Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Special studies

7

The pathologist's H&E is like the clinician's H&P (history and physical)—basic examinations that are performed on every specimen or patient to form the cornerstone of diagnosis. However, the pathologist is no longer limited to H&E: a wide variety of special studies are available to evaluate pathologic processes, from simple histochemical stains to global gene expression patterns. Pathologists are now clinical cell biologists. Familiarity with the types of special studies available is important as the initial processing of the gross specimen may limit the types of studies that can be performed.

HISTOCHEMISTRY

Almost all histochemical stains are suitable for use on formalin-fixed tissues. Common stains and their uses are listed in Table 7-1. However, numerous other types of stains and modifications are used and pathologists must be aware of individual laboratory practices.

Table 7-1 Histochemical stains

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
AFOG (acid fuchsin orange G; modified Masson's trichrome)	Nuclei: brown Connective tissue: blue Basement membrane: blue Proteins, fibrin, readsorption droplets in cells, immune complexes: red/orange/yellow RBCs: yellow	Evaluation of renal biopsies
Alcian blue	Acid mucins: blue (e.g., normal intestinal glands) Nuclei: red Cytoplasm: pink	Sometimes used to identify mucosubstances in mesotheliomas or intestinal metaplasia; affected by pH; hyaluronidase digestion can be used to identify hyaluronic acid
Alcian blue–PAS	Intestinal metaplasia: dark purple Normal stomach: pink	Demonstrates both acid and neutral mucins
Alcian yellow	Free mucus: yellow Bacteria: dark blue	Identification of *H. pylori* in gastric biopsies
Acid-fast bacilli stains (Fite–Faraco, Ziehl–Neelsen, Kinyoun)	TB: red and beaded MAI: red *Nocardia*: pink Tissue: blue	Identification of mycobacteria; modifications are used to demonstrate *M. leprae* or *Nocardia*; tissues fixed in Carnoy's cannot be used, and B5 is suboptimal; slides must be examined under oil

Table 7-1 Histochemical stains—*cont'd*

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
Alizarin red S	Calcium: orange red, polarizes	Identifies calcium in tissues
Bile	Bile: dark green on a yellow background	Identification of bile
Bodian's	Nerve fibers and neurofibrils: black Nuclei: black Tissue: blue	Neural tumors, identification of axons
Chloroacetate esterase (CAE; Leder)	Mature myeloid cells, mast cells: red granules Nuclei: blue	Evaluation of leukemias; identification of mast cells; cannot be used for tissue fixed in Zenker's or B5
Congo red	Amyloid: orange-red with apple-green birefringence after polarization Nuclei: blue	Detection of amyloid; immunoperoxidase studies can be used to identify specific types; overstaining can result in false positives
Dieterle	Spirochetes, *Legionella*, other bacteria: brown to black Tissue: pale yellow or tan	Infectious lesions; melanin, chromatin, formalin pigment, and foreign material may also stain
Diff-Quik® (a modified Giemsa stain)	*H. pylori*: dark blue Other bacteria: blue Nuclei: dark blue Cytoplasm: pink	Evaluation of chronic gastritis
Elastic stains (Verhoeff–van Gieson) (= ET)	Elastic fibers: blue black to black Nuclei: blue to black Collagen: red Other tissue: yellow	Identification of arteries and veins, vasculitis, invasion of lung tumors into visceral pleura, abnormal elastic fibers in elastofibromas
Fibrin (see phosphotungistic acid–hematoxylin [Mallory's PTAH])		To demonstrate fibrin in renal biopsies
Fontana–Masson	Melanin, argentaffin granules, chromaffin granules, some lipofuscin: black Nuclei: red	Identification of melanin in melanomas and secretory granules in neuroendocrine tumors; use of this stain has largely been replaced by immunohistochemistry
Giemsa (May–Grünwald)	Bacteria (e.g., *H. pylori*): blue Parasites (*Leishmania, Plasmodium*) Mast cells: red to purple granules Nuclei: blue Cytoplasm of leukocytes: pink to blue depending on cell type and differentiation	Lymphoproliferative disorders (good nuclear and cytoplasmic detail); identification of bacteria, rickettsias, and *Toxoplasma gondii*
Gram (Brown–Hopps, Brown–Brenn)	Gram-positive bacteria: blue Gram-negative bacteria: red Nuclei: red Tissue: variable	Identification of bacteria, some cases of actinomycetes, *Nocardia*, coccidioidomycosis, blastomycosis, cryptococcosis, aspergillosis, rhinosporidiosis, and amebiasis
Grimelius	Argentaffin and argyrophil granules: dark brown to black Nuclei: red Background: pale yellow-brown	Evaluation of neuroendocrine tumors (largely replaced by the use of immunohistochemistry for chromogranin)
Hematoxylin and eosin (H&E)	Nuclei: dark blue or purple Cytoplasm: pink to red	Standard stain for the routine evaluation of tissues
Iron (colloidal iron)	Ferric iron (e.g., hemosiderin): blue Nuclei: red Background: pink	Bone marrow (iron stores, myelodysplasias), liver (hemochromatosis); chromophobe renal cell carcinomas are positive

Table 7-1 Histochemical stains—*cont'd*

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
Melanin bleach		Removes melanin from tissue, usually for IHC; melanin can be difficult to distinguish from IHC positivity
Methyl green–pyronin Y	DNA (nuclei): green to blue-green RNA: red Goblet cells: mint green Plasma cell and immunoblast cytoplasm: pink to red Mast cells: orange Background: pale pink to colorless	Plasma cell lesions (largely replaced by immunohistochemistry); does not work well on tissues decalcified with formic acid
Mucicarmine (Mayer)	Mucin: deep rose to red Capsule of cryptococcus: deep rose to red Nuclei: black Tissue: blue or yellow	Identification of adenocarcinomas, identification of cryptococcus
Oil red O	Fat: red Nuclei: blue	Requires frozen sections (lipids are dissolved by most fixatives or during processing); tissue fixed in formalin can be used if tissue is frozen
Periodic acid–Schiff (PAS)	Glycogen: red Basement membranes (BM): red Mucins: red Colloid: red Fungi: red	Classification of tumors with glycogen (e.g., Ewing's/PNET, rhabdomyosarcoma, renal cell carcinoma), glomerular diseases (BM), identification of adenocarcinomas (mucin), fungal diseases (especially in argentophilic areas: neutrophils and debris), spironolactone bodies in adrenal adenomas treated with this drug
Periodic acid–Schiff with diastase digestion (PAS-D)	As above, except glycogen has been digested and will not be stained	Identification of glycogen in tumors; identification of fungus in glycogen-rich tissue (e.g., skin); PAS-D resistant deposits in liver are present in α_1-antitrypsin deficiency
Phosphotungistic acid–hematoxylin (Mallory's PTAH)	Glial fibers: blue Nuclei: blue Neurons: salmon pink Myelin: blue Skeletal muscle cross-striations: blue Fibrin: blue Collagen: red-brown	Identification of neural lesions; skeletal muscle differentiation (Zenker's fixative is preferred); this stain has been replaced by IHC for muscle markers
Reticular fibers (Gomori, Gordon and Sweets, Snook) (RETIC)	Reticulin: black Mature collagen, type 1: brown Immature collagen, types 3 and 4: black	Bone marrow (myelophthisis), liver (fibrosis, veno-occlusive disease), carcinoma versus sarcoma (reticular network); largely replaced by IHC
Silver stain (Grocott methenamine–silver nitrate—GMS) (GMS or MMS)	Fungi: black *Pneumocystis carinii*: black Mucin: taupe to gray Tissue: green	Evaluation of infectious diseases; bacteria will also stain black
Steiner	Spirochetes, *H. pylori*, *Legionella*, other bacteria: dark brown to black Tissue: light yellow	Evaluation of infectious diseases
Toluidine blue	Mast cells: deep violet Background: blue	Mast cell diseases, chronic cystitis

Table 7-1 Histochemical stains—*cont'd*

STAIN	COMPONENTS STAINED	POSSIBLE USES AND COMMENTS
Trichrome (Gomori's trichrome, Masson) (= TRI)	Mature collagen, type 1: dark blue Immature collagen, types 3 and 4: light blue Mucin: green or blue Nuclei: black Cytoplasm, keratin, muscle fibers: red	Liver (fibrosis)
Von Kossa calcium	Calcium: black Tissue: red	Demonstration of phosphate and carbonate radicals with calcium in tissues, identification of malakoplakia (Michaelis–Gutmann bodies)
Warthin–Starry	Spirochetes: black Cat scratch bacillus and *Bartonella henselae* (bacillary angiomatosis): black Other bacteria: black Tissue: pale yellow to light brown	Infectious lesions
Wright's	Eosinophilic granules: pink Neutrophilic granules: purple Lymphocytic cytoplasm: blue Nuclei: blue to purple	Blood smears

The WebPath section of the University of Utah site (http://medlib.med.utah.edu) has useful descriptions of special stains and illustrative photographs.

IMMUNOPEROXIDASE STUDIES

The development of methods to detect antigens on tissue sections with antibodies was a major advance in surgical pathology. Immunohistochemical (IHC) studies are most frequently used for the following purposes:

- Classification of tumors (e.g., carcinoma versus lymphoma, B-cell versus T-cell lymphoma)
- Identification of in situ lesions versus invasion (e.g., myoepithelial markers in breast cancers, basal cell markers in prostate)
- Prognostic factors (e.g., Ki-67 in glioblastomas)
- Predictive factors to guide specific therapy (e.g., c-kit, estrogen and progesterone receptors, HER2/neu)
- Identification of extracellular material (e.g., β_2-microglobulin amyloid)
- Identification of infectious agents (e.g., cytomegalovirus).

Use of immunohistochemistry

A differential diagnosis is generated after examination of the H&E stained slides. Immunohistochemistry is then used to gain evidence for or against diagnostic possibilities. "Trolling" cases through an immunohistochemistry laboratory by ordering numerous antibody studies without a clear reason in mind is more likely to lead to misguided diagnosis due to aberrant immunoreactivity than to provide an unexpected correct diagnosis.

A very useful website has been developed by Dr. Dennis M. Frisman (http://www.immunoquery.com): it tabulates published literature on the immunoreactivity profiles for numerous tumors. There is also a comprehensive list of the included references with web links.

Panels

There are no absolute rules for immunoreactivity in cells and tissues. Aberrant positive immunoreactivity (or absence of immunoreactivity) is occasionally observed for all antibodies, either due to biologic variability (e.g., occasional keratin-positive melanomas) or technical factors (impure antibodies, cross-reaction with other antigens, failure to preserve antigenicity). Thus, immunohistochemical markers are used most effectively as panels of markers, with interpretation based on an immunohistochemical profile.

Slides for immunohistochemistry

Tissue is often dislodged from normal glass slides during the treatments required for IHC. Slides must be coated (e.g., with glue, poly-L-lysine, gelatin, albumin) or special commercial slides must be used. If slides are being prepared by another laboratory, the type of glass slide to be used must be specified.

Factors affecting immunogenicity

Numerous variables can affect antigenicity. The most common are described below. Each laboratory must optimize its procedures for each antibody used. Studies on tissues or slides not prepared in the routine fashion for a laboratory must be interpreted with caution.

Type of fixative.

Some fixatives destroy some antigens (e.g., Bouin's diminishes ER immunoreactivity, keratins are not well

preserved in B5).[1] Most studies are based on formalin-fixed tissue. Results cannot be assumed to be equivalent for other fixatives.

Length of time of fixation in formalin.

Protein cross-linking and antigenicity generally decrease with fixation times over 24 hours. To some extent, this effect can be reversed using antigen-retrieval methods.

Prior decalcification in hydrochloric acid.

This decreases the antigenicity of some epitopes (predominantly nuclear) but not others (predominantly cytoplasmic).[2] Decalcifying agents using EDTA do not alter immunogenicity.

Decreased: ER, PR, Ki-67, p53, Ber-EP4 (tumor cells).
Not affected: calcitonin, chromogranin, GCDFP-15, HMB-45, thyroglobulin, S100, PSA, keratins (CK 20, CAM 5.2, AE1/AE3), others.

Length of time since the glass slide was cut.[3–6] The immunoreactivity of the majority of antigens declines over days to weeks and may be lost completely at 1 month.[3–6] The loss may be due to oxidation of amino acids with exposure of tissue to air, as the immunogenicity of tissue deeper in the block can be preserved for many years. Antigen-retrieval methods do not completely restore the antigenicity of old slides. Coating slides with paraffin, storing the slides in a nitrogen desiccator, and/or storing at lower temperatures can partially preserve antigenicity. However, studies should be performed on newly cut slides, if possible.

Antigen-retrieval procedures. These include proteolysis, heating (microwave, steam), and special incubation fluids. To some extent these methods reverse the effects of formalin fixation. Variable effects are observed for different antibodies.

Type of antibody (polyclonal versus monoclonal versus mixture of different monoclonals), epitope detected. Very different results can be obtained with different antibodies to the same protein or different commercial sources of the same antibody.

Incubation time, incubation temperature, dilution of antibody.

Methods of signal amplification.

Temperature of baking the slide.

Controls

Controls are essential for the appropriate interpretation of immunohistochemical studies and to ensure that all steps of this complicated procedure have been adequately performed.

Positive controls. Tissues known to be immunoreactive should be included each time an antibody is used for a test case. Internal positive controls should always be evaluated when present as they control not only for the technique used but also for the antigenicity of the tissue under investigation. Table 7-30 (see pp. 103) lists normal cells that are generally immunoreactive for each antibody. Some laboratories have used vimentin as a control for immunogenicity as almost all tissue should demonstrate positivity.[7] Given the wide and nonspecific distribution of vimentin, smooth muscle α-actin may be more useful in this context as pericytes, vascular smooth muscle, and myoepithelial cells present in most tissues are immunoreactive.

Examples of internal controls are:

- S100: Normal nerves, melanocytes and Langerhans' cells in epidermis, cartilage, some myoepithelial cells, skin adnexa
- Estrogen and progesterone receptors: Normal luminal cells in ducts and lobules of the breast
- CD31, FVIII: Vascular endothelium
- c-kit: Mast cells
- Smooth muscle α-actin: Blood vessel walls, myoepithelial cells in the breast
- Vimentin: Blood vessels, stromal cells
- High-molecular-weight keratin: Squamous epithelium
- Low-molecular-weight keratin: Glandular epithelium
- CD15: polymorphonuclear leukocytes.

Negative controls. The primary antibody is replaced with non-immune animal serum diluted to the same concentration as the primary antibody for a negative control. No positive reaction should be present. If multiple primary antibodies that are reactive with different target antigens are used, then they may serve as negative controls for each other. Although the best negative control would be to use antibody preabsorbed against the target antigen, this is rarely practical in a diagnostic laboratory. Diagnostic slides should also be evaluated for internal negative controls. Aberrant immunoreactivity of tissues that should not be immunoreactive indicates that the immunoreactivity is nonspecific and should not be used for interpretation.

Evaluation of immunoperoxidase studies

The following features must be taken into consideration when evaluating immunoperoxidase studies:

Examples:
Nuclear: ER, TTF-1, P63, Myf4, Ki-67 (MIB-1)
Membranous: EMA, HER2/neu, e-cadherin, EGFR
Cytoplasmic: actin, keratin
Stromal: amyloid (β_2-microglobulin, calcitonin, lambda chain)

In rare cases, immunoreactivity in an unusual location is of diagnostic importance:

- TTF-1: Cytoplasmic (instead of nuclear) positivity in hepatocellular carcinomas.
- Ki-67 (MIB-1): Cytoplasmic and membrane (instead of nuclear) positivity in trabecular hyalinizing

adenomas of the thyroid and sclerosing hemangiomas of the lung.

- Beta-catenin: Nuclear (instead of cytoplasmic) positivity in solid-pseudopapillary tumors of the pancreas and pancreaticoblastomas. Both nuclear and cytoplasmic positivity is seen in the majority of colon carcinomas. Nuclear positivity is present in approximately 20% of endometrioid endometrial carcinomas.

Identification of immunoreactive cells. Immunoreactivity of tumor cells must be distinguished from immunoreactivity of normal entrapped cells (e.g., desmin-positive skeletal muscle cells infiltrated by tumor, S100-positive Langerhans' cells in tumors, smooth muscle α-actin-positive blood vessels, etc.). Plasma cells have large amounts of cytoplasmic immunoglobulin and can react nonspecifically with many antibodies.

Intensity of immunoreactivity. Some weak immunoreactivity may be present as a nonspecific finding. It is important to compare positive cells with control slides and with normally non-immunoreactive cells to determine whether the immunoreactivity is significant.

Number of immunoreactive cells. In some cases, the number of positive cells may be important as a criterion for positivity or as a prognostic marker (e.g., markers of proliferation such as Ki-67). In other cases, rare weakly positive cells must be distinguished from intermingled normal cells or just nonspecific immunoreactivity.

Criteria for a "positive" result. Specific criteria for evaluating IHC have been developed for a few antibodies (see Tables 15-3, 15-4, and 7-28). However, criteria do not exist for most antibodies or are not universally used by all pathologists. The significance of immunoreactivity varies with the type of lesion, the antibody, and the specific assay. Strong positivity in the majority of cells is easily interpreted as a positive result. As the number of positive cells decreases, and the intensity of immunoreactivity weakens, the lower threshold of a "positive" result becomes more difficult to determine.

Time. Alkaline phosphatase chromogens (red color) fade over time. DAB (brown color) is more permanent. This is not a problem in evaluating current pathology specimens. However, if immunoperoxidase slides are reviewed after a period of time, some chromogens may have faded and once-positive results may appear to be negative.

Location of immunoreactivity (Fig. 7-1). Antigens are present in specific sites. Some antigens may be present in more than one location or be extracellular.

Artifacts. Nonspecific positivity should be suspected when immunoreactivity is present in atypical locations:

Background: Suspect nonspecific positivity if normal cells or stroma are positive. This can occur with suboptimal performance of the assay or suboptimal antibodies.

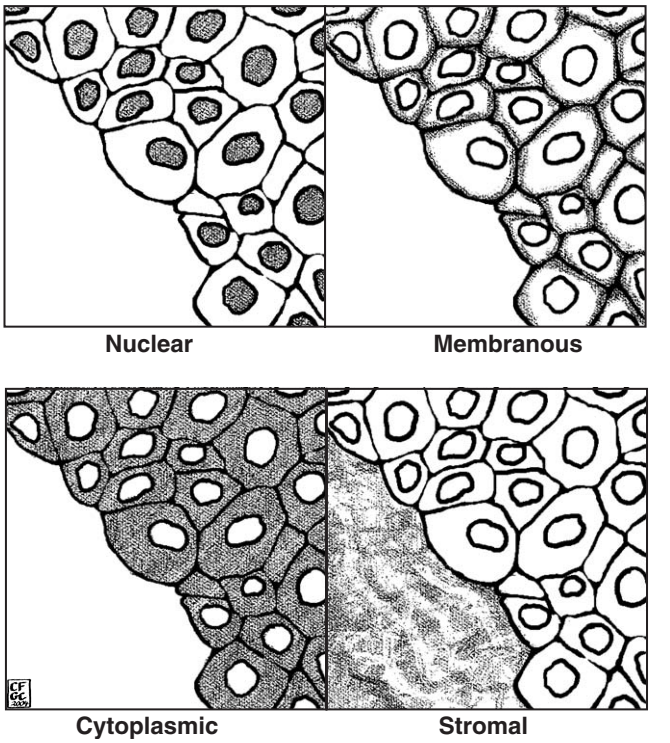

Figure 7-1 Location of immunoreactivity.

Edge artifact: Antibodies can pool at edges or in holes in tissue. True positivity should also be present in the center of the tissue.

Necrosis or crushing of cells: Nonspecific positivity can be seen in disrupted cells. Although keratin is generally reliable in necrotic tumors, other markers generally should not be interpreted.

Inappropriate location (e.g., cytoplasm instead of nucleus): Occasionally ER or PR is present in the cytoplasm instead of the nucleus. This is not interpreted as a positive result.

Common panels for immunohistochemical studies

Tables 7-3 to 7-30 include information from the literature as well as the personal experiences of the staff at Brigham and Women's Hospital. Because of the many differences in specific antibodies, laboratory assays, and criteria for considering a result "positive," results may vary among institutions. The results have been divided into five categories for general markers and four categories for hematopathology markers (Table 7-2). Note that "%" refers to the number of tumors reported to be positive, not the number of cells positive within a tumor.

The actual markers used to evaluate a case depend upon the differential diagnosis based on the H&E appearance. In some cases, an initial panel that is often used for typical cases has been suggested. Not all markers listed would be used for all cases, and some markers are

Table 7-2 Evaluation of positivity of immunohistochemical studies

GENERAL MARKERS			HEMATOPATHOLOGY MARKERS		
CATEGORY	% OF TUMORS	INTERPRETATION	CATEGORY	% OF TUMORS	INTERPRETATION
Positive (POS)	>90%	Almost always positive; a negative result would be unusual	+	>90%	Almost always positive
High	60–90%	Most tumors are positive	+/–	>50%	Majority positive
Moderate (Mod)	40–60%	May or may not be positive – usually the least useful type of marker	–/+	<50%	Minority positive
Low	10–40%	Most tumors are negative	–	<10%	Rarely positive
Negative (neg) or rare	<10%	Almost all tumors are negative; a positive result would be unusual	Blank		Results unknown or too few cases to quantify
Blank		Results unknown or too few cases to quantify ? = Results based on very few cases (e.g., <10)			

included to indicate when they would not be useful for distinguishing the tumors listed in the table.

Cytokeratin 7 and Cytokeratin

The combination of these two cytokeratins has been found to be useful to divide carcinomas into four main groups (Ck7+/Ck20+, Ck7+/Ck20–, Ck7–/Ck20+, Ck7–/Ck20–).[8–10]

In Tables 7-3 to 7-7, other commonly used antibodies have been included to show differences within each group. The most useful additional antibodies depend on the specific differential diagnosis.

Spindle cell lesions, soft tissue lesions, and sarcomas See Table 7-8.

Small blue cell tumors See Table 7-9.

Myoepithelial markers in breast cancer
See Table 7-10.

Epidermal lesions of the nipple See Table 7-11.

Endocervical carcinoma versus endometrial carcinoma See Table 7-12.

Endometrial stromal sarcoma versus leiomyosarcoma See Table 7-13.

Primary ovarian carcinoma versus metastatic carcinomas See Table 7-14.

Ovarian carcinoma versus mesothelioma
See Table 7-15.

Trophoblastic lesions See Table 7-16.

Tumors of germ cells and sex-cord stromal tumors See Table 7-17.

Adrenal and kidney tumors See Table 7-18.

Tumors of bladder, prostatic, or renal origin
See Table-7.19.

Prostate carcinoma versus other lesions
See Table 7-20.

Hepatic tumors See Table 7-21.

Thyroid and parathyroid lesions See Table 7-22.

B-cell neoplasms See Table 7-23.

T-cell neoplasms See Table 7-24.

Hodgkin's lymphoma See Table 7.25.

Metastatic tumors of unknown origin
See Table 7-26.

Poorly differentiated tumors See Table 7-27.

Estrogen and progesterone receptor evaluation and HER2/neu score
See Chapter 15, pages 240-243.

EGFR (HER1) score See Table 7-28.

