The Newsletter of PhenoPath

phenomena™

Laboratories ● 1-888-92-PHENO ● www.phenopath.com ● October 2008 ● Volume11 No.3

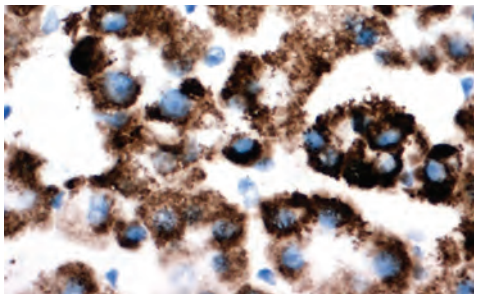
We are pleased to announce that Dr. Harry Hwang joined PhenoPath as a staff pathologist and Director of Molecular Biology on July 1, 2008.

Dr. Hwang is not new to PhenoPath, as he completed a one-year Immunohistochemistry (IHC) Fellowship at PhenoPath in 2002-3, and has served as an Adjunct Pathologist since that time. As Director of Molecular Biology, Dr. Hwang brings to PhenoPath the combined strengths of extensive diagnostic pathology experience, coupled with intensive knowledge of molecular biology. His responsibilities include directing the FISH service, participating in immunohistochemistry and fluorescence in situ hybridization studies of solid tumor and hematopathology cases, and helping develop new molecular assays of diagnostic and therapeutic importance. Dr. Hwang received his Bachelor of Science degree from Massachusetts Institute of Technology. Upon graduating from the University of Pennsylvania School of Medicine, he completed his AP/CP residency training at the University of Washington Medical Center, where he trained under Dr. Allen M. Gown, who was Professor of Pathology at that time. Following his IHC Fellowship, he was an Associate in Clinical Research in the Clurman Laboratory at FHCRC, where he studied cell cycle regulation in cancer. In particular, he worked on identifying novel oncogenes and micro RNAs that collaborate with loss of the cell cycle regulatory protein p27, which is frequently lost in human cancers.

Please join us in welcoming Dr. Hwang to the PhenoFamily of pathologists, all of whom are here to assist you with your challenging cases.

Carcinomas of Unknown Primary -

Nuclear Transcription Factors v. Cytoplasmic Differentiation Markers



HepPar1
Hepatocellular carcinoma identified by espression of the HepPar1 antigen, CPS1

This is the second installment in a series of articles concerning issues related to the use of immunohistochemistry to identify the primary site of carcinomas presenting as metastases. Determination of the primary site of metastatic carcinomas, as explained in the first piece in this series (Phenomena, Vol 11, No. 2), can prove challenging, but armed with an intelligently composed antibody armamentarium, in the vast majority of cases such determination can be made.

The original organ/tumor-specific markers, such as prostatic specific antigen (PSA) as a marker of prostatic adenocarcinoma, and thyroglobulin as a marker of thyroid carcinoma, represent a class of markers that can be described as "cytoplasmic differentiation markers," which generally represent markers of terminal differentiation of a given cell type. While such markers have proven useful over the past three decades, and form a core of the antibodies useful in identification of carcinoma primary site, these markers have a fundamental shortcoming: with few exceptions, they are least likely to be expressed in the clinicopathologic settings where they are most needed, i.e., in settings where the tumor is poorly differentiated. In the latter setting, these cytoplasmic markers are often expressed at low levels, both in terms of the intensity of positivity in individual tumor cells, as well as the fraction of tumor cell population that is positive. One advantage of these cytoplasmic markers, however, is their sheer diversity. Markers exist for a wide range of carcinomas, including those in the table to the right.

Examples of cytoplasmic markers of carcinomas

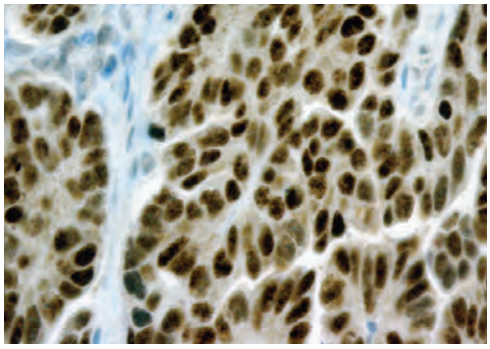
Surfactant ApoA1	*Lung*
Thyroglobulin	*Thyroid*
GCDFP-15 (Brst2)	*Breast*
Mammaglobin	*Breast*
Prostate specific antigen (PSA)	*Prostate*
Uroplakin	*Bladder*
HepPar1 Antigen (carbamoyl phosphate synthetase 1)	*Liver*
Villin	*Colon and GI Tract*
Cytokeratin 17	*Pancreas*
c-kit	*Thymus*
Inhibin-alpha	*Adrenal*

The sensitivity and specificity of these markers vary tremendously, ranging from those with very high specificity and low sensitivity (e.g., uroplakin) to those with very high specificity and high sensitivity (e.g., PSA). As with all markers, it is important to know the sensitivity and specificity of markers when interpreting results of such markers. For example, villin, a marker of GI adenocarcinomas, is expressed in up to 10% of lung adenocarcinomas, and surfactant ApoA can be expressed in 5-10% of thyroid carcinomas. The predictive value (PV) of a negative study can vary tremendously; in the case of PSA, with 95% sensitivity for prostatic adenocarcinoma, the "negative PV" in ruling out prostate as a primary source is high. In contrast, in the case of uroplakin, with a relatively low sensitivity of <50% in the metastatic setting, its "negative PV" is quite low. As positive and negative PVs of these markers are to some degree a moving target, as more data are published, it is critical to keep up with the published literature.

