Molecular Diagnostics in Lung Cancer

Mutations in lung carcinomas and their impact on diagnosis and treatment

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• Laboratory professionals may earn 1.0 contact hour for participation in today’s program

• Learning objectives: upon completion of this program, participants will be able to
  ✓ Discuss the current and emerging roles of molecular profiling in the treatment of NSCLC
  ✓ Differentiate between genetic mutations often found in patients with NSCLC and communicate the effects that these mutations have on patient management
  ✓ Describe the clinical utility for assays that identify mutations of EGFR, KRAS and EML4-ALK
Lung Cancer Epidemiology

- 219,440 new cases diagnosed and 159,390 deaths in 2009
- 15% survival at 5-years

(NCCN Practice Guidelines in Oncology v.2.2010)

**FIGURE 7–21A** Cancer incidence and mortality by site and sex. Excludes basal cell and squamous cell skin cancers and in situ carcinomas, except urinary bladder.

(Adapted from Jemal A et al.: Cancer statistics, 2008. CA Cancer J Clin 58:2, 2008.)

**FIGURE 7–21B** Cancer incidence and mortality by site and sex. Excludes basal cell and squamous cell skin cancers and in situ carcinomas, except urinary bladder.

(Adapted from Jemal A et al.: Cancer statistics, 2008. CA Cancer J Clin 58:2, 2008.)
Non-small Cell Lung Carcinoma

Adenocarcinoma
- Gland formation & mucin
- More common in non-smokers
- Worse outcome in general
- BAC type has better outcome

Squamous cell carcinoma
- Keratinization & necrosis
- Strongly associated w/smoking
- Arises in airway (bronchial) cells
- Difficult to cure in part due to in situ lesions (“field” effect)
Histologic Differences Reflected in Immunophenotype

Lung cancer or not

Adenocarcinoma vs Squamous Cell Carcinoma

CK7

CK20

TTF-1

P63

CK20 ctrl

P63 ctrl
How Lung Cancer is Treated

- If caught early (usually by incidental radiology) → surgery
  - High cure rate; adjuvant radiotherapy or maintenance chemo may help

- If found at advanced stage (III/IV) → 3 main options
  - Chemotherapy: 2 drugs including carboplatin
    - ~4-6 months prolongation of overall survival
  - New drugs that (mostly) don’t kill cells but block growth
    - Kinase inhibitors against receptor tyrosine kinases (RTKs)
    - Antibody immunotherapy against RTKs (e.g. cetuximab)
  - Clinical Trials using a combination of chemo & targeted approaches
    - SWOG S0342: paclitaxel/carboplatin plus cetuximab
      (J Clin Oncol, 2010;28:4747)
EGFR and Its Downstream Effectors Drive Cancer Growth

Mechanisms of Action of Anti-EGFR Drugs

EGFR Kinase Inhibitors Work in a Subset of Lung Cancer

Kaplan-Meier Curve

Maemondo et al., *N Engl J Med.* 2010;362:2380-8. ©2010 Massachusetts Medical Society. All rights reserved. Used with permission.
Activating Point Mutations in EGFR Predict Response to EGFR Inhibitors

1. Only 5-10% of US patients with lung cancer respond to EGFR kinase inhibitors
2. Response to EGFR KI is correlated with point mutation or deletions in EGFR kinase domain
3. Range of mutations (exons 18-21)
4. Largely confined to never-smokers, ACA

Signals from multiple cell surface receptor families converge at the level of Ras.
EML4-ALK: A Newly Identified Targetable Change

- Novel gene fusion produced by chr 2 paracentric inversion large restricted to NSCLC
  - EML (Echinoderm Microtubule-associated protein Like 4) drives dimerization of ALK
    - Alternate fusions contain various amounts of EML
  - ALK (Anaplastic Lymphoma Kinase, CD246) gene drives growth signaling

- 4-7% of all Non-Small Cell Lung Cancers
  - Mouse model generates multiple tiny lung adenoacarcinomas
  - ALK gene rearrangements are not unique to lung cancer but found in anaplastic large cell lymphoma, inflammatory myofibroblastic tumor, neuroblastoma…
    - Higher levels of ALK in ALCL than in NSCLC (Ab clone matters)

- Interest in detection is due to availability of KIs with high activity against ALK kinase
  - Seminal Phase I-II trial on activity of the Ki crizotinib (Pfizer, PF-02341066) in lung cancer published last month (NEJM, 10/28/10)
  - Crizotinib, a well-tolerated oral MET/ALK inhibitor, now in Phase 3 clinical trials
Moving Beyond EGFR Mutations: ALK Targeted Therapy in Lung Cancer

Crizotinib in ALK rearranged (EML4-ALK) NSCLC

57% response rate (47 of 82 pts, 46 PRs, 1 CR)

