Lung cancer remains the most common cancer in the world today, accounting for almost 13% of new cancer diagnoses and over 17% of cancer deaths every year. Historically the classification of lung carcinomas has been based on histologic type, with the two major categories being non-small cell lung carcinomas (NSCLC) and small cell carcinoma. NSCLC is further subclassified into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. These histologic categories have been used to make major therapeutic decisions regarding chemotherapeutic regimens for patients with metastatic disease. For example, squamous cell histology is considered a contraindication to the use of the anti-vascular endothelial growth factor bevacizumab because of an association with life-threatening hemorrhage [1].

However, there is now increasing data that NSCLC can also be classified according to specific genetic mutations. In fact, over half of all lung adenocarcinomas can be classified based on the presence of a known genetic abnormality. In some cases, these genetic mutations have important therapeutic implications, with specific targeted therapeutic drugs that can be more effective and less toxic than traditional chemotherapies. As more mutations with therapeutic relevance are discovered, molecular diagnostic tests are becoming vital to the classification and treatment of NSCLC. Currently, the most important mutations in NSCLC are epidermal growth factor receptor (EGFR) mutations and fusions involving the anaplastic lymphoma kinase (ALK) gene. KRAS gene mutations are also being actively evaluated in many trials. Diagnostic testing for these genetic abnormalities in NSCLC is becoming standard of care in patients suffering from these malignancies because they offer opportunity for personalized medicine regimens in which these patients can receive therapy tailored to their tumor.

**EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MUTATIONS**

EGFR mutations remain the most important genetic mutation to accurately identify in NSCLC patients, representing up to 40% of adenocarcinomas in some populations. EGFR mutations are most common in female patients who are never smokers or are light smokers (less than 10 pack-years). The mutation is also more common in Asian patients. Although EGFR mutations can be seen in all histologic subtypes of ade-
nocarcinoma, some studies have shown an increase in \textit{EGFR} mutations in non-mucinous bronchioloalveolar carcinoma (BAC) or invasive adenocarcinomas with extensive bronchioloalveolar features as well as papillary and micropapillary carcinomas [2].

Mutations in the \textit{EGFR} gene in NSCLC mainly affect the ATP binding pocket in the tyrosine kinase domain. The most common mutations are in-frame deletions in exon 19 and the L858R substitution mutation in exon 21 [3]. Tumors harboring either of these mutation types are more likely to respond to \textit{EGFR} tyrosine kinase inhibitors (\textit{EGFR}-TKI), such as gefitinib and erlotinib, both of which have become standard chemotherapeutic regimens for the treatment of advanced \textit{EGFR}-mutant NSCLC. In large clinical trials, patients with a new diagnosis of \textit{EGFR}-mutant metastatic lung adenocarcinoma treated with gefitinib had higher response rates, prolonged progression-free survival, and better quality of life than those treated with standard chemotherapy [4]. In addition, patients without an \textit{EGFR} mutation did worse when treated with this drug. Thus knowing a tumor’s \textit{EGFR} status has become essential in the effective treatment of lung adenocarcinoma.

There are several methods available for detecting \textit{EGFR} mutations. The MLabs \textit{EGFR} Mutation Analysis test utilizes a novel multiplex polymerase chain reaction (PCR) assay to detect exon19 deletion mutations and the L858R substitution mutation (see Figure 1), which together account for almost 90% of \textit{EGFR} mutations. MLabs developed this novel assay to offer a superior combination of test sensitivity, specificity, and time to result. This test can be performed on fresh, frozen, or formalin-fixed paraffin-embedded tissue. Fresh tissue should be sent on saline-soaked gauze or in RPMI within 24 hours of collection; frozen tissue should be stored at -70 degrees C and should not thaw at any time. Paraffin-embedded tissue blocks or 5 slides (unstained and unbaked) can be sent at room temperature. This test can also be performed on cytologic preparations, including cell blocks containing at least 5% tumor nuclei and non-coverslipped Diff-Quik stained direct smears.

\textbf{KRAS MUTATIONS}

\textit{KRAS} gene mutations are present in between 10 and 30% of resected lung adenocarcinomas. \textit{KRAS} mutant tumors tend to be seen in male smokers in an older age group, and typically present with high stage disease. Although \textit{KRAS} mutations can be seen in all histologic subtypes of lung adenocarcinoma,
similar to \textit{EGFR} mutant tumors, some studies have shown them to be associated with poorly differentiated adenocarcinomas with solid growth and tumors with mucinous differentiation, including mucinous BAC [5]. \textit{KRAS} mutations tend to be associated with a poor prognosis.

