Deploying lung cancer molecular pathology guidelines in real life

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AMP Companion Meeting
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I have no conflicts of interest

But there’s always hope...

2013 Lung Cancer Testing Guidelines

- Why?
  - Questions still raised regarding who, what, and how to test?
    - Which patients to test? Clinical selection? Pathologic selection?
    - Which samples to test: cytopathology? FNA? Small samples?
    - Which genes to test: EGFR, ALK, KRAS?
  - What methods to use: IHC, IHC-PCR, NS5?
  - How to operationalize: TAT, validation, QA?
  - Evidence had been accumulated to answer most of these

- Who?
  - AMP, CAP, and IASLC
    - each sent a co-chair, a “steerer,” and three experts
    - strict confidentiality policy

- When?
  - This took 2.5 years: Dec, 2010 - Apr, 2013

2013 Guidelines: Who?

- Who & When to test?
  - Clinical presentations, Baseline characteristics
  - Pathologic presentations, Epithelial cell architecture

- Why?
  - Sample selection, handling, and processing
    - Tumor architecture
    - Tumor content
    - Fresh tumor tissue
    - FFPE slides

- EGFR testing
  - IHC, IHC-PCR: Lindeman, Ladanyi
  - PCR, PCR-PCR: Chitale, Saldivar

- ALK Rearrangement FISH
  - IHC, PCR: Saldivar, Thunnissen
  - FISH: Enou, Ladanyi

- 2013 Guidelines: What were the recommendations?

- Enough about the process: What were the recommendations?
Did you get all that?

A summary of the salient points...

2013 Guideline: Major Points

- Who to test:
  - Advanced stage lung cancers with an adenocarcinoma component
  - Regardless of age, gender, smoking history, ethnicity
  - Early stage testing is an institutional policy decision

- What to test:
  - EGFR and ALK
  - Optimal sample according to quantity and quality of cancer DNA
  - Cytology samples (cell block preferred) or tissue samples
  - Fresh, frozen, or fixed (avoid acids and heavy metals)
  - Primary or metastasis
  - Each of multiple primaries if histologically distinct

- How to test:
  - EGFR by Molecular Diagnostics, ALK by FISH
  - Platform selected by performance characteristics, not technology
  - Sensitivity > 50% malignant cell content
  - Must make available more sensitive methods (>10%)
  - All testing completed within 10 working days

Lung Guidelines: Real world challenges

- Selecting patients
- Communicating orders
- Selecting samples
- Limited samples
- Sensitivity for EGFR mutations
- Scope of genetic alterations tested
- Turnaround time
- IHC vs FISH for ALK

Selecting Patients: who to test

- No-brainer: stage IV adeno, Rx candidate
- What about?
  - Early stage adeno
  - Squamous cell
  - Miscellaneous/ambiguous NSCLC
  - Small cell
  - Smokers?

Selecting Patients

- Clinical criteria: inadequate predictors

- AGE
- GENDER
- TOBACCO
- ETHNICITY
Selecting Patients

- Histology
  Test any tumor with lung adenocarcinoma
  - May be mixed (adenosq, adeno/small cell)
  - NO pure squamous, small cell, neuroendocrine
  - Except maybe incomplete small biopsies
  - If clinically high risk, and requested by oncologist

- Poorly differentiated tumors are tested

- Stage
  - Generally, advanced stage patients

Order communication

Challenges: Paperwork/Communication

Custom IS solution needed: LIMS did not provide needed function

Courtesy of Frank Kuo
Selecting Samples

- Pathologist is needed!
- Oncologist cannot tell from reports
- Surgeons cannot tell from procedure
- Radiologists cannot tell from imaging

Selecting Samples

- Quality and quantity are key determinants
  - A cellular FNA is better than a necrotic resection
- Primary vs. metastasis
  - Quality is determinant, tempered by interval therapy
  - If metastasis after initial TKI response, then test metastasis
- Multiple primaries
  - If histologies differ, then test BOTH/ALL
  - Patients will benefit even if 1 of multiple tumors responds
- Testing multiple areas in a tumor is unnecessary

Fixation: the weekend problem

- A satellite hospital noticed that tests often failed on samples obtained on Fridays
- All other days → no problem
- Started scheduling procedures for earlier in the week

The weekend problem

- Unbuffered formalin oxidizes to formic acid
- Acid hydrolyzes DNA
- Samples in acid fail PCR

Limited samples

- 5 x 5 micron sections
  - 80% fail rate
  - 20% fail rate

Solution

- Buffered Formalin
- Friday samples worked
- Headache gone

*A cellular cytology specimen is as good or better than a small biopsy. In a pinch, even a single, stained slide can suffice...*
Limited Samples

**EGFR mutation?**

- 100 ng DNA needed
  - Sensitive to 50% tumor
- 200 ng DNA needed
  - Sensitive to 30% tumor

Challenges: Limited samples

- **Total Consent and Requisition: 9727**
- **Oncomap, 8/11-12/12**
  - Requires 250 ng, 30% tumor

<table>
<thead>
<tr>
<th>Gene</th>
<th>Withdrawn</th>
<th>Insufficient</th>
<th>OSH</th>
<th>Into Lab</th>
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<td>EGFR</td>
<td>816</td>
<td>368</td>
<td>0</td>
<td>956</td>
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<tr>
<td>18S6</td>
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<td>31%</td>
<td>16%</td>
<td>4%</td>
</tr>
<tr>
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<td>31%</td>
<td>4%</td>
<td>615</td>
<td>16%</td>
</tr>
<tr>
<td>1856</td>
<td>49%</td>
<td>31%</td>
<td>16%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Limited Samples

- **Heterogenous samples:** 30% tumor

Ultrasensitive EGFR for resistance:

- **Cell-free circulating DNA**
  - “Liquid biopsy”

- Flow cytometry distinguishes mutant from normal DNA in blood samples
- Can detect very low amounts of tumor DNA
- Agrees with biopsy - few false positives, but some false negatives

WHEN should results be available?

