New developments with PD-L1 testing continue at a rapid pace, with important implications for pathologists. During the past few months, the FDA approved new indications for existing checkpoint inhibitor drugs, particularly nivolumab [Opdivo'] and pembrolizumab [Keytruda']. At the end of September 2017, the FDA approved nivolumab for the treatment of hepatocellular carcinoma (in patients previously treated with sorafenib), without a requirement for companion PD-L1 tissue testing. Also at the end of September, the FDA approved pembrolizumab for the treatment of patients with recurrent or metastatic adenocarcinoma arising in the stomach or gastroesophageal junction. In the latter tumors, not only was there a requirement for PD-L1 tissue testing, but also a unique scoring system, completely different from the “tumor proportion score” (TPS) previously approved, e.g., for lung non-small cell carcinomas. The new scoring system, the “combined positive score” (CPS), is based largely on the results of the KEYNOTE-059 trial of gastroesophageal adenocarcinomas presented at the June 2017 ASCO meeting. In contrast to the TPS, the CPS incorporates the scoring of PD-L1 expression on the tumor cell population as well as the accompanying immune cells (i.e., lymphocytes and macrophages). CPS scores range from 0 to 100; in the KEYNOTE-059 trial, employing the CPS and using a threshold for positivity of CPS ≥ 1, optimal identification of the subset of patients who would respond to pembrolizumab was possible.

The practicality of determining the CPS on a section of gastroesophageal adenocarcinoma makes it a much more laborious procedure than performing the TPS. At PhenoPath, worksheets have been created that permit the pathologist, working side by side with IHC technologists, to score up to ten different 20x fields, in each field estimating the total number of tumor cells and counting the number of PD-L1 positive cells. As shown in the accompanying images, the same tumor which might be scored with a TPS of <1, could easily manifest a CPS of 20 or more, if PD-L1 expression is found exclusively on the accompanying macrophage and lymphocyte population. Please contact PhenoPath pathologists should you have more detailed questions about the use of the CPS scoring system in gastroesophageal adenocarcinomas.

(Left) H&E stained section of gastric adenocarcinoma. (Right) Gastric adenocarcinoma immunostained with antibody 22C3 to PD-L1 showing expression confined to the immune cell component. There is no signal at all on the tumor cell population. Thus a “TPS” of this tumor would be <1, while the “CPS” was calculated at 15, though the latter was assessed by examining 10 different fields.
New IHC Antibody: MUC4

MUC4 (Mucin 4) is a high molecular weight glycoprotein that has been found to play various roles in the progression of cancer, particularly due to its signaling and anti-adhesive properties that contribute to tumor development and metastasis. While MUC4 can be overexpressed in various carcinomas, its principal use in diagnostic surgical pathology is as a very sensitive and specific marker of low-grade fibromyxoid sarcoma (LGFMS).

LGFMS is a distinctive fibroblastic tumor that often displays a whorled architecture, alternating collagenous and myxoid areas, and deceptively bland spindle cell morphology. Most of these tumors can be identified via fluorescence in situ hybridization (FISH) looking for a translocation involving the FUS gene on chromosome 16; typically, this involves the fusion partner CREB3L2 on chromosome 7.

MUC4 overexpression can also be used as a marker of two other sarcomas thought to be related to LGFMS. These are hyalinizing spindle cell tumor with giant rosettes (HSCTGR) and sclerosing epithelioid fibrosarcoma (SEF), both of which can share some of the histologic features of LGFMS. HSCTGR is a morphological variant of LGFMS that shares the same FUS-CREB3L2 gene fusion and is also immunoreactive for MUC4. SEF is a rare tumor that generally arises in the deep soft tissues of the lower extremities, limb girdles or trunk; approximately 70% of these tumors show MUC4 immunoreactivity. Those tumors with histology suggestive of LGFMS have been shown to harbor the identical FUS-CREB3L2 fusion transcripts; however, the majority of SEFs harbor a different EWSR1 rearrangement. PhenoPath offers both MUC4 IHC and FUS FISH given the incompletely overlapping sensitivity and specificity of these tests.


New FISH Assay: TFE3(Xp11) Breakapart

Tumors manifesting a translocation involving Xp11 include alveolar soft part sarcoma, Xp11 translocation renal cell carcinoma (as well as the melanotic Xp11 translocation variant of renal cell carcinoma), and the Xp11 translocation perivascular epithelioid cell tumor (PEComa). Immunohistochemistry (IHC) employing antibodies to TFE3 has traditionally been used to identify the overexpression of this gene product, which is generally overexpressed in these translocation tumors. (Another IHC marker helpful in this context is cathepsin K.) However, these are a family of tumors in which there are different “partner genes” for the Xp11 translocation, such as ASPL, PRCC, PSF, NONO, CLTC, and several others. We have found that the existing antibodies to TFE3 are suboptimal in their performance characteristics, and recent published data suggest that, owing to the presence of fusion partners in some translocation renal cell carcinomas that do not involve the TFE3 gene, direct detection of the Xp11 translocation using fluorescence in situ hybridization (FISH) is a superior method to identify this important subset of tumors.

