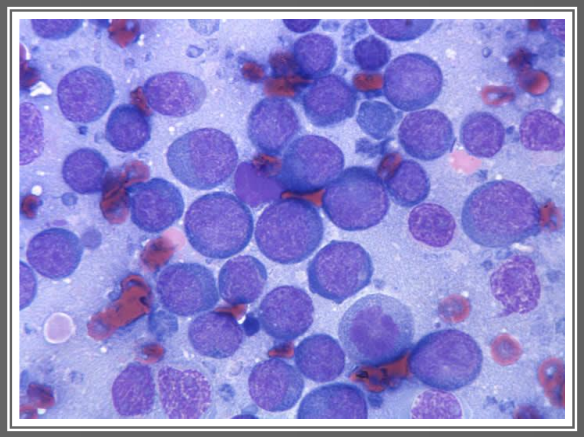
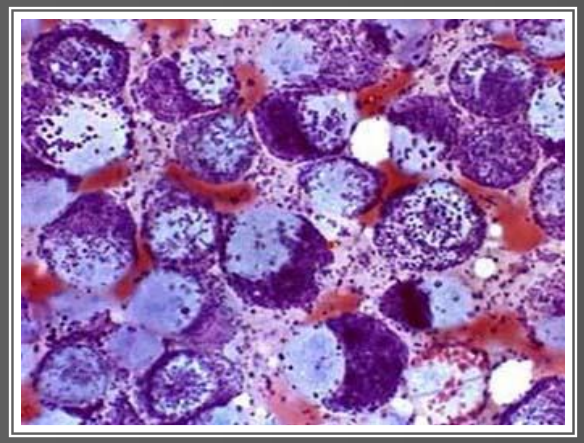
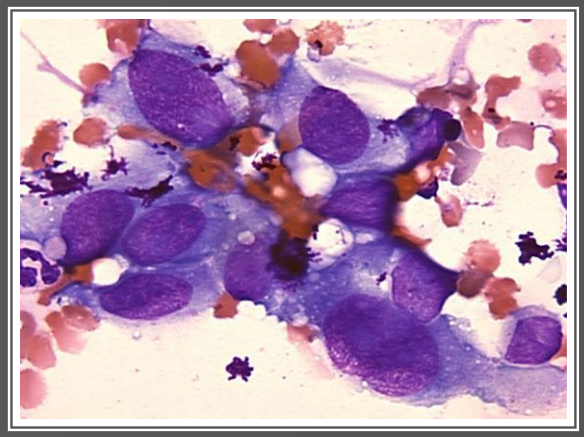
Oncology Diagnostics:
how to proceed to get what you need

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3/4/23

Goals of Veterinary Oncology

- Quality of life
- Balanced treatment plans
  - Effective treatment against cancer while minimizing side effects
- Cure usually not feasible or realistic
  - Can we stabilize disease for a longer life?
  - Help owners set realistic expectations
- Our first step: obtaining a diagnosis

Overview

- Cytology
- Histopathology
- Flow cytometry, PARR
- BRAF
- MDR1 genotyping
- New cancer blood tests

IndyVet
Emergency & Specialty Hospital
Where care comes first.

Why wait? Aspirate ©

- See Something, Do Something.
- See Something
  - When a skin mass is the size of pea or larger or has been present for 1 month,
- Do Something
  - Aspirate or biopsy, and treat.
- Early detection saves lives.

Obtaining a sample

- Needle aspirate
  - Needle only, no syringe attached
    - Woodpecker method
    - 22-25 gauge needle, 5-10 cc syringe
  - Needle with syringe
    - Many tumor cells are too fragile for this method
    - Useful for a cystic or fluid filled mass
- Impression smear, touch prep from tissue

Making slides

- Inject material on slide.
- Lightly touch material with perpendicular slide.
- Smear on to new slide.
- You can make several slides from one aspirate.
- Material will be thin enough for cytologic interpretation.

Staining the sample

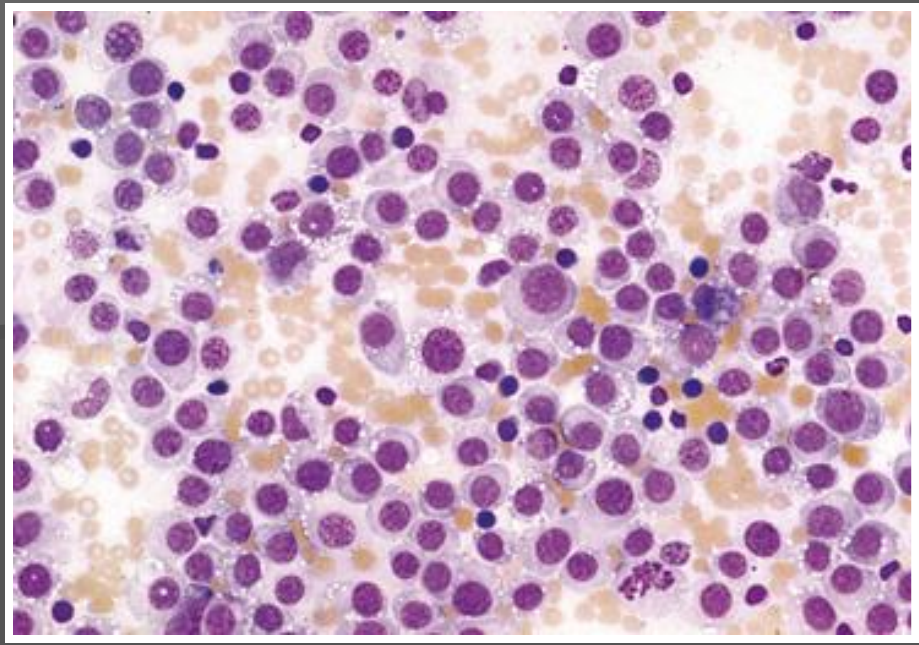
- Romanowsky stains
  - Diff-Quik
    - Variant of Wright-Giemsa
    - Readily available, fast
  - Wright-Giemsa
    - Commercial labs
- Special stains performed at the lab
  - Immunophenotyping
  - Characterization of tissue of origin

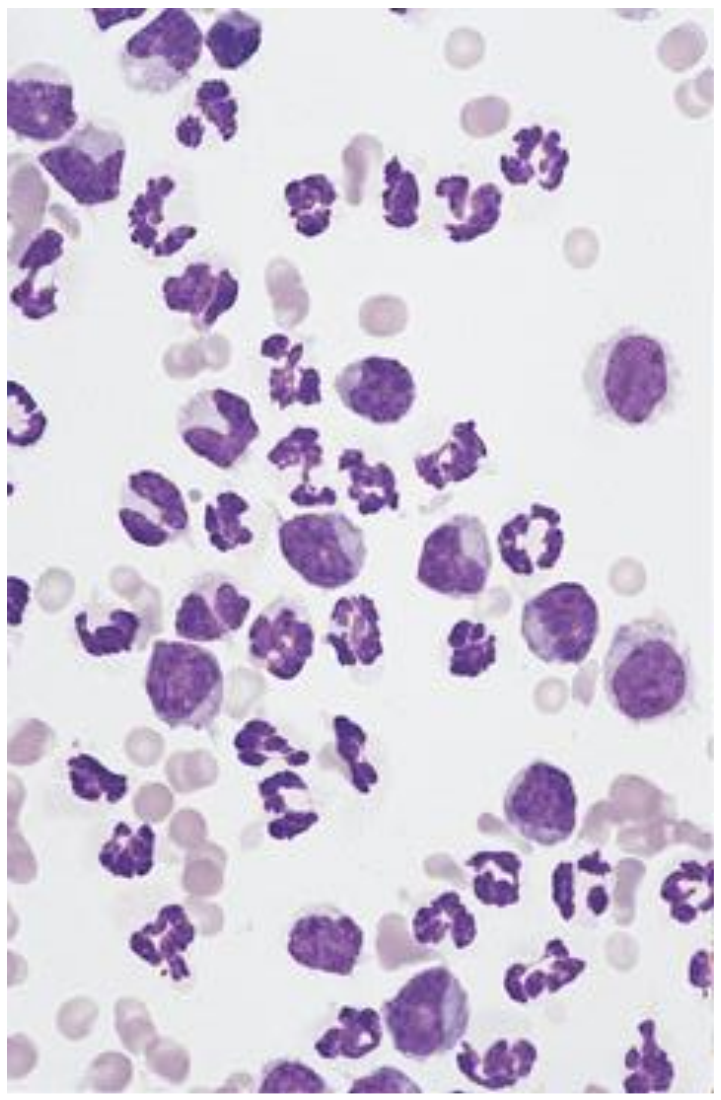
Sending samples to the lab

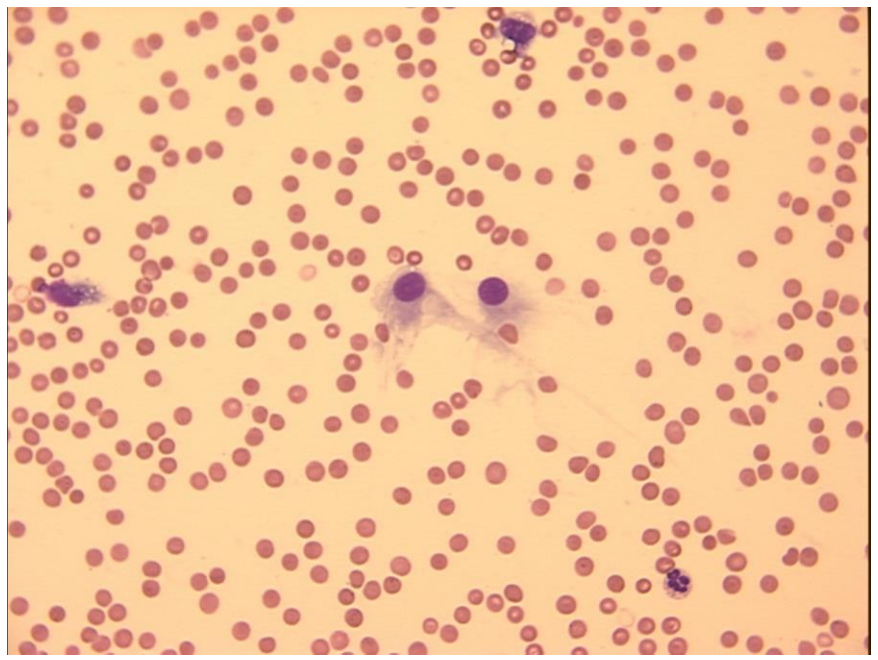
- Always recommended
  - Less invasive, faster to perform and to get results back.
- Don’t stain all of the slides.
- Don’t put oil all over them.
- Do evaluate a slide to make sure you have a diagnostic sample.
- Do give your pathologist an accurate history.

