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Early progression during or after cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy indicates poor outcome with rescue protocols in dogs with multicentric lymphoma

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Abstract

Background: Dogs with lymphoma that fail cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy (CHOP) before completion of their protocol are commonly thought to have poor long-term outcome, but no previous studies have evaluated the effect of early relapse on progression-free interval (PFI) or overall survival time (OST) for patients undergoing rescue chemotherapy.

Objective: Correlate rescue treatment outcomes in dogs with multicentric lymphoma with outcomes after 1st-line CHOP chemotherapy.

Methods: Data were collected from 6 previous retrospective or prospective studies in 187 dogs with multicentric lymphoma that received 1st-line CHOP chemotherapy and then received either lomustine (CCNU), L-asparaginase and prednisone (LAP), or rabacfosadine (RAB, Tanovea), with or without prednisone or L-asparaginase.

Results: The PFI after initiation of CHOP chemotherapy was significantly associated with response rate postprogression, PFI, and postrescue survival time (ST) for both rescue protocols. Immunophenotype (B- vs T-cell) was not significantly associated with response, PFI or OST for LAP but was significantly associated with response and PFI for RAB.

Conclusion: Dogs that experience short PFI during or after 1st-line CHOP chemotherapy had lower response rates to rescue treatment, with shorter PFI and ST. Immunophenotype did not significantly affect outcome with LAP but was associated with PFI for RAB.

Abbreviations: AGT, O^6-alkylguanine-DNA alkyltransferase; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CR, complete response; HR, hazard ratio; JAK, Janus kinase; LAP, lomustine, asparaginase, prednisone; MGMT, O^6-methylguanine-DNA methyltransferase; ORR, overall response rate; OST, overall survival time; PD, progressive disease; PFI, progression free interval; P-GP, P-glycoprotein; PI3K, phosphatidylinositol 3-kinase; PR, partial response; PRST, postrescue survival time; RAB, rabacfosadine; SC, subcutaneously; SD, stable disease; ST, survival time; STAT, signal transducer and activator of transcription; VCOG, Veterinary Cooperative Oncology Group.

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KEYWORDS
canine, hematopoietic, multicentric, remission, species

1 | INTRODUCTION

Lymphoma is a common neoplasm in dogs, comprising approximately 83% of all hematopoietic neoplasms.[1] A discontinuous multidrug chemotherapy protocol utilizing cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) is the gold standard treatment for multicentric lymphoma in dogs, and remission rates of approximately 80% to 95% have been reported with overall median survival times of 10 to 12 months.[1]

The duration of 1st remission achieved with CHOP has been shown to affect the duration of subsequent remissions after CHOP reinduction. One study showed that dogs that relapsed after completion of a protocol based on CHOP could be retreated with CHOP for a 2nd remission duration of approximately half the duration of the 1st remission.[2] The median 2nd remission duration in that study was 159 days, and 78% of dogs achieved complete remission with retreatment.[2] Despite these results, many dogs develop disease progression during their CHOP protocol or relapse within 6 months of completion, and additional chemotherapy protocols are necessary for treatment of such dogs. Multiple rescue protocols have been described for dogs with lymphoma that relapse, including lomustine (CCNU), L-asparaginase and prednisone (LAP) and rabacfosadine (RAB). A previous study found an overall response rate (ORR) of 87% for dogs with relapsed or refractory lymphoma treated with a LAP protocol and a median time to progression of 63 days.[3] A recent study of RAB and L-asparaginase for the treatment of refractory or relapsed lymphoma showed an ORR of 67% with a median progression-free survival time of 63 days.[4]

Dogs with multicentric lymphoma that develop disease progression during initial CHOP chemotherapy commonly have been thought to have overall worse long-term outcome when compared with patients that completed their initial protocols, but no previous studies have evaluated the effect of early disease progression on response rate, progression-free interval (PFI), or survival time (ST) for patients undergoing rescue chemotherapy protocols.

Our aim was to correlate PFI in response to initial CHOP chemotherapy with treatment outcomes in response to rescue treatment using LAP or RAB.

2 | MATERIALS AND METHODS

2.1 | Study population

Raw data were retrospectively evaluated from 6 previous multi-institutional retrospective and prospective studies, including 2 retrospective studies and 1 prospective study evaluating chemotherapy based on CHOP as well as 3 VetDC-sponsored prospective studies evaluating RAB as a rescue agent.[4-9] Patients eligible for inclusion included dogs with a cytologic or histologic diagnosis of multicentric lymphoma that underwent 1st-line CHOP chemotherapy and then received either CCNU, LAP, or RAB with or without L-asparaginase and with or without prednisone as 1st rescue treatment. The duration of 1st-line CHOP chemotherapy was variable depending on institution and clinician preference and included 25-, 19-, and 15-week protocols.[6,7,10] Patient variables such as age, breed, sex, and weight, as well as PFI with 1st-line CHOP chemotherapy, PFI with 1st rescue treatment, and best response to 1st rescue treatment were recorded for all dogs. Immunophenotype, maximal response to initial chemotherapy, and overall survival time (OST) were recorded when available. Individual patient staging data were not recorded. Exclusion criteria included use of other rescue protocols or radiotherapy as part of 1st rescue treatment.