(Continued on page 2)

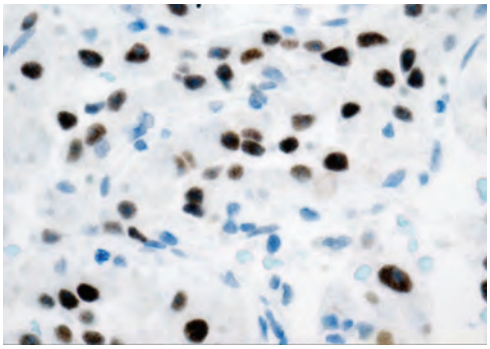
(Continued from page 1)

Nuclear transcription factors, in contrast, tend to be expressed in the entire tumor population, and often independent of the state of differentiation of the tumor. Indeed, remembering that nuclear transcription factors (e.g., TTF-1 in the lung) regulate the "downstream" expression of cytoplasmic markers (e.g., such as surfactant ApoA in the lung), helps explain at least some of the theoretical advantages of nuclear transcription factors. Nuclear transcription factors that can be exploited as markers of carcinomas of unknown primary exist for many organs, although as shown in the table below, the range is more limited than that of cytoplasmic markers. Many of these markers display what can best be described as "contextual" specificity. TTF-1 can point to the diagnosis of lung, in the setting of lung v. breast, but not assist in the distinction of thyroid v. lung. The significance of WT-1-positive cells in the pleural fluid can mean something very different in a male v. female patient. Nuclear transcription factors such as TTF-1 and CDX-2 display a curious loss of fidelity when they are expressed by high-grade neuroendocrine carcinomas; TTF-1, for example, while rarely expressed in non-neuroendocrine carcinomas primary to the bladder or cervix, can frequently be expressed in high-grade neuroendocrine carcinomas of these latter sites. Thus, the positive and negative PVs of nuclear transcription factors may be a function of the expression of neuroendocrine markers such as synaptophysin.



WT-1
The Wilms tumor gene product (WT-1) in the context of carcinomas of unknown primary is a highly sensitive and specific marker of serous carcinomas of ovarian surface epithelial origin

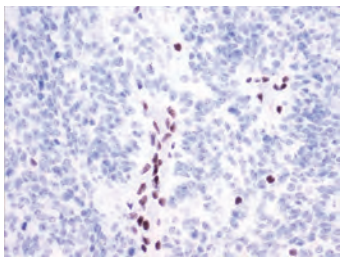
Examples of nuclear transcription factors as markers of carcinomas	
TTF-1	*Lung, thyroid*
CDX-2	*Colon, pancreatobiliary tract, stomach*
PAX-2	*Kidney, GYN tract*
ER	*Breast ovary, cervix, endometrium*
WT-1	*Mesothelioma, ovarian serous*



PAX-2
Expression of the nuclear transcription factor, PAX-2, in a case of metastatic renal cell carcinoma

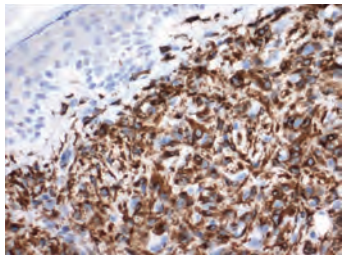
NEW ANTIBODIES RECENTLY VALIDATED AT PHENOPATH LABORATORIES

The **INI-1** gene (also known as SMARCB1) is a candidate tumor suppressor gene located on 22q11.2; in the majority of malignant rhabdoid tumors, there is homozygous inactivation of the gene through deletions and/or mutations, with the "common final pathway" of loss of expression of this protein. Loss of INI-1 gene expression has also been found in cases of the proximal variant of epithelioid sarcoma (which, curiously, also display some rhabdoid changes), and in atypical teratoid/rhabdoid tumors of the brain, typically found in the pediatric setting. Very recently, INI-1 loss has also been described in a variant of extraskeletal myxoid chondrosarcoma, as well as renal medullary carcinoma, a rare aggressive tumor that typically occurs in young patients with sickle cell trait or disease. The image on the right shows the loss of INI-1 observed in a recently studied case of atypical tetroid/rhabdoid tumor (AT/RT) of the cerebellum of a 12-year-old male. (References: *Hoot AC et al., Am J Surg Pathol 28:1485-91, 2004; Haberler C et al., Am J Surg Pathol 30:1462-8, 2006; Kohashi K et al., Am J Surg Pathol 32:1168-74, 2008; Cheng JX et al., Mod Pathol 21:647-52, 2008*)



INI-1
AT/RT showing loss of expression of INI-1 (note: endothelial cells serving as positive internal controls)

CD163, a hemoglobin scavenger receptor, is a cell surface protein expressed almost exclusively in monocytes and macrophages. As shown by Nguyen and colleagues (*Am J Surg Pathol 29:617-24, 2005*), CD163 is expressed in the cells of Rosai-Dorfman disease, histiocytic sarcoma, littoral cell angioma, and Langerhans cell histiocytosis. As a lineage-restricted marker, it has some advantages over CD68, a more commonly employed monocyte-macrophage marker, as the latter is a cytoplasmic marker that has a slightly wider range of specificity compared with CD163. (Reference: *Lau SK et al., Am J Clin Pathol 122:794-801, 2004*)



