Since its inception in 2005, the PhenoPath clinical flow cytometry service has been committed to thorough assessment of potential therapeutic targets at the time of both new and recurrent malignant hematopoietic diagnoses. This service lets treating oncologists consider the broadest possible range of potential targeted therapies for their patients. As shown in the figures right, PhenoPath’s 9- and 10-color flow methodology allows multiple potential therapeutic targets to be assessed in a single assay. Figure 1 shows two-dimensional flow dot-plots from a recent low-grade follicular lymphoma diagnosed in our laboratory (the neoplastic B cells are colored black), in which a single 9-color assay allows the assessment of one antigen (CD20) targetable by multiple FDA-approved therapies, and four additional antigens (CD19, CD22, CD38, CD52) targetable by potential experimental therapies. Figure 2 shows two-dimensional flow dot-plots from a recent ALK-positive anaplastic large cell lymphoma diagnosed in our laboratory (the neoplastic T cells are colored black), in which a single 9-color assay allows the assessment for one antigen (CD30) targetable by an FDA-approved therapy, and two additional antigens (CD25, CD52) targetable by potential experimental therapies.

Flow Cytometry Testing for Therapeutic Targets

Figure 1

Figure 2

Celine Jacquemont, PhD

Dr. Celine Jacquemont is an accomplished scientist with expertise in molecular and cellular biology of cancer. As PhenoPath's CRO Senior Scientist, she leads the design, development and execution of preclinical and clinical research projects for PhenoPath's CRO clients, and provides scientific and technical expertise and assistance to CRO projects, including clinical trials. Dr. Jacquemont also designs and leads the development, testing, validation, and implementation of new assays and technologies, such as RNAScope, multiplexed IHC and fIHC for tumor immunophenotyping on cancer specimens, digital imaging quantitation, and drug occupancy assays by flow cytometry.

Before joining PhenoPath’s CRO team, Dr. Jacquemont conducted research on genomic instability, DNA repair and tumor sensitivity/resistance to chemotherapeutics in the Fred Hutchinson Cancer Research Center/HHMI Lab in Seattle, aiming at identifying and validating new drug targets and small molecule therapeutics for cancer therapy using high-throughput drug and siRNA library screenings, as well as innovative cell-based assays involving cellular imaging, flow cytometry and gene targeting. Dr. Jacquemont studied the molecular functions of the BRCA1/BRCA2 genes in breast cancer and Fanconi Anemia at the Curie Institute in Paris. She received her MS in Drug Toxicology and her PhD in Cellular Biology and Physiology from the University of Paris V, France.
PD-L1: The Story Continues

In the previous issue of Phenomena (vol 19 no. 1), we introduced the novel drugs targeting the “immune checkpoints” of the PD-1/PD-L1 axis, and the two different FDA-approved tests required or recommended for patients whose oncologists are contemplating the use of pembrolizumab (KEYTRUDA®) or nivolumab (OPDIVO®). This is a rapidly progressing area of oncology; however, more recent clinical trials using these drugs, as well as others targeting PD-1/PD-L1, have shown exciting positive results. The FDA approval process is progressing rapidly, with new drugs and new tumor targets being approved, many with required or recommended immunohistochemical (IHC) tests. The chart below summarizes the current status of FDA approval of these drugs for various tumor types and the appropriate IHC testing required or recommended for specific indications. Note that there are three drugs now approved, and it is expected that a fourth will soon be approved.

Update on pembrolizumab: In the case of the FDA-approved 22C3-based IHC assay to determine the level of expression of PD-L1 in NSCLC, while the original data (“KEYNOTE-001”) suggested a positive response to pembrolizumab only in those tumors showing high ‘TPS’ (tumor proportion scores) of 50% or more, more recent data from the KEYNOTE-010 trial showed survival benefit of this drug, compared to platinum-based chemotherapy, but there was clinical benefit seen even in patients with a ‘low’ TPS of 1-49%, even though the magnitude of the benefit was significantly higher in the patients with high TPS (Herbst RS et al. Lancet 387:1540-50, 2016). All PhenoPath reports for PD-L1 testing now include the actual percentage of tumor cells positive, as cutoffs may change with the publication of new data.

This information is up to date as of early August, 2016. We recommend you check the PhenoPath website for the most current information regarding PD-L1 testing.

