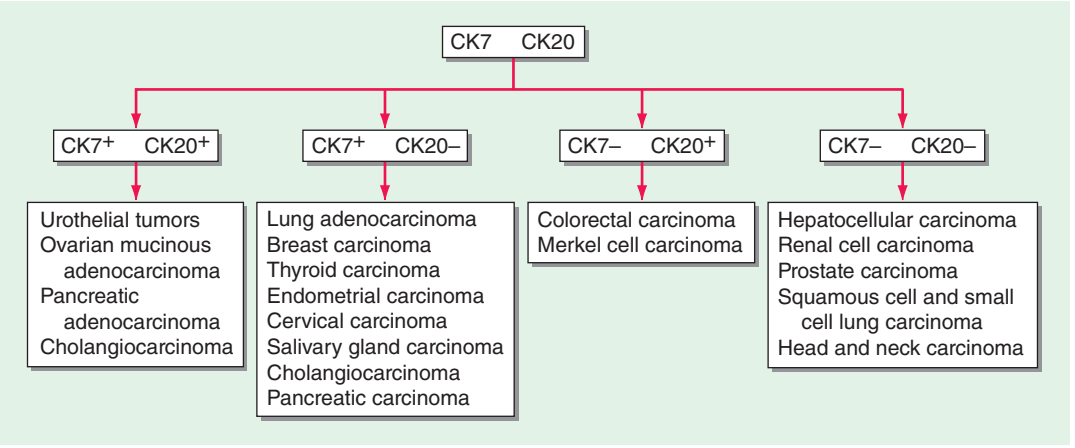

FIGURE 120e-1 Approach to cytokeratin (CK7 and CK20) markers used in adenocarcinoma of unknown primary.

ROLE OF IMMUNOHISTOCHEMICAL ANALYSIS Immunohistochemical stains are peroxidase-labeled antibodies against specific tumor antigens that are used to define tumor lineage. The number of available immunohistochemical stains is ever-increasing. However, in CUP cases, more is not necessarily better, and immunohistochemical stains should be used in conjunction with the patient's clinical presentation and imaging studies to select the best therapy. Communication between the clinician and pathologist is essential. No stain is 100% specific, and overinterpretation should be avoided. PSA and thyroglobulin tissue markers, which are positive in prostate and thyroid cancer, respectively, are the most specific of the current marker panel. However, these cancers rarely present as CUP, so the yield of these tests may be low. Fig. 120e-1 delineates a simple algorithm for immunohistochemical staining in CUP cases. Table 120e-2 lists additional tests that may be useful to further define the tumor lineage. A more comprehensive algorithm may improve the diagnostic accuracy but can make the process complex. With the use of immunohistochemical markers, electron microscopic analysis, which is time-consuming and expensive, is rarely needed.

There are >20 subtypes of cytokeratin (CK) intermediate filaments with different molecular weights and differential expression in various cell types and cancers. Monoclonal antibodies to specific CK subtypes have been used to help classify tumors according to their site of origin; commonly used CK stains in adenocarcinoma CUP are CK7 and CK20. CK7 is found in tumors of the lung, ovary, endometrium, breast, and upper gastrointestinal tract including pancreaticobiliary cancers, whereas CK20 is normally expressed in the gastrointestinal epithelium, urothelium, and Merkel cells. The nuclear CDX-2 transcription factor, which is the product of a homeobox gene necessary for intestinal organogenesis, is often used to aid in the diagnosis of gastrointestinal adenocarcinomas.

Thyroid transcription factor 1 (TTF-1) nuclear staining is typically positive in lung and thyroid cancers. Approximately 68% of adenocarcinomas and 25% of squamous cell lung cancers stain positive for TTF-1, which helps differentiate a lung primary tumor from metastatic adenocarcinoma in a pleural effusion, the mediastinum, or the lung parenchyma.

Gross cystic disease fibrous protein-15, a 15-kDa monomer protein, is a marker of apocrine differentiation that is detected in 62–72% of breast carcinomas. UROIII, high-molecular-weight cytokeratin, thrombomodulin, and CK20 are the markers used to diagnose lesions of urothelial origin.

IHC performs the best when used in groups that give rise to patterns that are strongly indicative of certain profiles. For example, the TTF-1/CK7+ and CK20+/CDX-2+/CK7– phenotypes have been reported as very suggestive of lung and lower gastrointestinal cancer profiles, respectively, although these patterns have not been validated prospectively in the absence of a primary cancer. IHC is not without its limitations; several factors affect tissue antigenicity (antigen retrieval, specimen processing, and fixation), interpretation of stains in tumor (nuclear, cytoplasmic, membrane) versus normal tissue, inter- and intraobserver variability, and tissue heterogeneity and inadequacy (given small biopsy sizes). Communication with the pathologist is critical to determine if additional tissue will be beneficial in the pathologic evaluation.

ROLE OF TISSUE OF ORIGIN MOLECULAR PROFILING In the absence of a known primary, developing therapeutic strategies for CUP is challenging. The current diagnostic yield with imaging and immunochemistry is ~20–30% for CUP patients. The use of gene expression studies holds the

TABLE 120e-2 SELECT IMMUNOHISTOCHEMICAL STAINS USEFUL IN THE DIAGNOSIS OF CARCINOMA OF UNKNOWN PRIMARY (CUP)

Likely Primary Profile	Commonly Considered IHC to Assist in Differential Diagnosis of CUP[a]
Breast	Estrogen receptor (ER), gross cystic disease fibrous protein-15 (GCDFP-15), mammaglobin, Her-2/neu
Ovarian/mullerian	Estrogen receptor (ER), Wilms' tumor gene (WT-1), CK7, PAX8, PAX2
Lung adenocarcinoma	Thyroid transcription factor (TTF-1; nuclear staining), napsin A, surfactant protein A precursor (SP-A1)
Germ cell	β-hCG, AFP, OCT3/4, CKIT, CD30 (embryonal), SALL4
Prostate	PSA, α-methylacyl CoA racemase/P504S (AMACR/P504S), P501S (prostein), and prostate-specific membrane antigen (PSMA)
Intestinal	CK7, CK20, CDX-2, carcinoembryonic antigen (CEA)
Neuroendocrine	Chromogranin, synaptophysin, CD56
Sarcoma	Desmin (desmoid tumors), factor VIII (angiosarcomas), CD31, smooth muscle actin (leiomyosarcoma), MyoD1 (rhabdomyosarcoma)
Renal	RCC, CD10, PAX8
Hepatocellular carcinoma	Hep par-1, arginase-1 (Arg-1), TTF-1 (granular cytoplasmic staining)
Melanoma	S100, vimentin, HMB-45, tyrosinase and melan-A
Urothelial	CK7, CK20, thrombomodulin
Mesothelioma	Calretinin, WT-1
Lymphoma	Leukocyte common antigen (LCA), CD3, CD4, CD5, CD20, CD45
Squamous cell carcinoma (SCC)	p63, p40 (lung SCC), CK5/6

[a]Patterns emerging from coexpression of stains are better than individual stains to suggest putative primary site. Even with optimization, no IHC panel is 100% sensitive or specific (e.g., ovarian mucinous carcinoma can exhibit positivity with intestinal markers).

Abbreviations: AFP, α fetoprotein; β-hCG, β human chorionic gonadotropin; CUP, carcinoma of unknown primary; IHC, immunohistochemistry; PSA, prostate-specific antigen.