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The Newsletter of PhenoPath
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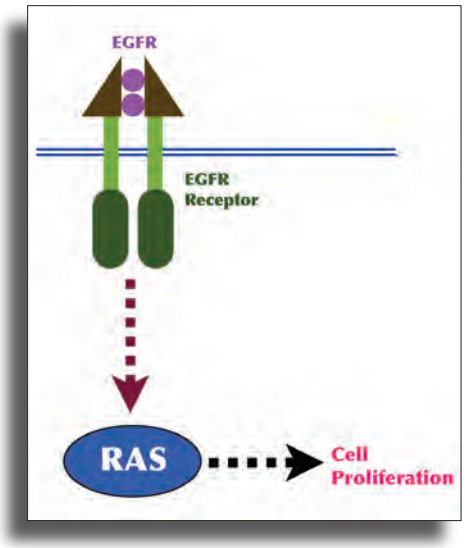
KRAS Testing For Colorectal Tumors

Now Available at PhenoPath Laboratories



Testing for the presence of mutations in the KRAS gene, a finding present in 30-40% of colorectal adenocarcinomas, has become a critical and essential test to determine which patients will benefit from the addition of cetuximab (Erbitux™) therapy.

KRAS is a G-protein that is a key component of the EGFR signal transduction pathway. Mutated KRAS protein is constitutively activated, and tumors carrying these activating mutations have been shown to be resistant to anti-EGFR therapy. In controlled clinical trials, patients with colorectal tumors having mutated KRAS were resistant to anti-EGFR antibody therapy and had similar progression-free survival (PFS) and overall survival (OS) compared to untreated control patients. In contrast, patients with unmutated KRAS did benefit from anti-EGFR therapy with improved PFS and OS compared to controls. Therefore, KRAS mutation status is a key predictor of patient responsiveness to anti-EGFR antibody therapy.

The most common activating mutations in KRAS have been identified in human cancers involving codons 12 and 13 in exon 2. These account for >98% of all known KRAS mutations. In the assay we have developed and validated at PhenoPath Laboratories, *designed to perform optimally in deparaffinized, formalin-fixed tissue*, these KRAS mutations are detected by the allele-specific PCR method (Amplification Refractory Mutation System [ARMS]). ARMS exploits the fact that oligonucleotide primers must be perfectly annealed at their 3' ends for a DNA Polymerase to extend these primers during PCR. Oligonucleotide primers that match only a specific DNA point mutation in the KRAS gene and not the wild-type allele can therefore be designed. The high sensitivity of PhenoPath Laboratories' ARMS assay for KRAS means that mutations can be detected even if mutated tumor accounts for ≤ 1% of the total DNA extracted from the tissue. You can be assured that slides from every case sent to PhenoPath Laboratories will be reviewed and tissue microdissected by one of our pathologists to ensure that the most appropriate portions of the tissue are analyzed, resulting in fewer quantity not sufficient (QNS) results.

KRAS FAQs

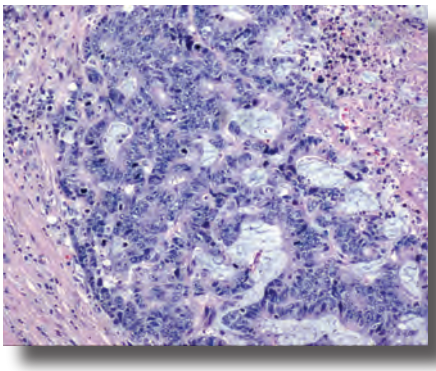
What kind of specimen do I need to submit to PhenoPath Laboratories to permit KRAS mutational analysis?

Paraffin blocks of formalin-fixed tissue, or cut sections from blocks, are most appropriate. Please contact PhenoPath Laboratories for recommendations regarding cut sections or the handling of scant or fresh specimens, including FNAs. Our staff will work with you to ensure proper handling of any type of specimen. We also have experience with DNA extractions performed on specimens fixed in non-formalin-based fixatives.

What is the turnaround time for KRAS mutational analysis?

Turnaround time is usually seven days or less from receipt of specimen.

(Continued on page two)



Colorectal adenocarcinoma

(KRAS Testing, continued from page one)

How has PhenoPath Laboratories validated this assay?

