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Respiratory Cytology

WHO System

Diagnostic Category	Risk of Malignancy (ROM)	
	Brush, Wash, Sputum	FNA
Insufficient/ Inadequate/ Non-diagnostic		~50%
Benign	~40%	~40%
Atypical	~80%	~50%
Suspicious for malignancy	~90%	~80%
Malignant	~100%	~95%

Modified from: WHO Reporting System for Lung Cytopathology

Note: The cited ROM ranges are very broad (for nondiagnostic sputum it's literally 0-100%!), so I've chosen to give simplified approximate numbers. But generally, it's close to 100% for "malignant" and still almost 50% for "Benign" (indicating a high pretest probability of malignancy). These numbers will likely be refined in future editions.

Brief Commentary

Many lung tumors, other than classic Squamous cell carcinoma, Adenocarcinoma, and Small cell carcinoma, require immunohistochemistry (e.g., carcinoids) for a more definitive diagnosis. So, its helpful to get a cell block or tissue biopsy. This is also helpful for ancillary testing as it's pretty standard to get PD-L1 and molecular testing to guide treatment.

So, <u>two things to remember:</u>
1) Try to **maximize tissue in cell block** or biopsy size for ancillary testing
2) Try to **preserve this tissue for clinical testing** (i.e., don't do unnecessary studies)

Since many rare tumors are difficult, if not impossible, to diagnose on a small biopsy, I'm going to focus these notes on the common tumors/conditions that can be diagnosed by cytology.

For additional information on these other tumors, please refer to my general "Lung Tumor" notes.

Insufficient/Inadequate/Non-diagnostic

A specimen that for quantitative or qualitative reasons does not explain the targeted lesion.
Possible reasons: low cellularity, necrosis, poor preparation. Any atypia precludes this Dx.

These terms can be used interchangeably and labs should pick one. Generally though, "Insufficient" and "Inadequate" are used for cases where there is a lack of cellular material, while "Non-diagnostic" is used in cases where there are benign findings that just don't explain the mass lesion.

If respiratory specimens have no macrophages and/or respiratory epithelial cells→ Inadequate

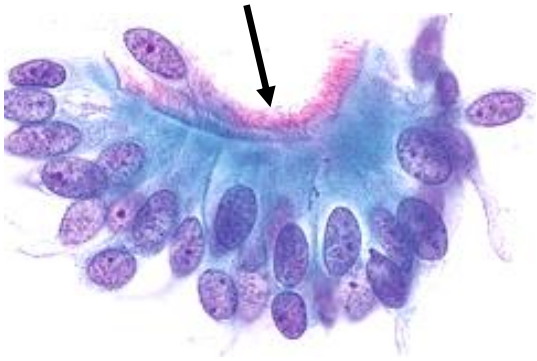
<u>Common scenario:</u> An FNA of a lung mass shows only benign bronchial epithelium→ can report as either "*Non-diagnostic*"(as doesn't explain a mass lesion) <u>or</u> "*Benign*" with a caveat that this may not be representative and that repeat biopsy may be necessary.

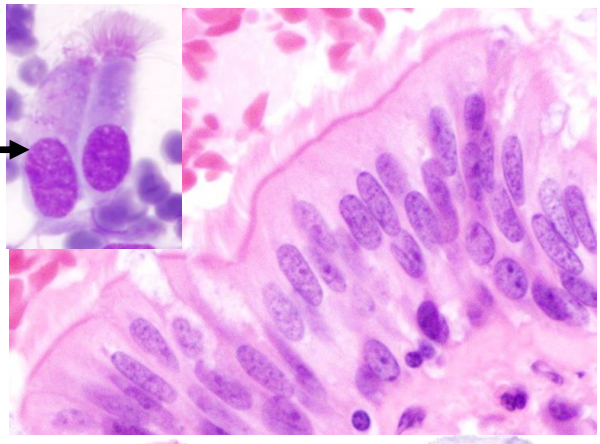
Kind of goes without saying, but I often say, *"Correlation with clinical and imaging findings is required."*

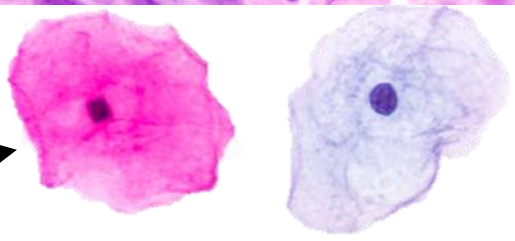
Normal Tissue

Ciliated respiratory epithelium

Predominant epithelial cells that line the trachea and bronchi--**Ciliated with a terminal bar**
looks like "Beaker" from the Muppets
Basal round/oval nuclei with even chromatin

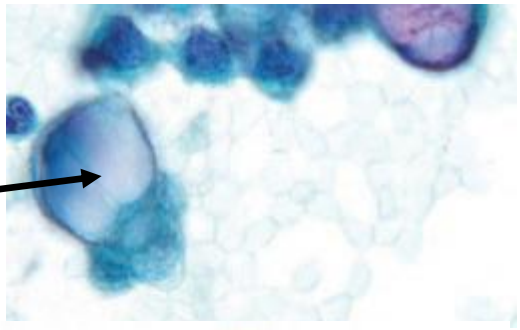






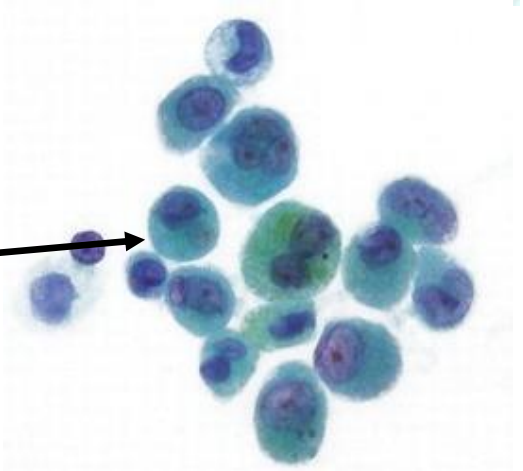
Squamous cells

Often represent oral contamination!
Identical to squamous cells at other sites
Abundant keratinizing cytoplasm
Can see squamous metaplasia with irritation/inflammation



Goblet cells

Mixed in with ciliated respiratory cells
Mucin-filled cytoplasmic vacuole
Basal oval to flat nucleus
Commonly increased in chronic conditions like: COPD, Asthma, Bronchiectasis.
(so be careful not to over interpret as mucinous neoplasm!)

