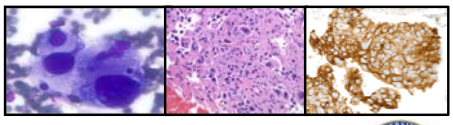
Minimally Invasive Biopsies of the Lung & Mediastinum in the Era of Personalized Medicine



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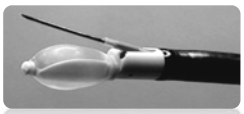
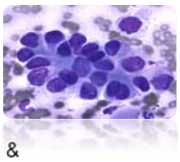
Outline

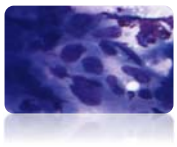
- **Introduction**
  - ❑ **Why minimally invasive biopsies?**
  - ❑ **Current Approach to Diagnosis of Mediastinal/Lung Lesions**
- **Interesting Cases**
  - ❑ **Pitfalls**
  - ❑ **Morphological challenges impacting ancillary studies**
- **Conclusion**



Introduction



- **Why minimally invasive techniques?**
  - Over half of NSCLC patients present with metastases→ Dx & Stage with EBUS-TBNA
  - Minimally invasive biopsies→ Shorter length of stay & less cost
  - Need for subtyping and molecular studies/theranostic data, without the need for complete surgical excision
- **Advantages of Cytological Specimens**
  - Better nuclear & cytoplasmic detail
  - Less fixation artifact
  - Ability to have ROSE for triage & to allocate material for appropriate testing



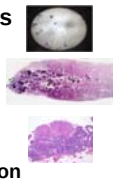
Introduction

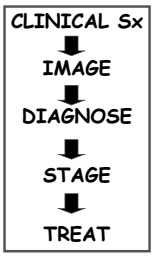
- **Increase in small biopsies and cytology specimens for lung & mediastinal lesions has led to…**
  - New approaches with improved biopsy techniques
  - Variety of different needles to choose from
  - New classification systems for small biopsies & cytology specimens
  - Expanding use for biomarker testing in the era of personalized medicine
    - Not enough to just make a diagnosis anymore
    - Do more with less
    - *EGFR, ALK→ EGFR, ALK, ROS*, PD-L1, and more

Imaging & Diagnosis of Mediastinal/Lung Lesions

- **Imaging Modalities**
  - Chest Xray
  - CT Scan
  - PET CT Scan

- **Minimally Invasive Diagnostic Modalities**
  - Sputum/BAL/BB/BW/Pl Fl
  - CT-Guided FNA
  - Transbronchial FNA (Wang biopsy)
  - **Supernavigational EMN biopsy**
  - **EBUS & EUS guided FNA**
  - **Endobronchial biopsy with touch preparation**

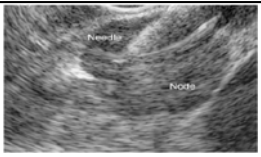




Small Biopsies of Lung/Mediastinum: The Power of EBUS TBNA

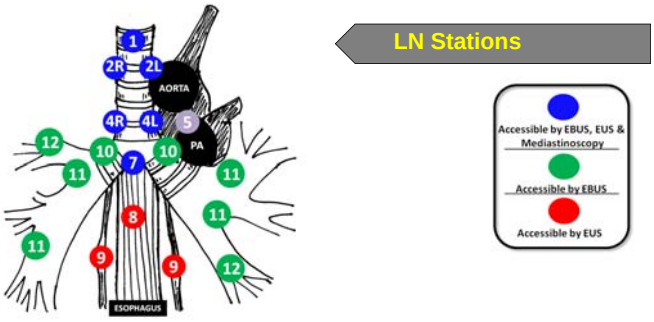
- **Advantages:**
  - Minimally invasive
  - Image guidance
  - Tissue confirmation of +PET/CT findings & evaluation of LNs <1 cm
  - Broad sampling capability
  - On-site evaluation→ triage
  - Lower cost
- **Disadvantages:**
  - Inability to access all LNs
  - Not universally available
  - Time & experience requirement
  - Non-diagnostic specimens

Among patients with clinical stage IIIA, 40% of patients were down-staged with EBUS-FNA
Gilbert S et al. JTCVS 2009



Varela-Lema L et al., *Eur Repir J*, 2009

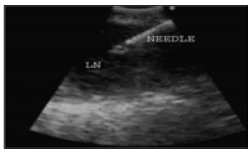
Sampling Capability

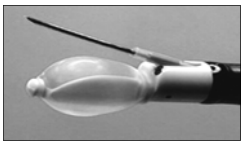


Monaco SE. Khalbuss WE, Pantanowitz L. EBUS-TBNA: A Practical Approach. Karger 2014

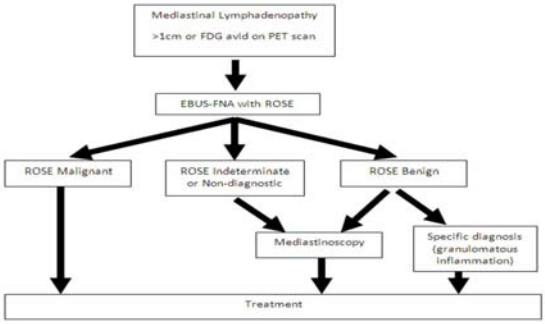
Advantages of Minimally Invasive Small Biopsies

- **Restaging**
- **Small LNs < 1 cm**
- **Poor Operative Candidates**
- **Non-Surgical Diseases**
- **Biomarker testing**





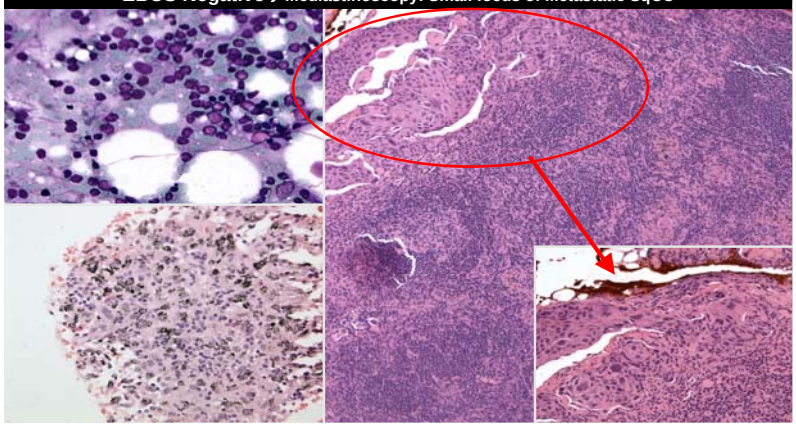
What is the management?



Gilbert S et al. JTCVS 2009

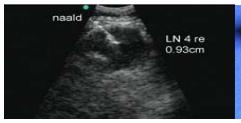
Monaco SE et al, *Cytojournal* 2012

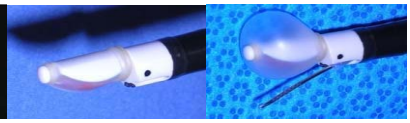
EBUS Negative→ Mediastinoscopy: Small focus of Metastatic SqCC

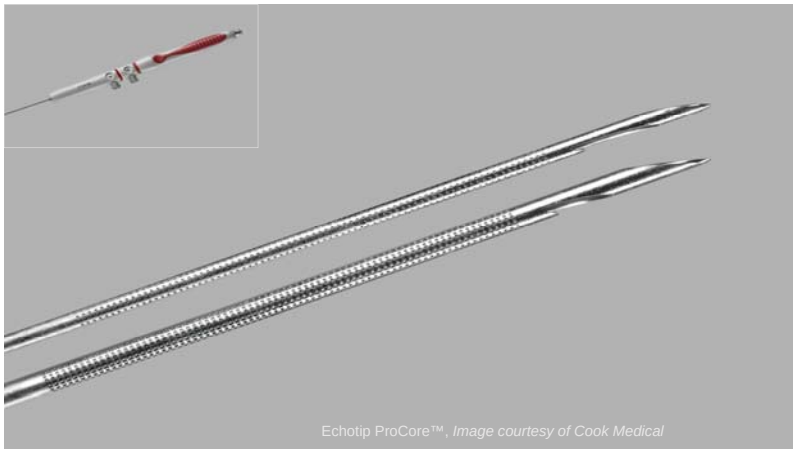


What is new with EBUS TBNA?

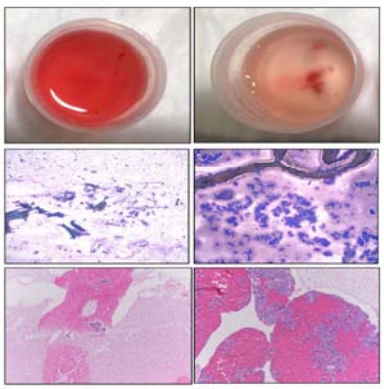
- **Equipment**
  - **New needle sizes:** 19-22G FNA needle
  - **New types of needles:** traditional TBNA needle vs Pro-core
  - **New designs:** superior echogenic design
- **Increased demand**
  - **Managing ROSE:** Telecytology vs Traditional
  - **Weekend and late procedures:** Cytology On-Call or Not?
  - **Optimizing tissue:** Doing more with less





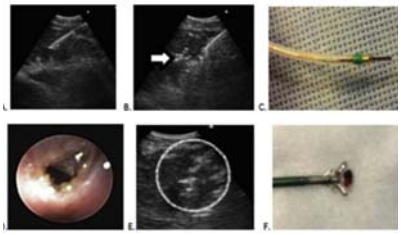


EBUS Procore



Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration: A Pilot Study to Evaluate the Utility of the ProCore Biopsy Needle for Lymph Node Sampling

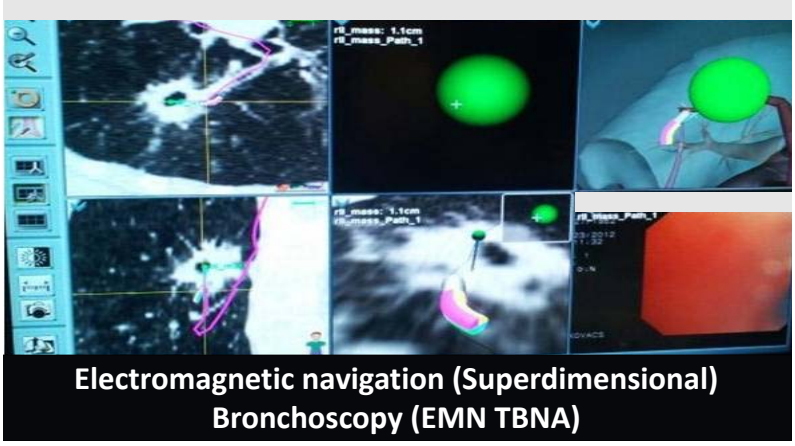
ca-TBFB



Endobronchial Ultrasound-Guided Cautery-Assisted Transbronchial Forceps Biopsies: Safety and Sensitivity Relative to Transbronchial Needle Aspiration

Kyle Bramley, MD, Margaret A. Pisani, MD, MPH, Terrence E. Murphy, PhD, Katy L. Arazujo, MPH, Robert J. Homer, MD, PhD, and Jonathan T. Puchalski, MD, MEd

Olympus ViziShot Flex 19G™, http://medical.olympusamerica.com/products/vizishot-flex-19g-ebus-tbna-needle



Electromagnetic navigation (Superdimensional) Bronchoscopy (EMN TBNA)

