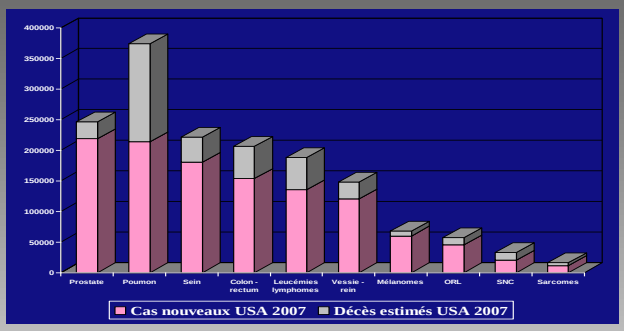
Histopathological classification and molecular pathology in lung cancers: the challenge of small specimens in routine practice

Prof. Sylvie Lantuéjoul,
Département de Pathologie- Plateforme de Génétique Moléculaire des cancers
CHU Grenoble

Lung cancers are the first cause of death by cancer worldwide



75% are Non-Small Cell lung carcinoma- 25% are Small Cell Lung Carcinoma

75% are diagnosed at advanced stage diseases

Improvement of the early detection of lung cancer

Definition of new biomarkers as therapeutic targets

→ ***New molecular approaches, taking into account the histological heterogeneity of lung tumors***

from Herbst, NEJM 2008

	Squamous cell carcinoma	Adenocarcinoma
Mutation KRAS	Rare	10-30%
Mutation BRAF	3%	2%
EGFR		
Mutation	Rare	10-40%
Amplification	30%	15%
Variant III mutation	5%	rare
HER2		
Mutation	Rare	4%
Amplification	2%	6%
ALK (fusion)	Rare	7%
Met		
Mutation	12%	14%
Amplification	21%	20%
TTF1 amplification	15%	15%
LKB1 mutation	19%	34%
PIK3CA		
Mutation	2%	2%
Amplification	33%	6%

Why and how using the WHO classification of lung tumors on small specimens ?

- **WHO classification was made from and for surgical specimens**
- **85% of specimens for diagnosis are non-surgical ones (biopsies or cytological specimens)**
- **Need to identify some entities with specific prognosis**
- **Targeted therapies are restricted to non-squamous carcinoma (TKI), as well as Pemetrexed and Bevazucimab**

WHO classification of lung tumors 2004

1.3.1 Squamous cell carcinoma

1.3.2 Small Cell Lung carcinoma

1.3.3 Adenocarcinoma

1.3.4 Large cell carcinoma

1.3.5 Adenosquamous carcinoma

1.3.6 Sarcomatoid carcinoma

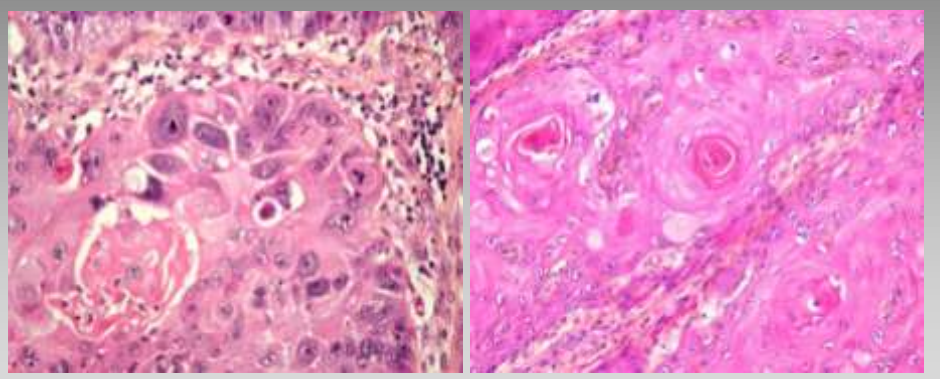
1.3.7 Carcinoid tumors

1.3.8 Salivary gland type carcinoma

1.3.9 Unclassified carcinoma

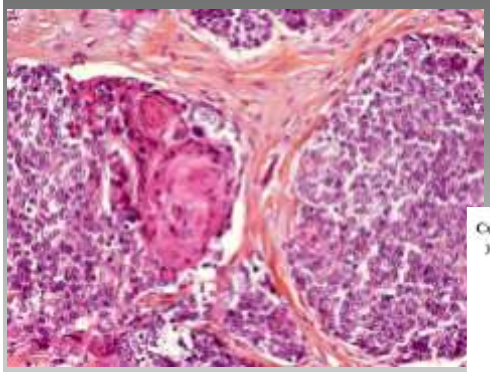
Squamous cell carcinoma

Malignant epithelial tumor with squamous differentiation (pearls of keratin and/or intercellular bridges)

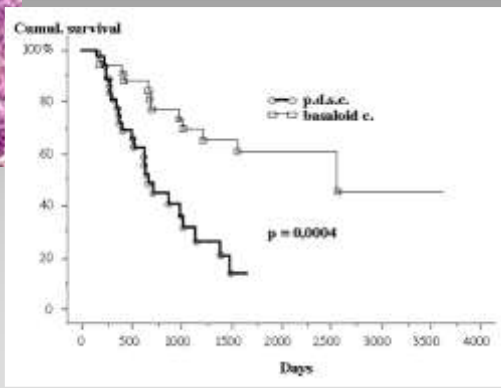


(Variants: papillary, clear cells, small cells,)

Basaloid carcinoma: SCC variant



Moro-Sibilot et al ERJ 2007



IASLC/ATS/ERS International Multidisciplinary Classification of Lung Adenocarcinoma

Preinvasive lesions
Atypical adenomatous hyperplasia
Adenocarcinoma in situ (≤3 cm formerly BAC)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Minimally invasive adenocarcinoma (≤3 cm lepidic predominant tumor with ≤5 mm invasion)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Invasive adenocarcinoma
Lepidic predominant (formerly nonmucinous BAC pattern, with >5 mm invasion)
Acinar predominant
Papillary predominant
Micropapillary predominant
Solid predominant with mucin production
Variants of invasive adenocarcinoma
Invasive mucinous adenocarcinoma (formerly mucinous BAC)
Colloid
Fetal (low and high grade)
Enteric

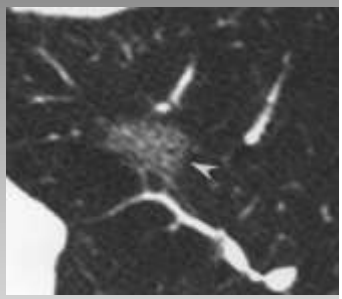
BAC, bronchioloalveolar carcinoma; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

Journal of Thoracic Oncology
February 2011

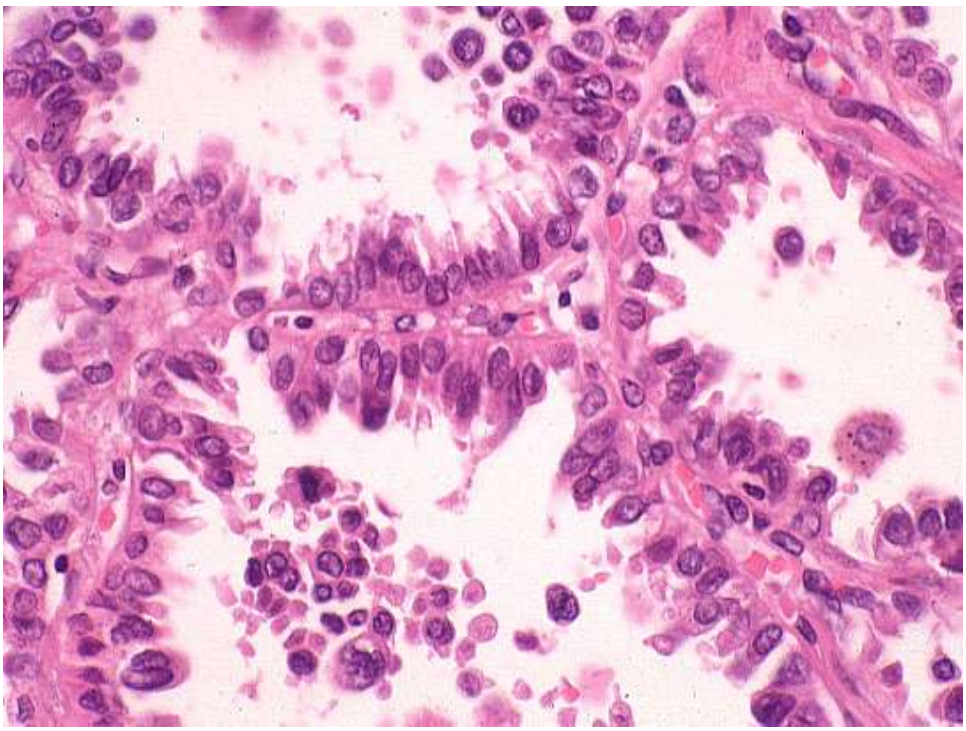
Adenocarcinoma *In situ* AIS with lepidic growth pattern (ex bronchiolo-alveolar carcinoma BAC)

2 to 6 % of NSCLC

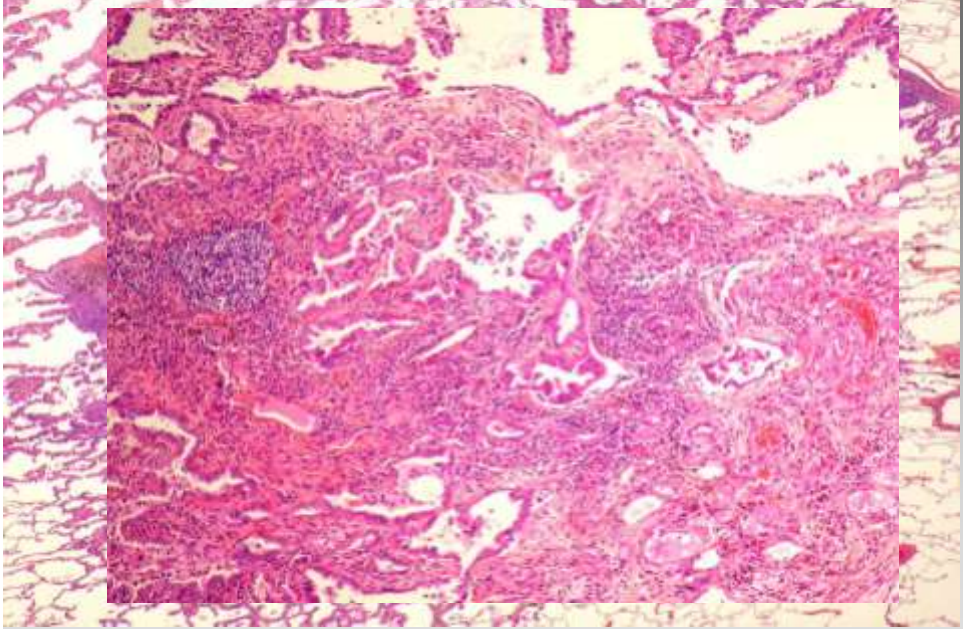
- **≤ 3 cm**
- **respect of pulmonary architecture/lepidic growth**
- **Tis**
- **stades I (p < 0,001)**
- **No lymph node metastasis (p < 0,001)**
- **100% 5 yr survival (p < 0,005)**

