evaluate whether dogs have an increased risk of AEs when treated with RAB in combination with L-ASP versus RAB alone. However, only 10 dogs (19%) required a dose reduction in this current study. This is at least similar to a previous study where 34 dogs with relapsed B-cell lymphoma were treated with RAB at 1.0 mg/kg IV every 3 weeks, and 12 of these dogs (35%) were reported to have had a dose reduction.⁴

In conclusion, RAB/L-ASP appears to be both safe and efficacious for dogs with relapsed multicentric lymphoma. Further studies are necessary to evaluate whether RAB/L-ASP offers any clinical benefit over RAB alone as a rescue protocol.

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CONFLICT OF INTEREST DECLARATION

Douglas H. Thamm is a consultant for and shareholder in VetDC. Karri Meleo, Gerald S. Post, Kathryn R. Vickery, David M. Vail, and Philip J. Bergman are members of VetDC's Clinical Advisory Board.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Colorado State University IACUC approval, number 15-5572A.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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