Updates in lung cancer classification and the role of cytological specimens

Andre L. Moreira MD, PhD

Role of Biopsy in the diagnosis of Lung cancer

- Approximately 70% of patients with lung cancer are diagnosed at advanced stages.
- Cytology (FNAB, EBUS-TBNA, etc) and small biopsies (endobronchial, transbronchial, Core biopsy) are the only available diagnostic material for these patients.

Role of Biopsy in the diagnosis of Lung cancer

- **Accurate diagnosis and tissue preservation:**
  1. Directs patient clinical management (Small cell carcinoma versus NSCLC)
  2. Directs chemotherapeutic regimen in NSCLC (adenocarcinoma versus squamous cell carcinoma)
  3. Guides the Identification of targetable mutations in adenocarcinoma
  4. Tissue preservation for future discoveries of new targetable mutations and appropriate biomarkers for prognosis and therapies

- **Approach:**

1. How to subtype Non-Small Cell Carcinoma in small biopsy specimen including cytology specimen?
2. Diagnosis of neuroendocrine tumor
3. Challenging and unusual lung cancer cases

The WHO classification of lung cancer 5th edition

- **Adenocarcinoma**

✓AIS and MIA

✓Invasive non-mucinous and mucinous

✓Enteric type, colloid and fetal type

- **Squamous cell carcinoma**

✓Keratinizing and non-keratinizing

✓Basaloid

✓Lymphoepithelial carcinoma

- **Adenosquamous carcinoma**
- **Sarcomatoid carcinoma**

✓Pleomorphic

✓Spindle and giant cell

✓Carcinosarcoma

✓Pulmonary blastoma

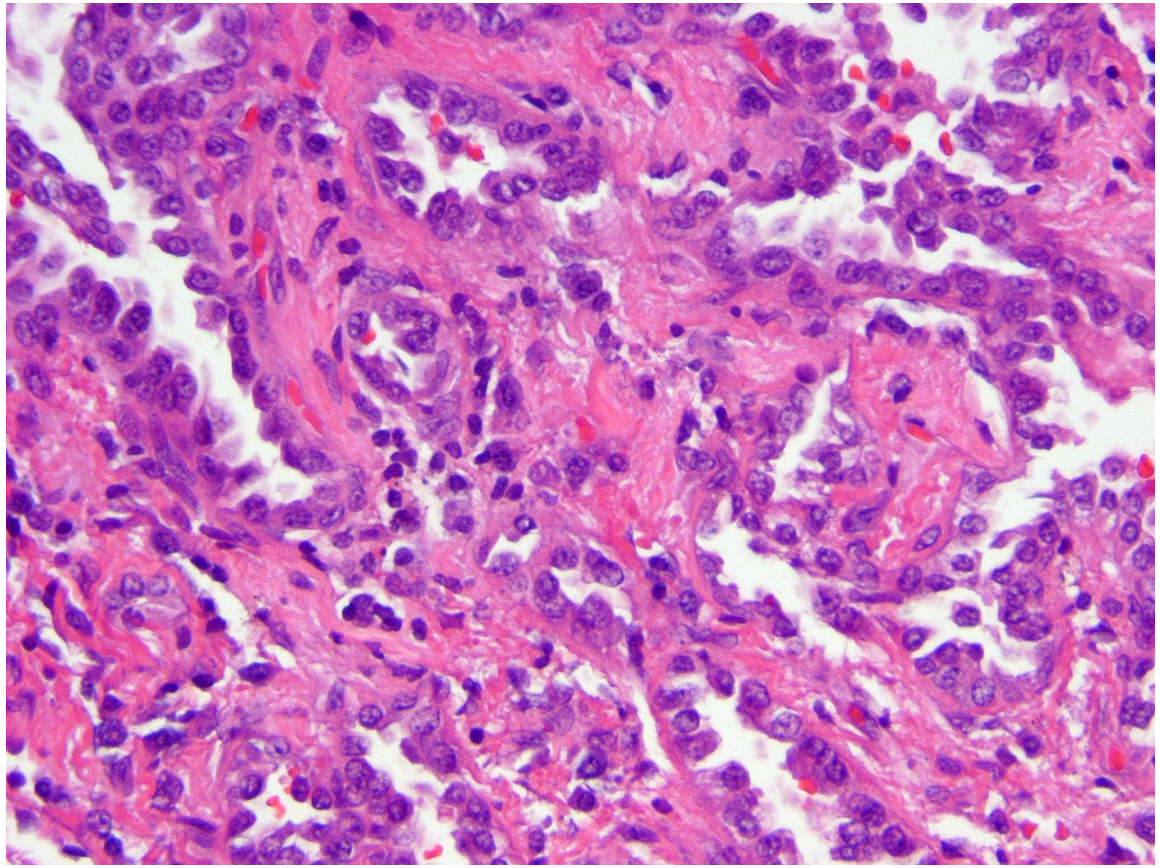
- **Other**

✓NUT carcinoma

✓SMARCA4 deficient tumor

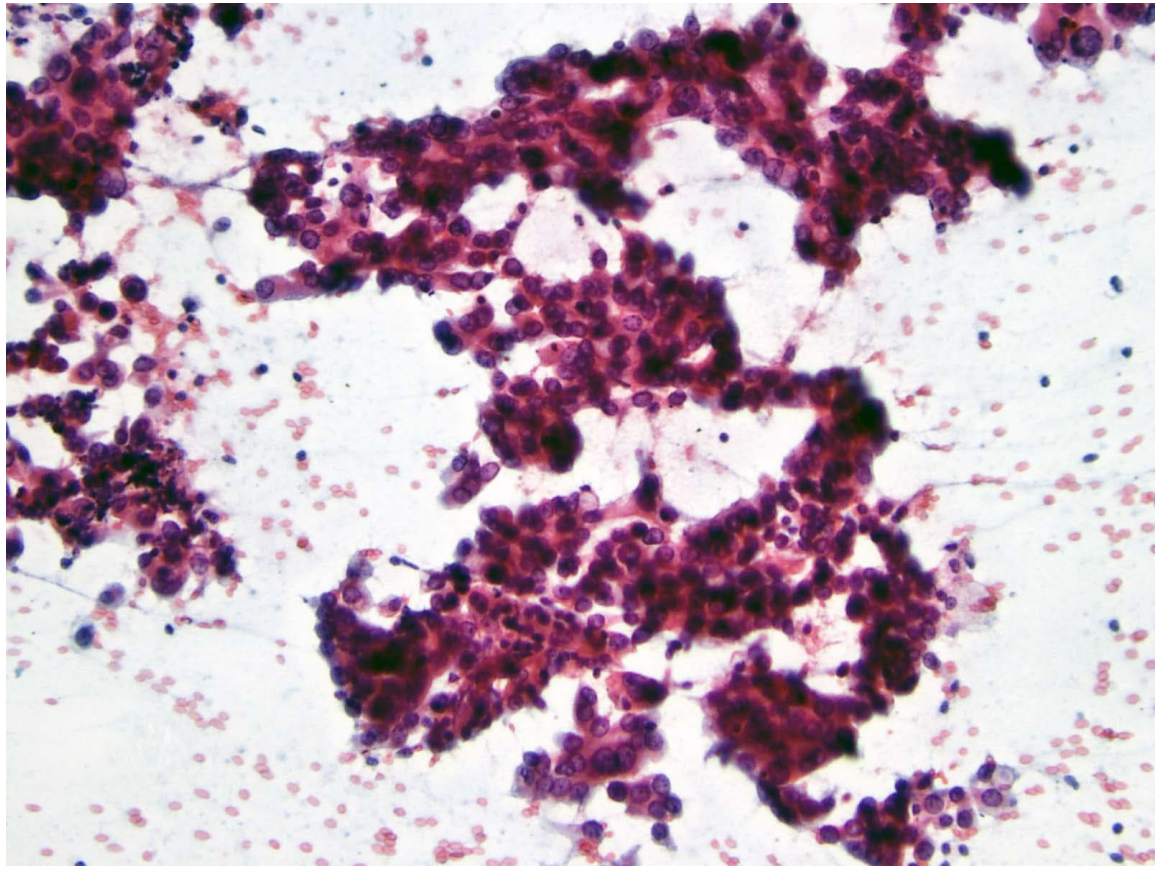
- **Salivary gland type**
- **Neuroendocrine tumor** (carcinoid, small cell carcinoma and Large cell neuroendocrine carcinoma and combined tumors)

Adenocarcinoma



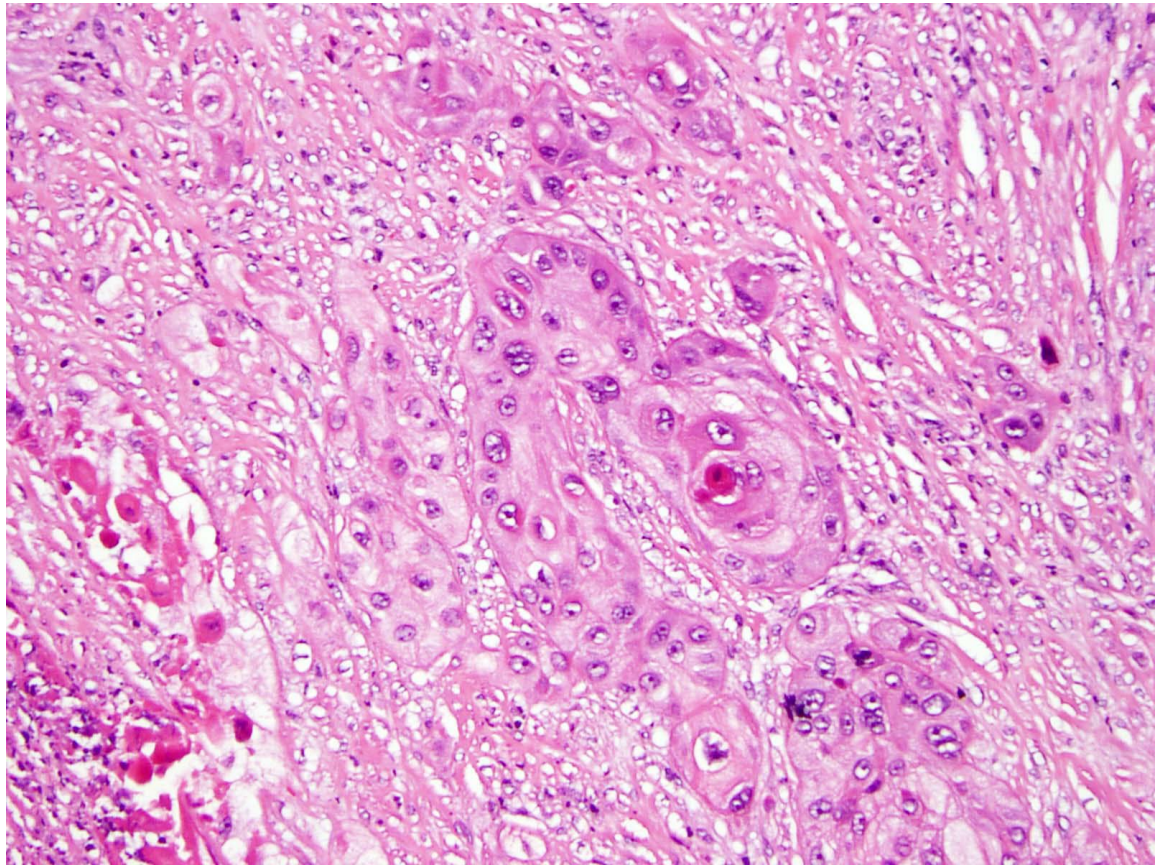
- **Small biopsy:**
- lepidic
- acinar,
- papillary,
- Micropapillary
- Complex glandular patterns

Adenocarcinoma



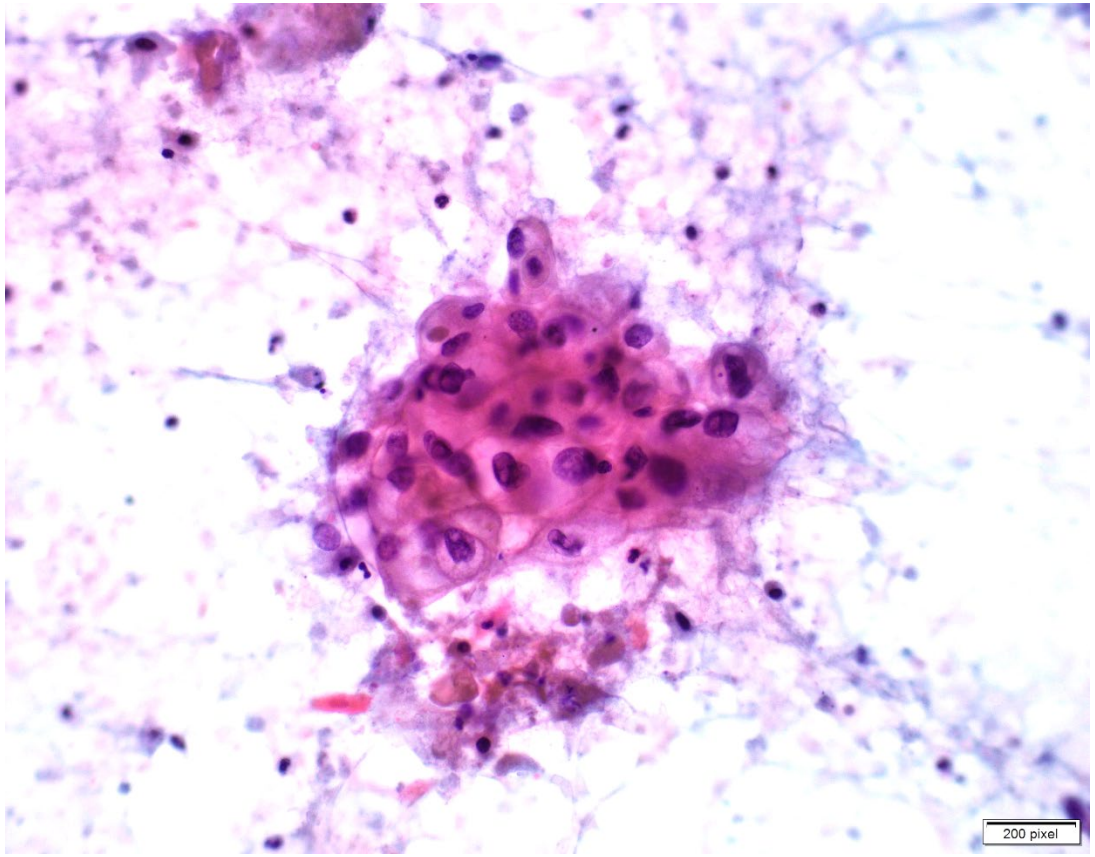
- **Cytology**:
  - tridimensional clusters
  - delicate/vacuolated cytoplasm
  - fine chromatin
  - prominent nucleoli

