HARVARD MEDICAL SCHOOL

Pulmonary Cytology:

Workup of Lung Cancer on FNA and Small Biopsy Specimens

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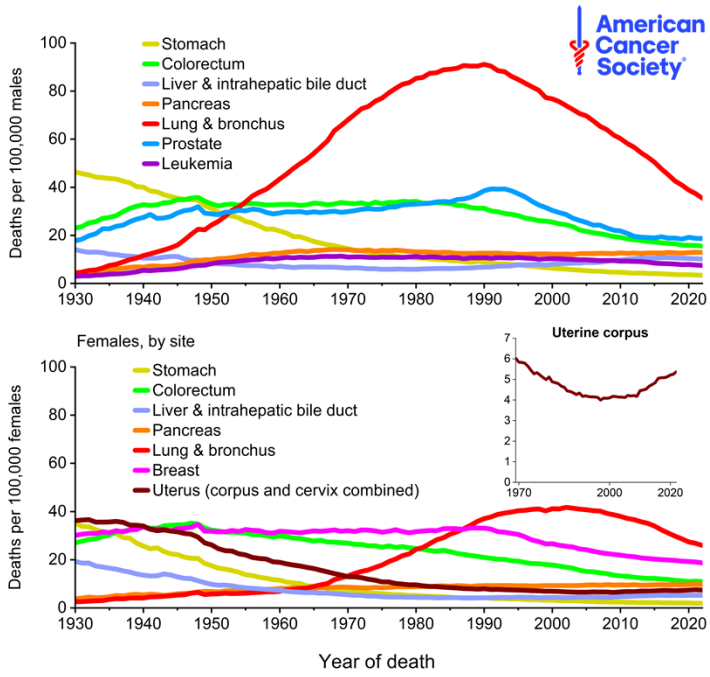
Disclosure of Relevant Financial Relationships

Paul VanderLaan reports the following relevant financial relationship(s) during the content development process for this activity, unrelated to the content of this talk:

Consulting fees:
- Gala/Galvanize Therapeutics
- Intuitive Surgical
- Ruby Robotics
- Veracyte
- Agilent Technologies



Siegel RL, et al. Cancer statistics, 2025. *CA Cancer J Clin*. 2025;75(1):10-45.

Lung cancer - Progress!

Prevention

✓ ↓ Smoking rates
USA: ~43% 1965 → ~10.8% 2023*

Early Detection

✓ CT Lung Cancer Screening
NLST & NELSON: ↓ 20-25% mortality

Diagnostic Procedures

✓ EBUS-TBNA
✓ ENB / Robotic Bronchoscopy

Treatment

✓ Surgery/SBRT
✓ Targeted therapies
✓ Immunotherapy

*** CDC - National Center for Health Statistics.**
Percentage of current cigarette smoking for adults aged 18 and over, United States, 2019-2023. National Health Interview Survey.
https://wwwn.cdc.gov/NHISDataQueryTool/SHS_adult/index.html

Patient presentation

Symptoms	Risk factors → Screening
+ Cough, hemoptysis, wt loss	+ Smoking

Imaging / Radiologic staging

CXR → Chest CT / PET-CT

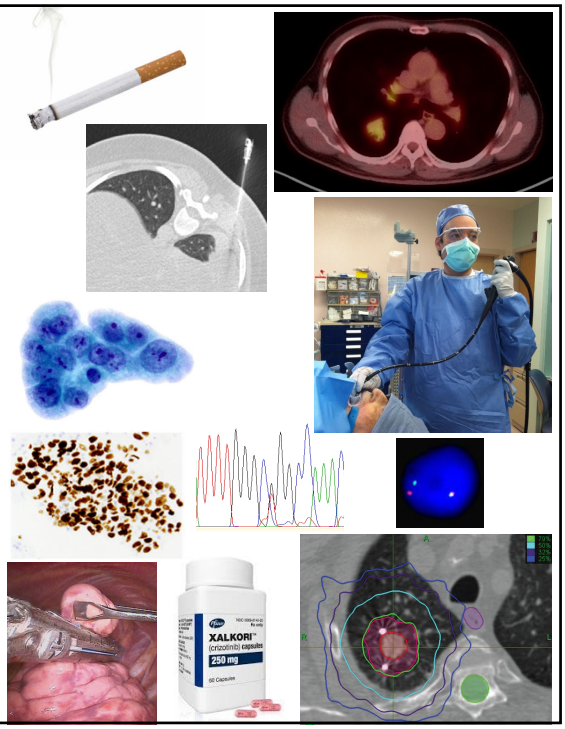
Tissue acquisition

Bronchoscopic Bx, Transthoracic Bx, EBUS-TBNA, Pleural fluid, Metastasis Bx

Pathology Diagnosis / Ancillary Testing

Tumor characterization (Proper specimen handling) →	Ancillary/Molecular testing

Treatment



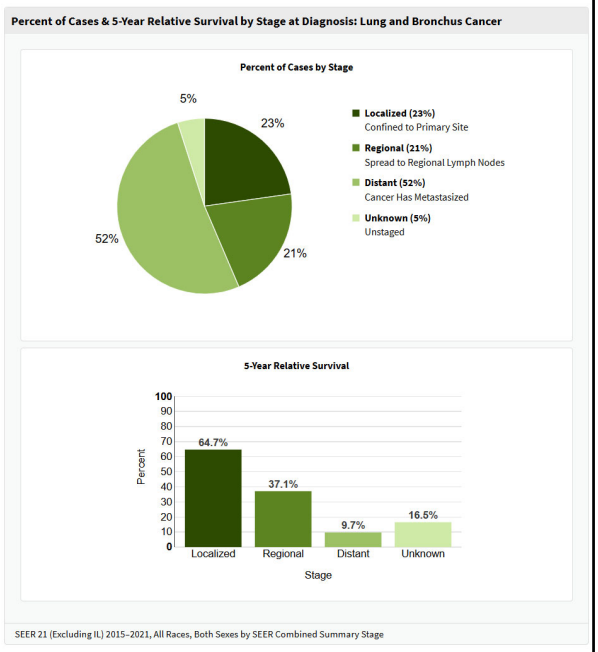
Pulmonary cytology and small biopsies

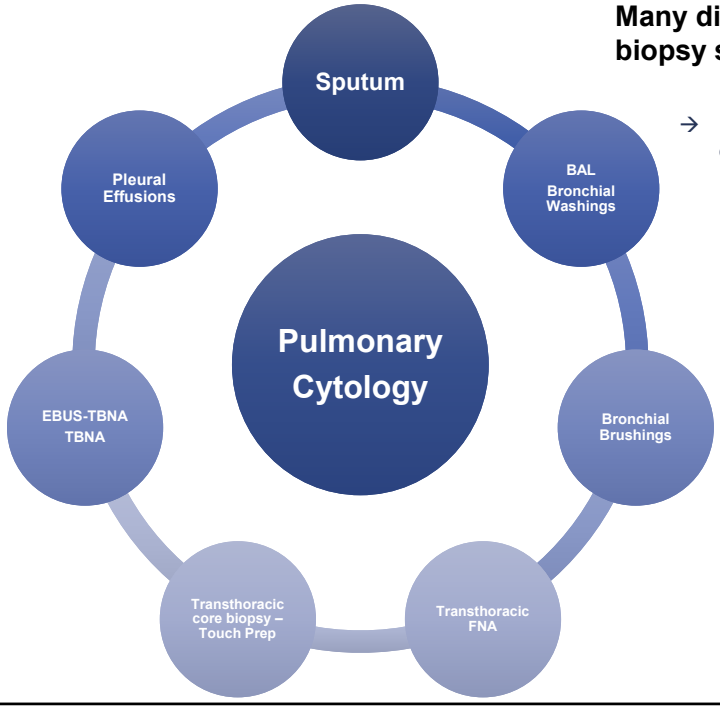
>2/3 of lung cancer patients present with advanced stage disease
- ✓Dx: cytology / small biopsy specimen

Minimally invasive sampling techniques have become the preferred diagnostic modality
- ✓Increase in cytology/small biopsy samples

Cytology/small biopsy specimens are used for diagnosis *and* ancillary molecular testing
- ✓Multiple objectives for the pathologist

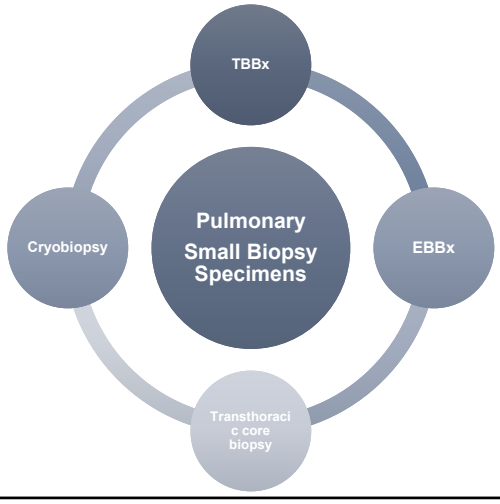
With resistance mechanisms following TKI therapy, new paradigm of *repeated* biopsies to guide therapy
- ✓Several biopsies from a patient over time

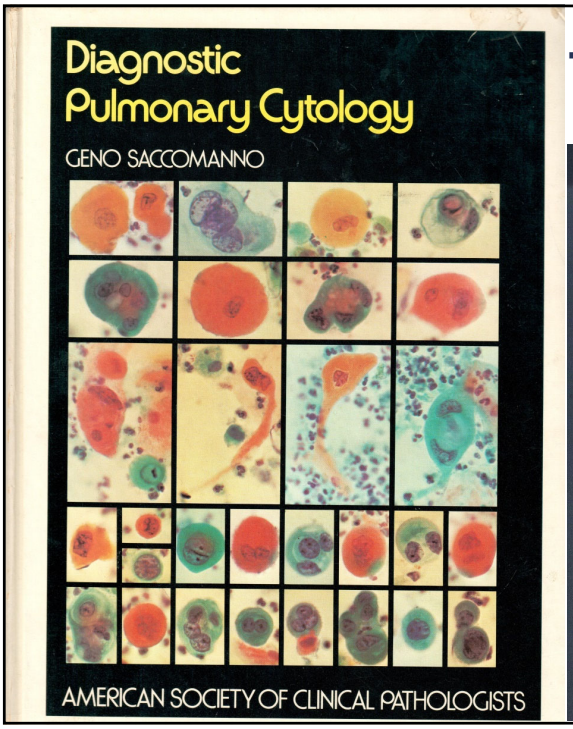




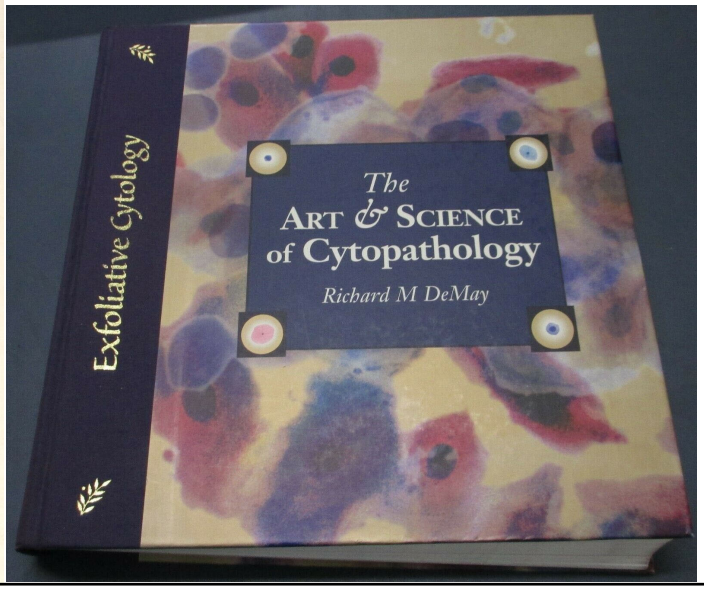
Many different ways to obtain cytology/small biopsy specimens from lung lesions

→ For the most part, cytomorphologic/histologic features of lesions similar regardless of acquisition method



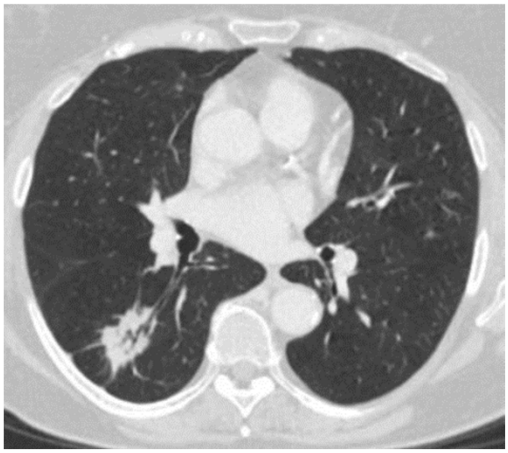


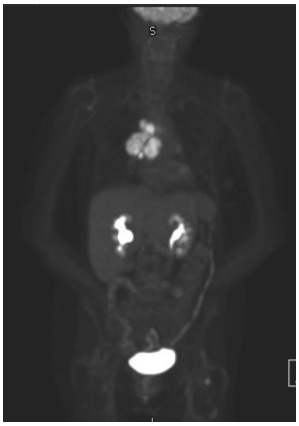
The cells still look the same…

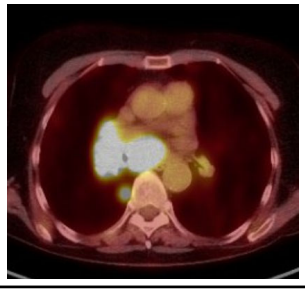


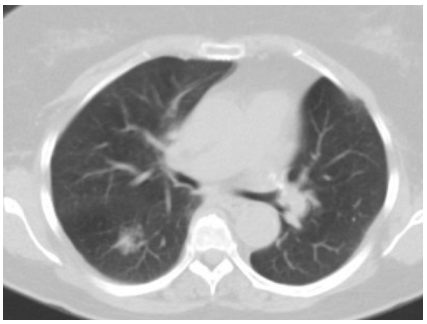
Question:

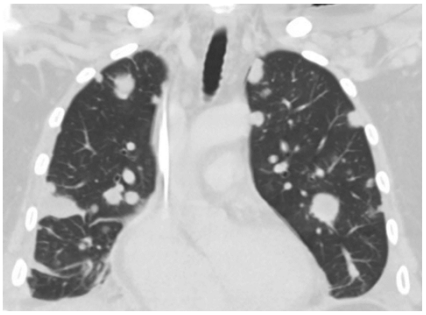
Patient with lung mass, is it cancer or not?





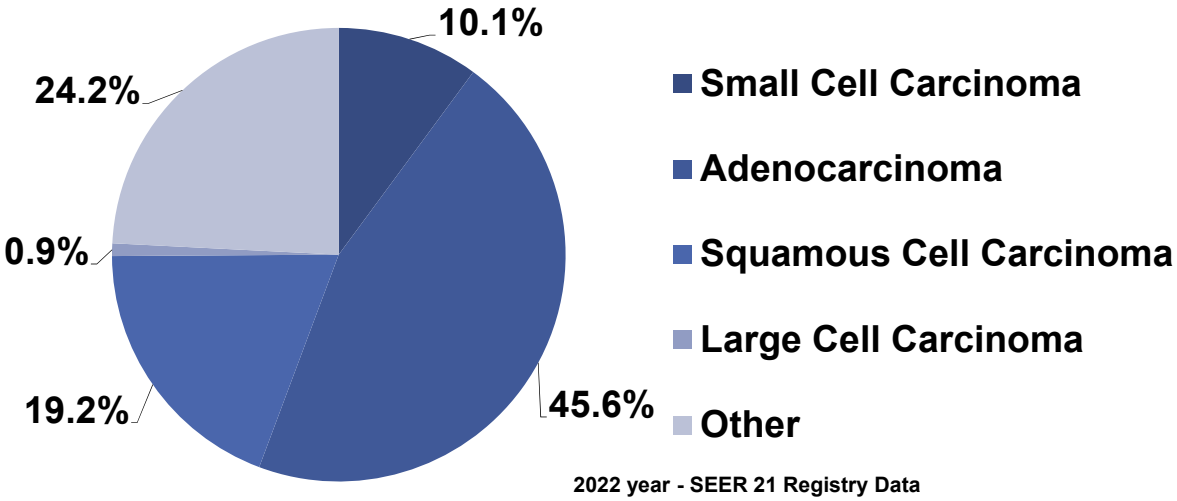






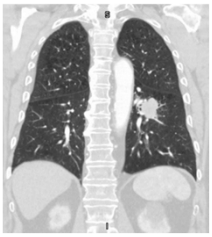
NATIONAL CANCER INSTITUTE
Surveillance, Epidemiology, and End Results Program

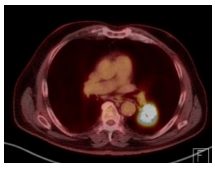
Cancer of the Lung and Bronchus

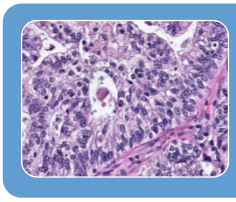


2022 year - SEER 21 Registry Data
(Subtype, Both Sexes, All Races, All Ages, All Stages, Observed Rates)

Diagnostic work-up of lung cancer

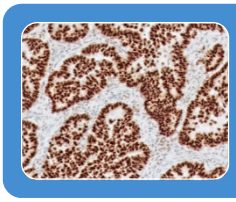






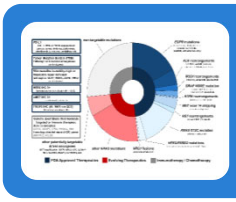
Malignant or not?

- Based on cytomorphologic assessment of cells, architectural cues, cellularity, background



Tumor type

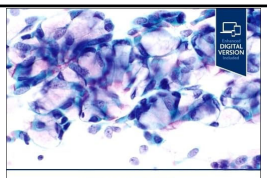
- Immunohistochemistry helps determine lineage of the cells in question
  - Adeno vs. Squam vs. Neuroendocrine vs. other



Ancillary testing

- Tissue preservation strategies are key!
  - Minimize levels/IHC stains; separate cores into 2 blocks (diagnosis vs testing); maximize cell block yield; use smears

Lung Cancer Cytomorphology



Journal of the American Society of Cytopathology (2020) 9, 332–345

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

Lung cancer cytology and small biopsy specimens: diagnosis, predictive biomarker testing, acquisition, triage, and management

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REVIEW

OPEN

Common Differential Diagnostic Issues in Lung Cytopathology: 3 Case Reports and a Review

Rachel Fanaroff, MD, Teklu B. Legesse, MD,† and Kim R. Geisinger, MD‡*

AJSP: Reviews & Reports 2021;26: 155–161

Diagnosis of Lung Carcinoma on Small Biopsy

Jian Jing, MD, PhD[a], Kristine E. Konopka, MD[b,*]

Surg Pathol Clin. 2020;13(1):1-15.

KEYWORDS

- Lung nodule • Core needle biopsy • Fine needle aspiration • Small biopsies
- Specimen management

Key points

- Early detection of lung cancer significantly reduces mortality, and small biopsies play a key part in allowing for early medical and/or surgical intervention.
- Pathologists are encouraged to build awareness for appropriate tissue triage of small biopsies to preserve material for possible ancillary molecular testing.
- Differentiating benign from malignant epithelial changes on small biopsies may require correlation with the clinical and radiographic context to avoid an erroneous overcall of malignancy.

Chapter 1

Small diagnostic samples

Travis WD
Al-Dayel FH
Bubendorf L
Chung JH
Rekhtman N
Scagliotti GV

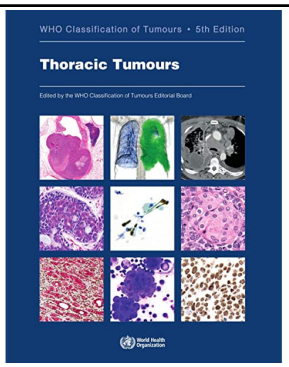
Rationale for classification in small biopsy and cytology specimens

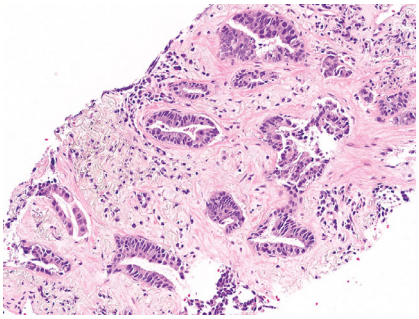
Pathological diagnosis is key to the management of lung cancer, in addition to careful consideration of risk factors and signs and symptoms, assessment of the extent of the disease (locally and outside the thoracic boundaries), and – in the case of resectable disease – evaluation of the cardiopulmonary and metabolic status of the patient. For the 70% of lung cancer patients who present with advanced-stage, unresectable disease, diagnosis must be based primarily on small biopsy and cytology specimens [3074].

Precise histological classification (and in many cases molecular and/or biomarker testing) of lung cancer is essential because of the clinical need for tailoring systemic therapies according to histological type as well as molecular/biomarker profiles [3068,3071,2760,1161]. However, achieving this goal can be

Box 1.01 Guidelines for good practice of small biopsies and cytological preparations [3073,3074]

1. For small biopsies and cytology, non-small cell carcinoma should be further classified into a more specific type, such as adenocarcinoma or squamous cell carcinoma, whenever possible.
2. The term "non-small cell lung carcinoma NOS" should be used as little as possible, and only when a more specific diagnosis is not possible.
3. When a diagnosis is made in a small biopsy or cytology specimen in conjunction with special studies, it should be clarified whether the diagnosis was established on the basis of light microscopy alone or whether special stains were required.
4. The term "non-squamous cell carcinoma" should not be used by pathologists in diagnostic reports. This categorization is used by clinicians to define groups of patients whose tumours comprise several histological types and who can be treated in a similar manner; in small biopsies and cytology, pathologists should classify non-small cell lung carcinoma as adenocarcinoma, squamous cell carcinoma, non-small cell lung carcinoma NOS, or other terms (see Table 1.04, p. 30).
5. The above classification of adenocarcinoma vs other histologies and the terminology in Table 1.04 (p. 30) and Table 1.05 (p. 31) should be used in routine diagnosis, future research, and clinical trials, to ensure a uniform classification of disease cohorts in relation to tumour subtypes, stratified according to diagnoses made by light microscopy alone vs diagnoses requiring special stains.
6. When paired cytology and biopsy specimens exist, they should be reviewed together to achieve the most specific and concordant diagnosis.
7. The terms "adenocarcinoma in situ" and "minimally invasive adenocarcinoma" should not be used for diagnosis of small biopsies or cytology specimens. If a non-invasive pattern is present in a small biopsy, it should be referred to as a lepidic growth pattern. Similarly, if a cytology specimen has the attributes of adenocarcinoma in situ, the tumour should be diagnosed as an adenocarcinoma, possibly with a comment that this may represent, at least in part, adenocarcinoma in situ.
8. The term "large cell carcinoma" should not be used for diagnosis in small biopsy or cytology specimens and should be restricted to resection specimens where the tumour is thoroughly sampled to exclude a differentiated component.
9. In biopsies of tumours that show sarcomatoid features (marked nuclear pleomorphism, malignant giant cells, or spindle cell morphology), these should be initially classified as above in relation to adenocarcinoma; non-small cell carcinoma, favour adenocarcinoma; squamous cell carcinoma; or non-small cell carcinoma, favour squamous cell carcinoma, because this is apt to influence management, with an additional statement that giant and/or spindle cell features (depending on what feature) are present. If such features are not present, the term "non-small cell carcinoma NOS" should be used, again with a comment on the sarcomatoid features.
10. Staining for neuroendocrine immunohistochemical markers should be performed only in cases where there is suspected neuroendocrine morphology.