Introduction

- Increase in small biopsies and cytology specimens for lung & mediastinal lesions has led to…
  - New approaches with improved biopsy techniques
  - Variety of different needles to choose from
- New classification systems for small biopsies & cytology specimens
- Expanding use for biomarker testing in the era of personalized medicine
  - Not enough to just make a diagnosis anymore
  - Do more with less
  - *EGFR, ALK→ EGFR, ALK, ROS*, PD-L1, and more

Diagnostic Shift in Lung Cancer

PAST

- NSCLC vs. SCC
- Treatment
  - Surgical: NSCLC
  - Non-surgical: SCC

PRESENT

- Need for subtyping and molecular studies
- Theranostic data

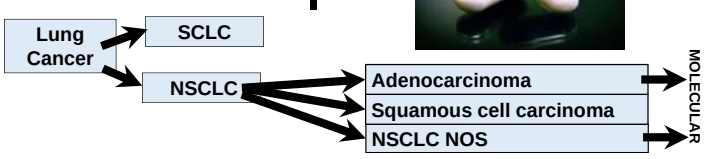


TABLE 2. Terminology and Criteria for Adenocarcinoma, Squamous Cell Carcinoma, and NSCC NOS in Small Biopsies and Cytology Compared with Terms in Resection Specimens[a]

New Small Biopsy/Cytology Terminology	Morphology/Stains	2015 WHO Classification in Resection Specimens
Adenocarcinoma (describe identifiable patterns present)	Morphologic adenocarcinoma patterns clearly present	Adenocarcinoma predominant pattern: lepidic, acinar, papillary, solid, and micropapillary
Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)		Minimally invasive adenocarcinoma, adenocarcinoma in situ, or an invasive adenocarcinoma with a lepidic component
Invasive mucinous adenocarcinoma (describe patterns present; use term mucinous adenocarcinoma with lepidic pattern if pure lepidic pattern)		Invasive mucinous adenocarcinoma
Adenocarcinoma with colloid features		Colloid adenocarcinoma
Adenocarcinoma with fetal features		Fetal adenocarcinoma
Adenocarcinoma with enteric features[b]		Enteric adenocarcinoma
NSCC, favor adenocarcinoma[c]	Morphologic adenocarcinoma patterns not present but supported by special stains (i.e., TTF-1 positive)	Adenocarcinoma (solid pattern may be just one component of the tumor)
Squamous cell carcinoma	Morphologic squamous cell patterns clearly present	Squamous cell carcinoma
NSCC, favor squamous cell carcinoma[c]	Morphologic squamous cell patterns not present but supported by stains (i.e., p40-positive)	Squamous cell carcinoma (nonkeratinizing pattern may be a component of the tumor)
NSCC NOS[d]	No clear adenocarcinoma, squamous or neuroendocrine morphology or staining pattern	Large cell carcinoma

2011 2015

TABLE 3. Diagnostic Terminology for Small Biopsy/Cytology Compared with the 2015 WHO Terms in Resection Specimens with Small Cell Carcinoma, LCNEC, Adenosquamous Carcinoma, and Sarcomatoid Carcinoma[a]

Small Biopsy/Cytology Terminology/Criteria	2015 WHO Classification in Resections
Small cell carcinoma	Small cell carcinoma
NSCC with NE morphology and positive NE markers, possible LCNEC	LCNEC
NSCC with NE morphology If negative NE markers comment: This is a NSCC where LCNEC is suspected, but stains failed to demonstrate NE differentiation.	Large cell carcinoma with NE morphology (LCNEM)
Morphologic squamous cell and adenocarcinoma patterns present: NSCC, NOS Comment that adenocarcinoma and squamous components are present and this could represent adenosquamous carcinoma.	Adenosquamous carcinoma (if both components ≥10%)
Morphologic squamous cell or adenocarcinoma patterns not present but immunostains favor separate glandular and adenocarcinoma components: NSCC, NOS Specify the results of the immunohistochemical stains and the interpretation and comment this could represent adenosquamous carcinoma.	Adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma or large cell carcinoma with unclear immunohistochemical features
NSCC with spindle cell and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)	Pleomorphic, spindle cell, and/or giant cell carcinoma

[a]Modified from the articles by Travis et al.[1,2,3]
LCNEC, large cell neuroendocrine carcinoma; NOS, not otherwise specified; NSCC, non–small cell carcinoma; NE, neuroendocrine; WHO, World Health Organization.

2011 2015

Standardized Terminology and Nomenclature for Respiratory Cytology

The Papanicolaou Society of Cytopathology Guidelines

Lester J. Layfield, M.D.,[1]* Zubair Baloch, M.D., PhD,[2] Tarik Elsheikh, M.D.,[3] Leslie Litzky, M.D.,[4] Natasha Rekhtman, M.D.,[5] William D. Travis, M.D.,[6] Maureen Zakowski, M.D.,[7] Matthew Zarka, M.D.,[8] and Kim Geisinger, M.D.[1]

Diagn Cytopathol 2016;44:399-409

2016

Table I. Proposed Pulmonary Cytology Specimen Terminology and Classification Scheme

Diagnostic category	Risk of malignancy[a](%)
Nondiagnostic	40
Negative for malignancy	24–43
Atypical	54
Neoplastic, benign neoplasm, low-grade carcinoma	N/A
Suspicious for malignancy	82
Malignant	77–100[b]

Table II. Proposed Pulmonary Cytology Specimen Diagnostic Categories and Definitions

Diagnostic Category	Definition
Nondiagnostic	A specimen that provides no useful diagnostic information about the pulmonary nodule, cyst or mass lesion identified by imaging studies. Any degree of cellular atypia beyond that clearly associated with changes associated with inflammation or repair excludes an interpretation of nondiagnostic for that sample.
Benign[a]	Specimen containing adequate cellular and/or extracellular material to evaluate or define a lesion that has been defined on imaging studies.
Neoplastic, benign neoplasm, low-grade cancer[b]	Specimen is sufficiently cellular and representative to be diagnostic of the benign or low-grade neoplasm
Atypical	Specimen demonstrates cytologic features of greater dysmorphology than those assigned to the negative for malignancy category but falling short of those assigned to the suspicious for malignancy category. Specimens displaying some, but an insufficient number of features for diagnosis of a benign or low-grade neoplasm.
Suspicious for malignancy[a]	A specimen displaying some but an insufficient number of features characteristic of a primary pulmonary or metastatic carcinoma, lymphoma or other malignancy. The cytologic features raise a strong suspicion for malignancy but they are either qualitatively or quantitatively insufficient for a conclusive diagnosis on purely cytomorphometric grounds.
Malignant[a]	Specimens representing a group of neoplasms that unequivocally display malignant cytologic features

Rapid On-Site Evaluation of Endobronchial Ultrasound–Guided Transbronchial Needle Aspirations for the Diagnosis of Lung Cancer

A Perspective From Members of the Pulmonary Pathology Society

Dhanpati Jain, MD, DNB, FIAC; Timothy Craig Allen, MD, JD; Diana L. Aisner, MD; Mary Beth Beasley, MD; Philip T. Cagle, MD; Irma Luisa Capellozzi, MD, PhD; Lida P. Hariri, MD, PhD; Sylvie Lantuejoul, MD; Ross Miller, MD; Mari Mino-Kenudson, MD; Sara E. Monaco, MD; Andre Moreira, MD; Kimie Nagata, MD; Natasha Rekhtman, MD; Anja Christiane Roden, MD; Sinchita Roy-Chowdhuri, MD, PhD; Célia da Cunha Santos, MD, PhD; Erik Thunnissen, MD, PhD; Giancarlo Troncone, MD, PhD; Marina Vivero, MD

• Context.—Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a very useful tool in the field of diagnostic respiratory cytology. Rapid on-site evaluation (ROSE) of EBUS-TBNA not only has the potential to improve diagnostic yield of the procedure but also to triage samples for predictive molecular testing to guide personalized treatments for lung cancer.

Objective.—To provide an overview of the current status of the literature regarding ROSE of EBUS-TBNA in the diagnosis of lung cancer.

Data Sources.—An electronic literature search in PubMed and Google databases was performed using the following key words: cytology, lung cancer, on-site evaluation, rapid on-site evaluation, and ROSE EBUS-TBNA. Only articles published in English were included in this review.

Conclusions.—Rapid on-site evaluation can ensure that the targeted lesion is being sampled and can enable appropriate specimen triage. If available, it should be used with EBUS-TBNA in the diagnosis of lung cancer because it can minimize repeat procedures for additional desired testing (ie, molecular studies). Some studies have shown that while ROSE does not adversely affect the number of aspirations, total procedure time of EBUS-TBNA, or the rate of postprocedure complications, it is helpful in providing a preliminary diagnosis that can reduce the number of additional invasive procedures, such as mediastinoscopy. As EBUS technology continues to evolve, our knowledge of the role of ROSE in EBUS-TBNA for the diagnosis of lung cancer will also continue to grow and evolve.

(Arch Pathol Lab Med; doi: 10.5858/arpa.2017-0114-SA)

2017

Table 2. Adequacy Criteria of Rapid On-Site Evaluation Specimens of Endobronchial Ultrasound (EBUS)–Guided Transbronchial Needle Aspirate Samples of Lymph Node

	Alsharif et al,[44] 2008	Nayak et al,[45] 2012	Jeffus et al,[13] 2015	Choi et al,[46] 2016
Overview	Lymphocytes in the most cellular areas on ×40 magnification	Scanning the slide at the low power	Scanning the slide at the low power	Procedure-related parameters and microscopic findings number of punctures (>3 per node), length of core tissue (>2 cm), the gross appearance of aspirates (pusllike or anthracotic), and microscopic findings
Criteria	Score 0: <40 lymphocytes per HPF Score 1: 41–200 lymphocytes per HPF Score 2: >200 lymphocytes (nonconfluent) per HPF Score 3: >200 lymphocytes per HPF (confluent) or germinal center fragments Any score >1 is adequate Or Pigment-laden macrophages Or Diagnostic material (cancer cells or granulomas) Airway contamination has no bearing on adequacy	>5 fields with at least 100 lymphocytes per low-power field (×100) in a smear Plus <2 groups of bronchial cells per low-power field (×100) Or Germinal fragments present	Presence of diagnostic material, germinal center fragments, >5 fields at ×100 magnification with at least 100 lymphocytes per field, and <2 groups of contaminating bronchial cells per field	Tissue core in EBUS needle ≥2 cm Or malignant cells Or microscopic anthracotic pigment Or lymphocyte density >40 per 10 high-power fields, at ×40 magnification
Assigned categories	a. Nondiagnostic b. Negative for malignancy c. Atypical d. Suspicious for malignancy e. Positive for malignancy	a. Nondiagnostic b. Negative for disease c. Granulomatous d. Suspicious for malignancy e. Positive for malignancy	a. Unsatisfactory b. Adequate, negative c. Adequate, benign d. Adequate, atypical e. Adequate, suspicious f. Adequate, positive	Objective algorithm proposed for clinicians

Abbreviation: HPF, high-power field.

