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
Guideline Publication


Dr. Lindeman has disclosed the following:

- Partners Health Care has filed a patent on EGFR Mutation Testing. NIL is not a patent holder.
<table>
<thead>
<tr>
<th>Role</th>
<th>CAP</th>
<th>IASLC</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steering Committee</td>
<td>Jan Nowak, MD, PhD</td>
<td>Paul A. Bunn, Jr, MD</td>
<td>Neal I. Lindeman, MD</td>
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<tr>
<td></td>
<td>North Shore University Health System</td>
<td>University of Colorado</td>
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<td></td>
<td>Evanston, Illinois</td>
<td>Denver, Colorado</td>
<td>Massachusetts</td>
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<tr>
<td>Co-chair</td>
<td>Philip T. Cagle, MD</td>
<td>Marc Ladanyi, MD</td>
<td>Neal I. Lindeman, MD</td>
</tr>
<tr>
<td></td>
<td>The Methodist Hospital</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
<td>Brigham and Women’s Hospital Boston</td>
</tr>
<tr>
<td></td>
<td>Houston, Texas</td>
<td>New York City, New York</td>
<td>Massachusetts</td>
</tr>
<tr>
<td>Expert Panelist</td>
<td>Sanja Dacic, MD, PhD</td>
<td>David J. Kwiatkowski, MD, PhD</td>
<td>Dhananjay Chitale, MD</td>
</tr>
<tr>
<td></td>
<td>University of Pittsburgh Medical Center</td>
<td>Brigham and Women’s Hospital Boston</td>
<td>Henry Ford Hospital</td>
</tr>
<tr>
<td></td>
<td>Pittsburgh, Pennsylvania</td>
<td>Massachusetts</td>
<td>Detroit, Michigan</td>
</tr>
<tr>
<td>Expert Panelist</td>
<td>Robert Brian Jenkins, MD, PhD</td>
<td>Giuseppe Giaccone, MD, PhD</td>
<td>Juan-Sebastian Saldivar, MD</td>
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<td>Mayo Clinic</td>
<td>National Institutes of Health</td>
<td>City of Hope National Medical Center</td>
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<td>Rochester, Minnesota</td>
<td>Bethesda, Maryland</td>
<td>Duarte, California</td>
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<tr>
<td>Expert Panelist</td>
<td>Mary Beth Beasley, MD</td>
<td>Erik Thunnissen, MD, PhD</td>
<td>Jeremy Squire, PhD</td>
</tr>
<tr>
<td></td>
<td>Mt Sinai Medical Center</td>
<td>VU University Medical Center, Amsterdam,</td>
<td>Kingston General Hospital</td>
</tr>
<tr>
<td></td>
<td>New York City, New York</td>
<td>the Netherlands</td>
<td>Kingston, Ontario</td>
</tr>
</tbody>
</table>
Advisory Panel

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## 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?
Disclosures

Dr. Cagle has disclosed the following:

• Archives of Pathology & Laboratory Medicine, Editor-in-Chief (Recused from the journals’ approval process of this guideline)
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>

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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>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>EGFR mutation Positive</strong></td>
<td><strong>EGFR mutation Negative</strong></td>
</tr>
<tr>
<td>Time to Progression/Progression Free Survival (months)</td>
<td>12.0 ± 7.86</td>
<td>3.4 ± 2.59</td>
</tr>
<tr>
<td>Median Survival Time (months)</td>
<td>23.3 ± 18.4</td>
<td>12.1 ± 13.9</td>
</tr>
</tbody>
</table>
Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

Eunice L. Kwak, M.D., Ph.D., Yung-Jue Bang, M.D., Ph.D., D. Ross Camidge, M.D., Ph.D., Alice T. Shaw, M.D., Ph.D., Benjamin Solomon, M.B., B.S., Ph.D., Robert G. Maki, M.D., Ph.D., Sai-Hong I. Ou, M.D., Ph.D., Bruce J. Dezube, M.D., Pasi A. Jänne, M.D., Ph.D., Daniel B. Costa, M.D., Ph.D., Marileila Varella-Garcia, Ph.D., Woo-Ho Kim, M.D., Thomas J. Lynch, M.D., Panos Fidias, M.D., Hannah Stubbs, M.S., Jeffrey A. Engelman, M.D., Ph.D., Lecia V. Sequist, M.D., M.P.H., Wei Wei Tan, Ph.D., Leena Gandhi, M.D., Ph.D., Mari Mino-Kenudson, M.D., Greg C. Wei, Ph.D., S. Martin Shreeve, M.D., Ph.D., Mark J. Ratain, M.D., Jeffrey Settleman, Ph.D., James G. Christensen, Ph.D., Daniel A. Haber, M.D., Ph.D., Keith Wilner, Ph.D., Ravi Salgia, M.D., Ph.D., Geoffrey I. Shapiro, M.D., Ph.D., Jeffrey W. Clark, M.D., and A. John Iafrate, M.D., Ph.D.
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>

