The Newsletter of PhenoPath

phenomena™

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The pathologists and team at PhenoPath

couldn't be more pleased that USCAP is being held in Seattle this year, PhenoPath's hometown! Seattle is a beautiful city and there are a lot of fun and exciting things to do. We are involved in many activities at USCAP, some of which are described below. In addition, see below for an exciting event being held at PhenoPath.

PhenoPath at the USCAP

March 12-18, 2016
Seattle, Washington

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 13, 2016, 7:30 PM-10:30 PM, Room CC Hall 4E / International Society of Breast Pathology: Allen M. Gown, MD will speak on HER2 testing in breast cancer at the symposium entitled, ***"Second Opinions and Diagnostic Concordance in Breast Pathology":*** Moderated by Aysegul Sahin, MD, The University of Texas MD Anderson Cancer Center, Houston, and Gelareh Farshid, MD, The University of Adelaide, Australia

Monday, March 14, 2016, 9:30 AM-12:30 PM, Room CC Hall 4ABC / Poster Session I #289
"Programmed Cell Death 1 Biomarker Testing: RNA and Protein Assessment" (Abstract #1919), Presented by Brandon S Sheffield, MD, University of British Columbia

Tuesday, March 15, 2016, 9:00 AM-12:00 PM, Room CC Hall 4ABC / Poster Session III #295
"TFE3 Immunohistochemistry: A Comparison of Two Methods, with Discussion of the Implications for the Diagnosis of TFE-3-Rearranged Neoplasms" (Abstract #2069), Presented by Rosalind Sandell, MD, Mayo Clinic

Tuesday, March 15, 2016, 1:00-4:30 PM, Room CC Hall 4ABC / Poster Session IV #54
"Increased HER2 FISH-IHC Discordance and Decreased FISH Equivocals Result from 2013 ASCO-CAP HER2 Scoring Guidelines: A Study of 11,813 Cases" (Abstract #157), Presented by Regan Fulton, MD, PhD, PhenoPath

Tuesday, March 15, 2016, 1:00-4:30 PM, Room CC Hall 4ABC / Poster Session IV #96
"Use of a Novel Rabbit Monoclonal Phospho-Histone H3 (Ser10) Versus H&E Mitotic Count in Invasive Melanoma" (Abstract #499), Presented by Regan Fulton, MD, PhD, PhenoPath

Visit PhenoPath booth #1026 in the Exhibit Hall and meet our staff and pathologists.

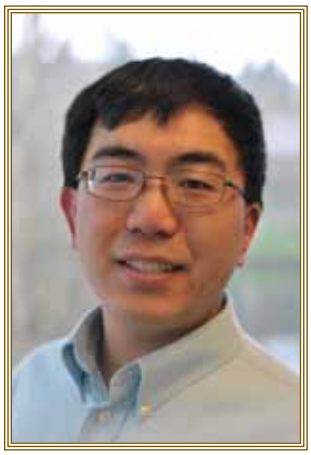
Come to PhenoPath for an Open House event on Tuesday, March 15 from 6:30 to 8:00 pm

Check out our state-of-the art lab and meet the team (pathologists, lab supervisors, & client services representatives) while you are visiting Seattle for the USCAP 2016 Annual Meeting! Learn about and view some of the exciting pathology technologies PhenoPath offers.

View some interesting cases. Visit with your colleagues, enjoy music , light dinner, wine, and beer!

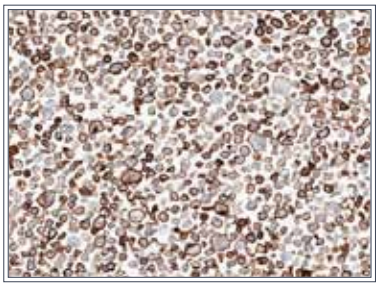
RSVP required at marketing@phenopath.com. Contact us regarding transportation

