

# Pitfalls in Cytology

## Kopana 2024

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# Disclosure

- I have no relevant financial or non-financial interests to disclose. The authors have no conflicts of interest to declare that are relevant to the content of this presentation.

### Education:

- PhD: Northwestern University (Chemistry)
- Medical School: Rush University Medical College
- Residency: University of Massachusetts Medical School
- Fellowship: Washington University School of Medicine in St. Louis (Molecular Genetics Pathology)
- Fellowship: University of Kansas Medical Center (Cytology)



Current Position & Institution: Associate professor @ University of Cincinnati College of Medicine

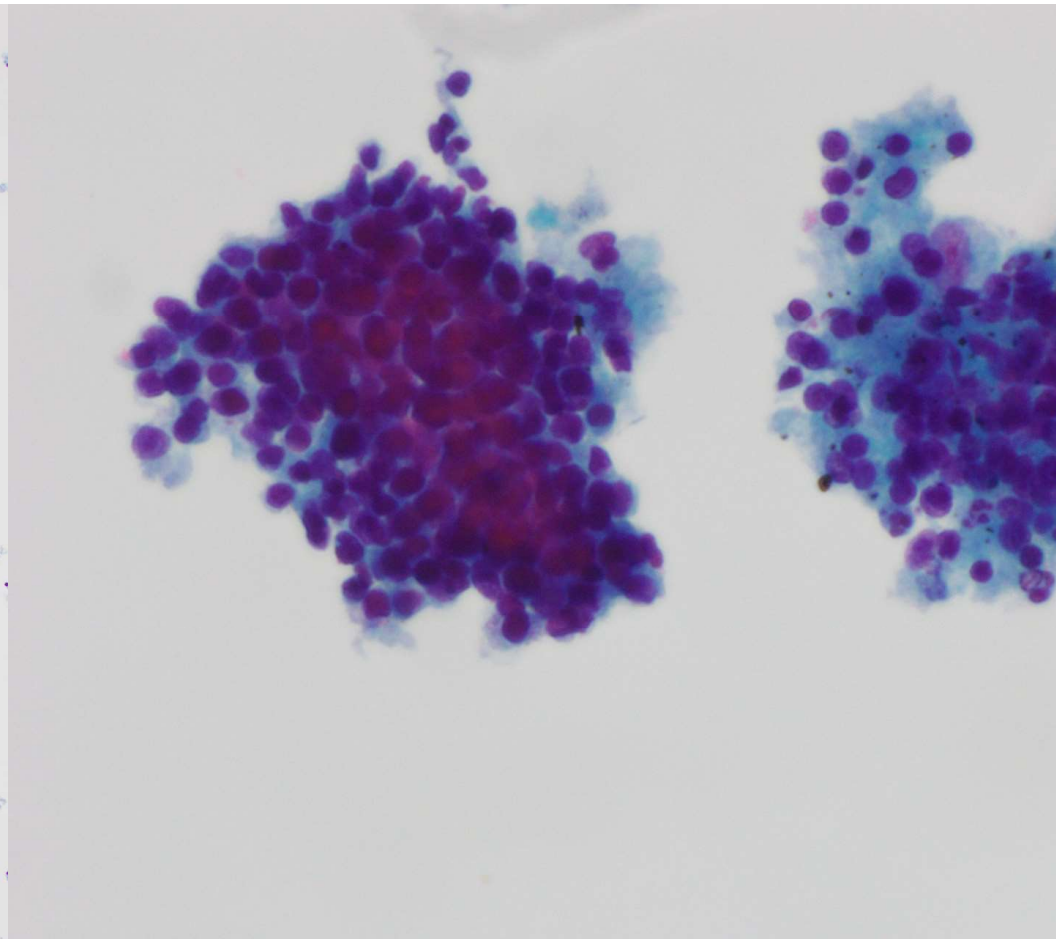
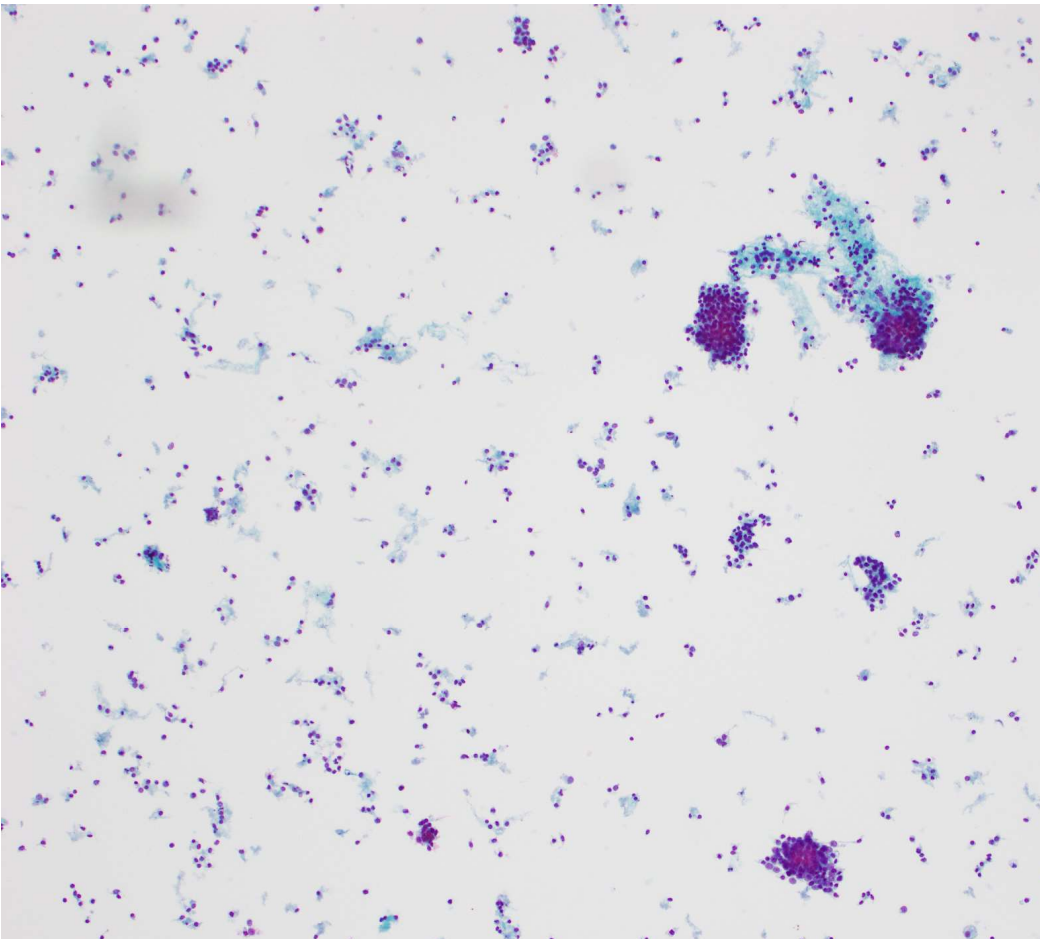
Subspecialty area: Molecular Pathology, GYN Pathology, Medical Renal, Cytopathology.



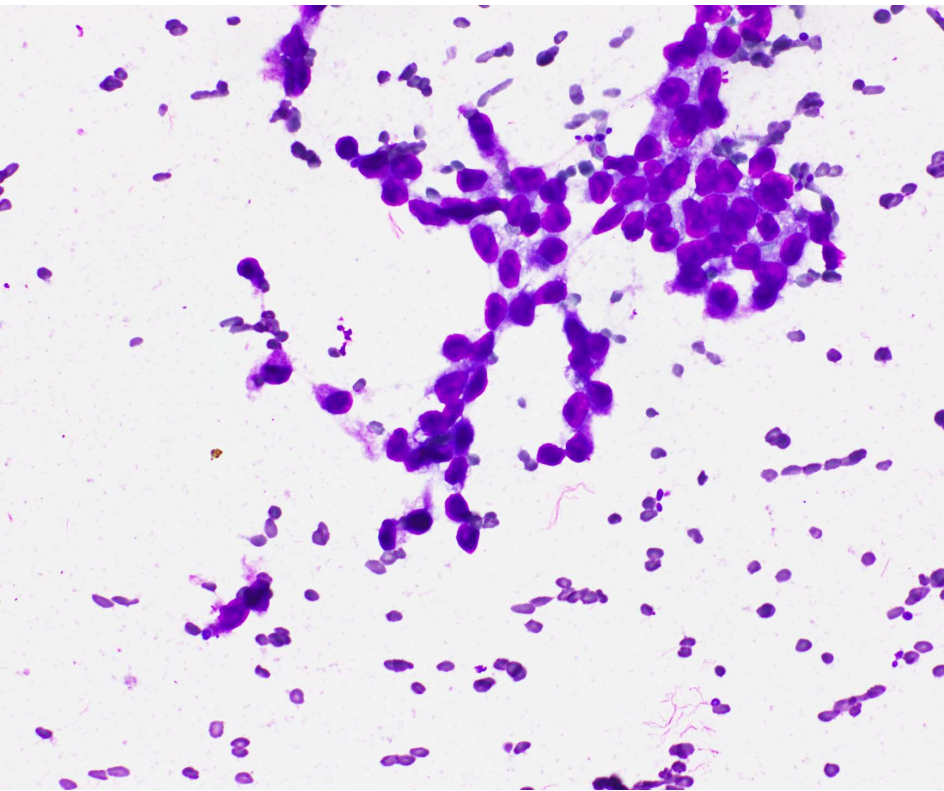
38 y/o M

- History of military service (exposure to uranium?)
- Presenting persistent bilateral hip pain.
- Imaging revealed multiple lesions in his hips, skull, ribs, and right lung.
- Underwent EBUS at OSH (VA) with a diagnosis of “small blue cell tumor”
- Came here got another EBUS of right lung nodule