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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.
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
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., 2006</td>
<td>Asian</td>
<td>136/322 (42.2)</td>
<td>0/102</td>
</tr>
<tr>
<td>Tsao, et al., 2006</td>
<td>North American</td>
<td>14/96 (14.6)</td>
<td>0/63</td>
</tr>
<tr>
<td>Tsao, et al., 2011</td>
<td>North American</td>
<td>32/231 (13.9)</td>
<td>8/162 (4.9)</td>
</tr>
<tr>
<td>Bae, et al., 2007</td>
<td>Asian</td>
<td>20/55 (36.4)</td>
<td>0/60</td>
</tr>
<tr>
<td>Lee, et al., 2010</td>
<td>Asian</td>
<td>36/117 (30.8)</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>0/95</td>
</tr>
<tr>
<td>TCGA, 2012</td>
<td>North American</td>
<td>-</td>
<td>2/178 (1.1)</td>
</tr>
</tbody>
</table>
Rekhtman N, Paik PK, Arcila ME, Tafe LJ, Oxnard GR, Moreira AL, Travis WD, Zakowski MF, Kris MG, Ladanyi M.

“Clarifying the Spectrum of Driver Oncogene Mutations in Biomarker-Verified Squamous Carcinoma of Lung: Lack of EGFR/KRAS and Presence of PIK3CA/AKT1 Mutations.”
RESULTS:

- 95 biomarker-verified SQCCs revealed no EGFR/KRAS mutations
- Detailed morphologic and immunohistochemical reevaluation of EGFR/KRAS-mutant 'SQCC'
- 10 (63%) cases reclassified as AD-SQCC
- 5 (31%) cases reclassified as poorly differentiated adenocarcinoma morphologically mimicking SQCC (i.e., adenocarcinoma with "squamoid" morphology)
- 1 (6%) case had no follow-up.
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>Metastatic lesions</th>
<th>Primary tumor</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>EGFR +</strong></td>
<td><strong>EGFR -</strong></td>
<td></td>
</tr>
<tr>
<td><strong>EGFR +</strong></td>
<td>108</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>EGFR -</strong></td>
<td>11</td>
<td>183</td>
<td></td>
</tr>
</tbody>
</table>

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

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

• 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.
Goldstraw et al.
Journal of Thoracic Oncology.
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.

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Neal I. Lindeman, MD
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
  o Need efficiency after diagnosis established

• Some patients can wait; some cannot
  o Untreated stage IV lung cancer survival: ~4 mos
    - Treatment is delayed pending test result

• Our opinion: 2 weeks or less is reasonable and feasible
  o 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 (eg, **acidic or heavy metal fixatives**, or **decalcifying 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
  o EGFR mutation: adequate if suitably cellular
  o ALK FISH: interpretive challenges
    - Overlapping nuclei
    - Identification of malignant cells with DAPI stain

http://www.eurocytology.eu
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

**UNMODIFIED Sanger**

*EGFR wild type*
Rx: platinum doublet
1-yr survival: 5%

**PNA-enriched Sanger**

*EGFR exon 21 mutation*
Rx: erlotinib
1-yr survival: 30%
### 6.3 Opinion: Test for all EGFR mutations accounting individually for at least 1% of all EGFR mutations