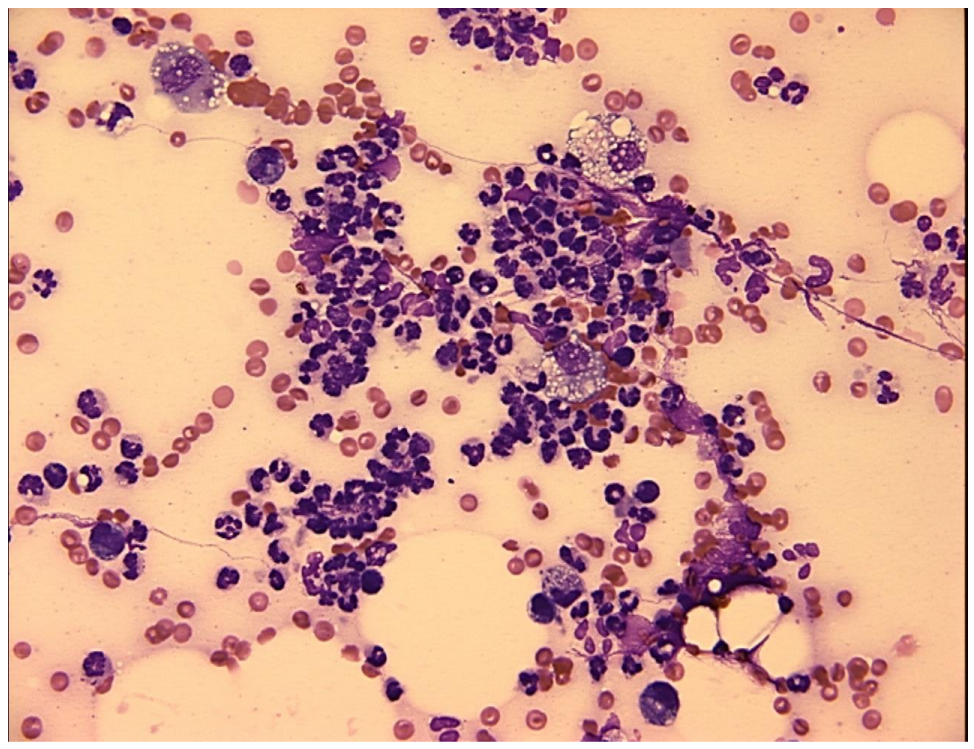
Cellularity

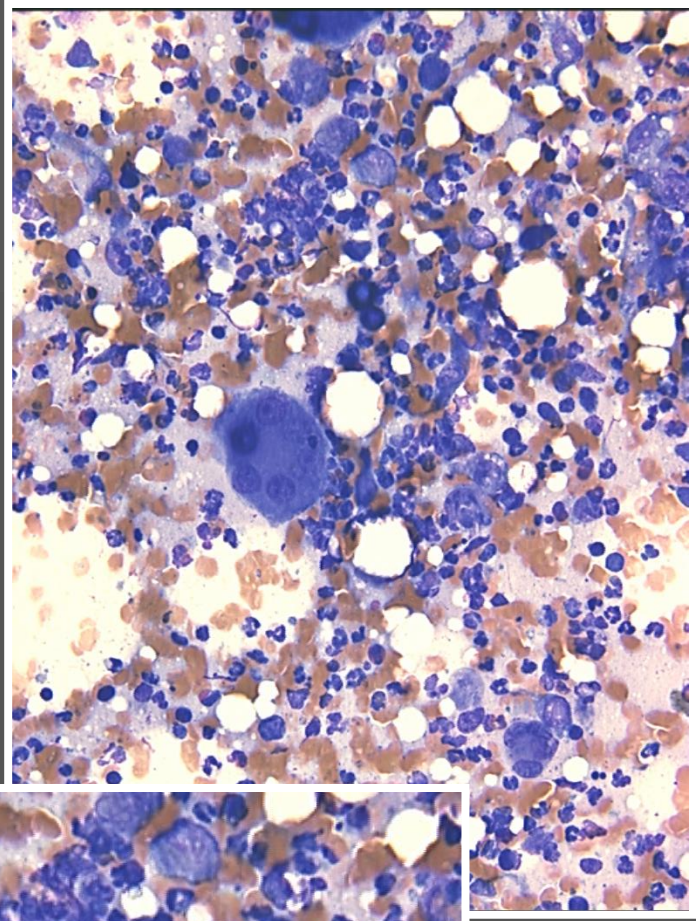
- First step to evaluation of cytology
- Can determine on low power evaluation
- Can assist with diagnosis
  - Highly cellular
    - Suppurative inflammation
    - Epithelial and round cell neoplasms
  - Low cellularity
    - Granulomatous inflammation
    - Mesenchymal neoplasia

