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The Newsletter of PhenoPath
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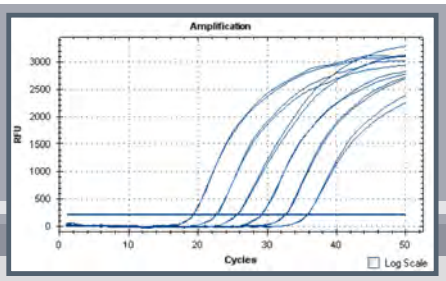
Introducing BCR-ABL1:



Major & Minor BCR-ABL1 RNA Testing Using Int'l Scale (IS) Reporting by Quantitative PCR

PhenoPath Laboratories now offers quantitative detection of BCR-ABL1 RNA containing the major (p210) and minor (p190) breakpoints by real time PCR for use in monitoring disease status in chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). All CML cases as defined by the 2008 WHO classification of hematolymphoid neoplasms, as well as a significant subset of ALL cases, are characterized by the presence of the Philadelphia (Ph) chromosome or Ph variants which are products of a reciprocal translocation between the long arms of chromosomes 9 and 22 resulting in the fusion oncogene, BCR-ABL1. The BCR-ABL1 fusion oncogene comprises major and minor breakpoints. The major breakpoint is seen in the majority of CML cases and results in the BCR-ABL1 p210 fusion oncogene, while the minor breakpoint accounts for the majority of Ph chromosome positive ALL. Both p210 and p190 BCR-ABL fusion oncogenes code for a mutant tyrosine kinase with elevated activity that is susceptible to inhibition by the tyrosine kinase inhibitor imatinib (Gleevec). Treatment of CML with such tyrosine kinase inhibitors has resulted in greatly improved clinical outcomes in CML and quantitative BCR-ABL1 transcript monitoring is important in documenting therapeutic milestones such as Major Molecular Response (MMR) as well as following relapse and drug resistance. The MMR treatment milestone is critical in establishing therapeutic response and clinical decision-making in patients with major BCR-ABL1 fusion gene positive CML. Our major BCR-ABL1 quantitative assay uses a standardized international scale (IS) in reporting of transcript levels as recommended by the 2005 NIH clinical consensus panel[3]. Such reporting allows for comparison of major BCR-ABL transcript levels regardless of laboratory and also has the advantage of providing a constant set value for MMR, defined as 0.1% IS. A standardized IS scale has not been established for minor BCR-ABL1, which is reported as a ratio to the reference gene ABL1. Quantitative major and minor BCR-ABL testing is performed on peripheral blood or bone marrow specimens in EDTA or heparin. Please contact PhenoPath at lab@phenopath.com for more details.

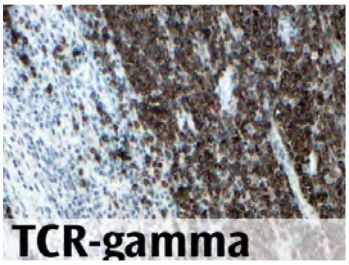
JAK2 V617F

mutation testing by real time PCR

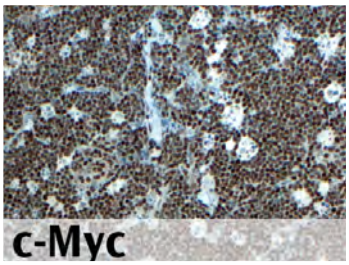
PhenoPath Laboratories also offers a highly sensitive and specific real time PCR assay for detection of the V617F mutation in the JAK2 gene, which has been identified in non-CML myeloproliferative neoplasms (MPN), including polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (IMF). The JAK2 gene codes for a tyrosine kinase that when containing the V617F mutation results in a hyperactive kinase which is believed to confer erythropoietin hypersensitivity and erythropoietin-independent survival of the myeloid stem cell. The majority of non-CML MPD and the great majority of PV contain a JAK2 V617F mutation. JAK2 V617F and BCR-ABL1 PCR testing should be considered complementary in the workup of MPNs. Because the literature contains reports of rare cases containing BOTH the JAK2 V617F mutation and the Philadelphia chromosome, we recommend performing both JAK2 V617F and BCR-ABL1 PCR testing in the molecular evaluation for MPNs. The JAK2 assay can be performed on both fresh and FFPE specimens. Please contact PhenoPath for more details.