<table>
<thead>
<tr>
<th>EGFR exon</th>
<th>EGFR codon</th>
<th>Mutations(^a) (amino acid)</th>
<th>Nucleotide substitutions</th>
<th>Approximate % of all EGFR mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G719</td>
<td>G719S, G719A, G719C, G719D</td>
<td>c.2155G&gt;A, c.2156G&gt;C, c.2155G&gt;T, c.2156G&gt;A</td>
<td>2-5%</td>
</tr>
<tr>
<td>19</td>
<td>K739, I740, P741, V742, A743, I744</td>
<td>Insertions 18 bp ins</td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>E746, L747, R748, E749, A750, T751, S752, P753</td>
<td>Deletions 15bp del, 18bp del, 9 bp del, 24bp del, 12bp del</td>
<td></td>
<td>45%</td>
</tr>
<tr>
<td>20</td>
<td>A763, A767, S768, V769, D770, N771, P772, H773, V774</td>
<td>Insertions 3 bp ins, 6 bp ins, 9 bp ins, 12 bp ins</td>
<td></td>
<td>5-10%</td>
</tr>
<tr>
<td></td>
<td>S768</td>
<td>S768I</td>
<td>c.2303G&gt;T</td>
<td>1-2%</td>
</tr>
<tr>
<td></td>
<td>T790</td>
<td>T790M</td>
<td>c.2369C&gt;T</td>
<td>2(^b)</td>
</tr>
<tr>
<td>21</td>
<td>L858</td>
<td>L858R, L858M</td>
<td>c.2573T&gt;G, c.2572C&gt;A (rare)</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>L861</td>
<td>L861Q, L861R</td>
<td>c.2582T&gt;A, c.2582T&gt;G</td>
<td>2-5%</td>
</tr>
</tbody>
</table>

\(^a\) Mutations denote substitutions in nucleotide sequences as compared to the wild-type sequence.
Question 6: How Should EGFR Testing Be Performed?

- **6.4: Recommendation:** Immunohistochemistry 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 (ie, **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: Arqule / Daiichi Sankyo (April 2010), NanoString (September 2012)

• Lecture Fees Paid by Entity: Genzyme (March 2010), Infinity (July 2010), Sequenom (November 2009), Remedica Medical Education (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.
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
**KRAS Mutations: A Negative Predictor for Response to EGFR TKIs**

Table 1. Retrospective Analyses of EGFR Tyrosine Kinase Inhibitors in Lung Adenocarcinoma

<table>
<thead>
<tr>
<th>Author</th>
<th>Drugs</th>
<th>Patients tested for KRAS mutations (mutant/WT)</th>
<th>Response rate KRAS mutant</th>
<th>Response rate KRAS WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackman\textsuperscript{12}</td>
<td>Erlotinib</td>
<td>41 (6/35)</td>
<td>0%</td>
<td>14%</td>
</tr>
<tr>
<td>Zhu\textsuperscript{13}</td>
<td>Erlotinib</td>
<td>206 (30/176)</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>Miller\textsuperscript{9}</td>
<td>Erlotinib</td>
<td>80 (18/62)</td>
<td>0%</td>
<td>30%</td>
</tr>
<tr>
<td>Massarelli\textsuperscript{14}</td>
<td>Erlotinib/Gefitinib</td>
<td>70 (16/54)</td>
<td>0%</td>
<td>7%</td>
</tr>
<tr>
<td>Hirsch\textsuperscript{10}</td>
<td>Gefitinib</td>
<td>138 (36/102)</td>
<td>1%</td>
<td>7%</td>
</tr>
<tr>
<td>Hirsch\textsuperscript{15}</td>
<td>Gefitinib</td>
<td>152 (12/140)</td>
<td>0%</td>
<td>8%</td>
</tr>
<tr>
<td>Han\textsuperscript{16}</td>
<td>Gefitinib</td>
<td>69 (9/60)</td>
<td>0%</td>
<td>27%</td>
</tr>
</tbody>
</table>

WT: wild type (non-mutated).

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

### Outcome

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percentage</th>
<th>n (N)</th>
<th>RR [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KRAS Mutations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No KRAS Mutations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response rate (%)</td>
<td>3%</td>
<td>24%</td>
<td>12(1041)</td>
<td>0.33 [0.18, 0.60]</td>
</tr>
</tbody>
</table>

### Outcome

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mean ± SD</th>
<th>n (N)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KRAS Mutations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No KRAS Mutations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to Progression/ Progression Free Survival (months)</td>
<td>3.4 ± 2.7</td>
<td>5 ± 3.7</td>
<td>7(918)</td>
</tr>
<tr>
<td>Median Overall Survival time (months)</td>
<td>9.2 ± 5.6</td>
<td>13.2 ± 7.1</td>
<td>7(737)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; RR, Relative Risk, Mantel-Haenszel, Random Effects model, [95% CI]

- ... but a lack of **KRAS** mutation is only associated with a 24% response rate to EGFR TKI because most (approx. 70%) of **KRAS-non-mutated** cases also lack **EGFR** mutations.
- A rapid and inexpensive **KRAS** assay may be performed to exclude **KRAS-mutated** tumors from **EGFR** mutation testing as part of an algorithm designed to maximize testing efficiency.
Question 8: What Additional Testing Considerations Are Important in the Setting of Secondary or Acquired EGFR TKI Resistance?