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https://tumourclassification.iarc.who.int/welcome/index.html
WHO Classification of Tumours
Genetic Tumour Syndromes 5th ed. details
Skin Tumours 5th ed. details
Eye Tumours 5th ed. details
Haematolymphoid Tumours 5th ed. details
Endocrine Tumours 5th ed. details
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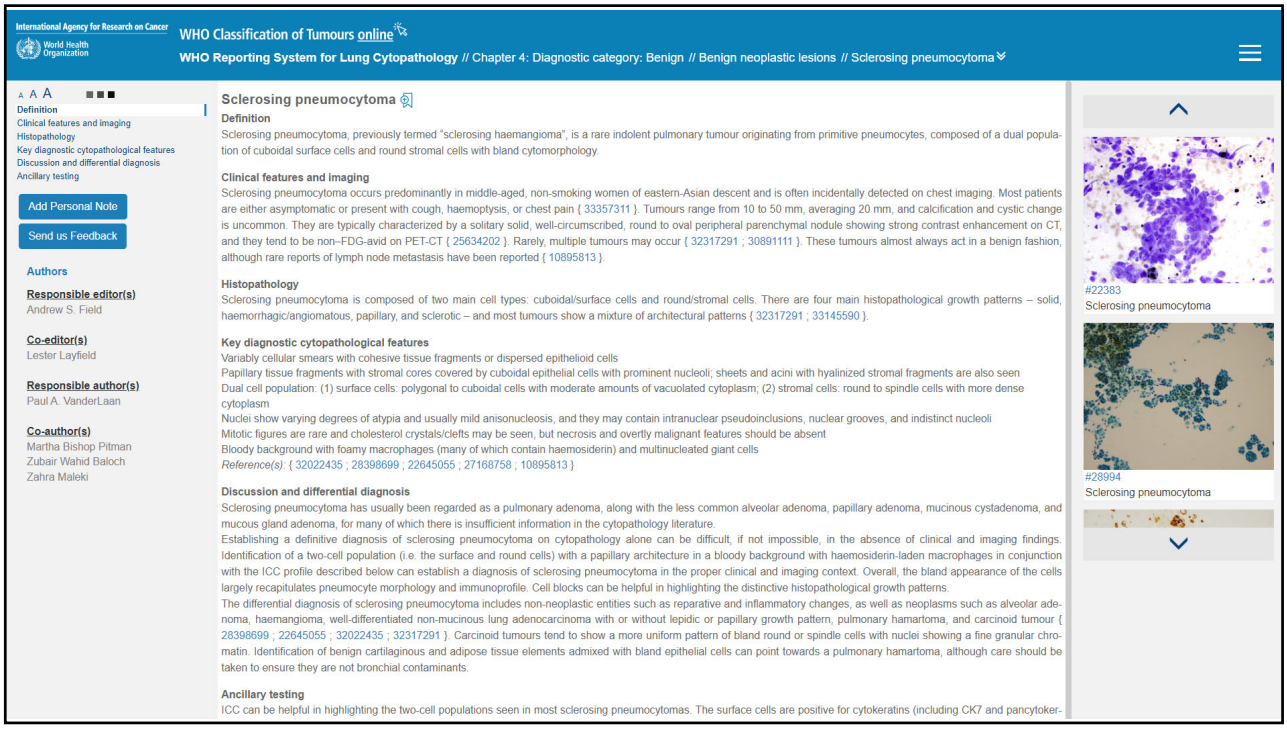
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WHO Reporting System for Lung Cytopathology // Chapter 4: Diagnostic category: Benign // Benign neoplastic lesions // Sclerosing pneumocytoma
Definition
Clinical features and imaging
Histopathology
Key diagnostic cytopathological features
Discussion and differential diagnosis
Ancillary testing
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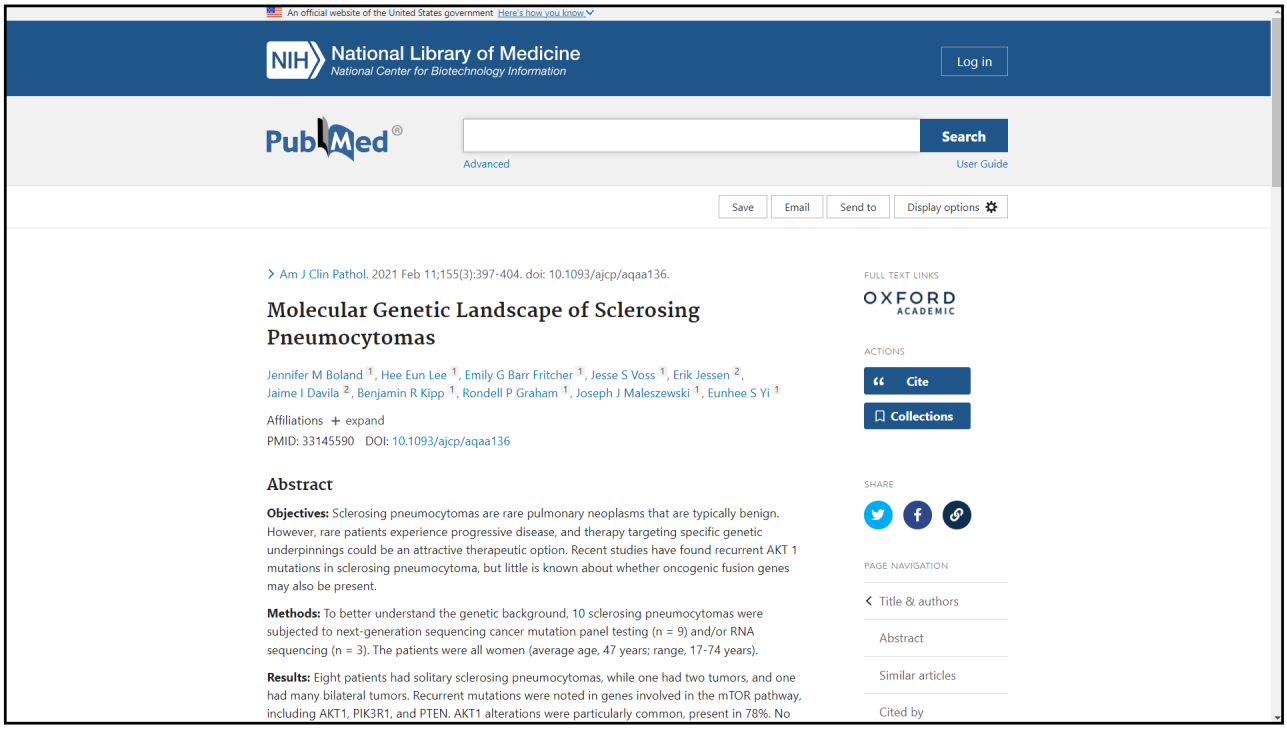
Paul A. VanderLaan
Co-author(s)
Martha Bishop Pitman
Zubair Wahid Baloch
Zahra Maleki
Sclerosing pneumocytoma
Definition
Sclerosing pneumocytoma, previously termed "sclerosing haemangioma", is a rare indolent pulmonary tumour originating from primitive pneumocytes, composed of a dual population of cuboidal surface cells and round stromal cells with bland cytomorphology.
Clinical features and imaging
Sclerosing pneumocytoma occurs predominantly in middle-aged, non-smoking women of eastern-Asian descent and is often incidentally detected on chest imaging. Most patients are either asymptomatic or present with cough, haemoptysis, or chest pain { 33357311 }. Tumours range from 10 to 50 mm, averaging 20 mm, and calcification and cystic change is uncommon. They are typically characterized by a solitary solid, well-circumscribed, round to oval peripheral parenchymal nodule showing strong contrast enhancement on CT, and they tend to be non–FDG-avid on PET-CT { 25634202 }. Rarely, multiple tumours may occur { 32317291 ; 30891111 }. These tumours almost always act in a benign fashion, although rare reports of lymph node metastasis have been reported { 10895813 }.
Histopathology
Sclerosing pneumocytoma is composed of two main cell types: cuboidal/surface cells and round/stromal cells. There are four main histopathological growth patterns – solid, haemorrhagic/angiomatous, papillary, and sclerotic – and most tumours show a mixture of architectural patterns { 32317291 ; 33145590 }.
Key diagnostic cytopathological features
Variably cellular smears with cohesive tissue fragments or dispersed epithelioid cells
Papillary tissue fragments with stromal cores covered by cuboidal epithelial cells with prominent nucleoli; sheets and acini with hyalinized stromal fragments are also seen
Dual cell population: (1) surface cells: polygonal to cuboidal cells with moderate amounts of vacuolated cytoplasm; (2) stromal cells: round to spindle cells with more dense cytoplasm
Nuclei show varying degrees of atypia and usually mild anisonucleosis, and they may contain intranuclear pseudoinclusions, nuclear grooves, and indistinct nucleoli
Mitotic figures are rare and cholesterol crystals/clefts may be seen, but necrosis and overtly malignant features should be absent
Bloody background with foamy macrophages (many of which contain haemosiderin) and multinucleated giant cells
Reference(s): { 32022435 ; 28398699 ; 22645055 ; 27168758 ; 10895813 }
Discussion and differential diagnosis
Sclerosing pneumocytoma has usually been regarded as a pulmonary adenoma, along with the less common alveolar adenoma, papillary adenoma, mucinous cystadenoma, and mucous gland adenoma, for many of which there is insufficient information in the cytopathology literature.
Establishing a definitive diagnosis of sclerosing pneumocytoma on cytopathology alone can be difficult, if not impossible, in the absence of clinical and imaging findings. Identification of a two-cell population (i.e. the surface and round cells) with a papillary architecture in a bloody background with haemosiderin-laden macrophages in conjunction with the ICC profile described below can establish a diagnosis of sclerosing pneumocytoma in the proper clinical and imaging context. Overall, the bland appearance of the cells largely recapitulates pneumocyte morphology and immunoprofile. Cell blocks can be helpful in highlighting the distinctive histopathological growth patterns.
The differential diagnosis of sclerosing pneumocytoma includes non-neoplastic entities such as reparative and inflammatory changes, as well as neoplasms such as alveolar adenoma, haemangioma, well-differentiated non-mucinous lung adenocarcinoma with or without lepidic or papillary growth pattern, pulmonary hamartoma, and carcinoid tumour { 28398699 ; 22645055 ; 32022435 ; 32317291 }. Carcinoid tumours tend to show a more uniform pattern of bland round or spindle cells with nuclei showing a fine granular chromatin. Identification of benign cartilaginous and adipose tissue elements admixed with bland epithelial cells can point towards a pulmonary hamartoma, although care should be taken to ensure they are not bronchial contaminants.
Ancillary testing
ICC can be helpful in highlighting the two-cell populations seen in most sclerosing pneumocytomas. The surface cells are positive for cytokeratins (including CK7 and pancytoker-
#22383
Sclerosing pneumocytoma
#28994
Sclerosing pneumocytoma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Display options
Am J Clin Pathol. 2021 Feb 11;155(3):397-404. doi: 10.1093/ajcp/aqaa136.
Molecular Genetic Landscape of Sclerosing Pneumocytomas
Jennifer M Boland [1], Hee Eun Lee [1], Emily G Barr Fritcher [1], Jesse S Voss [1], Erik Jessen [2], Jaime I Davila [2], Benjamin R Kipp [1], Rondell P Graham [1], Joseph J Maleszewski [1], Eunhee S Yi [1]
Affiliations + expand
PMID: 33145590 DOI: 10.1093/ajcp/aqaa136
Abstract
Objectives: Sclerosing pneumocytomas are rare pulmonary neoplasms that are typically benign. However, rare patients experience progressive disease, and therapy targeting specific genetic underpinnings could be an attractive therapeutic option. Recent studies have found recurrent AKT 1 mutations in sclerosing pneumocytoma, but little is known about whether oncogenic fusion genes may also be present.
Methods: To better understand the genetic background, 10 sclerosing pneumocytomas were subjected to next-generation sequencing cancer mutation panel testing (n = 9) and/or RNA sequencing (n = 3). The patients were all women (average age, 47 years; range, 17-74 years).
Results: Eight patients had solitary sclerosing pneumocytomas, while one had two tumors, and one had many bilateral tumors. Recurrent mutations were noted in genes involved in the mTOR pathway, including AKT1, PIK3R1, and PTEN. AKT1 alterations were particularly common, present in 78%. No
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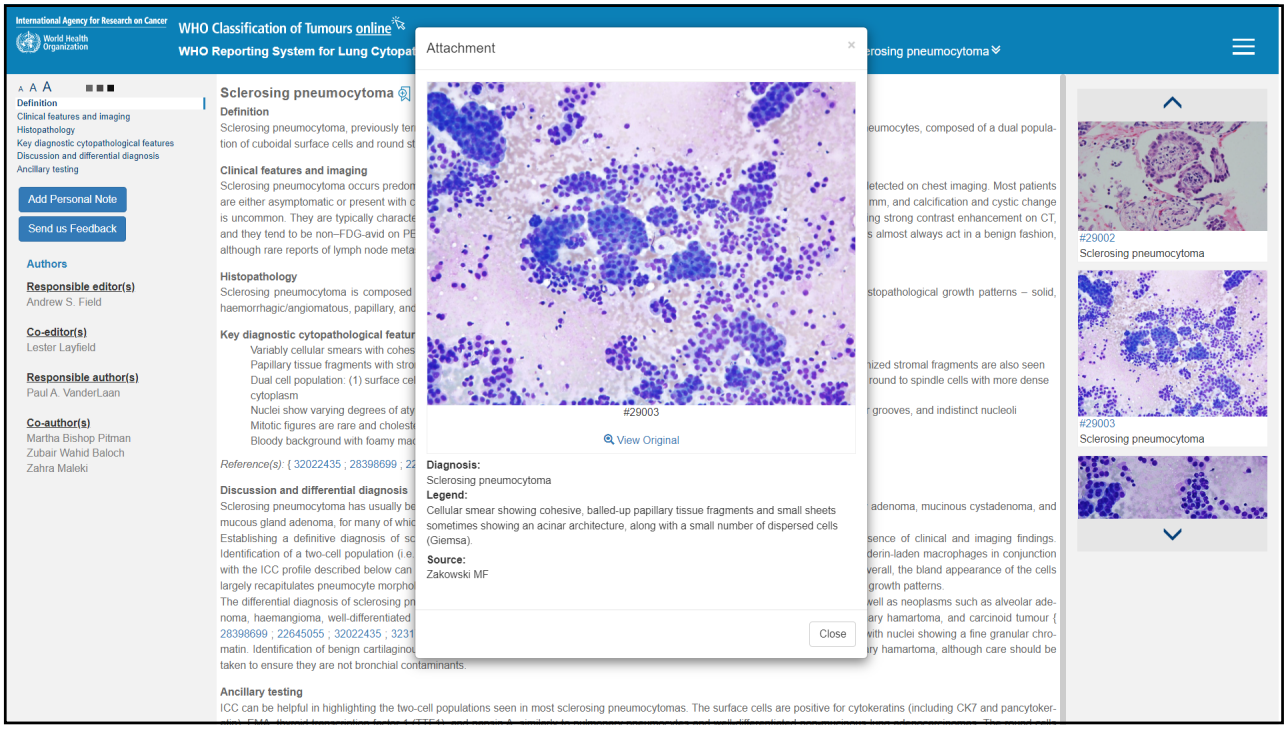
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Zahra Maleki
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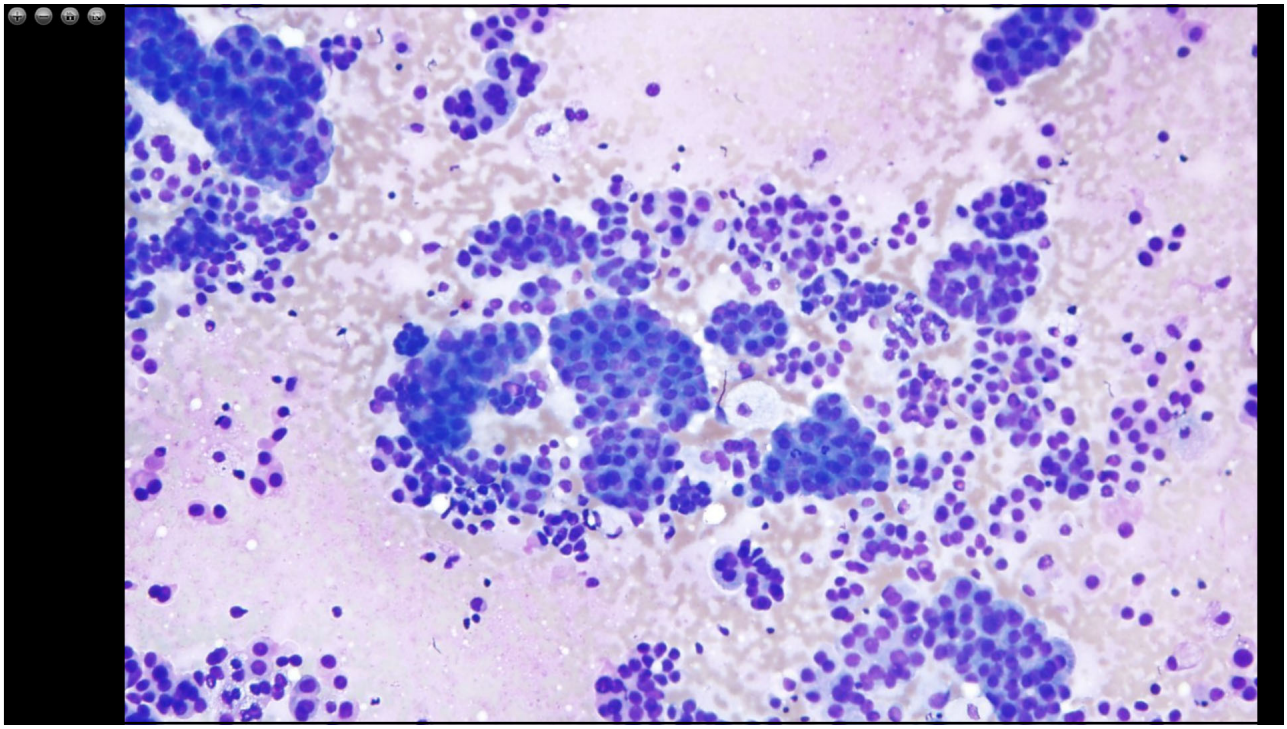
Clinical features and imaging
Histopathology
Key diagnostic cytopathological featur
Discussion and differential diagnosis
Ancillary testing
Attachment
#29003
View Original
Diagnosis:
Sclerosing pneumocytoma
Legend:
Cellular smear showing cohesive, balled-up papillary tissue fragments and small sheets sometimes showing an acinar architecture, along with a small number of dispersed cells (Giemsa).
Source:
Zakowski MF
Close
#29002
Sclerosing pneumocytoma
#29003
Sclerosing pneumocytoma



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Thoracic Tumours (5th ed.) // Tumours of the lung // Epithelial tumours // Adenomas // Sclerosing pneumocytoma

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Fernando Augusto Soares
Teh-Ying Chou
En-Hua Wang

Sclerosing pneumocytoma

Definition
Sclerosing pneumocytoma is a tumour of pneumocytic origin composed of a dual population of surface cells resembling type II pneumocytes and round cells. The tumour demonstrates varying amounts of solid, papillary, sclerotic, and haemorrhagic patterns.

ICD-O coding
8832/0 Sclerosing pneumocytoma

ICD-11 coding
2F0Z & XH7436 Benign neoplasms of respiratory and intrathoracic organs, unspecified & Sclerosing pneumocytoma

Related terminology
Not recommended: sclerosing haemangioma (obsolete).

Subtype(s)
None

Localization
Sclerosing pneumocytoma is typically solitary and peripheral. Rarely, tumours are multiple; occur as an endobronchial mass; or are situated in the hilum, visceral pleura, or mediastinum { 14704717 ; 30985653 }.

Clinical features
Patients are typically asymptomatic, with the tumour often discovered incidentally. Radiographs show a solitary circumscribed mass, which may rarely be calcified or cystic { 12016362 ; 22832056 ; 15176609 ; 21529795 ; 25634202 }.

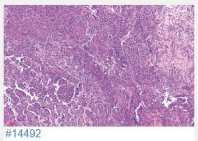
Epidemiology
Sclerosing pneumocytoma occurs in a wide age range (11–80 years), with female predominance. The incidence is higher in eastern Asian populations and rare among individuals of European descent { 23587094 ; 10895813 ; 20123460 ; 12728972 ; 30691016 }.

Etiology
The tumour is thought to derive from primitive respiratory epithelium { 23587094 ; 10895813 ; 20123460 ; 11075855 ; 17873892 }.

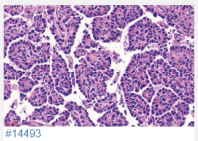
Pathogenesis
Although sclerosing pneumocytoma is presumably derived from primitive pulmonary epithelium, the pathogenesis is unknown. However, molecular data have demonstrated the same monoclonal pattern in both the round and surface cells, consistent with a true neoplasm { 17873892 }. *AKT1* alterations are present in nearly all sclerosing pneumocytomas and may be useful for diagnosis in selected cases.

Macroscopic appearance
The tumours are well-circumscribed masses that are solid and show a variegated grey-tan to yellow on cut section, with foci of haemorrhage. Cystic degeneration and calcification may be evident.

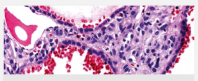
Histopathology
The key feature of sclerosing pneumocytoma is the presence of two cell types: cuboidal surface cells and round stromal cells, both of which are considered neoplastic. The sur-



#14492
Sclerosing pneumocytoma



#14493
Sclerosing pneumocytoma



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The World Health Organization Reporting System for Lung Cytopathology

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Diagnostic category	Estimated risk of malignancy, %	Clinical management options
Insufficient/Inadequate/Non-diagnostic	43–53	Correlate with CLIN-IMG-MICRO, ideally discuss at a MDT meeting, and perform repeat FNAB with or without CNB
Benign/negative for malignancy	19–64	Correlate with CLIN-IMG-MICRO, and if these confirm benign diagnosis, then routine follow up at 3–6 months. If no correlation, then perform repeat FNAB with or without CNB
Atypical	46–55	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all show a benign diagnosis, then routine follow up at 3–6 months. If no correlation, then perform repeat FNAB with ROSE with or without CNB
Suspicious for malignancy	75–88	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all support a diagnosis of malignancy, consider definitive treatment. If no correlation that lesion is Malignant, perform repeat FNAB with ROSE with or without CNB
Malignant	87–100	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all support a diagnosis of malignancy, provide definitive treatment. If no correlation that lesion is Malignant, consider repeat FNAB with ROSE with or without CNB

Journal of the American Society of Cytopathology (2023) 12, 251–257

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A brief review of the WHO reporting system for lung cytopathology

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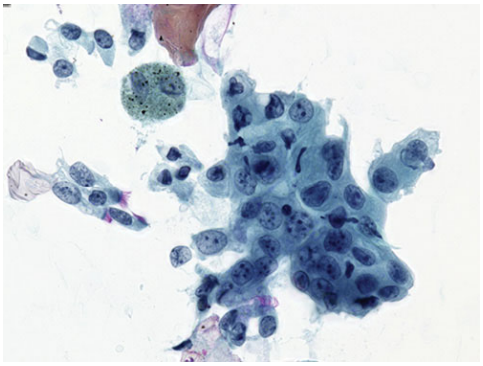
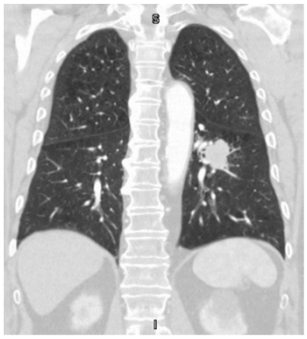
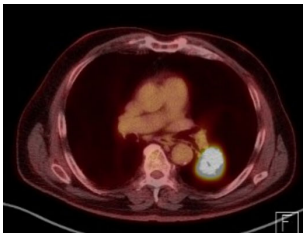
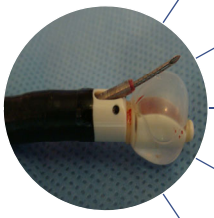


Our job

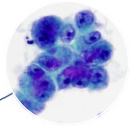
Cytology/small biopsy specimens



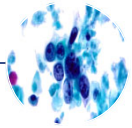




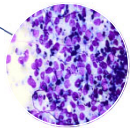
- NSCLC
  Favor SqmCC



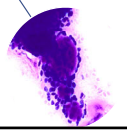
- NSCLC
  Favor ACA



- NSCLC - NOS

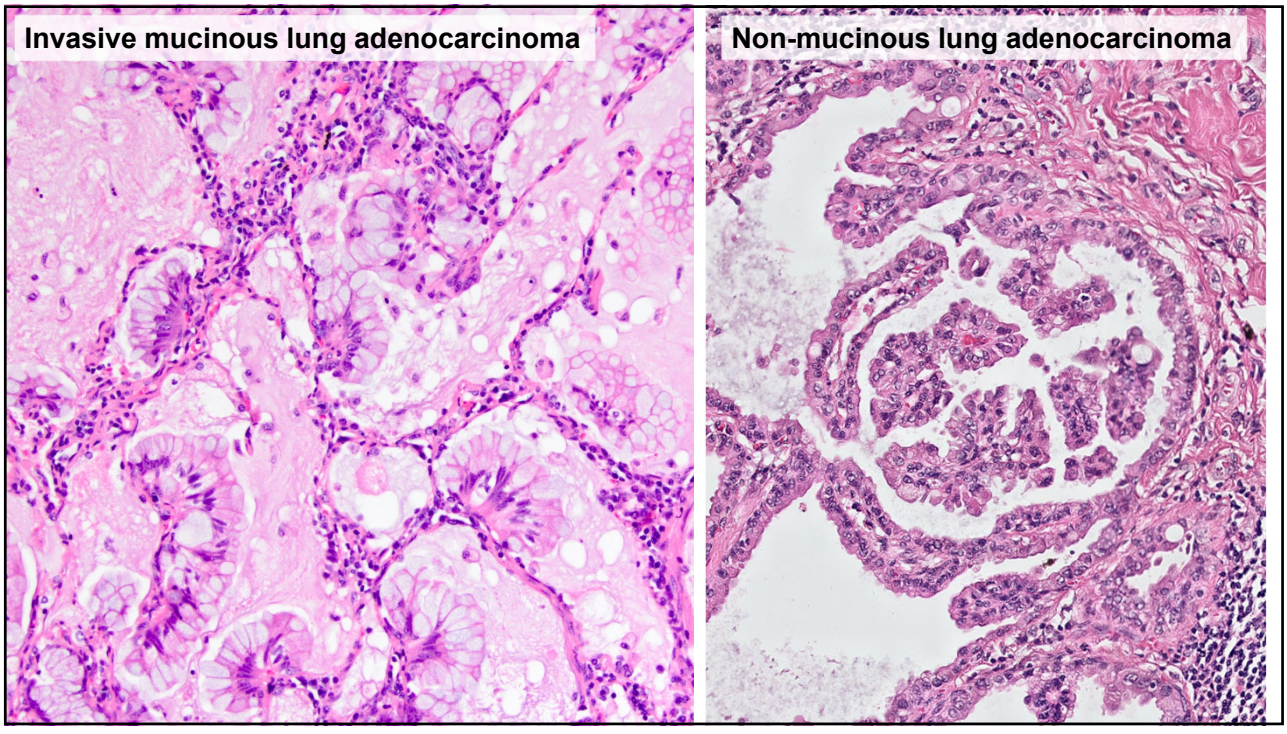


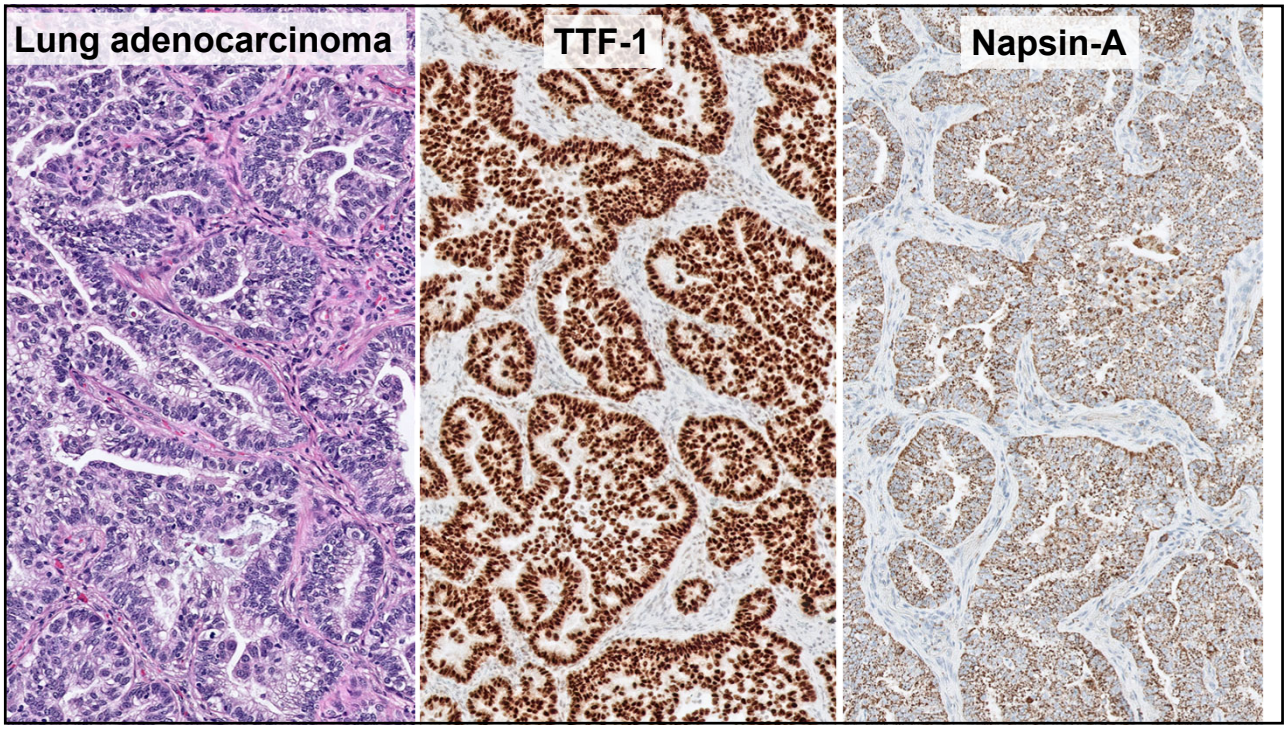
- Carcinoid NOS
- SCLC
- LCNEC



- Other:
  Salivary tumor,
  Sarcoma, etc…

Lung Adenocarcinoma





Lung Adenocarcinoma
Cytologic features

Nuclei:

- Eccentric nuclei with prominent nuclei
- WD: Intranuclear inclusions, pale chromatin, smooth nuclear membranes
- PD: Irregular nuclear membrane and clumped chromatin

Cytoplasm:

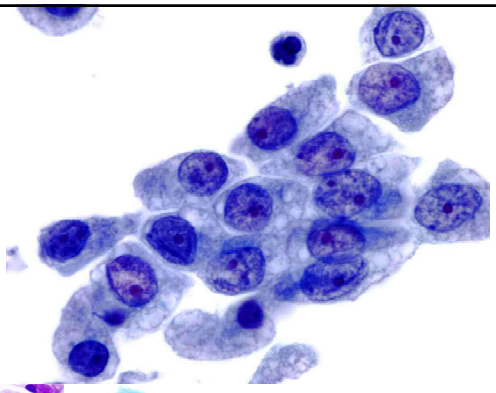
- Pale, foamy, or vacuolated cytoplasm
- Intracytoplasmic mucin/globules

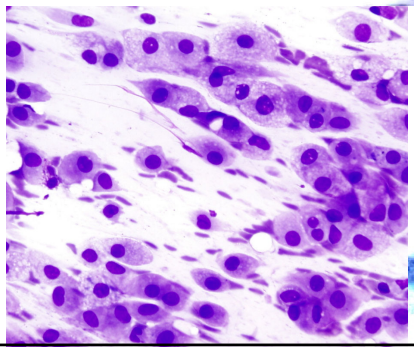
Architecture:

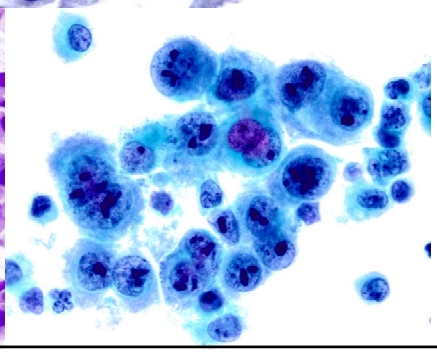
- Glandular or tubular structures
- Honeycomb sheets
- Three-dimensional cell clusters

Other:

