PhenoPath Laboratories is pleased to announce that we are now offering the FDA-approved Abbott Vysis LSI ALK dual-color breakapart probe for the detection of rearrangements involving the ALK (Anaplastic Lymphoma Kinase) in non-small cell lung carcinoma (NSCLC). This IVD ALK probe from Vysis will be used on all cases of NSCLC that we receive for ALK testing. Patients testing positive for an ALK rearrangement using this FDA-approved IVD reagent are eligible for treatment with the ALK inhibitor drug Xalkori.

In August, 2011, the U.S. Food and Drug Administration approved Xalkori (crizotinib) to treat certain patients with late-stage (locally advanced or metastatic) NSCLC whose tumors contain an abnormal anaplastic lymphoma kinase (ALK) gene that is translocated to the EML4 gene resulting in an EML4-ALK translocation gene. Xalkori has been approved with a companion diagnostic test that will help determine if a patient has the abnormal ALK gene, the Vysis ALK Break Apart FISH Probe Kit.

New PhenoPath HER2 testing protocol may identify additional patients who will benefit from HER2-targeted therapy

A new fluorescence in situ hybridization (FISH) testing protocol developed at PhenoPath Laboratories may more accurately identify an important group of breast cancer patients who are likely to benefit from targeted therapies such as trastuzumab, which have been shown to dramatically improve breast cancer survival.

Standard HER2 FISH studies employ a set of probes to both HER2 as well as a ‘reference gene’ on chromosome 17, CEP17. According to the ASCO-CAP guidelines, when the HER2/CEP17 ratio exceeds 2.2, the tumor is classified as amplified. However, when both HER2 and CEP17 signals are increased in tandem, it has been assumed that this represents polyplody of chromosome 17, and if the ratio is below 1.8, such patients are not considered candidates for HER2-targeted therapy. As recent studies have raised questions as to the true incidence of polyplody involving chromosome 17, PhenoPath pathologists-scientists hypothesized that in many cases the amplicon (region of chromosome involved in amplification) included both HER2 as well as CEP17 (explaining the non-increased ratio of HER2 to CEP17).

In a paper published in the Journal of Clinical Oncology, specimens from 171 patients, whose breast cancers were shown by FISH testing to have elevated copy numbers of both HER2 and CEP17, had FISH studies examining the status of three other genes on chromosome 17: SMS, RARA, and p53. Out of 132 cases classified as negative (non-amplified) based on the HER2 to CEP17 ratios using current testing protocols, PhenoPath’s protocol reclassified 58 (43.9%) of these negative cases as positive, or amplified, and also reclassified 13 (92.9%) of 14 “equivocal” cases as positive, or amplified.

PhenoPath’s demonstration of what appears to be true amplification of the HER2 gene in a subset of patients previously thought to be negative might alter treatment for many breast cancer patients in the United States. Many patients whose breast cancers are misinterpreted as negative for amplification under current testing protocols would no longer be excluded from receiving trastuzumab and other HER2-targeted drugs. Although the ultimate response rate of these patients to therapy cannot yet be known, access to targeted therapy could have a profound impact upon their survival.

To learn more, refer to the JCO article:
New Diagnostic Hematopathology Markers

**B Cell Lymphoma:**

PhenoPath has recently validated two antibodies to help distinguish chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) from mantle cell lymphoma (MCL) using flow cytometry. The ability to distinguish these two CD5-positive B cell neoplasms is important clinically, as CLL/SLL typically has a relatively indolent course and is usually treated relatively conservatively, while MCL usually behaves in a more aggressive manner and is therefore usually treated more intensively.

The first antibody we have validated detects CD200, or OX-2 membrane glycoprotein, a cell surface antigen with an immunomodulatory function that is expressed on subsets of both B and T cells. It is believed that CD200 expression on tumor cells helps diminish the immune response to those cells. Several studies (see references 1 and 2, among others) have shown that CD200 is expressed in the large majority of CLL/SLL cases, but is only expressed in a small minority of MCL cases and, when expressed, is typically seen at low levels on a small subset of cells.

The second antibody detects CD79b, or immunoglobulin-associated beta, a cell surface antigen that complexes with CD79a and immunoglobulin to enable B cell signaling in response to antigen. A number of studies (see references 3 and 4, among others) have shown that CD79b is expressed at very low to negative levels on CLL/SLL cells, and is expressed at significantly higher levels in most non-CLL/SLL mature B cell neoplasms, including MCL.