The KRAS mutational assay has been rigorously validated by direct DNA sequence verification on control cell lines as well as on a large number of colorectal cancer specimens to ensure accurate results. Our assay can detect KRAS mutations when cells containing the mutation comprise ≤ 1% of the specimen, which is an industry standard for this assay. Additionally, the assay has been cross-validated with other methodologies.

How does PhenoPath Laboratories' KRAS mutational assay differ from other laboratories' assays?

We employ a robust and rapid proprietary DNA extraction method that maximizes DNA yields without sacrificing sensitivity and specificity, and allows for more rapid PCR assay turnaround time without sacrificing accuracy. Microdissection of all specimens is performed by a pathologist. Unlike other ARMS-based mutational assays, the design of our assay employs direct visualization of mutant and control KRAS gene PCR products, which decreases the likelihood of false positive results. The ARMS-based KRAS assay employed at PhenoPath Laboratories is more sensitive and specific than sequencing alone.

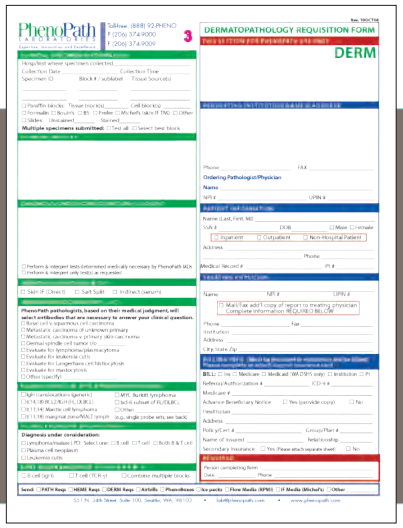
If you or your clinicians have any questions regarding PhenoPath Laboratories' KRAS mutational assay, contact us at 206-374-9000, or by email at lab@phenopath.com.

PhenoPeople Profile
Doug Sites

PhenoPath Laboratories is pleased to welcome Doug Sites as Director of Sales and Marketing. He has worked closely with pathologists in both clinical and research laboratories throughout the U.S. and Canada and has been involved in launching multiple services and products. Doug's goal at PhenoPath is to heighten the awareness of the company's quality services and expand the sales force across the U.S.

Doug brings a well-rounded knowledge of the reference laboratory industry, having worked with US Labs/LabCorp and PLUS Diagnostics, developing marketing programs and selling in both hospital and physician offices. In addition to his reference laboratory experience, Doug has been Vice President of Sales and Marketing for several medical distribution companies and was a Regional Sales Manager with Ventana Medical Systems. He has developed and taught a number of sales training courses, and introduced new products designed specifically for the pathology suite.

Doug earned his BS from The Ohio State University and completed various classes in sales management at the University of Michigan business school. He lives in Cincinnati with his wife, Laura, and has two daughters, Alexis and Emily. Doug's interests include cooking, travel and any outdoor adventures. When he isn't working for PhenoPath, he might be found canoeing down a stream somewhere in the world with his family.


PhenoPath LABORATORIES

DERMATOPATHOLOGY REQUISITION FORM

DERM

Dermatopathology Requisition Form

We have introduced a new requisition form specifically for dermatopathology. You can download this requisition form directly from our website, www.phenopath.com, or call us at 206-374-9000, and we will be happy to send you these forms pre-printed with your information.

Common dermatopathology settings include, but are not limited to:

- Basal cell v. squamous cell carcinoma
- Metastatic carcinoma of unknown primary
- Dermal spindle cell tumors
- Evaluation for lymphoma/plasmacytoma
- Evaluation for leukemia cutis
- Evaluation for Langerhans cell histiocytosis
- Evaluation for mastocytosis

If you have any questions or would like to discuss other situations, please contact us.

Visit us at the USCAP Annual Meeting

John B. Hynes Convention Center, Boston, MA, March 7-13, 2009

PhenoPath Laboratories is actively participating in the 2009 Annual Meeting of the United States and Canadian Academy of Pathology in Boston, MA from March 7-13, 2009. Dr. Allen M. Gown is among the speakers for the Special Course: Basic Principles & Practice of Molecular Pathology in Cancer. In addition, several abstracts are being presented highlighting our ongoing clinical research studies. PhenoPath will also be exhibiting at the meeting, showcasing new immunohistochemistry and molecular tests, along with our 9-color flow cytometry service. Be sure to visit us at Booth 1011, where we will be discussing our new tests and marketing programs for 2009. Ask for our Director of Sales and Marketing, Doug Sites, to see what changes are happening at PhenoPath.

