

Canine High Grade Lymphoma Made Easy

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Outline

- Cell biology
- Helpful terminology
- Presentation
- Pathophysiology, progression and how it relates to clinical signs
- Diagnosis, staging and testing
- Prognostic indicators
- T versus B cell
- Treatment options & cost estimates for common protocols
- Expected outcomes

Size (and maturity) matters

- Large cell and intermediate cell lymphoma both come from immature forms
 - greater ability to divide (pluripotent)
 - More aggressive disease course
- Lymphocyte (small cell)
 - More mature (lymphocytic lymphoma)
 - Less ability to divide
 - Less aggressive disease course



Large cell (blast)



Intermediate-large cell



Small Lymphocyte

Aggressive behavior 

Grade versus stage

- Grade
 - Grade in lymphoma is aggressiveness of the cells under the microscope
 - Intermediate to large cell lymphoma is high grade
 - Lymphoma is one of the few cancers whose grade can be shown with cytology (not biopsy)
- Stage is where it is in the body and how affected the patient is

High grade lymphoma stages

- Substage is how clinically affected the patient is
 - Substage A- not ill
 - Substage B- clinically ill
- Stage I - one lymph node
- Stage II- two or more nodes on one side of the diaphragm
- Stage III- Nodes on both sides of the diaphragm
- Stage IV- Liver and or Spleen +/- nodes
- Stage V- ANYWHERE ELSE: bone marrow, eye, lungs, GI, CNS....

Stage V lymphoma (involving the bone marrow) versus leukemia

The difference is where the disease starts first... and the prognosis

- Leukemia:
 - Bone marrow pluripotent cells that then move out to the circulation
 - MST with treatment → days
- Stage V lymphoma
 - starts in lymphoid organs with pluripotent cells and goes back to the bone marrow
 - MST with treatment → 1 year

Helpful features:

-Lymphoma patients → usually have bigger nodes first

-Leukemia patients → smaller nodes, clinically ill, more significant cytopenias

High grade lymphoma immunophenotype (B & T cell)

- B cell
 - Born and mature in the bone marrow (B)
 - Antibody production and memory
 - Lymphoma breeds
 - Rottweilers (Europe), Bernese mtn dog, Dobermans +/- goldens

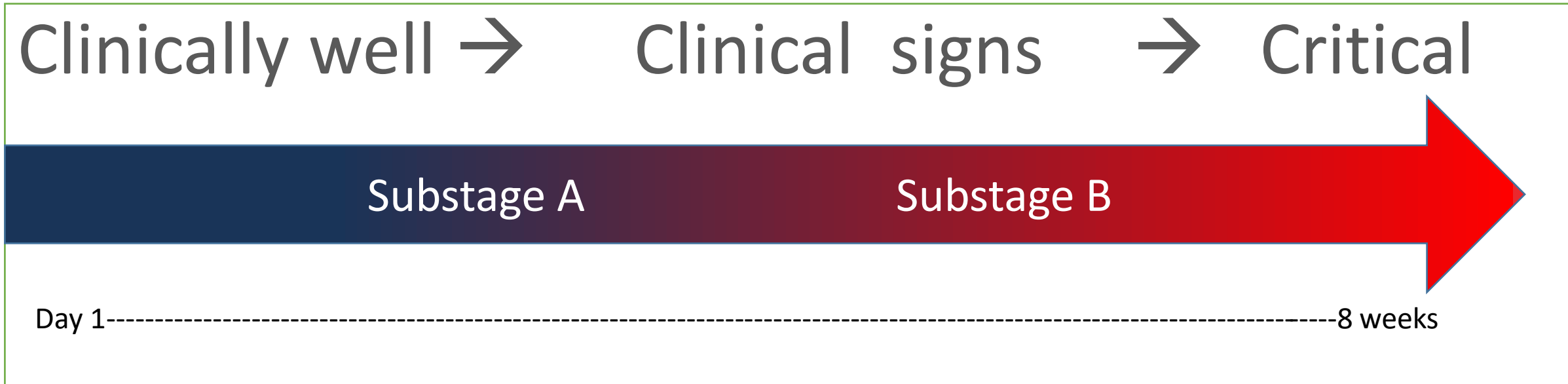
High grade lymphoma immunophenotype (B & T cell)

- T cell
 - Born in the bone marrow
 - Mature in the thymus (T)
 - Cytotoxic, memory, helper, regulatory cells
 - Calcium signaling
 - Lymphoma breeds
 - Boxers, labs, goldens

Presenting complaint

- “Lumps” → lymphadenopathy
 - DDX generalized or “multicentric” lymphadenopathy....
- ADR → vague waxing & waning lethargy
- Gastrointestinal signs → hyporexia, diarrhea, vomiting

