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Feature Story

Quantitative Analysis of *MGMT* Promoter Methylation in Glioblastoma p2

Quantitative Analysis of *MGMT* Promoter Methylation in Glioblastoma

By Bin Yang, MD, PhD

Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. It has a very poor prognosis with a median overall survival of only 12 to 15 months. Current treatments of GBM include surgical resection, radiotherapy, and administration of alkylating agents such as temozolomide (1). Alkylating agents inhibit DNA replication and induce tumor cell apoptosis by producing cross-links between adjacent DNA strands. The most common site for alkylation is the O^6 position of guanine (2). Methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme, specifically removes promutagenic alkyl groups from the O^6 position of guanine in DNA. Repair of O^6-alkylguanine adducts in tumor DNA reduces the cytotoxicity of alkylating chemotherapeutic agents, resulting in tumor chemoresistance (3).

Preclinical and clinical evidence showed that the expression of MGMT is regulated primarily at the epigenetic level by promoter methylation (4). Under normal circumstances, the CpG sites spanning a large portion of the promoter and exon 1 of the *MGMT* gene are unmethylated. In some tumor cells, however, the cytosines at certain CpG sites are methylated. This prevents transcriptional factors from binding and eventually leads to the reduced expression of MGMT protein, which sensitizes the cancer cells to alkylating agents.

On the Cover: Director of PLMI's Cancer Epigenetics Core Laboratory Bin Yang, MD, PhD, (center) Kelly Lyon, laboratory manager, Molecular Pathology, (l) and Maureen Jakubowski, medical technologist (r) are the first to validate pyrosequencing on FFPE tissue for identifying *MGMT* methylation status in glioblastoma patients. *MGMT* status provides valuable molecular-based guidance for treatment planning for these patients.

Many studies have established a significant correlation between methylation of *MGMT* and a positive clinical outcome for patients with glioblastoma treated with alkylating agents such as temozolomide, carmustine, and procarbazine with or without radiotherapy (2, 5-7). Additionally, significant differences were demonstrated among varying levels of methylation, with a higher *MGMT* methylation level associated with longer overall survival (8). These studies suggested that *MGMT* methylation can predict glioblastoma chemosensitivity to alkylating agents and help determine the patient population likely to benefit from such treatment.

Several methods have been used to identify *MGMT* methylation status (3, 9-12). Methylation-specific PCR (MSP) has high sensitivity and is widely used for assessing *MGMT* methylation in the research setting, but it is a qualitative technique with a high rate of false positives. The application of MSP in the clinical setting is limited by its mediocre compatibility with formalin-fixed paraffin-embedded (FFPE) tissue specimens and requirement for high quality DNA (11).

Pyrosequencing is a highly reproducible and quantitative method for confirming methylation status and has been shown to be the best approach for assessing *MGMT* methylation status in GBM patients and correlating with clinical outcomes (3). However, no studies have been done to validate this method in the clinical setting for its compatibility with FFPE tissue specimens, analytical quality, and clinical feasibility. In this study, we aimed to validate a quantitative *MGMT* methylation assay using pyrosequencing on FFPE GBM tissue to determine its analytical sensitivity and precision and clinical feasibility for pathologic diagnosis and prediction of glioblastoma chemosensitivity.

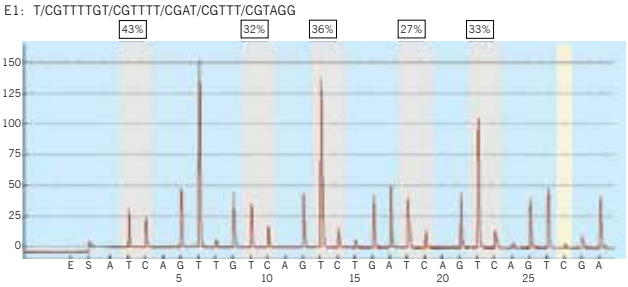

Figure 1

Materials and Methods

We selected 43 cases of brain tissue for quantitative analysis of *MGMT* promoter methylation status. Tissue specimens came from 10 patients with epilepsy as non-neoplastic controls, 17 patients with resected GBM, and 16 patients with stereotaxic biopsy for GBM. All specimens were FFPE tissue transported and stored at room temperature.

We first extracted genomic DNA from a 10 μm tissue section of FFPE tissue samples using the Gentra Puregene tissue kit (QIAGEN Inc., Valencia, Calif.). After extraction, we further purified the DNA using the QIAamp MiniElute column (QIAGEN Inc.) and performed the procedure for genomic DNA cleanup as given in the manufacturer's manual. Approximately 100-200 ng DNA was subjected to bisulfite conversion using the EZ DNA Methylation Gold kit (Zymo Research, Orange, Calif.) according to the manufacture's protocol. In this step, sodium bisulfite converts unmethylated cytosine to uracil, while methylated cytosine remains unchanged before PCR.