Introducing Dr. David Ng



PhenoPath is delighted to welcome Dr. David Ng to the hematopathology team. Dr. Ng received his B.S. in Electrical Engineering from the University of Illinois at Urbana-Champaign, and his M.D. from the University of Illinois at Chicago. He completed his residency in Anatomic and Clinical Pathology at Dartmouth Hitchcock Medical Center, and his fellowship in Hematopathology at the University of Washington. Prior to coming to PhenoPath, Dr. Ng was a Senior Hematopathology Fellow in the Department of Laboratory Medicine at UWMC, where he developed algorithms for automated analysis in flow cytometry, leading to first authorship on a recent publication in the American Journal of Clinical Pathology (see reference below). Dr. Ng is board certified in Anatomic Pathology, Clinical Pathology, and Hematopathology. Dr. Ng brings a passion for Hematopathology to PhenoPath, and participates in our full range of services, including flow cytometry, immunohistochemistry, FISH, PCR, cytogenetics, and full case consultation. Dr. Ng has already made significant contributions to PhenoPath's hematopathology service, taking a leading role in the optimization and validation of the CD138-based plasma cell enrichment procedure, and participating in the development and validation of several new flow cytometric and immunohistochemical assays. Dr. Ng is an excellent addition to the PhenoPath team!

Reference: Ng DP, et al. Computer-Aided Detection of Rare Tumor Populations in Flow Cytometry: An Example With Classic Hodgkin Lymphoma. AJCP 144(3):517-24, 2015



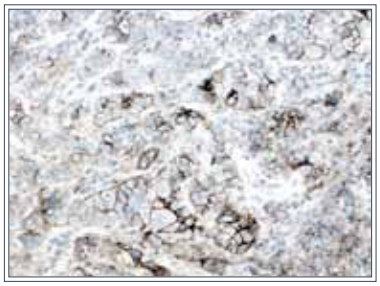
PD-L1 Expression on Cell Membrane of Positive Control Cell Line for 28-8 Assay

PhenoPath at the Forefront: PD-L1 Immunohistochemical Assays for Non-Small Cell Lung Cancer and Other Malignancies

The approval by the Food and Drug Administration (FDA) of the anti-PD1 targeted monoclonal antibodies, pembroluzimab [KEYTRUDA] and nivolumab [OPDIVO] follows the publication of several studies documenting efficacy of these drugs in melanoma, non-small cell lung carcinoma (NSCLC), renal cell carcinoma, and others. To review, programmed cell death protein 1 (PD-1) is an immune inhibitory receptor expressed on several immune cells, particularly cytotoxic T cells. PD-1 interacts with two ligands, programmed cell death ligand 1 (PD-L1) (B7-H1, CD274) and PD-L2 (B7-DC). PD-L1 may be expressed on tumor cells in addition to immune cells. The interaction of these ligands with PD-1 inhibits T cell activation and cytokine production. In the context of infection or inflammation in normal tissues, the interaction of PD-1 with PD-L1/2 is critical for maintaining homeostasis of the immune response and preventing autoimmunity. In tumor microenvironments, however, the interaction of PD-1 with PD-L1/2 provides an immune escape for tumor cells by turning off cytotoxic T cells. Thus, blocking these interactions may subject the tumor cells to attack from cytotoxic T cells.

Which PD-L1 Immunohistochemical Assay Should I Request?

The enclosed insert summarizes criteria for different PD-L1 assays. It is important to note that the FDA has approved pembrolizumab [KEYTRUDA] with a "companion diagnostic" (22C3) to determine patient eligibility for treatment with this drug only in the case of NSCLC. While the FDA approved pembrolizumab [KEYTRUDA] for melanoma, that approval did not come with a companion diagnostic. Other indications are in clinical trials or undergoing FDA review. Watch our web site for updates.



PD-L1 Expression in Non-Small Cell Lung Carcinoma, 22C3 clone

In the case of nivolumab [OPDIVO], the FDA approved this drug with a recommendation for a "complementary" diagnostic in the setting of NSCLC and melanoma. Nivolumab [OPDIVO] has also been approved by the FDA for treatment of renal cell carcinoma, but without the complementary diagnostic. In the case of the FDA-approved assays for NSCLC, the choice of test depends upon the drug that is being contemplated for the patient: if it is pembrolizumab [KEYTRUDA], the test is the 22C3-based assay (pharmDx by Dako), and if it is nivolumab [OPDIVO], the appropriate test is the 28-8-based assay (also a pharmDx by Dako).

In the case of the FDA-approved assays for melanoma, the choice of test depends upon the drug that is being contemplated for the patient: if it is nivolumab [OPDIVO], the appropriate test is the 28-8-based assay (also a pharmDx by Dako), and if it is pembrolizumab [KEYTRUDA], testing is not required.