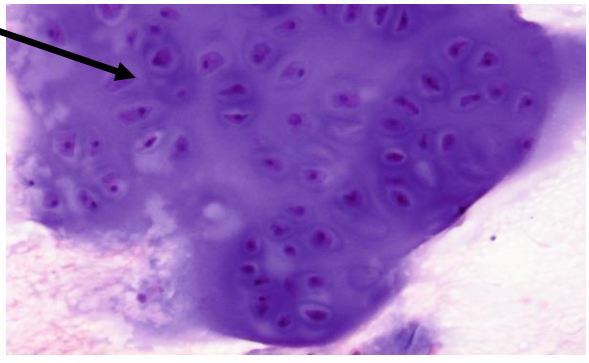


Alveolar macrophages

Abundant cytoplasm, often with granular debris/pigment
- Can have anthracotic pigment (black) or Hemosiderin
Vesicular nuclei with fine chromatin

Cartilage (and other bronchial tissue)

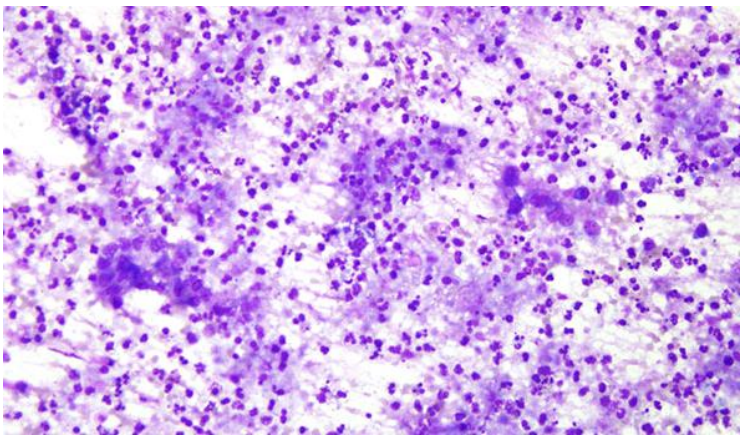
Extracellular thick material with chondrocytes in lacunae
Can also see other bronchial tissue like smooth muscle/fibrous tissue, bronchial glands, lymphocytes, etc…



Benign Lesions

Unequivocal benign cytologic findings which may or may not be diagnostic of a specific inflammatory process or benign neoplasm. Correlation with imaging and clinical findings (the "triple test," with pathology), is necessary to make sure the cytology findings match.

Acute Inflammation

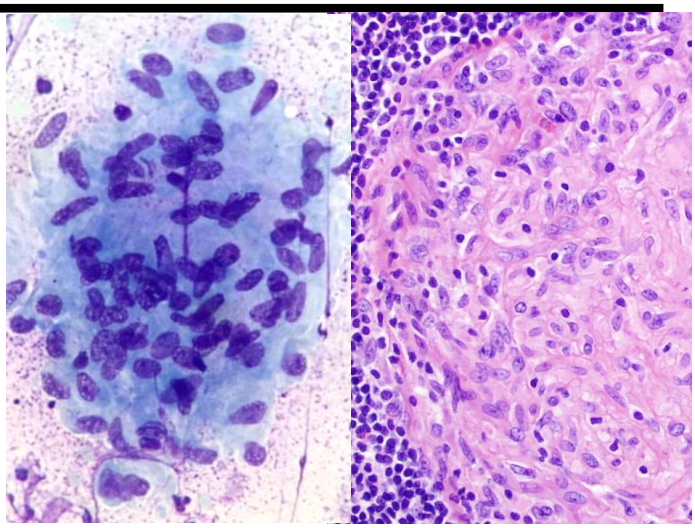


Inflammation dominated by **neutrophils**.
Often varying degrees of degeneration/**necrosis**.
May see bacteria and/or fungus.
Other inflammatory cells/debris may be present.

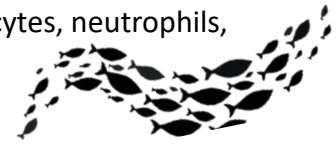
DDX: Often infectious (pneumonia)
Also: Aspiration, infarction, vasculitis,
Unlikely to be malignancy

If seen at ROSE → send cultures!

Granulomatous Inflammation



Collections of epithelioid histiocytes and giant cells
Often admixed lymphocytes, neutrophils, eosinophils
±Necrosis



Histiocytes have **syncytial, epithelioid cytoplasm**
Elongated, often indented ("barefoot") nuclei
Look like a school of fish swirling around

DDX: **Infection** (esp. TB and Fungus)→*Bug stains!*
Autoimmune (**Sarcoidosis**, vasculitis),
Hypersensitivity pneumonitis, drug reaction, aspiration

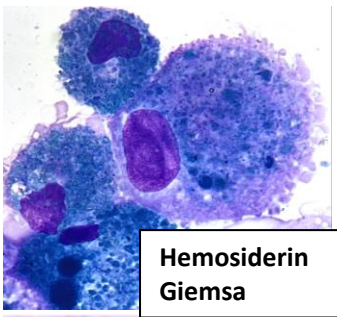
Other inflammation

Lymphocytes
DDX: Lymphocytic interstitial pneumonia, Hypersensitivity pneumonitis, Sarcoidosis, Bechet disease, Lymphoma, Organizing pneumonia

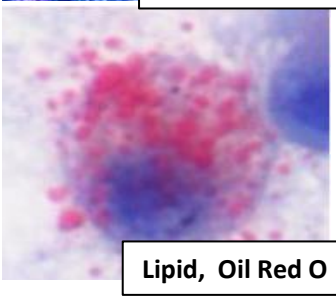
Eosinophils: Lots of eosinophils, Charcot-Leyden crystals,
DDX: Eosinophilic pneumonia, Asthma, Allergic bronchopulmonary aspergillosis, Churg-Strauss disease, Parasitic infections