2.2 | Rescue protocols

All dogs were treated using either a LAP protocol or RAB with or without L-asparaginase and with or without prednisone protocol after disease progression after initial chemotherapy based on CHOP. Treatment with LAP was based on a previously described rescue protocol,[3] with CCNU given at a target dosage of 70 mg/m^2 PO every 21 days for 5 treatments. L-asparaginase was administered SC concurrently with the 1st 2 doses of CCNU at a dosage of 10 000 IU/m^2 or 400 IU/kg based on clinician preference, and prednisone was administered at a tapering dosage throughout the protocol. Typically, prednisone was administered PO at 2 mg/kg/d for the 1st week, then 1 mg/kg/d for 2 weeks, then 1 mg/kg q48h until protocol completion. Rabacfosadine[4] was administered at the label dosage and schedule (0.82-1 mg/kg as a 30-minute IV infusion once every 21 days for up to 5 treatments). L-asparaginase (10 000 IU/m^2 or 400 IU/kg SC) with or without prednisone was administered concurrently as previously described.[4]

2.3 | Response assessment

Response to treatment was reported by the Veterinary Cooperative Oncology Group (VCOG) v1.0 response evaluation criteria for lymphoma.[4-9,11] A complete response (CR) was defined as return to normal of all measurable peripheral lymph nodes. Partial response (PR) was defined as at least a 30% decrease in the sum of diameters of peripheral lymph nodes measurable at time of rescue treatment initiation. Stable disease (SD) was defined as neither a 30% decrease nor a 20% increase in the sum of measurable peripheral lymph node diameters, and progressive disease (PD) was defined as a >20% increase in the sum of peripheral

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lymph node diameters or development of new lesions. Stable disease needed to persist for a minimum of 21 days to be considered clinically relevant.

2.4 | Statistical analysis

Continuous data were expressed as median and range, and categorical data as frequencies and percentages. Predictors of response were evaluated using a 2-tailed Fisher exact test. Initial PFI was calculated from the date of CHOP treatment initiation, and OST from this date to the date of disease progression or death, respectively. Postrescue treatment PFI and survival time (PRST) were calculated from the time of rescue protocol initiation to disease progression or death, respectively. The PFI and ST were calculated via the Kaplan-Meier method. The impact of variables on PFI and PRST was calculated using logrank, Gehan Breslow Wilcoxon and Cox proportional hazards analysis. In addition to evaluating initial treatment PFI as a continuous variable, we dichotomized this variable by 2 different cutoffs. Because a 15-week CHOP protocol is 105 days long, the 1st cutoff was set at 100 days as an approximation for identification of patients that progressed during CHOP chemotherapy, rather than after the completion of treatment. This early cutoff accounts for the variable amount of time required to complete chemotherapy based on CHOP, given the different CHOP durations employed as well as variability in the use of dose delays. We also used an unbiased Web-based algorithm (X-tile: http://www.yalepath.org/edu//PathCamp/x-tile/) for dichotomizing continuous data to optimize statistical power to select alternate cut points. Significant univariate predictors of PFI and PRST were incorporated into a forward stepwise multivariable Cox model with an alpha of 0.1 and 0.05 for model entry and retention, respectively. All statistical analyses were performed by commercial statistical software (Prism v9.4, GraphPad Software, San Diego, California; SPSS v28.0.1.0, IBM, Armonk, New York). A *P* value of .05 was considered significant for all evaluations.

3 | RESULTS

3.1 | Patient population

One hundred eighty-seven dogs met the inclusion criteria and were divided into 2 rescue treatment treatment groups: LAP (72 dogs) and RAB (115 dogs). Information regarding signalment, weight, and immunophenotype is presented in Table 1. Multiple breeds were represented with the most common being mixed breed dogs (n = 56) and golden retrievers (n = 21). Median age for both groups was approximately 8 years old. A majority of dogs in both treatment groups were either castrated males (n = 100) or spayed females (n = 75). Sixty-six dogs in the LAP treatment group initially were treated using a 15-week CHOP chemotherapy protocol, 3 dogs were treated by a 19-week CHOP protocol, and 3 dogs were treated by a 25-week CHOP protocol.[6,7,10] Duration of initial CHOP treatment protocol was not known for patients in the RAB rescue treatment group.

Immunophenotype data were available for all 115 dogs in the RAB rescue treatment group with B-cell as the predominant immunophenotype (n = 105, 91%). Immunophenotype information was only available for 44 dogs in the LAP treatment group with 28 of these dogs having a B-cell immunophenotype (63%).

3.2 | Patient outcomes

The median CHOP PFI for patients in the LAP rescue group was 157 days (range, 22-469). The best response to initial CHOP treatment was recorded for all patients in the LAP rescue group with 83.3% obtaining CR, and only 1 patient was refractory to treatment with PD as the only response. The median LAP PFI was 61 days (range, 4->1092) with median PRST of 116 days (range, 2->1092). The median OST for LAP treated dogs was 290 days (range, 37->1140). The median CHOP PFI for patients in the RAB rescue group was 201 days (range, 15-1144). The best response to CHOP chemotherapy was not known for patients in the RAB rescue group. The median

TABLE 1 Signalment data.