Karunamurthy A et al, Cancer Cytopathology 2014

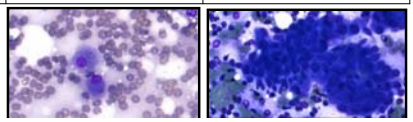
FNA Adequacy	Overall Total # (%)	Total with follow-up # (%)	False Negatives # (%)
Unsatisfactory	66 (11%)	30 (46%)	7 (23%)
Less than optimal	107 (18%)	55 (51%)	6 (11%)
Satisfactory	420 (71%)	118 (28%)	4 (3%)
TOTAL	593	203 (34%)	17 (8%)

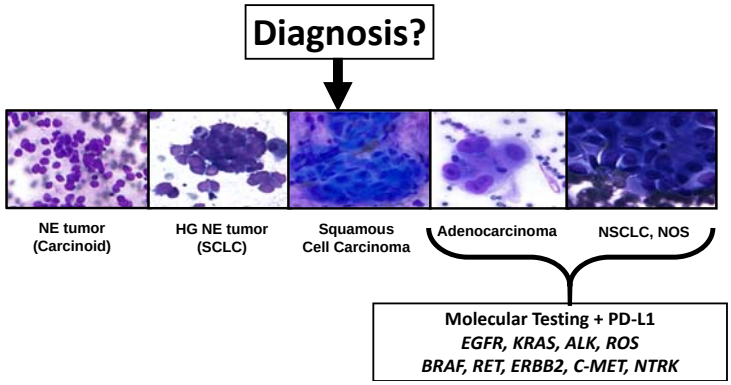
UPMC EBUS Data (2007-2010)

593 EBUS FNAs from 357 patients
34% with histological follow-up

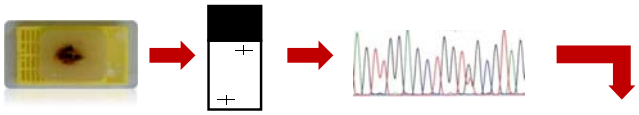
EBUS TBNA & CTG FNA: Quantity & Quality

	CTG FNA	EBUS TBNA
Target	Anterior Mediastinum or Lung	Usually mediastinal lymph nodes
Baseline cellularity (negative case)	Low	High
Cells present (negative case)	Few bronchial cells, macrophages & red blood cells. No background mucus present.	Numerous lymphocytes & contaminating bronchial epithelial cells or squamous cells. Background mucus present in EBUS.
Abnormal case	Quantitative abnormality. Increase in inflammatory cells or tumor cells.	Qualitative abnormality. Metastatic tumor, granulomas, or malignant lymphoid cells identified among benign/reactive cells.
Screening	Faster (less cells to examine)	Longer (more cells to examine)
Location	Radiology	OR or bronchoscopy suite



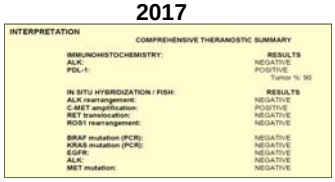


Biomarker Testing in Lung Cancer



2012

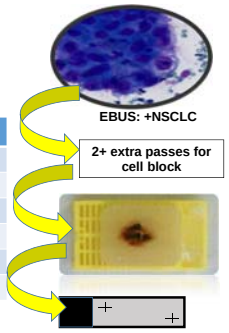
2017



Optimizing Small Samples

- **Rose with Dedicated passes (FNA) or core biopsies**
- **Upfront blanks cut to avoid trimming of block**
- **Unstained charged slides numbered in order**
- **Limited IHC**

Blank #	Triage
1	H&E to case pathologist
2	H&E to FISH & Molecular Labs
3, 4, 5	First 3 IHC markers (i.e. TTF-1, p40, synapto)
6-11	Blanks to Molecular Lab (NGS testing)
12-15	Blanks to FISH Lab (ALK, ROS, CMET, KRET)
16	IHC for PD-L1 (SP263)



Outline

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  - ❑ Current Approach to Diagnosis of Mediastinal/Lung Lesions
- **Interesting Cases**
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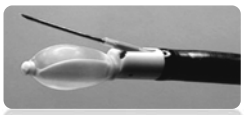
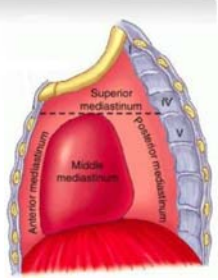
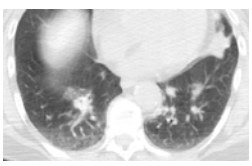
- **Conclusion**

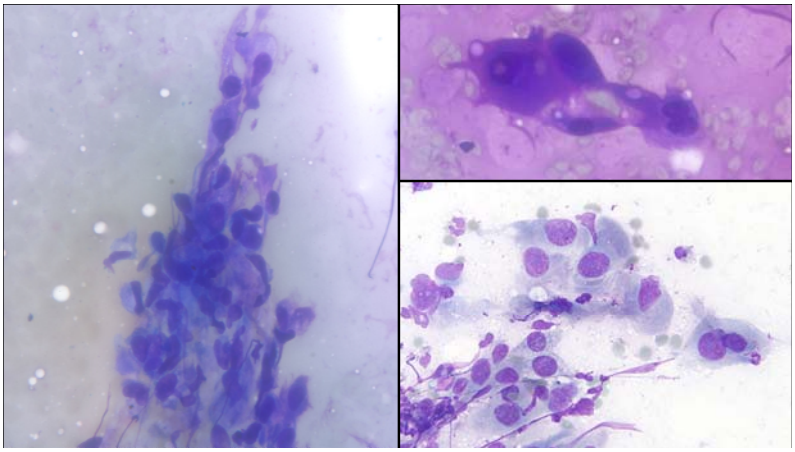


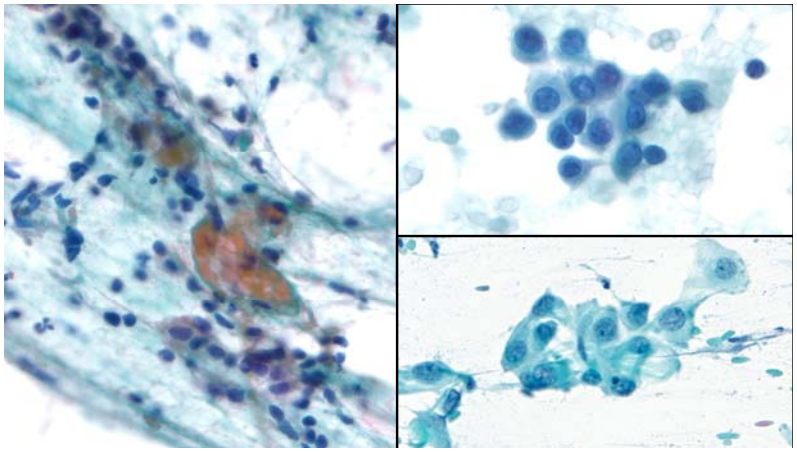


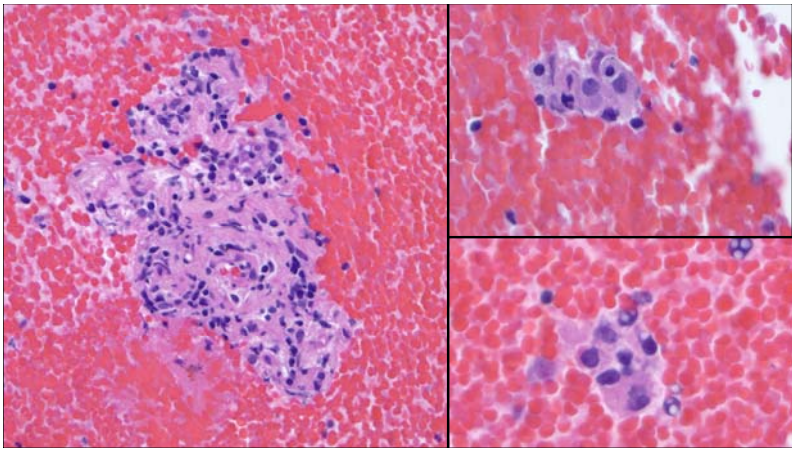
Case 1: CTG-FNA of Lung Lesion

- 63 year old woman with a history of melanoma in 2012, with metastases in 2015 to the liver
  - Primary was a right lateral vaginal wall tumor in 2012
  - *BRAF* V600E positive, *NRAS* negative, *NF1* unknown
- Currently on Nivolumab treatment (started 18 months prior to biopsy)
- CT scan: bilateral consolidative pulmonary opacities
  - ?infection
- CTG FNA of lung mass/opacity sampled





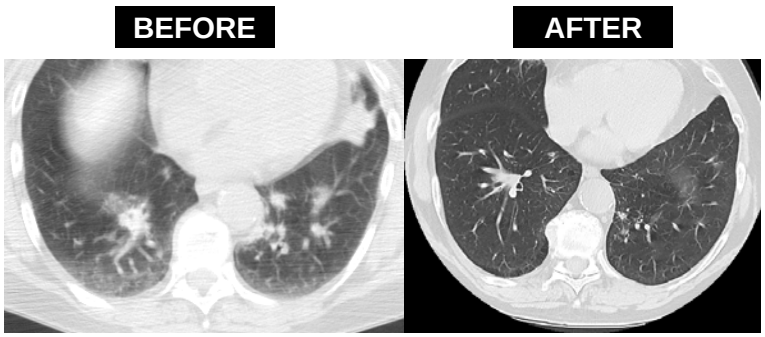




Case Diagnosis

- **Final Diagnosis**
  - Less than optimal- scant cellularity
  - Atypical cells present.
  - Atypical epithelial cells and chronic inflammation, favor reactive.

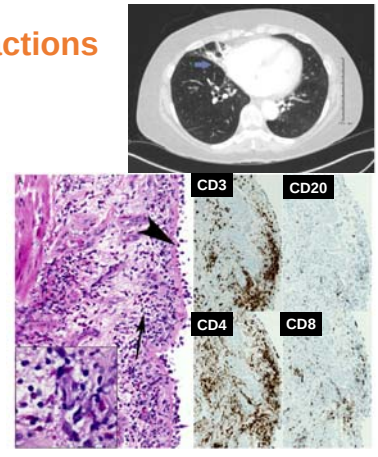
- No viral or infectious etiology seen, including special stains.
- Nivolumab was discontinued.
- On follow-up (1 month later), most of lung lesions completely resolved.
- Changes attributed to reactive pneumocyte atypia in the setting of PD-1 inhibitor toxicity (pneumonitis)



Adverse Pulmonary Reactions with PD-1 inhibitors

- Thought to be an autoimmune related pneumonitis
- Seen in 1-14% patients on phase 2 and 3 trials
  - Smaller % with high-grade toxicity
  - Larger % with low-grade toxicity
  - Rare respiratory failure & death (3)
- Sx: Pneumonia-like symptoms with bilateral lung infiltrates (rarely unifocal)
- Onset about 7-24 mo after Tx

Sehgal S et al. Respir Med Case Rep 2016;19:118-20.



Anti–PD-1–Related Pneumonitis during Cancer Immunotherapy

TO THE EDITOR: The use of antibodies against programmed cell death 1 (PD-1), which block inhibitory T-cell checkpoints, is a promising new therapy for advanced cancers.[1] Recent trials have shown substantial clinical activity of anti–PD-1 antibodies in advanced cancers and led to the approvals of these agents, including pembrolizumab for melanoma and nivolumab for melanoma and squamous-cell lung cancer.[2-4] Pneumonitis related to the use of antibodies against PD-1 is an immune-mediated toxic effect that resulted in three drug-related deaths in a phase 1 trial.[5] Clinical identification and management of pneumonitis are contingent on radiographic assessment. We report three cases of pneumonitis associated with the use of anti–PD-1 antibodies in patients with melanoma.

A 70-year-old man (Patient 1) received nivolumab and ipilimumab sequentially. A 56-year-old woman (Patient 2) and a 58-year-old man (Patient 3) were treated with nivolumab alone. Patient 2 had previously received ipilimumab before starting the nivolumab trial. The onset of pneumonitis occurred 7.4 to 24.3 months after the initiation of therapy (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Computed tomographic (CT) imaging of pneumonitis at the time of diagnosis showed a spectrum of findings that are typically seen in interstitial pneumonias.[6] These conditions were morphologically classified as acute interstitial pneumonia–acute respiratory distress syndrome (ARDS) in Patients 1 and 2 and as nonspecific interstitial pneumonia in Patient 3 (Table S2 in the Supplementary Appendix).