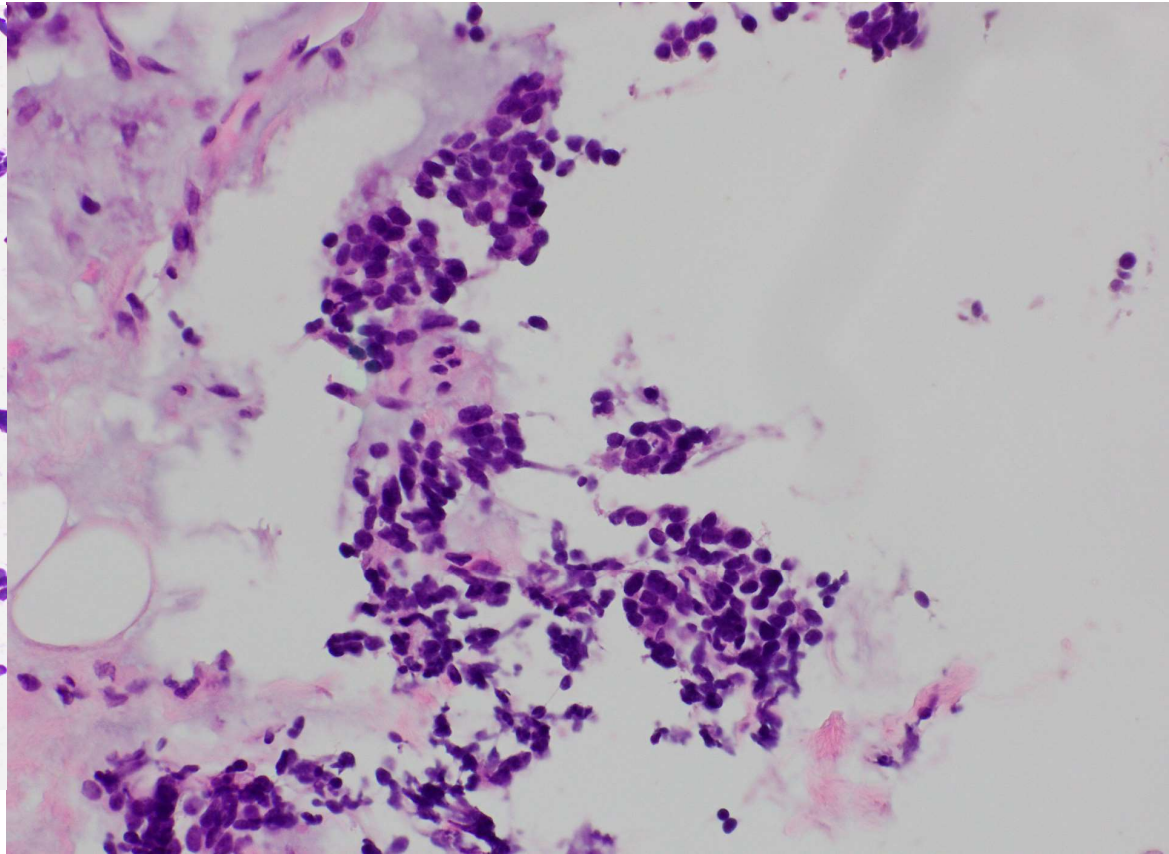
# Thin Prep



Diff-quick



Cell Block



# Differential

- Non-small cell carcinoma
  - Pulmonary adenocarcinoma
  - Squamous cell carcinoma
- Small cell carcinoma
- Other neuroendocrine tumors (i.e. large cell carcinoma)
- Melanoma
- Other small blue cell tumors (Ewing's)
- Sarcomas?

- A panel of immunohistochemical stains performed on the cell block is resulted as the following:

- Pan-keratin – positive (weak)
- TTF-1 – negative
- P40 - negative
- CK7 – negative
- CK20 – negative
- Chromogranin – negative
- Synaptophysin – negative
- WT-1 – negative
- HMB-45 – negative
- Desmin – negative
- CD99 – negative
- CD34 – negative
- BCOR – equivocal
- BRG-1 – retained
- INI1- retained
- Ki-67 index – 40-50% (high)

#### FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ANALYSIS

EWSR1 (Ewing Sarcoma) BreakApart Final Report

INTERPRETATION: NORMAL for the probe tested.

KARYOTYPE: nuc ish(EWSR1x2)[189]

PROBE: Vysis LSI EWSR1(22q12)Dual Color, Breakapart Rearrangement Probe

RESULTS: Interphase Analysis

PATIENT RESULTS

EWSR1(22q12) BreakApart = 1.0 %

Total EWSR1 Nuclei = 200

CONTROL RESULTS

EWSR1(22q12) BreakApart = 2.0 %

Total EWSR1 Nuclei = 200

COMMENTS:

SUMMARY: Fluorescence in situ hybridization (FISH) analysis using the dual color, break-apart rearrangement probe specific to the EWSR1 gene localized to chromosome 22q12 showed a signal pattern consistent with an EWSR1 gene rearrangement in 1.0 % of cells from this patient's specimen versus 2.0 % of cells from a normal control.

- Signed out as “Basaloid malignant neoplasm”. Sent to outside institution for further



Many thanks for asking me to look at this man's transbronchial biopsies from hilar lymph nodes. I note the clinical evidence of a lesion in the region of the left hip. I am returning your original stained sections and the blocks herein.

In both the specimens from the 11R and 7 lymph nodes, there is, as you have rightly described, a malignant neoplasm consisting of small closely packed cells with a high N/C ratio and ovoid or more tapering nuclei. I wondered initially about the possibility of small cell carcinoma but stains for CAM5.2, synaptophysin, INSM1 and TTF1 are negative. Stains for desmin, LCA, CD99 and S100 protein are also negative - but there is strong and diffuse nuclear positivity for SOX10. Stains for MART-1, tyrosinase and PNL2 are negative. In these circumstances, I would label this as an **small cell malignant neoplasm, most suggestive of metastatic malignant melanoma**. There are virtually no other tumor types that would show this type of morphology combined with strong and diffuse nuclear positivity for SOX10. Careful clinical correlation to check for a primary lesion elsewhere, including possible mucosal locations, may be informative in this regard and I would be interest to hear if the primary can be identified.

## Second consultation

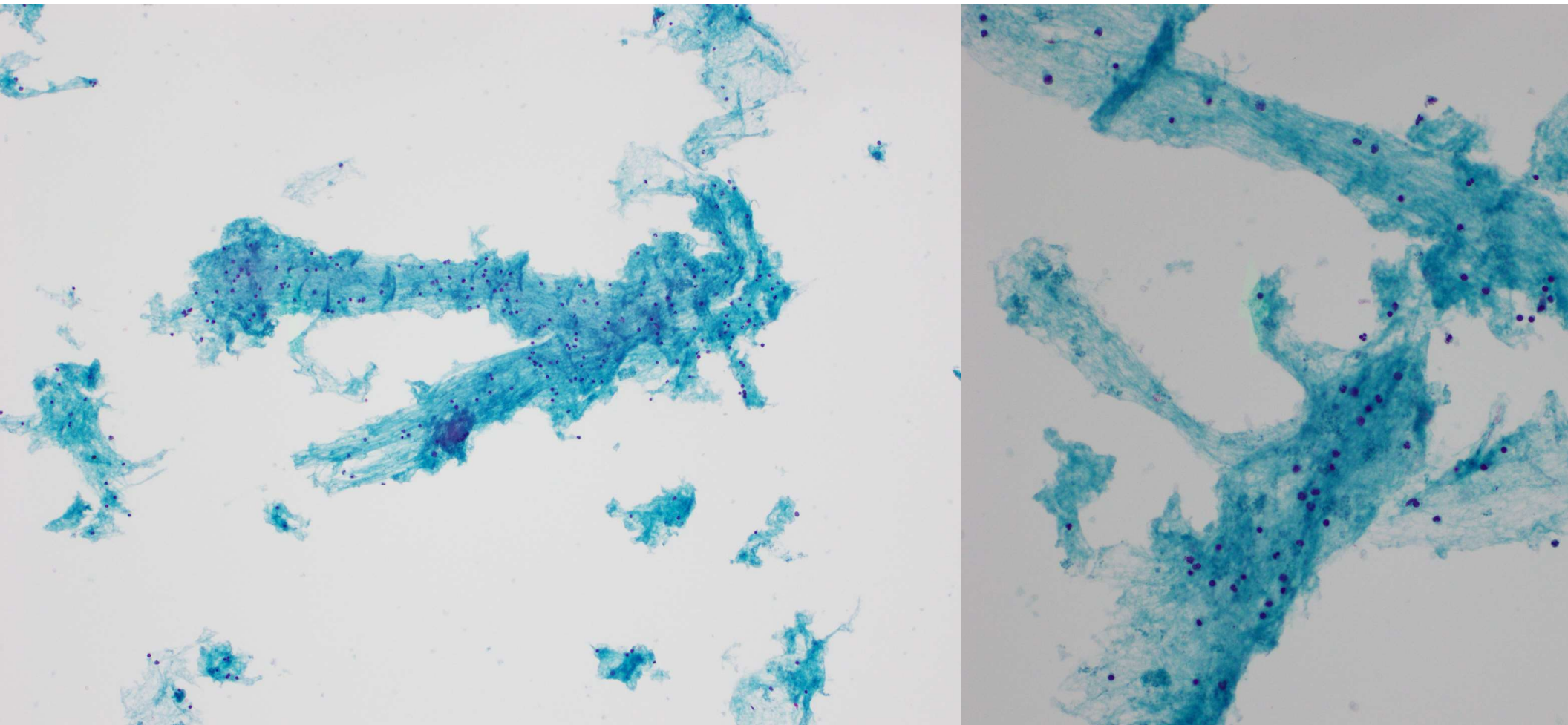
The submitted immunohistochemical stains [REDACTED] show that the neoplastic cells are positive for pan-cytokeratin, while negative for TTF-1, HMB-45, chromogranin, synaptophysin, WT-1, BCOR, CD99, desmin, CD45, and CD34. BRG-1 and INI-1 (BAF-47) are retained. The Ki-67 proliferation index is 40-50%. IHC performed at [REDACTED] show that the neoplastic cells are positive for SOX-10, pan-cytokeratin (focal, weak), and EMA (focal, weak), while negative for S100 and BRAF. Per report, FISH was performed for *EWSR1* breakapart and the results are negative. The lymph node biopsies [REDACTED] have a similar morphology to the EBUS FNA of the lymph nodes. The neoplastic cells form pseudoglandular spaces with some basophilic matrix. The submitted IHC stains show that the neoplastic cells are positive for TdT (weakly), while negative for CD45, PAX-5, chromogranin, CD3, TTF-1, CK20, and synaptophysin. The Ki-67 proliferation index is 50%. Per [REDACTED] report, the neoplastic cells are negative for MART-1, tyrosinase, and PNL2. Additional immunohistochemical stains performed in our lab on the lymph node, 11R, [REDACTED] shows that the neoplastic cells are focally positive for CAM 5.2 and CD117 (weak), while negative for calponin, p40, and SMA. Also, FISH was performed at [REDACTED] in a research setting using BAC probes for MYBL1 inversion, MYB, and NFIB; the results are positive for MYBL1 inversion.

In summary, the lymph node FNAs and biopsies are consistent with an adenoid cystic carcinoma with a solid growth pattern. This morphology raises the differential diagnosis of Ewing sarcoma, melanoma, and myoepithelial carcinoma. The reported negative FISH for *EWSR1* breakapart and the negative CD99 argues against an Ewing sarcoma.