References for articles on this page:
1.Gabert J et al. Leukemia 2003: 17(12):2318-57
2.Hughes TP et al. NEJM 2003: 349(15):1423-32
3.Hughes T et al. Blood 2006: 108(1):28-37
4.White HE et al. Blood 2010: 116(22):e111-7
5.Baxter, EJ et al. Lancet 2005: 365(9464): 1054-61. Erratum in: Lancet 2005: 366(9480):122
6.Levine RL et al. Cancer Cell 2005: 7(4): 387-97
7.Kralovics R et al. NEJM 2005: 352(17):1779-90
8.Jones AV et al. Blood 2005: 106(6):2162-8
9.Campbell PJ et al. Lancet 2005: 366(9501):1945-53
10.Larsen TS et al. Eur J Haematol 2007: 79(6):508-15
11.Nussenzveig RH et al. Exp Hematol 2007: 35(1):32-8
12.JAK2 MutaScreen™ Kit – Instructions for Use (Ipsogen). Version 05, November 2008

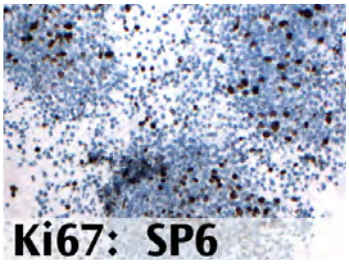
PhenoPath has recently validated three new monoclonal antibodies for clinical use in hematopathology diagnosis



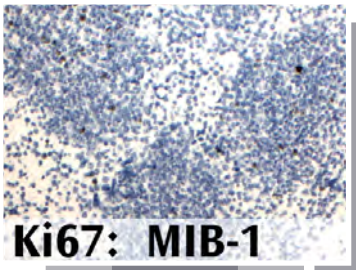
Our new monoclonal antibody, clone γ3.20, to the T cell receptor-gamma chain (TCR-gamma) allows for the specific identification of both benign and neoplastic gamma-delta T cells.[1] The pattern of immunoreactivity is cytoplasmic and membranous. WHO-defined T cell neoplasms that may be of gamma-delta type include the following: hepatosplenic T cell lymphoma, primary cutaneous gamma-delta T cell lymphoma, enteropathy-associated T cell lymphoma, and peripheral T cell lymphoma, not otherwise specified.[2] In addition, rare NK/T cell lymphomas of gamma-delta T cell type have recently been described.[3]



Our new monoclonal antibody to the c-Myc protein, clone Y69, allows for the specific identification of this critical growth promoter in both benign and neoplastic cells of a variety of cell types.[4] The pattern of immunoreactivity is nuclear. In addition to representing the defining genetic feature of Burkitt lymphoma (BL), c-Myc gene rearrangements have been described in approximately 10% of diffuse large B cell lymphomas (DLBCL) and 30% of cases of B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL.[2] The presence of c-Myc gene rearrangements in DLBCL has been associated with an inferior prognosis in the current era of Rituxan-CHOP (R-CHOP) chemotherapy. A recent study of 219 DLBCLs and BLs suggests that the presence of greater than 70% c-Myc positive cells in B cell lymphomas by immunohistochemistry is associated with a high likelihood of a chromosomal rearrangement involving the c-Myc gene.[5] Therefore, the main usage of this antibody in our hematopathology laboratory will be the identification of aggressive B cell lymphomas with a high likelihood of c-Myc rearrangement, for subsequent definitive c-Myc gene fluorescence in situ hybridization (FISH) studies.



A third antibody recently validated at PhenoPath, for both hematolymphoid and non-hematolymphoid processes, is clone SP6, an alternative for determination of the Ki-67-defined cell proliferation index.[6] Clone SP6 will mainly be used for those rare cases in which the standard antibody, MIB-1, exhibits suboptimal performance. In our laboratory, SP6 has been slightly more robust than MIB-1 for identifying Ki-67 in suboptimally fixed tissue, particularly decalcified tissue.



