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Multicenter, randomized, double-blinded, placebo-controlled study of rabacfosadine in dogs with lymphoma

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Abstract

Background: Rabacfosadine (RAB, Tanovea-CA1) is a novel chemotherapy agent conditionally approved for the treatment of lymphoma in dogs.

Hypothesis/Objectives: To determine the efficacy and safety of RAB in dogs with lymphoma.

Animals: One hundred and fifty-eight client-owned dogs with naïve or relapsed multicentric lymphoma were prospectively enrolled from January to October 2019.

Methods: Dogs were randomized to receive RAB or placebo at a 3 : 1 ratio. Treatment was given every 21 days for up to 5 treatments. Study endpoints included progression-free survival (PFS), overall response rate (ORR) at a given visit, best overall response rate (BORR), and percent progression free 1 month after treatment completion. Safety data were also collected.

Results: The median PFS was significantly longer in the RAB group compared to placebo (82 vs 21 days; $P < .0001$, HR 6.265 [95% CI 3.947-9.945]). The BORR for

Abbreviations: AE, adverse event; BORR, best overall response rate; CC, clinical chemistry; CR, complete response; HR, hazard ratio; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; PMEG, 9-(2-phosphonylmethoxyethl) guanine; PR, partial response; RAB, rabacfosadine; SAE, serious adverse event; SD, stable disease; UA, urinalysis; VCOG-CTCAE, Veterinary Comparative Oncology Group Common Terminology Criteria for Adverse Events.

RAB-treated dogs was 73.2% (50.9% complete response [CR], 22.3% partial response [PR]) and 5.6% (0% CR, 5.6% PR) for placebo-treated dogs ($P < .0001$). One month after the last treatment, 37 RAB-treated dogs (33%) were progression free compared with no placebo-treated dogs ($P < .0001$). The most common adverse events observed in the RAB group were diarrhea (87.5%), decreased appetite (68.3%), and vomiting (68.3%) and were generally low grade and reversible. Serious adverse events were reported in 24 RAB-treated (20%) and 5 placebo-treated dogs (13%).

Conclusions and Clinical Importance: Rabacfosadine demonstrated statistically significant antitumor efficacy in dogs with lymphoma when administered every 21 days for up to 5 treatments as compared to placebo.

KEYWORDS

canine, chemotherapy, multicentric, neoplasia

1 | INTRODUCTION

Multicentric lymphoma is 1 of the most common cancers diagnosed in dogs, with an estimated annual incidence of 13 to 114 per 100 000 dogs at risk.[1] Multiagent chemotherapy protocols (eg, CHOP-based protocols using a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone) are the most common first-line therapies used for dogs with lymphoma. These protocols have high remission rates (80%-95%), but the majority of dogs will have relapse of the disease at some point either during or after treatment, with progression free intervals of approximately 5 to 7.5 months in most studies.[1-5] There is an unmet need for novel anticancer agents for the treatment of lymphoma in dogs.

Rabacfosadine (previously referred to as VDC-1101 or GS-9219; VetDC, Fort Collins, Colorado) is a double prodrug of the nucleotide analog 9-(2-phosphonylmethoxyethl) guanine (PMEG). It preferentially targets lymphoid cells and inhibits DNA synthesis via inhibition of DNA polymerases.[6] TANOVEA-CA1 (rabacfosadine for injection) (RAB) was conditionally approved for the treatment of lymphoma in dogs by the FDA-CVM under NADA 141-475 in December 2016. The label dose is 1.0 mg/kg IV every 21 days for up to 5 treatments.

Rabacfosadine has been used successfully in dogs with both B- and T-cell lymphoma as well as naïve and relapsed disease, with response rates of 74% to 100% when used as a single agent.[6-9] Observed response rates and durations are improved in dogs with B-cell lymphoma and in dogs that have received fewer prior treatment protocols for their disease.[6-9]

Rabacfosadine is well tolerated with mostly low grade adverse events (AEs) observed.[6-13] Unique AEs associated with RAB have included dermatopathies and pulmonary fibrosis.[6,8-13] Dermatopathies usually present as focal erythematous, alopecic, and pruritic lesions, most commonly on the dorsum, inguinal region, and ear canal. These lesions typically resolve with supportive care and can be mitigated by dose delays, dose reductions, or a combination of both. Pulmonary fibrosis has been diagnosed in approximately 4% of dogs treated with RAB and can be life-threatening. The cause of pulmonary fibrosis is unknown, but is suspected to be idiosyncratic.[6-13]

In order to obtain full FDA approval of RAB, a pivotal clinical field study demonstrating substantial evidence of effectiveness must be completed and approved within 5 years of conditional approval. This study was conducted to fulfill this requirement. The primary objective of this study was to determine the efficacy and safety of RAB in dogs with multicentric lymphoma. The primary outcome was progression-free survival (PFS). Secondary outcomes evaluated included overall response rate (ORR), best overall response rate (BORR), and progression-free percentage 1 month after the last treatment. Our hypothesis was that RAB would have superior efficacy, as evidenced by prolonged PFS and higher ORR and BORR, when compared to placebo.

2 | MATERIALS AND METHODS

2.1 | Eligibility criteria

To be eligible for inclusion in the study, dogs had to be at least 1 year old with a confirmed histological or cytological diagnosis of lymphoma. Peripherally accessible and measurable disease with at least 1 peripheral lymph node ≥20 mm in size was required. Both newly diagnosed and previously treated dogs were eligible. Adequate organ function was necessary, as defined as absolute neutrophil count >2000 cells/μL, hematocrit >25%, platelet count >75 000/μL, serum creatinine <2.5 mg/dL, bilirubin ≤ the upper normal limit, and transaminases ≤3 times the upper normal limit, or if >3 times the upper normal limit, then fasting and postprandial serum bile acids needed to be ≤ the upper normal limit. A Constitutional Clinical Signs General Performance Score of 0 or 1 was also required.[14] For dogs with

relapsed disease, a washout period of 2 weeks for chemotherapy, 6 weeks for radiation therapy, 4 weeks for long-acting corticosteroids, and 1 week for short-acting corticosteroids was necessary. Dogs were excluded from the clinical trial if they had received immunotherapy to treat their lymphoma, had any previous treatment with bleomycin, had pulmonary fibrosis or a history of chronic pulmonary disease that could lead to fibrosis, were of the West Highland White Terrier breed, had concurrent malignancy or serious systemic disorder that could result in a life expectancy of less than 3 months, or were pregnant, lactating, or intended for breeding.