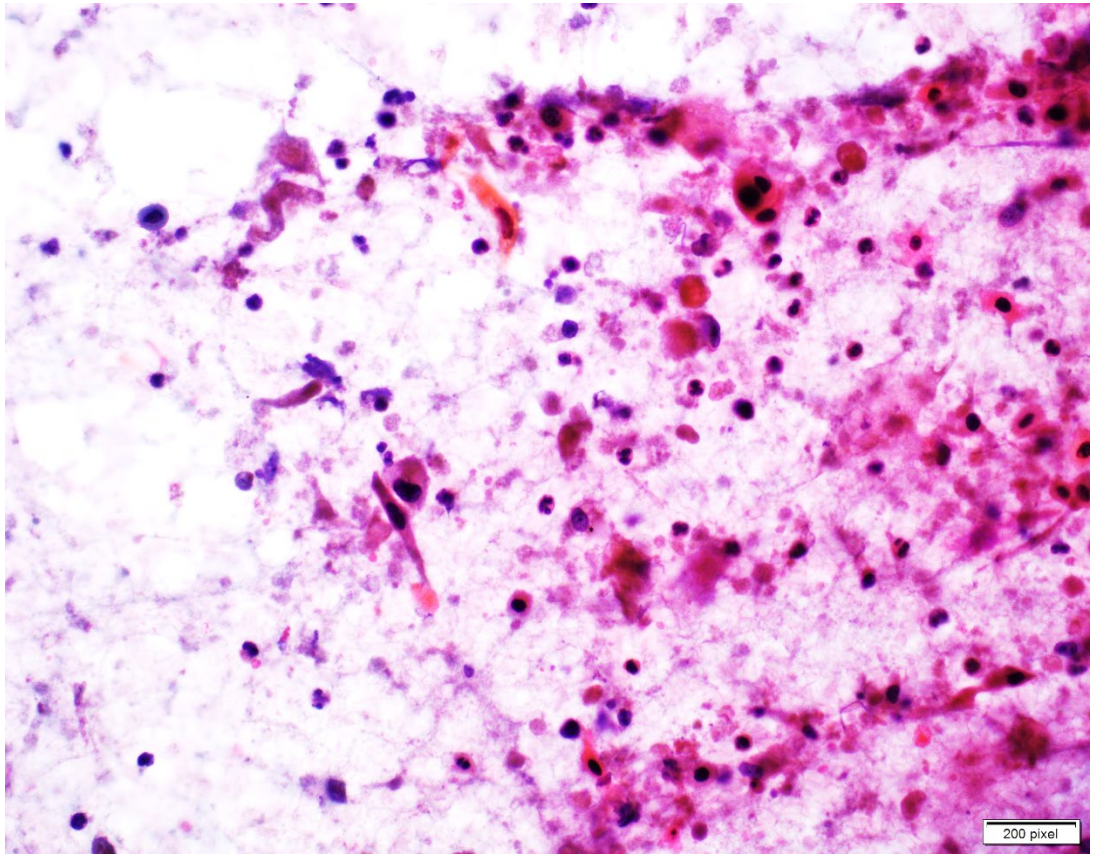
Squamous cell carcinoma



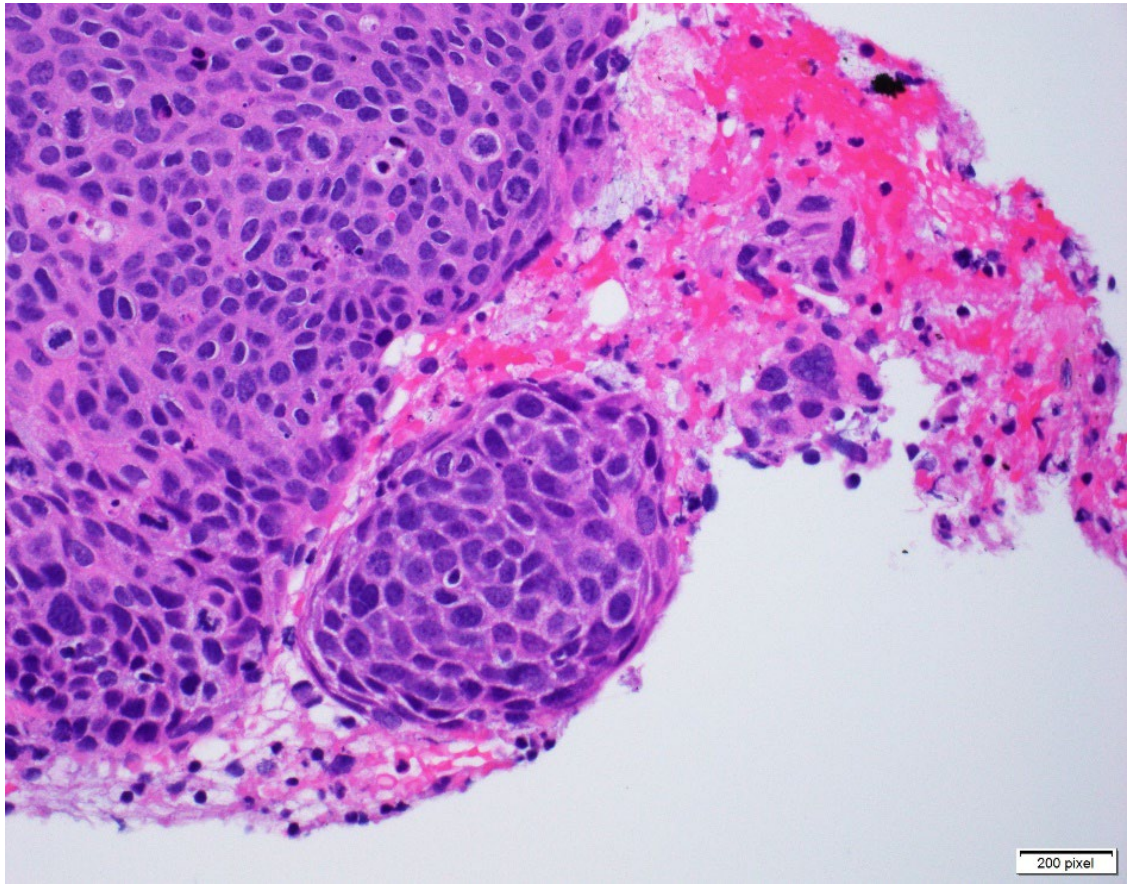
- **Biopsy**:
- Evidence of keratinization
- Presence of intracellular bridges

Squamous cell carcinoma, smear





Squamous cell carcinoma



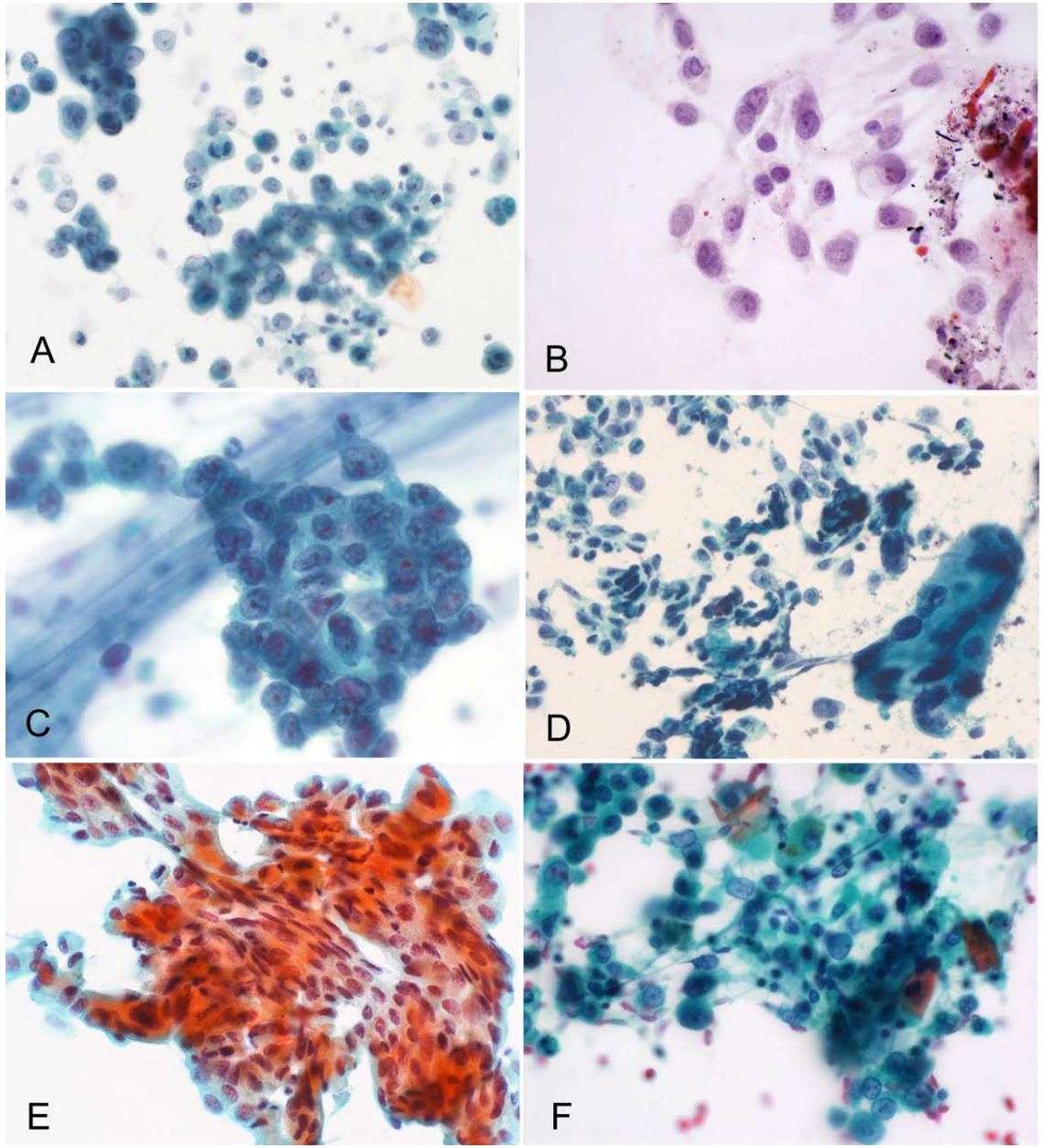
- **Cell Block (Cytology):**
  - Evidence of keratinization
  - Presence of intracellular bridges (cannot be seen on smears)
  - Dense cytoplasm
  - Flat sheets
  - Delicate nucleoli

Limitations of cytomorphology

A-C = squamous cell carcinoma misclassified as adenocarcinoma

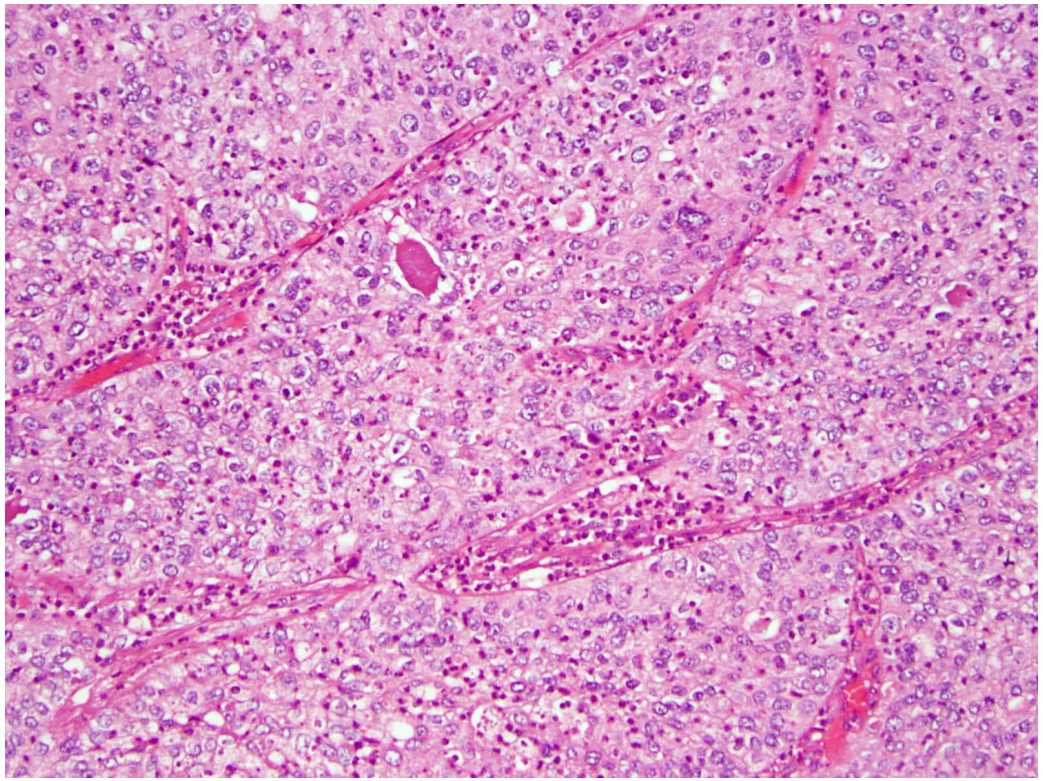
D-F= adenocarcinomas misclassified as squamous cell carcinoma

