Molecular testing of lung carcinoma

Los Angeles Society Of Pathologists
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OUTLINE

- Clinical testing for predictors of therapy response
  - $EGFR$, $ALK$, other

- Role of surgical pathologists in targeted therapies
Treatment of advanced NSCLC

**ADENOCA**
- EGFR
- EML4/ALK
- BRAF
- Her2
- VEGFR

**SQC**
- FGFR1
- DDR2
- IGF-R

**NSCLC-NOS**
- Same as ADC

Platinum based therapies
Diagnosis of lung carcinoma

- Before 2005.
  - Small cell carcinoma vs. non-small cell carcinoma

- From 2005-present
  - Small cell carcinoma vs. non-squamous cell carcinoma
EGFR mutations and EGFR-TKI responders

- Women; never smokers
- Adenocarcinoma
- Non-squamous carcinoma

Pretreatment

Posttreatment

Genetic alterations in lung adenocarcinoma

WT

KRAS

EGFR

ALK

NF1

METexon14

HER2-mut

BRAF

PI3KCA

MET amp

AKT

MAP2KI

ROS1

KIF5B-RET

HRAS

NRAS

WT
COMMON QUESTIONS

- What assay to choose?

- What type of sample to send for molecular analysis?

- What histologic subtype of NSCLC should be tested?

- What is the future of molecular testing?
SPECIAL ARTICLE

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,* Philip T. Cagle,† Mary Beth Beasley,‡ Dhananjay Arun Chitale,§ Sanja Dacic,¶ Giuseppe Giaccone,** Robert Brian Jenkins,*** David J. Kwiatkowski,†† Juan-Sebastian Saldivar,††† Jeremy Squire,¶¶ Erik Thunnissen,¶¶¶ and Marc Ladanyi,¶¶¶
Iressa Pan-Asian Study (IPASS)

**EGFR-Mutation-Positive**

**EGFR-Mutation-Negative**

Mok TS et al. NEJM 2009;361:947-957
Frequency of *EGFR* mutations

- **Exon 19 del**: 44%
- **Exon 21 L858R**: 41%
- **Exon 20 insertions**: 6%
- **Exon 18 G719X**: 5%
- **Exon 18-21 rare missense mutations**: 5%

Multiple test platforms are acceptable for EGFR mutation testing.

A mutation method must be at least as sensitive as Sanger sequencing.

EGFR mutation analysis should capture all mutations that individually account for 1% or more of mutant case.

Assay for the $T_{790}^{M}$ should have sensitivity in the 1-5% range.
DETECTION OF EGFR ABNORMALITIES

- Immunohistochemistry

- FISH/CISH

- Detection of mutations
  - DNA sequencing or other mutation detection techniques
EGFR IHC

- IHC for total EGFR
  - Not acceptable
- IHC for phosphorylated EGFR
  - Limited experience, unreliable
- IHC for mutant forms of EGFR
EGFR MUTATION SPECIFIC ANTIBODIES

H&E

EGFR IHC

EGFR exon 19 del IHC

 Courtesy of Lucian Chirieac, MD, Brigham and Women's Hospital

Clin Cancer Res 2009;15(9):3023-3028
**EGFR exon 19 and L858R mutation specific antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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</thead>
<tbody>
<tr>
<td>Exon 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 bp</td>
<td>100</td>
<td>98.8</td>
</tr>
<tr>
<td>&lt;15 bp</td>
<td>74.2</td>
<td>98.8</td>
</tr>
<tr>
<td>Exon 21</td>
<td>95.2</td>
<td>98.8</td>
</tr>
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</table>

CAP/IASLC/AMP recommendation

- EGFR IHC is NOT recommended test for EGFR TKI treatment selection

- Mutant EGFR allele-specific IHC is too insensitive to be used as a stand alone assay for EGFR-TKI treatment selection
Response to Crizotinib in ALK-Positive Tumors

ALK and fusion products in NSCLC
**ROS1** rearrangements

- receptor tyrosine kinase of the insulin receptor family
- 6q22
- 2% lung ADC
- Young, never smokers
- Respond to crizotinib

METHODS OF DETECTION

- Classical cytogenetics
- FISH
- Immunohistochemistry
- RT-PCR
ALK- FISH

Dept of Pathology UPMC

Normal

A

B

Translocation

A/B

B/A

Diagn Mol Pathol 2004; 13(4):197-206
# ALK-IHC

<table>
<thead>
<tr>
<th>CLONE</th>
<th>PROVIDER</th>
<th>SPECIFICITY (%)</th>
<th>SENSITIVITY (%)</th>
</tr>
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<tbody>
<tr>
<td>D5F3</td>
<td>Ventana Medical System, Inc. Cell Signaling Technology, Danvers, MA</td>
<td>75-99</td>
<td>91-100</td>
</tr>
<tr>
<td>5A4</td>
<td>Novocastra, New Castle, UK</td>
<td>87.5-98</td>
<td>100</td>
</tr>
<tr>
<td>ALK1 M7195</td>
<td>Dako, Carpinteria, CA</td>
<td>91-99</td>
<td>64-100</td>
</tr>
</tbody>
</table>

