


**Pathological Assessment of
Diagnostic Specimens**



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Tumours of the Lung: WHO 2004

- Squamous cell carcinoma
- Small cell carcinoma
- Adenocarcinoma
- Large cell carcinoma
- Adenosquamous carcinoma
- Sarcomatoid carcinoma
- Carcinoid tumour
- Salivary Gland tumours
- Squamous Dysplasia/CIS
- AAH
- DIPNECH
- Mesenchymal tumours
- Benign Epithelial tumours
- Lymphoproliferative tumours
- Miscellaneous tumours
- Secondary tumours

Tumours of the Lung: WHO 2004

- Squamous cell carcinoma
- Small cell carcinoma
- Adenocarcinoma
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- Adenosquamous carcinoma
- Sarcomatoid carcinoma
- Carcinoid tumour
- Salivary Gland tumours

Classification 'assumes' whole tumour examined

Presence or absence of features somewhere in the lesion

Minority components exceeding 10% of the lesion

Importance of patterns

Applicability of full classification to small diagnostic samples?

- Secondary tumours

'Biopsy' techniques in lung cancer diagnosis

- Sputum cytology
- Bronchial brushings and washings
- Fluids
- FNA cytology – primary or mets
- Transbronchial biopsy
- Bronchial biopsy
- Core biopsy – primary or mets
- Liver biopsy
- **Mediastinoscopy**
- **Lymph node excision**
- **VATS biopsy / resection**
- **Thoracotomy & tumour excision**

Increase in Cell number and Tissue architecture

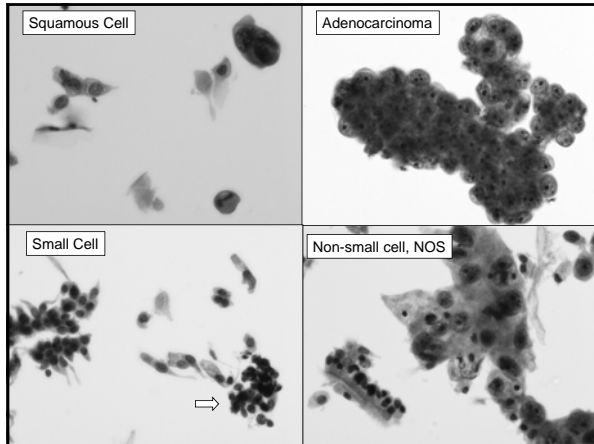
Limitations of small sample diagnosis

80-90% of lung cancers are NOT resected

- Heterogeneity
 - undifferentiated areas
 - mixed differentiation
- Lack of architecture
- Few individual cytological features
- Limited material for further investigation
 - Mucin stains
 - Immunohistochemistry
 - Molecular biology

} Sampling error?

Squamous Cell	Adenocarcinoma
Small Cell	Non-small cell, NOS



Limitations of small sample diagnosis

80-90% of samples are rejected

Cytology

- Absent architecture, invasion issues
- Performance and outcome sample dependant
- Very experience dependant
- Unavoidable false positive rates (1-2% minimum)
- Further tests before definitive surgery?
 - Immunohistochemistry
 - Molecular biology

Compare Cytology with Biopsy:
Does the technique yield a diagnosis of malignancy?

Diagnostic Hierarchy

- Confirm malignancy
- Carcinoma vs other tumours
- Primary carcinoma vs mets
- NSCLC vs SCLC
- Subclassify NSCLC
 - Adenocarcinoma
 - Squamous
 - Other

Bronchoscopy for Central tumour

- Bronchial Washings – 37-48% sensitivity
- Bronchial Brushings – 59-64% sensitivity
- Bronchial Biopsy – 74-83% sensitivity

...for a diagnosis of 'carcinoma', not further specified

Figures poorer and differences smaller for peripheral tumours: targeting problems

Transthoracic FNAC & core biopsy

- 90% sensitivity for FNAC. Core biopsy reported to be 'better'. Better targeting.
- Few data

Detterbeck et al, 2001
Schreiber & McCrory, 2003
Rivera et al, 2003
Chojniak et al, 2006

Small samples: How good is the pathologist – SCLC vs NSCLC?