2.2 | Study design

This was a randomized, blinded, placebo-controlled clinical trial with 2 treatment groups. Dogs were prospectively enrolled in the clinical trial from January 2019 to October 2019 at 7 participating sites (Colorado State University [Fort Collins, Colorado], Katonah Bedford Veterinary Center [Bedford Hills, New York], Hope Veterinary Specialists—BluePearl Malvern [Malvern, Pennsylvania], the Veterinary Cancer Center [Norwalk, Connecticut], Veterinary Cancer Group [Culver City, California], Animal Diagnostic Clinic [Dallas, Texas], and Veterinary Specialty Hospital of San Diego [San Diego, California]). The clinical trial was approved by the Colorado State University Institutional Animal Care and Use Committee (IACUC) and Clinical Review Board, and signed informed consent was obtained from all owners. This document clearly stated that dogs could receive a placebo. A copy of the consent form is provided in the Supporting Information. Dogs were randomly allocated to receive either RAB or placebo at a 3 : 1 ratio in groups of 4 dogs based on order of presentation. Randomization occurred within a study site and was done using the Plan Procedure in SAS (SAS Institute, Cary, North Carolina; version 9.4 or later). Stratification was not used in the randomization process. All study personnel conducting observations, collecting data, and administering treatment were blinded to the treatment group. Each site had a designated Treatment Dispenser who was responsible for preparing the infusion and was therefore unblinded.

2.3 | Diagnostics

A physical examination and CBC, clinical chemistry (CC), and urinalysis (UA) were performed within 7 days of enrollment, at each treatment visit (Days 21, 42, 63, and 84), and every 4 weeks after treatment completion. Physical exam and CBC were performed 7 days after each treatment (Days 7, 28, 49, 70, and 91). Blood and urine samples were submitted to the clinical pathology laboratory at IDEXX BioAnalytics (Columbia, Missouri) within 24 hours of sample collection. These assays could also be performed at the study site on the day of the examination in order to minimize delay in enrollment or treatment; however, the results from testing performed by the study clinical pathology laboratory were used for the final determination of enrollment, for treatment decisions at each visit, and for evaluation for adverse events.

A fine needle aspirate of an affected lymph node was collected before enrollment and submitted to the Colorado State University Clinical Immunology Laboratory (Fort Collins, Colorado) for immunophenotyping by flow cytometry as previously described.[15]

Abdominal (2 views) and thoracic (3 views: left lateral, right lateral and ventrodorsal) radiographs were obtained preenrollment (Day −7 to 0) and on study days 42, 84, 140, 196, 252, 308, and 364. All radiographs were evaluated by a single radiologist (EKR) by subject sequentially by date. The radiologist had access to only the radiographs and was blinded to treatment group and clinical status but not to the day of radiography. These interpretations were made after study enrollment occurred, and were not provided to the Investigators nor used to make treatment decisions. Sites were able to use the radiographs at the time they were obtained at their discretion.

Clinical stage was determined based on peripheral lymph node palpation, imaging, and the results of the CBC before enrollment. Stage 5 disease was assigned if there was evidence of nonlymphoid organ involvement, including the presence of circulating lymphoblasts on a blood smear made from the sample submitted for CBC to IDEXX. Substage was determined based on clinical assessment.

2.4 | Treatment

TANOVEA-CA1 (rabacfosadine succinate for injection commercial formulation; manufactured according to Current Good Manufacturing Practice regulations) was provided by the sponsor as a sterile, white to off-white lyophilized powder in the form of a cake contained in a 3 mL amber glass vial. Each single-use vial contained 16.4 mg of rabacfosadine, present as the succinate salt, along with 20 mg of mannitol and 1.6 mg of citrate as excipients. Dogs were treated with either TANOVEA-CA1 (1 mg/kg diluted in 0.9% NaCl to yield a total infusion volume of 2 mL/kg) or placebo (2 mL/kg 0.9% NaCl) given IV over 30 minutes. Dose administration occurred every 21 days for up to 5 treatments. Immediately after dose administration and at each subsequent visit, the injection site was evaluated for the presence of erythema, bruising, and swelling, and all observations were recorded. Stepwise dose modifications (of 0.8 and 0.66 mg/kg) and delays of up to 14 days were allowable in the event of dose-limiting toxicosis, or both, which was defined as any nonhematologic grade 3 or 4 toxicosis or uncomplicated grade 4 neutropenia or thrombocytopenia per the Veterinary Comparative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v1.1.[14]

2.5 | Response assessment

Lymph node measurements and response assessment were performed at each treatment visit (Days 0, 21, 42, 63, and 84) and then every 4 weeks after treatment was complete. Measurements were made in accordance with the VCOG Response Criteria for Peripheral Nodal Lymphoma v1.0.[16] Target and nontarget lesions were assessed by 2 evaluators at each visit where response evaluations occurred, and

remeasurement was required if the 2 evaluator measurements diverged by >20%. Evaluators were blinded to the measurements recorded at prior visits, as well as the measurements recorded by the other evaluator at that visit. Dogs experiencing a complete response (CR), partial response (PR), or stable disease (SD) were eligible to continue on the study.

