Testing Guidelines for Lung Cancer and Colon Cancer

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Importance of Guidelines for Pathologists

- Sets standards of performance
- Promotes uniform quality
- Establishes oversight responsibility within profession
- Establishes scope of medical practice for pathologists
- Potential value in developing PQRS measures for pathologists
- Payment
Guidelines for NSCLC

- NCCN
- IASLC/ATS/ERSI
- IASLC/CAP/AMP
Preinvasive lesions
  - Atypical adenomatous hyperplasia
  - Adenocarcinoma in situ (<3 cm formerly BAC)
    » Nonmucinous
    » Mucinous
    » Mixed mucinous/nonmucinous

Minimally invasive adenocarcinoma (<3 cm lepidic predominant tumor with <5 mm invasion)
  - Nonmucinous
  - Mucinous
  - Mixed mucinous/nonmucinous
IASLC/ATS/ERSI Multidisciplinary Classification of Lung Adenocarcinoma

Travis, et al, J Thorac Oncol, 2011; 6:244-285

- Invasive adenocarcinoma
  - Lepidic predominant (formerly nonmucinous BAC pattern, with >5 mm invasion)
  - Acinar predominant
  - Papillary predominant
  - Micropapillary predominant
  - Solid predominant with mucin production

- Variants of invasive adenocarcinoma
  - Invasive mucinous adenocarcinoma (formerly mucinous BAC)
  - Colloid
  - Fetal (low and high grade)
  - Enteric
“Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.”
Pathological Diagnosis and classification of lung cancer in small biopsies and cytology: Strategic management of tissue for molecular testing
Travis, WD and N Rekhtman,  *Sem Respir Crit Care Med* 2011; 31:22 - 31

- Minimize use of “non-small cell carcinoma”
- Use IHC judiciously
- Reserve sufficient tissue for molecular studies
• **Principles of pathologic review**
  – Purpose of pathologic evaluation
    » Classify
    » Determine extent of invasion
    » Evaluate margins
    » Determine molecular abnormalities to predict response to EGFR-TKI
Non-Small Cell Lung Cancer

THERAPY FOR RECURRENCE OR METASTASES

- Adenocarcinoma
- Large Cell
- NSCLC NOS

Establish histologic subtype

EGFR mutation or ALK negative or unknown

- EGFR mutation testing (category 1)
- ALK testing

EGFR mutation positive

EGFR mutation discovered prior to first-line chemotherapy

Erlotinib (category 1)

EGFR mutation discovered during first-line chemotherapy

Switch maintenance: erlotinib or may add erlotinib to current chemotherapy (category 2B)

ALK positive

Crizotinib

EGFR mutation and ALK testing are not routinely recommended

See First-Line Therapy

NSCL-15

FIRST-LINE THERAPY

See First-Line Therapy

NSCL-14
EGFR recommended
  - Exon 19 del
  - Exon 21 L858R

KRAS not recommended

ALK Gene Fusion recommended

ERCC1 Expression not recommended

Others (BRAF, HER2, MEK1, MET, ROS) not recommended
The Center Process for Developing Guidelines and Consensus Statements

The Center develops evidence-based guidelines and consensus statements related to the practice of pathology and laboratory medicine. Through them, we continually improve the quality of diagnostic medicine and patient outcomes.
Center Guiding Principles

• Evidence-based
• If insufficient evidence, use established method for consensus
• Transparent
• Rigorous conflict of interest
CAP/IASLC/AMP Molecular Testing Guidelines for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Steering Committee
Co-chairs:
• Paul Bunn
• Jan Nowak
• Neal Lindeman

Expert Panel
Co-chairs:
• Philip Cagle, CAP
• Marc Ladanyi, IASLC
• Neal Lindeman, AMP

Staff: Megan Wick

- Mary Beth Beasley
- Dhananjay Chitale
- Sanja Dacic
- Giuseppe Giaccone
- Robert Jenkins
- David Kwiatkowski
- Jeremy Squire
- Juan-Sebastian Saldivar
- Erik Thunnissen

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Kickoff

• Symposium (Chicago, Dec, 2010) Panel, co-chairs, advisory board
  – “interested parties” Pharma, biotech, NCI, FDA, CMS

• Day 1: public
  – Presentations about content, preliminary recommendations, feedback solicitation
  – 3 key questions: Who, When, How to test?