Histiocytes
Hemosiderin-laden→ Alveolar hemorrhage
Lipid-laden macrophages (foamy, Oil Red O+)→ Aspiration/Lipoid pneumonia, EVALI, Amiodarone toxicity, Organizing pneumonia

Note: BAL from pediatric patients are often sent for ORO staining to look for lipid as it's thought to be indicative of aspiration, but this isn't actually all that sensitive or specific. (PMID: 36998564)



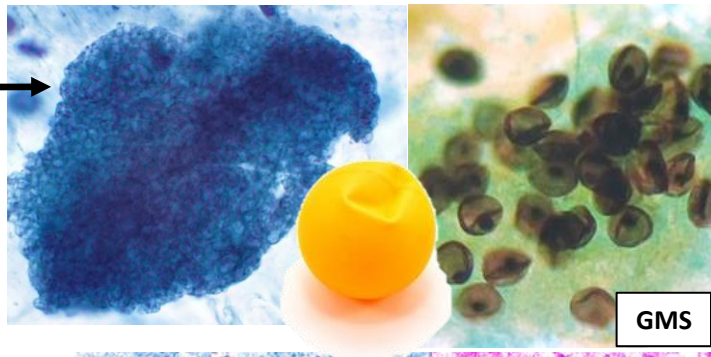
Hemosiderin Giemsa



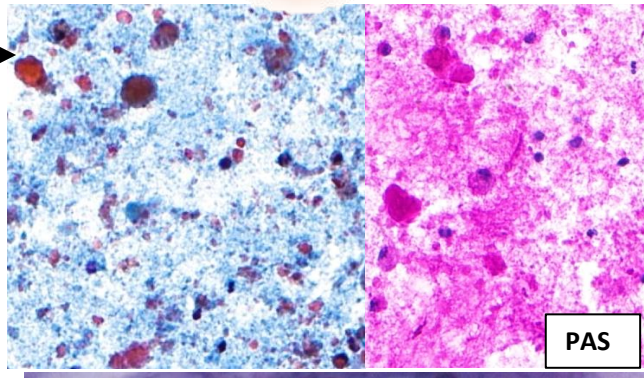
Lipid, Oil Red O

Extracellular material

Pneumocystis jirovecii
Infects immunocompromised hosts (classically AIDS).
Frothy, proteinaceous casts
"Crushed ping pong balls" with central dot
(+) GMS (can see negative impression on conventional stains); (-) PAS

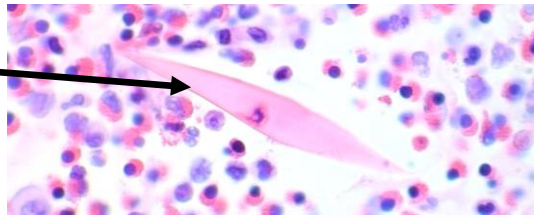


Alveolar proteinosis
Abnormal accumulation of surfactant protein in alveoli. Can be Congenital, Primary, or Secondary.
Dense, amorphous globules in a dirty, frothy background
(+)PAS/D, Oil Red O; (-) GMS

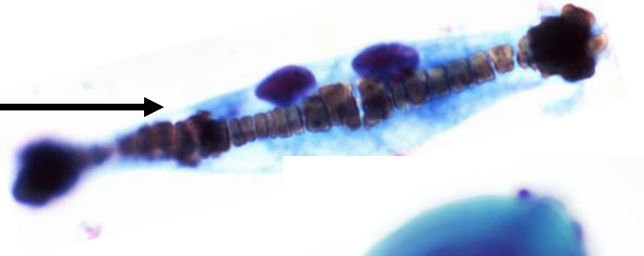


Curschmann spirals
Coils of inspissated mucus. Non-specific, but frequently associated with asthma and smoking.

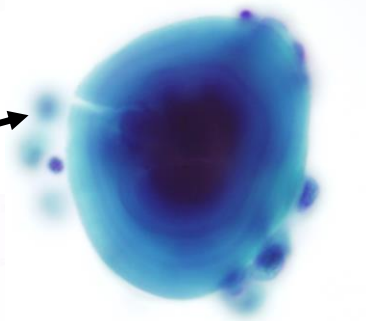
Charcot-Leyden Crystals
Orangeophilic rhomboid/needle-shaped crystals formed from the granules within degenerating eosinophils. Seen in allergic conditions e.g., Asthma



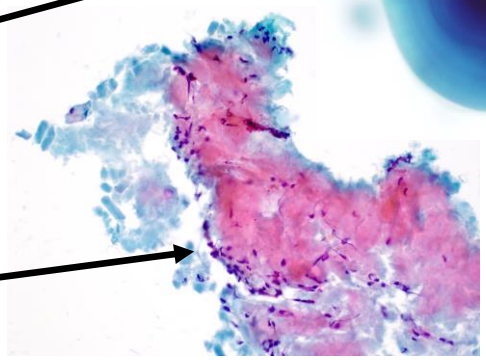
Ferruginous bodies
Fiber particles, usually asbestos, encrusted with proteins containing iron salts. Important to mention given possible mesothelioma risk.
Look like dumbbells.



Corpora amylacea
Spherical, concentrically laminated, noncalcified casts composed of glycoproteins. Non-specific.



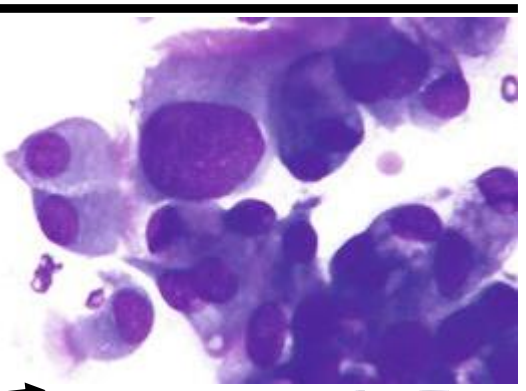
Amyloid
Dense, amorphous, waxy material with classic "apple green" birefringence under polarized light with Congo red.



Reactive Epithelial Changes

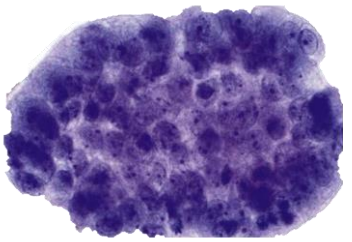
Warning!!