Adverse events and concomitant medications were recorded at each study visit. Adverse events were graded prospectively according to the VCOG-CTCAE v1.1[14] and then coded according to the Committee for Medicinal Products for Veterinary Use Veterinary Dictionary for Drug Regulatory Activities (VeDDRA 2017, EMA/CVMP/PhVWP/10418/2009-Rev.9). A serious adverse event (SAE) was defined as an AE that is fatal or life-threatening, requires or prolongs hospitalization, or causes prolonged or permanent disability. Additionally, neutropenia, grade 4 or higher, or grade 3 with fever and clinical signs of systemic disease, or both, or human exposure to the

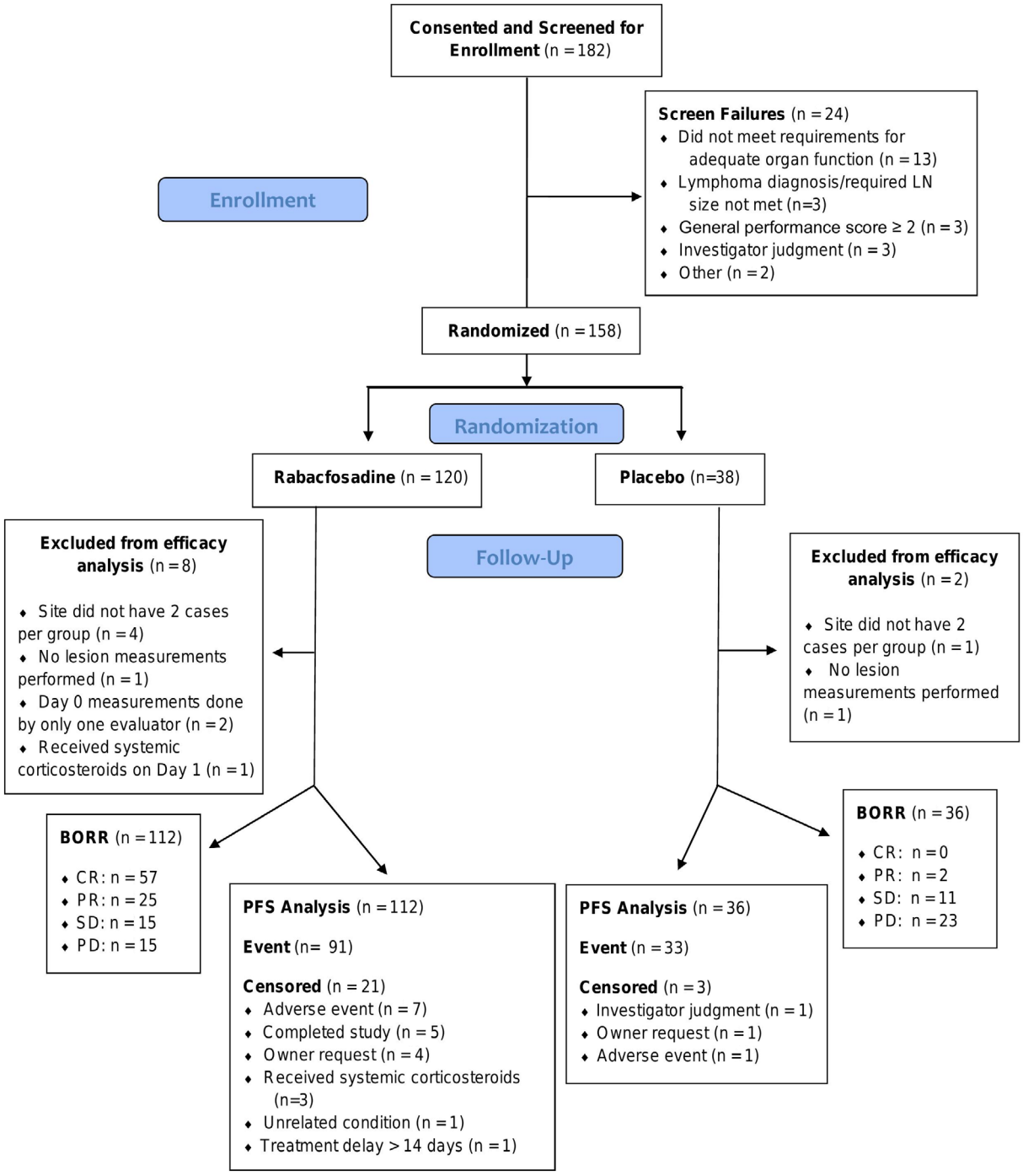

FIGURE 1 Flow diagram showing the progression of dogs through the study. BORR, best overall response rate; CR, complete response; LN, lymph node; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease

TABLE 1 Characteristics of 158 dogs treated with either rabacfosadine or placebo

Treatment group	Rabacfosadine (n = 120)	Placebo (n = 38)	*P* value
Age (years)			
Median (range)	7.9 (1-15)	7.1 (3.2-16)	.34
Sex			
Male intact	9 (15%)	6 (22%)	.1
Male neutered	51 (85%)	21 (78%)	
Female intact	3 (5%)	1 (9%)	
Female spayed	57 (95%)	10 (91%)	
Body weight (kg, Day 0)			
Median (range)	27.0 (3.3-65)	28.1 (3.4-63)	.26
Immunophenotype			
B-cell	100 (83%)	32 (84%)	>1
T-cell	20 (17%)	6 (16%)	
Stage[a]			
I	1 (0.8%)	0	.78
II	6 (5%)	1 (2.6%)	
III	71 (59.2%)	20 (52.6%)	
IV	33 (27.5%)	14 (36.8%)	
V	9 (7.5%)	3 (8%)	
Substage[a]			
a	107 (89.2%)	29 (76.3%)	.06
b	13 (10.8%)	9 (23.7%)	
Number of previous treatment protocols for lymphoma			
0	57 (48%)	20 (53%)	.62
1	39 (33%)	14 (37%)	
2	17 (14%)	3 (8%)	
>2	7 (6%)	1 (3%)	

[a]Stage and substage reported at the time of study enrollment.

study treatment were considered SAEs for this study. Concomitant medications that were not permitted during the study period included: homeopathic or alternative therapies, chemotherapy other than RAB, immunotherapy or radiation therapy for lymphoma, investigational medications, systemic corticosteroids, and nonsteroidal anti-inflammatory drugs not in use before the start of the study. Topical corticosteroids for the treatment of otic and dermatologic disease were allowed.

