New WHO classification: Putting it into practice

Small biopsy and cytology diagnosis

ELCC, Geneva

17th April 2015

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Professor of Respiratory Pathology National Heart and Lung Division Imperial College, London, United Kingdom
## Disclosure slide

<table>
<thead>
<tr>
<th>Type of affiliation / financial interest</th>
<th>Name of commercial company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receipt of grants/research supports:</td>
<td>Astra Zeneca</td>
</tr>
<tr>
<td>Receipt of honoraria or consultation fees:</td>
<td>Glaxo Smith Klein Ltd, Astra Zeneca, Eli Lilly Ltd, Pfizer, Boehringer Ingelheim, Novartis, Bristol Myers Squib, Merck</td>
</tr>
<tr>
<td>Participation in a company sponsored speaker's bureau:</td>
<td>Astra Zeneca, Roche</td>
</tr>
</tbody>
</table>
IASLC/ATS/ERS ADENOCARCINOMA MULTIDISCIPLINARY PANEL
New York, 2008
Rationale For New ADC Classification
IASLC/ATS/ERS sponsored meeting(s)

Multidisciplinary criticisms in relation to 2004 classification...

• Bronchioloalveolar carcinoma (BAC) – confusing used many different ways despite 99/04 WHO; mucinous and non-mucinous

• No classification for biopsies

• Greater clinical relevance (too “for pathologists by pathologists”…)

• Take into account rapid evolving molecular advances (EGFR)
1-1B Rationale for classification in small biopsies and cytology and 1-1C Terminology and criteria in non-resection specimens:

(1999/2004) Malignant \(\rightarrow\) Ca \(\rightarrow\) NSCLC

- (2008-9) NSCLC \(\rightarrow\) SQCa

- Poorly diff NSCLC
  - If clinically relevant
    - IHC (CK5/6, 34BE12, p63, TTF-1)
    - Mucin stain
    - Expert referral
    - Another sample

- ADC
  - Architecture (BAC/pap/acinar)
  - Grade

TTF-1, mucin +ve, others -ve ..... "favours ADC"
TTF-1, mucin -ve others +ve ..... "favours SQCa"
ADENOCARCINOMA CLASSIFICATION
Travis WD et al. JTO Feb 2011;6:244-286

• PREINVASIVE LESIONS
  • AAH
  • ADC-in-situ (formerly pure BAC) *most non-mucinous (NM) (30mm or less)

• INVASIVE
  • Minimally invasive (< 5mm invasion) (30mm or less)
  • Lepidic pattern predominant
  • Acinar pattern predominant/pure
  • Papillary pattern predominant/pure
  • Micropapillary pattern predominant/pure
  • Solid pattern predominant/pure
  • Invasive mucinous carcinomas
  • Colloid
  • Fetal (low and high grade)
  • Enteric

... A multidisciplinary approach
  Respiratory Physician
  Imaging
  Surgery
  Oncology
  Pathology
  Molecular Biology
1-1: Introduction
   1-1A Lung cancer staging and grading
   1-1B Rationale for classification in small biopsies and cytology
   1-1C Terminology and criteria in non-resection specimens
   1-1D Molecular testing for treatment selection in lung cancer

1-2: Adenocarcinoma
   1-2A Invasive adenocarcinoma
   1-2B Variants of invasive adenocarcinoma
   1-2C Minimally invasive adenocarcinoma
   1-2D Preinvasive lesions
      1-2D-i: Atypical adenomatous hyperplasia
      1-2D-ii: Adenocarcinoma in situ

1-3: Squamous cell carcinoma
   1-3A: Keratinizing and nonkeratinizing squamous cell carcinoma
   1-3B: Basaloid carcinoma
   1-3C: Preinvasive lesion: Squamous carcinoma in situ

1-4: Neuroendocrine Tumours
   1-4A: Small cell carcinoma
   1-4B: Large cell neuroendocrine carcinoma
   1-4C: Carcinoid tumors
   1-4D: Preinvasive lesion: Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

