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

Philip T. Cagle, MD, Marc Ladanyi, MD, Neal I. Lindeman, MD
April 24, 2013
cap.org

Guideline Publication


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Neal I. Lindeman, MD
Dr. Lindeman has disclosed the following:

- Partners Health Care has filed a patent on EGFR Mutation Testing. NIL is not a patent holder.
Definition of grades of recommendations

<table>
<thead>
<tr>
<th>Grade of Recommendation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Body of evidence can be trusted to guide practice</td>
</tr>
<tr>
<td>B</td>
<td>Body of evidence can be trusted to guide practice in most situations</td>
</tr>
<tr>
<td>C</td>
<td>Body of evidence provides some support for recommendation(s) but care should be taken in its application</td>
</tr>
<tr>
<td>D</td>
<td>Body of evidence is weak and recommendation must be applied with caution</td>
</tr>
</tbody>
</table>

Clinical Practice Guideline Questions

I. When should molecular testing for NSCLC be performed?

II. How should EGFR testing be performed?

III. How should ALK testing be performed?

IV. Should other genes be routinely tested in lung adenocarcinoma?

V. How should molecular testing of lung adenocarcinomas be implemented and operationalized?

Philip T. Cagle, MD, FCAP
Question 1: Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

- 1.1a: Recommendation: EGFR molecular testing should be used to select patients for EGFR-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

- 1.1b: Recommendation: ALK molecular testing should be used to select patients for ALK-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

Different Outcomes in All Stages of Non-Small Cell Lung Cancer Patients With and Without EGFR Mutations, Treated With Tyrosine Kinase Inhibitor

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percentage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFR mutation Positive</td>
<td>EGFR mutation Negative</td>
</tr>
<tr>
<td>Response rate</td>
<td>68%</td>
<td>11%</td>
</tr>
<tr>
<td>Disease control rate</td>
<td>86%</td>
<td>42%</td>
</tr>
</tbody>
</table>
### Different Outcomes in All Stages of Non-Small Cell Lung Cancer Patients With and Without EGFR Mutations, Treated With Tyrosine Kinase Inhibitor

<table>
<thead>
<tr>
<th>Outcome</th>
<th>EGFR mutation Positive</th>
<th>EGFR mutation Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to Progression/Progression Free Survival (months)</td>
<td>12.0 ± 7.86</td>
<td>3.4 ± 2.59</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Median Survival Time (months)</td>
<td>23.3 ± 18.4</td>
<td>12.1 ± 13.9</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

### Outcomes in advanced adenocarcinoma patients with ALK rearrangements at a mean treatment duration of 6.4 months with crizotinib

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Response rate (%)</td>
<td>57%</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>33%</td>
</tr>
<tr>
<td>Disease control rate (%) at 8 weeks</td>
<td>87%</td>
</tr>
<tr>
<td>Estimated 6 month probability of Progression free survival</td>
<td>72%</td>
</tr>
</tbody>
</table>
Question 1: Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

- **1.1a: Recommendation:** EGFR molecular testing should be used to select patients for EGFR-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

- **1.1b: Recommendation:** ALK molecular testing should be used to select patients for ALK-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

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Which Patients Should be Tested for EGFR Mutations: Clinical Features?

- EGFR mutations more common in
  - women than men
  - never-smokers than former or current smokers
  - Asians than other ethnic groups

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Which Patients Should be Tested for ALK Fusion Genes: Clinical Features?

- ALK rearrangements more common in
  - never/light smokers versus former or current smokers
  - Average age of patients is younger
Clinical Criteria Excludes Too Many Potential Recipients Who Might Benefit

- Not recommended to use these clinical characteristics to exclude patients for EGFR mutation or ALK rearrangement testing
- Despite associations, there are many exceptions
- Excludes significant numbers of patients who might benefit from treatment

Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

1.2: Recommendation.—
In the setting of lung cancer resection specimens, EGFR and ALK testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade.

Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

1.2: Recommendation.—
In the setting of fully excised lung cancer specimens, EGFR and ALK testing is NOT recommended in lung cancers that lack any adenocarcinoma component, such as “pure” squamous cell carcinomas, “pure” small cell carcinomas, or large cell carcinomas lacking any immunohistochemistry (IHC) evidence of adenocarcinoma differentiation.
### Major studies specifically reporting EGFR mutation analysis in surgically resected squamous cell carcinomas as compared to adenocarcinomas

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Predominant Ethnic Origin of Study Population</th>
<th>EGFR Mutations in Resected Adenocarcinomas, No. (%)</th>
<th>EGFR Mutations in Resected Squamous Cell Carcinomas, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marchetti, et al., 2005</td>
<td>European</td>
<td>39/375 (10.4)</td>
<td>0/454</td>
</tr>
<tr>
<td>Sugio, et al., 2005</td>
<td>Asian</td>
<td>136/322 (42.2)</td>
<td>0/202</td>
</tr>
<tr>
<td>Bao, et al., 2006</td>
<td>North-American</td>
<td>14/96 (14.6)</td>
<td>0/63</td>
</tr>
<tr>
<td>Bao, et al., 2007</td>
<td>Asian</td>
<td>20/95 (21.0)</td>
<td>0/102</td>
</tr>
<tr>
<td>Lee, et al., 2010</td>
<td>Asian</td>
<td>36/127 (28.4)</td>
<td>0/56</td>
</tr>
<tr>
<td>Miyamae, et al., 2011</td>
<td>Asian</td>
<td>-</td>
<td>3/87 (3.4)</td>
</tr>
<tr>
<td>Rekhtman, et al., 2012</td>
<td>North-American</td>
<td>-</td>
<td>8/162 (4.9)</td>
</tr>
<tr>
<td>TCGA, 2012</td>
<td>North-American</td>
<td>-</td>
<td>2/178 (1.1)</td>
</tr>
</tbody>
</table>

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CONCLUSIONS:

- Our findings suggest that EGFR/KRAS mutations do not occur in pure pulmonary SQCC.
- and occasional detection of these mutations in samples diagnosed as “SQCC” is due to challenges with the diagnosis of AD-SQC and adenocarcinoma,
- which can be largely resolved by comprehensive pathologic assessment incorporating immunohistochemical biomarkers.

Studies Specifically Reporting Outcome of ALK Rearrangement
Studies in Squamous Cell Carcinomas

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>ALK Rearrangement Positive, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takeuchi, et al., 2008</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>Takahashi, et al., 2010</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Inamura, et al., 2008</td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: n, number of squamous cell carcinoma samples tested.

Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

1.3: Recommendation:

In the setting of more limited lung cancer specimens (biopsies, cytology) where an adenocarcinoma component cannot be completely excluded,

EGFR and ALK testing may be performed in cases showing squamous or small cell histology

but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing.
Which Patients Should Be Tested for EGFR Mutations and ALK Rearrangements?

1.4: Recommendation:
To determine EGFR and ALK status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.

<table>
<thead>
<tr>
<th>Primary tumor</th>
<th>EGFR+</th>
<th>EGFR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor 1</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Tumor 2</td>
<td>11</td>
<td>183</td>
</tr>
</tbody>
</table>

1.5: Expert consensus opinion:
For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.

Question 2: When Should a Patient Specimen Be Tested for EGFR Mutation or ALK Rearrangement?

2.1a: Recommendation:
EGFR mutation testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV) who are suitable for therapy
or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.
Question 2: When Should a Patient Specimen Be Tested for EGFR Mutation or ALK Rearrangement?

• 2.1b: Suggestion:

ALK rearrangement testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV) who are suitable for therapy or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.

• 2.2a: Expert Consensus Opinion:

EGFR testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.

• 2.2b: Expert Consensus Opinion:

ALK testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged, but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.
Question 2: When Should a Patient Specimen Be Tested for EGFR Mutation or ALK Rearrangement?

- 2.3: Recommendation:
  Tissue should be prioritized for EGFR and ALK testing.
Question 3: How Rapidly Should Test Results Be Available?

- 3.1: Expert Consensus Opinion: EGFR and ALK results should be available within 2 weeks (10 working days) of receiving the specimen in the testing laboratory.
- 3.2: Expert Consensus Opinion: Laboratories with average turnaround times beyond 2 weeks need to make available a more rapid test — either in-house or through a reference laboratory — in instances of clinical urgency.
- 3.3: Expert Consensus Opinion: Laboratory departments should establish processes to ensure that specimens that have a final pathologic diagnosis are sent to outside molecular pathology laboratories within 3 working days of receiving requests and to intramural molecular pathology laboratories within 24 hours.