Progression and pathophysiology of clinical signs in untreated patients



Pathophysiology & Clinical Signs

Clinically well



Clinical signs



Critical

Clonal expansion of large lymphocytes/blasts

Structural changes of infiltrated lymphatic organs

Pathophysiology & Clinical Signs

Clinically well



Clinical signs



Critical

Clonal expansion of large lymphocytes/blasts

Structural changes of infiltrated lymphatic organs

Further expansion of lymphatic centers (organomegally/lymphadenopathy)

Lymphatic obstruction: edema/effusion

Cytokine release: lethargy/fever

PTHrP: hypercalcemia

Immune mediated disease

Vasculitis

Pathophysiology & Clinical Signs

Clinically well



Clinical signs



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Immune mediated disease

Vasculitis

Target organ dysfunction/failure;

Bone marrow

Gastrointestinal

Lung

Liver

Kidney (infiltration, Ca)

Brain

Hemorrhage,
coagulopathy, DIC, ARDS
+/- sepsis if cytopenia

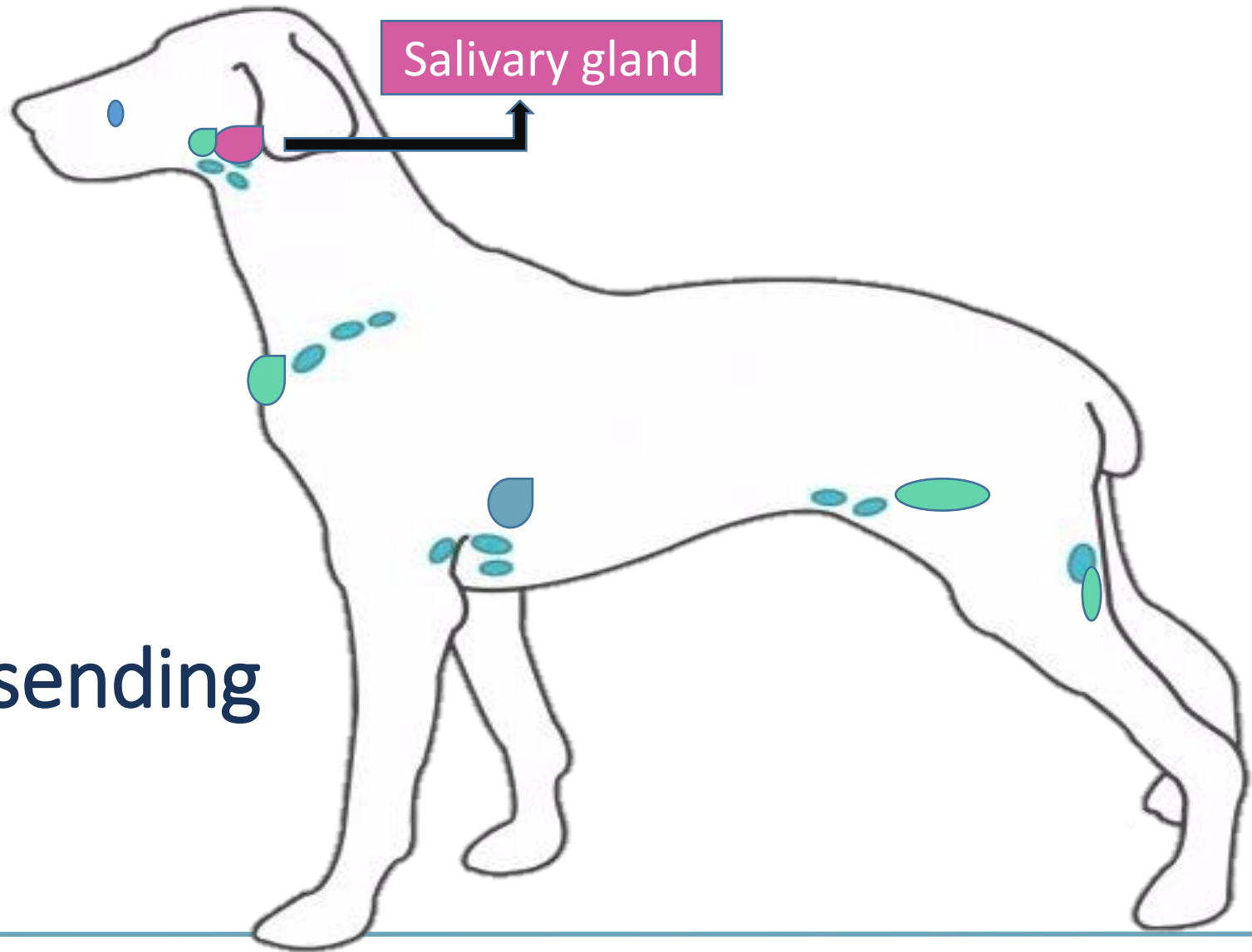
Next steps...