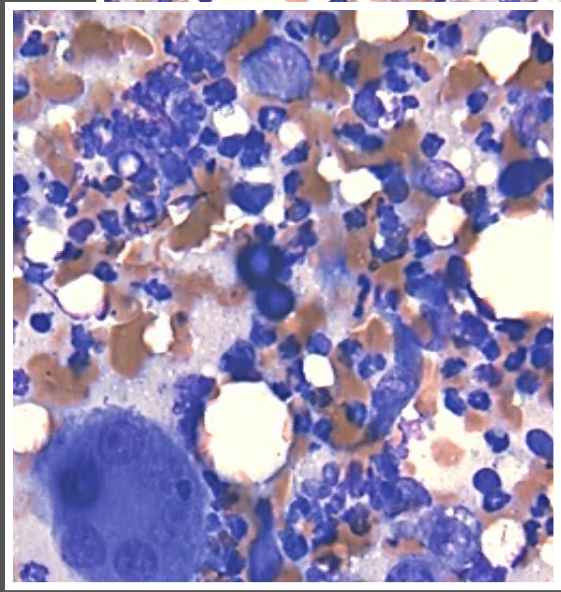






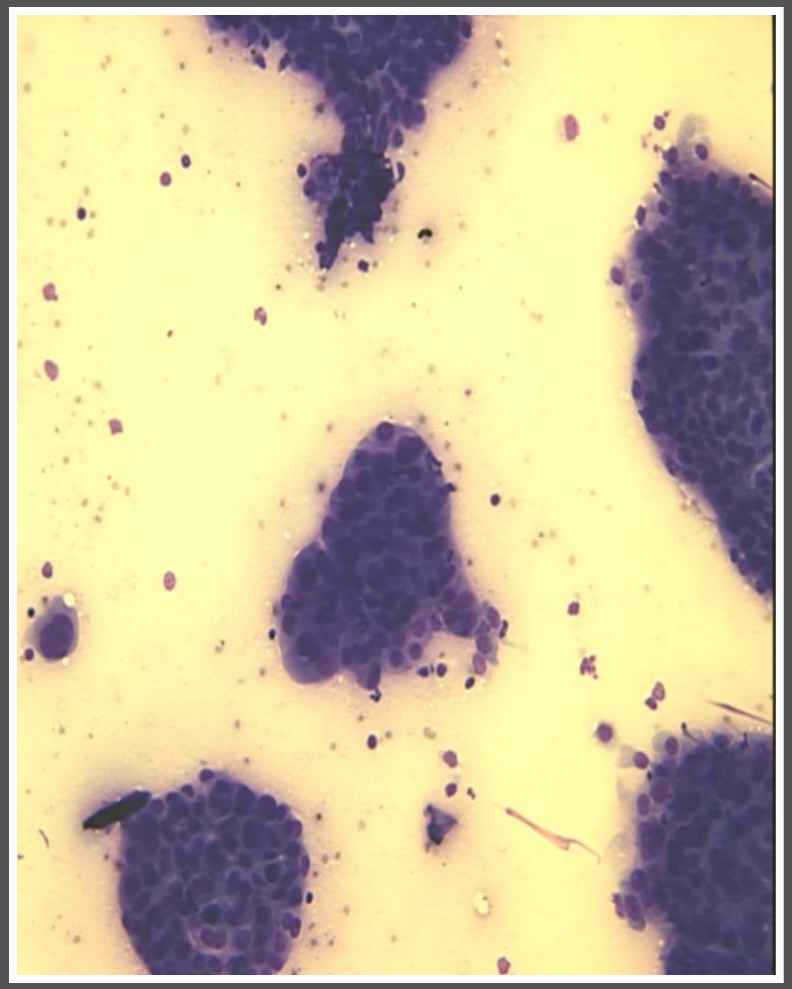


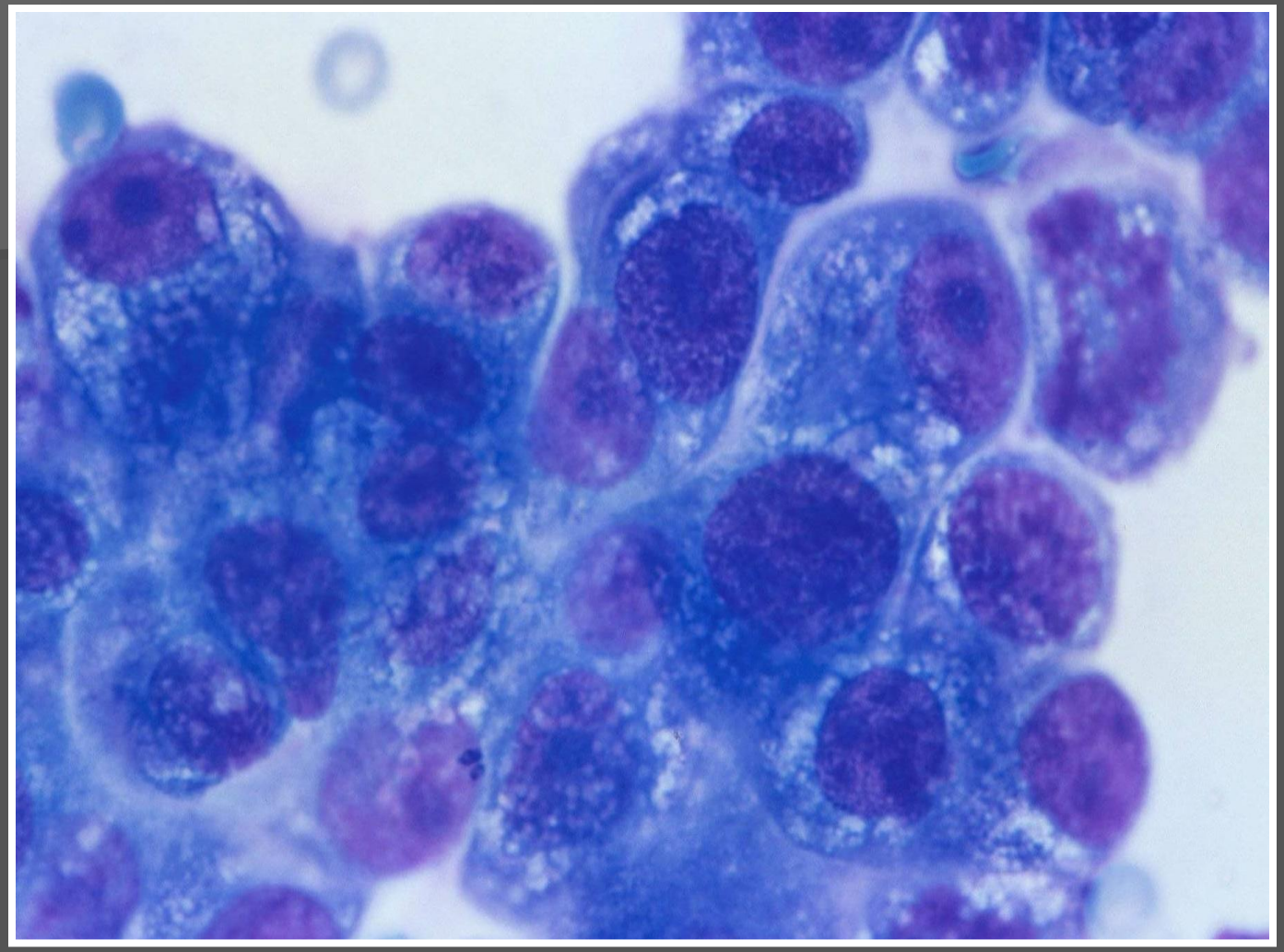




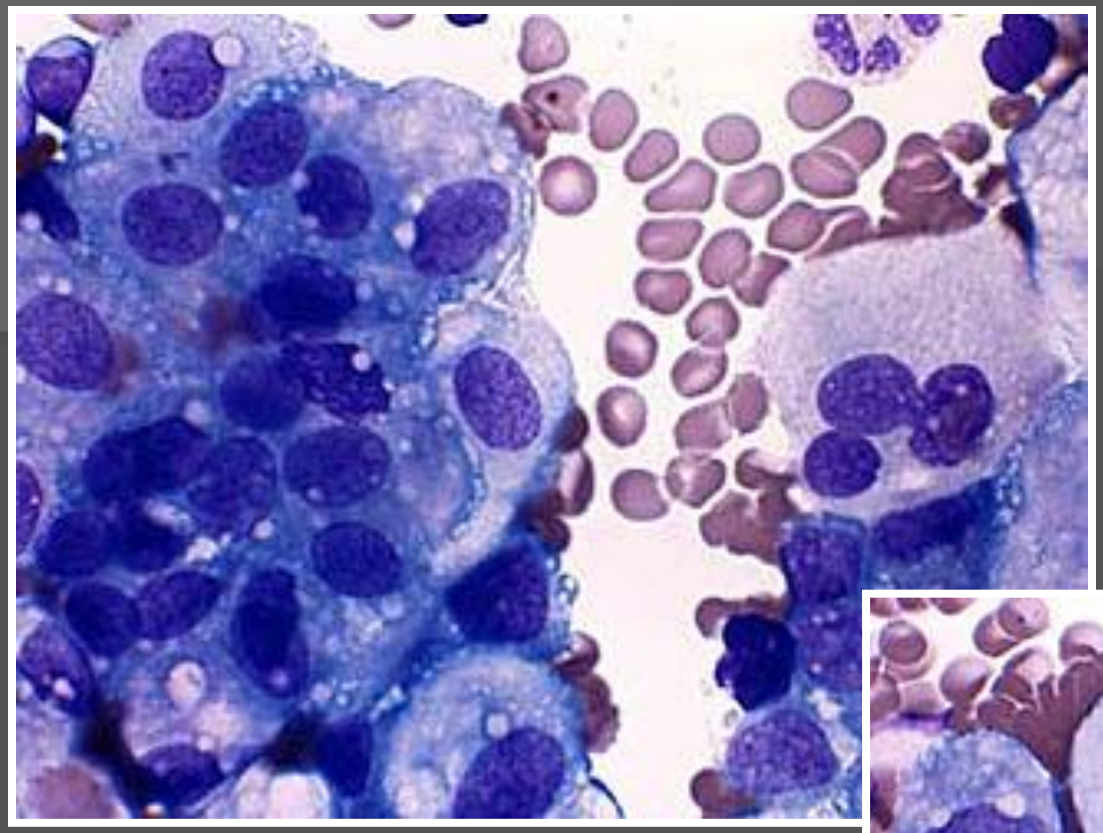
Epithelial cells

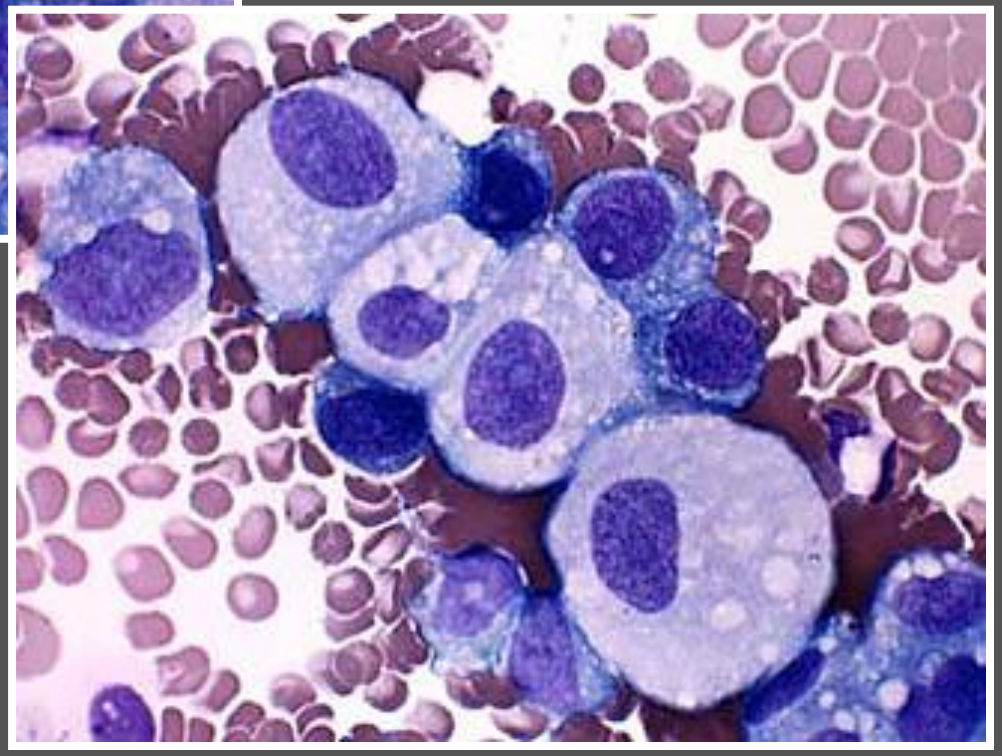
- Squamous, glandular, non grandular
- Usually in clusters
- Distinct cell borders
- Round to polygonal in shape
- Round nucleus



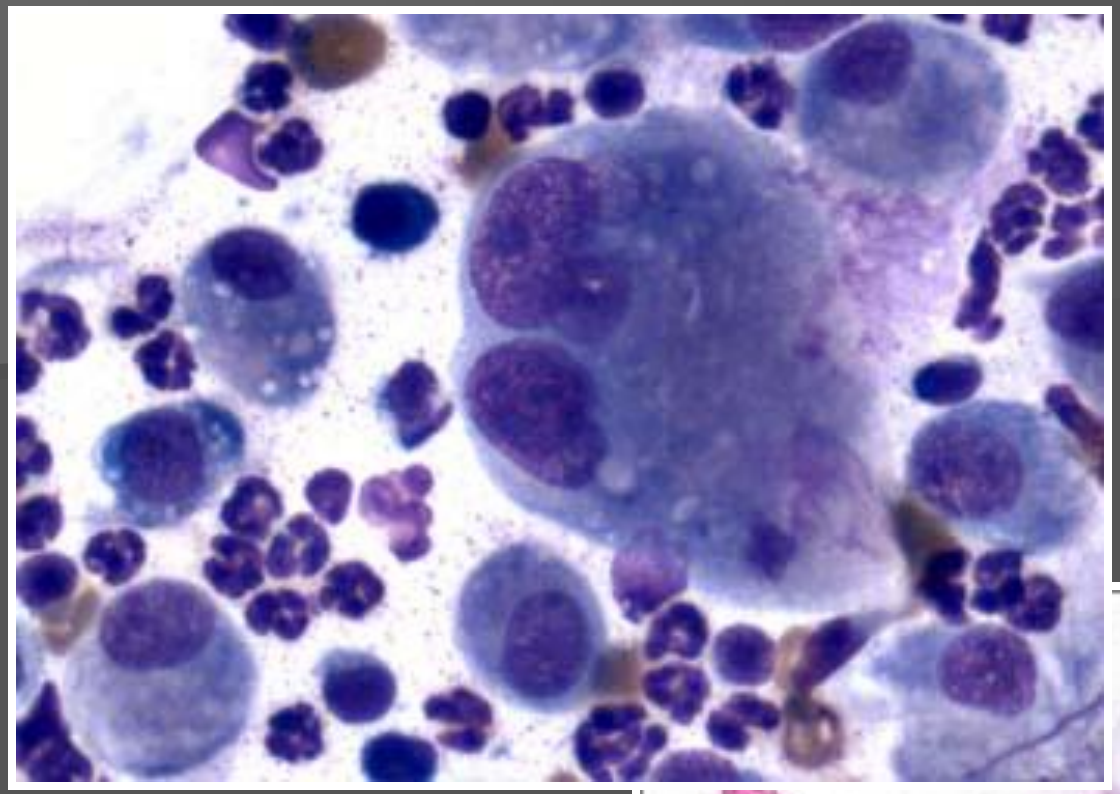


Lung mass aspirates: sample the periphery to avoid necrotic center, mass must be against the thoracic wall to visualize with ultrasound.

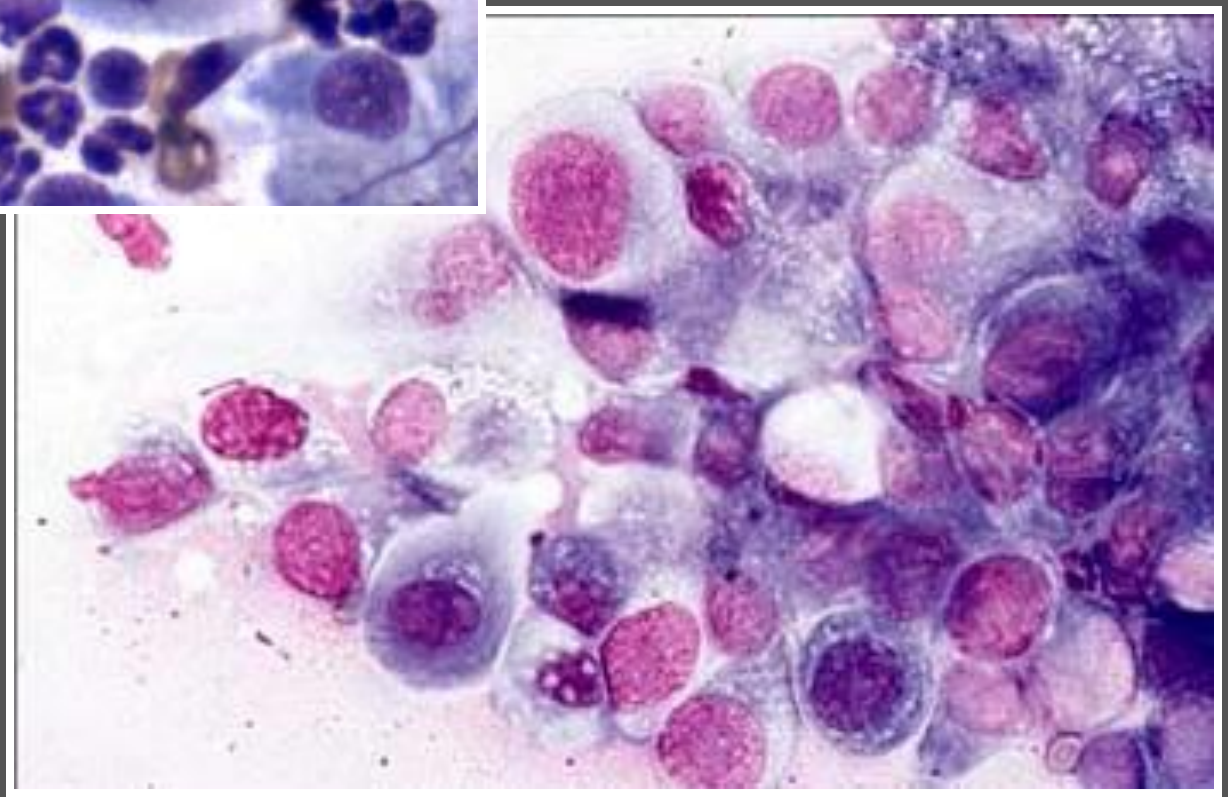




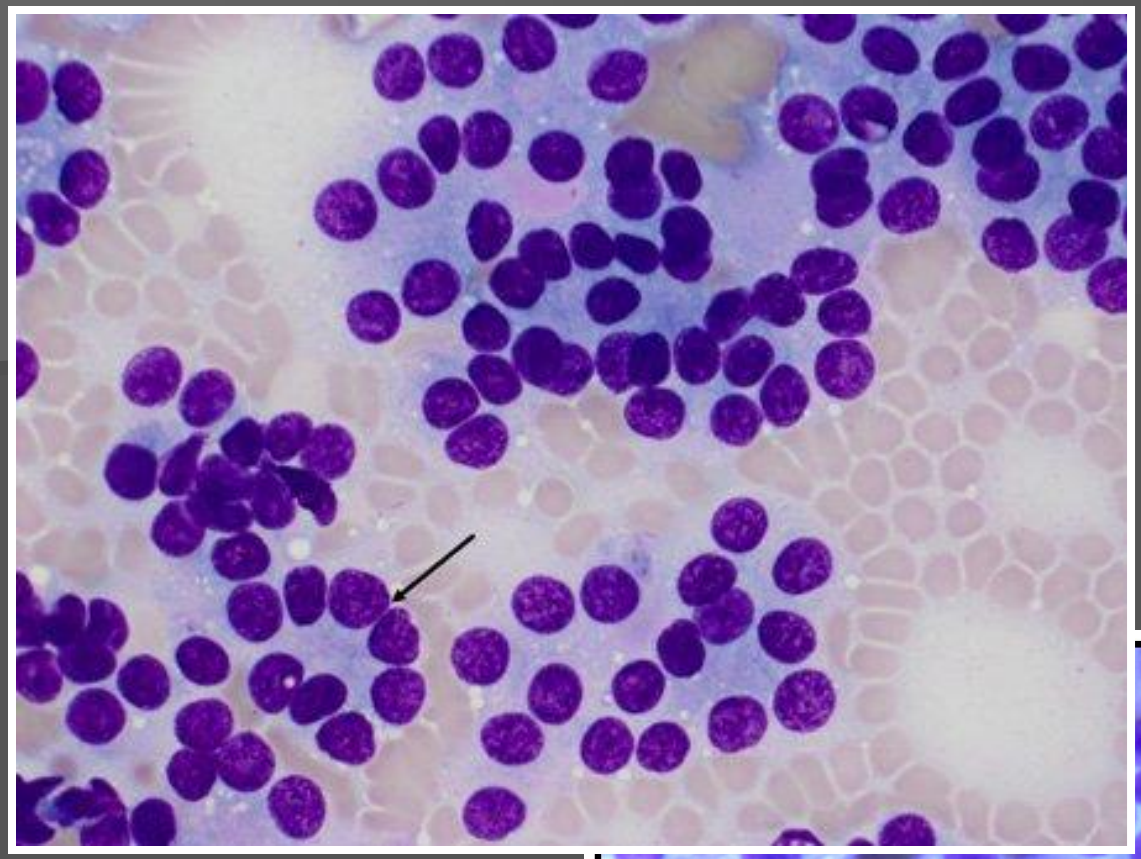
Make slides immediately for urine cytology

