Canine Lymphoma

Molecular diagnostics and treatment strategies

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Consultant to Heska

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Presentation Outline

- Canine lymphoma overview
- Molecular diagnostics
  - Nu.Q® Vet Cancer Screening Test
  - Immunohistochemistry
  - Flow cytometry
  - PCR for antigen receptor rearrangement
- Impact of immunophenotype on treatment and prognosis

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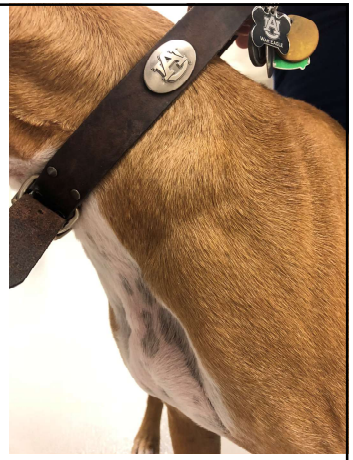
Canine Lymphoma

- Incidence and demographics
- 30+ different forms
  - Diffuse large B-cell lymphoma (65%)
  - Peripheral T-cell lymphoma (15-20%)
  - Low grade lymphoma (5-29%)
- Multicentric most common
  - Typically stage III+



Source: Valli et al. Classification of canine malignant lymphomas according to the World Health Organization Criteria. Vet Path 48(1) 198-211, 2011.

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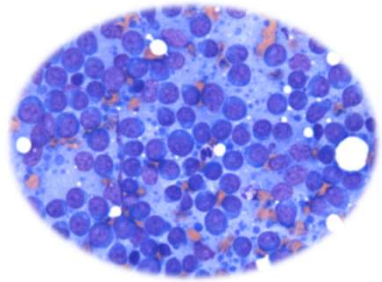
Diagnosis

- Intermediate to large > small cell
- Frequently achieved by cytology
  - Impaired by steroids
- Molecular diagnostics and/or histopathology for challenging cases



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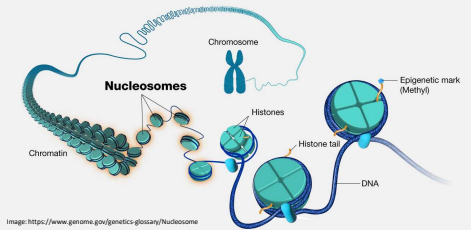
Nu.Q® Vet Cancer Screening Test

- ELISA-based form of liquid biopsy
  - Circulating nucleosomes
- Annual or biannual screening for older or at-risk breeds
- Fasted serum sample



Nu.Q® should be used for screening *healthy* dogs. Severe inflammation (sepsis, trauma, IMHA, acute and chronic gastroenteropathies) can also elevate circulating nucleosomes.

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Low Suspicion	≤ 50 ng/mL	• Nu.Q results are consistent with those found in healthy animals over 1 yr, and all genders. Retest at next wellness visit.
Moderate Suspicion	51-80 ng/mL	• Nu.Q results are in the "gray zone" and further testing should be considered. • Patient fasted and sample handling procedures followed? Re-test in 2-4 weeks, consider further diagnostics
High Suspicion	≥ 81 ng/mL	• Nu.Q results are consistent with increased risk of cancer in otherwise healthy animals over 1 yr, and all genders. Pursue further diagnostics to screen for cancer (chest x-rays, abdominal US)

HESKA

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Circulating Nucleosomes in Dogs with Cancer

- Compared dogs with cancer to healthy controls
- Cut-off for detection (AUC 68%)
  - 97% specificity
  - 49.8% sensitivity

- **Good ability to detect:**
  - Lymphoma*
  - Hemangiosarcoma*
  - Histiocytic sarcoma
  - Malignant melanoma

Wilson-Robles H, Bygott T, Kelly TK. Evaluation of plasma nucleosome concentrations in dogs with a variety of common cancers and in healthy dogs. *BMC Vet Res* 2022.