Inflammation can cause significant reactive changes/atypia. Always reconsider ("pull back") if there is a lot of inflammation. Frequently diagnose as ***"Atypical."***

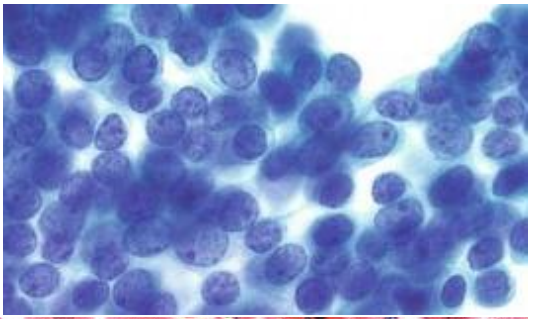


Causes of inflammation: Pneumonia, ARDS, Infarction, Bronchiectasis, Abscess, Intubation, Radiation, Chemotherapy, Viral/fungal infections, Oxygen therapy.

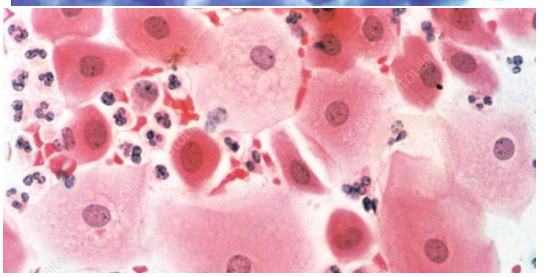
Respiratory epithelium:



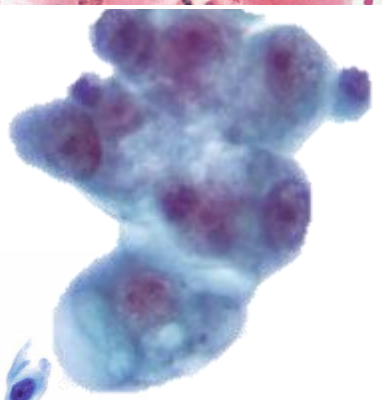
Enlarged nuclei, Papillary architecture,
Often retain terminal bars and cilia (good clue!)
Relatively uniform oval nuclei, single nucleolus.
Abundant cytoplasm.
"*Creola body*"—3-D clusters of bronchial epithelium enlarged nuclei, vesicular chromatin, and smooth nuclear membranes.



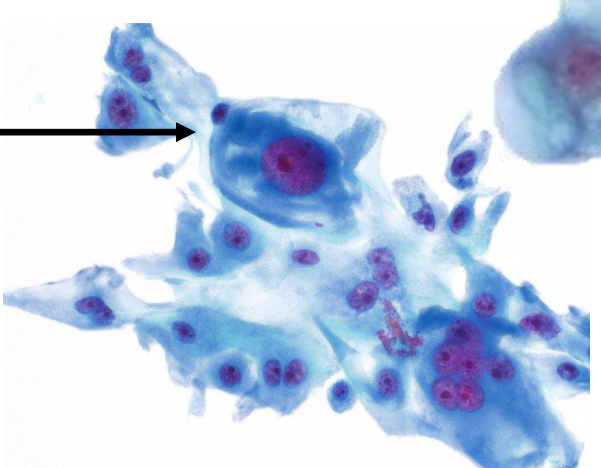
Basal (reserve) cell hyperplasia:
(mimic of small cell carcinoma)
Abnormal multiplication of basal cells (form several layers)
Often see with squamous metaplasia
Cohesive sheets. Uniform high N:C ratio cells.
Round to oval nuclei. Even chromatin (similar to respiratory cells).
Small/absent nucleolus. Clean background. No mitoses.
[vs small cell carcinoma with irregular contours/molding, lots of mitoses/necrosis]



Squamous metaplasia:
Dense cytoplasm with some keratinization.
Variable N:C ratio. Round to slightly irregular nuclei.



Type 2 pneumocyte hyperplasia
Large cells with cuboidal cytoplasm.
Large nuclei with prominent micronucleoli.
Sometimes vacuolated.
Can see viral inclusions.

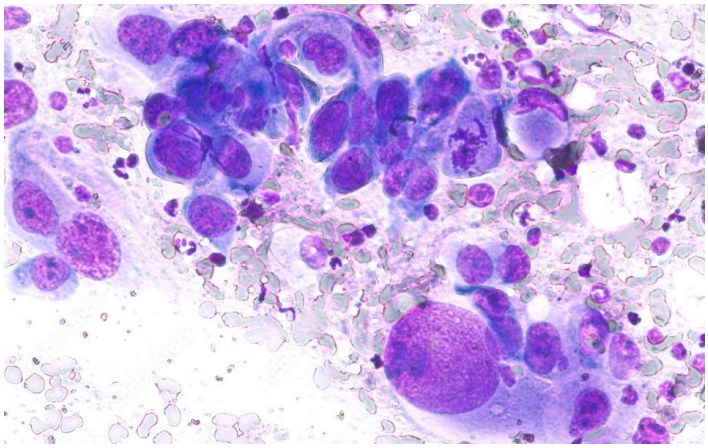


Chemotherapy/Radiation changes
Enlarged cells with big nuclei (cytomegaly)
Maintained, low N:C ratios.
Smudged, hyperchromatic chromatin.
Often vacuolated cytoplasm.
Can be multinucleated.

Malignant

Unequivocal features of malignancy.

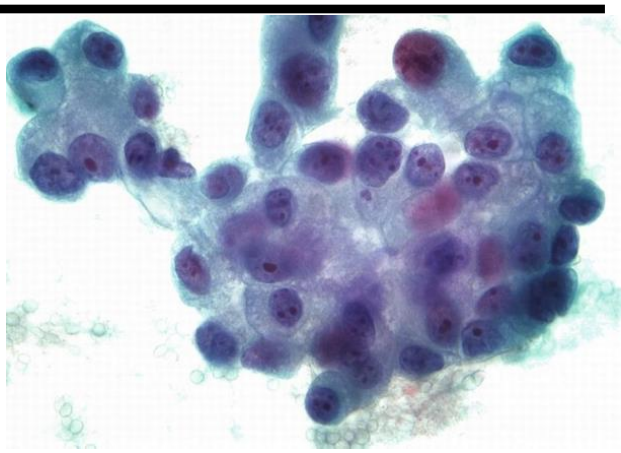
Non-small Cell Carcinoma "NOS"



Used for FNAs/biopsies without morphologic squamous or glandular morphology and indeterminate/negative IHC stains.