CD163
Dermal histiocytic process identified with anti-CD163 antibody

Antibody Validations at PhenoPath: Winners and Losers

Before an antibody is brought online at PhenoPath, extensive validation studies are performed, in which tissues, sometimes including microarrays of up to hundreds of different tumors and tissues, are critically evaluated to assess sensitivity and specificity, and to compare results with those of the published literature and/or the claims of the antibody vendor. In some cases, this validation fails, and despite the presence of published studies we choose not to offer the test. An example of this is the marker GLUT1 (Reference: *Kato Y et al., Mod Pathol 20:215-20, 2006*), a glucose transport protein that has been suggested to represent a marker that can distinguish mesothelioma (positive) from reactive mesothelium (negative). After immunostaining a large panel of mesotheliomas, as well as specimens containing reactive mesothelium, we concluded that this antibody, contrary to the published literature, could not reliably make this distinction, as a significant subset of the reactive mesothelial proliferations were positive as well. Thus, PhenoPath Laboratories will not be offering GLUT1 to distinguish mesothelioma from reactive mesothelium. There are existing, albeit imperfect, markers to assist in this differential, including p53 (overexpressed in a subset of mesotheliomas, but rarely in reactive mesothelium) and desmin (positive in the vast majority of reactive mesothelial cells, and in a small but significant percentage of mesotheliomas). However, the search for more definitive markers will continue.

VISIT US AT THE FOLLOWING MEETINGS:

For up-to-date information, visit our website: www.phenopath.com

23rd Annual Clinical Cytometry Society Meeting and Course:
10/10/08 - 10/14/08, Hilton Portland & Executive Tower, Portland, OR
Steven J. Kussick, MD, PhD was a featured speaker and presented *"Flow Cytometry in the Diagnosis of Myelodysplastic Syndromes & Myeloproliferative Disorders."*

The American Society of Dermatopathology:
ASDP 45th Annual Meeting
10/16/08 - 10/19/08, Hyatt Regency San Francisco
PhenoPath was represented at an exhibit booth.

USC Pathology & Laboratory Medicine Grand Rounds:
10/24/08, LAC + USC Medical Center & the Keck School of Medicine of Southern California, Los Angeles, CA
Allen M. Gown, MD was a featured speaker and presented *"Current Controversies in HER2 Testing."*

Washington State Medical Oncology Society:
Fall Membership Meeting
11/21/08, Grand Hyatt Seattle
Allen M. Gown, MD is a featured speaker and will present *"The Role of Tumor Gene & Protein Expression in Customizing Cancer Treatment."*

California Society of Pathologists:
61st Annual Convention, "Seminars in Pathology"
12/3/08 - 12/6/08, Hyatt Regency Century City, Los Angeles, CA
PhenoPath will be represented at an exhibit booth.

San Antonio Breast Cancer Symposium:
31st Annual San Antonio Breast Cancer Symposium
12/10/08 - 12/14/08, Henry B. Gonzalez Convention Center, San Antonio, TX
Christopher Tse, MBBS will present a poster entitled *"Amplification involving the CEP17 region may lead to false negative results of HER2 gene amplification by FISH"* at poster session #1079 on Thursday, December 11, 2008.

34th Annual Review & Recent Advances in Pathology:
1/26/09 - 1/30/09, The Alexander Hotel, Miami Beach, FL
Allen M. Gown, MD is a featured speaker and will present two courses entitled, *"Molecular Versus Immunohistochemical Classification of Breast Cancers"* at 9:00 AM and *"New Markers in Diagnostic Immunohistochemistry"* at 11:30 AM on January 28, 2009.

NEW REQUISITION FORMS

In order to better accommodate your needs and to facilitate ordering from the increasingly complex menu of test offerings at PhenoPath Laboratories, we are pleased to introduce updated versions of our requisition forms.
We have increased space and readability throughout the pathology and hematopathology requisition forms. You will also notice we have added new FISH and immunohistochemistry tests, and have made some changes to the information requested for billing. This release is version "Rev.03OCT08" as noted in the upper right corner. Please discard all previous versions.

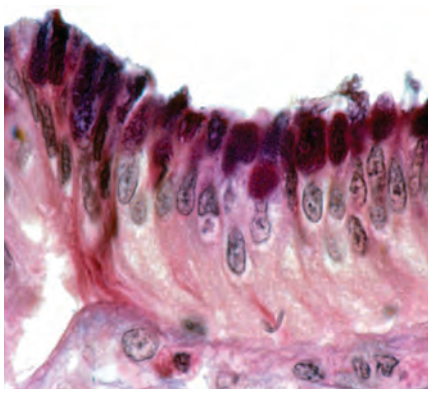
Many of you have already received pre-printed copies of the new requisition forms. For your convenience, these new forms can also be downloaded from the PhenoPath website, filled out electronically, printed and mailed with your specimens. If you need additional requisition forms, or if we can be of further assistance, please call Client Services at 1-888-92-PHENO.