Diagnostic Hierarchy

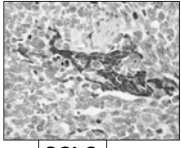
- Confirm malignancy
- ↳ Carcinoma vs other tumours
- ↳ Primary carcinoma vs mets
- ↳ NSCLC vs SCLC ☆
- ↳ Subclassify NSCLC
 - Adenocarcinoma
 - Squamous
 - Other

NSCLC, not further specified

Interobserver consistency was excellent
K = 0.86

Burnett RA et al, J Clin Pathol, 1994

Diagnostic accuracy is good
NSCLC - 98%
SCLC - 90%



SCLC

Dettlerbeck F et al, Diagnosis and Treatment of Lung Cancer, 2001
Schreiber G & McCrory DC, Chest 2003; 123, 115-128

Small samples: How good is the pathologist at subtyping NSCLC?

Diagnostic Hierarchy

- Confirm malignancy
- ↳ Carcinoma vs other tumours
- ↳ Primary carcinoma vs mets
- ↳ NSCLC vs SCLC
- ↳ Subclassify NSCLC ☆
 - Adenocarcinoma
 - Squamous
 - Other

Interobserver consistency was poor
K = 0.25 - 0.39

Burnett RA et al, J Clin Pathol 1994 & 1996

Reported Diagnostic accuracy is variable

Squamous cell carcinoma:

Adenocarcinoma:

Large cell carcinoma:

Accuracy of Bronchial Biopsy Diagnosis			
Reference	Squamous	Adenocarcinoma	Large Cell carcinoma
Payne et al, 1981	97%	58%	56%
Chuang et al, 1983	67%	93%	12%
Matsuda et al, 1986	73%	56%	55%
Thomas et al, 1993	70%	50%	<50%
Cataluna et al, 1996	89%	86%	50%

Cytology: 43% correct specific diagnosis
Squamous carcinoma: 64% accuracy
Adenocarcinoma: 32% accuracy

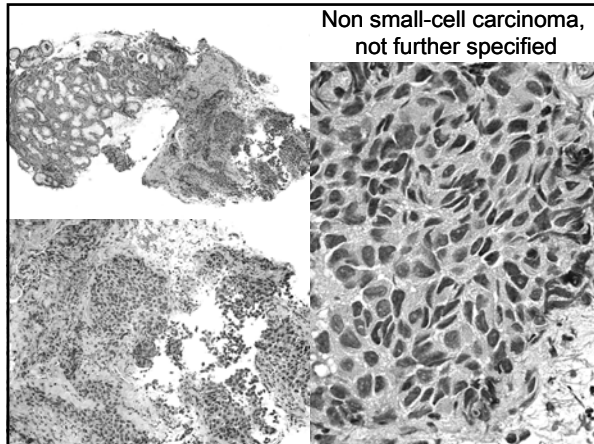
Tissue biopsy: 63% correct specific diagnosis
Squamous carcinoma: 79% accuracy
Adenocarcinoma: 36% accuracy

Dettlerbeck F et al, Diagnosis and Treatment of Lung Cancer, 2001
Schreiber G & McCrory DC, Chest 2003; 123, 115-128
Riviera P et al, Chest 2003; 123, 129-136

⇒ Apply WHO in full?