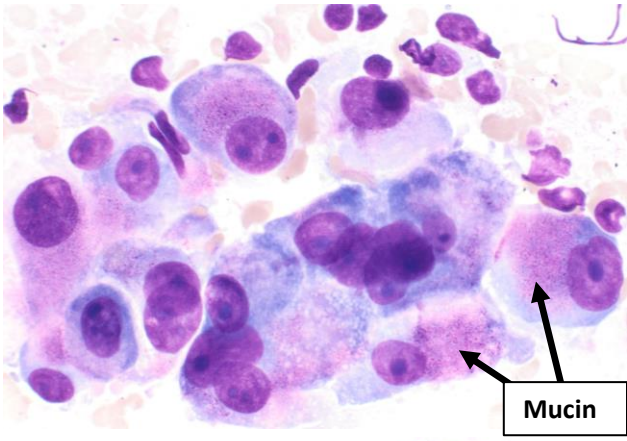
If a case requires stains to make a definitive Dx, say "Non-small cell carcinoma, favor .... [adenocarcinoma or squamous cell carcinoma]"

Adenocarcinoma



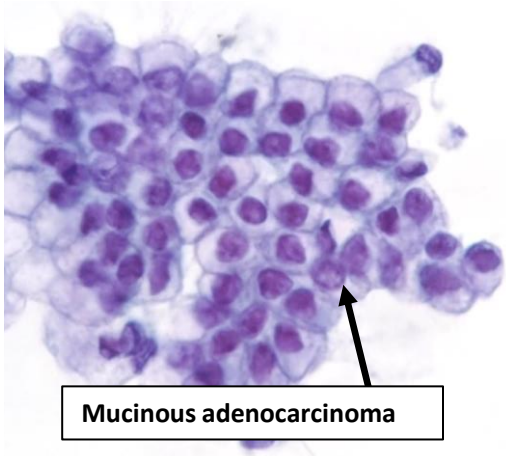
Most common type of primary lung cancer.
Glandular differentiation

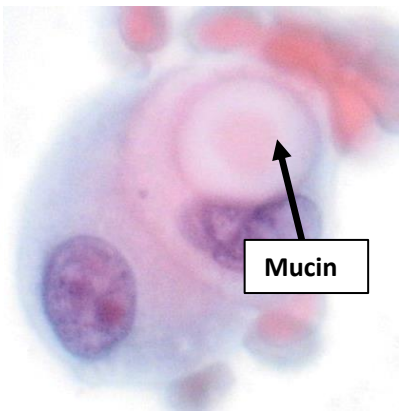
Delicate, granular to vacuolated to foamy cytoplasm
Frequent prominent nucleoli.
Arranged in **flat sheets, papillae, acini,** etc…
Acini= nuclei at periphery, cytoplasm in center
Eccentric nucleus.
Can see intracytoplasmic (or extracellular) mucin
Coarse to fine chromatin (usu. NOT hyperchromatic)
Nuclear membrane irregularities
Well-diff: Intranuclear inclusions and grooves
Can see background necrosis.

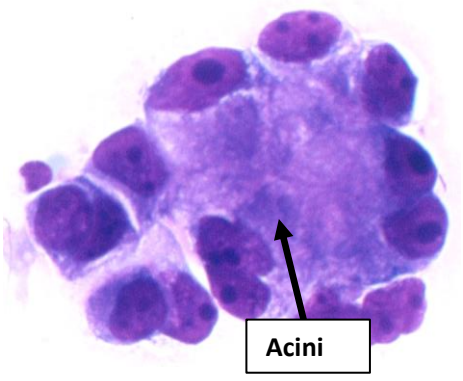


IHC: (+) TTF1, NapsinA; (-) p40, CK5/6
Note: Mucinous adenocarcinomas may *not* express TTF1 (see lung tumor notes for more info)

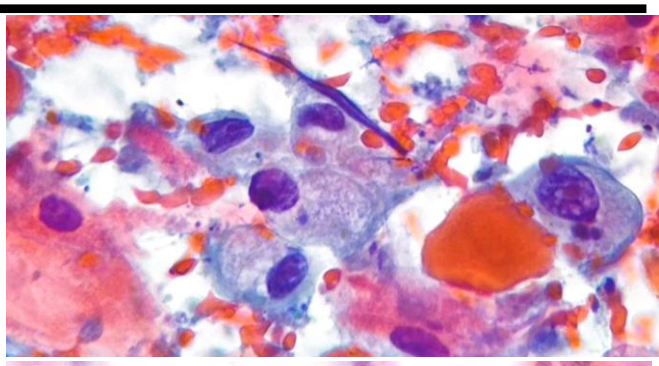
Distinction between adenocarcinoma in situ, minimally invasive, and invasive adenocarcinoma is not accurate on FNA (and small biopsy).

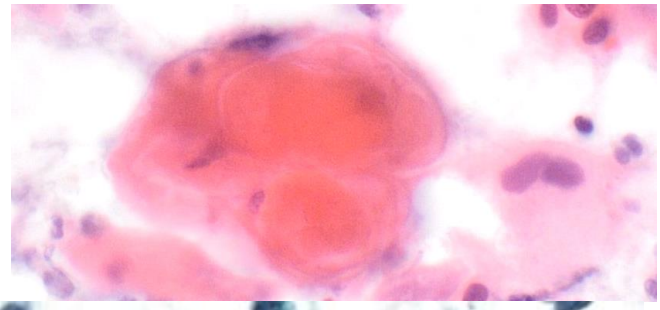


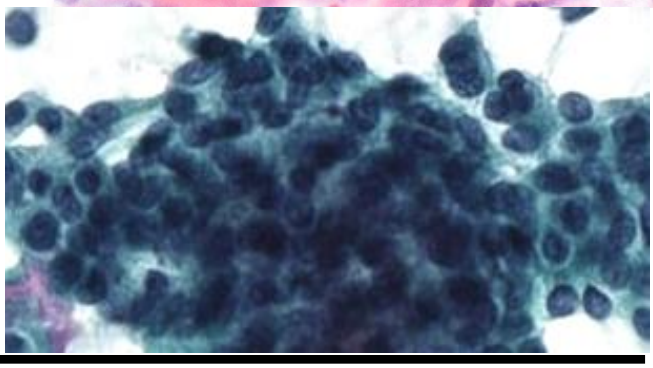




Squamous Cell Carcinoma







Malignant epithelial tumor with keratinization and/or intercellular bridges.