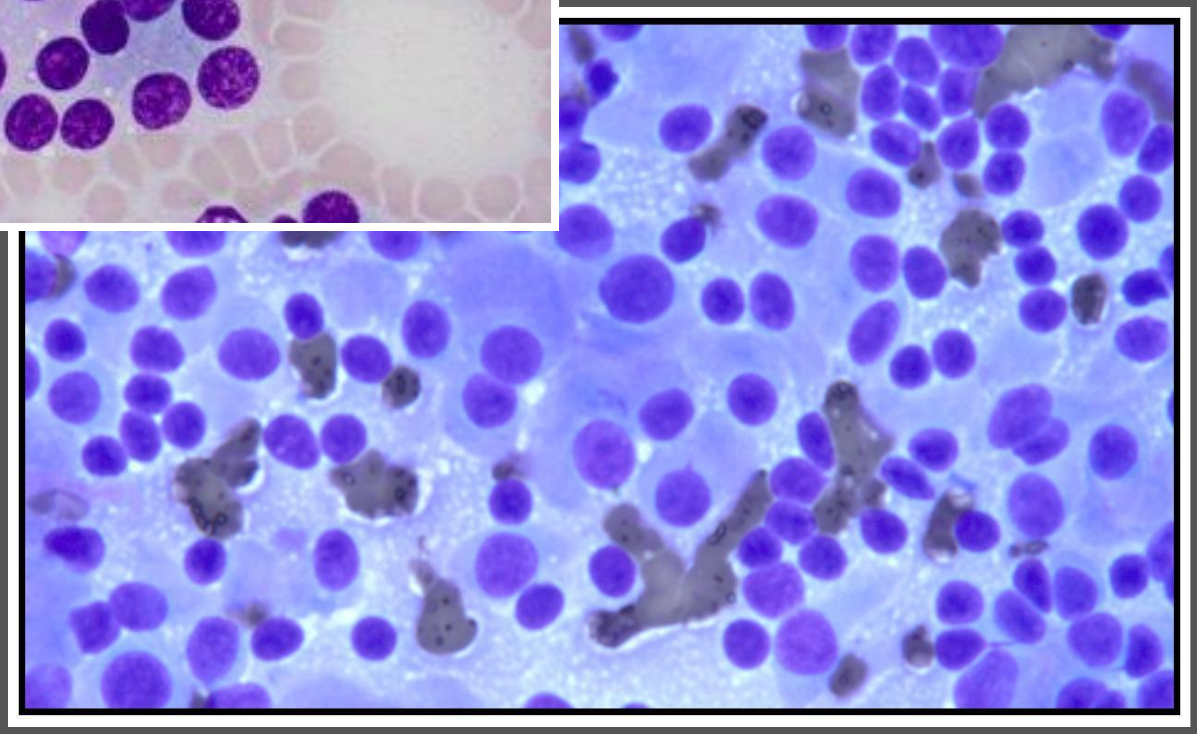


Mammary masses:
•Cytology usually insufficient to determine benign vs. malignant.
•Rule out other tumor types.



Dog: 50% benign, 50% malignant.
Cat: >95% malignant
Check regional lymph nodes!

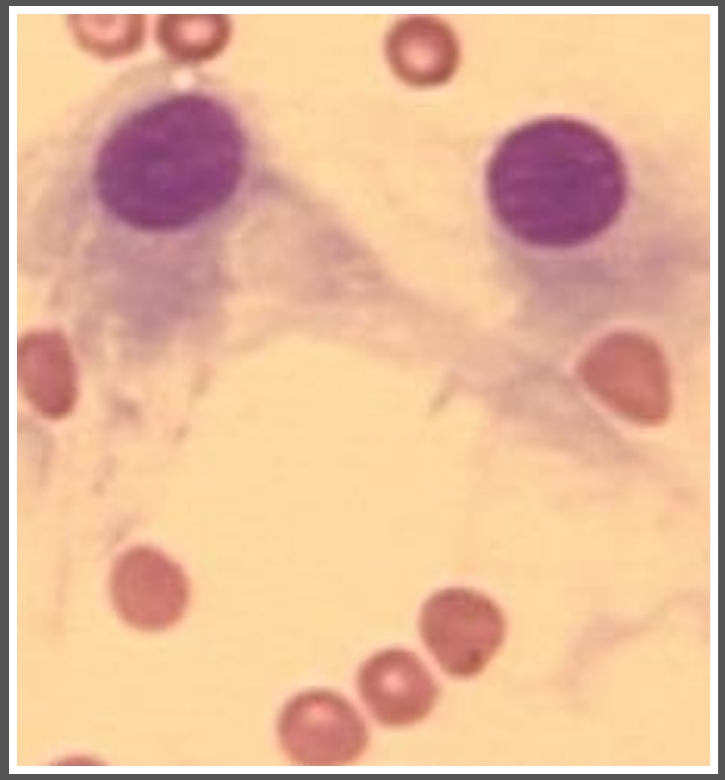


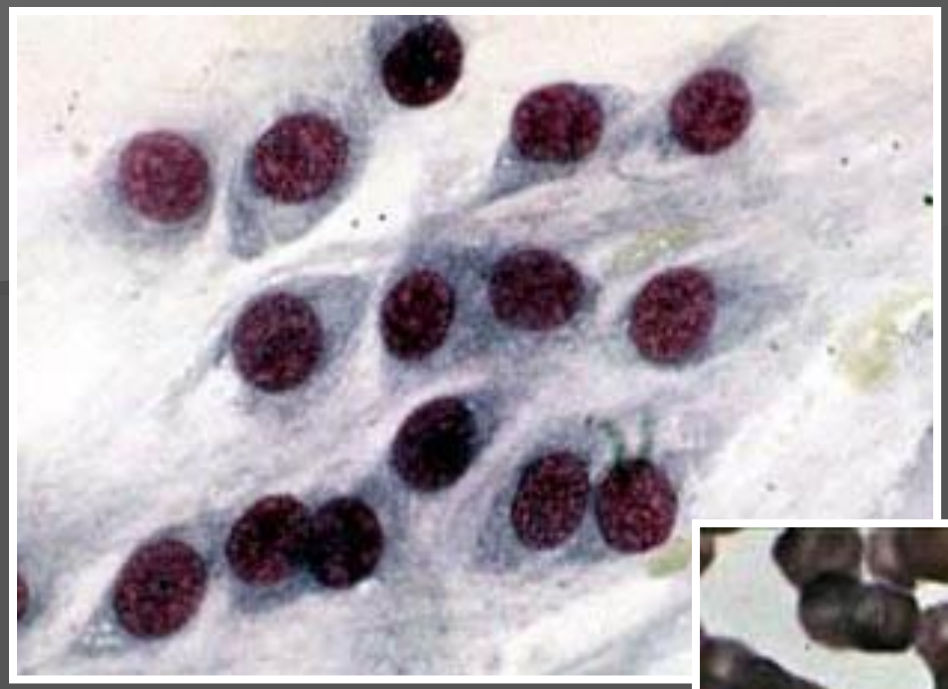


Neuroendocrine tumors: anal sac carcinoma, thyroid carcinoma

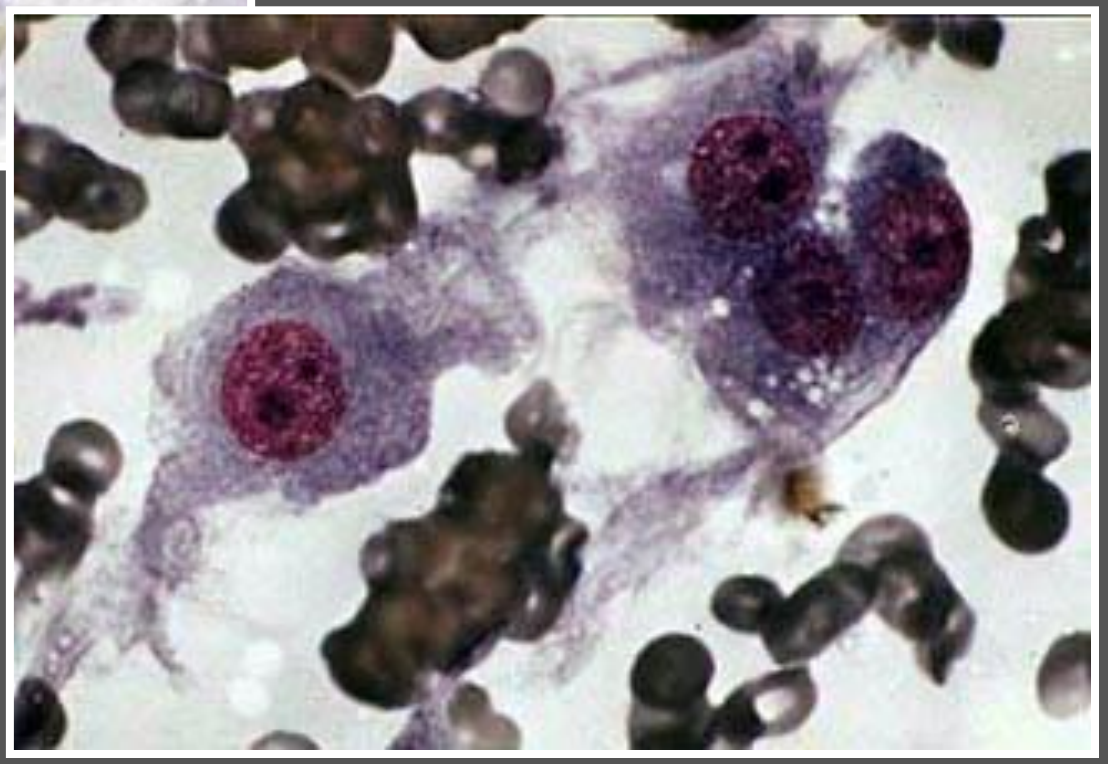
Mesenchymal cells

- Connective tissue origin
- Individualized cells
- Spindiloid in shape
- Indistinct to wispy cell borders
- Oval nucleus
- Exfoliate poorly