If the patient's tumor is something other than NSCLC and melanoma, PhenoPath offers a 'generic' assay employing the E1L3N mouse monoclonal antibody (see previous Phenomena) if desired. In addition to the use of different antibodies, the PD-L1 assays for pembrolizumab [KEYTRUDA] and nivolumab [OPDIVO] employ different scoring systems. For testing with the 'generic' E1L3N clone, there is no prescribed scoring system. At PhenoPath, we provide the percent of positive cells and the staining intensity for the E1L3N assay.

The New FDA-Approved Roche cobas® EGFR Mutation Test (Version 2) Detects the EGFR T790M Mutation as Well as Other Activating EGFR Mutations

PhenoPath is pleased to offer the **Roche cobas® EGFR Mutation Test (v2)**, an FDA-approved companion diagnostic for ***both*** first- and second-line therapy decisions for patients with non-small cell lung carcinoma (NSCLC). This assay is FDA approved for identifying NSCLC patients with EGFR exon 19 deletions and exon 21 (L858R) substitution mutations for whom treatment with the tyrosine kinase inhibitor (TKI) Tarceva® (erlotinib) may be effective as a first-line therapy, and for identifying NSCLC patients who harbor a T790M mutation, indicating eligibility for treatment with Tagrisso® (osimertinib) as a second-line TKI therapy. Per the FDA news release of November 13, 2015, Tagrisso® is the only approved medicine indicated for patients with metastatic EGFR T790M mutation-positive NSCLC who have progressed on or after EGFR tyrosine kinase inhibitor therapy.

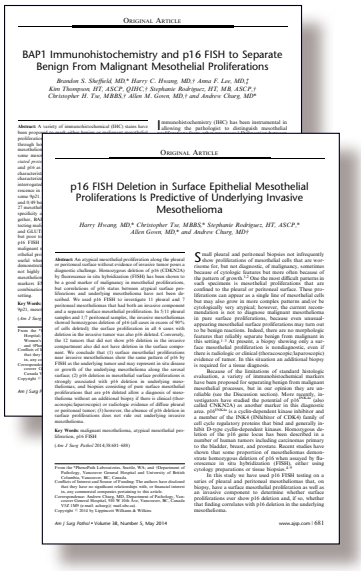
Although patients with lung non-small cell adenocarcinoma may initially respond to first-line TKI therapies such as Tarceva® (erlotinib), acquired resistance is common. The development of a T790M mutation accounts for over half of such acquired resistance cases (J Thorac Dis 2011;1:10-18). It should be noted that eligibility for Tagrisso® (osimertinib), the first FDA-approved therapy for T790M positive tumors, requires identification of T790M in a post-therapy biopsy specimen.

Effective November 17, 2015, the Roche cobas® EGFR Mutation Test (v2) replaced the EGFR used previously. The Roche cobas® assay similarly uses qualitative real-time PCR to detect clinically relevant mutations in exons 18, 19, 20, and 21 of the EGFR gene and provides ***improved sensitivity***, as only 5% mutant allele is needed for reliable mutation detection in a background of wild-type DNA. In addition, the cobas EGFR assay covers a ***greater number of mutations***, identifying 42 mutations in exons 18-21, as compared to the 21 mutations covered by PhenoPath's previous assay. Furthermore, this new EGFR test ***requires less tissue*** than our previous assay, which is particularly advantageous for small biopsy specimens.

For more information about EGFR and PD-L1 testing at Phenopath, including pricing and ordering information, please contact our Client Services department at lab@phenopath.com or 888-927-4366; or visit us at www.phenopath.com.

For more information about the FDA's approval of the Roche cobas® EGFR Mutation test or the FDA's approval of Tagrisso®, see www.cobasegfrtest.com and www.astrazeneca.com.

PhenoPath Collaboration Investigates Utility of BAP1 Immunohistochemistry and P16 FISH in the Diagnosis of Malignant Mesothelioma



ORIGINAL ARTICLE

BAP1 Immunohistochemistry and p16 FISH to Separate Benign From Malignant Mesothelial Proliferations

Brandon S. Sheffield, MD, Harry C. Hwang, MD,† Anna F. Lee, MD,‡ Kim Thompson, HT, ASCP, QIHC,† Stephanie Rodriguez, HT, MB, ASCP,† Christopher H. Yu, MBBS,† Allen M. Gown, MD,† and Andrew Churg, MD**

ORIGINAL ARTICLE

p16 FISH Deletion in Surface Epithelial Mesothelial Proliferations Is Predictive of Underlying Invasive Mesothelioma

Harry Hwang, MD, Christopher Yu, MBBS,* Stephanie Rodriguez, HT, ASCP,* Allen Gown, MD,* and Andrew Churg, MD†*

The evaluation of mesothelioma versus atypical reactive mesothelial proliferations remains a challenging area in diagnostic surgical pathology. Recently alterations of the BRCA1-associated protein-1 (BAP1) and p16INK4a genes have been described in mesothelioma. In particular, identification of BAP1 mutations in familial mesothelioma has spurred studies to examine the use of BAP1 IHC to diagnose sporadic mesothelioma in surgical pathology specimens. Homozygous loss of the cell cycle regulatory protein P16INK4a detected by FISH is also an active area of study for this differential diagnosis.