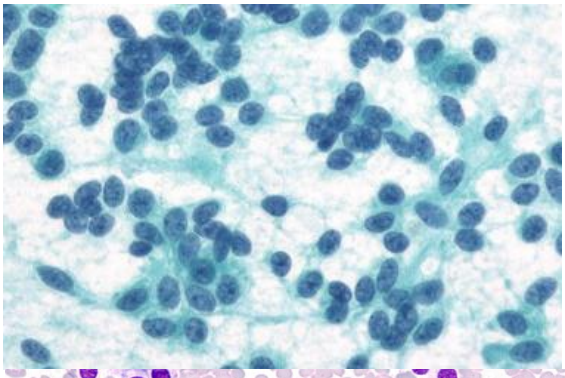
Keratinization (Orangeophilia on Pap, Robin's egg on Giemsa)
Dense cytoplasm. Well-defined cell borders.
Central large, hyperchromatic, irregular nucleus.
Usually NO prominent nucleoli.
Polygonal, elongated, or bizarre shapes.
Keratin Pearls.
Dirty, **necrotic** background.

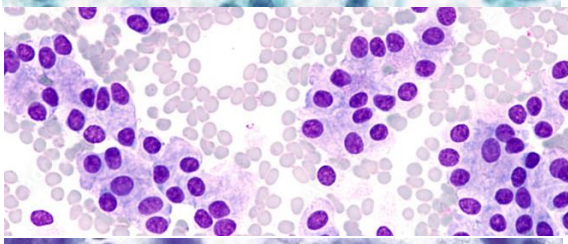
Basaloid SCC has less specific morphology without keratinization. Clusters of high N:C ratio cells with coarse chromatin→ requires IHC to confirm Dx and exclude small cell carcinoma

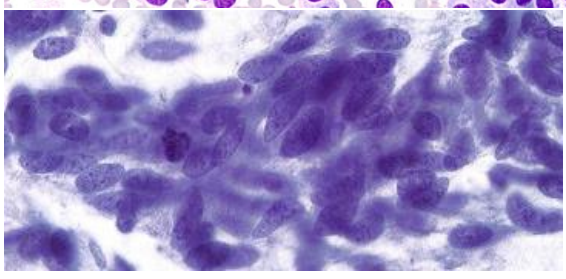
Cannot distinguish primary lung SCC from SCC metastasis unless other primary is HPV+ and lung tumor is HPV-, or, potentially, by molecular signature.

IHC: (+)p40, CK5/6;
(-) TTF1, NapsinA, HPV ISH, NUT,

Carcinoid tumor







Well-differentiated tumors with pure neuroendocrine differentiation.

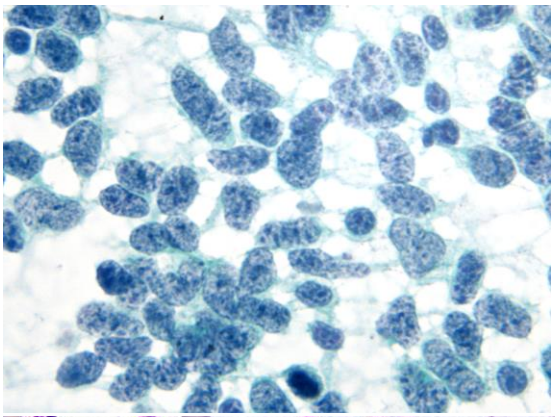
Small, loosely cohesive cells.
Epithelioid to plasmacytoid. Sometimes spindled.
Round/oval nuclei with stippled "salt and pepper" chromatin.
Dense, granular cytoplasm.
Cellular smears. No necrosis.

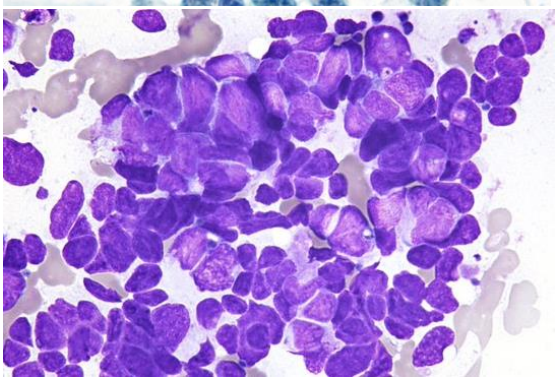
Bx: Nested and/or trabecular architecture

IHC: **(+)Synaptophysin, Chromogranin, CD56, ISNM1**
Ki67 low (<10%), but not officially used for grading.

Unless you see necrosis or mitoses, it is often challenging to distinguish between typical and atypical carcinoid tumors on small biopsies. It is recommended to usually just report as "Carcinoid/Well-differentiated neuroendocrine tumor, NOS" and defer final subtyping to a resection specimen.

Small Cell Carcinoma





Small cell size (usually smaller than 3 resting lymphocytes)
Scant cytoplasm. Unclear borders.
Angulated nuclei. Frequent nuclear molding.
Densely cellular sheet-like growth. Cells fusiform to round.
Finely granular chromatin (*no* nucleoli)
High mitotic rate [>10 mitoses per 2 mm^2 (median 80)]
Frequent **necrosis** (often large zones) and apoptoses.
Often discohesive with many single cells.
Frequent crush artifact→ chromatin smearing.

Technically a morphologic diagnosis, but IHC is useful to exclude other diagnoses (lymphoma, SCC, sarcoma), and it's reassuring to have NE marker staining (up to 10% will be negative though!).