1-5: Large cell carcinoma

1-6: Adenosquamous carcinoma

1-7: Sarcomatoid carcinoma
   1-7A: Pleomorphic, spindle cell and giant cell carcinoma
   1-7B: Carinosarcoma
   1-7C: Pulmonary blastoma

1-8: Other carcinomas
   1-8A: Lymphoepithelioma-like carcinoma
   1-8B: NUT-carcinoma
<table>
<thead>
<tr>
<th>New Small Biopsy/Cytology Terminology</th>
<th>Morphology/Stains</th>
<th>2015 WHO Classification in resection specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma (describe identifiable patterns present)</td>
<td>Morphologic adenocarcinoma patterns clearly present</td>
<td>ADENOCARCINOMA (Predominant pattern) Acinar Papillary Solid Micropapillary</td>
</tr>
<tr>
<td>Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)</td>
<td></td>
<td>Lepidic (nonmucinous)</td>
</tr>
<tr>
<td>Invasive mucinous adenocarcinoma (describe patterns present; use term mucinous adenocarcinoma with lepidic pattern if pure lepidic pattern – see text)</td>
<td></td>
<td>Invasive mucinous adenocarcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma with mucinous features</td>
<td></td>
<td>Colloid adenocarcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma with fetal features</td>
<td></td>
<td>Fetal adenocarcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma with enteric features ‡‡</td>
<td></td>
<td>Enteric adenocarcinoma</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Morphologic squamous cell patterns clearly present</td>
<td>SQUAMOUS CELL CARCINOMA</td>
</tr>
</tbody>
</table>

Adapted from: Travis WD et al. IASLC/ATS/ERS classification of ADCs J Thor Oncol 2011;6:244-285
Classification of sampled tissue

“Adenocarcinoma with a purely lepidic pattern in this sample”

“Adenocarcinoma with both lepidic and micropapillary patterns”
Pure Ground Glass Nodules $\geq 10\text{mm}$ and solitary: Recent Data: Pre-invasive vs invasive adenocarcinoma

46 pure GGNs $\geq 10\text{mm}$ resected

- 41% In Situ
- 20% Minimally invasive
- 39% Invasive

14.4mm 18.9mm

Lim HJ et al. Persistent pure ground-glass nodules $\geq 10\text{mm}$ at CT: histopathologic comparisons. Chest 2013;144

Courtesy of Dr A Devaraj
<table>
<thead>
<tr>
<th>SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS</th>
<th>2015 WHO Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small cell carcinoma</td>
<td>SMALL CELL CARCINOMA</td>
</tr>
<tr>
<td>Non-small cell carcinoma with</td>
<td>Large cell neuroendocrine carcinoma (LCNEC)</td>
</tr>
<tr>
<td>neuroendocrine (NE) morphology and</td>
<td></td>
</tr>
<tr>
<td>positive NE markers, possible LCNEC</td>
<td></td>
</tr>
<tr>
<td>Morphologic squamous cell and</td>
<td>ADENOSQUAMOUS CARCINOMA</td>
</tr>
<tr>
<td>adenocarcinoma patterns present:</td>
<td></td>
</tr>
<tr>
<td>Non-small cell carcinoma, NOS,</td>
<td>No counterpart in 2015 WHO classification</td>
</tr>
<tr>
<td>(comment that adenocarcinoma and</td>
<td></td>
</tr>
<tr>
<td>squamous components are present and</td>
<td></td>
</tr>
<tr>
<td>this could represent adenosquamous</td>
<td></td>
</tr>
<tr>
<td>carcinoma).</td>
<td></td>
</tr>
<tr>
<td>Morphologic squamous cell or</td>
<td>Pleomorphic, spindle and/or giant cell carcinoma</td>
</tr>
<tr>
<td>adenocarcinoma patterns not present</td>
<td></td>
</tr>
<tr>
<td>but immunostains favor separate</td>
<td></td>
</tr>
<tr>
<td>glandular and adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>components</td>
<td></td>
</tr>
<tr>
<td>Non-small cell carcinoma, NOS,</td>
<td></td>
</tr>
<tr>
<td>(specify the results of the</td>
<td></td>
</tr>
<tr>
<td>immunohistochemical stains and the</td>
<td></td>
</tr>
<tr>
<td>interpretation)</td>
<td></td>
</tr>
<tr>
<td>Comment: this could represent</td>
<td></td>
</tr>
<tr>
<td>adenosquamous carcinoma.</td>
<td></td>
</tr>
<tr>
<td>NSCC with spindle and/or giant cell</td>
<td></td>
</tr>
<tr>
<td>carcinoma (mention if adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>or squamous carcinoma are present)</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from: Travis WD et al. IASLC/ATS/ERS classification of ADCs J Thor Oncol 2011;6:244-285
Small cell carcinoma