Jain D et al. JTO , March 2022 (Epub ahead of print) PMID: 35331963



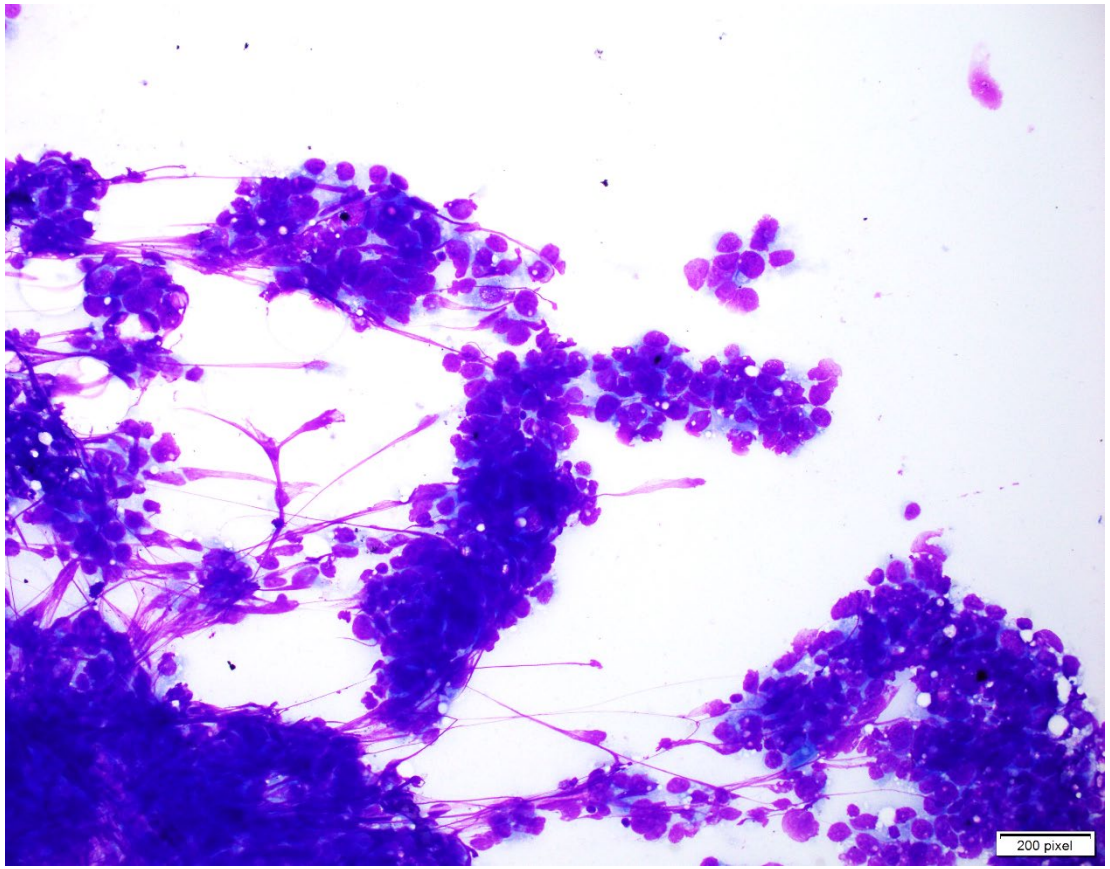
NSCLC, Poorly differentiated

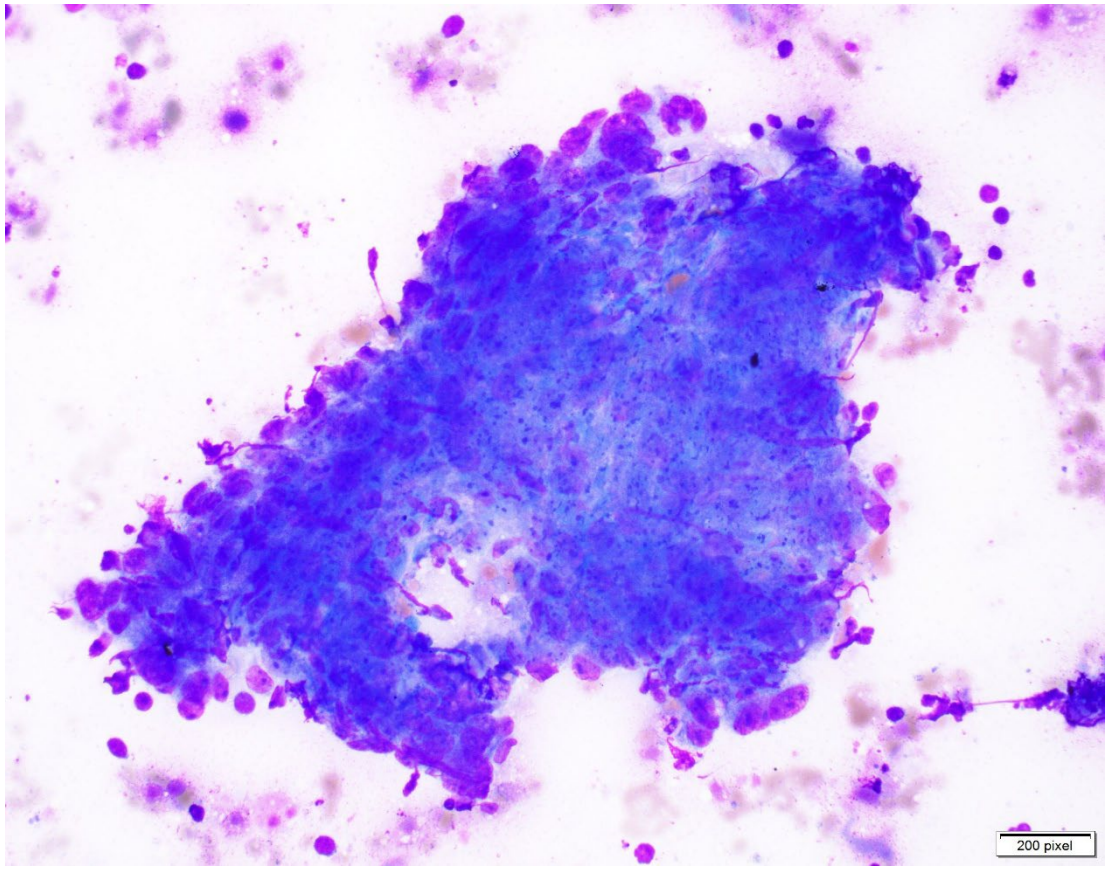
Core biopsy



- No evidence of gland formation
- No evidence of keratinization
- The diagnosis of Large Cell carcinoma cannot be made in small biopsy material/cytology

NSCLC





What to do next?

- A. diagnose NSCLC
- B. IHC for TTF-1 and p40
- C IHC for CK7, CK20, TTF-1, p40, CDX-2, PAX-8
- D wait for the results of core biopsy

B. IHC for TTF-1 and P40

Rekhtman N, Ang DC, Sima CS, Travis WD, Moreira AL.

Mod Pathol. 2011 Oct;24(10):1348-59

Travis DW, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of tumors of the Lung, Pleura, Thymus and Heart. 2014

Literature on the subject

- There have been a lot of publications on an IHC panel that can efficiently differentiate adenocarcinoma from squamous cell carcinoma
- Most common markers: CK7, TTF-1, Napsin-A, p63, p40, 34be12 CK 5/6 etc.
- Use of many markers defeats the idea of tissue preservation for prognostic/predictive markers

Travis WD, Brambilla E, Noguchi M, et al. Arch Pathol Lab Med 2013; 137: 668-684.

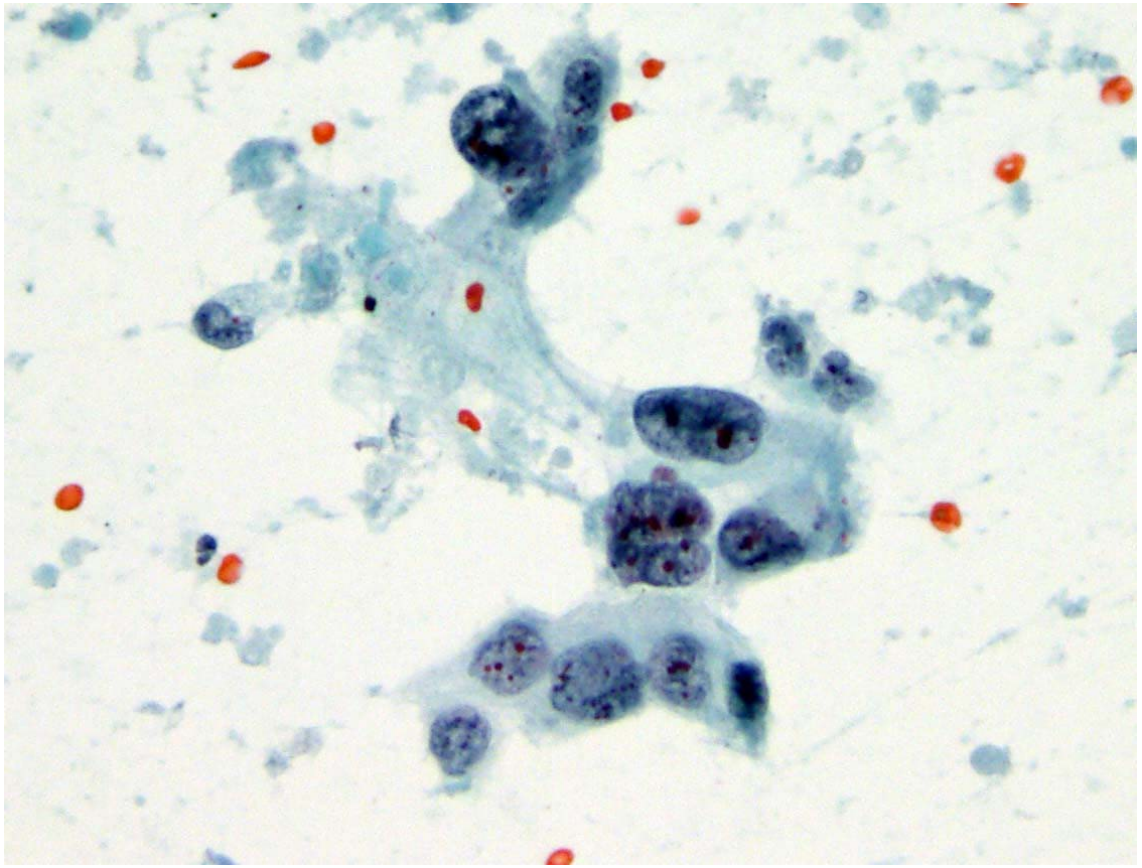
Minimal panel

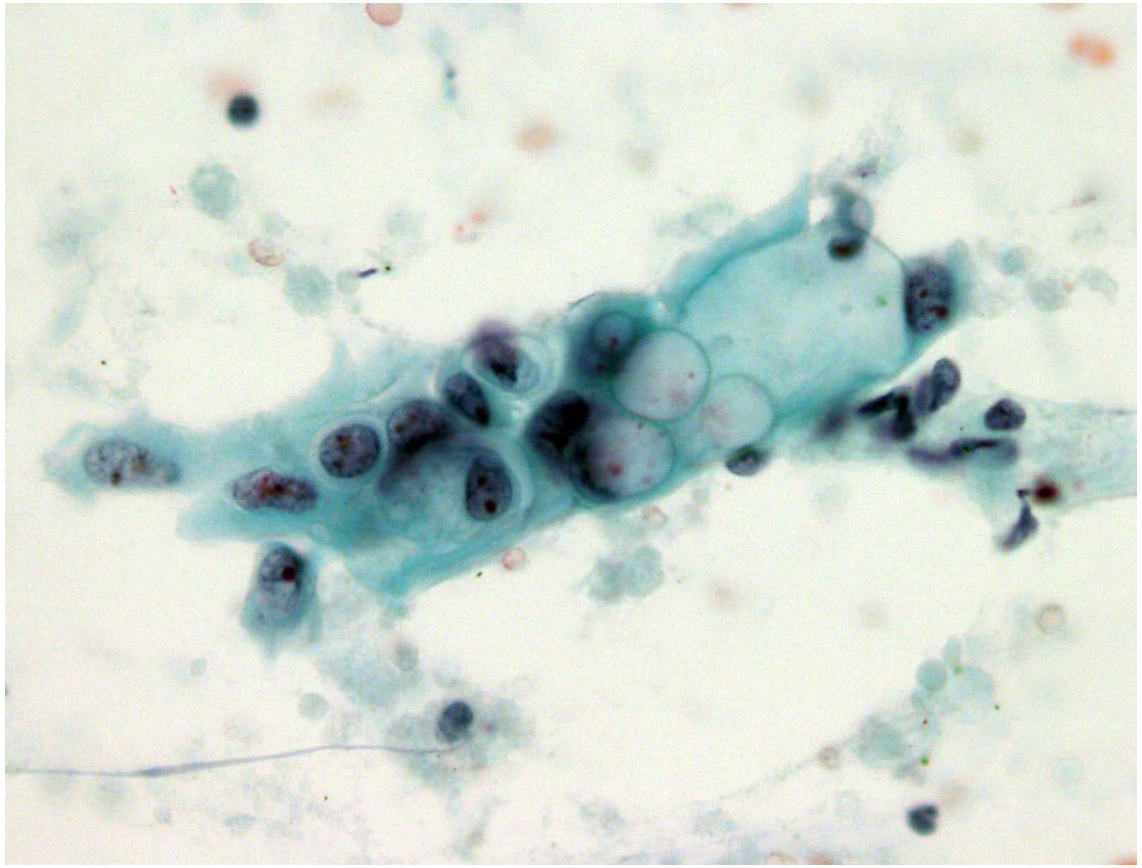
	p40-	p40+
TTF-1+	**ADC**	ADC*
TTF-1-	NSCLC* (favors ADC)	**SQCC (diffuse)**

- *If tissue is available other markers such as napsin-A and CK 5/6 can be added to the panel
- Always compare TTF-1 to p40. Performing one stain has very low predictive value

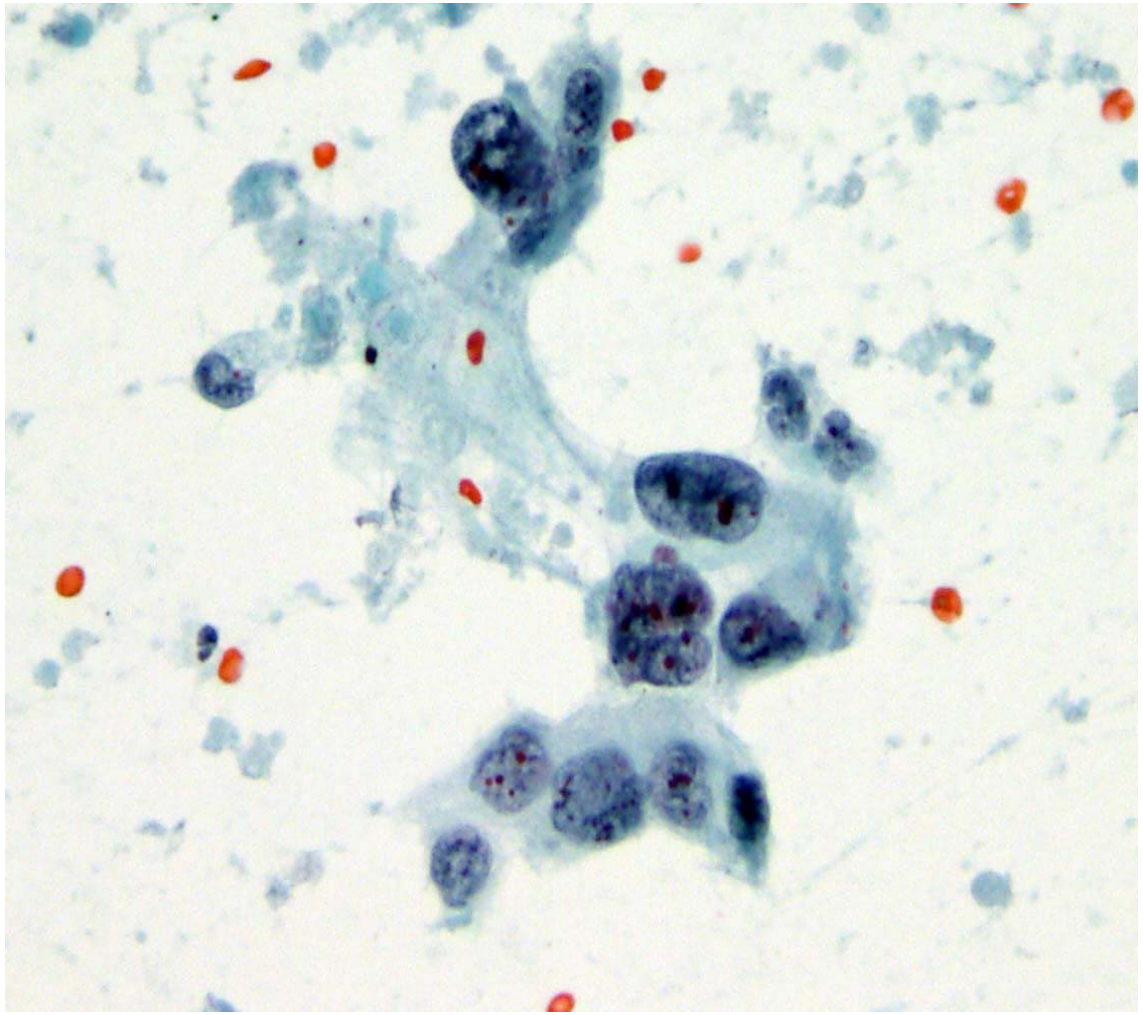
- Rekhtman N et al. Mod Pathol. 2011 Oct;24(10):1348-59