⇒ Issues with accuracy in adenocarcinoma

⇒ Use the term 'Non-small cell carcinoma' (not otherwise specified)



Non small-cell carcinoma,
not further specified

Small samples: How good is the pathologist at subtyping NSCLC? Comparison with resected tumour

Diagnostic Hierarchy

- Confirm malignancy
- ↳
- Carcinoma vs other tumours
- ↳
- Primary carcinoma vs mets
- ↳
- NSCLC vs SCLC
- ↳
- Subclassify NSCLC ☆
 - Adenocarcinoma
 - Squamous
 - Other

Diagnostic accuracy is better

Squamous – 87% correct

Adenocarcinoma – 80%

Edwards S et al, J Clin Pathol 53: 2000

Squamous cell carcinoma:

Adenocarcinoma:

'Non-small cell carcinoma, not possible to further classify'

About 30% of cases

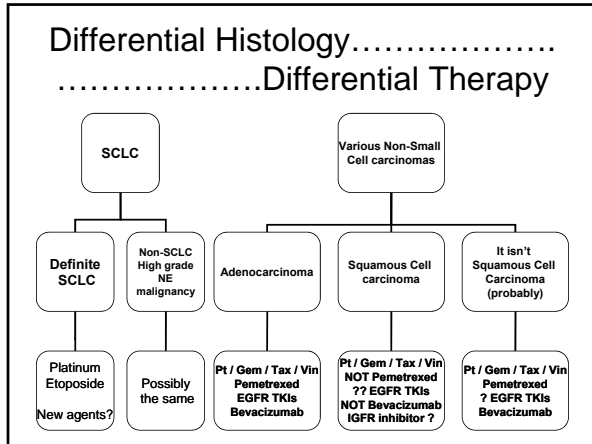
Non-small cell carcinoma, NOS:

- Diagnosis in resected cases?

- All small samples (bronchial and transthoracic biopsy and cytology diagnoses)
 - 64% Adenocarcinoma

(Edwards S et al, J Clin Pathol 53: 2000)
- Bronchial biopsies only
 - 46% Squamous cell carcinoma
 - 24% Adenocarcinoma
 - 16% Large cell carcinoma
 - 14% Others

(Loo PS et al, unpublished, 2009)



NSCLC, not further specified?

- Can we refine this group?
 - 'probably squamous cell'
 - 'probably adenocarcinoma'
 - 'probably not squamous cell'
- Immunohistochemistry for 'lineage markers'
 - TTF1, Surfactant proteins, Napsin A (Favour Adenocarcinoma) Mucin stains
 - CK5/6, HMWCK, p63 (Favour Squamous cell carcinoma)
 - S100A7, Desmocollin3
- Don't 'rewrite' the classification....