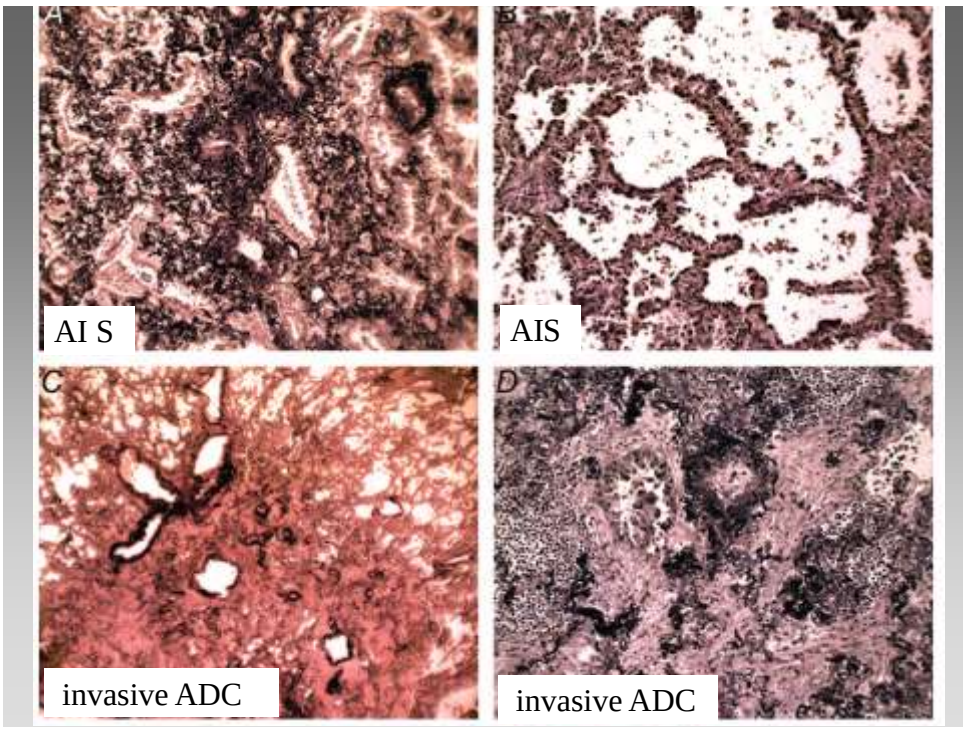


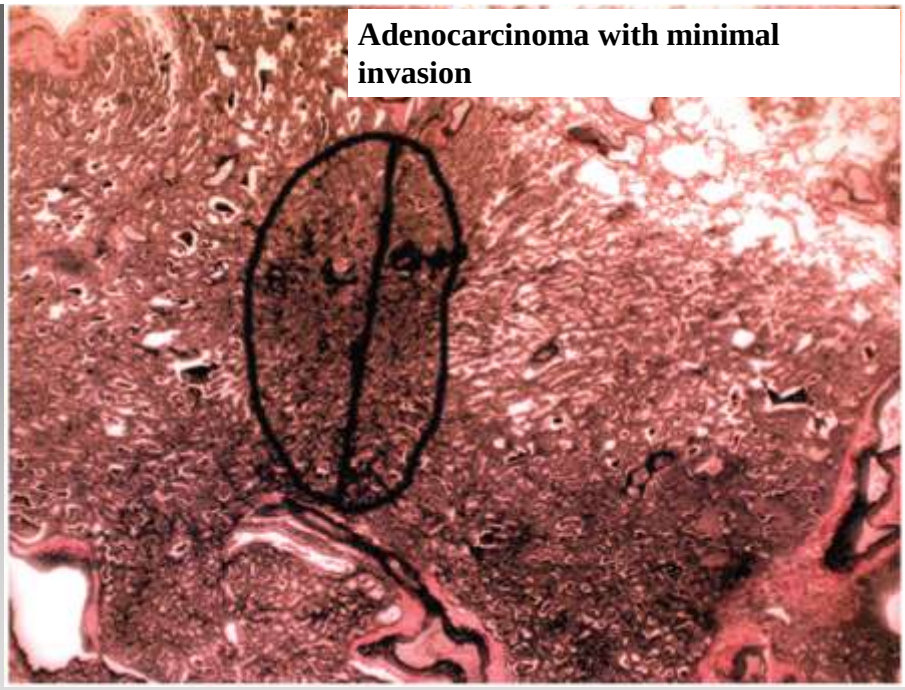
Noguchi et al Cancer 1995
Yokose et al Lung Cancer 2000
Suzuki et al Ann Thorac Surg 2002



Minimally invasive adenocarcinoma MIA (≤5mm or invasion < 10%) T1 mi: 100% 5yr survival



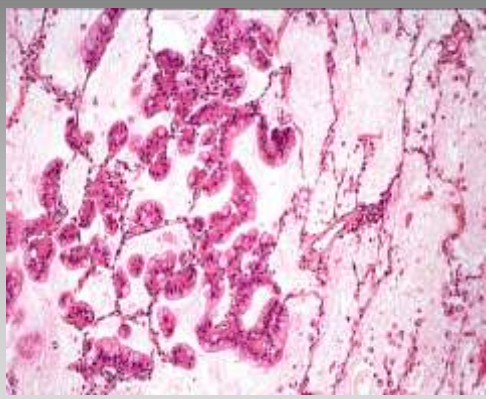


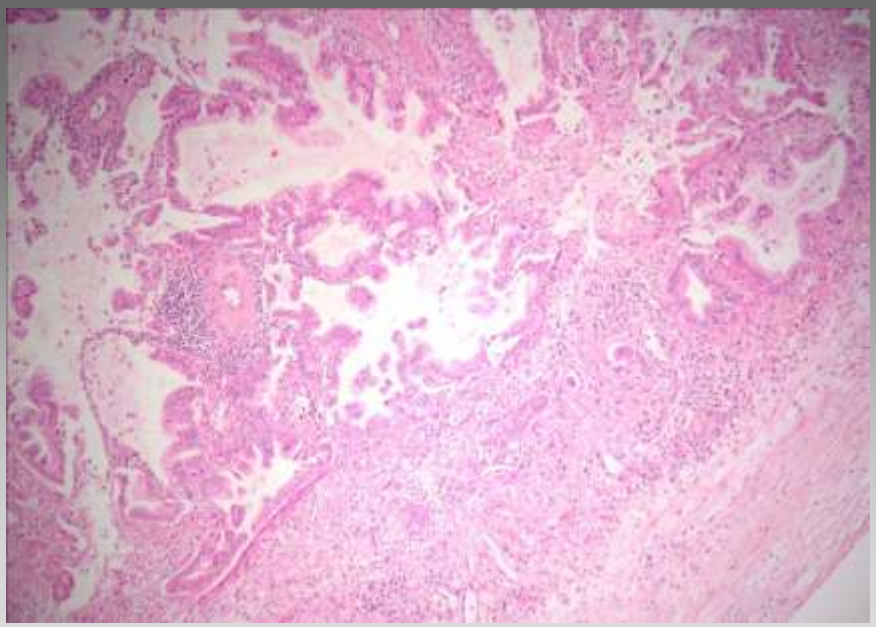


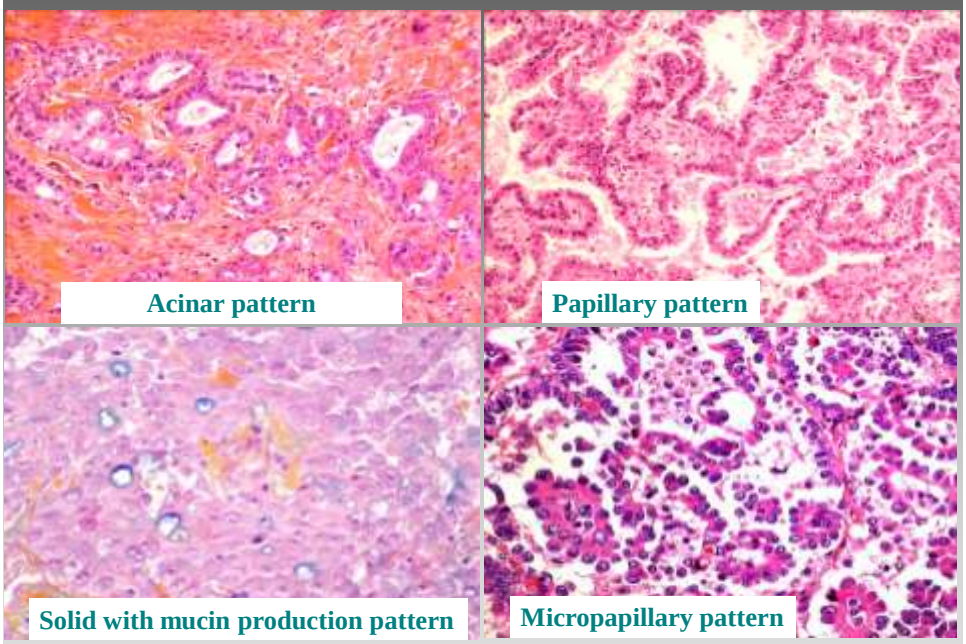
Russell et al 2011

Mucinous adenocarcinoma (ex mucinous BAC)

- **Frequently invasive**
- **58% women**
- **45% smokers**
- **often multifocal**
- **CK7, CK20 +**
- **TTF1 neg**
- **76% K-ras mutations**



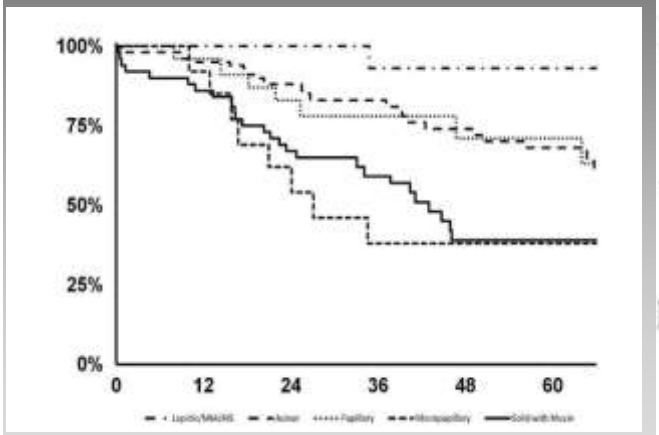


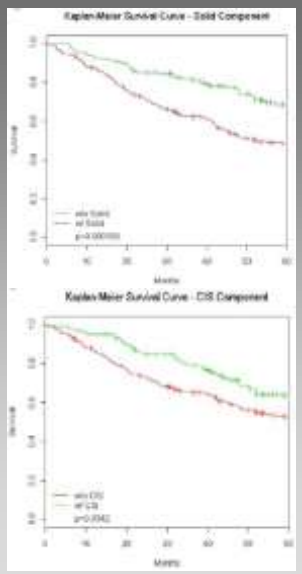

Acinar pattern
Papillary pattern
Solid with mucin production pattern
Micropapillary pattern

Does Lung Adenocarcinoma Subtype Predict Patient Survival?

