



# Challenging Cases and Lessons Learned Virtual Microscopy 3

Moderator: Jeffrey Mito, MD, PhD  
Lisa Zhang, MD, Kristine Wong, MD, Sarah Wu, MD, PhD,  
and Marina Vivero, MD

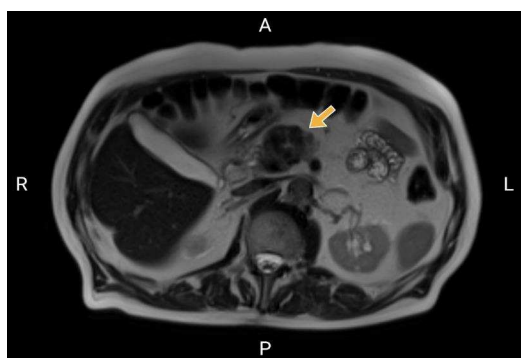
## Virtual Microscopy Case

M. Lisa Zhang, MD  
Massachusetts General Hospital  
Harvard Medical School

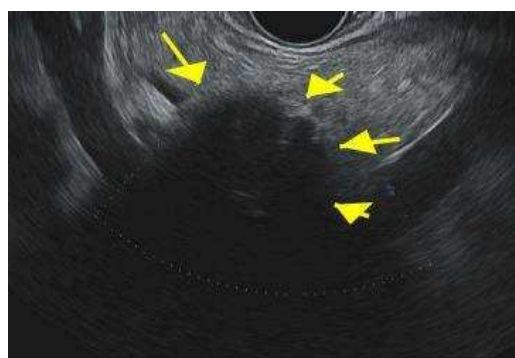
## Clinical History



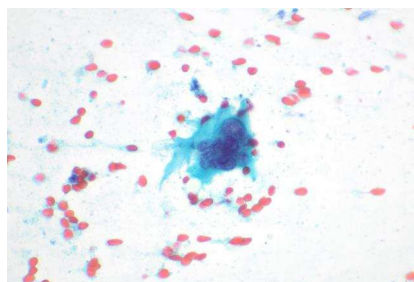
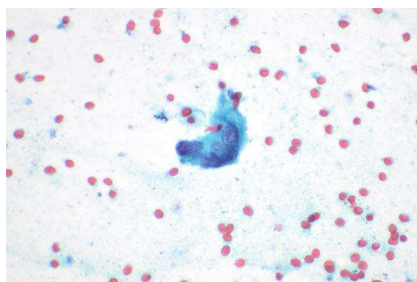
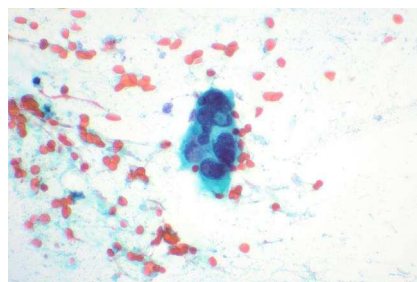
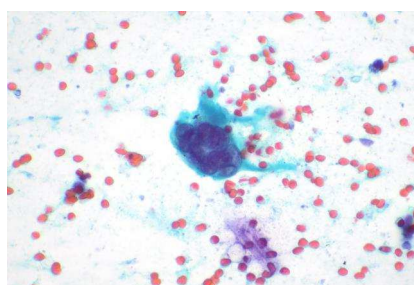
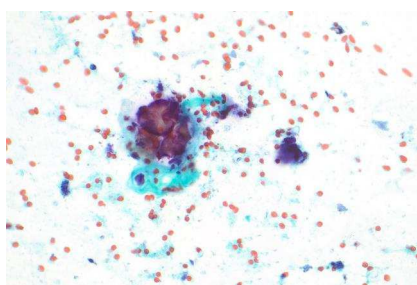
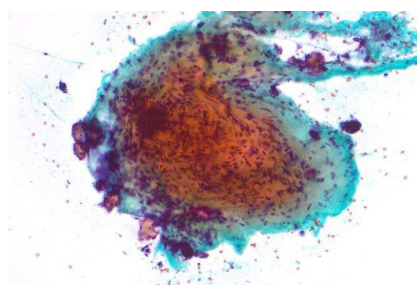
- 83-year-old man with incidentally found pancreatic neck mass

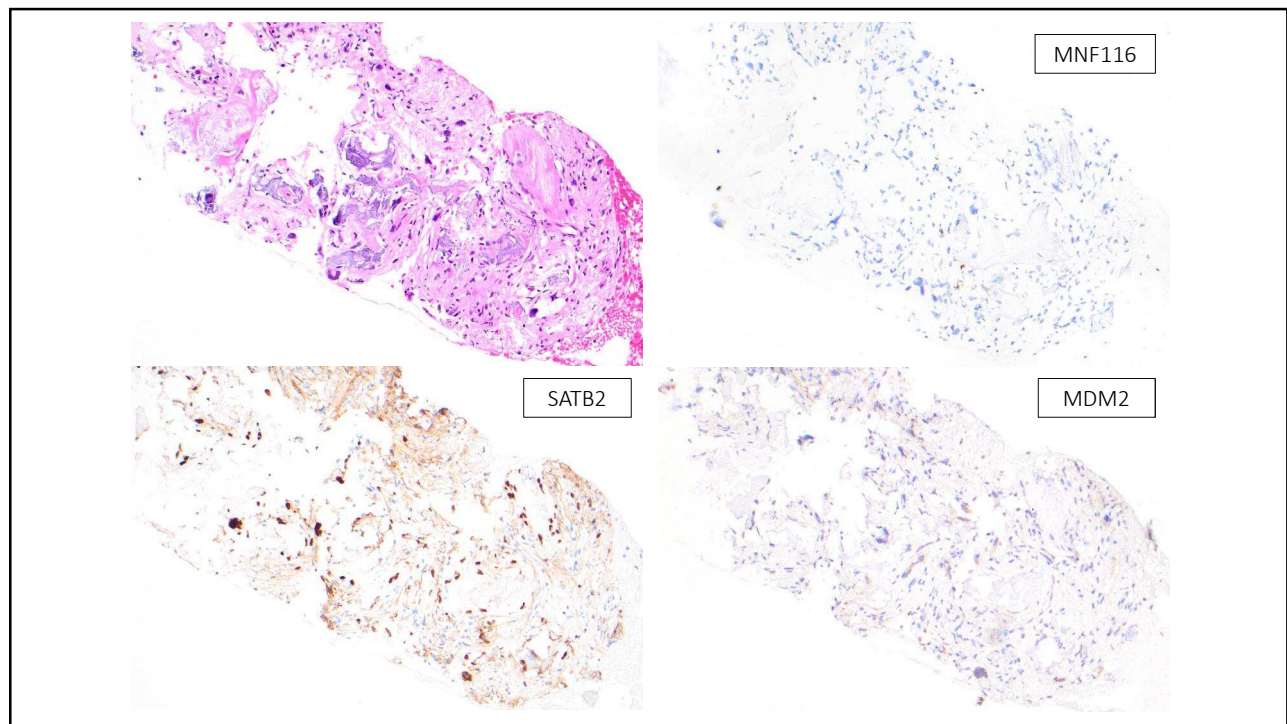


MRCP



EUS

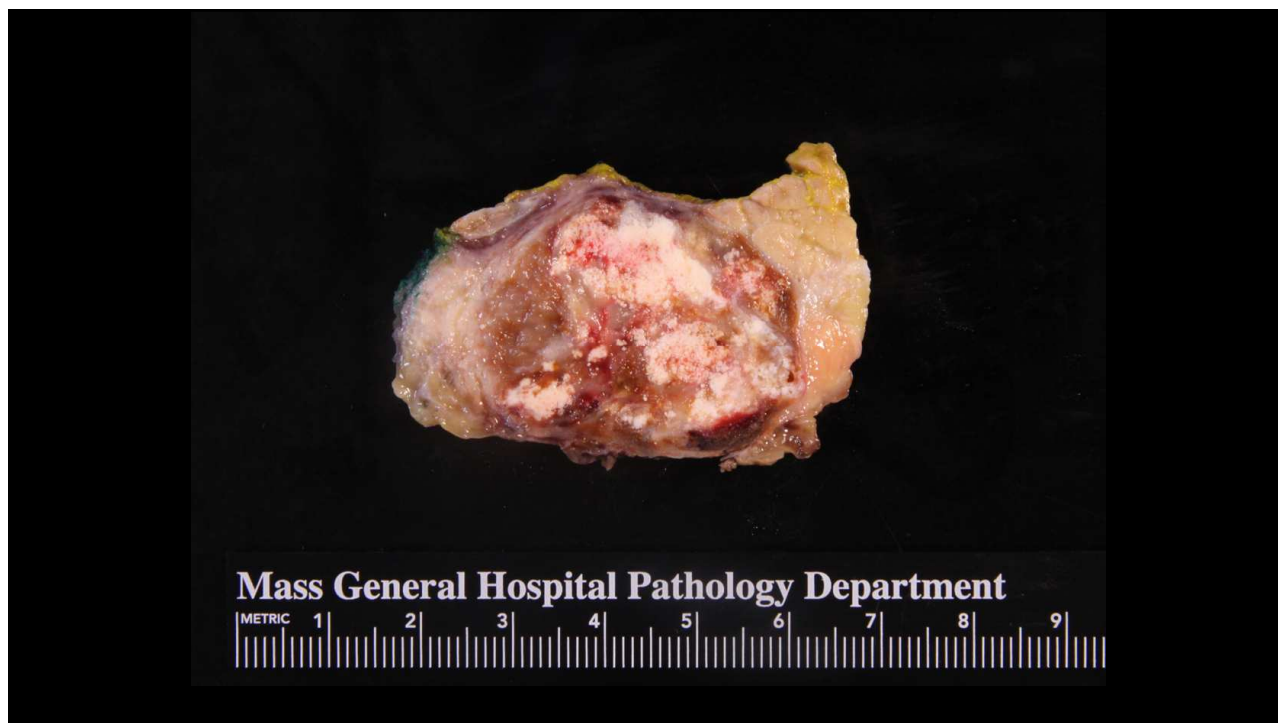
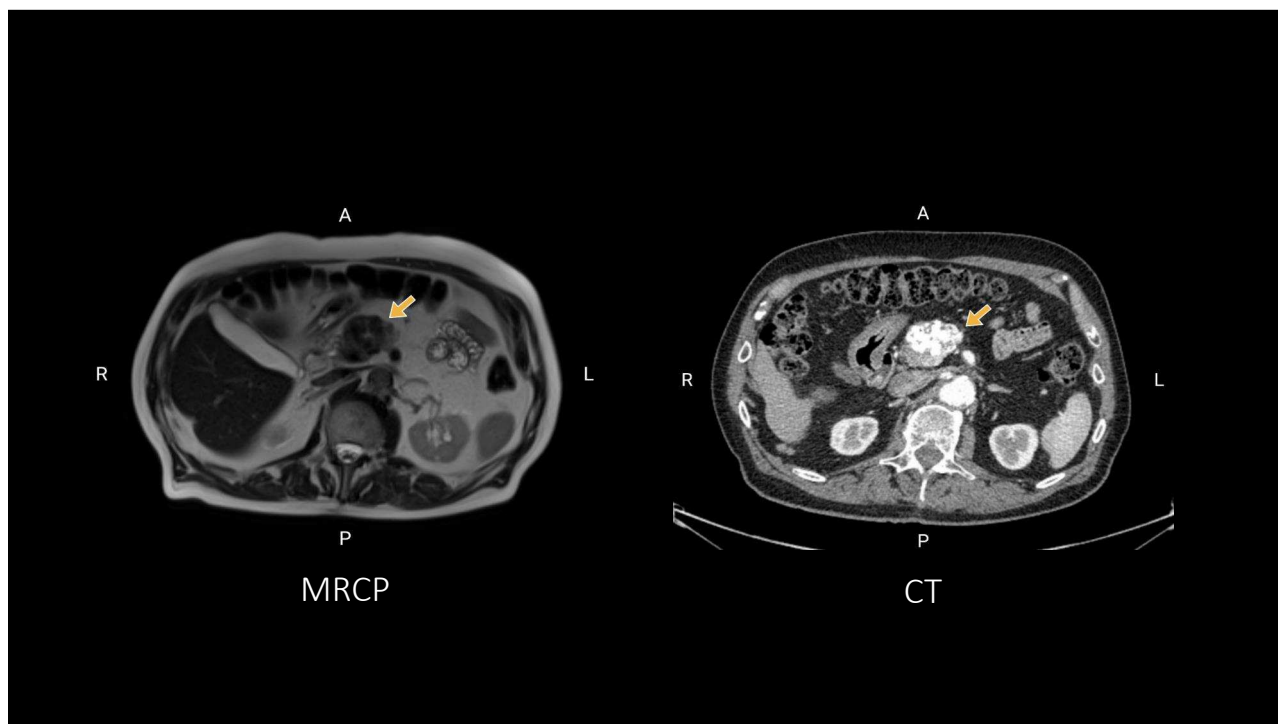