	LAP (n = 72)		RAB (n = 115)	
Age (years)	Median (range)	8 (2-13.5)	Median (range)	8.3 (2-14)
Sex	MC	37 (51%)	MC	63 (55%)
	MI	3 (4%)	MI	8 (7%)
	FS	31 (43%)	FS	44 (38%)
	FI	1 (1%)	FI	0 (0%)
Weight (kg)	Median (range)	29.7 (3.5-66.7)	Median (range)	31.4 (4.2-66.7)
Immunophenotype	B-cell	28 (39%)	B-cell	105 (91%)
	T-cell	16 (22%)	T-cell	10 (9%)
	Not available	28 (39%)	Not available	0 (0%)

Note: Immunophenotype was determined with flow cytometry, PCR for antigen receptor rearrangement (PARR), immunocytochemistry, immunohistochemistry or some combination of these.
Abbreviations: FI, female intact; FS, female spayed; LAP, lomustine, L-asparaginase, and prednisone; MC, male castrated; MI, male intact; RAB, rabacfosadine.

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RAB PFI was 82 days (range, 7-546). Data regarding survival after RAB treatment were only available for 34 of the 115 dogs (30%). For these 34 dogs, the median PRST was 131 days (range, 13-1093) and the median OST was 337 days (range, 76-1128). Seven dogs were censored from PFI analysis in the LAP group, and 29 dogs were censored in the RAB group. Reasons for censorship included withdrawal because of owner perception of unacceptable adverse events (15), nonprogressive at time of last follow-up (13), owner noncompliance (5), and development of an unrelated medical issue (3). The median follow-up times in censored patients were 63 and 123 days for LAP and RAB patients, respectively.

3.2.1 | LAP outcomes

The duration of CHOP PFI was significantly associated with outcomes for LAP rescue treatment. Summaries of all factors evaluated for effect on PFI and PRST in the LAP treatment group are included in Table 2. When

TABLE 2 Summary of factors evaluated for effect on progression-free interval (PFI) and postrescue treatment survival time (PRST) for dogs treated with LAP rescue treatment.

LAP (n = 72)						
Factors affecting PFI						
Factor		**n**	**MPFI (days)**	***P* value**	**HR**	**95% CI**
CHOP PFI	<100 d	26	42	.03 (LR) .004 (GBW)	1.91	1.08-3.37
	≥100 d	46	75			
CHOP PFI	Continuous	…	…	.01 (Cox)	0.90	0.83-0.98
CHOP PFI	<86 d	18	35.5	.007 (LR) <.001 (GBW)	2.09	1.03-4.26
	≥86 d	54	74.5			
Immunophenotype	B-cell	28	75	.88 (LR) .19 (GBW)	0.95	0.49-1.85
	T-cell	16	49			
Best CHOP response	CR	60	72	.33 (LR) .03 (GBW)	1.37	0.66-2.82
	Not CR	12	30.5			
Best LAP response	CR	46	83	<.001 (LR test for trend)[a]	…	…
	PR	17	33			
	CR/PR	63	73			
	NR	9	21			
Multivariate	LAP response	…	…	<.001	10.9	5.47-21.8
Factors affecting PRST						
Factor		**n**	**MST (days)**	***P* value**	**HR**	**95% CI**
CHOP PFI	<100 d	26	76	.05 (LR) .003 (GBW)	1.78	1.01-3.13
	≥100 d	46	141			
CHOP PFI	Continuous	…	…	.07 (Cox)	0.93	0.85-1.01
CHOP PFI	<86 d	18	49	.03 (LR) <.001 (GBW)	2.10	1.07-4.14
	≥86 d	54	141			
Immunophenotype	B-cell	28	137	.75 (LR) .32 (GBW)	0.90	0.46-1.77
	T-cell	16	110			
Best CHOP response	CR	60	130	.85 (LR) .11(GBW)	0.94	0.49-1.83
	Not CR	12	62.5			
Best LAP response	CR	46	152	<.001 (LR test for trend)[a]	…	…
	PR	17	76			
	CR/PR	63	137			
	NR	9	42			
Multivariate	LAP Response	…	…	<.001	3.65	2.13-6.26

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CI, confidence interval; Cox, Cox proportional hazards; CR, complete response; GBW, Gehan Breslow Wilcoxon; HR, Hazard ratio; LAP, lomustine, L-asparaginase, and prednisone; LR, Logrank; MPFI, median progression-free interval; MST, median survival time; NR, no response; PR, partial response.

[a]Comparison of CR versus PR versus NR.