A total of 10-20 ng bisulfite-treated DNA was carried on for PCR using the PyroMark PCR Kit (QIAGEN Inc.). PCR conditions for the MGMT gene were 95°C for 15 minutes; 45 cycles of 95°C for 20 seconds, 53°C for 20 seconds, and 72°C for 20 seconds; 72°C for five minutes, and then on hold at 4°C. The PCR products were immobilized to beads and strand separation. We then conducted the pyrosequencing methylation assay using the sequencing primer provided in the PyroMark CpG MGMT Kit on the PyroMark Q96 ID pyrosequencer (QIAGEN Inc.) The Pyromark CpG MGMT kit detected the level of methylation on 5 CpG sites located in exon 1 of the *MGMT* gene. A cytosine not followed by a guanine served as an internal control for completion of bisulfite conversion. The percent methylation (percent of Cs present) was reported for each CpG site. A sample with methylation above 10% was interpreted as positive. A sample below 10% methylation was interpreted as negative.

We determined the analytical sensitivity of this methylation test by pyrosequencing using a series of DNA dilution tests. In these tests, SW40, a colon cancer cell line with a mean *MGMT* methylation of 95%, was used as the positive control; MRC5, a fibroblast cell line with 0% *MGMT* methylation, was used as the negative control. We diluted SW40 DNA with MRC5 DNA from a ratio of 1:10 to 1:1000. The most diluted concentration with detectable methylation was recorded as the analytical sensitivity, which was compared with the standard MSP method. In addition, we tested 50, 100, 150, and 200 ng DNA obtained from FFPE tissues in *MGMT* methylation pyrosequencing for the minimal requirement of DNA volume. To measure the biopsy specimen size needed for *MGMT* methylation pyrosequencing, we reviewed 50 consecutive GBM biopsy specimens in 2010 at our institution and documented the size of these specimens. We chose 16 biopsy specimens that represent the biopsy size below 30th percentile for testing. Genomic DNA was extracted from eight sections of 15 μm-thick FFPE blocks. The concentration of DNA was measured in duplicate in each case.

Statistical analysis for this study was descriptive in nature. All the statistical analyses were performed using Stata 12 (StataCorp LP, College Station, Texas).

Results

We optimized the experimental conditions for the *MGMT* methylation pyrosequencing assay. After extraction, genomic DNA was further purified to generate highly purified DNA for pyrosequencing. The amount of DNA sample loss was less than 15%. We used the EZ DNA Methylation Gold kit for bisulfite conversion, which converts greater than 95% of unmethylated cytosines in the genome to satisfy the requirement of the PyroMark Q96 ID pyrosequencer for consistency.

We determined the level of methylation on five CpG sites in exon 1 of the *MGMT* gene from the brain tissues of controls and patients with glioblastoma. Typical examples of pyrograms are shown in Figure 1. Shaded boxes encompass T/C pairs with methylation of five CpG sites measured quantitatively. In a typical tissue specimen from an epilepsy patient, most of the CpG sites are unmethylated with occasional sites exhibiting less than 10% methylation. In contrast, a typical tumor specimen from a glioma patient often has more than 25% methylation in all the CpG sites.

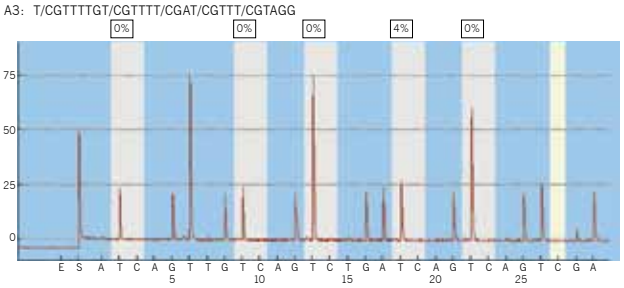

Figure 2

As shown in Figure 2, we measured *MGMT* gene methylation in 10 non-neoplastic epilepsy patients. None of the epilepsy brain tissues exhibited more than 10% *MGMT* methylation. We observed a low level of *MGMT* methylation in two cases (4% and 8%). In contrast, among the glioblastoma tissues from 33 patients, the range of methylation percentage of any CpG island in the *MGMT* gene was between 33 and 95% with a mean of 65%. We observed greater than 10% *MGMT* methylation in 33% of patients (11/33) and greater than 5% MGMT methylation in 46% patients (15/33). We determined that 10% methylation is the cutoff to distinguish *MGMT* methylation-positive cases from negative cases.

By a series dilution of a methylated cancer cell line with an unmethylated normal cell line, pyrosequencing can detect one tumor cell harboring *MGMT* methylation out of 80 to 100 cells without *MGMT* methylation. Pyrosequencing has a comparable sensitivity to MSP with fewer false-positive cases and provides the quantitative methylation value of each CpG island. We determined that 100 ng (approximately 3,000 cells) is the minimum amount of genomic DNA required for successful detection of *MGMT* methylation by pyrosequencing.