- Measure those nodes
- Baseline exam and vitals
- Cytology → don't delay

Aspirate easiest node

Aspirate 2-3 times

Review 1 slide before sending



Understanding lymph node cytology

- Get to know the normal population
 - 80-90% small lymphocytes
 - Intermediate to large lymphocytes <10-15 % are ok
 - Vary with lymph node inflammatory status
 - Loss of monomorphic small population
 - Potential visitors in reactivity 5%
 - Macrophages
 - Neutrophils
 - Plasma cells (Mott's cells)
 - Mast cells

Hate lymph node cytology? Don't panic- Find a friendly neutrophil...

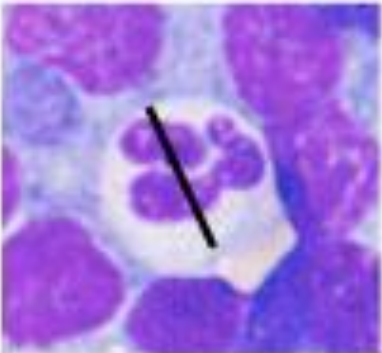
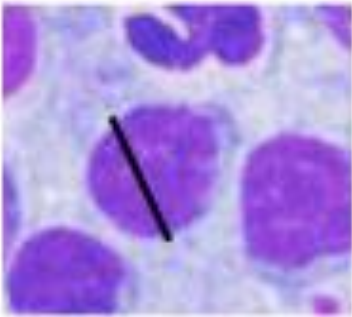
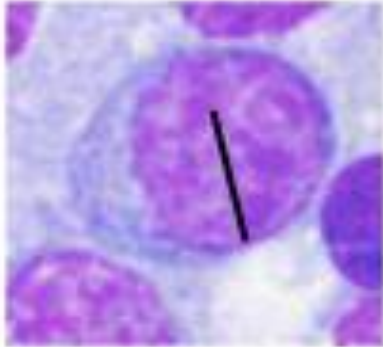
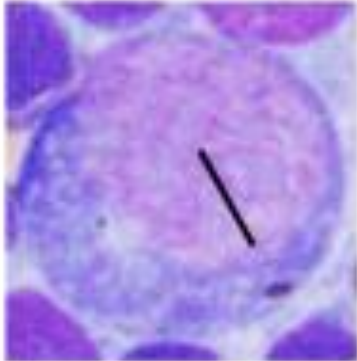
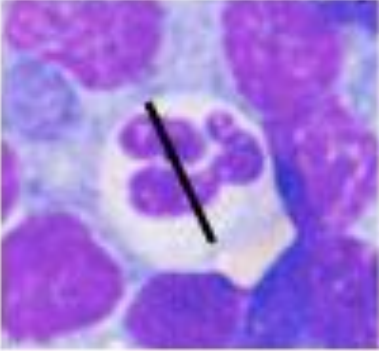
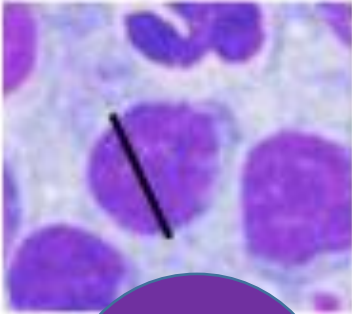
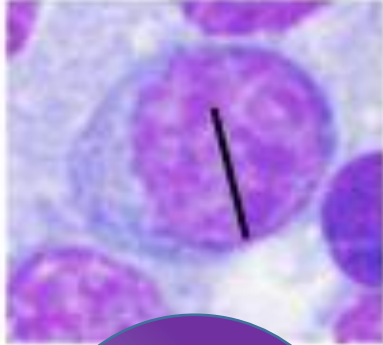
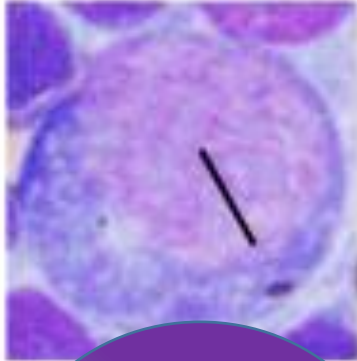
	Small Lymphocyte	Medium Lymphocyte	Large Lymphocyte
Intact Neutrophil Diameter	Nucleus < 1.0x	Nucleus 1.0-2.0x	Nucleus >2.0x
			