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Dogs with Lymphoma Have Elevated Circulating Nucleosomes

- 6.8X increase in dogs with LSA
  - Increases with stage
  - B-cell > T-cell
- Cut-off for detection (AUC 87.8%)
  - 94.8% specificity
  - 80.2% sensitivity

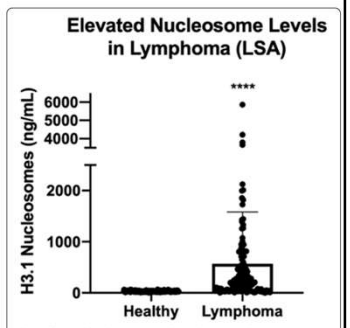

Fig. 1 Elevated Nucleosome Levels in LSA. Mean plasma nucleosome concentrations (ng/mL) were significantly higher in LSA dogs compared to healthy controls. Boxes represent the mean and the bars represent the standard deviation. Dots represent individual data points. **** indicates a *p*-value < 0.0001

Dolan C, Miller T, Jill J. Characterizing circulating nucleosomes in the plasma of dogs with lymphoma. *BMC Vet Res* 2021.

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Point-of-Care Element i+

- Exclusive to Heska
  - In-clinic Nu.Q® results in ~ 10 minutes
- Wellness screening
- Monitoring response to cancer treatment
  - LSA data coming soon

HESKA

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To Stage or Not to Stage?

- Minimum database
- Thoracic radiographs
- Abdominal ultrasound
  - Aspirate liver, spleen, etc.
- Bone marrow aspirate
- Lymph node biopsy
- → Immunophenotyping

Minimum to treat:

- Diagnosis
- Minimum database

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Immunophenotyping

- B-cell vs. T-cell
  - "B is better, T is terrible" does not always apply
- Guides treatment and prognosis
  - Prognosis ultimately dependent on decision to pursue treatment and response

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Immunophenotyping

- Utility
  - Confirm diagnosis in otherwise ambiguous sample
  - Determine subtype → alters treatment and prognosis
- Modalities
  - Immunohistochemistry (IHC)
  - Flow cytometry (FC)
  - PCR for antigen receptor rearrangement (PARR)

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Histopathology with IHC

- "Gold standard"
  - Assigns WHO subtype and immunophenotype
- Disadvantages
  - Heavy sedation or anesthesia
  - Increased cost
  - Delay in diagnosis
  - Cannot dx all LSA subtypes
  - Lack of certain prognostic markers

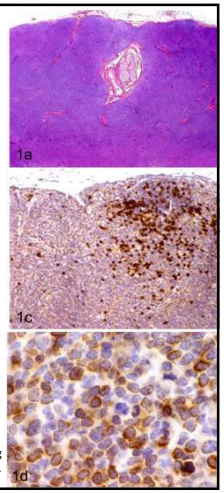

Image source: Valli et al. Classification of canine malignant lymphomas according to the World Health Organization Criteria. Vet Path 48(1) 198-211, 2011.

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Flow Cytometry

- Individual cells sorted by size, complexity, antibody labeling
  - Live cells from LN, blood, marrow, effusion, etc.
- Classification of hematopoietic neoplasms
  - Lymphomas
  - Leukemias
  - Mediastinal masses

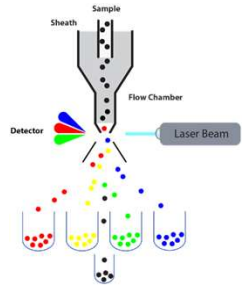

Image source: https://www.iop.nia.nih.gov/branches/img/fcf/new-index.htm

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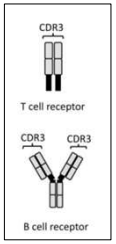
PARR

- Establishes clonality
- Highly specific
  - Ehrlichia can be clonal
- Moderately sensitive
  - No standardized protocols
  - Polyclonal background
- Immunophenotype may be inaccurate



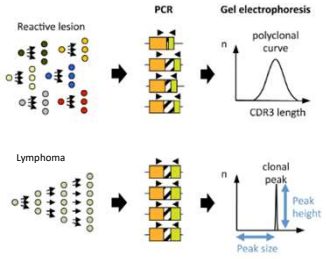


Image source: Keller SM, Vernau W, Moore PF. Clonality testing in veterinary medicine: a review with diagnostic guidelines. Vet Path 53(4) 711-725, 2016.