Most of the clinical GBM biopsy specimens ranged from 0.1 to 3.0 cm in size with a median of 1.0 cm. We chose the 30% smallest biopsy tissues for testing. As shown in Figure 3, the mean DNA yield increase correlated with the increase in biopsy sample size. Four 15 μm-thick tissue sections should yield more than 300 ng genomic DNA, corresponding to a 0.3 cm biopsy specimen with less than 15%. To fulfill the clinical requirement of duplicate tests, 150 ng DNA samples could be used for each run.

After the testing protocol was finalized, we ran a precision test with a known *MGMT* methylation case along with positive and negative controls. Results of duplicated tests were consistent and compatible with expected results.

Discussion

Results from several studies demonstrate that pyrosequencing is the preferred approach to stratify patients treated with temozolomide into different prognostic groups based on their measured *MGMT* methylation status.

The reliability and reproducibility of most of the previously developed methods such as MSP require high-quality DNA, often from a fresh frozen specimen (7). In most clinical settings, FFPE specimens are needed for easy transport and storage, but MSP can provide reliable MGMT methylation status on only two-thirds of FFPE tumor specimens (7). We solved this problem by further purifying the genomic DNA extract from FFPE tissue samples using a Qiagen MiniElute column. After elution with 100 μl AE buffer, we were able to generate highly purified genomic DNA for the subsequent steps with less than 15% DNA loss.

Bisulfite conversion of cytosine is a crucial step toward success. Complete conversion of unmethylated cytosine to uracil is the basis of methylation quantification. Incomplete conversion leads to overestimation of *MGMT* methylation and severely compromises methods used on clinical samples with low DNA content. In our study, we compared two bisulfite treatment methods: the EZ DNA Methylation Gold kit and the Qiagen DNA methylation kit. The former method converts greater than 95% of unmethylated cytosines in the genome, which satisfies the requirement of the PyroMark Q96 ID pyrosequencer for consistent results. However, the latter method used in some of the previous studies converts only about 90% of unmethylated cytosines, which does not fulfill this requirement.

The cutoff of 10% methylation that we chose to stratify patients was based on the maximum level of methylation (8%) in the control group. The 10% cutoff and the prevalence of *MGMT* hypermethylation in the tumor tissue of glioblastoma patients who met this criterion were consistent with previous reports (4, 11).

The analytical sensitivity of our method for *MGMT* methylation detection ranges from 1:80 to 1:100, approximately five times less than MSP (1:500). Although MSP is more sensitive than pyrosequencing, has a false-positive rate of approximately 10% (10). This concern with false-positivity using MSP was mentioned in the literature due to its “super-sensitivity” and mispriming (PCR bias), which can be further aggravated by high cycle number and nested primers used for decreased

sample quality of PPFE specimen (13). Since most testing samples will be GBM material from FNAB and are less diluted with normal brain tissue, sensitivity of 1:80-1:100 should be adequate for clinical usage.

Further analysis in this study demonstrated agreement in 95% of cases between pyrosequencing and MSP assays. Pyrosequencing has higher specificity and provides a quantitative value of both average and individual CpG island methylation status, which may have higher predictive value for clinical outcomes (11). In contrast, MSP is not quantitative and cannot tell methylation status of individual CpG islands.

In our study, 100 ng DNA is adequate to repeatedly detect *MGMT* methylation by pyrosequencing, which is much less than the Qiagen kit's recommended more than 300 ng genomic DNA for research purposes. The biopsy sample size requirement (0.3 cm) of our method is considerably smaller than the current median sample size of 1.0 cm. DNA yielded from even the smallest GBM biopsy size (0.1 cm) at our institution in 2010 was enough for duplicated assays, demonstrating that our tissue requirement is reasonable clinically and is significantly less demanding than commercially available MSP kits that requires more than 1.0 cm tissue with minimal 500 ng input.

To complete our technique, we created positive and negative report templates compatible with CAP MP reporting guidelines.

Conclusion

We described the first validated pyrosequencing-based *MGMT* methylation test on clinical FFPE biopsy tissue from 33 patients with glioblastoma and 10 patients with epilepsy as controls. We demonstrated that pyrosequencing detection of *MGMT* methylation has an analytical sensitivity and DNA and biopsy tissue requirements that are suitable for routine clinical application. The *MGMT* methylation assay provides pathologic diagnosis and a useful molecular biomarker for prediction of chemosensitivity in GBM patients.

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Personalizing the Testing Paradigm for Selection of NSCLC Treatment: *ALK* Rearrangement

By Raymond Tubbs, DO

The August 2011 FDA approval of the targeted therapy crizotinib (Pfizer, New York, N.Y.) and the companion diagnostic assay Vysis *ALK* Break Apart FISH Probe Kit (Abbott Molecular, Inc., Chicago, Ill.) opened the door to a new era in the treatment of late-stage non-small cell lung cancer (NSCLC).