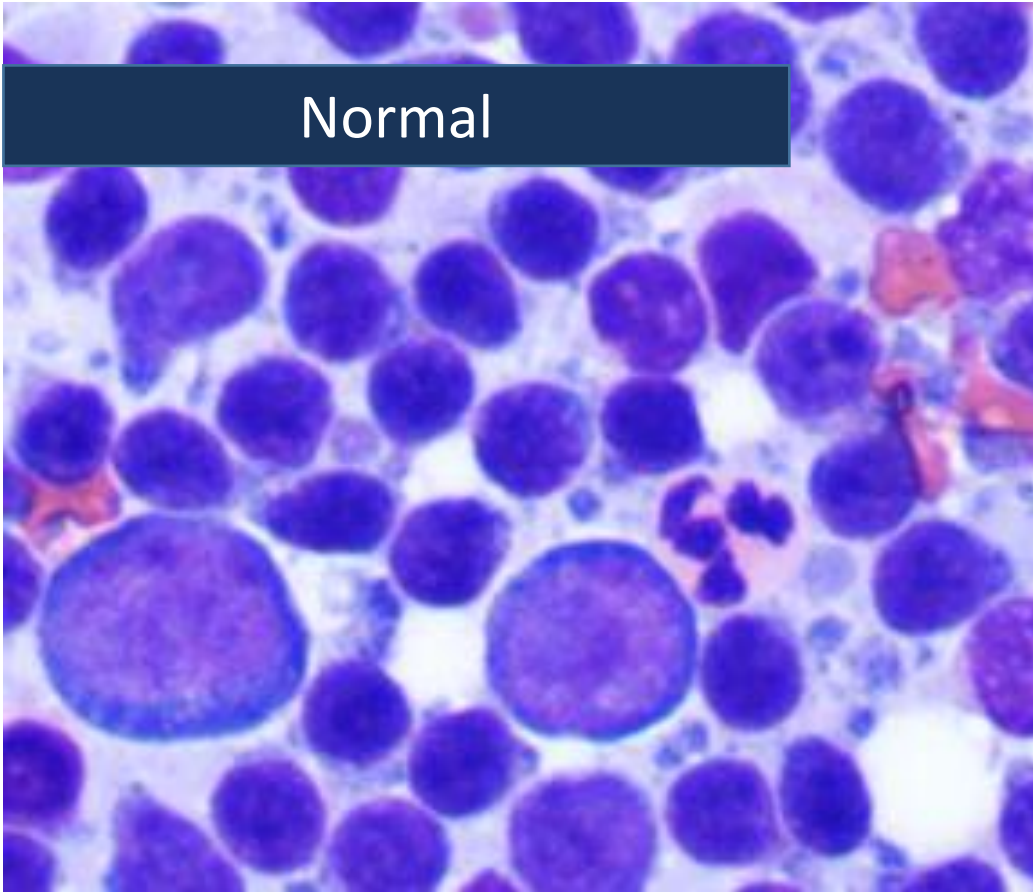
Image Source: https://www.ksvdl.org/resources/news/diagnostic_insights/march2020/cytologic-evaluation-peripheral-lymph-node-aspirates.html

Remember population numbers in a normal node

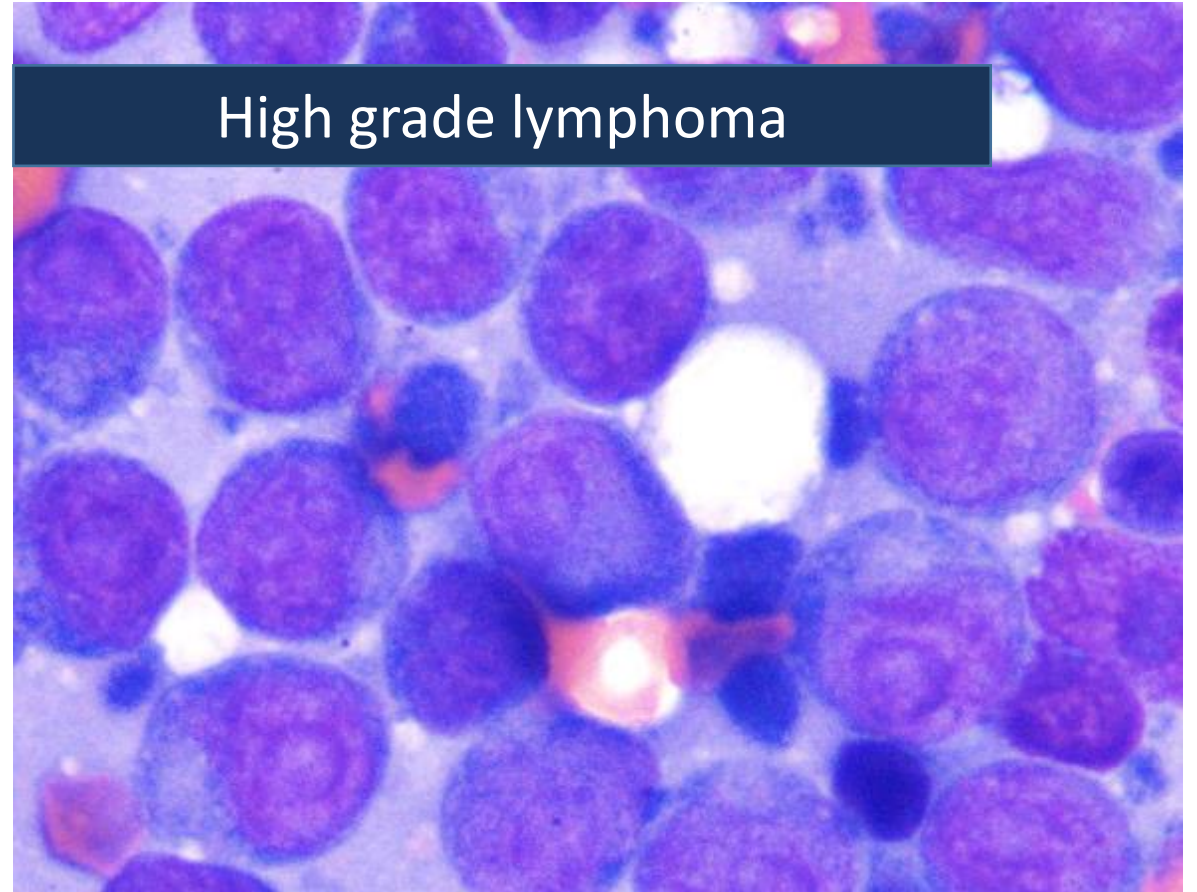
	Small Lymphocyte	Medium Lymphocyte	Large Lymphocyte
Intact Neutrophil Diameter	Nucleus < 1.0x	Nucleus 1.0–2.0x	Nucleus >2.0x
			