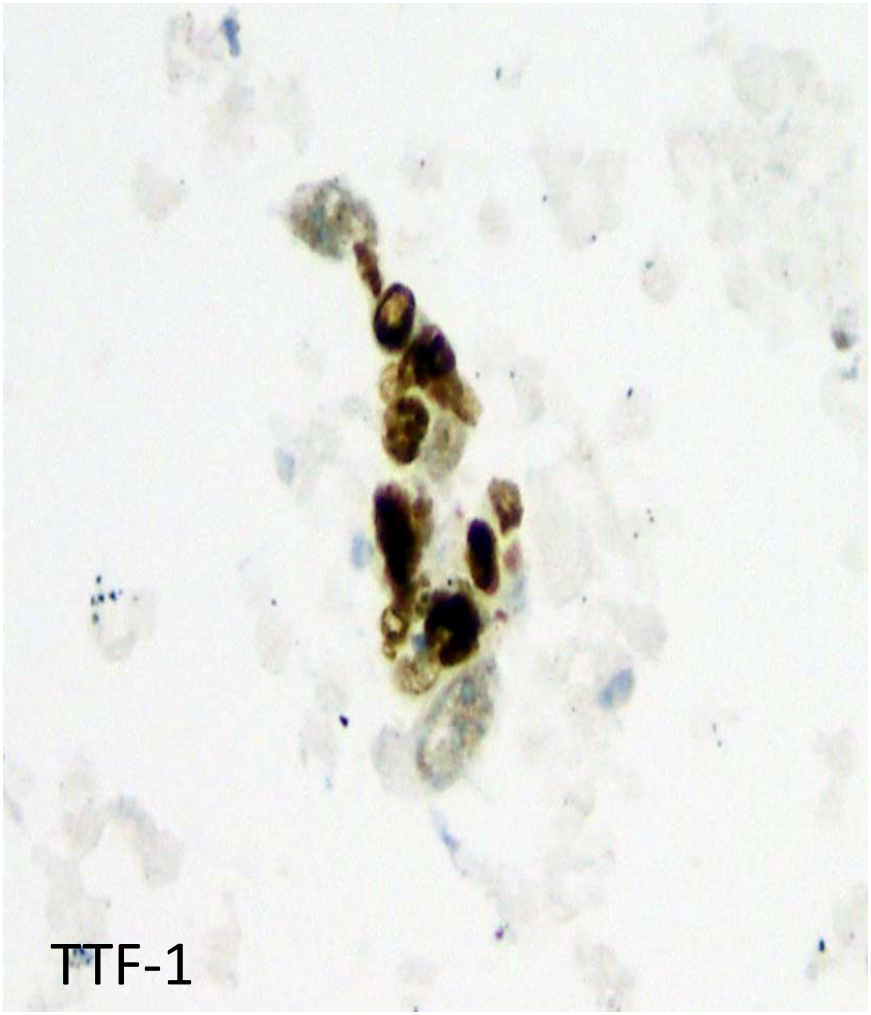
Adenocarcinoma versus Squamous cell carcinoma





Adenocarcinoma X SQCC





IASLC/ATS/ERS classification of NSCLC in small biopsy

	Histologic Criteria	2 markers IHC profile
Adenocarcinoma	Presence of acinar, papillary, lepidic, or micropapillary patterns	Not recommended
Squamous cell carcinoma	Presence of keratinization and intracellular bridges	Not recommended
NSCC, favor adenocarcinoma	**Solid pattern, no other evidence of differentiation**	Any TTF-1 positivity, negative for p63/p40
NSCC, favor squamous cell carcinoma	**Solid pattern, no other evidence of differentiation**	Diffuse and strong positivity for p63 or p40. Negative for TTF-1
NSCLC, not otherwise specified (NOS)	**Solid pattern, no other evidence of differentiation**	Double positivity for TTF-1 and p63 or p40 either focal or diffuse Double negative stain for TTF-1 and p40

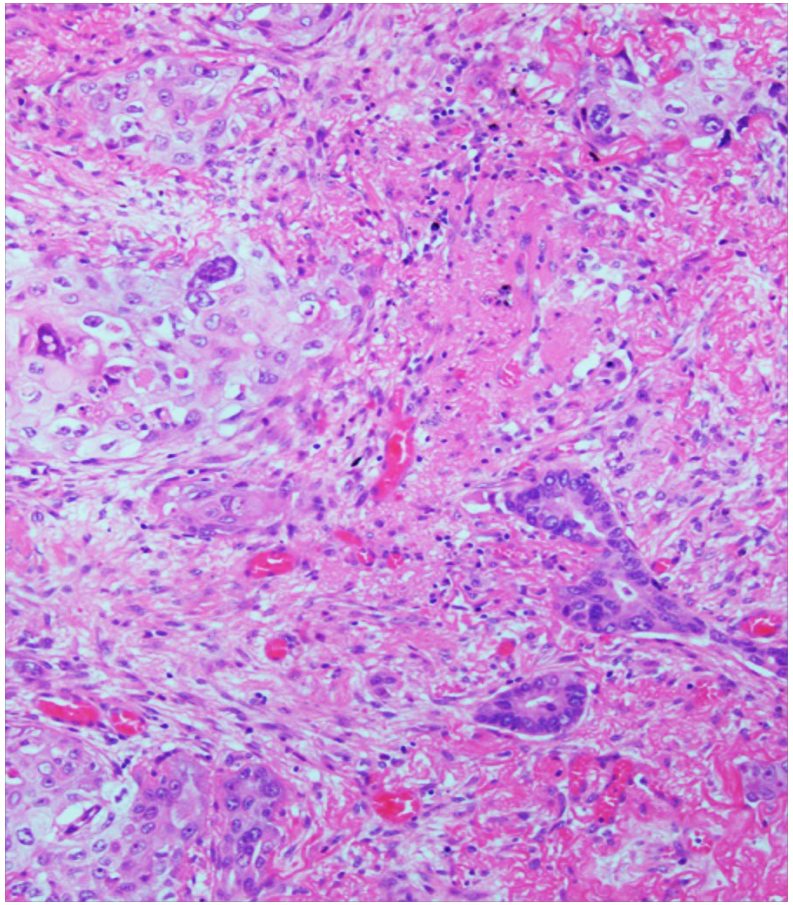
Pitfalls

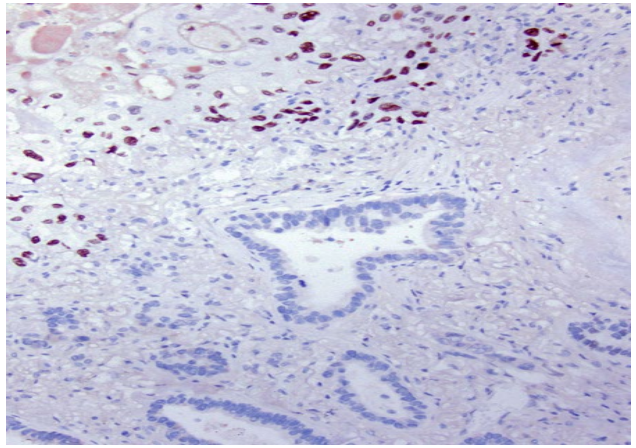
- Double positive tumors
- Double negative tumors

Double positive tumors

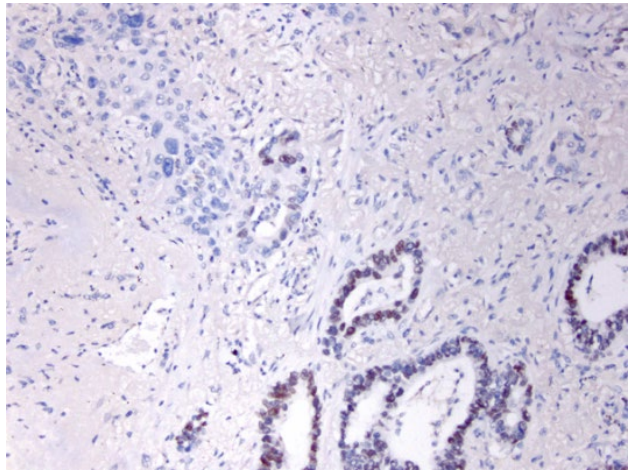
- **Pay attention to where the double positive cells are!!**
- -Double positivity for TTF-1/p40 in the same cells
- **P40 Diffuse:** NSCLC- NOS
- **P40 Focal:** NSCLC- favor adenocarcinoma
- -Double positivity in different areas of the tumor
- NSCLC, favor adenosquamous carcinoma

Adenosquamous carcinoma





p40



TTF-1

Images provided by Dr Saqui, Columbia University, USA

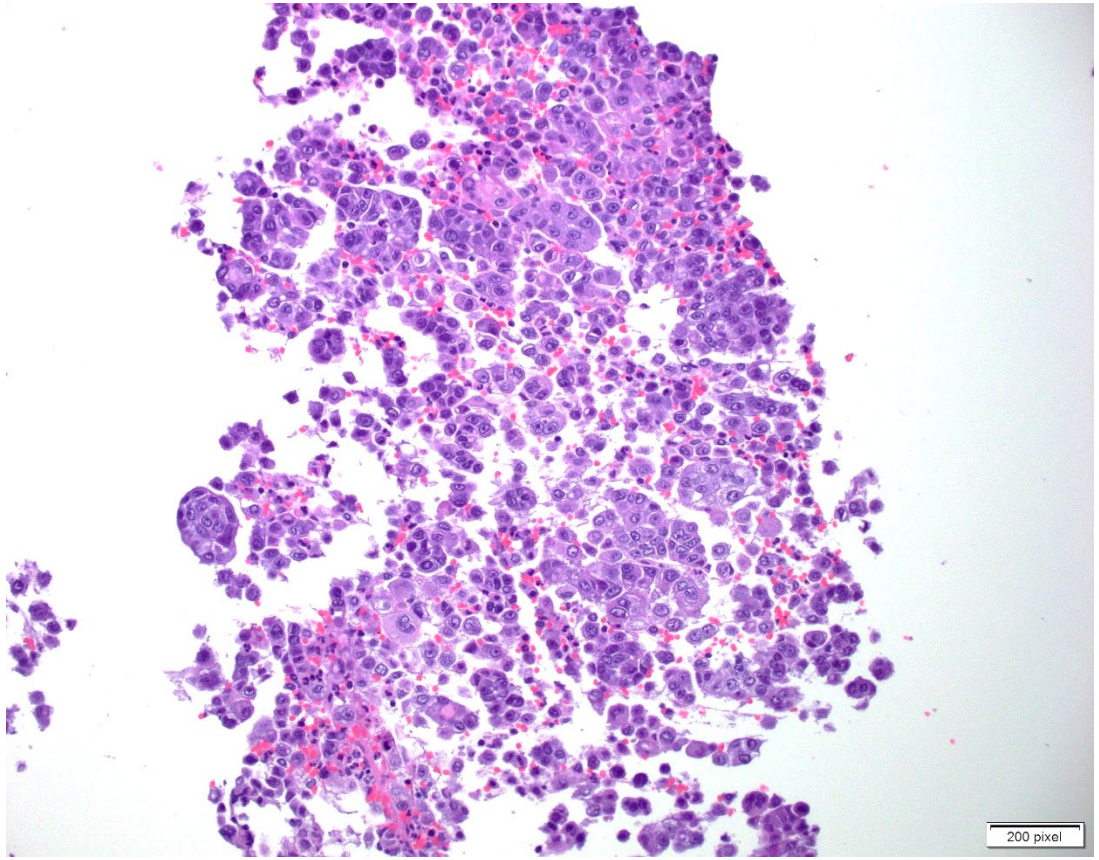
- The diagnosis of adenosquamous carcinoma is difficult in biopsy/cytology specimens
- In a biopsy/cytology both components need to be present
- More frequently the diagnosis can be suspected in a biopsy diagnosis of SQCC in a non-smoker. In this case, molecular testing is recommended!

Double negative tumors

- >20% of lung adenocarcinomas are negative for TTF-1
- Add other markers such as napsin-A, other organs specific markers and **keratins**

- In double negative tumor, consider other primaries including non-epithelial tumors

- 64 y.o. old woman, remote history of smoking and histerectomy.
- Presented with cough
- CT scan showed a single pulmonary nodule
- A FNA of the mass was performed



Tumor is negative for TTF-1 and p40. What to do next?

- A- include a pan keratin (AE1/AE3, CAM 5.2)
- B- include CDX-2, PAX-8
- C- include S-100 and Melan-A
- D- all of above

D- all above

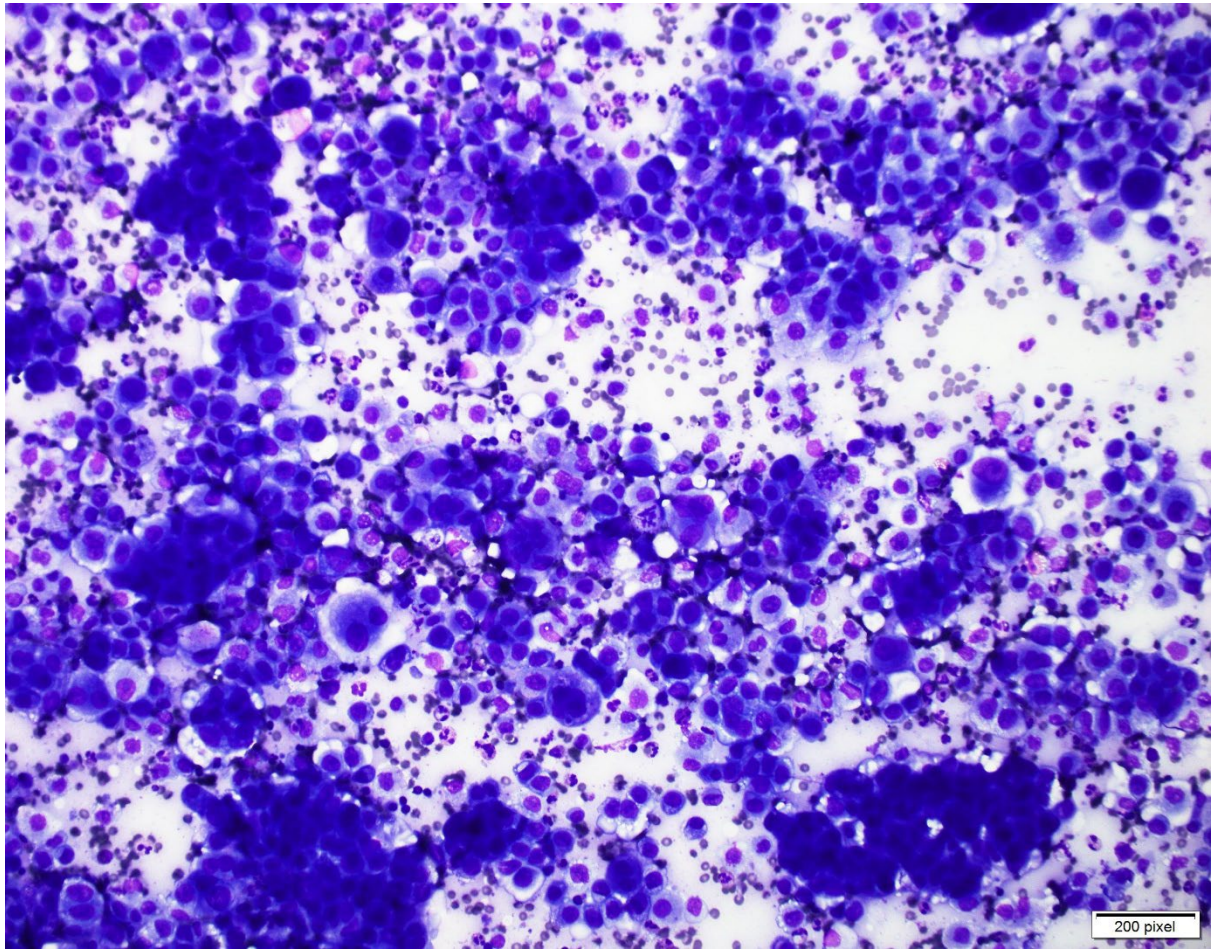
- Travis DW, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of tumors of the Lung, Pleura, Thymus and Heart. 2014

- IHC
- Negative for p40, TTF-1, napsin A, and other organ specific marker. Positive for keratin

- DX: Non-small cell carcinoma, NOS
- Sent for molecular pathology, KRAS G12c was identified

- 81 y.o, man, smoker, history of leukemia
- S/P chemotherapy
- Presents with a lung nodule