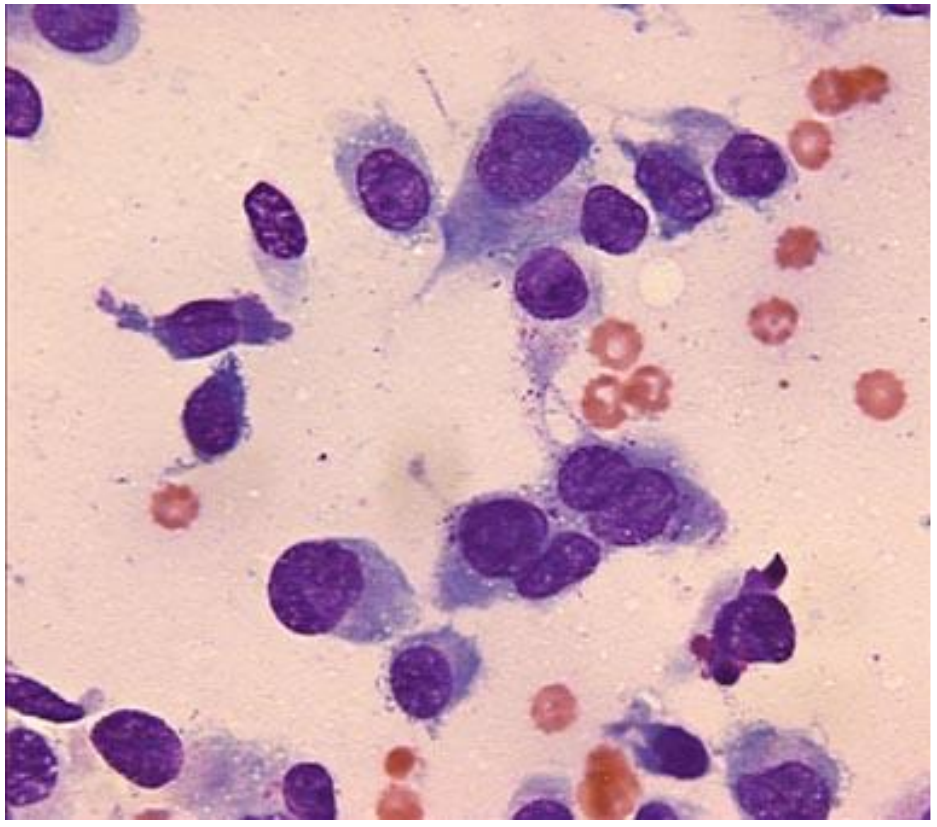




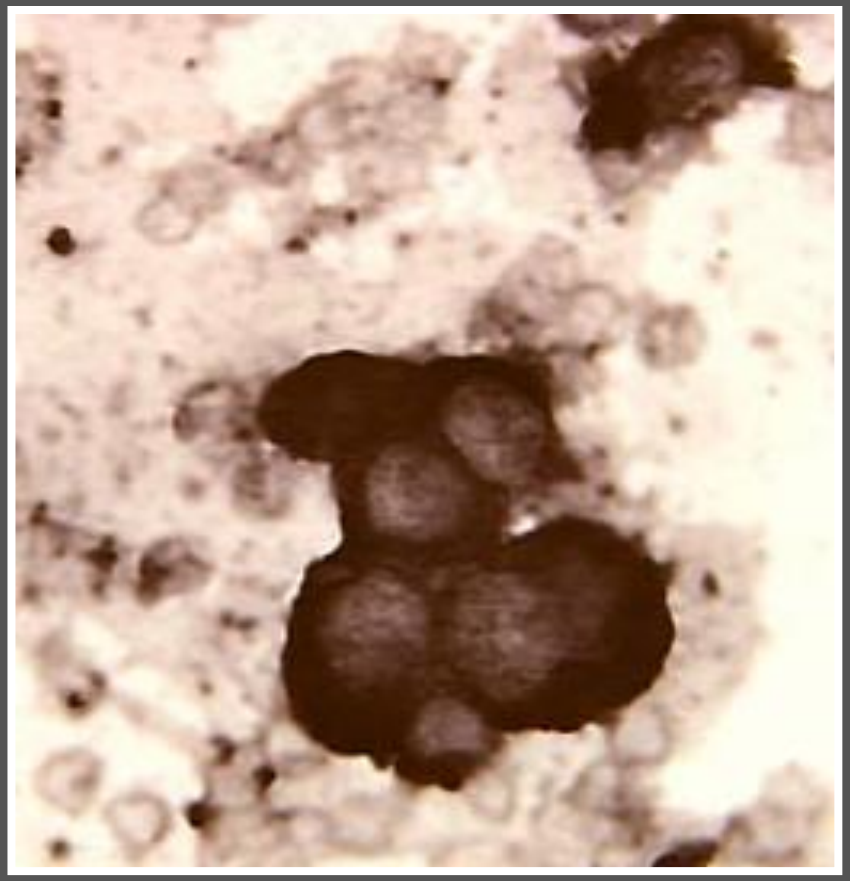
Soft tissue sarcomas: aspirate or biopsy at periphery to avoid necrotic center



Hemangiopericytoma, peripheral nerve sheath tumor, fibrosarcoma



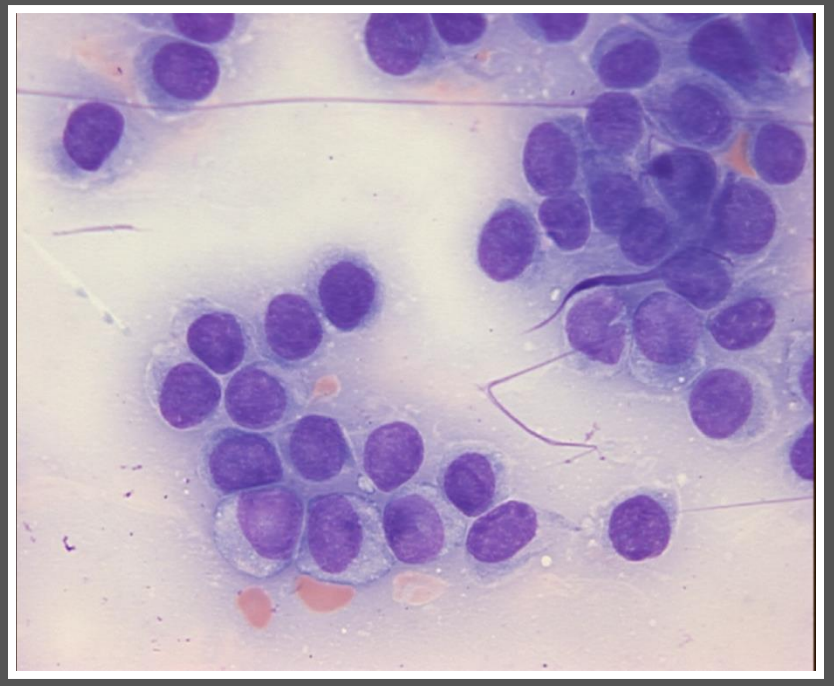
Bone tumors: aspirate or biopsy near center to avoid reactive bone at periphery

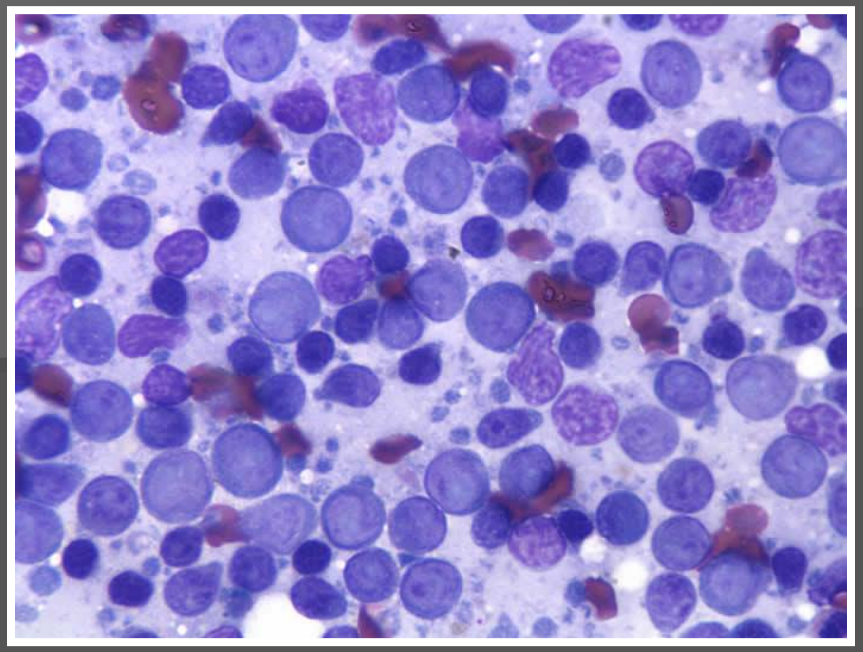


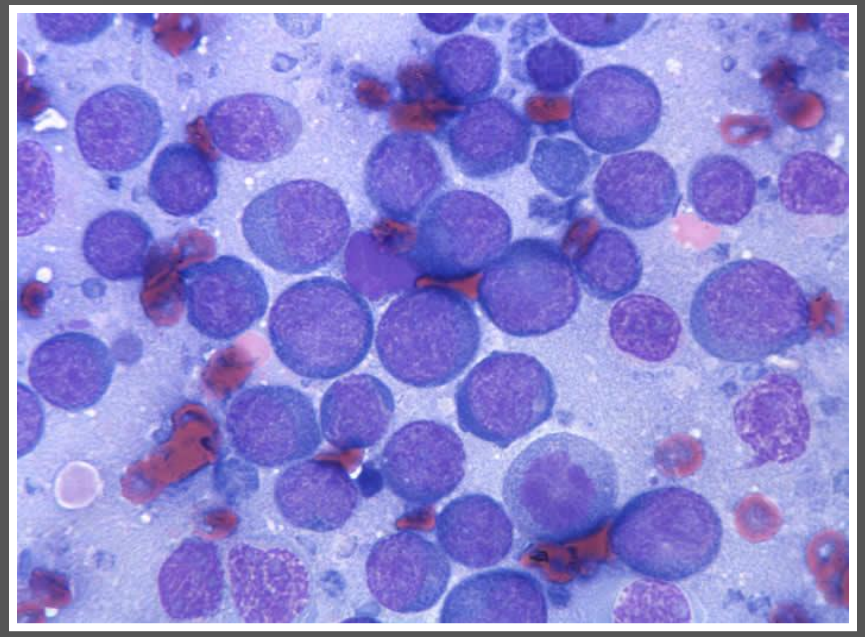
Alkaline phosphatase stain to differentiate osteosarcoma, 100% sensitivity, specificity 89%

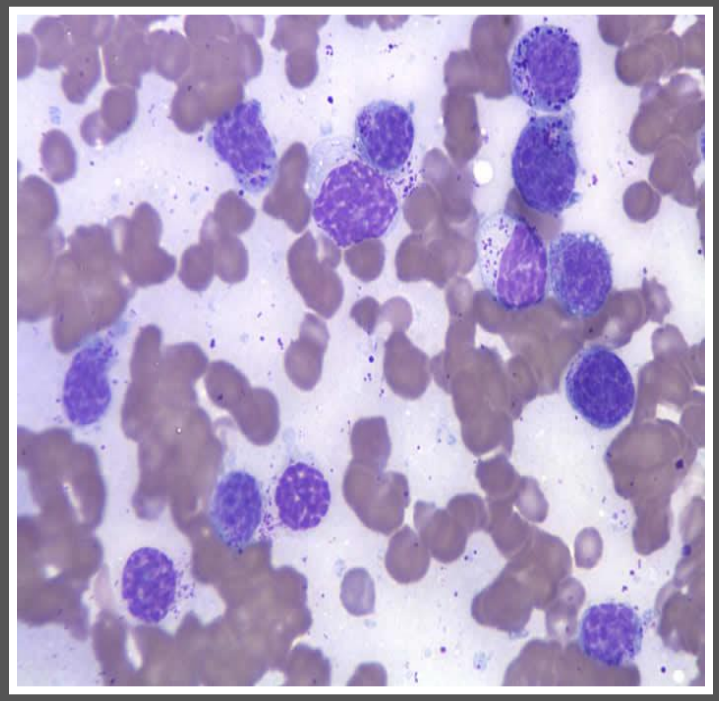
Round cells

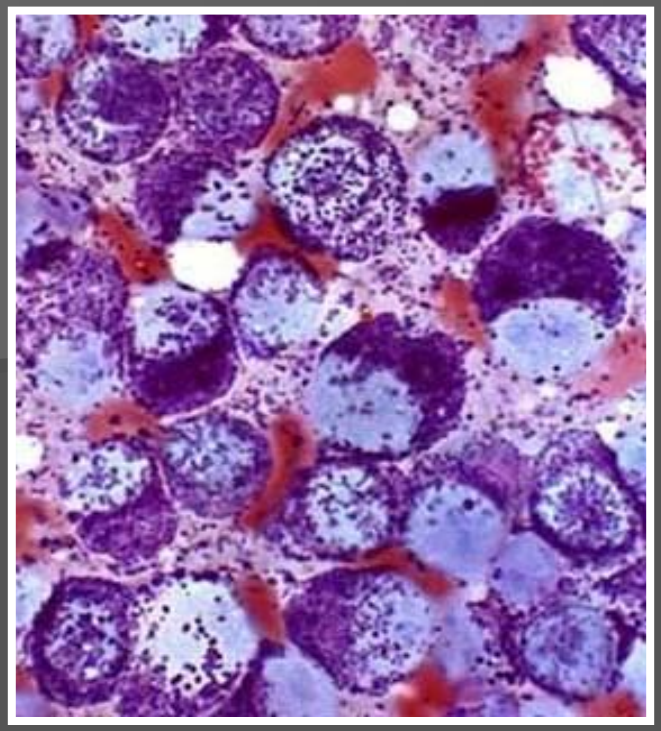
- Round in shape
- Discrete cells
- Occur individually or in a sheet
- Generally highly cellular

