The Newsletter of PhenoPath

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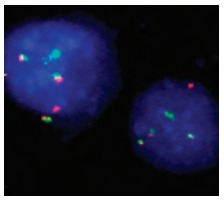
Harvey A. Greisman, MD, PhD, Joins PhenoPath

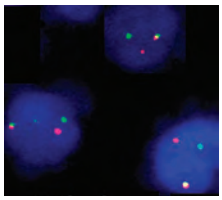
We are pleased to announce that Harvey Greisman, MD, PhD, hematopathologist, joined PhenoPath on June 17, 2013. Dr. Greisman brings extensive experience in the use of morphologic, immunophenotypic and molecular methods in the diagnosis of hematolymphoid neoplasms. He is involved in the entire range of PhenoPath diagnostic hematopathology testing, including IHC, flow cytometry, FISH, and PCR.

Dr. Greisman received his MD from Harvard Medical School and PhD from the Massachusetts Institute of Technology, followed by completion of his Anatomic Pathology Residency and Hematopathology Fellowship at the Brigham and Women's Hospital. He is board certified in Anatomic Pathology and Hematopathology. While an Attending Hematopathologist at the Brigham and Women's Hospital, Dr. Greisman also performed postdoctoral research at Harvard Medical School in the laboratories of Klaus Rajewsky (lymphoma pathogenesis) and Charles Lee (molecular cytogenetics, FISH, array-CGH). Prior to joining PhenoPath, Dr. Greisman was an Attending Hematopathologist and Associate Director of the Hematopathology and Molecular Hematopathology Laboratories at the University of Washington. At the UW, he oversaw the validation of many new molecular diagnostic assays related to hematopathology and developed a microarray-based method for identifying recurrent balanced translocations (patent pending). In addition to over ten years of clinical experience in diagnostic hematopathology, Dr. Greisman has a broad research background, with multiple peer-reviewed research publications in areas such as flow cytometry, molecular diagnostics, and the pathogenesis of lymphoma translocations. His publications have appeared in several high-impact journals including *Science*, *New England Journal of Medicine, Blood,* and *Journal of Molecular Diagnostics*. ***Please join us in welcoming Dr. Greisman!***

ACUTE MYELOID LEUKEMIA (AML) PANEL BY FISH



t(8;21) dual color dual fusion



CBFB breakapart

The AML FISH panel detects the most common recurrent chromosomal alterations in AML, allowing for the proper WHO classification of AML, and also providing important prognostic information to help guide therapy. AML FISH testing may also be indicated for follow-up studies to monitor disease progression and response to therapy. The AML FISH panel at PhenoPath detects the following AML-associated chromosomal alterations:

- ***t(8;21)(RUNX1T1/RUNX1)***: This translocation is the most common abnormality associated with AML. Patients with this abnormality have a high complete remission rate with conventional chemotherapy.
- ***Inv 16/t(16;16)/CBFB rearrangements***: This FISH assay detects any rearrangement/inversions involving the CBFB gene on 16q22. Such alterations are found in about 10% of AML patients, with 50% being AML M4 subtype with abnormal eosinophilia.
- ***t(15;17)(PML/RARA)***: This translocation is associated with AML M3, or acute promyelocytic leukemia (APL). Patients are typically younger, have better prognosis, and almost always have a degree of disseminated intravascular coagulation (DIC), which can become life-threatening. They are usually treated with ATRA (all-trans retinoic acid) therapy, which is relatively well tolerated.
- ***t(11q23:var)/MLL gene rearrangements***: This FISH assay detects any rearrangement involving the MLL gene on 11q23, independent of translocation partner. MLL gene rearrangements are found in a subset of AMLs, and a group of ALL (acute lymphoblastic leukemia/lymphoma) patients. In addition, they can be seen in treatment-related leukemias, associated with anti-TOP2A therapy, as well as conventional alkylating or radiotherapy. The detection of these translocations is important, because they predict a poor overall prognosis.
- ***Deletion of 5q31/Monosomy of chromosome 5***: These alterations are associated with poor outcome.
- ***Deletion of 7q31/Monosomy of chromosome 7***: These alterations are associated with poor outcome.
- ***Trisomy 8***: This alteration is associated with an intermediate prognosis.