References:
1.Garcia-Herrera A, et al. AJSP 2011: 35(8):1214-25
2.Swerdlow SH, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Fourth Edition. IARC Press, Lyon, 2008
3.Pongpruttipan T, et al. AJSP 2012: 36(4):481-499
4.Ruzinova MB, et al. AJSP 2010: 34(6):882-91
5.Green TM, et al. AJSP 2012: 36(4):612-9
6.Zabaglo L, et al. J Clin Pathol 2010: 63(9):800-4

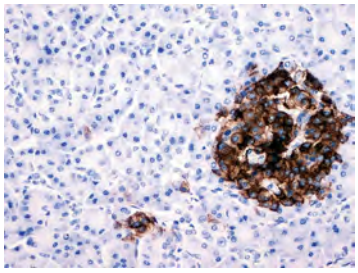
Book Chapter: Flow cytometric principles in hematopathology

Dr. Steven Kussick, PhenoPath Associate Medical Director and Director of Hematopathology and Flow Cytometry, has just published a new book chapter entitled "Flow cytometric principles in hematopathology" in the second edition of Hematopathology, edited by Dr. Eric Hsi and published by Saunders, Elsevier in 2012. The chapter focuses exclusively on flow cytometry in the diagnosis of hematolymphoid neoplasms, and includes an introductory section on basic principles of clinical flow cytometry as applied to 9- and 10-color flow cytometry, followed by detailed descriptions of the major hematolymphoid neoplasms identified by flow cytometry. The chapter includes 20 full-color figures primarily incorporating 9- and 10-color flow cytometry from diagnostic cases at PhenoPath, to illustrate the common antigenic abnormalities in these neoplasms.

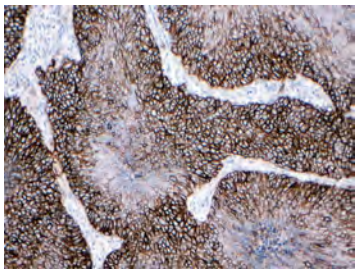
Recently validated monoclonal antibodies for clinical use in solid tumors

Somatostatin Receptor 2a

Somatostatin receptors are highly expressed in neuroendocrine tumors of the gastrointestinal tract and pancreas. The presence of somatostatin receptors has been exploited by radiologists wishing to visualize these tumors for staging and tumor follow-up using octreotide, which is an octapeptide that mimics somatostatin pharmacologically. While there are five subtypes of somatostatin receptor, SSTR2a is the one with the highest levels of expression, and for which a new monoclonal antibody, UMB-1, has been validated at PhenoPath Laboratories for IHC studies. As demonstrated recently by Korner and colleagues, IHC staining correlates with in vitro ^{125}I-[Tyr^{3}]-octreotide radiography. Immunostaining for SSTR2a shows a membranous and cytoplasmic pattern (see images).



Pancreatic islet cells positive for expression of SSTR2a

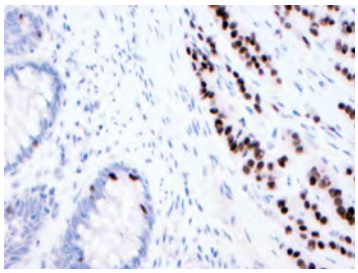


Neuroendocrine carcinoma positive for expression of SSTR2a

In addition to its utility as an IHC correlate of octreotide positive tumors, our extensive validation studies also confirmed the potential of SSTR2a to serve as an alternative marker of neuroendocrine tumors, complementing markers such as synaptophysin and chromogranin A, although without the complete specificity of the latter markers.

Reference: Korner M et al., Am J Surg Pathol 36:242-52, 2012

PAX-6 (and PAX-8 and PAX-5)



Antibodies to PAX-6 showing nuclear positivity in scattered neuroendocrine cells of normal intestine (left) and in carcinoid tumor (right)

The "paired box" gene family encodes the nuclear transcription proteins, PAX-1 through PAX-9. Several of these transcription factors, particularly PAX-2, PAX-5, and PAX-8, have been employed as cell or tissue-type specific markers that can be identified by immunohistochemistry. PAX-5, for example, is an excellent B cell marker that can complement CD20 and CD79a in the diagnosis of B-lymphoid neoplasms including Hodgkin lymphoma and B-lymphoblastic leukemia/lymphoma. PAX-2 and particularly PAX-8 have had their major applications as markers of renal cell and GYN carcinomas, proving to be far more sensitive and specific than prior markers (e.g., CD10 for renal cell carcinoma, or WT-1 and ER for ovarian carcinoma) in this regard. PAX-8 has also been described recently as a marker of pancreatic islets and neuroendocrine tumors, but it has now been demonstrated that these latter studies employed anti-PAX-8 antibodies that cross-react with another member of the paired box gene family, PAX-6. Using new monoclonal anti-PAX-8 antibodies, as well as antibodies to PAX-6, it is clear that these neuroendocrine cells and tumors do not express PAX-8 but do express PAX-6. PhenoPath Laboratories has validated a new monoclonal anti-PAX-6 antibody that is a sensitive, albeit not completely specific, marker of pancreatic islet cell tumors, as well as some low-grade neuroendocrine tumors of the GI tract (e.g., carcinoid tumors).

