LUNG CYTOLOGY

Malignant

Prof. Fernando Schmitt
Director of Department of Pathology and Medicine
Laboratoire National de Santé, Luxembourg
General-Secretary of the International Academy of Cytology

LUNG CYTOLOGY

- The importance of cytological techniques for investigation of respiratory conditions has been recognized since the earliest days of clinical cytology.
- The last few decades have shown a clear demonstration of the sensitivity and predictive value of cytodiagnosis of lung tumors and an acceptance of all cytological modalities as a basis for management.
- Nowadays, in around 40% of lung cancer cases, the only material available for diagnosis and molecular testing is the cytological material.

Schmitt FC. Cytopathology 2011

ACCURACY OF LUNG CYTOLOGY FOR TUMOR DIAGNOSIS AND MANAGEMENT

- Sensitivity and predictive value of malignant diagnosis.
- Tumor histological typing
- Select markers for targeted therapy

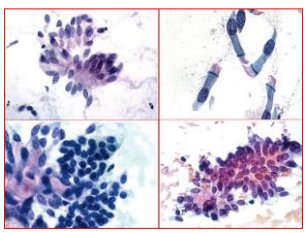
LUNG CYTOLOGY
Sensitivity

SAMPLING	LOCATION OF THE LESION	
	CENTRAL	PERIPHERAL
SPUTUM	70-85%	30-60%
BRONCHIAL WASHING	70-90%	61-76%
BRONCHIAL BRUSHING	77-90%	50-70%
BAL	80-90%	70-80%
FNA	80-95%	80-95%

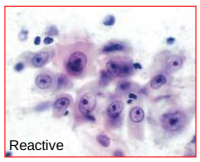
Gray and Kocjan, 2010; Bibbo M, 2008; Layfield et al, 1996; Rosenthal DL, 1988

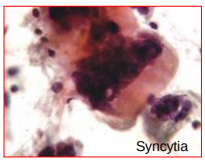
LUNG CYTOLOGY
Morphological Aspects

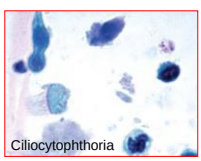
- Normal Bronchial Cells
  - May be hypercellular
  - Often elongated
  - Round nuclei
  - Fine chromatin
  - Cilia



