LUNG CYTOLOGY
Predictive markers and molecular tests

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Pathologists’ role

Historically
• Guiding the surgeons’ hands
• Pattern recognition
• Tumour burden
• Basements (hiding behind a microscope)

2016
• Oncologists’ best mate
• Understanding disease
• Disease biology
• Integral part of the multidisciplinary team

Ancillary Studies in Cytology
Challenges

• To select the correct test for a limited sample quantity.

• Avoid jumping from a histological adapted technique directly to cytological material.

• Use appropriate controls for cytological material.

Pre-analytical issues

• The variability in material and fixatives is a major factor preventing standardization of some procedures using cytology.

• Spray and ethanol fixation results in better DNA quality than air drying (but both give reliable results in clinical specimens).

• Due the similarities with histological material, cell-blocks are more easy to handle on the molecular lab.

MOLECULAR TESTING WORKFLOW

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

Simple protocol for DNA extraction from archival stained FNA smears

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Enrichment by macrodissection if necessary.
Simple protocol for DNA extraction from archival stained FNA smears

3. Collecting the Tissue

4. Tissue Lysis and DNA Extraction

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.

Rapid On-Site Evaluation

- The increasing need to assess specific targetable mutations and/or genetic aberrations implicates in a detailed evaluation of the collected material with formulation of a preliminary diagnosis followed by extra passes or adequate triage.
- Only an experienced cytopathologist can consistently and correctly decide on handling of the specimens and failure to do so, may result in direct negative consequences to patient care.

 EGFR mutation status testing workflow

KEY POINTS TO USE MOLECULAR TECHNIQUES IN CYTOLOGY

- Collect good and well-preserved material.
- Validate molecular studies on cytological material.
- Control the cases morphologically.

THE PETHALS AND THORNS OF ROSE Disadvantages

- Need of an experienced on-site cytopathologist because relies only on morphology.
- Equivocal on-site diagnosis may prematurely end a procedure.
- Need of extra-time from the cytopathologist with financial under compensation of pathologist’s time.
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.

LUNG CYTOLOGY and TUMOR TYPING

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

LUNG CYTOLOGY and TUMOR TYPING

TABLE 3. Sensitivity and Specificity of Cytologic Tumor Subtyping

<table>
<thead>
<tr>
<th>Subtyping</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
<th>Accuracy, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC vs. nsSCLC</td>
<td>100 (97.9-100)</td>
<td>100 (97.9-100)</td>
<td>100 (97.9-100)</td>
<td>100 (97.9-100)</td>
<td>100 (97.9-100)</td>
</tr>
<tr>
<td>Squamous vs. SqCC</td>
<td>87 (66.4-97.2) / 70 (53.2-86.6)</td>
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<tr>
<td>Adenocarcinoma vs. squamous malignancies</td>
<td>91 (83.9-96.4) / 84 (78.3-90.8)</td>
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Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

TABLE 2. Common Immunohistochemistry markers used in lung cytology

<table>
<thead>
<tr>
<th>Tumor Subtype</th>
<th>TTF-1</th>
<th>Napsin A</th>
<th>CK7</th>
<th>Probes</th>
<th>EGFR</th>
<th>ALK</th>
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<tbody>
<tr>
<td>ADC</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>SqCC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCLC</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCLC/NSCLC</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metastatic</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

* Especially metastatic ADC.

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

Cancer Cytopathology 2011
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)

Diagnostic algorithm for ALK testing

Genomic Analysis in Cytology

- Next generation sequence (NGS) has recently entered in the routine clinical molecular diagnostics by providing a highly multiplexed platform for simultaneous screening of multiple genes at a high analytical sensitivity with minimal amounts of DNA.
- Cytologic specimens offer a number of advantages in terms of molecular testing and minimally invasive procedures such as FNA are often used to establish a diagnosis.

Genomic Analysis in Cytology

- However, cytology specimens are still underutilized for molecular testing because most molecular testing requests are directed reflexively to the histology and cytology is used only when there is no concurrent core biopsy (CB) or the CB is insufficient for testing.
- The other issue is that molecular laboratories are not always validated all the different types of cytologic preparations (smears, cytopsins, LBC, cell-blocks) and prefer to treat all specimens as they do with the FFPE histology blocks.
Pre-analytical challenges for genomic analysis in cytology

- Pre-analytical factors are determinant for the success of NGS sequencing and recognizing these factors can enable better processing or triaging of specimens to further improve NGS success.
- In cytology specimens, these factors may be especially important because of the wide variations in sample types, preparations and adequacy criteria.

Pre-analytical factors that affects NGS analysis in cytology

- Specimen cellularity.
- Type of preparation.
- Type of fixative and stains.
- Type of glass slides.
- Tumour fraction.
- DNA yield
- Input DNA

Role of the Cytopathologists

- There is a wide interobserver variation among cytopathologists in estimating tumor fraction, even with predefined adequacy criteria.
- Some cytopathologists demonstrate high cancellation rates, resulting in low NGS failure rates, whereas others have low cancellation rates with high NGS failure rates.
- The variation among cytopathologists in tumor fraction estimation and selecting cases for NGS is also seen in the selection of substrate (cell blocks versus smears) and the number an type of glass slides sent for testing.