IHC ASSAY IMPLEMENTATION

CHALLENGES

- Tissue quality and quantity
- Antibody clone
- IHC protocol (antigen retrieval method, detection system)
- Interpretation criteria
## ALK IHC agreement with FISH

<table>
<thead>
<tr>
<th>Agreement Rate</th>
<th>Agreement Between IHC and FISH</th>
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</thead>
<tbody>
<tr>
<td><strong>Overall Percent Agreement</strong></td>
<td>n/N (%)</td>
</tr>
<tr>
<td>92/98 (93.9)</td>
<td>87.3, 97.2</td>
</tr>
<tr>
<td><strong>Positive Percent Agreement</strong></td>
<td>39/43 (90.7)</td>
</tr>
<tr>
<td><strong>Negative Percent Agreement</strong></td>
<td>53/55 (96.4)</td>
</tr>
</tbody>
</table>
A commercial break-apart FISH assay developed by Abbott Molecular is recommended.

DAKO ALK1 antibody is not reliable for ALK rearrangement screening.

RT-PCR is not currently recommended as a first-line diagnostic method for ALK fusion status.
WHAT TYPE OF TISSUE SAMPLE SHOULD BE TESTED?
SAMPLE FOR \textit{EGFR/ALK} TESTING

- Sample processing
- Primary vs. metastatic tumor
- Selecting a block for analysis
- Multiple primary lesions
SAMPLE PROCESSING

- **ACCEPTABLE fixatives**
  - 10% neutral-buffered formalin (NBF)
  - Alcohol (70% ethanol)

- **UNACCEPTABLE fixatives**
  - Heavy metal fixatives (e.g. Zenker’s, B5, AZF, B plus)
  - Acidic solutions (Bouin’s solution, bone decalcifying solutions)
SAMPLE PROCESSING
CAP/IASLC/AMP recommendation

- Specimen should be fixed in 10% NBF for no less than 6 hours and no more than 48 hours before processing

- Cell block is recommended for cytology specimens
Paraffin-embedded tissue sample: Core biopsy or FNA cell block preparation

- Level 1: Hematoxylin & Eosin
  - Pathologist
  - Levels 2-5 Immunohistochemistry

- Level 6: Hematoxylin & Eosin
  - Pathologist
  - Levels 7-17 H&E and unstained slides Mutation testing EGFR/KRAS
  - Levels 18-23 H&E and FISH (ALK, ROS, RET, MET)

- Level 24: Hematoxylin & Eosin (core biopsies only)
  - Pathologist
  - Levels 25-26 Immunohistochemistry
  - Level 27 Negative control for immunohistochemistry
WHAT TUMOR SAMPLE TO TEST
PRIMARY TUMOR OR METASTASES?
Heterogeneity of \textit{EGFR} mutations


- Discrepancy in EGFR mutations between primary and recurrent NSCLC

- Role of chemotherapy (Bai H. JCO 2012;30:3077)
The distribution of the *EGFR* mutations

Three small areas were selected from each of 50 ADCs carrying the *EGFR* mutation

Identical *EGFR* mutation among the three areas

Five ADCs with the *EGFR* mutation were dissected into more than 100 pieces

Identical *EGFR* mutation among the pieces
EGFR mutation and amplification

EGFR mutation +/- amplification +

EGFR mutation +/- amplification -
Pseudoheterogeneity of *EGFR* mutations in lung adenocarcinoma

Yatabe Y et al. JCO 2011;29:2972-2977
Tumor heterogeneity revisited by deep sequencing
HIGH CONCORDANCE BETWEEN PRIMARY TUMORS AND METASTASES

Table 4. Concordance Between Primary Tumor and Matched Metastasis for Recurrent Somatic Alterations and Likely Passenger Alterations

<table>
<thead>
<tr>
<th>Alterations</th>
<th>No. of Evaluated Alterations</th>
<th>Shared</th>
<th>Unshared</th>
<th>Concordance Rate (%)</th>
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</thead>
<tbody>
<tr>
<td>Mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>28</td>
<td>26</td>
<td>2</td>
<td>93</td>
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<tr>
<td>Passenger</td>
<td>144</td>
<td>88</td>
<td>56</td>
<td>61</td>
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<tr>
<td>Large structural alterations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Passenger</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Global</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>33</td>
<td>31</td>
<td>2</td>
<td>94</td>
</tr>
<tr>
<td>Passenger</td>
<td>159</td>
<td>95</td>
<td>64</td>
<td>63</td>
</tr>
</tbody>
</table>
PRIMARY TUMOR VS. METASTASES
CAP/IASLC/AMP recommendation