LUNG CYTOLOGY
Bronchial cell atypia

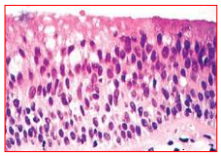

Reactive


Syncytia

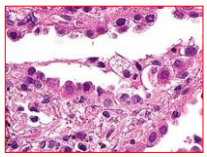

Ciliocytophthoria


Creola bodies: hyperplastic reactive epithelium

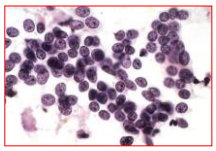
LUNG CYTOLOGY
Bronchial cell atypia

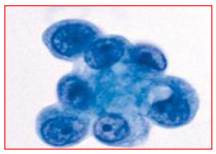


Basal cell hyperplasia of Bronchial epithelium

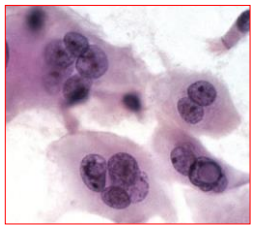


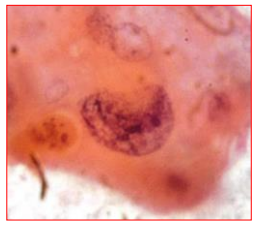
Hyperplasia of type II pneumocytes





LUNG CYTOLOGY
Morphological Aspects

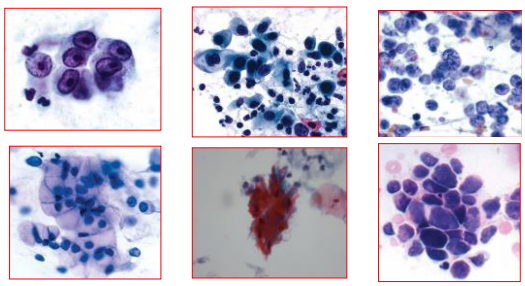




Acute irradiation effect

LUNG CYTOLOGY
Morphological Aspects

ADENOCARCINOMA **SqCC** **SCC**



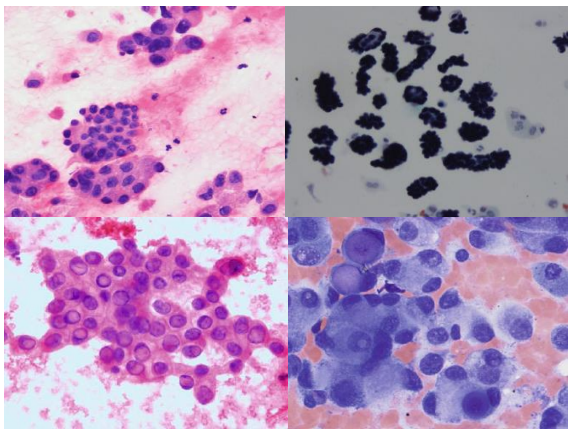
LUNG CYTOLOGY and TUMOR TYPING

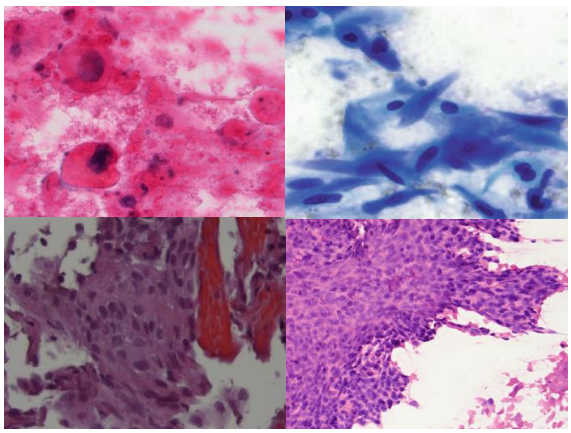
TABLE 3. Sensitivity and Specificity of Cytologic Tumor Subtyping

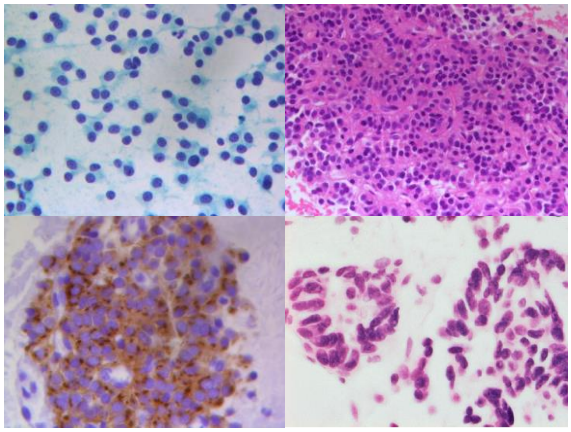
	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV,[a] % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
SCLC vs. non-SCLC	100 (29.2–100)	100 (97.9–100)	100 (29.2–100)	100 (97.9–100)	100 (98.1–100)
Squamous vs. nonsquamous	87 (66.4–97.2)	98 (94.6–99.6)	87 (66.4–97.2)	98 (94.6–99.6)	97 (93.2–98.6)
Adenocarcinoma vs. nonadenocarcinoma	98 (93.9–99.6)	79 (63.2–89.7)	94 (88.7–97.2)	92 (77.5–98.3)	93 (89.1–96.3)

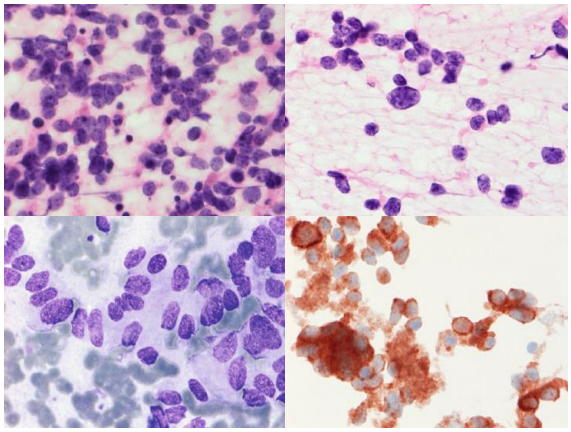
- Cytology is a powerful tool in the diagnosis of lung cancer, in particular in the distinction of adenocarcinoma from squamous cell carcinoma.

J Thorac Oncol. 2011;6: 451–458

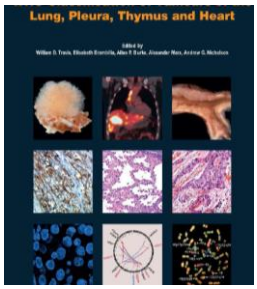








The 2015 WHO Classification on Lung Tumors



What is new?

•A new classification for small biopsies/Cytology samples

•Wide use of IHC for classification

•Emphasis on genetics for predictive biomarkers in advanced lung cancer

•The issue of LCC, SqCC and NET

•A different approach to lung adenocarcinoma (AIS, MIA…)

•Reclassifying other tumors (NUT, hamartoma, myxoid sarcoma…)

Pathology recommendations applicable to small biopsy and cytology specimens

- For small biopsies and cytology, we recommend that NSCLC be further classified into a more specific type, such as adenocarcinoma or SqCC, whenever possible.
- We recommend that the term NSCL-NOS be used as little as possible and it be applied only when a more specific diagnosis is not possible by morphology and/or special stains.
- When a diagnosis is made in a small biopsy or cytology in conjunction with special studies, it should be clarified whether the diagnosis was established based on light microscopy alone or if special stains were required.