2.6 | Statistical analysis

Based on the results of previous studies, a hazard ratio (HR) of 5 (PFS of 77 days in RAB group [50% response rate] vs PFS ≤14 days in the placebo group [<10% response rate]) was anticipated. A minimum of 48 evaluable dogs in the RAB group was deemed necessary to provide at least 90% power to detect the anticipated HR at a 2-sided significance level of 0.05. However, to ensure the ability to appropriately characterize the adverse event spectrum of treated dogs, a target of 100 evaluable RAB treated dogs was planned. Demographic features of the RAB and placebo groups were compared using unpaired Mann-Whitney tests and Fisher exact tests for continuous and categorical variables, respectively. All dogs that received treatment (either RAB or placebo) were included in the safety evaluation. Dogs meeting all inclusion criteria and having none of the exclusion criteria were included for the evaluation of effectiveness. In order to be included in the effectiveness analysis, a site needed at least 2 evaluable cases in each treatment group. PFS was defined as the interval between the date of randomization and the first date that the criteria for progressive disease (PD) were met. An HR for treatment group was calculated using a Cox regression analysis. Progression-free survival was also estimated using the Kaplan-Meier method with median time to progression and 95% confidence intervals (CIs). Dogs not showing progression were censored from PFS analysis at the date of the last visit when response assessment was performed. The ORR was determined as the proportion of dogs with CR or PR at a given visit based on target, nontarget and new lesions, in relation to total number of dogs. The BORR was defined as the proportion of dogs with CR or PR at any time, based on target, nontarget and new lesions, in relation to total number of dogs. The percent progression free was determined as the proportion of dogs that had not developed PD 1 month after the last treatment, in relation to total number of dogs. Within the RAB group, potential factors influencing PFS and response were assessed using log rank analysis or Cox regression. A multivariable forward stepwise logistic regression model incorporating all variables that were significant on univariable analysis was built. Entry and exit alpha values for the model were set at 0.1 and 0.05, respectively. Factors influencing response and incidence of serious AEs were interrogated using unpaired Mann-Whitney tests and Fisher exact tests for continuous and categorical variables respectively. All statistical tests were 2-sided and the significance level was set at $P < .05$. Statistical analyses were performed using SAS/STAT software (Version 9.4 of the SAS System for Windows, SAS Institute, Inc, Cary, North Carolina), Prism 9 (GraphPad Software, San Diego, California), and SPSS v26 (IBM, Armonk, New York).

This study was conducted and reported in compliance with US FDA CVM Guidance for Industry 85 (VICH GL9) Good Clinical Practice. Electronic data collected abided by 21 CFR Part 11 for electronic records and electronic signatures.

3 | RESULTS

3.1 | Enrolled cases

A total of 182 dogs were screened for enrollment, with 158 dogs confirmed to be eligible and randomized for treatment. Of these 158 dogs, 120 (75.9%) were randomized to receive RAB and 38 (24.1%) to placebo. Ten dogs were excluded from the assessment of efficacy: 1 study site did not have 2 cases from each group and therefore was excluded from the efficacy evaluation (n = 5 dogs), 2 were excluded

Classification by flow cytometry	Rabacfosadine (N = 112)	Placebo (N = 36)	*P* value
B cell			
Medium to large cell B cell	80 (71.4%)	27 (75%)	.83
Small cell B cell	10 (8.9%)	2 (6%)	.73
T cell			
Peripheral T cell	19 (17%)	4 (11%)	.6
T zone	1 (0.9%)	2 (6%)	.15
Other			
Flow results inconclusive[a]	1 (0.9%)	1 (3%)	.43
No flow data available[b]	1 (0.9%)	0 (0%)	>.99

TABLE 2 Subgroups of lymphoma diagnosed via flow cytometry by treatment group for the 148 dogs included in the efficacy analysis

[a]The dog treated with rabacfosadine with inconclusive results from the flow cytometry had immunophenotyping performed outside of the study using immunocytochemistry that was consistent with B cell lymphoma. The flow cytometry results for the placebo-treated dog were diagnostic for neoplasia, most likely B cell in origin, but a definitive diagnosis of lymphoma vs plasma cell neoplasia could not be made. Lymphoma was considered the most likely diagnosis based on the results of other diagnostics.

[b]The dog with no flow cytometry data available had immunophenotyping performed outside of the study using flow cytometry, the results of which were consistent with B cell lymphoma.

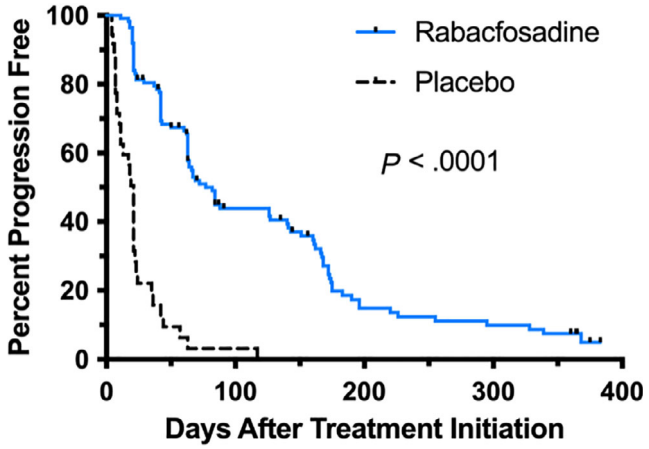

FIGURE 2 Kaplan-Meier curve depicting progression-free survival (PFS) of dogs treated with rabacfosadine (RAB) (n = 112) vs placebo (n = 36). Dogs treated with RAB had a significantly prolonged PFS compared to placebo-treated dogs (*P* < .0001). Tick marks indicate censored dogs

since there was no lesion measurement performed after initiation of study treatment, 2 were excluded because Day 0 measurements were conducted by only 1 evaluator, and 1 was excluded because a systemic corticosteroid was administered on Day 1. Of the 148 dogs included in efficacy analysis, 112 (75.7%) were treated with RAB and 36 (24.3%) with placebo. Figure 1 contains a flow diagram detailing the progression of dogs through the study.

Demographic information for dogs in both groups is displayed in Table 1. A total of 38 different breeds were enrolled in the treatment group and 19 in the placebo group. The most common breed in both groups was large mixed breed, with 33 (27.5%) in the RAB group and 15 (39.5%) in the placebo group. Complete breed information by treatment group is available in the Supporting Information (Table S1). Detailed data about the results of flow cytometry for each treatment group are shown in Table 2.