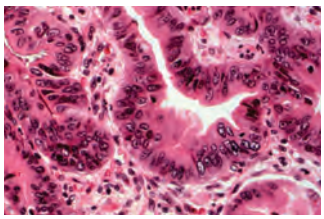
PhenoPath®
LABORATORIES
Expertise, Innovation and Excellence

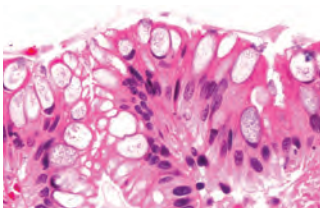
551 North 34th Street, Suite 100
Seattle, Washington, 98103
P (206) 374-9000
F (206) 374-9009

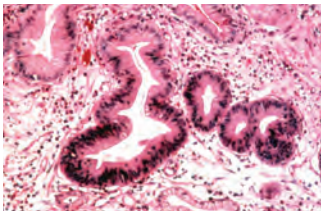
Featuring Dr. John Goldblum

At Our Winter Quarterly Conference









John Goldblum, M.D., of the Cleveland Clinic in Cleveland, OH, will present "Controversies in the Diagnosis of Barrett's Esophagus and Barrett's-Related Dysplasia" at the Quarterly Pathology/ Immunohistochemistry Conference on *Thursday, December 4, 2008*. The format of the conference is a social hour commencing at 6:30 p.m., followed by Dr. Goldblum's lecture at 7:30 p.m. A light catered dinner will be served during the social hour. All pathologists are welcome to attend.

Dr. Goldblum is the Chair of the Department of Anatomic Pathology at the Cleveland Clinic. He is board-certified by the American Board of Pathology in anatomic pathology. Dr. Goldblum received his M.D. degree from the University of Michigan, followed by a residency in Anatomic Pathology and a Surgical Pathology Fellowship at the University of Michigan Health Systems.

Dr. Goldblum specializes in the interpretation of biopsy and resection specimens in the fields of soft tissue pathology and gastrointestinal pathology for Cleveland Clinic and non-Cleveland Clinic patients throughout the U.S. and foreign countries. He is the co-author of the world's highest selling textbook on soft tissue tumors with Dr. Sharon Weiss, and a GI pathology textbook with Dr. Robert Odze. In addition, he has published over 200 peer-reviewed articles. Dr. Goldblum is a renowned speaker who has lectured extensively nationally and internationally in the field of anatomic pathology. A recipient of numerous awards, Dr. Goldblum was distinguished with The Arthur Purdy Stout Annual Prize Award in 2004.

✂ *Clip and Save*

Optimal Handling of Specimens

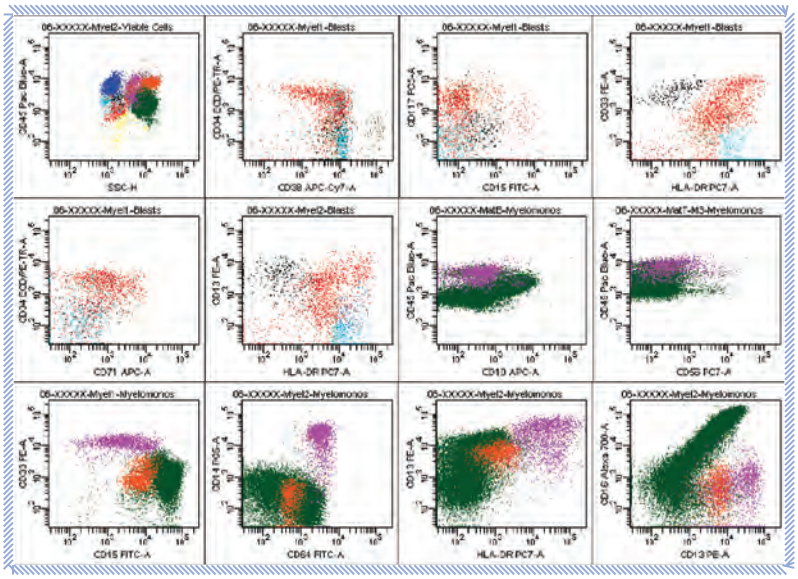
Evaluated for Myeloid Stem Cell Neoplasms

Summary:

Rapid advances in our understanding of the myeloid stem cell neoplasms (MSCNs) – including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative disorders (MPD), and myelodysplastic/myeloproliferative overlap syndromes (MDS/MPD) - have generated a growing set of diagnostic and prognostic tests applicable to these disorders[1]. The work-up for MSCNs is frequently even more complex than the work-up for lymphoma and/or plasma cell dyscrasia, again requiring careful planning by the pathologist to ensure optimal patient care.

The following principles should be applied to EVERY work-up for MSCNs in patients lacking a firm prior diagnosis:

Clinical Information: As much clinical information as possible about the patient should ALWAYS be obtained by the pathologist, including current and prior blood counts with leukocyte differential, the results of serum iron and vitamin B12 and folate evaluation, information about any underlying illnesses or medications/therapies that may affect blood counts, and information about organomegaly, particularly splenomegaly.



9-color flow cytometric evaluation of normal myeloid maturation in the bone marrow.

Morphologic Evaluation: The morphologic evaluation should ALWAYS be based on both peripheral blood and bone marrow, including freshly-made, Romanowsky-stained peripheral blood and bone marrow aspirate smears or, in cases of inaspirable bone marrows, Romanowsky-stained marrow core biopsy touch imprints. Whenever possible, formalin-fixed clot or particle preparations should be made from marrow aspirate remaining after smears are made, and after portions are allotted for flow cytometric and cytogenetic testing. H&E-stained sections of BOTH the marrow clot/particle preparation and core biopsy should ALWAYS be evaluated. Routine special stains should ALWAYS include a Perl's iron stain or equivalent on the marrow aspirate, and a reticulin stain on the marrow core biopsy. For inaspirable bone marrows, an iron stain should ALWAYS be performed on the core biopsy, with the caveat that decalcification typically decreases the amount of stainable iron identified[2].