In Patients 1 and 2 with ARDS-pattern pneumonitis, diffuse ground-glass opacities, reticular opacities, consolidation, and traction bronchiectasis involved all lobes, with decreased lung volumes and effusions (Fig. 1A, 1B, and 1C). In Patient 1, the symptoms rapidly progressed during the 2 weeks before the diagnosis (Fig. 1A and 1E). The two patients were admitted to the intensive care unit and received intravenous antibiotic agents, glucocorticoids, and infliximab. Patient 1 required intubation, and his condition improved over the course of 10 weeks. Patient 2 died 4 weeks after the diagnosis of pneumonitis.

Patient 3 had ground-glass opacities and reticular opacities in the peripheral and lower lungs, indicative of nonspecific interstitial pneumonia (Fig. 1D). He discontinued nivolumab for 8 weeks and received oral glucocorticoids as an

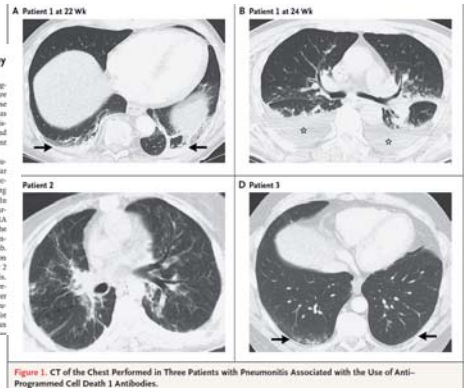
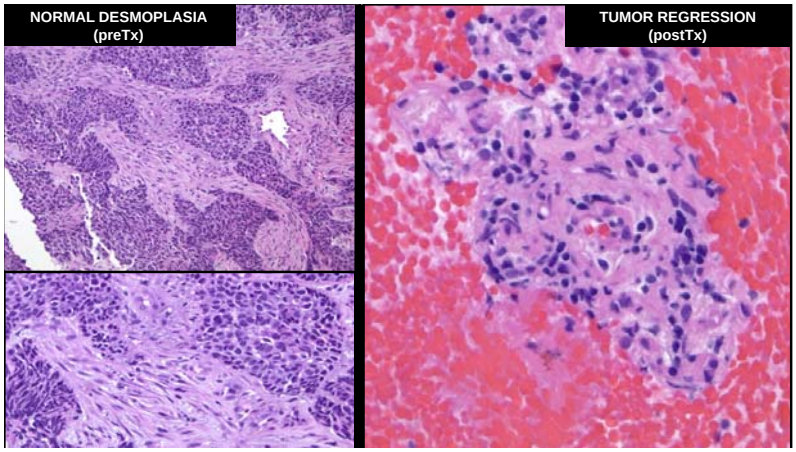
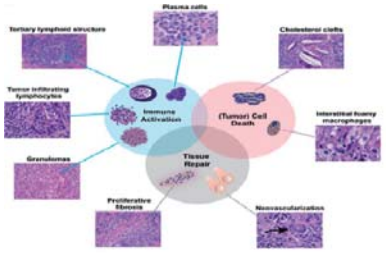
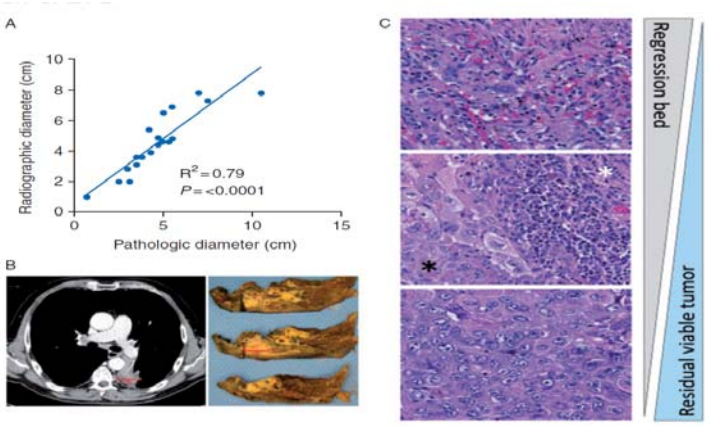


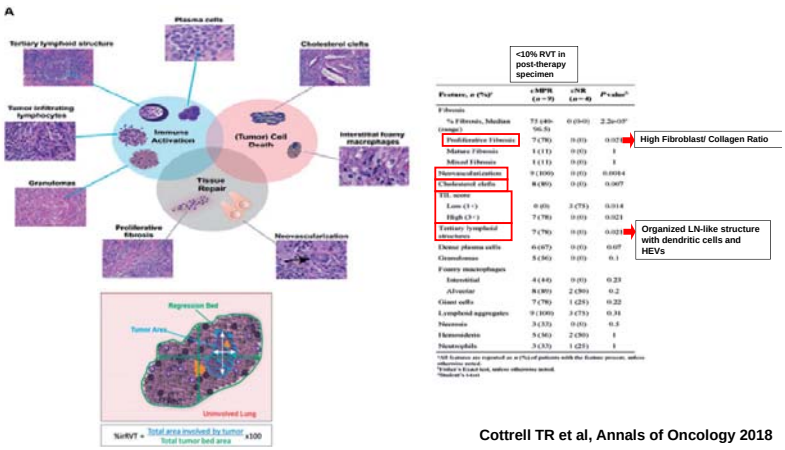
Figure 1. CT of the Chest Performed in Three Patients with Pneumonitis Associated with the Use of Anti–Programmed Cell Death 1 Antibodies.



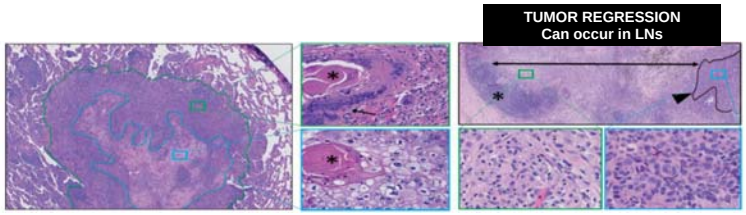




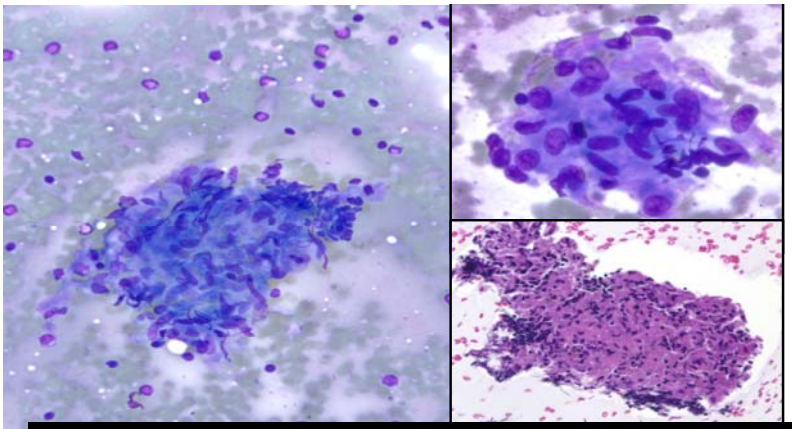
Cottrell TR et al, Annals of Oncology



Cottrell TR et al, Annals of Oncology 2018



Cottrell TR et al, Annals of Oncology 2



61 yo man with Hx of Melanoma, s/p Pembrolizumab, now LAD. EBUS FNA (subcarinal)

Nivolumab-Induced Sarcoid-Like Granulomatous Reaction in a Patient With Advanced Melanoma

François-Xavier Danlos, MD; Cécile Pagès, MD; Barouyr Baroudjian, MD; Laetitia Vercellino, MD; Maxime Battistella, MD, PhD; Maurice Mimoun, MD, PhD; Majdi Jebali, MS; Martine Bagot, MD, PhD; Abdellatif Tazi, MD, PhD; and Céleste Lebbé, MD, PhD

To our knowledge, we report the first case of sarcoid-like granulomatous reaction induced by nivolumab, a fully human IgG4 anti-programmed death 1 (PD-1) immune checkpoint inhibitor antibody. A 57-year-old man was treated with nivolumab 3 mg/kg for 2 weeks for a desmoplastic melanoma stage III American Joint Commission on Cancer, with no *BRAF*, *NRAS*, and *cKit* mutations. At 10 months, although melanoma complete response was achieved, he developed sarcoid-like granulomatous reaction in the mediastinal lymph node and skin, which resumed after nivolumab arrest. Melanoma did not relapse after 12 months of follow-up. Considering the recently demonstrated role of activated PD-1/PDL-1 axis in sarcoidosis, granulomatous reaction in the patient seems to be a paradoxical reaction, but similar observations have been reported with ipilimumab, another immune checkpoint inhibitor. Sarcoid-like granulomatous reaction during immunotherapy treatment could be a manifestation of cell-mediated immunity induced by these drugs. Impact of granulomatous reaction induced by immune checkpoint inhibitor on melanoma progression is not known and requires further study. Melanoma patients treated by immunotherapy (anti-cytotoxic T-lymphocyte-associated protein-4/anti-PD-1) should be considered for developing sarcoid-like granulomatous reaction that must not be confused with tumor progression.

CHEST 2016; 149(5):e133–e136

KEY WORDS: granulomatosis; melanoma; nivolumab; PD-1

We report a case of sarcoid-like granulomatous reaction induced by nivolumab, a fully human IgG4 anti-programmed death 1 (PD-1) the right nasolabial fold (Breslow thickness, 4.0 mm; Clark level, 5), for which he underwent wide local resection.

