PhenoPath is proud to announce the one-year anniversary of our Cytogenetics Laboratory, providing reliable and reproducible karyotyping for our pathology and oncology clients nationwide.

PhenoPath offers comprehensive chromosomal studies for all of the hematological malignancies, including acute leukemia, myeloproliferative neoplasms (including chronic myeloid leukemia, CML), myelodysplastic syndromes (MDS), multiple myeloma, and lymphoma / chronic lymphoproliferative disorders (including chronic lymphocytic leukemia/small lymphocytic lymphoma, CLL/SLL). PhenoPath also performs chromosomal studies on non-hematopoietic neoplasms. Testing can be performed on a variety of fresh specimens, listed below. To increase the sensitivity for detecting abnormalities, each specimen is uniquely cultured according to specimen type, clinical indication, and flow cytometric findings (if applicable).

The Cytogenetics Laboratory combines state-of-the-art cell preparation techniques with comprehensive interpretation of test results by experienced, board-certified cytogenetics experts. Karyotyping is aided by MetaSystems image analysis, which increases the ability to identify metaphases when the mitotic index is low. If indicated, FISH will be performed over standard Giemsa (G)-banded metaphase spreads to confirm karyotype results. Our laboratory, is accredited by the College of American Pathologists (CAP), has CLIA certification, and is licensed in the states of Washington, Florida, California, Maryland, and Rhode Island.

**Service Features**
- **Specimens:** heparin-anticoagulated peripheral blood or bone marrow aspirate; non-anticoagulated body fluid; bone marrow core biopsy in RPMI/fetal bovine serum; finely minced lymph node, spleen or other tissue in RPMI/fetal bovine serum
- **Turnaround time** for karyotyping to rule out hematopoietic neoplasms: 5 to 7 business days
- **Turnaround time** for karyotyping to rule out non-hematopoietic neoplasms: 10 to 14 business days (due to more fastidious culture requirements)
- **Culture success rates:** > 95%
- **Minimum number of cells analyzed:** 20 (if available)
- **Minimum target resolution:** 350-400 bands (Giemsa banding)
- **Automated metaphase identification with MetaSystems scanning platform**
- **Initial independent analysis by two cytogenetic technologists, plus an additional quality control step for each case**
- **Final analysis and report verification by a board-certified cytogeneticist, with references to the literature as appropriate**

**Our Cytogenetics Team**

**Director:** Shawna Pyott, PhD
Board certified in Cytogenetics by the American Board of Medical Genetics (ABMG), and a Fellow of the American College of Medical Genetics (FACMG)

**Cytogenetic technologists:** Certified by the American Society of Clinical Pathologists (ASCP) Board of Certification (BOC) in cytogenetics (CG) or medical technology (MT); over 40 years of cumulative experience

- Tricia Makin, CG (ASCP)
- Ashlie Cindric, MT (ASCP)
- Ray Clemes
- Karishma Hendrickson, CG (ASCP) - per diem
- Melissa Chiu, CG (ASCP)
- Itu Mohapatra, CG (ASCP) (*not pictured*) - per diem

PhenoPath’s highly trained and knowledgeable team is the key to our quality cytogenetics service. They continually evaluate PhenoPath’s assay performance and meet regularly with PhenoPath scientists and pathologists to ensure the highest quality service.

If you would like more information about PhenoPath’s Cytogenetics service, please contact your sales representative or any of our pathologists (888.927.4366).
New IHC Markers In Hematologic Diseases

HGAL
HGAL - also known as GCET2, GCAT2, and GCSAM – is a cytoplasmic protein expressed by benign and neoplastic follicle center (germal center)-derived B cells. As part of a panel of IHC antibodies, HGAL is useful to distinguish follicle center-derived B cell non-Hodgkin lymphoma (see Figure 1) from other B cell proliferations, particularly marginal zone lymphomas. Note that one study of germinal center B cell markers (reference 1) reported that, in a series of 29 nodal and extranodal follicular lymphomas, HGAL was a more sensitive immunohistochemical marker of follicle center origin than CD10, bcl-6, or LMO2.