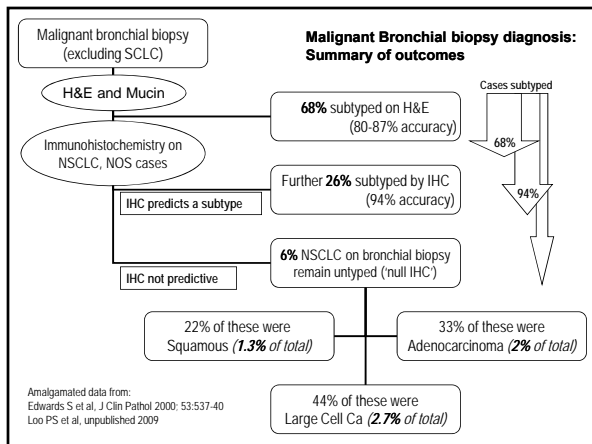
NSCLC, NOS but.....	CK5/6
HMWCK	P63

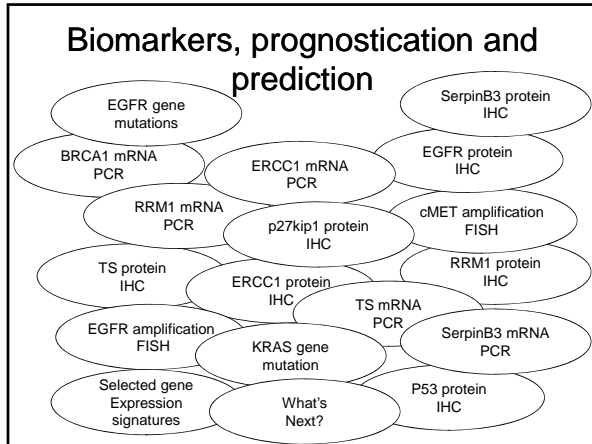
NSCLC, NOS but.....	
TTF1	

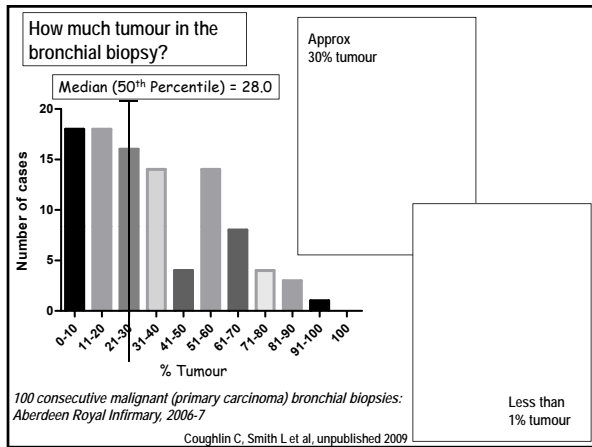
Immunohistochemistry for 'lineage markers'
In the context of the 'NSCLC, NOS' biopsy.....

	PPV	NPV	Sensitivity	Specificity
'Adenocarcinoma'				
ABPAS	100%	76%	23%	100%
TTF1	86%	83%	54%	97%
ABPAS OR TTF1	90%	88%	69%	97%
'Squamous'				
High CK5/6	84%	79%	84%	79%
High HMWCK	81%	82%	88%	74%
High p63	82%	88%	92%	74%
Any S100A7	64%	47%	36%	74%
'Not squamous'				
ABPAS OR Low/Neg p63	88%	85%	79%	92%

(Loo PS, Thomas SC et al, unpublished, 2009)

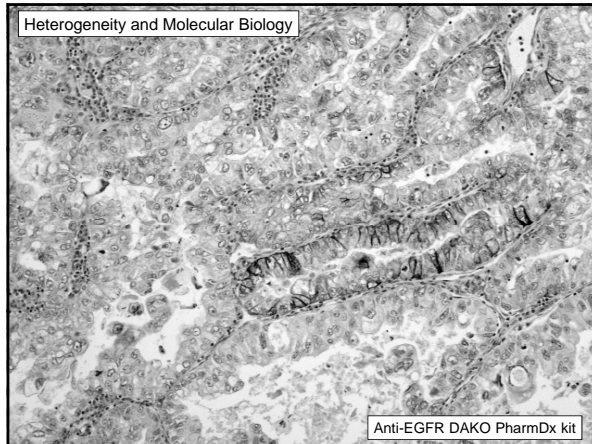






How much of a bronchial biopsy sample is tumour ?

Tumour type	Mean tumour area %	Median tumour area %	% of frags with tumour
Small cell	46	49	85
All NSCLC	29	23	64
Squamous	32	28	68
Adenoca	32	23	69
NSCLC,NOS	24	15	57



Pathological Assessment of Diagnostic Specimens

Challenges posed by:

- The classification
 - Heterogeneity
 - Patterns and tumour components
- Small samples
 - Lack of material
 - Limited cytological and architectural features

Solutions

- Reporting strategies
- Immunohistochemistry

Non-small cell carcinoma, NOS: Diagnosis in resected cases?