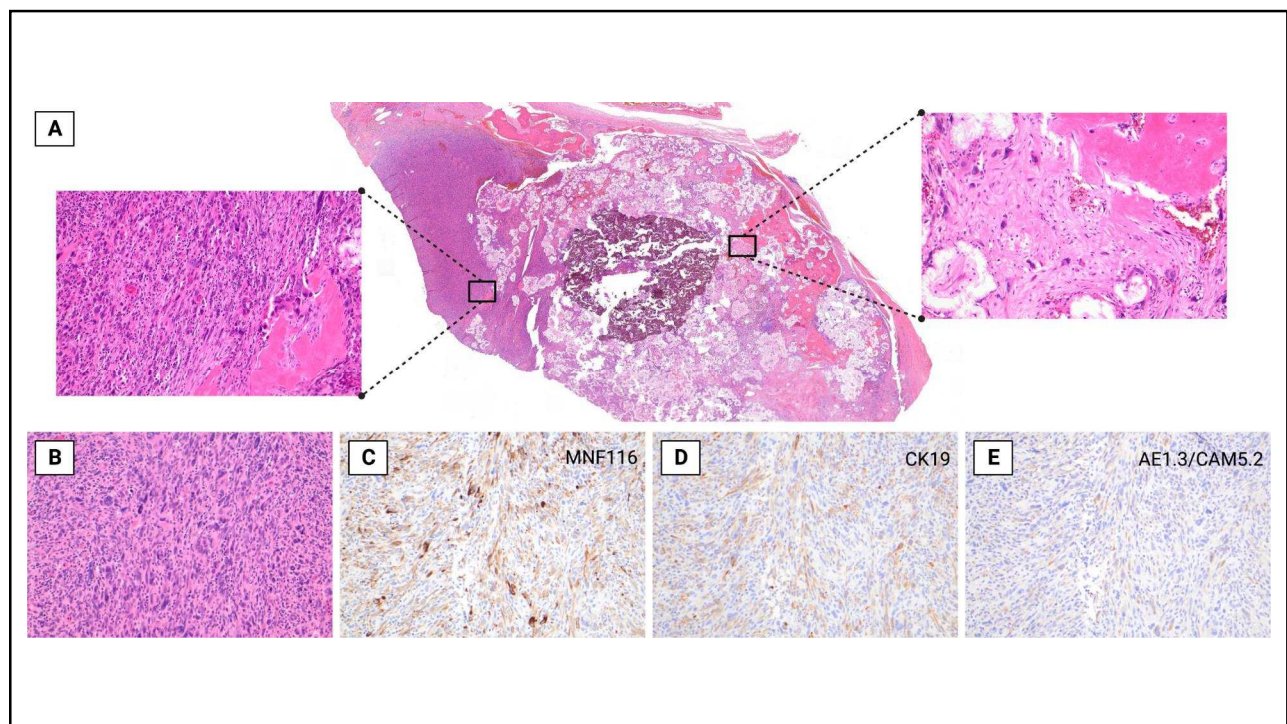
## “Malignant neoplasm with osteosarcomatous differentiation”

Differential:

- Sarcomatoid carcinoma (with carcinoma not sampled)
- Retroperitoneal liposarcoma with osteosarcomatous differentiation
- Intimal sarcoma arising from nearby large vessel (such as abdominal aorta)
- Metastatic osteosarcoma
- Primary extraskeletal osteosarcoma







## Histologic subtypes of pancreatic carcinoma

- Histologic subtypes
  - Adenosquamous
  - Colloid
  - Hepatoid
  - Medullary
  - Invasive micropapillary
  - Signet-ring cell
  - Undifferentiated
    - Anaplastic
    - Sarcomatoid
    - Carcinosarcoma
    - Osteoclast-like giant cells

“Sarcomatoid undifferentiated carcinoma” is defined by the WHO as a tumor composed of  $\geq 80\%$  spindle cell morphology that may contain heterologous elements such as bone and cartilage

## “Sarcomatoid undifferentiated carcinoma with heterologous elements (osteosarcomatous differentiation)”

### Sarcomatoid undifferentiated carcinoma (pancreas)

- Literature search showed 16 case reports (1987-2021)
  - *Anaplastic* and *osteoclast-like with giant cells* a little more common
- Case descriptions include: “spindle cells,” “pleomorphic giant cells,” and “adenocarcinoma” component
  - None describe specific heterologous elements
- Median overall survival ~9 months (range 0-27 months)
  - 5-year survival: 41%
  - 10-year survival: 21%
- Early diagnosis with R0 resection was the main predictor of long-term survival

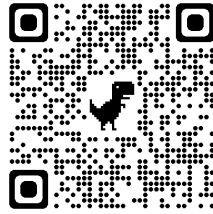
## Molecular findings (NGS)

- *KRAS* p.G12V (55% VAF)
  - *NTRK3* p.T261A (48% VAF)
  - *EPHA2* p.V69M (46% VAF)
  - *TP53* p.P191Sfs\*17 (22% VAF)
  - *TP53* p.Q192\* (21% VAF)
  - *BRCA2* p.L698P (21% VAF)
  - *MYD88* p.L302I (14% VAF)
  - *CBL* p.Q367K (9% VAF)
- Gkoutakos et al. 2022: NGS findings in 10 patients with pancreatic sarcomatoid undifferentiated carcinoma
    - Genetic alterations similar to those seen in conventional PDAC
    - All cases had *KRAS*, 90% had *TP53*, and 60% had *CDKN2A* mutations

## Summary

- EUS-FNA has relatively high sensitivity (73%-97%) and specificity (72%-100%) for solid pancreatic tumors
- Vast majority are conventional ductal adenocarcinomas (PDAC)
- Rare histologic subtypes of carcinoma can be challenging to diagnose on FNA/small biopsies
  - In this case, no conventional PDAC component was seen on biopsy
- However, FNA/B can provide an **early malignant diagnosis and prompt early resection**, which is most important for favorable patient outcomes
- This patient has been followed in clinic for almost 10 months without evidence of recurrent disease or metastasis

# Thank you!



Zhang ML et al. Diagn Cytopathol 2023;51(5):E164–9.