**Summary of FDA Approval of PD-1/PD-L1 Targeted Therapies and IHC Testing Required or Recommended**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Pembrolizumab (KEYTRUDA®)</th>
<th>Nivolumab (OPDIVO®)</th>
<th>Atezolizumab (TECENTRIQ™)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>FDA approved; testing optional with E1L3N</td>
<td>Complementary diagnostic testing with FDA-approved test using 28-8</td>
<td>Not FDA approved; testing optional with E1L3N</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Companion diagnostic testing with FDA-approved test using 22C3</td>
<td>Complementary diagnostic testing with FDA-approved test using 28-8</td>
<td>Not FDA approved; testing optional with E1L3N</td>
</tr>
<tr>
<td>Hodgkin Lymphoma</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
</tr>
<tr>
<td>Urothelial Carcinoma</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>Complementary diagnostic testing with FDA-approved PD-L1 test using SP142</td>
</tr>
<tr>
<td>Renal Cell CA</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
</tr>
<tr>
<td>Head and Neck Squamous Cell CA</td>
<td>FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
</tr>
<tr>
<td>Other tumors</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
<td>Not FDA approved; testing optional with E1L3N</td>
</tr>
</tbody>
</table>

**Markers of metastatic carcinoma of breast origin**


This timely review summarizes and compares the three major breast-associated markers that can be of assistance in evaluating metastatic carcinomas for which a breast primary diagnosis is entertained. As reviewed in the paper, these markers include gross cystic disease fluid protein-15 (GCDFP-15), mammaglobin, and GATA3. Although the first two show comparable sensitivities for breast cancer, relatively few of the published studies have employed the same antibodies against the target molecule, making direct comparisons challenging. GATA3 shows superior sensitivity to GCDFP-15 and mammaglobin; however, the specificity of GATA3 can pose challenges, inasmuch as carcinosmas of the bladder and other sites can show significant levels of positivity. Determination of the optimal panel of antibodies employed in a given clinical setting, therefore, will depend on which non-breast tumors are included in the differential diagnosis.

**Practical Applications in Immunohistochemistry: Carcinomas of Unknown Primary Site**


Identification of the site of origin of carcinoma of unknown primary using immunohistochemistry is a frequent requirement of anatomic pathologists. Diagnostic accuracy is crucial, particularly in the current era of targeted therapies and smaller sample sizes. In this state-of-the-art review, practical guidance is provided for classifying carcinomas of unknown primary using both legacy and new antibodies, as well as targeting panels based on integration of morphologic and clinical features. As demonstrated in the article, it is crucial to understand not only the diagnostic uses of the many available antibodies but also their potential limits and pitfalls.
Kappa / Lambda ISH

PhenoPath is pleased to introduce kappa/lambda assessment by in situ hybridization (ISH) for formalin-fixed, paraffin-embedded tissue (FFPE). This assay complements our existing immunohistochemistry (IHC)-based kappa/lambda assay for FFPE. Because RNA-targeted ISH is often associated with lower background staining than protein-targeted IHC in FFPE, in some specimens ISH may allow more definitive assessment of kappa/lambda expression. In our consultative hematopathology practice, we typically use kappa/lambda IHC first due to its lower cost, but reflex to ISH if background staining or other technical issues limit the IHC interpretation. Note that our clients may order kappa/lambda ISH as a global study to be interpreted by PhenoPath hematopathologists, or as a “tech only” study in which the client renders the interpretation.

Molecular Testing in Colorectal Carcinoma (CRC)

Testing for RAS gene mutations is very important in determining the best form of treatment for patients with metastatic colorectal carcinoma (CRC). Approximately 30-50% of colorectal tumors harbor a mutated RAS gene. These patients are unlikely to respond to treatment with anti-epidermal growth factor receptor therapies such as cetuximab and panitumumab. Therefore, it is recommended that all patients with metastatic CRC should be tested for the presence of a RAS mutation prior to therapy (including testing for clinically relevant mutations in both the KRAS and NRAS genes). KRAS exon 2 mutations are the most common; therefore, initial testing using the FDA-approved KRAS exon 2 assay is suggested, with more extensive testing performed on a reflexive basis.