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How to Select the "Best" Test

- Flow: 94% agreement with IHC
- PARR: 69% agreement with IHC
  - Ability to confirm diagnosis of LSA: 74%
  - Incorrect immunophenotype: 33% of B-cell and 25% of T-cell lymphomas
- **Flow is better for immunophenotype**
- **PARR is better for clonality**

Thalheim L, Williams LE, Borst LB. Lymphoma immunophenotype of dogs determined by immunohistochemistry, flow cytometry, and polymerase chain reaction for antigen receptor rearrangements. *J Vet Intern Med* 2013;27(6):1506-16.

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Immunophenotype Impacts Prognosis

Type of Lymphoid Neoplasia		Median Survival Time
B-cell LSA	Multicentric large cell B-cell LSA	10-12 months* *Low MHC class II, large cell size = 4-5 months
	Nodal small cell B-cell LSA	7-9 months
T-cell LSA	Peripheral T-cell LSA	5-6 months
	T-zone LSA	1.7 years
	Cutaneous epitheliotropic T-cell LSA	2 months to 2 years
Leukemias	Chronic lymphocytic leukemia	T-cell: 2.5 years B-cell: 10-16 months Atypical: 22 days
	$CD4^{-}CD8^{-}$ T-cell leukemia (English Bulldogs)	26 days
	Acute myeloid or lymphocytic leukemia	1-5 weeks

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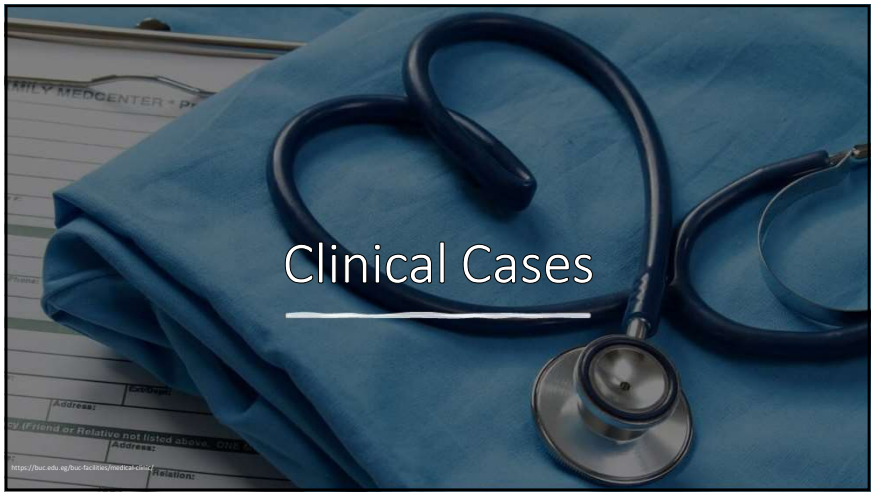