## *Advances in Cytology and Small Biopsies*

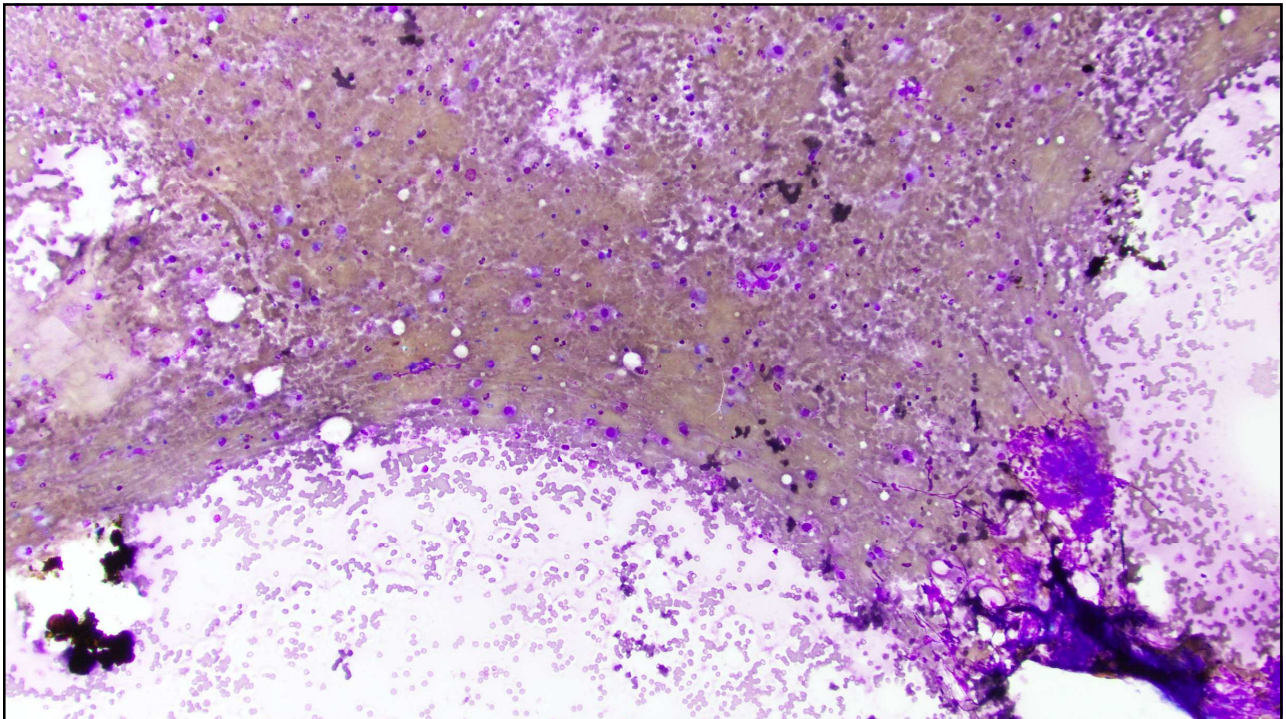
### Challenges and Lessons Learned

Kristine Wong, MD

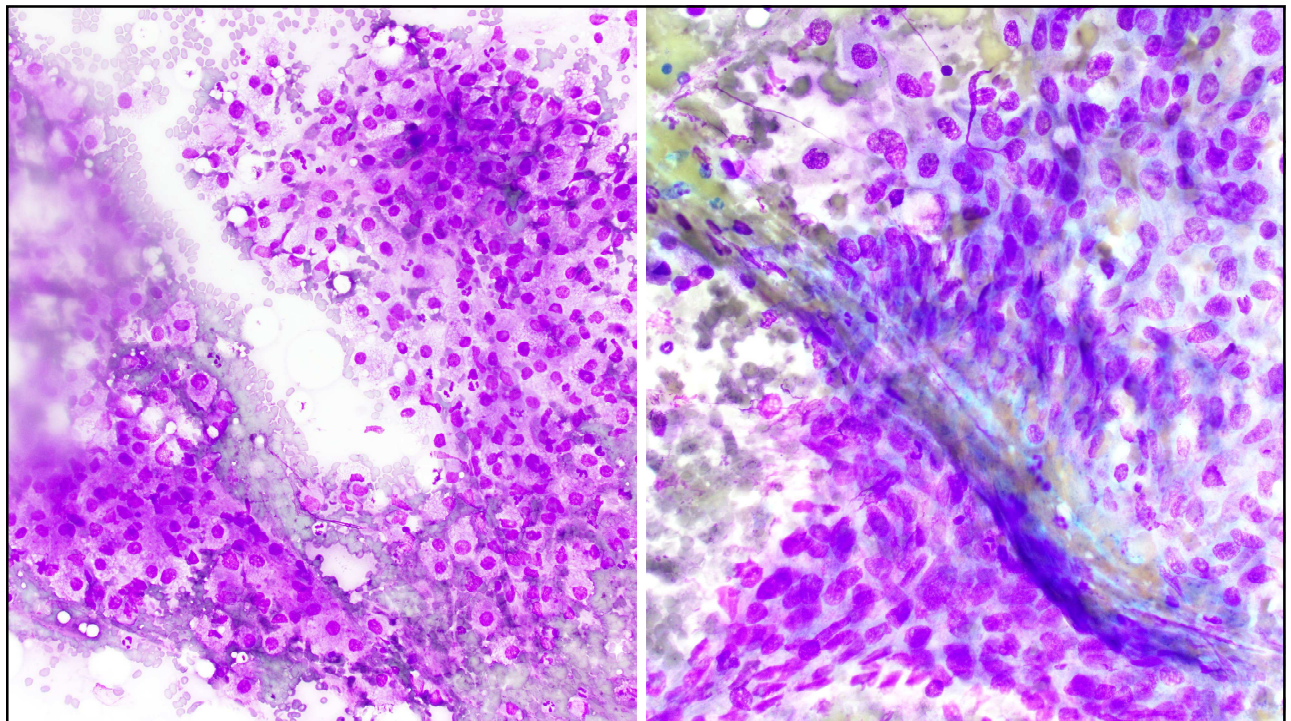
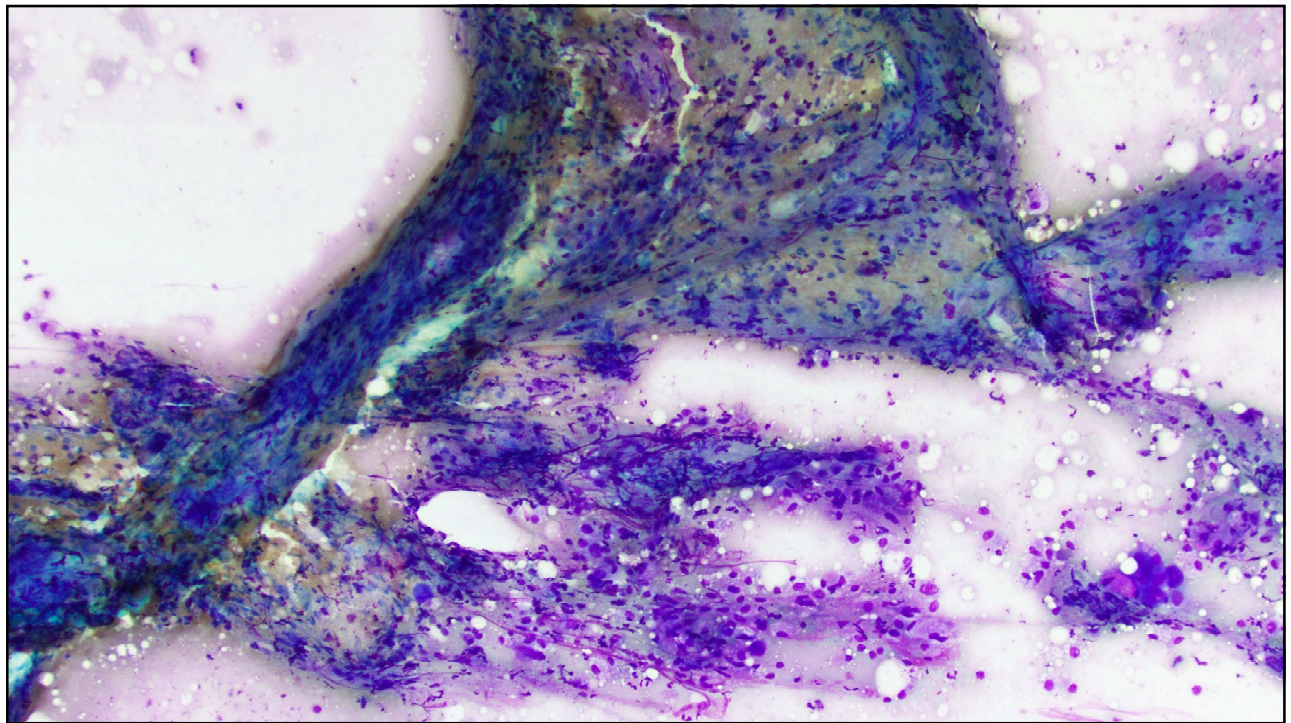
Department of Pathology, Brigham and Women's Hospital and  
Harvard Medical School

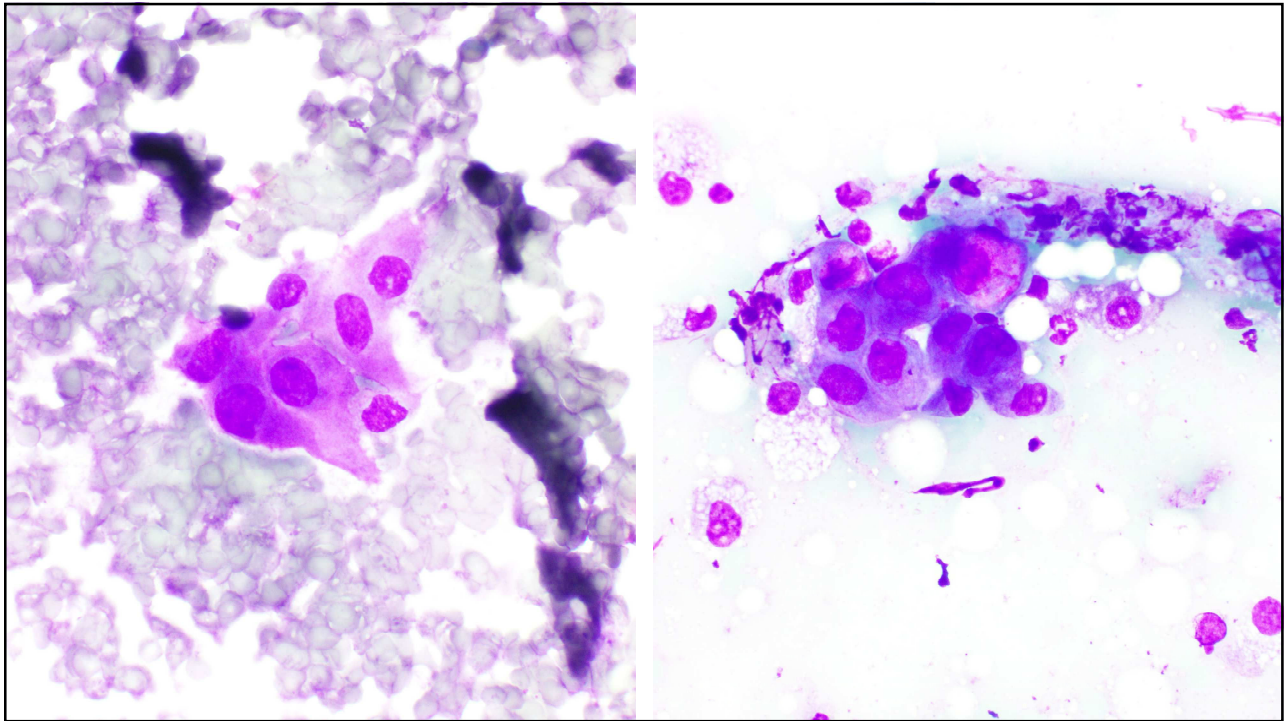
## Clinical history

- 70F with a history of invasive SCC of the oral cavity and Merkel cell carcinoma of the face
- Presented with a palpable mass near the angle of the mandible which had increased in size and was associated with pain
- Imaging showed a mass-like lesion in the parotid with possible bone erosion
- FNA was recommended









Diagnosis?



## Diagnosis?

- Milan system
  - Non-diagnostic
  - Non-neoplastic
  - AUS
  - Neoplasm
    - Benign
    - Uncertain malignant potential
  - Suspicious for malignancy
  - Malignant

## FINE NEEDLE ASPIRATION, ANGLE OF MANDIBLE:

### INTERPRETATION:

Suspicious for malignancy.