Turnaround Time (TAT)

- No publications relate TAT to outcome
- Diagnosis must be established first
  - Need efficiency after diagnosis established
- Some patients can wait; some cannot
  - Untreated stage IV lung cancer survival: ~4 mos
    - Treatment is delayed pending test result
- Our opinion: 2 weeks or less is reasonable and feasible
  - Slowest recommended method: Sanger

Question 4: How Should Specimens Be Processed for EGFR Testing?

- 4.1: Expert Consensus Opinion — Pathologists should use formalin-fixed, paraffin-embedded (FFPE) specimens or fresh, frozen, or alcohol-fixed specimens for polymerase chain reaction (PCR)-based EGFR mutation tests. Other tissue treatments (e.g., acidic or heavy metal fixatives, or decalifying solutions) should be avoided in specimens destined for EGFR testing.
Question 4: How Should Specimens Be Processed for EGFR Mutation Testing?

- 4.2: Expert Consensus Opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.

- Smear preparations
  - EGFR mutation: adequate if suitably cellular
  - ALK FISH: interpretive challenges
    - Overlapping nuclei
    - Identification of malignant cells with DAPI stain

Question 5: What Are the Specimen Requirements for EGFR Testing?

- 5.1: Expert Consensus Opinion: Pathologists should determine the adequacy of specimens for EGFR testing by assessing cancer cell content and DNA quantity and quality.

- 5.2: Expert Consensus Opinion: Each laboratory should establish the minimum proportion and number of cancer cells needed for mutation detection during validation.

- 5.3: Expert Consensus Opinion — A pathologist should assess the tumor content of each specimen and either perform, or guide a trained technologist to perform, microdissection for tumor cell enrichment as needed.

Question 6: How Should EGFR Testing Be Performed?

- 6.1: Recommendation: Laboratories may use any validated EGFR testing method with sufficient performance characteristics.

- 6.2: Expert consensus opinion: Laboratories should use EGFR test methods that are able to detect mutations in specimens with at least 50% cancer cell content, although laboratories are strongly encouraged to use (or have available at an external reference laboratory) more sensitive tests that are able to detect mutations in specimens with as little as 10% cancer cells.
Question 6: How Should EGFR Testing Be Performed?

- Sanger sequencing is OK
  - Initial discoveries that showed EGFR mutations were clinically useful used Sanger sequencing

- BUT...

- A lot of patients have samples that are too small or too heterogeneous for Sanger sequencing
  - Sanger labs should make a more sensitive test available for these patients
    - PNA/LNA enrichment, COLD-PCR, second test, sendout

Sample with 30% Tumor content

<table>
<thead>
<tr>
<th>UNMODIFIED Sanger</th>
<th>PNA-enriched Sanger</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR wild type</td>
<td>EGFR exon 21 mutation</td>
</tr>
<tr>
<td>Rx: platinum doublet</td>
<td>Rx: erlotinib</td>
</tr>
<tr>
<td>1-yr survival: 5%</td>
<td>1-yr survival: 30%</td>
</tr>
</tbody>
</table>

6.3 Opinion: Test for all EGFR mutations accounting individually for at least 1% of all EGFR mutations
Question 6: How Should EGFR Testing Be Performed?

- 6.4: Recommendation: Immunohistochemistry (IHC) for total EGFR is not recommended for selection of EGFR TKI therapy.

Mutation vs. response rate
RR=5.2

IHC vs. response rate
RR=1.3

- 6.5: Recommendation: EGFR copy number analysis (e.g., FISH or CISH) is not recommended for selection of EGFR TKI therapy.

Mutation vs. PFS
WMD=7.5

ISH vs. PFS
WMD=0.22

Marc Ladanyi, MD
**Disclosures**

Dr. Ladanyi has disclosed the following:

- Consultancy: Ample / Dako Inc. (April 2010), NanoString (September 2012)
- Lecture Fees Paid by Entity: Genzyme (March 2010), Infinity (July 2010), Sequenom (November 2009), Medscape CME (June 2012)
- Family and Business Partners: Wife: Continuing Medical Education (CME) activities for Abbott
- Institutional Financial Interest: Memorial Sloan-Kettering Cancer Center (MSKCC) licensed patent for EGFR T790M testing to MolecularMD. ML is not a patent holder.