Cell pellet

CD56

Cytokeratin

TTF-1

High proliferation rate on Ki-67
### SPECIFIC TERMINOLOGY AND CRITERIA FOR ADENOCARCINOMA, SQUAMOUS CELL CARCINOMA AND NON-SMALL CELL CARCINOMA-NOS IN SMALL BIOPSIES AND CYTOTOLOGY †

*(continued)*

<table>
<thead>
<tr>
<th>New Small Biopsy/Cytology Terminology</th>
<th>Morphology/Stains</th>
<th>2015 WHO Classification in resection specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell carcinoma, favor adenocarcinoma‡</td>
<td>Morphologic adenocarcinoma patterns not present, but supported by special stains, i.e. +TTF-1</td>
<td>Adenocarcinoma (solid pattern may be just one component of the tumor) ‡</td>
</tr>
<tr>
<td>Non-small cell carcinoma, favor squamous cell carcinoma‡</td>
<td>Morphologic squamous cell patterns not present, but supported by stains i.e. +p40</td>
<td>Squamous cell carcinoma, (nonkeratinizing pattern may be just one component of the tumor) ‡</td>
</tr>
<tr>
<td>Non-small cell carcinoma, not otherwise specified NSCLC-NOS‡‡</td>
<td>No clear adenocarcinoma, squamous or neuroendocrine morphology or staining pattern</td>
<td>LARGE CELL CARCINOMA</td>
</tr>
</tbody>
</table>

‡† Metastatic carcinomas should be carefully excluded with clinical and appropriate but judicious immunohistochemical examination.

‡ The categories do not always correspond to solid predominant adenocarcinoma or non-keratinizing squamous cell carcinoma respectively. Poorly differentiated components in adenocarcinoma or squamous cell carcinoma may be sampled.

‡‡ NSCLC-NOS pattern can be seen not only in large cell carcinomas but also when the solid poorly differentiated component of adenocarcinomas or squamous cell carcinomas are sampled but do not express immunohistochemical markers or mucin.

Thyroid transcription factor-1 (TTF-1), WHO – World Health Organization

Adapted from: Travis WD et al. IASLC/ATS/ERS classification of ADCs *J Thor Oncol* 2011;6:244-285
What IHC should I do....?