The \textit{KRAS} gene encodes a protein which is a GTPase and plays a role in many signal transduction pathways, functioning as a molecular “on/off” switch. \textit{KRAS} mutations in lung carcinomas lead to constitutive activation of the \textit{KRAS} protein. These mutations most commonly occur as single nucleotide substitutions at codon 12, with occasional mutations in codon 13 and 61 [6]. \textit{KRAS} and \textit{EGFR} mutations in lung adenocarcinomas are strictly mutually exclusive. Whereas patients with \textit{EGFR}-mutant tumors have improved response to \textit{EGFR}-TKIs, lung cancers with \textit{KRAS} mutations may be resistant to these same drugs [6, 7]. Thus, \textit{KRAS} mutation status in lung carcinomas may have both prognostic and therapeutic implications.

MLabs currently offers \textit{KRAS} mutation testing by PCR followed by sequence analysis to detect all mutations within codons 12, 13, and 61 (see Figure 2). This test can be performed on fresh, frozen, or formalin-fixed paraffin-embedded tissue. Fresh tissue should be sent on saline-soaked gauze or in RPMI within 24 hours of collection; frozen tissue should be stored at -70 degrees C and should not thaw at any time. Paraffin-embedded tissue blocks or 5 slides (unstained and unbaked) can be sent at room temperature.

**ANAPLASTIC LYMPHOMA KINASE (ALK) TRANSLOCATIONS**

One of the most recently described genetic alterations with therapeutic implications in the management of NSCLC is the \textit{ALK} family of translocations. \textit{ALK} is a receptor tyrosine kinase which is normally expressed in the developing brain. In NSCLC, most \textit{ALK} rearrangements result from an inversion within chromosome 2p, leading to the fusion of exons 20 through 29 of \textit{ALK} with a portion of the echinoderm microtubule-associated protein-like 4 (\textit{EMLA}) creating the \textit{EML4-ALK} gene fusion [8]. \textit{ALK} fusions involving non-\textit{EMLA} partners including \textit{TFG} and \textit{KIF5B} have also been described. Collectively, 3-4% of NSCLCs harbor \textit{ALK} rearrangements, although this increases to approximately 15% in patients who are light or never smokers with adenocarcinoma. Patients with \textit{ALK} rearrangements also tend to be about 10-15 years younger than those without \textit{ALK}.

Spotlight on
Lindsay Schmidt, M.D.

Dr. Lindsay Schmidt completed her undergraduate degree at Marquette University and her M.D. at the Medical College of Wisconsin, both in Milwaukee, before matriculating in the anatomic and clinical pathology residency program at The University of Michigan in 2005. Following her residency Dr. Schmidt did an additional year of training as a fellow in pulmonary pathology. She joined our faculty as Assistant Professor of Pathology in July 2010 with clinical responsibilities in surgical and pulmonary pathology. In just the last three years she has authored or co-authored nine papers in the peer-reviewed literature and spoken at regional, national and international meetings.

You may have met Lindsay at our 28th Annual MLabs Symposium hosted in Ann Arbor, Michigan in March 2011 when she focused on \textit{New Advances in the Diagnosis and Treatment of Lung Carcinoma}. Here at MLabs we are very excited about the work that Dr. Schmidt is doing in collaboration with Drs. Bryan Betz (Technical Director) and Kojo Elenitoba-Johnson (Director) in the Molecular Diagnostics Laboratory as well as Departmental and MLabs leadership to ensure our place at the cutting edge of molecular testing in lung cancer.
rearrangements [9]. *EML4-ALK* positive NSCLC also tends to be associated with adenocarcinomas, especially those with signet ring histology. The translocation site within the *ALK* gene is at exon 20, just prior to the kinase domain; there are numerous truncation sites in the *EML4* portion of the translocation, all of which lead to a biologic gain of function.

Although *ALK* gene rearrangements are not associated with prognosis, there is significant therapeutic implication. In a recent early-phase clinical trial of the small molecule crizotinib, which is an inhibitor of *ALK* that competes for an ATP binding site, the overall response rate was 57%, including one complete response [10]; 33% of patients had stable disease without progression after 6 months.

Based on these results, clinical testing for the *EML4-ALK* translocation has rapidly become in high demand. Because there are numerous translocations sites within *EML4*, and also alternate *ALK* fusion partners, PCR-based tests are limited in detecting the wide variety of *ALK* rearrangements. Fluorescence in situ hybridization (FISH) testing with an *ALK* dual-color breakapart probe has become a preferred method and is currently required for entry into clinical trials. FISH has the ability to detect the variety of *ALK* translocations and can be performed on relatively small formalin-fixed paraffin embedded tissues. MLabs will begin offering FISH testing for the *ALK* rearrangements in fall 2011 (see Figure 3). This test will be available for formalin-fixed paraffin-embedded tissues.