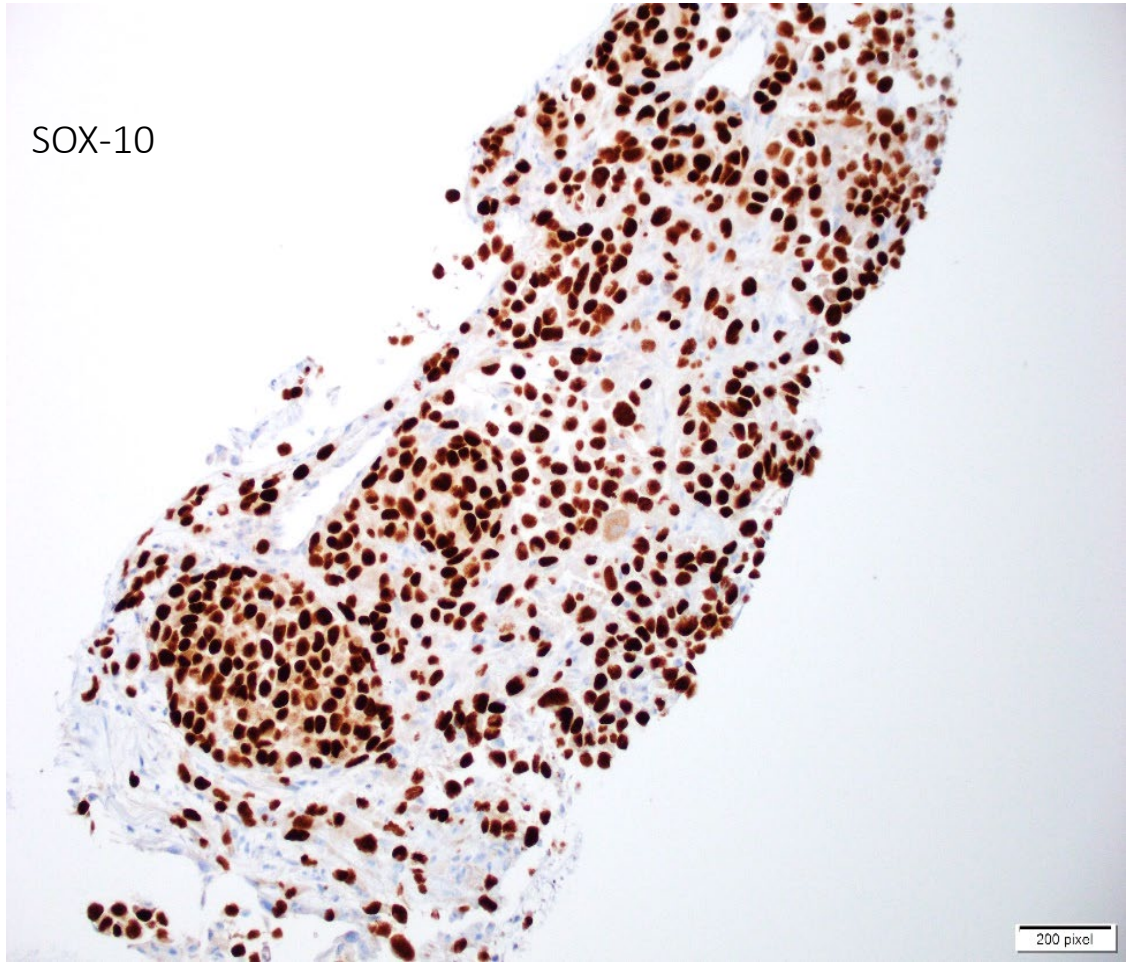
- A biopsy of the nodule was obtained



- IHC
- Negative for TTF-1, p40, NE markers

- Keratin- NEGATIVE

- Metastatic melanoma.
- A remote history of melanoma was discovered after the diagnosis



Take Home message

- Solid adenocarcinomas of the lung can have a squamoid appearance, which may lead to misdiagnosis.
- All adenocarcinomas or NSCLC, favor adenocarcinoma or NSCLC, NOS should be considered for molecular diagnosis **(save tissue)**
- Squamous cell carcinoma or NSCLC, favor squamous cell carcinoma do not need to be sent for molecular studies, **unless in certain clinical conditions, such as non-smoker** (possibly a component of adenosquamous carcinoma)
- Double negative add more markers

Neuroendocrine tumors groups

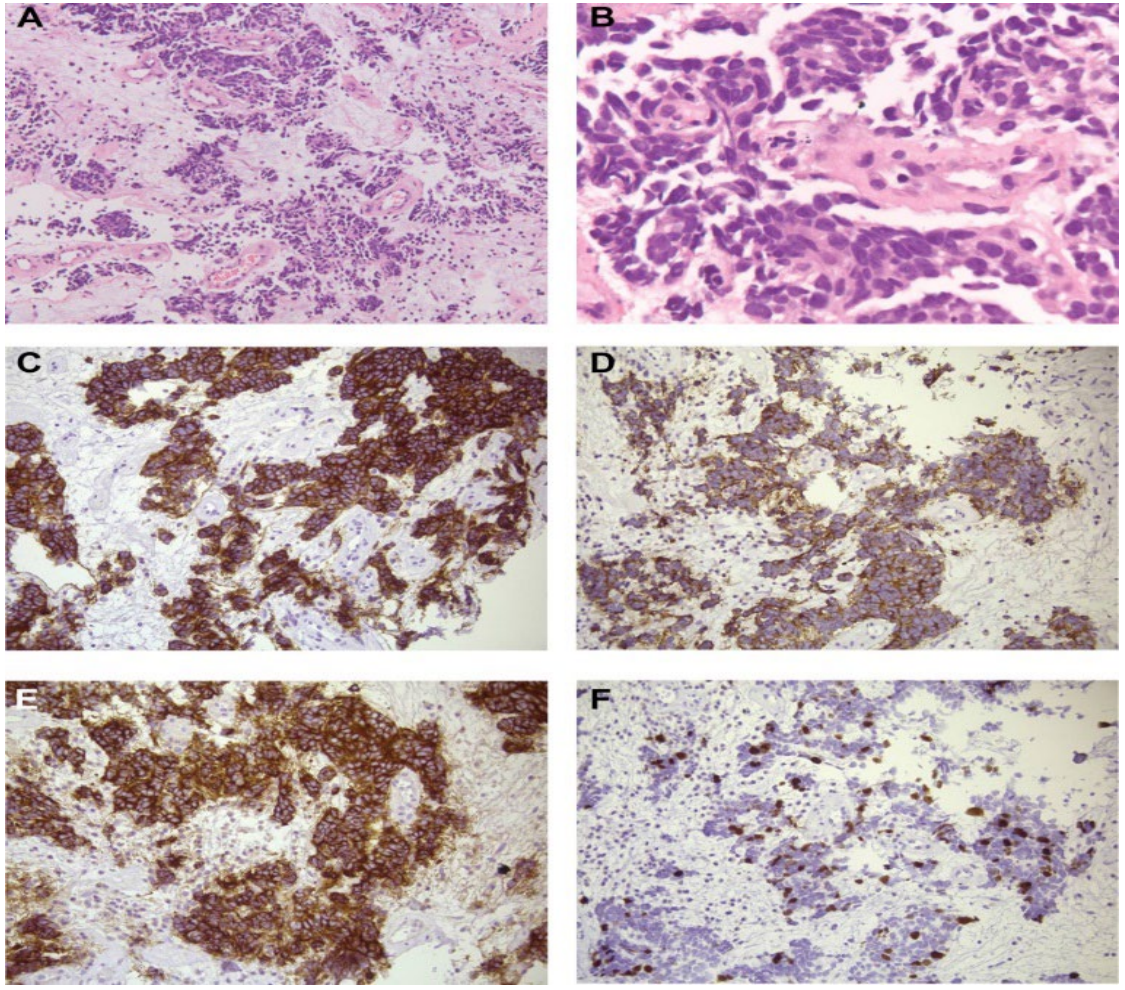
- **High grade NET**
  - Small cell carcinoma
  - Large cell Neuroendocrine carcinoma (LCNEC)

- **Low-intermediate grade NET (difficult to classify in small biopsy/cytology)**
  - Typical Carcinoid tumor
  - Atypical carcinoid tumor

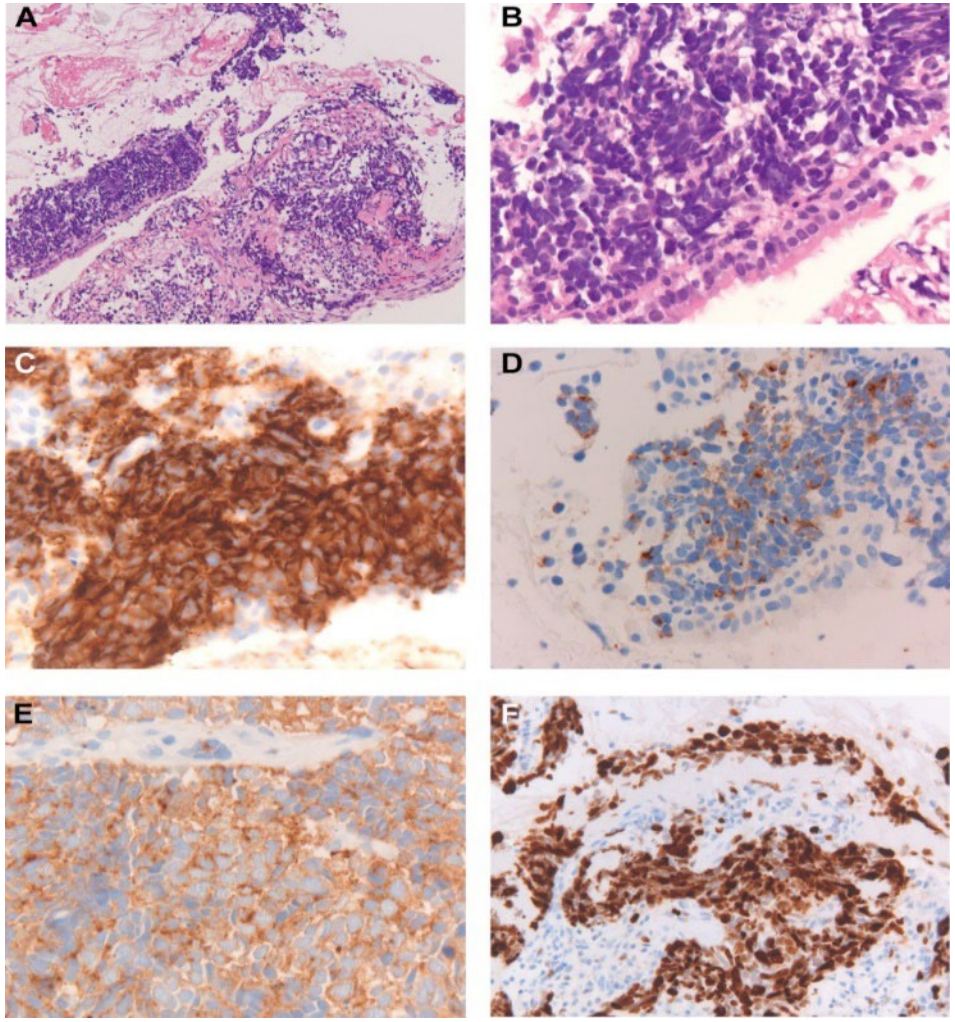
What is important to know for cytopathologists!

- Reproducibility issues!
- Moderate reproducibility among expert pathologists! Mostly due to small biopsies with crush artifact.
- Use of IHC is recommended to confirm diagnosis and avoid pitfalls with other NET
- IHC work up should include KI-67 proliferation rate.
- Thunissen E et al. J Thorac, Pathol. 2017; 12:334-46.

Carcinoid tumor



Small Cell Carcinoma



Journal of Thoracic Oncology 2017 12, 334-346

Journal of Thoracic Oncology 2017 12, 334-346DOI: (10.1016/j.jtho.2016.12.004)

LCNEC

- **Definition**: NSCLC with **NE morphology** with IHC evidence of NE differentiation.

- There is growing evidence that LCNEC is a heterogeneous group of tumors within a spectrum of small cell and adenocarcinoma
- Biopsy diagnosis is difficult but can be made with caution.
- There are overlapping features with adenocarcinoma and small cell carcinoma

Carcinoid tumors

- **Typical carcinoid**: less than 2 mitotic figures per $2mm^2$, no necrosis.
- **Atypical Carcinoid**: 2 to 10 mitotic figures per $2mm^2$, punctate necrosis

- Ki-67 index is not helpful in differentiating Typical from atypical carcinoid, but is good to separate High grade NE tumors
- Difficult to separate the 2 entities in biopsy or cytology material, focal mitotic figures

Combined SCC and NSCLC

- Small cell carcinoma can be combined with Sqcc, adenocarcinoma, and other types
- Incidence is unreliable, in biopsies only one component may be sampled
- SCC component may have the same mutation as the counterpart.
- Can be diagnosed in cytology specimen!!!

Take home message

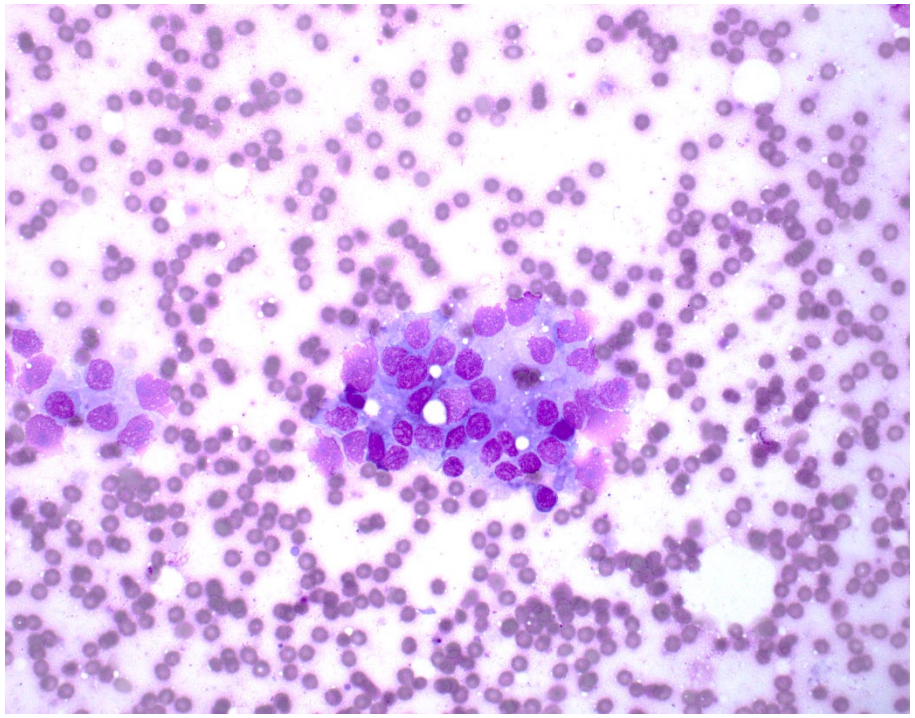
- IHC is a very powerful method of identifying cell type of NSCLC
- Awareness of the exact algorithm and its pitfalls is essential!
- The minimal panel is only useful when the diagnosis is a primary lung carcinoma
- **Use of a limited panel of IHC markers saves tissue for molecular diagnosis**
- **Be aware of crush artifact in NE tumors, include KI 67 in your work up panel**