Kwak, EL., N Engl J Med. 2010;363:1693-1703. ©2010 Massachusetts Medical Society. All rights reserved. Used with permission.
Mutations in NSCLC and Their Clinical Importance

- **EGFR Mutations**
  - 10-15% Caucasians, 30-40% Asians
  - Non-smokers, women, BAC morphology as well as small proportion of squamous cell carcinoma

- **KRAS Mutations**
  - 30% North American population
  - Smokers, men
  - Nearly always adenocarcinoma

- **ALK Rearrangements**
  - 4-7% in US and Asia
  - Non/light smokers, younger men,
  - Mucinous, signet ring or cribriform adenocarcinoma

RTK Pathways in Lung Adenocarcinoma
NCCN Guidelines for NSCLC*

- **PRINCIPLES OF PATHOLOGIC REVIEW**
  - Determine status of predictive molecular biomarkers of lung cancer
  - EGFR and KRAS mutations are mutually exclusive in lung cancer
  - EGFR mutations, especially exons 19 and 21 predict response to TKIs
  - KRAS mutations predict resistance to TKIs

- **SYSTEMIC THERAPY FOR ADVANCED DISEASE**
  - First-line treatment with erlotinib is indicated for EGFR mutation positive patients
  - If known KRAS mutation, treatment other than erlotinib should be given first
  - Second, third-line, maintenance

**ASCO Guidelines**
- First-line treatment with gefitinib is indicated for EGFR mutation positive patients
- Second, third-line, maintenance

*National Comprehensive Cancer Network  V.2.2010*
Logistics of Testing for Lung Cancer Mutations

- **Preferred specimen:**
  - Formalin-fixed paraffin-embedded (FFPE) tissue block

- **Specimen types that are acceptable:**
  - Primary or metastatic lung tumors (including squamous differentiation)
  - Pleural fluid or other cytologic cell block

- **Slide preparation:**
  - H&E is made and reviewed for tumor adequacy
    - >20% tumor cells (nuclei-to-nuclei): report without caveat
    - 5-20% tumor cells: report positive result and non-mutated result with caveat on sensitivity
  - DNA extracted from 1-4 sections depending on size of the biopsy
  - FISH performed on entire or selected area of slide

- **Transport temperature:** room temp or refrigerated acceptable, frozen unacceptable
What Results Can You Expect From EGFR Mutation Tests?

- **EGFR mutation**: Not detected (in exons 18-21)…at a sensitivity level of 10-20% mutation-bearing cells

- **EGFR mutation**: Detected

- **Mutation Type, Level**: T790M (c.2369C>T, exon 20), mixed/heterozygous

- **Comment**: This mutation is pan-resistant to EGFR-directed kinase inhibitors
EGFR Point Mutations Are Scattered Throughout The Kinase Domain

G696E
Exon 18
Insertion-deletions Mutations (Indels) Are Mostly in Exon 19

AGAGGTTAACATCTCTGAGAACGAAATTAAGAGAAGCAACATCTCTGGAAACCCAAACAAAGGAAATCTCTGAT

2205 2215 2225 2235 2245 2255 2265 2275

agaaggtgagaagtcctccgctcgtactcagagaagctaagagagcgacacatctctggaaagccaaacaaaggaagatctctgat

P E G K V K I P V A I K E L R E A T S P K A N K E I L D

GGACCTCTGATCCCCAGGAGTCAGAAAAATTCCCTGCTGATCTAACAAAACATCTCTGGAAAACCCAAACAGGAAATCTCTGAT

GGACTYKGATCCCCAGGAGTCAGAAAAATTCCCTGCTGATCTAACAAAACATCTCTGGAAAACCCAAACAGGAAATCTCTGAT

Using in Vitro Data to Predict Response of Lung Tumors With Specific EGFR mutations

<table>
<thead>
<tr>
<th>EGFR mutation</th>
<th>gefitinib</th>
<th>erlotinib</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td></td>
<td>IC50 (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E709G</td>
<td>70⁵</td>
<td></td>
<td>Low-Mid</td>
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<tr>
<td>G719S</td>
<td>68/90⁵</td>
<td>16</td>
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<td>65</td>
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<tr>
<td>S768I</td>
<td>315/90⁵</td>
<td>250</td>
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<td>S784F</td>
<td>193</td>
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<tr>
<td>T790M</td>
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<tr>
<td>G810S</td>
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<tr>
<td>L838V</td>
<td>187/&lt;20⁵</td>
<td>160</td>
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<tr>
<td>L858R</td>
<td>12/20⁵</td>
<td>6</td>
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<tr>
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<td>170/80⁵</td>
<td>103</td>
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<tr>
<td>A864T</td>
<td>75</td>
<td>49</td>
<td>Mid</td>
</tr>
</tbody>
</table>

References


b. *Oncogene*, 2006;25:1205, IC50 for inhibition of kinase activity through EGFR autophosphorylation
Q: Does Detection of a Prototypic EGFR Mutation Mean That a Carcinoma is of Lung Origin?