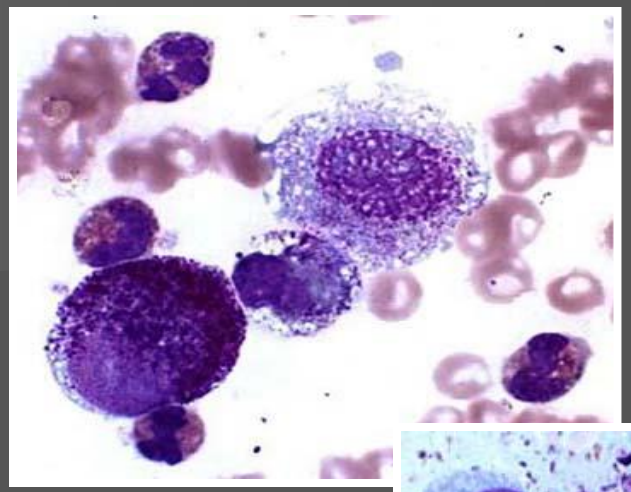


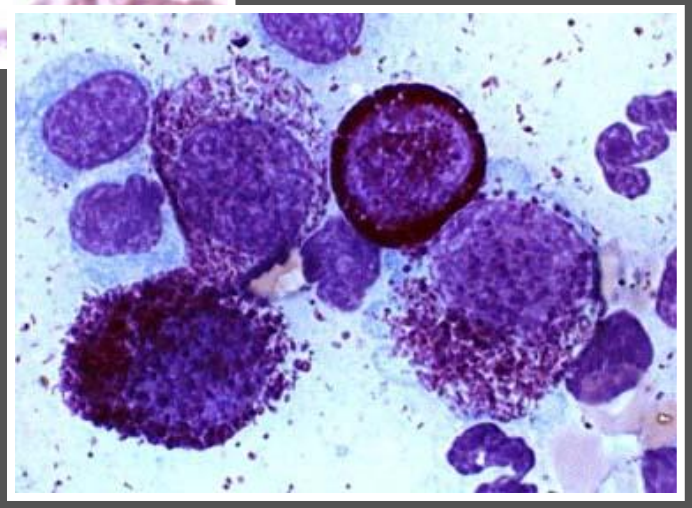


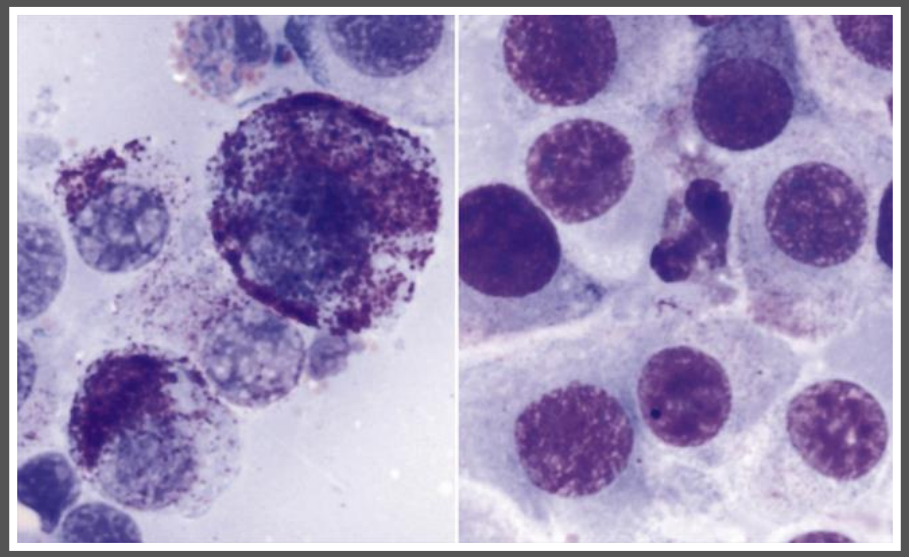






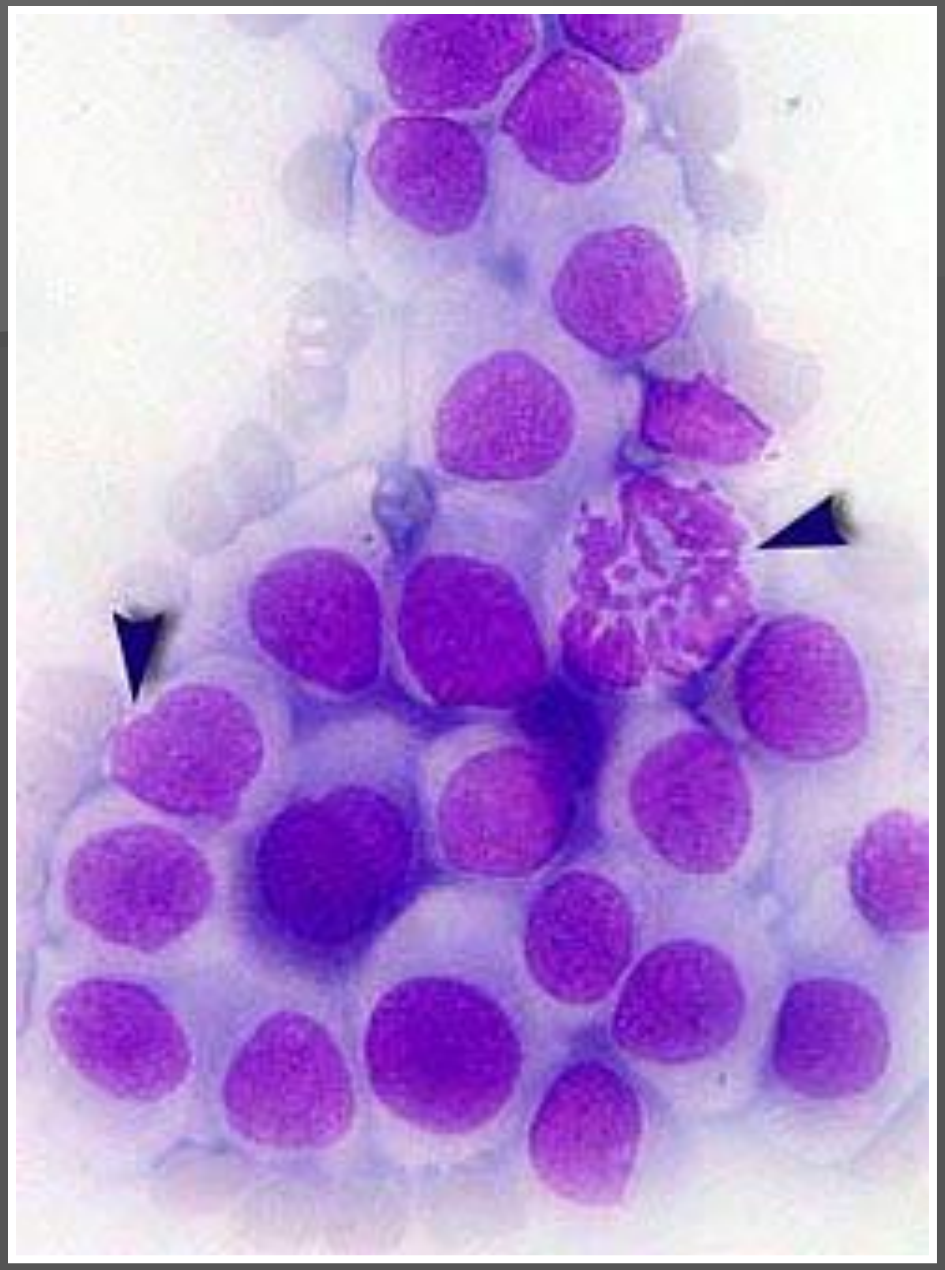


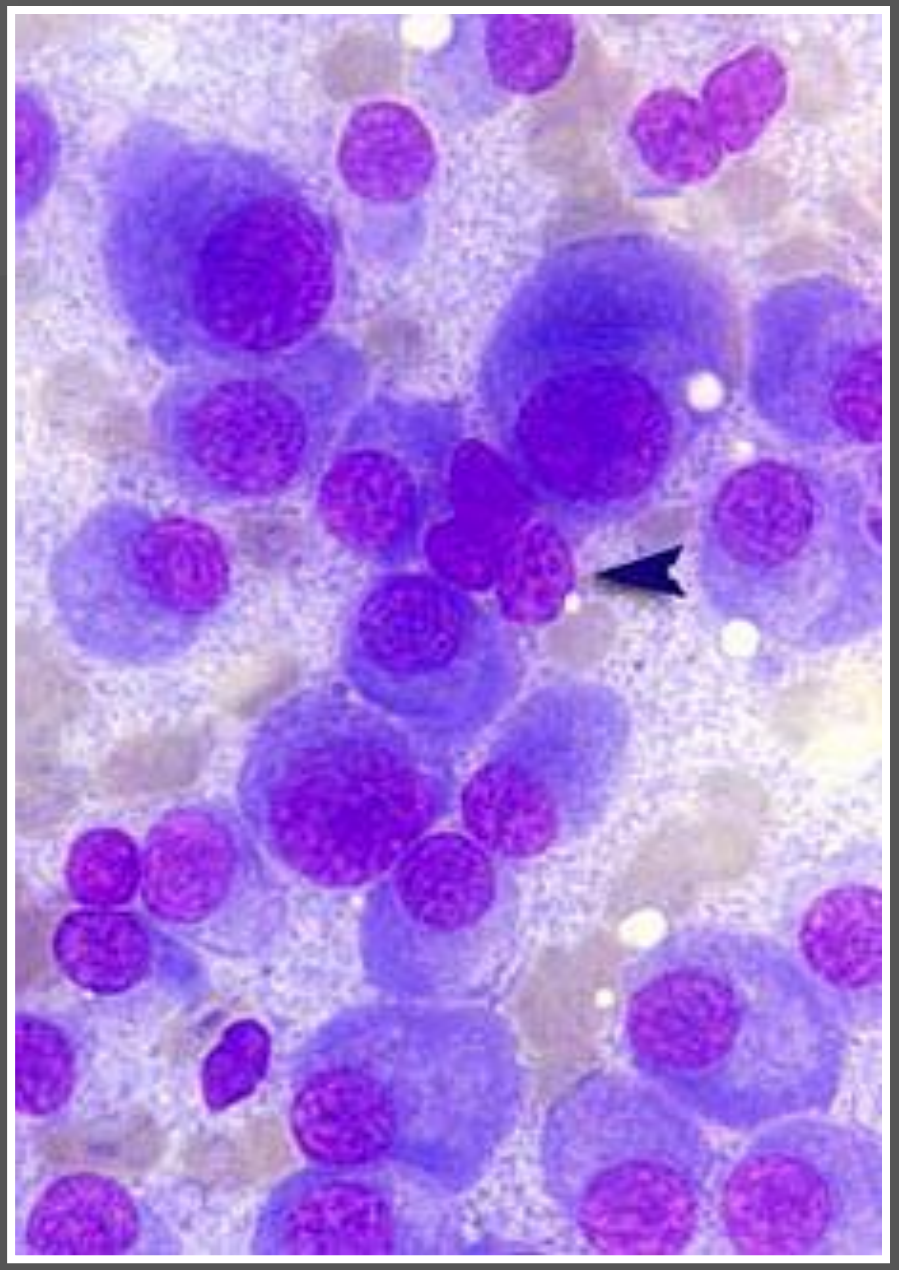


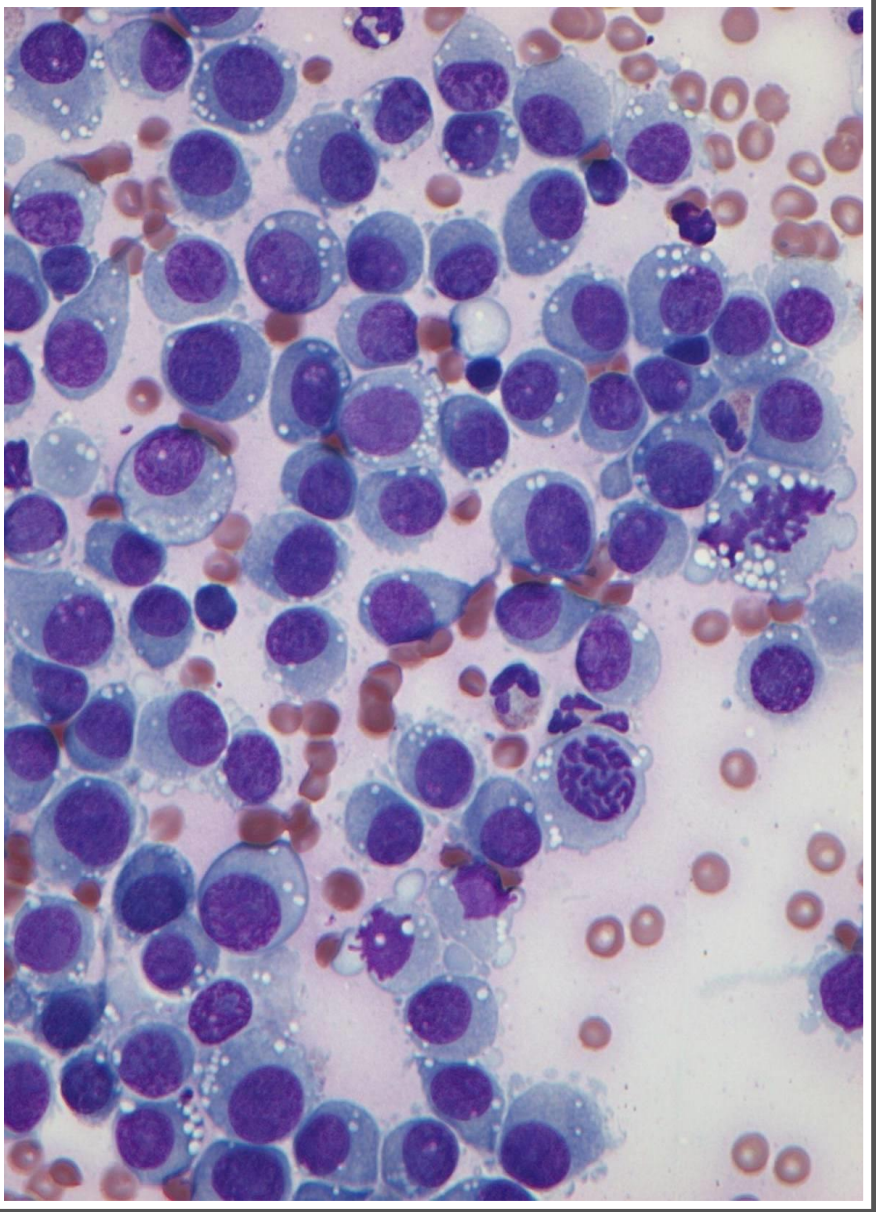


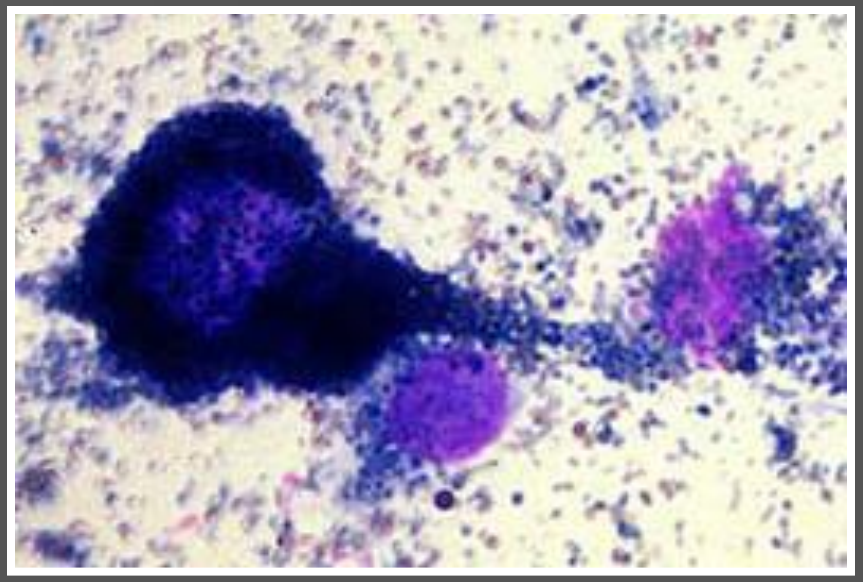
Cytologic grading?

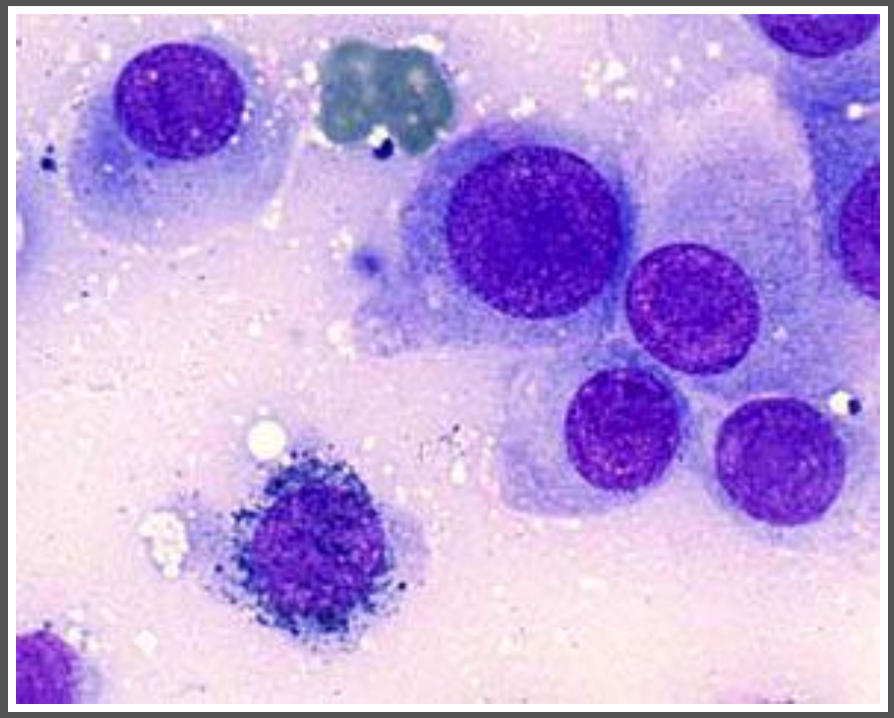
Check regional lymph nodes!

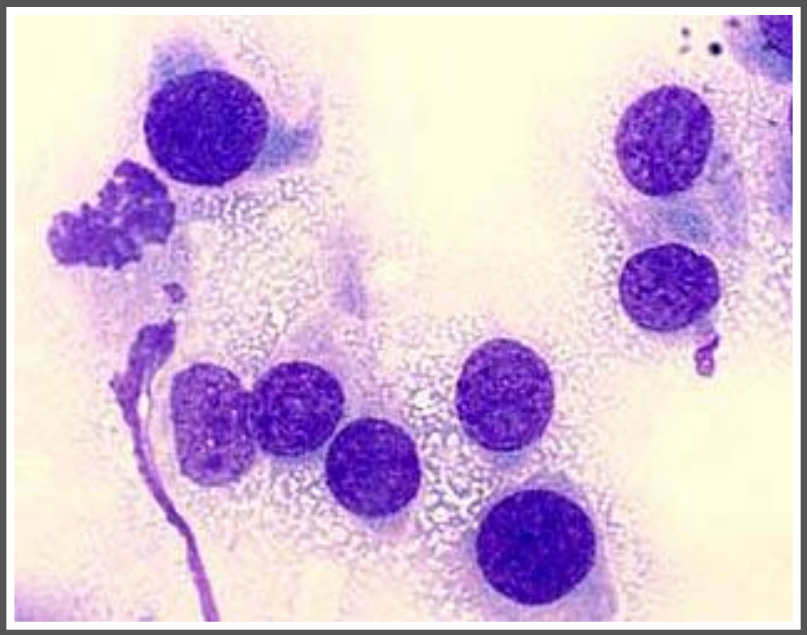












Cytologic interpretation

- The interpretation is the diagnosis the pathologist feels comfortable making
  - Neoplasia vs. Carcinoma
  - Suspect or probable
  - Make friends with your pathologist!
- Comments
  - May recommend additional tests or list differentials.

Histopathology

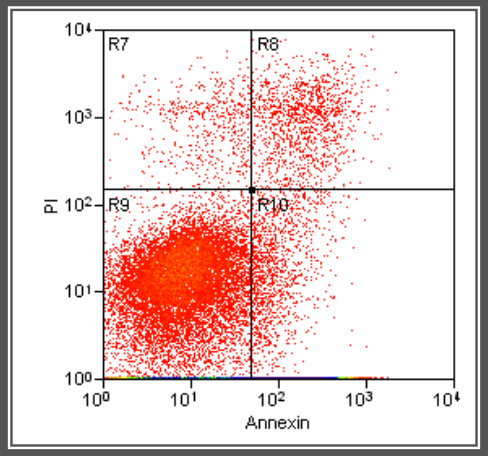
- Incisional vs. excisional biopsy
- Able to excise biopsy site/ scar if second surgery needed?
- Immunohistochemistry (IHC)
  - Special stains: labeled antibodies bind to target antigens on cells
  - Aids in diagnosis and prognosis, can take 10-14 days so recommend adding as soon as possible
    - I'm always going to recommend IHC!

Considerations for lymphoma

- Leukemia is the proliferation of neoplastic hematopoietic cells in the bone marrow. These cells may or may not be circulating in the peripheral blood.