Sarcoid-like Reactions with PD-1 checkpoint inhibitor therapy

- **Treatment:** Steroids may not be required (systemic steroids needed in about 40% cases).
- **Removal of the PD-1 checkpoint inhibitor (e.g. Nivolumub) usually causes granulomatous lesions to regress.**
- **No cases have been refractory to treatment, thus far.**

ELSEVIER

Human PATHOLOGY

Original contribution

Immune checkpoint blocker-related sarcoid-like granulomatous inflammation: a rare adverse event detected in lymph node aspiration cytology of patients treated for advanced malignant melanoma☆,☆☆

Erika F. Rodríguez MD, PhD[a], Evan Lipson MD[b], Karthik Suresh MD[c], Laura C. Cappelli MD[d], Sara E. Monaco MD[e], Zahra Maleki MD[a]

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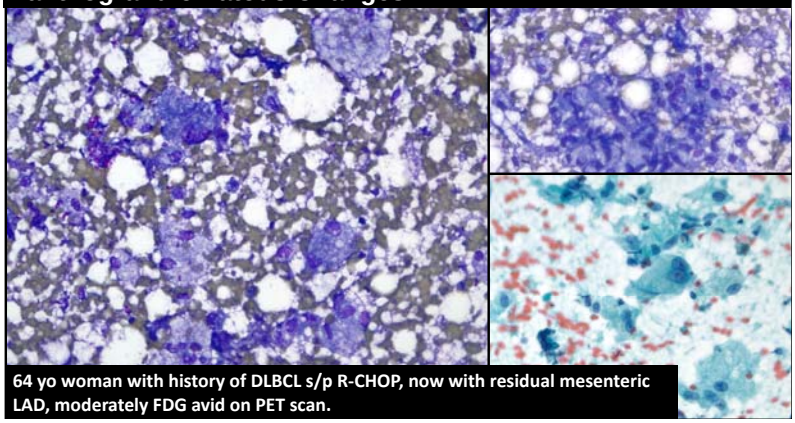
Table 2 The cytomorphologic features of granulomas in sarcoidosis and sarcoid-like reaction induced by immunotherapy

Cytological finding	Sarcoid	Sarcoid-like reaction with immunotherapy
Overall cellularity	Variable depending on amount of hyalinizing fibrosis	Cellular, given lack of hyalinizing fibrosis
Granulomas	Discrete clusters of epithelioid histiocytes of low to intermediate cellularity and scattered mature small lymphocytes	Cellular and numerous granulomas mostly very large, coated with mature small lymphocytes with a peripheral cuff of crushed lymphocytes, or pockets of small lymphocytes
Multinucleated giant cells	Variable, but typically less than infectious type granulomas	Rare or none
Background	Clean, no necrosis	Clean, no necrosis

Xanthogranulomatous Changes

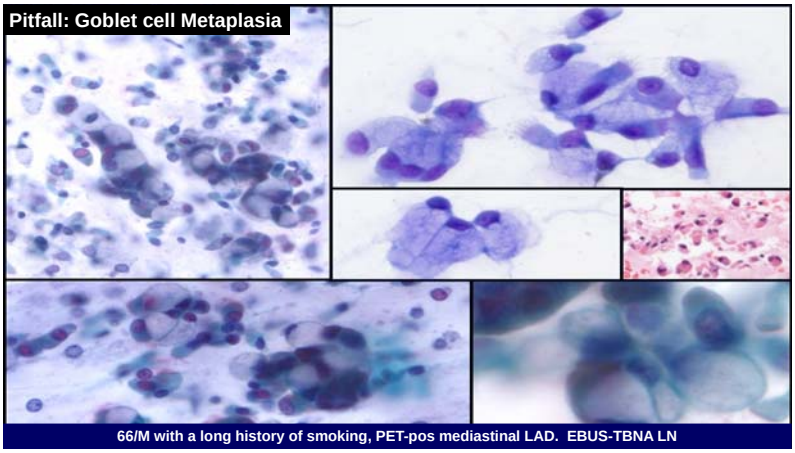


64 yo woman with history of DLBCL s/p R-CHOP, now with residual mesenteric LAD, moderately FDG avid on PET scan.

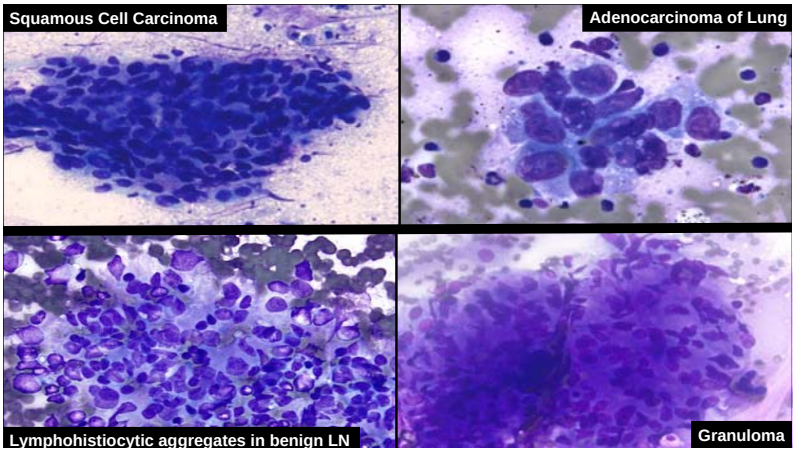
Benign/Reactive Changes that Mimic Malignancy

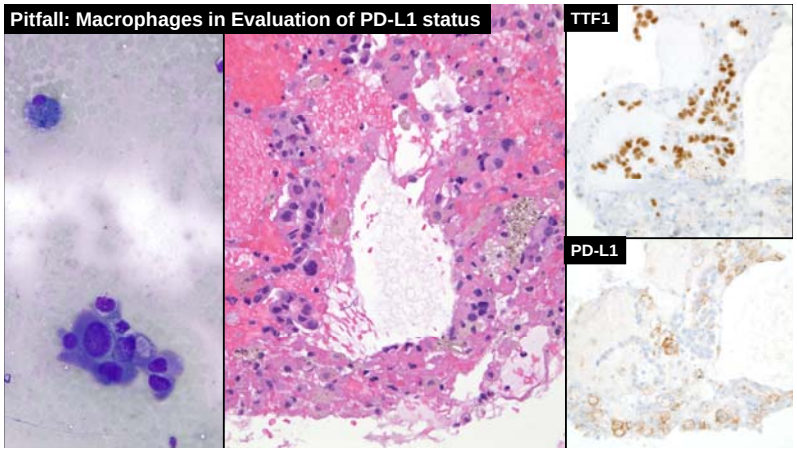
- **Benign/reactive epithelial cells**
  - Therapy-related atypia (chemotherapy, radiation, medication)
  - Metaplastic changes (goblet cell or squamous metaplasia)
- **Mesothelial hyperplasia**
- **Granulomatous inflammation/epitheloid histiocytes**
  - Cellular granulomas
- **Contamination of the FNA needle**
  - Primary luminal dysplasia (Barrett's esophagus) in EUS-FNA

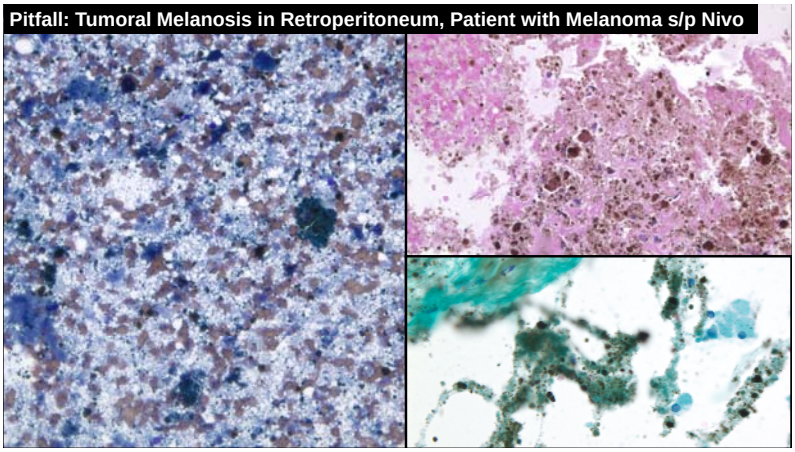
Pitfall: Goblet cell Metaplasia

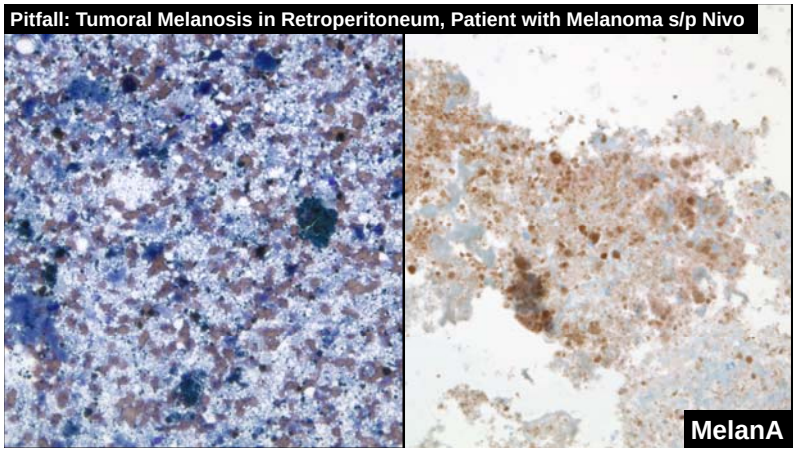


66/M with a long history of smoking, PET-pos mediastinal LAD. EBUS-TBNA LN







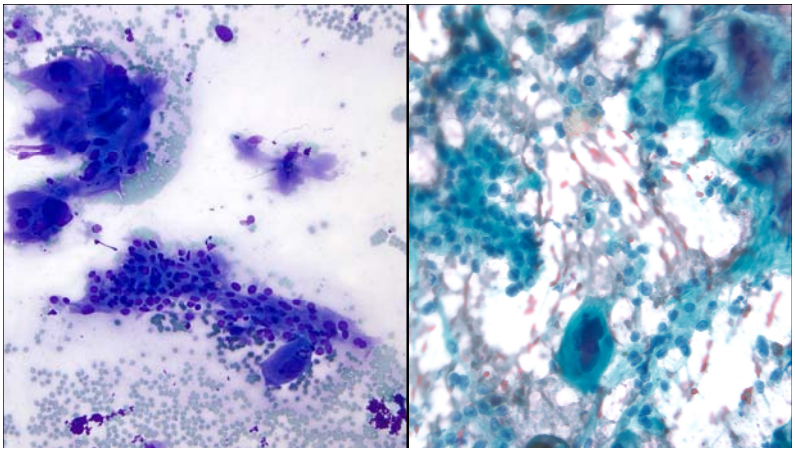


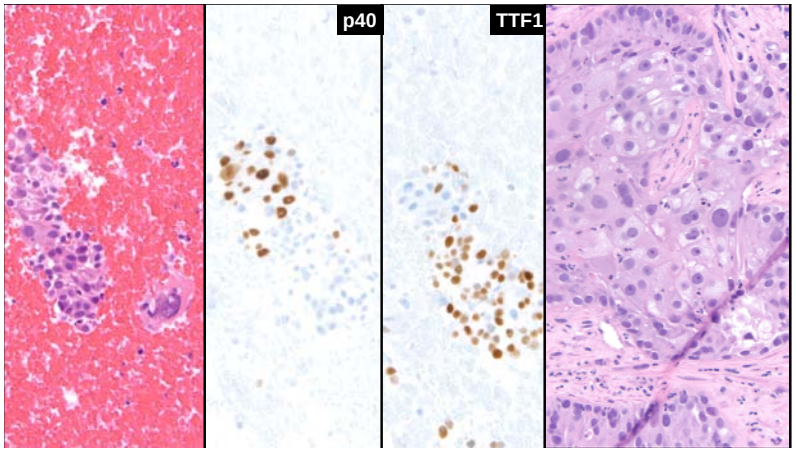
Take Home Messages

- **In the era of personalized medicine with new therapeutic agents, think about treatment related changes**
  - Expanding number of protocols & targeted or other therapies
  - Neoadjuvant therapy for down-staging prior to resection
  - PD-1 inhibitors (Lung cancer, Melanoma, Other)
  - Other radiation/chemotherapy-related changes
  - Avoid False Positive Diagnoses
  - **Cytology findings:** pneumocyte/squamous atypia, granulomatous, fibrotic, inflammatory, or necrotic changes in the lymph node and lung
- **Important to consider drug-induced toxicity for treatment**
  - Test of time: Withdrawal of agent leading to improvement
  - Sometimes the best medicine is no medicine

Case 2: Lung EMN biopsy

- 40 year old woman with right lung mass
  - Non-smoker
  - 3.0 cm lung mass identified at an OSH
- Previously biopsied at an OSH and called squamous cell carcinoma
- Clinicians requested repeat biopsy given that she is non-smoker
- EMN biopsy of lung mass





Case Diagnosis

- **Final Diagnosis:**
  - Satisfactory for interpretation.
  - Positive for malignant cells.
  - Non-small cell carcinoma.