Differential diagnosis of epithelial mesothelioma and lung adenocarcinoma[13]
See Table 7-29.

Table 7-3 Predominantly CK7+/CK20+

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Cholangiocarcinoma	High	Low	Low	neg	High	POS	Low	POS	POS	High	POS	neg	Low				rare	
Transitional cell carcinoma	POS	Low	neg	neg	Mod	POS	High	POS		Mod	Mod	neg	High	neg	neg	neg	neg	
Pancreas	High	Low	Low	neg		POS	Low	POS	POS	High	POS	neg	Mod		neg	neg	Low	DPC4 lost in 55%
Ovarian mucinous	POS	Low	neg	neg		POS	neg	POS		Mod	Low			neg			Low	
Esophageal adenocarcinoma	POS	neg	neg	neg								neg	Low	neg			Mod	

Table 7-4 CK7–/CK20+

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Merkel cell carcinoma	Rare	neg	High	Low		High	neg	High	POS		POS	neg			Low	High	neg?	NSE High
Colon adenocarcinoma	Low	neg	High	Low	neg	POS	neg	High	POS	POS	POS	neg	Low	neg	Low	neg	neg	CDX2 POS

Table 7-5 Predominantly CK7+/CK20–

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Acinic cell carcinoma	neg	POS	neg	neg	POS	POS		Mod			Low				POS	Low		
Adenoid cystic carcinoma	neg	POS	neg	neg	POS	High	POS	Mod		POS	Low		POS		Mod	neg		GFAP Low
Breast ductal carcinoma	Low	High	neg	neg	neg[a]	POS	Low	POS	High	High	Mod	neg	Low[a]	High	Mod	Low	neg	ER/PR[b] GCDP Mod
Breast lobular carcinoma	Low	POS	neg	neg		POS	neg	POS	Mod	Mod	Mod	neg	Low			Low	neg	ER/PR[b] GCDP Mod E-cadherin neg
Brenner tumor	neg	POS	neg	neg				POS			High			Low	neg?	POS		Calretinin Low NSE POS
Cervical squamous cell carcinoma	neg	High	neg	Low	POS	neg	POS	POS		POS	Low	neg	POS			neg	neg	HPV POS p16 High
Choroid plexus	neg	High	neg	Low		POS		Low	neg						Mod			GFAP High
Chordoma	neg	POS	neg	neg	Mod	POS		POS	neg	neg	neg				POS	neg		GFAP neg
Craniopharyngioma	neg	POS	neg	neg	POS		POS											
Embryonal carcinoma	neg	POS	neg	neg	neg	POS		Low		Low	Low	neg?	neg		neg	neg	neg	PLAP High CD30 High
Endometrial carcinoma	Low	High	neg	neg		POS	Low	POS	POS	Low	Low	neg		neg?	High	neg	neg	Vimentin POS ER High
Lung: adenocarcinoma	Low	High	neg	Low	Mod	POS	neg	POS	POS	High	High	High	High?	Low	Low	neg	Low	
Lung: BAL[c] non-mucinous	neg	POS	neg	neg	POS	POS				High	High	High	Mod	neg	Mod	neg		
Meningioma: secretory type[d]	neg	POS	neg	neg		neg	High	POS		POS	POS				Low	neg		PR Mod ER neg
Mesothelioma	neg	High	neg	Low	High	POS	High	High	neg	neg	neg	neg	neg	High	neg	Low	neg	Calretinin High

Table 7-5 Predominantly CK7+/CK20—*cont'd*

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Mixed tumor[e]	neg	POS	neg	neg		POS	POS	Low		Low	neg?		POS		POS		neg	GFAP High SMA POS Calponin POS
Ovarian: endometrioid	neg	POS	neg	neg		POS	Low	POS	POS	Low	Low	neg?	Low	High	Low		neg	ER Mod
Ovarian: serous carcinoma	neg	POS	neg	neg		POS	Low	POS	POS	neg	neg	neg?	Low	POS	High		neg	ER High Calretinin Low
Renal cell: papillary and chromophobe	neg	POS	neg	neg	POS			POS						Mod[f]				
Thyroid: papillary	neg	POS	neg	neg	POS	POS	Mod	High		neg	Mod	POS	High		High	neg	neg	Thy POS Calci neg
Thyroid: follicular	neg	POS	neg	neg	neg		neg	Mod		neg	Low	POS			Mod	neg	neg	Thy POS Calci neg
Thyroid: medullary	neg	POS	neg	neg	neg		neg	neg		POS	Mod	POS				POS		Thy rare Calci POS

[a] p63 may be positive in breast "basal like" carcinomas, some spindle cell metaplastic carcinomas, squamous cell carcinomas, and some papillary carcinomas. These subtypes may also have less typical keratin subsets such as CK14 (detected by 34β E12), CK17 (detected by MNF-116), or CK5/6.

[b] Most well and moderately differentiated ductal carcinomas, and carcinomas of special type (except for medullary) will be positive for hormone receptors. Poorly differentiated carcinomas, metaplastic carcinomas, and medullary carcinomas are usually negative. Well and moderately differentiated lobular carcinomas are almost always positive for ER, and usually positive for PR. Poorly differentiated lobular carcinomas may be negative for these markers.

[c] Non-mucinous bronchiolo-alveolar carcinomas (BAL) have an immunophenotype similar to lung adenocarcinomas. Mucinous BALs are more likely to be CK20 positive (approximately 70% positive) and less likely to be TTF-1 positive (approximately 30% positive).

[d] Secretory meningiomas are frequently positive for CK7 and CEA, whereas other subtypes are usually negative for CK7 and CEA. The majority of all types of meningiomas are positive for PR (including meningiomas in males).

[e] Mixed tumors (pleomorphic adenomas) occur most frequently in the salivary glands, but can also arise in soft tissues (myoepithelial tumors of soft tissue). These tumors have a similar immunophenotype with keratin (AE1/AE3 77%) or PANK (68%) or EMA (63%) present in the majority of tumors and frequent expression of markers associated with myoepithelial cells (e.g., calponin, GFAP, SMA, S100, p63). However, p63 is seen less frequently (23%) as compared to salivary tumors (100%).

[f] Chromophobe renal cell carcinomas may be positive for WT-1. Other types are negative.

Table 7-6 Predominantly CK7–/CK20–

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Adrenal cortical adenoma	neg	neg	neg	POS		neg	neg	neg		neg	Low	neg			neg	neg	Low	Melan-A103 POS Inhibin POS
Carcinoid	neg	Low	Low	High	neg	POS	Low	Low		Mod	Mod	VAR[a]	neg		VAR[b]	POS	Low	
Epithelioid sarcoma	neg	Low	neg	POS	Mod	High	Low (focal)	POS (focal)					Low (foc)		neg		neg	
Esophageal squamous cell carcinoma	neg	Low	neg	High	POS	High?	POS	POS	High?	Low?	Low	neg	POS		neg	neg	neg?	
Seminoma	neg	Low	neg	High	neg	Low	Low	neg		neg	neg				neg	neg	neg	PLAP POS CD117 POS
Head and neck squamous cell carcinoma	neg	Low	Low	High	POS	neg	POS	POS			neg	neg	POS			neg	neg	
Hepatocellular carcinoma	Low	Low	neg	High	Low	POS	neg	Low	Low	neg	High[c]	High[d] (cyt)	Low		neg	neg	High	AFP Mod
Lung: squamous cell carcinoma	neg	Low	Low	High	POS	High	POS			Mod	Low	neg	POS			neg	Low	
Lung: small cell carcinoma	neg	Low	neg	High	neg	High	neg	POS	POS	Mod	High	POS	rare		neg?	Mod	neg	
Pheo/paraganglioma	Rare	Rare	Rare	POS	neg	neg	neg	neg				neg			High	POS		Inhibin neg Melan-A103 rare
Prostatic carcinoma	neg	neg	Low	High	neg	POS	neg	Low	POS	neg	Mod	neg	neg	neg	neg	Low	neg	PSA POS
Renal cell carcinoma: clear cell	neg	Low	neg	High	neg	High	neg	POS	Low	Low	neg	neg	Low	neg?	Low	neg	neg	Vime POS

Table 7-6 Predominantly CK7–/CK20––*cont'd*

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Squamous cell carcinoma	neg	Low		High	POS	Low	POS	POS	neg	Mod	Low	Low	POS		neg	neg	neg	
Thymic carcinoma					POS	POS	POS	Mod	High	Low	neg?	neg	POS	neg	neg	Low		CD5 Mod
Thymoma	neg	Low	neg	High		High	High	Mod		Low	neg?	neg	POS	neg	neg	neg?	neg?	CD5 neg

[a] Non-pulmonary carcinoid tumors are negative for TTF-1. Some pulmonary carcinoids may be positive.
[b] Sustentacular cells may be positive for S100 and positivity can vary with site.
[c] CEA has a canalicular pattern in hepatocellular carcinoma, a diffuse cytoplasmic pattern in other carcinomas.
[d] TTF-1 immunoreactivity in hepatocellular carcinoma is cytoplasmic (not nuclear as in lung and thyroid carcinomas). Positivity can vary with the antibody used to detect TTF-1.

Table 7-7 No dominant CK7/CK20 pattern or pattern unknown

TUMOR	CK7+ CK20+	CK7+ CK20–	CK7– CK20+	CK7– CK20–	34β E12	CAM 5.2	CK 5/6	EMA	BER-EP4	CEA m	CEA p	TTF-1	P63	WT-1	S100	CHRO	HEP	OTHER
Gastric adenocarcinoma	Low	Low	Low	Low	neg	POS	neg	High	POS	High	High	neg	Low	neg?	Low	neg	Low	
Ameloblastoma/ Adamantinoma[a]					POS	neg		neg							neg?	neg?		
Lymphoepithelial carcinoma[b]					POS			High		Mod?	Mod?		POS		neg[c]			

[a] Approximately 15% of ameloblastomas are positive for CK7.
[b] Approximately 50% of nasopharyngeal carcinomas are positive for CK7. Many cases in Asian and North African patients (less commonly in US patients) are associated with EBV. EBV can be demonstrated by in situ hybridization, PCR, or occasionally by immunohistochemistry. These carcinomas are also positive for broad-spectrum keratins (AE1/AE3 and PANK).
[c] S100-positive dendritic cells are present.

Table 7-8 Spindle-cell/soft-tissue lesions and sarcomas

	AE1/AE3	CAM 5.2	EMA	S100	HMB-45	HHF-35	SMA	DESMIN	H-CALDESMON	CD34	CD31	FVIII	c-kit CD117	CD99	OTHER
Neural															
Perineurioma	neg	neg	POS	Low	neg	Mod	Low	neg		neg	neg	neg	neg	Mod	CLAUD-1 POS[a]
Neurofibroma	neg	neg	POS[b]	POS	neg	neg	neg	neg		High	neg			neg	
MPNST	Low	Low	Low	Mod	neg	Low	Low	neg	neg	Low			neg		GFAP Mod
Schwannoma	Low	neg	Neg[c]	POS	neg	neg	neg	neg		Mod	neg	neg	neg		CD68 POS
Granular cell tumor[d]	neg	neg	neg	POS	neg	neg	neg	neg		neg					CD68 POS Calretinin POS Inhibin POS
Melanoma	rare	rare	neg	POS	High[e]		neg	neg	neg	neg	neg	neg	Mod	Low	Melan-A High FLI-1 neg
Clear cell sarcoma	neg	neg	neg	High	POS	Low	neg	neg		neg	neg	neg	Low	Low	Melan-A Mod
PEComa[f]	neg	neg	neg	Low	POS	POS	POS	High	Mod	Low	neg	neg	VAR[g]		Melan-A POS
Gastrointestinal stromal tumor	neg	neg	neg	Low		Mod	Low	neg	High	High	neg		POS	POS	
Muscle															
Rhabdomyosarcoma	Low	Low	Low	neg	neg	High	Mod	High	neg	Low	neg	neg	neg	Low	Myf4 POS WT-1 Mod FLI-1 neg
Glomus tumor	neg	neg	neg	neg	neg	POS	POS	Low	High	Low	neg	neg	neg		
Leiomyoma or leiomyosarcoma	Low	Low	Mod	neg	neg	POS	POS	High	POS	Low	neg	neg	neg	Low	ER/PR High CD10 Low
Endometrial stromal sarcoma	Mod (focal)	Low (focal)			neg		High	Mod	neg	neg				neg	ER/PR High CD10 High
Vascular															
Angiosarcoma	Low[h]	Low[h]	rare	neg	neg	Low	Low	neg		High	High	High	Low		FLI-1 POS
Kaposi's sarcoma	neg	neg	neg	neg		neg	POS	neg		POS	High	Mod	neg		FLI-1 POS HHV 8 POS

Table 7-8 Spindle-cell/soft-tissue lesions and sarcomas—*cont'd*

	AE1/AE3	CAM 5.2	EMA	S100	HMB-45	HHF-35	SMA	DESMIN	H-CALDESMON	CD34	CD31	FVIII	c-kit CD117	CD99	OTHER
Epithelioid hemangioendothelioma	High	neg	neg	neg	neg	neg	Low	neg		High	High	POS	neg		FLI-1 POS
"Fibrous" Fibrosarcoma	neg	Low	neg	neg		neg	neg	neg		neg				Low	
Solitary fibrous tumor	neg	neg	Low	neg		neg	Low	neg	neg	POS	neg	neg	neg	High	
DFSP	neg	neg	neg	neg	neg	High	Low	neg	neg	POS	neg	neg	neg		
Dermatofibroma	neg	neg		neg	neg	High	High	neg[i]		neg	neg			neg	
Fibromatosis	neg	neg	neg	Mod		High	High	Mod	neg	neg	neg		neg		ER Low
Postoperative spindle cell nodule	Mod	Mod	Low	neg		High	High	Mod	neg	neg			neg		
Myofibroblastic tumors		neg	neg	neg		POS	High	Mod	neg	Mod		neg			ER High PR POS
Atypical fibroxanthoma	neg	neg	neg	neg	neg		Low	neg		Low					CD68 Mod
Other Osteosarcoma	neg	neg	Low	Low		Mod	High	neg	neg					Low	
Chondrosarcoma	neg	neg	Low	POS	neg	neg	neg	neg		neg				Low	
Chondroblastoma	neg	neg	neg	POS		Mod	Low	neg	neg?					POS	
Mesenchymal chondrosarcoma	neg	neg	neg	POS	neg		rare	Low						POS	My4 neg
Extraskeletal myxoid chondrosarcoma	neg	neg	Low	Low		neg	neg	neg		Low			Low	neg	
Alveolar soft part sarcoma	neg	neg		Low	neg	Low	Low	Low		Low	neg		neg	Low	myoD1 neg myogenin neg TFE3 POS[j]
Epithelioid sarcoma	POS	POS	POS	neg	neg	Low	Low	neg		Mod	neg	neg	neg	Low	FLI-1 neg
Synovial sarcoma[k]	High	High	High	Mod	neg	neg	Low	neg	neg	neg	neg	neg	neg	High	WT-1 neg

Table 7-8 Spindle-cell/soft-tissue lesions and sarcomas—*cont'd*

	AE1/AE3	CAM 5.2	EMA	S100	HMB-45	HHF-35	SMA	DESMIN	H-CALDESMON	CD34	CD31	FVIII	c-kit CD117	CD99	OTHER
Adenomatoid tumor	POS	POS	POS							neg	neg			neg	Ber-EP4 High Calretinin POS
Mesothelioma sarcomatoid type[m]	High	POS	Low			POS	High	Low		neg				Low	WT-1[n] Calretinin Low
Meningioma	neg[o]	neg[o]	High	Low	neg	Low	Low	neg		Low	neg	neg		POS	ER neg PR POS PANK Low
Carcinoma: spindle cell[p]	VAR	VAR	VAR	VAR	neg	rare	rare	neg		neg	neg	neg			

[a] Some claudin-1-positive perineurial cells can be present in neurofibromas and schwannomas.
[b] Perineural cells are positive for EMA in neurofibromas.
[c] EMA may be positive in capsule and perineural cells of schwannomas.
[d] Congenital granular cell tumors are positive for CD68 but negative for S100 and NSE.
[e] HMB-45 is less frequently present in spindle cell melanomas and usually negative in classic desmoplastic melanomas. Other markers for melanoma are also less frequently positive in these subsets.
[f] PEComas (perivascular epithelioid cell tumors) include angiomyolipoma, lymphangioleiomyomatosis, clear cell sugar tumor of the lung, clear cell myomelanocytic tumor of ligamentum teres/falciform ligament, and abdominopelvic sarcoma of perivascular epithelioid cells.
[g] Results in the literature are conflicting. Angiomyolipomas are likely not positive for CD117.
[h] Keratin positivity in angiosarcomas is more common in epithelioid types.
[i] Cellular dermatofibroma may show focal desmin immunoreactivity.
[j] Alveolar soft-part sarcomas are characterized by a translocation that fuses the *TFE3* transcription factor gene at Xp11 to a novel gene at 17q25 called *ASPL*. These sarcomas demonstrate nuclear immunoreactivity for TFE3 (as do rare pediatric renal tumors with the same translocation) and this immunoreactivity is not present in other tumors or normal tissues. The characteristic cytoplasmic crystals are composed of monocarboxylate transporter 1 (MCT1) and its chaperone CD147. However, these proteins are found in many other cell types and are not specific for this tumor.
[k] Keratin and EMA positivity are usually only focal in monophasic synovial sarcomas.
[l] Claudin-1 is positive in glandular areas of synovial sarcoma but less so in spindle cell areas.
[m] The immunohistochemical pattern for epithelioid mesotheliomas is given in Table 7-30.
[n] WT-1 may be positive in a minor epithelioid component of sarcomatoid mesotheliomas, but is generally negative in the spindle cells.
[o] Secretory meningiomas are typically cytokeratin 7 positive (CK20 negative) and also positive for CEA. Other subtypes are generally negative for keratin. However, malignant meningiomas may be positive for keratin.
[p] Squamous cell carcinomas with a spindle cell morphology are generally strongly positive for AE1/AE3 (less commonly for CAM 5.2), EMA, and p63. Spindle cell carcinomas of the breast often express markers expressed by myoepithelial cells such as "basal keratins" (including cytokeratin 14 which is included in the group detected by PANK or MNF-116), smooth muscle α-actin, and p63. Poorly differentiated carcinomas with spindle cell morphology may only show focal positivity for keratins and EMA.

Table 7-9 Small blue cell tumors

TUMOR	PANK	CAM 5.2	CK20	EMA	S100	HMB-45	NSE	SYN	CHRO	CD99	SMA	HHF 35	DES MIN	MYF-4	LCA	NFP	WT-1[a]	PAS[b]
Melanoma	rare	rare	neg	neg	POS	High[c]	High	Low	neg	Low	Low	neg	neg		neg	neg		
Esthesioneuroblastoma	Low	Mod		Low	POS		POS	High	Mod	Low			neg	neg?	neg	Mod		
Neuroblastoma	neg	neg	neg	Low	Mod	neg	POS	High	High	neg	neg	neg	neg		neg	High	Low	neg
Small cell carcinoma[d]	POS	Mod	neg	POS	neg	neg	High	Mod	Mod	Low		neg			neg	neg		neg
Merkel cell carcinoma[e]	POS	POS	POS	POS	neg	neg	High	Mod	High	Low			neg?		neg	Mod		neg
Desmoplastic small round cell tumor	POS	POS	neg	POS	Low	neg	High	Low	Low	Mod	Low	Low	POS	neg		neg	POS	POS
Ewing's sarcoma (PNET)	Low	Low		Low	Low	neg	Mod	Low	neg	POS[f]	neg	Low	neg		neg	Low	neg	POS
Medulloblastoma	neg			neg?	Low		POS	POS		Low			Low			neg		
Rhabdomyosarcoma	neg	Mod		neg	Low	neg	Mod	neg	neg	Low	Low	POS	POS	POS	neg	Low	Mod	POS
AML	neg	neg	neg	neg	Low	neg	POS?			Mod					High			
Lymphoma	neg	neg	neg	neg	neg	neg	neg	neg	neg	Var	neg	neg	neg	neg	POS			neg[g]

[a] Polyclonal WT-1.
[b] PAS is a histochemical stain for glycogen. A PAS-D stain confirms the presence of glycogen by treatment of the tissue with diastase, which digests the glycogen and eliminates the positivity. Although used for these tumors in the past, these studies are currently not usually performed.
[c] MART-1 is also frequently positive in melanomas.
[d] Small cell carcinomas of the lung are positive for TTF-1.
[e] Merkel cell carcinomas demonstrate a dot-like perinuclear pattern for most markers.
[f] Significant immunoreactivity is a membrane pattern in the majority of the cells.
[g] Some plasma cell lymphomas may be positive.
Ewing's sarcoma (PNET), desmoplastic small round cell tumor, rhabdomyosarcoma, neuroblastoma, and medulloblastoma have characteristic cytogenetic changes (see Table 7-33).
EM has some advantages over immunohistochemistry in the evaluation of childhood small round blue cell tumors.[11]
Initial panel. Keratin, S100, LCA.
Additional studies may be helpful depending on the histologic appearance and the results of the initial studies.

Table 7-10 Myoepithelial markers in breast carcinoma

MARKER	LOCATION	NORMAL LUMINAL CELLS	MYOEPITHELIAL CELLS	BLOOD VESSELS	MYOFIBROBLASTS	CARCINOMAS[a]	COMMENT
p63	Nucleus	neg	POS	neg	neg	rare	Only nuclear marker Clean background
SMA	Cytoplasm	neg	POS	POS	POS	rare	Positive in most myoepithelial cells
CD10	Membrane	neg	POS	neg	POS	rare	
SMM-HC	Cytoplasm	neg	POS	POS	High	rare	
Calponin	Cytoplasm	neg	POS	POS	Mod	rare	

[a] Rare carcinomas with myoepithelial features (adenoid cystic carcinomas, some spindle cell carcinomas, some basal-like carcinomas, some carcinomas associated with *BRCA1* mutations) can show focal to diffuse positivity for myoepithelial markers.

Myoepithelial markers can be useful for the evaluation of breast lesions:

- Invasive carcinoma versus sclerosing adenosis (frequently involved by DCIS, LCIS, or apocrine metaplasia).
- DCIS versus DCIS with microinvasion. Double immunolabeling with p63 (brown nucleus) and cytokeratin (AE1/AE3—red cytoplasm) can be useful to highlight small nests of tumor cells lacking myoepithelial cells.
- DCIS versus carcinoma invading as circumscribed tumor nests versus lymphovascular invasion.

S100 protein and cytokeratins (e.g. 34β E12) are not recommended for this purpose, as fewer myoepithelial cells are positive and luminal cells are also sometimes positive.

p63 is a good general marker for myoepithelial cells and is particularly helpful in cases with prominent myofibroblasts (e.g., sclerosing lesions) or with blood vessels closely apposed to tumor cells (e.g., papillary fronds in papillary DCIS). In some cases, SMA may be positive in more cells than p63.