Case

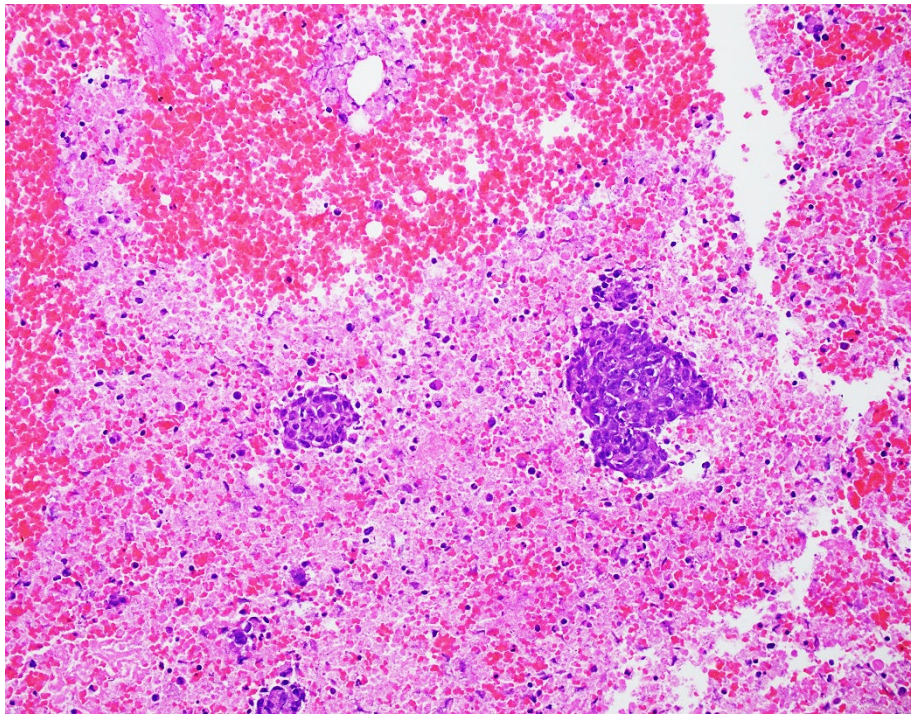
- 42 year old woman, never smoker
- Presented with cough and weight loss
- A CT scan of the chest showed a large mass involving the hilum of the lung, mediastinum and lymphadenopathy
- A EBUS-TBNA was performed

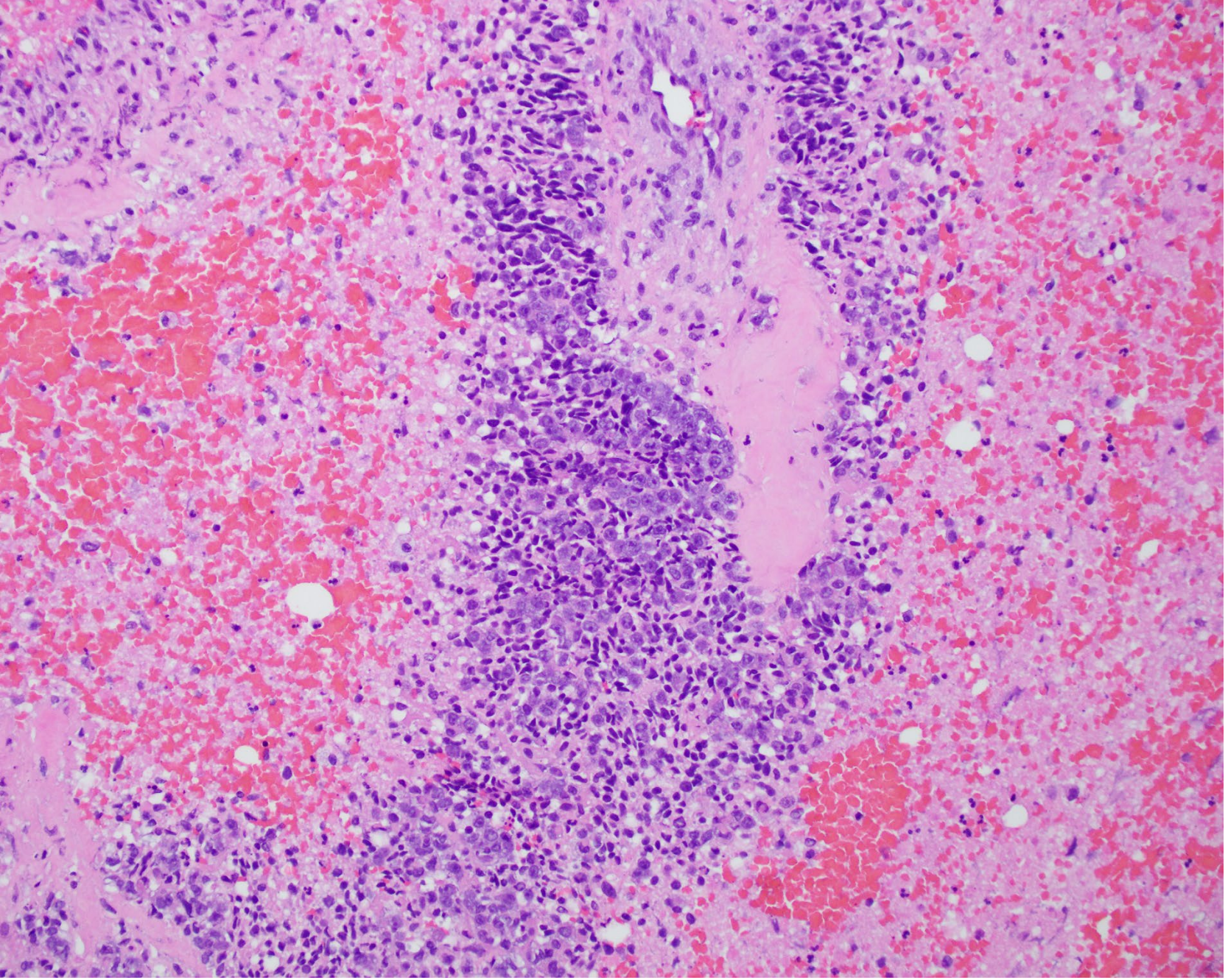
EBUS TBNA- level 7 lymph node

Smear



Cell block





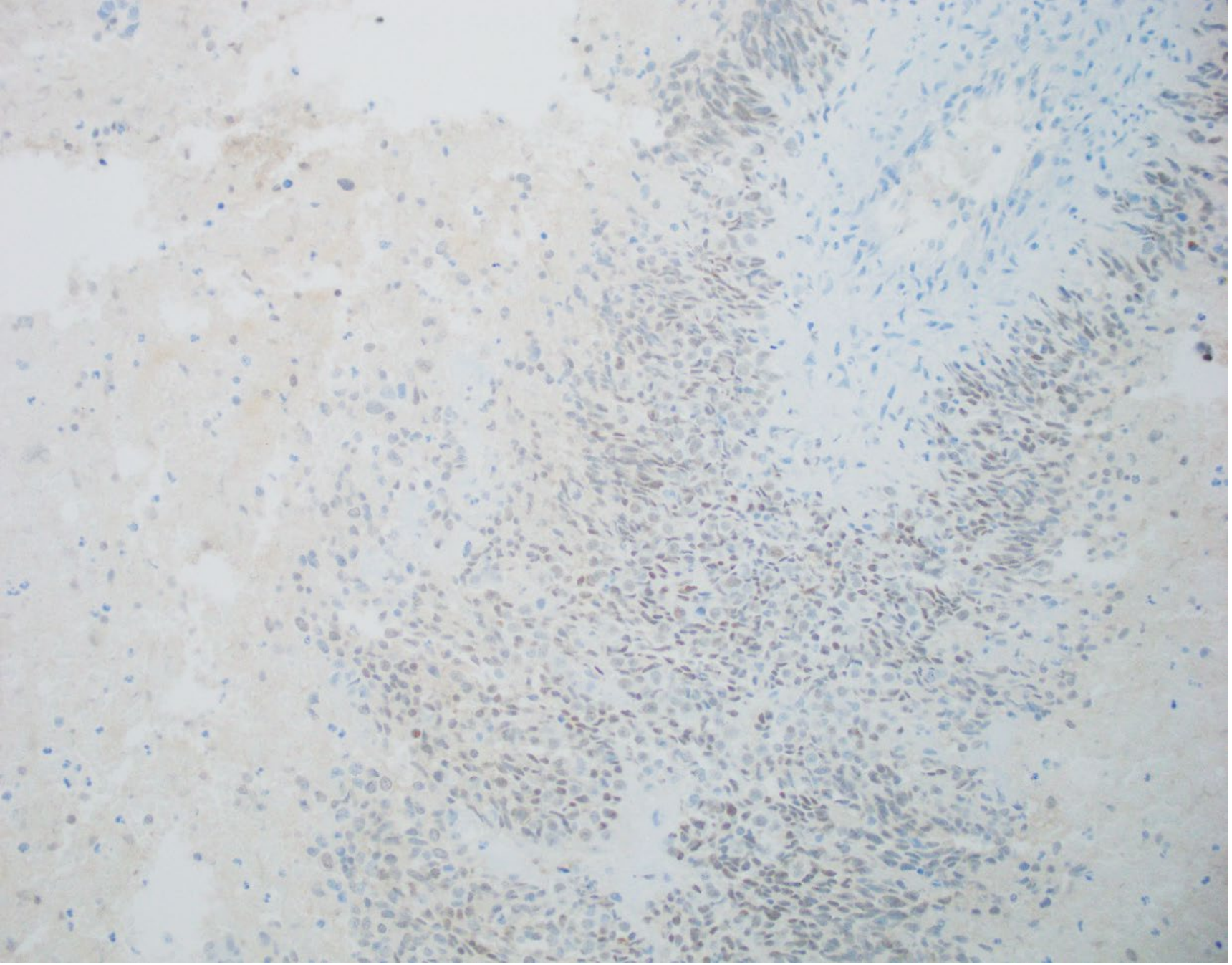
The tumor cells are negative for TTF-1, focally positive for p40 and positive for p63

A diagnosis of NSCLC-favor squamous cell carcinoma was made

Poorly-differentiated carcinoma
With homogeneous appearance

Presence of necrosis in the background

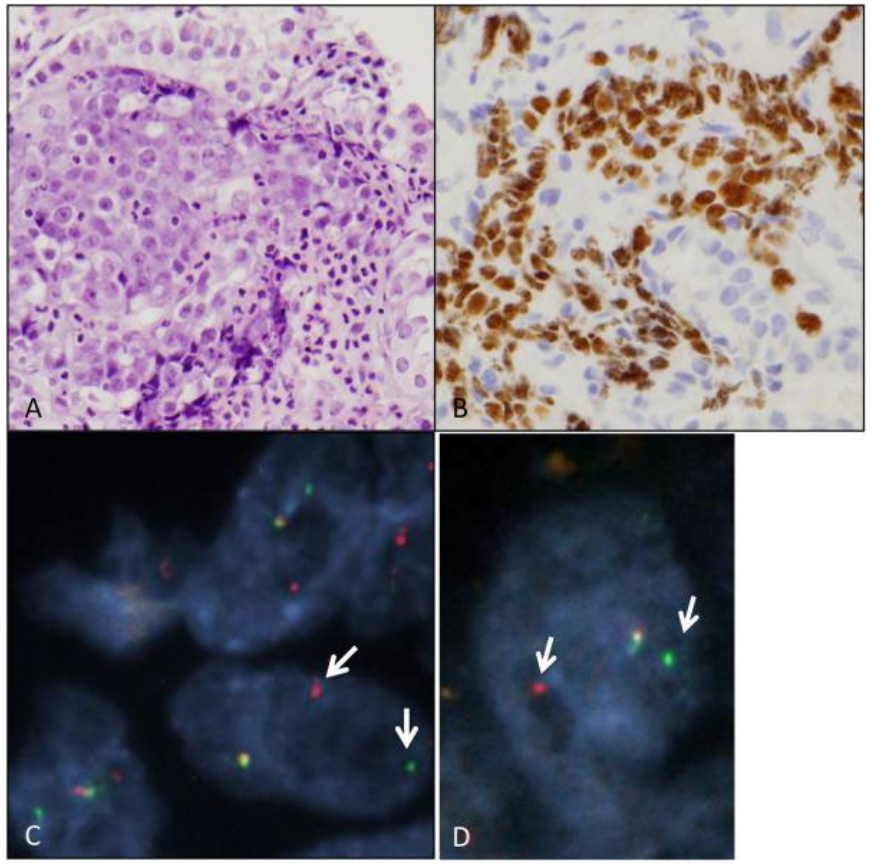
The patient is non-smoker!



NUT IHC

Speckled nuclear stain

Other carcinomas: Nut carcinoma



Poorly-differentiated with characteristic t(15;19) rearrangement leading to NUT fusion
Rare tumor
Poor prognosis
Often monotonous features with abrupt keratinization's
Include in the differential diagnosis of poorly-differentiated carcinomas
Difficult in biopsy material!

Scholl LM et al. JTO. 2015, 10:951-9

Pitfalls

- Cytological and histological features are not very specific!
- If you do not include NUT carcinoma in your differential diagnosis, you will not make the diagnosis!!!!
- Include NUT carcinoma in a any NSCLC-NOS and NSCLC-favor squamous cell carcinoma, specially in a non-smoker!!!
- The diagnosis can be made by IHC (NUT) or by molecular analysis
- More common in younger patients but has a large age distribution

NUT carcinoma

- Can show focal positivity for p40 and diffuse positivity for p63
- If using the minimal panel (TTF-1/p40 or p63) to classify NSCLC, this tumor can be confused with a non-keratinizing Squamous cell carcinoma
- Can also be confused with small cell carcinoma!
- NUT is **not** associated with cigarette smoking!
- Germ cell tumor and thymic carcinoma may enter the differential diagnosis (mediastinal mass)

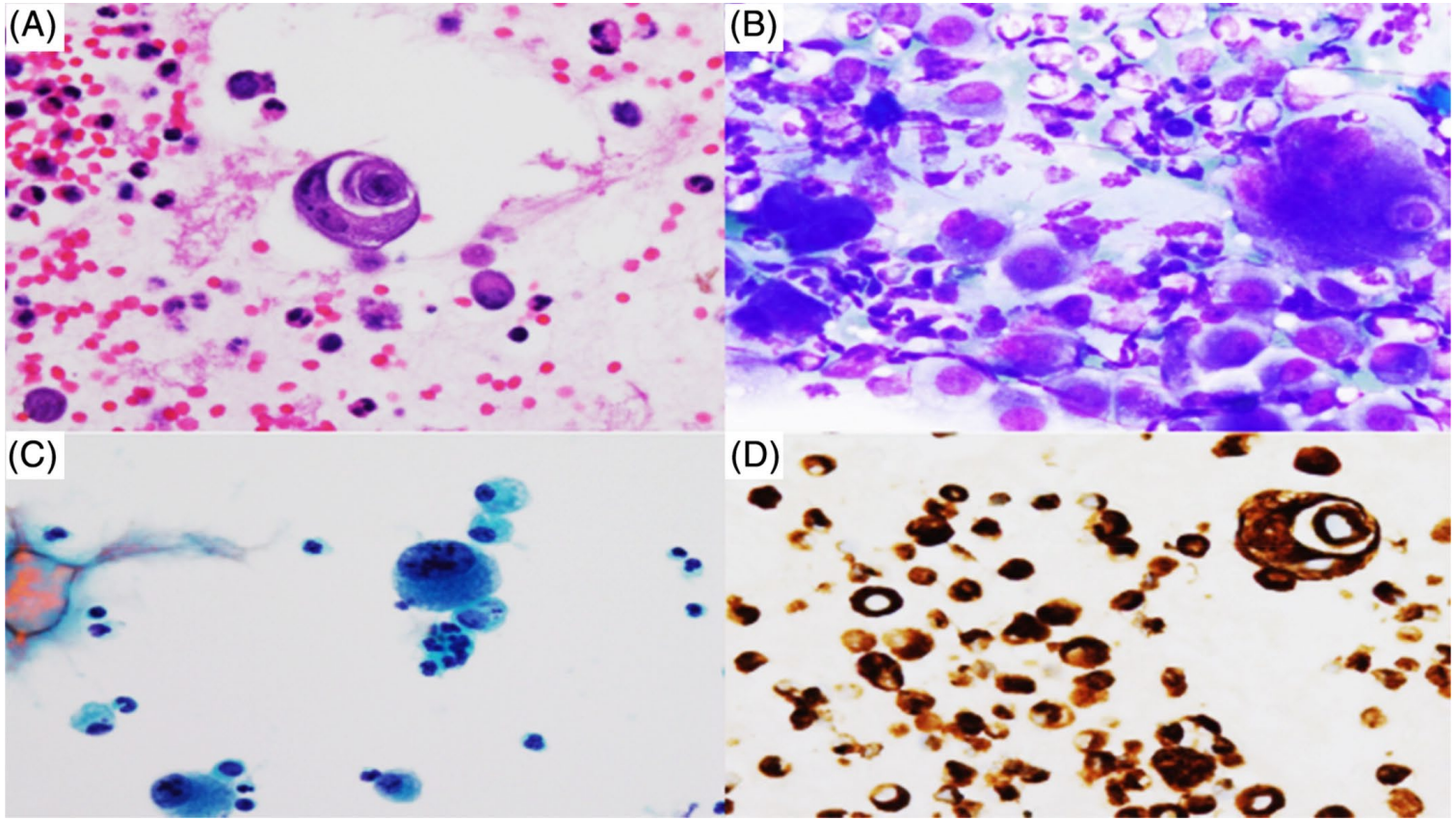
Sarcomatoid carcinoma

- **Overreaching term in cytology that encompass all tumor in this category**
- -Pleomorphic carcinoma
- - Carcinosarcoma (if heterologous element is present)
- -Pumonary blastoma
- -Spindle or Giant cell carcinoma
- Aggressive tumor with poor prognosis, often associated with smoking history!