Flow Cytometry: When an MSCN is in the clinical differential, bone marrow aspirate should ALWAYS be sent for flow cytometry, for three main reasons: 1) to look for immunophenotypic aberrancy on the myeloid cells; 2) to estimate the myeloid blast (or blast equivalent) percentage after lysis of the erythroid elements; and 3) to rule out lymphoid neoplasms that may mimic MSCNs clinically, such as hairy cell leukemia and large granular lymphocytic leukemia. In cases of inaspirable bone marrow, strong consideration should be given to submitting a fresh marrow core biopsy in tissue culture medium for mechanical disaggregation and flow cytometric evaluation. If neither a marrow aspirate nor core biopsy is available for flow cytometric evaluation, and blasts or blast equivalents are present in the peripheral blood, then flow cytometric evaluation of blood should ALWAYS be performed, with the caveat that the myeloid blast percentage in the blood is often lower than that in the bone marrow, and myeloid blast enumeration in the marrow may require immunohistochemical determination of the percentage of CD34 and CD117/c-kit positive cells in the paraffin-embedded marrow core biopsy.

Toll-free: (888) 92-PHENO
www.phenopath.com
lab@phenopath.com
551 N. 34th Street, Suite 100
Seattle, WA 98103

PhenoPath LABORATORIES
Expertise, Innovation and Excellence

Cytogenetics: A fresh, sterile specimen, ideally bone marrow aspirate in preservative-free heparin, should ALWAYS be sent for cytogenetic evaluation. In cases of inaspirable bone marrow, cytogenetic evaluation may be attempted on a fresh marrow core biopsy or peripheral blood specimen.

PCR In AML: At the time new diagnoses of AML are made, the relevant clinician(s) should be queried about potential interest in polymerase chain reaction (PCR) studies to look for internal tandem duplications of the Flt3 gene and point mutations of the nucleophosmin gene, both of which significantly influence prognosis in normal-karyotype AML[3].

BCR/ABL Testing: For patients in whom the clinical and morphologic features of the peripheral blood strongly suggest chronic myelogenous leukemia (CML), a fresh blood specimen should ALWAYS be sent for RT-PCR evaluation for BCR/ABL mRNA BEFORE a bone marrow evaluation is performed. If **qualitative** BCR/ABL RT-PCR is positive, then **quantitative** RT-PCR should be strongly considered[4], again on a fresh specimen, to assess the peripheral blood burden of disease prior to the initiation of therapy. With the diagnosis of CML established from the blood, evaluation of the bone marrow may be useful to help determine the phase of disease, i.e., chronic vs. accelerated vs. blast phase.

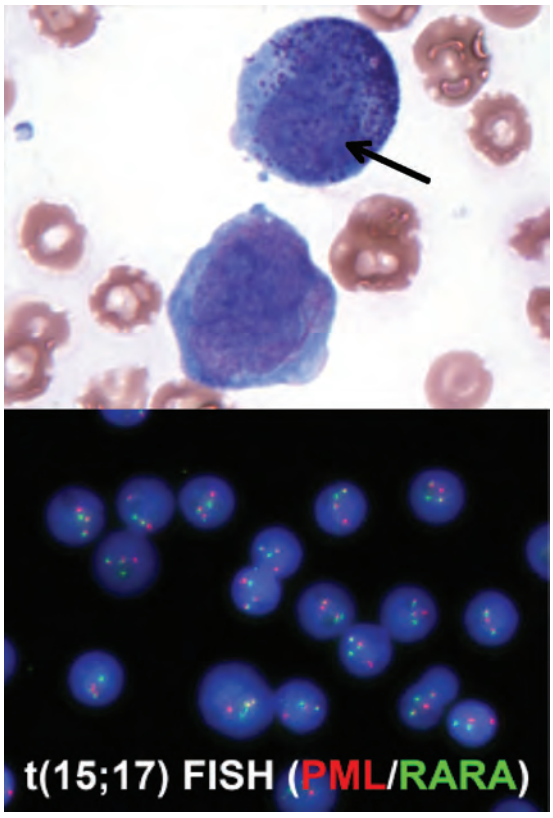
JAK2 Testing: For patients in which there is a concern for MPD, but the clinical and morphologic figures do not strongly suggest CML, PCR testing of the peripheral blood for the JAK2 V617F mutation common in non-CML MPDs[5] should ALWAYS be performed in parallel to BCR/ABL RT-PCR.

Hypereosinophilia/Mastocytosis Testing: Cases of hypereosinophilia, with or without evidence of concurrent systemic mastocytosis (SM), should have fresh material sent for FISH or PCR testing to look for rearrangement of the PDGFR-alpha gene, since positive cases may respond to imatinib therapy[6,7]. Cases of SM should also be evaluated for the c-kit D816V mutation, which may confer non-responsiveness to imatinib[8].

Microbiological Studies: If intramedullary infection is a consideration, then a portion of the marrow specimen should be aliquoted into appropriate culture media under sterile conditions.

Background:

Multiple diagnostic modalities are employed in the evaluation of MSCNs in the modern hematopathology laboratory, as the 2000 WHO classification of tumors of the hematopoietic and lymphoid tissues[1] bases such diagnoses on a combination of morphologic, immunophenotypic, and, in many cases, molecular findings deriving from classical cytogenetics, fluorescence in situ hybridization (FISH), and/or PCR studies. Therefore, careful handling of specimens from patients in whom these diagnoses are being considered is IMPERATIVE to maximize the yield of diagnostic information. This article reviews the optimal utilization of these diagnostic modalities in the evaluation for MSCNs. Please see our previous pamplet for information on "Optimal Handling of Specimens Evaluated for Lymphoma and/or Plasma Cell Dyscrasia."