<table>
<thead>
<tr>
<th>Marker</th>
<th>Result</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE1/AE3</td>
<td>Positive</td>
<td>Keratins: 40, 48, 50, 52, 54, 56.5, 58, 59, 64, 65, 67</td>
</tr>
<tr>
<td>CK7</td>
<td>Positive-Diffuse/Strong</td>
<td>Keratin-54 kD, Subset of Carcinomas (OV-TL 12/30)</td>
</tr>
<tr>
<td><strong>TTF-1</strong></td>
<td>Positive-Diffuse/Strong</td>
<td>Thyroid Transcription Factor-1, Lung and Thyroid Carcinomas</td>
</tr>
<tr>
<td>Napsin A</td>
<td>Positive-Diffuse/Strong</td>
<td>Lung Adenocarcinomas</td>
</tr>
<tr>
<td>ER (1D5)</td>
<td>Negative</td>
<td>Estrogen Receptor (1D5)</td>
</tr>
<tr>
<td>Mammaglobin</td>
<td>Negative</td>
<td>Breast, Uterine, Salivary Gland and Skin Appendage Tumors</td>
</tr>
<tr>
<td>S100</td>
<td>Negative</td>
<td>S100 Protein, Nerve Sheath Tumor, Melanomas, Chondrocytes</td>
</tr>
<tr>
<td>WT-1 (N-terminus)</td>
<td>Negative</td>
<td>Mesothelial &amp; Mullerian Tumors, DSRCT</td>
</tr>
<tr>
<td>CA125</td>
<td>Positive-Few Cells</td>
<td>Ovarian, Breast, other Adenocarcinomas</td>
</tr>
<tr>
<td>CEA (p)</td>
<td>Positive</td>
<td>Carcinoembryonic Antigen (Polyclonal), Adenocarcinomas</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Negative</td>
<td>Synaptophysin, Neural, Neuroendocrine Tumors</td>
</tr>
<tr>
<td>CA19.9</td>
<td>Negative</td>
<td>Pancreas, GI, Ovary, Lung and Bladder Carcinoma</td>
</tr>
<tr>
<td>CDX2</td>
<td>Negative</td>
<td>Colorectal Carcinoma</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>Positive-Rare Cells</td>
<td>Gross Cystic Disease Fluid Protein-15, Breast, Salivary Gland</td>
</tr>
<tr>
<td>MOC 31</td>
<td>Positive</td>
<td>Epithelial Cells, Adenocarcinoma</td>
</tr>
<tr>
<td>PR (PgR 636)</td>
<td>Negative</td>
<td>Progestosterone Receptor (PgR 636)</td>
</tr>
<tr>
<td><strong>p63</strong></td>
<td>Positive-Rare Cells</td>
<td>Prostatic basal cells, breast myoepithelial cells, squamous carcinoma</td>
</tr>
<tr>
<td>CK5,6</td>
<td>Negative</td>
<td>Keratin: Mesothelial &amp; Squamous Cells, some Adenocarcinomas</td>
</tr>
</tbody>
</table>

Courtesy of Bill Travis
BIOPSY SUBCLASSIFICATION VALIDATION 2010-2011


• Immunohistochemistry by Means of Widely Agreed-Upon Markers (Cytokeratins 5/6 and 7, p63, Thyroid Transcription Factor-1, and Vimentin) on Small Biopsies of Non-small Cell Lung Cancer Effectively Parallels the Corresponding Profiling and Eventual Diagnoses on Surgical Specimens. Pelosi G et al. J Thorac Oncol. 2011;6:1039-1049


• Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. Rekhtman N et al. Mod Pathol. 2011

• Rapid Multiplex Immunohistochemistry Using the 4-antibody Cocktail YANA-4 in Differentiating Primary Adenocarcinoma From Squamous Cell Carcinoma of the Lung. Yanagita E et al. Appl Immunohistochem Mol Morphol. 2011


• Role of fine needle aspiration cytology, cell block preparation and CD63, P63 and CD56 immunostaining in classifying the specific tumor type of the lung. Kim DH, Kwon MS. Acta Cytol. 2010;54:55-9


NSCLC, favouring ADC

NSCLC, favouring SQCC

TTF-1

P63 or P40

Fix quickly

Only cut tissue once unless absolutely necessary (take spare sections)

H&E (diagnosis in ~60% of cases)

TTF-1 and P40, P63, or CK5/6 (diagnosis in ~90% of cases)

NSCLC-NOS rates are mainly below 15% in the UK (aim for 10%)
If there is diffuse positivity for TTF-1 and staining P63 (or P40) in the same cells, then this should be classified as:

Non-small cell carcinoma favouring adenocarcinoma on IHC

If there is diffuse positivity for TTF-1 and staining P63 (or P40) in the different cells, then consider adenosquamous carcinoma
IHC typing of CK + morphologically undifferentiated NSCLC (mucin stains already undertaken to exclude solid pattern ADC**). Focal = 0-10% of cells positive, Diffuse = >10% of cells positive