The FISH method employs a pair of conjugated DNA probes that flank the Xp11 gene. In the native (nontranslocated) form, the tumor nuclei will manifest pairs of red-green signals within the nuclei. In tumors harboring an Xp11 translocation, however, there will be unpaired red and green signals (see accompanying image). As with our other FISH probes, PhenoPath employs a Metasystems image analysis platform, with thresholds for positivity determined by extensive testing during the probe validation. PhenoPath no longer offers TFE3 IHC, given the superior sensitivity and specificity of the FISH technique. PhenoPath pathologists are happy to answer specific questions about this methodology change.

**What is amyloid?**

Rudolph Virchow, the great 19th century German pathologist, introduced and popularized the term “amyloid” to refer to extracellular deposits in human tissues that exhibited a positive blue-violet staining reaction to iodine and dilute acid. Subsequent microscopic studies have shown that amyloid deposits exhibit an affinity for Congo red dyes, which also yield a property known as “dichroic birefringence” in which crossed polarizing filters produce apple-green birefringence. In the 20th century, X-ray diffraction analysis revealed amyloid to be composed of fibrils ordered in a beta pleated sheet conformation. Using the criteria of “congophilia,” dichroic birefringence, and fibrillar morphology, more than 30 biochemically distinct forms of amyloid have been identified, many associated with unique clinical syndromes.

**Why is it important to identify the amyloid protein?**

Amyloidosis is not a single entity, but a disease state that depends upon the composition of the amyloid deposits. Physicians are most familiar with immunoglobulin light-chain related amyloidosis (AL amyloid), generally associated with an underlying plasma cell disorder, and amyloid A amyloidosis, generally associated with chronic inflammatory disorders. However, as noted in a seminal study, a surprising percentage of amyloidosis cases contain amyloid that is derived from genetic variants of transthyretin, apolipoprotein A-I, lysozyme, and fibrinogen A α-chain (1). Identification of the source of the amyloid protein can have important diagnostic, prognostic, and therapeutic implications.

**Confirmation and Identification of Amyloid at PhenoPath**

The presence of amyloid is often suggested by the histologic appearance on H&E. Confirmation of the presence of amyloid, however, is best accomplished using a Congo red stain on an 8 micron section. Experience dictates that Congo Red stains are imperfect and sometimes difficult to interpret, particularly when other tissue components, such as collagen, show birefringence with polarized light, albeit not dichroism. However, a recent innovation has dramatically improved the sensitivity and specificity of Congo red stains. Tissue sections are examined in a fluorescence microscope using a “Texas red” filter, with the sites of amyloid deposition producing a bright red fluorescence (2). Additional confirmation of the presence of amyloid can be made by immunohistochemistry demonstrating positivity for the amyloid P component, which is found in most forms of amyloid.

Once the presence of amyloid is confirmed, using a limited number of immunohistochemistry antibodies can positively identify an important subset of amyloids, including those composed of AA protein, immunoglobulin light chains, transthyretin (prealbumin), and others (e.g., beta amyloid). The information derived from immunohistochemistry often points to further investigation that might be required to refine the diagnosis. For example, transthyretin-related amyloidoses encompass two forms of disease: familial (arising from misfolding of a mutated or variant transthyretin), and a sporadic (“senile”) variant (caused by misaggregation of wild-type transthyretin).

However, owing to the limited numbers of antibodies available, it is not possible to identify all of the amyloidogenic proteins by immunohistochemistry. A subset of light chain-derived amyloids from the “variable” portion of the immunoglobulin molecule might not be recognized by the antibody reagents. Nonetheless, immunohistochemistry can provide a major “first pass” at subclassification of tissue amyloids and identification of underlying disease; as noted by Linke in a highly recommended review article, immunohistochemistry also has considerable advantages over other techniques (3).

We welcome any questions you have about amyloid detection analysis at PhenoPath.

Achim Jungbluth, MD, PhD will present “How to Set Up an IHC Protocol in the Times of PD-L1 (Without the Need to Consult a Psychiatrist)” at the PhenoPath Conference at 7:00 pm on Thursday, January 25, 2018. It promises to be an interesting and entertaining talk. Dr. Jungbluth will be giving another talk “Melanocyte Differentiation Antigens” at noon at PhenoPath, to which you are also invited. Please inquire for more information.

Dr. Jungbluth is the Director of the IHC Developmental Lab in the Department of Pathology at Memorial Sloan Kettering Cancer Center (MSKCC) in New York, where his focus is the development, standardization and initialization of novel IHC protocols for patient care and translational research. For over twenty years, Dr. Jungbluth was Director of Pathology at the New York branch of the Ludwig Institute for Cancer Research (LICR) at MSKCC, a renowned international cancer research foundation dedicated to discovery and development of immunological and biological-based cancer therapies. At the LICR, Dr. Jungbluth was leader in the seminal discovery, identification and characterization of tumor-associated antigens, development of therapeutic and diagnostic antibodies and other immunological therapies, which were the basis for some of the current established cancer immunotherapies.

Dr. Jungbluth received his MD/PhD at the University of Giessen and Wurzburg in Germany and performed his pathology training at the University Hospital of Mainz and Darmstadt, Germany.