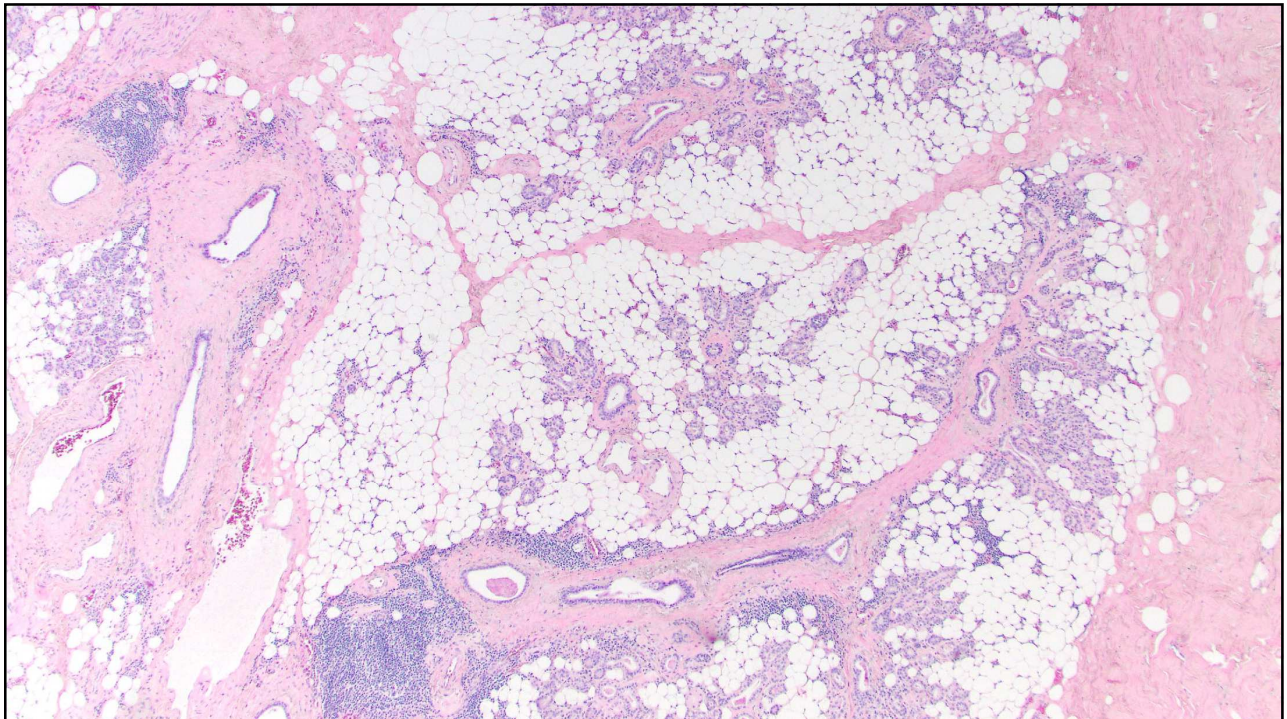
### DIAGNOSIS:

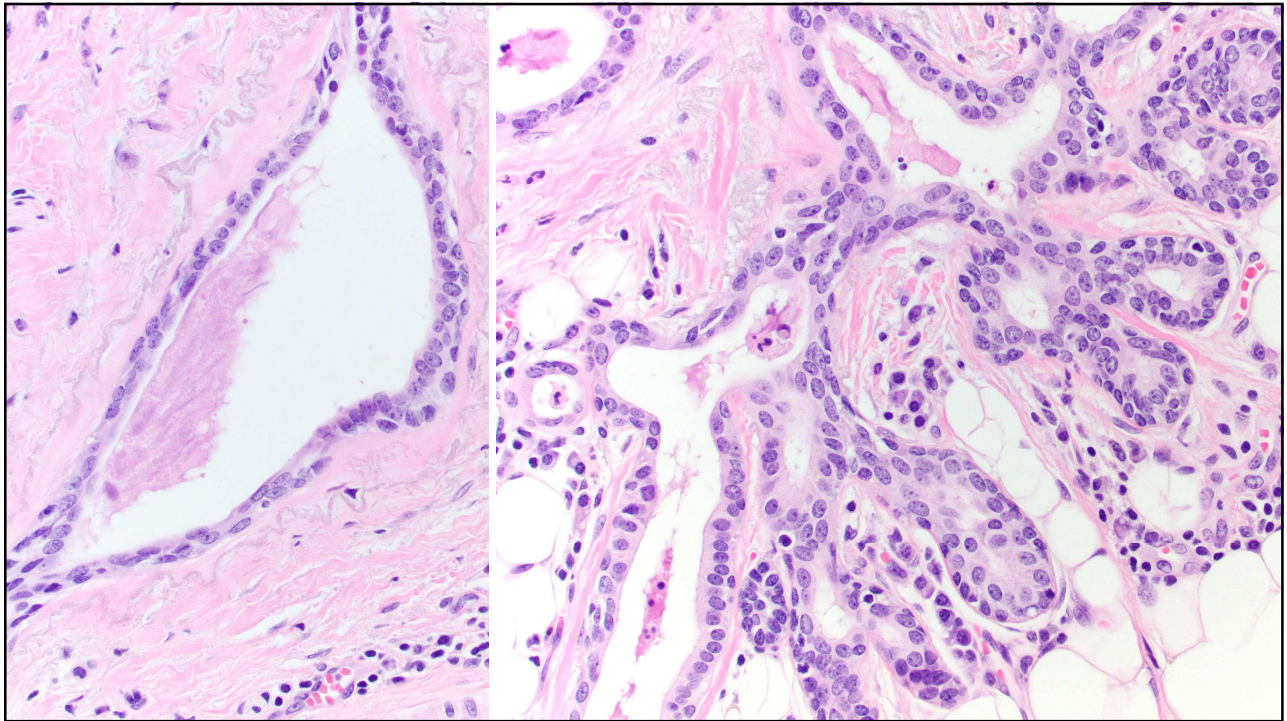
Suspicious for metastatic carcinoma, see note.

Note: **The suspicious cells are concerning for poorly differentiated carcinoma.** However, sparse cellularity precludes definitive diagnosis.

## Clinical history

- Given the concern for malignancy, resection was recommended
- The patient underwent a parotidectomy—intraoperatively, an ill-defined mass associated with scar was identified





#### LEFT PAROTIDECTOMY:


Chronic sialadenitis, see note.

Note: The findings are consistent with a non-specific chronic sialadenitis. **The findings of inflammation, reactive ductal atypia, and fibrous stroma would account for the findings in the prior fine needle aspiration biopsy.**



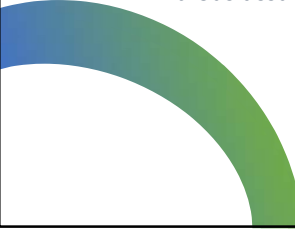


## Chronic sialadenitis

- Can present with pain, swelling, and/or an ill-defined mass
  - May be autoimmune (i.e. IgG4-related disease) or other etiology (commonly related to sialolithiasis)
  - Histologically can see florid fibrosis/sclerosis and lymphoplasmacytic inflammation, often with atrophy of acini and reactive ductal changes
- 



## Chronic sialadenitis on FNA

- Sparsely cellular
  - Inflammatory cells (including lymphocytes and histiocytes)
  - Scant epithelial elements (usually ductal, as acini are often atrophic)
    - May see metaplastic changes in ductal cells (i.e. squamous or mucinous metaplasia)
  - Fibrous tissue
- 

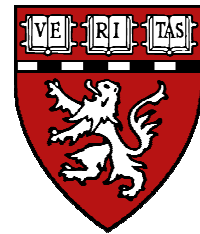
## Chronic sialadenitis on FNA

- Differential diagnosis
  - Normal salivary tissue (i.e. sampling error)
  - Neoplasms
    - Lymphocyte-rich tumors (i.e. Warthin tumor)
    - Sparsely cellular or cystic tumors (i.e. mucoepidermoid carcinoma)
    - Low-grade-appearing tumors (i.e. basaloid neoplasms)
    - High-grade carcinomas may also be considered if there is prominent reactive atypia

## Chronic sialadenitis on FNA

- Potential categorization using the Milan System (although will depend on cellularity and degree of atypia)
  - Non-diagnostic
  - Non-neoplastic
  - Atypia of undetermined significance

Questions?



# Virtual Microscopy

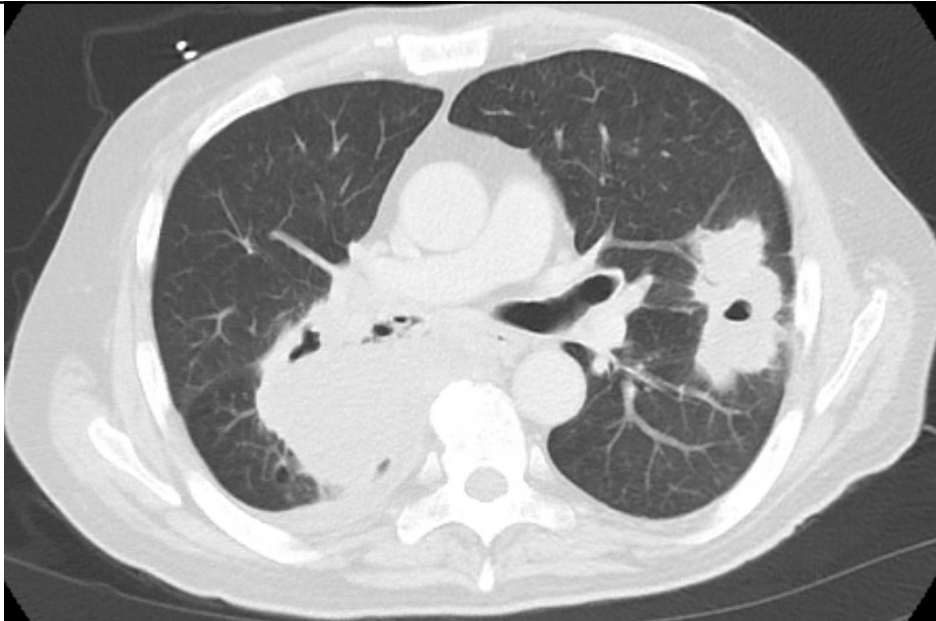
Sarah Wu

Advances in Cytology and Small Biopsies

June 14<sup>th</sup>, 2023

## Case Vignette

- 65M, never smoker, with a past medical history significant for A. Fib (on Eliquis), hypotension, iron deficiency anemia, COPD, Crohn's disease, GERD and asthma.
- He presented with unintentional weight loss, fatigue, weakness, chronic cough with blood-tinged sputum, and dyspnea. Imaging demonstrated bilateral pulmonary nodules.
- Two prior biopsies were attempted.
  - #1: Lung parenchyma with lymphohistiocytic chronic inflammation and focal necrosis with acute inflammation.
  - #2: Necrotic debris only, no viable cellular material for evaluation.



CT Chest: In addition to the large right hilar mass, which extends into the central right lower lobe, there are multiple additional bilateral pulmonary nodules and masses.

You are paged to perform ROSE on the EBUS-guided FNA of the right hilar mass...



What is your impression at ROSE?

- A. Non-diagnostic
- B. Negative
- C. Atypical
- D. Suspicious
- E. Positive for malignancy



You receive the slides for the concurrent biopsy the next day...

What stains would you like?