Case 2

- 67 year old man, heavy smoker
- Presented with cough and left side pleural effusion
- CT scan showed 6 cm subpleural mass, hilar and mediastinal lymphadenopathy and an adrenal mass
- Pleural effusion was collected.

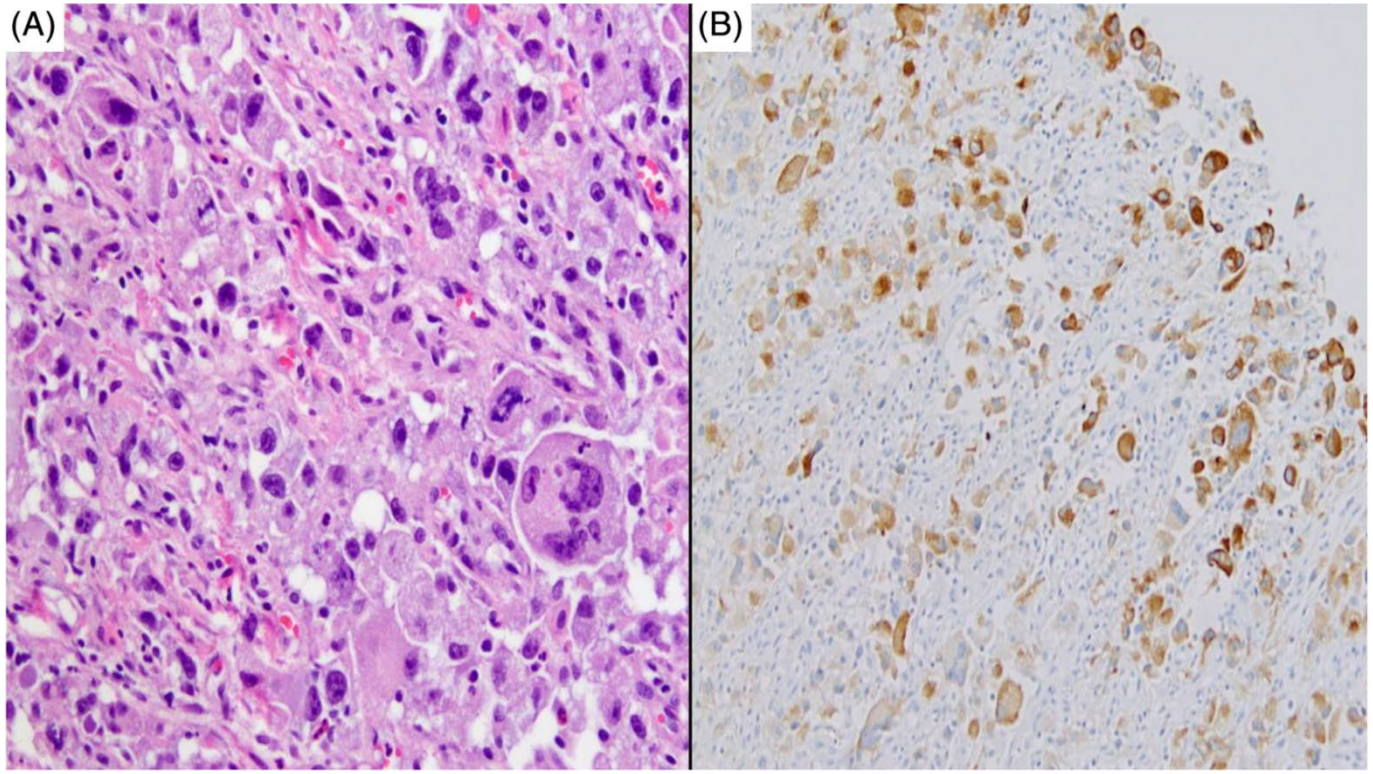
Cytology of sarcomatoid carcinoma



Cytologic features

- Pleomorphic malignant cells
- Multinucleation may be present
- Irregular nuclear contours
- Coarse chromatin pattern
- Prominent nucleoli
- Immunohistochemical pattern: often the tumor cells are positive for **keratin only!**
- **Tumor cells may be positive for TTF-1 or p40, but are often double negative.**

Associated histology



Brandler T et al. Diag. Cytopathol. 2019. 47:813-16.

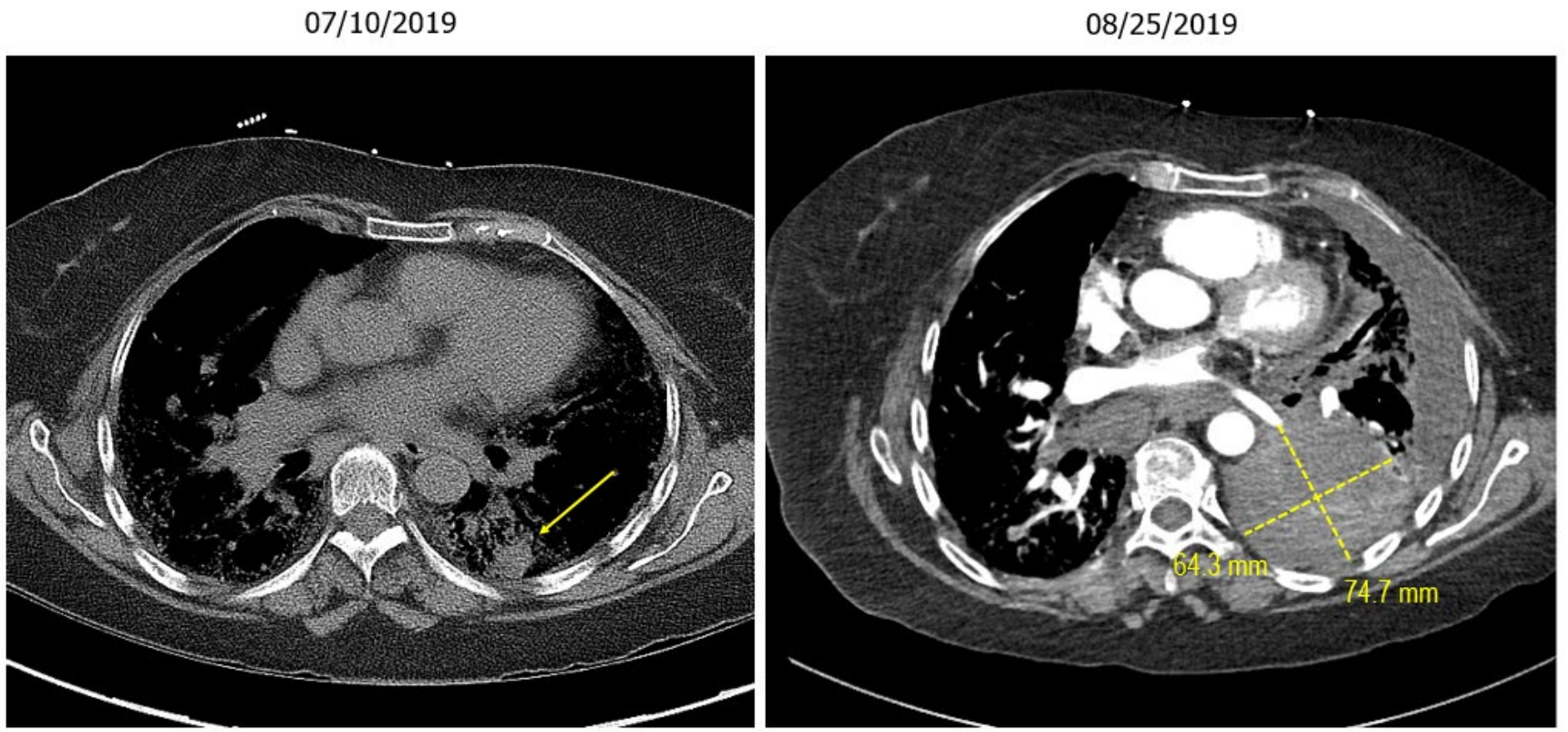
Considerations in sarcomatoid carcinoma

- Sarcomatoid carcinoma can occur in a background of adenocarcinoma or squamous cell carcinoma. This is important for selection of chemotherapy.
- Targetable mutations including EGFR, ALK and ROS-1 have been found.
- Therefore, it is important to consider molecular analysis if TTF-1 is positive or if the tumor is double negative (TTF-1/p63-p40 negative).
- MET exon 14 skipping is frequently associated with this tumor type.
- KRAS and p53 mutations are also common.

Case 3

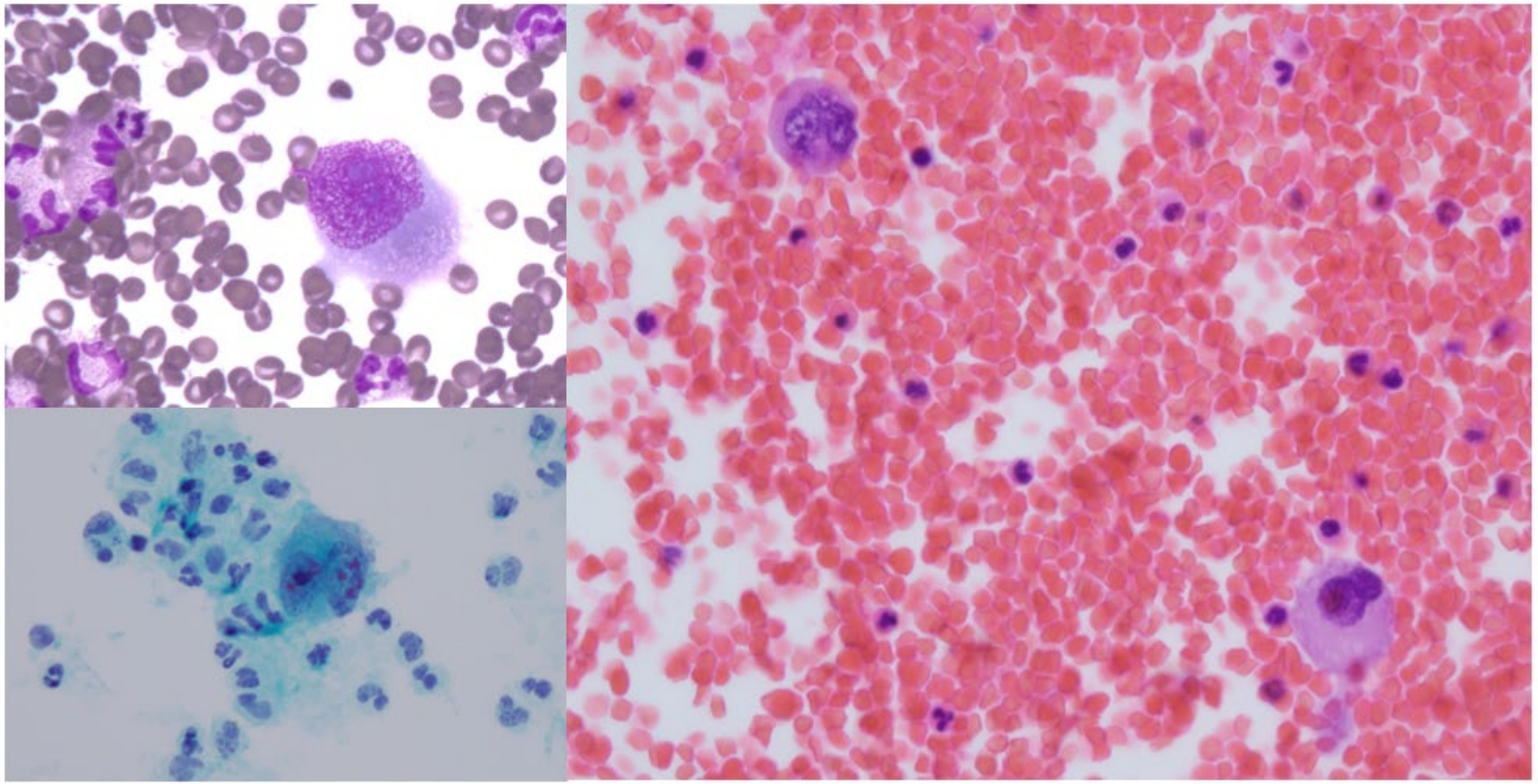
- 70 year old woman with severe progressive interstitial lung disease with significant decline in pulmonary functions was listed for lung transplant.
- A CT scan showed a new consolidation within the left lower lobe superimposed on pulmonary fibrosis and mediastinal lymphadenopathy.
- The patient clinical condition deteriorated rapidly and she was admitted to the hospital