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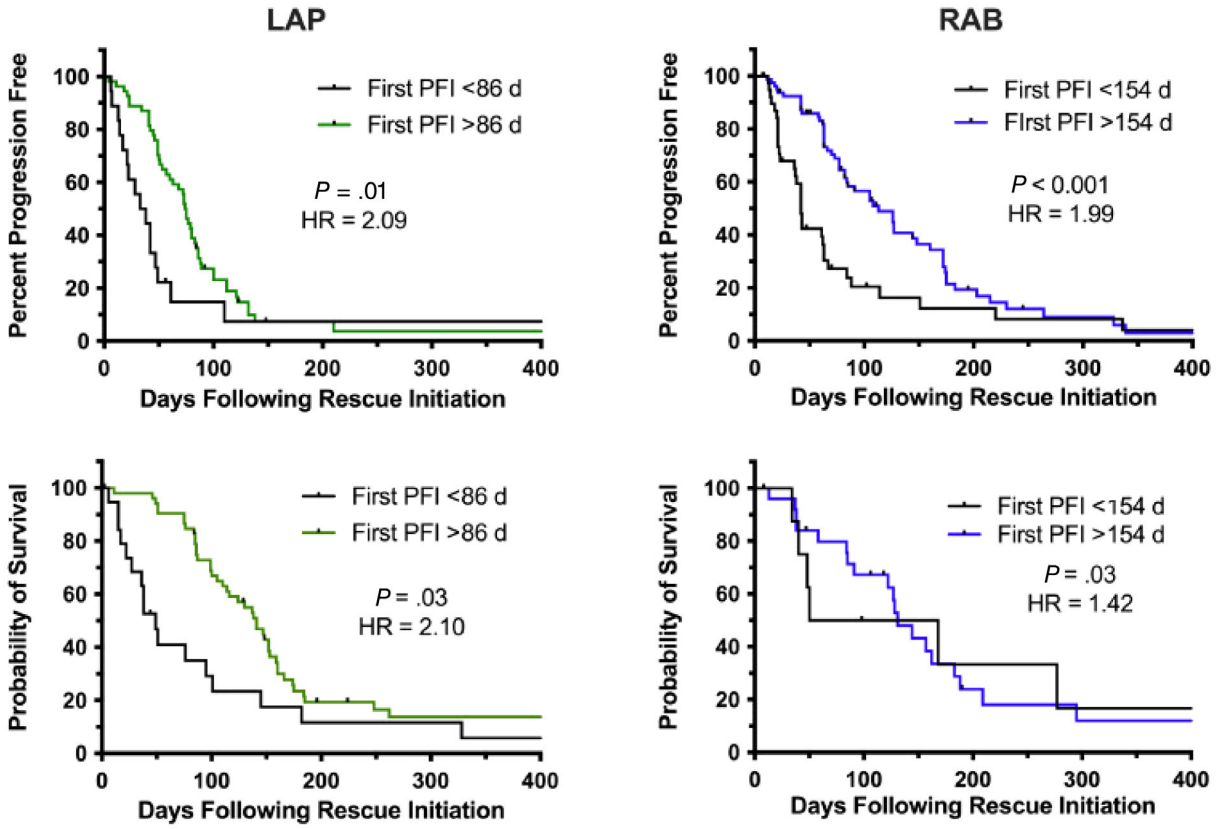


FIGURE 1 Kaplan-Meier curve depicting effects of initial cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment progression-free interval (PFI) on rescue PFI after lomustine, L-asparaginase, and prednisone (LAP) (top left) and rabacfosadine (RAB) (top right) rescue treatment. Kaplan-Meier curves depicting effects of initial treatment PFI on postrescue treatment survival time (PRST) after LAP (bottom left) and RAB (bottom right) rescue treatment. *P*-values represent univariate log rank values. Tick marks indicate censored patients. HR, hazard ratio.

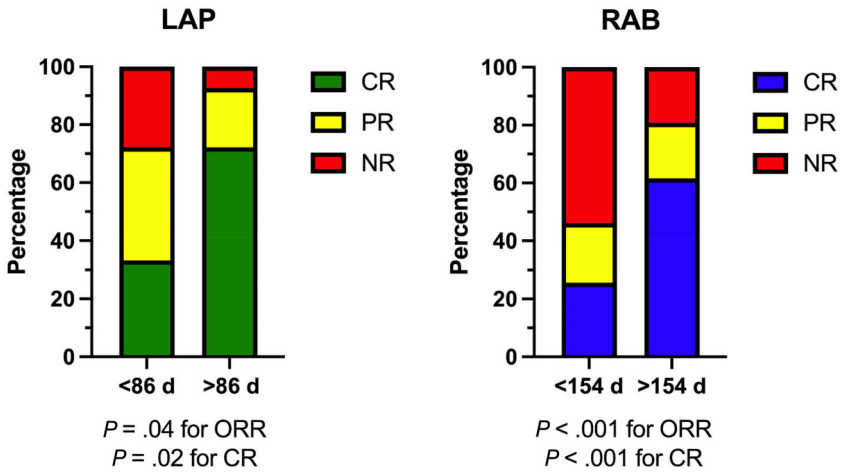


FIGURE 2 Stacked bar graph depicting effects of initial cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment progression-free interval (PFI) on overall response rate (ORR) and complete response (CR) rate to lomustine, L-asparaginase, and prednisone (LAP) (left) and rabacfosadine (RAB) (right) rescue protocols. Partial response (PR) and no response (NR). HR, hazard ratio.