In a series of studies in the American Journal of Surgical Pathology (AJSP), Drs. Allen Gown and Harry Hwang with PhenoPath technical staff, in collaboration with Drs. Andrew Churg and Brandon Sheffield (UBC Department of Pathology), evaluated the combined use of BAP1 IHC and p16 FISH in diagnosing mesothelioma versus atypical mesothelial proliferations. In the May 2014 issue of AJSP, we showed that P16 deletion detected by FISH can be predictive of detecting an underlying invasive mesothelioma in a subsequent biopsy specimen (ref 1). In the July 2015 issue of AJSP, using tissue microarrays, we showed that BAP1 IHC and P16 FISH in combination is a specific marker of mesothelioma, though with somewhat limited sensitivity (ref 2). In the January 2016 issue of AJSP, we further showed that in matched cytology and tissue biopsy specimens, that the combined use of BAP1 IHC and P16 FISH is both sensitive and specific in detecting mesothelioma (ref 3). Overall these studies highlight PhenoPath's continuing commitment and interest in refining the use of both immunohistochemical and molecular tools in diagnostic surgical pathology through collaborations with academic colleagues.

References:

1. Hwang HC, et al. P16 FISH Deletion in Surface Epithelial Mesothelial Proliferations Is Predictive of Underlying Invasive Mesothelioma. AJSP 38(5):681-8, 2014

2.. Sheffield BS, et al. BAP1 Immunohistochemistry and P16 FISH to Separate Benign from Malignant Mesothelial Proliferations. AJSP 39(7):977-82, 2015

3. Hwang HC, et al. Utility of BAP1 Immunohistochemistry and p16 (CDKN2A) FISH in the Diagnosis of Malignant Mesothelioma in Effusion Cytology Specimens. AJSP 40(1):120-6, 2016

Meet Our Pathologists at the Upcoming ASCP Course in Chicago, May 11-12!

Allen M. Gown, MD and Regan Fulton, MD, PhD of PhenoPath, and Dr. Jason L. Hornick, MD, PhD, FASCP of Brigham and Women's Hospital present the ASCP course, **Diagnostic Immunohistochemistry: Basics, Clinical Applications, and Challenges**, on Wednesday, May 11 - Thursday, May 12, 2016 in Chicago, IL. For the complete program, please visit www.ascp.org. Please check http://phenopath.com/#/upcoming-events for detailed information about this and other upcoming events at which PhenoPath pathologists are speaking.

PhenoPath
Diagnoses you can count on®

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FEATURED
At the PhenoPath Conference

Martin "Mac" Cheever, MD
Fred Hutchinson Cancer Research Center, Seattle, WA

PhenoPath, Thursday, March 31, 2016, 6:00PM (light dinner), 7:00PM (talk)