CT scan

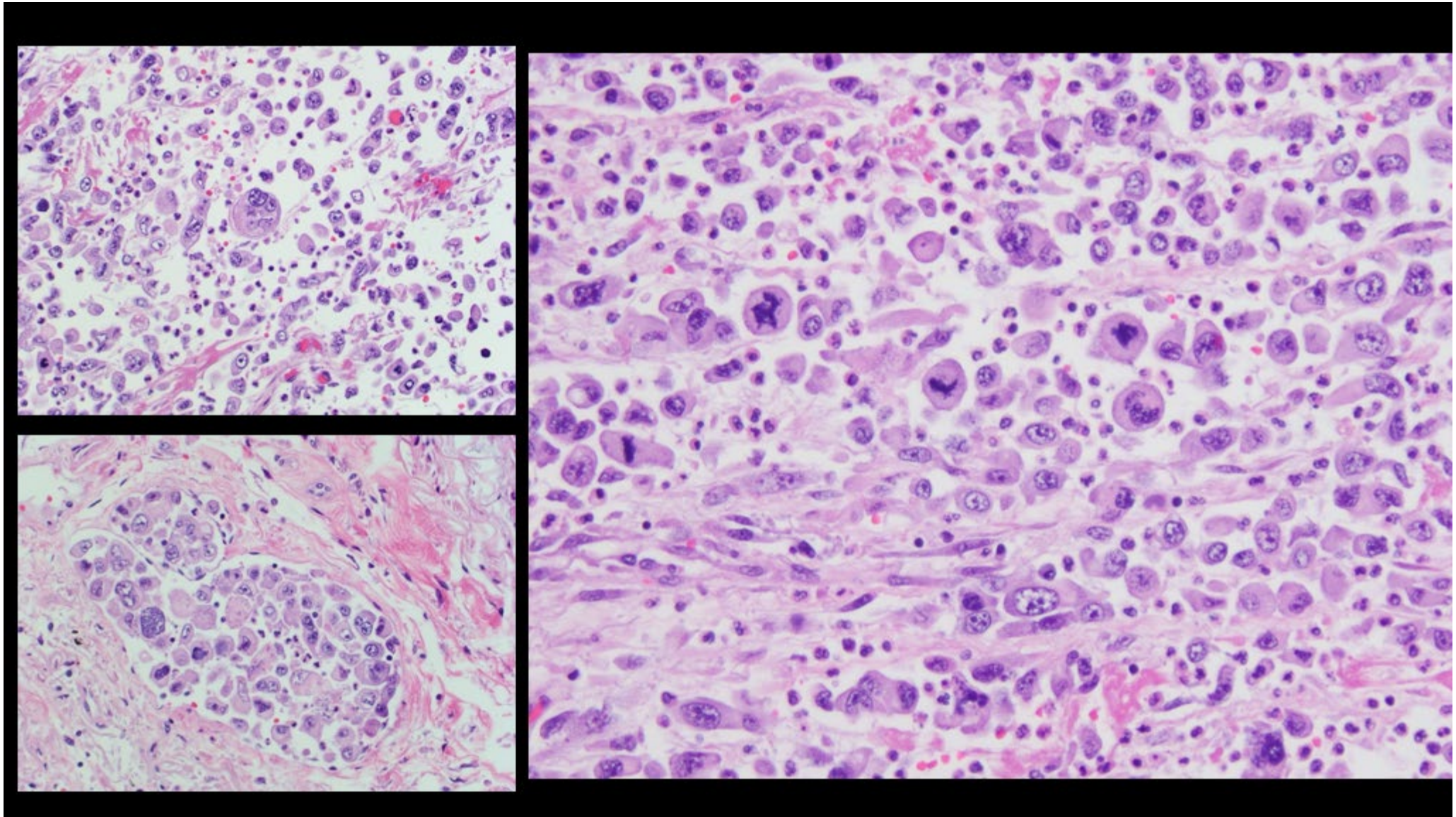


- Fast growth of the nodule
- Mixed attenuation, concerning fro necrosis
- Presence of loculated pleural effusion

Pleural effusion was obtained



Cell block



Case 3

Cytological features

- Large pleomorphic cells
- Multinucleation
- Multiple nucleoli
- Rhabdoid cells

❑Differential diagnosis

- Sarcomatoid carcinoma
- Melanoma
- Lymphoma
- Mesothelioma

Immunocytochemical stain

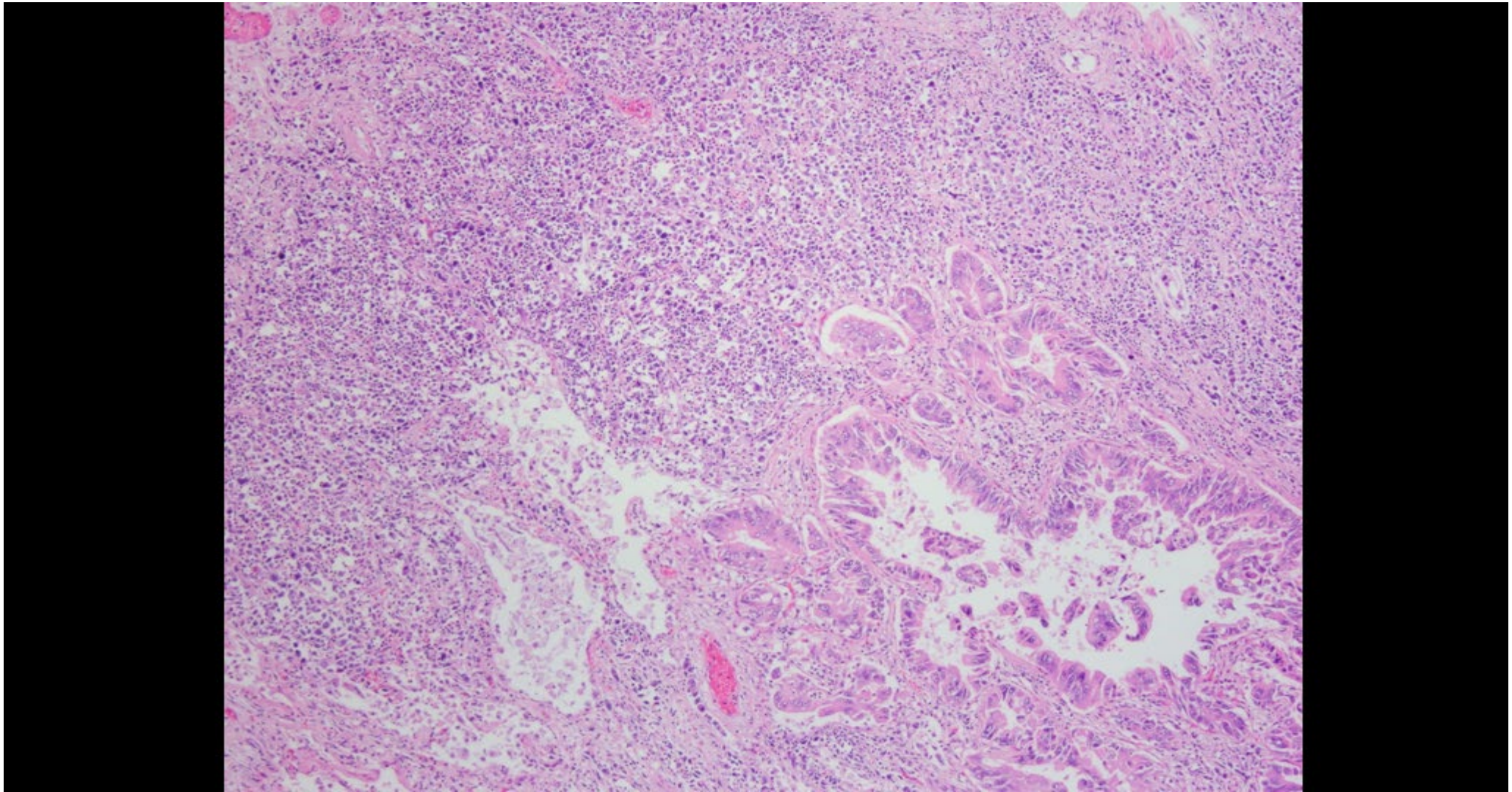
- Negative for multiple keratin markers
- Negative for lymphoid marker
- Negative for melanoma markers
- negative for mesothelioma marker

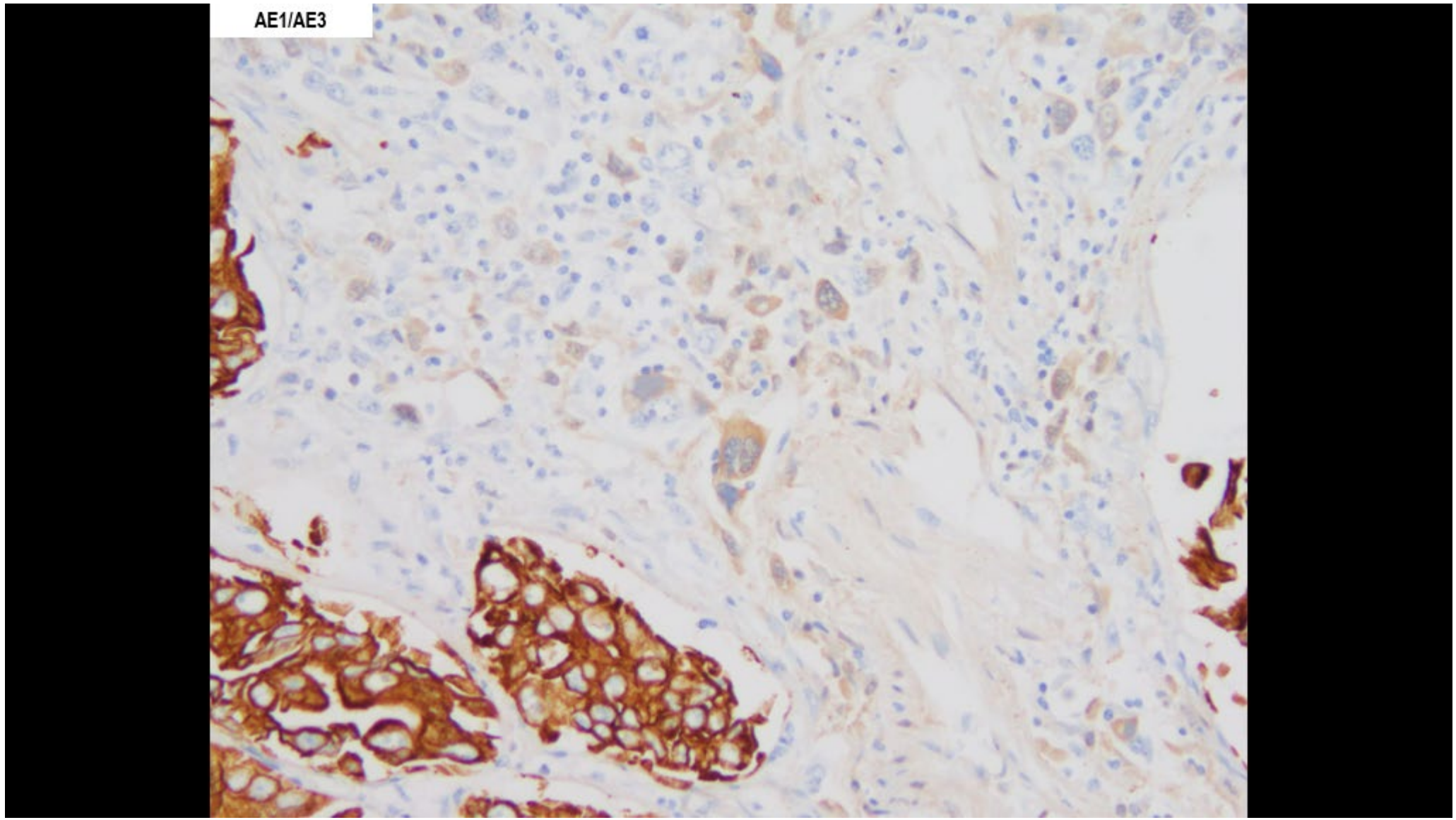
- Diagnosis: **Malignant neoplasm**

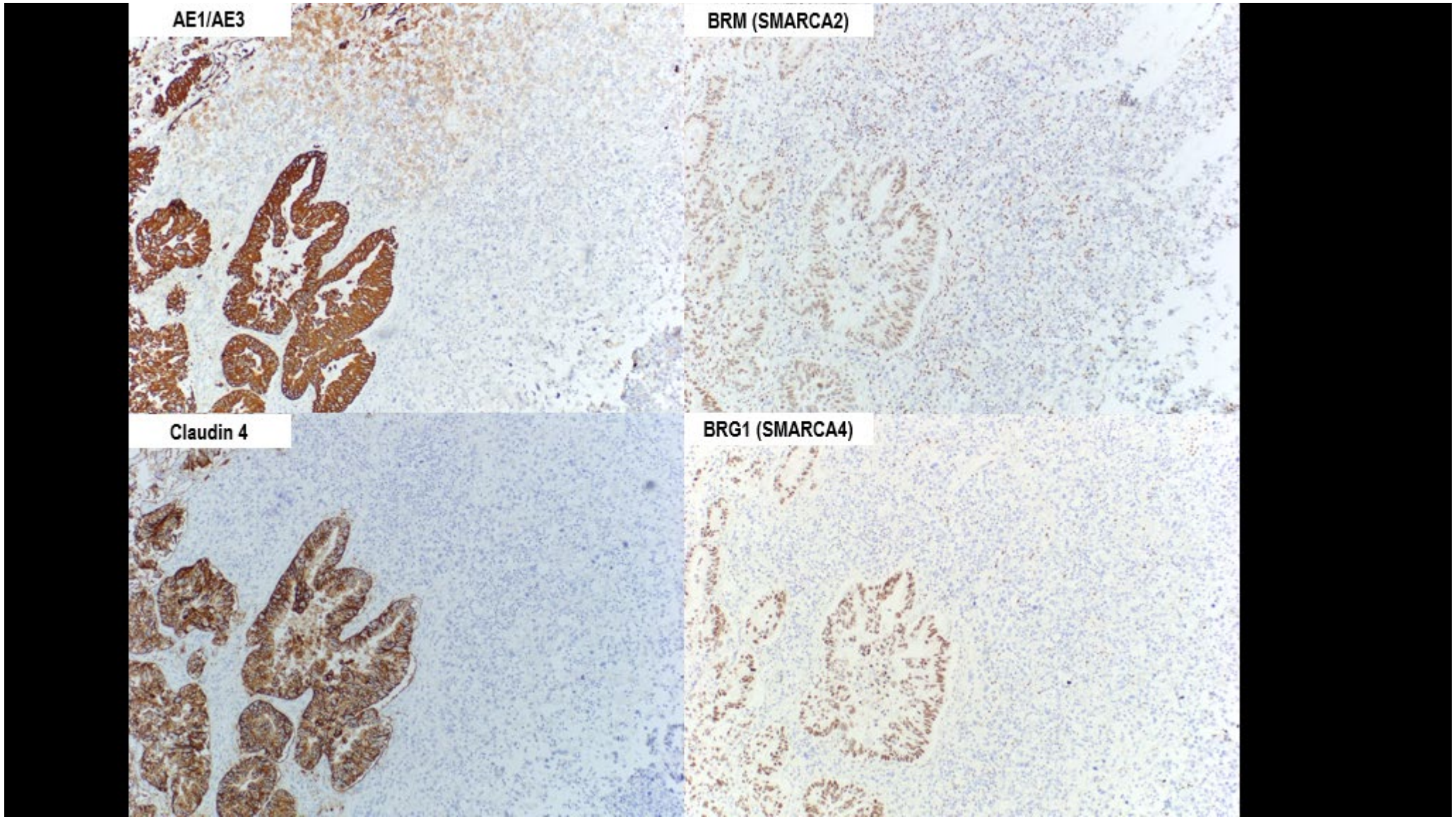
Case 3

- The patient passed way, and autopsy was performed.

Lung




AE1/AE3


AE1/AE3
BRM (SMARCA2)
Claudin 4
BRG1 (SMARCA4)

Thoracic SMARCA4-deficient tumors

- Strong association with smoking history
- More common in men
- Aggressive tumor
- BRG1 (SMARCA4) lost is most common, but partial loss can be seen in approximately 25% of cases.
- BRM (SMARB1) partial loss is common

Other uncommon tumors

- **Intestinal type adenocarcinoma, colloid carcinoma and other mucinous adenocarcinomas.**
- Pitfall:
- These tumors may express CDX2, a marker of intestinal differentiation, and may be confused with metastases from the gastrointestinal tract, including pancreas.

- Suggestion:
- Indicate that the tumor may be of pulmonary origin, provided that a metastasis from the gastrointestinal tract can be excluded.

Take home message

- Apply the minimal panel of TTF-1/P63-p40 to all NSCLC.
- Consider expanding the panel for tumors that are double negative

1. Check clinical history for other tumors
2. Consider using IHC for molecular markers (NUT and BRG1, BRM) for poorly-differentiated tumors
3. The incidence and prevalence of NUT and SMARCA4-deficient tumors is not well known as many remain undiagnosed.