A Clinicopathologic Study Based on the New International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society International Multidisciplinary Lung Adenocarcinoma Classification

Russell et al, Journal of Thoracic Oncology Sept 2011

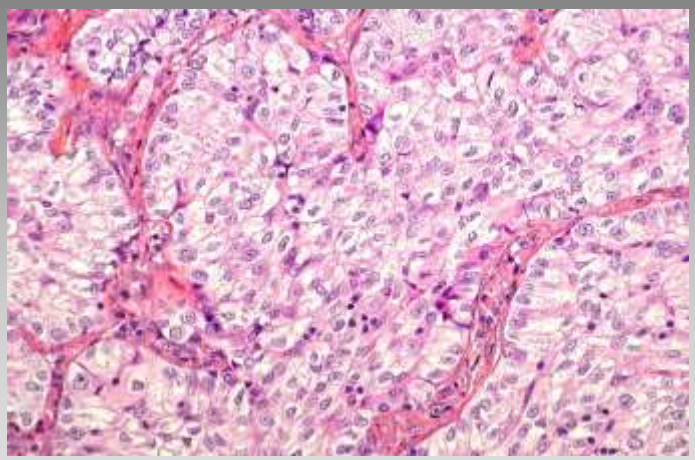




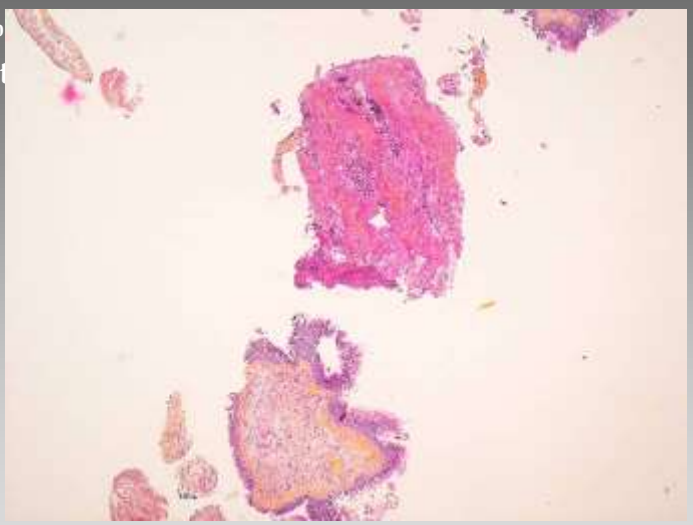
Bryant et al, PLoS One 2010

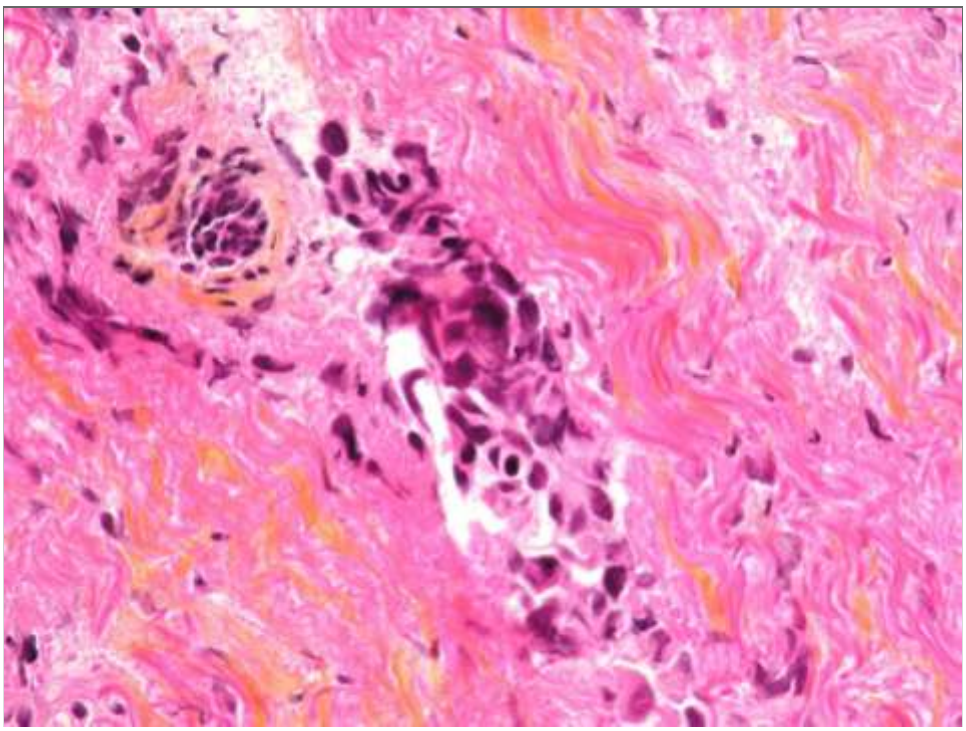
Large cell carcinoma

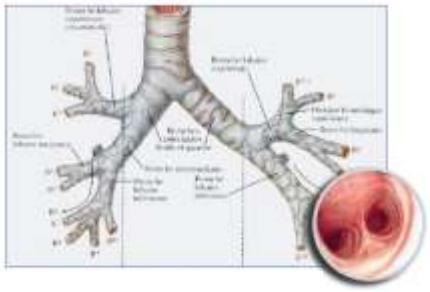
Diagnosis of exclusion (absence of squamous, glandular or ne neuroendocrine differentiation)



- 2%
- 20 t

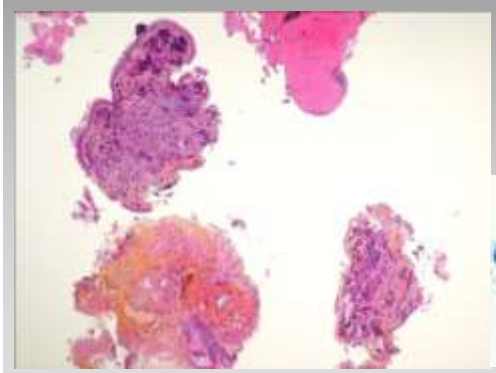


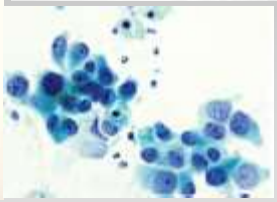


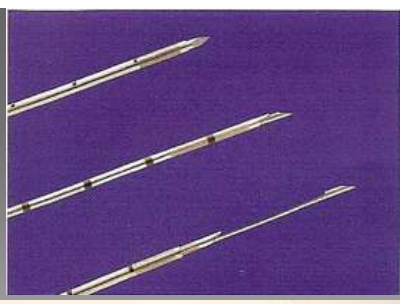


Bronchoscopy

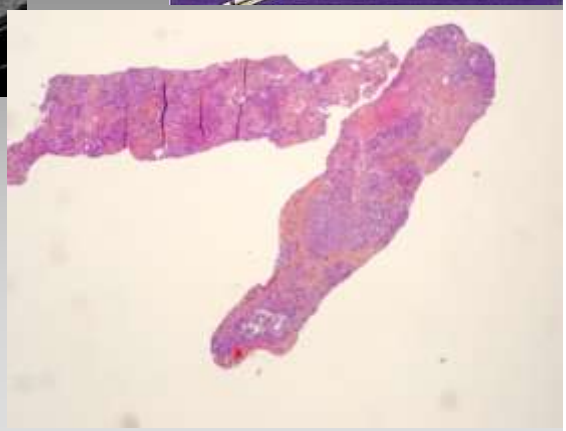
EBUS TBNA







CT scan guided transthoracic biopsy (or FNA)



Number of biopsies and % of malignant cells

TABLE 2. Flexible Bronchoscopic Biopsy Samples: Tissue Fragment Numbers, Area of Sample (%) Replaced by Tumor, and Histological Type

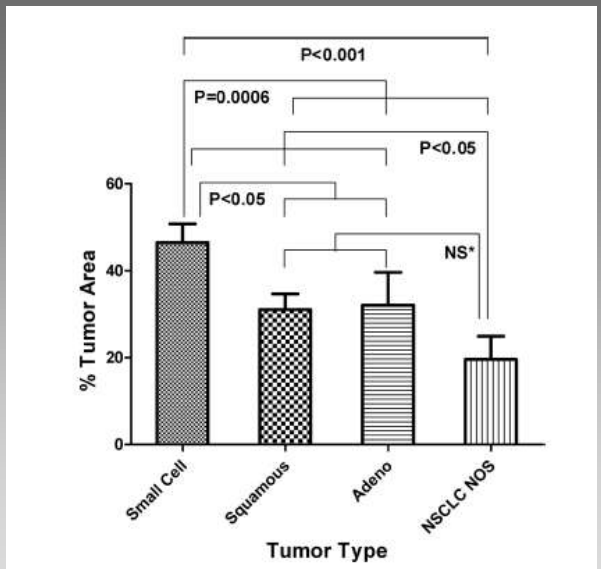
	All Cases	Small Cell	All NSCLC	Adenocarcinoma	Squamous	Non-small Cell, NOS
No. of cases	98	28	70	11	37	20
Total no. of fragments						
Mean (range)	3.9 (1–14)	3.4 (1–5)	4.2 (1–14)	4.6 (3–8)	4.1 (1–14)	3.9 (1–7)
Median number	4	4	4	4	4	4
Sum	386	96	290	51	152	79
Fragments with tumor						
Mean (range)	2.8 (1–8)	2.9 (1–5)	2.7 (1–8)	3.3 (1–6)	2.8 (1–8)	2.0 (1–5)
Median number	3	3	2	3	2	2
Sum	272	83	189	36	105	40
Area of tumor						
Mean (range)	33.4% (0.1–94)	46.5% (0.1–90)	28.2% (0.1–94)	32.1% (3–94)	31.1% (1–89)	19.6% (0.1–74)
Median	28%	40%	22%	23%	27%	10%

The "all NSCLC" group includes the adenoid cystic and large cell neuroendocrine carcinoma case.
NSCLC, non-small cell lung cancer; NOS, not otherwise specifiable.

Median nb of fragments : 4 (1- 14!)
Median nb of fragments with tumor: 2 to 3
% of malignant cells: 10 to 49%

Coghlin CL et al. J Thorac Oncol. 2010

% of malignant cells and histological subtyping

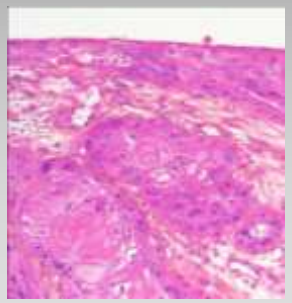


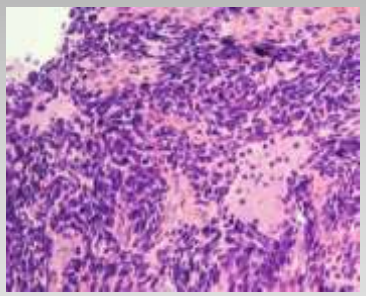
Coghlin CL et al. J Thorac Oncol. 2010

How using the WHO classification on small specimens

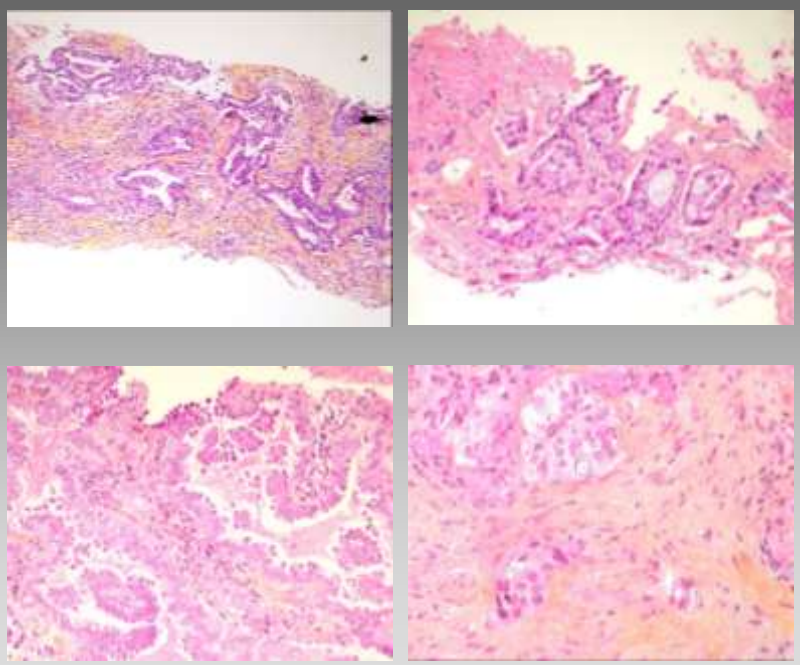
- **if criteria of differentiation (squamous, glandular) are present:**
  - **how reporting the case ?**
- **if criteria are absent:**
  - **how do we call the proliferation ?**
  - **what are the additional tools we could use ?**

SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	*Morphologic squamous cell patterns clearly present:* Squamous cell carcinoma
No 2004 WHO counterpart	*Morphologic squamous cell patterns not present (supported by stains):* Non-small cell carcinoma, favor squamous cell carcinoma
SMALL CELL CARCINOMA	Small cell carcinoma

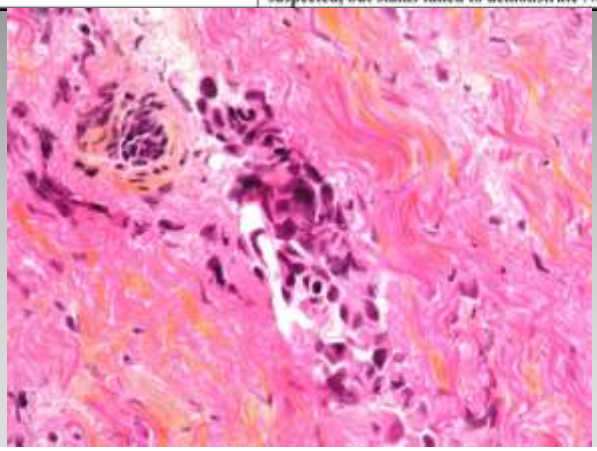




2004 WHO Classification	SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	*Morphologic adenocarcinoma patterns clearly present:* Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification) Comment: If pure lepidic growth – mention an invasive component cannot be excluded in this small specimen
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloid pattern
Signet ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
No 2004 WHO counterpart – most will be solid adenocarcinomas	*Morphologic adenocarcinoma patterns not present (supported by special stains):* Non-small cell carcinoma, favor adenocarcinoma



LARGE CELL CARCINOMA	Non-small cell carcinoma, not otherwise specified (NOS)
Large cell neuroendocrine carcinoma (LCNEC)	Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNEC
Large cell carcinoma with NE morphology (LCNEM)	Non-small cell carcinoma with NE morphology (negative NE markers) – see comment Comment: This is a non-small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.



Aditional tools for subtyping NSCLC

Mucin stains (D PAS, Alcian Blue)
Immunohistochemical markers: TTF1, napsin, CK5-6, P63, desmocollin 3, desmogleine, etc....

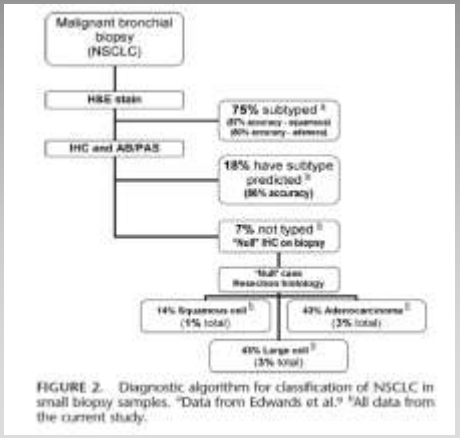
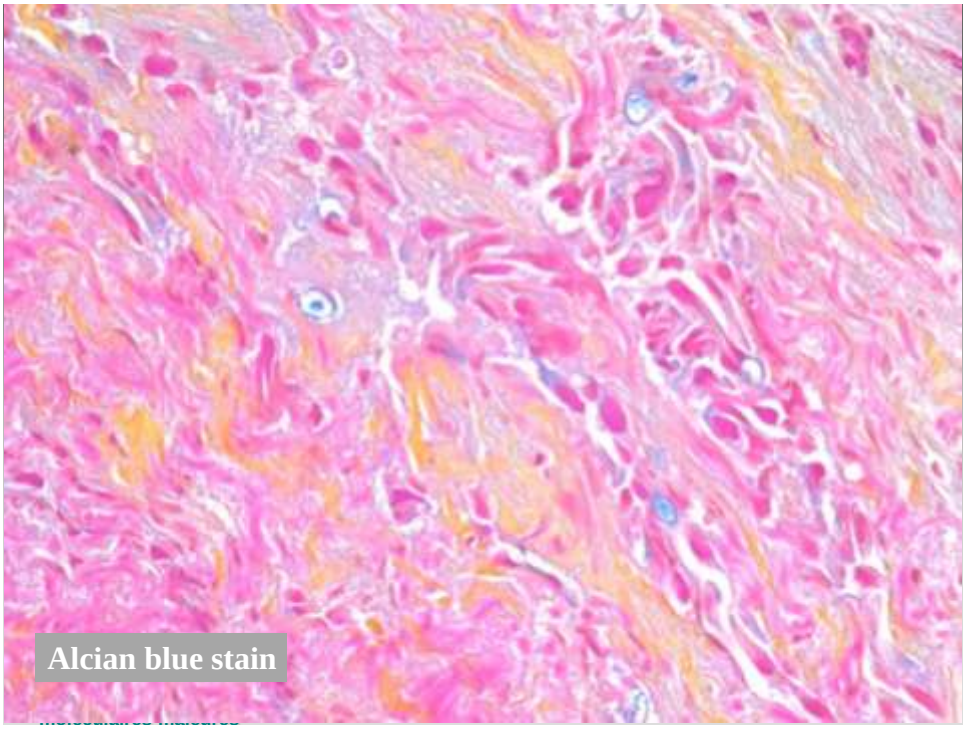


FIGURE 2. Diagnostic algorithm for classification of NSCLC in small biopsy samples. [a]Data from Edwards et al.[9] [b]All data from the current study.

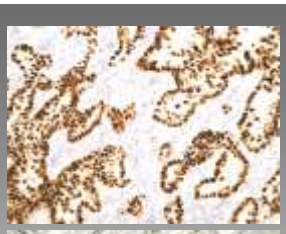
75% → 93% of sub-typed NSCLC

Loo, PS et al. J Thoracic Oncol, 2010

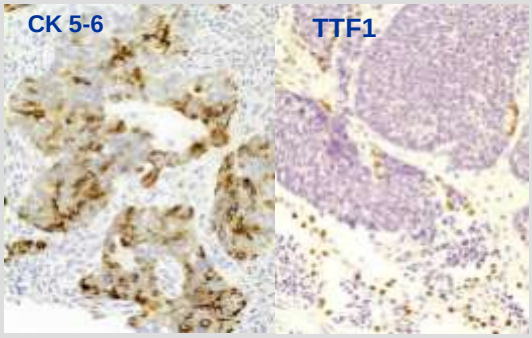


Panel of immunohistochemical markers

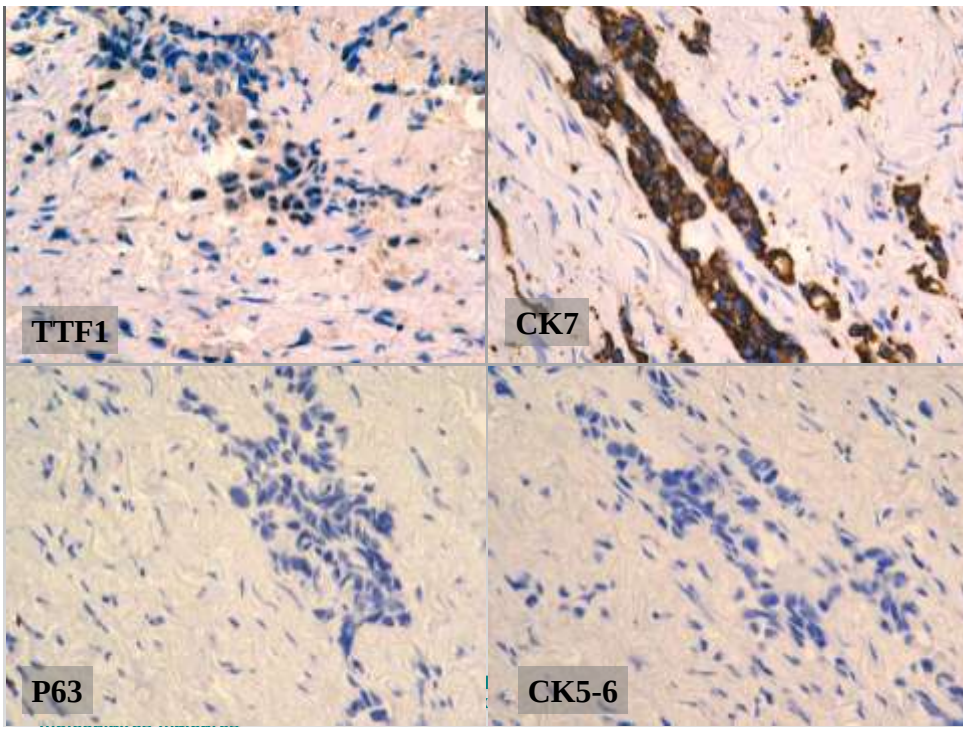
- **TTF1 (Thyroid Transcription Factor - one)**
  for surfactant proteins and thyroglobulin
  85% of pulmonary adenocarcinoma
- **P63**
  Development and differentiation of stratified squamous epithelium
- **Cytokeratines 5-6:**
  Basal cells of squamous epithelium



- **Adenocarcinoma**
  - 0 - 30 % P63 +
  - 20% CK 5 - 6 +
  - 80% TTF1 +
- **SCC**
  - 97 % P63 +
  - 100% CK 5 - 6 +
  - < 5% TTF1 +