Arch Pathol Lab Med. 2012;136:1–17

Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

Fernando Schmitt, MD, PhD; [illegible]; José Carlos Machado, PhD

Key Points
Cytologic Diagnosis of Lung Carcinoma

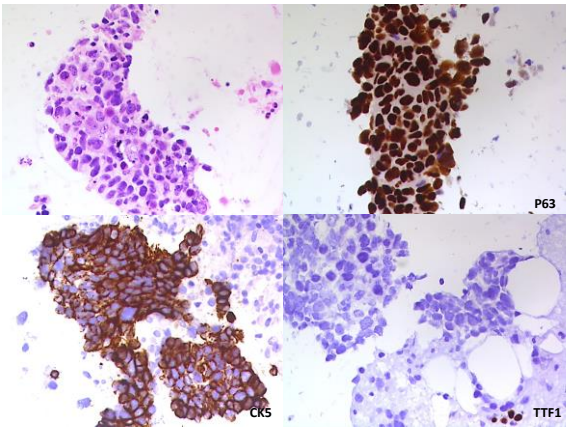
1. If definite squamous or ADC features are present by morphologic criteria, a tumor can be diagnosed on cytology as SqCC or ADC.
2. Keratinization, pearls, and intercellular bridges in cells with dense cytoplasm and hyperchromatic nuclei are characteristics of SqCC.
3. Cell balls, papillary fronds, acinar structures, and individual cells with large cytoplasm and nuclei with centrally located nuclei are characteristics of ADC.
4. Small cells in loose clusters and single cells, nuclear molding, and salt-and-pepper chromatin are characteristics of SCLC.
5. Large cells, isolated or in groups, with ill-defined cytoplasm, large nuclei, and variable nucleoli and without any morphologic features of squamous or glandular differentiation should be classified as NSCLC-NOS.
6. The term, NSCLC-NOS, should be used as little as possible (ie, only when a more specific diagnosis is not possible by morphology and/or ICC).
7. ADC in situ, minimally invasive carcinoma, and large cell carcinoma are diagnoses that should not be rendered in cytology cases.
8. Cytologic diagnosis of LCNEC requires a combination of morphology and use of neuroendocrine markers.
9. If pleomorphic cells are present on cytology together with any other pattern (ADC, SqCC, or NSCLC-NOS), this should be reported in a comment implying the possibility of pleomorphic carcinoma.

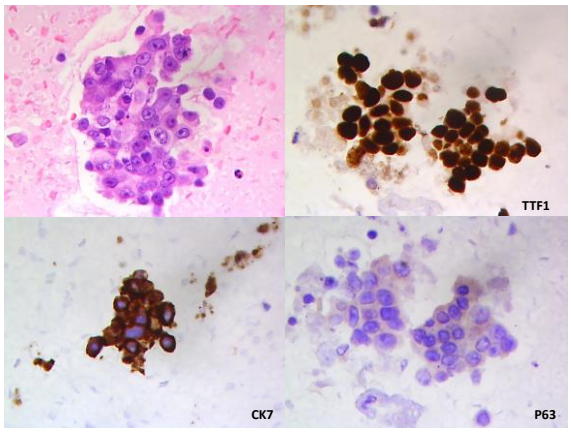
LUNG CYTOLOGY
Primary vs. Metastasis

- Metastases in lung may be single, large, multiple or lymphangitic.
- In most instances a previous diagnosis of malignancy is available, and confirmation of the clinically suspected lesion is all that is required from the cytopathologist.
- However, some pulmonary metastases present before the primary tumour has been identified and may require careful investigation, if inappropriate diagnosis and treatment of presumed lung primary carcinoma are to be avoided.
- Adenocarcinoma, squamous cell carcinoma and small cell carcinoma are histological types that can be difficult to distinguish between primary or secondary tumours.

LUNG CYTOLOGY
Primary tumours

- In recent years, cytology have been increasingly used for establishing the diagnosis of lung cancer and classifying the specific tumour type.
- Accurate distinction between SCLC and NSCLC, as well as further distinction between SQCC and Adenocarcinoma has crucial therapeutic significance.
- However, sometimes morphologic distinction can be difficult and ancillary techniques can help the cytopathologist in this task.





