

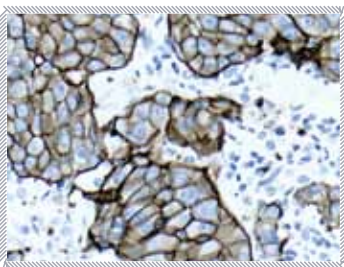
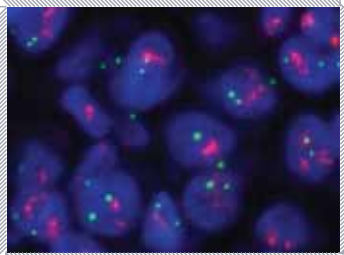


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PhenoPath

2013 Updated ASCO-CAP HER2 Testing Guidelines in Breast Cancer



Breast cancer positive for HER2 amplification by FISH and HER2 3+ overexpression by IHC

PhenoPath is one of the first labs in the US to adopt all the complex changes of the 2013 American Society of Clinical Oncology and College of American Pathologists (ASCO-CAP) HER2 Testing Guideline¹ updates in breast cancer. PhenoPath offers both IHC and FISH—including options for alternative Chromosome 17 probes² that have been recommended as a means of resolving equivocal cases—and has performed over 10,000 assays of each during the past five years alone. As demonstrated in several publications, PhenoPath's HER2 IHC and FISH concordance rate exceeds the 95% concordance bar. Indeed, our most recent concordance data for 2013 is 97.3% (positive concordance) and 99.2% (negative concordance). Our pathologists are available to discuss or explain these new guidelines by phone (888-927-4366) or by email (lab@phenopath.com).

PhenoPath has implemented the new ASCO-CAP guidelines and incorporated all the reporting recommendations.

These are some of the significant differences between the new 2013 guidelines and the 2007 guidelines.

The acceptable duration of tissue fixation has been modified to >6 and <72 hours, which is consonant with the ER testing recommendations from 2010.

Patients who develop metastatic disease must now have the metastatic tumor retested for HER2 status, if specimen is available.

HER2 test results, and treatment decisions, may be based on either immunohistochemistry (IHC) or in situ hybridization (ISH; fluorescence or chromogenic).

The definitions of 3+, 2+, 1+ and 0 IHC scoring have been modified from the 2007 guidelines. In particular, the criterion for designation of 3+ positivity has been changed from 30% to 10% (strong membranous immunostaining).

The definitions of ISH positivity have been significantly changed. Under the new guidelines, using a dual (HER2 and CEP17) probe system (as employed at PhenoPath), HER2 amplified cases include those with a HER2:CEP17 ratio ≥ 2.0 , independent of the absolute copy number, and also include cases with HER2:CEP17 ratios < 2.0 , but with absolute HER2 copy numbers ≥ 6.0 .

The definition of an equivocal HER2 ISH test has been significantly changed. The 2007 definition of an equivocal ISH test—a HER2:CEP17 ratio between 1.8 and 2.2—is no longer operative. An equivocal ISH test is now defined as one showing a HER2:CEP17 < 2.0 and an average absolute HER2 signal count per cell of ≥ 4.0 and < 6.0 .

Equivocal HER2 tests must be reflexed to either an alternative ISH chromosome 17 probe, or to the test methodology not originally performed (i.e., run FISH if IHC was run originally and was equivocal, and vice versa). PhenoPath has options for alternative ISH chromosome 17 probe tests: SMS, RARA and p53.

Tumor heterogeneity: If there is a subpopulation of tumor cells corresponding to more than 10% of the total tumor population with a different HER2:CEP17 ratio, results on this subpopulation should also be reported.

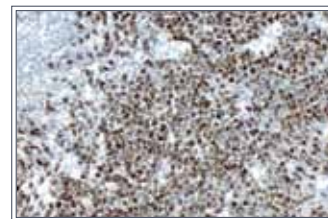
Requirements for initial HER2 test validation changed to be consonant with the 2010 ER and PR testing guidelines.

There are some slight modifications to the elements required in HER2 IHC and ISH reports.