<table>
<thead>
<tr>
<th>TTF-1</th>
<th>P63</th>
<th>P40</th>
<th>CK5/6</th>
<th>DIAGNOSIS (RESECTION)</th>
<th>DIAGNOSIS (BIOPSY/CYTO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive focal or diffuse</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>ADC</td>
<td>NSCLC, favour ADC</td>
</tr>
<tr>
<td>Positive focal or diffuse</td>
<td>Positive, focal or diffuse</td>
<td>Negative</td>
<td>Negative</td>
<td>ADC</td>
<td>NSCLC, favour ADC</td>
</tr>
<tr>
<td>Positive focal or diffuse</td>
<td>Positive, focal or diffuse</td>
<td>Positive, focal</td>
<td>Negative</td>
<td>ADC</td>
<td>NSCLC, favour ADC</td>
</tr>
<tr>
<td>Positive focal or diffuse</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive, focal</td>
<td>ADC</td>
<td>NSCLC, favour ADC</td>
</tr>
<tr>
<td>Negative</td>
<td>Any one of above diffusely positive</td>
<td></td>
<td></td>
<td>SQCC</td>
<td>NSCLC, favour SQCC</td>
</tr>
<tr>
<td>Negative</td>
<td>Any one of above focally positive</td>
<td></td>
<td></td>
<td>LCC-unclear#</td>
<td>NSCLC-NOS</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>LCC-null***</td>
<td>NSCLC-NOS</td>
</tr>
<tr>
<td>No stains available</td>
<td>No stains available</td>
<td>No stains available</td>
<td>No stains available</td>
<td>LCC with no additional stains</td>
<td>NSCLC-NOS (no stains available)</td>
</tr>
</tbody>
</table>

*Napsin may be used as an alternative to TTF-1. Although a sensitive marker, CK7 is not recommended as a marker of adenocarcinomatous differentiation due to a lack of specificity.

** Positive for mucin is defined as (5 or more droplets in 2 consecutive high power fields in resections {2004 WHO book} and mucin droplets in two or more cells within a biopsy). Fewer positive cells are regarded as negative.

*** Sarcomatoid carcinoma and neuroendocrine tumours should be excluded (i.e. undifferentiated morphology with no spindle/giant cells).

# Negativity for TTF1 and focal positivity for p63/p40/CK5-6 point to adenocarcinoma cell lineage once neuroendocrine tumours are excluded.
Adapted from:
Travis WD et al. IASLC/ATS/ERS classification of ADCs *J Thor Oncol* 2011;6:244-285
Putting into practice...

Beware of pitfalls....
“Pseudosquamoid” solid ADC


Table 5. Summary of reassessment of 16 EGFR/KRAS-mutant SQCCs identified by routine clinical genotyping

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen type</td>
<td></td>
</tr>
<tr>
<td>Small specimen (biopsy or cytology)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Surgical resection</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
</tr>
<tr>
<td>Lung primary</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Metastasis (lymph node, adrenal, bone, skin)</td>
<td>6 (38)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Interpretation after morphologic and</td>
<td></td>
</tr>
<tr>
<td>immunohistochemical reassessment</td>
<td></td>
</tr>
<tr>
<td>Reclassified as AD-SQCb</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Reclassified as solid adenocarcinoma by IHC</td>
<td>5 (31)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Smoking status by mutation</td>
<td></td>
</tr>
<tr>
<td>EGFR-mutant &quot;SQCC&quot;</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Never</td>
<td>7</td>
</tr>
<tr>
<td>Current or former</td>
<td>3</td>
</tr>
<tr>
<td>KRAS-mutant &quot;SQCC&quot;</td>
<td>6 (37)</td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
</tr>
<tr>
<td>Current or former</td>
<td>5</td>
</tr>
</tbody>
</table>
TBNA lymph node aspirate....

Originally reported as negative, lymphocytes
Metastatic small cell carcinoma

Cell pellet

CD56
EBUS TBNA parabronchial mass
“low grade epithelioid tumour”
Carcinoid tumour

Bland plasmacytoid cells

Rosettes
“Metastatic non-small cell carcinoma/epithelioid tumour”
Metastatic melanoma

S100
Non-neoplastic pathology
Diagnostic Algorithm for EBUS TBNA

Is the specimen adequate?