82 y/o F

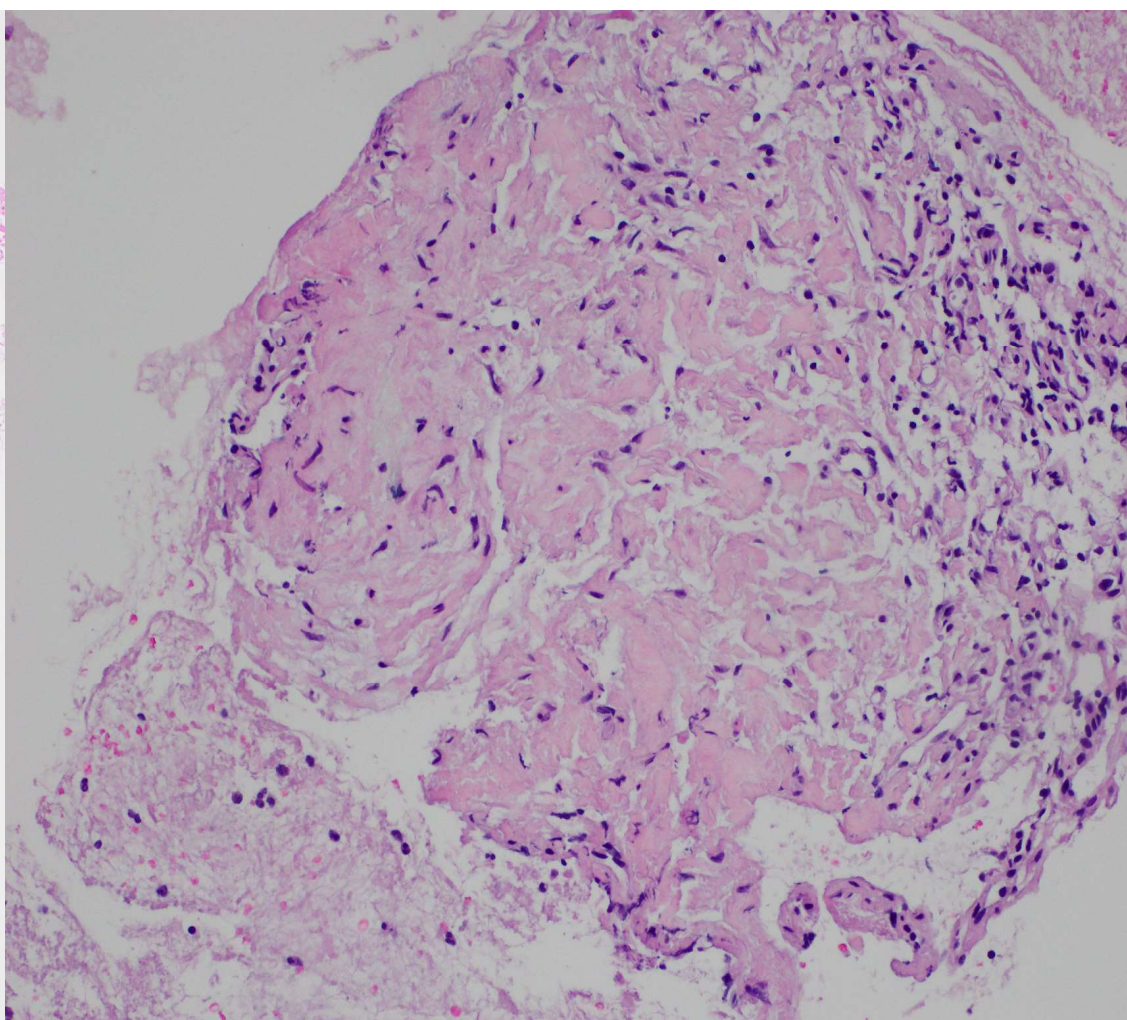
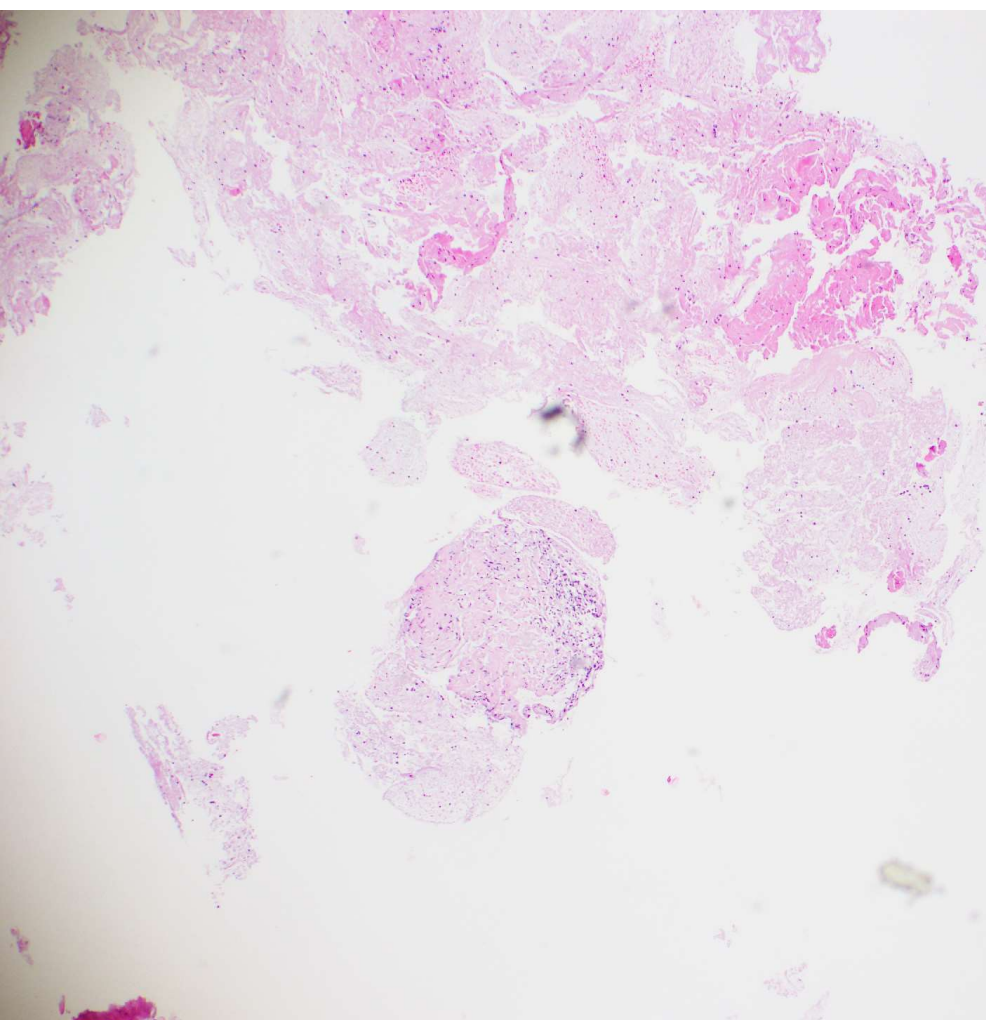
- **With histories of a “low-grade epithelial neoplasm of lung” s/p lobectomy (2001), renal cell carcinoma s/p nephrectomy (1993), skin cancer, thyroid carcinoma s/p thyroidectomy (1987), and sarcoidosis.**
- **Presenting with a mediastinal lymphadenopathy.**
- **CT scan summary - Enlarging subcarinal lymph node, highly concerning for nodal metastatic disease. Sarcoidosis-related lymphadenopathy would be another consideration. PET/CT and/or tissue sampling is recommended at this time.**
- **Stable postsurgical changes of right lower lobectomy.**