- A. AE1/AE3
- B. TTF-1
- C. CD45
- D. SOX10
- E. Desmin

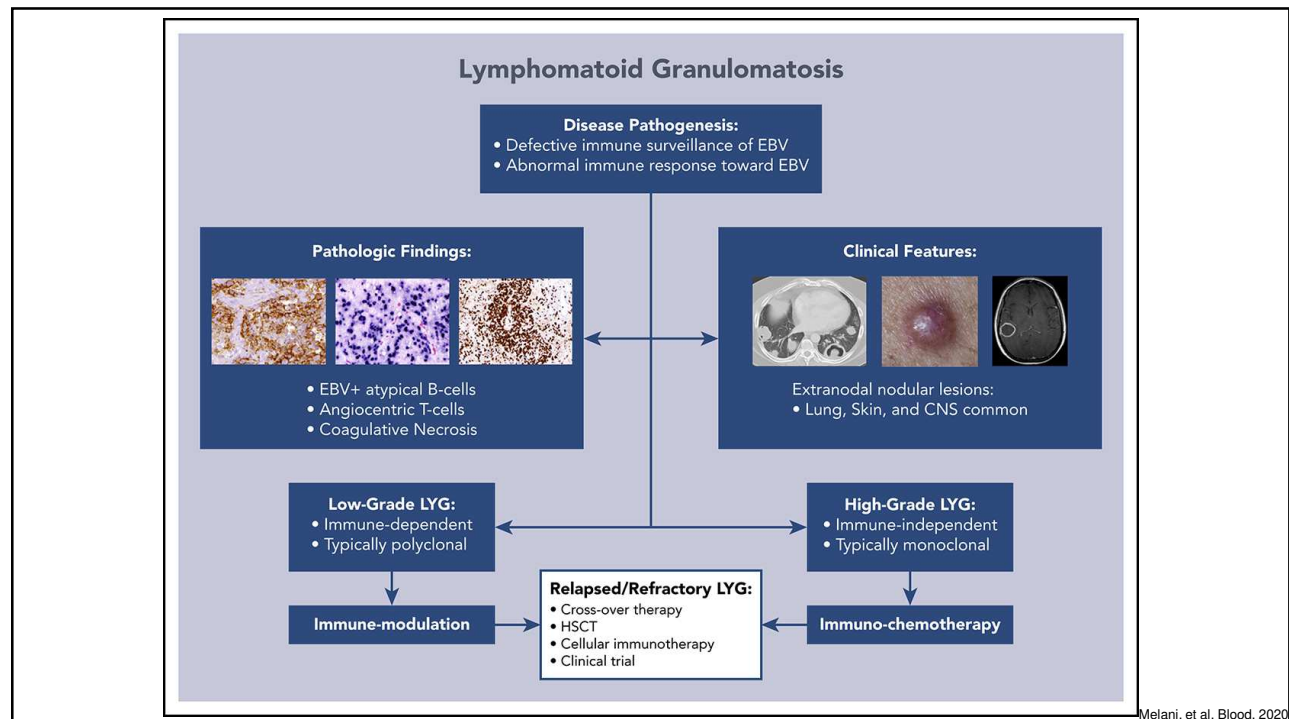
Diagnosis:

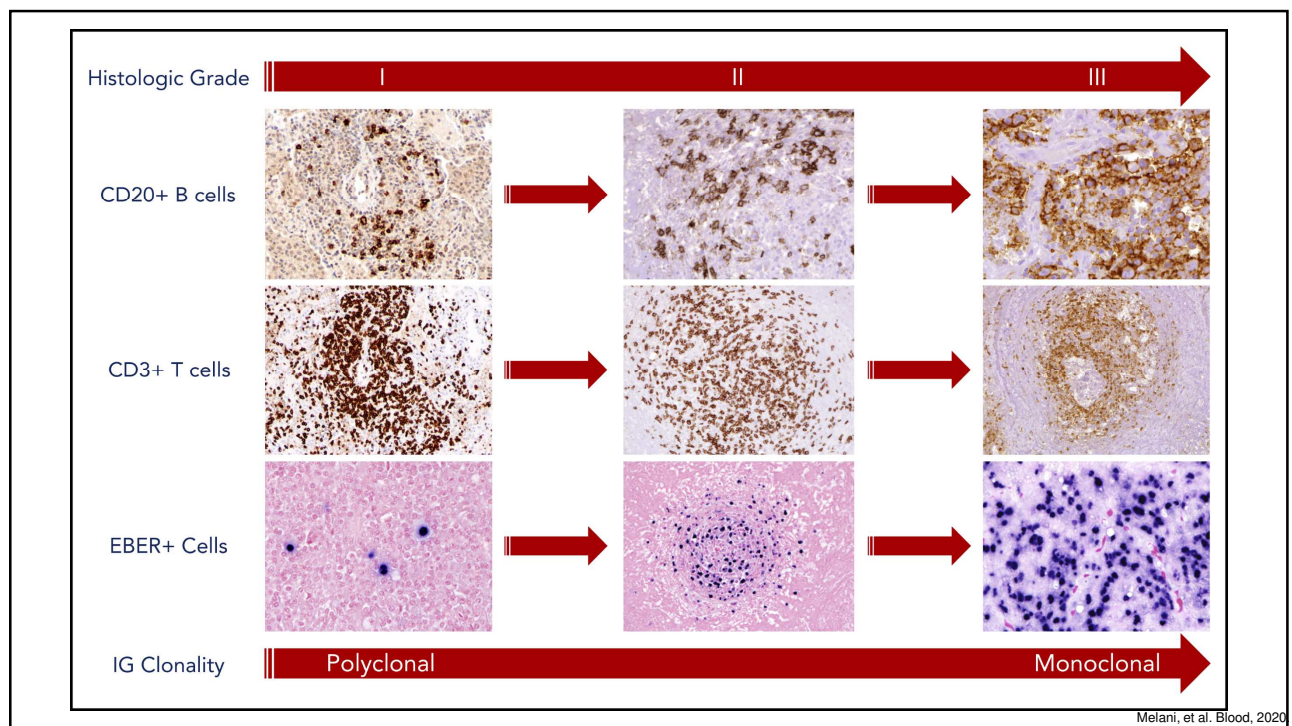
Consistent with EBV-positive B cell  
lymphoproliferative disorder.

A wedge resection was performed...

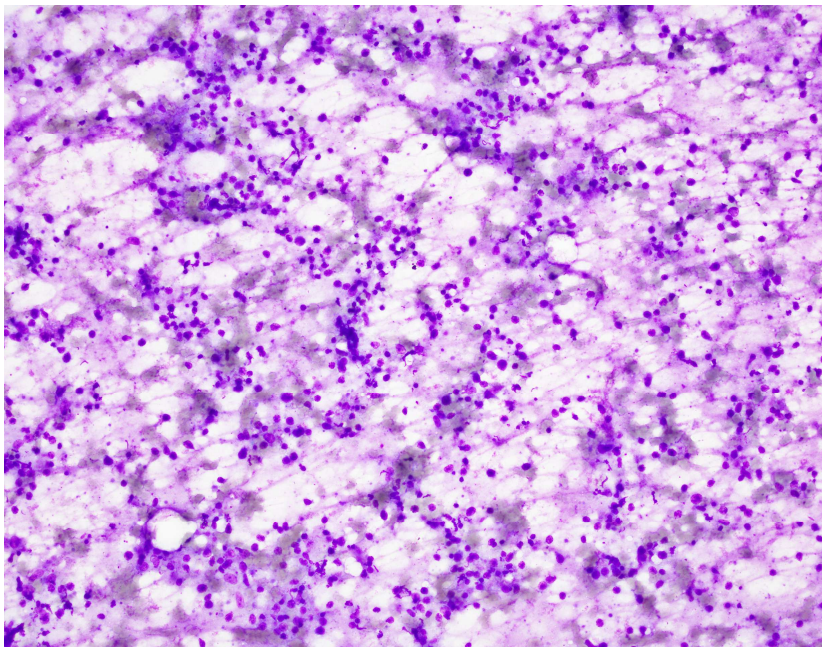
## Diagnosis:

EBV-positive B cell lymphoproliferative disorder with extensive necrosis, in clinicopathologic context most in keeping with Lymphomatoid Granulomatosis



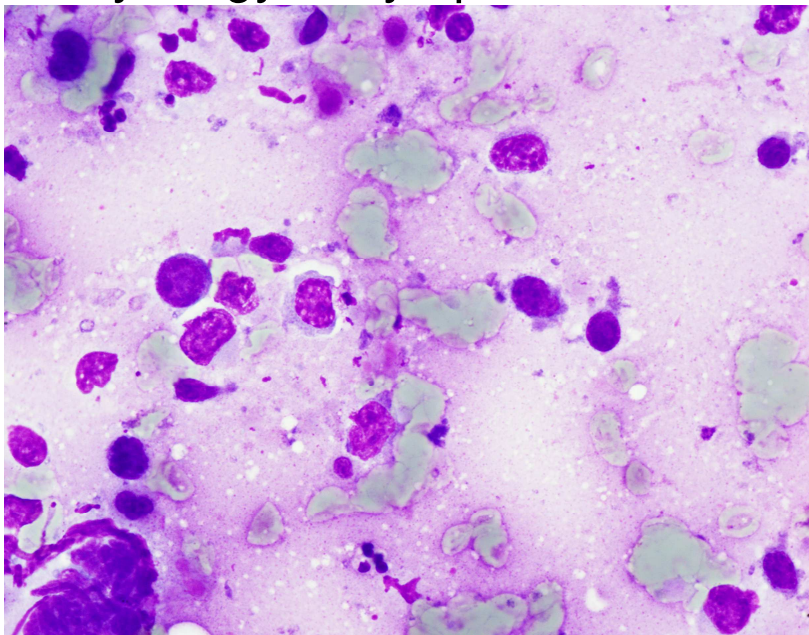


## Cytology of Lymphomatoid Granulomatosis



- Necrotic background
- Scattered lymphocytes

## Cytology of Lymphomatoid Granulomatosis



- Admixture of lymphocytes, some with pronounced atypia

<b>Infectious lung diseases</b>	
Mycobacteria	Tuberculosis and nontuberculous mycobacteriosis
Fungal infection	<i>Cryptococcus</i> , <i>Coccidioides</i> , <i>Histoplasma</i> , <i>Blastomyces</i> and <i>Aspergillus</i>
Aspiration pneumonia	
Others	Syphilis, Hansen's disease (leprosy), tularemia, cat scratch disease, parasitic infections and Whipple's disease
<b>Noninfectious lung diseases</b>	
Inflammatory	Sarcoidosis
	Necrotising sarcoid granulomatosis
	Bronchocentric granulomatosis
	Inflammatory bowel disease
Exposure/toxins	Hypersensitivity pneumonitis
	Drugs (methotrexate, interferon, Bacillus Calmette-Guérin, infliximab, etanercept, leflunomide, mesalamine and sirolimus)
	Hot tub lung
	Berylliosis
	Talc
	Metals (aluminium and zirconium)
	Foreign body reaction
Vasculitis	Granulomatosis with polyangiitis
	Eosinophilic granulomatosis with polyangiitis
Autoimmune diseases	Rheumatoid nodule
Malignancy	Sarcoid-like lesions
	Lymphomatoid granulomatosis
Others	Pulmonary Langerhans cell histiocytosis Granulomatous-lymphocytic interstitial lung disease

## Lessons Learned

- The differential for cytology of a necrotic lung nodule can be broad.
- Patient demographics and history are important in forming your differential diagnosis.
- Lymphomatoid granulomatosis is a rare diagnosis and the presentation can be non-specific with sparse lesional cells.
- Requesting additional biopsy tissue can be helpful during rapid on-site evaluation of these lesions.