NSCLC remains a major public health concern, accounting for a large portion of cancer diagnoses and deaths in the United States. Mortality from this disease remains high despite current advances in therapeutics. However, the advent of targeted therapies and testing for the corresponding genetic alteration now provides opportunity for improved outcomes. Testing for *ALK* gene rearrangements and mutations in *EGFR* and *KRAS* enables a more personalized and rational approach to therapy selection. While genetic alteration of these three genes represents approximately a third of NSCLCs, mutations in a number of additional genes have already been discovered. These too are being explored for their therapeutic relevance with the hope of further improvement in therapy optimization.

**TO SEND A SPECIMEN**

EGFR mutation and *KRAS* mutation testing can be performed on fresh, frozen, or formalin-fixed paraffin-embedded tissues. Fresh tissue should be sent on saline-soaked gauze or in RPMI within 24 hours of collection; frozen tissue should be stored at -70 degrees C and should not thaw at any time. Paraffin-embedded tissue blocks or 5 slides ( unstained and unbaked) can be sent at room temperature.

For assistance 24 hours per day, 7 days per week, call MLabs at 800-862-7284 or visit our website at www.mlabs.umich.edu.

**REFERENCES**


Test Updates

New Tests

CMA ABERRATION CONFIRMATION BY PCR

Effective June 15, 2011, the MLabs Molecular Genetics Laboratory began offering a relative-quantitative PCR (rqPCR) assay for the confirmation of an aberrant Chromosomal Microarray (CMA) result (that is too small or that cannot be confirmed by FISH) and to determine the origins of inheritance of an aberration by testing parental samples. In addition, this rqPCR assay can also be used for a symptomatic sibling(s) to screen for a known familial copy number aberration previously detected by CMA testing at the Molecular Genetics Laboratory. There is a separate charge for parental and sibling rqPCR testing.

COAGULATION ANTI-IIA ASSAY, DABIgATrAN

The MLabs Coagulation Laboratory began offering a new test for the determination of Dabigatran in plasma effective May 31, 2011. The methodology is an anti-IIa chromogenic assay reported in µg/mL. Dabigatran is an oral direct thrombin inhibitor that is used for the prevention of venous thromboembolism. In certain clinical situations such as serious bleeding into critical organs (e.g. intracerebral bleeding), potential overdose, and emergency surgery, clinicians will need to make an assessment of the anticoagulant status of a patient receiving dabigatran before deciding on future management strategies.

Normal individuals have no dabigatran activity. A correlation of anti-IIa activity and therapeutic benefit has not been demonstrated. If this test result is greater than 0.04, then the patient has some anticoagulant effect from dabigatran.

METHADOnE METABOLITE (EDDP), urInE

In response to the need for better compliance drug testing, the MLabs Chemical Pathology Laboratory began offering urine methadone metabolite testing by Enzymatic Immunoassay effective July 6, 2011. This test is for the qualitative detection of methadone metabolite (2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine or EDDP) in urine at a cutoff concentration of 100 ng/mL. Methadone is a synthetic opiate commonly used to detoxify and maintain heroin addicts. Methadone...
treatment compliance can be effectively monitored by urine screening of methadone and its metabolite. Methadone is quickly metabolized by the liver into normethadone which is hard to detect due to its rapid dehydration to EDDP (primary metabolite). If methadone testing only is performed, only the parent drug is being measured leading to possible false positive (urine adulteration by the addict) or negative (only metabolite present in high enough quantity to measure) results.

Test Methodology, Reference Range, and Specimen Handling Changes

**BRAF MUTATIONS**

Effective July 25, 2011, the MLabs Molecular Diagnostics Laboratory replaced the BRAF V600E Mutation test with the BRAF V600E/V600K Mutation test. This new test adds the ability to detect and distinguish the V600K mutation which accounts for approximately 15% of BRAF mutations in melanoma.