evaluated as a continuous variable via Cox proportional hazards, CHOP PFI was associated with LAP PFI ($P = .01$, hazard ratio [HR] = 0.899). As depicted in the Kaplan-Meier curve in Figure 1, dogs with a CHOP PFI <86 days had a median rescue PFI of 35.5 days versus 74.5 days for dogs with a CHOP PFI >86 days ($P = .01$, HR = 2.09). The CHOP PFI dichotomized at 86 days also was significantly associated with PRST after LAP rescue treatment with a median PRST of 49 days for dogs with a shorter CHOP PFI versus 141 days for dogs with a longer CHOP PFI ($P = .03$, HR = 2.10) as depicted in Figure 1. A 100-day CHOP PFI was also significantly associated with both PFI and PRST after LAP rescue treatment as presented in Table 2.

As shown in Figure 2, dogs with a CHOP PFI >86 days were significantly more likely to have a response to rescue treatment (CR or PR, $P = .04$; Table 3). These dogs also had a significantly increased

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TABLE 3 Summary of factors evaluated for effect on overall response (ORR) and complete response (CR) rates for dogs treated with LAP rescue treatment.

LAP (n = 72)					
Factors affecting response (CR/PR)					
Factor		**n**	**No. of responding**	**Percentage**	***P* value**
Immunophenotype	B-cell	28	27	96.4%	.54
	T-cell	16	14	87.5%	
CHOP PFI	<100 d	26	21	80.8%	.27
	≥100 d	46	42	91.3%	
CHOP PFI	<86 d	18	13	72.2%	.04
	≥86 d	54	50	92.6%	
Best CHOP response	CR	60	54	90.0%	.17
	Not CR	12	9	75.0%	
Factors affecting CR					
Factor		**n**	**No. of CR**	**Percentage**	***P* value**
Immunophenotype	B-cell	28	19	67.9%	.53
	T-cell	16	9	56.3%	
CHOP PFI	<100 d	26	11	42.3%	.01
	≥100 d	46	35	76.1%	
CHOP PFI	<86 d	18	6	33.3%	.02
	≥86 d	54	39	72.2%	
Best CHOP response	CR	60	43	71.7%	.01
	Not CR	12	3	25.0%	

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; LAP, lomustine, L-asparaginase, and prednisone; PFI, progression-free interval; PR, partial response.

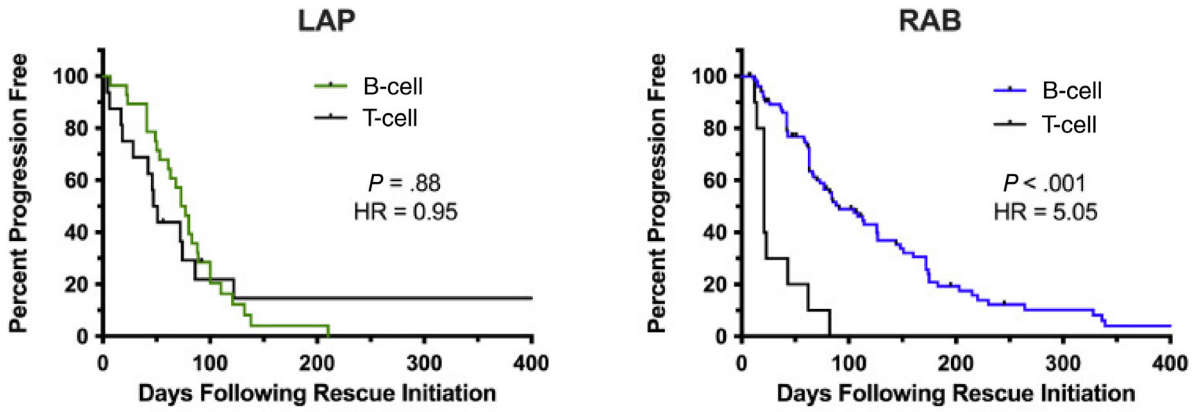


FIGURE 3 Kaplan-Meier curves depicting effects of lymphoma immunophenotype (B- vs T-cell) on progression-free interval (PFI) for dogs undergoing either lomustine, L-asparaginase, and prednisone (LAP) (left) or rabacfosadine (RAB) (right) rescue protocols. *P*-values represent univariate log rank values. Tick marks indicate censored patients. HR, hazard ratio.

rate of CR over dogs that had a CHOP PFI <86 days ($P = .02$; Table 3). Immunophenotype (B-cell vs T-cell) was not significantly associated with ORR ($P = .54$), PFI ($P = .88$), or PRST ($P = .75$) for LAP rescue treatment (Figure 3).

Summaries of all factors evaluated for effect on response rate in the LAP treatment group are presented in Table 3. Achieving a CR with the initial CHOP protocol was not significantly associated with ORR but was significantly associated with obtaining a CR with LAP rescue treatment. On multivariate analysis, best response to rescue treatment was the sole predictor of PFI and PRST for patients treated with LAP (Table 2).