## Challenges and Lessons Learned: Virtual Microscopy

Marina Vivero, MD

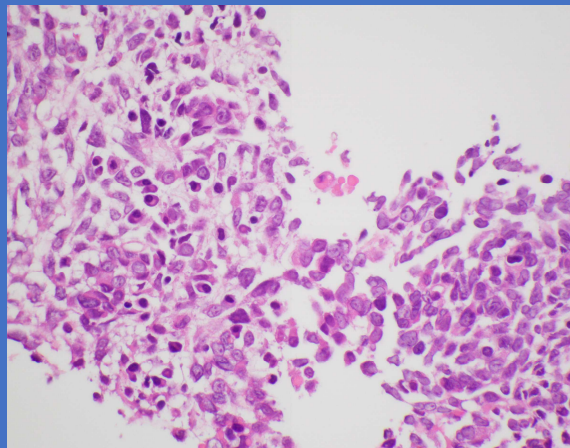
June 14, 2023



## Case 4

- 62 year old female never-smoker with a 2.2 cm intramural esophageal mass
- No clinical history of malignancy
- No evidence of disease in any other organs
- Initial outside biopsy showed the following IHC profile:
  - POSITIVE: Pan-K (focal), AE1/AE3 (focal), Cam5.2 (focal), EMA (focal), CD56, synaptophysin (weak), Ki-67 (15%)
  - NEGATIVE: CK5/6, CK20, p40, chromogranin, Cd1a, INSM1, NUT, TTF-1, DOG1, SOX10
- Initial diagnosis: **neuroendocrine tumor**, intermediate to high grade.
- Repeat biopsy and esophageal brushing 5 months later.

## Case 4



IHC:

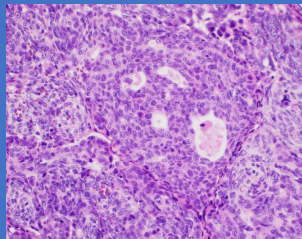
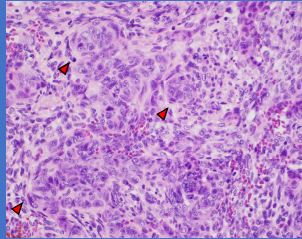
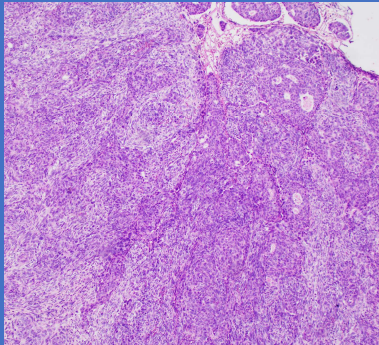
POSITIVE: p40 (rare cells), Cyclin D1 (weak, patchy)

NEGATIVE: p63, CD5, C-Kit



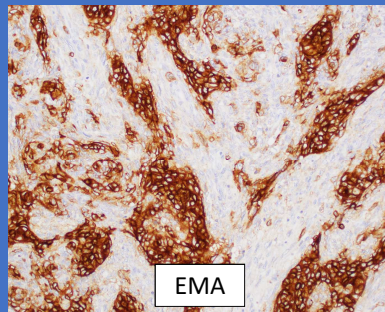
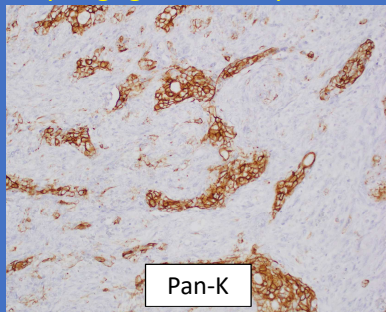
## Case 4

Esophagogastrectomy:



## Case 4

Esophagogastrectomy:



IHC:

POSITIVE: Pankeratin, EMA

NEGATIVE: p40, p63, chromogranin, synaptophysin,  
INSM1, SOX10, ERG, S100, GFAP, B-catenin

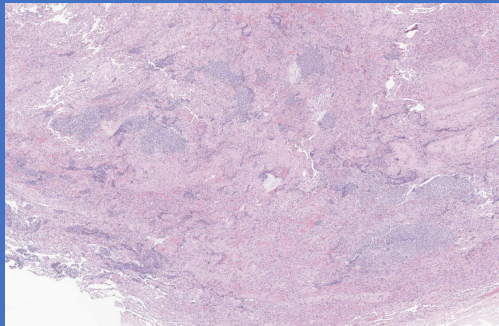
## Case 4

Diagnosis?

- A) Sarcomatoid adenocarcinoma
- B) Primary sarcoma
- C) Metastatic porocarcinoma
- D) Sarcomatoid squamous cell carcinoma

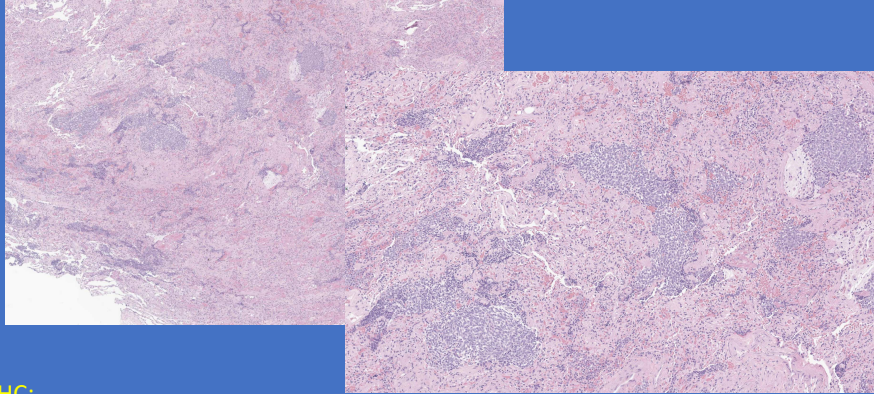
## Case 4

- Follow-up CT scan showed a 0.4 cm right lower lobe nodule.



## Case 4

- Follow-up CT scan showed a 0.4 cm right lower lobe nodule.

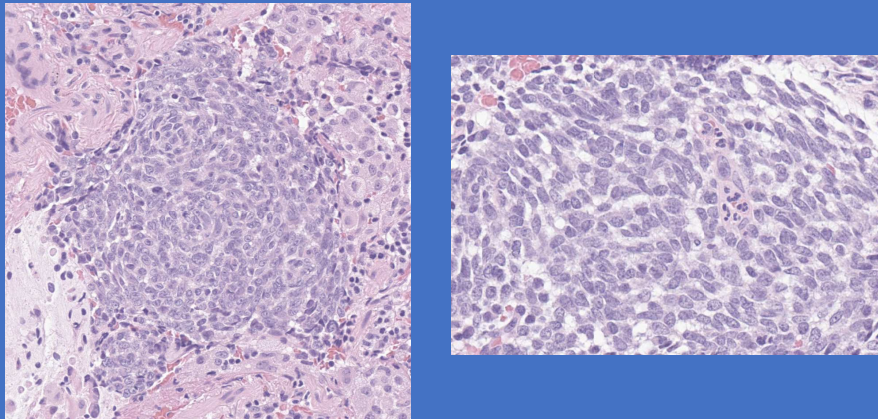


IHC:

POSITIVE: AE1/AE3 (focal), Pan-K (focal), CAM5.2 (focal), EMA (patchy)

NEGATIVE: TTF-1, INSM1, synaptophysin, chromogranin

## Case 4

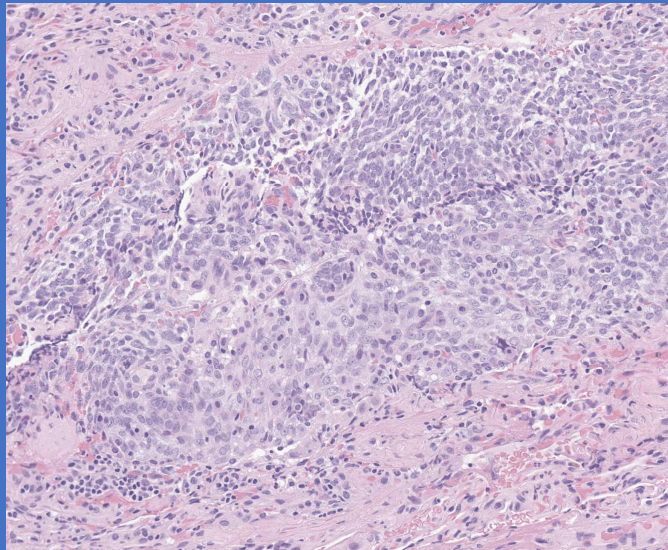


IHC:

POSITIVE: AE1/AE3 (focal), Pan-K (focal), CAM5.2 (focal), EMA (patchy)

NEGATIVE: TTF-1, INSM1, synaptophysin, chromogranin

## Case 4



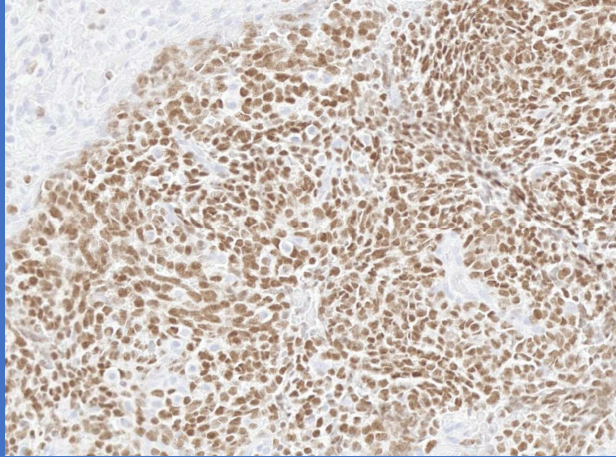
## Case 4

Should we do one more stain?