# Thin Prep

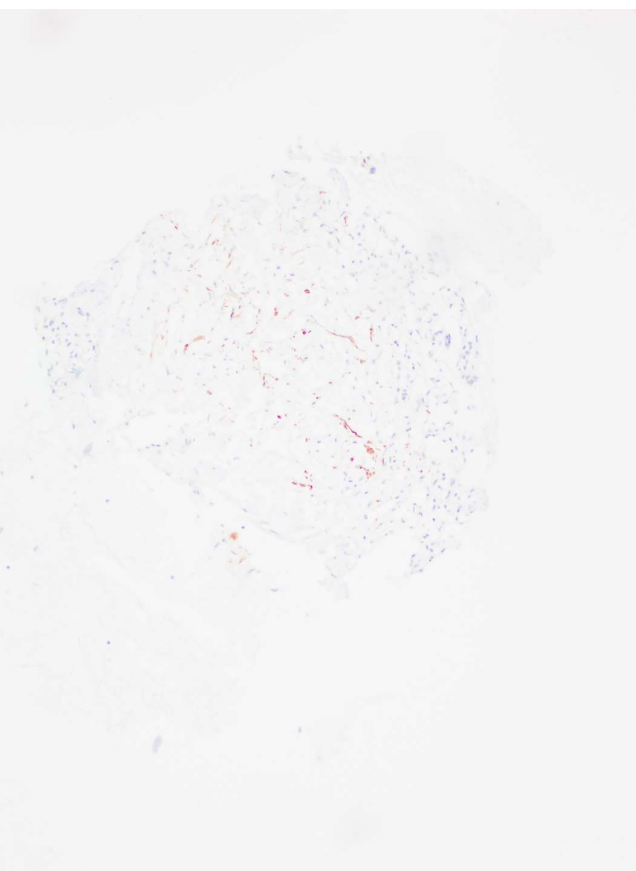




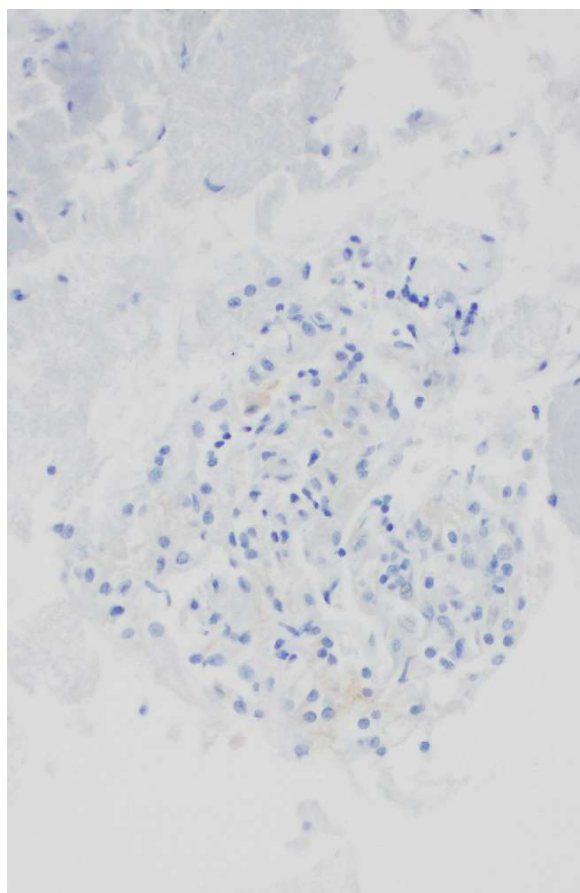
# Cell block



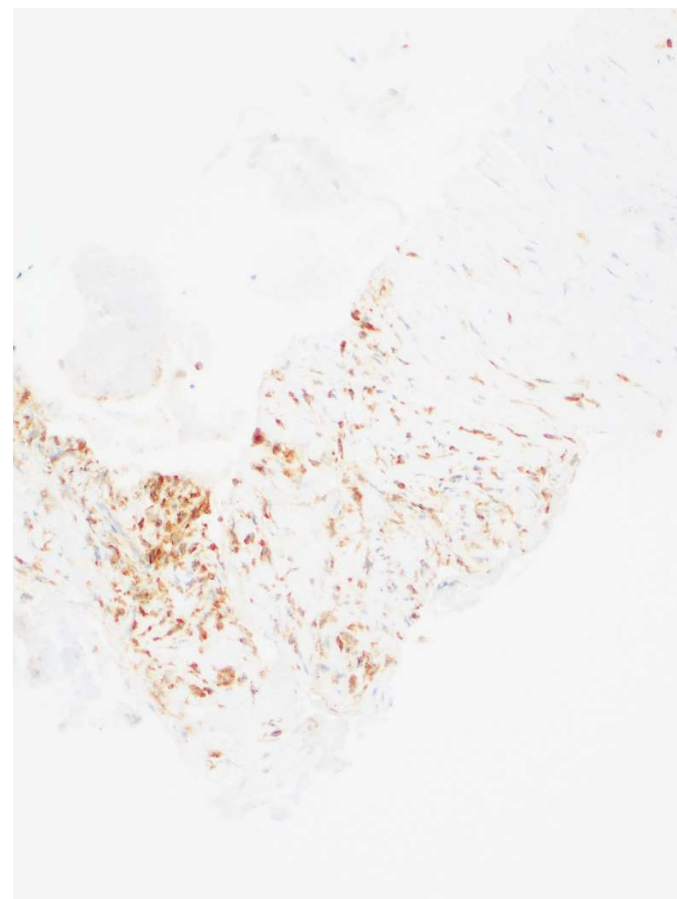
CK7



Pan-Keratin



CD68

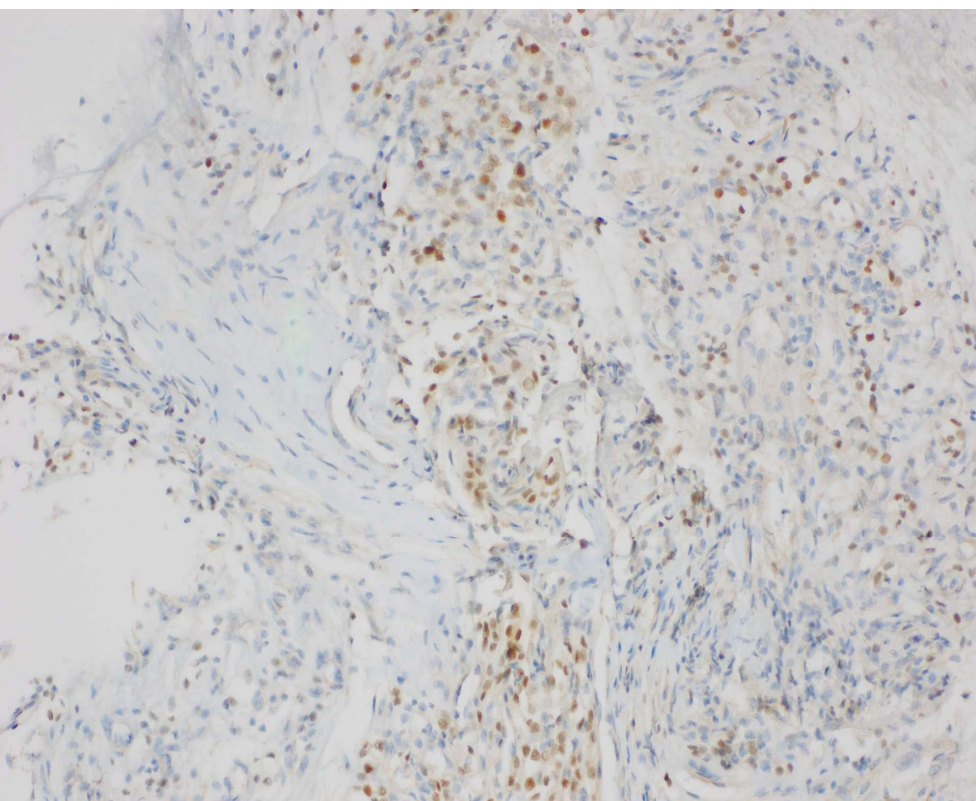


# Signed out

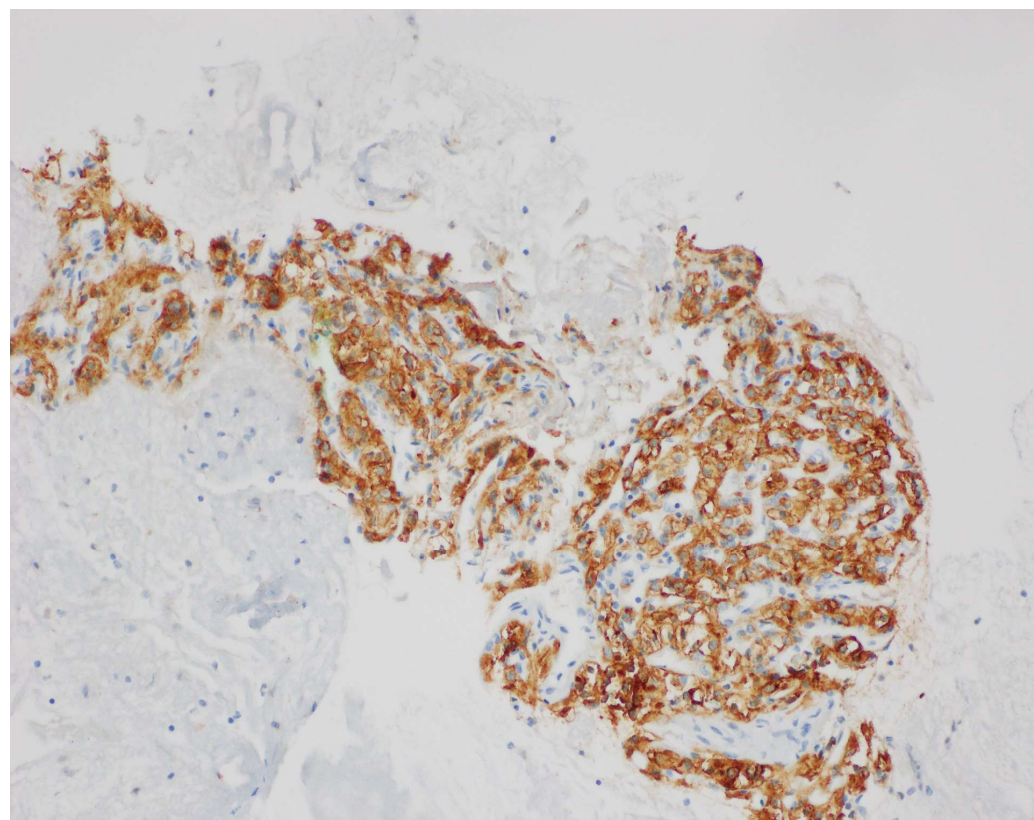
- **St 7 Lymph Node, EBUS-Fine Needle Aspiration:**
- - Scant polymorphous population of lymphocytes.
- - No definitive evidence of carcinoma or granulomas in the sampled specimen.
- - Scant benign appearing lymphocytes, histiocytic aggregates, and bronchial cells present.
- - See comment.
  
- **Comment:**
- The patient's histories of a low grade epithelial neoplasm of lung, renal cell carcinoma, and sarcoidosis are noted. In the background of polymorphous population of lymphocytes, there are clusters of bland appearing cells with bubbly cytoplasm. The immunohistochemical stains performed on the cell block are positive in these cells for CD68; and negative for CK7, mammaglobin, S100, p63, CD56, and pan-Keratin. These cells represent histiocytic aggregates. The morphology does not demonstrate definitive granulomas. This immunohistochemical profile and morphology support the diagnosis.