Lung cancer is the leading cause of cancer death in men and women worldwide. Approximately 85% of all lung cancers are of non-small cell type. Advances in surgical treatment and combination therapies have improved the average one-year survival rate (38% for stage IA-IIB, Source: National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database) only marginally from the period 1975-1979 to the present; the five-year survival rate for all stages of lung cancer combined remains at 15%. The primary reason is that nearly 85% of cases present at an advanced stage.

Targeting *ALK* Gene Rearrangements

Crizotinib is highly effective in treating patients whose NSCLC tumors harbor a rearrangement of the anaplastic lymphoma kinase (*ALK*) gene. The *ALK*-FISH assay allows for selection of the subgroup of NSCLC patients who are potentially therapy-responsive based on identification of *ALK* rearrangements at the 2p23 chromosome in the tumor genome. This genetic alteration occurs in 2 to 7% of NSCLC patients.

The *ALK* gene encodes for a transmembrane glycoprotein with tyrosine kinase activity that normally is expressed only in select neuronal cell types. The constitutive kinase activity of ALK is essential for cellular proliferation in this subset of NSCLC. Gene rearrangements in the presence of known fusion partners, including *EML4*, *TFG* and *KIF5B*, result in a chimeric protein with tyrosine kinase activity. In particular the *EML4*/*ALK* fusion appears to be a key driver of tumorigenesis in NSCLC. The *EML4*/*ALK* fusion also has been identified in about 2.5% of breast and colon carcinomas using exonic sequencing.

ALK rearrangements are typically mutually exclusive from *EGFR* and *KRAS* mutations, and the majority are found in adenocarcinomas. Patients with tumors exhibiting *ALK* rearrangements are usually younger, male and light or never-smokers. Lung adenocarcinomas with *ALK* rearrangement occur in 15% of this population with advanced stage NCSLC.

ALK+ Response to Crizotinib

ALK FISH is a qualitative fluorescence *in situ* hybridization (FISH) test that detects *ALK* gene rearrangements with all

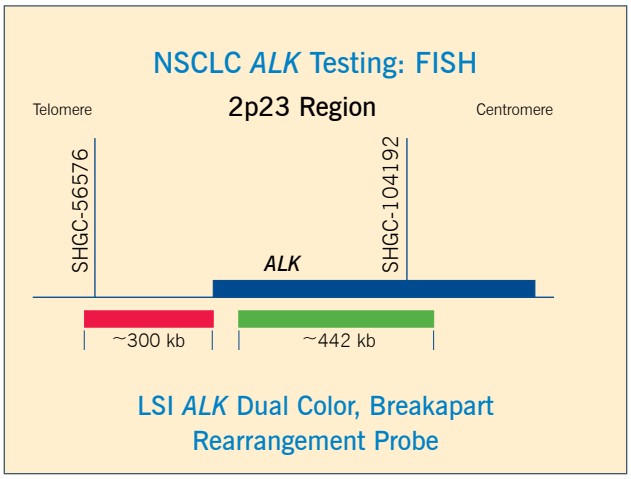

Figure 1
Probe map, breakapart format probe set, FDA-approved fluorescence *in situ* hybridization (FISH) assay for identification of rearrangements at the 2p13 locus that includes the *ALK* gene.

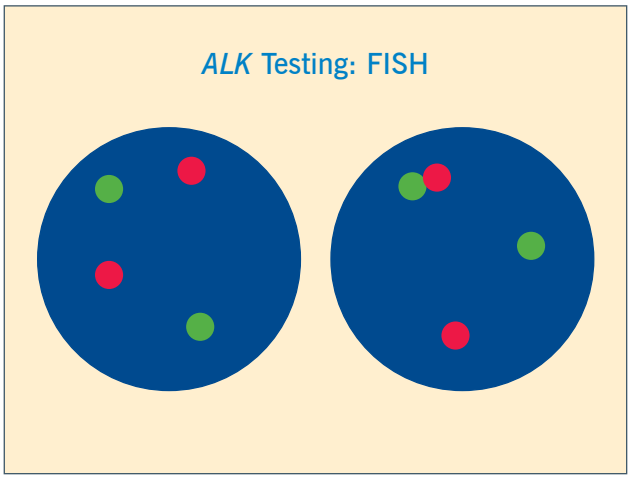

Figure 2
Diagram of typical monoallelic *ALK* rearrangement. One allele (right) is uninvolved and demonstrates a green and a red signal in apposition. Biallelic rearrangments (left) are much less common.