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**Question 7: What Is the Role of KRAS Analysis in Selecting Patients for Targeted Therapy With EGFR TKIs?**

- **7.1: Recommendation:** KRAS mutation testing is not recommended as a sole determinant of EGFR TKI therapy.
  - KRAS mutations are mutually exclusive with EGFR mutations (and ALK fusions)
  - KRAS mutations are the most common oncogene mutations in lung adenocarcinoma (approx. 30-35%)
  - KRAS mutations are “easy” to study: >95% are in codons G12 and G13 so can be detected by sequencing just exon 2 of KRAS
  - KRAS mutations predict lack of response to EGFR TKIs

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**KRAS Mutations: A Negative Predictor for Response to EGFR TKIs**

<table>
<thead>
<tr>
<th>Author</th>
<th>Drugs</th>
<th>Patients tested for KRAS</th>
<th>Response rate reported (%)</th>
<th>Response rate (KRAS mutant)</th>
<th>Response rate (wild type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jemal et al.</td>
<td>Erlotinib</td>
<td>45 (68%)</td>
<td>0%</td>
<td>14%</td>
<td>2%</td>
</tr>
<tr>
<td>Druce et al.</td>
<td>Erlotinib</td>
<td>50 (80%)</td>
<td>0%</td>
<td>15%</td>
<td>5%</td>
</tr>
<tr>
<td>Marikonda et al.</td>
<td>Erlotinib</td>
<td>70 (12%)</td>
<td>0%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Politi et al.</td>
<td>Erlotinib</td>
<td>108 (18%)</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td>Najbauer et al.</td>
<td>Erlotinib</td>
<td>80 (15%)</td>
<td>0%</td>
<td>6%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Wild type (n=18)*

Impact of KRAS mutations on outcomes in patients for treated with EGFR Tyrosine Kinase Inhibitors

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No KRAS Mutations</th>
<th>KRAS Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (%)</td>
<td>3%</td>
<td>24%</td>
</tr>
<tr>
<td>95% CI</td>
<td>[0.18, 0.60]</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

**Outcome Mean ± SD**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No KRAS Mutations</th>
<th>KRAS Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPF/DFS (months)</td>
<td>3.4 ± 2.7</td>
<td>5 ± 3.7</td>
</tr>
<tr>
<td>Median Overall Survival (months)</td>
<td>9.2 ± 5.6</td>
<td>13.2 ± 7.1</td>
</tr>
</tbody>
</table>

**Recommendation:** If a laboratory performs testing on specimens from patients with acquired resistance to EGFR kinase inhibitors, such tests should be able to detect the secondary EGFR T790M mutation in as few as 5% of cells.

**Question 8:** What Additional Testing Considerations Are Important in the Setting of Secondary or Acquired EGFR TKI Resistance?

- As a secondary, acquired mutation, the T790M is not present in every tumor cell.
- Biopsies of previously treated, recurrent tumors often have low tumor cell content, further increasing the need for more sensitive mutation detection.
- In vitro studies suggest that cell population level EGFR TKI resistance becomes detectable in the presence of as little as 5% T790M-bearing cells.

Detection of EGFR T790M in tumors from patients with relapse after initial response to EGFR TKI treatment

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>T790M Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen HJ, et al., 2009</td>
<td>14 10</td>
<td>68%</td>
</tr>
<tr>
<td>Kosaka T, et al., 2006</td>
<td>7 10</td>
<td>90%</td>
</tr>
<tr>
<td>Onitsuka T, et al., 2010</td>
<td>7 10</td>
<td>70%</td>
</tr>
<tr>
<td>Oxnard, et al., 2011</td>
<td>58 93</td>
<td>62%</td>
</tr>
<tr>
<td>Total</td>
<td>86 146</td>
<td>53%</td>
</tr>
</tbody>
</table>

- The EGFR tyrosine kinase domain mutation, T790M, is caused by a single base substitution, C to T, at nucleotide 2369.
- This mutation is found as a second mutation on the EGFR allele harboring the initial "sensitizing" EGFR mutation.
9.1: Recommendation: Laboratories should use an ALK FISH assay using dual-labeled break-apart probes for selecting patients for ALK TKI therapy; ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for ALK FISH testing.

- FISH was the methodology used in the initial studies that demonstrated major clinical responses of patients with ALK-rearranged tumors to treatment with crizotinib, a targeted ALK TKI.
Comparing ALK FISH with Immunohistochemistry (IHC)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Concordance</th>
<th>Discordance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FISH+/IHC+</td>
<td>FISH+/IHC-</td>
</tr>
<tr>
<td>IHC - CD246</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>IHC - D5F3/D9E4</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>IHC - 5A</td>
<td>28</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: n, Number of studies; N, Number of patients

- A properly validated ALK IHC method may be used as a screening modality, and tumors that fail to demonstrate ALK immunoreactivity with a sensitive IHC method may not need to be tested by ALK FISH.