MNDA
Although the expression of MNDA was initially thought to be restricted to the myelomonocytic lineage, several studies (reference 2) have shown expression of this protein in normal and neoplastic B lymphocytes of marginal zone derivation (see Figure 2, from normal spleen), but not in follicle center-derived B cells (see Figure 3, from normal tonsil). MNDA has been shown to be expressed in splenic marginal zone lymphomas, mantle cell lymphomas, nodal marginal zone lymphomas, and hairy cell leukemia. MNDA can be used as part of a panel of IHC antibodies to help distinguish marginal zone B cell lymphoma (MZL) from follicle center-derived B cell lymphoma, particularly CD10-negative FL.


New IHC Markers In Solid Tumors

PD-L1
PD-L1 (Programmed Death-Ligand 1), also known as CD274, is a 40 kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease, and cancer. In the non-neoplastic setting, PD-L1 is expressed on activated T cells, B cells, dendritic cells and macrophages, in addition to some immune-privileged non-hematopoietic tissues such as retina and placenta. Binding of PD-L1 to its ligand PD-1, which is expressed by various immune cell types including T cells, transmits an inhibitory signal that attenuates T cell function, expansion, and survival.

More recently, it has been discovered that the neoplastic cells of many human tumors can express PD-L1, including breast, ovarian, gastric, pancreatic, lung and renal cell carcinomas, and classical Hodgkin lymphoma (CHL). PD-L1 expression by tumor cells is thought to inhibit the local immune response to the tumor, at least in part by binding to T cell PD-L1 and protecting the tumor from T-cell-mediated immunity.

Blockade of the PD-1/PD-L1 axis by humanized monoclonal antibodies against PD-1 and PD-L1 has emerged as a promising new cancer therapy. In recent clinical trials, anti-PD-1 therapy has been associated with significant clinical responses in patients with refractory non-hematopoietic tumors including melanoma, renal cell carcinoma, and non-small cell lung carcinoma (NSCLC), as well as CHL and diffuse large B cell lymphoma. As a result of these trials, the anti-PD-1 antibody nivolumab and pembrolizumab have received FDA approval for treating metastatic squamous NSCLC and metastatic melanoma, respectively.

PhenoPath scientists and pathologists are at the forefront in working with pharmaceutical companies looking for the features of the tumor which might predict response to these anti-PD-L1 agents, particularly the expression of PD-L1 on the tumor cell population, as determined by immunohistochemistry. From these studies, we have optimized PD-1 and PD-L1 protocols, which have been analytically validated in our laboratory. While at the current time, no universally agreed-upon scoring system for PD-L1 exists, we are employing the same scoring system used in several of the clinical trials presented at the 2015 ASCO meeting, in which the percent of tumor cell positivity and the intensity are recorded.


STAT6
In the past, markers such as CD34 have been employed in the immunohistochemical confirmation of the diagnosis of solitary fibrous tumor (SFT) and the tumors formerly known as hemangiopericytoma (now known to be one and the same). However, CD34 is by no means a specific marker of SFT, and there do not appear to be any lineage-specific markers that define this tumor. The molecular alteration that characterizes solitary fibrous tumors is a fusion between NAB2 and STAT6, adjacent genes on chromosome 12q13. While there are several fusion variants, all result in formation of a NAB2-STAT6 chimeric protein that relocates to the nucleus. Nuclear STAT6 immunoreactivity has been reported as an excellent surrogate marker for the NAB2-STAT6 gene fusion, and STAT6 is a highly sensitive and specific immunohistochemical marker for SFT.

New Markers of Basal-Like Breast Cancer, Nestin and INPP4B

Basal-like breast cancers constitute 10-15% of all breast cancers. These were first defined by the gene expression studies in the early 2000s, with outcome data showing these tumors to have the poorest prognosis amongst the various molecular subtypes. While the ‘triple-negative’ immunophenotype (ER, PR and HER2 negative) is known to overlap strongly with this group of basal-like breast cancers, there have been several efforts to look for other positive or negative immunohistochemical markers which might better define the group. In a collaborative study published in 2004 (in which PhenoPath participated), a panel of four antibodies (ER, EGFR, HER2, and keratin 5) was found to be the optimal panel. But in a study from 2013, some of the same authors updated this analysis, employing a wider panel of potential markers. The two markers which, in conjunction, displayed the highest specificity (96%) and sensitivity (83%) were nestin and INPP4B, with the latter a negative marker of basal-like tumors. Nestin is a major intermediate filament protein of embryonic central nervous system progenitor cells, and INPP4B encodes the inositol polyphosphate 4-phosphatase type II, one of the enzymes involved in phosphatidylinositol signaling pathways. PhenoPath has validated the two markers in the context of breast cancers and recommends their use instead of the older panels.