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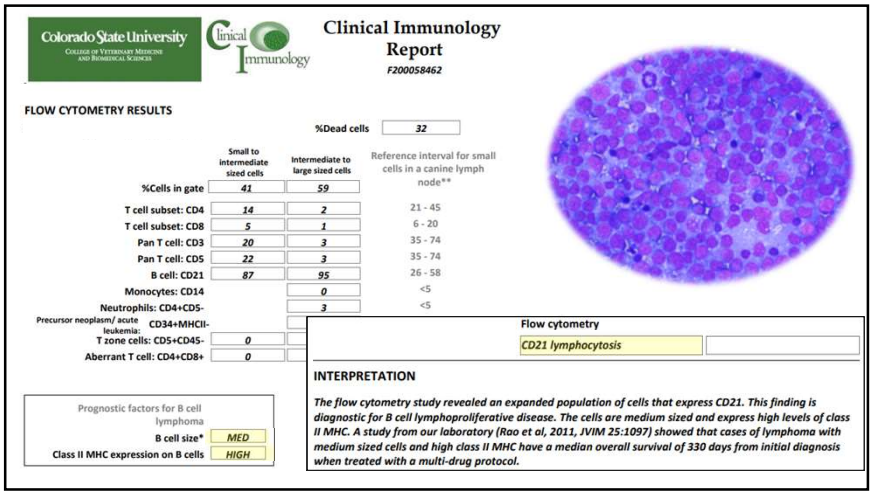
Colorado State University
College of Veterinary Medicine and Biomedical Sciences

Clinical Immunology

Clinical Immunology Report

F200058462

FLOW CYTOMETRY RESULTS

%Dead cells: *32*

	Small to intermediate sized cells	Intermediate to large sized cells	Reference interval for small cells in a canine lymph node**
%Cells in gate	*41*	*59*	
T cell subset: CD4	*14*	*2*	21 - 45
T cell subset: CD8	*5*	*1*	6 - 20
Pan T cell: CD3	*20*	*3*	35 - 74
Pan T cell: CD5	*22*	*3*	35 - 74
B cell: CD21	*87*	*95*	26 - 58
Monocytes: CD14		*0*	<5
Neutrophils: CD4+CD5-		*3*	<5
Precursor neoplasm/ acute leukemia: CD34+MHCII-			
T zone cells: CD5+CD45-	*0*		
Aberrant T cell: CD4+CD8+	*0*		

Flow cytometry: *CD21 lymphocytosis*

INTERPRETATION

The flow cytometry study revealed an expanded population of cells that express CD21. This finding is diagnostic for B cell lymphoproliferative disease. The cells are medium sized and express high levels of class II MHC. A study from our laboratory (Rao et al, 2011, JVIM 25:1097) showed that cases of lymphoma with medium sized cells and high class II MHC have a median overall survival of 330 days from initial diagnosis when treated with a multi-drug protocol.

Prognostic factors for B cell lymphoma

B cell size*	*MED*
Class II MHC expression on B cells	*HIGH*

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Treatment Plan

- Initiation of 15-week CHOP chemotherapy

	Week											
	1	2	3	5	6	7	9	10	11	13	14	15
Vincristine 0.7 mg m^{-2} IV	a			a			a			a		
Cyclophosphamide 250 mg m^{-2} PO or IV		a			a			a			a	
Doxorubicin 30 mg m^{-2} IV[a]			a			a			a			a
Prednisone (mg kg^{-1} per day) PO	2.0	1.5	1.0	0.5								

[a] Patients with body weight <15 kg received doxorubicin at 1 mg kg^{-1}.

- Generally, MST 10-12 months
  - Does age play a role?

Curran K and Thamm DH. Retrospective analysis for treatment of naïve canine multicentric lymphoma with a 15-week, maintenance-free CHOP protocol. *Vet Comp Oncol* 2016 Aug;14 Suppl 1:147-55.

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Small Animals

Usefulness of chemotherapy for the treatment of very elderly dogs with multicentric lymphoma

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OBJECTIVE
To evaluate factors for associations with duration of first remission and survival time in dogs ≥ 14 years of age with stage III to V multicentric lymphoma.

DESIGN
Retrospective cohort study.

ANIMALS
29 dogs ≥ 14 years of age with multicentric lymphoma treated with a chemotherapy protocol at dosages used for younger dogs (n = 22) or with prednisolone alone (7).

PROCEDURES
Various data were collected from the medical records, including treatment response and related adverse events. Survival analysis was performed to determine duration of first remission and survival time (from start of chemotherapy), and these outcomes were compared between various groupings.

