

Which antibodies should be run?

PhenoPath Laboratories' pathologists utilize a focused approach to evaluating cases of carcinoma of unknown primary site. After careful review of the H&E stained section and clinical history, the most appropriate antibodies are selected for a given case based on our pathologists' extensive experience. We believe the extra tests included in large panels promoted by certain labs are neither necessary nor helpful in determining carcinoma of unknown primary site.

For example, in the setting of a carcinoma in the lung presenting in a patient with a history of breast cancer, the principal differential diagnosis is metastatic breast vs. primary lung carcinoma, and the antibodies most useful will be those to estrogen receptors (assuming the primary tumor was ER positive), GCDFP-15, mammaglobin A, TTF-1 and NapsinA. In this setting, markers such as CEA and cytokeratin 7 will not be informative. Additional markers, however, could prove useful if all the above markers are negative and other organs were entertained as possible primary sites.

Carcinomas of unknown primary may present in enlarged lymph nodes, as nodules in the liver, or as malignant ascites or pleural effusion, among other sites. Analysis of every case at PhenoPath always starts with the pathologist reviewing the H&E and the clinical history, and speaking with the referring pathologist when necessary to obtain additional relevant clinical information. With the foundation of the morphologic and clinical data, the immunophenotype of the tumor as revealed by IHC studies can generally 'fingerprint' the primary site with reasonable certainty, ruling out other potential primary sites, or at least narrowing the field of potential primary sites to a short list.

Harnessing the power of IHC techniques in this setting requires a comprehensive knowledge of the available markers, and based on their relative sensitivities and specificities, the most sensitive and specific markers to run in a given clinicopathologic setting.

There are three classes of antibody markers that can be of assistance in analyzing carcinomas of unknown primary: (a) antibodies to cytokeratins and cytokeratin subsets; (b) antibodies to organ-specific markers, recognizing that this 'specificity' is generally not absolute; and (c) antibodies to squamous and neuroendocrine carcinomas. In any given clinical situation, antibodies from more than one of these classes may be employed.

Antibodies to Cytokeratins

Cytokeratins are a complex set of proteins, expression of which is the hallmark of epithelium and their corresponding tumors, i.e., carcinomas.

It is useful to employ a 'pan-keratin' anti-cytokeratin antibody, if necessary, to confirm the diagnosis of carcinoma in histologic settings where this diagnosis is not obvious. We generally employ the OSCAR anti-cytokeratin monoclonal antibody, developed by PhenoPath Laboratories' scientists, which typically outperforms the AE1/AE3 monoclonal antibody cocktail, identifying many (but not all) of the 20 principal members of the cytokeratin protein family. 'Pan-keratin' antibodies can confirm that a tumor is a carcinoma, but cannot provide additional evidence of possible primary site.

Cytokeratins 7 and 20 are two individual cytokeratin proteins that have a partially overlapping but unique distribution among normal epithelium and corresponding carcinomas. When studied concurrently, cytokeratins 7 and 20 expression patterns can help guide the identification of the most likely primary site; for example, renal cell carcinomas typically manifest a cytokeratin 7-negative, cytokeratin 20-negative immunophenotype, in contrast to bladder transitional cell carcinomas which typically manifest a cytokeratin 7-positive, cytokeratin 20-positive immunophenotype. However, it is important not to base interpretation of the most likely primary site solely on the CK7 and CK20 results.

Cytokeratin 5 is expressed along with p63 in squamous and transitional cell carcinomas, but can also be expressed in mesotheliomas as well as a significant subset of adenocarcinomas of the lung, pancreas, and other sites.