Yes

Assess cell types present

Malignant cells present

Small cell

Non-small cell ca

Other

Non

Squam

Adeno

NOS

Immuno

TTF-1, CK5/6, p63

Could it be non-thoracic?

Beware small cell mimicking lymphocytes

Beware necrotic tumour cells

Granulomas in LN draining tumours

DOES IT FIT CLINICAL CONTEXT?

Are there granulomas?

Necrosis

Cell pellet

Special stains
Tissue Handling
Separate embedding of multiple cores

“Diagnostic” core

“Molecular” core

Slide provided by Natasha Rekhtman
Molecular testing on cytology: smears vs cell blocks

- Smears:
  - Pros:
    - No formalin fixation (no DNA cross-linking) → better DNA quality
    - FISH: no nuclear truncation → true number of FISH signals/nucleus
  - Cons:
    - Destroys original slide (medico-legal issues – CLIA slide retention policy, though exemption in CAP’14)
    - IHC suboptimal on smears → cell block still usually needed
    - Some commercial labs only accept paraffin-embedded material

- Cell blocks:
  - Pros:
    - Operational simplicity: same workflow as surgical specimens (serial recuts)
    - No destruction of diagnostic slides
  - Cons:
    - ...

Arch Pathol Lab Med 2013

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

Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Neal I. Lindeman, MD; Philip T. Cagle, MD; Mary Beth Beasley, MD; Dhananjay Arun Chitale, MD; Sanja Dacic, MD, PhD; Giuseppe Giaccone, MD, PhD; Robert Brian Jenkins, MD, PhD; David J. Kwiatkowski, MD, PhD; Juan-Sebastian Saldivar, MD; Jeremy Squire, PhD; Erik Thunnissen, MD, PhD; Marc Ladanyi, MD

- Cytologic samples are suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.”

Slide provided by Natasha Rekhtman
**Tissue Handling**

**Step 1.** In the initial block sectioning, sufficient unstained spares should be prepared for potential IHC and molecular testing, the necessity of latter which should be determined with discussion with clinicians.

**Step 2.** According to the histological status, further procedures should be followed.

<table>
<thead>
<tr>
<th>Clear morphology (ADC, SQCC, SCLC) -&gt; Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSCLC, NOS:</strong> Examined with a panel of at least one but no more than two ADC-specific (e.g. TTF-1, CK7) and SQCC-specific (e.g. p40 and CK5/6) marker using the u/s sections</td>
</tr>
<tr>
<td><strong>Looks like SCLC:</strong> Be confirmed by a panel of cytokeartin, CD56 and TTF-1 if required, using the unstained sections</td>
</tr>
<tr>
<td><strong>Looks like NSCLC with NE morphology:</strong> Be confirmed by a panel of CD56 and/or chromogranin and/or synaptophysin.</td>
</tr>
<tr>
<td><strong>If there is no evidence of tumour on initial levels, Undertake further sectioning</strong></td>
</tr>
<tr>
<td><strong>If tumour is only present in the first two levels,</strong> Discuss with clinician and molecular biologist about what testing may be needed and what is feasible on the sections should be undertaken. Re-biopsy may be required</td>
</tr>
</tbody>
</table>

**Step 3.** When molecular testing is needed, the adequacy of specimens for molecular testing in terms of cancer cell content and DNA quantity and quality should be accessed.
Failure and cellularity (CRUK):  

**ASSESSMENT CATEGORIES:**

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>v low</td>
<td>&lt;100</td>
</tr>
<tr>
<td>v low - low</td>
<td>100-700</td>
</tr>
<tr>
<td>low</td>
<td>~1,000</td>
</tr>
<tr>
<td>low-intermediate</td>
<td>1,500-4,000</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4,000-10,000</td>
</tr>
<tr>
<td>High</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>v high</td>
<td>&gt;50,000</td>
</tr>
</tbody>
</table>

14_1002 100x VERY LOW <0.01ng/uL NGS FAIL  
14_1010 40x LOW <0.01ng/uL NGS FAIL  
14_1039 20x INTERMEDIATE 5.3ng/uL NGS PASS  
14_1026 20x HIGH 47.5ng/uL NGS PASS 

Courtesy of CRUK
Best Practice for Usage of Tissue – Everyone has a Role to Play!