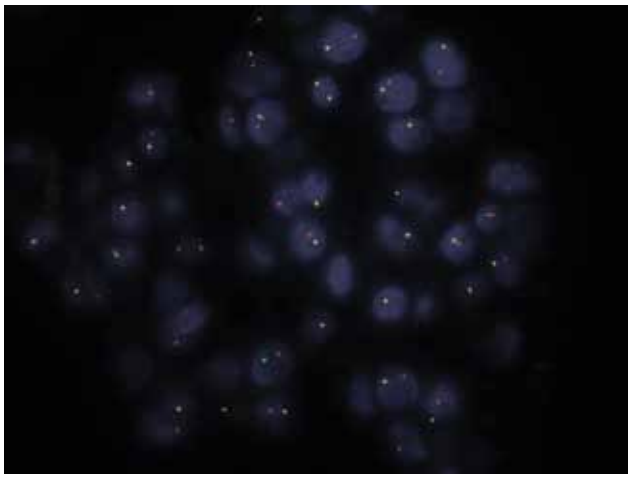

Figure 3
An atypical 2p23 *ALK* rearrangement (one green/red fusion signal + an isolated red signal). This pattern is also considered a positive result qualifying NSCLC patients for crizotinib-based therapy.

potential fusion partners, including *EML4*, *TFG* and *KIF5B*. It is the only diagnostic assay approved by the U.S. Food & Drug Administration for *in vitro* diagnostic (IVD) use to predict response to crizotinib. Other available molecular testing methodologies such as RT-PCR detect only the fusion target for which they have been constructed (e.g. *EML4*/*ALK* fusion product), and, of course, other potentially clinically significant fusion targets will not be detected. The Abbott Molecular Vysis (AMV) *ALK* FISH kit was used in one of the two single-arm trials (Study A) leading to the FDA approval of crizotinib and has become the gold standard for detecting *ALK* rearrangement in NSCLC.

Study A included 136 patients; Study B, 119 patients. All patients had locally advanced or metastatic *ALK*-positive NSCLC. Patients in both studies received 250 mg. crizotinib orally twice daily. The combined median age for both trials was 52 years; 63% of patients were Caucasian; 30% were Asian; 48% were male; 84% percent had an ECOG performance status of 0 or 1. Fewer than 3% of patients were current smokers. Ninety-six percent had adenocarcinoma, 95% had metastatic disease, and 9% had received prior systemic treatment for NSCLC.

The primary endpoint of both trials was objective response rate (ORR) as assessed by the investigator. In Study A, the ORR was 50% (95% CI: 42%t, 59%) with a median response duration of 42 weeks. In Study B, the ORR was 61% (95% CI: 52%, 70%) with a median response duration of 48 weeks. A complete response was observed in 1% of patients. Performance status, number of prior chemotherapeutic regimens, or percentage of cells found to have the *ALK* gene rearrangement did not influence ORR.

Patient Selection Using *ALK* FISH

The AMV *ALK* Break Apart FISH Probe mixture consists of two fluorophore-labeled DNA probes in hybridization buffer containing dextran sulfate, formamide, and SSC with blocking DNA. The two probes used are Vysis LSI 3 *′-ALK* SpectrumOrange and LSI 5 *′-ALK* SpectrumGreen.

The FDA-approved version of the test is performed on a formalin-fixed paraffin-embedded NSCLC tissue specimen. Alternative approaches using cytopathology ThinPrep slides for probe hybridization with morphologic correlation are under active development. Following appropriate specimen preparation, the specimen is hybridized using the probe mixture described above at 37°C for 14 to 24 hours. After washing and counterstaining, slides are evaluated for adequate hybridization. Slides are then assessed for the quality of the *ALK* signal and the tissue morphology. Signals from 50 tumor cells from representative areas of the slide are recorded.

When hybridized with the *ALK* FISH probes, the 2p23 *ALK* region in its native state will be observed as two immediately adjacent or fused (overlapping) orange/green (yellow) signals. However, if a chromosome rearrangement at the 2p23 *ALK* breakpoint region has occurred, one orange and one green signal separated by at least two signal diameters will be identified. Alternatively, a single orange signal (deletion of green signal) in addition to a fused or broken apart signal may be observed.

Cells are considered negative (non-rearranged) when orange and green signals are adjacent or fused (appear yellow under the Orange/Green V2 filter). A single green signal without a corresponding orange signal is also abnormal, but represents a rearrangement at the 2p23 locus that is not predictive of over-expressed ALK protein. Orange and green signals that are less than two signal diameters apart are classified as a single fused signal.

Cells are considered positive for a rearrangement when at least one set of orange and green signals are two or more signal diameters apart, or a single orange signal occurs without a corresponding green signal in addition to fused and/or broken apart signals. Multiple 2p23 signals are often observed as well, indicative of either genomic gain at 2p23 or an aneusomic state for chromosome 2.

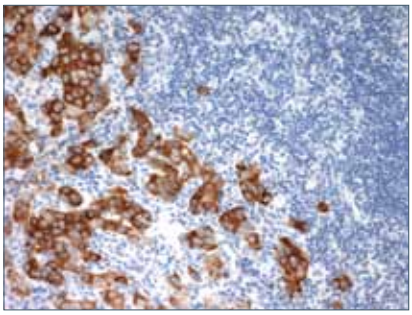

Figure 4
ALK overexpression in NSCLC tumor cell cytoplasm detected by immunohistochemistry. D5F3 primary antibody (Cell Signaling Technology, Beverly, Mass.) with detection via OptiView (Ventana Medical Systems, Tucson, Ariz.).