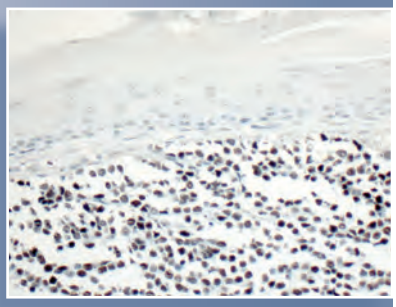
FISH testing for these chromosomal abnormalities has certain advantages over conventional cytogenetic karyotyping, including more rapid turnaround time, ability to detect alterations in a small number of target cells, and ability to analyze interphase or non-dividing cells. Also, FISH testing can directly detect these abnormalities in the setting of atypical breakpoints or complex karyotypes. Contact PhenoPath Client Services for more information.

References: 1. Mancini M et al. Leukemia 14:364-368, 2000; 2. Acute Myeloid Leukemia: The Challenge of Capturing Disease Variety. Hematology 2008 Ham Wasserman Lecture; 3. McKenna RW. Acute myeloid leukemia. In Practical diagnosis of hematologic disorders, 4th Ed. C Kjeldsberg, Ed. 2006; Chicago: ASCP Press, 457–98; 4. Chromosomal and molecular genetic aberrations of tumor cells. In Cancer Cytogenetics, 3rd Ed. S Heim and F Mitelman, Eds. 2009; Hoboken, New Jersey: Wiley-Blackwell; 5. Swerdlow SH et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. 2008; Lyon, France: International Agency for Research on Cancer, 109–47.

New Crop of IHC Markers Now Available

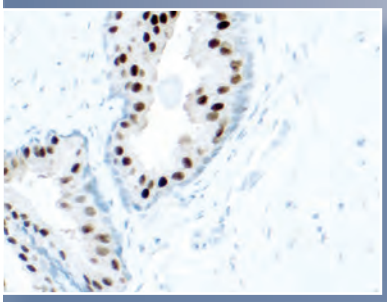
Merkel cell polyomavirus

Merkel cell polyomavirus (MCPyV) is now a suspected etiologic agent in the majority of Merkel cell carcinomas of the skin, with the virus identifiable in ~80% of cases, where it is clonally integrated into the carcinoma genome, along with truncating mutations in the viral large T antigen gene. The presence of the integrated virus can be detected by immunohistochemistry using a monoclonal antibody directed at the Merkel MCPyV T antigen. As this polyomavirus is not associated with other high-grade neuroendocrine carcinomas (e.g., lung small cell carcinoma), identification of MCPyV can be a useful adjunct in the identification of Merkel cell carcinoma and its distinction from metastatic small cell carcinoma. The 'dot-like' expression of cytokeratin 20 has typically been employed as a marker of Merkel cell tumor to help distinguish it from metastatic small cell carcinoma of lung or other origin, although this finding can also be seen in small cell carcinomas of salivary gland origin. Despite this similarity, MCPyV is not present in these latter tumors.



References: Busam KJ et al. AJSP 33:1378-85, 2009, Chernock RD et al. AJSP 35:1806-11, 2011

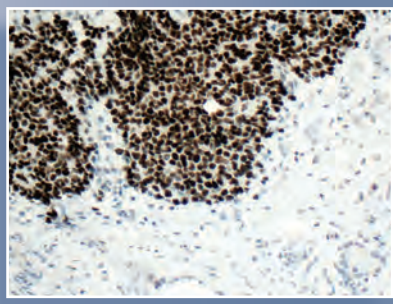
NKX3.1 - Even better than PSA?



NKX3.1 is an androgen-regulated NK-class homeobox gene on chromosome 8p, primarily expressed in prostatic epithelium. In its initial description, NKX3.1 proved to be a marker of prostatic adenocarcinoma, as well as a subset of breast carcinoma. In a larger study of prostatic and other carcinomas, NKX3.1 was found to be expressed in virtually all primary and metastatic prostatic adenocarcinomas, with a slightly higher sensitivity than PSA. In studying 349 nonprostatic tumors, the specificity of NKX3.1 was found to be 99.7%, with only breast lobular carcinomas showing any significant immunostaining. In our experience, NKX3.1 has proven most useful in settings of poorly differentiated and/or poorly preserved carcinoma, e.g., in bone marrow, showing superior immunostaining compared with antibodies to PSA in selected cases. NKX3.1 may be a useful adjunct to PSA as a marker of metastatic prostatic adenocarcinoma.