Table 7-11 Epidermal lesions of the nipple

	AE1/AE3	CAM 5.2 OR CK7	CK20	EMA	S100	HMB-45	GCDFP-15	CEA p	CEA m	HER2	ER OR PR	MUCICARMINE STAIN
Paget's disease of the nipple	POS	POS	neg	POS	Mod	neg	Mod	Mod	Low	POS	Low	High
Squamous cell carcinoma	POS	Low	Low	POS	Low	neg	neg	Low	Mod	Low	neg	neg
Melanoma	Low	Low	neg	Low	POS	POS	neg	Mod	neg	neg	neg	neg

Most cases of Paget's disease of the nipple are associated with DCIS deeper in the breast and involve the lactiferous sinuses, and approximately half will also have areas of invasion. Rare cases may be difficult to interpret due to the absence of associated disease in the breast or if the initial biopsy is shallow. In some cases, Paget cells may take up melanin and may be difficult to distinguish from melanoma. Toker cells in nipple epidermis are CAM 5.2 and CK7 positive but negative for HER2/neu.

Initial panel. CAM 5.2 (or CK7), HER2, and S100 with additional antibody studies based on these findings, if necessary.

Cases of extramammary Paget's disease are more likely to be CK20 positive and less likely to be positive for HER2/neu (less than 40%).

Table 7-12 Endocervical carcinoma versus endometrial carcinoma

	CK7	CK20	VIM	CEA m	CEA p	P16	HPV (IN SITU)	ER	PR
Endocervical carcinoma	POS	rare	rare	POS	High	POS (difffuse, strong)	High	Low (focal)	Low
Endometrial carcinoma	POS	rare	POS	Low[a]	Mod	Low (patchy, weak)	neg	High (diffuse)	High

[a] 27% of cases have some positivity but primarily in squamous areas and only focally in glandular areas.

Table 7-13 Endometrial stromal sarcoma versus leiomyosarcoma

	CD10	DESMIN	H-CALDESMON	ER/PR
Endometrial stromal sarcoma	High	Mod	neg	High
Leiomyosarcoma	Low	High	POS	High

Table 7-14 Primary ovarian carcinoma versus metastatic carcinomas

	CK7	CK20	DPC4 (SMAD4)	CDX2	ER	CEA m	CEA p
Endometrioid ovarian carcinoma	POS	neg		Low	Mod	Mod	Low
Clear cell ovarian carcinoma	POS	neg		Mod?	Mod		neg
Mucinous ovarian carcinoma	POS (diffuse)	High (patchy)	POS	Mod	Low	Mod	Low
Mucinous breast carcinoma	POS	Low	POS	neg[a]	POS	Mod	Low
Pancreatic carcinoma	POS	High	Mod	Mod	neg	High	POS
Appendiceal carcinoma	Low (patchy)	POS	POS			High	Low
Mucinous colon carcinoma	Low (patchy)	POS (diffuse)	High	POS	neg	POS	POS

[a] Breast cancers, in general, are negative for CDX2. Results for mucinous breast carcinomas have not been reported.

Table 7-15 Ovarian carcinoma versus mesothelioma

	CK7	CK20	CK5/6	CEA m	CEA p	CD15 (LEUM1)	ER	WT-1	CALRET	BER-EP4
Peritoneal mesothelioma	High	neg	POS	neg	neg	rare	rare	High	High	neg
Ovarian serous carcinoma	POS	Low	neg	neg	neg	Mod	POS	POS	Low	POS
Ovarian endometrioid carcinoma	POS	neg	Low	Mod	Low		High	High	Low	POS
Ovarian mucinous carcinoma	POS	High	neg	Mod	Low		Low	neg	Low	

Table 7-16 Trophoblastic lesions

	KERATIN	ALPHA-INHIBIN	HPL[a]	HCG[a]	CD146 (MELCAM)	KI-67[b]	P57[c]	DNA PLOIDY[d]
Choriocarcinoma	POS	POS	Weak (focal)	Strong (diffuse)	POS	69%		
Placental site trophoblastic tumor	POS	POS	Mod (greater than hCG)	Focal (less than HhPL)	POS	>14%		
Epithelioid trophoblastic tumor	POS	POS	Focal	Focal	Focal	>14%		
Placental site nodule	POS	POS	Weak (focal)	Focal	Focal	<1%		
Exaggerated placental site			POS (diffuse)	Focal	POS	0%		
Partial mole	POS	POS	Weak[e] (diffuse)	Weak (diffuse)			POS	Triploid
Complete mole	POS	POS	Weak[e] (focal)	Strong (diffuse)			rare[f]	Diploid (paternal)
Hydropic fetus	POS	POS					POS	Diploid (60%) Triploid (40%)

[a] Evaluated in syncytiotrophoblast.
[b] Implantation-site intermediate trophoblastic cells are evaluated for the number of Ki-67-positive cells. CD146 can be used to help identify these cells using a double label technique. Lymphocytes can also be positive for Ki-67 and should not be counted.
[c] p57 is a paternally imprinted gene, expressed from the maternal gene, which shows decreased expression in complete moles, whose DNA is completely derived from paternal DNA.
[d] Ploidy is usually determined by flow cytometry.
[e] Increases with advancing pregnancy.
[f] In complete moles, p57 positivity is present in villous stromal cells and extravillous trophoblast but absent in intermediate trophoblast lining the villi.
Cytokeratin and alpha-inhibin (present in syncytiotrophoblastic cells and some intermediate trophoblastic cells) are not useful for the differential diagnosis of these lesions, but may be helpful if other types of tumors are in the differential diagnosis.

Table 7-17 Tumors of germ cells and sex-cord stromal tumors

	CK7	CK20	AE1/AE3	CAM5.2	NSE	EMA	PLAP[a] (mem)	AFP	CD30 (Ki-1, Ber-H2)	CD117 (c-kit)	VIM	hCG	HPL	INHIBIN	MELAN-A103	OTHER
Seminoma	Mod	neg	Mod	Low[b]	High	neg	POS	neg	Low	POS	Mod	Low[c]	neg	neg	neg	
Embryonal carcinoma	High	neg	POS	POS	High	Low	High	Low	High[d]	neg	Low	Low	neg	neg	neg	
Yolk sac tumor			POS	POS	High	neg	Mod	High	Low		Low	neg	neg	neg	neg	
Choriocarcinoma			POS	POS	Mod	Mod	Mod	neg	neg		neg	POS	POS	POS	neg	
Intratubular germ cell neoplasia				neg			POS			POS	neg					
Spermatocytic seminoma				Mod (focal)			neg		Mod							
Leydig cell tumor	Mod	Mod	Mod	Mod		Low	Low	neg			POS			POS	High	
Granulosa cell tumor	Mod	Low	Low	Mod	Low	neg	neg	neg		neg	POS			POS	High	WT-1 High HHF35 High S100 Mod
Sertoli cell tumor			Mod	Mod		POS	neg				High			POS		

[a] PLAP is expressed in embryonic germ cells, but not in normal spermatogonia, spermatocytes, and spermatids.
[b] CAM5.2 is present as a strong dot-like paranuclear positivity. 80% of mediastinal seminomas are positive for CAM5.2 compared to 20% to 30% of testicular seminomas.
[c] hCG may be positive in trophoblasts in seminomas.
[d] Only 35% of embryonal carcinomas metastatic to lymph nodes after chemotherapy are positive for CD30.

Table 7-18 Adrenal and kidney tumors

	AE1/AE3	CK7	CK20	PANK	CAM5.2	MUC-1 (EMA)	S100	CHROM	SYN	MELAN-A103[a]	INHIBIN	NSE	NFP	AMACR	VIM	OTHER	IRON STAIN
Adrenal tumors[b]																	
Cortical adenoma	neg	neg	neg	Low	Low	neg	neg	neg	POS	POS	High	High	neg	neg	High	TTF-1 neg CD10 neg	
Cortical carcinoma					neg		neg	neg	High	POS	POS		neg				
Pheo/ paraganglioma		neg	neg	neg	neg	neg	High[c]	POS	POS	neg	neg	POS	POS		Mod	GFAP mod	
Kidney tumors																	
Renal cell carcinoma: clear cell	High	Low	neg	High	High	High (diff)	Low	neg	neg	neg	neg	Mod	neg		High	p63 neg TTF-1 neg GFAP low RCC POS CD10 POS	Focal, course
Papillary	POS	High	Low	POS		Mod (mem)								POS		RCC POS CD10 POS	Focal, coarse
Chromophobe	High	High	neg	POS		POS (mem)								neg	neg	RCC Mod CD10 neg	Diff, strong
Oncocytoma[d]	Mod	High	neg	POS												RCC neg CD10 low	Focal, weak
Transitional cell carcinoma	Mod	POS	High	POS	POS	POS	neg	neg	neg	neg		Low		Low	Low	p63 POS CD10 mod	

diff, diffuse positivity; mem, positivity located on membrane.

[a] Positivity is also present with MART-1.

[b] Clear cell renal cell carcinoma (RCC) metastatic to the adrenal can sometimes be confused with an adrenal cortical tumor (thus, the older term for clear cell carcinoma of "hypernephroma"). RCC has clear cytoplasm (compared to the bubbly cytoplasm of the adrenal cortex) and blood lakes are typically present. Glycogen is present in RCC and absent in adrenal lesions (demonstrated by PAS with and without diastase). Cytokeratin and EMA are useful IHC markers.

[c] Positivity is present in sustentacular cells. These cells may be absent in malignant tumors.

[d] 50% of oncocytomas have a punctate/dot-like pattern for CK8 or CK18 which is not seen in RCC. EM may be helpful to distinguish oncocytoma from chromophobe RCC (see Table 7-32).

Renal cell carcinoma subtypes have typical cytogenetic abnormalities (see Table 7-33).

CD117 (c-kit) has been reported to be positive in almost all papillary renal cell carcinomas (cytoplasmic) and chromophobe carcinomas (membrane) but is not present in clear cell carcinomas. Mutations in c-kit were only found in papillary carcinomas.

Table 7-19 Tumors of bladder, prostatic, or renal origin

	CK7	CK20	KERATIN HMW	PSA	PAP	AMACR	CEA m	CEA p	P63	CA125	MUCI
Prostatic carcinoma	Low	Low	neg	High	POS	POS	neg	Mod	neg	neg	neg
Transitional cell carcinoma	POS	High	Mod	neg	neg	Low	Mod	Mod	High	neg	neg
Bladder adenocarcinoma	High	High	neg	neg	neg		Mod	High		Low	POS
Renal cell carcinoma: clear cell	Low	neg	neg	neg			Low	neg	Low	neg	neg
Rectal adenocarcinoma	Low	POS	neg	neg	neg		POS	POS		neg	POS
Seminal vesicle carcinoma	High	neg		neg	neg		VAR	POS		High	

Table 7-20 Prostate carcinoma versus other lesions

	34β E12 (BASAL CELLS)	P63 (BASAL CELLS)	AMACR (504S) (GLANDULAR CELLS)
Benign glands	POS	POS	neg
PIN	POS	POS	High
Invasive carcinoma	neg	neg	POS

Antibody cocktails. These antibodies can be combined to facilitate the evaluation of small lesions:
34β E12 + p63 labels a greater number of basal cells than either marker alone
AMACR + p63 and/or 34β E12 facilitates the identification of small foci of invasive carcinoma.

Table 7-21 Hepatic tumors

	CK7	CK20	AE1/AE3	CAM 5.2	KERATIN HMW	CEA m	CEA p	TTF-1	HEP	AFP	CD10	CHROM	MUCI	BILE	CIRRHOSIS	HBV
Hepatocellular carcinoma (HCC)	Low	neg	Low	POS	neg	neg	High[a]	High[b] (cyt)	High	Mod	High[a]	neg	rare	May be present	65–90%	50%
Hepatoblastoma			Low	POS		Low	High[a]		POS	High		Low			absent	rare
HCC: fibrolamellar	Mod ?	neg					POS[a]		POS	neg ?			neg	May be present	absent	rare
Cholangiocarcinoma	POS	Mod	POS	POS	High	High	POS	neg	neg	neg	neg		75–100	neg	rare	rare
Metastatic carcinoid tumor	Low	Low	High	POS		Mod	Mod	Low[c] (nuc)	neg		Low	POS		neg	absent	absent

cyt = cytoplasmic immunoreactivity; nuc = nuclear immunoreactivity.
[a] Bile canalicular pattern. Other carcinomas have a membrane or cytoplasmic pattern.
[b] TTF-1 is seen in the cytoplasm (unlike the nuclear pattern seen in lung and thyroid carcinomas).
[c] Carcinoids arising at sites other than lung are very unlikely to be positive for TTF-1. Lung carcinoids may be positive and are more likely to express CK7.
Sinusoids of HCC show diffuse CD34 positivity in 80% to 90% of cases, but this is not seen in normal liver. CD34 positivity can also be seen in focal nodular hyperplasia. Metastatic carcinomas can show diffuse positivity in 20% of cases, but the positive endothelial cells are present throughout the tumor and the cells do not surround nests of tumor cells, as is seen in HCC.
Reticulin stains can be helpful in the evaluation of fine needle aspirates or core needle biopsies of liver lesions. HCC has an abnormal pattern of absent, decreased, or expanded trabecula, whereas benign lesions show a normal trabecular pattern.
Metastatic carcinomas can usually be distinguished from HCC by frequent expression of CK7, only rare expression of HepPar1, the absence of a bile canalicular pattern for CEA p and CD10, and the absence of cytoplasmic positivity for TTF-1.
Metastatic carcinomas to the liver often cannot be reliably distinguished from cholangiocarcinomas by histologic appearance or immunohistochemical pattern, with the exception of colorectal carcinomas. If the patient has a known primary carcinoma, it is most helpful to compare the two tumors.

Table 7-22 Thyroid and parathyroid lesions

	KER HMW	CK19	HBME[a]	GALECTIN-3	CALCITONIN	SYN	CHRO	RET	P27	PPAR GAMMA	THY	TTF-1	S100[b]	CEA M	CEA P	CD57	RB PROTEIN	VIM	OTHER
Thyroid lesions																			
Hyperplastic nodule		Mod	Low	Low	neg				POS	neg	POS	POS				Low	POS		
Follicular adenoma	neg	Mod	Low	Low	neg	Mod	neg	neg	POS	Low 10%	POS	POS	Low			Low	POS	POS	
Follicular carcinoma	neg	Mod	Mod	Low	neg	High	neg	neg	POS	Low 30%	POS	POS	Mod	neg	Low	Mod	neg?	POS	
Papillary carcinoma: follicular variant		POS	POS	Mod	neg			Low	Mod	Low 10%	POS	POS				High	neg		
Papillary carcinoma	POS	POS	High	POS	neg	High	neg	Low	POS	Low 10%	POS	POS	High	neg	Mod	POS	neg	POS	p63 POS
Medullary carcinoma	neg			Mod	POS	POS	POS			neg	Low	POS		POS	Mod	Mod	Mod	High[c]	
Anaplastic carcinoma[d]		Mod									rare	rare							
Parathyroid lesions																			
Parathyroid adenomas and carcinomas					Low	Low	POS		High[e]	neg	neg	neg	Low				POS	neg/ weak	PTh POS RCC POS Cyclin D1 POS

[a] Tumors with Hürthle cell changes may be negative for HBME.
[b] Hürthle cells (both benign and neoplastic) are positive for S100 (nuclear and cytoplasmic).
[c] Spindle cells may be positive for vimentin.
[d] Anaplastic thyroid carcinomas are frequently negative for TTF-1, thyroglobulin, and CK20.
[e] p27 is low in parathyroid carcinomas.

Thyroid adenomas, follicular carcinomas, papillary carcinomas, and medullary carcinomas are CK7 positive and CK20 negative. Variable immunoreactivity has been reported for CK7 in anaplastic carcinomas.

Metastatic carcinomas to the thyroid will be negative for thyroglobulin, TTF-1 (except for lung carcinomas), and calcitonin.

DDIT3 and ARG2 are new markers that may prove helpful for distinguishing follicular carcinoma (approximately 70% to 80% positive) from adenoma (90% negative).

Table 7-23 B-cell neoplasms

	B cell markers															
	CD45 LCA	CD19 B4	CD20 L26	CD22	CD79a	sIg	cIg	CD5 Leu 1	CD10 CALLA	CD23	CD43 Leu 22	CD34	bcl-2	bcl-6	CD138 SYNDECAN	CYCLIN D1	OTHER
Precursor lymphoblastic lymphoma/ leukemia	+/–	+	+/–	+/–	+ cyt	–	+M	–	+[a]	–	+/–	+/–	–	–	–	–	TdT + CD99 +
Small lymphocytic lymphoma/CLL	+	+	+ wk	+ wk	+	+M/D wk	–/+	+	–	+	+/–	–	+	–	–	–	CD11c+ wk CD79b – FMC7 –
Mantle cell lymphoma	+	+	+	+	+	+M/D	–	+	–	–	+	–	+	–	–	+	CyclinD + FMC +
Marginal zone lymphoma (MALT)	+	+	+	+	+	+	+/–	–	–	–	+/–	–	+	–	–/+[b]	–	CD11c +/– CD21+ CD35+
Follicular lymphoma	+	+	+	+	+	+M	–	–	+	–/+	–/+	–	+/–	+	–	–	CDw75 +
Burkitt lymphoma and Burkitt-like lymphoma	+	+	+	+	+	+M	+/–	–	+	–	+/–	–	–	+		–	TdT- MIB-1 100% EBER in situ in 52% MYC[c]
Mediastinal large B-cell lymphoma	+	+	+	+	+/–	–	–	–	–			–		+	–	–	CD30+/– wk
Large B-cell lymphoma	+/–	+	+	+	+	+/–	+/–	–/+	–/+		–/+	–	–/+	+/–		–	CD30 +/– MIB–1 >40%
Lymphoplasmacytic lymphoma	+/–	+	+	+	+	+M/D	+M/G st	–	–	–	+/–				–/+[b]	–	

Table 7-23 B-cell neoplasms—*cont'd*

B cell markers																	
	CD45 LCA	CD19 B4	CD20 L26	CD22	CD79a	sIg	cIg	CD5 Leu 1	CD10 CALLA	CD23	CD43 Leu 22	CD34	bcl-2	bcl-6	CD138 SYNDECAN	CYCLIN D1	OTHER
Hairy cell leukemia	+	+	+	+	+	+		–	–	–	–	–	–	–	–	–/+	DBA.44+ CD79b– CD11c+ CD103+ CD25+ st FMC7+
Primary effusion lymphoma	+	–	–		–	–	–					–		–	+	–	CD30 (Ki–1)+ HHV8+ EBER +/–
Plasmacytoma/ myeloma	–/+	–	– /+	–	+	–	+G/A st	–	–/+	–	+/–		–		+	–/+	CD56+ CD38 + EMA +

cyt = cytoplasmic immunoreactivity; M, D, G, A, type of heavy Ig chain present; st = strong immunoreactivity; wk = weak immunoreactivity.
[a] Lymphoblasts in t(4;11)(q21;q23) ALL are CD10 negative and frequently CD24 negative.
[b] Positive in plasma cell component.
[c] The *myc* gene (8q24) is translocated to Ig genes:
t(8;14) (heavy chains) 85% of cases
t(2;8) (kappa light chain)
t(8;22) (lambda light chain).

Table 7-24 T-cell neoplasms

	CD45 LCA	TCR	CD2 TE/T11	CD3 T3	CD43 Leu 22	CD5 Leu 1	CD7 LEU 9	CD4 T4	CD8 T8	CD25 IL2R	TIA-1	Granzyme B	CD56 NCAM	CD30 Ki-1	TdT	ALK	OTHER
Precursor lymphoblastic lymphoma/ leukemia	+	–	+/–	+	+/–	+/–	+	+/–	+/–	+/–	–	–	–	–	+	–	CD34+ CD99+ CD1a +/–
T-cell prolymphocytic leukemia	+	+	+	+wk	+	+	+	+/–	–/+	+/–	–	–	–		–	–	CD1a–
Adult T-cell lymphoma/ leukemia	+	+	+	+	+	+	–/+	+	–	+	–	–	–	+/–		–	
Mycosis fungoides and Sézary syndrome		TCRβ+	+	+	+	+	–	+	–/+	–/+	–/+	+/–	–	–	–	–	HECA+
Peripheral T-cell lymphoma, NOS	+	+	+/–	+/–	+	+/–	–/+	+/–	–/+		+	+/–	–/+	+ (large cells)	–	–	
Hepatosplenic T- cell lymphoma		TCRδ1+ TCRαβ-	+	+	+	–	+/–	–	–	–	+	–	+/–	–	–	–	CD57– CD16–/+ LMP–1– Perforin –
Panniculitis-like T-cell lymphoma CD56+		–	+ CD3ε	+	+			–	–	–	+	+	+	–	–	–	CD95+
CD56–		+	– CD3ε	–	+			–	+	–	–/+	+	–	–	–	–	CD95–
Angioimmunoblastic lymphoma	+	+	+	+	+	+	+	+	–/+	–	+	+	–	–	–	–	CD10+/– CD57+ bcl-6+–
Enteropathy-type T-cell lymphoma	+			+	+	–	+	–	–/+	–	+/–	+/–	+ (small cells)	+ (large cells)	–	–	CD103+

Table 7-24 T-cell neoplasms—*cont'd*

	CD45 LCA	TCR	CD2 TE/T11	CD3 T3	CD43 Leu 22	CD5 Leu 1	CD7 LEU 9	CD4 T4	CD8 T8	CD25 IL2R	TIA-1	Granzyme B	CD56 NCAM	CD30 Ki-1	TdT	ALK	OTHER
Anaplastic large cell lymphoma (Ki-1 lymphoma)	+/–	+/–	+/–	–/+	+/–	–/+	–/+	+/–	–/+	+/–	+/–	+/–	–/+	+ (mem, Golgi)	–	+/–[a] (cyt, nuc)	Clusterin+[b] EMA+/– Perforin +/– EBER– BSAP–
Extranodal NK/ T-cell lymphoma, nasal type	+	–	+	– CD3ε+ (cyt)	+	–	–/+	–	–	–	+	+	+	–/+	–	–	EBER+ CD16+ CD57–
Blastic NK-cell lymphoma		–	–/+	–	+/–		–/+	+/–		–			+	–	+/–	–	CD33– Myelo–

cyt = cytoplasmic; nuc = nuclear; wk = weak immunoreactivity.
[a] Only positive in systemic ALCL (subset); negative in primary cutaneous ALCL.
[b] Expressed in all cases of systemic ALCL but less commonly in primary cutaneous ALCL and very rarely in diffuse large B-cell lymphoma, peripheral T cell lymphoma, and NS HD.

Table 7.25 Hodgkin's lymphoma

	CD45 LCA	CD20 L26	CD3 T3	CD15 LEUM1	CD30 Ki–1	EMA	sIg	CD79a	CDw75	Oct2	BOB.1	BSAP	LMP1	OTHER
Classical Hodgkin lymphoma (HL)	–	–/+	–	+/–	+	– Rare	–	–/+	–	–	–/+	+	+/–	traf–1 + bcl–2 +
Nodular sclerosis HL	–	–/+	–	+/–	+	– Rare	–	–/+	–	–	–/+	+	–/+	
Lymphocyte-rich HL	–	–/+	–	+/–	+	– Rare	–	–/+	–	–	+/–	+	+/–	
Mixed cellularity HL	–	–/+	–	+/–	+	– Rare	–	–/+	–	–	–/+	+	+/–	
Lymphocyte-depleted HL	–	–/+	–	+/–	+	– Rare	–	–/+	–	–	–/+	+	+ (if HIV +)	
Nodular lymphocyte predominant HL	+	+	–	–	–/+	+/–	+	+ wk	+/–	+	+	+	–	bcl-6 + bcl-2 –

wk = weak.