• Day 2: writing panel only
  – Outline of content and recommendations
  – Assignment of writing tasks

*Note this was done BEFORE the literature review really began
Outlines and Assignments

• **Who & When to test?**
  – Clinical determinants: Giaccone, Kwiatkowski
  – Pathology determinants: Cagle, Thunnissen

• **How to test?**
  – Sample selection, handling and processing General: Beasley, Chitale
  – Molecular: Lindeman, Saldivar, Dacic
  – FISH: Squire, Jenkins
  – EGFR testing Molecular: Lindeman, Ladanyi
  – IHC, FISH: Dacic, Ladanyi
  – Resistance testing: Ladanyi, Kwiatkowski
  – ALK testing: FISH: Squire, Jenkins
  – IHC: Cagle, Beasley
  – RT-PCR: Ladanyi
  – Other genes: Lindeman, Ladanyi, Giaccone
  – Logistics Turnaround time: Thunnissen, Saldivar, Giaccone
  – Testing algorithms: Thunnissen, Chitale, Saldivar
  – Reporting: Lindeman, Kwiatkowski, Cagle, Jenkins
  – QA, Validation: Thunnissen, Squire, Saldivar
Manuscript Preparation

• version 1: Jan – Jun 2011 Reviewed by expert panel

• version 2: Jul – Aug, 2011 Reviewed by advisory board

• version 3: Oct – Nov, 2011 Reviewed by public

• version 4: Feb – Apr, 2012 Reviewed by organizations

• Final: August, 2012
1. Clinical characteristics (e.g., age, gender, ethnicity, smoking history) are not sufficiently sensitive to be used to select patients for EGFR or ALK testing.

2. EGFR and ALK testing should be performed on all NSCLC that contain an adenocarcinoma component, regardless of histologic grade or histologic subtype.

3. EGFR and ALK Testing are not recommended for pure squamous cell carcinomas, pure small cell carcinomas, or pure neuroendocrine carcinomas.

4. Small biopsies or incomplete excisions showing only squamous or small cell histology may be tested for EGFR and ALK mutations.

5. Poorly differentiated tumors or tumors that otherwise cannot be classified as pure squamous, pure small cell, or pure neuroendocrine carcinomas should be tested for EGFR and ALK mutations.

6. "Non small cell" lung carcinoma (NSCLC) is no longer acceptable as a pathologic diagnosis or as an operational category for clinical management.

7. In the absence of interceding therapy with a targeted inhibitor, primary tumors and metastatic lesions are equally suitable for testing.

8. Both tumors should be tested in patients with apparently separate primary cancers.

9. Testing multiple different areas within a tumor is not recommended.

10. Patients with stage III or stage IV disease should be tested at the time of diagnosis.

11. The decision whether to test patients with stage I or II disease for EGFR/ALK should be made locally by each institution, in collaboration with its oncology care team.

12. Tissue must be allocated for EGFR and ALK testing in all patient samples.

13. EGFR and ALK results should be available within two weeks (10 working days) of receiving the sample in the molecular diagnostics laboratory.

14. Laboratories with typical turnaround times beyond two weeks need to make available a more rapid test – either in house or by send out – in instances of clinical urgency.

15. Samples should be sent to the molecular pathology laboratory within 3 working days of receiving requests.

16. Formalin-fixed, paraffin-embedded samples are adequate for PCR-based EGFR mutation tests, as are fresh, frozen, or alcohol-fixed samples.