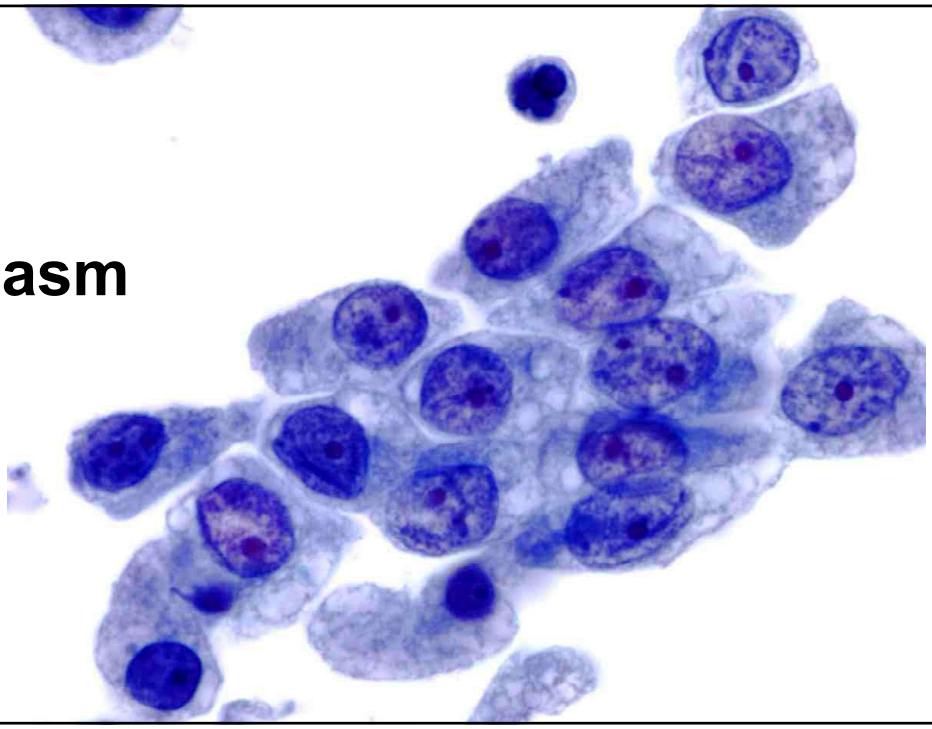
- Hypercellular
- Extracellular mucin



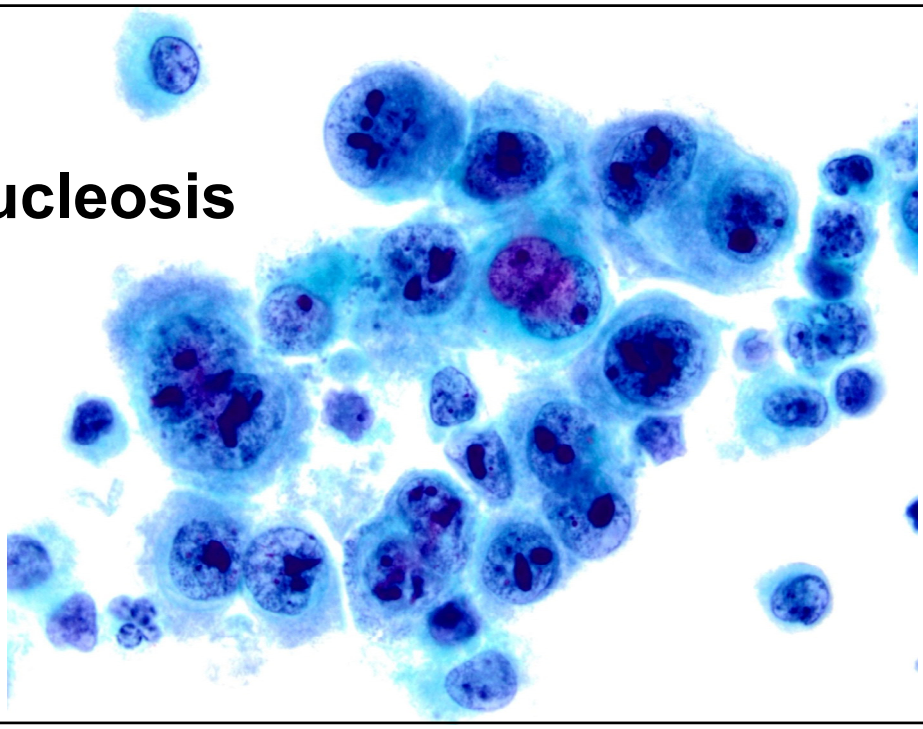


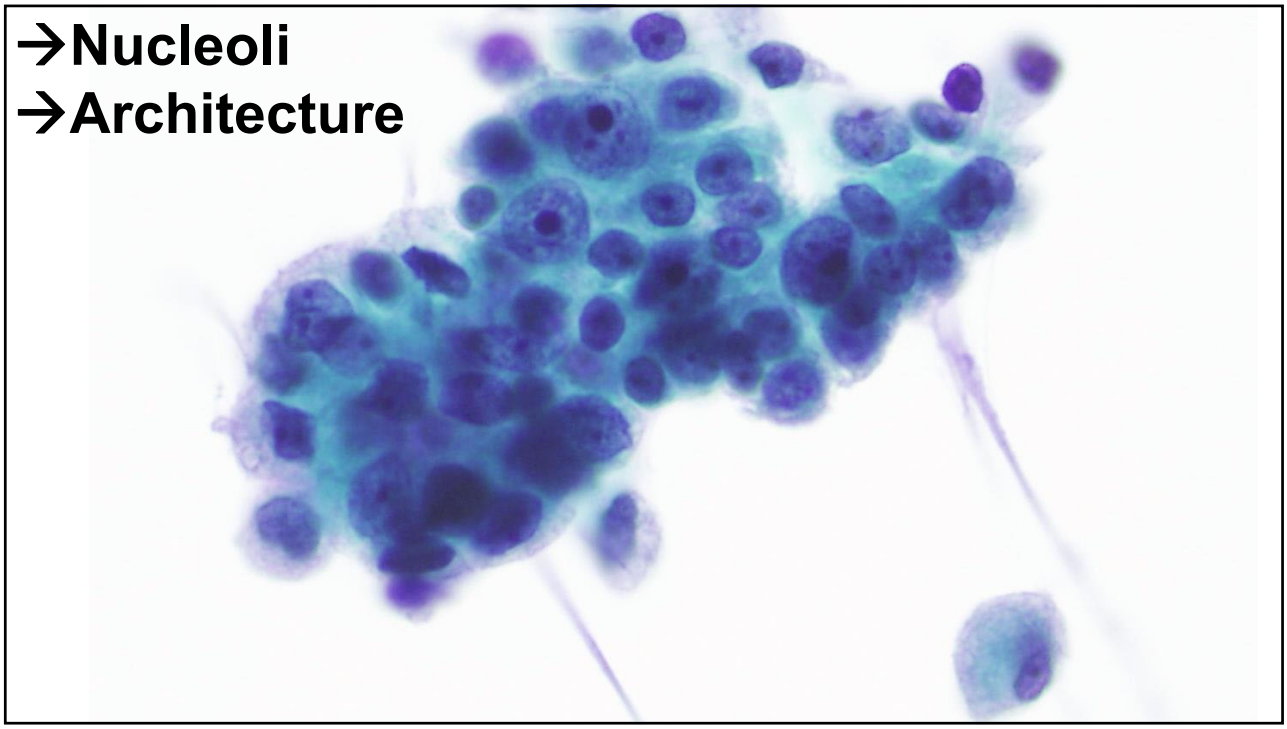


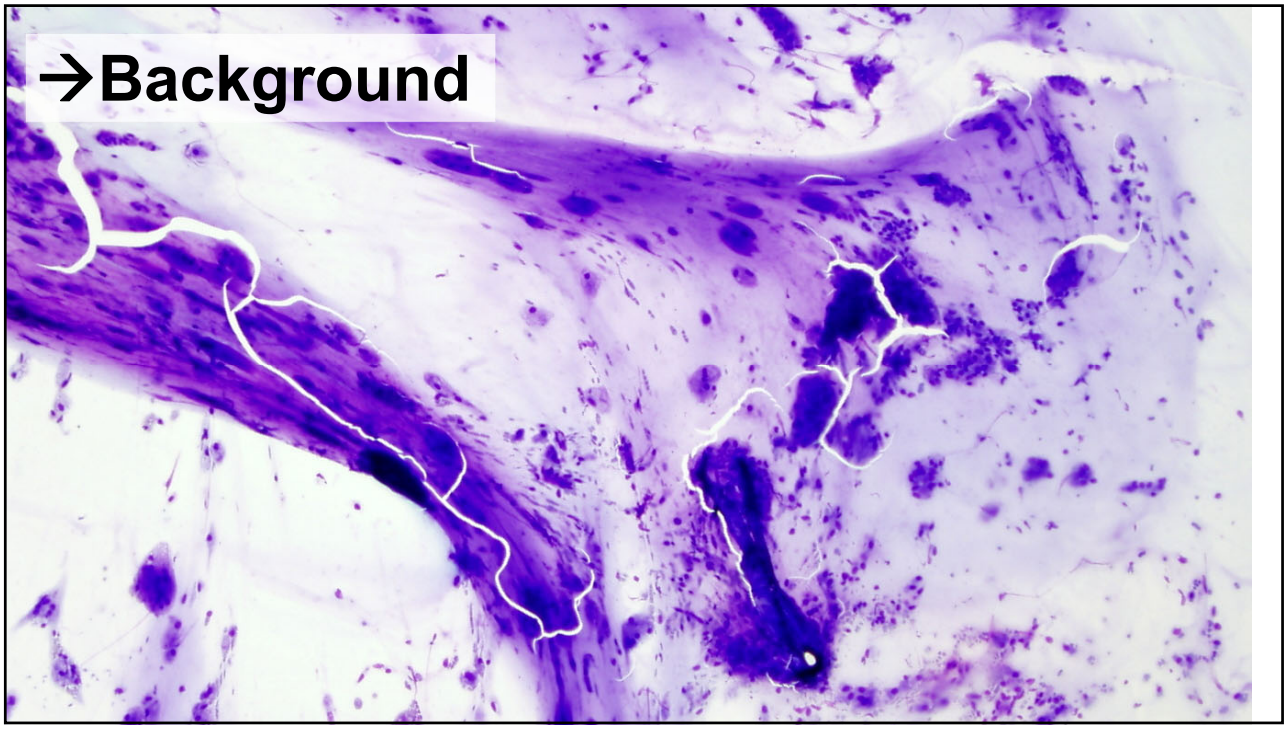
→Nuclei
→Cytoplasm

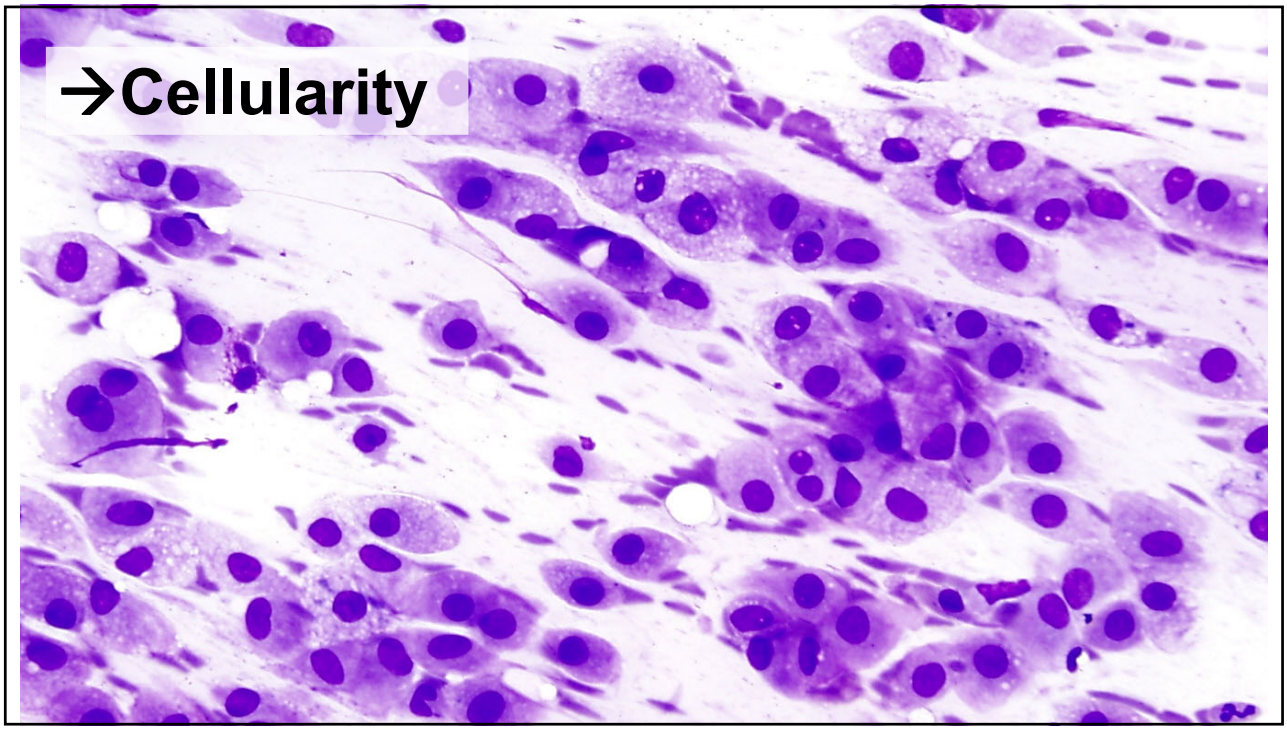


→Anisonucleosis

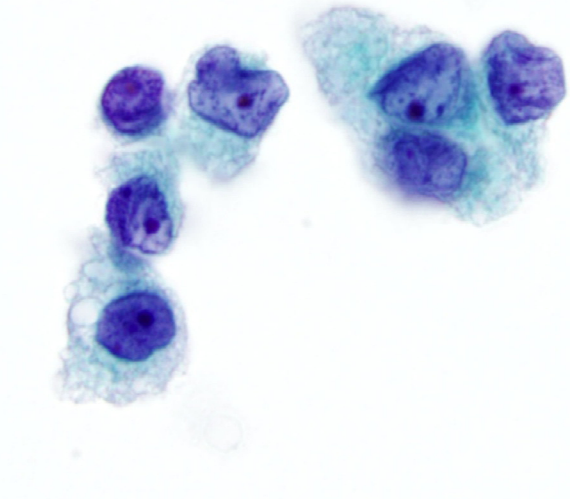


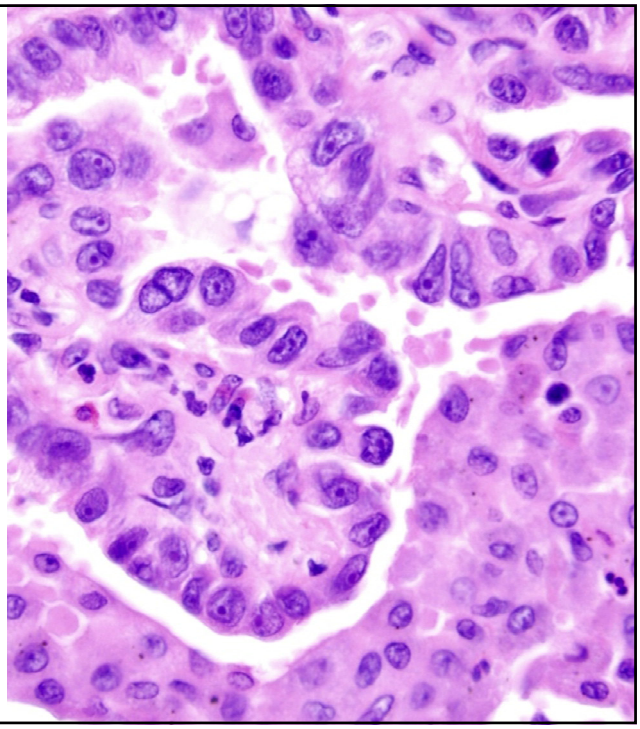

→Nucleoli
→Architecture


→Background

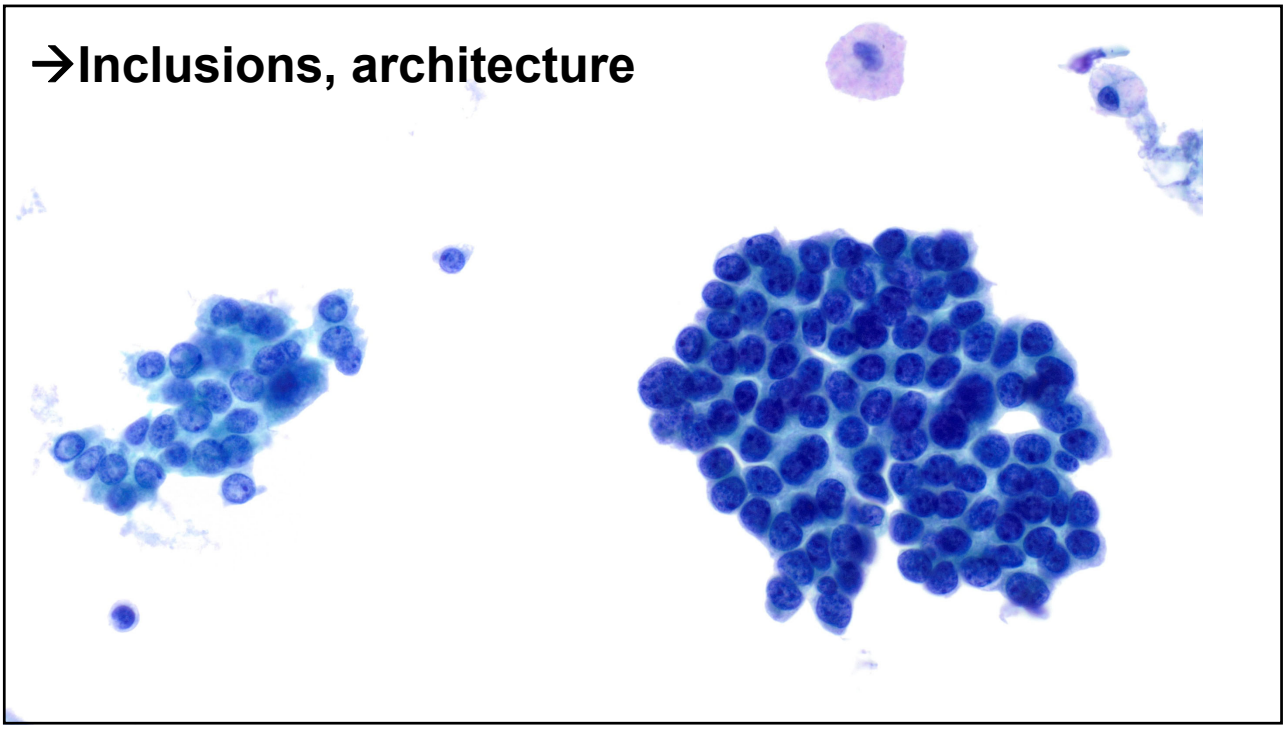


→Nuclear membranes

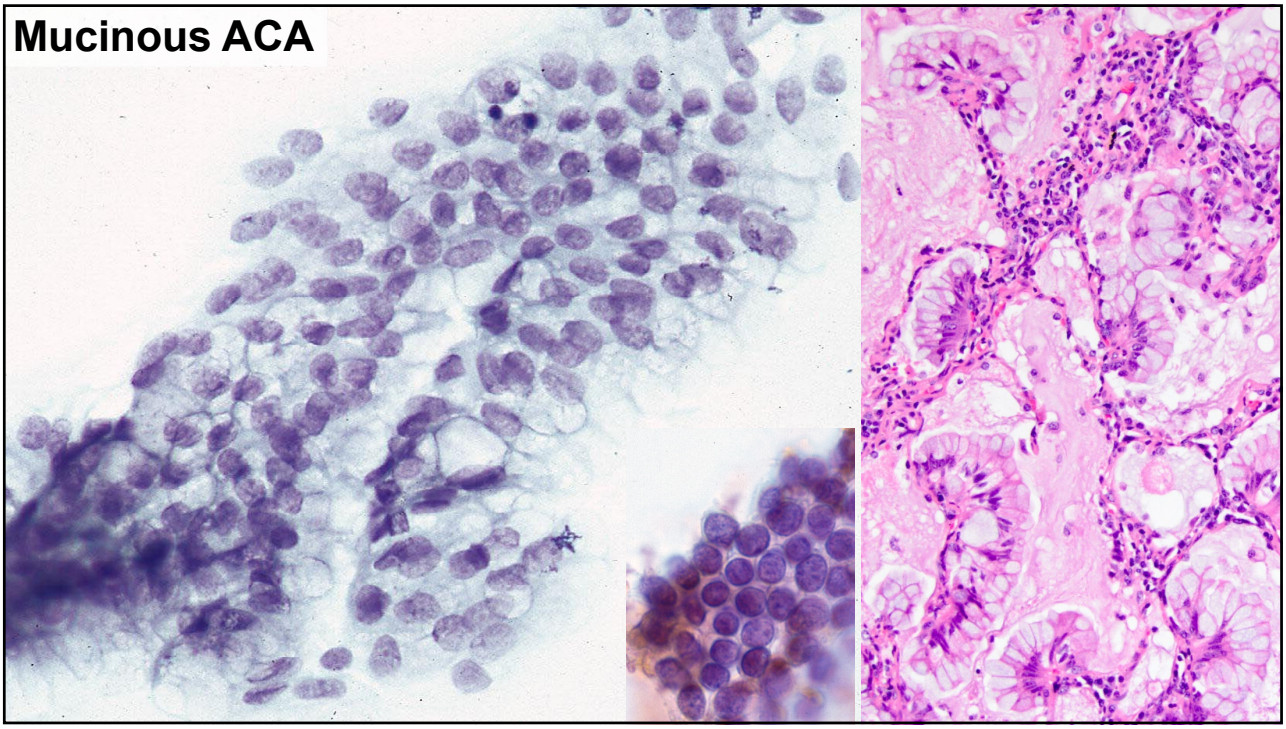


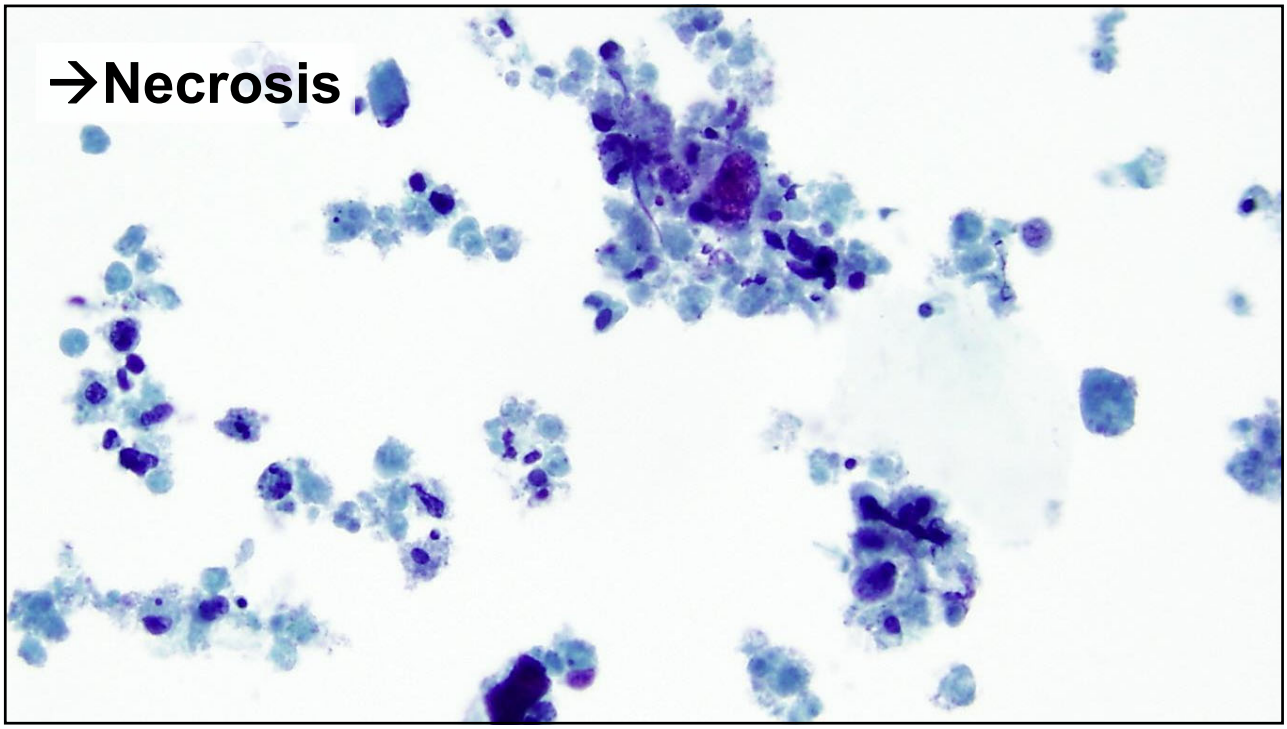


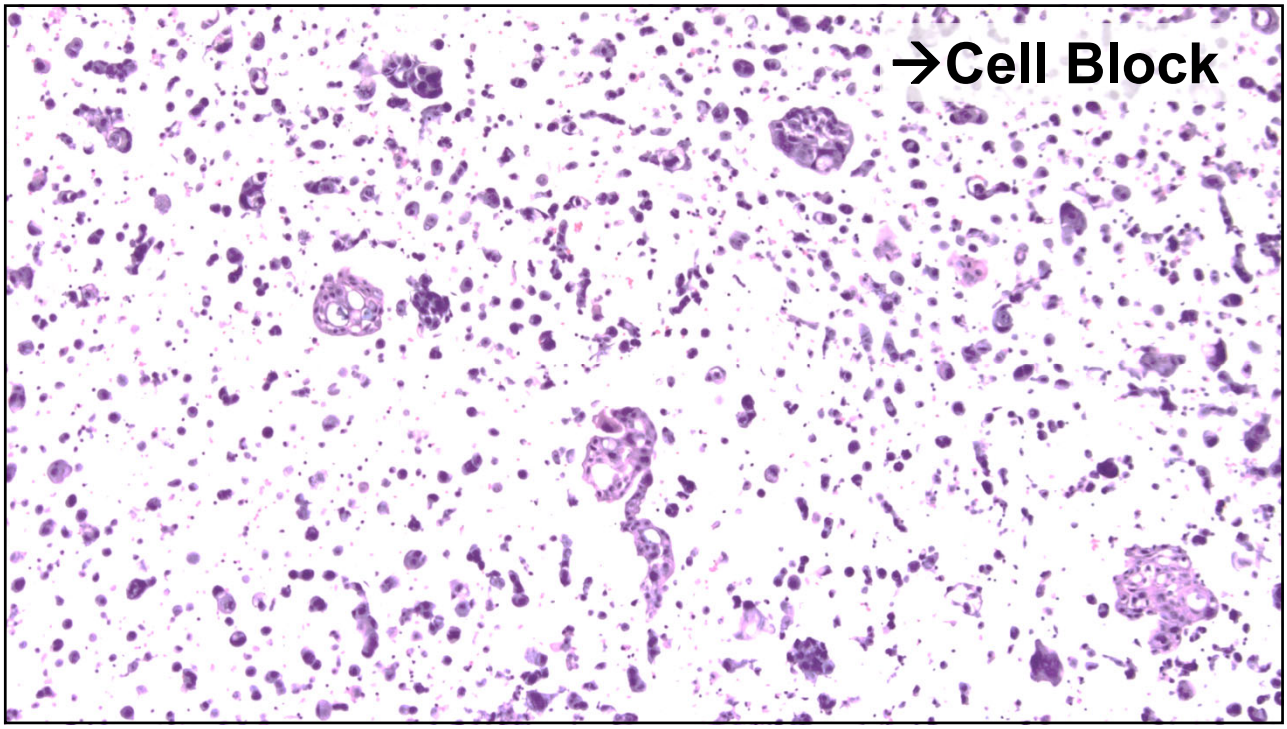
→Inclusions, architecture

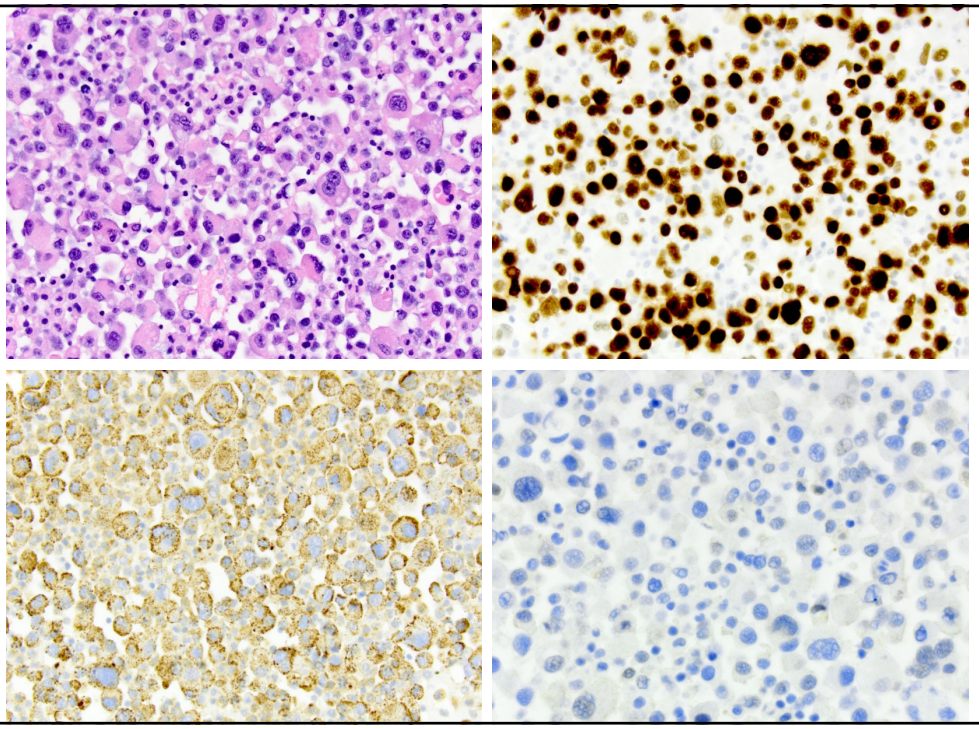


Mucinous ACA

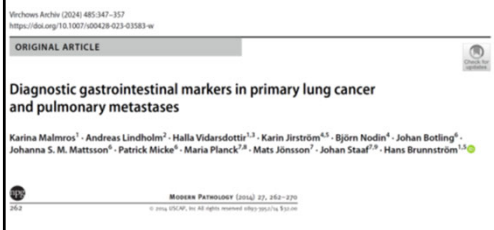



→Necrosis


→Cell Block



Mucinous Adenocarcinoma
Lung primary vs Metastasis



Virchows Archiv (2024) 485:347–357
https://doi.org/10.1007/s00428-023-03583-w

ORIGINAL ARTICLE

Diagnostic gastrointestinal markers in primary lung cancer and pulmonary metastases

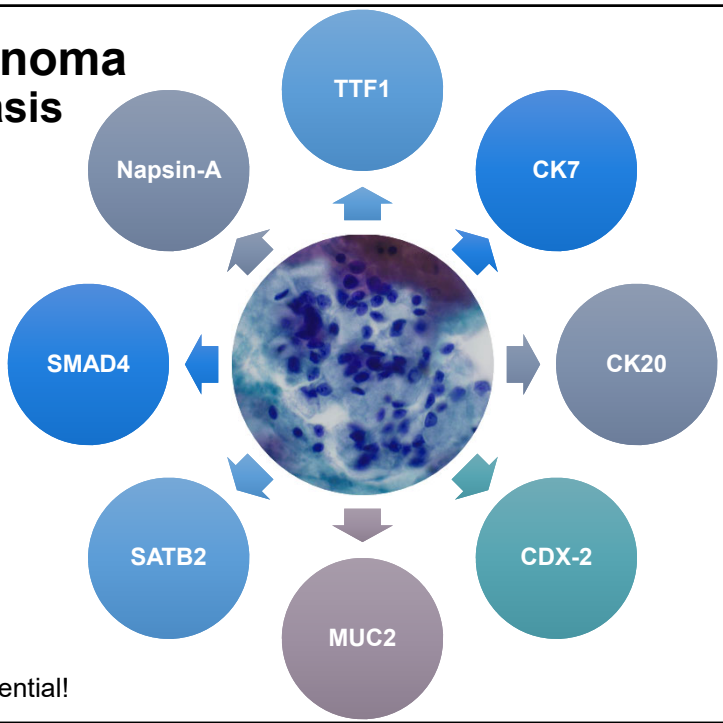
Karina Malmros[1] · Andreas Lindholm[2] · Halla Vidarsdottir[1,3] · Karin Jirström[4,5] · Björn Nodin[4] · Johan Botling[6] · Johanna S. M. Mattsson[6] · Patrick Micke[6] · Maria Planck[7,8] · Mats Jönsson[7] · Johan Staaf[7,9] · Hans Brunnström[1,3]

Modern Pathology (2014) 27, 262–270

KRAS mutational analysis and immunohistochemical studies can help distinguish pancreatic metastases from primary lung adenocarcinomas

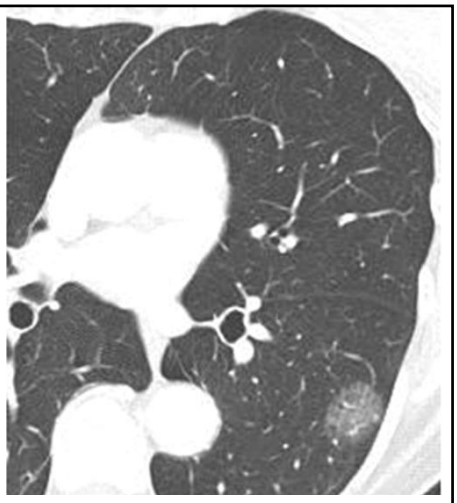
Alyssa M Krasinskas, Simon I Chiosea, Timothy Pal and Sanja Dacic

Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA



→ Clinical and Radiologic correlation is essential!

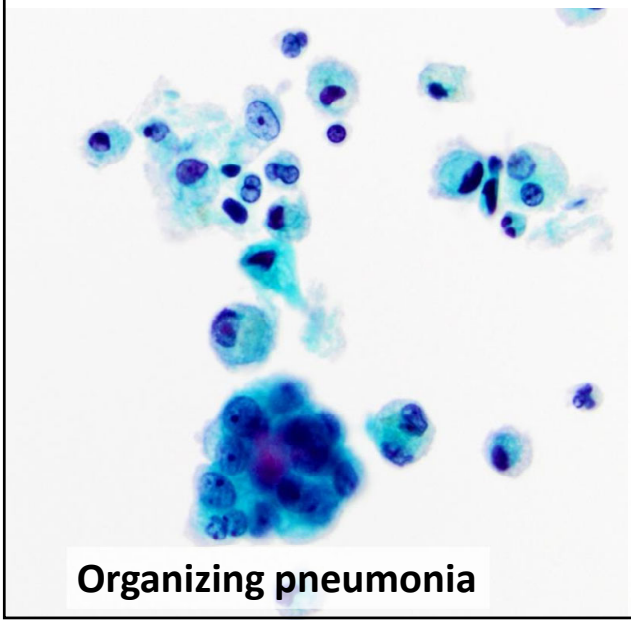
Pitfalls
Lung Adenocarcinoma



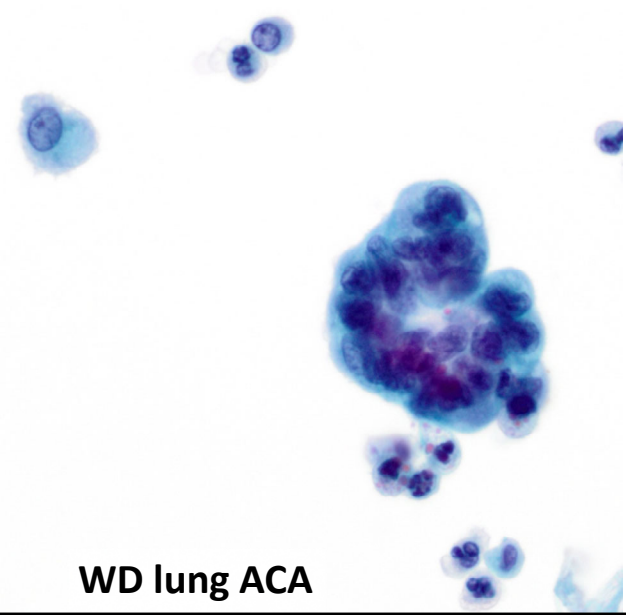
Travis et al. *J Thor Onc* 2011;6:244-285.

- ? WD Lepidic ACA
- vs. reactive pneumocytes, bronchiolar metaplasia/bronchiolar adenoma