The recent addition of CD200 and CD79b antibodies to our routine flow cytometric evaluation of CD5-positive B cell neoplasms is particularly useful in those occasional CLL/SLL cases that, based on levels of expression of CD20, CD23, FMC7, and surface light chains, have an MCL-like immunophenotype (the latter cases are described in reference 5). In these cases, the CLL/SLL cells show the expected patterns of CD200 and CD79b, in addition to absence of the t(11;14) and/or cyclin D1 protein expression, despite other immunophenotypic features of MCL.

A third antibody recently validated at PhenoPath is a rabbit monoclonal against the B cell-associated transcription factor BOB-1. This antibody offers performance superior to our previous polyclonal antibody against BOB-1, and replaces the polyclonal antibody. As with the previous antibody, the new antibody shows mainly nuclear reactivity, with a small amount of cytoplasmic activity in a subset of the positive cells. The new BOB-1 antibody will be used in the same two ways as previously: 1) to look for low to absent nuclear expression in classical Hodgkin lymphoma (CHL), to help distinguish CHL from both nodular lymphocyte predominant Hodgkin lymphoma and large B cell non-Hodgkin lymphoma, as the latter two entities typically express BOB-1 in a uniformly strong manner; and 2) as a marker of B-lineage in unusual B cell neoplasms, such as plasmablastic lymphoma or primary effusion lymphoma, in which more common B-lineage-associated antigens are frequently not expressed.

**T Cell Lymphoma:**

PhenoPath has recently validated three immunohistochemical assays to aid in the diagnosis of three different mature T cell neoplasms.

The TCL-1 (T cell lymphoma-1) gene is fused to the T cell antigen receptor gene by the inv(14)(q11;q32) or t(14;14)(q11;q32) in the large majority of T cell prolymphocytic leukemia (T-PLL) cases. It encodes a cytoplasmic and nuclear antigen that promotes cell survival by enhancing signaling through the AKT protein serine/threonine kinase (see reference 6). In addition to its causal role in T-PLL, TCL-1 expression is a key feature that can help distinguish blastic plasmacytoid dendritic cell neoplasms (TCL-1-positive) from otherwise similar monocytic leukemias (TCL-1-negative, reference 7). TCL-1 is also expressed in a variety of B cell neoplasms, and its expression confers an adverse prognosis in CLL/SLL.

PD-1 (programmed death-1, or CD279), is a transmembrane protein thought to help negatively regulate T cell signaling. Among other cells, PD-1 is expressed by follicular helper T cells, a subset of mature CD4+ T cells populating germinal centers and assisting in the germinal center response to antigen. Because angioimmunoblastic T cell lymphoma (AITL) is a tumor of follicular helper T cells, in the appropriate clinical and histologic setting, documentation of PD-1 coexpression on an atypical CD4+ T cell population can provide strong support for the diagnosis of AITL (reference 8). PD-1 expression has also been described in CLL/SLL (reference 9).

Clusterin is a heterodimeric cytoplasmic glycoprotein implicated in intercellular and cell matrix interactions, regulation of the complement system, lipid transport, stress responses, and apoptosis. It is expressed in a Golgi pattern in the large majority of anaplastic large T cell lymphomas (ALCLs) of systemic type, and...
in many primary cutaneous ALCLs (references 10 and 11). Importantly, clusterin identification in CD30-positive large T cell lymphoma provides strong evidence against the prognostically inferior entity peripheral T cell lymphoma, not otherwise specified (PTCL, NOS).

**Langerhans Cell Histiocytosis:**

PhenoPath has also recently validated an immunohistochemical assay for Langerin. Langerin (CD207) is a type II transmembrane C-type lectin associated with the formation of Birbeck granules in Langerhans cells, and is therefore a very specific marker for mature Langerhans cells. Langerin is most useful in the differentiation of Langerhans cell histiocytosis (LCH) from non-LCH histiocytic/dendritic cell tumors, especially from indeterminate dendritic cell tumors, which are also S100 and CD1a positive.

FEATURED
At Our Winter Conference

Matt van de Rijn, MD, PhD of Stanford University Medical Center will present “Gene Expression Profiling Studies Leading to Novel Diagnostic Markers in Sarcoma” at the PhenoPath Winter Conference at 7:30 PM on Thursday, February 16, 2012. Dr. van de Rijn will also be giving a daytime lecture at 1PM the same day entitled, “Gene Expression Profiling Studies Leading to Novel Therapeutic Targets in Leiomyosarcoma.”

Dr. van de Rijn completed his MD and PhD at the University of Amsterdam, and completed his residency and fellowship training and rose to the rank of full Professor of the Department of Pathology at Stanford University Medical Center. He is a member of the editorial boards of several major pathology journals, publishes extensively, is a sought-after speaker both nationally and internationally, and is a renowned expert in the fields of gene expression profiling and sarcoma studies.

We look forward to welcoming Dr. van de Rijn to PhenoPath.