Testing for Lynch Syndrome has also become an important part of the diagnostic work-up of CRC patients. The hallmark of Lynch Syndrome is a germline mutation in one of the enzymes involved in DNA mismatch repair (MMR). As many sporadic cancers exhibit MMR deficiency, the goal of current testing algorithms is to identify such patients so that genetic sequence testing is performed only when Lynch Syndrome remains a reasonable possibility. In colorectal cancers, BRAF V600 mutation testing is recommended if the tumor shows loss of MLH1 by immunohistochemistry. The presence of a BRAF mutation indicates that the loss of MLH1 expression is likely due to methylation of the MLH1 promoter and not due to a germline mutation, arguing against Lynch Syndrome. BRAF testing is not indicated when loss of MSH2 and/or MSH6 is observed. Results of any testing should be correlated clinically, and genetic counseling should be considered where appropriate. Please see the enclosed Colorectal Adenocarcinoma Recommended Molecular Testing and Lynch Syndrome Screening algorithms.

PhenoPath and Horizon Discovery Joint Webinar

Thursday, Sept 15, 2016, 8:00 - 8:40 AM (PST)

Dr. Regan Fulton (Pathologist & Director of Contract Research, PhenoPath) and Dr. Farah Patell-Socha (Senior Product Manager, Horizon Discovery) will present “Antibody and Testing Validation in IHC” at 8:00AM (PST) on Thursday, Sept 15, 2016.

Attend the webinar and learn about:
- Methods for optimization, verification and validation of slide-based assays
- Importance of standardized reference material
- Usefulness of on-slide controls; in particular, HER2 and PD-L1 assays will be discussed

Please check http://phenopath.com/#/upcoming-events for more information about the webinar.
Richard Levenson, MD
UC Davis Medical Center, Sacramento, CA

PhenoPath, Thursday, September 8, 2016, 5:30PM (light dinner), 6:30PM (talk)

Richard Levenson, MD will present “Path, Present and Future, 150-year-old histology is getting an update.” at the PhenoPath Conference at 6:30pm on Thursday, September 8, 2016. Dr. Levenson will give a related lecture at noon the same day.

Dr. Levenson is the Professor and Vice Chair for Strategic Technologies in the Department of Pathology and Laboratory Medicine at the University of California, Davis. He received his BA in History and Literature at Harvard College, and MD at the University of Michigan. A pathology residency at Washington University, St. Louis, was followed by a Cancer Research fellowship at the University of Rochester, and an Asst. Professorship in Pathology at Duke University. He became active in the optics field while at the NSF Center for Light Microscope Imaging and Biotechnology (Carnegie Mellon) and then joined Cambridge Research and Instrumentation, Inc. (CRI - now part of PerkinElmer) in Boston, eventually as Vice President for Research. Following that, he consulted in optics, software, instrumentation, and digital pathology until joining UC Davis in 2012.

Dr. Levenson’s research activities include development of mass-tag-based multiplexed ion-beam imaging for up to 100-plex immunohistochemistry (Nat Med, 2014) and RNA in-situ detection; artificial intelligence for image analysis and informatics (leading a graduate-level health-informatics course); cancer immunotherapy; digital autopsy, and real-time, slide-free ex-vivo tissue microscopy. He writes, “A recent project evaluating the capabilities of pigeons to detect breast cancer on pathology and radiology images was published in PLoS ONE and has received greater attention than any of my more mainstream efforts.”
Colorectal Adenocarcinoma
Recommended Molecular Testing

Colorectal Adenocarcinoma

KRAS-IVD PCR Test (Exon 2, FDA Approved)

- Ineligible for Cetuximab

Extended RAS Test (KRAS Exons 3, 4) (NRAS Exons 2, 3, 4)

+ Eligible for Cetuximab

Deficient Mismatch Repair / Microsatellite Testing (Go to dMMR/MSI Testing Algorithm)

Questions? Contacts:
Harry Hwang, MD
or Sandra Bohling, MD
206-374-9000
Lynch Syndrome Screening

Clinical assessment and/or Bethesda criteria supports MSI Testing

MMR by IHC (MLH1, MSH2, MSH6, PMS2)

Loss of MSH2/MSH6 (or all 4 markers)

CONSIDER:
Germline MMR gene sequencing and EPCAM genetic testing with genetic counseling

Loss of expression (MLH1/PMS2)

Endometrial carcinoma

BRAF V600 by PCR

MLH1 Methylation

STOP

Colorectal carcinoma

STOP

STOP

STOP

STOP = No further testing recommended, unless significant clinical suspicion for Lynch Syndrome

Questions? Contacts:
Harry Hwang, MD
or Sandra Bohling, MD
206-374-9000

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