3.2 | Efficacy

Of the 148 dogs included in efficacy analysis, 44 dogs in the RAB group (36.7%) received all 5 cycles of treatment compared to 1 dog (2.6%) in the placebo group (*P* < .0001). Five dogs in the RAB cohort (4.2%) completed the study (defined as CR at Day 365 of the study) as compared to no dogs in the placebo group.

The majority of dogs were withdrawn for progressive disease (90 [75%] in the RAB group, 31 [81.6%] in the placebo group). Seven dogs in the treatment group (5.8%) were withdrawn due to AEs compared to 1 dog (2.6%) in the placebo group. Ten dogs (8.3%) in the treatment group and 2 dogs (5.3%) in the placebo group were withdrawn due to death/euthanasia, and the reason for study withdrawal was listed as Other (includes owner request, Investigator judgment, and protocol noncompliance) in 8 dogs (6.7%) that received RAB and 4 dogs (10.5%) that received placebo.

Twenty-one treated dogs and 3 placebo dogs were censored in PFS analysis. In the RAB group, reasons for censorship included AEs (n = 7), alive and in CR at study completion (n = 5), owner request (n = 4), received systemic corticosteroids while on study (n = 3, censored at day of first corticosteroid administration), unrelated medical or surgical condition (n = 1), and delay in treatment of more than 14 days (n = 1). In the placebo group, reasons for censorship included investigator judgment (n = 1), owner request (n = 1), and AE (n = 1). The median (range) follow-up time in censored dogs was 89 days (28-383) and 7 days (6-18) for RAB and placebo, respectively.

The median PFS was significantly longer in the RAB group compared to the placebo group (82 vs 21 days; *P* < .0001, HR 6.265 [95% CI 3.947-9.945]). Figure 2 displays a Kaplan-Meier plot of PFS by

TABLE 3 Effect of various factors on progression-free survival in dogs treated with rabacfosadine

Factor		N	Median PFS (*d*)	*P* value	HR (95% CI)
Immunophenotype	B-cell	**92**	**126**	**.0005**	**2.419 (1.166-5.019)**
	T-cell	**20**	**29**		
Prior chemotherapy	No	**53**	**151**	**.01**	**1.668 (1.097-2.536)**
	Yes	**59**	**63**		
Number of prior treatment lines	0	**53**	**143**	**.002**	...
	1	**37**	**82**		
	2	**15**	**60**		
	3+	**7**	**41**		
Response	CR/PR	**82**	**151**	**<.0001**	**5.627 (2.56-12.37)**
	NR	**30**	**21**		
Best response	CR	**57**	**168**	**<.0001**	...
	PR	**25**	**63**		
	SD	**15**	**41.5**		
	PD	**15**	**21**		
Stage	I/II	7	196	.12	...
	III	68	84		
	IV	28	63.5		
	V	9	168		
Substage	a	99	69	.24	0.6583 (0.3555-1.219)
	b	13	183		
Sex	F	**57**	**126**	**.02**	**1.633 (1.073-2.485)**
	M	**55**	**64**		
Neuter status	Neutered	100	77	.62	0.835 (0.4251-1.64)
	Intact	12	84		
Age	Continuous			>1	1.00 (0.041-24.627)
Weight	Continuous			.29	0.992 (0.978-1.007)
Grade 3/4 AE	Yes	32	127	.32	1.268 (0.805-1.999)
	No	80	66		
Dose delay	Yes	11	161	.06	1.926 (1.093-3393)
	No	101	66		
Dose reduction	Yes	**27**	**151**	**.03**	**1.727 (1.107-2.693)**
	No	**85**	**64**		

Note: Statistically significant results are shown in bold.
Abbreviations: AE, adverse event; CR, complete response; HR, hazard ratio; NR, no response (SD or PD); PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

treatment group. Dogs with naïve lymphoma treated with RAB had a significantly prolonged median PFS compared to placebo-treated dogs (151 vs 19 days; $P < .0001$, HR 6.142). In dogs that had received prior chemotherapy, the RAB-treated dogs had a median PFS of 63 days, compared to 21 days in those treated with placebo ($P < .0001$, HR 3.595). The impact of various prognostic factors on PFS in RAB treated dogs is shown in Table 3, and an HR event plot for these factors is presented in Figure 3. Significant differences in PFS were found when comparing immunophenotype, prior chemotherapy treatment (as a yes/no variable and by number of prior treatment protocols), response (CR/PR vs no response), best response, sex, and dose reduction. When subjected to multivariate analysis, factors that remained significant included immunophenotype ($P < .001$; HR 2.481, 95% CI 1.445-4.262), prior chemotherapy status (yes/no) ($P = .025$; HR 2, 95% CI 1.092-3.66), and sex ($P = .02$; HR 1.675, 95% CI 1.086-2.584). Kaplan-Meier curves for these factors are presented in Figure 4.

The ORR was calculated for both treatment groups by study visit. Depending on the study visit, dogs treated with RAB had an ORR ranging from 48.2% to 63.4%, vs 0% to 5.6% in the placebo group. The data for all dogs by treatment group are displayed in Table S2. For dogs treated with RAB, factors affecting response are displayed in

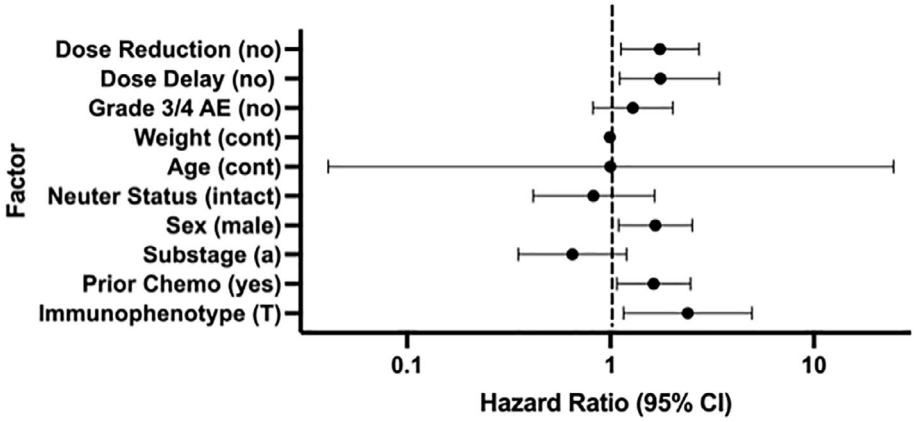

FIGURE 3 A hazard ratio event plot illustrating the effects of various prognostic factors on progression-free survival in dogs treated with rabacfosadine. AE, adverse event

Table S3 and those affecting CR rate are in Table S4. Immunophenotype and number of prior treatment lines significantly influenced both ORR and CR rate, and stage was also significant for CR rate.