3.2.2 | RAB outcomes

The CHOP PFI was significantly associated with outcomes for patients treated with RAB rescue treatment. A summary of all factors evaluated for effect on PFI and PRST in the RAB treatment group is presented in

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TABLE 4 Summary of factors evaluated for effect on progression-free interval (PFI) and post rescue treatment survival time (PRST) for dogs treated with rabacfosadine (RAB) rescue treatment.

RAB (n = 115)						
Factors affecting PFI						
Factor		**n**	**MPFI (days)**	***P* value**	**HR**	**95% CI**
CHOP PFI	<100 d	25	61	.17 (LR) .02 (GBW)	1.39	0.81-2.39
	≥100 d	90	105			
CHOP PFI	Continuous	…	…	.05 (Cox)	0.99	0.99-1.0
CHOP PFI	<154 d	39	43	<.001 (LR) <.001 (GBW)	1.99	1.21-3.3
	≥154 d	76	126			
Immunophenotype	B-cell	105	91	<.001 (LR) <.001 (GBW)	5.05	1.32-19.4
	T-cell	10	21			
L-asparaginase included	L-asparaginase	18	85	.92 (LR) .66 (GBW)	1.03	0.59-1.79
	No L-asparaginase	97	82			
RAB dose	0.82 mg/kg	21	63	.16 (LR) .12 (GBW)	1.46	0.79-2.71
	1.0 mg/kg	94	85			
Best RAB response	CR	55	160	<.001 (LR test for trend)[a]	…	…
	PR	22	63			
	CR/PR	77	127			
	NR	35	26			
	RAB response	…	…	<.001	9.93	5.60-17.6
Factors affecting PRST						
Factor		**n**	**MST (days)**	***P* value**	**HR**	**95% CI**
CHOP PFI	<100 d	8	109	.75 (LR) .64 (GBW)	1.14	0.49-2.61
	≥100 d	28	131			
CHOP PFI	Continuous	…	…	.89 (Cox)	1.00	0.99-1.00
CHOP PFI	<154 d	13	50	.33 (LR) .03 (GBW)	1.42	0.64-3.16
	≥154 d	21	157			
Immunophenotype	B-cell	31	131	.76 (LR) .99 (GBW)	1.20	0.33-4.36
	T-cell	5	122			
L-asparaginase included	L-asparaginase	11	144	.68 (LR) .65 (GBW)	1.18	0.54-2.62
	No L-asparaginase	23	127			
RAB dose	0.82 mg/kg	4	36	.22 (LR) .01 (GBW)	2.05	0.41-10.3
	1.0 mg/kg	30	144			
Best RAB response	CR	16	188	<.001 (LR test for trend)[a]	…	…
	PR	4	121			
	CR/PR	20	183			
	NR	13	48			
Multivariate	RAB response	…	…	<.001	11.7	3.82-35.9

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CI, confidence interval; Cox, Cox proportional hazards; CR, complete response; GBW, Gehan Breslow Wilcoxon; HR, hazard ratio; LR, Logrank; MPFI, median progression-free interval; MST, median survival time; NR, no response; PR, partial response.

[a]Comparison of CR versus PR versus NR.

Table 4. When evaluated as a continuous variable using Cox proportional hazards, CHOP PFI was associated with RAB PFI ($P = .05$, HR = 0.943). When dichotomized by X-tile, a CHOP PFI of <154 days was significantly associated with a decreased PFI after RAB rescue treatment (Figure 1). Dogs with a CHOP PFI <154 days had a median rescue PFI of 43 days versus 126 days for dogs with a CHOP PFI >154 days ($P = .0001$, HR = 2.0). The CHOP PFI was also significantly associated with PRST after RAB rescue treatment as depicted

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TABLE 5 Summary of factors evaluated for effect on overall response (ORR) and complete response (CR) for patients treated with rabacfosadine (RAB) rescue treatment.

RAB (n = 115)					
Factors affecting response (CR/PR)					
Factor		**n**	**No. of responding**	**Percentage**	***P* value**
Immunophenotype	B-cell	102	75	73.5%	.001
	T-cell	10	2	20.0%	
CHOP PFI	<100 d	24	15	62.5%	.47
	≥100 d	88	62	70.5%	
CHOP PFI	<154 d	39	18	46.2%	<.001
	≥154 d	73	59	80.8%	
L-asparaginase included	L-asparaginase	18	13	72.2%	>.99
	No L-asparaginase	94	64	68.1%	
RAB dose	0.82 mg/kg	21	12	57.1%	.30
	1.0 mg/kg	89	63	70.8%	
Factors affecting CR					
Factor		**n**	**No. of CR**	**Percentage**	***P* value**
Immunophenotype	B-cell	102	53	52.0%	.09
	T-cell	10	2	20.0%	
CHOP PFI	<100 d	24	8	33.3%	.04
	≥100 d	88	52	59.1%	
CHOP PFI	<154 d	39	10	25.6%	<.001
	≥154 d	73	45	61.6%	
L-asparaginase included	L-asparaginase	18	11	61.1%	.31
	No L-asparaginase	94	44	46.8%	
RAB dose	0.82 mg/kg	21	9	42.9%	.63
	1.0 mg/kg	89	45	50.6%	

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; PFI, progression-free interval; PR, partial response.

in Figure 1. Dogs with a CHOP PFI <154 days had a median PRST of 50 days versus 157 days for dogs with a longer initial CHOP PFI ($P = .03$, HR = 1.42). A 100-day CHOP PFI was significantly associated with PFI after RAB rescue treatment but was not associated with PRST, as presented in Table 4.