Table 7-26 Markers for tumors of unknown origin

TYPE OF TUMOR	IMMUNOHISTOCHEMICAL MARKER(S)	POTENTIAL TREATMENT AND COMMENTS
Breast	ER/PR HER-2/neu GCDFP-15	ER/PR+ tumors can be palliated with hormonal treatment HER-2/neu+ carcinomas can be treated with Herceptin[a] GCDFP-15 is not very sensitive, as many breast carcinomas are negative The most common type of breast carcinoma to present as an occult primary is invasive lobular carcinoma Rare women will present with positive axillary nodes with no known primary. Most of these women will have breast cancer. The prognosis is the same, whether or not the primary is detected
Carcinoid tumor	Chromogranin	Chromogranin positivity should be strong and diffuse. Focal and/or weak positivity can be seen in many carcinomas Metastatic breast cancer and prostate cancer can closely resemble carcinoid tumor and both can be positive for chromogranin Carcinoid tumors can be palliated with tumor-directed pharmaceuticals
Germ cell tumors	PLAP	PLAP is not specific but a germ cell tumor is unlikely if it is negative. Inhibin is more likely to be positive in choriocarcinomas Chemotherapy for possible cure
GIST	c-kit (CD117)	Treatment with Gleevec[b]
Lung adenocarcinoma	TTF-1	10% to 20% of patients will have specific activating mutations in EGFR (detected by PCR) and these patients may respond well to treatment with gefitinib[c]
Lymphoma	LCA, B- and T-cell markers	Treatment for cure or long-term palliation
Prostate	PSA or PrAP	Hormonal therapy effective for palliation
Small cell carcinoma	TTF-1 (if of lung origin)	Diagnosis made by H&E appearance Neuroendocrine markers are often positive. p63 is usually negative Chemotherapy for palliation

Table 7-26 Markers for tumors of unknown origin—*cont'd*

TYPE OF TUMOR	IMMUNOHISTOCHEMICAL MARKER(S)	POTENTIAL TREATMENT AND COMMENTS
Squamous cell carcinomas	CK5/6, p63 p16 or HPV	Not specific, but characteristic. H&E appearance usually sufficient to reveal keratin production or intercellular bridges Radiation therapy often effective HPV or p16 is most commonly present in carcinoma of the cervix, but may be seen in carcinomas at other sites (e.g., basaloid carcinomas of the tonsil) Approximately 26–38% of patients with a cervical lymph node metastasis of unknown primary will have an occult tonsillar carcinoma. Complete sampling of the tonsil may be necessary to identify these small carcinomas
Thyroid: papillary or follicular carcinoma	Thyroglobulin and TTF-1	Lung carcinomas are also TTF-1 positive, but will be thyroglobulin negative Highly effective treatment for cure with radioactive iodine
Thyroid: medullary carcinoma	Calcitonin	Palliative treatment with tumor-directed radionucleotides If familial, important for counseling other family members
Trophoblastic tumors	Inhibin	Inhibin is not specific, but a trophoblastic tumor is unlikely if it is negative Chemotherapy for possible cure

[a] Trastuzumab (Herceptin) is a monoclonal antibody directed against the HER-2/neu receptor.
[b] Imatinib mesylate (STI571, Gleevec™, Glivec™) is a small molecule tyrosine kinase inhibitor used for CML, ALL (Ph+), and GIST.
The KIT protein is encoded by the *c-KIT* proto-oncogene and is a transmembrane receptor protein with tyrosine kinase activity. Mutated proteins may or may not respond to therapy with Imatinib. Mutations that render KIT independent of its ligand, SCF (stem cell factor), have been found in GIST, AML, germ cell tumors and systemic mastocytosis. Wild-type KIT and KIT with mutations in the juxtamembrane domain (the intracellular segment between the transmembrane and tyrosine kinase domains) are found in GISTs and are sensitive to imatinib. Other tumor types are associated with mutations in the enzymatic domain and the altered protein is generally not sensitive to imatinib.
[c] Gefitinib (Iressa) is a tyrosine kinase inhibitor that is effective against a small subset of lung adenocarcinomas with specific activating mutations.
Pathologists frequently receive specimens with metastatic tumors.[12] Often, the site of origin is known to the clinician but this information is not provided to the pathologist. A good clinical history is frequently more successful for correct classification than a battery of immunoperoxidase studies. The CK7/CK20 pattern is generally helpful to narrow down the potential site of origin of carcinomas (see Tables 7-3 to 7-7). Additional studies can then be used to identify specific types of carcinoma. The most important tumors to identify are those with specific therapeutic treatments for cure or palliation.

Table 7-27 Markers for poorly differentiated tumors

TYPE OF TUMOR	IMMUNOHISTOCHEMICAL MARKER	COMMENTS
Carcinoma	Broad-spectrum keratins AE1/AE3 or PANK (MNF-116)	Some carcinomas may express unusual keratin subtypes. If negative, try other keratin types (e.g., CAM 5.2). The CK7/CK20 pattern may be helpful in determining the likely site of origin Some non-carcinomas can have an epithelioid appearance and strongly express keratins (e.g., epithelioid angiosarcoma, epithelioid sarcoma, mesothelioma)
Melanoma	S100 protein	S100 is strongly positive in the vast majority of melanomas Some carcinomas (esp. breast) and sarcomas are also positive for S100 and additional markers may be required HMB-45 and MART-1 are expressed by most epithelioid melanomas but may be focal or absent in non-epithelioid melanomas (e.g., spindle cell or desmoplastic melanomas)
Lymphoma	Leukocyte common antigen (LCA)	Present in almost all non-Hodgkin's lymphomas. May be absent in 30% of anaplastic (Ki-1) large cell lymphomas. These lymphomas are keratin negative but may express EMA. These tumors will be positive for CD30 (Ki-1) and ALK

Table 7-28 Scoring of the EGFR (HER 1) test

SCORE	INTENSITY OF MEMBRANE STAINING	% OF CELLS POSITIVE
0	No staining	0
1+ (Positive)	Weak	≥1%
2+ (Positive)	Moderate	≥1%
3+ (Positive)	Strong	≥1%

The EGFR pharmDx™ assay has been approved by the FDA to select patients with colorectal carcinoma for treatment with a monoclonal antibody to EGFR (cetuximab or Erbitux). This test has not been shown to be superior to other comparable tests.

Unlike HER2/neu, the mechanism of overexpression of EGFR does not appear to be gene overexpression.

Table 7-28 illustrates the suggested method for scoring this test.

Immunoreactivity can be membrane or cytoplasmic. Only membrane immunoreactivity is scored, but can be partial or complete.

In clinical trials, 75% to 85% of colorectal carcinomas have been positive (1+ to 3+). Patients with positive results treated with cetuximab alone or in combination with other agents have shown clinical responses (11% to 23%). Patients with carcinomas with scores of 0 for EGFR were not treated.

No correlation has been found between the degree of tumor response and the percentage of EGFR-positive cells or the intensity of staining.

Note: Many normal cells are also positive for EGFR (notably hepatocytes and basal squamous cells).

Table 7-29 Differential diagnosis of epithelial mesothelioma and lung adenocarcinoma

	EPITHELIAL MESOTHELIOMA	LUNG ADENOCARCINOMA
Immunohistochemistry		
AE1/AE3 keratin	POS (perinuclear)[a]	POS (membrane)[b]
Calretinin	POS	neg
WT1 (clone 6F-H2)	POS (nuclear)[c]	neg[d]
CEA (polyclonal)	neg	High[e]
LeuM1 (CD15)	neg	High
TTF-1	neg	High
Mucins		
Mucicarmine	3–4%	60%
PAS-D	<3%	65%
Alcian blue	30%	POS
Alcian blue + hyaluronidase	Staining lost	Staining preserved
Ultrastructure (EM)		
Microvilli	Elongated, serpiginous, and branched	Short, blunt, rigid appearing
Length to diameter ratio	10 to 16:1	4 to 7:1
Cytogenetics		
	Deletions of 1p, 3p, 17p, loss of 9 and 22	Deletions of 3p, highly variable changes

[a] Keratin immunoreactivity is accentuated around the nucleus and is present in the cytoplasm, without prominent membrane accentuation.
[b] Keratin immunoreactivity is diffusely present in the cytoplasm with membrane accentuation in some cells.
[c] WT1 immunoreactivity is nuclear.
[d] Metastatic adenocarcinomas are generally negative for WT1 except for ovarian serous carcinomas and some renal carcinomas (see Table 7-5).
[e] Most metastatic adenocarcinomas will be positive for CEA, but there are some exceptions (see Table 7-5).
Tissue should be obtained for EM and cytogenetics, if possible.
Initial panel. AE1/AE3, calretinin, WT-1 (clone 6F-H2), CEA, Leu-M1, and TTF-1 with additional studies ordered in difficult cases.
Other antibodies generally reported as negative in epithelial mesotheliomas and positive in lung adenocarcinomas include the following: MOC-1, B72.3, Ber-EP4, and BG-8. Cytokeratins 5 and 6 are reported to be positive in mesotheliomas and negative in lung carcinomas. However, in our experience, these markers have proven less useful than those listed above. The use of EMA is controversial. Strong membrane positivity is characteristic of epithelial mesothelioma, whereas cytoplasmic positivity is characteristic of adenocarcinomas.
Less is known about the immunophenotype of pure sarcomatoid mesotheliomas. The spindle cells are positive for cytokeratin, but are less frequently positive for the other markers as compared to the epithelioid cells. Tumors that can, on occasion, resemble mesotheliomas are generally negative for cytokeratins, with the notable exceptions of some cases of angiosarcoma, epithelioid hemangioendothelioma, synovial sarcoma, epithelioid sarcoma, and leiomyosarcoma (see Table 7-8).

Table 7-30 Antibodies for immunohistochemistry

General markers

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Alpha fetoprotein* *(AFP, α_1-fetoprotein)*	Glycoprotein present in fetal liver *(cytoplasm, granular)*	Fetal liver, regenerating liver cells	HCC (but not the fibrolamellar variant), hepatoblastomas, yolk sac tumors, embryonal carcinoma (but less commonly)	HCC (+/–) versus other cell types (however, AFP is rarely present in other carcinomas such as breast and ovary) Yolk sac tumors (+) versus other germ cell tumors (–/+).	Correlates with extracellular hyaline eosinophilic globules in yolk sac tumors
Alpha-1-antitrypsin *(AAT, α_1-AT)*	Glycoprotein inhibiting proteolytic enzymes produced in the liver *(cytoplasm)*	Histiocytes, reticulum cells, mast cells, Paneth cells, salivary gland	HCC, germ cell tumors, true histiocytic neoplasms, colon and lung carcinoma, others	Accumulates in liver cells in AAT deficiency	Not specific for tumor type. CD68 is somewhat more specific for macrophages
Alpha smooth muscle actin* *(SMA, SM-ACT)*	Smooth muscle isoform of actin *(cytoplasm)*	Smooth muscle, myoepithelial cells, blood vessel walls, pericytes, some stromal cells of intestine, testis, and ovary, myofibroblasts in desmoplastic stroma Not in striated muscle or myocardium	Smooth muscle tumors, myofibroblastic tumors, PEComas, glomus tumors, KS, some spindle cell carcinomas (e.g., with features of myoepithelial cells)	Identification of smooth muscle differentiation (muscle or myofibroblasts) in tumors Sclerosing lesions (myoepithelial cells present) versus invasive carcinoma, in the breast	Good marker for myoepithelial cells of the breast but also positive in myofibroblasts in stroma. p63 is only positive in myoepithelial cells
AMACR* *(P504S, alpha-methylacyl-CoA racemase)*	Mitochondrial and peroxisomal enzyme involved in the metabolism of branched-chain fatty acid and bile acid intermediates *(cytoplasm)*	Not present in normal tissues	Colorectal carcinoma (92%), colonic adenomas (75%), prostate carcinoma (83%), PIN (64%), breast cancer (44%), ovarian carcinoma, TCC, lung carcinoma, RCC, lymphoma, melanoma	Can be combined with p63 to distinguish prostate carcinoma (AMACR +, p63 absent in basal cells) from benign mimics (AMACR –, p63 present in basal cells)	
Androgen receptor *(AR)*	Mediates the function of androgens *(nucleus)*	Prostate, skin, oral mucosa	Osteosarcoma, prostatic carcinoma, breast carcinoma, ovarian carcinomas, others		
B72.3 *(Tumor-associated glycoprotein 72, TAG-72, CA 72-4)*	Oncofetal glycoprotein, may be a precursor of the MN blood group system, sialosyl-Tn antigen *(cytoplasm, membrane)*	Not present in most benign adult epithelial cells (may be present in secretory endometrium), apocrine metaplasia, and fetal GI tract	Adenocarcinomas (esp. ovary, colon, breast)	Adenocarcinoma (+ >90%) versus mesothelioma (5%) or mesothelial cells (–)	Other markers are more useful for this purpose

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
bcl-2*	Protein involved in inhibition of apoptosis *(membrane, cytoplasm)*	Medullary lymphocytes and epithelial cells of the normal thymus, mantle and T zone small lymphocytes	Synovial sarcoma, solitary fibrous tumor, myofibroblastic tumors, schwannoma, neurofibroma, granular cell tumor, GIST, KS, melanoma Small lymphocytic lymphoma/CLL, mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma (MALT), some large B-cell lymphoma	Synovial sarcoma (+/–) versus mesothelioma (–) Thymic carcinomas strongly express bcl-2 compared to thymomas Small lymphocytic lymphoma, mantle cell lymphoma, and marginal zone lymphoma (MALT) (+) vs reactive follicles (–)	The *bcl-2* gene is involved in the t(14;18) found in follicular lymphomas
Ber-EP4 *(Epithelial specific antigen (ESA), Ep-CAM)*	Glycoprotein *(membrane)*	All epithelial cells except superficial layers of epidermis	Most carcinomas	Adenocarcinoma (+; strong and diffuse in 60–100%) versus mesothelioma (– or focal in 26%)	Other markers are better for distinguishing adenocarcinoma from mesothelioma
Beta-amyloid *(6F/3D)*	Amyloid present in Alzheimer's disease (AD) and in cerebral amyloid angiopathy *(extracellular)*	None	Senile plaque core in AD, amyloid cores, neuritic plaques, neurofibrillary tangles	Diagnosis of AD, other diseases	Found in AD, Lewy body dementia, Down's syndrome, hereditary cerebral amyloidosis (Dutch type)
Beta-catenin	Component of the adherens junction that binds to e-cadherin and functions in cell adhesion and anchoring the cytoskeleton; signaling molecule of the Wnt/wingless pathway *(membrane, cytoplasm)*	Urothelium, breast epithelium, colon, esophagus, stomach, thyroid	TCC, colonic adenocarcinomas and adenomas, breast carcinoma, esophageal squamous cell carcinoma, head and neck squamous cell carcinomas, gastric carcinoma, ovarian carcinoma, thyroid carcinoma, prostate carcinoma, HCC, brain neoplasms	Aberrant nuclear expression in solid-pseudopapillary tumors of the pancreas (95%) and pancreatoblastomas (78%)	
Beta-2 micro-globulin	Immunoglobulin associated protein *(extracellular deposits of amyloid)*	Plasma cells		Identification of amyloid in patients on dialysis	Amyloid tends to accumulate around joints and in the GI tract

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
BG8	Lewis blood group y antigen *(cytoplasm)*	Red blood cells, endothelial cells	Adenocarcinomas (95%), rare mesotheliomas (about 5%)		Other markers are better for distinguishing adenocarcinoma from mesothelioma
Blood group antigens	A, B, and H antigens *(membrane)*	Epithelial cells and red blood cells, endothelial cells	Lost or abnormally expressed in many carcinomas	Can be helpful to identify potentially misidentified specimens	
CA125* *(OC125)*	Mucin-like glycoprotein, antibody to ovarian carcinoma antigen *(luminal surface)*	Epithelial cells, mesothelial cells	Adenocarcinomas of ovary, breast, lung (bronchioloalveolar), and others (rarely colon), TCC, the uterus, squamous cell carcinoma, seminal vesicle carcinoma, anaplastic lymphoma	Seminal vesicle carcinoma (+) versus prostate carcinoma (–)	Used as a serum marker for monitoring ovarian cancer
CA19-9 *(Carbohydrate antigen 19-9)*	Antigen of sialyl Lewis[a]-containing glycoprotein; antibody to colon carcinoma *(cytoplasm)*	Epithelial cells of breast, colon, kidney, liver, lung, pancreas, salivary gland, others	Adenocarcinomas of GI tract, pancreas, ovary, lung, and bladder, rare in mesotheliomas Chronic pancreatitis		Used as a serum marker for monitoring gastrointestinal and pancreatic carcinomas
Calcitonin*	Peptide hormone produced by C cells *(cytoplasm and extracellular amyloid)*	C cells of the thyroid	Medullary carcinoma of the thyroid (within tumor cells and in amyloid)	ID of C-cell hyperplasia ID of medullary thyroid carcinoma	Used as a serum marker for medullary carcinoma
Caldesmon* *(h-caldesmon)*	Actin and calmodulin binding protein in smooth muscle *(cytoplasm)*	Vascular and visceral smooth muscle cells, some myoepithelial cells of the breast	Smooth muscle tumors, PEComa, GIST	Smooth muscle tumors (+) vs myofibroblastic lesions (–) or endometrial stromal tumors (–)	
Calponin *(CALP)**	Protein that binds to calmodulin, F-actin, and tropomyosin to regulate smooth muscle contraction *(cytoplasm)*	Vascular and visceral smooth muscle cells, myoepithelial cells of the breast, periacinar and periductal myoepithelial cells of the salivary gland	Myoepithelioma, some smooth muscle tumors, myofibroblastic lesions	May be helpful to identify myoepithelial cells in breast lesions	SMA is a better marker of myofibroblasts

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Calretinin*	Intracellular calcium-binding protein of the troponin C superfamily with an EF-hand domain *(cytoplasm, nucleus)*	Subsets of neurons, pineal cells, germinal epithelium of ovary, mesothelial cells, keratinocytes, breast, sweat glands, neuroendocrine cells, thymus	Epithelial mesotheliomas (less + in sarcomatoid type), adenomatoid tumor, some lung squamous cell carcinomas, rare adenocarcinomas, mesenchymal tumors (e.g., synovial sarcoma), granular cell tumor, Leydig cell tumor, granulosa cell tumor	Epithelial mesotheliomas (>90%) versus adenocarcinoma (<10%)	Useful marker in that it is positive in mesothelioma and usually negative in carcinomas
Carcinoembryonic antigen* *(CEA, CD66e)*	Glycoproteins with immunoglobulin-like regions found in fetal tissues *(cytoplasm)*	Fetal tissues	Adenocarcinomas (liver, colon, pancreas, bile duct, and lung more than breast, liver, ovary), TCC, medullary carcinoma of the thyroid Usually absent in RCC, prostate carcinoma, and papillary or follicular thyroid carcinomas	Adenocarcinoma (+) versus mesothelioma (–) HCC: polyclonal CEA has a canalicular pattern	Different reactivity patterns occur with different antibodies and with polyclonal versus monoclonal antibodies
CD5* *(Leu 1)*	Transmembrane glycoprotein *(membrane)*	T cells and B-cell subsets (mantle zone)	Thymic carcinoma, adenocarcinomas, mesothelioma (cytoplasmic). T-cell leukemias and lymphomas, aberrantly expressed in low-grade B-cell lymphomas (CLL or mantle cell lymphoma)	Thymic carcinoma (+/–) versus thymoma (–). Thymic carcinoma (+/–) versus metastatic squamous carcinoma (–) Classification of low-grade B-cell lymphomas. Evaluation of T-cell lymphomas (this marker is frequently lost)	
CD10* *(CALLA [**c**ommon **a**cute **l**eukemia **a**ntigen], J5)*	Cell surface metalloendopeptidase that inactivates peptides *(membrane)*	Precursor B cells, granulocytes, rare cells in reactive follicles, myoepithelial cells of breast, bile canaliculi, fibroblasts, brush border of kidney and gut	Endometrial stromal sarcoma, RCC (clear cell and papillary types), HCC, TCC, rhabdomyosarcoma, pancreatic carcinoma, schwannoma, melanoma Precursor lymphoblastic lymphoma/leukemia, follicular lymphoma, Burkitt's lymphoma, CML, angioimmunoblastic lymphoma	Myoepithelial cell marker in breast Endometrial stromal sarcoma (+) versus leiomyosarcoma (–/+) (but caldesmon is preferred for this purpose) Evaluation of low-grade lymphomas Evaluation of leukemias	Not specific for nonlymphoid neoplasms

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD15* *(LeuM1)*	3-fucosyl-N-acetyllactosamine, X-hapten—CHO moiety linked to cell membrane protein *(membrane and cytoplasm)*	Granulocytes, monocytes	Adenocarcinomas CMV-infected cells RS cells (not LP HD) in a membranous and Golgi pattern, some large T-cell lymphomas, MF, some leukemias	Adenocarcinomas (+) versus mesotheliomas (–) Evaluation of HD	
CD30* *(Ber-H2, Ki-1)*	Single chain transmembrane glycoprotein homologous to the nerve growth factor superfamily *(cytoplasm, membrane, and Golgi)*	Activated B and T cells, some plasma cells, immunoblasts, interdigitating cells, histiocytes, follicular center cells, decidualized endometrium, reactive mesothelial cells, most other tissues negative	Embryonal carcinoma, some vascular tumors (not KS), some mesotheliomas Anaplastic large cell (CD30+) lymphomas, mediastinal large B-cell lymphoma, primary effusion lymphoma, HD (but not LP HD), some other B- and T-cell lymphomas, EBV-transformed B cells	Evaluation of anaplastic large cell (CD30+) lymphomas Evaluation of HD (RS cells are positive except in LP HD) Evaluation of peripheral T-cell lymphoma (large cells may be positive)	
CD31* *(PECAM-1, platelet-endothelial cell adhesion molecule)*	Transmembrane glycoprotein functioning in cell adhesion *(cytoplasm, membrane)*	Endothelial cells, platelets, megakaryocytes, plasma cells, histiocytes, other hematopoietic cells	Vascular tumors (>80% of angiosarcomas), KS, histiocytic neoplasms, PEComa, very rarely other tumors	ID of endothelial differentiation in tumors Evaluation of angiogenesis	Most sensitive and specific marker for endothelial cells
CD34* *(HPCA-1, hematopoietic progenitor cell, class 1, QBEnd10)*	Single chain transmembrane glycoprotein, leukocyte differentiation antigen *(cytoplasm, membrane)*	Hematopoietic progenitor cells (decreases with maturation), endothelial cells, fixed connective tissue cells (e.g., in skin), fibroblasts	Acute leukemia, sarcomas of vascular origin, KS, epithelioid sarcoma, GIST, DFSP, solitary fibrous tumor, neurofibroma, schwannoma, spindle cell lipoma	Identification of endothelial or fibroblastic differentiation in tumors Evaluation of angiogenesis Evaluation of the number of blasts in bone marrow in acute leukemia Solitary fibrous tumor (+) versus sarcomatoid mesothelioma (–) DFSP (+) versus dermatofibroma (–)	Not specific but can be useful in context with other features
CD44v3 *(CD44 variant 3, H-CAM)*	Transmembrane glycoprotein that mediates cell adhesion *(membrane)*	Many, including myometrium	Many, including endometrial carcinomas	Possibly helpful to distinguish cellular leiomyoma (+) from endometrial stromal sarcoma (–)	Many splice variants of CD44 are present in normal and malignant cells