RESULTS
The 7 (24%) dogs that received prednisolone alone had a median survival time of 27 days and were excluded from further analysis. Complete clinical remission was achieved in 21 of the 22 (95%) remaining dogs; 1 (5%) achieved partial remission. Median duration of first remission was 181 days. Anemic dogs had a briefer remission period (median, 110 days) than nonanemic dogs (median, 228 days). Median survival time for all 22 dogs was 202 days, with estimated 1- and 2-year survival rates of 31% and 5%, respectively. Six (27%) dogs had adverse events of chemotherapy classified as grade 3 or worse.

CONCLUSIONS AND CLINICAL RELEVANCE
Survival time was substantially longer in dogs treated with a chemotherapy protocol versus prednisolone alone. Findings suggested that the evaluated chemotherapy protocols for lymphoma were beneficial for and tolerated by very elderly dogs, just as by younger dogs, and need not be withheld, or dosages adjusted, because of age alone. (*J Am Vet Med Assoc* 2018;252:852–859)

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Case Progression

- Entered remission after Week 2
- Clinical relapse after 6 months, feeling well
- Rescue chemotherapy options?
  - CHOP: 80-90% response rate
  - Alternatives?

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Tanovea® for Relapsed B-Cell Lymphoma

- IV once every 3 weeks x 5
- 74% response rate, median PFS 6.7 months
- Side effects
  - GI upset, dermatopathy*, myelosuppression, proteinuria, nephrotoxicity, liver injury, pulmonary fibrosis*
  - Strong irritant

Saba CF, Vickery KR, Clifford CA, et al. Rabacfosadine for relapsed canine B-cell lymphoma: Efficacy and adverse event profiles of 2 different doses. *Vet Comp Oncol*, 2018 Mar;16(1):E76-E82.

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Other Uses for Tanovea® (Full FDA Approval)

- Substitute for doxorubicin or in addition
  - T-CHOP: 93% response, median PFS 6 months[1]
- Cutaneous epitheliotropic T-cell lymphoma
  - 45% response, median PFS 38 days[2]
- Multiple myeloma
  - 73% response, median PFS 6 months[3]

No established dose (yet) in cats

[1] Curran K, Vail D, Donoghue A, et al. Safety and efficacy of rabacfosadine, vincristine, cyclophosphamide, doxorubicin, and prednisone (T-CHOP) combination in dogs with treatment-naïve lymphoma [abstract]. In: Veterinary Cancer Society Annual Conference; 2022;. Norfolk, VA.
[2] Morges MA, Burton JH, Saba CF, et al. Phase II evaluation of VDC-1101 in canine cutaneous T-cell lymphoma. *J Vet Intern Med.* 2014;28(5):1569-74.
[3] Thamm D, Saba C, Rebhun R, et al. Rabacfosadine for the treatment of canine plasma cell neoplasia [abstract]. In: Veterinary Cancer Society Annual Conference; 2022;. Norfolk, VA.

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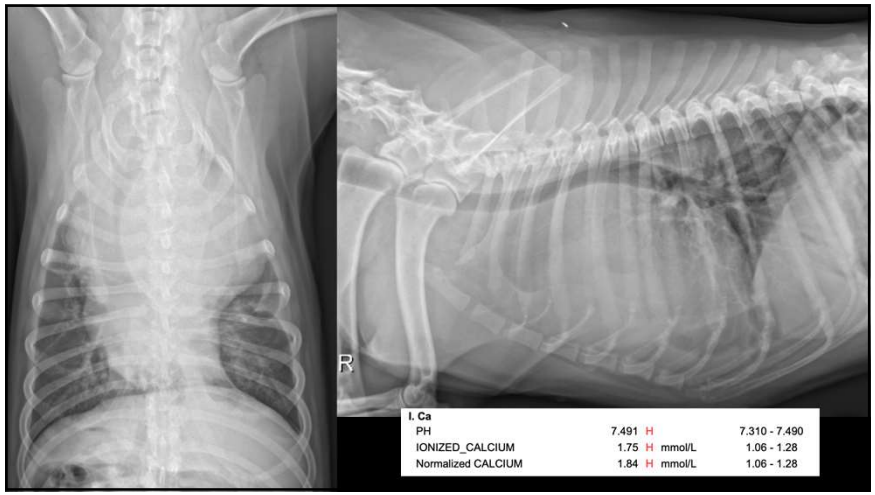