LUNG CYTOLOGY and TUMOR TYPING

TABLE 5. Frequency and Accuracy of Morphologic vs. Immunohistochemistry-Aided Diagnoses of Adenocarcinoma and Squamous Cell Carcinoma in Cytology

	Frequency	Accuracy
Diagnosis based on morphology	146 (88%)	96%
Diagnosis based on IHC	14 (9%)	100%
Diagnosis that needs IHC but cellularity insufficient (diagnosis: NSCLC-NOS)	5 (3%)	na

J Thorac Oncol. 2011;6: 451–458

Expanded use of immunocytochemistry

- In 1999 and 2004 WHO classification IHC was limited to LCNEC, sarcomatoid carcinoma and dd with mesothelioma.
- In 2015 WHO classification, IHC is recommended:
  - ✓ In biopsy/cytology samples
  - ✓ Resected specimens (solid adenocarcinoma, LCC, nonkeratinizing SqCC, sarcomatoid carcinoma, NET, NUT…)
  - ✓ More defined subtyping (for therapy)
- Minimalist antibody panel approach (the more the stains, the more difficult the diagnoses).

Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

Fernanda Schmitt, MD, PhD, FIAC[a,b,c,*], José Carlos Machado, PhD[d]

Table 2
Common immunocytochemistry markers used in lung cytology

	Markers					
Lung Cancer Subtype	TTF-1	Napsin A	P63	P40	CK5/6	Neuroendocrine Markers
ADC	+/−[a]	+	−/+	−	−	−
SqCC	−	−	+	+	+	−
SCLC	+	−	−	−	−	+
NSCLC-NOS	+/−	−	−	−	−	−
Metastatic carcinoma	−/+	−	−/+	−/+	−/+	−/+

[a] Especially mucinous ADC.

Review

p40: A p63 Isoform Useful for Lung Cancer Diagnosis – A Review of the Physiological and Pathological Role of p63

Ana Rita Nobre[a,b] André Albergaria[a,c] Fernando Schmitt[a,c]

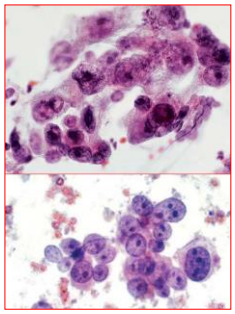
Table 1. Distribution of TTF-1, p63, and p40 in lung cancer

	Histological diagnosis					SE	SP	PPV	NPV	Ref.
	ADC	SCC	LCC/SC	ADSC	LCL					
TTF-1	115/150 (77)	0/50 (0)	n.a.	n.a.	n.a.	0.77	1.00	1.00	0.59	45
p63	27/150 (18)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.82	0.65	1.00	
p40	0/150 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1+/p63+	26/180 (14)	0/50 (0)	n.a.	n.a.	n.a.	0.14	1.00	1.00	0.25	
TTF-1+/p63-	115/180 (64)	0/50 (0)	n.a.	n.a.	n.a.	0.64	1.00	1.00	0.43	
TTF-1-/p63+	4/180 (2)	50/50 (100)	n.a.	n.a.	n.a.	1.00	0.98	0.93	1.00	
TTF-1-/p63-	35/180 (19)	0/50 (100)	n.a.	n.a.	n.a.	0.19	1.00	1.00	0.26	
TTF-1+/p40+	0/180 (0)	0/50 (0)	n.a.	n.a.	n.a.	0.00	1.00	0.00	0.22	
TTF-1+/p40-	141/180 (78)	0/50 (0)	n.a.	n.a.	n.a.	0.78	1.00	1.00	0.56	
TTF-1-/p40+	0/180 (0)	50/50 (100)	n.a.	n.a.	n.a.	1.00	1.00	1.00	1.00	
TTF-1-/p40-	39/180 (22)	0/50 (0)	n.a.	n.a.	n.a.	0.00	0.78	0.00	0.74	
TTF-1	28/30 (93)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.93	1.00	1.00	0.83	46
p63	0/30 (30)	10/10 (100)	1/1 (100)	5/5 (100)	n.a.	1.00	0.70	0.53	1.00	
p40	3/30 (17)	10/10 (100)	0/1 (0)	5/5 (100)	n.a.	1.00	0.83	0.67	1.00	
TTF-1+/p63+	8/30 (27)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.27	1.00	1.00	0.31	
TTF-1+/p63-	20/30 (67)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.67	1.00	1.00	0.50	
TTF-1-/p63+	1/30 (3)	10/10 (100)	1/1 (100)	2/5 (40)	n.a.	1.00	0.97	0.91	1.00	
TTF-1-/p63-	1/30 (3)	0/10 (0)	0/1 (0)	0/5 (0)	n.a.	0.00	0.97	0.00	0.74	
TTF-1+/p40+	4/30 (14)	0/10 (0)	0/1 (0)	3/5 (60)	n.a.	0.13	1.00	1.00	0.28	
TTF-1+/p40-	24/30 (80)	0/10 (0)	0/1 (0)	2/5 (40)	n.a.	0.80	1.00	1.00	0.56	
TTF-1-/p40+	1/30 (3)	10/10 (100)	0/1 (0)	2/5 (40)	n.a.	0.03	0.97	0.91	0.76	
TTF-1-/p40-	1/30 (3)	0/10 (0)	1/1 (100)	0/5 (0)	n.a.	0.00	1.00	0.00	0.74	
p63	74/257 (31)	81/81 (100)	n.a.	n.a.	82/152 (54)	1.00	0.69	0.52	1.00	47
p40	7/205 (3)	81/81 (100)	n.a.	n.a.	0/152 (0)	1.00	0.97	0.92	1.00	
TTF-1	51/66 (77)	0/26	3/12	0/1	n.a.	0.77	1.00	1.00	0.62	49
p63	13/66 (20)	24/24 (100)	7/12	1/1 (100)	n.a.	1.00	0.80	0.65	1.00	
p40	1/29 (3)	15/15 (100)	3/12 (25)	1/1 (100)	n.a.	1.00	0.97	0.94	1.00	
Napsin A	11/29 (38)	0/15 (0)	1/12 (8)	0/1 (0)	n.a.	0.38	1.00	1.00	0.45	