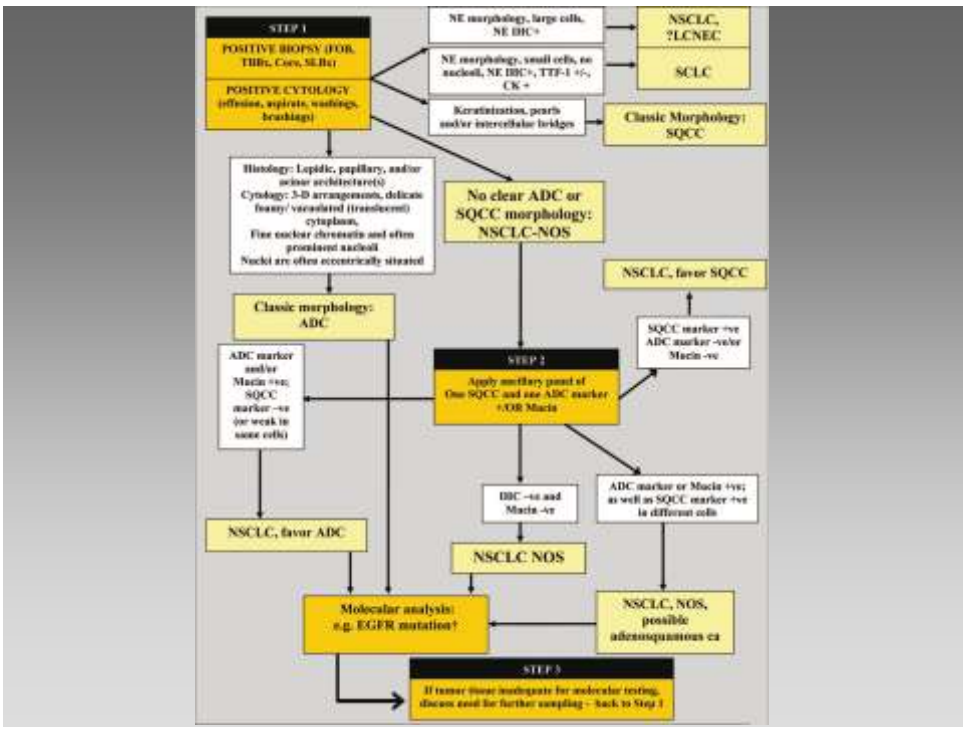


100% of specificity and 82% of sensitivity Kargi et al 2007



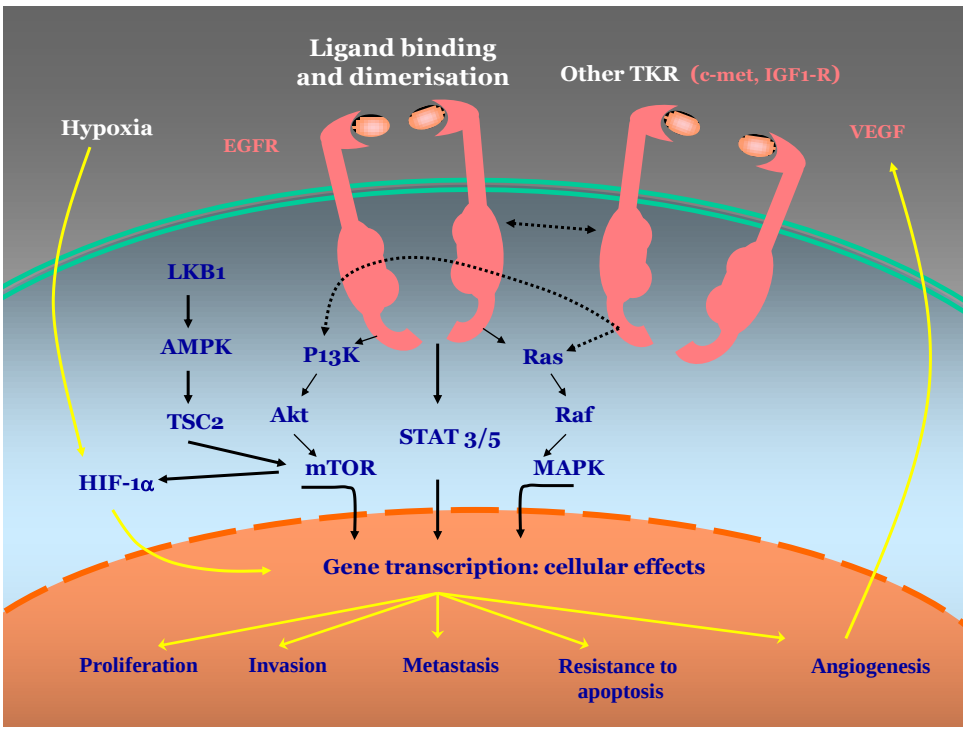
Usefulness of cytology in subtyping ?

- **Good when associated with histology ++++:**
  - **11% of non- subtyped NSCLC with cytology alone**
  - **6 % with histology alone**
  - **4% using both**
- **Cell blocks +++: 98% are available for EGFR/ras mutations**



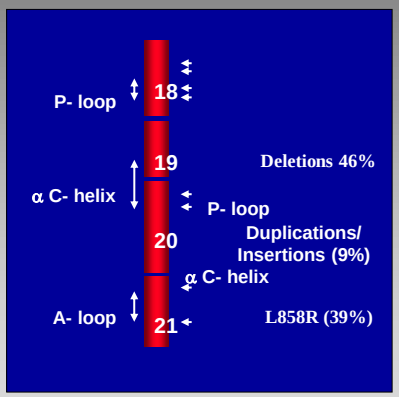
from Herbst, NEJM 2008

	SCC	ADC
Mutation KRAS	Rare	10-30%
Mutation BRAF	3%	2%
EGFR		
Mutation	Rare	10-40%
Amplification	30%	15%
Variant III mutation	5%	rare
HER2		
Mutation	Rare	4%
Amplification	2%	6%
ALK (fusion)	Rare	7%
Met		
Mutation	12%	14%
Amplification	21%	20%
TTF1 amplification	15%	15%
LKB1 mutation	19%	34%
PIK3CA		
Mutation	2%	2%
Amplification	33%	6%



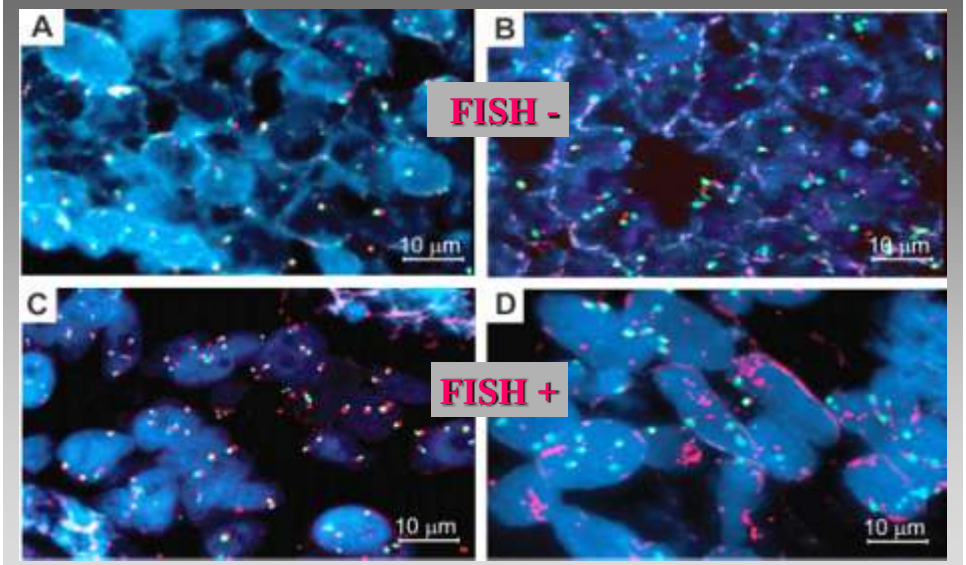
Epidermal Growth Factor Receptor EGFR

- **EGFR mutation : exon 19 deletion and L858R exon 21 mutation**
- **Predictive of TKI response in adenocarcinoma, in women, non-smokers (Lynch et al, Paez et al 2004)**
- **10 to 16% NSCLC USA-Europe- 30-50% East Asia**
- **Lepidic or papillary histology (TRU origin)**
- **Resistance mutations T790M, exon 20insertion**



Response to Gefitinib correlated to the EGFR copy nb

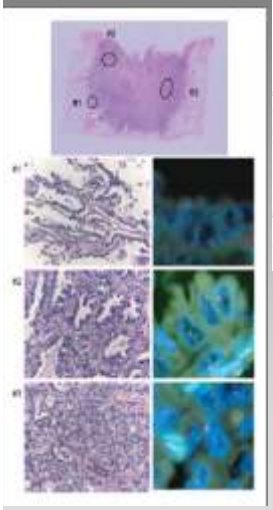
Cappuzzo et al 2005



High chromosome 7 polysomy **Gene amplification**

Epidermal Growth Factor Receptor Gene Amplification Is Acquired in Association with Tumor Progression of *EGFR*-Mutated Lung Cancer

Yasushi Yatabe,[1] Takashi Takahashi,[3] and Tetsuya Mitsudomi[2] Cancer Res 2008

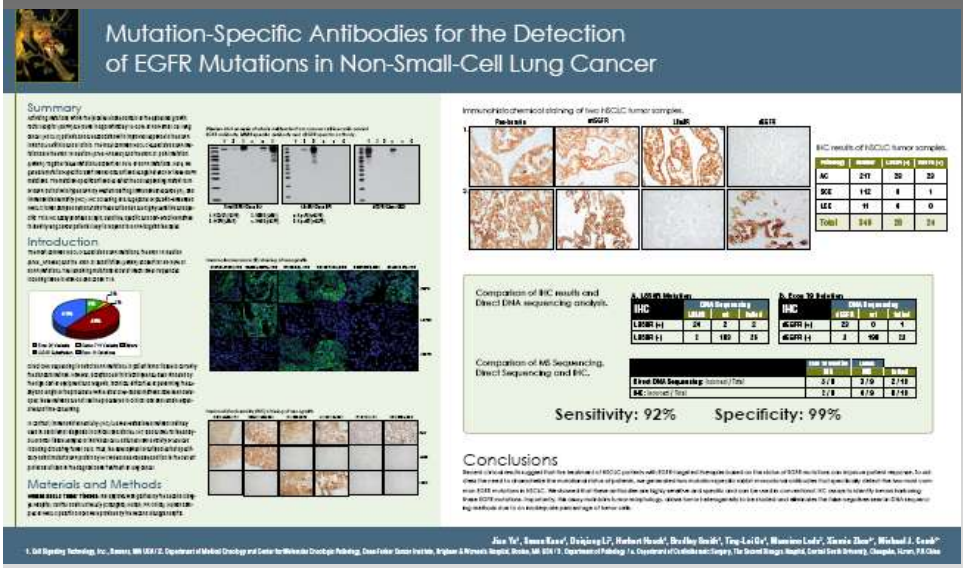


		EGFR	
	Total	Mutation (%)	Amplification (%)
Preneoplastic lesion (AAH)	17	3 (18)	0 (0)
In situ lesion (BAC)	21	7 (40)	1 (5)
Minimally invasive adenocarcinoma	23	14 (61)	2 (9)
Overtly invasive adenocarcinoma*	46	28 (61)	4 (9)

*For this category, adenocarcinomas with subsequent recurrence were selected.

- **EGFR amplification associated with EGFR mutation (Del 19)**
- **with EGFR hyper-expression**
- **with invasive phenotype and solid areas**
- **with a poor prognosis** Sholl et al 2009, Soh et al 2008

Abs 2085 (del 746-750) et 3197 (L858R exon 21) Cell Signaling Technology®



Yu et al, Clin Cancer Res 2009

	Nb de patients	Pays H:F, Fumeurs	Del 19 Se. %	Del 19 Spe. %	L858R Se. %	L858R Spe. %
Yu et al **	340	Chine	92	99	100	88
Brevet et al *	194	USA	85	99	95	99
Kato et al 2010 *	70	Japon	82	100	75	96
Kitamura et al *	238	Japon 36:34 41%	40	99	36	97
Kosu et al 2010 &	577	Japon 319:258 40%	42	99	76	98
Simonetti et al 2010 @	78	Espagne 49:29 42%	63	100	100	100

* TMA; & HRMA; @ microdissection avant seq.; ** Direct seq / MS based seq.