The BORR for all RAB-treated dogs was 73.2% (50.9% CR and 22.3% PR), while the BORR for placebo-treated dogs was 5.6% (0% CR and 5.6% PR), $P < .0001$. A summary of BORR data by treatment group is shown in Table 4. Additional information describing response and PFS for different subgroups in dogs treated with RAB is presented in Table S5.

At the time of the Month 4 visit (1 month after the last treatment), 37 RAB-treated dogs (33%) were progression free compared with no placebo-treated dogs ($P < .0001$).

3.3 | Dose delays and reductions

There were 29 dogs in the RAB group that received dose reductions. Of these, 25 received 1 stepwise reduction (from 1.0 to 0.8 mg/kg), and 4 dogs received 2 stepwise reductions (from 1.0 to 0.8 to 0.66 mg/kg). Fourteen dogs had dose reductions due to some form of gastrointestinal toxicosis (hyporexia, nausea, diarrhea, or vomiting, or a combination of these symptoms), 7 due to dermatopathies, 2 due to weight loss, and 1 due to neutropenia. The dose was reduced in 5 dogs due to a combination of various signs. All dogs continued on the reduced dose(s) for the remainder of the study until withdrawn.

There were 14 dogs for which a dose delay was implemented, typically 1 week beyond the scheduled treatment visit (median 7 days; range 5-15). The primary reason cited for dose delay in all cases was dermatopathy, although weight loss and signs of gastrointestinal disease were also given as a reason in 1 dog each. Eight treated dogs had both a dose reduction and a dose delay, the majority of which were attributable to dermatopathies.

3.4 | Adverse events

Every dog treated with either RAB or placebo had at least 1 AE reported during the course of the study. Of the AEs observed in RAB-treated dogs, 83.9% were grade 1, 12.5% grade 2, 2.9% grade 3, 0.2% grade 4, and 0.3% grade 5. For placebo-treated dogs, 84.8% of AEs were grade 1, 11.2% grade 2, 1.3% grade 3, 1.9% grade 4, and 0.6% grade 5. The most commonly observed AEs are summarized in Table 5. Some AEs were observed only in the RAB-treated dogs, including dermatopathies, neutropenia, and hematochezia. At least 1 dermatopathy-related AE was reported in 67 (55.8%) RAB-treated dogs. Dermatologic AEs typically did not appear until the third cycle of treatment and persisted to a lesser extent in cycles 4 and 5. There were combined clinical and radiographic pulmonary changes reported in 5 dogs treated with RAB that resulted in death/euthanasia. The diagnosis of pulmonary fibrosis was confirmed via histopathology in 2 of these dogs. The median time from randomization to first detection of clinical or radiographic pulmonary changes was 88 days (range 84-140). In the 5 dogs that died or were euthanized either on or off study as a result of pulmonary dysfunction, the median time from randomization to death was 127 days (range 112-172). No pulmonary changes attributable to fibrosis were reported in dogs that received placebo.

Serious adverse events were reported in 24 dogs treated with RAB (20%) and 5 placebo-treated dogs (13.2%). The SAEs in the RAB-treated dogs included dermatopathy ($n = 6$), pulmonary fibrosis ($n = 5$), euthanasia due to PD ($n = 4$), euthanasia due to unrelated neoplasia/comorbidities ($n = 3$), and 1 each of hepatopathy, renal insufficiency, neutropenia, nausea, colitis, and hematochezia. In the placebo group, SAEs included lameness, pneumonia, progressive dyspnea, euthanasia due to renal failure, and euthanasia due to an unknown cause. Various factors were assessed for their impact on SAE occurrence in the RAB cohort, and none were found to be significant (Table S6).

3.5 | Concomitant medications

Adverse events were treated with a variety of supportive measures and concomitant medications at the study clinic, at home, or a combination of both. Concomitant medications were used primarily for diarrhea, decreased appetite and dermatopathy (bacterial skin infections) and occurred in both RAB and placebo treated dogs, indicating that