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Cytology and PARR

- Lymph node cytology = medium to large lymphocytes consistent with lymphoma
- PARR:

TEST RESULTS

PCR for antigen receptor rearrangements (PARR)

Immunoglobulin gene: POLYCLONAL
T cell receptor gene: CLONAL

INTERPRETATION

The sample was not viable for flow cytometry so the PARR assay was performed. The results indicate the presence of a clonally rearranged T cell receptor gene. This finding is 94% specific for neoplasia, most commonly T cell in origin although acute myeloid leukemias can also harbor a clonally rearranged T cell receptor gene. T cell neoplasia can have a broad variety of outcomes, from acute T cell leukemia/lymphoma, with a median survival time of days to weeks, to peripheral T cell lymphoma (median survival time measured in months) to indolent T zone lymphoma with a prolonged clinical course that may not require treatment. Flow cytometry or histopathology can distinguish between some (but not all) of these subtypes and provide prognostic information.

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Treatment Plan

- L-asparaginase (Elspar) while awaiting flow cytometry
- Initiated LOPP

Drug	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	29
Vincristine 0.7 mg m^{-2} IV	X														X	X
Lomustine 60 mg m^{-2} PO	X															X
Procarbazine 50 mg m^{-2} PO		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Prednisolone 30 mg m^{-2} PO		X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Abbreviations: IV, intravenously; LOPP, lomustine, vincristine, procarbazine, and prednisolone; PO, per os.

- Expected survival?

Morgan E, O'Connell K, Thompson M, et al. Canine T cell lymphoma treated with lomustine, vincristine, procarbazine, and prednisolone chemotherapy in 35 dogs. *Vet Comp Oncol,* 2018;16(4):622-629.

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The Case for Treating T-Cells Differently

Protocol	Remission Rate	Median Survival	25% Survival
CHOP[1]	88%	6 months	9 months
LOPP[2,3]	82-90%	10-16 months	2 years
MOPP[4]	78%	9 months	2 years

- Alkylator-rich protocols
  - Higher risk of hospitalization

Outcome: Completed LOPP, relapsed 6 weeks later. Initiated rescue chemotherapy. Survived 9 months post diagnosis.

[1] Mrebhun RB, Kent MS, Borrofka SAEB, et al. CHOP chemotherapy for the treatment of canine multicentric T-cell lymphoma. *Vet Comp Oncol. 2011;*9(1):38-44.
[2] Morgan E, O'Connell K, Thompson M, et al. Canine T cell lymphoma treated with lomustine, vincristine, procarbazine, and prednisolone chemotherapy in 35 dogs. *Vet Comp Oncol,* 2018;16(4):622-629.
[3] Brown PM, Tzannes S, Nguyen S, et al. LOPP chemotherapy as a front-line treatment for dogs with T-cell lymphoma. *Vet Comp Oncol.* 2018;16:108-113.
[4] Brodsky EM, Maudlin GN, Lachowicz JL, et al. Asparaginase and MOPP treatment of dogs with lymphoma. *J Vet Intern Med.* 2009;23(3):578-84.