IHC: (+) AE1/AE3, Synaptophysin, ISNM1, CD56, TTF1
(-) p40, CK20, CD45

Ki67 often essentially 100% (if it's lower than 60% consider another Dx!)

Can be "combined" with other tumors, such as SCC.
Most common Neuroendocrine Neoplasm in the lung.

Large Cell Neuroendocrine Carcinoma

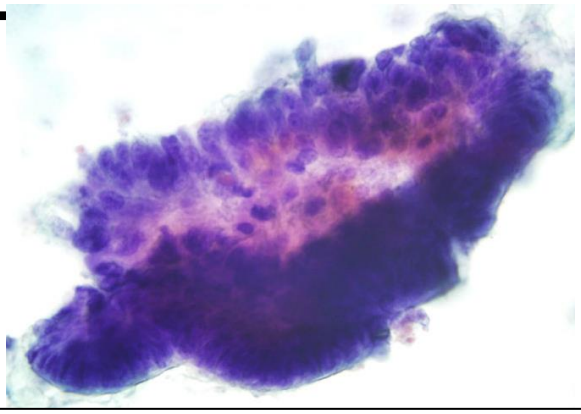
Difficult to diagnose by cytology, except perhaps if there is a good cell block as need to see neuroendocrine architecture of nesting, palisading, and/or rosettes (cytomorphology itself is non-specific).

	Typical carcinoid	Atypical carcinoid	Large cell neuroendocrine carcinoma	Small cell carcinoma
Smoking association	No	Maybe	Yes	Yes
Mitoses/2mm^2	0-1	2-10	>10 (median 70!)	>10 (median 80!)
Necrosis	No	Focal, if any	Yes	Yes, extensive
Neuroendocrine morphology	Yes	Yes	Yes	Yes
Ki-67 Proliferation index	Up to 5%	Up to 30%	40-80%	Often almost 100%
TTF1 expression	Usually No, sometimes in peripheral/spindled		Yes (70%)	Yes (85%)
Combined with NSCC component	No	No	Sometimes	Sometimes

Adapted from: WHO Classification of tumors of the lung, pleura, thymus, and heart. 2015 and 2021

Other Malignancies

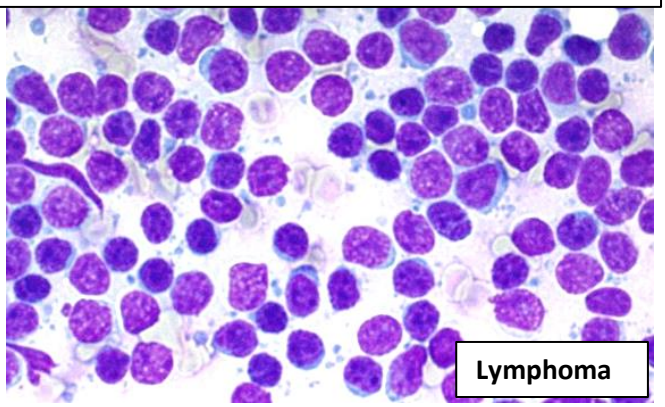
Metastases!!!
Always a consideration in the lung. Consider history!
Common sites: GI tract, Breast, Kidney, Melanoma.



Colon cancer metastasis with glands of columnar cells with hyperchromatic, penicillate nuclei. Background of mucin and necrosis.

Rare Carcinomas
(that are hard to Dx on FNA ;-)
NUT carcinoma
Thoracic SMARCA4-deficient undifferentiated tumor
Carcinosarcoma
Pulmonary blastoma
Adenosquamous carcinoma
Pleomorphic carcinoma

Lymphomas
Cellular smears of discohesive lymphocytes.
Lymphoglandular bodies (cytoplasmic fragments)
Can be low-grade (e.g., Extranodal marginal zone lymphoma) or high-grade (e.g., DLBCL)

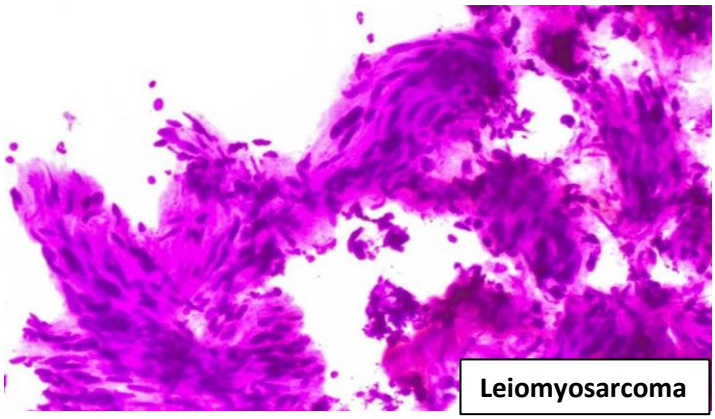


Lymphoma

Sarcomas
Intimal sarcoma
Leiomyosarcoma
Synovial sarcoma
Angiosarcoma

Salivary gland carcinomas
Mucoepidermoid carcinoma
Adenoid cystic carcinoma
Epithelial-myoepithelial carcinoma

Paraganglioma
Mesothelioma
Germ Cell tumors



Leiomyosarcoma

Atypical

A specimen with features *predominantly* seen in benign lesions and minimal features that raise the possibility of malignancy (either insufficient quality or quantity).

Often used in the setting of inflammation, where there can be considerable reactive changes to not "overcall" malignancy (thereby maintaining a higher negative predictive value of "Benign").