Monday, March 9, 2009

8:00 AM to 1:00 PM (location pending): ***SPECIAL COURSE: Basic Principles & Practice of Molecular Pathology in Cancer: Integration of the Molecular Classification of Breast Cancer into Current Practice***, Presented by ***Allen M. Gown, M.D.***, PhenoPath Laboratories, Seattle, WA and University of British Columbia, Vancouver, BC.

Tuesday, March 10, 2009

9:15 am (Platform Session: Section B, CC BR B): ***Comparative and Additive Sensitivities of Immunohistochemical Markers of Breast Cancer Using New Monoclonal Antibodies to GCDFP-15 and Mammaglobin***, Presented by ***AJ Shaw, LC Goldstein, PL Kandalaft, HC Hwang, SJ Kussick, AM Gown***, PhenoPath Laboratories, Seattle, WA and IMPRIS, Seattle, WA.

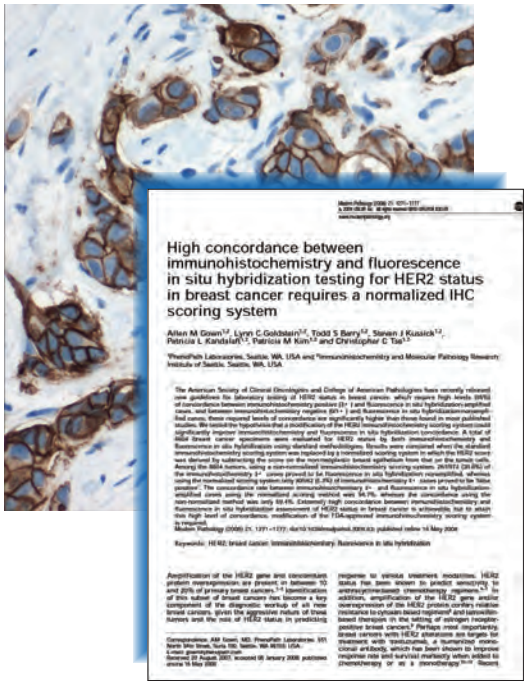
9:30 am (Poster Session III # 116, Exhibit Hall): ***Bcl-6 Coexpression in Mantle Cell Lymphoma: A Rare Finding Associated with a Lack of CD5 and/or CD3 Coexpression***, Presented by ***AJ Shaw, AM Gown, HC Hwang, PL Kandalaft, LC Goldstein, SJ Kussick***, PhenoPath Laboratories, Seattle, WA and IMPRIS, Seattle, WA.

Wednesday, March 11, 2009

1:00 pm (Poster Session VI # 131, Exhibit Hall): ***Carcinoma of Collecting Ducts of Bellini: Analysis of 27 Distinctive Cases of Renal Cell Carcinoma with Aggressive Clinical Behavior***, Presented by MB Amin, R Gupta, AO Osunkoya, O Hes, A Billis, CE Bacchi, D Hansel, M Zhou, MG deCastro, H Moch, P Salles, RA Cabrera, ***AM Gown***, Cedars Sinai Medical Center, LA, CA; Emory University Hospitals, Atlanta; Charles University Hospital, Pilsen, Czech Republic; State University of Campinas, Campinas, Brazil; Cunsultoria em Patalogia, Sao Paulo, Brazil; Cleveland Clinic Foundation, Cleveland; Santa Casa School of Medicine, Sao Paulo, Brazil; University Hospital Zurich, Zurich, Switzerland; Associao Mario Penna-Hospital, Belo-Horizonte, Brazil; Institute of Oncology, Lisbon, Portugal; PhenoPath, Seattle.

See our website for other talks our pathologists are involved in during this quarter outside of the USCAP Meeting.