PAX 8



CAIX



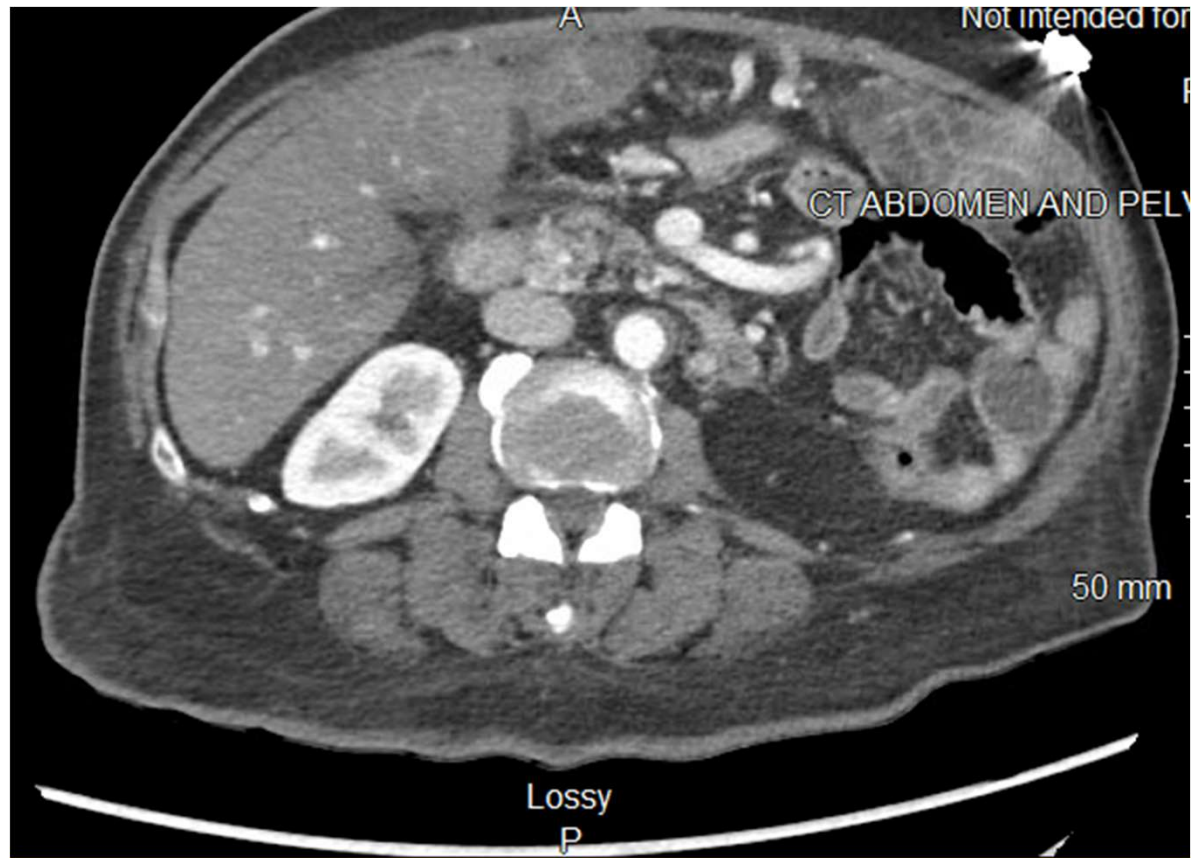


# **Amendment:**

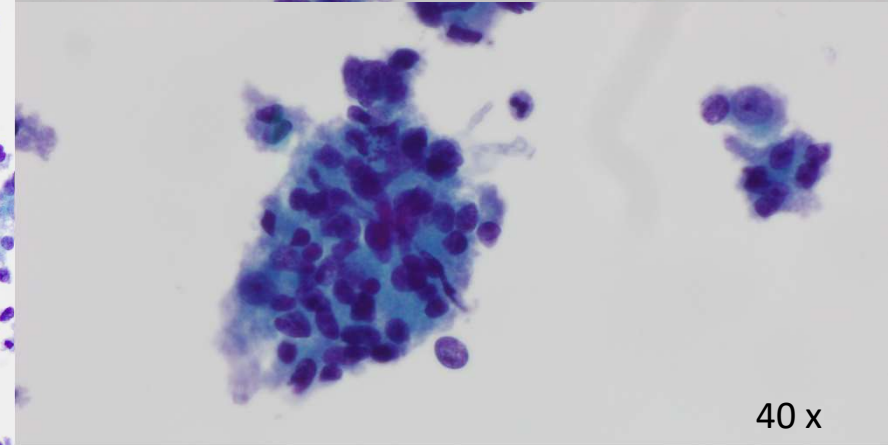
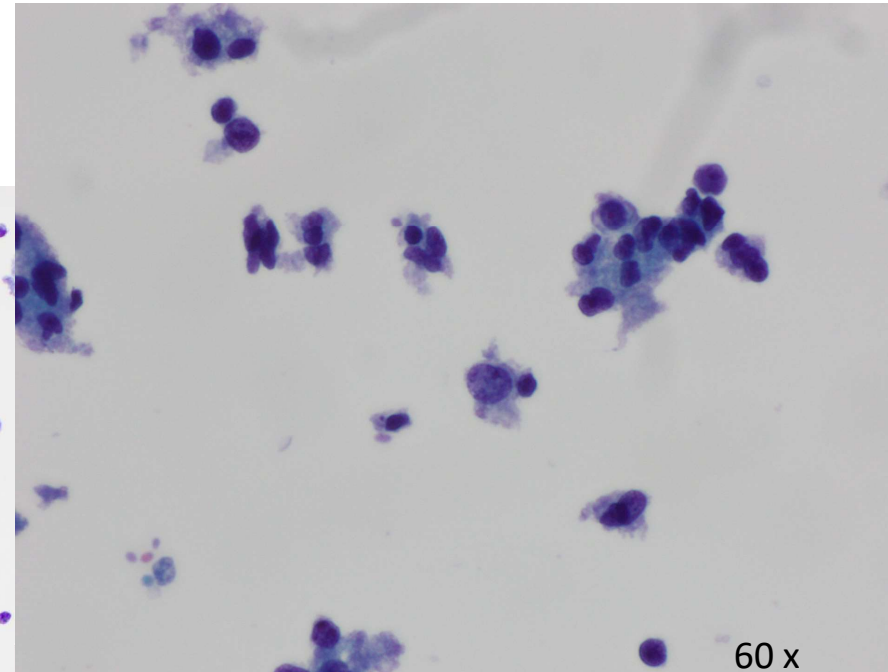
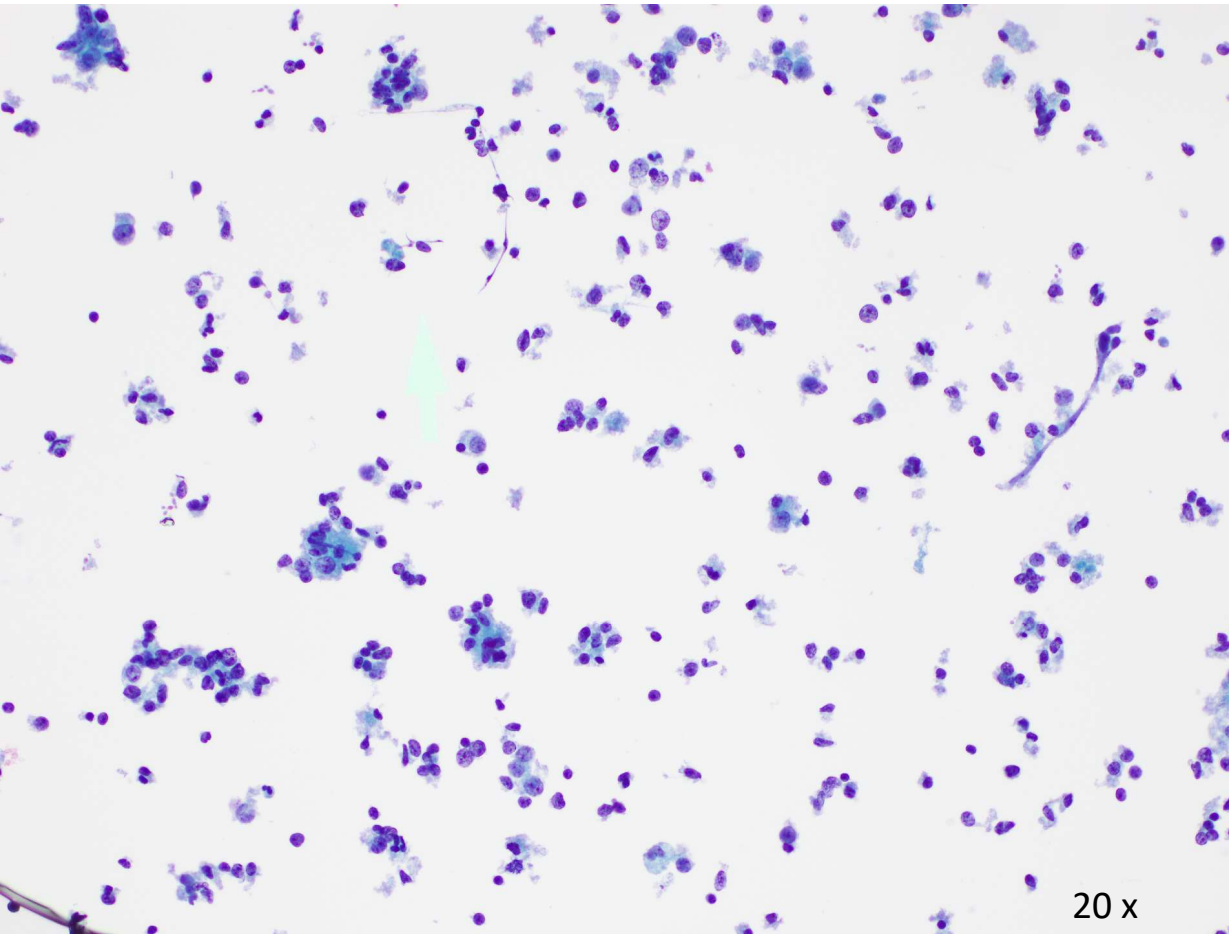
- **This amendment is created to change the diagnosis.**
- **After additional immunohistochemical stains (PAX-8 and Carbonic anhydrase IX) on cytology case, the diagnosis is changed. The diagnosis is corrected as "Scant specimen. Rare fragments of metastatic renal cell carcinoma."**

## 62 y/o F with mass in the pancreas

- CT scan showed a solid enhancing body mass which abuts the gastric wall and causing splenic vein occlusion. 4.1 x 3.2 cm mass.