Question 9: What methods should be used for ALK testing?

- 9.2: Recommendation: RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.

ALK fusions: multiplicity of EML4-ALK variants + rare other ALK fusion partners complicate comprehensive detection by RT-PCR.

Horn L, Pao W JCO 2009;27:4232-4235
Question 9: What methods should be used for ALK testing?

- 9.3: Expert consensus opinion: A pathologist should be involved in the selection of sections for ALK FISH testing, by assessing tumor architecture, cytology, and specimen quality
  - For ALK FISH, a pathologist should choose slides or indicate regions of slides for scoring in which tumor cells are most numerous and can be distinguished from admixed normal cells under fluorescence, typically through a combination of cytologic and architectural features that can be appreciated without stains or visualization of cytoplasm.

- 9.4: Expert consensus opinion: A pathologist should participate in the interpretation of ALK FISH slides, either by performing the analysis directly or by reviewing the interpretations of cytogeneticists or technologists with specialized training in solid tumor FISH analysis.
  - The FISH technologist should work closely with a pathologist who can identify tumor-rich areas.
  - The FISH technologist should also have had training on the morphologic appearance of lung cancer, and should have easy access to assistance from a pathologist with training in FISH.

- 9.5: Expert consensus opinion: Testing for secondary mutations in ALK associated with acquired resistance to ALK inhibitors is not currently required for clinical management.
  - A diverse set of secondary mutations in ALK have been reported to confer acquired resistance to crizotinib (L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, G1269A).
  - The spectrum of acquired resistance mechanisms and their implications for further management require further studies.
Question 10: Are Other Molecular Markers Suitable for Testing in Lung Cancer?

- 10.1a: Recommendation: Testing for EGFR should be prioritized over other molecular markers in lung adenocarcinoma.

- 10.1b: Suggestion.—After EGFR testing, testing for ALK should be prioritized over other proposed molecular markers in lung adenocarcinoma, for which published evidence is insufficient to support testing guideline development at the present time.
  - In advanced stage patients diagnosed by small biopsies, precious tumor tissue must be reserved for these analyses, before any other molecular analysis is considered.

Priority of Testing for EGFR and ALK in major clinical guidelines

Neal I. Lindeman, MD
Question 11: Must All Adenocarcinomas Be Tested for Both EGFR and ALK?

- 11.1: Expert consensus opinion: Laboratories may implement testing algorithms to enhance the efficiency of molecular testing of lung adenocarcinomas, provided the overall turnaround time requirements are met.

- EGFR, ALK, and KRAS are largely mutually exclusive
  - If a mutation is found in one, further testing is unnecessary
  - This may not apply to novel mutations

Question 12: How Should EGFR and ALK Results Be Reported?

- 12.1: Expert consensus opinion: EGFR mutation testing reports and ALK FISH reports should include a results and interpretation section readily understandable by oncologists and by nonspecialist pathologists.

Formal nomenclature should be used, but also translated

```
nuc ish(ALKx2)/(ALK sep 3’ALKx1)[56/100]
FISH for ALK showed a split (positive) signal in 56% of 100 cancer cells analyzed
This result demonstrates an ALK rearrangement and suggests that this lung cancer is likely to respond to treatment with a targeted inhibitor of the ALK kinase, such as crizotinib.
```

Question 13 & 14: How Should EGFR and ALK Testing Be Validated? How Should Quality Assurance Be Maintained?

- 13.1: Expert consensus opinion: EGFR and ALK testing validation should follow the same guidelines as for other molecular diagnostics and FISH tests.

- 14.1: Expert consensus opinion: Laboratories should follow similar quality control and quality assurance policies and procedures for EGFR and ALK testing in lung cancers as for other clinical laboratory assays. In particular, laboratories performing EGFR and ALK testing for TKI therapy should enroll in proficiency testing, if available.
Lung Adenocarcinoma molecular testing guidelines: what’s next

Mutually exclusive oncogene mutations in lung adenocarcinoma

- KRAS mutation: 29,000/yr
- EGFR mutation: 20,000/yr
- No known driver oncogene

(numbers based on approximate US annual incidence of 100,000)

Except for RAS genes, all have effective targeted agents available or in clinical development.

Markers in response to Crizotinib in a patient with ROS1-fusion-positive Lung Adenocarcinoma

Note: Crizotinib is a TKI for ALK/MET/ROS1.