FRED HUTCH
40 YEARS OF CURES 1975–2015

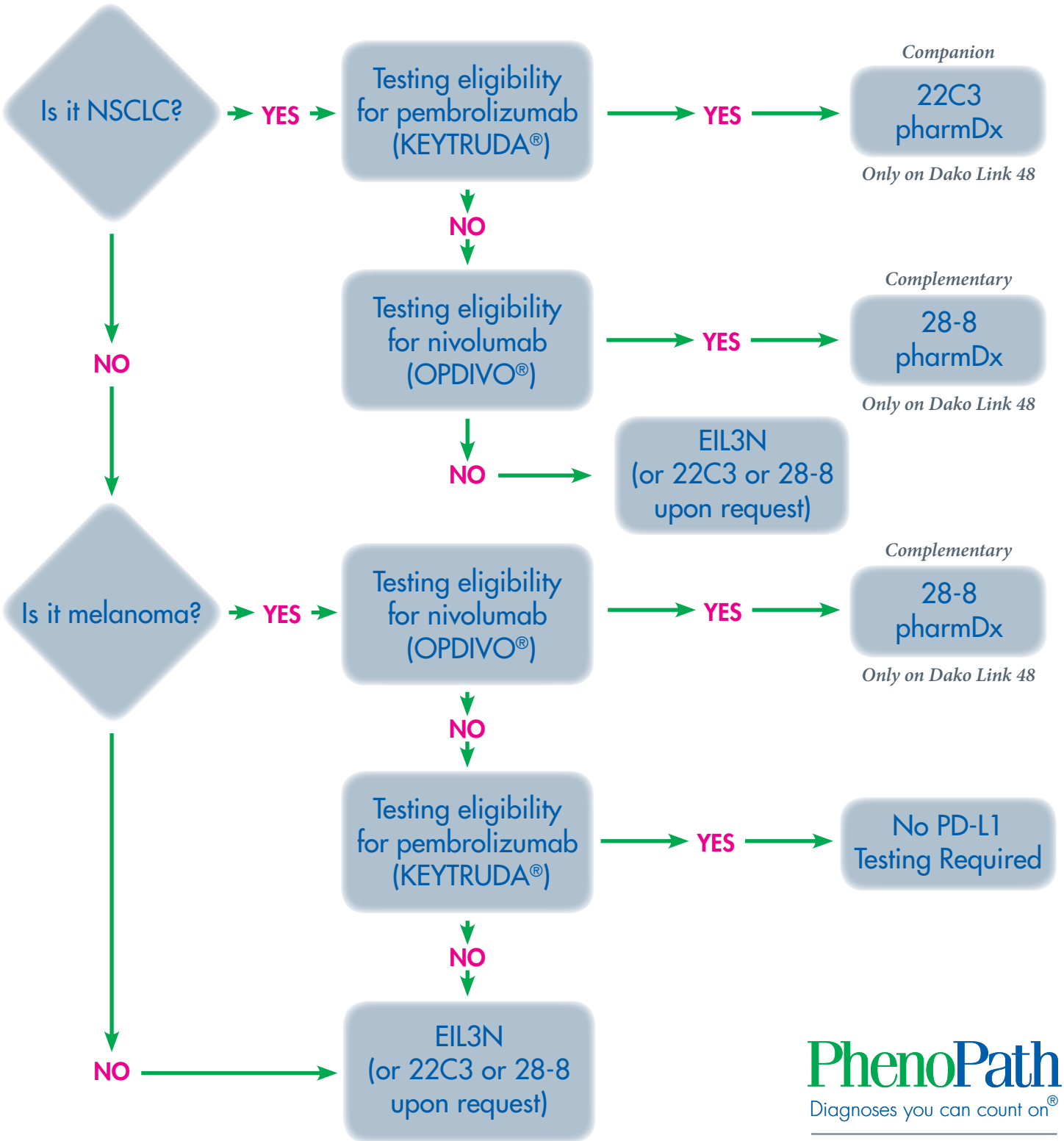
Martin "Mac" Cheever, MD will present "Cancer Immunotherapy During the First Half of 2016" at the PhenoPath Conference at *7:00pm on Thursday, March 31, 2016*. Dr. Cheever will give a related lecture at noon the same day.

Dr. Cheever is the Director of the NCI-funded Cancer Immunotherapy Trials Network (CITN), a Member of Fred Hutchinson Cancer Research Center (FHCRC) and a Professor of Medicine/Oncology at the University of Washington. The CITN has brought together leading immunotherapists from 32 foremost universities and cancer centers to design and conduct innovative early phase cancer immunotherapy trials. The CITN is currently conducting trials of a checkpoint inhibitor (anti-PD1), an antigen presenting dendritic cell growth factor (Flt3-Ligand), an antigen presenting dendritic cell activator (anti-CD40), the T cell growth factors (IL-7 and IL-15), and a small molecule inhibitor of the inhibitory enzyme, IDO.

Dr. Cheever has extensive experience with cancer immune therapy clinical trials, as well as cancer antigen discovery, vaccine development and principles of T cell therapy. He received his MD from the University of Michigan. He completed his internship and residency at the University of Washington, training and working as a faculty member in Dr. E. Donnall Thomas's bone marrow transplant program. In 1994 he co-founded a biotech company, Corixa Corporation, in order to develop cancer vaccines as therapeutic products. He served as Corixa's Vice President of Clinical Research and Medical Affairs from 1997 to 2005. From 2007 to 2013 he returned to the Fred Hutch to serve as Director of Solid Tumor research for the Fred Hutch/University of Washington Cancer Consortium. Currently he is focused full time on the CITN.