References: Gurel B et al. AJSP 34:1097-1105, 2010 , Gelman EP et al. The Prostate 55:111-7, 2003

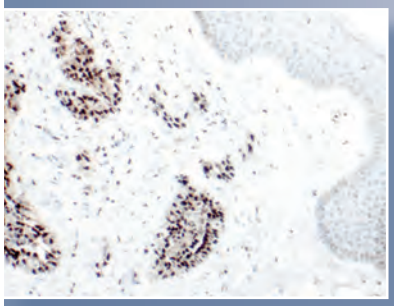
NKX2.2 - A sensitive marker for PNET/Ewing (PNET/ES) sarcoma



NKX2.2 is a transcription factor that plays a crucial role in differentiation of the central nervous system and pancreatic islets, and the NKX2.2 gene is a target of the EWS-FLI-1 fusion protein specific to PNET/ES sarcoma. A recent article by Yoshida and colleagues demonstrated nuclear expression of NKX2.2 in 93% of PNET/ES sarcoma cases with all of the positive cases exhibiting diffuse (>50%) staining. In contrast, NKX2.2 was not expressed in the vast majority (89%) of non-PNET/ES small blue round cell tumors. Positive expression was also identified, however, in olfactory neuroblastoma (100%) and a significant minority of mesenchymal chondrosarcomas, small cell carcinomas, and malignant melanomas. In our own validation studies, we found 100% sensitivity of NKX2.2 expression in PNET/ES sarcoma and a similar level of specificity to that identified in the Yoshida study. Thus, NKX2.2 is a very useful immunohistochemical tool for detecting PNET/ES sarcoma with an extremely high rate of sensitivity and typically robust immunostaining, and is best used in the context of an appropriate panel of immunostains to differentiate PNET/ES sarcoma from other small round cell tumors. This antibody is a much more robust and sensitive marker for PNET/ES sarcoma than FLI-1, and far superior to CD99. Nonetheless, NKX2.2 positive cases generally require confirmation of the diagnosis of PNET/ES sarcoma with FISH analysis looking for the presence of a translocation involving the EWSR gene.

Reference: Yoshida A et al. AJSP 36:993-9, 2012

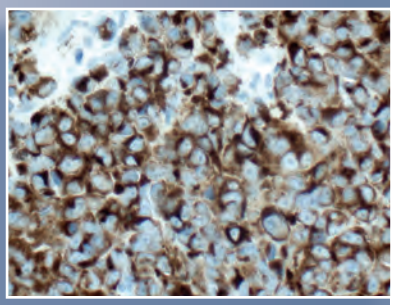
ERG - The new endothelial marker



ERG is a member of the ETS transcription factor gene family, whose members also include FLI-1 and ETS1. ERG is strongly expressed in endothelial cells and is a highly sensitive and specific marker of vascular neoplasms. This new endothelial marker shows superior sensitivity compared with older markers, including CD34, CD31, and vWF. Antibodies to ERG are important tools for the identification of angiosarcoma and other vascular neoplasms. Indeed, ERG appears to represent the most robust vascular marker to date. However, ERG is also expressed uniquely among carcinomas by the subset of prostatic adenocarcinomas displaying the TMPRSS2-ERG gene fusion. In fact, IHC determination of ERG expression can serve as a surrogate for FISH studies demonstrating the presence of this translocation.

References: Yaskiv O et al. AJCP 138:803-10, 2012 , Miettinen M et al. AJSP 35:432-41, 2011