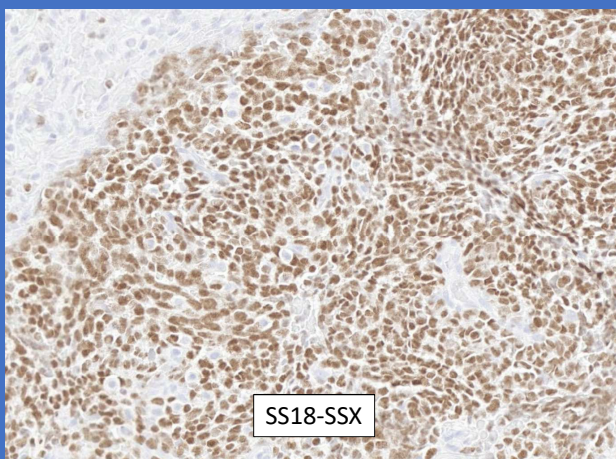
- A) YAP1 c-terminus (porocarcinoma)
- B) SS18-SSX (synovial sarcoma)
- C) Repeat p40
- D) Claudin-4



## Case 4



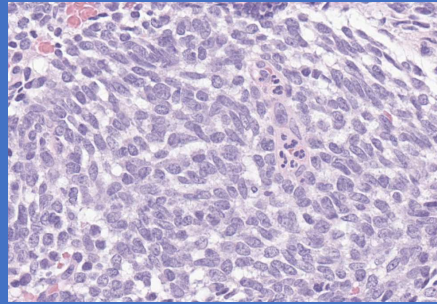
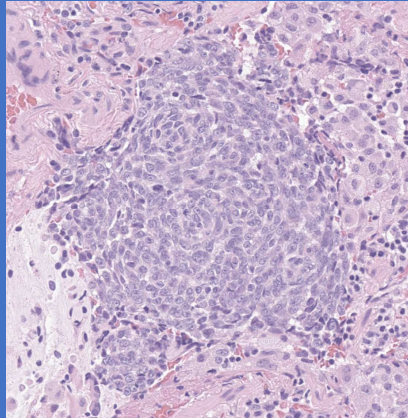
## Case 4



SS18-SSX



## Synovial Sarcoma



## Synovial Sarcoma

- Tumors in the upper digestive tract are rare
  - ~5% intrathoracic
  - <20 cases in the literature, primarily to case reports
- Differential diagnosis
  - Sarcomatoid carcinoma
  - GIST
  - Leiomyosarcoma
  - Mesothelioma
  - Solitary fibrous tumor
- t(X;18), SS18 fusion with SSX1, SSX2, SSX3
  - Cytogenetic or molecular testing
  - IHC: SS18-SSX, SSX C-terminus
- IHC: EMA (variable, focal), Keratin (variable); TLE1, BCL-2 less specific

## Case 5

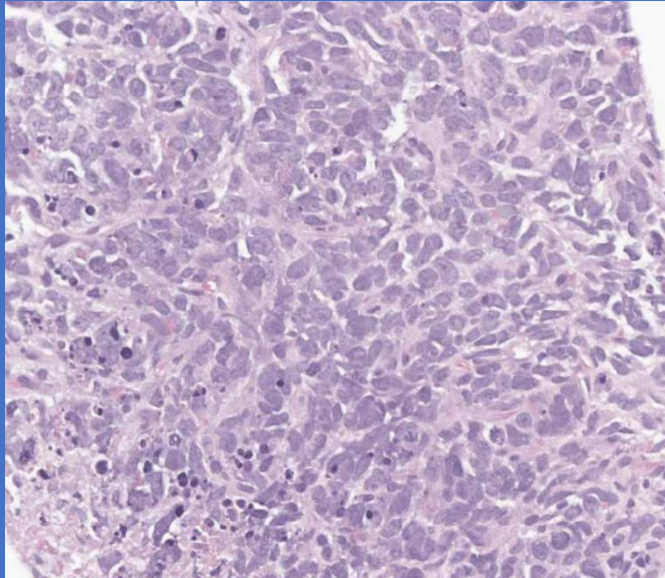
- 44 year old woman with a 16 pack-year smoking history and a 3.7 cm left upper lobe mass and mediastinal lymphadenopathy
- No prior history of malignancy
- Underwent core needle biopsy of lung mass and fine needle aspiration of an enlarged level 4 lymph node

## Case 5

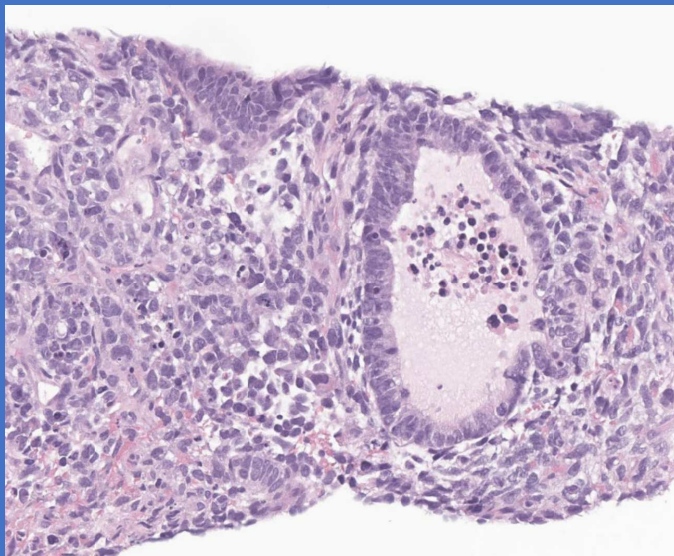
Favored Diagnosis?

- A) Sarcomatoid non-small cell carcinoma
- B) Combined NSCLC and SCLC
- C) Mesothelioma
- D) Sarcoma

## Case 5



## Case 5

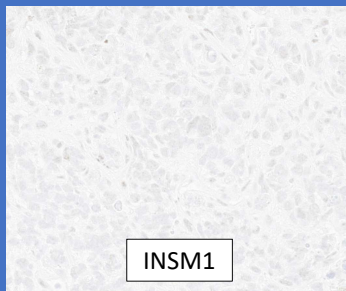
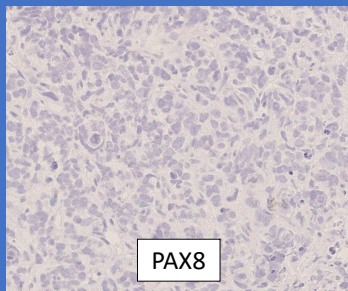
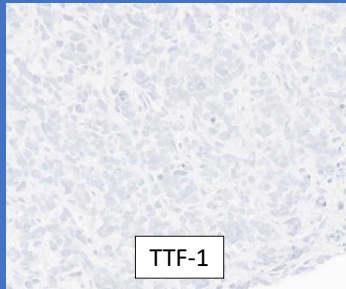
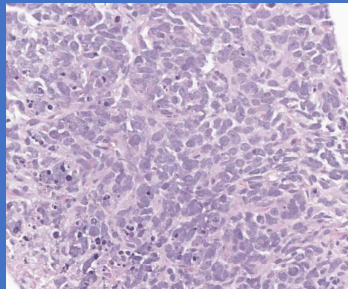


## Case 5

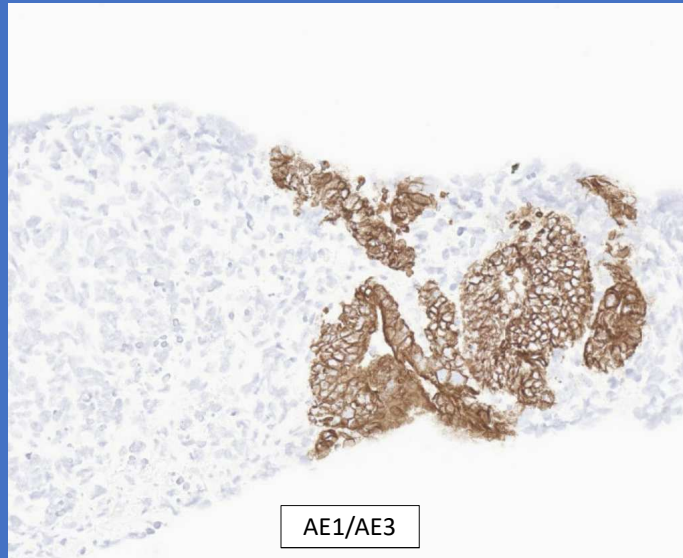
What stain to do next?

- A) INSM1
- B) TTF-1
- C) B-catenin
- D) PAX8

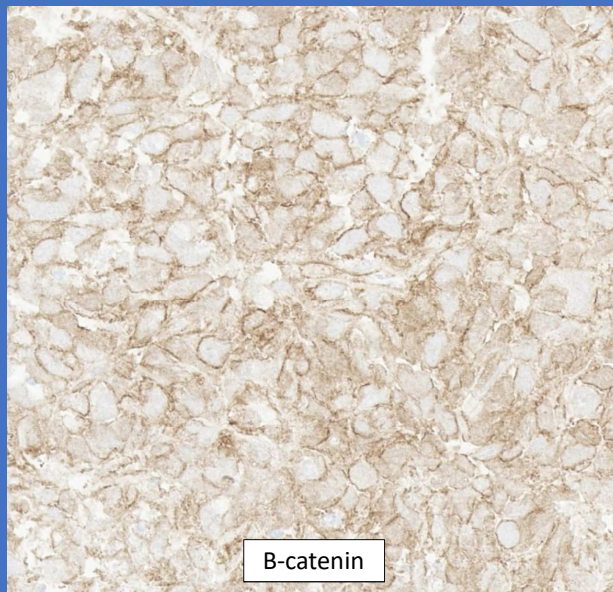
## Case 5



## Case 5



## Case 5





## Case 5

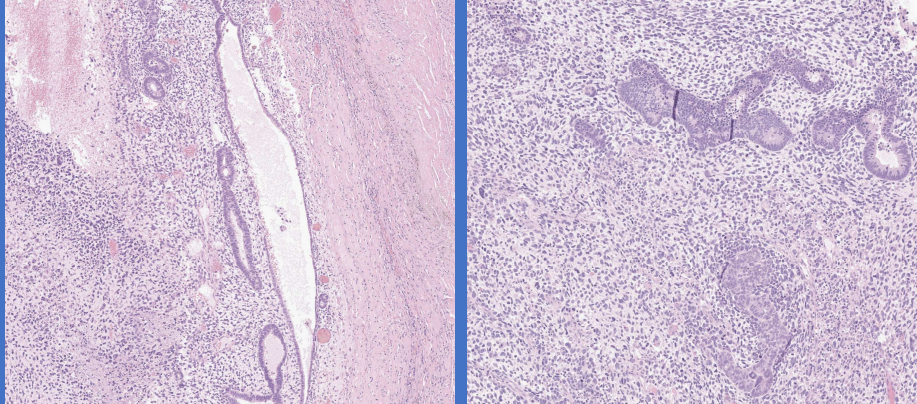
Favored diagnosis?

- A) Sarcomatoid carcinoma with adenocarcinoma component
- B) Synovial sarcoma
- C) Pulmonary blastoma
- D) Metastatic germ cell tumor with yolk sac component

## Case 5

- Patient underwent chemotherapy: cisplatin + pemetrexed + nivolumab
- Improved disease on subsequent CT scans with decreased size of primary tumor and decreased lymphadenopathy
- Underwent lobectomy...

## Case 5



## Case 5

**Mutational Burden:**  
Tumor Mutational Burden/Megabase: 0.365  
This is higher than 49% of all Non-Small Cell Lung Cancers sequenced by this version of OncoPrint  
This is higher than 70% of all Profile cases sequenced by this version of OncoPrint

**ACTIONABLE FINDINGS**

Mismatch Repair Status:  
Proficient (MMR-P / MSS)

Mutations:  
Tier 1 variants: None identified.  
Tier 2 variants: None identified.

Structural Variants:  
Tier 1 variants: None identified.  
Tier 2 variants: None identified.