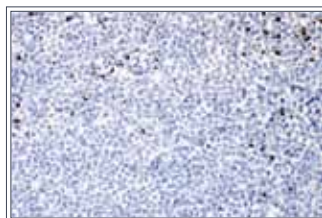
Three Robust Antibodies that Aid in the Classification of Low-Grade B Cell Lymphomas

SOX11

Gene expression profiling (GEP) has shown that rare mantle cell lymphoma (MCL) cases lack both the characteristic t(11;14) and cyclin D1 protein expression, with otherwise typical morphologic, immunophenotypic, and clinical features of MCL (Fu K, et al. Blood 106:4315-21, 2005). Such cases are commonly referred to as cyclin D1-negative MCL (CN-MCL). It has been discovered that the nuclear transcription factor SOX11 is overexpressed in the great majority of MCLs, including both cyclin D1-positive and negative forms, but not in potential MCL mimics such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), marginal zone lymphoma (MZL), and low-grade follicular lymphoma (LGFL). SOX11 immunohistochemistry (IHC) using anti-SOX11 mouse monoclonal antibody MRQ-58 can help identify CN-MCL without GEP studies in formalin-fixed paraffin-embedded (FFPE) tissue, and can also be used to confirm the diagnosis of typical cyclin D1-positive MCL (Soldini D, et al. Am J Surg Path 38:86-93, 2014). This antibody has been validated for clinical use in FFPE tissue at PhenoPath.



Positive SOX11 in MCL



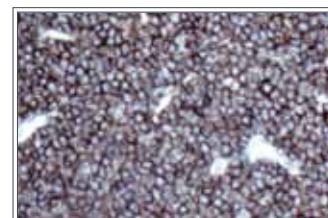
Negative LEF1 in MCL

LEF1

Lymphoid enhancer-binding factor 1 (LEF1), also known as T cell transcription factor 1-alpha (TCF1a), is a nuclear transcription factor in the WNT/beta-catenin signaling pathway that is normally expressed in T cells and immature pro-B cells, but not in normal mature B cells. LEF1 is aberrantly expressed in virtually all cases of CLL/SLL, but is almost always negative in MCL, MZL, and LGFL, and therefore may be helpful in distinguishing CLL/SLL from other low-grade B cell lymphomas (Tandon B, et al. Mod Pathol 24:1433-43, 2011). Anti-LEF1 antibody EPR2029Y has been validated for clinical use in FFPE tissue at PhenoPath.

CD200

CD200 is a cell surface protein that has been associated with immune evasion in a variety of hematopoietic and non-hematopoietic human malignancies. CD200 is expressed in the large majority of CLL/SLL cases, but very few MCL cases (Palumbo GA, et al. Leuk Res 33:1212-6, 2009); its expression in other low-grade B cell lymphomas such as MZL and LGFL is variable, and generally not useful diagnostically. The PhenoPath flow cytometry laboratory routinely evaluates CD200 to aid in the distinction between CLL/SLL and MCL. The PhenoPath IHC laboratory has now validated a robust, anti-CD200 rabbit polyclonal antibody for use in clinical IHC on FFPE tissue. Note that CD200 expression has also been associated with primary mediastinal large B cell lymphoma (Dorfman, DM, et al. Mod Pathol 25:1637-1643, 2012), and with angioimmunoblastic T cell lymphoma (Dorfman, DM, Shahsafaei A. Am J Surg Pathol 35:76-83, 2011).



Positive CD200 in CLL/SLL

PhenoPath at the USCAP

March 1-7, 2014
San Diego, California

Attendees of the United States & Canadian Academy of Pathology (USCAP) annual meeting might be interested in the following presentations by or involving PhenoPath pathologists:

Sunday, March 2, 1:30-5:00 PM, CC Ballroom 20 D / Rodger C. Haggitt Gastrointestinal Pathology Society, Companion Society Meeting, Rodger C. Haggitt Memorial Lecture (4:00 PM):

“Next Generation Immunohistochemistry: A Window Onto The Molecular Biology of Gastrointestinal Tumors”

Presented by Allen M. Gown, MD

Monday, March 3, 1:00-3:00 PM, CC Exhibit Hall DE / Poster Presentation

“GATA-3 Expression in Trophoblastic Tissues: An Immunohistochemical Study, Including Diagnostic Utility” (abstract #1136)

Presented by Russell Vang, MD, Johns Hopkins Medicine, Baltimore, MD

Tuesday, March 4, 8 AM to noon, CC Exhibit Hall DE / Poster Presentation

“Vascular Mesangial ‘Shunts’ in Nodular Diabetic Glomerulosclerosis” (abstract #1671), Presented by Stephen Bonsib, MD, MS,

Nephropath™, Little Rock, AR

Tuesday, March 4, 8 AM to noon, CC Exhibit Hall DE / Poster Presentation

“CD200 Is Expressed by Neuroendocrine Carcinoma” (abstract #1985), Presented by Mark Kilgore, MD, University of Washington, Seattle, WA

Tuesday, March 4, 1:00 PM, CC Ballroom 20 BC / Platform Presentation – Breast Pathology - Section B

“Comparative Sensitivities and Specificities of Antibodies to Breast Markers GCDFP-15, Mammaglobin A, and Different Clones of GATA-3” (abstract #225), Presented by Patricia Kandalaf, MD

Friday, March 7, 2014 from 1:00-4:30 PM, CC 25 C / Short Course #35

“Diagnostic Immunohistochemistry: Plagued with Potential Problems but Pregnant with Possibilities”, Presented by Allen M. Gown, MD

Visit PhenoPath booth #711 in the Exhibit Hall and meet our staff and pathologists.