Anti-PD-L1 antibodies 22C3, 28-8, and E1L3N

Which PD-L1 test should I order?



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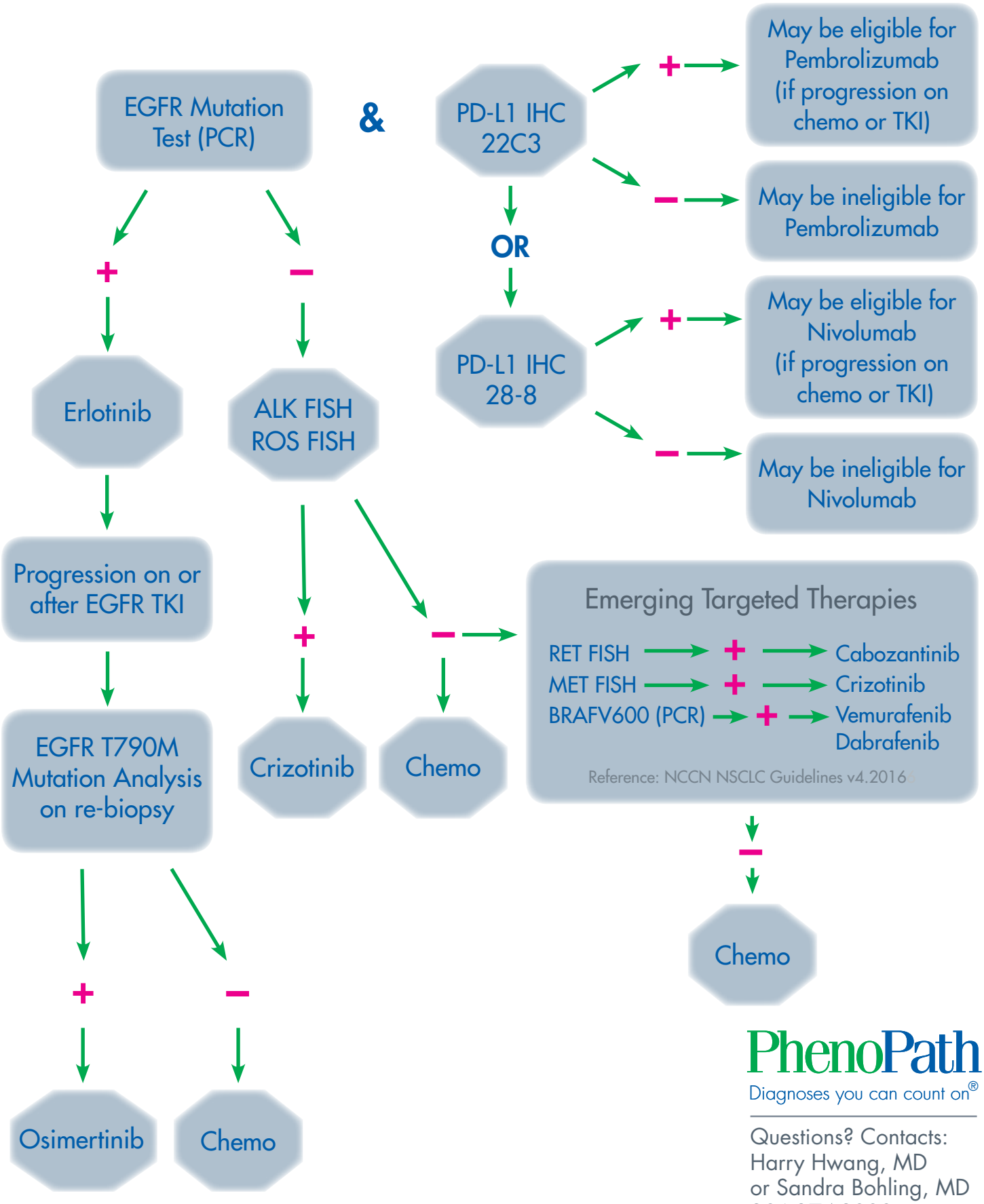
Questions? Contacts:
Allen M. Gown, MD or
Regan Fulton, MD, PhD
206-374-9000

Non-Small Cell Lung Carcinoma (NSCLC) Testing



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or Sandra Bohling, MD
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