Landmark HER2 Testing Concordance Study Published



Amplification of the HER2 gene and concomitant protein overexpression are both present in between 10 and 20% of primary breast cancers, and identification of this subset of breast cancers is key. In the October 2008 issue of *Modern Pathology*, the pathologists at PhenoPath Laboratories published a landmark study capping more than a decade of HER2 testing in breast cancer. Following publication of the ASCO-CAP Guidelines, questions were raised as to the ability of laboratories to attain the required 95% concordance between results of HER2 status, in positive and negative cases, as tested by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). This study demonstrates that the answer to that question is a resounding yes at PhenoPath Laboratories.

A total of 6,604 breast cancer specimens received over a four-year period were evaluated at PhenoPath Laboratories for HER2 status by both IHC and FISH using standard methodologies. Among the HER2 negative cases (as determined by IHC), the concordance with FISH was 99.2%; among the HER2 positive cases (as determined by IHC), the concordance rate was 94.7%. The tissues for this study were obtained prior to the ASCO-CAP Guidelines for HER2 testing, and thus the specimens, from hundreds of laboratories around the United States, had a wide range of fixation times. The study demonstrates that only when we modified the standard IHC scoring system with a "normalized" scoring system (in which the HER2 score was derived by subtracting the score on the non-neoplastic breast epithelium from that on the tumor cells), could we obtain such high levels of concordance between IHC and FISH for HER2 status. These results reflect the many years of experience of our pathologists and staff, our extensive and demanding quality assurance program for HER2 testing, and the overall dedication to quality testing at PhenoPath Laboratories.

Gown AM, Goldstein LC, Barry TS, Kussick SJ, Kandalaft PL, Kim PM, Tse CC. High concordance between immunohistochemistry and fluorescence in situ hybridization testing for HER2 status in breast cancer requires a normalized IHC scoring system. *Mod Pathol 21: 1271-1277, 2008.*

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Expertise, Innovation and Excellence

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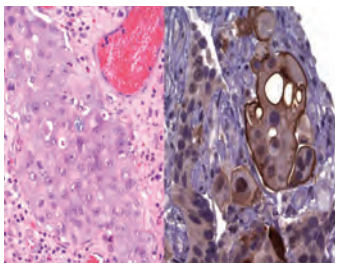
Featuring Dr. Mahul Amin

At Our Spring Conference

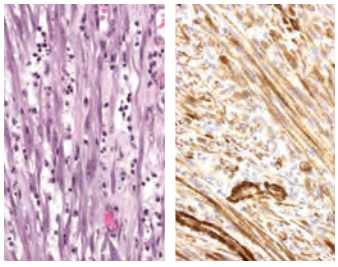
Mahul Amin, M.D., of Cedars-Sinai Medical Center, Los Angeles, CA, will present "Role of Immunohistochemistry in Urologic Pathology – Selected Topics" at the PhenoPath Pathology/Immunohistochemistry Conference on *Thursday, February 12, 2009*. The format of the conference is a social hour commencing at 6:30 PM, followed by Dr. Amin's lecture at 7:30 PM. A light catered dinner will be served during the social hour.

Currently Professor and Chairman of the Department of Pathology and Laboratory Medicine, and the Fellowship Director in Urologic Pathology at Cedars-Sinai Medical Center, Dr. Amin is also a Professor of Pathology at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA).

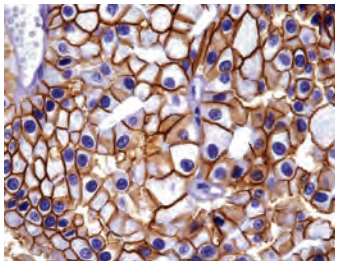
Recognized as one of the leading authorities in genitourinary pathology with extensive expertise in oncologic pathology of the genitourinary tract, Dr. Amin is also a national and international consultant in tumors of the genitourinary tract, including prostate, urinary bladder, kidney and testis. In addition to publishing over 219 peer-reviewed articles, Dr. Amin is also the co-author of the current classification systems for urothelial tumors and renal neoplasms, two Armed Forces Institute of Pathology fascicles, and a monograph on Gleason Grading of Prostate Cancer. He has authored two other books in genitourinary pathology including co-authoring a book on bladder biopsy interpretation. Dr. Amin is a highly sought-after speaker, having given over 200 national and international lectureships.



Uroplakin 3



SMA



Ksp-cadherin