- **Pre-examination phase**
  - Identify those who you would consider for targeted therapy
  - Handle tissue appropriately (right media, timely fixation etc)
  - Put core biopsies in separate pots

- **Examination phase** ("judicious use of tissue")
  - Consider separate blocks for different cores
  - Cut into the tissue carefully (if cutting levels, take spare sections)
  - Selection for testing based on histology
    - ADC versus SQCC
    - Apply immunohistochemistry appropriately (ideally only once)
    - Specific antibodies (ALK)
      - ALK IHC correlates with gene rearrangement

- **Post-examination phase**
  - Provide data on tumour load in the sample
  - Enhance tumour load by microdissection
1-1D Molecular testing for treatment selection in lung cancer

Guidelines for the good use of tissue for molecular studies (from WHO 2015: chapter 1-1D)

| Tissue specimens should be managed not only for diagnosis, but also to maximise the amount of tissue available for molecular studies. |
| Cell blocks should be prepared for cytology samples (including pleural fluids) when positive lung cancer, as it is not possible to predict whether other material is suitable for IHC or molecular analysis. |
| To guide therapy of patients with advanced lung cancer, each institution should have multidisciplinary team that coordinates the optimal approach to using tissue for molecular studies. |
| The pathology department should ensure that all molecular results become part of the records each individual specimen. |

- Issues in 2015
  - Integrating next generation sequencing into clinical practice
  - Prioritisation of which test first
  - Competition for IHC based tests (PD-L1, ALK IHC).
  - Getting complex results into the pathology report in understandable and timely fashion....
## Sampling and analysis

<table>
<thead>
<tr>
<th>Year</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>Sputum, Brushings, Washings, Biopsies, Core needle, Mediastinoscopy, Open resections</td>
</tr>
<tr>
<td>2015</td>
<td>Washings, Biopsies, Core needle, TBNA (cytology), VATS/open resection, Systematic nodal resection, No tissue (Cyber knife/RFA)</td>
</tr>
<tr>
<td>2020-30</td>
<td>Washings, Biopsies, Core needle, TBNA (biopsy), VATS/open resection, Systematic nodal resection, Circulating tumour cells, Blood, Breath, No tissue (Cyber knife/RFA)</td>
</tr>
</tbody>
</table>

Samples are getting smaller….  

At some point, will there be a rate limiting size?  
Will there be a rate limiting number of samples (heterogeneity)
Circulating tumour cells.....

CTCs were first described in 1869

Ashworth, T. R (1869). "A case of cancer in which cells similar to those in the tumours were seen in the blood after death". Australian Medical Journal 14: 146–7

As of 2013, only one system has FDA clearance for clinical usage.
Overview of Applications of Molecular Diagnostics in Current Practice: Lung

- Accurate and consistent classification using the WHO 2015 terminology for biopsies and cytology

- **Beware pitfalls**
  - Dispersed population tumour cells
  - Small cell carcinoma vs lymphocytes
  - Metastatic carcinoma from extrathoracic malignancies
  - Rare tumours
  - Low cellularity & necrosis
  - Review at high power, beware cellular necrosis
  - Reactive bronchial epithelial cells, goblet cells and seromucinous glands

- **Appropriate** use of immunohistochemistry will reduce error rate and rate of NSCLC-NOS
- **Do not use IHC indiscriminately as it wastes tissue that may be needed for molecular studies**

- **Tissue availability** – prioritising for best practice
  - All clinical staff should be thinking about it!
  - Standardisation of specimen handling

- **Molecular diagnostics**
  - More targets will appear leading to ever increasing competition for less tissue
Pathology in practice...
ADC with an ALK translocation

JULY 2011
MARCH 2012

Courtesy of Dr S Popat, RMH