The "promiscuous" nature of the older anti-PAX-8 polyclonal antibodies is also manifested in cross-reactivity with PAX-5, explaining why several published studies have described PAX-8 positivity of lymphocytes. In both cases, replacement of the rabbit anti-PAX-8 polyclonal antibodies with new anti-PAX-8 monoclonal antibodies has eliminated this spurious cross-reactivity with lymphocytes as well as neuroendocrine tumors. PhenoPath Laboratories now has a set of antibodies specific to many of the paired box gene family, including PAX-2, PAX-5, PAX-6, and PAX-8.

Reference: Lorenzo PI et al., Histochem Cell Biol 136:595-607, 2011

MEET OUR PATHOLOGISTS AT THE FOLLOWING MEETINGS

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

Dermpedia 2012 Course: Melanoma Update, Critical & Urgent Diagnoses and Alopecia

4/20/12 – 4/22/12, Hyatt Regency Scottsdale Resort and Spa at Gainey Ranch

Presentation

4/20/12, 4:45 – 5:30 PM: Allen M. Gown, MD presents ***"Immunohistochemistry in Diagnosis of Cutaneous Metastasis of Unknown Primary: Update on New Antibodies and Special Techniques of Interest in Dermatopathology"***

PhenoPath Booth Exhibit

www.dermpedia.org

Tallahassee Memorial Hospital Tumor Board

4/26/12, Tallahassee Memorial Hospital, Tallahassee, FL

Presentation

11:45 AM - 1:00 PM: Harry Hwang, MD presents ***"Determining True HER2 Gene Status in Breast Cancers with Polysomy"***

www.tmh.org

PSA Webinar

5/8/12, 11:00 AM - 12:30 PM PST: Steve Kussick, MD, PhD presents ***"10-color flow cytometry for leukemia, lymphoma, and myeloma immunophenotyping"***

Contact PSA at **www.psapath.com** for registration details.

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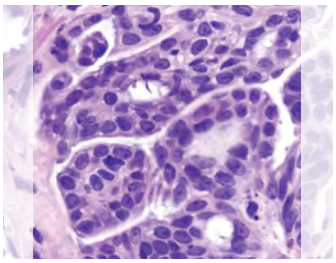
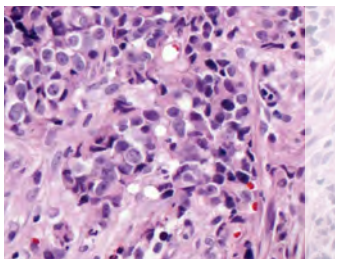
Blake Gilks, MD

UBC

Featured At Our Spring Conference





Blake Gilks, MD of the University of British Columbia, Vancouver, Canada will present "Ovarian Surface Epithelial Carcinomas: Importance of Accurate Subtype Diagnosis" at the PhenoPath Spring Conference at ***7:30 PM on Thursday, May 3, 2012***. Dr. Gilks will also be giving a daytime lecture at noon the same day entitled, "Sex Cord-Stromal Tumors of the Ovary: Recent Advances and an Algorithm for Diagnosis".

Dr. Blake Gilks is a Professor in the Department of Pathology and Laboratory Medicine, at the University of British Columbia, in Vancouver, Canada. He is Director of the Division of Anatomical Pathology and does clinical service work at Vancouver General Hospital, the site of the largest gynecological oncology program in the province of British Columbia. He graduated from Dalhousie University Medical School in 1982 and did a residency in anatomical pathology at the University of British Columbia, followed by fellowships in gynecological pathology and molecular pathology at Massachusetts General Hospital and Fox Chase Cancer Center, respectively. Dr. Gilks leads a research program focused on gynecological cancers and is co-founder of the Genetic Pathology Evaluation Centre, a laboratory that uses tissue microarrays of human tumor samples for cancer research, and OvCaRe, a multidisciplinary team studying ovarian cancer. He is also co-founder and co-director of the Canadian Immunohistochemistry Quality Control Program, which provides proficiency testing for Canadian diagnostic immunohistochemistry laboratories.