NGS in Lung Cancer Cytology

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Disclosures

Speakers honoraria and advisory boards:
Astra Zeneca, BMS, MSD, Novartis, Pfizer, Roche
NGS: Technical Revolution

$500,000,000,000

Sequencing costs per human genome (3000MB)

13 years

$1000

3 days
UNLEASHED!
Studying diseases across species advances dog and human health
2004: EGFR Mutations
Oncogenic drivers
- initial diagnosis
- acquired

TARGETED therapy

TMB (Mut/Mb)
- Checkmate 227

Immunotherapy

NGS

Lawrence MS et al., Nature 2013

Tsao AS et al., JTO 2016
NSCLC: 40% diagnosed by cytology
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Any cytology preparation suited for sequencing analyses

CAP/IASLC/AMP Guidelines 2018
NSCLC: 40% diagnosed by cytology

Any cytology preparation suited for sequencing analyses
CAP/IASLC/AMP Guidelines 2018

NGS
Stoy SP et al., Clin Lung Cancer. 2018
Piqueret-Stephan L et al., Cancer Cytopathol. 2016
Treece AL et al., Cancer Cytopathol. 2016
Vigliar E et al., Cytopathology 2015
de Biase D et al., PLoS One. 2013
Scarpa et al., PLoS One. 2013
Next Generation Sequencing (NGS)

- Many genes at the same time
  - Multigene panels: Oncomine Solid Tumor DNA Kit (22 genes)
- Fast
- Cost-effective (many samples/run)
Testing for predictive mutations in routine practice:
> 50% on cytology

**Sanger**
n=1295

- 777 (60%)
- 518 (40%)

**NGS**
n=493

- 276 (56%)
- 217 (44%)
Cytology for DNA based analyses

Any kind of specimen (TBNA, effusion, liquid based, cell block...)

Jain D and Roy-Chowdhuri S, Arch Pathol Lab Med. 2018
How much tumor cells do we need?

Depends on NGS platform

- Ion Torrent for targeted sequencing:
  - Analytic sensitivity: 20% → 20% of tumor cell content
  - For prestained, alcohol or air dryed conventional cytology specimens: ≈ 200 tumor cells
Tumor cell enrichment by laser capture microdissection

<table>
<thead>
<tr>
<th>Laser microdissection</th>
<th>Sanger</th>
<th>NGS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>31%</td>
<td>13%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cytology</td>
<td>87%</td>
<td>49%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Biopsy vs cytology: p<0.01
Collecting tumor cells for DNA extraction

Effusion

Tumor cells >20%

Manual collection

DNA extraction
Collecting tumor cells for DNA extraction

Effusion

Tumor cells >20%

Manual collection

DNA extraction
Collecting tumor cells for DNA extraction

- Effusion
  - Tumor cells >20%
  - Manual collection
  - DNA extraction
Collecting tumor cells for DNA extraction

**Effusion**
- Tumor cells >20%
- Manual collection 51%
- DNA extraction

**Bronchial secretion**
- Tumor cells <20%
- Laser microdissection 49%
- DNA extraction
Collecting tumor cells for DNA extraction

- **Effusion**
  - Tumor cells >20%
  - Manual collection 51%
  - DNA extraction

- **Bronchial secretion**
  - Tumor cells <20%
  - Laser microdissection 49%
  - DNA extraction
Individualized, under morphological control

→ Rejection rate < 5%

Effusion

- Tumor cells >20%
- Manual collection 51%
- DNA extraction

Bronchial secretion

- Tumor cells <20%
- Laser microdissection 49%
- DNA extraction

PD-L1 ICC
NGS: Correlation input DNA (ng/ul) und library output (pM)
NGS: From sample to report

- Sample
- DNA extraction
- Library preparation
- Sequencing
- Data generation
- Report to cytopathologist

![Turnaround time NGS (Median)](image)

- Cytology: 6 days
- Biopsie: 4 days
Cytology: Prevalence of oncogenic drivers in non-sq NSCLC

EGFR mutations in Europe: **11-20%**

*Midha A et al., Am J Cancer Res, 2015*

**Sanger (n=774)**
- No driver: 409 (53%)
- KRAS: 209 (27%)
- EGFR: 114 (15%)
- BRAF: 31 (4%)
- HER2: 11 (1%)

**NGS (n=276)**
- No driver: 109 (39%)
- KRAS: 88 (32%)
- EGFR: 51 (18%)
- BRAF: 24 (9%)
- HER2: 4 (1%)
NGS cytology: Distribution of EGFR mutations

Exon 18
- Cytology UHBS: 4%
- Kobayashi Y, Cancer Sci, 2016: 4%

Exon 19
- Cytology UHBS: 47%
- Kobayashi Y, Cancer Sci, 2016: 45%

Exon 20
- Cytology UHBS: 12%
- Kobayashi Y, Cancer Sci, 2016: 7%

Exon 21
- Cytology UHBS: 35%
- Kobayashi Y, Cancer Sci, 2016: 41%

n=51
1. Line treatment at times of personalized therapy

Advanced NSCLC

Morphology

Adeno-CA

Squamous-CA

PD-L1

Genomic Profiling

NGS

FISH/IHC

EGFR, KRAS, BRAF, HER2 + other Genes

ALK, ROS1, MET, RET, NTRK

CAP/IASLC/AMP Guidelines 2018
NCCN Clinical Practice Guidelines 2018
Integrated report
• Morphol. diagnosis
• All predictive markers:
  - ALK, ROS1 ICC
  - PD-L1 ICC
  - NGS
  - RET, MET, (NTRK)
• Methodology section

5 - 10 working days
Summery

• Need for comprehensive molecular testing of lung cancer
  – emerging targetable oncogenic alterations
  – TMB as predictor of response to immunotherapy (300-400 gene panels)

• Cytology specimens provide excellent DNA for molecular testing by NGS
  – well established in diagnostic routine

Cytology Specimens
A Goldmine for Molecular Testing

Natasha Rekhtman, MD, PhD; Sinchita Roy-Chowdhuri, MD, PhD

Arch Pathol Lab Med 2016
Do More With Less

The Past

Resection  Biopsy  Biopsy  Cytology  Laser microdissection  Liquid Biopsy

The Future

Courtesy of Luca Quagliata
Take Home Message

Cytopathologist need to be involved in predictive marker testing.
Thank you!

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