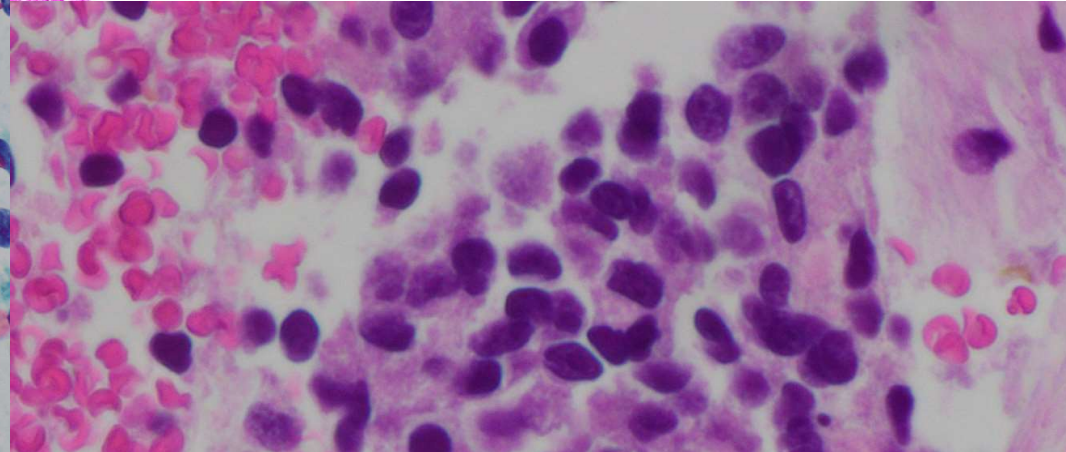
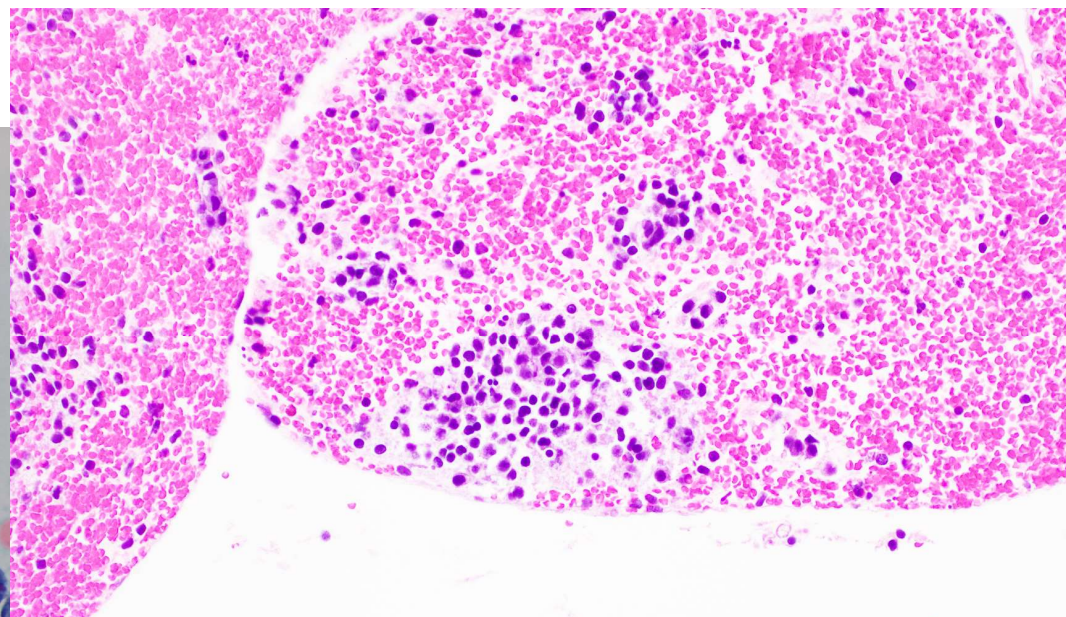
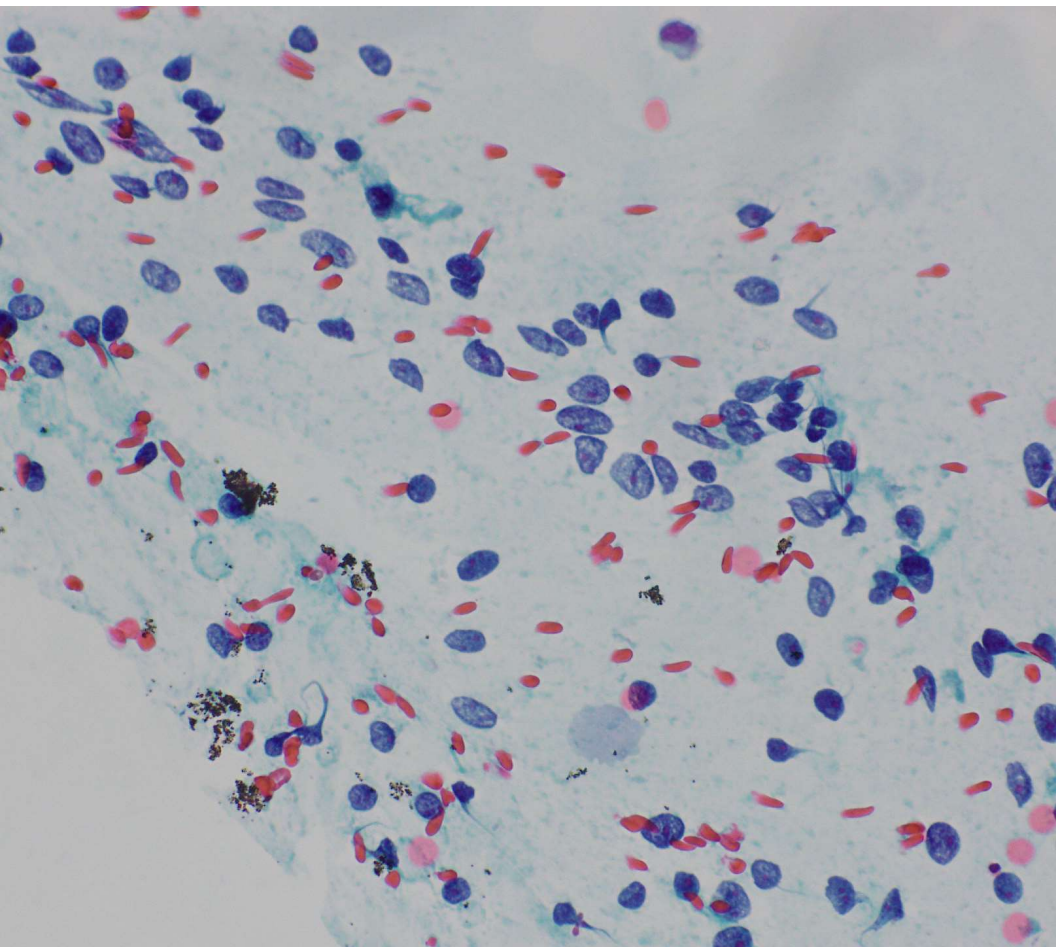


# Thin Prep





## Pap / Cell block H&E



# Differential

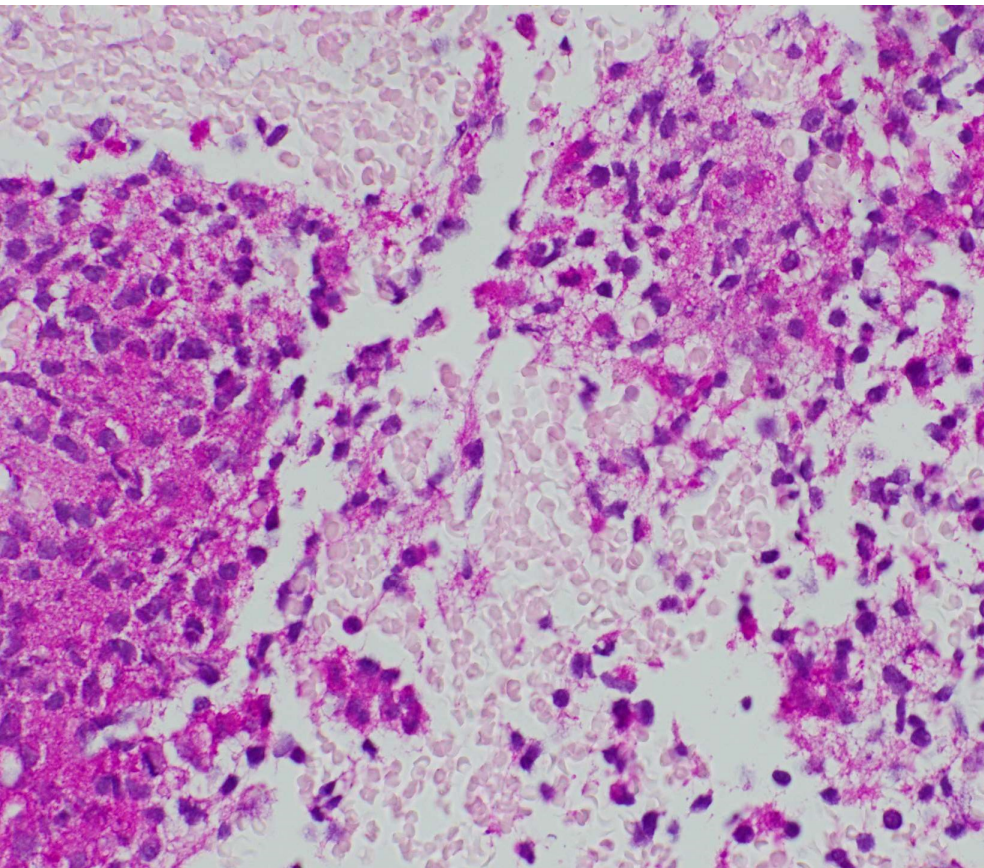
- Pancreatic neuroendocrine tumor (PNET)
- Non-Hodgkin lymphoma
- Solid pseudopapillary neoplasm (SPN)
- Islet cell hyperplasia
- Acinar cell carcinoma
- Melanoma
- Ectopic spleen
- Metastatic process

# IHCs - negative

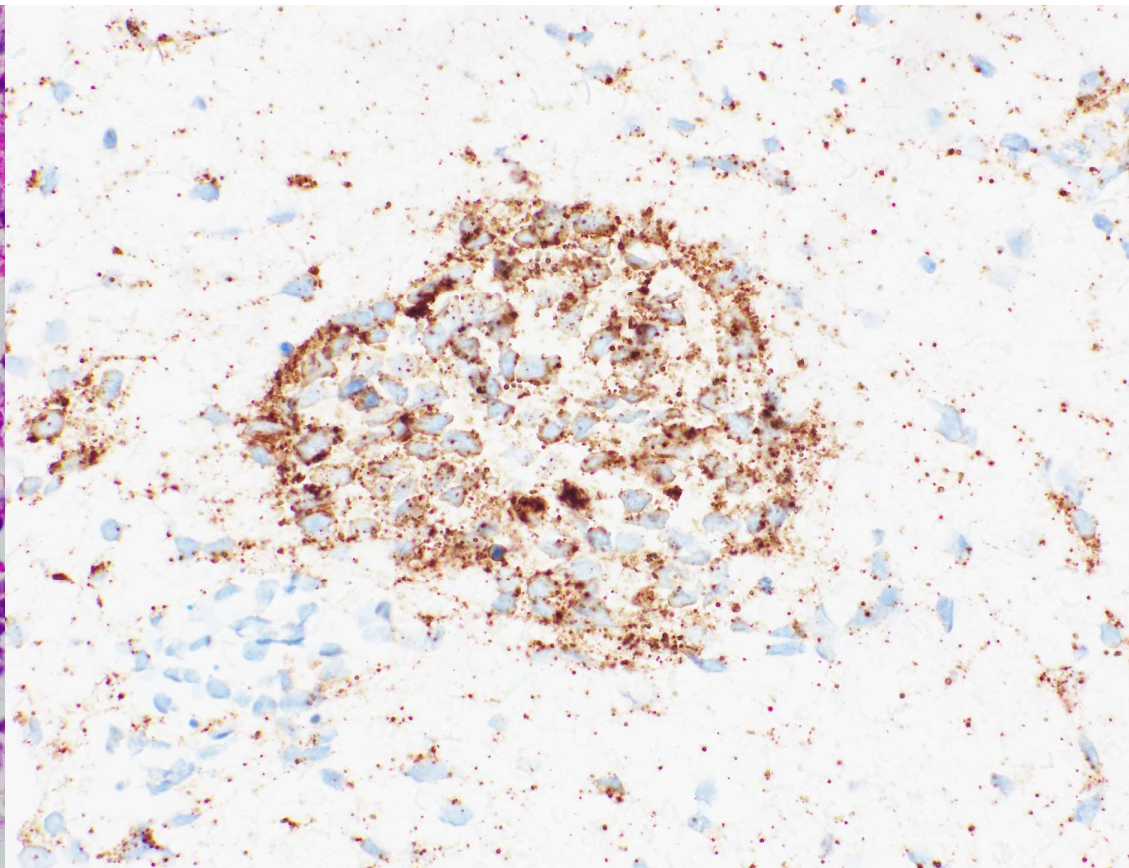
- **Epithelial markers:** Cam 5.2, Pankeratin (AE1/3), and 34betaE12 (high molecular weight keratin)
- **Neuroendocrine markers:** Synaptophysin, Chromogranin and CD56
- **Solid pseudopapillary neoplasm markers:** Beta-catenin (no nuclear stain), E-Cadherin (intact membrane stain)
- **Lymphocytic markers:** CD45 and CD30
- **Melanocytic markers:** SOX10, S-100 and Mart-1
- **Vascular marker:** ERG
- **Muscular marker:** Desmin
- **Mesenchymal marker:** Vimentin
- **Breast, urothelial and squamous marker:** Gata-3
- **Thyroid, kidney and ovarian marker:** PAX-8
- **Plasma cell marker:** CD138