Integrative data of the expression of TTF-1, p63, and p40 in subtypes of lung cancer and comparison of the sensibility and specificity of these markers for SCC. Values in parentheses are percentages. ADSC = Adenosquamous carcinoma; LCC = large cell carcinoma; LCL = large cell lymphoma; n.a. = not assessed; NPV = negative predictive value; PPV = positive predictive value; SC = sarcomatoid carcinoma; SE = sensitivity; SP = specificity; Ref. = reference.

LUNG CYTOLOGY
Morphological Aspects

- **NSCLC-NOS**
  - Hypercellular
  - Large groups
  - Large cells
  - Macronucleoli, sometimes multiple
  - Cytoplasm outline frequently ill-defined



Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

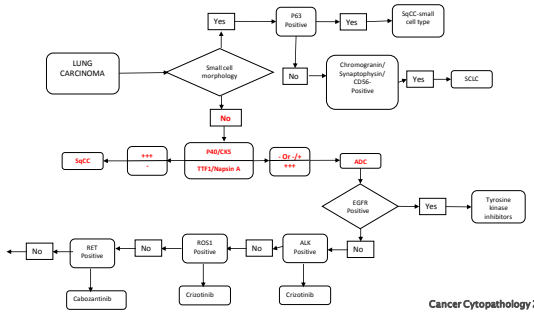
Fernando Schmitt, MD, PhD, Inês Vieira, MSc, José Carlos Machado, PhD

Key Points
IMMUNOCYTOCHEMISTRY IN LUNG CYTOLOGY

1. All cytology preparations can be used to perform ICC; however, cell blocks are more reproducible, allow use of controls, and are standardized for molecular studies.
2. A minimalist panel of markers should be validated to preserve material for eventual molecular studies.
3. TTF-1 and P40 are mutually exclusive and are helpful in distinguishing ADC from SqCC.
4. P40 is more specific than monoclonal P63 antibody in recognizing SqCC.
5. Napsin-A and CK5/6 can be used to distinguish ADC from SqCC, especially when used together in cocktails of nuclear and cytoplasmic markers.
6. Tumors that coexpress TTF-1 and P63 are usually ADCs, and this coexpression is associated with ALK-positive cases.
7. Mucinous lung ADCs are TTF-1 negative and can express CDX2.
8. TTF-1–negative nonmucinous ADC in the lung requires additional markers to exclude metastases.
9. Poorly differentiated tumors that are TTF-1 and P63 negative and lack squamous or glandular differentiation should be stained for cytokeratin to confirm epithelial differentiation.

Role of Ancillary Studies in Fine-Needle Aspiration From Selected Tumors

Fernando Schmitt, MD, PhD[1,2] and Helena Barroca, MD[3]



Cancer Cytopathology 2011

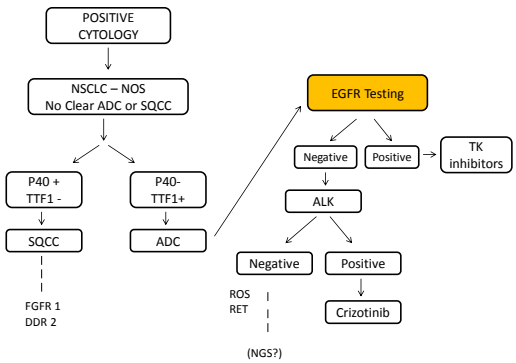
International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