17. Other tissue treatments (e.g., acidic or heavy metal fixatives, mordants, or decalcifying solutions) may hamper EGFR testing, and should be mentioned in the surgical pathology report.

18. Specimens should be fixed in 10% neutral buffered formalin for between 6–48 hrs. Prolonged fixation should be noted in the surgical pathology report.

19. Cell blocks are preferred over smear preparations for the analysis of cytology specimens.

20. Adequacy for EGFR testing is not determined by sample type, but rather by malignant cell content and DNA quality.

21. Each lab must establish during validation, and perform quality control in production, the minimum amount and concentration of cancer cells needed for precise mutation detection.

22. The absence of proven-dysny, more than one sample should be used to determine test sensitivity during validation.

23. Tumor content of each sample must be assessed by a pathologist; this should be included in test validation.

24. Cancer cell enrichment may be performed on heterogeneous samples by a pathologist or trained technologist under a pathologist’s guidance.

25. Manual dissection of unstained slides is recommended for cancer cell enrichment.

26. Any validated method with sufficient performance characteristics may be performed.

27. EGFR tests must be able to detect mutations in samples with at least 50% cancer cell content, with absolute precision.

28. Laboratories with tests based on unmodified Sanger sequencing only are strongly encouraged to employ a more sensitive method, or must make available sendout to a more sensitive method for patients.

29. Specificity of positive results must be confirmed, particularly for highly sensitive methods.

30. EGFR copy number analysis should be performed within each laboratory’s institution and within the two weeks (10 working days) recommendation set forth in this guideline.

31. EGFR mutation testing should capture all individual mutations reported in at least 1% of EGFR mutant lung adenocarcinomas.

32. EGFR Immunohistochemistry is not recommended for selection of EGFR TKI therapy.

33. EGFR copy number analysis (i.e., FISH or CISH) is NOT recommended for selection of EGFR TKI therapy.

34. KRAS mutation testing is not recommended as a sole determinant of anti-EGFR therapy.

35. KRAS mutation testing is not recommended as a sole determinant of anti-HER2 therapy.

36. ALK FISH requires the same slide pretreatment and hybridization procedures as other FISH tests performed on similar types of samples.

37. ALK FISH slides should be interpreted by two independent scorers who have specialized training in solid tumor FISH analysis, with guidance from a pathologist with training or experience in FISH.

38. ALK FISH should be interpreted in areas with clearly interpretable hybridization signals in the majority of cells.

39. For split apart ALK FISH assays, narrowly split signals can be seen in the absence of ALK rearrangement, and therefore interpretive criteria regarding the magnitude of signal splitting in positive samples must be established in validation.

40. Other abnormal FISH findings of uncertain significance should be reported as such.

41. Evidence of rearrangement in >15% of 50 analyzed cells should be interpreted as evidence of an ALK-positive tumor.

42. Each case should be reviewed by two independent observers and confirmed by a cytogeneticist or pathologist with training in FISH.

43. Quality control for ALK FISH should be performed similarly to quality control for other solid tumor FISH assay.

44. Immunohistochemistry is not currently recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.

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

46. Mutation testing for acquired resistance to ALK inhibitors is not currently recommended for clinical management.

47. EGFR and ALK tests must be able to detect T790M mutation in as few as 5% of small cell lung carcinomas.

48. Tumor cell enrichment may be performed on heterogeneous samples by a pathologist or trained technologist under a pathologist’s guidance.

49. Manual dissection of unstained slides is recommended for cancer cell enrichment.

50. Any validated method with sufficient performance characteristics may be performed.

51. EGFR tests must be able to detect mutations in samples with at least 50% cancer cell content, with absolute precision.

52. Laboratories with tests based on unmodified Sanger sequencing only are strongly encouraged to employ a more sensitive method, or must make available sendout to a more sensitive method for patients.

53. Specificity of positive results must be confirmed, particularly for highly sensitive methods.

54. EGFR copy number analysis should be performed within each laboratory’s institution and within the two weeks (10 working days) recommendation set forth in this guideline.