PAS

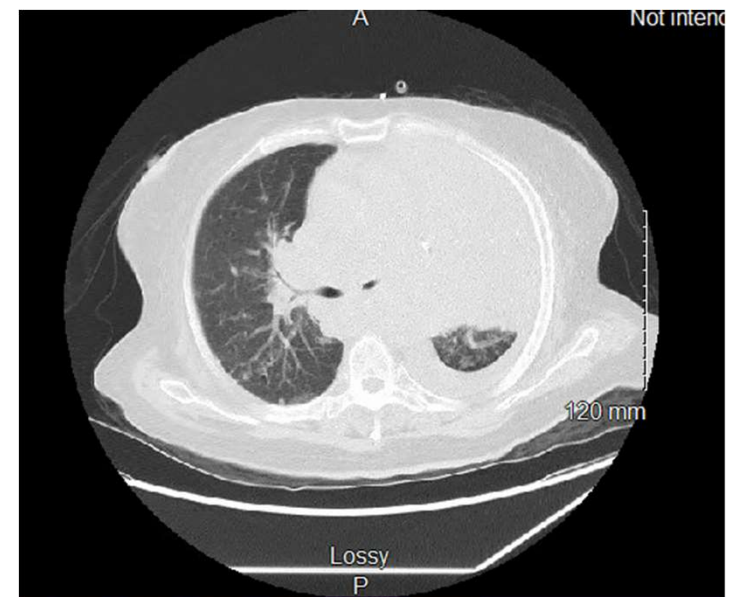
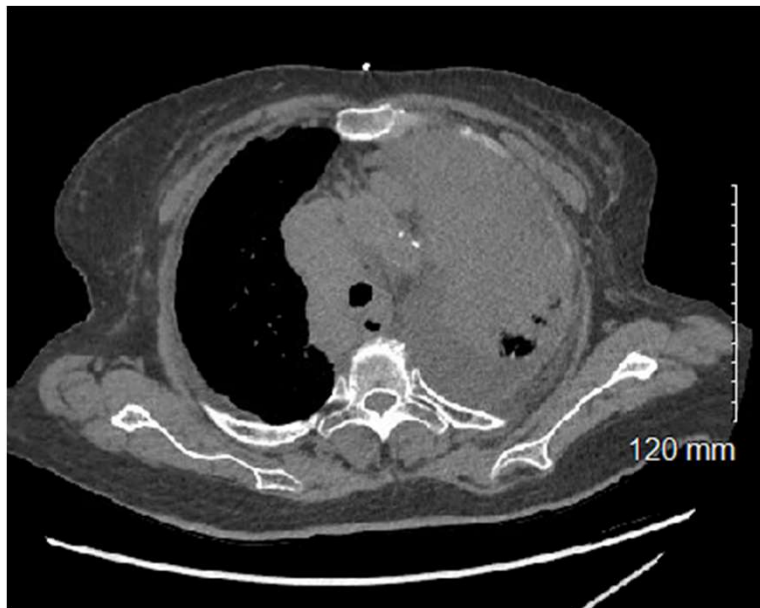


Trypsin



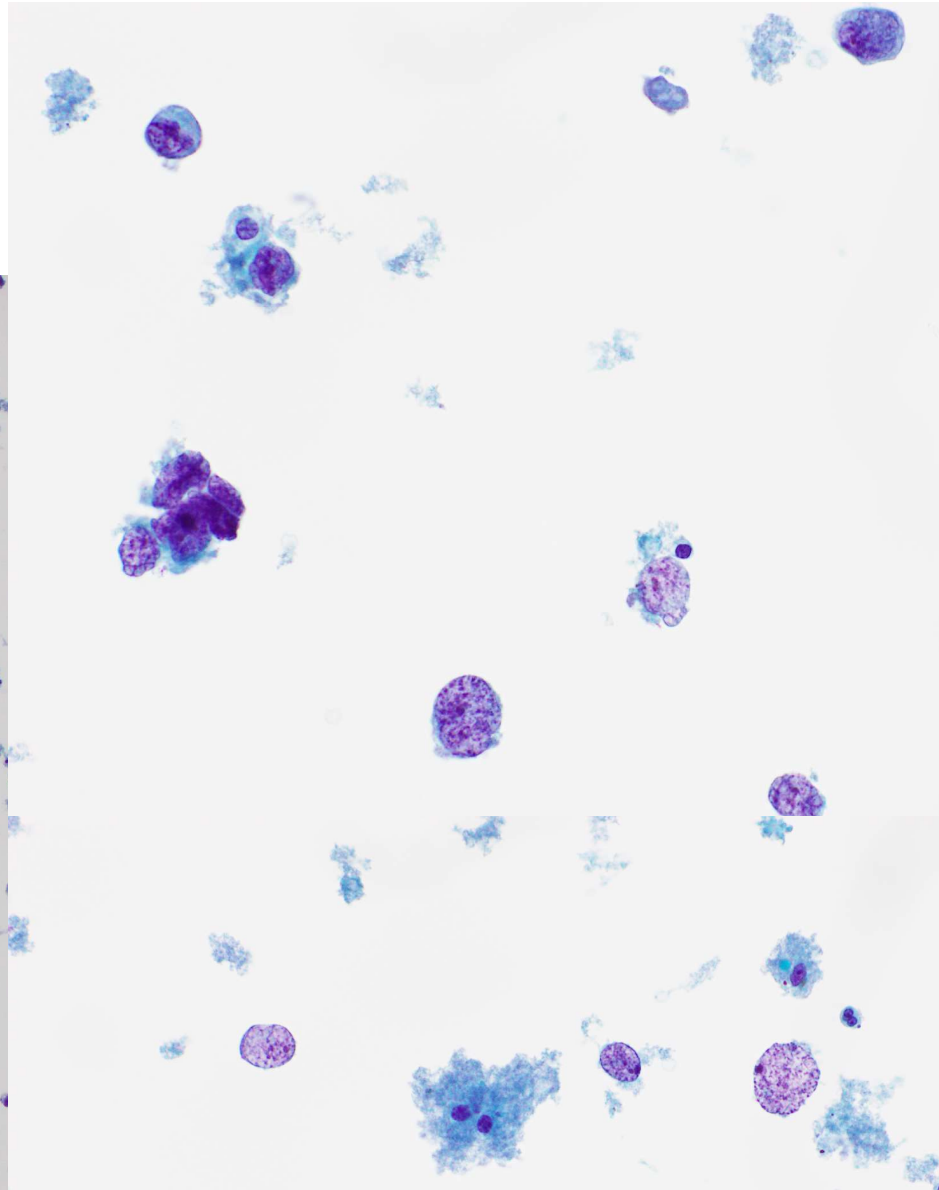
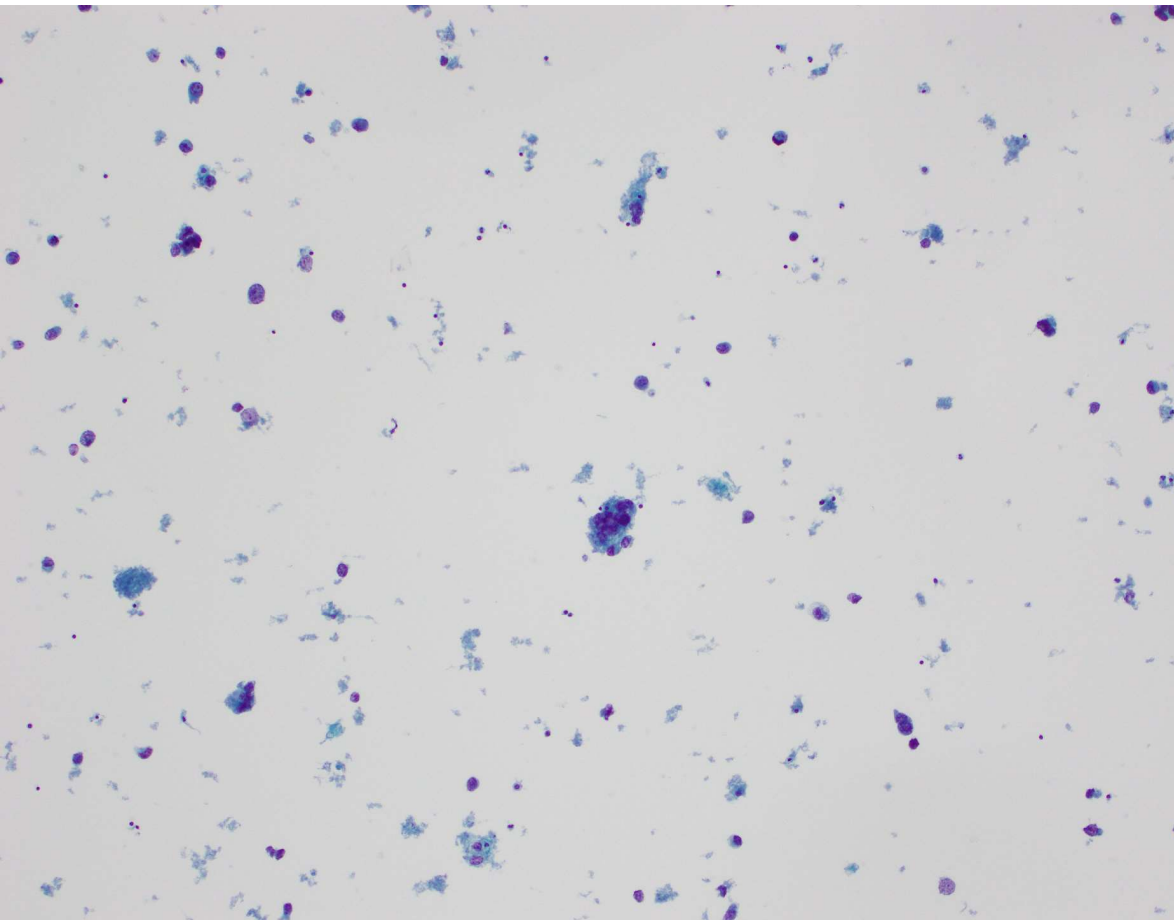
64 y/o F

- Presenting with left hilar mass and subcarinal adenopathy.
- CT scan shows multifocal disease in the right adrenal glands, bilateral kidneys, left retroperitoneal lymph nodes, omentum, abdominal mesentery, left chest wall, left paraspinal musculatures, right gluteal left.