Molecular Profiling of Lung Adenocarcinoma Prognostic and Predictive Biomarkers

Non-small cell lung carcinoma (NSCLC) accounts for approximately 85% of lung cancers and includes predominantly adenocarcinomas, the most common subtype in the US. The pathogenesis of lung adenocarcinoma is complex; however, over the past decade, it has become evident that subsets of NSCLC contain recurrent 'driver' mutations, which are detected in over 60% of lung adenocarcinomas (Figure 1). The most commonly tested molecular markers in lung adenocarcinoma are EGFR and ALK, and mutations involving these genes are predictive of responsiveness to erlotinib and crizotinib, respectively. Published consensus guidelines (NCCN, CAP, IASLC, and AMP) recommend that all new cases of lung adenocarcinoma be tested for EGFR and ALK. PhenoPath offers FDA-approved tests for both. As driver mutations in lung adenocarcinomas are believed to be mutually exclusive, a reflexive testing algorithm can be utilized (Figure 2). In addition to EGFR and ALK, recent data also support testing for ROS1 and RET. Although not as well established as EGFR and ALK, current NCCN Guidelines list these genes as well as others as having available targeted agents in NSCLC (see Table 1).

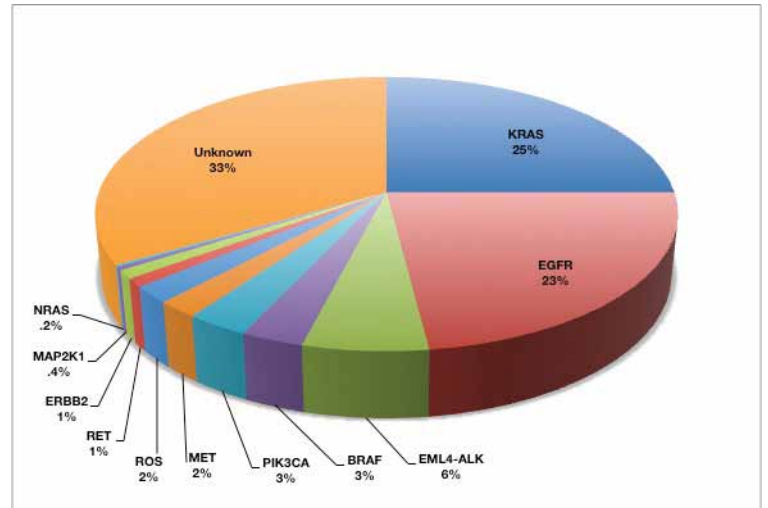


Figure 1: Mutations in Lung Adenocarcinoma

Source: Cheng L et al. *Mod Pathol* 25(3):347-69, 2012

At PhenoPath, we believe the pathologist plays a critical role in not only the histologic classification of lung cancers but also in the triaging and prioritization of precious tissue biopsy samples for necessary molecular testing. Involvement of the pathologist can optimize use of such tissues and therefore help clinicians and their patients make the best-informed clinical therapy decisions.

For cases of lung adenocarcinoma, PhenoPath recommends the following testing strategies (based on available guidelines and current literature):

- Molecular testing for EGFR and ALK mutations should be performed in all cases of lung adenocarcinoma regardless of clinical features.
- An FDA-approved testing method should be used to identify patients carrying EGFR or ALK mutations.
- We recommend EGFR and ALK testing utilizing a reflexive approach for lung adenocarcinoma (see Figure 2).
- Evaluation of KRAS mutation status is NOT recommended as a sole determinant of anti-EGFR therapy in lung adenocarcinoma.
- In addition to EGFR and ALK, we recommend performing ROS1 and RET FISH when EGFR and ALK are negative.
- Literature does not currently support routine screening for MET and PIK3CA. Early clinical data may support performing MET when ROS1 and RET are negative or in the setting of acquired resistance.