	80-90%	< 10%	< 5-10%

Lymph node cytology

Normal



High grade lymphoma



To stage or not to stage...

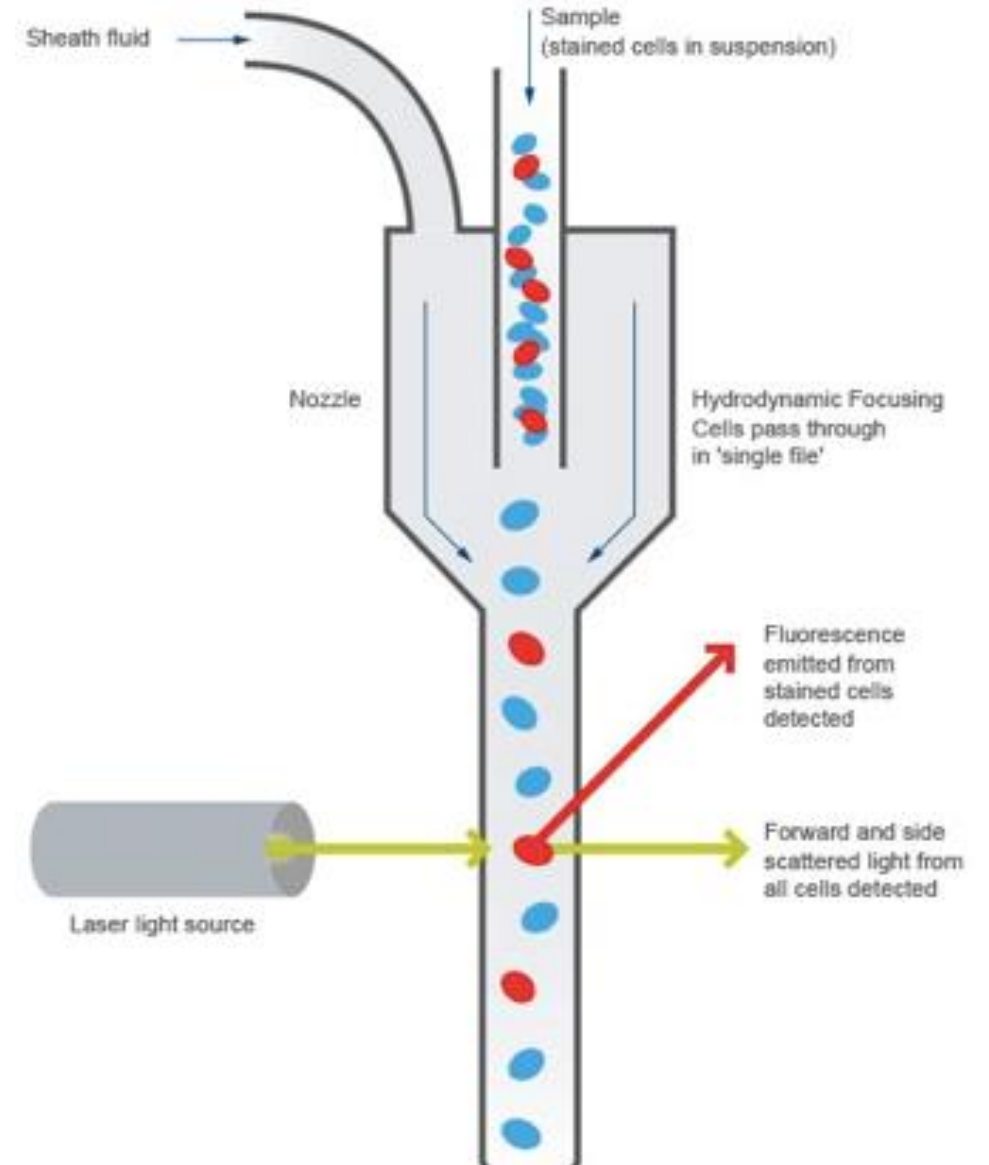
- Cytology
- CBC, Chem (Ica if PU/PD), UA
- +/-
- Chest x-rays
- Abdominal ultrasound
- Bone marrow
- Advanced cytologic or histopathology
 - Biopsy (rare in high grade lymphoma)
 - Molecular testing (PARR/FLOW)