- The choice of primary vs. metastatic tumor should be based on the sample qualities (tumor content and preservation)
MULTIPLE PRIMARY LESIONS
CAP/IASLC/AMP recommendation

- Both separate primary tumors should be tested if tissue available

- Testing of multiple areas of a single primary tumor is not recommended
**EGFR inhibitor acquired resistance**

- **EGFR T790M amplification**: 50%
- **Her2 amplification**: 12%
- **MAPK1 amplification**: 5%
- **PIK3CA amplification**: 5%
- **MET amplification**: 4%
- **SCLC transformation**: 6%
- **Unknown**: 15%
CAN HISTOLOGIC SUBTYPE OF LUNG ADENOCARCINOMA PREDICT MUTATION PROFILE?
MUTATION and MORPHOLOGY

**EGFR/BRAF-PAPILLARY**

**EGFR/BRAF-LEPIDIC**

**KRAS-MUCINOUS**

**ALK-SOLID WITH SIGNET RING**
PRIMARY HISTOLOGIC PATTERNS IN MIXED SUBTYPE ADENOCARCINOMAS AND MUTATION TYPE

AC- acinar; SOL-solid; BAC-bronchioloalveolar; MUC-mucinous; PAP-papillary

Dacic S. et al. Mod Pathol 2010;23(2):159-68.
MORPHOLOGIC PREDICTORS OF MUTATIONAL PROFILE

EGFR + predictor

Absence of solid growth pattern
OR 0.024; 95% CI 0.001-0.825
P=0.0388

KRAS + predictor

Mucinous growth pattern
OR 3.938; 95% CI 1.574-9.852
P=0.001

Dacic S. et al. Mod Pathol 2010;23(2):159-68.
WHAT ABOUT OTHER NSCLC WITH GLANDULAR DIFFERENTIATION?
KRAS/EGFR in adenosquamous carcinoma and sarcomatoid carcinomas

H&E

KRAS

EGFR-FISH

Tochigi et al. AJCP 2011
LARGE CELL CARCINOMA

- TTF-1
- P40
- Mucin
Genetic Alterations in SQC (mutations, amplifications)

Courtesy of Dr. Elisabeth Brambilla
Gene expression subtypes of SQC

Nature 2012
HISTOLOGY AND GENOTYPIC ANALYSIS
CAP/IASLC/AMP recommendation

- All NSCLC that contain an adenocarcinoma component, regardless of histologic grade

- Not recommended for pure squamous cell carcinoma, small cell carcinoma or large cell neuroendocrine carcinoma
RESEARCH ARTICLE

CANCER

A Genomics-Based Classification of Human Lung Tumors

The Clinical Lung Cancer Genome Project (CLCGP) and Network Genomic Medicine (NGM)*†
Genomic alterations and histology

A

q-value amplifications

AD
C
L
S
SQ

MYCL1
MYCN
FHIT
SOX2
PTPRD
CDKN2A
RB1
EGFR
FGFR1
MYC
CCND1
KRA5
MDM2
NKX2-1
ERBB2
CCNE1

1e-17 4e-21 0.02
1e-07 2e-06 5e-26
2e-02 2e-17 0.02
1e-00 1e-99 1e-99

q-value deletions

B

q value 0.05 1e-10

AD
CA
LC
S
SQ

C

KEAP1
STK11
NFE2L2
TP53
NKX2-1
KRAS
CCND1
EGFR
EGFR

Routine work up of lung adenocarcinomas

24 hrs

Tissue collection/Fixation

Fixation/Tissue Processing

Pathologists review and report

EGFR mutation testing

ALK-FISH

10 working days

Final report with tumor histology and mutation results

Final report

Molecular testing

Pathology

Biopsy
WHAT IS NEXT?
Next Generation Sequencing

Single gene assays
Multiplexed hotspots
Multigene panels
Whole exome
Whole genome

ABI
Sequenom MassArray
Ion Torrent PGM
454
Illumina HiSeq
A RECENT CASE – SUCCESS STORY

Pre-treatment

Post-treatment

FBXW7 p.R465H Point Mutation

TTF-1 +/- p40-
HOW MUCH IS ENOUGH?

Roychowdhury et al. Sci Transl Med 2011
Obstacles in molecular testing

- Payers
- Patent owners
- Physicians
- Drug companies
- FDA
What does a surgeon/oncologist expect from a pathologist?

- Close interactions, good communication, respect
- Accuracy ⇐ fast response
- Integration of molecular profiling and diagnostic work up of NSCLC
- Team approach!
SUMMARY

- Molecular testing for predictors of targeted therapy response in lung adenocarcinoma must include *EGFR* mutation analysis for exons 19-21 and *ALK*-FISH.

- Testing for other molecular biomarkers in NSCLC is not currently indicated for clinical management.

- Pathologist must make every effort to spare the tissue for molecular testing after histologic diagnostic evaluation.
Thank you