BRAF gene mutations occur in a variety of human malignancies including approximately 10% of colorectal cancers (CRC), 45% of papillary thyroid carcinomas (PTC), 50% of melanomas, and virtually all hairy-cell leukemias (HCL). The most common BRAF mutation is the c.1799T>A (V600E) substitution. In CRC, this mutation has been associated with a limited clinical response to epidermal growth factor receptor (EGFR) targeted therapies (cetuximab or panitumumab). As a complement to KRAS mutation analysis, BRAF V600E mutation detection may predict response to these therapies. In addition, BRAF V600E mutations are found in sporadic microsatellite instability high (MSI-H) CRC cases, but not in hereditary non-polyposis colorectal cancers (HNPCC). Therefore, determination of BRAF mutation status may help to differentiate sporadic vs. germline MSI-H colorectal cancers. BRAF V600E mutation testing may also aid in the diagnosis of papillary thyroid carcinoma, since benign thyroid neoplasms are not associated with BRAF mutation. In melanoma, the V600E and V600K mutations may predict response to BRAF targeted therapies. This test qualitatively detects the BRAF c.1799T>A (V600E) and BRAF c.1798_1799GT>AA (V600K) mutations in formalin fixed paraffin-embedded tissues, fresh/frozen tissue, peripheral blood, and bone marrow. BRAF mutations other than V600E and V600K will not be detected. Analytic sensitivity is 5% mutation.

Collection Instructions: Collect blood or bone marrow in a lavender top tube. Refrigerate and send intact blood or bone marrow specimen within 16 hours of collection. Alternatively, send fresh, frozen, or formalin-fixed paraffin-embedded tissue containing greater than 5% tumor. Fresh tissue should be sent on a piece of gauze in saline, or in RPMI, within 16 hours of collection; refrigerate. Frozen tissue should be stored at -80 degrees C; do not allow to thaw at any time. Paraffin-embedded tissue should be stored at room temperature. Please provide an estimate of tumor percentage with paraffin-embedded tissue specimens.

**FETAL LUNG MATURITY, AMNIOTIC FLUID**

The MLabs Special Chemistry Laboratory has discontinued Fetal Lung Maturity testing effective July 1, 2011, due to discontinuation of reagent from the manufacturer (Abbott) and low request volume. Requests for this test will be sent to Warde Medical Laboratory.

Collection Instructions: Collect 2 mL of amniotic fluid and refrigerate. Avoid contamination of the amniotic fluid with blood, meconium, or maternal urine. If possible, send specimen immediately. Store refrigerated up to 72 hours or freeze for longer storage. Do not centrifuge sample. Note that an additional 10 mL of amniotic fluid are needed if the FLM results indicate that the lung is immature and the clinician wishes to add the L/S Ratio.

**GROWTH HORMONE**

Please note that reference range for the Growth Hormone assay has changed effective July 19, 2011:

Reference Range: MALES: 0 - 3 ng/mL; FEMALES: 0 - 5 ng/mL; POST-STIMULATION: Pediatric (<18 yrs): >7 ng/mL; Adult (>18 yrs): >5 ng/mL.

**INTERLEUKIN 6**

Effective June 13, 2011, requests for Interleukin 6 are sent to IBT Laboratories.

Collection Instructions: Collect specimen in an SST or red top tube. Centrifuge, aliquot 1 mL of serum into a plastic vial within 30 minutes of collection, and freeze.
MYCOPLASMA PNEUMONIAE ANTIBODY

The MLabs Microbiology Laboratory has discontinued Mycoplasma pneumoniae Antibody testing effective May 9, 2011. Requests for this test will be sent to Mayo Medical Laboratories for Mycoplasma pneumoniae IgG & IgM by EIA. If this test is positive for IgM Antibodies, IgM by IFA will be performed at an additional charge.

PROSTATE CANCER ANTIGEN 3 (PCA3)

Please note that specimen collection instructions for the PCA3 assay have been revised to indicate that refrigerated specimens must be received by MLabs within 5 days of collection. If the combined time from original specimen collection and receipt date at MLabs will be greater than 5 days, freeze the specimen and ship on dry ice.

REFERENCE LABORATORY CHANGE

Please note that effective July 1, 2011, the following tests will be sent to Warde Medical Laboratories:

Calcitonin, Serum

Collection Instructions: Collect specimen in an SST or red top tube. Centrifuge, aliquot 1 mL (minimum 0.5 mL) of serum into a plastic vial and freeze. Refrigerated samples are not acceptable.

Reference Range: Males: <18 pg/mL; Females: <12 pg/mL.

Folic Acid, Erythrocytes

Collection Instructions: Collect specimen in a lavender top tube. Send 3 mL (minimum 0.5 mL) of EDTA whole blood, refrigerated or frozen. Protect specimen from light (place in brown plastic bag or aliquot into brown tube). If specimen is submitted frozen, include a Hematocrit result from a specimen drawn within 24 hours of the RBC Folate specimen.

Reference Range: 280 – 791 ng/mL

Levetiracetam, Serum

Collection Instructions: Collect specimen in a red top tube; do not use SST tube. Centrifuge, aliquot 0.2 mL (minimum 0.1 mL) of serum into a plastic vial and refrigerate.