50 cases: NSCLC, NOS on biopsy → Resection

- 46% were Squamous cell carcinoma
- 24% were Adenocarcinoma
- 16% were Large cell carcinomas

'Gold standard' Resection H&E diagnosis

Of the Squamous carcinomas:
91% correctly assigned, 9% 'null' IHC

Of the Adenocarcinomas:
58% correctly assigned, 25% 'null', 17% 'squamous'

Of the Large cell carcinomas:
50% 'correct 'null'', 37% 'squamous', 13% 'adeno'

Using IHC Biopsy Diagnosis

'Null' IHC on the 'NSCLC, NOS' biopsy

Final resection histology:
44% Large cell, 33% Adenocarcinoma, 22% Squamous

(Loo PS et al, unpublished, 2009)

**Malignant bronchial biopsies:
Primary carcinoma**

	% of NSCLC diagnoses
Squamous Cell Carcinoma	39% (53%)
Adenocarcinoma	11% (15%)
Small Cell Carcinoma	27%
 Non-small cell carcinoma, NOS	 23% (32%)

*100 consecutive malignant (primary carcinoma) bronchial biopsies:
Aberdeen Royal Infirmary, 2006-7*

Coughlin C, Smith L et al, unpublished 2009

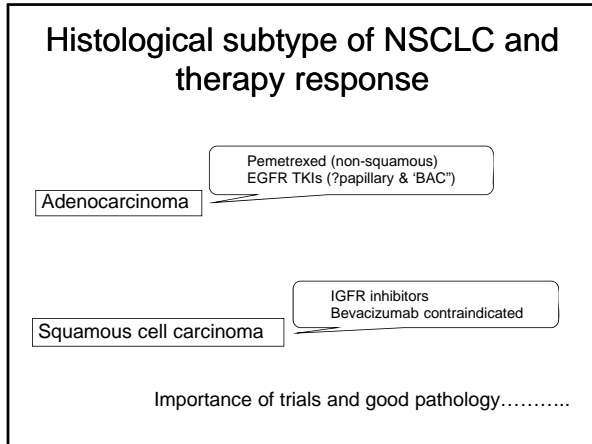
**How many of the fragments
contained tumour ?**

Tumour type	Mean tissue fragments per sample	Mean number of fragments with tumour	% of frags with tumour
Small cell	3.4 [1-5]	2.9 [1-5]	85
All NSCLC	4.14 [1-14]	2.7 [1-8]	64
Squamous	4.1 [1-14]	2.8 [1-8]	68
Adenoca	4.6 [3-8]	3.3 [1-6]	69
NSCLC,NOS	4.0 [1-7]	2.3 [1-5]	57

Neither inadequacy of biopsy nor inaccuracy of reporting predict non-response to small-cell carcinoma chemotherapy

T N Doig, W A Wallace, D M Salter, H Monaghan and E Brown
J. Clin. Pathol. 2006;59:887-888
doi:10.1136/jcp.2006.037432

76 cases of SCLC reviewed
6 non-responders
All good histology
Lack of response not related to poor biopsy
or uncertain diagnosis



How representative is biopsy immunohistochemistry?

Markers: p53, EGFR, HER2, Ki67, Bcl2

- Biopsy vs resection
 - Concordance 80 – 95%
 - PPV/NPV 80 – 92%
 - FP/FN rates 5 – 12%
- 'Good correlation'

Variable with marker studied

Meert AP et al, Lung Cancer 2004; 44, 295
Vibertti L et al, Hum Pathol 1997; 28, 189
Bozzetti C et al, Lung Cancer 2002; 35, 243

Immunohistochemical expression of biomarkers: a comparative study between diagnostic bronchial biopsies and surgical specimens of non-small-cell lung cancer

T. Saitoh¹, F. Haseki¹, S. Ito¹, S. Horiuchi¹, S. Matsuda¹, K. Tsuruta¹, G. Mizutani¹, F. Ueda¹, J. Dohi¹, F. Goto¹, O. Gotoh¹, T. Ito¹, S. Ochiai¹ & J.-C. Soria²

Annals of Oncology 18: 1043-1050, 2007
doi:10.1093/annonc/mdn012
Published online 12 March 2007

Correlation coefficients between biopsy and resection:

ERCC1	0.83
hTERT	0.55
Ki67	0.64
EGFR	0.24
p-Akt	0.29

'evaluation of biomarkers in bronchial biopsies can be misleading.'