- 10% of false positive with L858RmAb
- 25% of false negative with Del 19 mAb (E746-A750)
- 2 /7 detected mutations with L858R mAb are resistance exon 20 mutations

Kitamura et al 2010

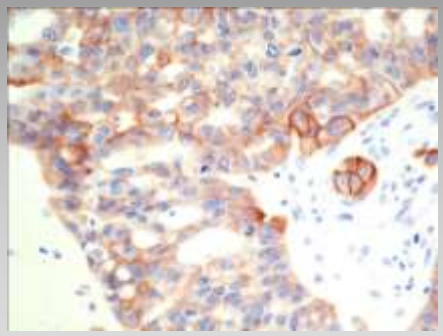
- Multivariate analysis of TKI response:
  - depending on IH or FISH: NS
  - depending on mutations (MB): p< 0,001

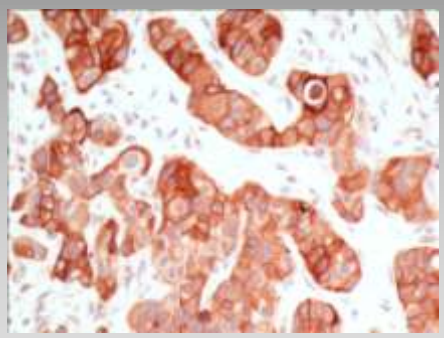
Li et al 2011

145 cases (biopsies and surgical specimens) : IH in comparison with EGFR mutations (pyrosequencing)

L858R	scores 2.3+	Mb + cytopl.
Se.	91%	62%
Spe.	62%	100%

Del 19	scores 2.3+	Mb + cytopl.
Se.	79%	50%
Spe.	70%	90%



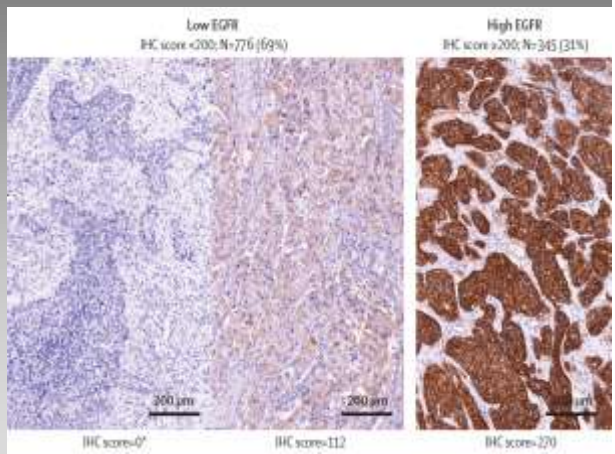


Which threshold and score: > 10% ? 3 + ? Which staining?

EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study

Pirker et al, Lancet Oncology 2011

- **1125 metastatic patients NSCLC (ADC and SCC)**
- **Linear mb staining (0-3 +) DAKO Pharm DX Kit**
- **Scoring of EGFR expression : 1(% cell +) + 2 (% cell ++) + 3(% cell +++)**
- **Score ≥ 200 = high level of EGFR niveau d'expression (31%)**
- **Correlated with increased survival with cetuximab**



C-met

- **Activation of c-Met and its ligands HGF favors proliferation, angiogenesis and invasion**
- **Its hyper-expression associated with advanced stage diseases and a shorter survival**
- **Papillary histology (TTF1, MUC1, CK7) et co-activated with EGFR and Her 2**
- **C-Met amplification in 3% adenocarcinoma and responsible for 10 to 20% of TKI resistance aux TKI via HER3 activation**

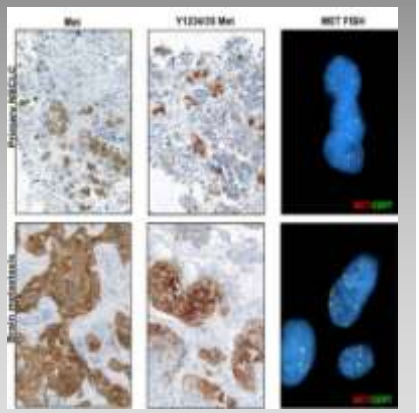
Engelman et al 2007, 2008, Matsubara et al 2010

Met activation in non-small cell lung cancer is associated with de novo resistance to EGFR inhibitors and the development of brain metastasis

Benedettini E, Sholl LM, Peyton M, Reilly J, Ware C, Davis I, Vena N, Bailey D, Yeap BY, Fiorentino M, Ligon AH, Pan BS, Richon V, Minna JD, Gazdar AF, Draetta G, Bosari S, Chirieac LR, Lutterbach B, Loda M

Am J Pathol 2010

- **Phopshorylated c- Met associated with progression under TKI**
- **Associated with de novo resistance**
- **Associated with brain metastases**
- **Interest of associating in p-C Met positive tumors TKI and c-met inhibitors ?**



HER2 - Braf

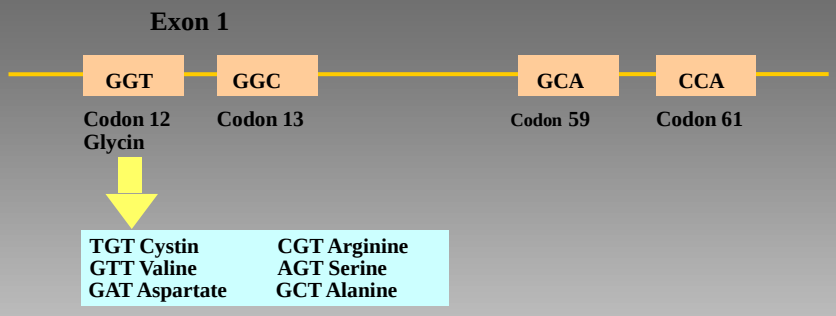
- **HER2**
  - **Mutations in 2% ADC**
  - **Non-smokers, Asia, Acinar and papillar pattern**
  - **Co-mutated /expressed with EGFR, with a shorter OS but a higher gefitinib response**

Brabender et al 2001

- **Braf**
  - **Mutations dans 3% NSCLC (3 à 4.9% ADC)**
  - **V600E (50%), G469A (39%), D594G (11%)**
  - **Smokers, solid and acinar histology : non V600E**
  - **Women, micropapillary histology and poor prognosis: V600E**
  - **Sensitivity to MEK inhibitors**

Capuzzo et al 2005, Daniele et al 2007; Marchetti et al 2011, Paik et al 2011

K-Ras mutations



Activating mutations in codons 12,13 or 61

Mutually exclusives with EGFR

Associated with smoking (transv. G to T ou C)

22% of ADC (very rare in SCC)

Can occur in 15% of non- smokers (transition G to A)

- **Ras mutations associated with primary TKI resistance**

TABLE 1. ANALYSES OF *KRAS* MUTATIONS AND EFFICACY OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS IN NON–SMALL CELL LUNG CANCER

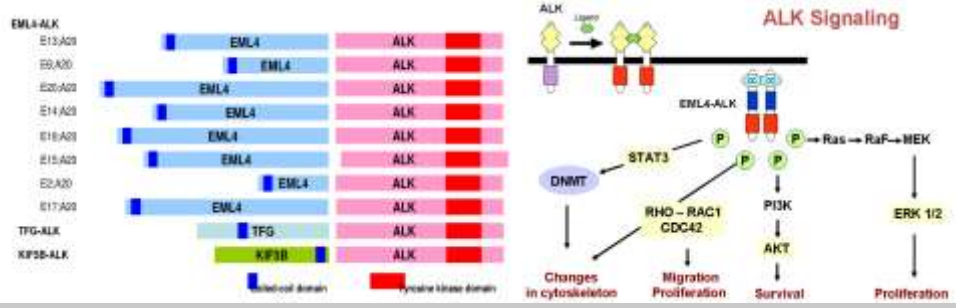
Author	Drugs	Patients Tested for *KRAS* Mutations (*Total Number Mutant*)	Response Rate in *KRAS* Mutant
Pao (2)	Gefitinib/erlotinib	59 (9)	0%
Jackman (47)	Erlotinib	41 (6)	0%
Zhu (48)	Erlotinib	206 (30)	5%
Miller (49)	Erlotinib	80 (18)	0%
Massarelli (50)	Gefitinib/erlotinib	70 (16)	0%
Hirsch (51)	Gefitinib	138 (36)	1%
Hirsch (52)	Gefitinib	152 (12)	0%
Han (53)	Gefitinib	69 (9)	0%
Van Zandwijk (54)	Gefitinib	15 (3)	0%
Fujimoto (55)	Gefitinib	31 (7)	0%
Felip (56)	Erlotinib	39 (7)	0%

- **Amplification at 12p12.1**
  - **Late event, in 12% of mutated ADC**
  - **Associated with a worse survival**

Riely et al 2008, Pao et al 2005, Marks et al 2008
Soh J et al, WCLC 2009, Dacic et al USCAP 2011

EML4-ALK fusion oncogene

2p 21 or p 23 inversion and fusion between Echinoderm Microtubule associated protein Like -4 and intracellular domaine of Anaplasic Lymphoma Kinase



3 - 7% NSCLC, ~30% ADC EGFR & KRAS non mutated

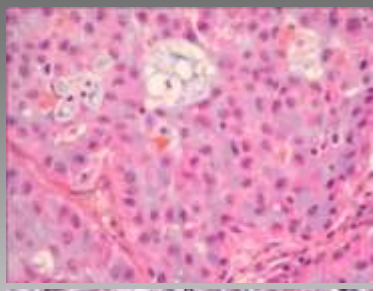
Young patients (M= F), non or light smokers (≤ 15PA), advanced stages

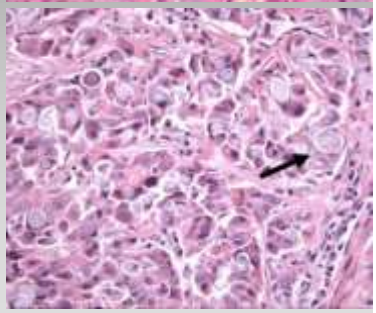
No response to TKI/ Response to crizotinib

D'après Horn and Pao, JCO 2009, Sasaki et al 2010

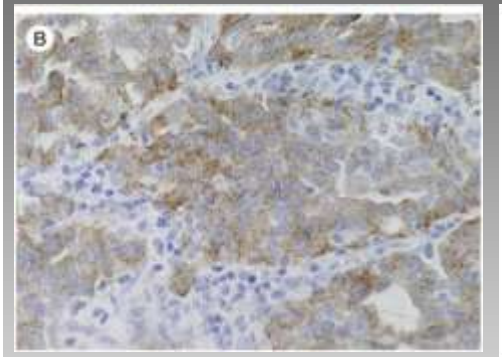
Histological findings of ALK rearranged ADC

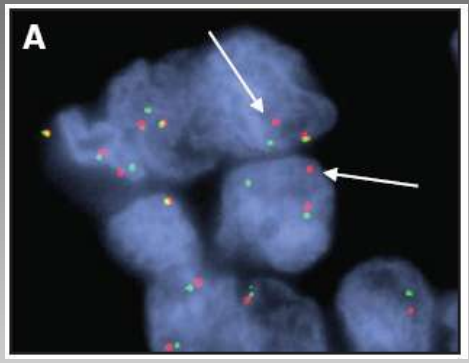
- 54 cases (Yoshida et al JTO 2011)
- Predominant architectural component:
  - 16 papillary or micropapillary
  - 25 acinar
  - 12 solid
- 80% cribriform features
- 69% with extracellular mucines
- 65%with « signet ring « cells
- Necrosis, embols, psammoma
- 72% TTF1+
- 54% P63 +(> 50% cells)