PARR (PCR for antigen receptor rearrangement)

- Clonal test of antigen receptors
- Can be run on any slide you make- dead cells allowed!
- Tests for two things:
 - Clonality
 - Normal lymph cells have receptors to look for specific antigens
 - If all the receptors are the same → all from same clone → lymphoma (clonal)
 - If all the receptors are different (healthy mix) → likely inflammation (polyclonal)
 - Immunophenotype
 - The receptor area being tested also helps decipher B versus T
 - Does not decipher B and T subtypes (helper vs cytotoxic)

FLOW cytometry

- Requires live cells in media
 - Serum, NaCl → you can make at your hospital
 - Blood, lymph, effusion, CSF
- Two main components
 - Adds anti-receptor antibodies
 - B versus T: plus other receptors for prognosis
 - Bone marrow progenitor markers for leukemia
 - Uses light refraction:
 - Size
 - Complexity
- Clinical immunology laboratory at CSU
 - Online specimen submission instructions
 - Sent overnight with a cold pack for delivery **M-F**
 - **Do not freeze!**



FLOW CYTOMETRY RESULTS

Date of flow cytometry assay **2/11/2021**

%Dead cells **12**

	Small to intermediate sized cells	Intermediate to large sized cells	Reference interval for small cells in a canine lymph node**
%Cells in gate	14	86	
T cell subset: CD4	28	95	21 - 45
T cell subset: CD8	6	1	6 - 20
Pan T cell: CD3	37	97	35 - 74
Pan T cell: CD5	37	97	35 - 74
B cell: CD21	60	2	26 - 58
Monocytes: CD14		0	<5
Neutrophils: CD4+CD5-		0	<5
Precursor neoplasm/ acute leukemia: CD34+MHCII-	0	0	0
T zone cells: CD5+CD45-	0	0	0
Aberrant T cell: CD4+CD8+	0	0	0

The flow cytometry study revealed a homogeneous population of CD4 T cells. These findings are diagnostic for T cell lymphoma/leukemia. Data from our laboratory indicates that the median survival for dogs with this phenotype of lymphoma is 150 to 180 days from diagnosis when treated with a multi-drug protocol (Avery et al, JVIM, 28:538, 2014).

FLOW versus PARR

- Costs similar (\$250-300 with shipping)
- Flow provides more info
 - Size, immunophenotype, additional molecular markers that helps prognosis
 - Helpful in stage V lymphoma versus leukemia
 - Higher sensitivity than PARR
 - Requires live cells that safely make the trip M-F
- PARR
 - Less prognostic information (no information on size, less specific markers)
 - Can be done on slides/dead cells so may be more feasible on weekends or with pre-existing samples

Diagnosed....



What to expect?

Prognostic indicators for your patients

- Stage V (back to the bone marrow and non-lymphatic organs)
 - Beware the weird ones → CNS, Intestinal etc....
- Substage B
 - Treat early, do better
- Immunophenotype
 - B versus T cell
 - NOT SO FAST!

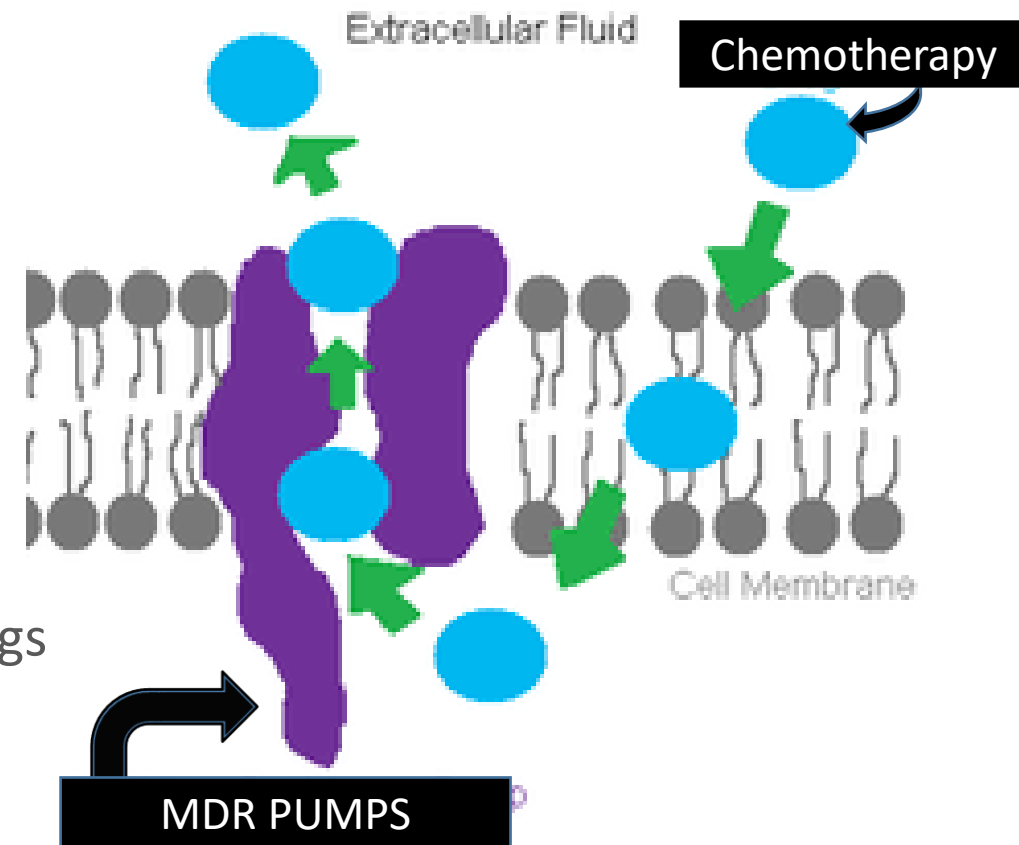
The B cell vs T cell conundrum in large cell lymphoma