Organ-Restricted Markers

These are a set of antibodies to proteins expressed in selected carcinomas. While many of these markers, when first discovered, were initially referred to as 'site-specific' markers (as indicated in names such as 'thyroid transcription factor-1,' or TTF-1), with time, the absolute specificity of virtually all of these markers has been disproven. However, the utility of these markers has been refined. For example, TTF-1 is expressed both in thyroid and lung carcinomas, as well as high-grade neuroendocrine carcinomas of various organs. When used in the context of other markers and with the knowledge of their generally limited cross reactivity, this class of markers can prove highly informative in the identification of primary site. There are two major categories of these organ-restricted markers: (a) cytoplasmic or membranous markers, usually a 'terminal differentiation' marker of a given organ or cell type; and (b) nuclear transcription factors, which are nuclear 'switch proteins' that control downstream expression of other cytoplasmic and membranous markers.

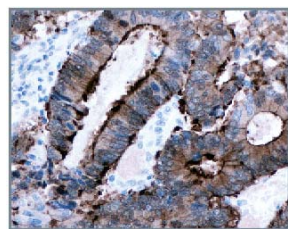
Some of the IHC markers that have been used for decades, such as PSA for prostatic adenocarcinoma, or the GCDFP-15 protein for breast cancer, are examples of cytoplasmic markers. PhenoPath pathologists have helped pioneer and validate the utility of some important new cytoplasmic or membranous carcinoma markers, including villin, an excellent marker of GI adenocarcinomas, including gastric and pancreatic carcinoma, and mammaglobin A, which along with GCDFP-15, is a sensitive and specific marker of metastatic breast cancer. Important nuclear transcription factors useful as organ-restricted markers include: (a) thyroid transcription factor-1 (TTF-1), a very sensitive and specific marker of lung and thyroid carcinomas; (b) CDX2, a marker of colorectal adenocarcinomas but also a subset of adenocarcinomas of the stomach and pancreatobiliary tree; and (c) PAX-8, an excellent marker of genitourinary, ovarian and other GYN carcinomas. PhenoPath pathologists have published and lectured extensively on the utility of IHC markers in the analyses of carcinomas of unknown primary.

Squamous Markers

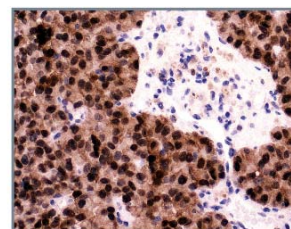
p63 and cytokeratin 5, particularly when co-expressed, are sensitive and specific markers of squamous (and transitional) cell differentiation. However, these proteins can also be expressed in tumors showing myoepithelial differentiation, many salivary gland carcinomas show strong p63 expression (p63 is also a marker of trophoblastic differentiation). Caution needs to be exercised in evaluating tissues for p63 expression, however, as lower levels of positivity can be seen in other tumors (e.g., the 'basal-like' breast cancer subtype as discussed in the Breast Marker Studies section). While there is strong immunophenotypic overlap of squamous cell carcinomas of various primary sites, additional markers (e.g., ER for cervical, CDX2 for esophageal) can help suggest the primary site.

Neuroendocrine Markers

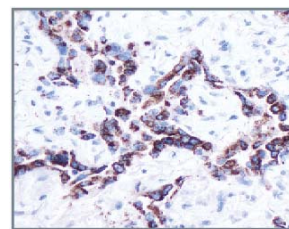
Chromogranin A and synaptophysin are cytoplasmic markers of neuroendocrine differentiation, expressed across the entire spectrum of neuroendocrine carcinomas, from well-differentiated tumors (e.g., carcinoid tumor, in which particularly chromogranin A is highly expressed) to poorly differentiated neuroendocrine carcinomas (e.g., small cell lung carcinoma, in which synaptophysin expression is more common). There is strong immunophenotypic overlap amongst neuroendocrine carcinomas of various organs, but expression of nuclear transcription factors (e.g., TTF-1, CDX2, PAX-6) can be helpful, particularly in the context of low-grade neuroendocrine carcinomas. Markers such as somatostatin receptor 2A not only identify neuroendocrine tumors, but expression may also correlate with in vivo somatostatin receptor (e.g., octreotide) targeting.