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD57* *(Leu 7, HNK-1)*	Lymphocyte antigen that cross reacts with a myelin-associated glycoprotein *(membrane)*	T-cell subsets, NK cells, myelinized nerves, neuroendocrine cells, prostate, pancreatic islets, adrenal medulla	Nerve sheath tumors (occasional), leiomyosarcoma, synovial sarcoma, rhabdomyosarcoma, neuroblastoma, gliomas, neuroendocrine carcinomas, neurofibromas, some prostate carcinomas Angioimmunoblastic lymphoma, T gamma lymphoproliferative disorder (large granular cell lymphocytic leukemia)	ID of neuroendocrine differentiation in tumors ID of angioimmunoblastic T-cell lymphoma Evaluation of NK neoplasms	Not very specific for solid tumors
CD63 *(NKI/C3, melanoma-associated antigen, ME491)*	Member of the tetraspanin or transmembrane 4 superfamily (TM4SF) found on lysosomes *(cytoplasm or membrane)*	Melanocytes, mast cells, histiocytes, salivary gland cells, sweat gland cells, pancreatic cells, islets of Langerhans, prostatic cells, Paneth cells, peribronchial glands, pituitary	Nevi, melanomas, carcinoids, medullary carcinomas of the thyroid, some adenocarcinomas	Cellular neurothekoma (NKI/C3 + and S100 –) versus melanocytic lesions (NKI/C3 and S100 +) ID of melanocytic lesions	May be negative in desmoplastic melanomas
CD68* *(KP1, CD68-PGM1, Mac-M)*	Intracellular glycoprotein associated with lysosomes *(cytoplasm, membrane)*	Macrophages, monocytes, neutrophils, basophils, large lymphocytes, Kupffer cells, mast cells, osteoclasts	Neurofibroma, schwannoma, MPNST, granular cell tumors, PEComa, melanomas, atypical fibroxanthoma, RCC Some lymphomas, histiocytic sarcomas, APML, Langerhans proliferative disorders	Best general marker for macrophages, although not specific to this cell type	The antibody PG-M1 does not react with granulocytes Not very specific for solid tumors

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD99* *(MIC-2, 12E7, Ewing's sarcoma marker, E2 antigen, HuLy-m6, FMC 29, O13 [different epitope])*	*MIC2* gene product—glycoproteins (p30 and p32) involved in rosette formation with erythrocytes *(membrane)* *Membrane immunoreactivity is more specific than cytoplasmic*	Cortical thymocytes, T lymphocytes, granulosa cells of ovary, pancreatic islet cells, Sertoli cells, some endothelial cells, urothelium, ependymal cells, squamous cells	PNET/Ewing's sarcoma, chondroblastoma, mesenchymal chondrosarcoma, synovial sarcoma, solitary fibrous tumors, GIST, some alveolar rhabdomyosarcomas, desmoplastic small cell tumors, small cell carcinomas, granulosa cell tumors, yolk sac components of germ cell tumors, Sertoli–Leydig cell tumors, atypical fibroxanthoma, meningioma B- and T-cell precursor lymphoblastic lymphoma/leukemia	Thymic carcinomas (lymphocytes +) versus other carcinomas. ID of PNET/Ewing's sarcoma (immunoreactivity should be clearly membranous in the majority of the cells) Evaluation of lymphoblastic lymphoma/leukemia	O13 is the most commonly used antibody Immunoreactivity is highly dependent upon the antigen retrieval system used
CD117* *(c-kit, stem cell factor receptor)*	Transmembrane tyrosine kinase receptor (ligand is stem cell factor)—apoptosis is inhibited when the ligand is bound *(cytoplasm, membrane)*	Mast cells, interstitial cells of Cajal (ICC—pacemaker cells of the GI tract found throughout the muscle layers and in the myenteric plexus), epidermal melanocytes, mononuclear bone marrow cells (4%), Leydig cells, early spermatogenic cells, trophoblast, breast epithelium	GIST (>95%), seminomas (>70%), intratubular germ cell neoplasia, mature teratomas (>70%), papillary RCC (cytoplasmic—associated with mutations), chromophobe RCC (membrane —not associated with mutations), some melanomas (focal), mast cell tumors, some carcinomas, some brain tumors, some PNET/Ewing's sarcoma, some angiosarcomas AML (>50%), CML in myeloid blast crisis	ID of GIST (+) versus leiomyomas (–) and schwannomas (–) ID of seminomas ID of mast cells (mastocytosis)	Mast cells are an excellent internal control CD117 positivity does not correlate with mutations and/or oncoprotein activity in tumors not known to have activating mutations and is, in general, not of clinical or therapeutic significance in this setting (e.g., to detect tumors likely to respond to therapy directed against the protein, e.g. Gleevec).

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD141 *(Thrombomodulin, TM)*	Transmembrane glycoprotein, receptor for thrombin *(cytoplasm [epithelial cells], membrane [mesothelial cells])*	Endothelium, platelets, monocytes, synovial cells, syncytiotrophoblast, mesothelial cells, dermal keratinocytes, islet cells, peripheral nerves	Mesotheliomas, TCC, KS, squamous cell carcinomas, choriocarcinomas, rarely adenocarcinomas, benign and malignant vascular tumors	Mesothelioma (+ 80%) versus adenocarcinoma (+ 10%) (but variable results have been reported in other studies)	Other markers are better for distinguishing adenocarcinoma from mesothelioma
CD146* *(melanoma cell adherin molecule, MELCAM, MCAM, MN-4, MUC18, A32 antigen, S-Endo-1)*	Membrane cell adhesion glycoprotein of the Ig gene superfamily *(membrane)*	Implantation site intermediate trophoblast, myofibroblasts, endothelium, pericytes, Schwann cells, ganglion cells, smooth muscle, cerebellar cortex, breast luminal and myoepithelial cells, external root sheath of hair follicle, subcapsular epithelium of thymus, follicular dendritic cells, basal cells of bronchus and parathyroid, subpopulations of activated T cells	Melanoma, angiosarcoma, KS, leiomyosarcoma, placental site trophoblastic tumor, choriocarcinoma May be focally positive in squamous cell carcinoma and small cell carcinoma of the lung, mucoepidermoid carcinoma, breast carcinoma, some leukemias, neuroblastoma	ID of placental site trophoblastic tumors	
CDX2* (CDX-88)	Homeobox nuclear transcription factor specific for the intestinal tract that regulates *MUC1* expression *(nucleus)*	Small intestine, colon, and endocrine pancreas	Colon carcinomas (usually strong and diffuse), small intestine carcinomas, mucinous ovarian carcinomas, bladder adenocarcinomas, some gastric, esophageal, pancreatic, and bile duct carcinomas HCC, breast, lung, and head and neck carcinomas are usually negative	ID of colon carcinomas and other carcinomas of the gastrointestinal tract However, other carcinomas (e.g., mucinous ovarian carcinoma) can also be positive	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Chromogranin A*	Acidic glycoprotein in neurosecretory granules *(cytoplasm, granular)*	Islet cells of pancreas, bronchial Kulchitsky cells, parathyroid, adrenal medulla, anterior pituitary, C cells of thyroid	Pheochromocytoma, carcinoids (not rectal), small cell carcinoma, neuroblastoma, some breast and prostatic carcinomas, Merkel cell tumors, islet cell tumors, medullary carcinoma of the thyroid, parathyroid lesions, Brenner tumor	ID of neuroendocrine differentiation in tumors Not present in pituitary prolactinomas Pheochromocytoma (+) versus adrenal cortical carcinoma (–) Parathyroid (+) versus thyroid (–)	Most specific marker of neuroendocrine differentiation Also can be detected in serum Bouin's solution or B5 fixation may increase immunogenicity
Claudin-1 *(CLDN1)*	Protein component of the tight junction complex *(membrane—not cytoplasmic)*	Epithelial cells, perineurial cells, some endothelial cells (venules)	Perineurioma, synovial sarcoma (epithelioid areas, lower in spindle cell areas) carcinomas Some perineurial cells may be present in neurofibromas and schwannomas	Perineurioma (+) versus DFSP (–), fibromatosis (–), low-grade fibromyxoid sarcoma (–)	
Collagen IV	Major constituent of basement membranes *(basement membrane)*	Mesangial cells within glomeruli, basement membranes, basal lamina of capillaries	Tumors with external lamina (schwannomas, smooth muscle tumors)	Absence or loss may be associated with stromal invasion by carcinomas	
Desmin*	Intermediate filament in muscle *(cytoplasm)*	All striated muscle (Z bands) and many smooth muscle cells, myofibroblasts, smooth muscle of some blood vessels	Rhabdomyosarcoma (80% +), leiomyosarcoma (50–70% +), PEComa, desmoplastic small round cell tumors (usually dot-like), some myofibroblastic tumors, endometrial stromal sarcoma	ID of muscle differentiation in tumors	
DPC4* *(homozygously deleted in pancreatic carcinoma, locus 4, Smad4)*	Transcriptional regulator interacting with the TGF-beta signaling pathway *(nucleus)*	Normal tissues	Expressed in most carcinomas Lost in 31% of Pan IN-3, 55% of pancreatic carcinomas, and 22% of stage IV colon carcinomas	Mucinous ovarian carcinoma (+) versus metastatic pancreatic carcinoma (– in 55%)	Mutated in familial juvenile polyposis in 25–60% of cases

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
E-cadherin	Transmembrane cell adhesion molecule that binds to catenins for cell polarization, glandular differentiation, and stratification *(membrane)*	Epithelial cells	Most carcinomas—may be lost in poorly differentiated carcinomas Not present in LCIS and invasive lobular carcinoma of breast or gastric signet ring cell carcinomas	Ductal (+) versus lobular (–) lesions of the breast	Diagnostic importance in the breast has not been established
EGFR *(Epidermal growth factor receptor, HER1)*	Transmembrane protein receptor of the type 1 growth factor family with tyrosine kinase activity *(membrane positivity scored, cytoplasmic positivity is not scored)*	Many types of epithelium, skin eccrine and sebaceous glands, mesenchymal cells, perineurium. The strongest membrane positivity is present in hepatocytes, bile ducts, basilar squamous cells, pancreatic ducts, breast epithelium, lung alveolar lining cells, mesothelial cells, prostate epithelium, endometrial glands and stroma	Adenocarcinomas (esp. colon), squamous cell carcinomas, TCC, neural tumors, sarcomas	Expression is increased in tumors of higher grade and poorer prognosis Colon carcinomas (80–90% positive): response to cetuximab does not appear to be related to IHC score (see Table 7-29)	Patients with colon carcinomas expressing EGFR are eligible for trials of targeted therapy (cetuximab).
Epithelial membrane antigen* *(EMA, MUC1, HMFG, DF3, CA 15-3, CA 27.29, PEM, many others)*	Episialin, glycoprotein found in human milk fat globule membranes *(cytoplasm [more common in malignant cells], membrane [more common in benign cells])*	Epithelial cells, perineurial cells, meningeal cells, plasma cells, usually negative in non-neoplastic mesothelial cells	Carcinomas, mesotheliomas (thick membrane pattern), some sarcomas (synovial sarcoma, epithelioid sarcoma, leiomyosarcoma, some osteosarcomas), adenomatoid tumor, chordoma, perineurioma, neurofibroma, meningioma, desmoplastic small round cell tumor, Sertoli cell tumor Some anaplastic large cell lymphomas (CD30 +), plasma cell neoplasms	ID of epithelial differentiation in tumors; however, keratin is more specific for this purpose Synovial sarcoma (typically focal positivity) versus other sarcomas	There are over 50 monoclonal antibodies recognizing different glycosylation patterns in normal tissues and tumors[16]

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Epstein-Barr virus* EBV-encoded nonpolyadenylated early RNAs *(EBERS)*	RNA produced by EBV *(nucleus)*	EBV-infected B cells	All EBV-related tumors	Most sensitive marker for EBV	Detected by in situ hybridization for RNA on paraffin sections
LMP-1	Latent membrane protein *(membrane)*	EBV-infected B cells	Nasopharyngeal carcinomas, RS cells (not LP HD), transplant lymphomas, AIDS related lymphomas, endemic Burkitt's lymphoma (rare in sporadic cases)	Evaluation of EBV related neoplasms	
EBNA 2 *(nuclear antigen 2)*	Nuclear protein (*Nucleus)*	EBV-infected B cells	Transplant-related lymphomas, AIDS-related lymphomas. Not present in Burkitt's lymphoma, nasopharyngeal carcinomas, or HD	Evaluation of transplant- and AIDS-related lymphomas	
Estrogen receptor* *(ER, 1D5, H222, others)*	Steroid binding protein *(nucleus)*	Breast epithelial cells (not myoepithelial cells), epithelial and myometrial cells of the uterus	Breast carcinomas (>70%), gynecologic carcinomas, some skin appendage tumors, rare in other carcinomas, present in some meningiomas, smooth muscle tumors, some melanomas, some thyroid tumors, desmoid tumors, myofibroblastomas of breast, vulvovaginal stromal tumor	Prognosis and prediction of response to hormonal therapy of breast cancer Only nuclear positivity is scored ID of metastatic breast cancer	Antibodies recognize different epitopes and have varying sensitivities in formalin-fixed tissue. Antigenicity may be diminished after decalcification or exposure to heat during surgery
Factor VIII-related antigen* *(VWF, FVIII:RAg, von Willebrand's factor)*	Glycoprotein involved in coagulation, part of FVIII complex *(cytoplasm)*	Endothelial cells, megakaryocytes, platelets, and mast cells, endocardium	Vascular tumors (often absent in angiosarcomas) Not present in KS, PEComa Megakaryocytic AML (M7) is positive	ID of endothelial differentiation in tumors (specific but not very sensitive) Evaluation of angiogenesis Evaluation of M7 (megakaryocytic) leukemias	May not detect smaller blood vessels (see CD 31 and 34). Present in Weibel–Palade bodies. Not a sensitive marker for vascular neoplasms

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Factor XIIIa *(Factor XIII subunit A)*	Transglutaminase involved in the coagulation pathway (cytoplasm)	Fibroblasts, dendritic reticulum cells in reactive follicles, dermal dendrocytes, liver, placenta, platelets, megakaryocytes, monocytes, macrophages	Fibroblastic neoplasms, dermatofibroma		Not very specific
Fascin	Actin binding protein thought to be involved in the formation of micro-filament bundles *(cytoplasm)*	Interdigitating reticulum cells in lymph nodes, dendritic cells of lymph node, thymus, spleen and peripheral blood, histiocytes, smooth muscle, endothelial cells, squamous mucosal cells, lining cells of splenic sinuses	RS cells and their variants (but not LP HD), rare non HD lymphomas Reticulum cell tumors Some sarcomas Some high-grade breast carcinomas		Not very specific
Fibronectin	Glycoproteins found in BMs and extracellular matrix, bind to integrins *(extracellular)*		Stroma of many tumors		
Fli-1* *(Friend leukemia integrin-site 1)*	Transcription factor (ETS family)—translocation in Ewing's can result in an EWS–Fli-1 fusion protein *(nucleus)*	Endothelial cells (hemangioblasts, angioblasts), small lymphocytes	Ewing's sarcoma/PNET, vascular tumors (including KS), Merkel cell carcinoma, melanoma Can also be weakly present in lymphomas, synovial sarcoma, some carcinomas	ID of vascular tumors (unlike other vascular markers, Fli-1 is nuclear) ID of Ewing's sarcoma/PNET	Reactivity can be variable with high background and may be difficult to interpret
Galectin-3* *(Gal-3)*	Lectin with anti-apoptosis function (galactoside-binding protein) *(nucleus, cytoplasm, membrane, extracellular matrix)*	Many epithelial cells, lymphocytes, mesenchymal cells, macrophages, activated endothelial cells	Many carcinomas, adenomas, lymphomas, soft tissue tumors	Thyroid carcinomas (papillary and to a lesser extent follicular) show higher expression than benign lesions In some carcinomas, expression is diminished in higher-grade lesions	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Glial fibrillary acidic protein *(GFAP)*	Intermediate filament *(cytoplasm)*	Normal and reactive astrocytes, developing and reactive ependymal cells, developing oligodendrocytes, choroid plexus, Schwann cells, enteric glial cells, pituitary cells, chondrocytes	Tumors of astrocytes, ependymal cells, and oligodendrocytes, MPNST, myoepitheliomas (salivary glands and soft tissue), sweat gland tumors, Merkel cell carcinomas, chordomas	ID of neural differentiation in tumors (30% of MPNSTs are +) Neuroblastomas are negative, schwannomas may be focally + Merkel cell carcinoma (+) versus small cell carcinoma (–) (but CK20 is a better marker for this purpose) ID of myoepithelial neoplasms	
GLUT-1 *(glucose transporter 1)*	Component of transmembrane glucose transport *(membrane)*	Erythrocytes, perineurium, blood vessels, trophoblasts, renal tubules, germinal center cells	TCC, lung carcinoma, squamous cell carcinoma, adenocarcinomas of colon, lung, bile ducts, kidney, ovary, pancreas, stomach, and endometrium, germ cell tumors		Not very specific
Gross cystic disease fluid protein-15* *(GCDFP, CDP, BR-2, BRST-2)*	Protein found in breast fluid *(cytoplasm)*	Apocrine sweat glands, apocrine metaplasia of the breast	Breast carcinomas (60%), sweat gland carcinomas, some salivary gland tumors, some prostate carcinomas	ID of apocrine differentiation in tumors ID of breast metastases (however, only positive in about 60%)	
HepPar-1* *(hepatocyte paraffin-1, HP1)*	Mitochondrial protein *(cytoplasm, coarsely granular)*	Liver	HCC, some cases of gastric adenocarcinoma, esophageal adenocarcinoma, others negative or rarely positive	HCC (80–95%) versus metastatic carcinomas to the liver	
HBME-1*	Antigen to microvilli on mesothelioma cells *(membrane and cytoplasm)*	Mesothelial cells, epithelial cells	Mesotheliomas (epithelial type—thick, membrane staining), adenocarcinomas, chordomas, chondrosarcomas	Positivity higher in thyroid carcinomas than in adenomas May be absent in thyroid carcinomas with Hürthle cell features	Not a specific marker for mesotheliomas

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
HER-2/neu *(c-erbB2)*	Growth factor receptor (tyrosine kinase) homologous to epidermal growth factor receptor *(membrane, some cytoplasm)*	Absent or rare in normal cells	Breast carcinomas (20–30%), Paget's disease of nipple (>90%), less frequently in other carcinomas (ovary, uterus, GI, pancreas), some synovial sarcomas	Poor prognostic factor in breast cancer Positivity used to select patients for treatment with Herceptin (scored from 0 to 3+) (see Chapter 15)	Only membrane positivity is scored Gene amplification (detected by FISH) correlates with strong complete membrane immunoreactivity in >90% of cases
HHF-35* *(Muscle-specific actin, MSA, muscle common actin, EM ACT)*	Alpha and gamma smooth muscle actins, recognizes a common epitope of alpha skeletal, cardiac, and smooth muscle *(cytoplasm)*	Smooth, striated, and cardiac muscle, smooth muscle of blood vessels, pericytes, myoepithelial cells, myofibroblasts	Numerous tumors including tumors of muscle, glomus tumor, PEComa, GIST, DFSP, dermatofibroma, myofibroblastic tumors, spindle cell carcinomas, salivary gland tumors, mesothelioma, others	ID of muscle differentiation in tumors	Sensitive but not specific. Present in tumors not of muscle origin
HHV8*	Latent nuclear antigen of human herpesvirus type 8 *(nucleus)*	Absent in normal tissue	KS (endothelial cells and some perivascular cells) Primary effusion lymphoma (PEL), AIDS-associated multicentric Castleman's disease	Evaluation of KS and PEL	
HMB-45* *(E-MEL, melanoma-specific antigen)*	Oligosaccharide side-chain of a melanosomal antigen, gp100/pmel17 *(cytoplasm)*	Fetal melanocytes and some normal adult superficial melanocytes, retinal pigment epithelium	Melanoma (epithelioid but not spindle cell or desmoplastic type), clear cell sarcoma, PEComa, tumors associated with tuberous sclerosis, melanotic schwannoma, others	ID of metastatic melanoma. Melanophages can also be positive Melan-A may be more specific ID of PEComa	NKI-betab detects the same protein Tissues fixed in B5 may have high background staining

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
hMLH1 *(human mutS homologue 2)* **and hMSH2** *(human mutL homologue 1)*	Proteins involved in mismatch repair of DNA (these genes account for 95% of HNPCC) *(nucleus)*	Most normal tissues May be lost in areas of chronic inflammation	Expression (or non-expression) is not specific for tumor type	Absence is associated with germline mutations in HNPCC patients and with gene silencing by methylation in 15% of sporadic colon carcinomas—correlated with characteristic clinical, pathologic, and treatment response features IHC will not detect the 5% of patients with mutations in other genes or rare patients with mutated gene products that are immunoreactive	Other assays for microsatellite instability utilize PCR (90% sensitive for microsatellite instability (MSI)
Hormones (ER and PR are listed separately)	Insulin, gastrin, glucagon, somatostatin, calcitonin, ACTH, FSH, LH, PRL, TSH, others *(cytoplasm)*	Hormone-producing cells	Hormone-producing tumors	ID of hormone products in tumors	May not correlate well with serum levels of the same markers May not correlate with response to hormonal therapies (e.g., ER in tumors other than breast and tamoxifen)
Human chorionic gonadotropin* *(hCG, β-hCG)*	Beta chain of the hormone *(cytoplasm)*	Syncytiotrophoblasts	Choriocarcinoma, giant cells in seminomas, placental site tumors (weak)	ID of trophoblastic differentiation in tumors	
Human placental lactogen* *(HPL, hPL)*	Hormone *(cytoplasm)*	Trophoblast	Choriocarcinoma (may be weak), complete moles (strong), partial moles (weak), some lung and stomach carcinomas	ID of trophoblastic differentiation in tumors	
Inhibin*: alpha subunit	Hormone produced by ovarian granulosa cells and prostate, inhibits FSH production *(cytoplasm)*	Ovarian granulosa cells, Sertoli cells, pregnancy luteomas, ovarian follicles, syncytiotrophoblast, adrenal cortex, hepatocytes	Granulosa cell tumors, juvenile granulosa cell tumors, Sertoli and Leydig cell tumors, ovarian stromal cells around other tumors, hydatidiform moles, choriocarcinoma, thecofibroma, adrenal cortical tumor, granular cell tumor	ID of sex cord stromal differentiation in ovarian tumors Distinguishes adrenal cortical tumors (>70% +) versus HCC (<5% +) and RCC (<5% +)	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Keratins*	Intermediate filaments *(cytoplasm)*	Epithelial cells	Carcinomas, mesotheliomas, desmoplastic small round cell tumors (dot-like pattern), thymomas, chordomas, synovial sarcomas, epithelioid sarcoma, leiomyosarcoma, trophoblastic tumors, some other sarcomas, rarely melanomas	ID of poorly differentiated carcinomas Site of origin of carcinomas	
AE1/AE3*	Two monoclonal antibodies. AE1 detects 10, 15, 16, and 19. AE3 detects 1 to 8. *(Cytoplasm)*		Most carcinomas. The only common carcinomas that are frequently negative are HCC (70% negative) and RCC, clear cell type (20% negative) Epithelioid hemangioendothelioma, epithelioid sarcoma, synovial sarcoma, mesothelioma, adenomatoid tumor	ID of epithelial differentiation in tumors. HCC (–/+) versus cholangiocarcinoma and metastatic carcinomas (+)	Good broad-spectrum keratin
CAM 5.2*	8, 18 *(cytoplasm)*	Simple and glandular epithelium	Most carcinomas including those usually negative for CK7 and 20: HCC, prostatic carcinoma, thymic carcinoma, gastric carcinoma, RCC small cell carcinoma Carcinoid tumor, thymoma, germ cell tumors, mesothelioma, dendritic cells Synovial sarcoma, epithelioid sarcoma Many squamous cell carcinomas are negative	ID of carcinomas that may be negative for CK7 and CK20 Paget's disease (+) versus squamous cell carcinoma (–) Positivity for dendritic cells in lymph nodes and elsewhere may be confused for micrometastases	May be positive when other keratins are negative