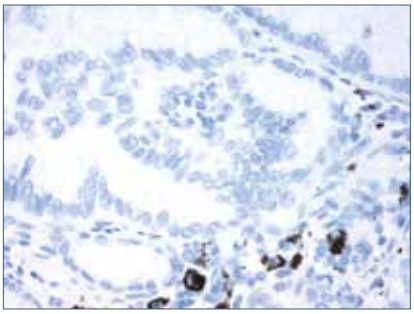

Figure 5
Absence of ALK overexpression in a NSCLC. Anthracotic pigment is present (lower right), but the tumor cells are negative.

A sample is considered negative if < 5 cells out of 50 (< 5/50 or < 10%) are positive. A sample is considered positive if > 25 cells out of 50 (> 25/50 or > 50%) are positive. A sample is considered equivocal if 5 to 25 cells (10 - 50%) are positive. If the sample is equivocal, a second technologist is required to evaluate the slide. The first and second cell count readings are added together, and a percent is calculated of 100 cells, the average percent positive cells. If the average percent positive cells is < 15% (< 15/100), the sample is considered negative. If the average percent positive cells is ≥ 15% (≥ 15/100), the sample is considered positive.

ALK FISH demonstrates consistency of results across all readers with an overall percent agreement of 97.64 (95% CI: 96.25, 98.52). The positive percent agreement is 96.46 (95% CI: 94.40, 97.78), and the negative percent agreement is 100.00 (95% CI: 98.42, 100.00).

Patients who are *ALK* positive are candidates for crizotinib therapy. For patients who are responsive to treatment, crizotinib yields significant clinical benefits. However, not all *ALK*-positive patients will respond, and those who do will eventually develop resistance to crizotinib. Time to drug resistance is unknown as yet; a variety of underlying molecular mechanisms are responsible for development of resistance

The National Comprehensive Cancer Network (NCCN Guidelines™) recognizes FISH as a specifically designed method for diagnosing *ALK*-rearranged adenocarcinomas. These guidelines recommend *ALK* testing concurrent with *EGFR* mutation testing in the diagnosis of adenocarcinoma, large cell, and other nonspecified histologic subsets of NSCLC.

Investigation into the role of IHC staining with monoclonal antibodies specific for ALK protein in identifying patients with *ALK* rearrangements continues. IHC can be performed on small biopsies, may be less sensitive to variations in fixation conditions than FISH and can be performed in routine daily practice.

Depending on the monoclonal antibody and concentration used in IHC, overall sensitivity of IHC for *ALK* rearrangements ranges from 80 to 95% with specificity of 100%. A testing algorithm using IHC as an initial screening in all cases of NSCLC has been proposed in which *ALK* IHC 2+ tumor cells would undergo *ALK*-FISH; 3+ cells would be reported as *ALK*-positive; 0 and 1+ cells would be reported as *ALK*-negative. However, no IHC assay for *ALK* rearrangements has been cleared by the FDA for IVD, and its use remains investigational.

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Cleveland Clinic Pathologists Present, Teach, Lead at USCAP

The Cleveland Clinic Pathology & Medicine Institute (PLMI) was a strong presence at the 2012 United States and Canadian Academy of Pathology (USCAP) annual meeting. PLMI faculty, fellows and residents presented 75 abstracts at the meeting, held in Vancouver, British Columbia, March 17-23. Residents and fellows were first authors on the majority of the PLMI presentations, giving them the opportunity to speak at the largest pathology meeting in the world.

PLMI continued its long-standing tradition of leadership in pathology education at USCAP with 12 PLMI faculty serving as short course directors:

Ana E. Bennett, MD, Thomas Plesec, MD, John R. Goldblum, MD — Biopsy Pathology of Esophageal and Coloanal Neoplasia

James R. Cook, MD, PhD, with colleagues from University of Texas Health Science Center and University of Pittsburgh School of Medicine — Diagnostic Hematopathology - A Roadmap for the Surgical Pathologist

John R. Goldblum, MD, and Scott E. Kilpatrick, MD, Novant Health Systems, Winston-Salem, NC — A Potpourri of Mesenchymal Delights: Pattern-Oriented Approach to the Diagnosis of Soft Tissue Tumors

Donna E. Hansel, MD, PhD, and Mahul B. Amin, MD, Cedar-Sinai Medical Center, Los Angeles — An Algorithmic Approach to the Diagnosis of Tumors and Tumor-Like Lesions of the Urinary Bladder

Eric D. Hsi, MD, Yaso Natkuna, MD, PhD, and Daniel A. Arber, MD, Stanford University School of Medicine, Stanford, Calif. — New Concepts in the Diagnoses and Classification of Extanodal Lymphomas

Rish K. Pai, MD, PhD, Lisa Yerian, MD, and **David Barnes, MD** — Interpretation of the Medical Liver Biopsy: What Do Clinicians Want?