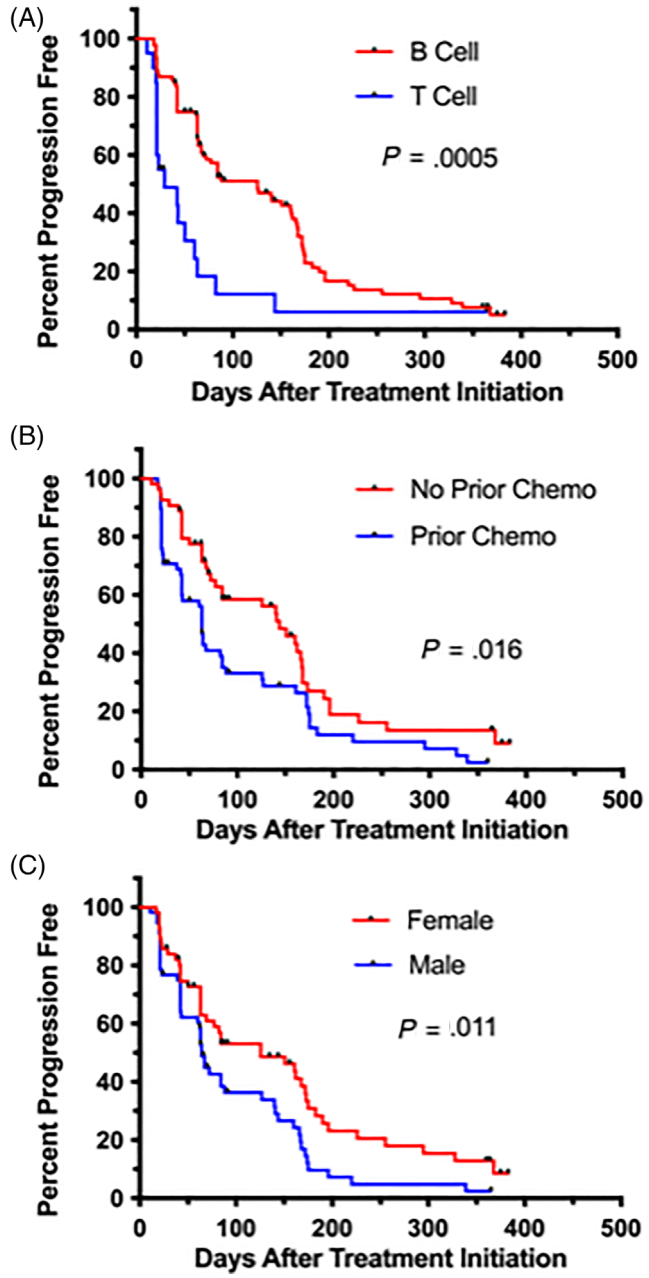

FIGURE 4 Kaplan-Meier curves depicting factors that were significant on multivariate analysis for progression-free survival in dogs treated with rabacfosadine. These included immunophenotype (A), prior chemotherapy status (yes/no; B), and sex (C). Tick marks indicate censored dogs

some of the clinical signs were associated with lymphoma as well as RAB treatment. The most commonly used concomitant medications are further detailed in Table S7.

4 | DISCUSSION

The results of this study suggest that RAB is safe and effective for the treatment of lymphoma in dogs. Rabacfosadine demonstrated a

TABLE 4 Comparison of best overall response rates of dogs treated with either rabacfosadine or placebo

Response	Rabacfosadine N (%)	Placebo N (%)	*P* value
CR	57 (50.9)	0	<.0001
PR	25 (22.3)	2 (5.6)	.02
SD	15 (13.4)	11 (30.6)	.02
PD	15 (13.4)	23 (63.9)	<.0001

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

statistically significant improvement in PFS compared to placebo (82 days vs 21 days, $P < .0001$). Median PFS was significantly longer in both naïve and previously treated dogs that were treated with RAB compared to placebo (naïve: 143 vs 19 days, $P < .0001$; relapsed: 63 vs 21, $P < .0001$). The median PFS in responding RAB treated dogs was 151 days (168 days for CR). The BORR was 73.2% for dogs treated with RAB and 5.6% for placebo. Importantly, 37 (33%) of RAB-treated dogs were progression free at 4 months vs none of the placebo dogs ($P < .0001$).

While a CHOP-based chemotherapy protocol is considered the standard of care for dogs with lymphoma, none of these cytotoxic drugs have been approved specifically for the treatment of lymphoma in animals. These are also intensive protocols that require weekly to biweekly visits for 15 to 26 weeks.[1-5] Less intensive, often single-agent protocols are also available as options, but tend to be associated with lower CR rates and shorter response durations.[17-19] Since the mechanism of action of RAB is different from standard chemotherapy drugs used to treat canine lymphoma, RAB could potentially be used in combination with other chemotherapy agents or integrated into CHOP-based protocols to prolong remission durations and time to drug resistance.

While the PFS reported in all dogs treated with RAB was relatively short, it is important to note that various characteristics of the enrolled dogs had an effect on PFS. Specifically, both naïve and pretreated dogs participated in this study, and prior chemotherapy treatment (as a yes/no variable and by number of prior treatment protocols) had a significant impact on PFS (Table 3, Figure 4). Other factors found to have a significant impact on PFS in dogs treated with RAB included immunophenotype, response (yes/no), best response, sex, and dose reduction. When subjected to multivariate analysis, immunophenotype, prior chemotherapy (yes/no), and sex remained significant. Of note, response was omitted as a variable in multivariate analysis given its intuitive association with outcome. The longer PFS observed in dogs with B-cell lymphoma compared to T-cell is not unexpected, as multiple prior studies have confirmed the presence of T-cell disease to be a negative prognostic factor after RAB treatment,[20-24] as is also seen with a variety of other drugs/protocols. Dogs with relapsed lymphoma have also been shown to have shorter response durations, both in studies using RAB as well as other conventional chemotherapy agents, likely due to the development of drug

TABLE 5 Summary of the most common adverse events (occurring in ≥10% of dogs treated with rabacfosadine) in dogs receiving rabacfosadine or placebo

	Rabacfosadine (n = 120)		Placebo (n = 38)		*P* value	
Adverse event	Any grade, N (%)	Grade 3 or 4, N (%)	Any grade, N (%)	Grade 3 or 4, N (%)	Any grade	Grade 3 or 4
Gastrointestinal						
Diarrhea	**105 (87.5)**	4 (3.3)	**19 (50)**	0	**<.0001**	.57
Decreased appetite	**82 (68.3)**	1 (0.8)	**15 (39.5)**	0	**.001**	>1
Emesis	**82 (68.3)**	0	**9 (23.7)**	0	**<.0001**	>1
Hyporexia	34 (28.3)	1 (0.8)	7 (18.4)	0	.22	>1
Increased appetite	24 (20)	0	6 (15.8)	0	.56	>1
Hematochezia	**20 (16.7)**	2 (1.6)	**0**	0	**.004**	>1
Nausea	15 (12.5)	1 (0.8)	2 (5.3)	0	.37	>1
Systemic disorders						
Lethargy	**76 (63.3)**	0	**24 (63.2)**	0	**.002**	>1
Weight loss	**58 (48.3)**	0	**4 (10.5)**	0	**<.0001**	>1
Adipsia	29 (24.2)	0	5 (13.2)	0	.15	>1
Dehydration	17 (14.2)	0	1 (2.6)	0	.08	>1
Laboratory abnormality						
Neutropenia	**55 (45.8)**	6 (5)	**0**	0	**<.0001**	.34
Hypoalbuminemia	24 (20)	1 (0.8)	5 (13)	0	.47	>1
Anemia NOS	20 (16.7)	1 (0.8)	3 (7.9)	0	.29	>1
Leucopenia	**14 (11.7)**	0	**0**	0	**.02**	>1
ALT	13 (10.8)	2 (1.6)	2 (5.3)	0	.52	>1
Monocytosis	13 (10.8)	0	3 (7.9)	0	.76	>1
CK	12 (10)	1 (0.8)	1 (2.6)	0	.19	>1
Other abnormal test result NOS	12 (10)	0	2 (5.3)	0	.52	>1
Dermatologic						
Alopecia	**30 (25)**	0	**2 (5.3)**	0	**.01**	>1
Dermatitis and eczema	**25 (20.8)**	1 (0.8)	**0**	0	**.0007**	>1
Otitis NOS	**19 (15.8)**	0	**0**	0	**.008**	>1
Erythema	15 (12.5)	0	1 (2.6)	0	.12	>1
Pruritus	15 (12.5)	0	1 (2.6)	0	.12	>1
Hyperpigmentation	**14 (11.7)**	0	**0**	0	**.02**	>1
Otitis externa	**12 (10)**	0	**0**	0	**.07**	>1
Renal/urinary						
Polydipsia	**40 (33.3)**	0	**6 (15.8)**	0	**.04**	>1
Polyuria	**29 (24.2)**	0	**2 (5.3)**	0	**.01**	>1
Proteinuria	13 (10.8)	0	2 (5.3)	0	.52	>1
Oliguria	12 (10)	0	1 (2.6)	0	.19	>1