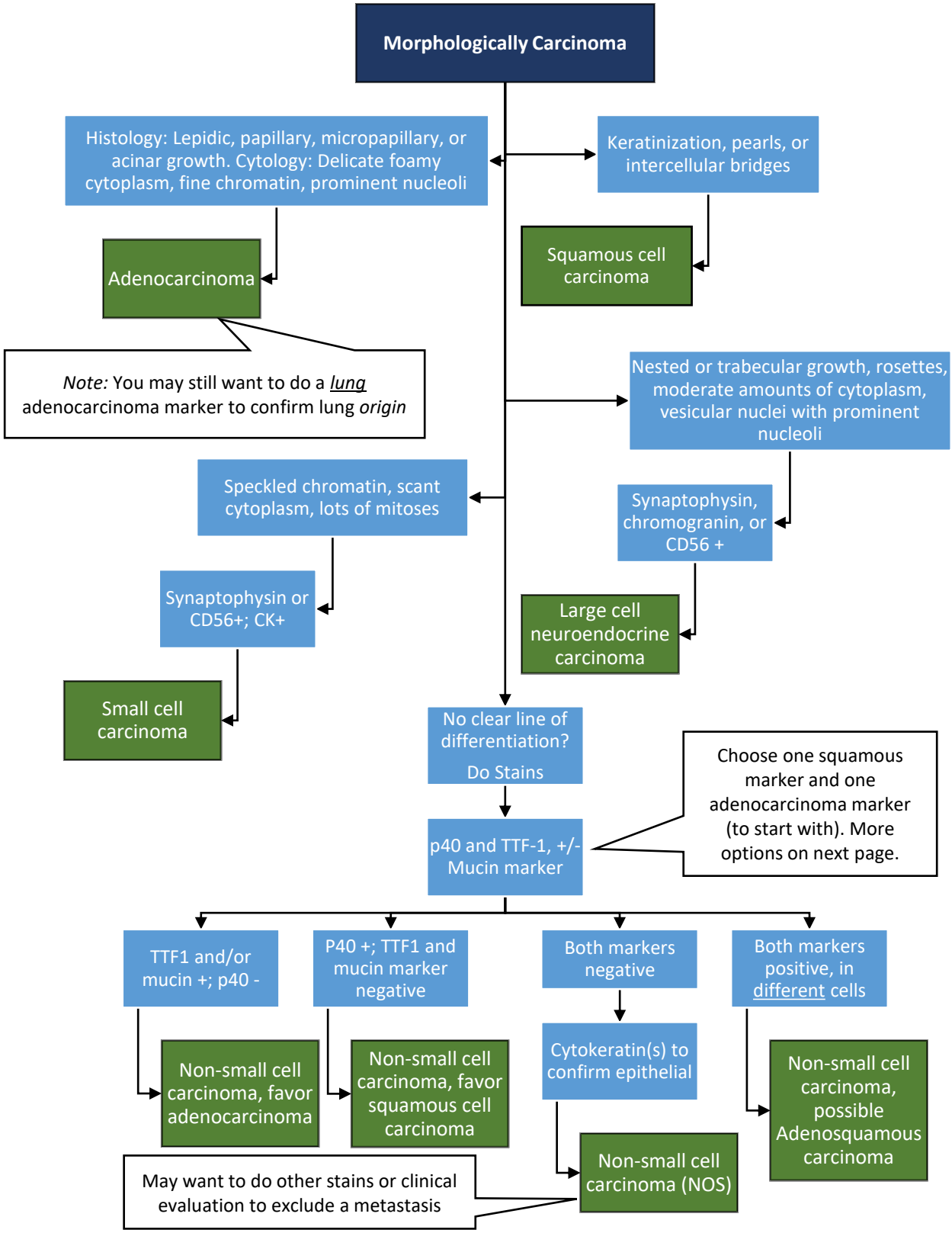
Suspicious

A specimen suggestive of malignancy but that has insufficient features for a definitive diagnosis (either qualitative or quantitative). [High degree of concern and risk of malignancy→ sometimes enough for next step in management depending on clinical setting.]

Classification of Lung Carcinomas with Limited Tissue

Diagnostic Algorithm

Try to be as *specific* as you can be, while also *sparing* as much tissue as you can for molecular testing (critical for lung cancer!)



Adapted from: WHO Classification of tumors of the lung, pleura, thymus, and heart. 2015.

Classification Guidelines

Some carcinomas can only be definitively diagnosed on ***resection*** (not on Bx): Adenocarcinoma in situ, Minimally invasive carcinoma, Adenosquamous carcinoma, Large cell carcinoma, Sarcomatoid carcinoma, Pleomorphic carcinoma, giant cell carcinoma, fetal adenocarcinoma, colloid adenocarcinoma, enteric adenocarcinoma.

If you can make the diagnosis morphologically → can call Adenocarcinoma or Squamous cell carcinoma

If can't tell *morphologically*, then do stains:
A simple panel of 2 stains (1 squamous and 1 adenocarcinoma) is usually adequate (e.g., p40 and TTF1)
A positive mucin stain (e.g., PAS-D, or mucicarmine) can also identify some adenocarcinomas.
→A mucin stain is supposed to highlight >5 intracytoplasmic mucin droplets in at least 2 HPFs
Report as "Non-small cell carcinoma, *favor*…." (either adenocarcinoma or squamous cell carcinoma)
Do not do neuroendocrine stains unless there are morphologic findings to suggest neuroendocrine differentiation (neuroendocrine differentiation in an SCC or Adeno doesn't impact treatment/prognosis).

Immunohistochemical Staining

Adenocarcinoma	Squamous cell carcinoma
TTF1	p40 (most specific)
Napsin A	CK5/6
CK7 (less specific)	p63 (less specific)

Note: Some primary lung adenocarcinomas, including Mucinous adenocarcinoma, Colloid carcinoma, and Enteric adenocarcinoma, can be TTF-1 negative. They can even stain with CK20 and CDX2. These cases require careful clinical correlation to exclude a metastasis from the GI tract.

IHC typing of a Cytokeratin-positive, morphologically undifferentiated non-small cell carcinoma (NSCC). Sarcomatoid carcinoma and neuroendocrine tumors should also be considered.

TTF1 Napsin-A	p63	p40	CK5/6	Resection Dx	Biopsy Dx
+	-	-	-	Adenocarcinoma	NSCC favor Adenocarcinoma
+	+	-	-	Adenocarcinoma	NSCC favor Adenocarcinoma
+	+	+ (focal)	-	Adenocarcinoma	NSCC favor Adenocarcinoma
+	-	-	+ (focal)	Adenocarcinoma	NSCC favor Adenocarcinoma
-	Any one of the above *diffusely* positive			Squamous cell carcinoma	NSCC favor SCC
-	Any one of the above *focally* positive			Large cell carcinoma	NSCC, NOS
-	-	-	-	Large cell carcinoma	NSCC, NOS

Adapted from: WHO Classification of tumors of the lung, pleura, thymus, and heart. 2015 & 2021.