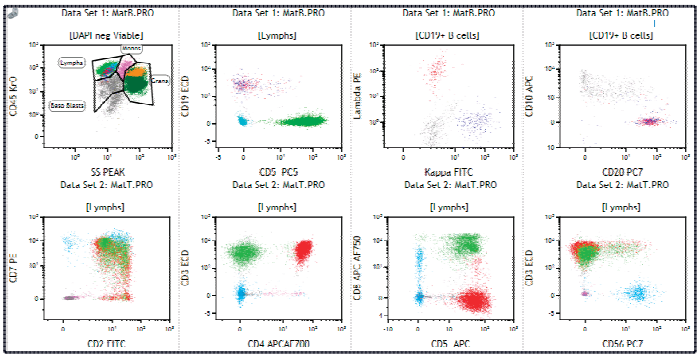
PNL2 - A new anti-melanoma antibody



The diagnosis of malignant melanoma, the great masquerader and histologic mimicker, can prove difficult, particularly in the metastatic setting. Melanomas may simulate the histologic appearance of carcinomas, lymphomas, or sarcomas, and it is important to have highly sensitive and specific markers of melanoma to assist in selected cases. There is a long history of melanoma-restricted markers, starting with antibody HMB-45 (developed by Drs. Gown and Vogel in the 1980s) to gp100 and antibodies to melan A (MART1) and microphthalmia transcription factor (MiTF) in the 1990s. All these markers show high degrees of sensitivity for melanoma, but display an incomplete sensitivity for metastatic melanoma (in the range of 85-90%), and low sensitivity in the context of spindle cell or desmoplastic variants. As a consequence, there has been a continual search for additional melanoma markers that can add to the sensitivity of existing ones. PNL2 is a monoclonal antibody generated by Rochaix and colleagues that seems to have a similar specificity to HMB-45, i.e., restricted to melanomas (and PEComas). A study comparing PNL2 to other melanoma markers was published by Busam et al. a number of years ago, and in this study PNL2 displayed the highest overall sensitivity. Nonetheless, PNL2 also displayed very low overall sensitivity in the context of desmoplastic melanoma. PNL2 appears to be a useful adjunct to existing melanoma markers in the context of the identification of melanoma, and is superior to markers such as MiTF and tyrosinase in this regard. The antibody does display cross-reactivity with a protein in neutrophils, which must be taken into consideration when evaluating the immunostained sections.

References: Busam KJ et al. AJSP 29:400-6, 2005, Rochaix P et al. Mod Pathol 16:481-90, 2003

PHENOPATH ANNOUNCES TWO NEW 10-COLOR FLOW CYTOMETERS



The Flow Cytometry Laboratory at PhenoPath has transitioned to the advanced technology of 3-laser/10-color Beckman Coulter (BC) Gallios flow cytometers. This transition was facilitated by extensive cross-validation, in which hundreds of specimens, encompassing all of the clinical flow assays offered by our laboratory, were analyzed in parallel.

Each Gallios instrument has an automated sample loader that can accommodate up to 32 tubes, enhancing specimen throughput. BC's Kaluza flow analysis software includes advanced features that speed the analysis process, including simple double-clicking to expanded windows for detailed cell analysis, plus a very fast "slidebar" system to adjust compensation for spectral overlap between fluorochromes.

The rigorous data analysis strategies, meticulous attention to detail, and clear, concise reporting of results that have always been employed in our laboratory have continued with our transition to Gallios-Kaluza methodology. Acquisition of these new flow cytometers has enhanced efficiency and throughput, while permitting PhenoPath to maintain the high level of quality our colleagues have come to trust.

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

FEATURED
At Our Summer Quarterly Conference
Jean F. Simpson, MD

PhenoPath Laboratories, September 12, 2013, 6:30 PM (light dinner), 7:30 PM (talk)

Jean F. Simpson, MD, of Vanderbilt University Medical Center, one of the most renowned breast pathologists in the US, will present ***"Therapeutic Implications of Ductal Carcinoma In Situ"*** at the PhenoPath Summer Conference at 7:30 PM on Thursday, September 12, 2013. Dr. Simpson will also give a daytime lecture at noon the same day titled, ***"Update on Proliferative Breast Disease."***

Dr. Simpson is a native of Columbus, Georgia. She completed residency training in AP/CP at Vanderbilt University Medical Center, and then served as Medical Staff Fellow at the National Cancer Institute. Returning to Vanderbilt, she completed a surgical pathology fellowship under the direction of Dr. David Page. She then moved to California, where she practiced anatomic pathology at The City of Hope National Medical Center. She returned to Vanderbilt in 1997, where she was the Director of Anatomic Pathology for eight years and is currently Professor of Pathology. Dr. Simpson leads the Breast Cancer Review Panel for the CAP Cancer Committee, is a member of the Board of Directors for the National Accreditation Program for Breast Centers, and was a member of the 2011 Consensus Conference for WHO classification of breast tumors. Her professional interests are in proliferative breast disease and risk assessment, and histopathology of breast lesions.

Please join us for an engaging event featuring Dr. Jean Simpson.