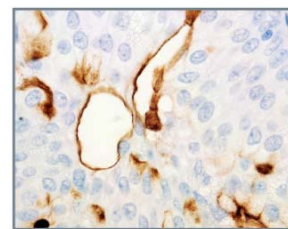
Villin



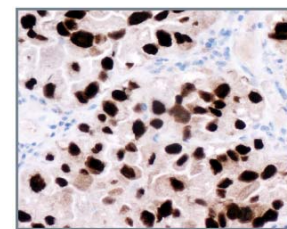
Arginase-1



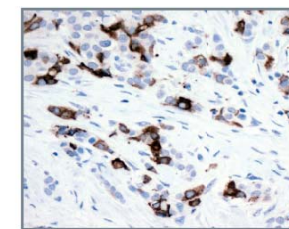
NapsinA



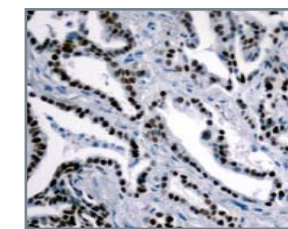
Uroplakin



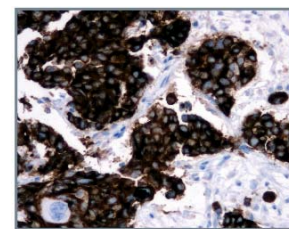
PAX-8



Mammaglobin A



TTF-1



Synaptophysin

Carcinoma Marker Heat Map

The following table summarizes the sensitivities and specificities of the most frequently employed ‘tumor specific’ markers employed at PhenoPath Laboratories in the analyses of carcinomas of unknown primary site. The data on which it is based represents a combination of the published literature as well as the decades of collective experience of PhenoPath’s pathologists. It should be considered a snapshot of a dynamic process in which new markers are constantly being added and our understanding of the specificity and sensitivity of current markers is continually being critically evaluated and modified. In addition, other markers, e.g., GATA3 and uroplakin (transitional cell markers), can be employed in selected clinical settings.

	GCDFP-15	Mammaglobin A	Estrogen receptor	TTF-1	NapsinA	Arginase-1	CDX2	Villin	PAX-2	PAX-8	PSA
Lung											
Breast											
Colorectal											
Non-Colorectal GI											
Hepatocellular											
Ovarian											
Endometrial											
Renal											
Prostate											
Thyroid											
Squamous											
Neuroendocrine											

Heatmap content available in the printed Pathology Reference Guide

Hormone Receptors

Estrogen and progesterone are often thought of as markers of adenocarcinomas of hormone-sensitive tissues, such as breast, ovary, and endometrium. In reality, ER expression differs in these tumors; it is expressed in about two-thirds of breast cancers, but more than 90% of serous carcinomas of the ovary. As such, documenting expression of ER can be helpful in distinguishing metastatic breast cancer from tumors in which ER and PR expression is rarely, if ever, seen (e.g., primary GI tract carcinomas). However, ER and/or PR have been found to be expressed in a number of ‘unexpected’ settings (e.g., lung adenocarcinoma, sweat gland carcinomas, papillary carcinomas of the thyroid, and even carcinoid tumors) and cannot be used as a ‘singular’ marker of breast cancer.

	P63	CK5

ROUNDING UP THE USUAL SUSPECTS....

In rounding up the ‘usual suspects’ of carcinoma markers, correct interpretation of IHC studies requires knowledge, critical evaluation, and integration of both past and more recently published studies. For example, estrogen receptors, once thought to absolutely distinguish at least a subset of metastatic breast cancers from metastatic lung cancers, have more recently (probably aided by more sensitive antibodies and detection systems) been shown to be expressed in up to 20% of lung adenocarcinomas. Furthermore, GCDFP-15, another ‘breast-specific’ marker, has been demonstrated to be expressed in ~10% of lung adenocarcinomas. TTF-1, a ‘lung-restricted’ IHC marker, has been demonstrated to be expressed in approximately 2.5% of breast cancers.

Almost always positive	Usually positive	Not helpful	Usually negative	Almost always negative

See introduction to heat maps page 15