Wright-stained morphology and positive t(15;17) FISH results in a case of acute promyelocytic leukemia.

Optimizing Morphologic Evaluation:

Careful evaluation of well-prepared and well-stained peripheral blood smears, and bone marrow aspirate smears and/or marrow biopsy touch imprints, remains a mainstay in the diagnosis of MSCNs, as such evaluation is the best way to evaluate for morphologic dysplasia. The Romanowsky stains include the Wright, Wright-Giemsa, May-Grünwald-Giemsa, and related stains. Histologic evaluation of marrow biopsies requires optimization of both fixation and decalcification. In terms of fixatives, we STRONGLY favor neutral buffered formalin (NBF), since adequate NBF fixation produces excellent histology, and preserves antigens for immunohistochemical studies. Some hematopathologists prefer B5 fixation because of the greater detail B5 imparts to nuclear chromatin, but we believe that the issues of B5 waste disposal and the compromised immunohistochemical staining of some antigens greatly outweigh the marginal improvement in the visualization of the chromatin. Similarly, we do not believe that other commercial fixatives (Prefer™, B-Plus™, etc.) have any advantages over formalin fixation. In terms of decalcification, a RAPID method should be used to minimize damage to biopsy morphology and antigenicity, and to optimize DNA preservation for potential FISH studies. A relatively recent publication describes an ultrasonic technique in an EDTA-based buffer that offers improved nucleic acid preservation compared to acid-based decalcification[9]. Finally, making formalin-fixed clot or particle preparations from unused marrow aspirate provides valuable additional material (that may be in significant excess of the material in the marrow core biopsy) for morphologic and, potentially, immunohistochemical and molecular evaluation, including both FISH and DNA-based PCR.

Optimizing Immunophenotypic Evaluation:

The two major methods for immunophenotyping myeloid cells are: 1) flow cytometric (FC) evaluation of fresh peripheral blood, bone marrow aspirate, and, in patients with inaspirable marrows, disaggregated marrow biopsies; and 2) immunohistochemical (IHC) evaluation of paraffin-embedded marrow biopsies and clots/particle preparations. For a detailed review of immunophenotyping in hematopathologic diagnosis, see the section on immunophenotypic markers in *Neoplastic Hematopathology, 2nd Edition*[10]. FC and IHC should be considered complementary studies in the evaluation for MSCNs, in that each offers diagnostic advantages and disadvantages.

Flow cytometry serves the following functions in the evaluation for MSCNs:

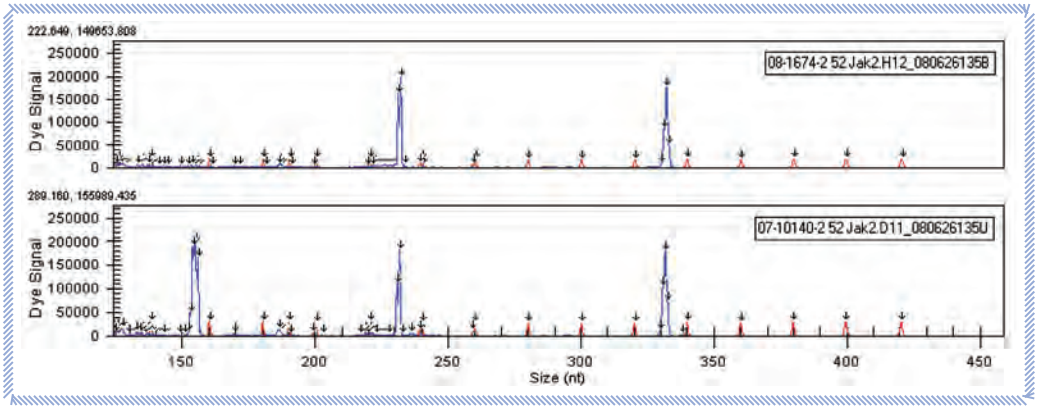
1. In acute leukemias, confirming myeloid lineage;
2. In AMLs, providing detailed immunophenotypic characterization of the leukemic blasts or blast equivalents (promyelocytes in acute promyelocytic leukemia and promonocytes in acute myelomonocytic and monocytic/monoblastic leukemias), for the dual purposes of:
   - Classifying the AML according to the type of differentiation, and providing immunophenotypic clues to specific genetic lesions, e.g., t(15;17), t(8;21), and inversion 16[11];
   - Following the patient's disease after therapy[12];
3. In the evaluation for MDS and MPD, providing objective evidence for antigenic aberrancy, which can be used in many cases as a surrogate for malignancy[13];
4. Ruling out lymphoid neoplasms that can mimic myeloid stem cell neoplasms by causing cytopenias (e.g., hairy cell leukemia and T cell large granulocytic leukemia).