8.1: 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.

- 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

- 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.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>T790M</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen HJ, <em>et al.</em>, 2009</td>
<td>14</td>
<td>29</td>
<td>48%</td>
</tr>
<tr>
<td>Kosaka T, <em>et al.</em>, 2006</td>
<td>7</td>
<td>14</td>
<td>50%</td>
</tr>
<tr>
<td>Onitsuka T, <em>et al.</em>, 2010</td>
<td>7</td>
<td>10</td>
<td>70%</td>
</tr>
<tr>
<td>Oxnard, <em>et al.</em>, 2011</td>
<td>58</td>
<td>93</td>
<td>62%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>86</strong></td>
<td><strong>146</strong></td>
<td><strong>59%</strong></td>
</tr>
</tbody>
</table>

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

- 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.
Figure 1. Negative for *ALK* rearrangement

Figure 2. Positive for *ALK* rearrangement (split 3’ ALK-5’ ALK)

Figure 3. Positive for *ALK* rearrangement (single 3’ ALK)

Figure 4. Negative for *ALK* rearrangement with ALK high copy number

Site of breaks
Crizotinib in *EML4-ALK* fusion positive lung adenocarcinoma

Approx. 70% have >30% decrease in tumor


Pre-treatment

After 2 cycles of crizotinib
Comparing ALK FISH with Immunohistochemistry (IHC)

<table>
<thead>
<tr>
<th>IHC Antibody</th>
<th>n(N)</th>
<th>FISH+/IHC+</th>
<th>FISH-/IHC-</th>
<th>FISH+/IHC-</th>
<th>FISH-/IHC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC - CD246</td>
<td>4(391)</td>
<td>25</td>
<td>344</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>IHC - D5F3/D9E4</td>
<td>3(148)</td>
<td>46</td>
<td>101</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IHC - 5A4</td>
<td>1(640)</td>
<td>28</td>
<td>602</td>
<td>0</td>
<td>10</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

---

**Diagram Description:**

- **ALK** gene
- **EML4** gene
- **Kinase** domain

**Variants:**

- E13;A20: V1 (45-55%)
- E20;A20: V2
- E6a/b;A20: V3a/b
- E14;ins11del49A20: V4
- E2;A20 & E2;ins117A20: V5a/b
- E13;ins69A20: V6
- E14;del12A20: V7
- E15del19;del20A20: “V4”
- E18;A20: “V5”

---

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

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

• 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.
  o A diverse set of secondary mutations in ALK have been reported to confer acquired resistance to crizotinib (L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, G1269A).
  o 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

\[ \text{nuc ish(ALKx2)(5'ALK sep 3'ALKx1)[56/100]} \]

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
- **BRAF** – 1500/yr
- **ERBB2** – 2000/yr
- **ALK** – 4000/yr
- **ROS1** – 1000/yr
- **RET** – 1000/yr

Others: **MET, MAP2K1, NRAS**

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.
Marked 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

Bergethon, Shaw, Ou et al., JCO 30(8): 863-70, 2012
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 \textit{BRAF V600E} Lung Cancer
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

*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.

*BRaf* – 1500/yr

*ERBB2* – 2000/yr

*ALK* – 4000/yr

*ROS1* – 1000/yr

*RET* – 1000/yr

Others: *MET, MAP2K1, NRAS*
Questions?
CAP Center Process-Guideline Development
### 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>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><strong>Response rate (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR mutation Positive</td>
<td>68%</td>
<td></td>
<td></td>
<td>51(3644)</td>
<td>5.16[4.41, 6.04]</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>EGFR mutation Negative</td>
<td>11%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disease control rate (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR mutation Positive</td>
<td>86%</td>
<td></td>
<td></td>
<td>28(2204)</td>
<td>1.99[1.73, 2.29]</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>EGFR mutation Negative</td>
<td>42%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mean ± SD</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><strong>Time to Progression/Progression Free Survival (months)</strong></td>
<td>12.0 ± 7.86</td>
<td></td>
<td></td>
<td>27(2347)</td>
<td>8.66 [6.31, 11.00]</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td><strong>Median Survival Time (months)</strong></td>
<td>23.3 ± 18.4</td>
<td></td>
<td></td>
<td>27(2489)</td>
<td>10.66 [8.36, 12.96]</td>
<td>P &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 in Months (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>NEJ 002</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>WJTOG 3405</td>
<td>117 (59 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

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