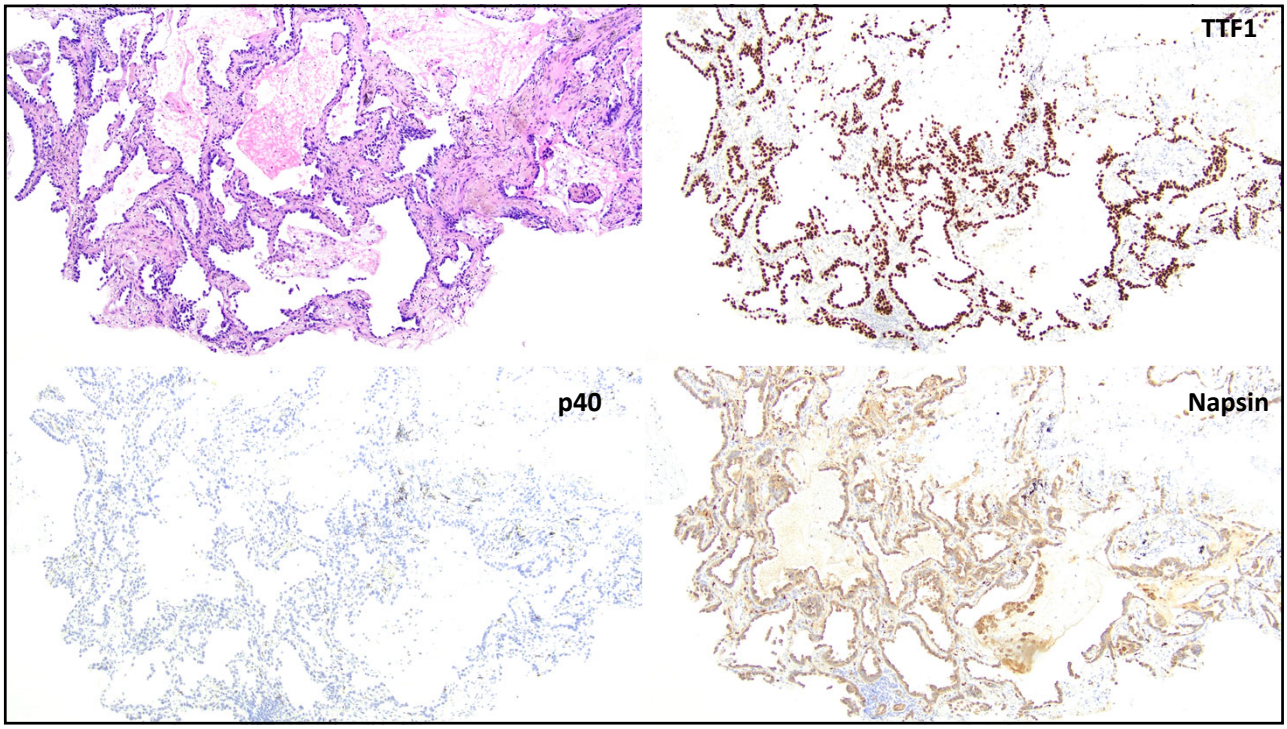
Bronchial Washings

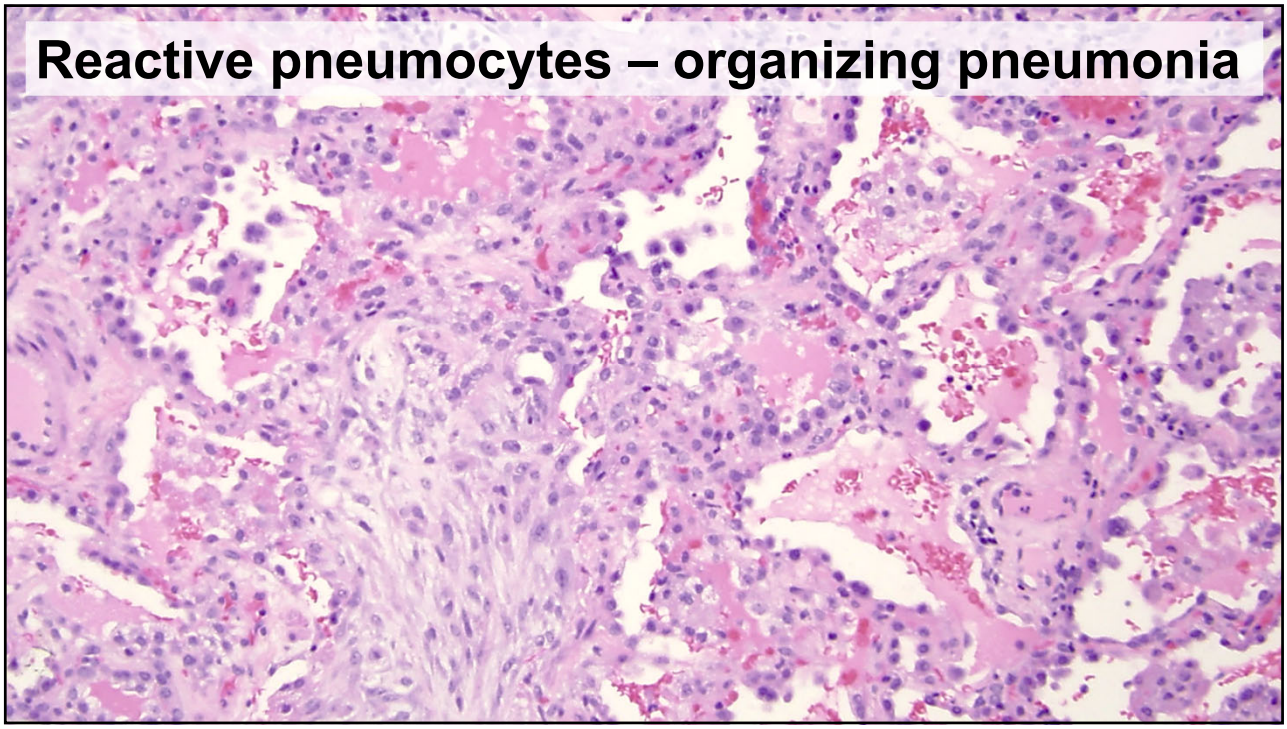


Organizing pneumonia

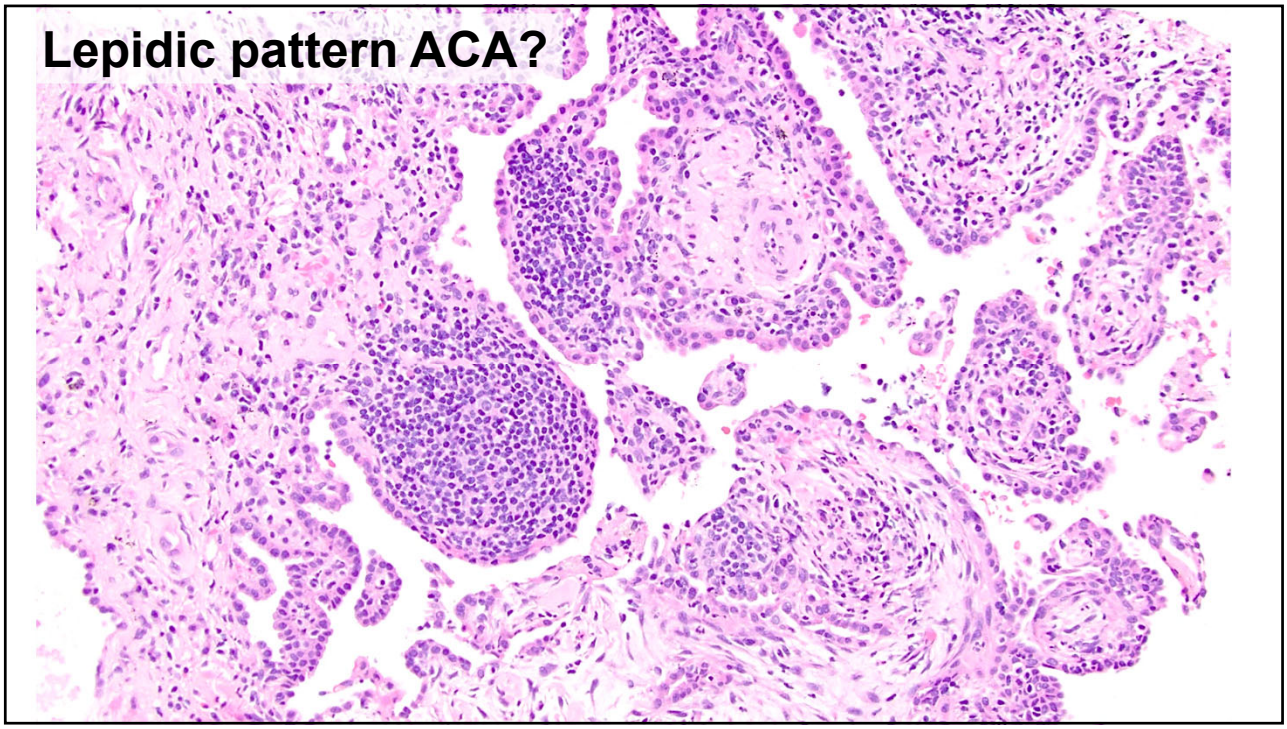


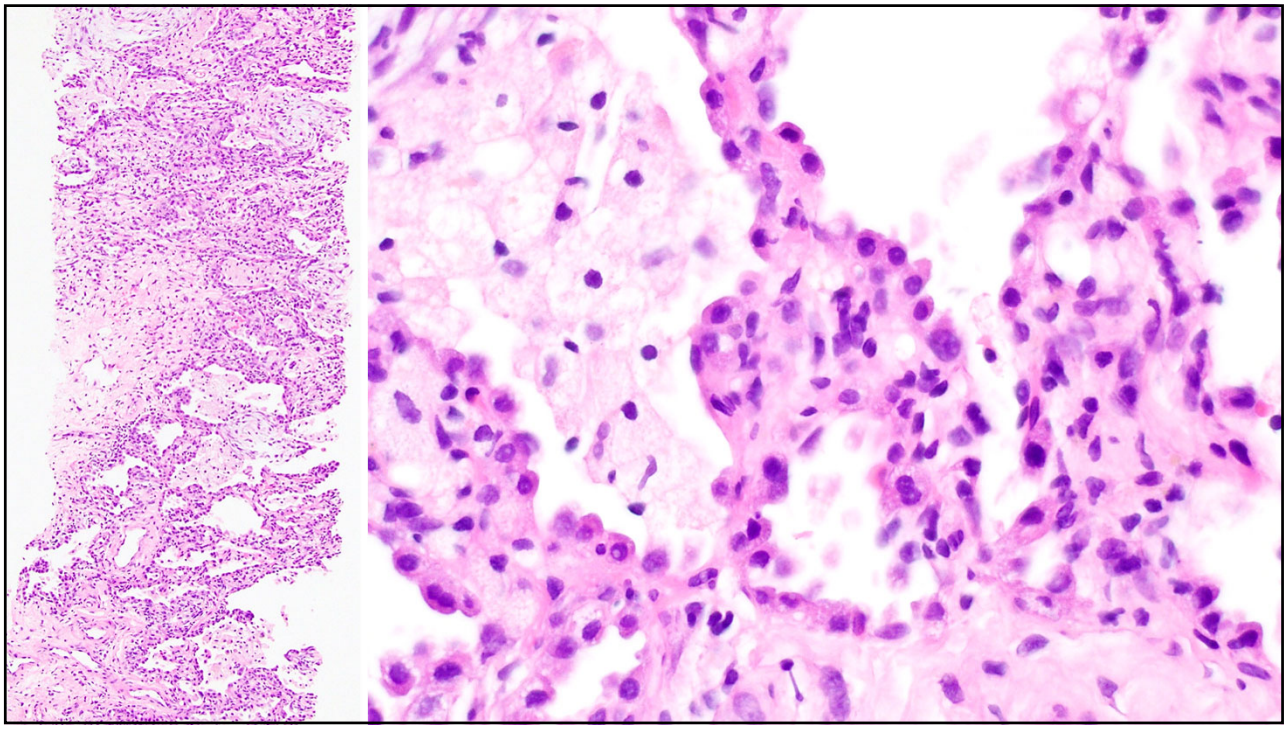
WD lung ACA

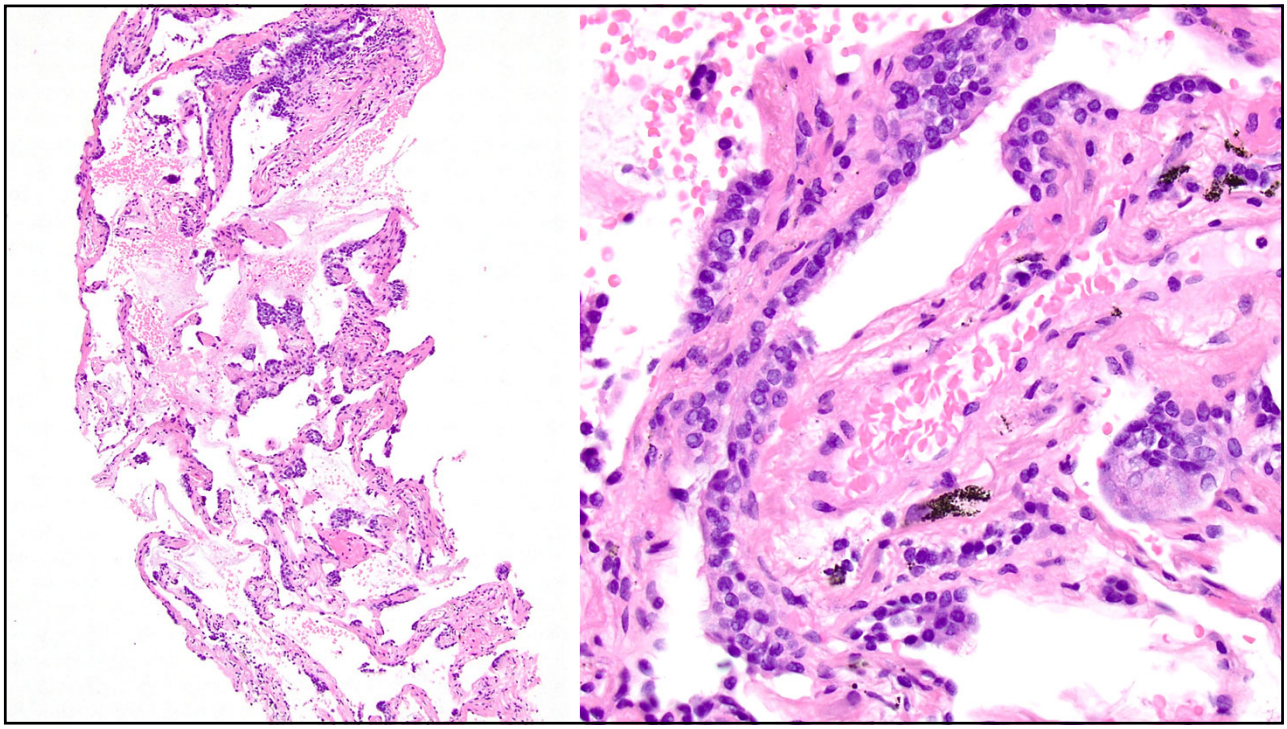

TTF1
p40
Napsin

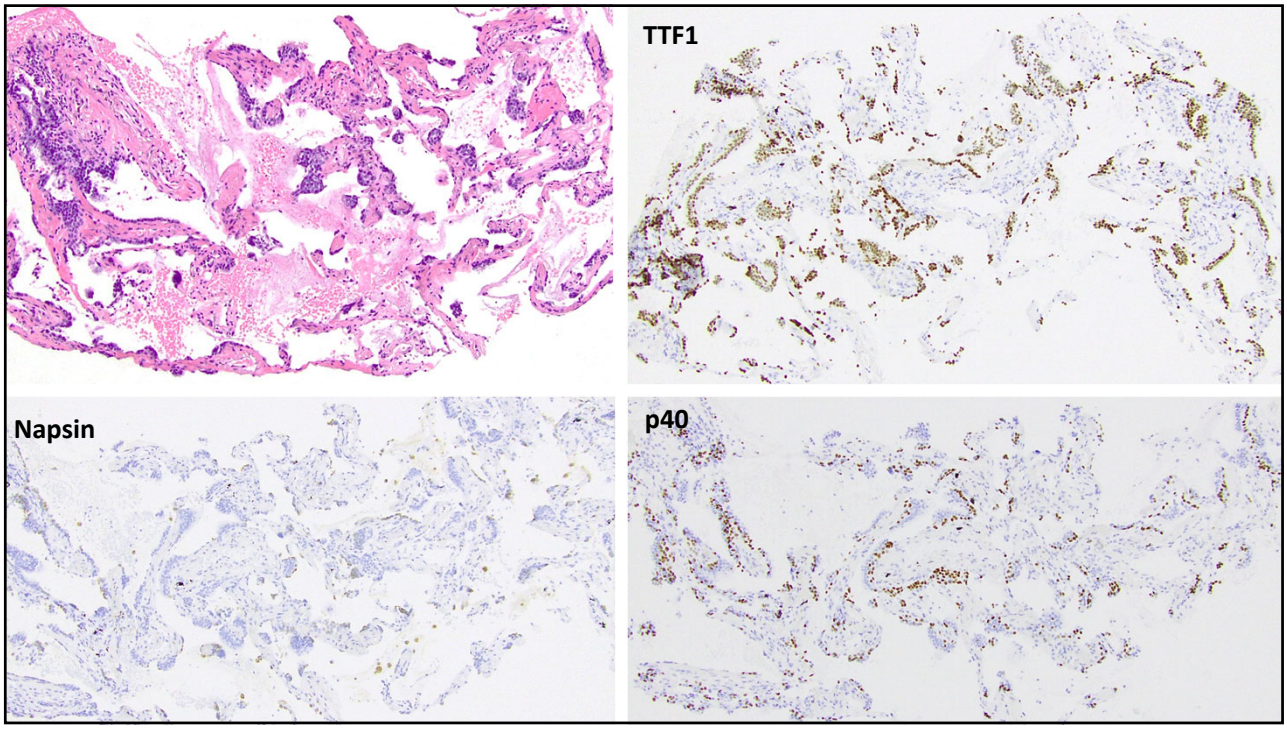

Reactive pneumocytes – organizing pneumonia

Lepidic pattern ACA?








TTF1
Napsin
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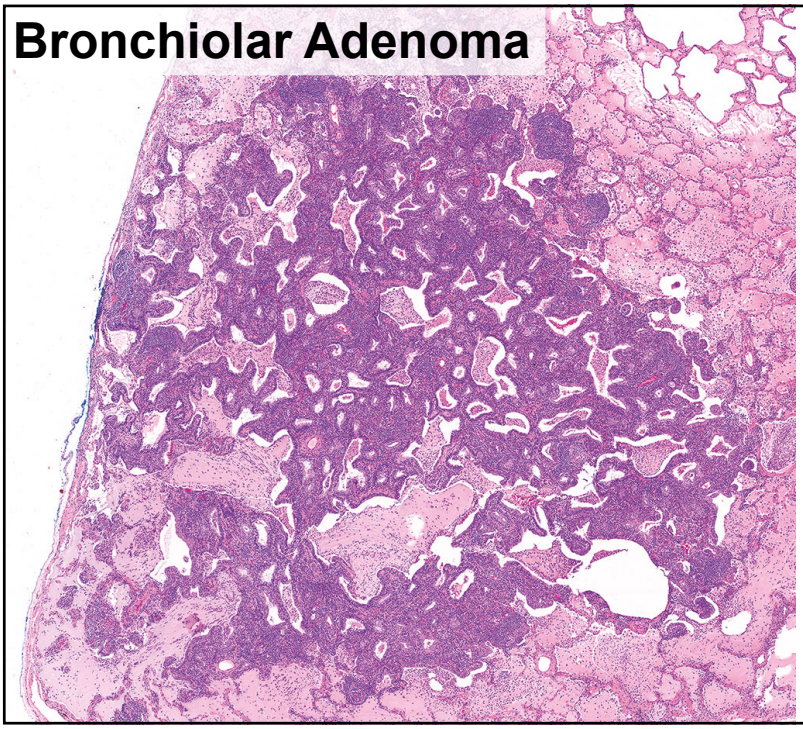
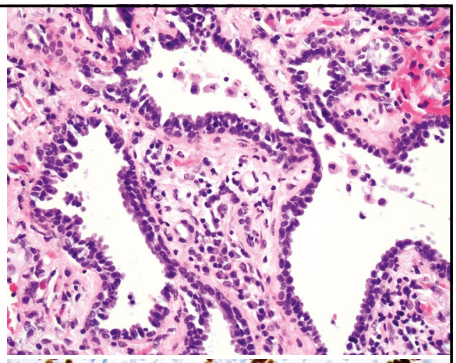
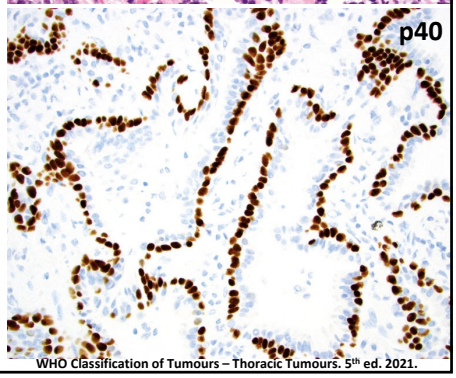
Bronchiolar Adenoma

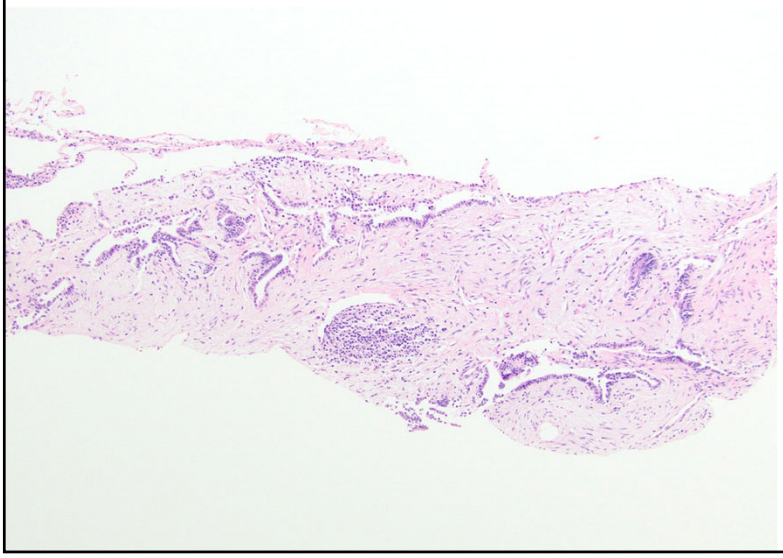




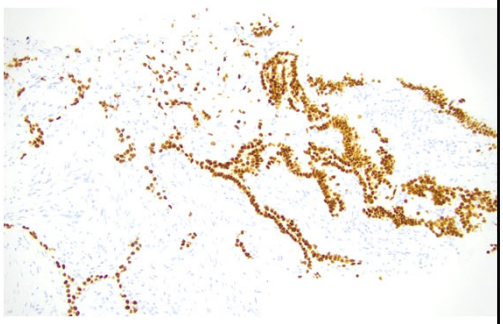


WHO Classification of Tumours – Thoracic Tumours. 5th ed. 2021.

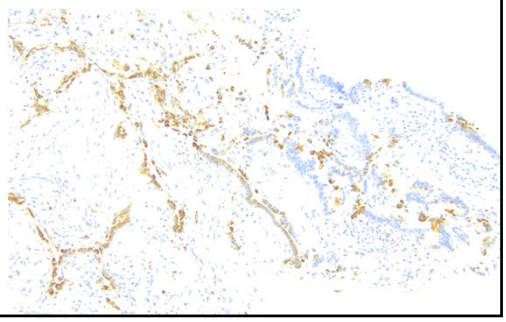
Lepidic adenocarcinoma?

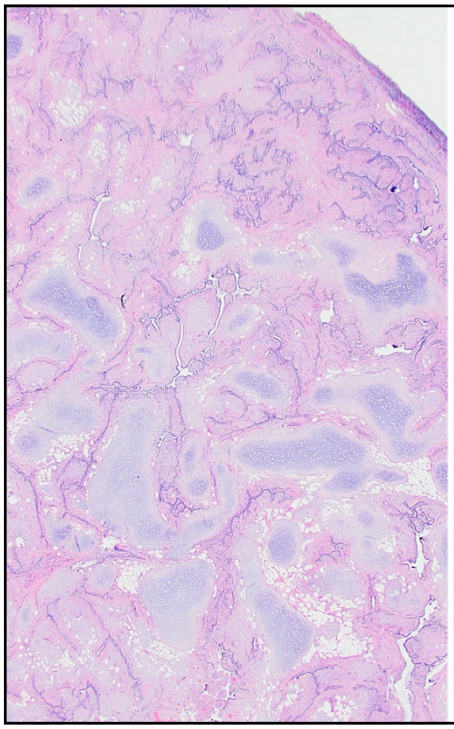


TTF1

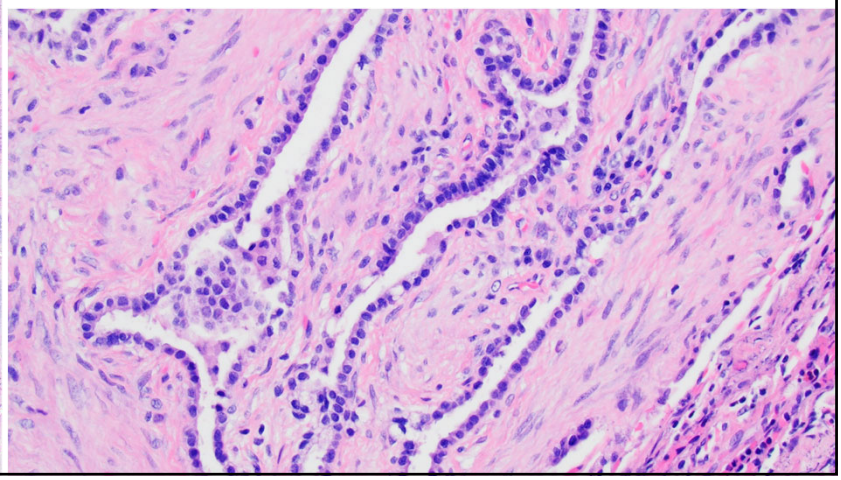


Napsin

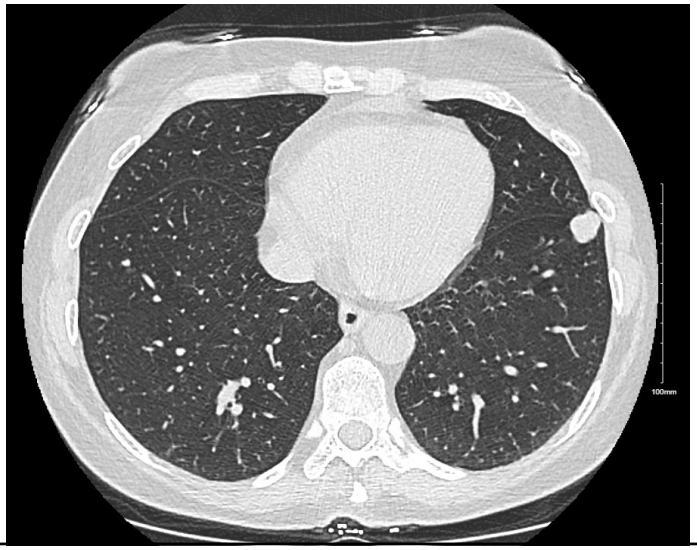


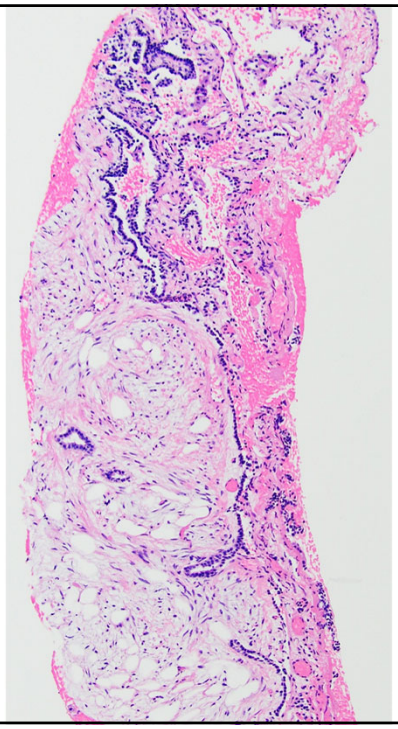


Pulmonary hamartoma



65F Breast cancer, LLL nodule
PET-non avid
→Pulmonary hamartoma



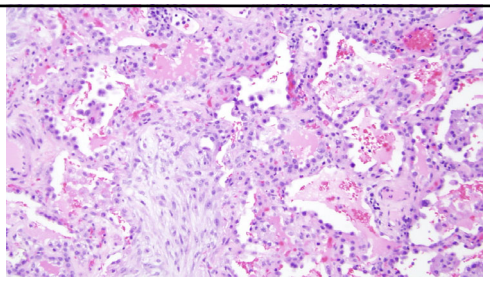


Suspicious/Atypical?

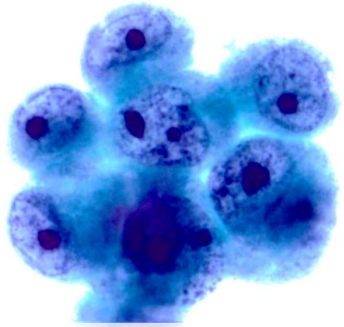
- ✓ Hypocellular aspirate
- ✓ Lack of 3-dimensional clusters
  - Reactive pneumocytes?
  - Mesothelial cells?
- ✓ Background inflammation/necrosis

Metastasis?
- IHC

→ Clinical correlation essential!



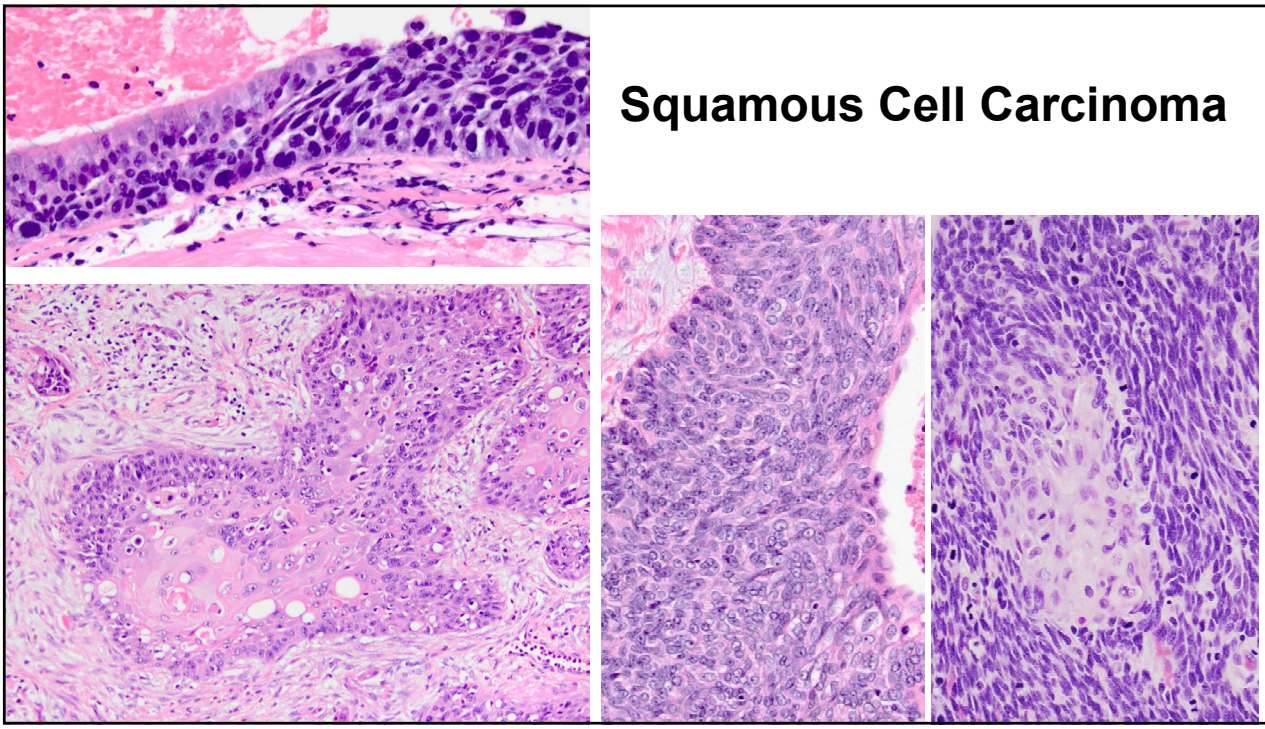
Organizing pneumonia



Bronchial Washings
- Reactive pneumocytes (ARDS/DAH)

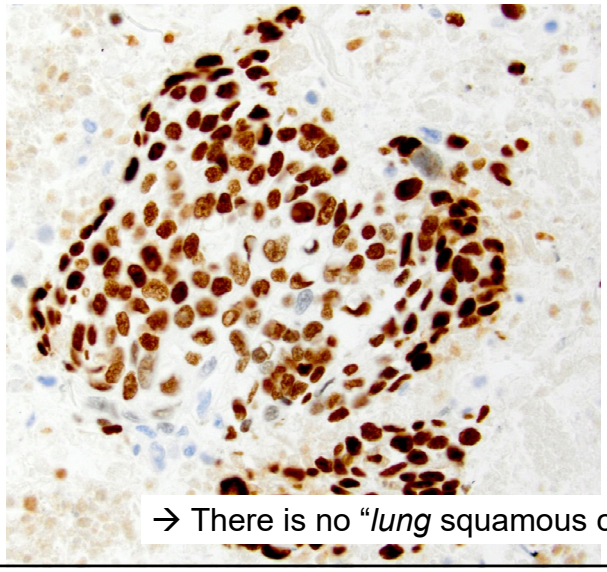
Squamous Cell Carcinoma of the Lung

Squamous Cell Carcinoma

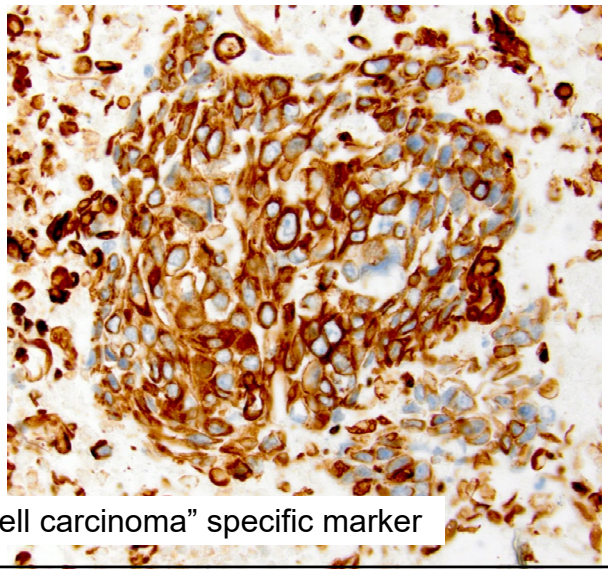


Squamous cell carcinoma markers

p40(p63)



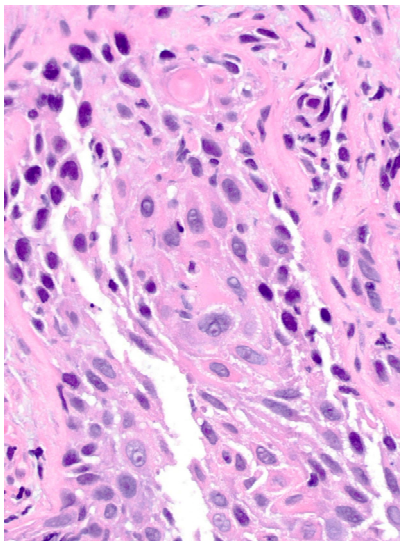
CK5/6



→ There is no "*lung* squamous cell carcinoma" specific marker

Squamous Cell Carcinoma

Cytologic features



Cytoplasm

- Well-differentiated/keratinizing
  - Abundant dense cytoplasm with sharp borders
    - Pap stain: orangeophilic
    - DiffQuik stain: robin-egg blue
    - H&E: eosinophilic
  - Variable cell shapes, polygonal, spindle shaped, elongated, tadpole shaped
  - Keratin pearls / intercellular bridges
- Poorly-differentiated
  - Moderate to scant amounts of cytoplasm, lack of keratinization (color)

Nuclei

- Dense ink-dot / lump-of coal chromatin
- Round-to-oval nuclei with prominent nucleoli: "russet-potato like"

Architecture

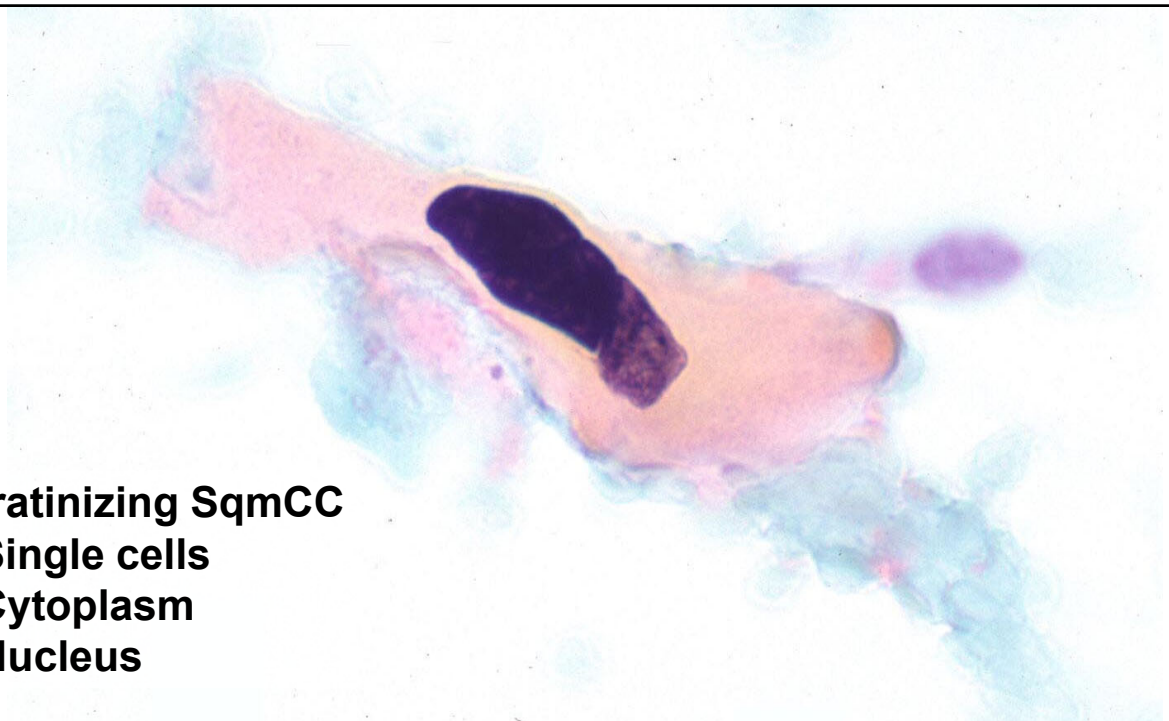
- Cohesive sheets
- Single atypical cells