- Previous literature
 - T cell terrible, B cell better
 - Treated patients
 - B cell MST 1 year
 - T cell MST 6 months
- New concepts:
 - Molecular identity testing (FLOW) is giving us more data
 - Best protocol for immunophenotypes (B vs T)

The B cell vs T cell discussion

- New concepts

- Best protocol for immunophenotypes (B vs T)
 - MDR pumps increased in T cell lymphomas
 - MDR pump utilizing drugs:
 - Doxorubicin, Vincristine, Prednisone (CHOP anyone?)
 - T cell protocols successful with less MDR sensitive drugs



Lymphoma myths not associated with prognosis

- Lymph node size/number
- “Metastasis”
 - (please don't use the “M” word in lymphoma)
- Spleen/Liver involvement (stage IV)

Alleviation of clinical signs → cytotoxic therapy

They say laughter is the best medicine, unless you have cancer, in which case chemotherapy is more effective



Goals with treatment

- Clinical remission
- Minimal adverse effects
- Developing a plan that fits both the patient and client
 - Finances
 - Travel
 - Comorbidities
 - Patient disposition

Time to treat: Prednisone

Prednisone

- MOA
 - Direct lympholytic via steroid receptor → CELL DEATH
- Understand the role of Prednisone in chemotherapy resistance
 - MDR pumps again...

What to know about starting prednisone

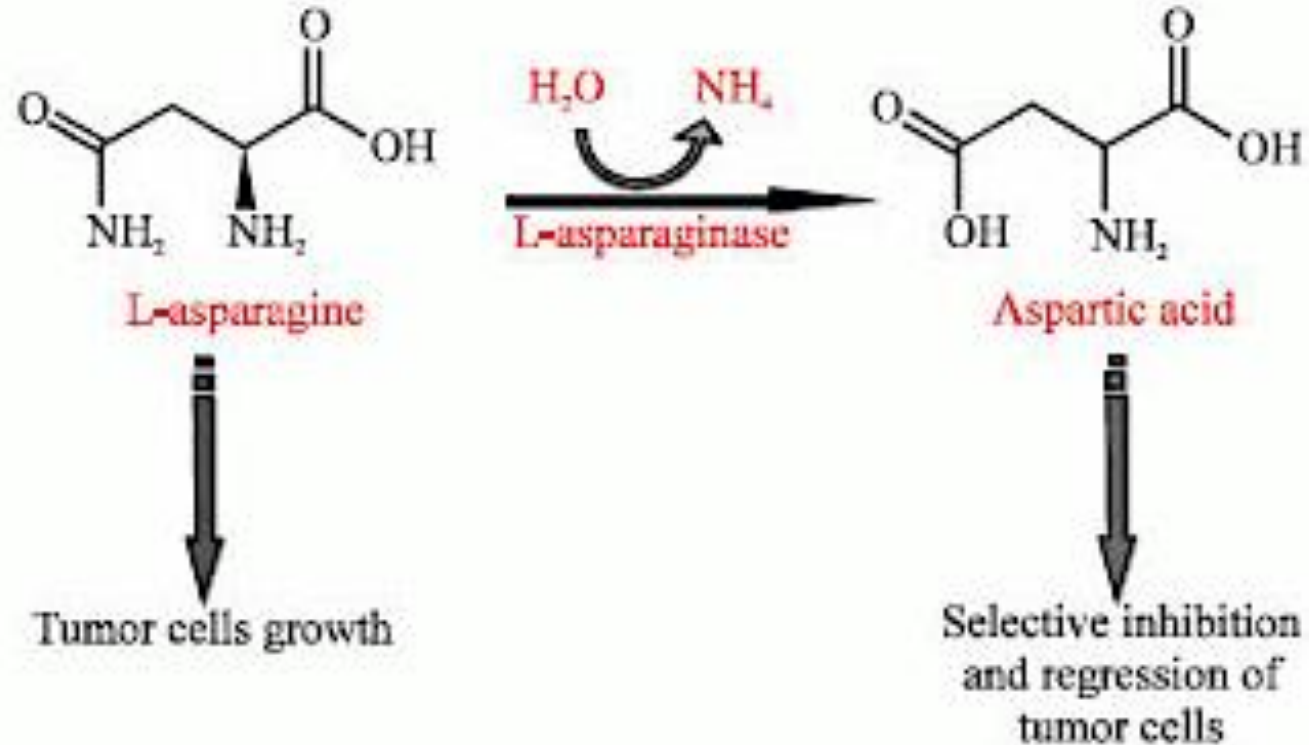
- Treat if diagnosed
- Treat if sick
- Treat if pursuing chemotherapy → soon
 - Two weeks or so...