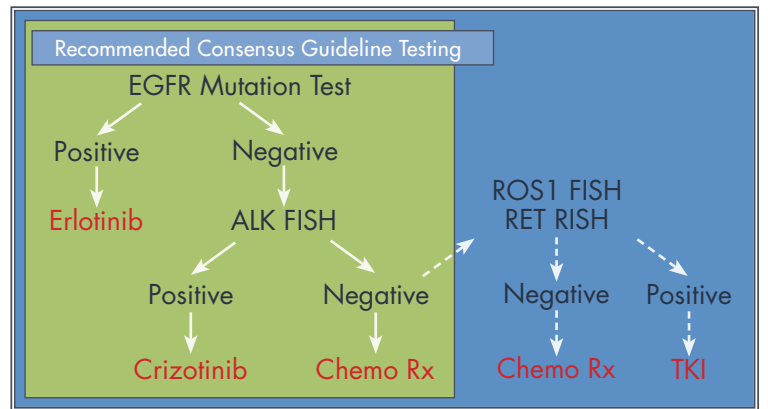


Figure 2: PhenoPath Lung Adenocarcinoma Testing Algorithm. Solid arrows represent consensus guideline recommendation testing. Dotted arrows represent potential testing that can also be performed if the patient is being considered for alternative tyrosine kinase inhibitor (TKI) therapy.

Molecular Marker	Possible Drugs / TKIs
EGFR mutations	erlotinib, gefitinib, afatinib
ALK rearrangements	crizotinib
ROS1 gene fusions	crizotinib
MET amplification	crizotinib
RET gene fusions	cabozantinib
BRAF mutations	vemurafenib, dabrafenib

Table 1: Targeted Agents for Patients with Other Genetic Alterations in NSCLC. This table shows a list of targeted therapy with the molecular marker for which responsiveness has been observed in the setting of NSCLC. **Note that only EGFR and ALK directed testing are recommended under current consensus guidelines and as having strong support in the literature.

Source: NCCN Guidelines, Non-Small Cell Lung Cancer, Version 2.2014

References:

1. NCCN Guidelines: Non-Small Cell Lung Cancer, Version 2.2014 (www.nccn.org/professionals/physician_gls/pdf/nscl.pdf)
2. CAP: Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors: Summary of Recommendations (www.cap.org/apps/docs/membership/cap_iaslc_amp_summary_of_recommendations.pdf)
3. My Cancer Genome: Molecular Profiling of Lung Cancer (www.mycancergenome.org/content/disease/lung-cancer)
4. Cheng L et al. *Mod Pathol* 25(3):347-69, 2012
5. Lindeman NI, et al. *J Thorac Oncol* 8(7):823-59, 2013
6. Bergethon K et al. *J Clin Oncol* 30:863-70, 2012
7. Drilon A et al. *Cancer Discov* 3:630-5, 2013
8. Abbreviations: NCCN (National Comprehensive Cancer Network), AMP (Association for Molecular Pathology), IASLC (International Association for the Study of Lung Cancer), CAP (College of American Pathologists)



FEATURED

At Our Winter Quarterly Conference

Christina Isacson, MD

CellNetix Pathology & Laboratories, Seattle, WA

PhenoPath Laboratories, February 13, 2014, 6:30 PM (light dinner), 7:30 PM (talk)

Christina Isacson, MD will present *“Practical Immunohistochemistry of the Female Genital Tract: Pearls and Perils”* at the PhenoPath Winter Conference at **7:30 PM on Thursday, February 13, 2014**. Dr. Isacson will also give a daytime lecture at noon the same day entitled, *“Update on Ovarian Serous Tumors: Classification and Pathogenesis.”*

Dr. Isacson is a gynecologic pathologist at CellNetix Pathology & Laboratories and Clinical Associate Professor at the University of Washington Medical Center, Department of Pathology, in Seattle. Following a two-year fellowship in gynecologic pathology at Johns Hopkins Hospital with Dr. Robert J. Kurman, she was Assistant Professor of Pathology at the Weill Medical College of Cornell University in New York, where her research interests included HPV-related disease and diagnostic immunohistochemistry. In 2000, she moved to Seattle where she has worked at Virginia Mason Medical Center and CellNetix Pathology. Dr. Isacson’s interests include teaching and women’s health issues. She has served on the Editorial Board of the International Journal of Gynecological Pathology and currently is Section Editor of Gynecologic Pathology for Archives of Pathology and Laboratory Medicine. She is actively involved with the United States & Canadian Academy of Pathology where she has served on the Education Committee and as Short Course Coordinator; she was appointed to Council in 2011. She is also a co-author of the widely used book *“An Illustrated Manual for the Dissection of Surgical Pathology Specimens.”*

Exciting addition to PhenoPath’s Quarterly Conference Series

Beginning in 2014, PhenoPath offers CME credits for conference attendees.