Other

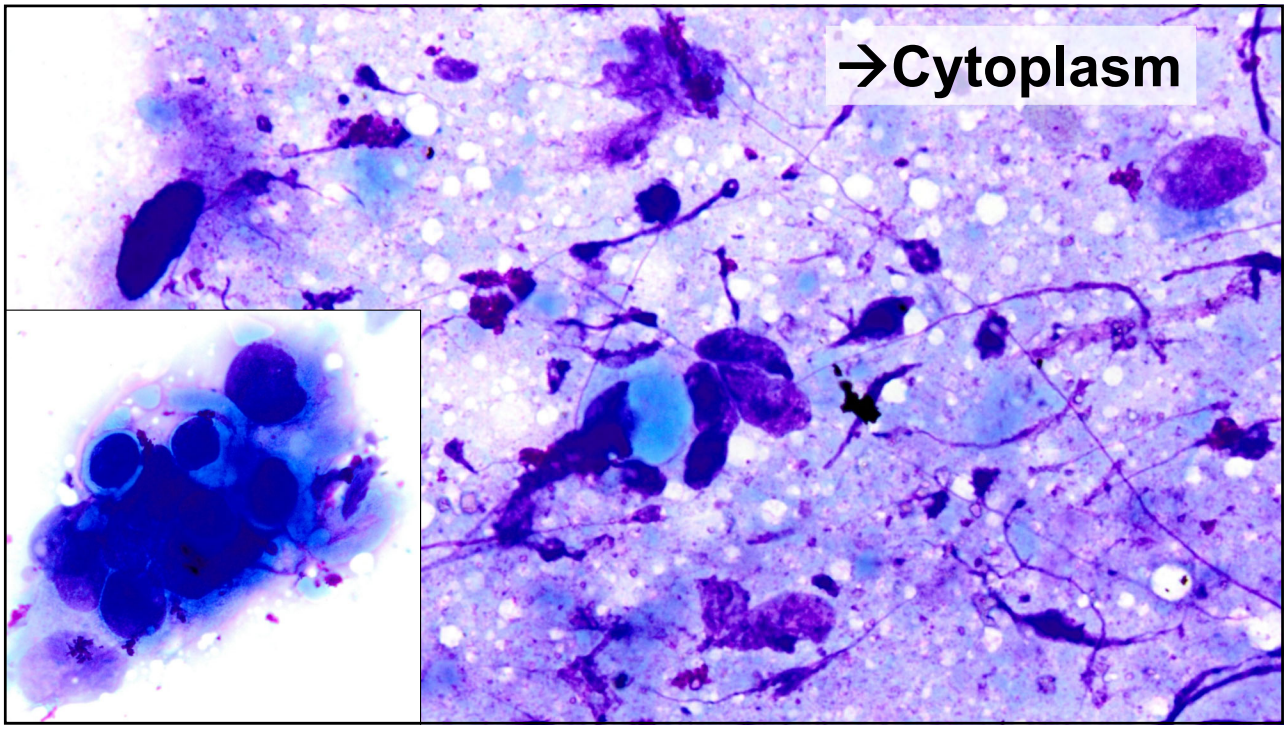
- Anucleate cells, keratin debris +/- FBGCR
- Necrosis / necroinflammatory debris

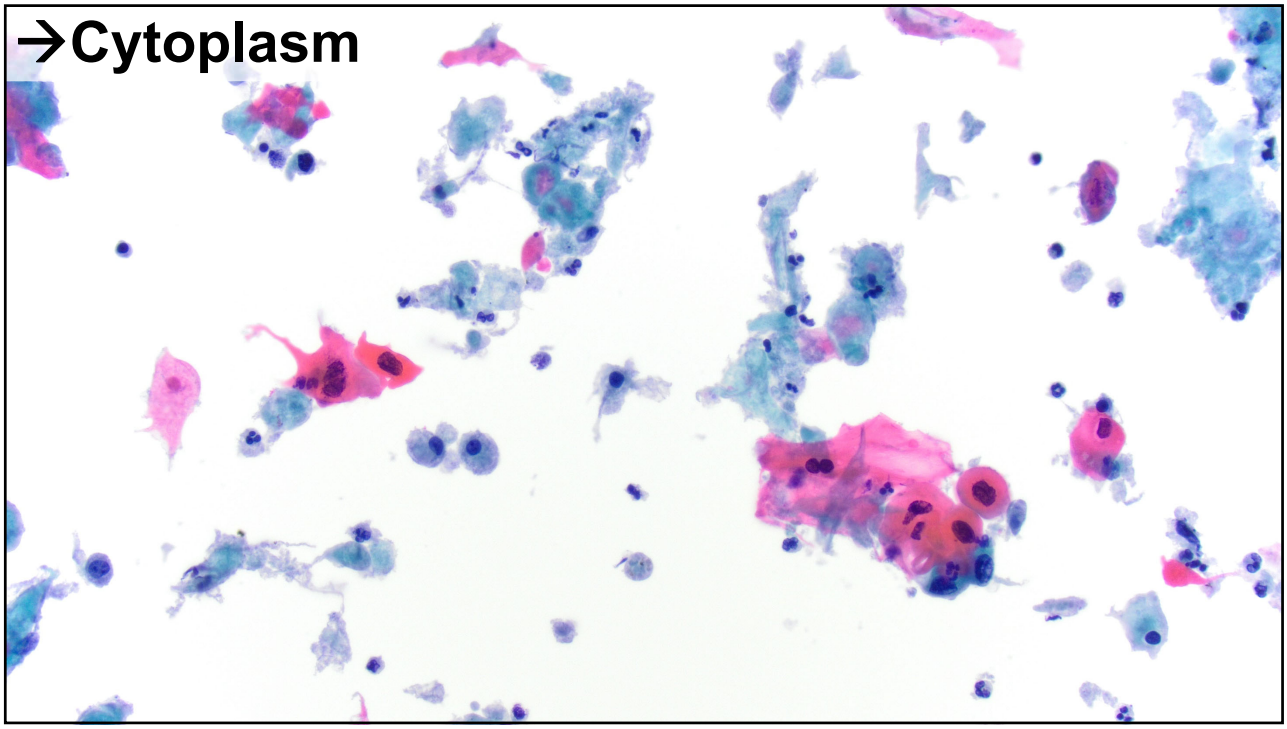
WHO emphasis:
1-Keratinization
2-Keratin pearls
3-Intercellular bridges

Cibas and Ducatman. Cytology Diagnostic Principles and Clinical Correlates, 6th ed. 2026.
H.A. Domanski. Atlas of Fine Needle Aspiration Cytology. 2nd ed. 2019.
WHO Classification of Tumours of the Lung, Pleura, Thymus, and Heart. 4th ed. 2015.

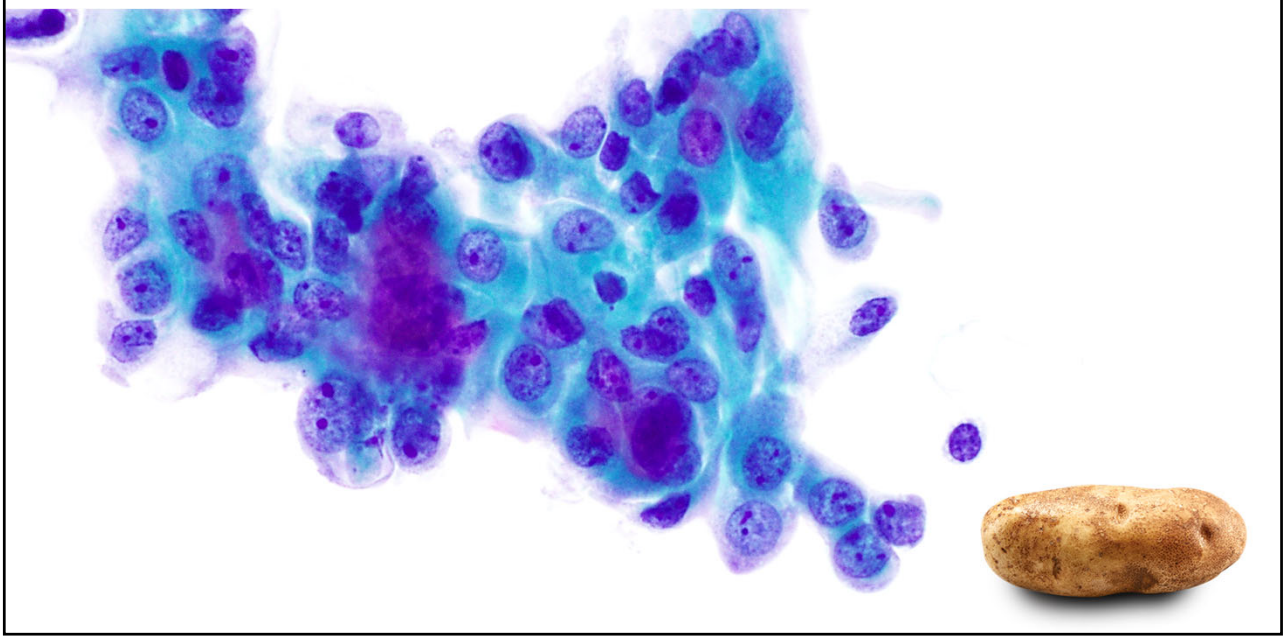


Keratinizing SqmCC
→Single cells
→Cytoplasm
→Nucleus

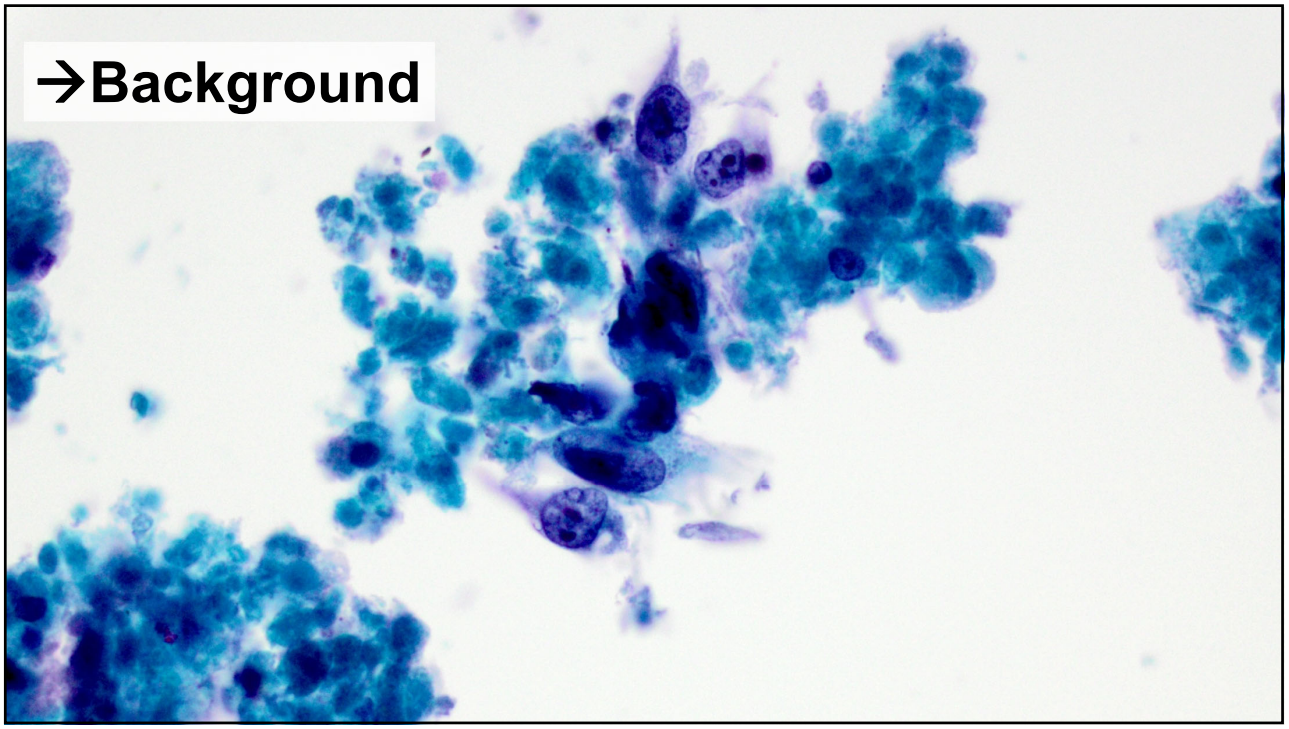

→Cytoplasm

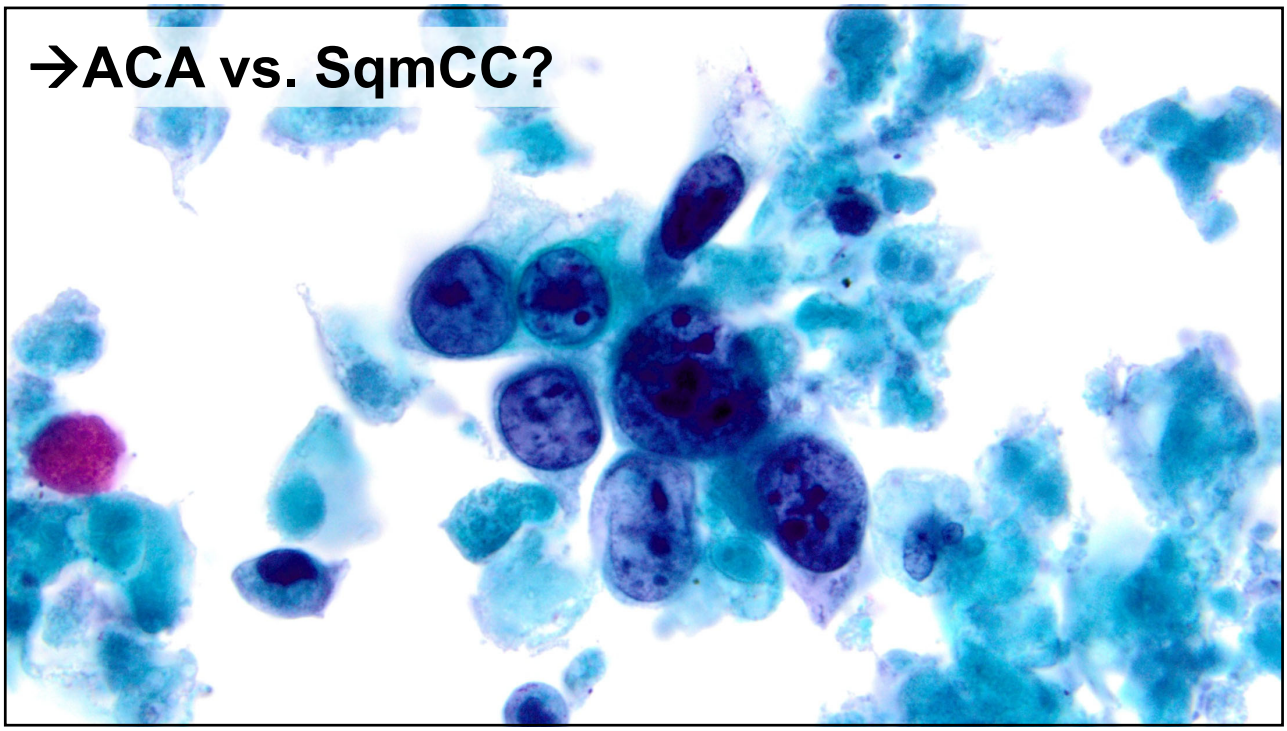

→Cytoplasm

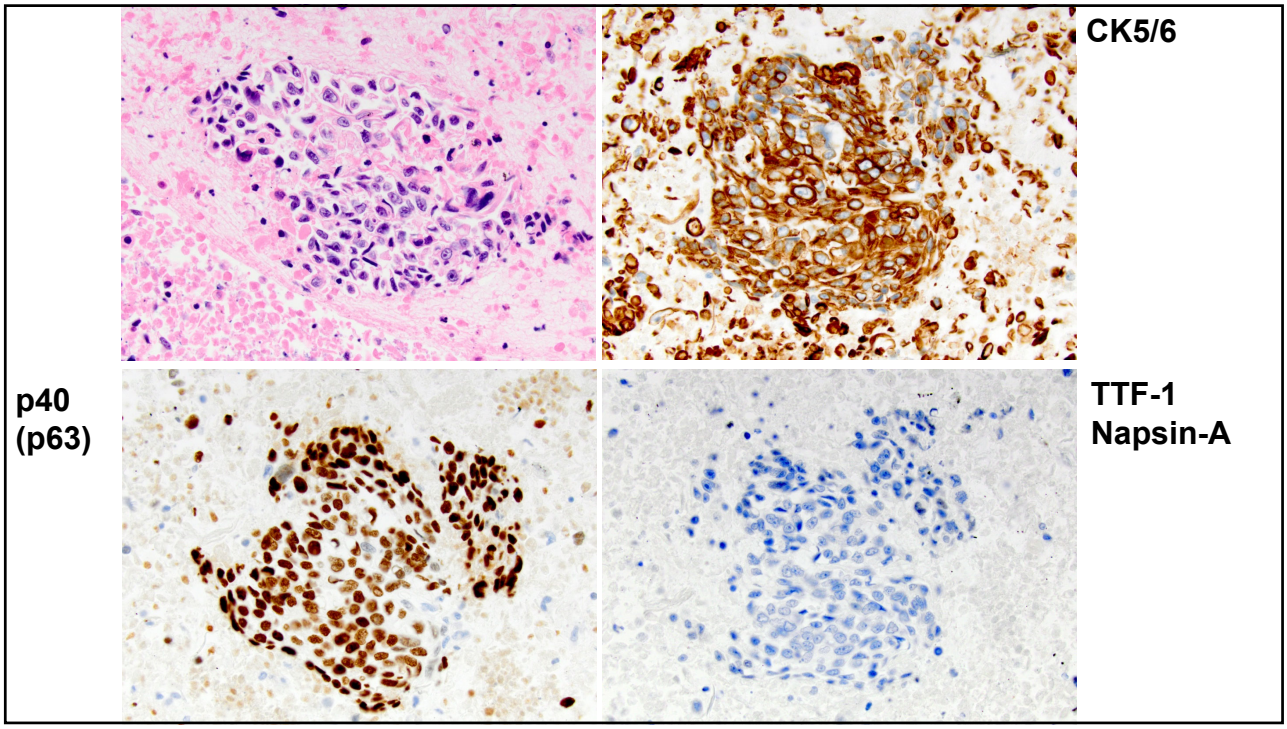
→Nucleus



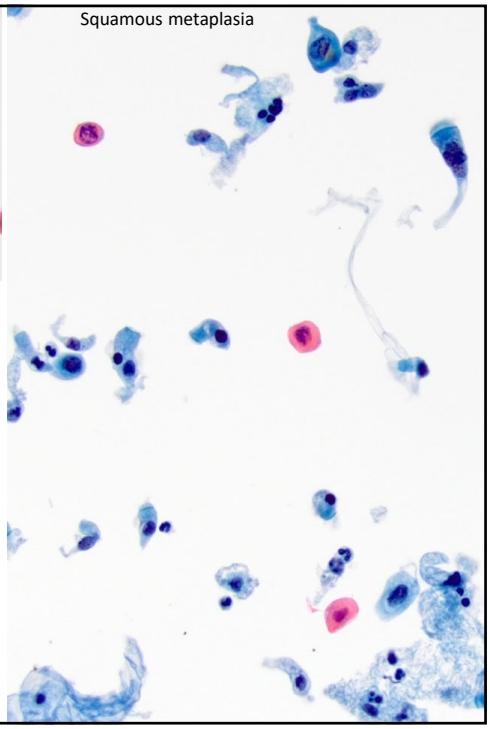
→Background



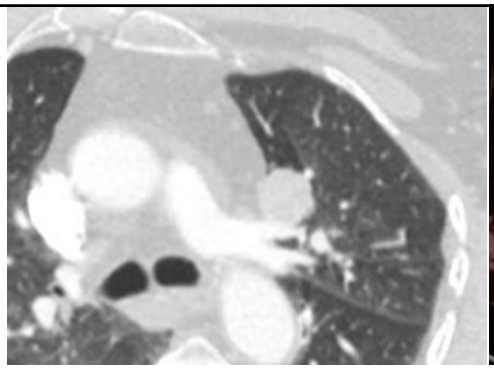

→ACA vs. SqmCC?

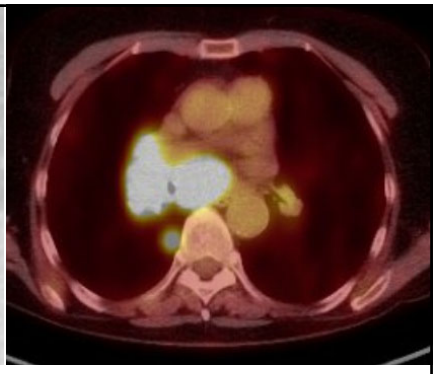

CK5/6
p40
(p63)
TTF-1
Napsin-A

Pitfalls:
Suspicious/Atypical?



- ✓ Squamous metaplasia
  - Trachea/bronchus 'contaminant'
  - Infectious/inflammatory parenchymal lesions
  - Infarction/ILD
- ✓ Mesothelial cells
  - Air-drying
  - → Red/orange cytoplasm on ThinPrep





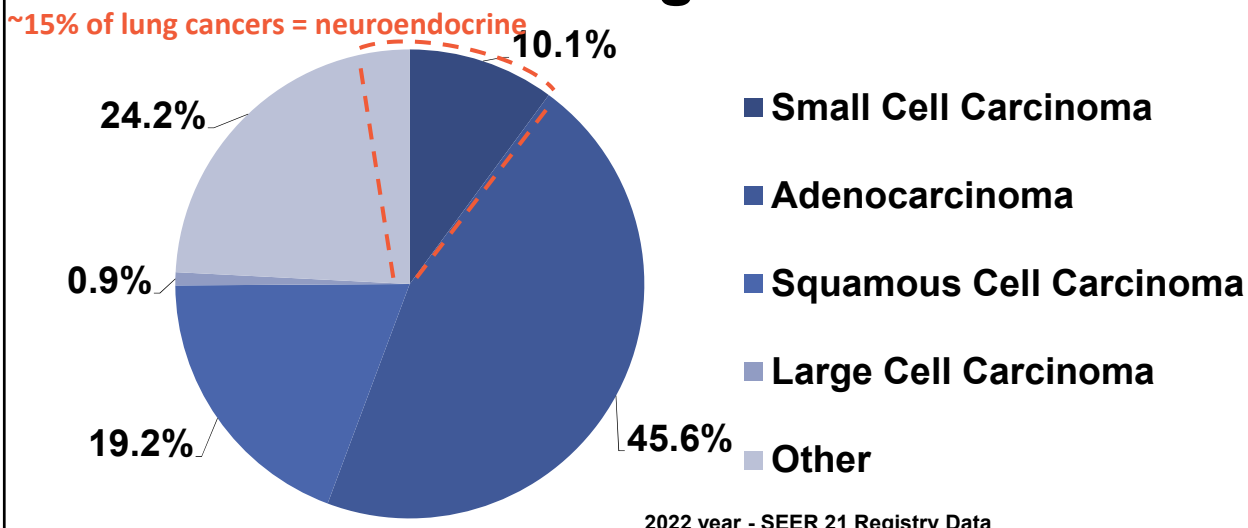
Neuroendocrine Tumors

Tips/pitfalls:

- Carcinoid tumors with spindled morphology
- Mib-1/Ki-67, p53, Rb, POU2F3
- Napsin IHC

NATIONAL CANCER INSTITUTE
Surveillance, Epidemiology, and End Results Program

Cancer of the Lung and Bronchus



2022 year - SEER 21 Registry Data
(Subtype, Both Sexes, All Races, All Ages, All Stages, Observed Rates)

Pulmonary Neuroendocrine Tumors

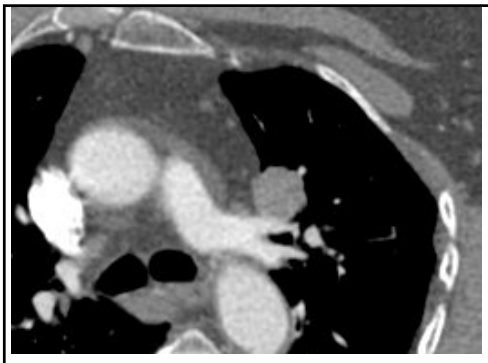
Grading →

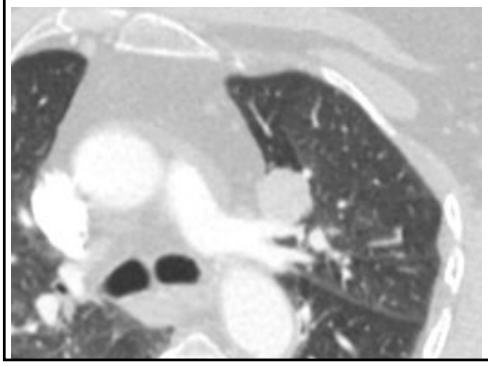
Cytology Small Biopsy →

	Typical carcinoid	Atypical carcinoid	LCNEC	SCLC
Average age	Sixth decade	Sixth decade	Seventh decade	Seventh decade
Sex predominance	Female	Female	Male	Male
Diagnostic criteria				
Mitoses per 2 mm^2	< 2	2–10	> 10 (median: 70)	> 10 (median: 80)
Necrosis	No	Focal, if any	Yes	Yes
Neuroendocrine morphology	Yes	Yes	Yes	Yes
Ki-67 proliferation index	Up to 5%	Up to 30%	30–100%	30–100%
TTF1 expression	Mostly positive in peripheral, mostly negative in central tumours	Mostly positive in peripheral, mostly negative in central tumours	Positive (70%)	Positive (85%)
p40 expression	Negative	Negative	Negative	Negative
Combined with NSCC component	No	No	Up to 25% of resected LCNEC	Up to 25% of resected SCLC

WHO Classification of Tumours of the Lung, Pleura, Thymus, and Heart. 5th ed. 2021.

Carcinoid Tumors





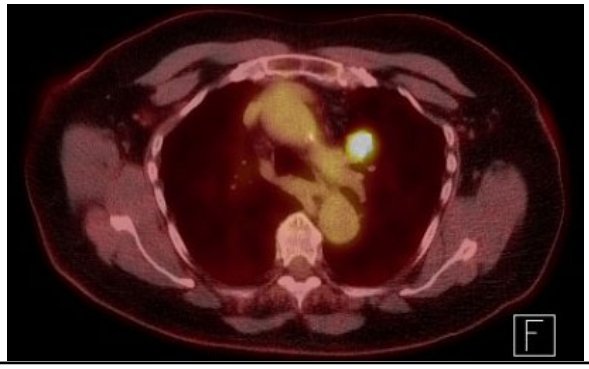
Carcinoid tumor

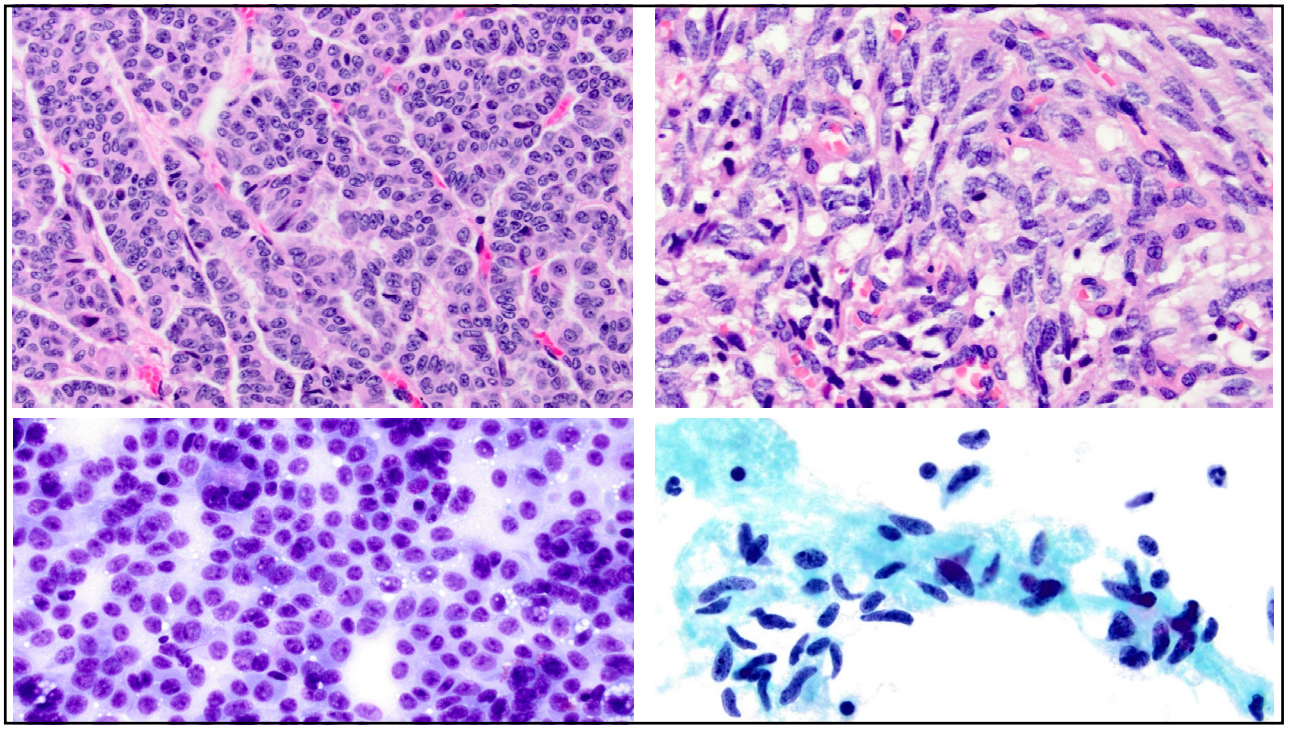
CT features:

- Central bronchial / peripheral round

PET features:

- Generally low FDG





Carcinoid tumors
Cytologic features

<u>Architecture</u>

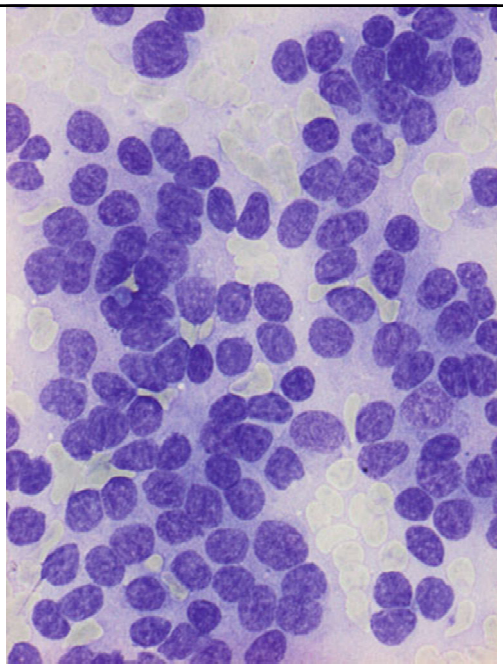
- Tight and loosely cohesive aggregates, dispersed cells
- Acinar and rosette-like structures
- Tumor aggregates might be associated with small capillaries