- Comment: There are squamous and glandular features.
- Histology: Adenosquamous lung carcinoma
- Material submitted for molecular testing.
  - *EGFR* mutation +
- **Pitfall:** Diagnosis of squamous cell carcinoma could exclude patient from molecular testing and potential targeted therapies.

Diagnosis Matters

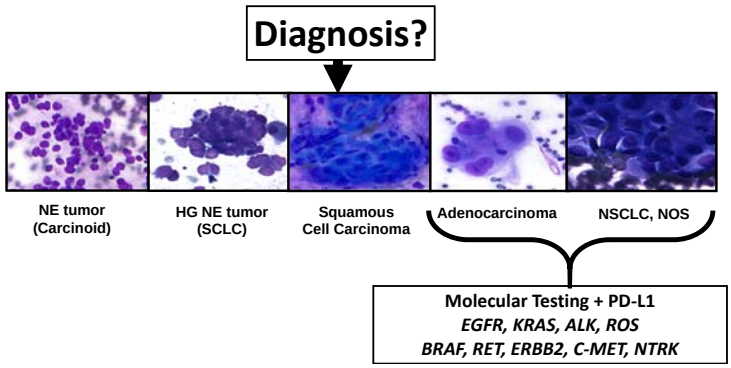


Table 1.05 Diagnostic terminology for small biopsy/cytology comparing the new IASLC/ATS/ERS terms with 2015 WHO terms in resections {2674,2676}

Small biopsy/cytology: IASLC/ATS/ERS classification	2015 WHO classification
Small cell carcinoma	Small cell carcinoma
Non-small cell carcinoma (NSCC) with neuroendocrine morphology and positive neuroendocrine markers, possible **large cell neuroendocrine carcinoma**	Large cell neuroendocrine carcinoma
Morphological squamous cell and adenocarcinoma patterns both present: **NSCC, not otherwise specified** Comment that adenocarcinoma and squamous components are present, and that this could represent adenosquamous carcinoma.	Adenosquamous carcinoma (if both components ≥ 10%)
Morphological squamous cell or adenocarcinoma patterns not present, but immunohistochemical stains favour separate squamous and adenocarcinoma components: **NSCC, not otherwise specified** Specify the results of the immunohistochemical stains and the interpretation, and comment that this could represent adenosquamous carcinoma	Adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma or large cell carcinoma with unclear immunohistochemical features.
NSCC with spindle cell and/or giant cell carcinoma Mention if adenocarcinoma or squamous carcinoma is present.	Pleomorphic, spindle cell, and/or giant cell carcinoma

ORIGINAL ARTICLE

Analysis of Major Known Driver Mutations and Prognosis in Resected Adenosquamous Lung Carcinomas

Rui Wang, MD,*† Yonjian Pan, MD,*† Chengyang Li, MD,PhD,*† Huibiao Zhang, MD,‡ David Garfield, MD,§ Yuan Li, MD, †‖ Ting Ye, MD,*† Haichuan Hu, MD,*† Xiaoyang Luo, MD,*† Hang Li, MD,*† Yang Zhang, MD,*† Jie Zhang, MD,*† Xianyan Zhou, MD,†‖ Lei Shen, MD,†‖ William Puo, MD,PhD,¶ Yihua Sun, MD,*† and Haiquan Chen, MD*†

(*J Thorac Oncol.* 2014;9: 760–768)

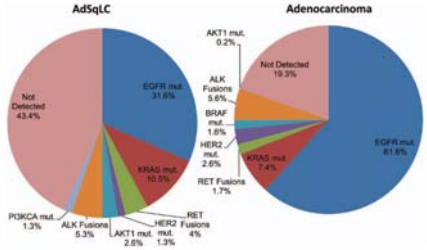
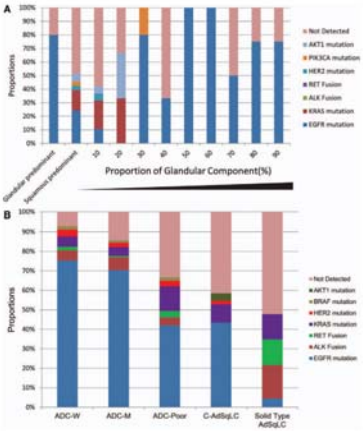


FIGURE 1. Frequency of driver mutations among adenosquamous lung carcinomas (AdSqLCs) and lung adenocarcinomas.



Pitfall: Combined tumors

- **Should I suggest adenosquamous lung carcinoma, or is one component benign/reactive?**
  - Look at nuclear grade
- **Combined tumors are rare (<5%), but could have important implications for testing**
  - If you falsely assume an adenosquamous cell carcinoma is a squamous cell carcinoma, then you could exclude the patient from important targeted therapies if you did not send it for molecular testing
  - When in doubt…
    - Check history
    - Err on the side of calling NSCLC, NOS to initiate molecular testing

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Summary of Recommendations

Section I. When Should Molecular Testing of Lung Cancers Be Performed?

Question 1. Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

1.1a: Recommendation: *EGFR* molecular testing should be used to select patients for EGFR- targeted tyrosine kinase inhibitor therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

1.1b: Recommendation: *ALK* molecular testing should be used to select patients for ALK-targeted tyrosine kinase inhibitor therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

1.2: Recommendation: In the setting of lung cancer resection specimens, *EGFR* and *ALK* testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade. In the setting of fully excised lung cancer specimens, *EGFR* and *ALK* testing is not recommended in lung cancers that lack any adenocarcinoma component, such as "pure" squamous cell carcinomas, "pure" small cell carcinomas, or large cell carcinomas lacking any immunohistochemistry (IHC) evidence of adenocarcinoma differentiation.

1.3: Recommendation: In the setting of more limited lung cancer specimens (biopsies, cytology) where an adenocarcinoma component cannot be completely excluded, *EGFR* and *ALK* testing may be performed in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing.

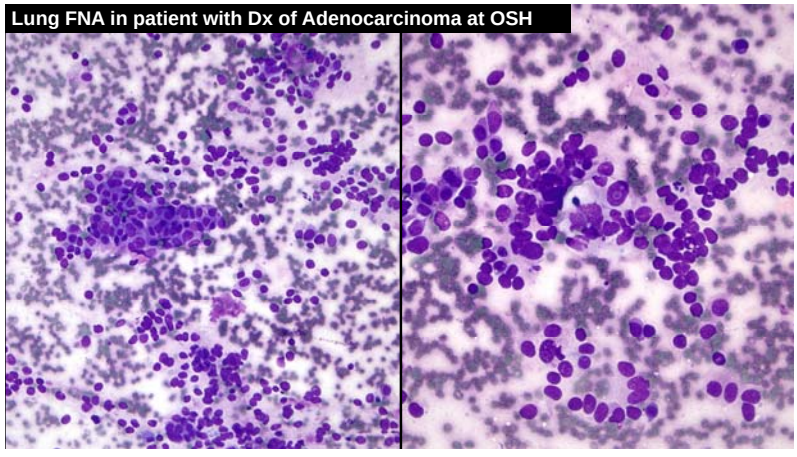
1.4: Recommendation: To determine *EGFR* and *ALK* status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.

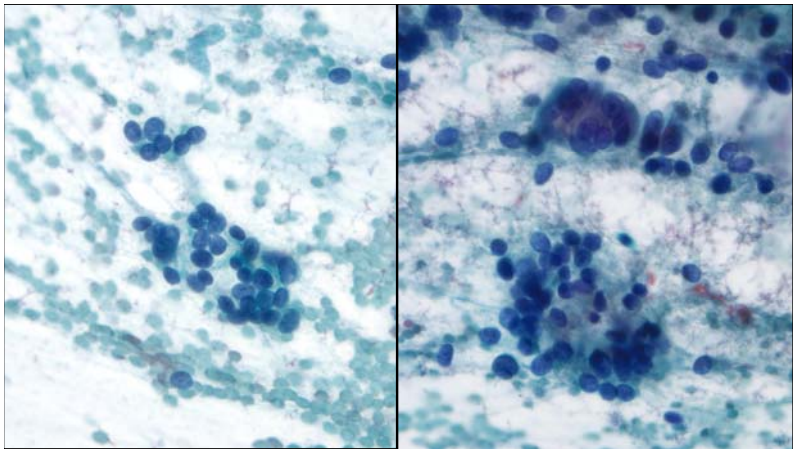
1.5: Expert consensus opinion: For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.

Misclassification of Lung AdenoCa

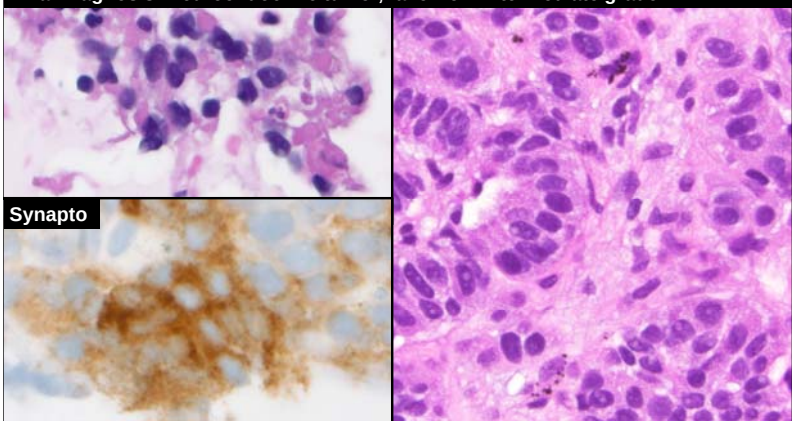
- **Benign/Reactive changes**
  - Goblet cell metaplasia, Treatment-related changes
- **Low-to-intermediate grade neuroendocrine tumors**
- **Poorly differentiated NSCLC without material for cell block/IHC**
  - SqCC, LCNEC
- **Salivary gland type tumors**
  - Mucoepidermoid carcinoma
- **Metastatic non-pulmonary carcinomas**
- **Metastatic melanoma**

Lung FNA in patient with Dx of Adenocarcinoma at OSH





Final Diagnosis: Neuroendocrine tumor, favor low-intermediate grade

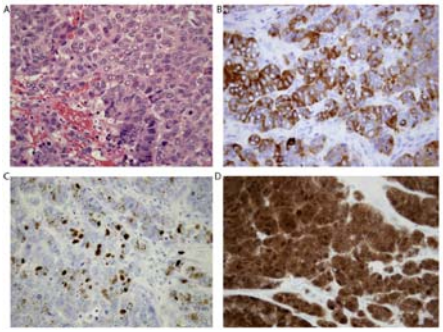


Synapto

Original article

Pulmonary neuroendocrine tumors with nuclear inclusion

Saori Kobayashi[a], Koji Tsuta[a,b], Shigeki Sekine[b], Akihiko Yoshida[a], Naoshi Sasaki[a], Yasuo Shibuki[a], Hiroyuki Sakurai[c], Shun-ichi Watanabe[c], Hisao Asamura[c], Hitoshi Tsuda[a]