- **TKI therapy no longer empirically started without evidence of a sensitizing mutation**

- **TAT for delivery of blocks/unstained slides:**
  - In-house: 24 hours
  - Outside: 3 working days

- **TAT once sample is received within the lab**
  - Goal: 10 working days
  - Slower labs: make a faster method available when needed

BWH experience:

- **2010 - ALK FISH launch:**
  - Oncologists requested it on pretty much everyone
  - Rapidly developed major backlog
    - 4-6 week TAT

- **2012 - Introduced sequential testing, including IHC screening step**
  - LUNG PANEL (Sequential EGFR, KRAS, ALK, and ROS1; testing stops after first positive result)
  - IHC results available in 2-3 days
  - Prompt FISH if positive
  - Eliminated 30-40% of ALK FISH volume
    - EGFR and KRAS mutated tumors culled out
Testing algorithms
- Critical to understand TAT needs
  - Testing should be completed within 10 days
- EGFR → ALK if neg
- EGFR screen first (i.e., melt curve)
  - Reflex to ALK or EGFR confirmation
- KRAS → EGFR if neg → ALK if neg

Lung Cancer Algorithm
- "Lung panel" requested
- 8 USS to IHC lab
- HE forwarded to pathologist for tumor adequacy review
- If NEGATIVE:
  - HE + 10 USS to lab for dissection/DNA extraction
  - 1 HE and 18 USS ordered
- If POSITIVE:
  - Immediately initiate FISH
    - Rapid EGFR analysis
    - Exons 19, 21
  - If NEGATIVE:
    - Continue molecular testing
    - KRAS pyrosequencing
  - If POSITIVE:
    - Report Results. Testing Done
- If NEGATIVE:
  - Sanger Sequencing:
    - EGFR
    - KRAS
    - BRAF
    - ERBB2
    - MET
    - NRAS
- ALK IHC
- ROS IHC
- RET IHC
- MET IHC
- ROS FISH
- RET FISH

Multiplex panels
- Query multiple variants at once
- Reduced DNA requirements
- Acceptable TAT
- Reduced administrative burden

DFCI/BWH
- "Oncomap"
- 470 mutations in 41 genes

Scope of genetic alterations:
Evidence regression
- EGFR: multiple Phase III RCTs, PFS
- ALK: Phase II, ORR
- RET: case reports
- NTRK1: mice
- MEK1: cell lines
Interpreting ~450 genes in a cancer sample

- 1999: PhD thesis
- 2013: 3 hours
- 2016: 5 minutes

New to POPv2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
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<tr>
<td>BCLL1</td>
<td>ERD</td>
</tr>
<tr>
<td>LINC0064</td>
<td>FGFR2</td>
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<tr>
<td>TPM1</td>
<td>MET</td>
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<tr>
<td>FANCM</td>
<td>PRDM1</td>
</tr>
<tr>
<td>JAK1</td>
<td>ZNF217</td>
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</tbody>
</table>

Scope of genetic alterations: Evidence regression

- EGFR: multiple Phase III RCTs, PFS
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Oncopanel interpretation

- 2016: 5 minutes
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- 1999: PhD thesis
Turnaround Time

TAT in Anatomic Pathology

- Develop core biopsy protocols:
  - Indicate when molecular testing needed on the req
    - BRIGHT PINK STICKERS!
  - Cut unstained slides up front to avoid refacing block
    - 1 H&E
    - 18 USS
    - 1 H&E
  - Minimize surg path workup as needed

- Accurate triaging
  - Too many unnecessary orders will sink histology

TAT: system improvement

Sept, 2011    Jan, 2012

How should ALK be tested?

- 2013: ALK is a FISH test
  - Reliable IHC antibodies not currently available
  - RT-PCR not recommended
    - Molecular and chromosomal variants
  - Mutation testing in resistance: not yet
2016: Is immunohistochemistry reliable for screening for ALK translocations?

YES

How to test for ALK?
- FISH is not perfect
- Neither is IHC
- Concordance:
- Either method can be performed
- We are not recommending both

FDA Approval 6-12-2015
- VENTANA ALK (D5F3) CDx Assay is intended for the qualitative detection of the ALK protein in FFPE NSCLC tissue stained with a BenchMark XT automated staining instrument
- It is indicated as an aid in identifying patients eligible for treatment with XALKORI® (crizotinib)

Lung Guidelines: Real world challenges
- Selecting patients → nonsquamous NSCLC
- Communicating orders → custom IS
- Selecting samples → pathologist job
- Limited samples → NGS
- Sensitivity for EGFR → NGS, ddPCR
- Scope of genes tested → liberal standards
- Turnaround time → never good enough
- IHC vs FISH for ALK → IHC works well

Q&A