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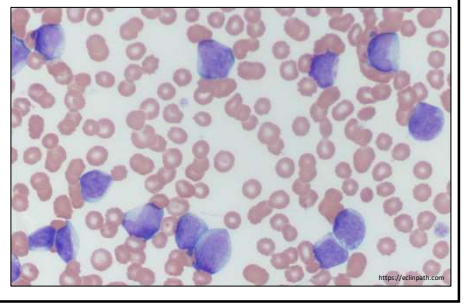
Test	Result		Units	Ref. Interval
RBC	5.60	L	x 10^6/uL	6.02 - 8.64
HGB	14.5		g/dL	13.1 - 20.1
HCT	40.7		%	38.7 - 59.2
MCV	72.7		fL	60.5 - 73.8
MCH	25.9	H	pg	20.4 - 25.7
MCHC	35.6		g/dL	32.0 - 37.2
RDW	14.1		%	11.2 - 14.4
PLATELET COUNT	77	L	x 10^3/uL	152 - 518
MPV	16.3	H	fL	8.0 - 14.6
RETIC_PCT	0.33			0.00 - 1.50
RETIC_ABS	18.3		x 10^3/uL	0.0 - 60.0
WBC	21.49	H	x 10^3/uL	5.09 - 17.41
Test	**Diff %**	**Result**	**Units**	**Ref. Interval**
SEG	(41%)	8.811	x 10^3/uL	2.600 - 10.400
BANDS	(0%)	0.000	x 10^3/uL	0.000 - 0.300
LYMPH	(58%)	12.464	H x 10^3/uL	0.390 - 6.730
MONO	(1%)	0.215	x 10^3/uL	0.160 - 1.160
EOS	(0%)	0.000	L x 10^3/uL	0.010 - 2.050
BASO	(0%)	0.000	x 10^3/uL	0.000 - 0.110
OTHER	(0%)	0.000	x 10^3/uL	0.000 - 0.000
NRBC		0	/100 WBC	No Ref Interval
PLT.EST	BELOW REFERENCE INTERVAL			



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Treatment Plan

- Induced with Elspar and vincristine pending flow…

Flow cytometry

CD34 leukocytosis

INTERPRETATION

The flow cytometry study revealed an expansion of CD34+ cells. This finding is diagnostic for a malignancy derived from precursor cells arising in the bone marrow, often referred to as acute leukemia, but which can also present with primary tissue involvement. The cells do not express lineage antigens (lymphocyte-specific or myeloid-specific) so we cannot determine if this is ALL or AML. Novacco, Comazzi et al (Vet Comp Onc, 2015) report a median survival of 9 days for acute leukemia of all types (range 1 - 120 days).

- Alternating vincristine/doxorubicin
  - Ultimately received cytarabine, L-asparaginase, Palladia…
- Euthanized ~2 months later

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Isabel

11-year-old spayed female dachshund mix

https://www.alphapaw.com/dog-breeds/dachshund-lab-mix/

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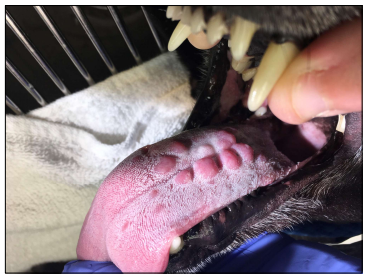

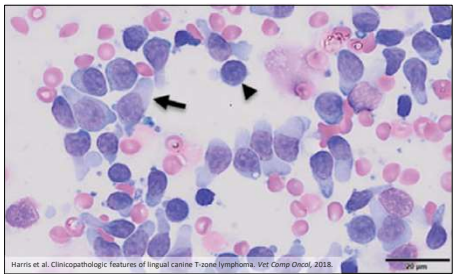

Harris et al. Clinicopathologic features of lingual canine T-zone lymphoma. *Vet Comp Oncol*, 2018.

- T zone lymphoma
  - CD3+, CD5+, low/moderate CD21+, loss of CD45

Harris LJ, Rout ED, Hughes KL, et al. Clinicopathologic features of lingual canine T-zone lymphoma. *Vet Comp Oncol.* 2018;16(1):131-139.