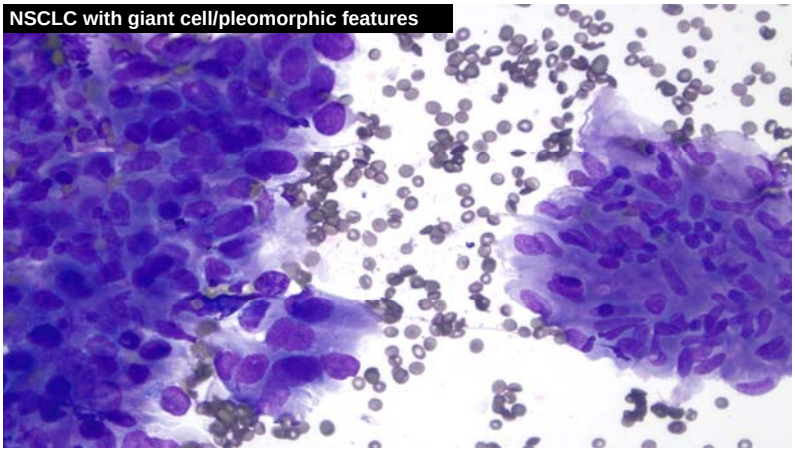


Pitfall

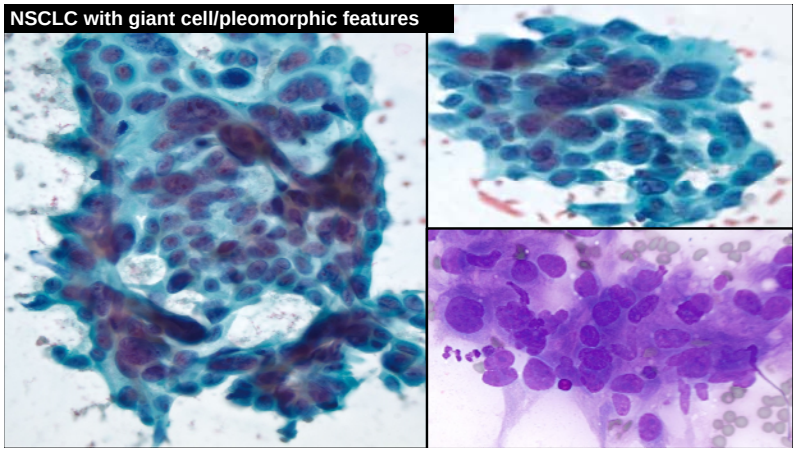
Neuroendocrine tumors can have:

- Pseudoglandular spaces/rosettes
- Intranuclear inclusions
  - Seen in 2/227 (0.9%) of pulmonary NETs
  - Usually higher-grade NETs

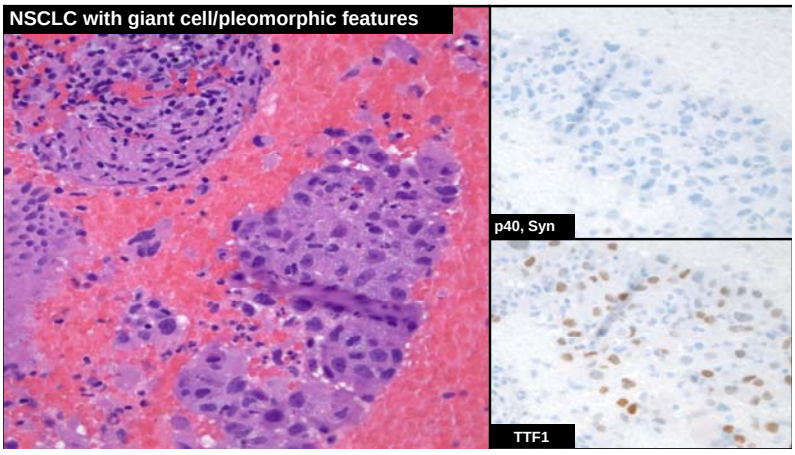
NSCLC with giant cell/pleomorphic features



NSCLC with giant cell/pleomorphic features



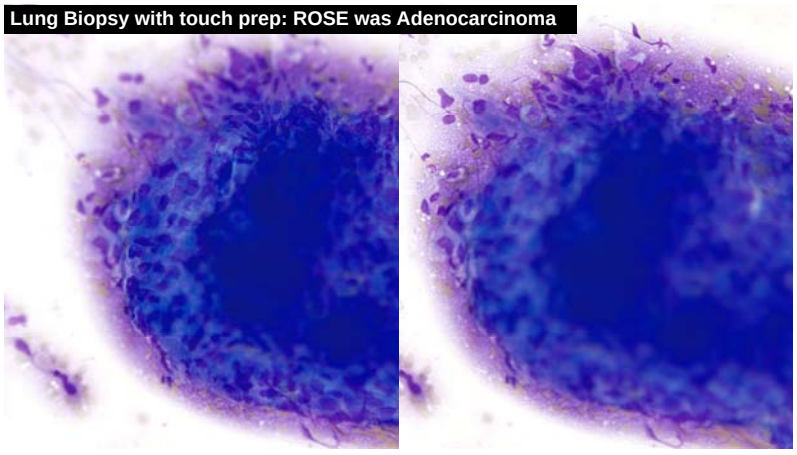
NSCLC with giant cell/pleomorphic features

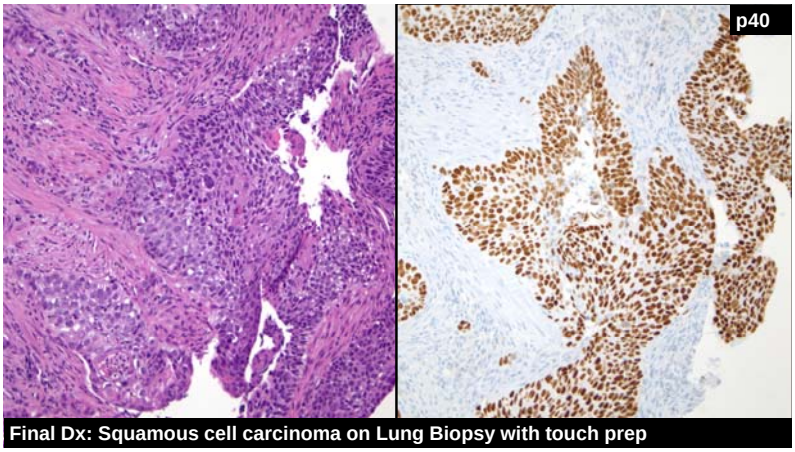


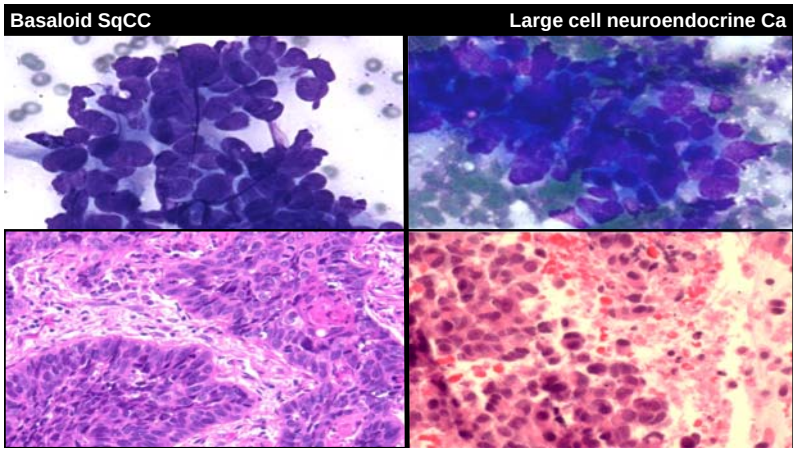
p40, Syn

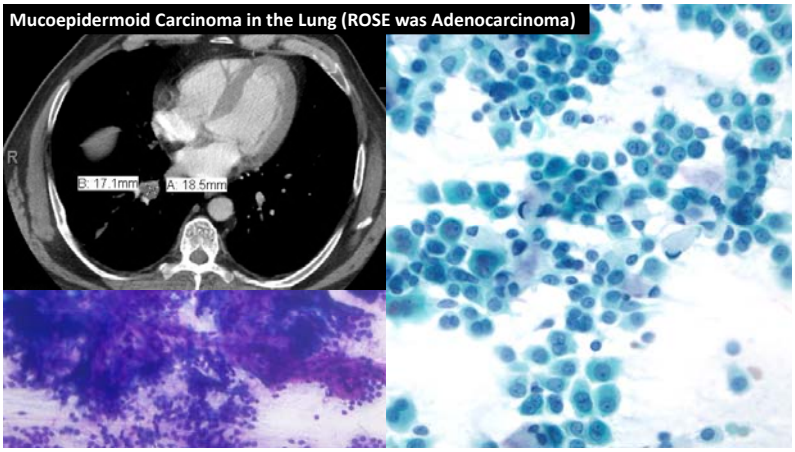
TTF1

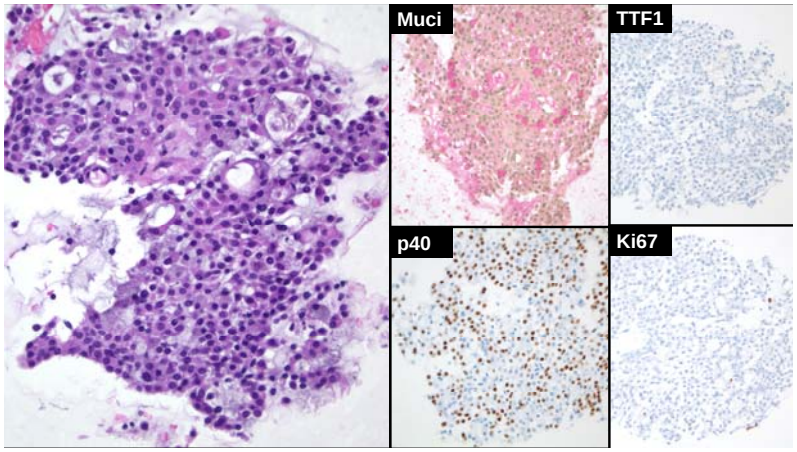
Lung Biopsy with touch prep: ROSE was Adenocarcinoma