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Keratin 5/6*	5/6 *(cytoplasm)*	Basal cells, stratum spinosum of epidermis, mesothelial cells	Squamous cell carcinomas, TCC, mesotheliomas, squamous metaplasia in adenocarcinomas, thymic carcinoma	Less frequently present in non-squamous cell carcinomas	Has limited use in routine practice
Keratin 7*	7 *(cytoplasm)*	Simple epithelia, respiratory epithelium, transitional epithelium, endothelial cells of small veins and lymphatics Not present in squamous epithelium	Most adenocarcinomas of glandular epithelial origin, TCC, mesothelioma, neuroendocrine neoplasms Not Merkel cell carcinoma or colon carcinoma Rare in clear cell RCC (but present in other variants), prostate carcinoma, HCC, lung small cell carcinoma, thymoma, carcinoid Not present in squamous cell carcinomas of the skin, but may be present in squamous cell carcinomas arising from non-keratinizing epithelium (e.g., cervical carcinoma)	The combination of CK7 and CK20 is used to distinguish carcinomas arising at different sites (see Tables 7-3 to 7-7)	
Keratin 20*	20 *(cytoplasm)*	Gastric foveolar epithelium, intestinal villi and crypt epithelium, Merkel cells, taste buds, umbrella cells of urothelium, subsets of epithelial cells Not present in non-epithelial cells	Colon carcinoma, Merkel cell carcinoma, TCC, adenocarcinoma of the bladder, pancreatic carcinoma, cholangio-carcinoma, mucinous ovarian carcinoma, esophageal adenocarcinoma	Merkel cell carcinomas CK20 positive, whereas most similar tumors are negative ID of metastatic colon carcinomas (the pattern of CK7–, CK20 + is most frequently seen in this carcinoma, but can rarely be seen in other types)	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
PAN-K* *(MNF-116)*	Broad spectrum detection of keratins including 5, 6, 8, 17, and 18 *(cytoplasm)*	Simple and squamous epithelial cells		Detection of keratin in all carcinomas, including poorly differentiated carcinomas (esp. spindle cell squamous cell carcinomas) May be more sensitive than AE1/AE3 for carcinomas with myoepithelial ("basal") features due to inclusion of the "basal" keratin CK17	
34βE12* *(903)*	High-molecular-weight keratins including 1, 5, 10, 14 *(cytoplasm)*	Complex epithelia, basal cells, myoepithelial cells	TCC, cholangiocarcinoma, squamous cell carcinoma, non-mucinous bronchiolo-alveolar lung carcinoma, RCC (papillary and chromophobe types), mesothelioma, papillary thyroid carcinoma, thymic carcinoma, lympho-epithelial carcinoma	TCC (+) versus prostate carcinoma (–) or RCC (–) Prostate carcinoma (no basal cells) versus benign lesions (with some + basal cells present). Can be combined with p63 for this use	
Ki-67* *(MIB-1)*	Protein found during the entire cell cycle but not in G0 *(nucleus)*	Any cycling cell	Any cycling tumor	Used as a prognostic marker for some tumors Detects number of cycling cells in Burkitt's lymphoma and large B-cell lymphoma Aberrant membrane and cytoplasmic immuno-reactivity is present in trabecular hyalinizing adenoma of the thyroid and sclerosing hemangioma of the lung	MIB-1 recognizes an epitope preserved in formalin-fixed tissue
Laminin	Component of basement membranes *(basement membrane)*	Basement membranes	Nerve sheath tumors, smooth muscle tumors	Loss associated with stromal invasion by carcinomas Present in microglandular adenosis of the breast	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Lysozyme *(Ly)*	Muramidase (mucolytic enzyme) *(cytoplasm)*	Circulating monocytes, some tissue macrophages, granulocytes, salivary gland, lacrimal gland, stomach and colon epithelial cells (inflamed or regenerative), apocrine glands, Paneth cells, some other epithelial cells	Salivary gland tumors, stomach and colon carcinomas AML with monocytic differentiation	Marker for histiocytes but not specific. May mark activated phagocytic macrophages Evaluation of myeloid leukemias	Not specific for identification of solid tumors
MAC 387 *(L1 antigen, calprotectin, calgranulin, cystic fibrosis antigen)*	Three polypeptide chains released with activation or death of neutrophils *(cytoplasm)*	Neutrophils, monocytes, some tissue macrophages, eosinophils, squamous mucosa, reactive skin, synovial lining cells	Lung carcinomas (not small cell or carcinoid), squamous cell carcinomas Histiocytic neoplasms (but not Langerhans cells)	Marker for macrophages (but not as specific as CD68)	Belongs to the S100 protein family Cells can passively take up antigen resulting in false positive results
Melan-A or MART-1* *(**m**elanoma **a**ntigen **r**ecognized by **T** cells), A103)*	Melanocyte differentiation antigen *(cytoplasm)* Melan-A (clone A103) and MART-1 are two different antibodies	Melanocytes of skin, uvea, and retina Melan-A is also positive in adrenal cortex, granulosa and theca cells of the ovary, Leydig cells	Melanomas (but <50% of spindle cell or desmoplastic melanomas), PEComas Melan-A is also positive in adrenocortical tumors, Leydig cell tumor, granulosa cell tumor	ID of melanomas. Melan-A is not positive in melanophages and may be more specific for the detection of micrometastases in lymph nodes Melan-A distinguishes adrenocortical tumors (–) (≥50% +) versus HCC and RCC (–)	More sensitive than HMB45 Peptides are used for melanoma immunotherapy Melan-A has a broader spectrum of immunoreactivity than MART-1
Myf-4* *(MRF4, myogenin)*	Human homologue of myogenin— muscle regulatory protein *(nucleus)*	Striated muscle	Rhabdomyosarcoma	ID of skeletal muscle differentiation in tumors	Better than MyoD1
MyoD1	Nuclear phosphoprotein, role in myogenic regulation *(nucleus)*	Developing muscle tissues (myoblasts), adult muscle is negative	Rhabdomyosarcoma (esp. poorly differentiated tumors), mixed mullerian tumors	ID of skeletal muscle differentiation in tumors	Background positivity is often high, making interpretation difficult

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Myoglobin	Oxygen binding protein *(cytoplasm)*	Striated muscle (including cardiac muscle), not smooth muscle	Tumors of striated muscle (rhabdomyosarcoma + 50%), but often negative in poorly differentiated tumors	ID of skeletal muscle differentiation in tumors	More specific but less sensitive than actin and desmin
Myosin Heavy Chain (fast) *(SM-MHC, Fast myosin)*	Contractile protein with 2 heavy and 4 light chains and many isoforms *(cytoplasm)*	Visceral and vascular smooth muscle, myoepithelial cells of the breast Striated muscle: type 2 fibers	Tumors with myoepithelial cells Rhabdomyosarcoma (some)	Marker for myoepithelial cells in the breast—may have less positivity in vascular endothelial cells and myofibroblasts ID of skeletal muscle differentiation in tumors	Antibodies to different isoforms will detect different types of muscle fibers
NEU N	*(Neuronal nuclei)*	Neuronal cells including cerebellum, cerebral cortex, peripheral ganglion cells			
Neurofilaments *(70 + 200 kD, NFP)*	Intermediate filaments with three subunits *(cytoplasm)*	Neuronal cells, adrenal medulla	Tumors of neuronal origin or with neuronal differentiation, neuroblastoma, medulloblastoma, retinoblastoma, Ewing's/PNET, esthesioneuroblastoma, Merkel cell carcinoma, some endocrine tumors (carcinoids, pheochromocytomas)	ID of neuronal differentiation in tumors ID of Merkel cell carcinomas	
Neuron-specific enolase* *(NSE—do not confuse with the enzyme non-specific esterase)*	Gamma-gamma enolase isoenzyme *(cytoplasm)*	Neuroectodermal and neuroendocrine cells, more weakly striated and smooth muscle, megakaryocytes, T cells, some platelets, neurons, pituitary cells, hepatocytes	Neuroectodermal and neuroendocrine tumors, melanomas (including desmoplastic melanomas), many breast carcinomas, germ cell tumors, alveolar soft part sarcoma	ID of neuronal or neuroendocrine differentiation in tumors	Lacks specificity

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
p16 *(MTS1, CDKN2)*	Binds to and inhibits the cyclin-dependent kinases cdk4 and cdk6 *(cytoplasm and nuclear)*	Absent	Cervical squamous cell carcinomas and adenocarcinomas (both in situ and invasive), endocervical carcinoma, endometrial carcinoma Some basaloid squamous cell carcinomas of the tonsil in young patients that are associated with HPV16	Evaluation of cervical lesions Possible use predicting tonsillar site for metastatic squamous cell carcinoma of the head and neck	Overexpression is due to HPV-induced cell cycle dysregulation
p27^{kip1}	A cyclin-dependent kinase inhibitor that regulates progression from G1 to S phase	Proliferating cells			
p53* *(Multiple antibodies to wild-type and mutant forms)*	Tumor suppressor gene product—probably most frequently mutated gene in malignancy *(nucleus)*	Overexpression uncommon or absent in normal cells or benign tumors	Many malignant tumors, but not specific for malignancy	Overexpression may be used as a prognostic factor	Different antibodies recognize different wild type and mutant forms of the protein and will give different results
p57 *(kip2, p57^{KIP2})*	Cyclin-dependent kinase inhibitor (CDKI) acting to inhibit cell proliferation, paternally imprinted *(nucleus)*	Cytotrophoblast, intermediate trophoblast, villous stromal cells, decidual stromal cells, absent in syncytiotrophoblast		Diploid complete moles show absent or low expression in cytotrophoblast and villous stromal cells (may be present in villous intermediate trophoblast and decidual stromal cells), partial moles and hydropic abortions have normal expression	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
p63*	Protein with at least six major isotypes, including deltaNp63, member of the p53 family *(nucleus)*	Proliferating basal cells of cervix, urothelium, prostate, and myoepithelial cells of breast, basal squamous cells, squamous metaplasia	Squamous cell carcinomas, TCC, adenomyoepithelioma, adenoid cystic carcinoma, nasopharyngeal carcinoma, "basal type" breast carcinomas, papillary carcinoma of the thyroid, others	ID of myoepithelial cells in sclerosing breast lesions Diagnosis of prostatic carcinoma by showing absence of basal cells (more sensitive when combined with 34βE12). Basaloid squamous lung cancer (+) versus small cell (–). ID of metastatic poorly differentiated squamous cell carcinomas	Easier to interpret than SMA as myofibroblasts are negative
Placental alkaline phosphatase* *(PLAP)*	Alkaline phosphatase secreted by trophoblast *(cytoplasm)*	Placenta (trophoblast)	Germ cell tumors (but not spermatocytic seminoma), intratubular germ cell neoplasia, partial moles, some carcinomas of breast, ovary, lung, stomach, and pancreas, some rhabdomyosarcomas (esp. alveolar type)	Absence of immunoreactivity makes a germ cell tumor unlikely. However, spermatocytic seminomas and immature teratomas are negative ID of intratubular germ cell neoplasia	
Progesterone receptor* *(PR, PgR)*	Steroid binding protein *(nuclear)*	Normal breast epithelial cells, endometrial cells, many smooth muscle cells, breast lobular stroma	Breast carcinomas, gynecologic carcinomas, some skin adnexal tumors, secretory meningiomas, endometrial stromal sarcomas, some leiomyomas, myofibroblastic tumors, rarely other tumors	Prognosis and treatment of breast cancer ID of metastatic breast cancer	
Prealbumin *(Transthyretin, TTR)*	Plasma transport protein for retinol and thyroxine *(cytoplasm)*	Pancreatic islet cells, choroid plexus, retinal pigment epithelium, liver	Pancreatic islet cell tumors, carcinoid tumors, choroid plexus papillomas, choroid plexus carcinomas (may be focal or absent)	ID of choroid plexus neoplasms Evaluation of some forms of amyloidosis	Major subunit protein in some forms of inherited systemic amyloidosis

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Prostate-specific antigen* *(PSA)*	Member of kallikrein family of serine protease isolated from human seminal plasma *(cytoplasm)*	Normal prostatic epithelium, urachal remnants, endometrium, transitional cells of bladder	Prostatic carcinomas, some breast carcinomas	ID of prostatic carcinomas (may be lost in some poorly differentiated carcinomas). Seminal vesicle carcinomas are negative	More specific than PAP Used as a serum screening test for prostate cancer
Prostate acid phosphatase* *(PrAP, PAP)*	Isoenzyme of acid phosphatase *(cytoplasm)*	Normal prostatic epithelium, periurethral glands, anal glands, macrophages	Prostatic carcinomas, TCC, rectal carcinoids	ID of prostatic carcinomas (may be lost in some poorly differentiated carcinomas)	
RCC* *(Renal cell carcinoma marker, gp200)*	Glycoprotein on surface of renal tubules, breast epithelial cells, epididymis *(cytoplasm, membrane)*	Renal tubules, breast, epididymis	Clear cell and papillary RCC, breast carcinoma, embryonal carcinoma	Clear cell and papillary RCC (+) versus chromophobe carcinoma (–/+) and oncocytoma (–)	
RET* *(Rearranged during transfection)*	*RET* proto-oncogene. Surface glycoprotein of the receptor tyrosine kinase family *(cytoplasm)*	Neurons, embryonic kidney	Papillary thyroid carcinomas (78%), follicular variant of papillary carcinoma (63%), Hürthle cell carcinoma (57%), insular carcinoma (50%), medullary carcinoma, not present in follicular carcinomas or benign lesions Neuroblastoma (+), pheochromocytoma (+)	Evaluation of thyroid tumors	Germline mutations occur in MEN2A and 2B (10q11.2), familial medullary thyroid carcinoma, and some cases of Hirschsprung's disease
S100 protein*	Calcium binding protein isolated from the CNS (member of the EF hand family) *(nucleus and cytoplasm)*	Glial and Schwann cells, melanocytes, chondrocytes, adipocytes, myoepithelial cells, Langerhans cells, macrophages, reticulum cells of lymph nodes, eccrine glands, others	Melanoma (including spindle cell and desmoplastic types), clear cell sarcoma, schwannoma, chordoma, ependymoma, astroglioma, Langerhans proliferative disorders, some carcinomas (e.g., breast, ovary endometrial, thyroid), granular cell tumor, histiocytic sarcoma, myoepithelioma	ID of melanoma (if negative, melanoma is highly unlikely) ID of Langerhans proliferative disorders Sustentacular cells in pheochromocytomas (loss may be poor prognostic factor) ID of neural tumors ID of cellular schwannomas (more strongly and diffuselypositive than in MPNST)	Langerhans cells and macrophages in tumors may be misinterpreted as positivity in the tumor itself S100 is very soluble and may be eluted from frozen tissues

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Synaptophysin*	Transmembrane glycoprotein found in presynaptic vesicles *(cytoplasm)*	Neuroectodermal and neuroendocrine cells, neurons	Medulloblastoma, neuroblastoma, pheochromocytoma, paragangliomas, carcinoids, small cell carcinoma, medullary carcinoma of the thyroid, neural neoplasms, pancreatic islet cell tumors	ID of neuroendocrine differentiation in tumors ID of neuronal differentiation in CNS tumors	
Synuclein-1	Neuron-specific protein associated with synaptosomes *(Lewy bodies)*	Brain	Present in Lewy bodies (Lewy body dementia and Parkinson's disease)		
Tau	Microtubule-associated protein *(cytoplasm, extracellular)*	Normal neuronal cell bodies and dendrites, neuropil, glial cells	Abnormal amounts in Alzheimer's disease in neurofibrillary tangles and senile plaques	Evaluation of Alzheimer's disease, Pick's disease, supranuclear palsy corticobasal degeneration, others	
Thyroglobulin*	Glycoprotein produced by thyroid follicular cells *(cytoplasm)*	Thyroid follicles	Thyroid carcinomas (papillary, follicular, and Hürthle cell types, rarely present in medullary carcinomas)	ID of metastatic thyroid carcinoma Loss may be a poor prognostic factor	Thyroglobulin can diffuse into metastatic tumors to the thyroid
TTF-1* *(Thyroid transcription factor 1)*	Transcription factor for thyroglobulin, thyroid peroxidase, Clara cell secretory protein, and surfactant proteins *(nucleus; aberrant cytoplasm positivity in HCC)*	Thyroid, lung, and some brain tissues	Thyroid carcinomas (including medullary carcinoma; may be negative in anaplastic carcinoma), lung adeno-carcinomas (75%, but lower in mucinous bronchioloalveolar carcinomas), small cell carcinoma of lung (>90%), HCC (cytoplasmic), absent or focal in most other adenocarcinomas	Mesothelioma (–) versus adenocarcinoma (+/–) Lung adenocarcinoma (+/–) versus metastatic breast carcinoma (–) Small cell carcinoma of lung (+) versus metastasis from other sites (–), but some extrapulmonary small cell carcinomas can also be + HCC (cytoplasmic 71%), rare in other tumor types	The detection of cytoplasmic TTF-1 may depend on the specific antibody used and the antigen-retrieval method

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Ulex *(Ulex europaeus I lectin, UEA 1)*	Lectin, fucose residues on blood group H *(cytoplasm)*	Endothelial cells	Vascular tumors, some carcinomas	Evaluation of angiogenesis	Not very specific
Vimentin*	Intermediate filament *(cytoplasm)*	Mesenchymal cells, fibroblasts, endothelial cells, chondrocytes, histiocytes, lymphocytes, many glial cells, myoepithelial cells, smooth muscle	All mesenchymal tumors, neural tumors, melanomas, meningiomas, chordoma, Leydig cell tumor, granulosa cell tumor, Sertoli cell tumor, adrenal cortical adenoma May be co-expressed with keratin in carcinomas of endometrium, thyroid, kidney (clear cell), adrenal cortex, lung, salivary gland, ovary, and liver	May be poor prognostic factor if co-expressed with keratin or GFAP	Can be used as an internal control for immunogenicity Not a specific marker for tumor type or line of differentiation
WT1* *(Wilms' tumor 1 protein)*	Zinc finger transcription factor *(cytoplasm, nucleus)*	Sertoli cells, decidual cells of uterus, granulosa cells of ovary, blood vessels, myelocytic cells	Wilms' tumors (epithelial and blastemal components), epithelial mesotheliomas (nuclei—80–90%), acute leukemia (nuclei), adenocarcinomas (cytoplasmic; esp. breast, ovary), desmoplastic small cell tumors (nuclear and cytoplasmic), rhabdomyosarcoma	Mesothelioma (+, nuclear) versus adenocarcinoma (adenocarcinoma usually negative for nuclear positivity except for ovarian)—monoclonal antibody used Desmoplastic small cell tumors—use polyclonal antibody	The gene is located on 11p13 and is inactivated in 5–10% of sporadic Wilms' tumors and nearly 100% of Denys–Drash syndrome patients Antibodies detect epitopes at different ends of the protein and may give different results. Not very specific

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

Hematopathology markers

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
ALK Protein* *(Anaplastic lymphoma kinase, ALK-1, p80)*	The ALK gene (2p23) is translocated to part of the nucleophosmin (NPM) gene (5q35) to form the fusion protein p80 and is overexpressed *(cytoplasm, nucleus)*	Nervous system	Anaplastic (CD30+) large cell lymphomas—approximately one third have t(2;5)(p23; q35) Some inflammatory myofibroblastic tumors	ID of anaplastic large cell lymphomas	The pattern of immunoreactivity varies with the translocation present
Alpha-1-antichymotrypsin *(ACH)*	Serine protease inhibitor *(cytoplasm)*	Histiocytes, granulocytes, others	Histiocytic tumors, many adenocarcinomas, melanomas, many sarcomas	Marker for histiocytes but CD68 is more specific	Not specific for tumor type
Alpha-1-antitrypsin *(AAT, α_1-AT)*	Glycoprotein synthesized in the liver that inhibits proteolytic enzymes (esp. elastase) *(cytoplasm)*	Histiocytes, reticulum cells, mast cells, Paneth cells, salivary gland	HCC, germ cell tumors, histiocytic neoplasms, colon and lung carcinomas, others	Accumulates in liver cells in AAT deficiency	Not specific for tumor type CD68 is a more specific marker for macrophages
bcl-2*	Protein involved in inhibition of apoptosis *(membrane, cytoplasm)*	Medullary lymphocytes and epithelial cells of the normal thymus, mantle and T zone small lymphocytes	CLL, mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma Synovial sarcoma, other soft tissue tumors	Follicular center cell lymphomas (+) versus reactive follicles (–). Hyperplastic marginal zones of the spleen, abdominal lymph nodes, and ilial lymphoid tissue are + Malignant thymomas may have greater reactivity than other thymomas Synovial sarcoma is more frequently positive compared to mesothelioma	Involved in the t(14;18) found in 90% of FCC lymphomas Not specific for ID of solid tumors

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Bcl-6*	Proto-oncogene—Kruppel-type zinc finger protein with homology to transcription factors *(nucleus)*	Normal germinal center B cells	Follicular lymphomas, diffuse large B-cell lymphomas, Burkitt lymphoma, mediastinal large B-cell lymphoma, LP HD Not present in B-CLL, hairy cell leukemia, mantle cell lymphoma, and marginal zone lymphomas	Evaluation of B-cell lymphomas	Involved in gene rearrangements at 3q27 in lymphomas
Blood group antigens	A, B, and H antigens *(membrane)*	Epithelial cells, endothelial cells, erythrocytes	Abnormally expressed or lost in many carcinomas	Sometimes helpful in identifying specimens	
BOB.1* *(B-cell Oct-binding protein 1)*	Coactivator that interacts with Oct transcription factors in B cells *(cytoplasm)*	B cells (including plasma cells)	B-cell lymphomas and leukemias, Reed–Sternberg cells in LP HD, usually absent in other HD types	Evaluation of HD	BOB.1 and Oct2 are necessary (but not sufficient) for Ig expression
BSAP* *(B-cell specific activator protein)*	Transcription factor encoded by the *Pax-5* gene that regulates B-lineage specific genes	B cells	All B-cell neoplasms and HD		Not reliable in Zenker's fixed tissue
CD1a* *(T6)*	Membrane glycoprotein *(membrane)*	Cortical thymocytes (immature T cells), Langerhans cells, dendritic cells	Langerhans proliferative disorders, lymphoblastic lymphoma	Evaluation of Langerhans proliferative disorders Evaluation of lymphoblastic lymphoma	
CD2* *(TE, T11, rT3, Leu 5a + b, LFA-2)*	Glycoprotein mediating adhesion of activated T cells and thymocytes with antigen-presenting cells and target cells, functions in E rosette formation *(membrane)*	T cells, NK cells, cortical thymocytes	T-cell neoplasms, may be aberrantly lost in peripheral T- cell neoplasms	Pan T cell marker	
CD3* *(T3)*	C3 antigen (five polypeptide chains) *(membrane, cytoplasm)*	T cells, cortical thymocytes	T-cell neoplasms, may be aberrantly lost in peripheral T-cell neoplasms Anaplastic large cell lymphoma is often negative	Best pan T cell marker	In paraffin sections, NK cells may also be positive