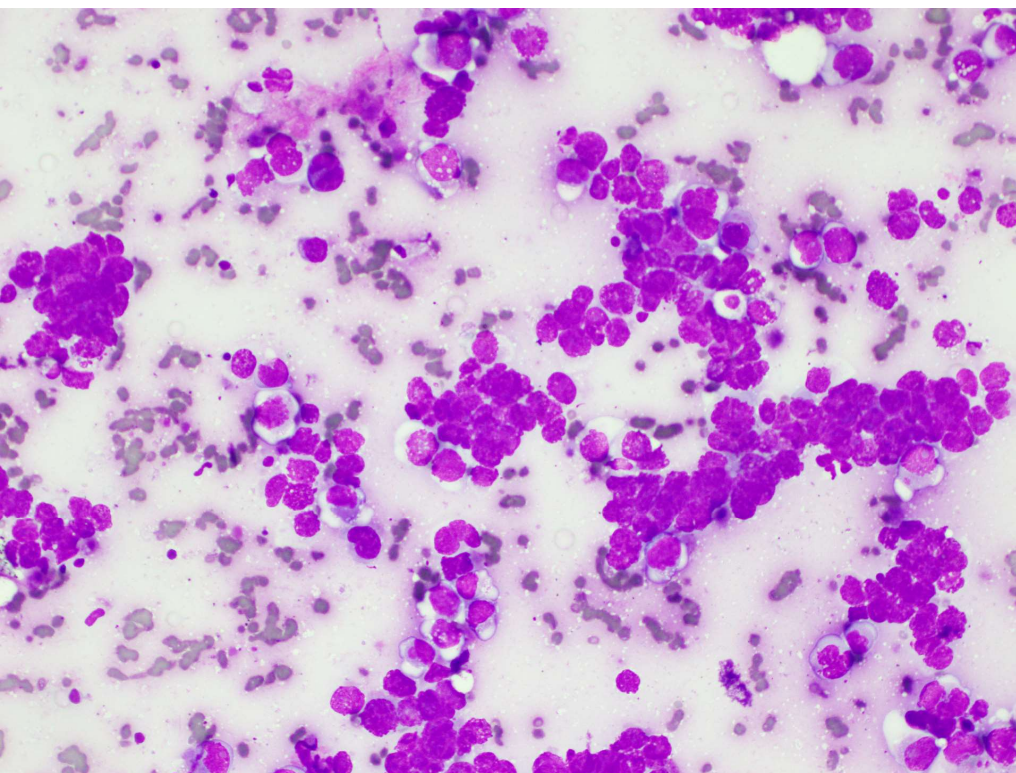




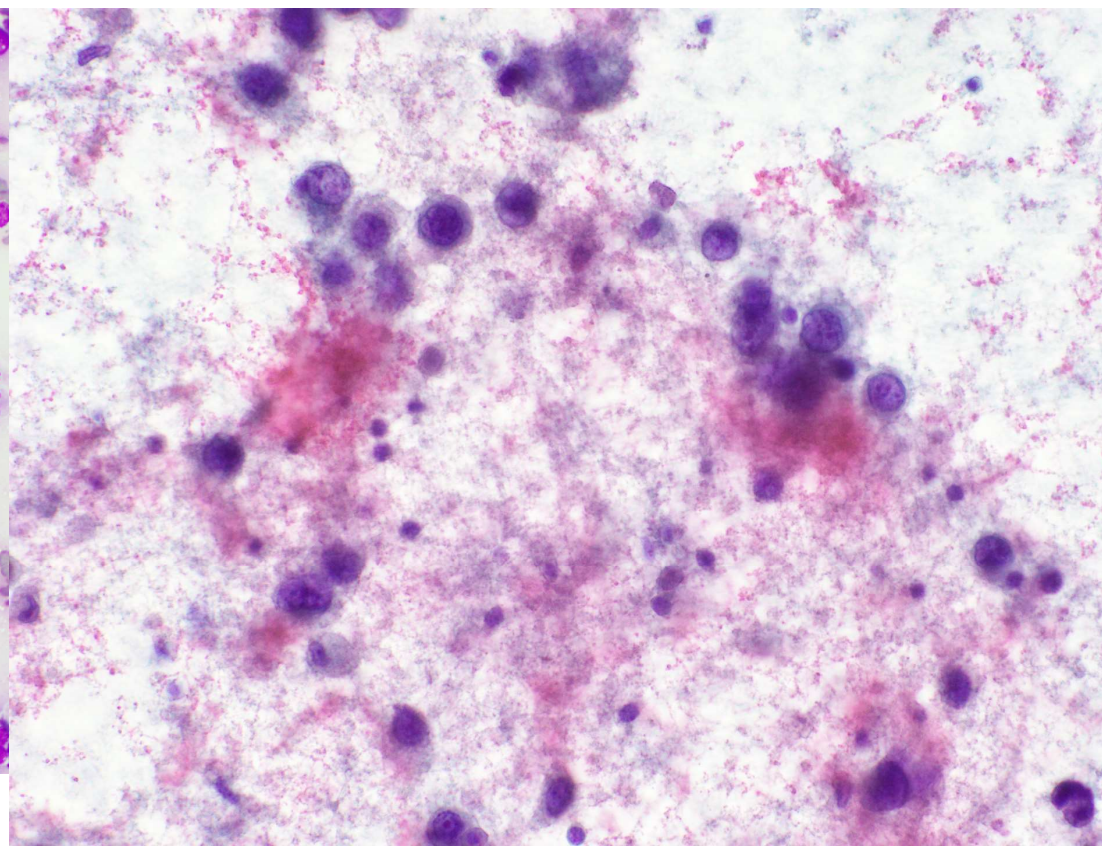
# Thin Prep



Diff-Quik

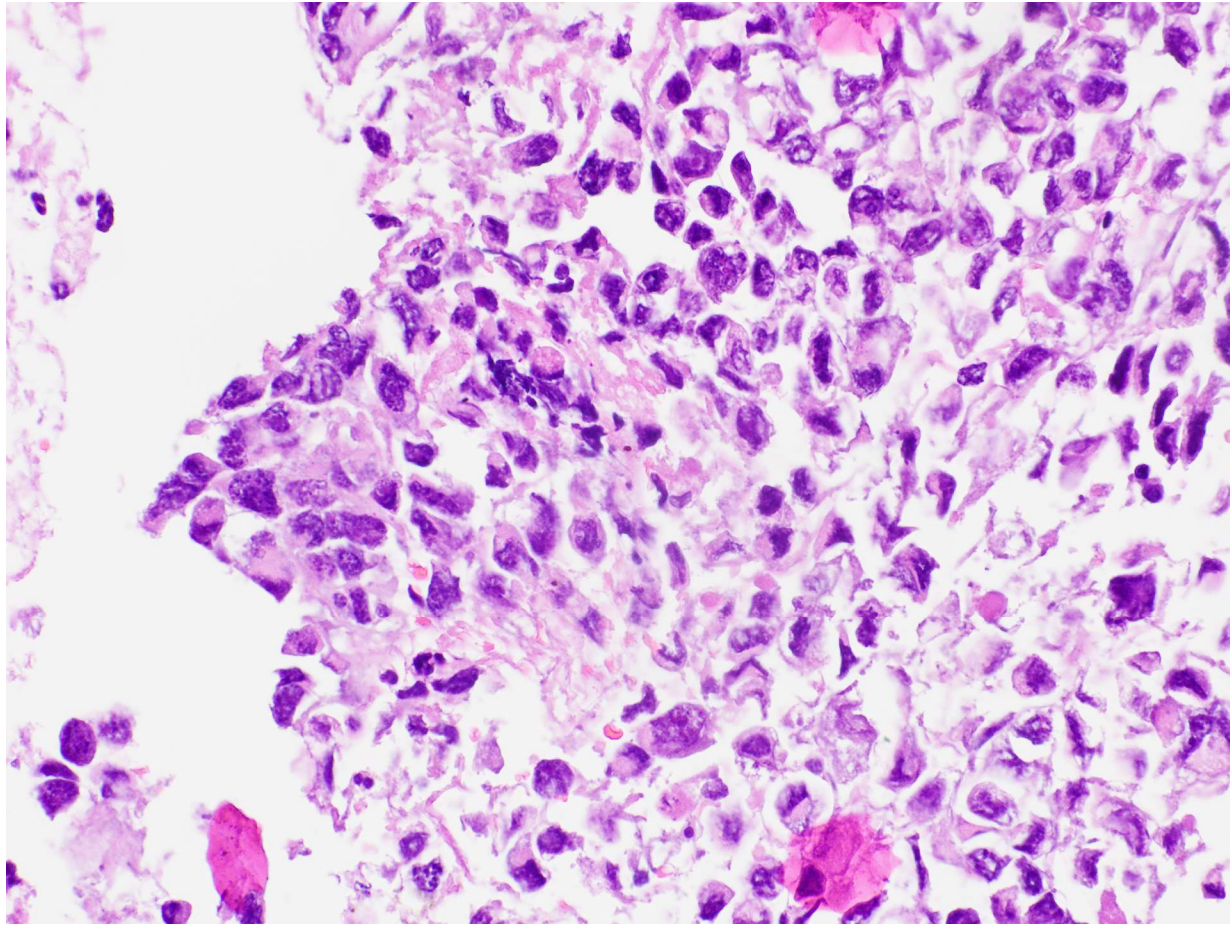


Pap





# Cell block



- The immunohistochemical staining pattern are as follows:
- Cam5.2 – Negative
- Pan-Keratin – Negative
- CK7 – Negative
- CK20 – Negative
- CK19 – Negative
- Synaptophysin – Negative
- Chromogranin – Negative
- CD56 – Negative
- TTF-1 – Negative
- P40 – Negative
- DOG-1 – Negative
- CD117 (c-KIT) – Negative
- CD99 – Negative
- CD30 – Negative
- CD45 – Negative in malignant cells /
  - highlights background lymphocytes
- Desmin – Negative
- HMB-45 – Negative
- S100 – Negative
- Mart-1 – Negative
- SOX-10 – Negative
- Myogenin – Negative
- MyoD1 – Negative
- Ki-67 – highlights >80% of the tumor cells (high index).
- CD31 – Negative in malignant cells / highlights blood vessels in tumor
- ERG – Negative
- CD34 – Positive (strong diffuse)
- BRG1 – loss of staining in malignant cells

**4R Lymph Node, (EBUS) Fine Needle Aspiration:**

- Positive for malignant cells.
- High grade sarcoma, most compatible with SMARCA4-deficient thoracic sarcoma.
- See comment.

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
SMARCA4	Seq	DNA-Tumor	Pathogenic Variant	c.2438+1G>C	1 /	c.2438+1G>C	88
TP53	Seq	DNA-Tumor	Pathogenic Variant	p.H179R	5	c.536A>G	92

# Lesson to learn

- Molecular testing will be much more routine in cytology specimens
- Trust yourself
- Every case is a TRAP!
- While common things being common, have to watch out for possibilities of rare entities after exclusion of everything else.