Copy Number Variants:  
No actionable copy number variants identified

**Tier 3 variants:**  
ARID1A c.1565dup (p.S523Ifs\*100), exon 3 - in 42% of 906 reads##  
DICER1 c.5428G>T (p.D1810T), exon 26 - in 43% of 461 reads##  
DICER1 c.2389A>T (p.R800\*), exon 16 - in 48% of 580 reads##  
GNAS c.1181\_1183delinsTT (p.A394Vfs\*296), exon 1 - in 56% of 714 reads##  
SHAD2 c.1002\_1005dup (p.V336Rfs\*14), exon 9 - in 37% of 520 reads##  
TP53 c.\*740C>T (p.R249W), exon 7 - in 93% of 304 reads##

**Tier 4 variants:**  
GLI3 c.1264G>T (p.D422Y), exon 8 - in 52% of 851 reads##  
JAK2 c.1818C>A (p.H606Q), exon 14 - in 4% of 303 reads##  
NF2 c.1253G>A (p.R418H), exon 12 - in 97% of 303 reads##  
NOTCH2 c.7418G>C (p.\*24725Ser\*7), exon 34 - in 4% of 567 reads##  
RAD21 c.1231G>A (p.D411N), exon 10 - in 92% of 443 reads##  
RAD54B c.692C>T (p.F231L), exon 5 - in 97% of 450 reads##

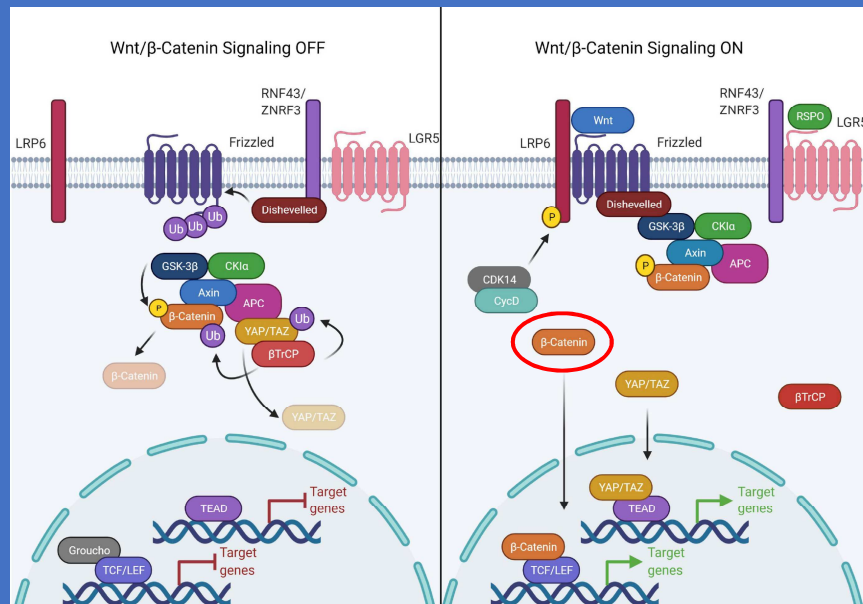
**Structural Variants:**  
Tier 3 variants:  
Rearrangement - PHOX2B exon 2 (chr4:41749538) :: PHOX2B exon 1 (chr4:41750415)  
Tier 4 variants: None identified.

16p12.2	Gain	FALSB
16q23.2-q24.3	Loss	MAF, CBFA2T3, FANCA
17p Arm level	Loss	FLCN, MAP2K4, AURKB, TP53, RPA1
17q11.2	Gain	NF1, RHO1, SUZ12
19p13.3	Loss	ELANE, GNA11, MAP2K2, STK11, TCF3
19q13.32	Loss	ARHGAP35
19q13.33-q13.41	Loss	BCL2L12, PNKP, POLD1, PPF2R1A
20p Arm level	Loss	MCM8
20q Arm level	Gain	ASXL1, BCL2L1, MAFB, AURKA, ZNF217,
GNAS,		
CDH4		
22q12.1	Two copy deletion	ZNRF3
22q12.2-q13.2	Loss	EWSR1, NF2, DMCL1, EP300, XRCC6

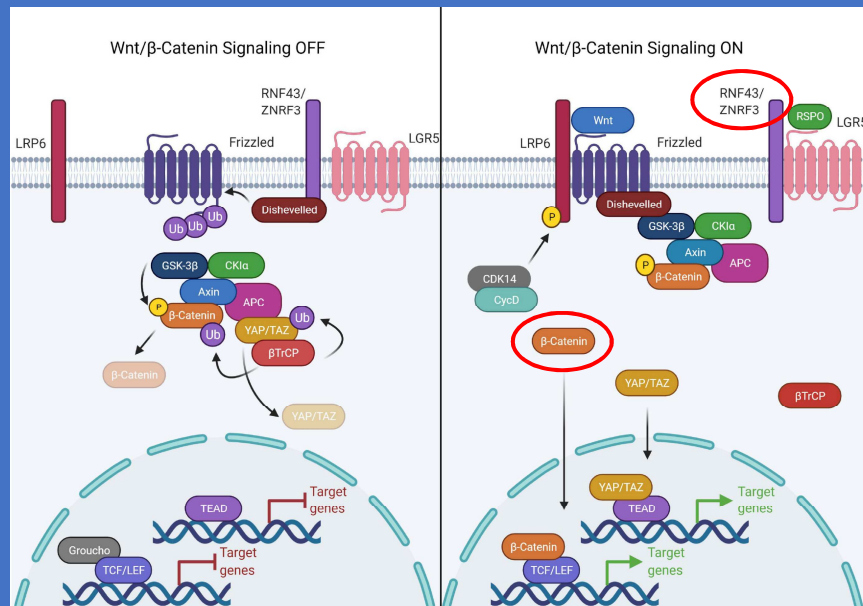
## Case 5

<p><b>Mutational Burden:</b></p> <p>Tumor Mutational Burden/Megabase: 0.345</p> <p>This is higher than 49% of all Non-Small Cell Lung Cancers sequenced by this version of OncoPanel</p> <p>This is higher than 70% of all Profile cases sequenced by this version of OncoPanel</p> <p><b>ACTIONABLE FINDINGS</b></p> <p>Mismatch Repair Status:</p> <p>Proficient (MMR-P / MSS)</p> <p>Mutations:</p> <p>Tier 1 variants: None identified.</p> <p>Tier 2 variants: None identified.</p> <p>Structural Variants:</p> <p>Tier 1 variants: None identified.</p> <p>Tier 2 variants: None identified.</p> <p>Copy Number Variants:</p> <p>No actionable copy number variants identified</p>		
<p>16p12.2 Gain</p> <p>16q23.2-q24.3 Loss</p> <p>17p Arm level Loss</p> <p>17q11.2 Gain</p> <p>19p13.3 Loss</p> <p>19q13.32 Loss</p> <p>19q13.33-q13.41 Loss</p> <p>20p Arm level Loss</p> <p>20q Arm level Gain</p> <p>GNAS,</p> <p>CDH4</p> <p>22q12.1</p> <p>22q12.2-q13.2</p>	<p>Gain</p> <p>Loss</p> <p>Loss</p> <p>Gain</p> <p>Loss</p> <p>Loss</p> <p>Loss</p> <p>Loss</p> <p>Gain</p> <p>Two copy deletion</p> <p>Loss</p>	<p><b>Tier 3 variants:</b></p> <p>ARID1A c.1565dup (p.S523Ife*100), exon 3 - in 42% of 506 reads##</p> <p>DICER1 c.5429G&gt;T (p.D1810Y), exon 26 - in 43% of 661 reads##</p> <p>DICER1 c.2398A&gt;T (p.R800*), exon 16 - in 48% of 590 reads##</p> <p>GNAS c.1181_1183delinsTT (p.A394Vfe*296), exon 1 - in 56% of 714 reads##</p> <p>SHAD2 c.1005_1005dup (p.V338Rfe*14), exon 9 - in 37% of 520 reads##</p> <p>TP53 c.742C&gt;T (p.R248W), exon 7 - in 93% of 304 reads##</p> <p><b>Tier 4 variants:</b></p> <p>GLI2 c.1264G&gt;T (p.D422Y), exon 8 - in 52% of 851 reads##</p> <p>JAK2 c.1818C&gt;A (p.R606Q), exon 14 - in 4% of 303 reads##</p> <p>NF2 c.1283G&gt;A (p.R418S), exon 12 - in 97% of 303 reads##</p> <p>NOTCH2 c.7415G&gt;C (p.*2472Sext*7), exon 34 - in 4% of 567 reads##</p> <p>RAD21 c.1231G&gt;A (p.D411N), exon 10 - in 52% of 443 reads##</p> <p>RADS4B c.652C&gt;T (p.F231L), exon 5 - in 97% of 450 reads##</p> <p><b>Structural Variants:</b></p> <p>Tier 3 variants:</p> <p>Rearrangement - PHOX2B exon 2 (chr4:41749538) :: PHOX2B exon 1 (chr4:41750415)</p> <p>Tier 4 variants: None identified.</p> <p><b>Pathway:</b></p> <p>MAF, CBFA2T3, FANCA</p> <p>FLCN, MAP2K4, AURKB, TP53, RPA1</p> <p>NF1, RHOT1, SUZ12</p> <p>ELANE, GNA11, MAP2K2, STK11, TCF3</p> <p>ARHGAP35</p> <p>BCL2L12, FNKP, FOLD1, PPP2R1A</p> <p>MCM8</p> <p>ASXL1, BCL2L1, MAFB, AURKA, ZNF217,</p> <p>ZNRF3</p> <p>EWSR1, NF2, DMC1, EP300, XRCC6</p>

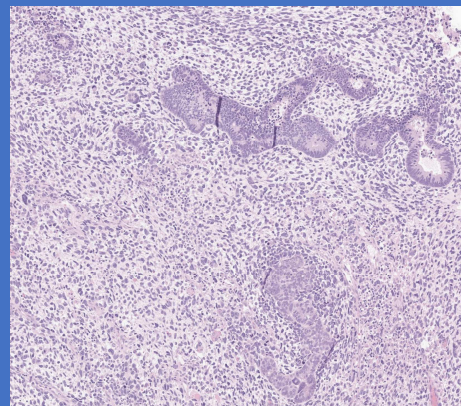
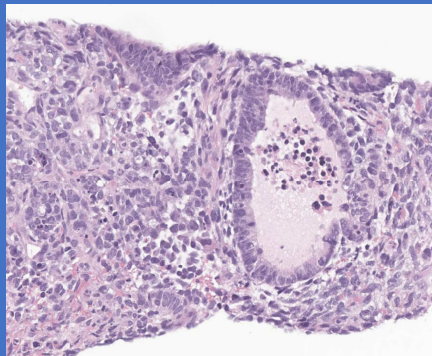
## Case 5



## Case 5



## Pulmonary Blastoma



## Pulmonary Blastoma

- Biphasic tumor of the lung
  - Primitive stromal component
  - Glandular component (histology = fetal adenocarcinoma)
    - Pseudostratified epithelium with sub- and supra-nuclear vacuoles
    - Morules
    - Focal neuroendocrine +
- M=F, 40-50's, smokers
- ~50% have CTNBB1 mutations and nuclear expression of B-catenin in both stromal and glandular components
- Rare tumors described with DICER1, EGFR, MET mutations
- TTF-1 positive in glandular component
- 5-year survival <20%