Baseline

After 3 months of crizotinib

Marked response to the ERBB2 TKI Dacomitinib in a patient with an ERBB2-mutated lung adenocarcinoma

MSKCC protocol #10-080, P.I.: Mark Kris, MD

Marked response to the BRAF kinase inhibitor Dabrafenib in a patient with BRAF V600E Lung Cancer

Baseline 6 weeks on Dabrafenib 4 months on Dabrafenib

Greg Riely, MD PhD, MSKCC

Marked response to the RET TKI Cabozantinib in a patient with RET fusion positive Lung Adenocarcinoma

Partial response (47% shrinkage) after 28 days of cabozantinib.

Lung Adenocarcinoma molecular testing guidelines: what's next

Mutually exclusive oncogene mutations in lung adenocarcinoma:

- **KRAS** mutation: 29,000/yr
- **EGFR** mutation: 20,000/yr
- **BRAF** - 1500/yr
- **ERBB2** - 2000/yr
- **ALK** - 4000/yr
- **ROS1** - 1000/yr
- **RET** - 1000/yr
- Others: MET, MAP2K1, NRAS

Except for RAS genes, all have effective targeted agents available or in clinical development.

Questions?

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By: CAP, ASCP, CLIA

4/24/2013
Different Outcomes in All Stages of Non-Small Cell Lung Cancer Patients With and Without EGFR Mutations, Treated With Tyrosine Kinase Inhibitor

<table>
<thead>
<tr>
<th>Outcome</th>
<th>EGFR Mutation Positive</th>
<th>EGFR Mutation Negative</th>
<th>n (N)</th>
<th>RR [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response Rate (%)</td>
<td>68%</td>
<td>11%</td>
<td>51(3644)</td>
<td>1.99 [1.73, 2.29]</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Disease Control Rate (%)</td>
<td>86%</td>
<td>42%</td>
<td>28(2204)</td>
<td>1.99 [1.73, 2.29]</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Time to Progression/Progression-Free Survival (months)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>EGFR Mutation Positive</th>
<th>EGFR Mutation Negative</th>
<th>n (N)</th>
<th>WMD [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to Progression</td>
<td>12.0 ± 7.86</td>
<td>3.4 ± 2.31</td>
<td>27(2347)</td>
<td>8.66 [6.31, 11.00]</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations:
- CI, Confidence interval;
- n, Number of studies;
- N, Number of patients;
- RR, Relative risk;
- SD, Standard deviation;
- WMD, Weighted mean difference.

Randomized Clinical Trial Data on EGFR Tyrosine Kinase Inhibitor (TKI) Therapy Versus Chemotherapy as First-Line Therapy for Patients With EGFR-Mutated Lung Cancers

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Patients With EGFR-Mutated Lung Cancers</th>
<th>Response Rate (EGFR TKI Versus Chemotherapy)</th>
<th>Progression-Free Survival (EGFR TKI Versus Chemotherapy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EURTAC</td>
<td>173 (86 erlotinib and 87 chemo)</td>
<td>58% vs. 15%</td>
<td>9.7 vs. 5.2 (HR 0.37)</td>
</tr>
<tr>
<td>OPTIMAL</td>
<td>154 (82 erlotinib and 72 chemo)</td>
<td>83% vs. 36%</td>
<td>13.1 vs. 4.6 (HR 0.16)</td>
</tr>
<tr>
<td>NEJ002</td>
<td>228 (114 gefitinib and 114 chemo)</td>
<td>74% vs. 31%</td>
<td>10.8 vs. 5.4 (HR 0.30)</td>
</tr>
<tr>
<td>WJTOG3405</td>
<td>117 (58 gefitinib and 59 chemo)</td>
<td>62% vs. 32%</td>
<td>9.2 vs 6.3 (HR 0.49)</td>
</tr>
<tr>
<td>IPASS</td>
<td>261 (132 gefitinib and 129 chemo)</td>
<td>71% vs. 47%</td>
<td>9.5 vs. 6.3 (HR 0.48)</td>
</tr>
<tr>
<td>LUX LUNG3</td>
<td>345 (230 afatinib and 115 chemo)</td>
<td>56% vs. 23%</td>
<td>11.1 vs. 6.9 (HR 0.58)</td>
</tr>
</tbody>
</table>

Abbreviations:
- Chemo, Chemotherapy; HR, Hazard ratio.