Note: Statistically significant results are shown in bold.
Abbreviations: ALT, alanine aminotransferase; CK, creatine kinase; NOS, not otherwise specified.

cross-resistance.[9,13] Sex is a prognostic factor in some prior studies with females having an improved prognosis,[25-27] as was the case in this study, but this is not a consistent finding. The relevance of this finding is not clear.

Stage was not found to be prognostic for PFS in the dogs treated with RAB. Since dogs were not required to have abdominal ultrasound examinations with aspirates or bone marrow aspiration cytology before enrollment, it is possible that some dogs might have been assigned a higher stage with more extensive diagnostics. This could have affected the impact of stage on prognosis.

There was 1 dog assigned to the placebo group that received all 5 cycles of treatment. This dog had a best response of SD reported and a

PFS of 117 days. Flow cytometry results for this dog were consistent with medium to large B cell lymphoma, but it is possible that this dog had a form of indolent lymphoma that was not detected by flow cytometry.

The AEs observed in this study were similar to those reported in previous studies of RAB and were generally low grade and reversible. The most common AEs seen in both treatment groups included diarrhea, decreased appetite, vomiting, lethargy, and weight loss. As these occurred both in dogs treated with RAB and placebo, it is assumed that some of these AEs were attributable to lymphoma. Additional AEs seen primarily in the dogs treated with RAB but not placebo included neutropenia, dermatopathies, hematochezia, and pulmonary fibrosis. Serious AEs occurred more frequently in RAB-treated dogs (20%) vs placebo-treated dogs (13.2%).

Pulmonary changes are an infrequent adverse event seen with RAB treatment, which range in impact from clinically silent to death.[6-13] The mechanism of action that leads to this is unknown. These changes tend to be observed later in the course of treatment, and in this study occurred at a median of 88 days (range 84-140) after randomization. Combined clinical and radiographic pulmonary changes were noted in 5 dogs after treatment and led to death or euthanasia either during or after the study. Of the 5 dogs, 2 were histologically confirmed as pulmonary fibrosis. Thoracic radiography might be performed in dogs treated with RAB, but changes that might be related to pulmonary fibrosis were not seen consistently or in advance of clinical signs in this study. Rabacfosadine should be avoided in dogs with a predisposition to or prior diagnosis of pulmonary fibrosis.

Dermatologic AEs were observed in over half of dogs treated with RAB, but these were predominantly low grade and manageable with dose delays and supportive care. Alopecia, dermatitis/eczema, otitis, erythema, pruritus, and hyperpigmentation were the most commonly reported events. The cause of these effects is also unknown. The majority of dermatopathies were observed during the third cycle of treatment, suggesting a cumulative effect over the course of treatment. This highlights the importance of careful and consistent monitoring of dogs for any early signs of skin effects and implementing dose reductions/delays and supportive care to help minimize potential progression of these effects with subsequent RAB treatment.

It is also important to note that, per study protocol, the use of systemic corticosteroids was prohibited due to the potential confounding effects on lymphoma response. Given that corticosteroids are widely utilized in veterinary practice for the prevention and treatment of dermatopathies, it is reasonable to conclude that the incidence and severity of RAB-associated dermatopathy, and possibly other AEs, could have been mitigated had corticosteroids been allowed.

While 32 dogs required dose delays, dose reductions, or both, treatment discontinuation owing to AEs was infrequent in this study, occurring in only 7 dogs in the RAB group and 1 dog in the placebo group. The primary reason for withdrawal/discontinuation was PD in both treatment groups (75% RAB vs 81.6% placebo) and the reported deaths on study, including euthanasia, appeared similar (8.3% RAB vs 5.3% placebo).

In conclusion, RAB demonstrated significant antitumor efficacy in dogs with lymphoma when administered at 1.0 mg/kg every 21 days for up to 5 treatments as compared to placebo. Adverse events were generally mild and manageable, although some serious AEs such as dermatopathy and pulmonary fibrosis occurred in dogs treated with RAB. Overall, the low incidence of dose limiting toxicosis suggests that dose reductions/delays can be an effective strategy to manage adverse effects, along with supportive care.

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CONFLICT OF INTEREST DECLARATION

Gerald S. Post, Brenda S. Phillips and Philip J. Bergman serve on the Clinical Advisory Board for VetDC, Inc. DHT is a shareholder in VetDC, Inc. Drs. Weishaar, Wright, Rosenberg, Post, McDaniel, Clifford, Phillips and Bergman were paid on a fee for service basis for seeing cases as part of this study.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Study protocol approved by the Colorado State University IACUC and clinical review board (protocol number 18-8096A).

HUMAN ETHICS APPROVAL DECLARATION

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

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SUPPORTING INFORMATION

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