Cathepsin K

Cathepsin K is a lysosomal cysteine protease which is a member of the peptidase C1 protein family. In the non-neoplastic setting, it is expressed predominantly in osteoclasts.

In malignant tumors, cathepsin K expression is quite restricted. Cathepsin K is expressed in alveolar soft part sarcoma, and among carcinomas, cathepsin K expression is highly specific for translocation renal cell carcinomas (RCCs), which include those tumors harboring translocations involving genes coding for TFE3 (Xp11 translocation RCCs) and TFE2 [t(6;11) RCCs]. In mesenchymal neoplasms, cathepsin K expression is characteristic of granular cell tumors and melanoma.


New Publications By PhenoPath Pathologists

An international study to increase concordance in Ki67 scoring

This is the most recent installment of an international concordance study involving 16 laboratories in eight countries (in which PhenoPath is participating) to determine optimal scoring methodologies for evaluation of Ki67-defined cell proliferation indices in breast cancer. Although it is widely recognized as an important biomarker in breast cancer, to date Ki67 has not had a standardized scoring method, which has limited its clinical use. In the present study, which required calibration to a common method using a web-based tool, based on tissue microarray samples of 18 breast cancers, an extremely high intra-class correlation of 0.94 was found. It was concluded that laboratories can achieve very high inter-laboratory reproducibility in Ki67 scoring of breast cancers. The next phase of the study will extend this approach to biopsies and whole sections rather than tissue microarrays, to account for staining variability, and link to outcomes.

GATA-3 expression in trophoblastic tissues: an immunohistochemical study of 445 cases, including diagnostic utility

GATA-3 is known mostly as a marker of breast and bladder carcinomas, in which it shows an extremely high sensitivity of >95%, but it is also a potential marker of trophoblastic tissues and tumors. In this collaborative immunohistochemical study of 445 cases, pathologists from Johns Hopkins Medical Center and PhenoPath examined GATA-3 expression in placentas, hydatidiform moles, implantation sites, and choriocarcinomas. GATA-3 was found to be frequently expressed in normal and lesional trophoblastic tissues, and differentially expressed in intermediate trophoblast, cytotrophoblast, and syncytiotrophoblast, which varies according to time during pregnancy. Recognition of GATA-3 expression in trophoblastic tumors is important to avoid the diagnostic pitfalls of examination of tumors involving the GYN tract.
Brigitte M. Ronnett, MD presented “Ovarian mucinous tumors: evolution, revolution, and adventures in masquerading” at the PhenoPath Conference at 7:00 pm on Thursday, September 17, 2015. Dr. Ronnett also gave a daytime lecture, “Hydatidiform moles: ancillary techniques to refine diagnosis” at noon the same day.

Dr. Ronnett is Professor of Pathology and Gynecology & Obstetrics at The Johns Hopkins Hospital, Baltimore, MD. She received her medical degree from the University of Chicago Pritzker School of Medicine and completed residency training in anatomic and clinical pathology at The Johns Hopkins Hospital, Baltimore, MD. She also completed a 1-year surgical pathology fellowship at Memorial Sloan-Kettering Cancer Center and a 1-year surgical pathology fellowship/chief residency at The Johns Hopkins Hospital. Dr. Ronnett then completed a 2-year subspecialty fellowship in gynecologic pathology at The Johns Hopkins Hospital, Baltimore, MD. She joined the faculty of the Department of Pathology at The Johns Hopkins Hospital in 1995 and achieved the rank of full Professor in 2007.

Her clinical efforts are focused on a large gynecologic pathology consultation practice at The Johns Hopkins Hospital. Her research has focused on ovarian mucinous tumors (distinction of primary and metastatic mucinous tumors in the ovaries, and the origin of pseudomyxoma peritonei in women), uterine, cervical and endometrial pathology (HPV-related cervical lesions, ancillary techniques for distinction of endocervical and endometrial adenocarcinomas and subtyping of endometrial adenocarcinomas), and hydatidiform moles (ancillary techniques for refined diagnosis).