55. EGFR mutation testing should capture all individual mutations reported in at least 1% of EGFR mutant lung adenocarcinomas.

56. EGFR Immunohistochemistry is not recommended for selection of EGFR TKI therapy.

57. KRAS mutation testing is not recommended as a sole determinant of anti-EGFR therapy.
Guideline Organization

- **WHO to test?** Which patients
  - Which cancers
  - Which samples

- **WHEN to test?** Disease stage
  - Who orders test?
  - Turnaround time

- **HOW to test?** Sample requirements
  - Pathologists’ roles
  - EGFR Mutation platform
  - Required mutations
  - IHC, ISH
  - Resistance testing
  - ALK FISH Assay design
  - IHC, RT-PCR
  - Other genes
  - Operational issues
Who to test?
Histology matters

• Test any tumor with lung adenocarcinoma

• May be mixed (adenosq, adeno/small cell)
  – NO pure squamous, small cell, neuroendocrine
  – Except maybe incomplete small biopsies

• Poorly differentiated tumors should be tested
  – Subtypes of adenocarcinoma not useful
  – Except maybe mucinous

• “NSCLC” is inadequate diagnosis for resection

• IHC may be needed to assess adeno component
What to test?

- 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
Minimum tumor content

• Two kinds of minima:
  – Absolute amount of cancer cells
  – Relative amount of tumor cells

• Each lab must determine during validation
  – GENERAL goals: 500 cells, 50% tumor

• Pathologist must review each section
• Enrichment: Gross dissection is recommended
• Laser capture, WGA are error-prone

• Low positive control should be included in each run
When to test?

- Stage IV: at diagnosis, ASAP
- Early stage: discuss with your oncologists
  - If testing not done now, save material!
How fast?

• 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
How to test?

- No specific platform is recommended

- Predicate method: Sanger sequencing. Acceptable methods must be AS SENSITIVE or better

- More sensitive (1-10%) testing must be available
  - Either in-house or send-out when needed
Which mutations to test for?

- KRAS wt status is not useful for EGFR therapy
  - KRAS mutant is informative: excludes EGFR and ALK mutations

- Exon 19 and Exon 21 alone are inadequate

- All mutations with >1% frequency
  - Exon 18: E709, G719 mutations
  - Exon 19: all deletions and insertions
  - Exon 20: insertions, T790M, S768
  - Exon 21: L858R, L861Q, T854

- Rare mutations (<5 hits): great caution! Confirm with repeat from a new DNA isolation

- T790M Ultrasensitive method required for resistance testing (5%)
  - Germline testing if T790M detected before treatment
How to test for ALK?

- ALK is currently a FISH test IHC may be used for pre-screening
  - RT-PCR not recommended
    - Molecular and chromosomal variants
  - Mutation testing in resistance: not yet
    - Too many translocations
How to test for ALK?

• Which samples? Tumor percentage less critical
  – At least 50 tumor nuclei
  – Non-overlapping cells

• Who to interpret? Pathologist trained in FISH
  – Technologist trained in pathology
  – Two independent reviewers?
Other genes?

- Clinical trials, but not standard care KRAS, BRAF, ERBB2, PIK3CA, MET

- Markers of chemotherapy response are not recommended ERCC1, TS, RRM1
Testing Algorithms?

• Critical to understand TAT needs
  – Testing should be completed within 10 days

• EGFR, if neg > ALK

• EGFR screen first (i.e., melt curve)
  – Reflex to ALK or EGFR confirmation

• EGFR, if neg > KRAS, if neg > ALK

• KRAS and EGFR, if neg > ALK
Colorectal Cancer - molecular pathways

- Hypermutability Pathway
- Chromosomal Instability Pathway
- FAP
- Lynch
What value is there in recognizing MMR (MSI) colorectal tumors?