A CHOP PFI >154 days for dogs treated with RAB rescue treatment was significantly associated with response to treatment. Dogs with a longer CHOP PFI were significantly more likely to have an objective response (CR or PR, $P = .0003$) to their RAB rescue protocol as well as a significantly increased rate of CR ($P < .0001$; Figure 2). Immunophenotype was significantly associated with PFI for RAB rescue treatment. Patients with B-cell lymphoma had a significantly longer median PFI (91 days) as compared with their T-cell lymphoma counterparts, which had a median PFI of 21 days ($P < .0001$, HR = 5.05; Figure 3). Rabacfosadine dose (0.82 vs 1.0 mg/kg) was significantly associated with PRST but not PFI in this cohort ($P = .011$, HR = 2.045).

Summaries of all factors evaluated for effect on response in the RAB treatment group are included in Table 5. Concurrent administration of L-asparaginase and RAB dose (1.0 mg/kg vs 0.82 mg/kg) were not significantly associated with ORR or CR to RAB rescue treatment. On multivariate analysis, best response to rescue treatment was the sole predictor of PFI and PRST (Table 4).

4 | DISCUSSION

In our study, dogs that had disease progression during or soon after completing their 1st-line CHOP chemotherapy protocol experienced shorter PFI and PRST when treated with LAP or RAB as rescue protocols. These results are not surprising because of the common expectation that patients that progress early in their initial chemotherapy treatment overall will have a worse prognosis, but to our knowledge, this outcome has not been demonstrated previously in the veterinary literature. The patient population for our study was consistent with previously reported populations of dogs with lymphoma in the United States with an even distribution of males versus females and a median age of approximately 8 years.[10,12-14] Treatment responses and outcomes for both the initial protocol based on CHOP and rescue protocols were also

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consistent with previous reports on lymphoma in dogs. The median initial CHOP PFI for our patient population was approximately 5 to 7 months for both LAP and RAB rescue treatment groups (157 days and 201 days, respectively). This time frame is consistent with previously reported median PFIs of 140 to 219 days for dogs treated with 15- to 25-week CHOP protocols.[10,12-17] Our study showed that a time to progression of <86 days was significantly associated with shorter PFI (35.5 days), PRST (49 days) and response to LAP rescue chemotherapy. Similarly, our study also showed that a time to progression of <154 days was significantly associated with shorter PFI (43 days), PRST (48 days), and response to RAB rescue treatment.

We originally evaluated an initial PFI cutoff of 100 days as a surrogate for patients that progressed during their initial CHOP chemotherapy protocol (<100 days; Tables 2–5). This cutoff time was chosen as the approximate end of treatment based on the 15-week CHOP chemotherapy protocol currently used at Colorado State University.[6] We found that the 100-day initial treatment PFI cutoff was significantly associated with PFI ($P = .03$), PRST ($P = .04$), and CR rate ($P = .01$) for the LAP rescue protocol (Tables 2 and 3), as well as PFI ($P = .02$) and CR rate ($P < .0001$) for RAB rescue treatment (Tables 4 and 5). However, using X-tile to determine alternate 1st treatment PFI cutoffs in an unbiased fashion allowed us to find time frames that were statistically significant and associated with HR across multiple variables. The X-tile calculated PFI cutoff of 86 days for LAP rescue treatment and 154 days for RAB rescue treatment were significantly associated with PFI, PRST, ORR, and CR percentage for both rescue protocols. Some arbitrariness is always associated with cutoff choice when dichotomizing a continuous variable, and clinicians should keep in mind that CHOP PFI was a significant predictor of rescue outcome when treated as a continuous variable.

The PFI and PRST results among groups were compared by means of both logrank and Wilcoxon analysis. Although both are statistically appropriate, the logrank test tends to place more emphasis on events occurring late in time, whereas the Wilcoxon test tends to be more powerful in detecting early differences. For the majority of factors, both tests yielded statistically significant results. The only exceptions were the effect of initial treatment PFI on RAB rescue PFI with a cutoff of 100 days and the effect of initial treatment PFI on RAB rescue PRST with a cutoff of 154 days. In both cases, differences were significant based on Wilcoxon but not logrank analysis.