Discuss with owner/caregiver

- May make advanced testing non-diagnostic
- May cause resistance if they wait > 1 month to treat
- Dose
 - Prednisone 1 – 1.5 mg/kg (don't go over 60 mg/dog) SID
 - Half the dose for BID
 - Higher dosages don't result in better response

Elspar (L-asparaginase)

- Enzymatic drug
 - Functions to remove food source from neoplastic lymphocytes



Elspar (L-asparaginase)

- May last for 1-3 weeks on average
- Low risk of side effects with SQ injection
- Uses
 - Sick patient/ER
 - While awaiting molecular testing for subtype (after confirming lymphoma)
 - To allow owner's time to consider chemotherapy
 - Palliatively as an add on to prednisone therapy
- Loses effectiveness after 2-3 dosages
- Cost \$350-450 per dose

Multi-agent chemotherapy (standard of care)

- CHOP or LOPP
 - Rotating drug protocol (weekly to every other week for LOPP) typically over 5 months
- Side effects seen in < 20% of patients
 - 3-5 days post therapy → mostly mild GI when observed
 - 7 days post therapy → increased risk of cytopenia
- 90% Response rate, MST 1+ years
- Average total cost \$5500-7000 (based on patient size), \$350-850 weekly over 5 months

Single-agent chemotherapy

- Doxorubicin, CCNU, Tanovea
 - one treatment every 3 weeks over 5 months
- Side effects seen in < 20% of patients
 - 3-5 days post therapy → mostly mild GI when observed
 - 7 days post therapy → increased risk of cytopenia
- 70- 90% Response rate, MST 6-9 months
- Average total cost \$3500-5500 (based on patient size/drug)
- Helpful to have immunophenotype when selecting drug

Overall prognosis with various treatment options

- CHOP/LOPP (+/- Elspar) 1- 1.5 year MST
- Single agent/drug (+/- Elspar) 6-9 months MST
- Elspar/Pred 2-4 months MST
- Prednisone 2 (+/-) months MST but improved QOL

QUIZ

1. The top differential for generalized lymphadenopathy in the dog is;
 - A. Lymphoma
 - B. Tick borne disease
 - C. Reaction/inflammatory

QUIZ

1. The top differential for generalized lymphadenopathy in the dog is;

A. Lymphoma

QUIZ

2. In general, what size lymph cells are associated with high grade lymphoma?

- A. Intermediate and large cells
- B. Small cells

QUIZ

2. In general, what size cells are associated with high grade lymphoma;

A. Intermediate and large cells

QUIZ

3. The difference between acute leukemia and stage V lymphoma is:
- A. The size of the cells
 - B. Where the cancer starts in the body

QUIZ

3. The difference between Leukemia and Stage V lymphoma is

B. Where the cancer starts in the body

QUIZ

4. In a normal lymph node, the cells that are present in the highest number (90%) are;

- A. Small lymphocytes
- B. Large lymphocytes/lympoblasts
- C. neutrophils

QUIZ

4. In a normal lymph node, the cells that are present in the highest number (90%) are;

A. Small lymphocytes

QUIZ

5. Do NOT give Prednisone if ;

- A. The patient is sick
- B. The patient is diagnosed
- C. The patient is starting treatment soon (< 2 weeks)
- D. The owner is hoping to perform FLOW prior to additional therapy

QUIZ

5. Do NOT give Prednisone if ;

D. The owner is hoping to perform FLOW prior to additional therapy

QUIZ

6. The benefit of PARR over FLOW is;

- A. You can use dead cells or a previous slide
- B. More prognostic information

QUIZ

6. The benefit of PARR over FLOW is;

A. You can use dead cells or a previous slide

I  questions...

MedVet Cleveland West

M-Th 216.362.6000