- **Answer**: Pretty much

A biased selection of EGFR mutation incidence data from the COSMIC database*

- Lung: 30% of samples tested (codon 858 in 17%, exon 19 indels in ~60%)
- H&N cancer: 3% (similar codons as lung)
- CNS: 6%; mostly glioma (including T790M)
- Thyroid, papillary & anaplastic: 5% (including L858R)
- Thymoma: <1%

- EGFR mutations in non-head & neck tumors are mostly scattered at non-lung codons
  - Prostate: 6%
  - Adrenal: 5%
  - Bone: 2%

**Conclusion**: The typical EGFR mutations in lung cancer are rare in other tumors

*www.sanger.ac.uk/genetics/CGP/cosmic*, queried 11/11/10
Single Mutations at Diagnosis: TKI Response
Additional Mutations at Relapse: TKI Resistance

The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP

Figure adapted from Yun et al, Proc Natl Acad Sci USA 105;2070
Q: Can I Avoid Doing EGFR Mutation by Doing EGFR FISH, CISH or IHC Instead?

- **Answer**: NO!

- EGFR amplification is common (+7) in many solid tumors and is increased in EGFR-mutated lung cancer (resulting in gain in copies of the mutated allele)

- Recent comparison studies have shown that EGFR mutation predicts erlotinib or gefitinib response much better than EGFR FISH or IHC (Sholl et al, Am J Clin Path 2010;133:922)
  - **Mutated EGFR**: 12/19 responded vs 1/20 wild-type (p = .0001)
  - **Amplified EGFR (FISH)**: 7/19 vs 4/17 (not significantly different)
  - **Amplified EGFR (CISH)**: 5/16 vs 6/21 (not significantly different)
  - **Overexpressed EGFR (IHC)**: 3/9 vs 7/22 (not significantly different)

- The range of testing needed for TKI-resistant lung cancer is still being investigated
  - Assess for MET, EGFR, HER2 RTK genomic amplification?
  - Multiple EGFR mutations may be observed (*Clin Cancer Res, 2008;14:7060*)
What Results Can You Expect From the KRAS Mutation Test?

- A range of amino acid changes at codons 12, 13 (exon 1) and 61 (exon 2)
  - All coding changes at these 3 codons are presumed to be equally relevant
  - Mutations outside of these codons are very rare
- KRAS mutation: NOT DETECTED…at a level of 10-20% mutation-bearing cells
- KRAS mutation: DETECTED, Q61H

- Non-overlapping with EGFR mutations
  - Best data on non-response to anti-EGFR therapy are for codon 12 and 13 mutations
- Most are heterozygous mutations; multiple KRAS mutations are rare
- Resistance mutations that overcome effects of KRAS mutations have not been described
What Results Can You Expect From the ALK FISH Test?

- No ALK rearrangement detected
  - With or without extra copies (polysomy)
- Rearrangement of the ALK locus at chromosome 2p23 detected
  - Probably EML4-ALK but can be definitively demonstrated with RT-PCR assay
- Other methods of demonstrating ALK activation (e.g. ALK IHC localization) may be comparable to FISH but are not yet the gold standard
- Important to review material submitted to assure that tumor cells are present in the analyzed material
Interpreting the Lung Cancer Mutation Panel (EGFR, KRAS, ALK)

**EGFR Mutations**
- Exon 19 indels & exon 21 PMs predict sensitivity to TKIs (erlotinib and gefitinib)
  - Rarer mutations may be predicted based on in vitro studies or checked in online clinical database
- T790M is the most mutation after resistance to KIs but amplification of MET is also seen

**KRAS Mutations**
- Largely predict resistance to EGFR KIs (gefitinib, erlotinib)
  - KRAS/EGFR double mutants may be KI-resistant (J Thorac Oncol 2010;5:399)
- Unlike in colorectal carcinoma, KRAS mutations may not indicate non-response to cetuximab (J Clin Oncol 11/1/10;28:4769)

**EML4-ALK Rearrangements**
- Common morphology (signet cell) & possible dual TTF-1/P63 by IHC
  - ALK detection by IHC can be tricky (Clin Cancer Res, 2010;16:1561)
- Predicts resistance to EGFR-targeted KIs
- Phase 3 clinical trial for combined ALK Inhibitor (crizotinib) open
Summary: The Take-Away

- **EGFR MUT**
  - **POSITIVE** → Treat with an EGFR inhibitor

- **KRAS MUT**
  - **POSITIVE** → Response to Anti-EGFR Unlikely

- **ALK FISH**
  - **POSITIVE** → Consider for ALK inhibitor clinical trial
  - **NEGATIVE** → Standard chemotherapy

  Investigational gene panels
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