The fact that early progression after CHOP chemotherapy impacted the efficacy of rescue treatment with mechanistically different antineoplastic agents is interesting and not necessarily intuitive. It is not possible to invoke the acquisition of multidrug resistance through P-glycoprotein (P-GP) upregulation as a mechanism of resistance, because asparaginase, CCNU and RAB are not P-GP substrates. Cyclophosphamide and CCNU are both alkylating agents, and some cross-resistance to these 2 agents has been identified inconsistently in vitro.[18,19] It is probable that corticosteroids and other antineoplastic drugs can induce drug resistance via mechanisms other than P-GP upregulation. For example, corticosteroid resistance in leukemias of humans can be associated with enhanced Toll-like receptor stimulation, resulting in enhanced Janus kinase/signal transducer and activator of transcription (JAK-STAT) and phosphatidylinositol 3-kinase (PI3K) signaling and epigenetic derepression of expression of the antiapoptotic molecule Bim, all of which theoretically could confer cross-resistance to a variety of antineoplastic agents.[20,21]

Rabacfosadine dose (1.0 mg/kg vs 0.82 mg/kg) had no significant effect on PFI or response to treatment (Table 3). These findings are consistent with a previous study evaluating the efficacy of 2 different RAB doses that showed similar PFIs and response rates for both treatment doses.[9] However, RAB dose did appear to have an impact on PRST in this population. We hypothesize that this finding is likely related to type 2 error, because very few dogs with PRST data were treated with 0.82 mg/kg of RAB ($n = 4$), and no effect was found on the primary outcome measures PFI or response. Our study also found no significant effect on PFI, PRST, or response to treatment with the addition of L-asparaginase to the RAB rescue protocol, but there was limited statistical power to detect any effect if present. A previous study determined that concurrent use of RAB and L-asparaginase was safe and effective,[4] but additional studies are needed to elucidate the survival benefit, if any, of the addition of L-asparaginase to this treatment protocol.

We found that immunophenotype was not predictive of overall response, PFI or PRST for the LAP rescue protocol, but immunophenotype was significantly associated with response and PFI for the RAB rescue protocol. Multiple previous studies have reported a significantly decreased PFI for dogs with T-cell lymphoma when treated with RAB compared with their B-cell counterparts.[4,8] More interesting is the fact that immunophenotype was not associated with outcomes for the patients in the LAP protocol. It is possible this result is because of T-cell lymphoma having a better response to alkylating agents such as CCNU. Neoplastic T-cells have been found to have low levels of specific DNA repair proteins O^6 alkylguanine-DNA alkyltransferase (AGT) and O^6 methylguanine-DNA methyltransferase (MGMT), which impairs their ability to repair DNA damage caused by alkylation.[22,23] These findings have led to the hypothesis that alkylating agents could be more effective in the treatment of T-cell lymphoma and to the consideration of using chemotherapy protocols based on alkylating agents such as CCNU, vincristine, procarbazine, and prednisone (LOPP) as 1st-line treatment for T-cell lymphoma in dogs.[24-26] It is also possible that immunophenotype was not a significant outcome predictor in dogs treated with LAP rescue treatment because of the small number of dogs in that treatment group with confirmed T-cell lymphoma, leading to limited statistical power to detect differences.

A major limitation of our study was variability in the initial CHOP protocol. Because of the retrospective nature of the study as well as the fact that data were collected from multiple institutions, not all patients received the same initial treatment protocol. A 15-week CHOP protocol typically is used at Colorado State University as previously described.[6] Therefore, the timing of this protocol was used as the basis for the original 100-day response cutoff for our study. Not all dogs in the study received a 15-week CHOP protocol, with the range of initial protocols based on CHOP also including 25-week and 19-week protocols. The variability in these protocols means that our 100-day cutoff calculations may not accurately represent patients that truly progressed during, versus after completion of their initial chemotherapy protocol based on CHOP. It is possible that this variability also affected when PD was noted as well as when rescue protocols were initiated.

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Another limitation of our study was that we did not have access to all patients' medical records because of the multi-institutional nature of the study. Therefore, we were unable to fully assess the best CHOP chemotherapy response for the RAB rescue treatment dogs. Also, we cannot account for patients that were refractory to initial CHOP chemotherapy, which would affect the population of the early progression group and could be a cause for these patients to not respond well to rescue treatment. We were also limited in our ability to fully assess survival after RAB rescue treatment because we only had survival data for 34 of the 115 dogs in that treatment group, which limited statistical power for making comparisons and correlations. However, it is acknowledged that ST is a relatively weak endpoint in veterinary cancer clinical trials, given substantial variability in owner choice to pursue subsequent rescue treatment and variable opinions on euthanasia timing. A final limitation is a lack of complete information on other patient factors that could have had prognostic relevance, such as initial patient stage and substage.

In conclusion, dogs with multicentric lymphoma that experience a short PFI with initial CHOP-based chemotherapy are likely to have shorter PFI and PRST with rescue treatments based on LAP or RAB. Immunophenotype may not substantially influence outcome for patients treated with LAP as a rescue treatment but was significantly associated with PFI for RAB rescue treatment.

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CONFLICT OF INTEREST DECLARATION

Douglas H. Thamm is an advisor for and shareholder in VetDC, Inc. and a paid consultant for Elanco Animal Health. No other authors declare a conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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

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