<u>Cellular features:</u>

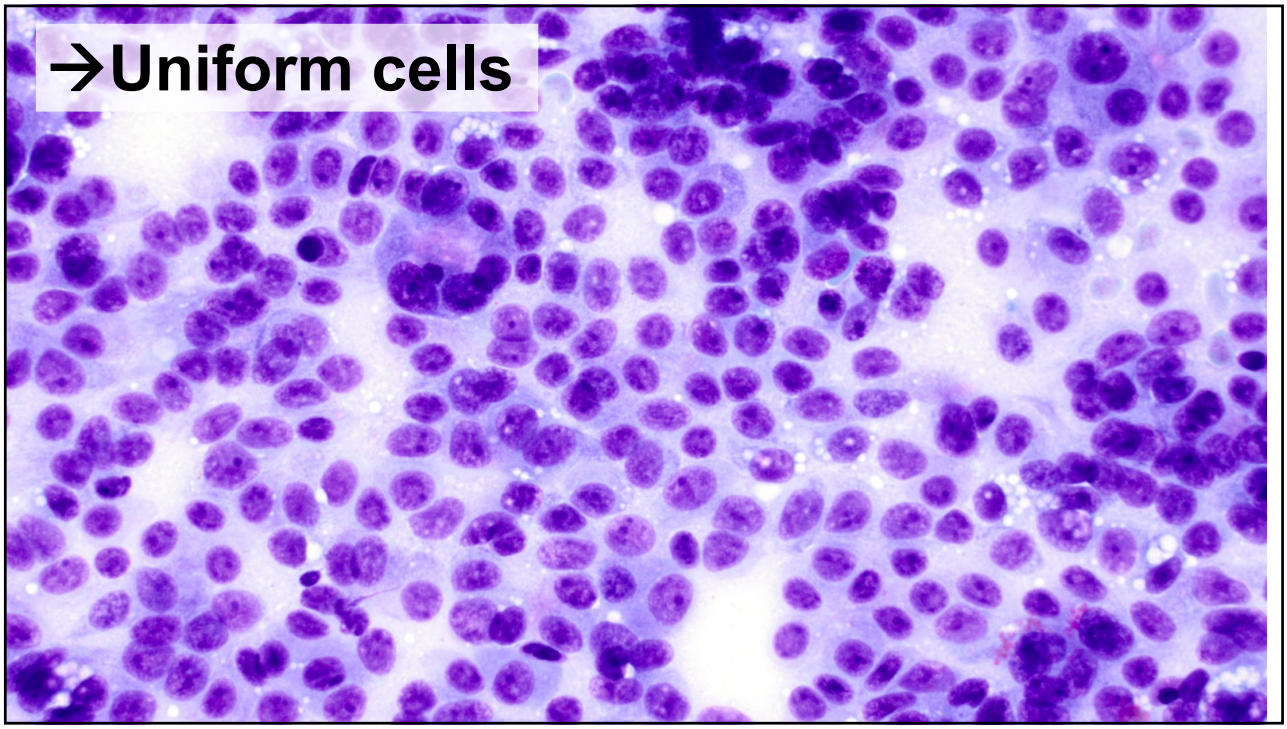
- Small, uniform, round-to-oval, cuboidal, or spindle cells
- Small nuclei, “salt and pepper” chromatin, inconspicuous nucleoli
- Scanty cytoplasm

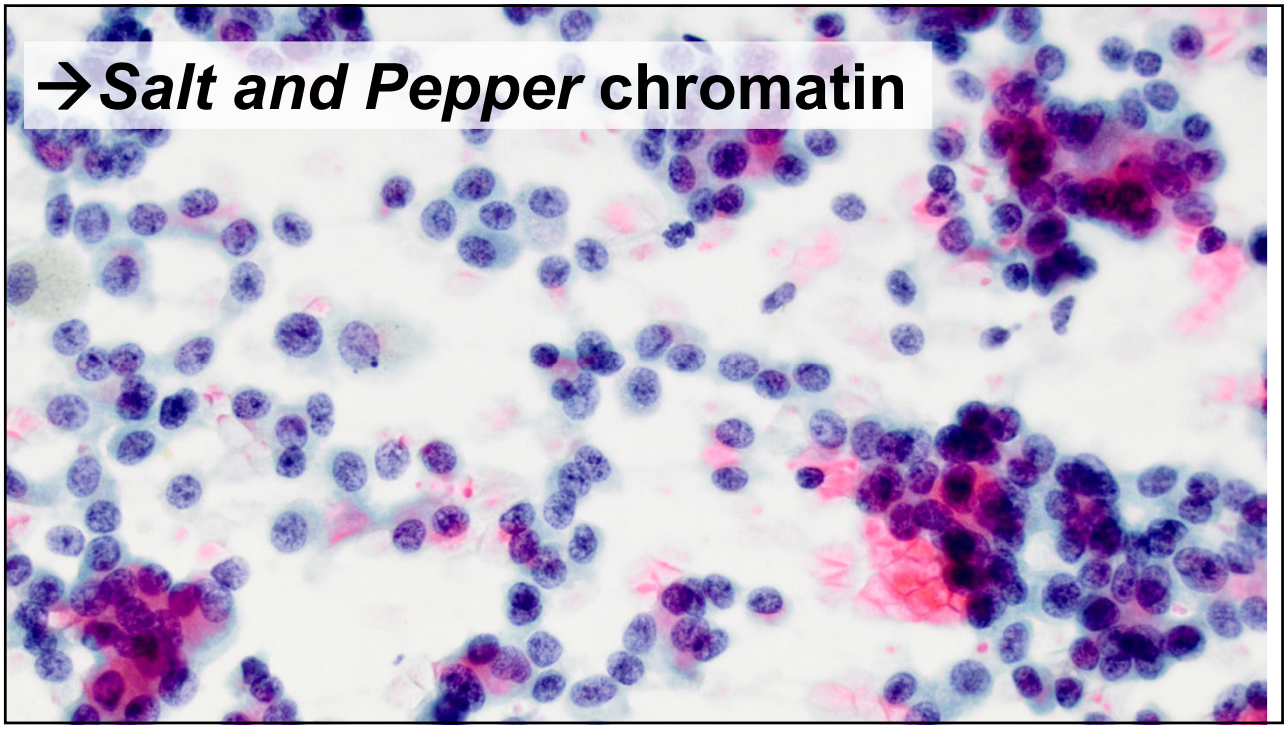
<u>Features seen in Atypical Carcinoid tumors:</u>

- Necrosis and increased cell size/nuclear pleomorphism
- Mitoses
- Occasionally prominent nucleoli in atypical carcinoid

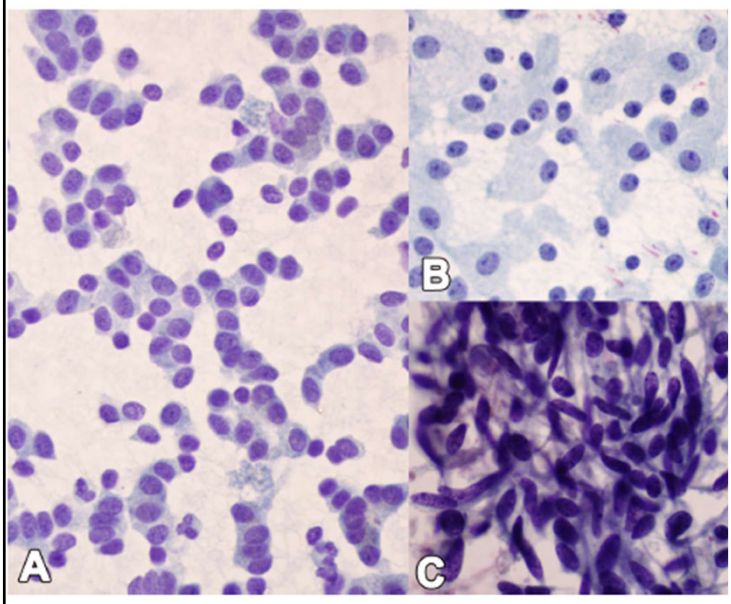


Cibas and Ducatman. Cytology, 6th ed. 2026.
H.A. Domanski. Atlas of Fine Needle Aspiration Cytology. 2nd ed. 2019.

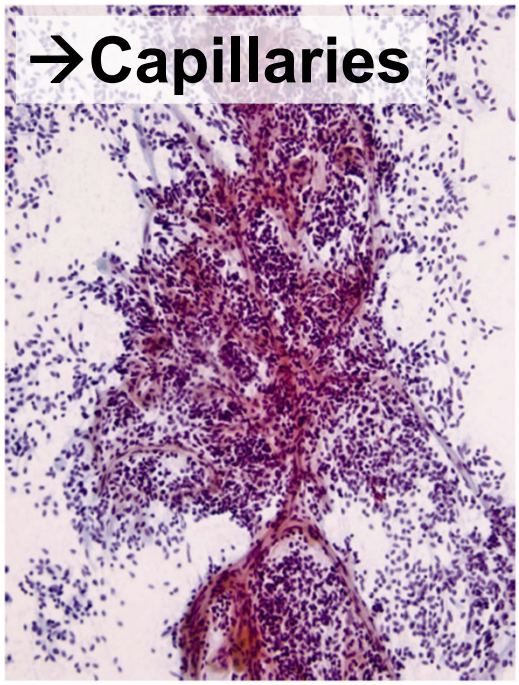

→Uniform cells


→*Salt and Pepper* chromatin

→Variable cell shape

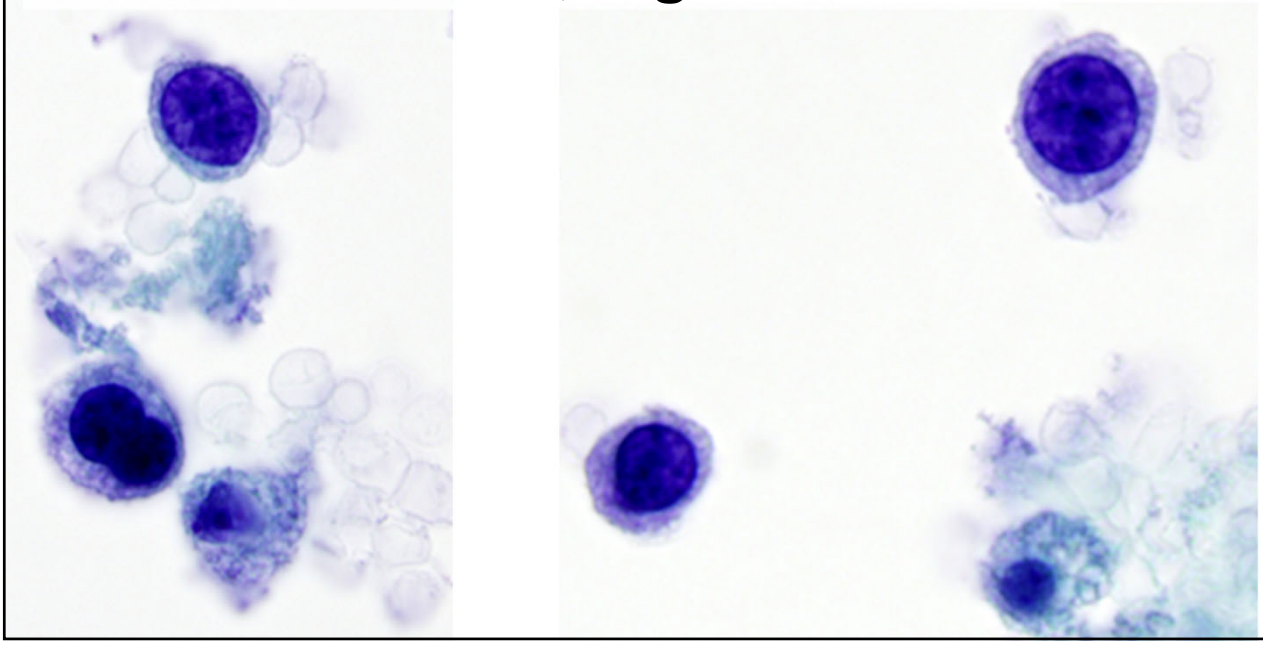


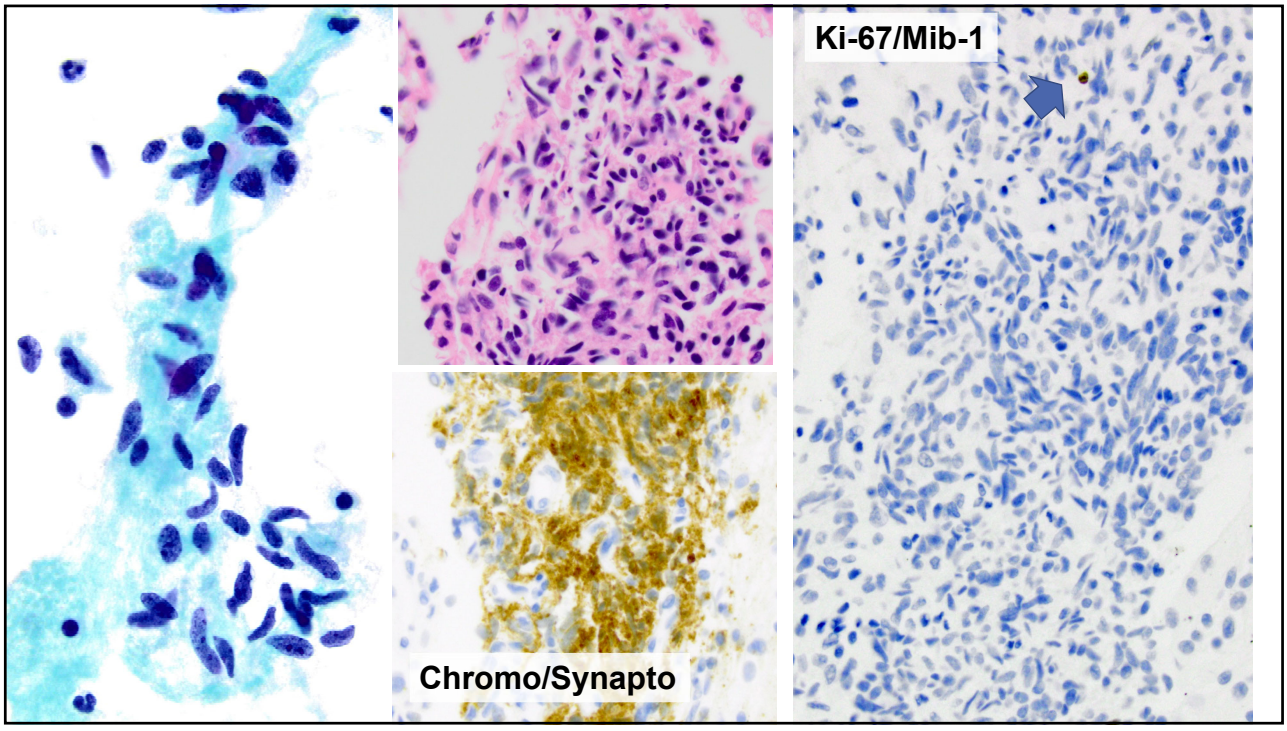
→Capillaries



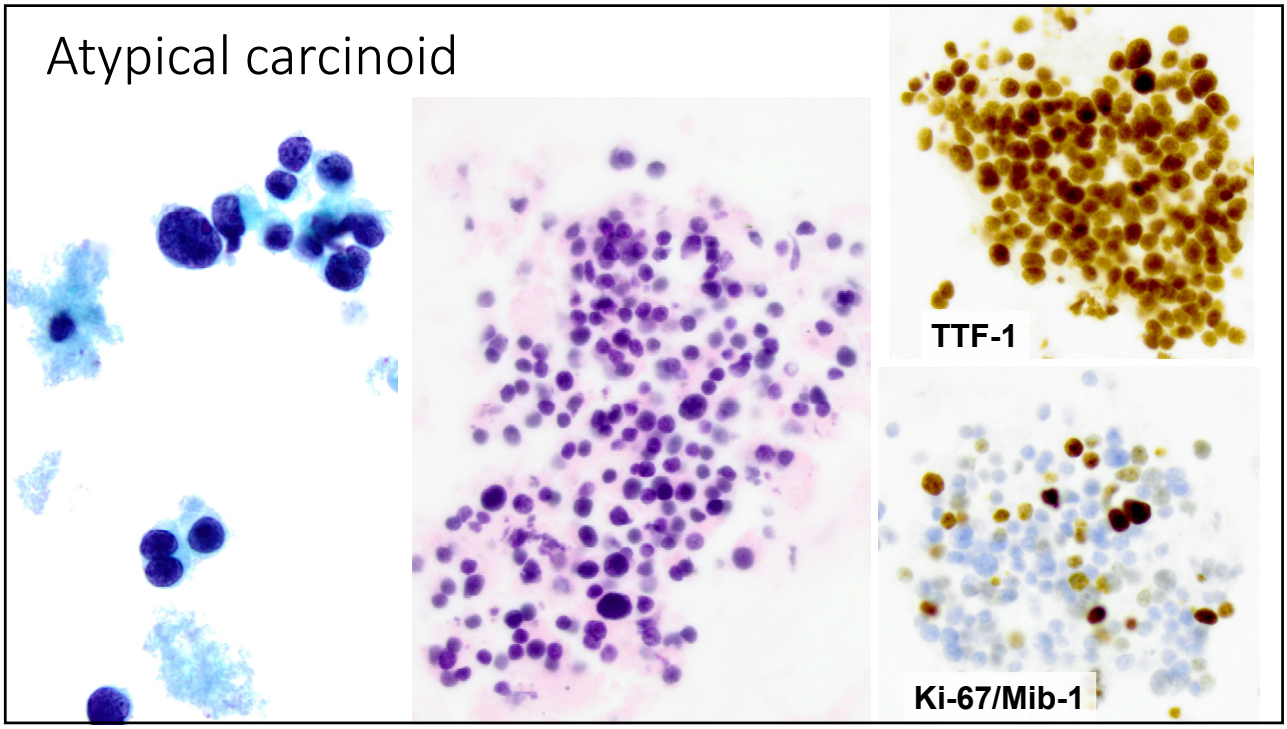
Huang et al. *Diagn Cytopathol*. 2013;41:689-696.

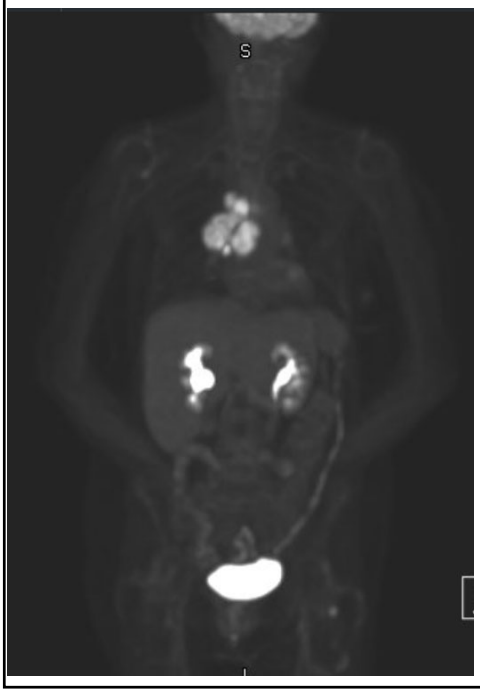
→Round nucleus, high N:C ratio





Atypical carcinoid



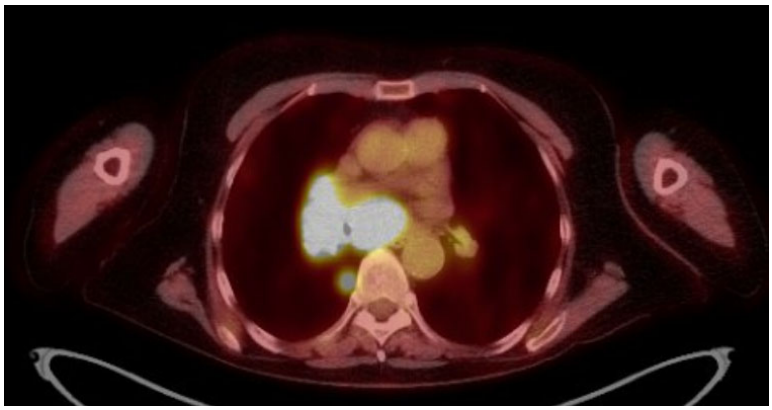


HIGH GRADE NEC

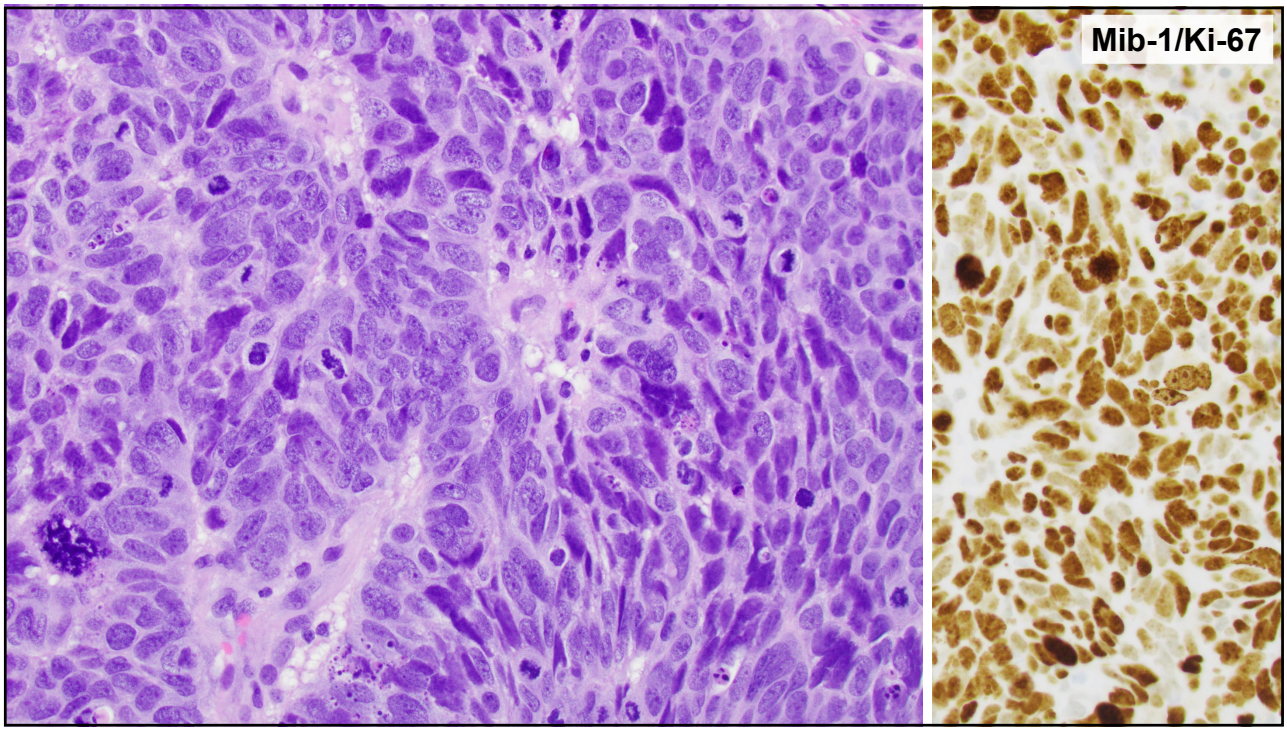
CT features:

- **Lobulated mass, hilar+LAD (SmCC) vs. peripheral+LAD (LCNEC)**

PET features: FDG-AVID!! +Metastasis

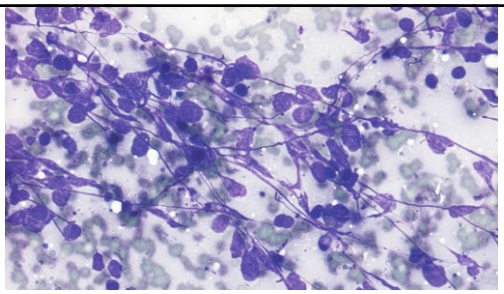


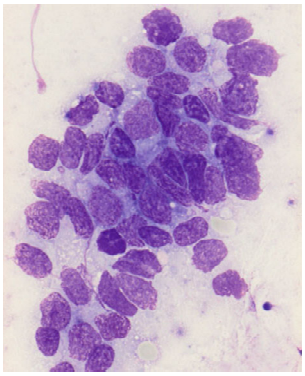
Small Cell Carcinoma

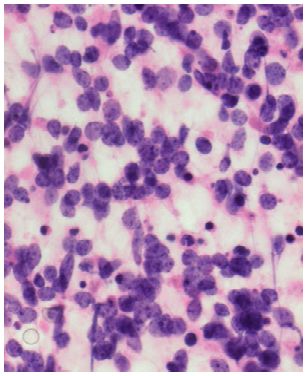


Small Cell Carcinoma

Cytologic features







Architecture:

- Loosely cohesive cells sheets or dispersed cells, rare cell clusters
- Nuclear molding (less in LBP)

Cellular features:

- Small to medium sized cells (twice the size of lymphocytes), occasionally larger
- Carrot/wedge shaped nuclei (less in LBP)
- High N/C ratio, scant cytoplasm

Nucleus/chromatin:

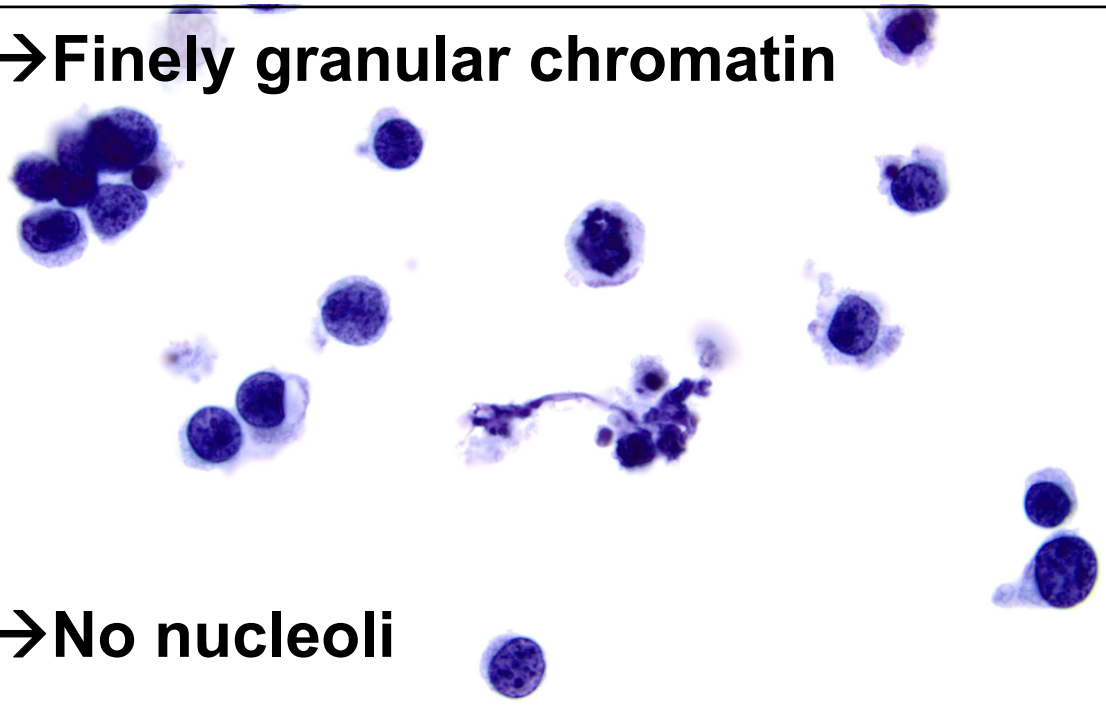
- Evenly dispersed, powdery chromatin
- NO or only indistinct nucleoli

Other:

- Frequent mitoses
- Nuclear debris
- Necrotic background
- Crush artifact (smears)

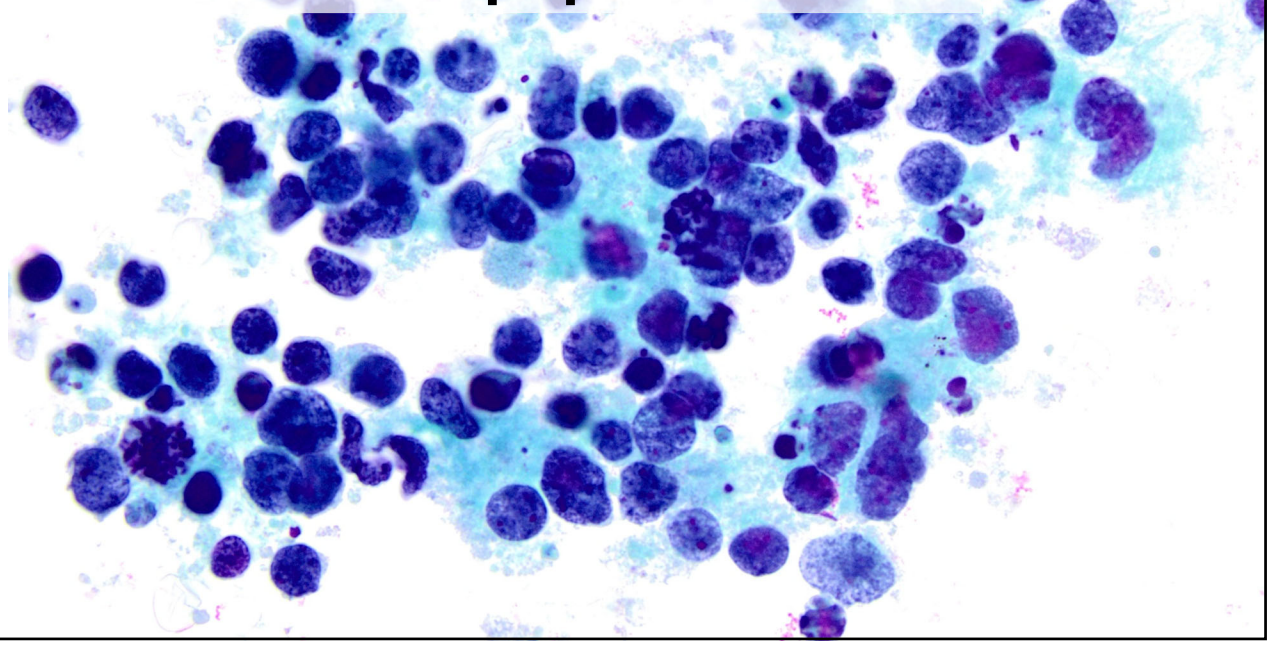
Cibas and Ducatman. Cytology, 5th ed. 2020.
H.A. Domanski. Atlas of Fine Needle Aspiration Cytology. 2nd ed. 2019.