- Lymphoma is neoplasia of lymphoid tissue.
- Immunocytochemistry
- Flow cytometry
- PARR testing for clonality

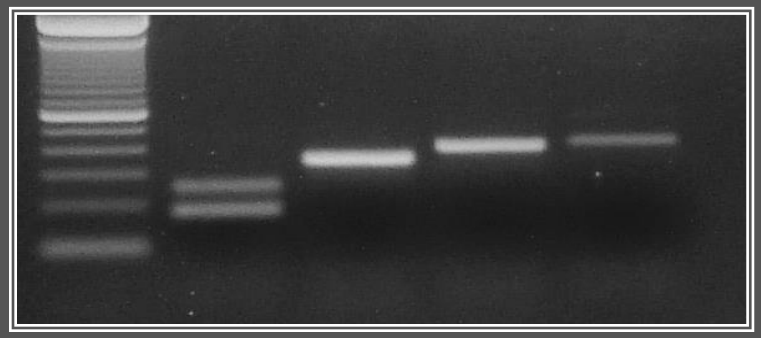
Molecular techniques

- Flow cytometry
  - Staining live cells with labeled Abs that bind to cell surface proteins
    - For examples T cells express CD3 protein
  - Determines the lineage of cells present
    - Homogeneous- neoplasia
    - Heterogeneous- reactive
  - Differentiation of lymphoid diseases, leukemias, thymoma



Molecular techniques

- PARR: PCR for antigen receptor rearrangements
  - PCR- polymerase chain reaction to amplify DNA
  - Detects clonal expansion of cells
    - Same clone- neoplasia
    - Multiple clones- reactive
  - High specificity >90%
  - Sensitivity
    - 75% dogs, 65% cats
    - Negative does not rule out lymphoma



Molecular techniques

- Which test should you choose?
  - Confirmed lymphoma patient: PARR
  - Flow cytometry usually best
    - Can provide specific phenotype information
      - High grade, indolent, thymoma vs lymphoma
  - Not helpful for neutrophilia/ CML
  - PARR formalin fixed tissues- nasal, GI
- Clinical Hematopathology Lab- Colorado St.