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD4* *(TH, T4, Leu 3)*	Transmembrane glycoprotein, HIV receptor *(membrane)*	T helper/inducer cells, macrophages, Langerhans cells	MF, other T-cell neoplasms	Evaluation of MF Evaluation of T-cell neoplasms	
CD5* *(Leu 1)*	Transmembrane glycoprotein *(membrane)*	T cells and B-cell subsets (mantle zone)	T-cell leukemias and lymphomas, aberrantly expressed in low grade B-cell lymphomas (CLL or mantle cell lymphoma) Thymic carcinoma, adeno-carcinomas, mesothelioma (cytoplasmic)	Classification of low-grade B-cell lymphomas Evaluation of T-cell lymphomas (this marker is frequently lost) Thymic carcinoma (~40%) thymoma (<10%) versus pulmonary squamous cell carcinoma (<5%)	
CD7* *(Leu 9)*	Membrane-bound glycoprotein *(membrane)*	Precursor T cells, T-cell subsets, NK cells, thymocytes	T-cell lymphomas and leukemias	Frequently (50%) lost in T-cell lymphomas versus reactive T cells (+) Evaluation of T-cell leukemias	
CD8* *(T8, Leu 2)*	Two glycoprotein chains *(membrane)*	T-cell subsets, NK cells, T cytotoxic/suppressor cells	T-cell lymphomas and leukemias	Evaluation of MF and T-cell lymphomas (this marker is frequently lost)	
CD10* *(CALLA [common acute leukemia antigen], J5, neprilysin)*	Cell surface metallo-endopeptidase that inactivates peptides *(membrane)*	Precursor B cells, granulo-cytes, rare cells in reactive follicles, myoepithelial cells of breast, bile canaliculi, fibroblasts, brush border of kidney and gut	Follicular lymphomas, pre-B-ALL, Burkitt's lymphoma, CML, angioimmunoblastic lymphoma RCC (clear cell and papillary), HCC, rhabdomyosarcoma, endometrial stromal sarcoma	Evaluation of follicular center cell lymphomas Evaluation of leukemias Myoepithelial cell marker in breast Endometrial stromal sarcoma (+) versus leiomyosar-coma (–) (but caldesmon is preferred for this purpose)	
CD11b *(Mac-1)*	Cell surface receptor for the C3bi complement fragment *(membrane)*	Granulocytes, monocytes, macrophages	Myelomonocytic leukemias		
CD11c*	Member of the beta(2) integrin family that mediates adhesion to vascular endothelium, transendothelial migration, chemotaxis, and phagocytosis *(membrane)*	Myeloid cells, NK cells, dendritic cells, activated lymphoid cells	Hairy cell leukemia, B-cell prolymphocytic leukemia, some B-CLL, marginal zone lymphoma (MALT)		

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD13 *(My 7)*	Aminopeptidase-N, a type II integral membrane metalloprotease functioning in cell surface antigen presentation, receptor for coronaviruses *(membrane, cytoplasm)*	Granulocytes, macrophages, bone marrow stromal cells, osteoclasts, renal tubules, intestinal brush border, cells lining bile duct canaliculi, endothelial cells, fibroblasts, brain cells	AML, CML with blast crisis, some ALL	Classification of leukemias	Requires frozen tissue
CD15* *(Leu-M1)*	3-fucosyl-N-acetyllactosamine, X-hapten—CHO moiety linked to cell membrane protein *(membrane and granular perinuclear)*	Granulocytes, monocytes	Reed–Sternberg cells (not LP HD), some large T-cell lymphomas, MF, some leukemias, some epithelial cells (adenocarcinomas), CMV-infected cells	Adenocarcinomas (+) versus mesotheliomas (–) Evaluation of HD	
CD16*	Low-affinity transmembrane Fc receptor for IgG *(membrane)*	NK cells, granulocytes, activated macrophages, subsets of T cells	Extranodal NK/T-cell lymphoma, some hepatosplenic T-cell lymphomas		
CD19 *(B4)*	B-cell type I integral membrane glycoprotein *(membrane)*	B cells, follicular dendritic cells, early myelomonocytic cells	pre-B-ALL and B-cell neoplasms (but not plasma cell lesions)	Good pan B cell marker	Fresh or frozen tissue required
CD20* *(L26, B1, Leu 16)*	B-cell non-glycosylated phosphoprotein functioning as a receptor during B-cell activation and differentiation *(membrane, cytoplasmic)*	B cells, monocytes, not plasma cells	B-cell lymphomas, Reed–Sternberg cells in LP HD, not plasmacytomas	Best pan B cell marker. Evaluation of B-cell lymphomas Evaluation of HD	Under investigation as a target for clinical treatment of B-cell lymphomas L26 is best for formalin-fixed tissue May be preserved in necrotic tissue
CD21* *(B2)*	Type I integral membrane glycoprotein functioning as the receptor for the C3d fragment of complement C3, CR2, receptor for EBV *(membrane)*	Follicular dendritic cells, mature B cells	Marginal zone (MALT) lymphomas, CLL (B cell), some T cell ALL, follicular dendritic cell tumors	ID of residual follicular structure in LP HD and other diseases Evaluation of low-grade B-cell lymphomas ID of follicular dendritic cell sarcoma	
CD22* *(BL-CAM)*	Type I integral membrane glycoprotein *(membrane, cytoplasm)*	B cells, precursor B cells	B-cell neoplasms (but not plasma cell lesions)	Pan B cell marker	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD23*	Membrane glycoprotein, low-affinity IgE receptor *(membrane)*	Subpopulation of peripheral B cells, follicular dendritic cells	CLL, but usually not mantle zone lymphoma, MALTomas, or follicular lymphomas	Evaluation of low-grade B-cell lymphomas	
CD25* *(IL-2 receptor)*	Interleukin-2 receptor *(membrane, cytoplasm)*	Subpopulation of T cells, myeloid precursors, oligodendrocytes HTLV-1 transformed T and B cells	Hairy cell leukemia, adult T-cell lymphoma/leukemia, some T-cell prolymphocytic leukemia, precursor lymphoblastic lymphoma, and anaplastic large cell lymphoma	Evaluation of cutaneous T-cell lymphomas for potential anti-CD25 therapy	
CD30* *(Ki-1, BERH2)*	Single chain transmembrane glycoprotein, homologous to the nerve growth factor superfamily *(cytoplasm, membrane and Golgi)*	Activated B and T cells, some plasma cells, immunoblasts, interdigitating cells, histiocytes, follicular center cells, decidualized endometrium, reactive mesothelial cells, most other tissues negative	Anaplastic (CD30+) large cell lymphomas, large B-cell lymphoma, primary effusion lymphoma, mediastinal large B-cell lymphoma, Reed–Sternberg cells (not LP HD), enteropathy T-cell lymphoma, peripheral T-cell lymphoma, EBV-transformed B cells Embryonal carcinoma, vascular tumors (not KS), some mesotheliomas, rarely carcinomas are positive	Evaluation of anaplastic (CD30+) lymphomas. Evaluation of HD (Reed–Sternberg cells are positive except in LP HD) Evaluation of peripheral T-cell lymphoma (large cells may be positive)	
CD33* *(My 9)*	Myeloid-specific receptor (sialic acid-binding immunoglobulin-like lectin or Siglec-3) *(membrane)*	Granulocytes, monocytes	AML	Evaluation of leukemias	Gemtuzumab ozogamicin is a humanized CD33 antibody linked to an antitumor antibiotic calicheaminin for the treatment of AML
CD34* *(HPCA-1, QBEnd10)*	Single chain transmembrane glycoprotein *(cytoplasm, membrane)*	Lymphoid and myeloid hematopoietic progenitor cells, endothelial cells, some skin cells, myofibroblasts	Acute leukemia Neurofibroma, angiosarcoma, KS, epithelioid hemangioendothelioma, solitary fibrous tumor, DFSP, epithelioid sarcoma, GIST, myofibroblastic tumors	ID of endothelial or myofibroblastic differentiation in tumors Evaluation of angiogenesis Evaluation of the number of blasts in bone marrow in acute leukemia.	Not specific for endothelial cells

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD35* *(CR1, C3b/C4b R)*	Transmembrane protein t that binds complement components C3b and C4b and mediates phagocytosis *(membrane)*	Erythrocytes, B cells, a subset of T cells, monocytes, neutrophils, eosinophils, glomerular podocytes, follicular dendritic cells	Marginal zone (MALT) lymphoma, follicular dendritic cell tumors	Detects follicular dendritic cells ID of follicular dendritic cell sarcomas	
CD38*	Type II transmembrane glycoprotein with enzymatic action for the formation and hydrolysis of cADPR *(membrane)*	Immature B and T lymphocytes, thymocytes, mitogen-activated T cells, Ig-secreting plasma cells, monocytes, NK cells, erythroid and myeloid progenitors, brain cells	Acute leukemias, plasma cell lesions Neurofibrillary tangles in Alzheimer's disease	ID of plasma cell lesions	Immunoreactivity may be a poor prognostic marker for patients with CLL
CD43* *(Leu 22, L60)*	Cell surface glycoprotein *(membrane)*	T cells, macrophages, granulocytes	AML (chloromas), T-cell neoplasms, aberrant expression in some low grade B-cell neoplasms (e.g. mantle cell lymphoma, SLL/CLL, marginal zone lymphoma), some MALT lymphomas	Evaluation of T-cell lymphomas and leukemias. Evaluation of low-grade B-cell lymphomas	Less specific than UCHL-1 for T cells
CD45, Leukocyte common antigen* *(LCA, CLA) Note:* CLA also refers to a different antigen, HECA-452	Five or more membrane glycoproteins *(membrane, cytoplasm)*	Lymphocytes, leukocytes, histiocytes, not plasma cells, erythrocytes, platelets	Non-Hodgkin's lymphomas, some anaplastic (CD30+) large cell lymphomas, Reed–Sternberg cells in LP HD (but not other types)	ID of poorly differentiated neoplasms as lymphomas. However, some anaplastic lymphomas and plasmacytomas may be negative	Preserved in necrotic tissue Best general marker for hematologic neoplasms
CD45RA *(DPB)*	Restricted form of leukocyte common antigen *(membrane, cytoplasm)*	B cells, monocytes, some T cells	B-cell neoplasms, hairy cells (not specific)	Pan B cell marker that can be used in Zenker's fixed tissue	Not completely specific—other B-cell markers are preferred
CD45RO *(UCHL-1)*	Isoform of CD45 (leukocyte common antigen) *(membrane, cytoplasm)*	T cells, granulocytes, monocytes	T-cell neoplasms, histiocytic sarcoma	Good pan T cell marker (CD3 is more specific)	
CD56* *(NCAM)*	Neural cell adhesion molecule—cell surface glycoprotein *(membrane)*	Neurons, astrocytes, Schwann cells, NK cells, subset of activated T cells	Some T/NK cell lymphomas, plasmacytomas Neuroblastoma	Evaluation of panniculitis-like T-cell lymphoma (both CD56+ and CD56–) and T/NK lymphomas	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD57* *(Leu 7, HNK-1)*	Lymphocyte antigen that cross-reacts with a myelin-associated glycoprotein *(membrane)*	T-cell subsets, NK cells, myelinized nerves, neuroendocrine cells, prostate, pancreatic islets, adrenal medulla	Angioimmunoblastic T-cell lymphoma Nerve sheath tumors (occasional), leiomyosarcoma, synovial sarcoma, rhabdomyosarcoma, neuroblastoma, gliomas, neuroendocrine carcinomas, neurofibromas, some prostate carcinomas	ID of T gamma lymphoproliferative disorder (large granular cell lymphocytic leukemia) ID of neuroendocrine differentiation in tumors Evaluation of NK neoplasms	Not very specific for solid tumors
CD61 *(GPIIIa, platelet glycoprotein IIIa)*	Glycoprotein, receptor for fibrinogen, fibronectin, von Willebrand factor, and vitronectin *(cytoplasm)*	Megakaryocytes, platelets	Megakaryocytic leukemias	ID of megakaryocytic differentiation	
CD68* *(KP1, CD68-PGM1, Mac-M)*	Intracellular glycoprotein associated with lysosomes *(cytoplasm, membrane)*	Macrophages, monocytes, neutrophils, basophils, large lymphocytes, Kupffer cells, mast cells, osteoclasts	Some lymphomas, histiocytic sarcomas, APML, Langerhans proliferative disorders Neurofibroma, schwannoma, MPNST, granular cell tumors, PEComa, melanomas, atypical fibroxanthoma, RCC	Best general marker for macrophages, although not specific to this cell type	The antibody PG-M1 does not react with granulocytes
CD74 *(LN2)*	Subunit of MHC II-associated invariant chain *(membrane)*	B cells, monocytes, histiocytes	B-cell neoplasms, hairy cell leukemia, plasma cell lesions	Pan B cell marker	
CDw75* *(LN1)*	Sialylated glycoconjugate present in surface Ig-positive B cells *(membrane, mytoplasm)*	Mature B cells, T-cell subsets, fetal colon, epithelial cells	Reed–Sternberg cells of LP HD (not other types), follicular lymphomas Colon carcinomas (50%), gastric carcinomas	Evaluation of HD	
CD77 *(BLA.36, PK antigen)*	Globotriaosylceramide, glycolipic membrane from Burkitt's lymphoma cell line (*cytoplasm, membrane*)	Tonsillar B cells, dendritic reticulum cells, sinus-lining cells, macrophages, endothelial cell, epithelial cells	HD, Burkitt's lymphoma, rarely other B- and T-cell lymphomas	Evaluation of RS cells	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD79a *(mb-1 protein)*	Heterodimer of mb-1 (CD79a) and B29 (CD79b) polypeptides, B-cell antigen receptor *(membrane)*	B cells, plasma cells	Precursor B-cell ALL, B-cell lymphomas, plasma cell lesions, but not primary effusion lymphoma	Evaluation of B-cell neoplasms (may be the only B-cell marker present)	
CD79b*	See above *(membrane)*			Absent from CLL, hairy cell leukemia	
CD95* *(Fas)*	Transmembrane glycoprotein member of the nerve growth factor receptor/ tumor necrosis factor superfamily—mediates apoptosis *(membrane)*	Activated T and B cells, epithelial cells	Panniculitis-like T-cell lymphoma (if CD56+)		
CD99* *(MIC-2, 12E7, Ewing's sarcoma marker, E2 antigen, HuLy-m6, FMC 29, O13 [different epitope])*	*MIC2* gene product—glycoproteins (p30 and p32) involved in rosette formation with erythrocytes *(membrane)* *Membrane immunoreactivity is more specific than cytoplasmic*	Cortical thymocytes, T lymphocytes, granulosa cells of ovary, pancreatic islet cells, Sertoli cells, some endothelial cells, urothelium, ependymal cells, squamous cells	B- and T-cell precursor lymphoblastic lymphoma/ leukemia PNET/Ewing's sarcoma, chondroblastoma, synovial sarcoma, solitary fibrous tumors, GIST, some alveolar rhabdomyosarcomas, desmoplastic small cell tumors, small cell carcinomas, granulosa cell tumors, yolk sac components of germ cell tumors, Sertoli–Leydig cell tumors, atypical fibroxanthoma, meningioma	Evaluation of lymphoblastic lymphoma/leukemia Thymic carcinomas (lymphocytes +) versus other carcinomas. ID of PNET/Ewing's sarcoma (immunoreactivity should be clearly membranous in the majority of the cells)	O13 is the most commonly used antibody Immunoreactivity is highly dependent upon the antigen retrieval system used
CD103*	Mucosal integrin $\alpha E\beta 7$ with specificity for e-cadherin *(cytoplasm)*	T cells	Enteropathy-type T-cell lymphoma, hairy cell leukemia		Requires frozen tissue or cell suspension

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
CD117* *(c-kit, stem cell factor receptor)*	Transmembrane tyrosine kinase receptor (ligand is stem cell factor)—apoptosis is inhibited when the ligand is bound *(cytoplasm, membrane)*	Mast cells, interstitial cells of Cajal (ICC—pacemaker cells of the GI tract found throughout the muscle layers and in the myemteroc plexus), epidermal melanocytes, mononuclear bone marrow cells (4%), Leydig cells, early spermatogenic cells, trophoblast, breast epithelium	GIST (>95%), seminomas (>70%), intratubular germ cell neoplasia, mature teratomas (>70%), some melanomas (focal), mast cell tumors, some carcinomas, some brain tumors, some PNET/Ewing's sarcoma, some angiosarcomas AML (>50%), CML in myeloid blast crisis	ID of GIST (+) versus leiomyomas (–) and schwannomas (–) ID of seminomas ID of mast cells (mastocytosis)	Mast cells are an excellent internal control CD117 positivity does not correlate with mutations and/or oncoprotein activity in tumors not known to have activating mutations and is, in general, not of clinical or therapeutic significance in this setting (e.g., to detect tumors likely to respond to therapy directed against the protein, e.g., Gleevec)
CD138* *(Syndecan-1)*	Transmembrane heparin sulphate glycoprotein that interacts with extracellular matrix and growth factors *(membrane)*	Pre-B cells, immature B cells, Ig-producing plasma cells, basolateral surface of epithelial cells, vascular smooth muscle, endothelium, neural cells	Plasma cell lesions, primary effusion lymphoma, plasma cell component of other B cell lymphomas Squamous cell carcinomas, other carcinomas	ID of plasma cells and their neoplasms Expression may be diminished or lost in poorly differentiated carcinomas	
CD207 *(Langerin)*	Langerhans cell specific C-type lectin (cytoplasm)	Langerhans cells of epidermis and epithelia	Langerhans cell histiocytosis		Induces formation of Birbeck granules
Clusterin* *(Apolipoprotein J, complement lysis inhibitor, gp80, SGP-2, SP40,TRPM2, T64, ApoJ)*	Multifunctional protein involved in lipid transport, complement regulation, immune regulation, cell adhesion, other functions *(membrane, cytoplasm, nucleus)*	Many tissues	Anaplastic large cell lymphoma (Golgi pattern) Alzheimer's disease—present in amyloid plaques and cerebrovascular deposits Many types of carcinomas		

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Cyclin D1* *(PRAD1, bcl-1)*	Cyclin-regulating cyclin-dependent kinases during G1 in the cell cycle, phosphorylates and inactivates the retinoblastoma tumor suppressor protein *(nucleus)*	Cycling cells (however, lymphocytes usually express only cyclins D2 and D3)	Mantle cell lymphoma Breast cancer (esp. lobular carcinomas and other ER positive carcinomas), esophageal cancer, bladder cancer, lung cancer, HCC, colon carcinoma, pancreatic carcinoma, head and neck squamous cell carcinomas, pituitary tumors, sarcomas Parathyroid adenomas (inversion involving cyclin D1 gene and the parathormone receptor)	ID of mantle cell lymphoma	Involved in t(11;14)(q13;q32) translocation in mantle cell lymphoma
DBA.44* *(HCL)*	B-cell antigen *(cytoplasm, membrane)*	Mantle zone B cells, some immunoblasts	Hairy cell leukemia (>95%), B-cell lymphomas (30%)	Evaluation of hairy cell leukemia	
Epithelial membrane antigen* *(EMA, MUC1, HMFG, DF3, CA 15-3, CA 27.29, PEM, many others)*	Episialin, glycoprotein found in human milk fat globule membranes *(cytoplasm [more common in malignant cells], membrane [more common in benign cells])*	Epithelial cells, perineurial cells, meningeal cells, plasma cells, usually negative in mesothelial cells, monocytes	Some anaplastic large cell lymphomas (CD30+), plasma cell neoplasms, malignant histiocytosis, erythroleukemia, AML (M4 and M5), LP HD Carcinomas, mesotheliomas, some sarcomas (synovial sarcoma, epithelioid sarcoma), adenomatoid tumor, chordomas, peri-neurioma, neurofibroma, meningiomas, desmoplastic small round cell tumor, Sertoli cell tumor	ID of epithelial differentiation in tumors—however, keratin is more specific for this purpose. Beware of EMA in some large cell lymphomas Synovial sarcoma typically shows focal positivity	There are over 50 monoclonal antibodies recognizing different glycosylation patterns in normal tissues and tumors[16]
Epstein–Barr virus EBV-encoded nonpolyadenylated early RNAs *(EBERS)*	RNA produced by EBV *(nucleus)*	EBV-infected B cells	All EBV-related tumors	Most sensitive marker for EBV	Detected by in situ hybridization for RNA on paraffin sections