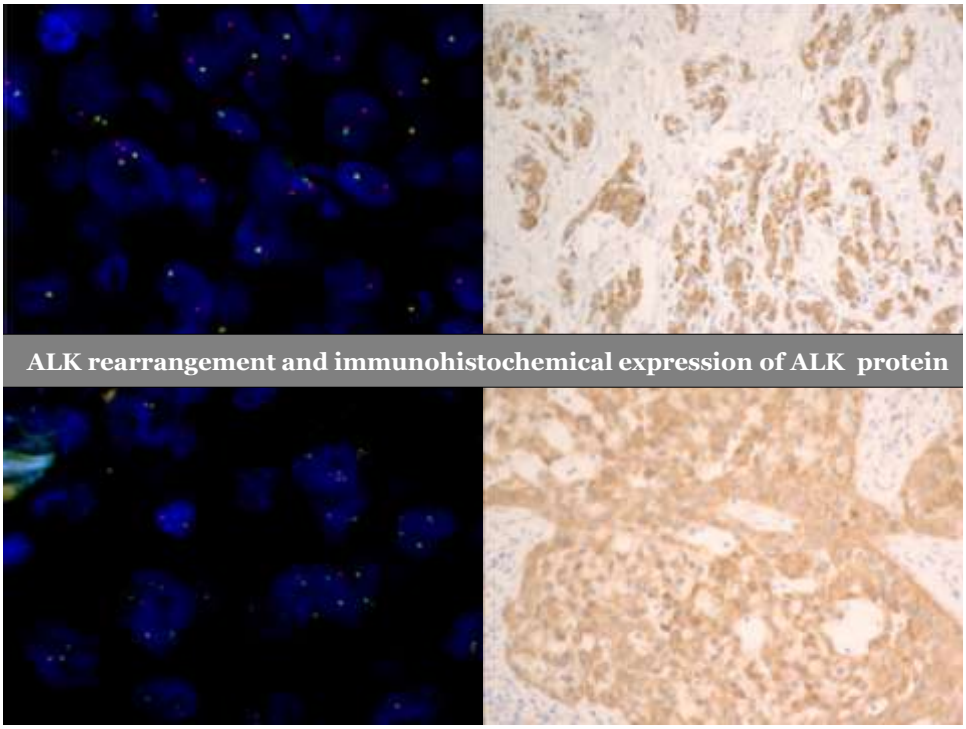


IHC, FISH or RT-PCR multiplex ?





⇨ **double -testing recommended** (Rodig et al, 2009; Shaw et al, JCO 2009)



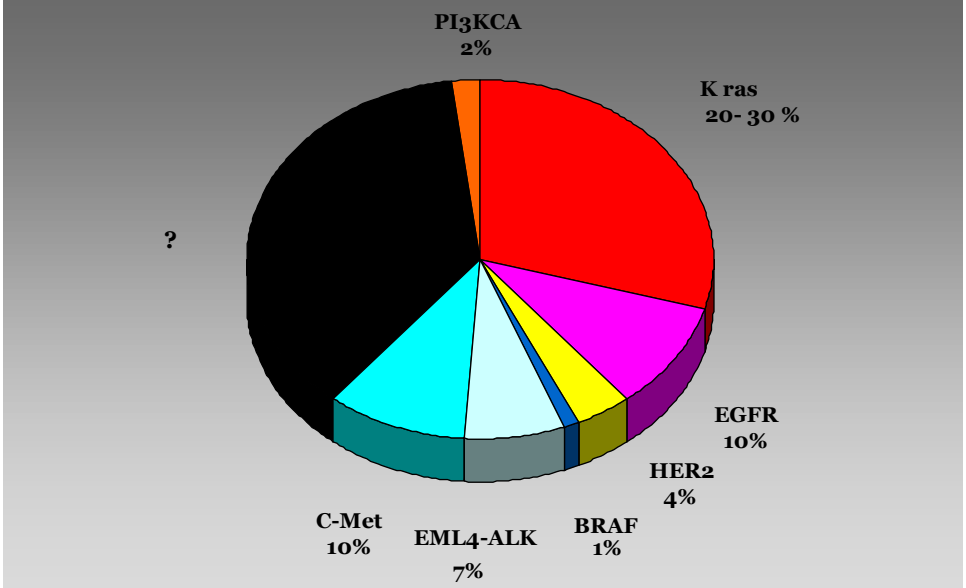
ALK rearrangement and immunohistochemical expression of ALK protein

PI3KCA

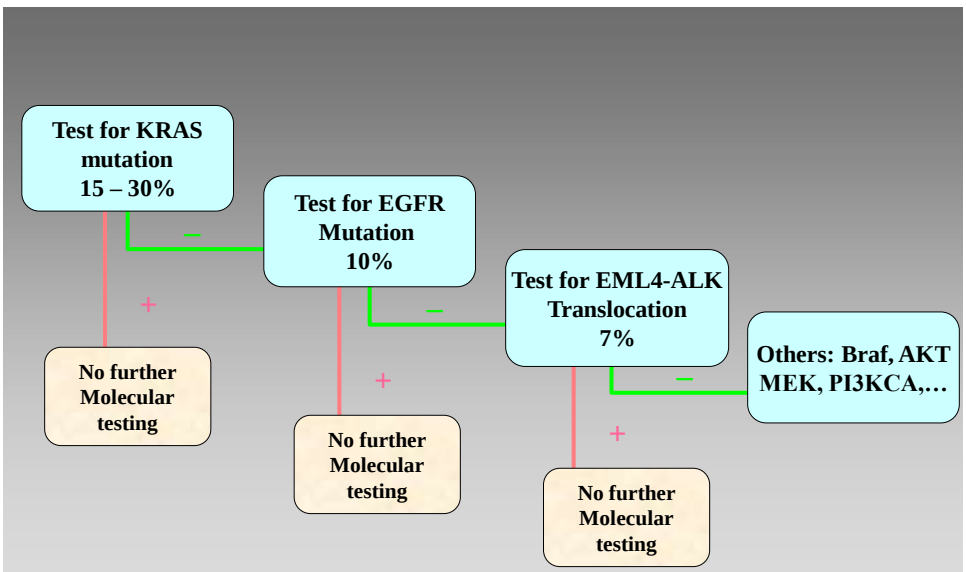
- PI3KCA mutations in 2 % NSCLC- Non smokers
- Gains in 3q22-q26 in 33% of SCC
- Acinar or papillary pattern in ADC
- PI3KCA hyperexpression associated with 3q26 amplification in 40% of SCC
- Associated with increased PI3KCA and AKT activities
- Overlap with EGFR (12%) or ras (40%) mutations

Quian and Massion, 2008, Angulo et al, 2008

Nearly all mutually exclusives (except EGFR and Her2 /C Met and PI3KCA)



Algorithm for sequential molecular analyses in ADC



A New Target for Therapy in Squamous Cell Carcinoma of the Lung

Kadoaki Ohashi and William Pao

Summary: Investigators report the identification of novel somatic mutations in the *DDR2* kinase gene in squamous cell carcinoma of the lung. Cellular, biochemical, and human data suggest that tumor cells harboring *DDR2* mutations have increased sensitivity to existing tyrosine kinase inhibitors, providing rationale for clinical trials of agents that inhibit DDR2 kinase in the disease. *Cancer Discovery 1(1). ©2011 AACR.*

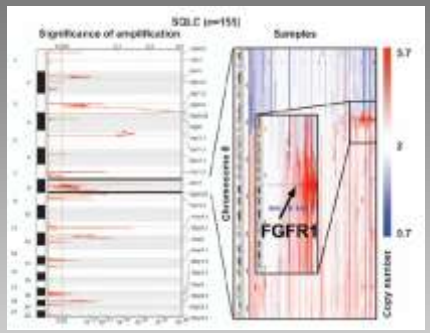
Commentary on Hammerman et al., p. OF7 (1)

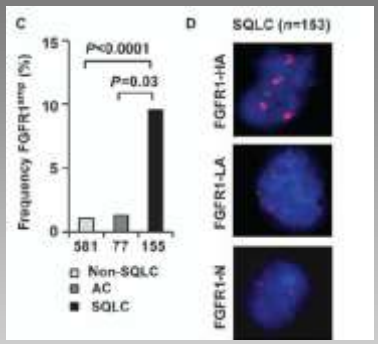
Table 1. Potential targetable driver mutations in squamous cell carcinoma of the lung

Gene	Frequency (%)	Drug	Reference
FGFR1 amplification	22	FGFR TKIs	Weiss et al. (6)
EGFRvIII mutations	5	EGFR TKIs	Ji et al. (10)
PIK3CA mutations	3.6	PI3K inhibitors	Yamamoto et al. (11)
EGFR kinase domain mutations	3.4	EGFR TKIs	Miyamae et al. (12)
DDR2 mutations	3.2	Dasatinib, nilotinib	Hammerman et al. (1)

Frequent and Focal *FGFR1* Amplification Associates with Therapeutically Tractable FGFR1 Dependency in Squamous Cell Lung Cancer

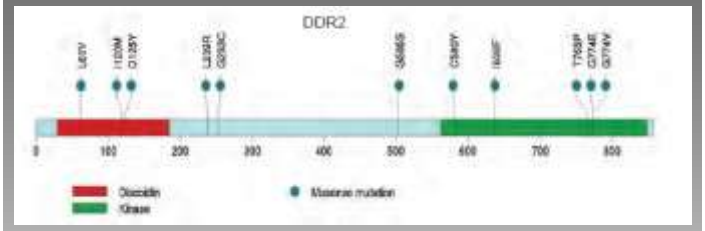
Jonathan Weiss,[1*] Martin L. Sos,[1*†] Danila Seidel,[1,2*] Martin Peifer,[1] Thomas Zander,[3]

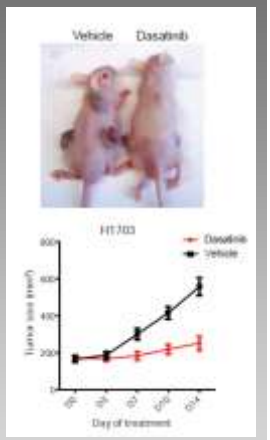




Amplification in 8p12 of FGFR1 in 22% SCC
Phosphorylation of FGFR activates MAPK ERK pathway
Associated with tumor growth and survival
Inhibiteur FGFR PD173074 decreases tumor size