→Finely granular chromatin

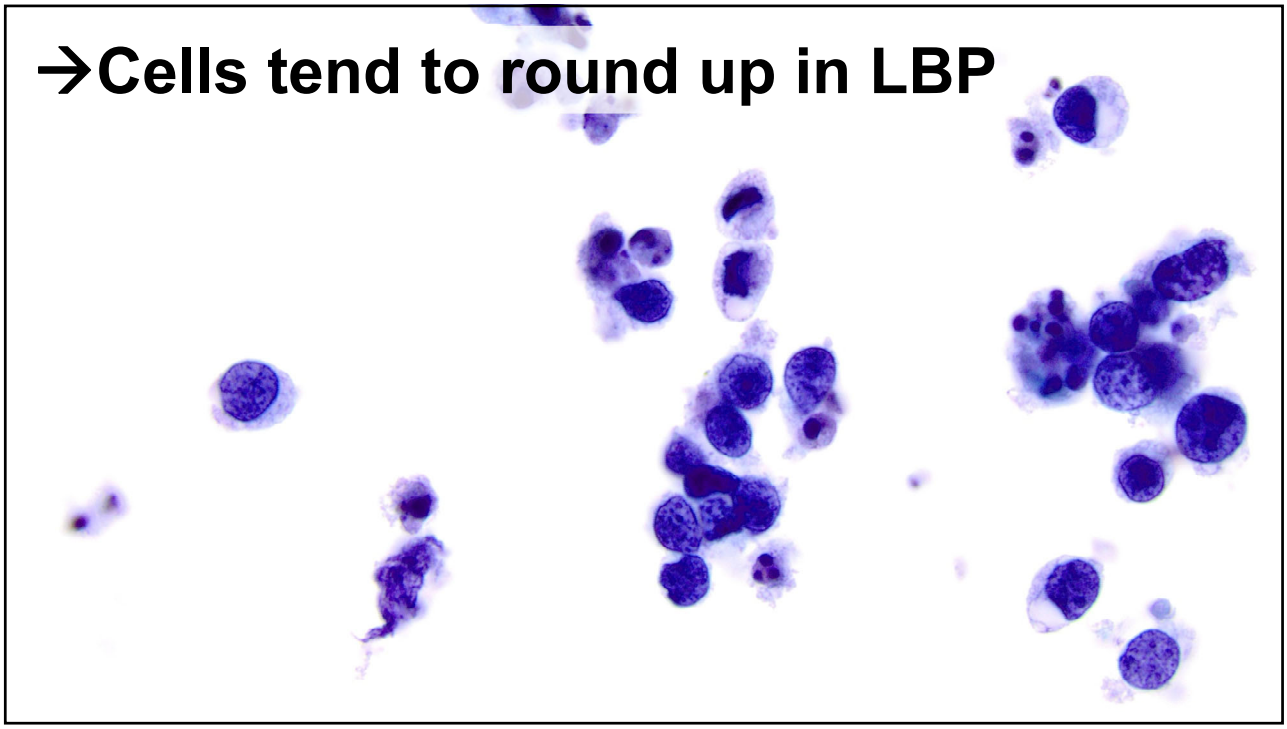


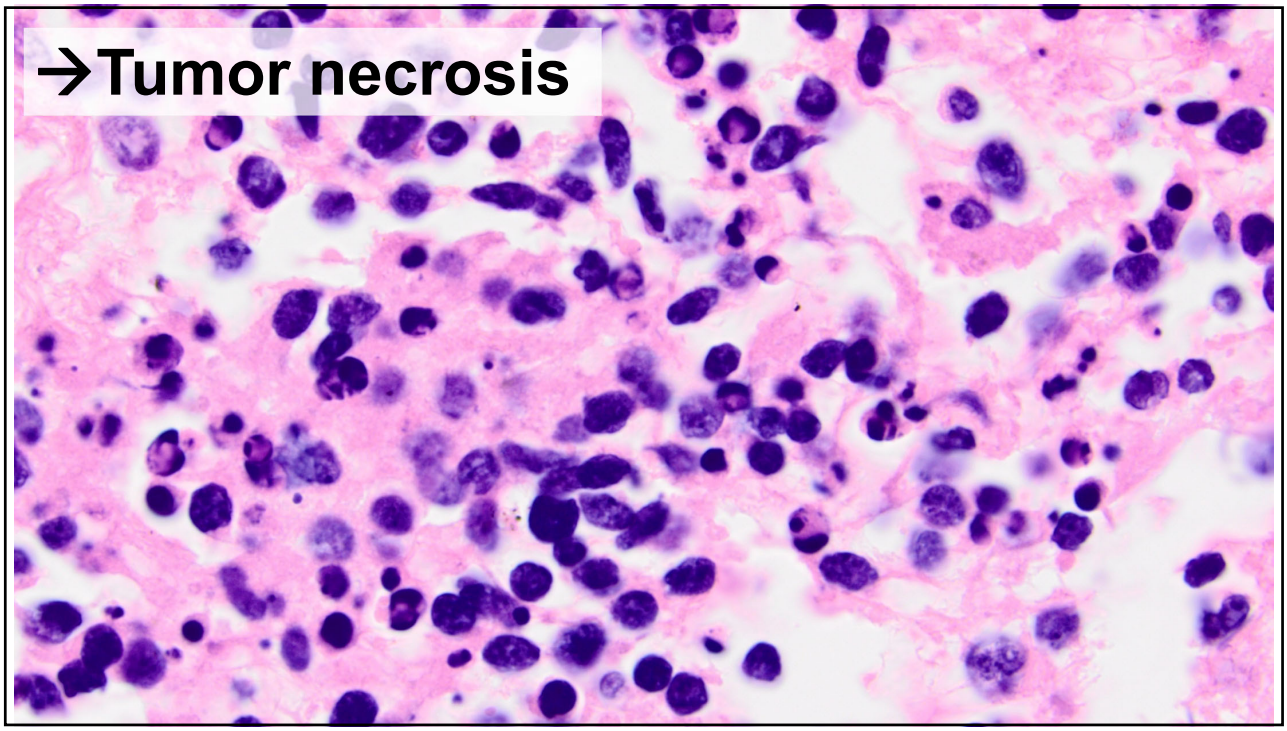
→No nucleoli

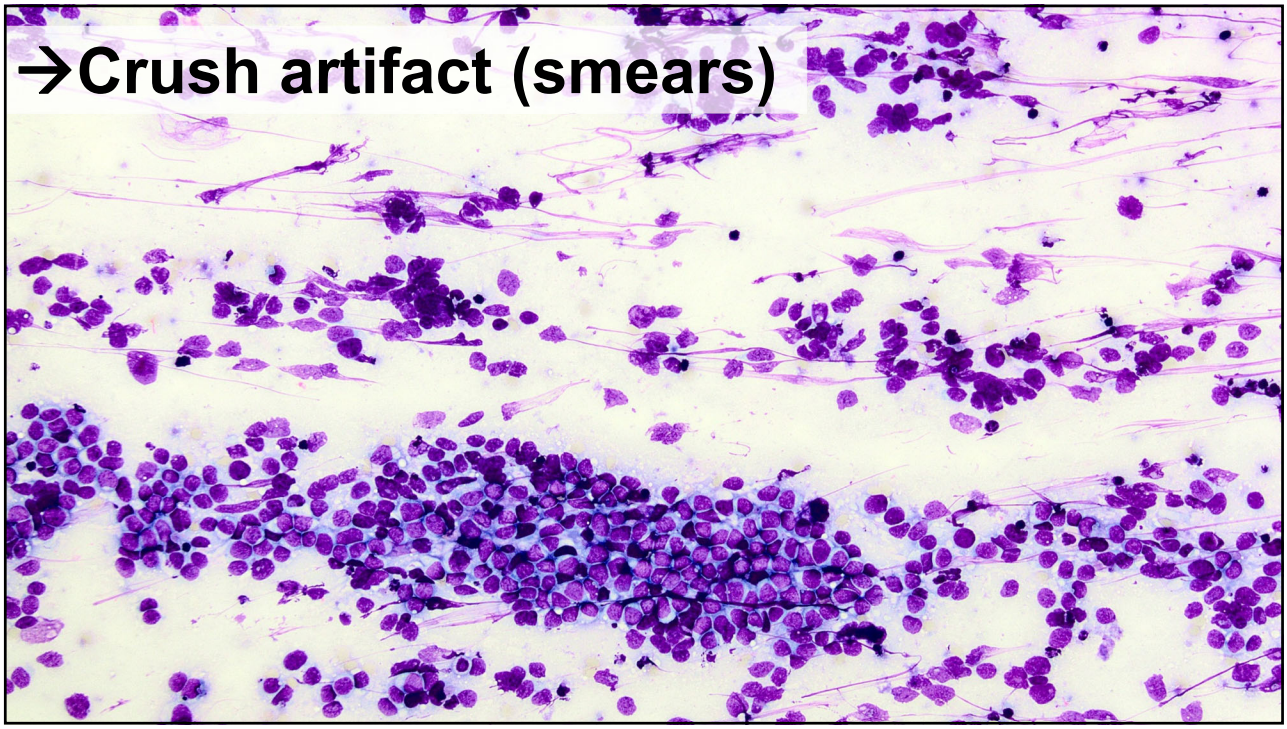
→Mitoses and apoptotic bodies

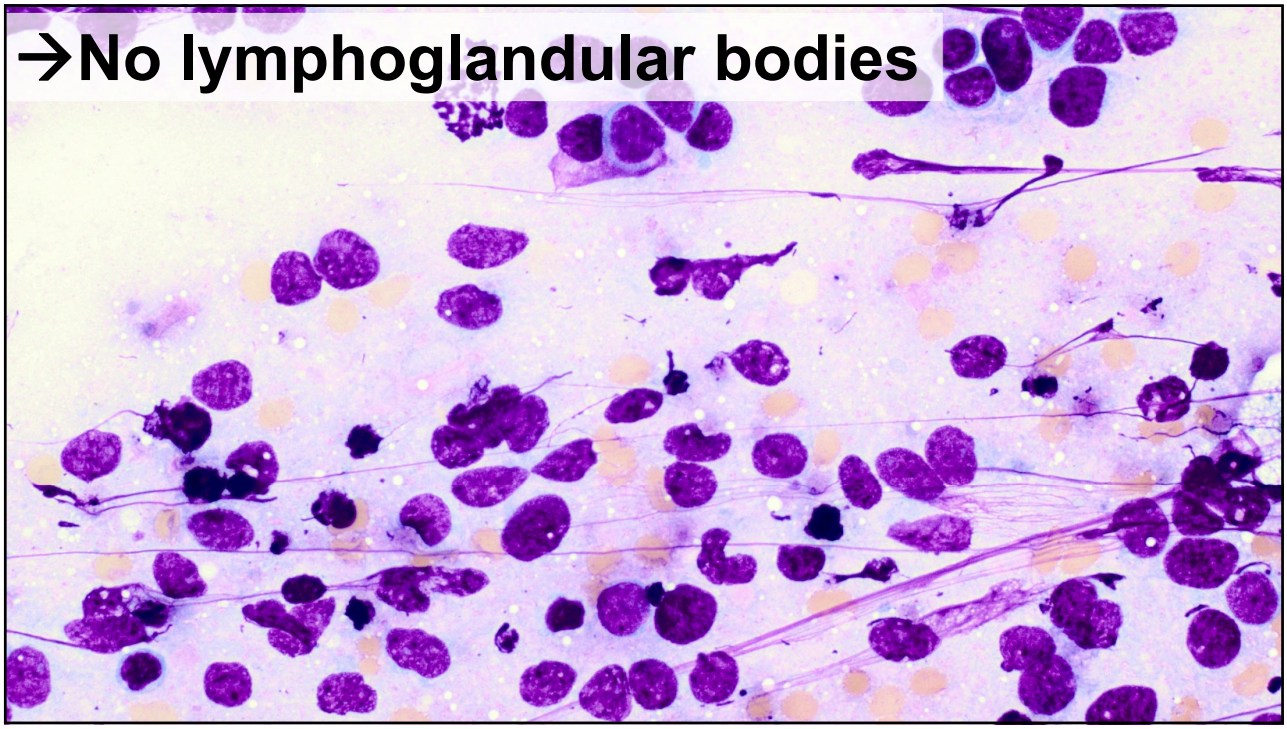


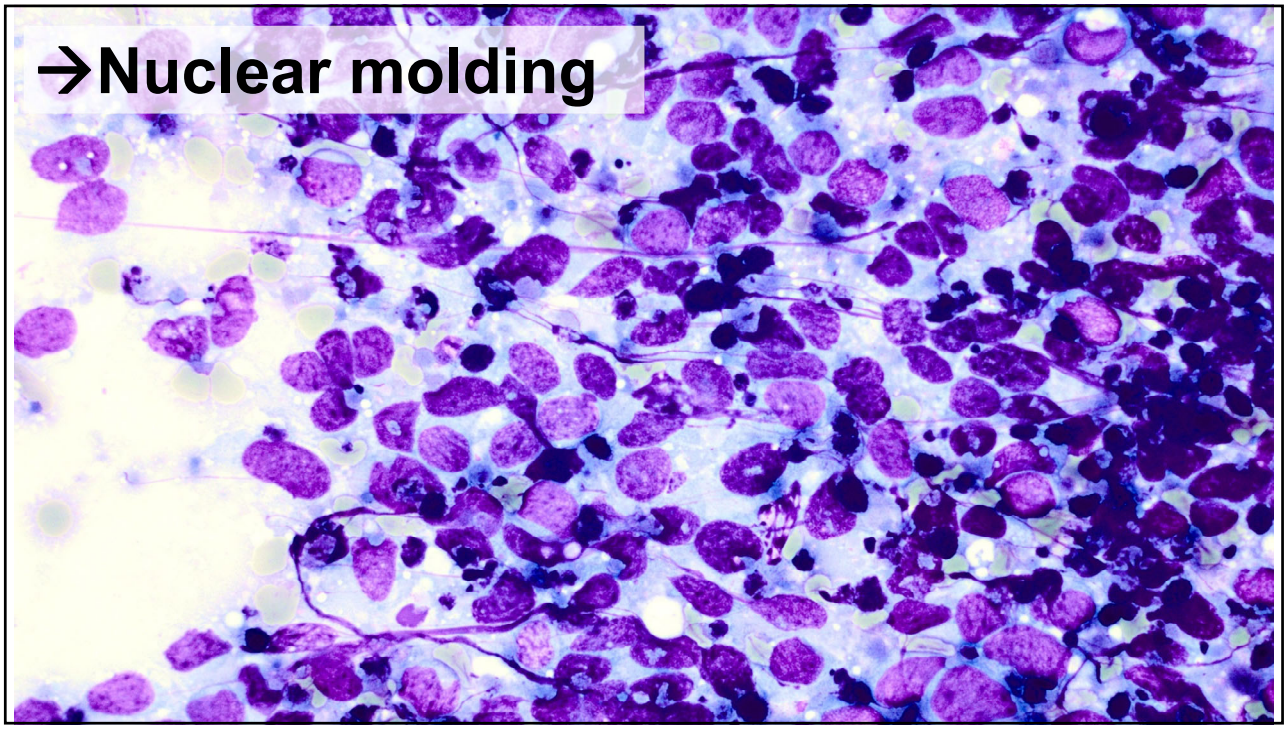
→Cells tend to round up in LBP

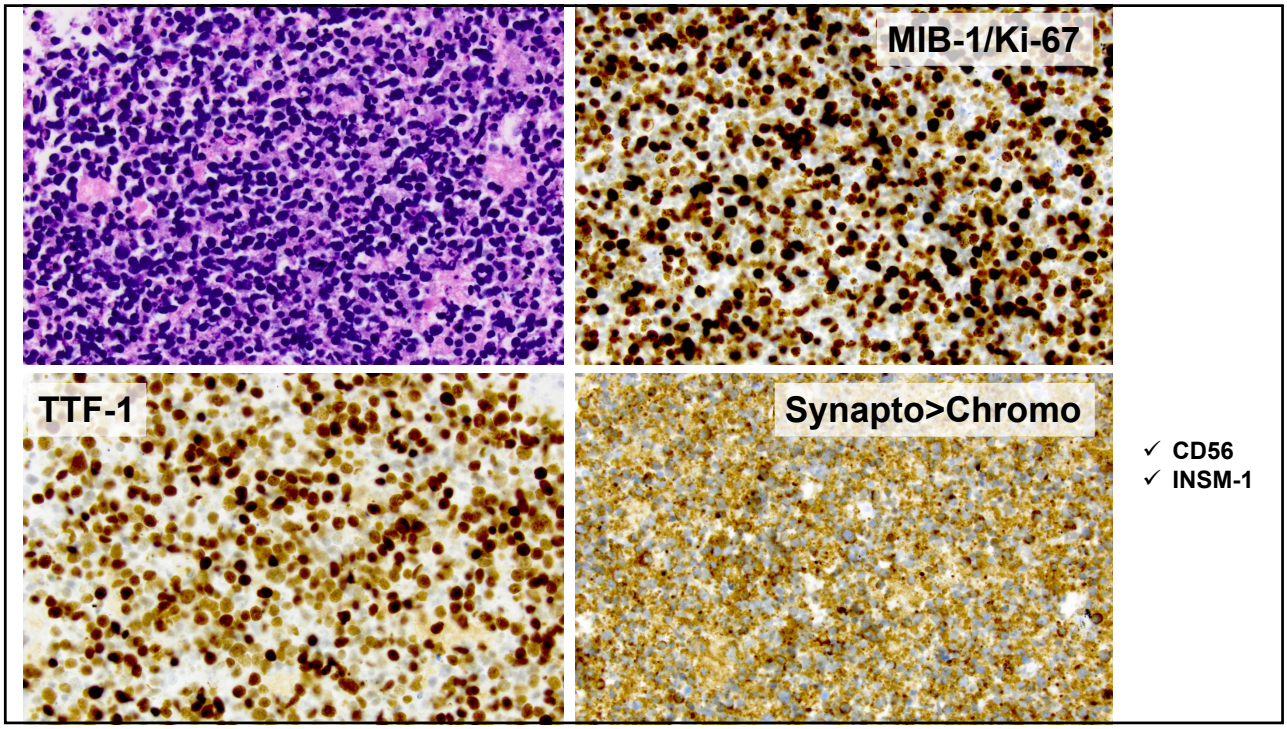





→Crush artifact (smears)


→No lymphoglandular bodies


→Nuclear molding


MIB-1/Ki-67
TTF-1
Synapto>Chromo
✓ CD56
✓ INSM-1

Beware the pitfalls on cytology/small biopsies

Case Report

Erroneous diagnosis of small cell lung cancer based on small biopsies with far-reaching consequences: case report of a typical carcinoid tumor

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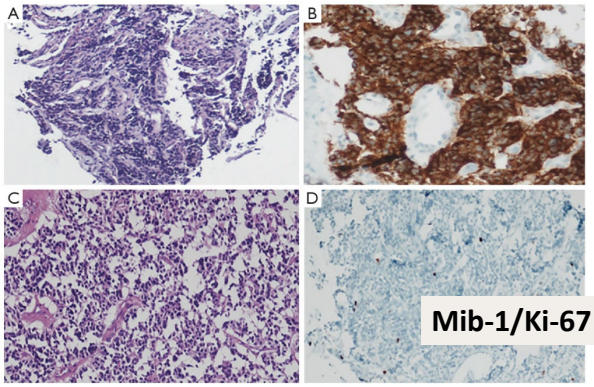
[1]Department of Thoracic Surgery and Endoscopy, Ruhrlandklinik, West German Lung Centre, University of Duisburg-Essen, Essen, Germany; [2]Department of Anesthesiology, Ruhrlandklinik, West German Lung Centre, University of Duisburg-Essen, Essen, Germany and Department of Anesthesiology and Intensive Care Medicine, University Hospital Magdeburg, Otto von Guericke University Magdeburg, Magdeburg, Germany; [3]Institute of Pathology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

J Thorac Dis 2017;9:E99-E102

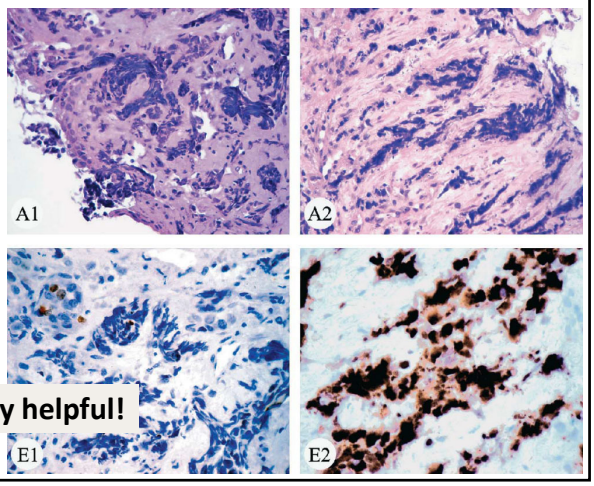


Typical and Atypical Pulmonary Carcinoid Tumor Overdiagnosed as Small-Cell Carcinoma on Biopsy Specimens

A Major Pitfall in the Management of Lung Cancer Patients

Giuseppe Pelosi, MD, Jaime Rodriguez, MD,† Giuseppe Viale, MD,* and Juan Rosai, MD†*

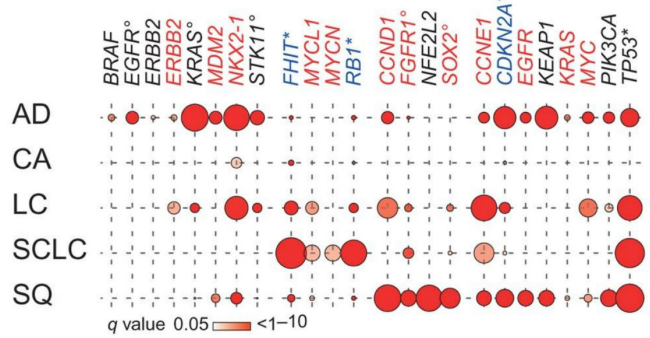
Am J Surg Pathol 2005;29:179–187



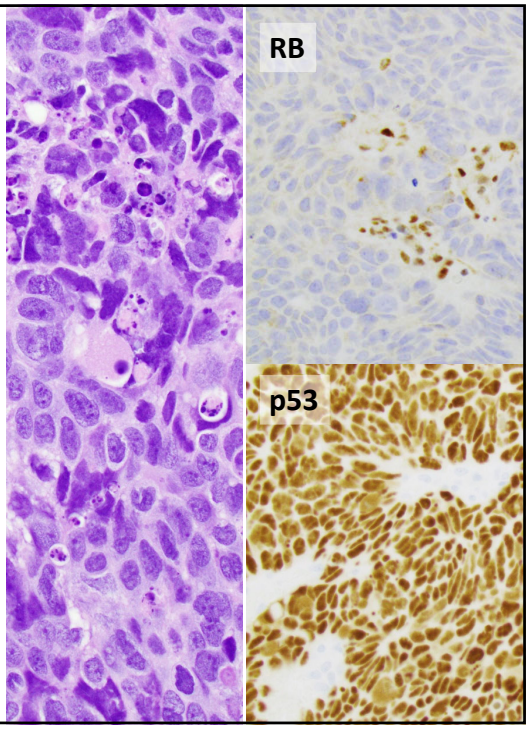
Mib-1/Ki-67 is very helpful!

Small cell carcinoma?

- ✓ MIB-1/Ki-67 staining
- ✓ pRB loss / p53+ / p16 + IHC
- ✓ Clinical presentation



Sci Transl Med. 2013. 30;5(209):209ra153.



ORIGINAL ARTICLE J Thorac Oncol. 2022;17(1):141-153. IASLC

Molecular Subtypes of Primary SCLC Tumors and Their Associations With Neuroendocrine and Therapeutic Markers

Song Qu, MD,[a] Patricia Fetsch,[b] Anish Thomas, MD,[c] Yves Pommier, MD, PhD,[c] David S. Schrump, MD,[d] Markku M. Miettinen, MD, PhD,[b] Haobin Chen, MD, PhD[d,*]

[a]Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland
[b]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
[c]Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
[d]Thoracic Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

SCLC Subtypes

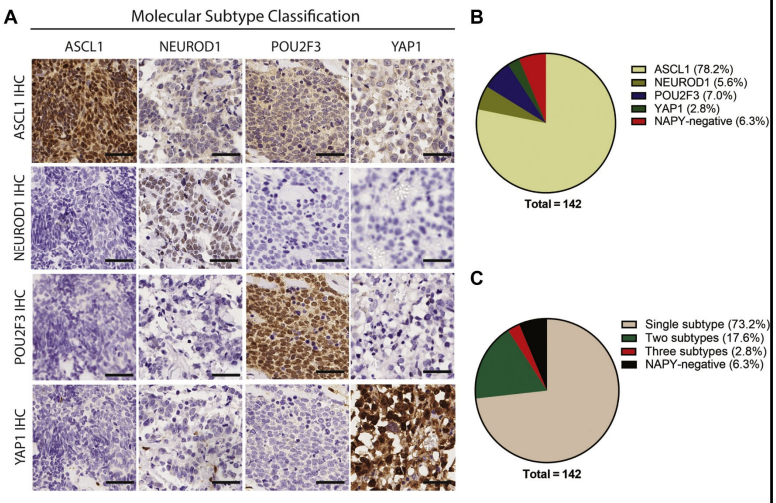
Transcription Regulator Expression:

SCLC-A: high expression of ASCL1, a regulator of neuroendocrine (NE) differentiation.

SCLC-N: Marked by NEUROD1 activation and frequent MYC amplification, leading to rapid proliferation and chemoresistance.

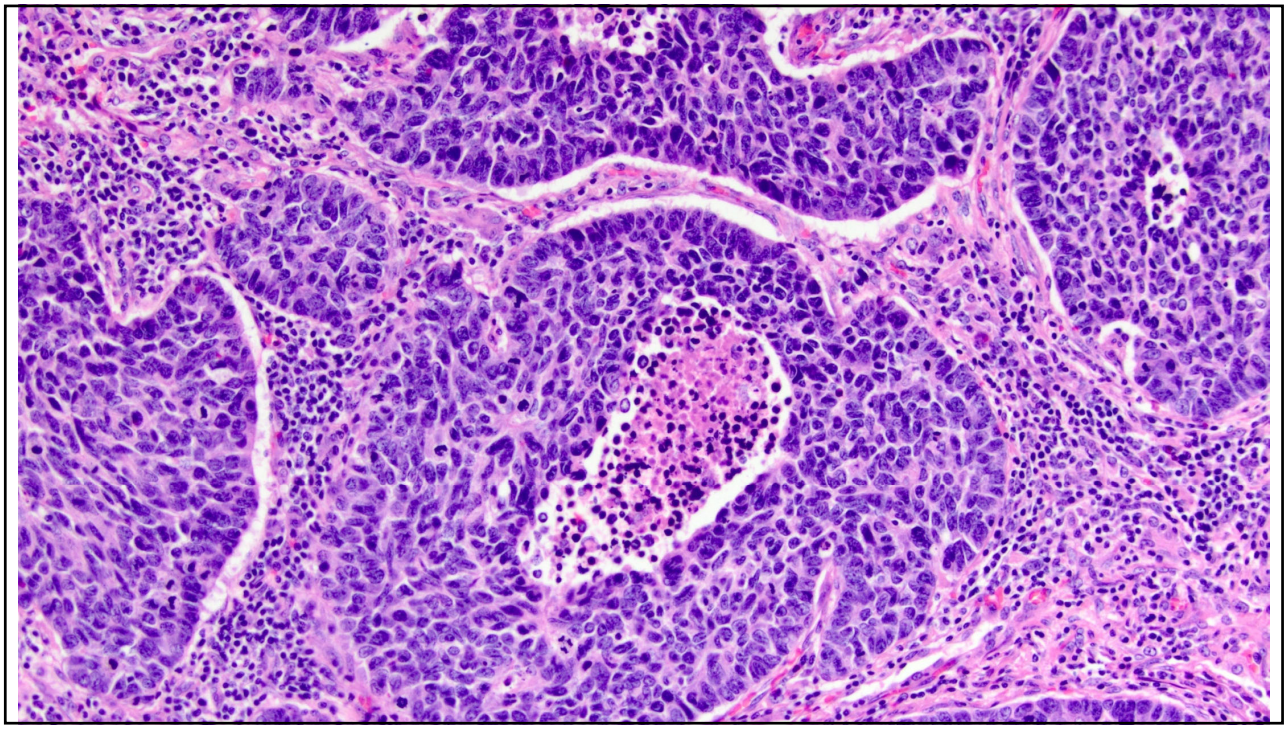
SCLC-P: Defined by POU2F3 expression and a lack of traditional NE features.

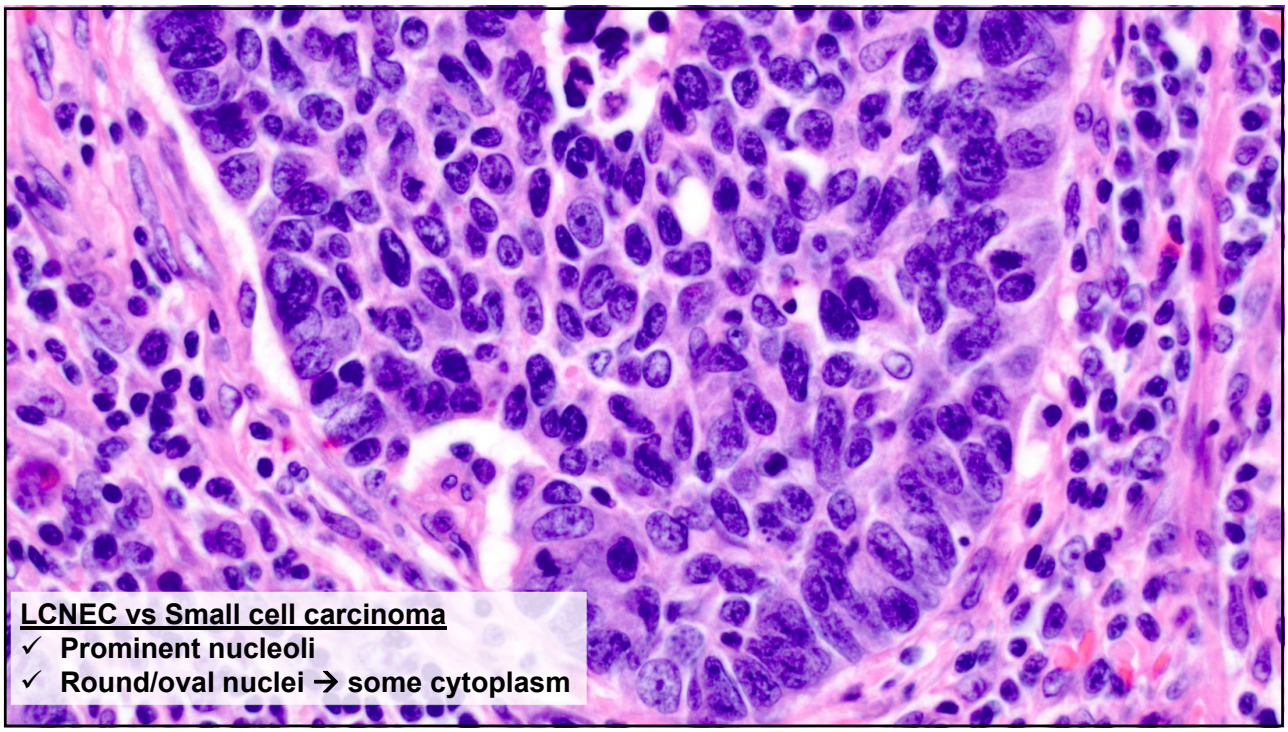
SCLC-I: Represents an immune-inflamed phenotype with high MHC class I/II expression, IFN-γ signaling, and immune checkpoint molecules. +/- YAP1.