Reference Range: 3.0 – 60.0 mcg/mL

Topiramate, Serum

Collection Instructions: Collect specimen in a red top tube; do not use SST tube. Centrifuge, aliquot 0.4 mL (minimum 0.125 mL) of serum into a plastic vial and refrigerate.

Reference Range: 2 – 20 µg/mL

Zonisamide, Serum

Collection Instructions: Collect specimen in a red top tube; do not use SST tube. Centrifuge, aliquot 0.3 mL (minimum 0.125 mL) of serum into a plastic vial and refrigerate.

Reference Range: 10 – 40 µg/mL; Toxic: >100 µg/mL

SELENIUM, SERUM

Please note the following reference range change for Mayo Medical Laboratories Selenium, Serum assay, effective May 26, 2011:

Reference Range: Age 0-2 months: 45 - 90 ng/mL; age 3-6 months: 50 - 120 ng/mL; age 7-9 months: 60 - 120 ng/mL; age 10-12 months: 70 - 130 ng/mL; age >1 year: 70 - 150 ng/mL.

STREPTOCOCCUS GROUP B

Effective August 1, 2011, the MLabs Microbiology Laboratory has replaced PCR with culture for assessment of Group B Streptococcus, Vaginal/Rectal samples. Using newly developed chromogenic media for culture has demonstrated equivalent sensitivity and specificity compared to PCR testing. Culture results will be available in 24 - 48 hours.

Collection Instructions: Swab lower vagina and anorectum and place in aerobic transport tube. Refrigerate and send within 24 hours of collection. Indicate specimen source and collection date/time. If an unacceptable specimen is received, the client will be notified and another specimen will be requested before disposal of the specimen.

Reference Range: Negative

Discontinued Test

BETA GLUCURONIDASE, CSF

Due to minimal use and utility, Mayo Medical Laboratories Beta Glucuronidase, CSF assay became obsolete effective April 12, 2011. Note that an alternative test that is useful in identifying meningeal invasion by peripheral tumors is the Carcinoembryonic Antigen (CEA) assay (CSF specimen).
MLabs News

U-M DEPARTMENT OF PATHOLOGY NEWS

MLabs welcomes Michael Bachman M.D., Ph.D., who joined our faculty July 1, 2011. Dr. Bachman was previously a Microbiology Fellow at the University of Pennsylvania. His area of academic and clinical focus is molecular microbiology and bacterial pathogenesis.

Congratulations are to be extended to Kojo S.J. Elenitoba-Johnson, M.D., Professor and Director, Division of Translational Pathology, who has been inducted into the American Society for Clinical Investigation, one of the oldest and most recognized organizations of physician scientists.

Amer Heider, M.D. who is currently a fellow in pediatric pathology at the University of Pittsburgh Medical Center, will be joining the faculty in Anatomic Pathology effective September 1, 2011. Dr. Heider will have service responsibilities in pediatric pathology and cytopathology.

MLabs is pleased to announce that David Keren, M.D. will be joining the Department as Clinical Professor of Pathology and Associate Director of the Clinical Laboratories effective January 1, 2012. Dr. Keren, who was on the faculty in the Department from 1978 to 1989, is currently Director of Warde Medical Laboratories in Ann Arbor. Dr. Keren has held a number of leadership positions in regional and national pathology organizations including serving as President of the Michigan Society of Pathologists, the Gastrointestinal Pathology Society and the American Society for Clinical Pathology. He currently serves as President of the American Board of Pathology. Dr. Keren has particular expertise in clinical immunopathology, chemistry and laboratory outreach and management and has served on numerous editorial boards. He has written a number of books and book chapters and has over 200 clinical and scientific publications.

Congratulations to Megan S. Lim, M.D., Ph.D., Associate Professor and Director, Hematopathology, who has been named Chair-Elect of the Hematopathology Subdivision of the Association for Molecular Pathology for 2011-2012.

NEW FRONTIERS IN PATHOLOGY

The New Frontiers in Pathology symposium will take place October 13 - 15, 2011, in Ann Arbor. This symposium is intended to address the educational requirements for practicing pathologists, residents and fellows.

Diagnostic problems in Anatomic Pathology will be identified and evaluated using current tools. At the end of this course, participants will have a better understanding of new methods of diagnosis using current tools and information technology; and will be presented with ideas for implementation of new methods in their current practice.

The impact of discoveries in molecular medicine and bioinformatics and the application of information technology to improve pathology practice are highlighted in plenary lectures and breakout sessions. Case-based presentations using virtual microscopy will address complex diagnostic challenges.

Visit the website at http://www.pathology.med.umich.edu/NewFrontiers/2011.php for more information or to register.