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T-Zone Lymphoma

- Indolent, small cell lymphoma
  - Hand-mirror lymphocytes
  - 93% stage V
- May or may not warrant treatment
  - Chlorambucil/prednisone
  - Survival of years

Died of unrelated disease 3+ years later

Martini V, Marconato L, Poggi A, et al. Canine small clear cell/T-zone lymphoma: clinical presentation and outcome in a retrospective case series. *Vet Comp Oncol.* 2016;14(1):117-26.

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Diagnostics and Treatment

- Histopathology with IHC: CD3+, Pax5- intermediate to large cell LSA
- Planned for L-LOPP
  - Chemotherapy sepsis, hypoglycemia, hypotension, AKI
  - But remission…
    - Persistent side effects despite substantial dose reductions

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When to use Laverdia®-CA1?

- Oral chemotherapeutic given twice weekly
  - Seemingly better response in T-cell lymphomas
- Side effects: GI upset (~50%)
  - Rarely myelosuppression, hepatopathy, dermatopathy
- 37% of dogs respond for median of 1 month
  - Compare to steroids alone?

Sadowski AR, Gardner HL, Borgatti A, et al. Phase II study of the oral selective inhibitor of nuclear export (SINE) KPT-335 (verdinexor) in dogs with lymphoma. *BMC Vet Res* 2018. 24;14(1):250.

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Survival time for dogs with previously untreated, peripheral nodal, intermediate- or large-cell lymphoma treated with prednisone alone: the Canine Lymphoma Steroid Only trial

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Nathan Yanda DVM
Chad M. Johannes DVM

OBJECTIVE
To evaluate survival times for dogs with previously untreated, peripheral nodal, intermediate- or large-cell lymphoma treated with prednisone alone.

ANIMALS
109 client-owned dogs recruited from 15 institutions in the United States.

PROCEDURES
Dogs were treated with prednisone at a dosage of 40 mg/m², PO, once daily for 7 days and at a dosage of 20 mg/m², PO, once daily thereafter. Quality of life (QOL) was assessed by owners with a visual analog scale when treatment was started (day 0), 1 and 2 weeks after treatment was started, and every 4 weeks thereafter. The primary outcome of interest was survival time as determined by the Kaplan-Meier method. Factors potentially associated with survival time were examined.

RESULTS
Median overall survival time was 50 days (95% CI, 41 to 59 days). Factors associated with survival time included substage (a vs b) and immunophenotype (B cell vs T cell). Owner-assigned QOL scores on days 0 and 14 were significantly positively correlated with survival time. When QOL score was dichotomized, dogs with day 0 or day 14 QOL scores ≥ 50 had significantly longer survival times, compared with dogs with day 0 or day 14 QOL scores < 50. No variables were predictive of long-term (> 120 days) survival.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that survival times were short for dogs with previously untreated, peripheral nodal, intermediate- or large-cell lymphoma treated with prednisone alone. Owner-perceived QOL and clinician-assigned substage were both associated with survival time. Findings provide potentially important information for clinicians to discuss with owners of dogs with lymphoma at the time treatment decisions are made. (*J Am Vet Med Assoc* 2021;259:62–71)

The median survival time with prednisone alone was **50 days** (95% CI, 41-59 days).

Only 7% of dogs survived > 6 months. Dogs with substage a disease, T-cell lymphoma, and those with a good Day 0 QOL score had a significantly longer survival time.

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A Change of Plan

- Pulse-dosed chlorambucil
  - Maintained remission for 6 months
- Relapse → CCNU/prednisone
  - Re-entered remission

When to use Laverdia®-CA1?
- Contraindication to steroids

Future directions – *in vitro* cytotoxicity:
- Melanoma
- Mammary carcinoma
- Transitional cell carcinoma
- Osteosarcoma

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Conclusions

- Diagnosis may be expedited with liquid biopsy
- PARR useful for clonality, flow cytometry determines immunophenotype
- Immunophenotype improves ability to subtype lymphoid neoplasia
  - Alters treatment and prognosis

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Questions?

Remember to
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NOTE: CE certificate not available
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Questions about CE?
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Thank you for joining us!

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