Journal of Thoracic Oncology • Volume 6, Number 2, February 2011

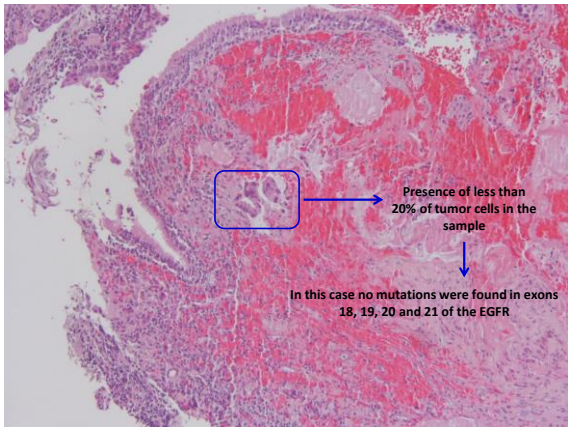
PATHOLOGY CONSIDERATIONS FOR GOOD PRACTICE

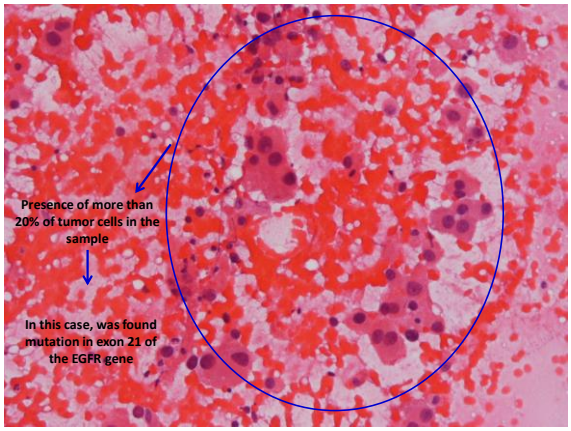
- Small biopsy and cytology samples should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

ALGORITHM FOR ANCILLARY TESTING IN NSCLC



Schmitt FC & Machado JC, 2013

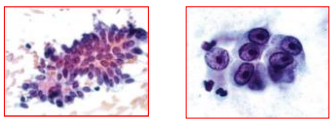




Mutation frequency and morphological control

	Mut	Wild-type	Total
Without control	463 (38%)	763 (62%)	1226
With control	309 (43%)	408 (57%)	717

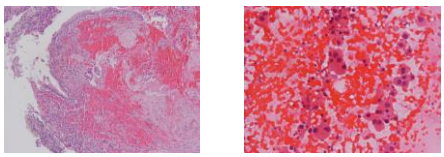
P<0.05



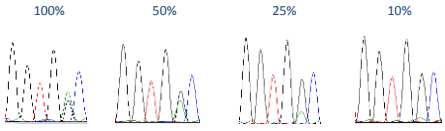
Mutation frequency and tumour cell content

	Mut	Wild-type	Total
<20% tumour cells	7 (26%)	20 (74%)	27 (4%)
>20% tumour cells	296 (44%)	378 (56%)	674 (96%)

P<0.05



% of tumour cells and mutation detection sensitivity



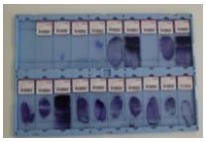
Normal – G, black
Mutation – A, green

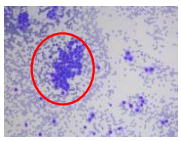
Method sensitivity set at 20% of tumoral cells

Simple protocol for DNA extraction from archival stained FNA smears

1. *Slide Selection and Assessment*
   . For cases with a high tumor content (>20%) the marking of areas of tumors is unnecessary. (1 cell = 6 pg DNA)

2. *Removing the Coverslip*
   . 48-72hs in xylene or substitute



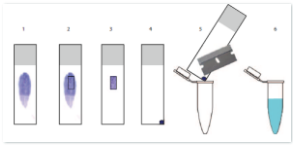


Enrichment by macrodissection if necessary.

Acta Cytologica

Simple protocol for DNA extraction from archival stained FNA smears

3. *Collecting the Tissue*



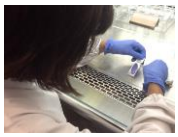
4. *Tissue Lysis and DNA Extraction*

Acta Cytologica 2012;56:632–635

Minimizing Delays in DNA Retrieval: The "Freezer Method" for Glass Coverslip Removal.

. The slide is placed flat in the freezer (at a temperature of -20ºC) for 1-3 minutes.

Minimizing Delays in DNA Retrieval: The "Freezer Method" for Glass Coverslip Removal.



. After removing the slide from the freezer and wearing eye protection, immediately place the tip of a scalpel blade under one corner edge of the coverslip, lift up the coverslip and remove it.