Richard A. Prayson, MD, and Bette K. Kleinschmidt-DeMasters, MD, University of Colorado at Denver and Health Sciences Center, Aurora, Colo. — A Practical Approach to Non-Neoplastic Surgical Neuropathology

J. Jordi Rowe, MD, and **Steven D. Billings, MD** — Mesenchymal Tumors of the Breast and their Mimics: An Update and Approach to Diagnosis

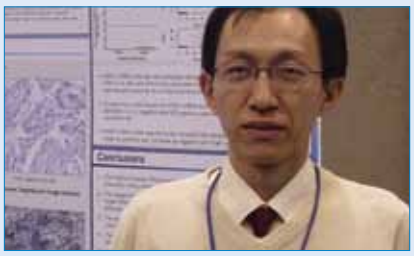
Brian P. Rubin, MD, PhD, and Jason L. Hornick, MD, PhD, Brigham and Women's Hospital, Boston — Classification and Prognostication of Mesenchymal Tumors of the Gastrointestinal Tract

Dr. Goldblum, Lynn Schoenfield, MD, and Carmela Tan, MD, were presenters for subspecialty evening conferences. In companion meetings held in advance of the USCAP meeting, Cristina Magi-Galluzzi, MD, PhD, moderated a session on GU pathology, and Gary Procop, MD, was a presenter in a panel on the multidisciplinary approach to diagnosing infectious diseases.

Dr. Magi-Galluzzi, Dr. Pai, Dr. Hansel, E. Rene Rodriguez, MD, and Jennifer Brainard, MD, served as moderators for various scientific platform sessions, and Dr. Cook was a presenter for the long course on malignant lymphomas. Two day-long special courses were led by PLMI faculty with Carol Farver, MD, as course director and a presenter, Dr. Yerian presenting a new course for residents and fellows on leadership and collaboration in health care, and Tarik Elsheikh, MD, course director and a presenter for a course on basic principles in cytology.

In addition to PLMI's leadership at the annual meeting, the institute also has a significant presence in the society's ongoing activities. Thirteen PLMI faculty are on the USCAP Abstract Review Board: Drs. Cook, Farver, Hsi, Pai, Plesec, Prayson, Rowe, Schoenfield and Tan, Andrea Arrossi, MD, Deborah Chute, MD, Walter Henricks, MD, and Deepa Patil, MD. Dr. Goldblum is Past President of the Arthur Purdy Stout Society of Surgical Pathologists and chairs the USCAP Education Committee with Drs. Elsheikh and Billings as active members, and Dr. Rubin is a senior editor for one of the two official USCAP journals, *Laboratory Investigation*.

Liz Severson, DO, AP Chief Resident



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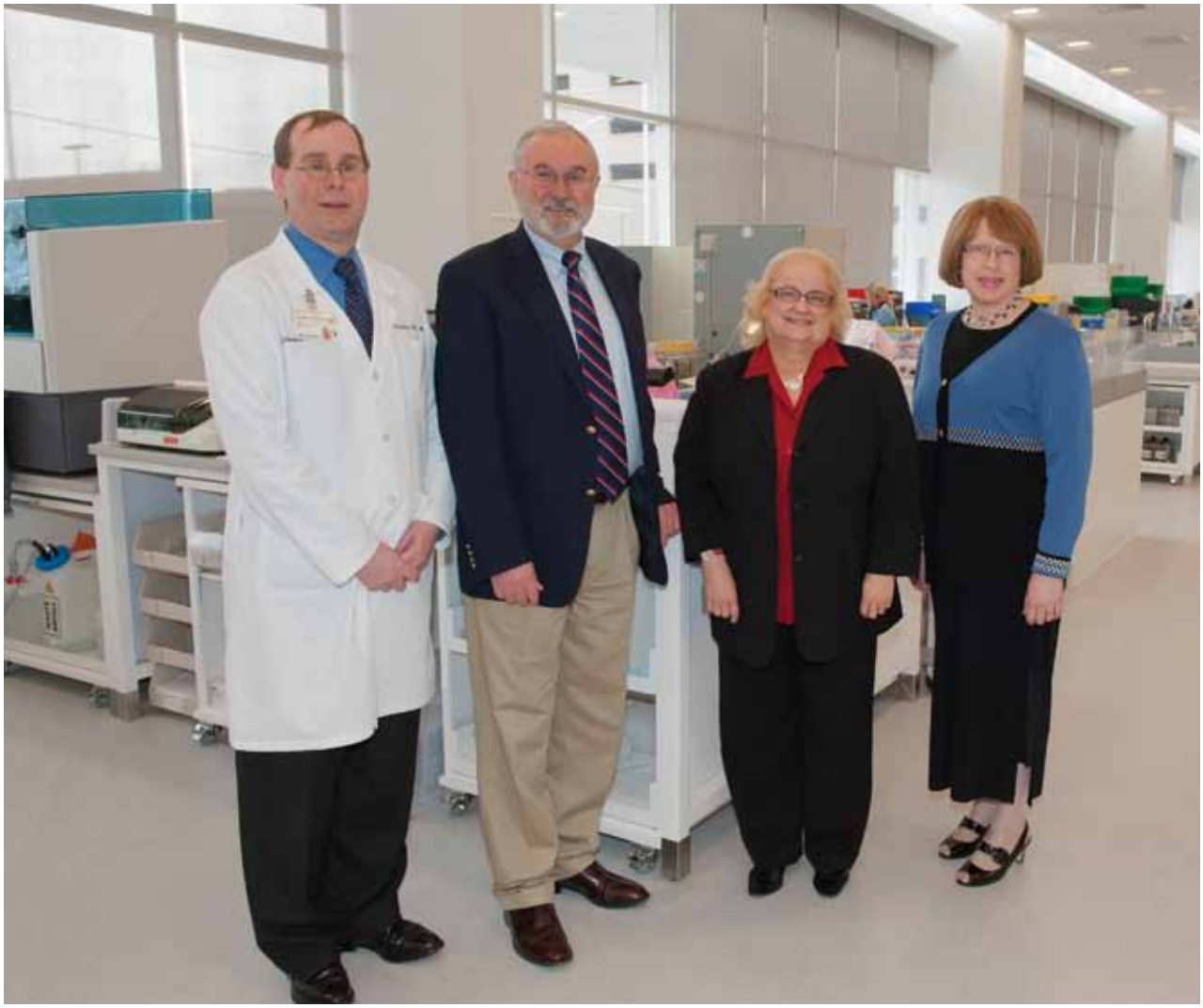