1. Prognosis
2. Response to chemotherapy
3. Screen for Lynch Syndrome (HNPCC)
• At least three family members with an \textit{HNPCC-associated cancer}, two of whom are first-degree relatives.
• At least two generations represented.
• At least 1 individual younger than 50 years at diagnosis.
• FAP should be excluded.
• Tumors should be verified by pathologic examination.

Vasen et al, Gastroenterology 1999;116:1453-56
Revised Bethesda Guidelines for testing colorectal tumors for MSI - 2004

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer in a patient less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other Lynch associated tumors, regardless of age.
3. Colorectal cancer with the MSI-H histology diagnosed in a patient less than 60 yr.
4. Colorectal cancer diagnosed in one or more first-degree relatives with an Lynch-related tumor, with one of the cancers being diagnosed under age 50 yr.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with Lynch-related tumors, regardless of age.

*Umar, et al., J Natl Cancer Inst 2004; 96:261-8*
“…found sufficient evidence to recommend offering genetic testing for Lynch syndrome to individuals with newly diagnosed colorectal cancer to reduce morbidity and mortality in relatives.”

= universal screening of all new CRCs
Prognostic significance of MSI in sporadic CRC

Gryfe, R et al, NEJM 2000; 342:69-77
MSI and response to chemotherapy?

Tumor Microsatellite-Instability Status as a Predictor of Benefit from Fluorouracil-Based Adjuvant Chemotherapy for Colon Cancer

Colorectal Cancer - molecular pathways
Role of BRAF testing?

- Hypermutability Pathway
- Chromosomal Instability Pathway
- FAP
- Lynch
Applications for BRAF mutation testing

• In MSI-H (MLH1) tumors, BRAF mutation correlates with hypermethylation, not Lynch Syndrome
  – No testing for Lynch needed

• Prognosis
  – BRAF mutation correlates with poor outcome in MSS and MSI-H tumors
Prognostic Role of Combined MSI and BRAF Mutation Status in Colorectal Cancer: Toward Routine Clinical Use

Jeanne Shen, Teppei Morikawa, Charles S Fuchs, Shuji Ogino. Brigham and Women’s Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA
Does BRAF preclude use of Anti-EGFR Rx?

*slide from Medscape commentary by Dr. J. Marshall*

Pooled Analysis: Overall Survival in Patients With K-ras Wild-Type/BRAF-Mutated Tumors

**K-ras Wild-Type/BRAF Wild-Type**

HR [95% CI]: 0.840 [0.710–0.99]; \( P = .041 \)

- FOLFIRI/FOLFOX4 + cetuximab: \( n = 349 \) median 24.8 mos
- FOLFIRI/FOLFOX4: \( n = 381 \) median 21.1 mos

**K-ras Wild-Type/BRAF Mutated**

HR [95% CI]: 0.633 [0.378–1.060]; \( P = .079 \)

- FOLFIRI/FOLFOX4 + cetuximab: \( n = 32 \) median 14.1 mos
- FOLFIRI/FOLFOX4: \( n = 38 \) median 9.9 mos

*OS = overall survival*

Relevance, pathogenesis, and testing algorithm for Mis-Match Repair-defective colorectal carcinomas

Guideline for Molecular Testing in Colorectal Cancer
Project Goals

• The project goal is to use evidence-based review to establish laboratory practices guidelines for the use of molecular markers in the evaluation of colorectal cancer.

  – KRAS

  – BRAF

  – Mismatch Repair (MSI and IHC)
Leadership

Expert Panel Co-chairs

• Wayne W. Grody, MD, ASCP Co-chair, University of California Los Angeles
• Stanley R. Hamilton, MD, CAP Co-chair, MD Anderson Cancer Center
• Federico A. Monzon, MD, AMP Co-chair, Baylor College of Medicine
• Monica M. Bertagnolli, MD, ASCO Co-chair, Brigham and Women’s Hospital

• Expert Panel (2 per organization)
  – TBD
• Advisory Panel (as needed)
  – TBD