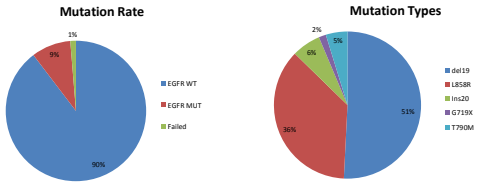
Minimizing Delays in DNA Retrieval: The "Freezer Method" for Glass Coverslip Removal.

. After remove the coverslip allow the now uncoverslipped slide to return to room temperature before soaking in xylene for 1 to 2 minutes until the remaining mounting media has been removed.

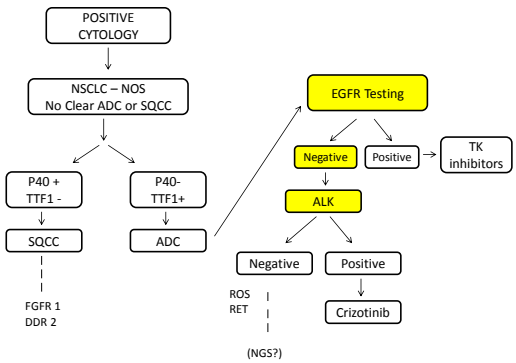
. The slide can be then sent for microdissection for collecting cells for DNA extraction.

EGFR testing on NSCLC at LNS

- 687 cases from 2013-2016



ALGORITHM FOR ANCILLARY TESTING IN NSCLC



Schmitt FC & Machado JC, 2013

ALK gene translocation in NSCLC

- 1-7% of NSCLC patients:
  - younger age and never or light smoking
  - high-stage disease
- Adenocarcinomas (ADCs)
- *ALK*+: no *EGFR* mutations
- Crizotinib: inhibitor of tyrosine kinase activity
  - potential treatment efficacy

Detection of *ALK* rearrangements

- Fluorescence *in situ* hybridization (FISH)
  - *ALK* dual colour break-apart probe (Abbott Molecular)
  - detect rearrangements in chromosome 2p23
- Immunohistochemistry (IHC)
  - detection of the chimeric ALK protein
  - clones: D5F3 (Cell Signaling); 5A4 (Novocastra)
  - rapid and relatively inexpensive
  - bright field microscopy: tumor morphology

Practical aspects of FISH testing for EML4-ALK

- At least 50 tumour cells should be counted for accurate results.
- FISH is considered positive when at least one set of orange and green signals is > 2 signal diameters apart or there is a single orange signal without a corresponding green signal in addition to fused (normal signals).
- A sample is considered negative for ALK rearrangement if there are <5 positive cells (<10%) and positive if there are >25 positive cells (>50%).
- A sample is considered equivocal if 10-50% of cells are positive. In this case a 2nd reader should evaluate the slide and if the average of 2 readings contains at least 15% of positive cells the case is considered positive.

Savic S and Bubendorf L, Acta Cytol 2012; 56: 611-621

Detection of ALK-Positive Non–Small-Cell Lung Cancers on Cytological Specimens

High Accuracy of Immunocytochemistry with the 5A4 Clone

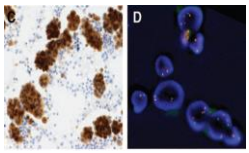
Spasenija Savic, MD, Beata Bode, MD,† Joachim Diebold, MD,‡ Ivo Tosoni, MD,§ Audrey Barascud,* Betty Baschiera,* Bruno Grilli,* Michelle Herzog,* Ellen Obermann, MD,* and Lukas Bubendorf, MD**

J Thorac Oncol. 2013;8: 1004-1011

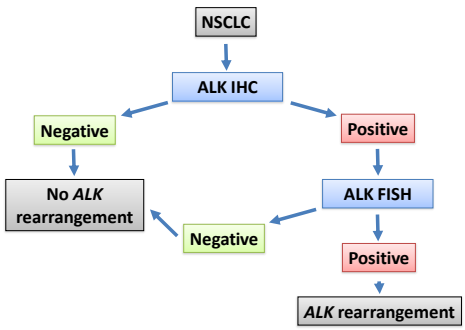
TABLE 1. Correlation between ALK ICC-Staining Intensity and the Final ALK FISH Results

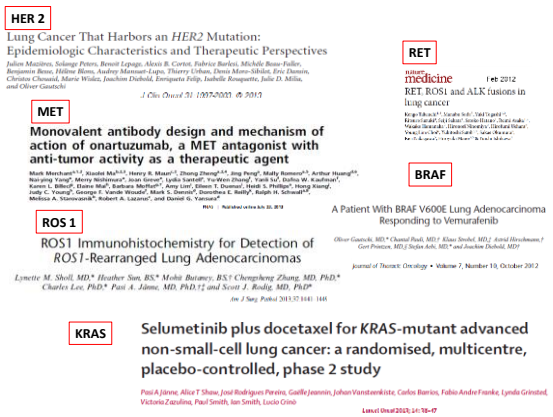
	FISH		
ICC-Staining Intensity	Positive (%)	Negative (%)	Total
0	1 (6.7)	24 (96.0)	25
1+	0	1 (4.0)	1
2+	2 (13.3)	0	2
3+	12 (80)	0	12
Total	15	25	40

ICC, immunocytochemistry; FISH, fluorescence in situ hybridization.