→ POU2F3 IHC can be helpful for SCLC without NE marker expression

Large Cell Neuroendocrine Carcinoma (LCNEC)




LCNEC vs Small cell carcinoma
✓ Prominent nucleoli
✓ Round/oval nuclei → some cytoplasm

Large Cell Neuroendocrine Carcinoma
(LCNEC) *Cytologic features*

Architecture:

- More cohesive sheets or clusters of cells
- May show rosette formation or nuclear palisading

Cellular features:

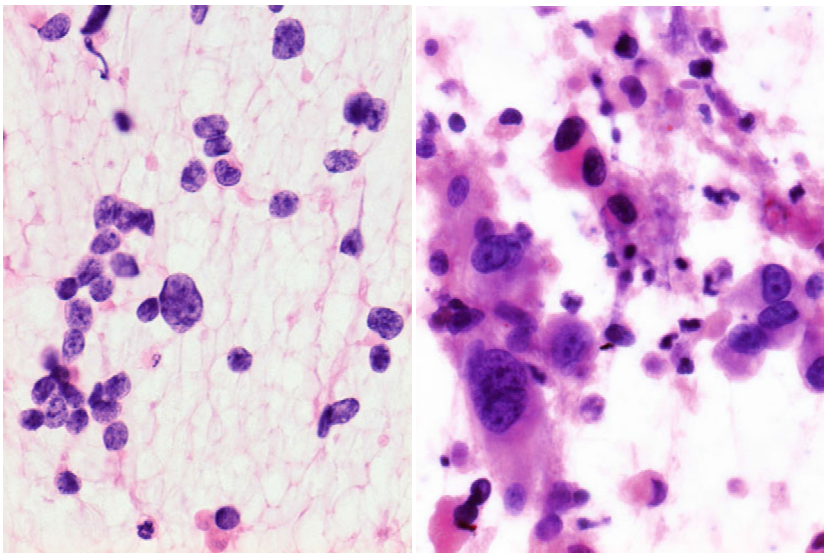
- Medium to large sized cells
- Round to oval nuclei
- High N/C ratio, moderate amounts of cytoplasm

Nucleus/chromatin:

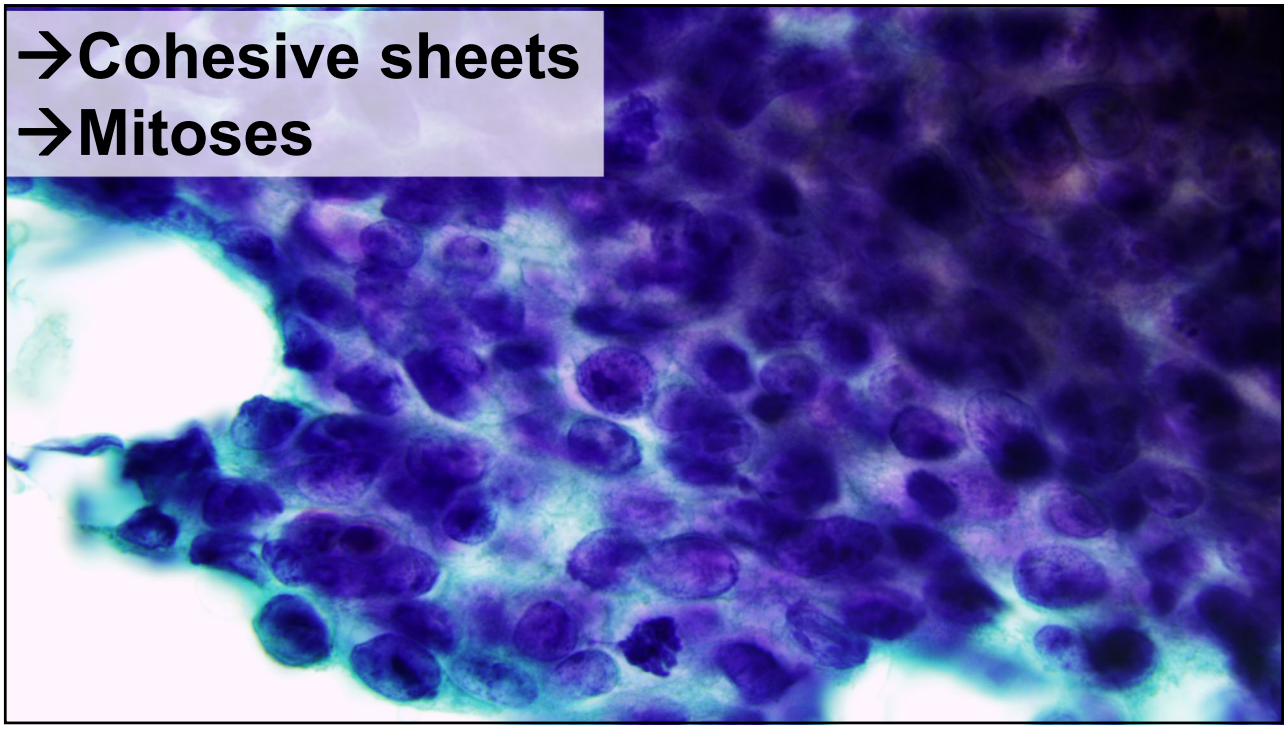
- Open, clumpy chromatin
- Often prominent nucleoli

Other:

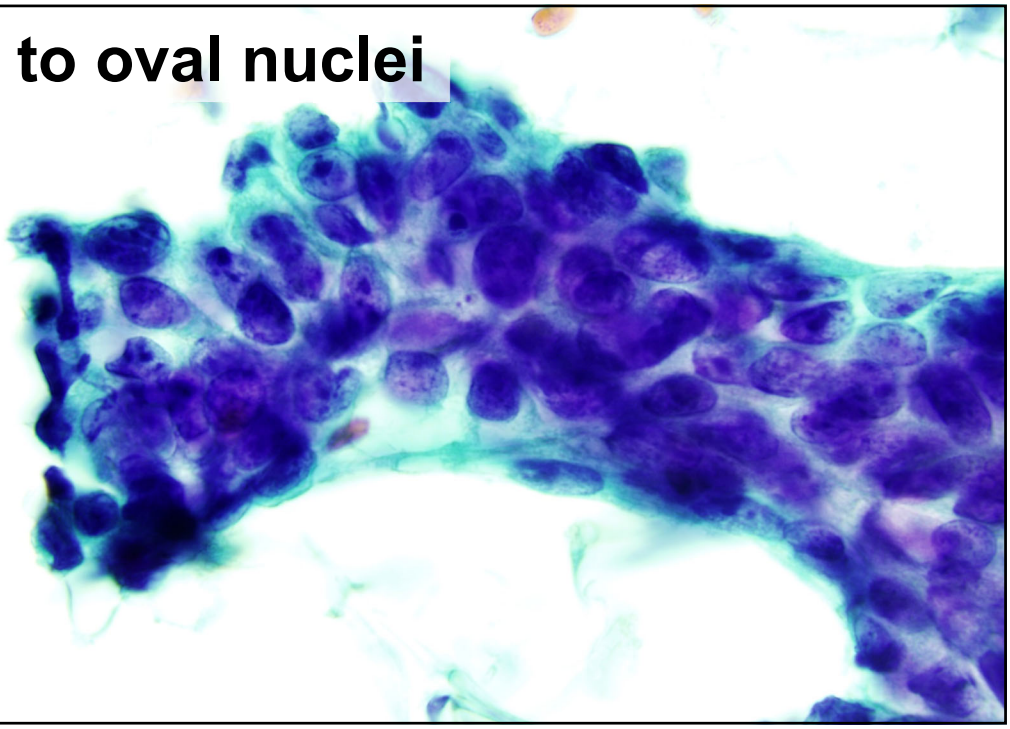
- Frequent mitoses
- Necrotic background
- Less nuclear molding
- Crush artifact (smears)



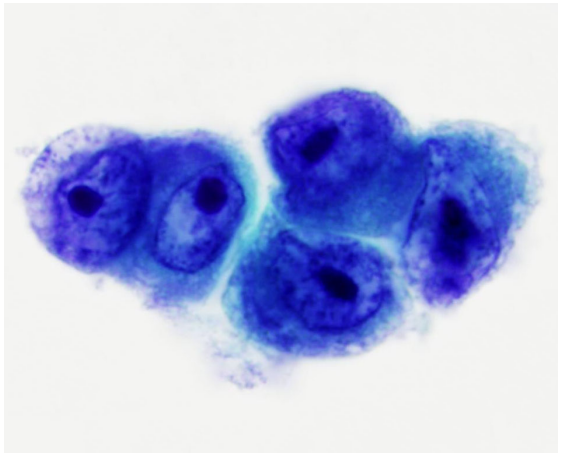
Cibas and Ducatman. Cytology, 6th ed. 2026.
H.A. Domanski. Atlas of Fine Needle Aspiration Cytology. 2nd ed. 2019.

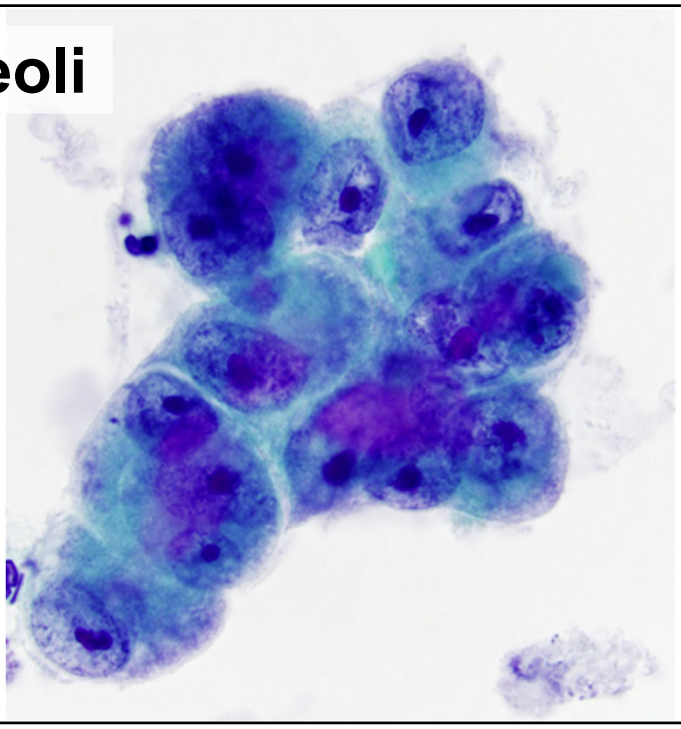


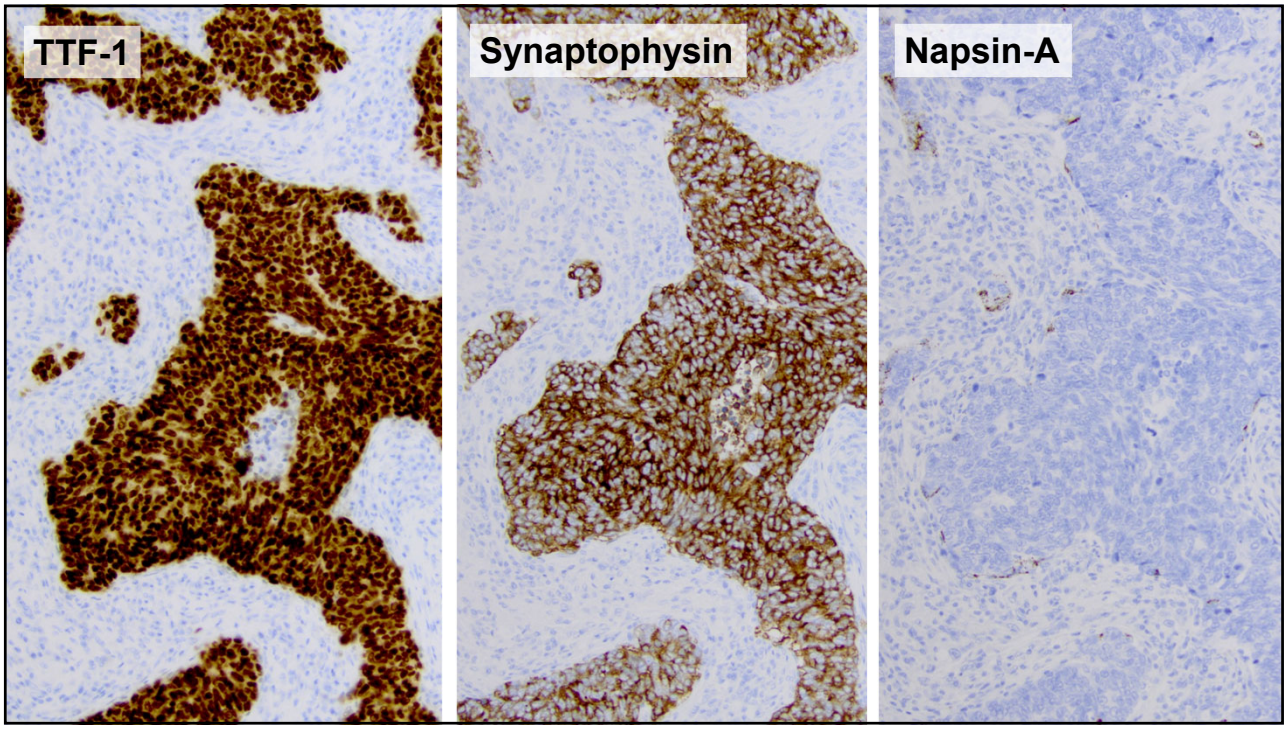
→Round to oval nuclei



→Prominent nucleoli







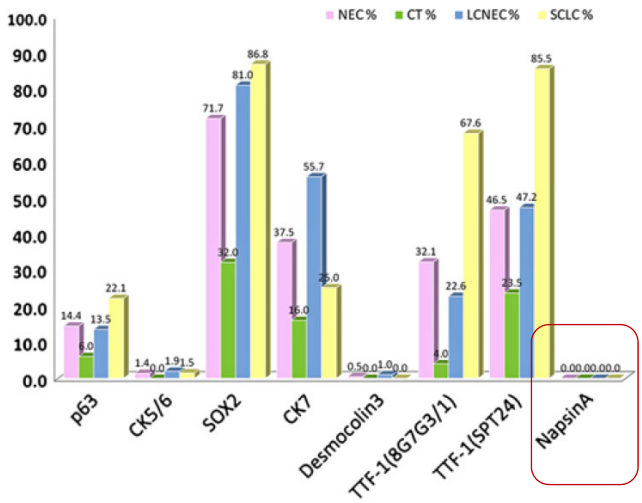
RESEARCH ARTICLE

Expression of Squamous Cell Carcinoma Markers and Adenocarcinoma Markers in Primary Pulmonary Neuroendocrine Carcinomas

Kyohei Masai, MD,† Koji Tsuta, MD, PhD,* Mitsumasa Kawago, MD,*† Takahiro Tatsumori, MD,* Tomoaki Kinno, MD,* Tomoko Taniyama, MD,* Akihiko Yoshida, MD, PhD,* Hisao Asamura, MD, PhD,† and Hitoshi Tsuda, MD, PhD**

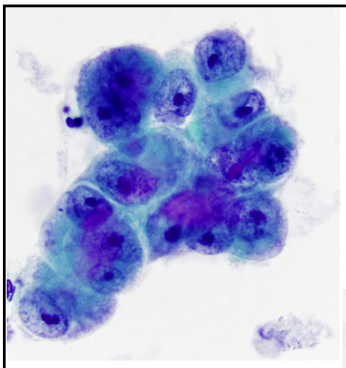
Tissue microarray study

- 52 Carcinoids
- 106 LCNECs
- 69 Small Cell carcinomas



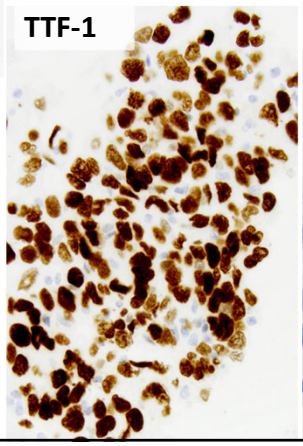
Napsin-A ab used: mouse monoclonal

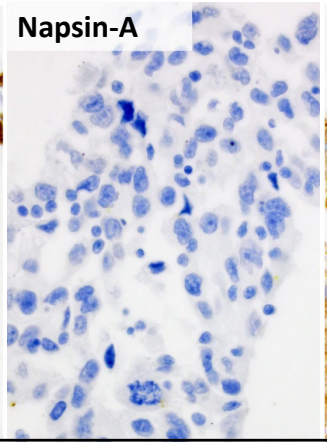
Appl Immunohistochem Mol Morphol 2013;21:292–297

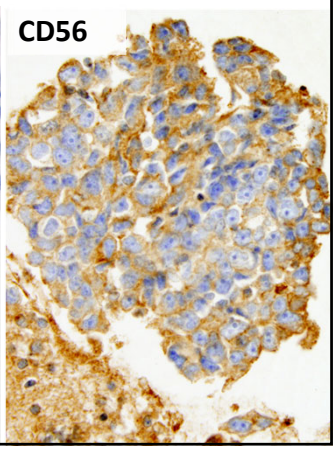


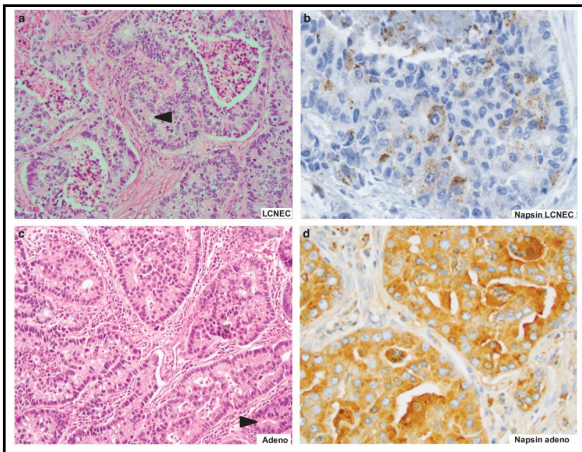
TTF-1+/Napsin- : *consider* neuroendocrine tumor

Carcinoid tumor
Large Cell Neuroendocrine Carcinoma (LCNEC)









Modern Pathology (2018) 31, 111–121

Pulmonary large cell neuroendocrine carcinoma with adenocarcinoma-like features: napsin A expression and genomic alterations

Natasha Rekhtman[1], Catherine M Pietanza[2,5], Joshua Sabari[2], Joseph Montecalvo[1], Hangjun Wang[1,6], Omar Habeeb[1,7], Kyuichi Kadota[1,8], Prasad Adusumilli[3], Charles M Rudin[2], Marc Ladanyi[1,4], William D Travis[1] and Philippe Joubert[1,9]

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [2]Thoracic Oncology Service, Department of Medicine, Division of Solid Tumor Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [3]Department of Thoracic Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA and [4]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Napsin-A abs used:

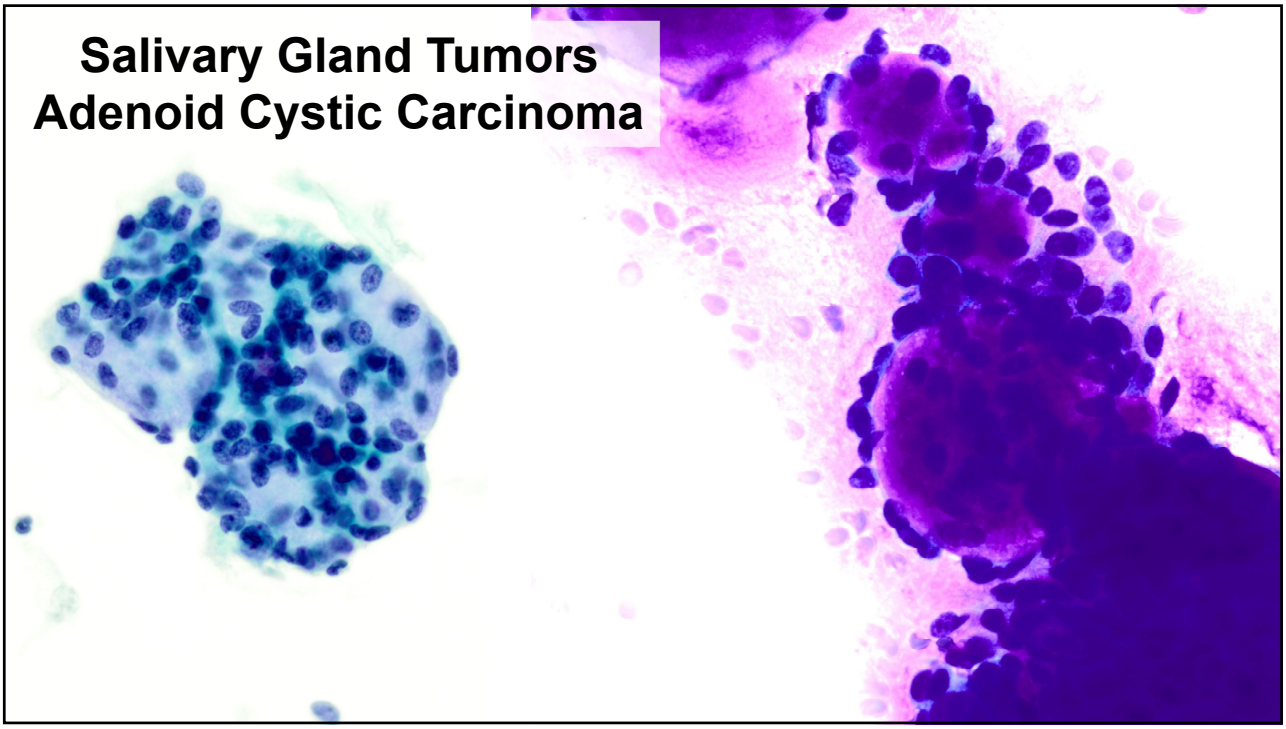
both rabbit polyclonal, mouse monoclonal

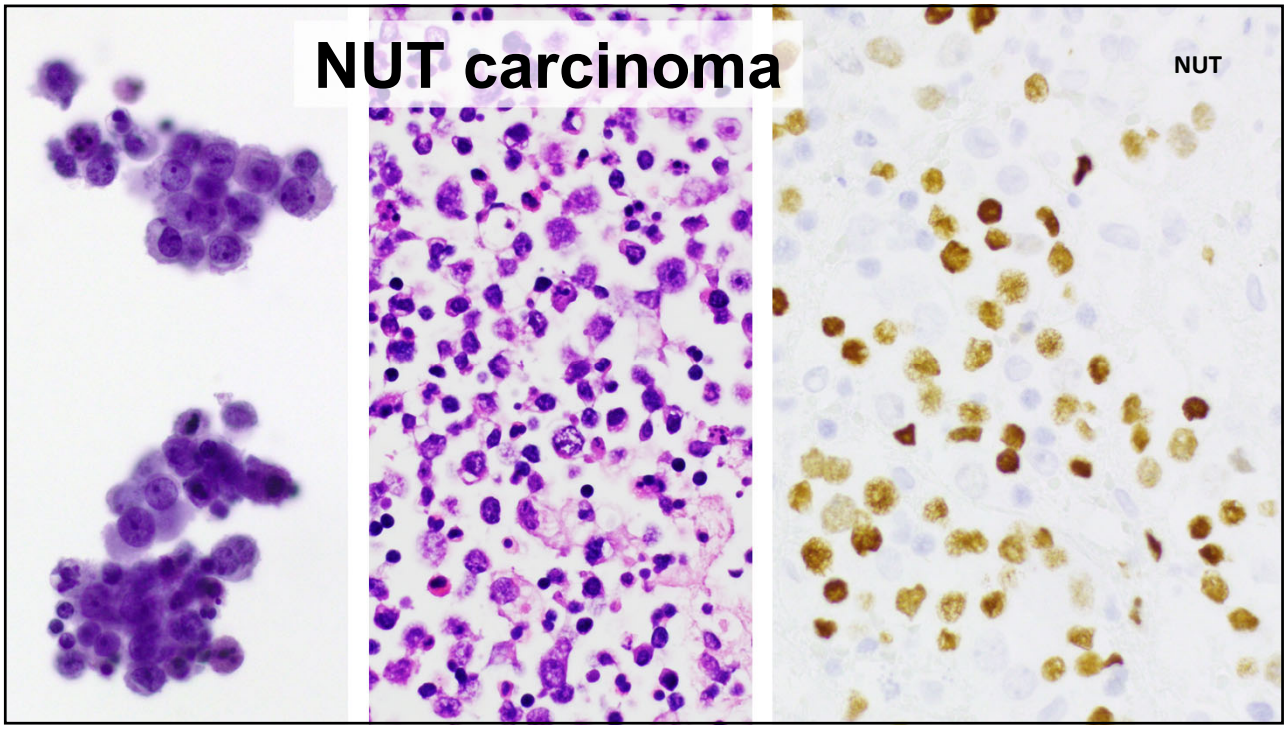
Table 3 Comparison of napsin A and TTF-1 expression in large cell neuroendocrine carcinoma *versus* lung adenocarcinoma

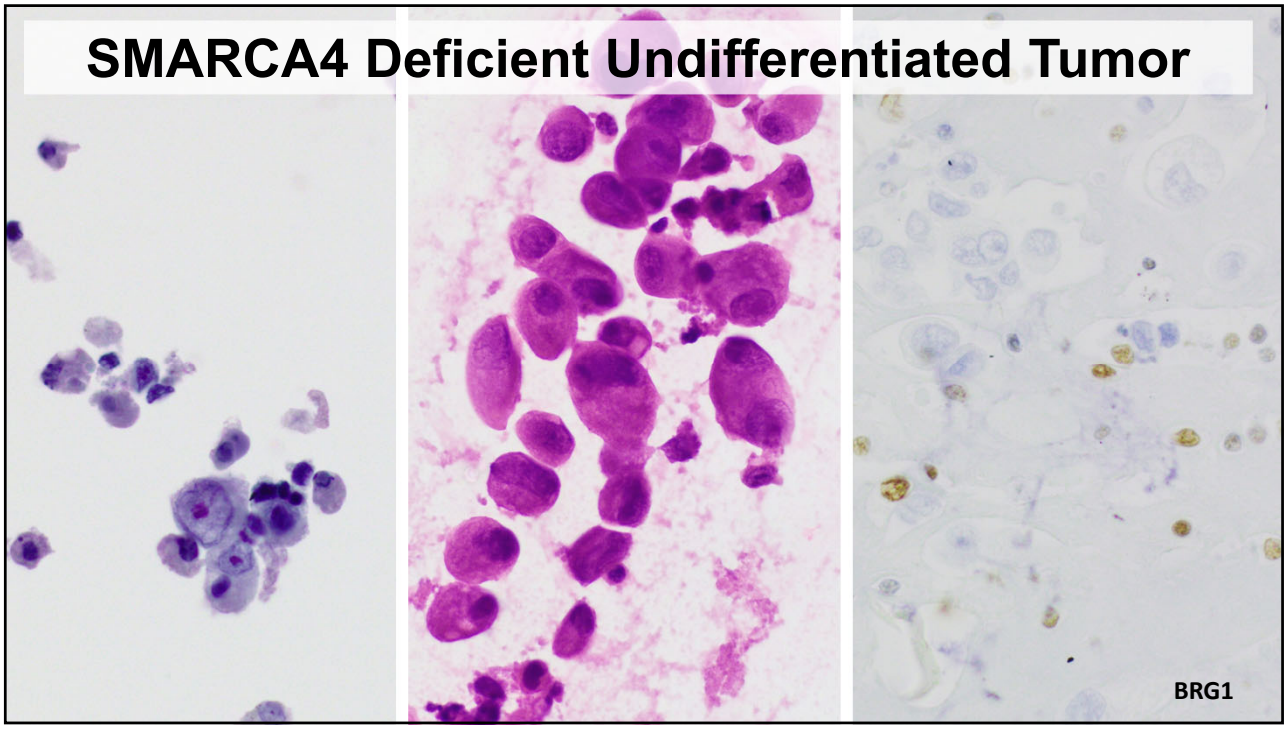
	LCNEC (n = *112*)	*Adenocarcinoma* (n = *60*)	P *value*
Napsin A expression			
Napsin A-positive: *n* (%)	17 (15%)	51 (85%)	0.0001
Extent of labeling in positive cases: mean ± s.d.	34 ± 32%	93 ± 34%	< 0.0001
Intensity of labeling in positive cases: mean ± s.d.	1.4 ± 0.4	2.6 ± 1.4	< 0.0001
Napsin A/TTF-1 joint expression[a]			
Napsin A(+)/TTF-1(+)	16 (15%)	51 (85%)	< 0.0001
Napsin A(−)/TTF-1(+)	47 (44%)[b]	2 (3%)[c]	
Napsin A(+)/TTF-1(−)	0	0	
Napsin A(−)/TTF-1(−)	43 (41%)	7 (12%)	

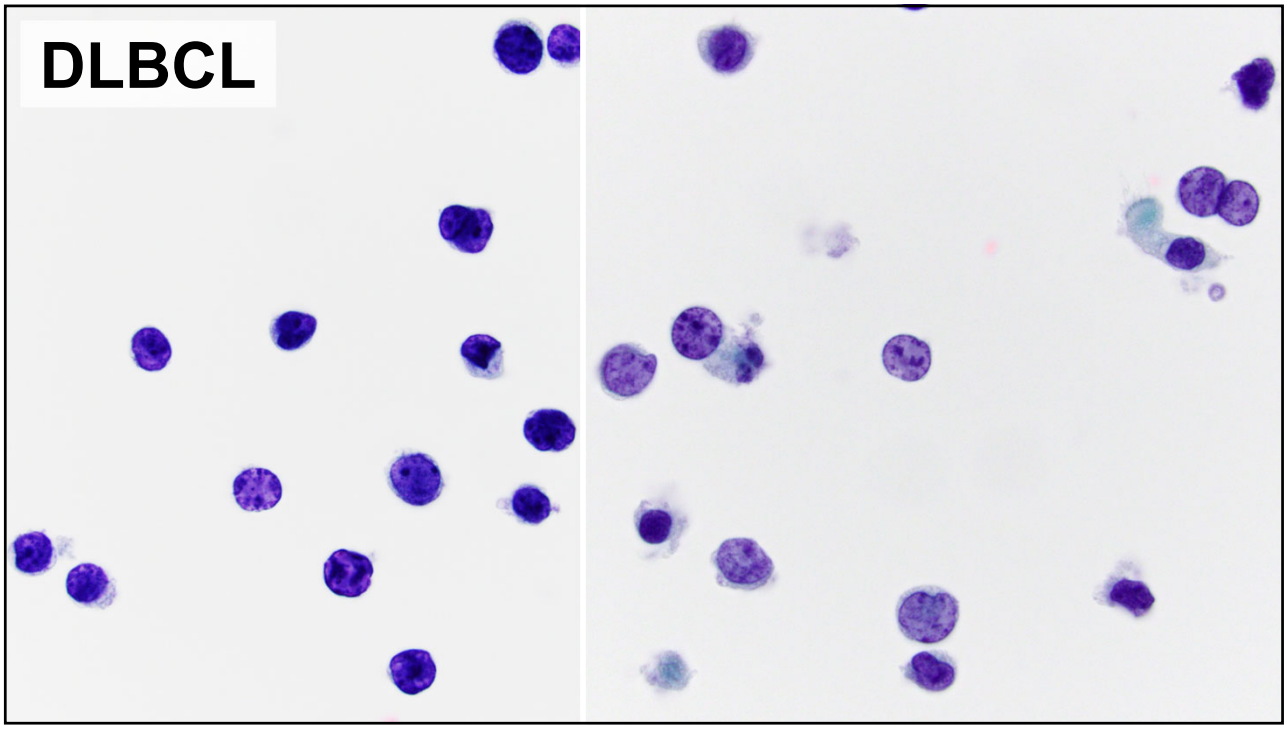
Rare Tumor Types

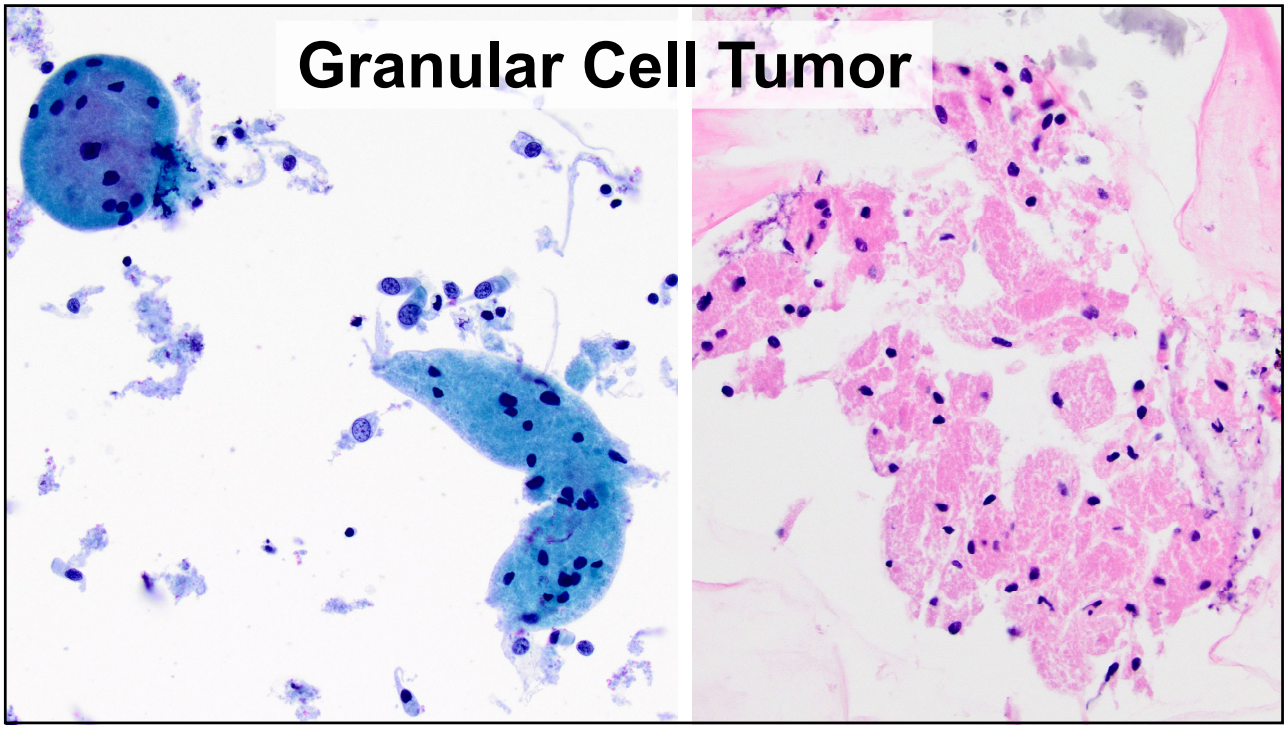
Salivary Gland Tumors
Adenoid Cystic Carcinoma




NUT carcinoma
NUT

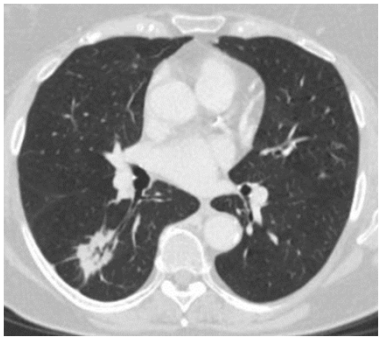

SMARCA4 Deficient Undifferentiated Tumor
BRG1


DLBCL


Granular Cell Tumor

PVL – DIAGNOSTIC APPROACH TO SMALL BIOPSIES

✓ **Review (PET)CT: Lung vs pleural vs thymic vs met**

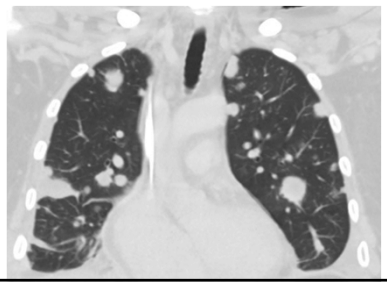


✓ **Assess histo/cytomorphology**

- Keratinizing squamous cell carcinoma: no stains needed
- Adenocarcinoma: TTF-1 and Napsin-A
- Squamous: p40, TTF-1
- PD NSCLC-NOS: TTF-1, Napsin-A, p40
- Neuroendocrine: TTF-1, Synaptophysin, Mib-1/Ki-67

✓ **Inconclusive / poorly differentiated tumor**

- Cytokeratins (CK5/6, CK7/20, cocktail)
- Mucicarmine stain
- Metastasis (CDX-2, PAX-8, GATA3, ER, NKX3.1, etc…)
- Consider salivary gland type tumor, etc…



✓ **Save material for subsequent ancillary testing!**

HARVARD MEDICAL SCHOOL

Pulmonary Cytology:

Workup of Lung Cancer on FNA and Small Biopsy Specimens

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