(from l) Dr. Myles, Dr. Hickey, Ms. Kennedy, and Dr. Marchant toured the new Cleveland Clinic Laboratories facilities during the Peer2Peer Practice Roundtable February 22.

CAP Connects with PLMI at Peer2Peer Roundtable

The Cleveland Clinic Pathology & Laboratory Medicine Institute (PLMI) hosted a College of American Pathologists (CAP) Peer2Peer Practice Roundtable February 22. CAP is conducting a series of these discussions at pathology practices across the country with the goal of understanding the challenges and opportunities facing pathology practices and strategies for addressing them. Each session includes the host pathologists, a CAP member and colleague and a representative from the CAP executive leadership team.

At the PLMI Peer2Peer Roundtable, PLMI Chair Kandice Kottke-Marchant, MD, PhD, Deborah Chute, MD, Carol Farver, MD, Walter Henricks, MD, Jonathan Myles, MD, Brian Rubin, MD, PhD, Paul Stagno, MD, PhD, and Lisa Yerian, MD, had the opportunity to meet with William F. Hickey, MD, CAP governor, and Mary Kennedy, CT (ASCP), MPH, manager, CAP Health IT Strategies.

CAP will use the information collected at the roundtables to formulate national strategic initiatives for pathology and laboratory medicine.

About the Authors

Pathology Innovations Magazine offers information from the medical staff in the Cleveland Clinic Pathology & Laboratory Medicine Institute about its research, services and laboratory technology.

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Dr. Yang earned his medical degree from Zhengzhou University School of Medicine in China and a PhD in Molecular Biology at Case Western Reserve University, Cleveland. He served a residency in pathology at Cleveland Clinic followed by a fellowship in surgical pathology at Washington University, St. Louis, Mo., and a fellowship in cytopathology and molecular pathology at Johns Hopkins, Baltimore, Md.

Dr. Yang is a two-time winner of the Society of Cytopathology Geno Saccomanno New Frontiers of Cytology Award for his research on DNA methylation profiling as biomarkers in cervical cancer. Molecular diagnosis and profiling of gynecologic cancers is his primary research interest.

He is a past president of the Chinese American Pathologist Association. He currently is the consultant pathologist for Preventive Oncology International and a consultant for the Sino-United States Cervical Cancer Programs for underserved areas in China. Dr. Yang is a frequent invited speaker at national and international conferences.

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Raymond Tubbs, DO, is Section Head for Molecular Oncologic Pathology and Professor of Pathology at the Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, serving on the medical school's Committee on Appointments and Promotions.

Dr. Tubbs is a molecular pathologist with a special interest in molecular oncology and hematopathology. He is board-certified in anatomic and clinical pathology, molecular genetic pathology and hematology.

A graduate of Kirksville College of Osteopathic Medicine, he served his residency in pathology at Cleveland Clinic and was named to the Cleveland Clinic professional staff in 1979. His former positions at Cleveland Clinic include Vice Chair of the Division of Pathology and Laboratory Medicine, Chair of the Department of Clinical Pathology and Section Head for Molecular Genetic Pathology, Department of Clinical Pathology.

Dr. Tubbs has published more than 375 research papers in peer-reviewed journals. His professional memberships include the College of American Pathologists, United States and Canadian Academy of Pathology, Association for Molecular Pathology. International Society for Analytical and Molecular Morphology and Society for Hematopathology.

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