Diagnostic algorithm for ALK testing





HER 2

Lung Cancer That Harbors an *HER2* Mutation: Epidemiologic Characteristics and Therapeutic Perspectives

RET

nature medicine

RET, ROS1 and ALK fusions in lung cancer

MET

Monovalent antibody design and mechanism of action of onartuzumab, a MET antagonist with anti-tumor activity as a therapeutic agent

BRAF

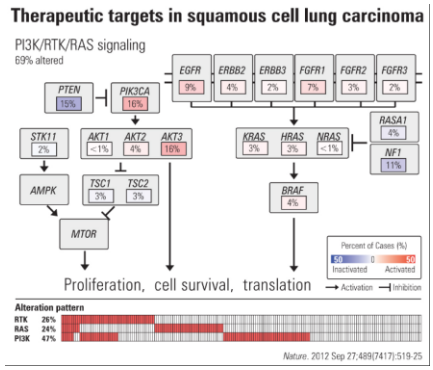
A Patient With BRAF V600E Lung Adenocarcinoma Responding to Vemurafenib

ROS 1

ROS1 Immunohistochemistry for Detection of *ROS1*-Rearranged Lung Adenocarcinomas

KRAS

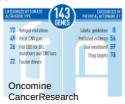
Selumetinib plus docetaxel for *KRAS*-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study



3 tier approach to NGS in oncology

- Clinical
  - Focused Panels detecting defined clinically relevant alterations (SNVs, CNVs, InDels, Fusion Transcripts)

- Translational
  - Broad Panels detecting a large number of defined alterations covering the drug development (oncology pipeline) spectrum (recruitment for clinical trials)



- Exploratory
  - Exome Sequencing (T/N)
  - RNAseq
  - WGS (T/N)

Cytology specimens are suitable for NGS

- Smears (both PAP & Diff-Quik), cell blocks and LBC.
- Studies have been successful using:
  - different sequencing platforms (Illumina & Life Technologies)
  - different enrichment strategies (PCR amplification & hybridization capture)
- Cytology specimens used for NGS capable of:
  - Identifying targets for therapeutic agents
  - Increase accuracy of diagnoses

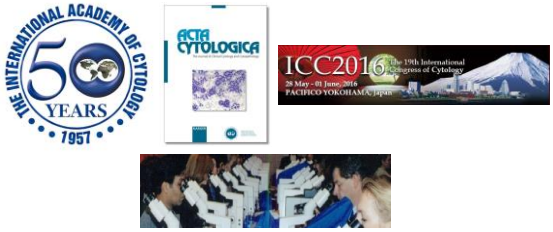
Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

Key Points
PRACTICAL APPLICATIONS OF MOLECULAR TESTING

1. All patients with lung ADC should be tested for EGFR activating mutations and ALK rearrangements. Patients should not be excluded from testing based on clinical features.
2. EGFR mutation and ALK rearrangement testing should be ordered at the time of diagnosis for patients presenting with advanced stage or at time of recurrence/progression in patients who originally presented with lower-stage disease but were not previously tested.
3. Where an ADC component cannot be completely excluded, for example, due to limited cellularity (tissue biopsy or cytology), EGFR and ALK testing may be performed in cases showing squamous or small cell histology.
4. To determine EGFR and ALK status, primary or metastatic tumors are equally suitable for testing.
5. Pathologists should determine the specimen adequacy for EGFR testing by assessing cancer cell content. Laboratories may use any validated testing methods, but tests that are able to detect DNA alterations in samples with as few as 20% tumor cells are recommended. Microdissection for tumor cell enrichment should be performed when needed.

Conclusions

- Small samples obtained by minimally invasive methods exploited morphological and molecular information are the model biopsies of the future.
- Various techniques using different types of cytological samples and preparations have shown promising results, with similar or higher accuracy and sensitivity when compared with surgical specimens.
- Presently, with the rapid development of personalized treatment every lung carcinoma should be tested for available biomarkers and every effort should be made to spare the tissue for molecular testing.



CENTRALOFFICE @CYTOLOGY-IAC.ORG