Species	Clinical sign	Site	First test to submit	Notes
Dog	Cytologically suspicious or confirmed neoplastic lymphocytes in any tissue (solid organs, bone marrow, blood, effusions).	Affected organ	Flow cytometry	Do not submit blood when there is no lymphocytosis or cytologically suspicious cells in the blood.
Dog	Differentiating thymoma from lymphoma.	Mediastinum	Flow cytometry	
Dog	Expanded population of lymphocytes in the peripheral blood that are cytologically normal.	Peripheral blood	Flow cytometry	
Dog	Enlarged lymph nodes/spleen with lymphocytes described as cytologically normal.	Affected organ	Flow cytometry	
Dog	Neurologic signs with significantly elevated white count in the CSF.	CSF	PARR	A minimum of 50,000 cells are needed for a diagnostic PARR result.
Dog	Flow cytometry results were equivocal.		PARR	Do not collect a new sample. PARR can be performed on the sample already submitted for flow cytometry.
Dog	Flow cytometry showed B cell chronic lymphocytic leukemia; looking for additional prognostic information.	Peripheral blood	Ki67	Ki67 expression indicates proliferation and is prognostic in B cell CLL.
Dog	Histologically suspicious cells in a lymphocyte-rich biopsy.	Request curls from the biopsy block.	PARR	
Dog	Histology suspicious but not definitive for lymphoma.	Affected organ	PARR on the biopsy block or flow cytometry of a fresh aspirate.	In cases where a precursor neoplasm is suspected (acute leukemia, which can also present with primarily solid organ involvement) flow cytometry of a fresh aspirate is preferred.

Cat	Lymphocytosis	Peripheral blood	Flow cytometry	Flow cytometry can be diagnostic and prognostic, but many cases require follow-up PARR testing. If a myeloid, erythroid or acute leukemia is suspected, flow cytometry is not useful.
Cat	Cytologically confirmed neoplastic lymphocytes in any tissue.	Affected organ	Flow cytometry	
Cat	Cytologically suspicious cells in any tissue or expansion of small lymphocytes.	Affected organ	PARR	
Cat	Equivocal evidence of neoplastic lymphocytes in a formalin-fixed biopsy.	Request curls from the biopsy block.	PARR	
Dog or Cat	Wanting to determine if plasma cells are neoplastic.	Any	PARR	We do not have antibodies to identify plasma cell by flow cytometry.
Dog or Cat	Suspicion for neutrophilic leukemia (CML) or eosinophilic leukemia.	Blood	None	We cannot distinguish neoplastic from reactive neutrophils or eosinophils by any currently available testing.

Case example: Enzo

- LN cyto: lymphoma, June 2021
  - Intermediate size, mild lymphocytosis on CBC
- Started chemo (CHOP) 7/9/21
  - PARR submitted (initial appt was on a Friday)
    - Clonally rearranged T cell receptor and immunoglobulin genes, flow recommended
  - LN remained enlarged, flow on blood submitted 8/5/21
    - CD8 lymphocytosis, CD5+CD45- lymphocytosis
- High grade B cell, indolent T zone

CADET® *BRAF*

- Available through Antech
- PCR test to detect the BRAF mutation in urothelial cells shed in the urine
  - Transitional cell carcinoma (TCC)/ urothelial carcinoma (UCC)
- Identifies 95% of TCC/UCC cases
  - *BRAF* identifies 85% (no mutation in 15%)
  - *BRAF-PLUS* increases diagnosed cases to 95%

Case example: Dillie

- Presented 6/27/22
  - History of stranguria since April 2022
- AUS and BRAF performed prior to referral
  - Consistent with TCC/UCC
- Started piroxicam
- Owners did not want to pursue standard chemotherapy protocols
- Elected targeted therapy through FidoCure

Pet Name	Owner	Species	Breed	Sex	Age	Chart#
Cordellia Dillie	[redacted]	Canine	Beagle	SF	13Y	N

Test Requested

CADET BRAF

BRAF Mutation Status DETECTED

Comment:

Result is diagnostic for transitional cell carcinoma/urothelial carcinoma.

The University of Wisconsin-Madison has a clinical study to determine whether household chemicals are linked to TCC / UC in dogs. Please provide the attached information link to owners of dogs with bladder cancer, so they can decide if they want to enroll in the study. The University of Wisconsin-Madison will handle all recruitment, consenting, and sample collection once owners contact them. For more information go to: https://Environmental risk for bladder cancer.pdf

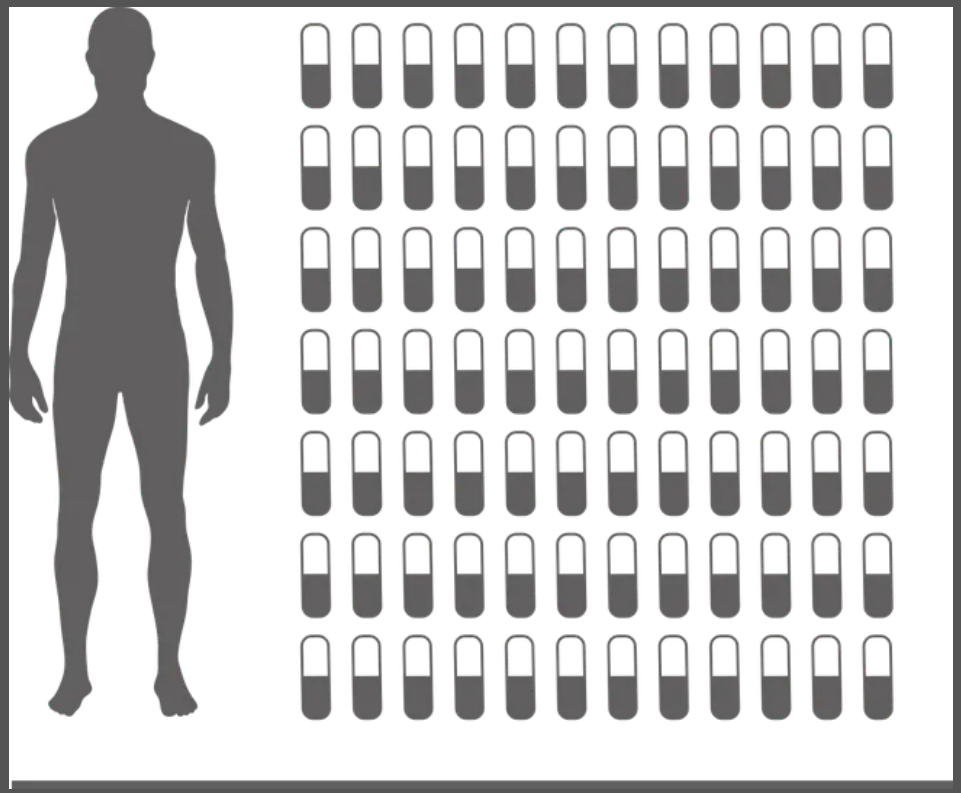
Volume of urine received	40	ml
Fract. abundance of BRAF mutation	28	%
% of cells with BRAF mutation	up to 56	%

Fractional abundance (FA) is the proportion of BRAF alleles in the specimen that are mutant.

BRAF mutation is generally detected in one of the two copies (alleles) of the gene in each cell. T
with the mutation is thus up to double the percentage of mutant alleles (fractional abundance) de

FidoCure®

Human Oncology
1.6M New Annual Cases
84+ Targeted Therapies in use



Veterinary Oncology
6M New Annual Cases
1 Targeted Therapy

FidoCure- how it works

1. Sequence DNA
Veterinarian submits a tissue sample to FidoCure® for DNA sequencing.

Tissue samples only, no aspirates

2. Identify Mutations
FidoCure® creates a personalized DNA report that helps identify genetic mutations that may affect treatment.

May not identify targetable mutations

3. Targeted Therapy
Informed by FidoCure®, veterinarian may prescribe targeted therapies (administered orally at home).

Compounded at Wedgewood

FidoCure

- DNA sequencing option
- Targeted therapy only option
  - Mast cell tumors, TCC/UCC, hemangiosarcoma, osteosarcoma
- Back to Dillie
  - Targeted therapy only option- lapatinib
    - Tyrosine kinase inhibitor EGFR, HER2
  - Started lapatinib June 2022, PD Jan 2023, euthanasia Feb 2023 (8 month survival)

Case example: Bilbo

- Lytic bone lesion R distal tibia
- Amputation March 2020
- Carboplatin chemotherapy
  - 6 doses, completed July 2020
- FidoCure genetic testing
  - Mutations: NOTCH1 and p53
  - Vorinostat- HDAC inhibitor, started Aug 2020
- Euthanasia Oct 2022- lung metastasis

Multidrug resistance

- *MDR-1* multidrug resistance gene
  - Also known as ABCB1 gene
  - Limits drug distribution to brain, enhances excretion
- Encodes P-glycoprotein
  - Transmembrane ATP-dependent drug efflux pump
  - Actively removes certain drugs from cells
- Upregulation in response to chemotherapy
- Increased expression may increase resistance
- *MDR-1* gene can be mutated
  - 1 or 2 copies of mutated gene

Multidrug sensitivity

- Breeds of concern: Collies (70%), Long-haired whippets (65%), Australian Shepherds (50%), shelties, OES, German shepherds
  - 4% of cats
- Drugs of concern: certain chemotherapeutics (vincas, doxorubicin, steroids), acepromazine, butorphanol, loperamide, ivermectin
- Blood test or cheek swab
- https://prime.vetmed.wsu.edu/

OncoK9® PetDx

- MCED test (multi cancer early detection)
  - Liquid biopsy using DNA sequencing
  - CANDiD study- 1,100 dogs
    - 98.5% specificity (few false positives)- ideal for a screening test
    - 54.7% sensitivity
    - Detection rate for lymphoma, HSA, OSA: 85.4%
    - Detection rate for 3 above + soft tissue sarcoma, MCTs, mammary carcinoma, anal sac carcinoma, melanoma: 61.9%
    - Detection rate for all cancers in study: 55%

OncoK9® PetDx

- Annual screening starting at 7 years
  - Higher risk dogs at a younger age
- Aid in diagnosis
- Limitations and risks
  - Does not provide a definitive diagnosis of cancer
  - False negative rate 45%
  - Should not be used as sole basis to recommend euthanasia or start treatment

Nu.Q® Volition Veterinary

- DNA within the cell's nucleus coiled around a histone protein core
  - Nucleosomes from cancer cells released into blood and can be measured using antibodies
  - Published study: 528 with cancer, 134 healthy
    - Specificity: 97%
    - Sensitivity: 49.8%
    - Top 4 cancers detected: lymphoma, HSA, histiocytic sarcoma, melanoma
    - Less likely to be detected: soft tissue sarcomas, OSA, MCTs

Nu.Q® Volition Veterinary

- Annual screening starting at 7 years
  - Higher risk dogs at a younger age
- Aid in diagnosis
- Limitations
  - Does not provide a definitive diagnosis of cancer
  - Must be fasted (at least 4 hours)
  - Inflammatory diseases, steroids can interfere

Nu.Q®

- Nucleosomes (DNA coiled around histone)
- 1 mL EDTA plasma
- Idexx- $59.95
- 1-4 day turnaround
- At least 4h fast
- Cancer suspicion level- low to very high
- Texas A&M GI Lab, Heska/ POC, Idexx

OncoK9®

- Cell free and genomic DNA, collection kit
- 14-17 mL whole blood
- Idexx- $399
- 10-15 day turnaround
- No fasting needed
- Cancer signal detected, not, failed
- Idexx, Antech, PetDx

What about cats?

- Usually need flow and PARR
  - GI lymphoma, PARR on biopsy samples
- MDR testing available
- No BRAF
- No cancer blood tests yet
  - Volition is working on it…
- FidoCure not available for cats
- We finally have Solensia™ (frunevetmab injection)

IndyVet Oncology Referrals

- Appointments Mon, Wed, Thurs
- Phone/ email consults prior to appointment not required
- Diagnosis recommended
  - We request records to review for scheduling
- Email: oncology@indyvet.com
- Records/ scheduling: referral@indyvet.com

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