Immunohistochemistry (IHC) serves the following functions in the evaluation for MSCNs:

1. Determining the marrow myeloid blast percentage in biopsy or clot sections, typically via CD34 IHC. In MSCNs with very low to negative CD34 expression on the blasts as determined by flow cytometry, CD117/c-kit evaluation can substitute for CD34 evaluation for determining the blast percentage. In such cases, CD117/c-kit should be performed in conjunction with CD15 and glycophorin A staining, in order to distinguish CD34-negative myeloid blasts (which typically are CD117+/CD15-negative/glycophorin A-negative) from promyelocytes (CD34-negative/CD117+/CD15+/ glycophorin A-negative) and proerythroblasts (CD34-negative/CD117+/CD15-negative/weakly glycophorin A+).
2. Helping enumerate more mature myeloid elements that may be neoplastic, including maturing granulocytic and monocytic cells (via CD15, a more sensitive marker of myeloid cells than myeloperoxidase), nucleated erythroid precursors (via glycophorin A or hemoglobin A), and megakaryocytes (via von Willebrand factor or CD61).
3. Particularly in the setting of myelofibrosis, ruling out marrow infiltration by non-myeloid neoplasms, such as metastatic carcinoma (pan-cytokeratin IHC), metastatic melanoma (S-100 and HMB45 IHC), or Hodgkin or non-Hodgkin lymphoma.

Optimizing Polymerase Chain Reaction (PCR) Evaluation:

In the evaluation for MSCNs, both DNA- and RNA-based approaches are used, the latter in the form of RT-PCR. Commonly-used DNA-based PCR studies include those to detect the JAK2 V617F mutation found in the majority of non-CML MPDs[5], and to detect the internal tandem duplication (ITD) of the Flt3 gene found in about 25% of all AMLs[3]. Importantly, DNA-based PCR studies may be applied successfully to either unfixed (e.g., fresh or frozen) or fixed material (generally formalin-fixed tissue); if both unfixed and fixed tissues are available for a particular specimen, it is GREATLY PREFERABLE that the PCR be performed on the unfixed tissue, because of the higher quality of the DNA. In contrast, RNA-based RT-PCR for chromosomal rearrangements - such as the t(9;22)/(BCR/ABL mRNA), t(15;17)/(PML/RARA mRNA), t(8;21)/(ETO/AML1 mRNA), and inv(16)/(CBFB/MYH11 mRNA) – is almost exclusively applied to unfixed tissue, and preferably to material less than 72 hours old, as the increased cell death in older specimens will result in decreased RNA yield and quality. Note that both DNA-based and RNA-based PCR techniques can be used to demonstrate exon 12 mutations in the nucleosphosmin (NPM) gene, which are identified in up to 35% of all AMLs, and are associated with a favorable prognosis in Flt3 ITD-negative, normal karyotype AMLs[3].



Representative PCR results for specimens containing two normal JAK2 gene alleles (upper, showing the two normal PCR products expected in this assay) or one normal and one V617F mutated allele (lower, showing a third, abnormal PCR product in addition to the two normal products.)

Optimizing Cytogenetic Evaluation:

A portion of all bone marrow aspirates from patients with known or suspected MSCNs should be sent immediately for classical cytogenetic evaluation, in preservative-free heparin, so that the cytogenetics laboratory receives the specimen within 24 hours of its being obtained from the patient. In most cytogenetic laboratories, the identification of the same karyotypic abnormality in two or more metaphase-arrested cells is interpreted as evidence for an abnormal, clonal stem cell population. Cytogenetic evaluation provides both diagnostic and prognostic information in patients with MSCNs. For example, the identification the t(9;22) is critical to both confirm the diagnosis of CML and to suggest the appropriateness of imatinib therapy[14]. Similarly, identification of a clonal deletion at chromosome 5q31-33 in a patient with macrocytic anemia, isolated dyserythropoiesis in the marrow, and a normal to increased platelet count confirms the diagnosis of MDS associated with isolated del(5q) chromosome abnormality[1], and suggests that such patients' anemia may respond to lenalidomide therapy[15]. Finally, in patients with a known history of an MSCN, including CML, the identification of a new cytogenetic abnormality may represent a marker of disease progression.

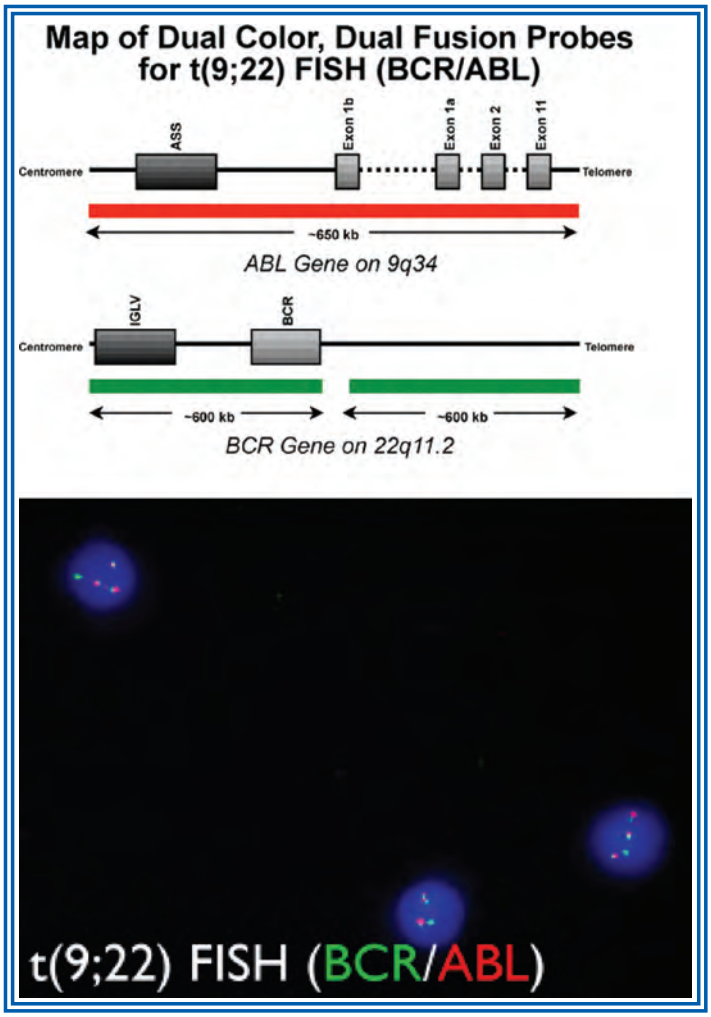
Optimizing Fluorescence In Situ Hybridization (FISH) Evaluation:

FISH evaluation, in which fluorescently labeled nucleic acid probes are applied to the nuclei of either whole or sectioned cells, has both diagnostic and prognostic application to MSCNs, particularly in cases in which standard cytogenetics was either not performed or did not yield adequate cell growth for evaluation (the latter event is relatively common in low-grade MDS, in which there is often an increased rate of apoptosis among the neoplastic stem cells). FISH probe sets are available for most of the common, recurrent chromosomal abnormalities in MSCNs. In the evaluation for MSCN, FISH is ideally performed on fresh blood or bone marrow containing enough neoplastic cells to enable a positive result to be distinguished from background fluorescence. However, if fresh material is not available, FISH can often be applied successfully to NBF-fixed, non-decalcified marrow clots or particle preparations. In our experience, fixation of marrow clots or particle preparations in alternative solutions such as B5 may damage the DNA to such an extent that FISH evaluation may not be possible. Regardless of the fixative, in our experience, FISH evaluation of decalcified tissue is often non-contributory due to a paucity of specific fluorescence signals.

The morphometric evaluation of FISH studies of hematolymphoid-associated chromosomal abnormalities, whether done manually or by automated image analysis, is a complex procedure requiring extensive assay validation. Therefore, such studies should only be done in specialized laboratories with extensive experience.

Optimizing Microbiological Studies:

If intramedullary infection is a consideration, as in a patient with unexplained neutrophilia in which the clinical differential includes occult infection vs. an MPD, then a portion of the marrow specimen should be aliquoted into appropriate culture media under sterile conditions.



Schematic illustration of the probes used for the t(9;22) FISH assay, and a representative example of a positive case

References:

1. ES Jaffe, et al. WHO Classification of Tumours: Pathology & Genetics: Tumours of the Haematopoietic and Lymphoid Tissues. IARC Press, Lyon, France, 2001.
2. DePalma L. The effect of decalcification and choice of fixative on histiocytic iron in bone marrow core biopsies. Biotech Histochem. 1996 Mar;71(2):57-60.
3. Gaidzik V, Döhner K. Prognostic implications of gene mutations in acute myeloid leukemia with normal cytogenetics. Semin Oncol. 2008 Aug;35(4):346-55.
4. Ou J, et al. Molecular diagnosis and monitoring in the clinical management of patients with chronic myelogenous leukemia treated with tyrosine kinase inhibitors. Am J Hematol. 2008 Apr;83(4):296-302.
5. Tefferi A. JAK and MPL mutations in myeloid malignancies. Leuk Lymphoma. 2008 Mar;49(3):388-97.
6. Tefferi A. Modern diagnosis and treatment of primary eosinophilia. Acta Haematol. 2005;114(1):52-60.
7. Florian S, et al. Systemic mastocytosis (SM) associated with chronic eosinophilic leukemia (SM-CEL): detection of FIP1L1/PDGFRalpha, classification by WHO criteria, and response to therapy with imatinib. 2006 Sep;30(9):1201-5.
8. Patnaik MM, et al. Systemic mastocytosis: a concise clinical and laboratory review. Arch Pathol Lab Med. 2007 May;131(5):784-91.
9. Reineke T, et al. Ultrasonic decalcification offers new perspectives for rapid FISH, DNA, and RT-PCR analysis in bone marrow trephines. Am J Surg Pathol. 2006 Jul;30(7):892-6.
10. Knowles DM, ed. Neoplastic Hematopathology, 2nd ed. Lippincott, Williams and Wilkins, Philadelphia, PA, 2001, pp. 93-226.
11. Hrusák O, Porwit-MacDonald A. Antigen expression patterns reflecting genotype of acute leukemias. Leukemia. 2002 Jul;16(7):1233-58.
12. San-Miguel JF, et al. Immunological evaluation of minimal residual disease (MRD) in acute myeloid leukaemia (AML). Best Pract Res Clin Haematol. 2002 Mar;15(1):105-18.
13. Kussick SJ, Wood BL. Using 4-color flow cytometry to identify abnormal myeloid populations. Arch Pathol Lab Med. 2003 Sep;127(9):1140-7.
14. O'Hare T, et al. Targeted CML therapy: controlling drug resistance, seeking cure. Curr Opin Genet Dev. 2006 Feb;16(1):92-9.
15. Kelaidi C, et al. The role of lenalidomide in the management of myelodysplasia with del 5q. Br J Haematol. 2008 Feb;140(3):267-78.

Toll-free: (888) 92-PHENO
www.phenopath.com
lab@phenopath.com
551 N. 34th Street, Suite 100
Seattle, WA 98103

PhenoPath LABORATORIES
Expertise, Innovation and Excellence