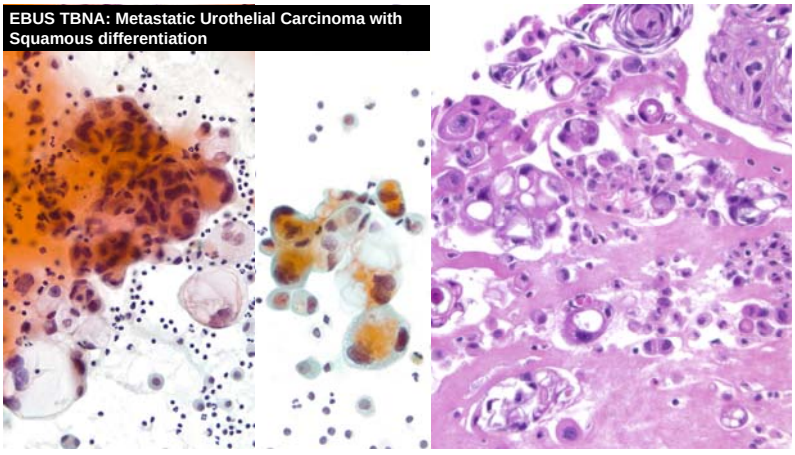










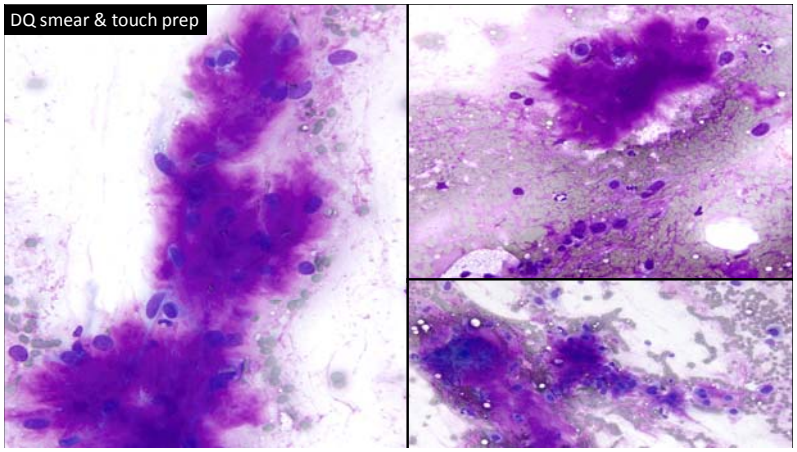


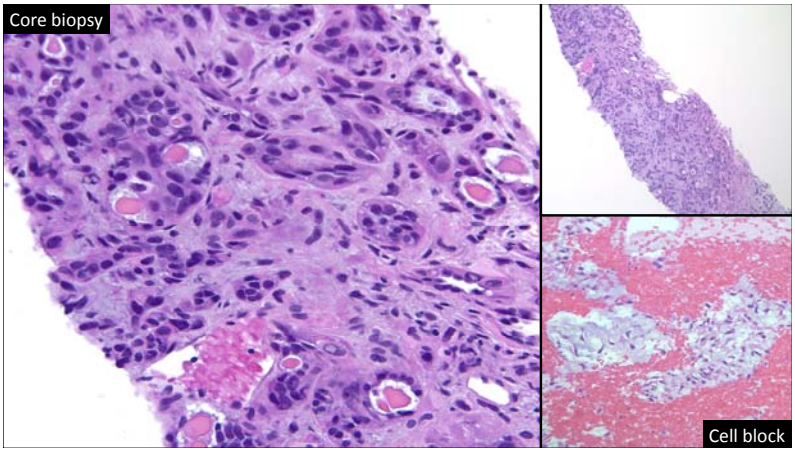
Take Home Messages

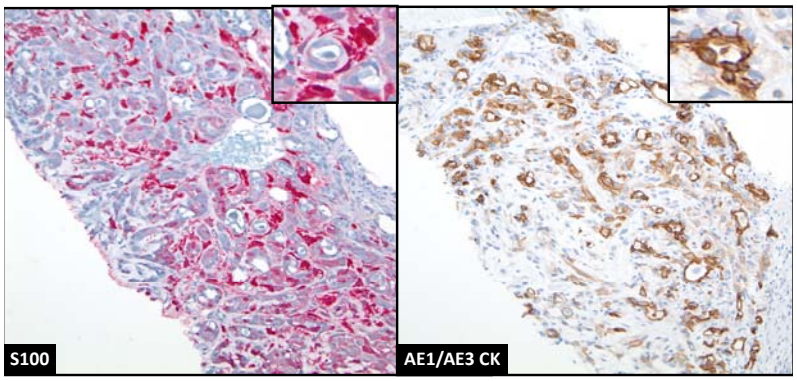
- **Subclassification can be difficult in a subset of lung tumors**
  - Obtain good clinical history (age, smoking status)
  - Look for history of prior malignancies
  - Use IHC to help
- **Avoid unnecessary testing & loss of material**
  - Morphological details can help guide selection
  - Do just enough to be correct

Case 3: CTG FNA and Core Bx with TP

- 82 year old woman with incidental well-circumscribed lesion in right upper lobe of the lung
- Prior CTG FNA showed features of a pulmonary hamartoma, but lesion was growing on imaging.
- CTG FNA and core biopsy with touch preparation.







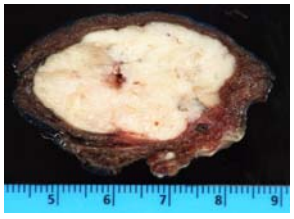
Case Diagnosis

- **Final Diagnosis:**
  - Satisfactory for Interpretation
  - Positive for neoplasm
  - Salivary gland-type tumor, favor Epithelial-Myoepithelial Carcinoma.

- **Challenges:**
  - Biphasic lesions in the lung: not always hamartoma
  - Salivary gland-type tumors: primary versus metastatic
  - Lung tumors that do not fall into SCLC vs NSCLC are challenging

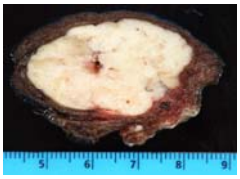
Follow-up

- No primary salivary gland lesion identified on CT-PET scan.
- Lobectomy showed a well-circumscribed, lobulated lung lesion grossly.
- Resection showed:
  - Carcinoma ex Pleomorphic adenoma
  - Carcinomatous component was an Epithelial-Myoepithelial carcinoma



Carcinoma ex Pleomorphic Adenoma in Lung

- **Rare in the lung, but arises from the bronchial glands**
- **Must exclude a head and neck primary**
- **Considered a low-grade malignancy with long interval to recurrence or metastasis**
- **Most common carcinomas in this setting:**
  - Poorly differentiated adenocarcinoma
  - Salivary duct carcinoma
  - Epithelial-myoepithelial carcinoma
- **Gross: well circumscribed, pushing border in an endobronchial location**



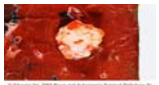
Carcinoma ex Pleomorphic Adenoma in Lung

- **Histologically: Malignant myoepithelial cells and duct-like structures in benign chondromyxoid stroma**
  - No mature cartilage
  - Biphasic cell population:
    - Large, clear myoepithelial cells (myoepithelial cells +S100, p63, SMMH, vim)
    - Small, dark ductal cells (epithelial cells +CK, EMA, +/-S100)
- **Cytomorphology:**
  - Cellular aspirates with cellular chondromyxoid-type material
  - Naked nuclei due to fragile clear cytoplasm of myoepithelial cells
  - Atypia
  - No mature cartilage

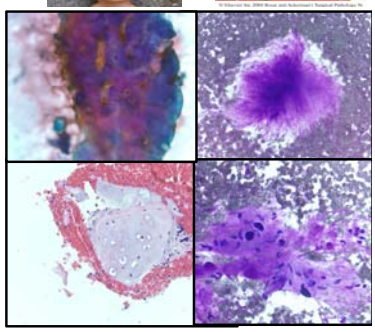
Differential Diagnosis

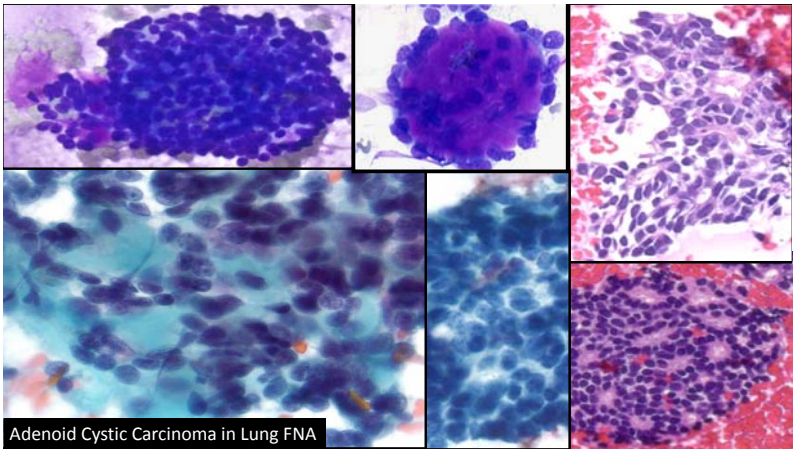
- **Benign: Granuloma, Amyloidoma**
- **Hamartoma**
- **Mesenchymal tumor** (e.g. solitary fibrous tumor, sarcoma)
- **Metastatic spindle cell tumor with myxoid change** (e.g. GIST)
- **Salivary gland-type tumor**
  - Primary (arising from the bronchial glands) vs. Metastatic
  - Benign (pleomorphic adenoma) vs. Malignant (epithelial-myoepithelial carcinoma)
  - Variable subtypes: pleomorphic adenoma, epithelial-myoepithelial carcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, basal cell neoplasm
- **Primary lung carcinoma with desmoplastic stroma or mucin** (Adenocarcinoma, Basaloid squamous cell carcinoma, Carcinosarcoma)

Pulmonary Hamartoma



- **Scant cellularity**
  - Due to dense nature of the lesion
  - Rubber eraser-like effect
- **Clean Background**
  - No necrosis or inflammation
- **Reactive bronchial cells**
- **Cartilaginous or Fibromyxoid fragments (metachromatic)**
- **Recurrent clonal rearrangements of HMGI(Y) gene on chr.6p21**



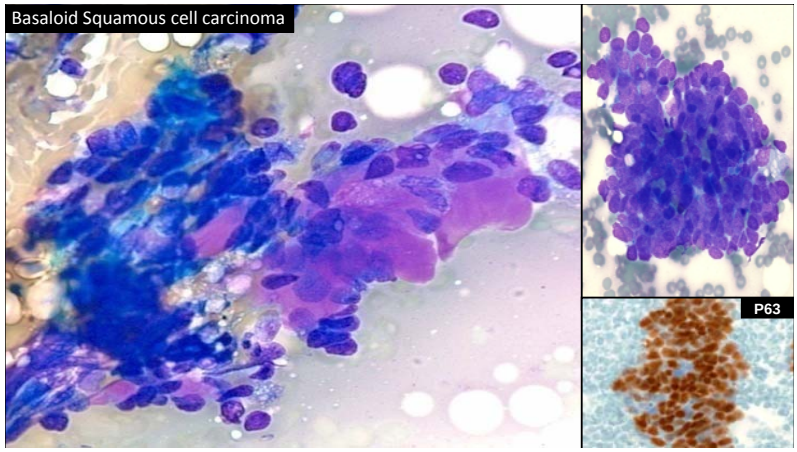


Adenoid Cystic Carcinoma in Lung FNA

Chromosomal abnormalities in salivary gland-like tumors that can be detected with FISH studies in small biopsies and cytology specimens.

*Note: These salivary gland tumors have only rarely been reported in the lung, and primarily are seen as metastases.

Salivary gland-type tumor	Gene(s)	Chromosome(s)
Mammary analogue secretory carcinoma*	*ETV6-NTRK3*	t(12;15)
Mucoepidermoid carcinoma	*MECT1-MAML2*	t(11;19)
Adenoid Cystic Carcinoma	*MYB-NFIB*	t(6,9)
Salivary duct carcinoma*	*Her2/neu*	17q
Hyalinizing clear cell carcinoma*	*EWSR1-ATF1*	t(12;22)

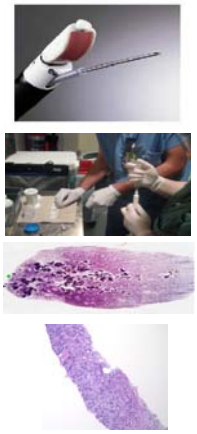


Take Home Messages

- **Pulmonary hamartomas typically do not grow rapidly.**
  - Increased growth on serial imaging is a **RED** flag.
- **Think of SGTTs in the lung when you see a biphasic tumor with chrondomyxoid material and basaloid or myoepithelial-type cells.**
  - Atypical features to look for in a fibromyxoid lesion in the lung: high cellularity, atypia, bilayered glandular structures, and lesional growth
- **Although SGTTs can occur as a primary in the lung (from the bronchial glands), a metastatic tumor should be excluded.**
- **FISH studies are becoming increasingly helpful in SGTTs for definitive classification.**

Conclusions

- **Minimally invasive biopsies & new FNA techniqes have changed the way that thoracic & mediastinal lesions are approached**
- **New Classification Systems for small biopsies provide a framework for how we should be formulating diagnoses**
- **Biomarker testing is crucial & growing**
- **Small biopsy & cytology diagnoses can be challenging**



Thank you!