DDR2 (Discoidin Domain Receptor 2) tyrosine kinase

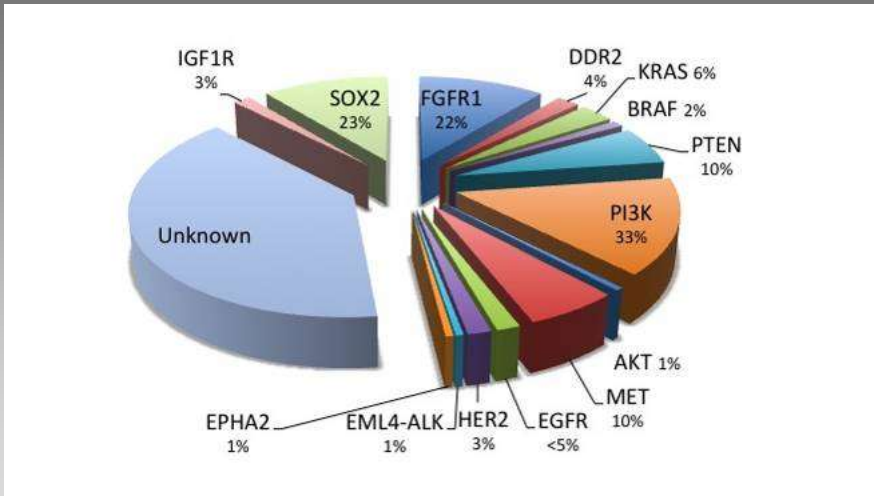




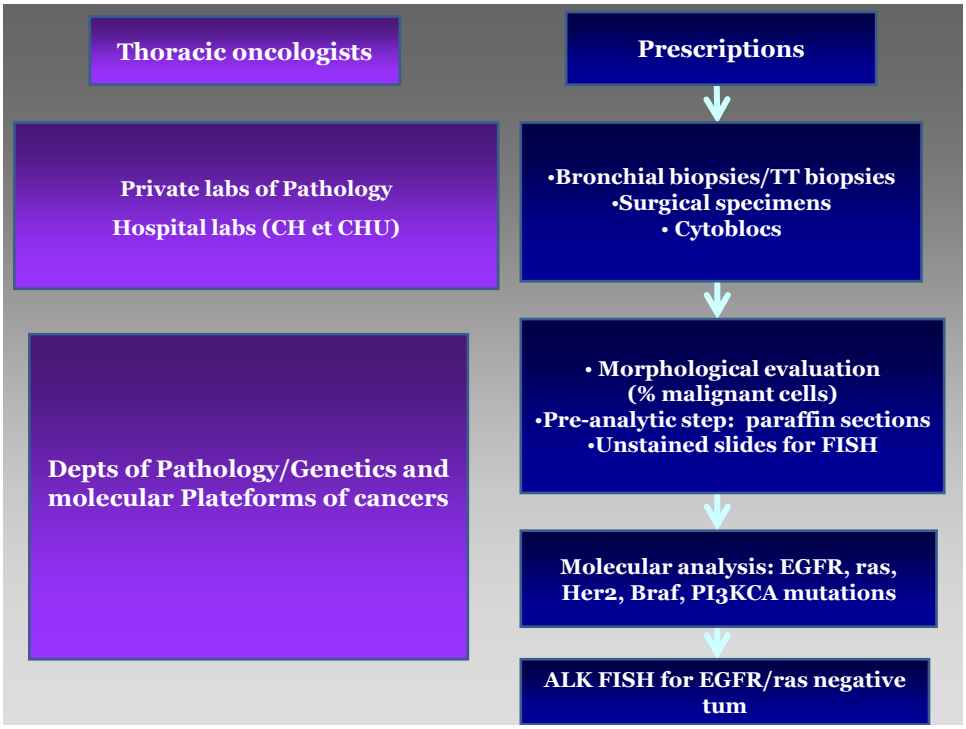
- **Mutations in 3.8% SCC**
- **Favor malignant transformation and tumor growth**
- **Associated with response to Dasatinib**

Hammermann et al , Cancer Discovery 2011

"druggable" molecular and genetic abnormalities in SCC (mutations, amplifications)

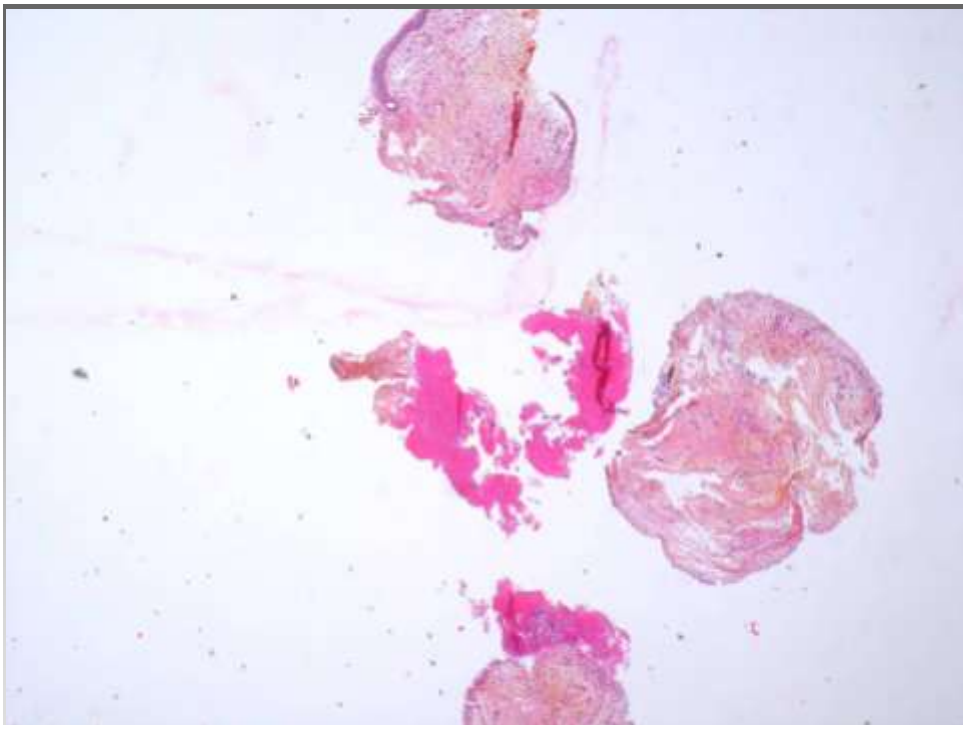


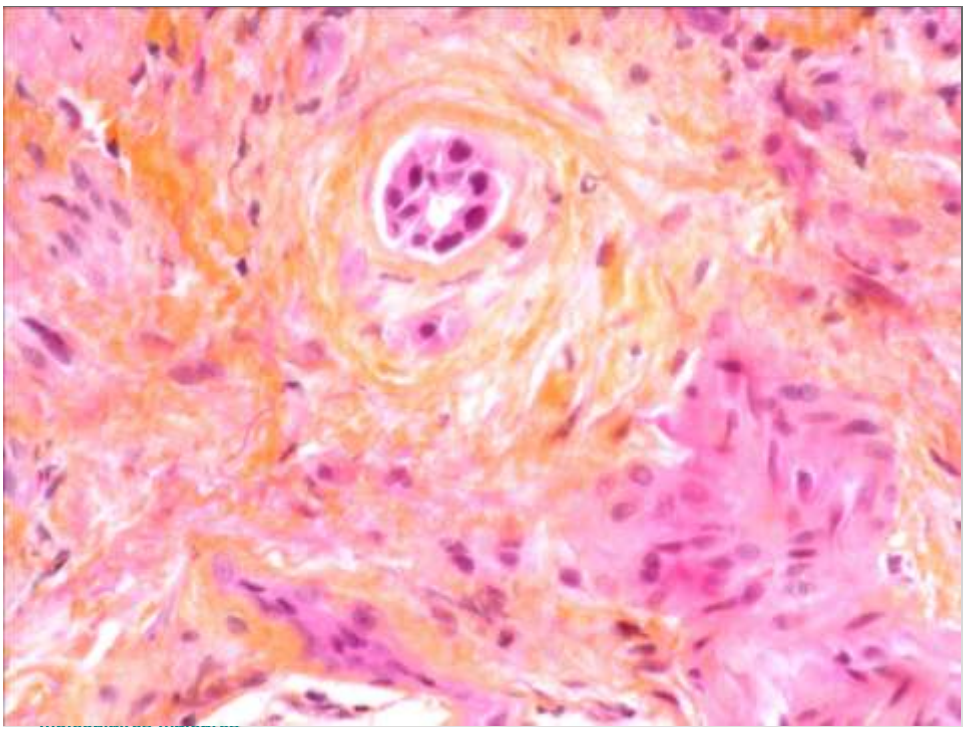
Soria J. C., Brambilla E. et al submitted



In routine practice for mutations (EGFR, Ras, ALK, Her2, Braf, PI3KCA) and FISH analyses:

- **Maximum material is required from the pneumologist/radiologist (3-6 br biopsies ou TT biopsy)**
- **Tissue specimens >>>> cytology / except cell blocks)**
- **If available, frozen material is the best for molecular analyses**
- **If not, formalin fixative recommended (no mercurics or picric acid)**
- **Morphological control by pathologist, with evaluation of the % of malignant cells ++ + (ideally > 10 - 20%)**

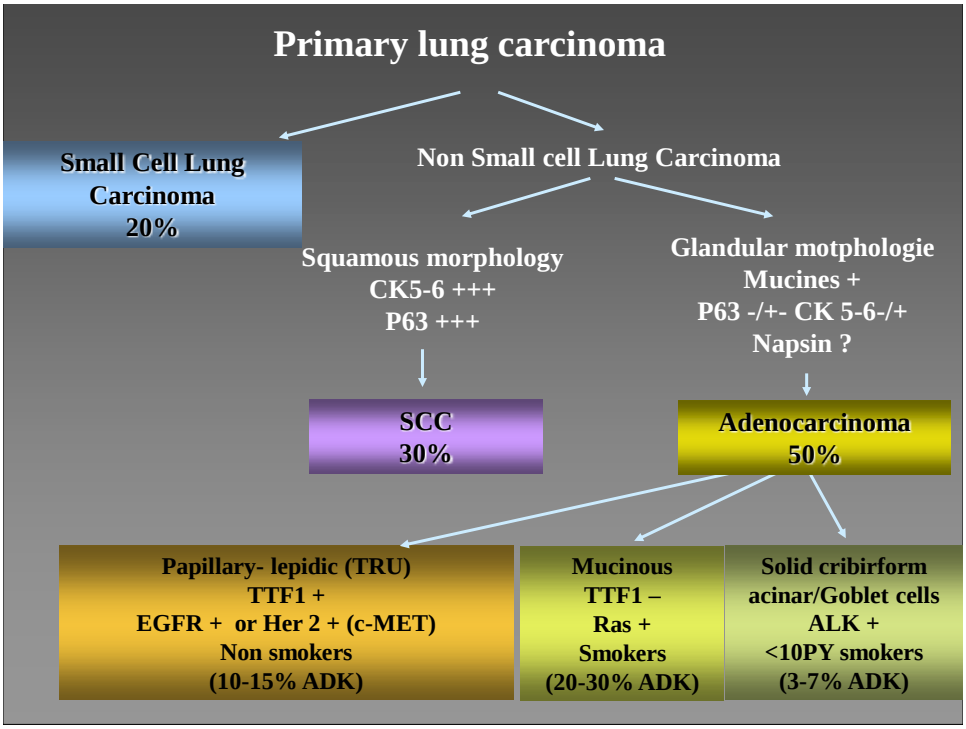




- Extraction (kit Qiagen)
  - 800ng for the 4 EGFR exons and duplicates
  - 1 µg
- Classical sequencing (sensitivity 30%)
- Pyrosequencing, PCR Q, HRM, Snapshot, ASO PCR…

The role of the pathologist is to manage specimens as economic as possible:

- **Determine the total nb of slides for diagnosis, Ipox and genetics (1 HE and 5 to 6 unstained slides for IH +/- FISH and molecular biology?)**
- **Choose the more efficient and economic panel of Abs for diagnosis (cocktails of Abs?)**
- **Micro or microdissect specimens ?**
- **Develop EGFR , ALK, and c.Met Abs, or combined Ipox -FISH ?**
- **Select cases histologically or clinically ??**



Inserm
Institut national de la santé et de la recherche médicale

UNIVERSITE JOSEPH FOURIER

CHU GRENOBLE