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
LMP-1*	Latent membrane protein *(membrane)*	EBV-infected B cells	Nasopharyngeal carcinomas, Reed–Sternberg cells (not LP HD), transplant lymphomas, AIDS-related lymphomas, endemic Burkitt's lymphoma (rare in sporadic cases)	Evaluation of EBV-related neoplasms	
EBNA 2 *(nuclear antigen 2)*	Nuclear protein *(nucleus)*	EBV-infected B cells	Transplant-related lymphomas, AIDS-related lymphomas. Not present in Burkitt's lymphoma or nasopharyngeal carcinomas	Evaluation of transplant- and AIDS-related lymphomas	
Fascin	Actin bundling protein regulated by phosphorylation *(cytoplasm)*	Interdigitating reticulin cells from the T-cell zones, dendritic cells, reticular network, histiocytes, smooth muscle, endothelium, squamous cells, splenic sinuses	Reed–Sternberg cells (but not in LP HD) High-grade breast carcinomas	ID of Reed–Sternberg cells in classical HD Fascin positivity has also been reported in anaplastic large cell lymphoma	
FMC7	Antigen on subgroups of mature B cells, epitope of CD20 *(cytoplasm)*	B cells	B-cell lymphomas	Not expressed by CLL	Pan B cell marker Epitope of CD20 but reactivity low in cells with low cholesterol
Glycophorin A *(GPA)*	A glycosylated erythrocyte membrane protein *(membrane)*	Erythroid elements at all stages	Erythroleukemia	ID of erythroid elements (normal and neoplastic)	
Granzyme B*	Neutral serine proteases stored in granules in cytotoxic T cells and in NK cells involved in target cell apoptosis by exocytosis *(cytoplasm)*	Cytotoxic T cells and NK cells	Some T-cell lymphomas, Reed–Sternberg cells of some cases of EBV-positive HD		
Heavy immunoglobulin chains* *(G, A, M, D)*	Heavy chain of immunoglobulins *(Cytoplasm [plasma cells], membrane [lymphocytes])*	Plasma cells (G>A>M>D)	Plasma cell tumors (monotypic expression of usually G or A), mantle zone lymphomas and WDLL/CLL may co-express M and D, lymphoplasmacytic lymphoma (M)	ID of monoclonal populations of plasma or plasmacytoid cells	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
HECA-452* *(Endothelial cell antigen, cutaneous lymphocyte-associated antigen, CLA)*	Cell surface glycoprotein *(membrane)*	T cells, more common in cutaneous T cells	Mycosis fungoides and other cutaneous T-cell lymphomas		*Note:* CLA is also used to refer to CD45
Hemoglobin *(Hb)*	Hemoglobin *(cytoplasm)*	Erythroid cells	Some leukemias	Marker for erythroid cells	
HHV8*	Latent nuclear antigen of human herpesvirus type 8 *(nucleus)*	Absent in normal tissue	Primary effusion lymphoma (PEL), AIDS-associated multicentric Castleman's disease Kaposi's sarcoma (endothelial cells and some perivascular cells)	Evaluation of Kaposi's sarcoma and primary effusion lymphoma	
HLA-DR	Major histocompatibility complex Class II gene	B lymphocytes, macrophages, Langerhans cells, dendritic cells, activated T cells, some endothelial and epithelial cells	Leukemic myoblasts		Not very specific for cell type.
Light immunoglobulin chains* *(lambda [L], kappa [K])*	Light chain of immunoglobulins *(cytoplasm)*	Plasma cells (normally K>L), B cells	Plasma cell tumors, B-cell lymphomas	ID of monoclonal populations of plasma c ells and B cells ID of some types of amyloid	May require frozen tissue for assessment of B lymphoid cells Excellent Ig preservation in plasma cells in B5 or Zenker's fixed tissue
Lysozyme *(Ly)*	Muramidase *(cytoplasm)*	Circulating monocytes, some tissue macrophages, granulocytes, salivary gland, lacrimal gland, stomach and colon epithelial cells (inflamed or regenerative), apocrine glands, some other epithelial cells	AML with monocytic differentiation, salivary gland tumors, stomach and colon carcinomas.	Marker for histiocytes but not specific. May mark activated phagocytic macrophages Evaluation of myeloid leukemias Strongly positive in monocytoid leukemias	Not specific for solid tumor identification
Mast cell tryptase	Serine protease *(cytoplasm)*	Mast cells	Mast cell neoplasms	ID of mast cell differentiation	
Myeloperoxidase* *(MPO)*	Enzyme in primary granules of myeloid cells *(cytoplasm)*	Myeloid cells, monocytes	AML, chloromas	Classification of leukemias	Can be used with tissue fixed in Zenker's fixative

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
Oct2* *(Octomer transcription factor)*	Transcription factor of the POU homeo-domain family binding to the Ig gene octomer sites regulating B-specific genes *(nucleus)*	B cells	B-cell lymphomas and leukemias Reed–Sternberg cells in LP HD (but not other types)	Evaluation of HD	Interacts with the transcriptional coactivator BOB.1 BOB.1 and Oct are necessary (but not sufficient) for Ig expression
Perforin*	Pore-forming protein in cytoplasmic granules of cytotoxic T cells *(cytoplasm)*	NK cells, large granular lymphocytes, gamma/delta T cells	NK cell lymphomas, anaplastic large cell lymphoma	Evaluation of T-cell lymphomas	
TCR* *(T-cell antigen receptor, JOVI 1)*	Two polypeptide chains (alpha and beta)	Peripheral T cells	Many T-cell lymphomas	Evaluation of T-cell lymphomas	Alpha/beta and gamma/delta T cell receptors can be evaluated in frozen tissue
Terminal deoxytransferase* *(TdT)*	Enzyme that catalyzes addition of nucleotides to ss DNA *(nucleus)*	Immature T and B cells	Lymphoblastic lymphoma/ ALL	Lymphoblastic lymphoma (+) versus Burkitt lymphoma (–)	
TIA-1 *(T-cell intracellular antigen)*	A cytolytic granule associated protein expressed in some CD8+ T cells *(cytoplasm)*	T cells, mast cells, polymorphonuclear leukocytes, eosinophils	Many T-cell lymphomas	Evaluation of T-cell lymphomas	

Table 7-30 Antibodies for immunohistochemistry—*cont'd*

NAME (ALTERNATE NAME)	ANTIGEN (LOCATION)	NORMAL CELLS AND TISSUES	TUMORS	USES	COMMENTS
traf-1* *(Tumor necrosis factor receptor-associated factor)*	Membrane-bound proteins that activate the nuclear factor-(kappa)B (NF-(kappa)B) transcription factor resulting in cell proliferation *(cCytoplasm)*		Hodgkin's lymphoma		May interact with LMP1

Abbreviations: AD, Alzheimer's disease; AIDS, acquired immunodeficiency syndrome; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; APML, acute promyelogenous leukemia; BM, basement membrane; CML, chronic myelogenous leukemia; CMV, cytomegalovirus; DFSP, dermatofibrosarcoma protuberans; EBV, Epstein–Barr virus; FISH, fluorescence in situ hybridization; GIST, gastrointestinal stromal tumor; HCC: hepatocellular carcinoma; HD, Hodgkin's disease; HNPCC, hereditary non-polyposis colorectal cancer; ID, identification; KS, Kaposi's sarcoma; LP HD, lymphocyte predominant Hodgkin's disease; MF, mycosis fungoides; MPNST, malignant peripheral nerve sheath tumor; NK, natural killer; PIN, prostatic intraepithelial neoplasia; PNET, primitive neuroectodermal tumor; RCC, renal cell carcinoma; RS, Reed–Sternberg cells; TCC, transitional cell carcinoma.

Notes:

NAME: The most common name used to refer to the marker; see also Box 7-1. The name may refer to the antigen, a CD number, or a specific antibody raised to the antigen. In some cases more than one name is commonly used. Antibodies with asterisks appear in the Tables. Most CD numbers correspond to a specific gene product. However, some correspond to antigens from post-translational modifications. For example, CD15 (LeuM1) is a carbohydrate side chain linked to a protein.

ALTERNATE NAME: This list includes abbreviations, antibody names (sometimes recognizing different epitopes), or other terms for the marker.

ANTIGEN: The antigen recognized by the antibody.

LOCATION: The normal location of the antigen. In some cases, only certain locations of the antigen are considered a positive result (e.g., nuclear immunoreactivity for estrogen receptor, membrane immunoreactivity for HER-2/neu).

NORMAL CELLS AND TISSUES: The presence of the marker in normal cells and tissues. These cells serve as important internal positive controls. Abnormal positive immunoreactivity is also an important control for the specificity of the study.

TUMORS: The tumors in which immunoreactivity is typically expected. Refer to the Tables for additional information.

USES: The most common uses for the marker. Different pathologists and institutions often have preferences for the use of certain markers.

COMMENTS: Additional comments regarding the marker.

Additional information on CD antigens can be found at http://www.ncbi.nlm.nih.gov/prow/guide/45277084.htm

Box 7-1 Alternative names for antigens

Looking for?	*Find it under:*
1D5	Estrogen receptor (G)
6F/3D	Beta-amyloid
12E7	CD99 (G, H)
34βE12	Keratins (G)
38.13	CD77 (H)
70 kD NF	Neurofilaments (G)
200 kD NF	Neurofilaments (G)
903	Keratins—34βE12 (G)
A (blood group antigen)	Blood group antigens (G)
A (Ig heavy chain α)	Heavy chain immunoglobulins (H)
A32 antigen	CD146 (G)
A103	MELAN-A (G)
AAT	Alpha 1-antitrypsin (G, H)
ACH	Alpha-1 antichymotrypsin (H)
AE1/AE3	Keratins (G)
AFP	Alpha-fetoprotein (G)
Alpha 1-antitrypsin	Alpha 1-antitrypsin (G, H)
Alpha 1-anti-chymotrypsin	Alpha 1-antichymotrypsin (H)
Alpha 1-fetoprotein	Alpha fetoprotein (G)
Alpha fetoprotein	Alpha fetoprotein (G)
Alpha-methylacyl-CoA racemase	AMACR (G)
Alpha smooth muscle actin	Alpha smooth muscle actin (G)
AMACR	AMACR (G)
Amyloid	Beta-amyloid (G)
Androgen receptor	Androgen receptor (G)
Apolipoprotein J	Clusterin (H)
AR	Androgen receptor (G)
B (blood group antigen)	Blood group antigens (G)
B1	CD20 (H)
B2	CD21 (H)
B4	CD19 (H)
B72.3	B72.3 (G)
bcl-1	Cyclin D1 (H)
bcl-2	bcl-2 (H, G)
B-cell specific activator protein	BSAP (H)
BER-EP4	BER-EP4 (G)
BERH2	CD30 (G, H)
Beta-amyloid	Beta-amyloid (G)
Beta-catenin	Beta-catenin (G)
Beta-2 microglobulin	Beta-2 microglobulin (G)
BG8	BG8 (G)
β-hCG	Human chorionic gonadotropin (G)
BLA.36	CD77 (H)
BL-CAM	CD22 (H)
Blood group antigens	Blood group antigens (G, H)
BR-2	Gross cystic disease fluid protein-15 (G)
BRST-2	Gross cystic disease fluid protein-15 (G)
C3b/C4bR	CD35 (H)
C5b-9	C5b-9 (G)
c-kit	CD117 (G)
CA 15-3	Epithelial membrane antigen (G, H)
CA 19-9	CA 19-9 (G)
CA 27.28	Epithelial membrane antigen (G, H)
CA 72-4	B72.3 (G)
CA125	CA125 (G)
CA19-9	CA19-9 (G)
Calcitonin	Calcitonin (G), Hormones (G)
Caldesmon	Caldesmon (G)
Calgranulin	MAC 387 (G)
CALLA	CD10 (G, H)
CALP	Calponin (G)
Calponin	Calponin (G)
Calprotectin	MAC 387 (G)
Calretinin	Calretinin (G)
CAM5.2	Keratins (G)
Carbohydrate antigen 19-9	CA19-9 (G)
Carcinoembryonic antigen	Carcinoembryonic antigen (G)
CD1a	CD1a (H)
CD2	CD2 (H)
CD3	CD3 (H)
CD4	CD4 (H)
CD5	CD5 (G, H)
CD7	CD7 (H)
CD8	CD8 (H)
CD10	CD10 (G, H)
CD11b	CD11b (H)
CD11c	CD11c (H)
CD13	CD13 (H)
CD15	CD15 (G, H)
CD16	CD16 (H)
CD19	CD19 (H)
CD20	CD20 (H)
CD21	CD21 (H)
CD22	CD22 (H)
CD23	CD23 (H)
CD25	CD25 (H)
CD30	CD30 (G, H)
CD31	CD31 (G)
CD33	CD33 (H)
CD34	CD34 (G, H)
CD35	CD35 (H)
CD38	CD38 (H)
CD43	CD43 (H)

Looking for?	*Find it under:*
CD44v3	CD44v3 (G)
CD45	CD45 (H)
CD45RA	CD45RA (H)
CD45Ro	CD45Ro (H)
CD56	CD56 (H)
CD 57	CD57 (G)
CD61	CD68 (G, H)
CD68	CD68 (G, H)
CD74	CD74 (H)
CDw75	CDw75 (H)
CD77	CD77 (H)
CD79a	CD79a (H)
CD79b	CD79b (H)
CD95	CD95 (H)
CD99	CD99 (G, H)
CD117	CD117 (G)
CD141	CD141 (G)
CDX	CDX (G)
CDKN2	p16 (G)
CDP	Gross cystic disease fluid protein-15 (G)
CEA	Carcinoembryonic antigen (G)
c-erbB2	HER-2/neu (G)
Chromogranin A	Chromogranin A (G)
c-kit	CD117 (G)
CLA	CD45 (H) or HECA-452 (H)
CLDN1	Claudin (G)
Clusterin	Clusterin (H)
Collagen IV	Collagen IV (G)
Common acute leukemia antigen	CD10 (G, H)
Complement lysis inhibitor	Clusterin (H)
CR1	CD35 (H)
Cyclin D1	Cyclin D1 (H)
Cystic fibrosis antigen	MAC 387 (G)
D (Ig heavy chain δ)	Heavy chain immunoglobulins (H)
DBA.44	DBA.44 (H)
Desmin	Desmin (G)
DF3	Epithelial membrane antigen (G, H)
DPB	CD45RA (H)
E2 antigen	CD99 (G, H)
EBERS	Epstein–Barr virus (G, H)
EBNA	Epstein-Barr virus (G, H)
E-cadherin	E-cadherin (G)
EGFR	EGFR (G)
EM ACT	HHF-35 (G)
EMA	Epithelial membrane antigen (G)
E-MEL	HMB-45 (G)
Endothelial cell antigen	HECA-452 (H)

Looking for?	*Find it under:*
Ep-CAM	BER-EP4 (G)
Epidermal growth factor receptor	EGFR (G)
Epithelial membrane antigen	Epithelial membrane antigen (G, H)
Epithelial specific antigen	BER-EP4 (G)
Epstein–Barr virus	Epstein–Barr virus (G, H)
ER	Estrogen receptor (G)
erbB2	HER-2/neu (G)
ESA	BER-EP4 (G)
Estrogen receptor	Estrogen receptor (G)
Ewing's sarcoma marker	CD99 (G, H)
Factor VIII related antigen	Factor VIII (G)
FVIII:RAg	Factor VIII (G)
Factor XIIIa	Factor XIIIa (G)
Fascin	Fascin (H)
Fast myosin	Myosin Heavy Chain (G)
Fibronectin	Fibronectin (G)
Fli-1	Fli-1 (G)
FMC7	FMC7 (H)
FMC 29	CD99 (G, H)
Friend leukemia integrin-site 1	Fli-1 (G)
FVIII:g	Factor VIII (G)
G (Ig heavy chain gamma)	Heavy chain immunoglobulins (H)
Gal-3	Galectin-3 (G)
Galectin-3	Galectin-3 (G)
Gastrin	Hormones (G)
GCDFP	Gross cystic disease fluid protein-15 (G)
GFAP	Glial fibrillary acidic protein (G)
Glial fibrillary acidic protein	Glial fibrillary acidic protein (G)
Glucagon	Hormones (G)
Glucose transporter 1	GLUT-1 (G)
GLUT-1	GLUT-1 (G)
GPIIIa	CD61 (H)
gp80	Clusterin (H)
gp200	RCC (G)
GPA	Glycophorin A (H)
Granzyme B	Granzyme B (H)
Gross cystic disease fluid disease-15	Gross cystic disease fluid protein-15 (G)
H (blood group antigen)	Blood group antigens (G)
H222	Estrogen receptor (G)
Hb	Hemoglobin (H)
HBME-1	HBME-1 (G)
h-caldesmon	Caldesmon (G)
H-CAM	CD44v3 (G)

Looking for?	*Find it under:*
hCG	Human chorionic gonadotropin (G)
HCL	DBA.44 (H)
HBME-1	HBME-1 (G)
Heavy chain immunoglobulins	Heavy chain immunoglobulins (H)
HECA-452	HECA-452 (H)
Hematopoietic progenitor cell, class 1	CD34
Hemoglobin	Hemoglobin (H)
HepPar-1	HepPar-1 (G)
Hepatocyte paraffin-1	HepPar-1 (G)
HER-2/neu	HER-2/neu (G)
HHF-35	HHF-35 (G)
HHV8	HHV8 (H)
HLA-DR	HLA-DR (H)
HMB-45	HMB-45 (G)
HMFG	Epithelial membrane antigen (G, H)
HNK-1	CD57 (G, H)
hMLH1	hMLH1 (G)
hMSH2	hMLH1 (G)
HNK-1	CD57 (G)
HP1	HepPar-1 (G)
HPCA-1	CD34 (G, H)
HPL	Human placental lactogen (G)
HuLy-m6	CD99 (G, H)
Human chorionic gonadotropin	Human chorionic gonadotropin (G)
Human herpesvirus 8	HHV8 (G, H)
Human mutL homologue 1	hMLH1 (G)
Human mutS homologue 2	hMLH1 (G)
Human placental lactogen	Human placental lactogen (G)
IL-2 receptor	CD25 (H)
Inhibin-alpha subunit	Inhibin-alpha subunit (G)
Insulin	Hormones (G)
J5	CD10 (G, H)
JOVI 1	TCR (H)
K (Ig light chain κ)	Light chain immunoglobulins (H)
Keratin 5/6	Keratins (G)
Keratin 7	Keratins (G)
Keratin 20	Keratins (G)
Keratins	Keratins (G)
Ki-1	CD30 (G, H)
Ki-67	Ki-67 (G)
kip2	p57 (G)
Kit	CD117 (G)
KP-1	CD68 (G, H)

Looking for?	*Find it under:*
L (Ig light chain lambda)	Light chain immunoglobulins (H)
L1 antigen	MAC 387 (G)
L26	CD20 (H)
L60	CD43 (H)
Laminin	Laminin (G)
LCA	CD45 (H)
Leu 1	CD5 (H)
Leu 2	CD8 (H)
Leu 3	CD4 (H)
Leu 5a + b	CD2 (H)
Leu 7	CD57 (G, H)
Leu 9	CD7 (H)
Leu 16	CD20 (H)
Leu 22	CD43 (H)
Leukocyte common antigen	CD45 (H)
LeuM1	CD15 (G, H)
Light chain immunoglobulins	Light chain immunoglobulins (H)
LFA-2	CD2 (H)
LMP-1	Epstein–Barr virus (G, H)
LN1	CDw75 (H)
LN2	CD74 (H)
Lysozyme	Lysozyme (H, G)
M (Ig heavy chain μ)	Heavy chain immunoglobulins (H)
Mac-1	CD11b (H)
MAC 387	MAC 387 (G)
Mac-M	CD68 (G, H)
MART-1	MELAN-A (G)
Mast cell tryptase	Mast cell tryptase (H)
mb-1	CD79a (H)
MCAM	CD146 (G)
ME491	CD63 (G)
MELAN-A	MELAN-A (G)
Melanoma antigen recognized by T cells	MELAN-A (G)
Melanoma-associated antigen	CD63 (G)
Melanoma cell adhesion molecule	CD146 (G)
Melanoma-specific antigen	HMB-45 (G)
MELCAM (or Mel-CAM)	CD146 (G)
MIB-1	Ki-67 (G)
MIC-2	CD99 (G, H)
MN-4	CD146 (G)
MNF-116	Keratin—Pan-K (G)
MPO	Myeloperoxidase (H)
MRF4	Myf-4 (G)
MSA	HHF-35 (G)
MTS1	p16 (G)

Looking for?	*Find it under:*
MUC1	Epithelial membrane antigen (G, H)
MUC18	CD146 (G)
Muscle common actin	HHF-35 (G)
Muscle-specific actin	HHF-35 (G)
My 7	CD13 (H)
My 9	CD33 (H)
Myeloperoxidase	Myeloperoxidase (H)
Myf-4	Myf-4 (G)
MyoD1	MyoD1 (G)
Myogenin	Myf-4 (G)
Myoglobin	Myoglobin (G)
Myosin Heavy Chain	Myosin Heavy Chain (G)
NCAM	CD56 (H)
Neprilysin	CD10 (G, H)
NEU N	NEU N (G)
Neurofilaments	Neurofilaments (G)
Neuron-specific enolase	Neuron-specific enolase (G)
NFP	Neurofilaments (G)
NKI-betab	HMB-45 (G)
NKI/C3	CD63 (G)
NSE	Neuron-specific enolase (G)
O13	CD99 (G, H)
OC125	CA125 (G)
Oct2	Oct2 (H)
Octomer transcription factor	Oct2 (H)
p16	p16 (G)
$p27^{kip1}$	$p27^{kip1}$ (G)
p53	p53 (G)
p57	p57 (G)
p63	p63 (G)
P504S	AMACR (G)
PAN-K	Keratins (G)
PAP	Prostate acid phosphatase (G)
PECAM-1	CD31 (G)
PEM	Epithelial membrane antigen (G, H)
Perforin	Perforin (H)
PGM1	CD68 (G, H)
PgR	Progesterone receptor (G)
PK antigen	CD77 (H)
Placental alkaline phosphatase	Placental alkaline phosphatase (G)
PLAP	Placental alkaline phosphatase (G)
Platelet glycoprotein IIIa	CD61 (H)
PR	Progesterone receptor (G)
PRAD1	Cyclin D1 (H)
PrAP	Prostate acid phosphatase (G)
Prealbumin	Prealbumin (G)
Progesterone receptor	Progesterone receptor (G)
Prostate acid phosphatase	Prostate acid phosphatase (G)
Prostate specific antigen	Prostate-specific antigen (G)
PSA	Prostate-specific antigen (G)
QBEnd10	CD34 (G, H)
Renal cell carcinoma marker	RCC (G)
ret	ret (G)
RCC	RCC (G)
rT3	CD2 (H)
S100	S100 (G)
S-Endo-1	CD146 (G)
SGP-2	Clusterin (H)
SMA	Alpha smooth muscle actin (G)
SM-ACT	Alpha smooth muscle actin (G)
Smad4	DPC4 (G)
SM-MHC	Myosin Heavy Chain (G)
Somatostatin	Hormones (G)
SP40	Clusterin (H)
Stem cell factor receptor	CD117 (G)
Synaptophysin	Synaptophysin (G)
Syndecan-1	CD138 (H)
Synuclein-1	Synuclein-1 (G)
T3	CD3 (H)
T4	CD4 (H)
T6	CD1a (H)
T8	CD8 (H)
T11	CD2 (H)
T64	Clusterin (H)
TAG-72	B72.3 (G)
Tau	Tau (G)
T cell antigen receptor	TCR (H)
T cell intracellular antigen	TIA-1 (H)
TCR	TCR (H)
TdT	Terminal deoxytransferase (H)
TE	CD2 (H)
Terminal deoxytransferase	Terminal deoxytransferase (H)
TH	CD4 (H)
Thrombomodulin	CD141 (G)
Thyroglobulin	Thyroglobulin (G)
Thyroid transcription factor 1	TTF-1 (G)
TIA-1	TIA-1 (H)
TM	CD141 (G)
traf-1	traf-1 (H)
Transthyretin	Prealbumin (G)
TRPM2	Clusterin (H)
TTF-1	TTF-1 (G)

Looking for?	*Find it under:*
TTR	Prealbumin (G)
Tumor-associated glycoprotein 72	B72.3 (G)
Tumor necrosis factor receptor-associated factor	traf-1 (H)
UCHL-1	CD45Ro (H)
UEA 1	Ulex (G)
Ulex	Ulex (G)
Vimentin	Vimentin (G)
von Willebrand's factor	Factor VIII (G)
VWF	Factor VIII (G)
Wilms' tumor 1 protein	WT1 (G)
WT1	WT1 (G)

G, General markers; H, hematopathology markers.

Results

The results of immunoperoxidase studies are incorporated into the surgical pathology report.[14] The following information is included:

- The type of tissue studied: formalin-fixed (or other fixatives) tissue, cryostat sections, cytology preparations, etc.
- The type of immunoagents used, being as specific as possible. For example, do not just list "keratin" but specify the type of keratin (e.g., AE1/AE3).
- The results of the studies in sufficient detail to allow interpretation: for example, the type of cell that is immunoreactive (e.g., tumor versus nontumor), intensity of immunoreactivity (e.g. weak, strong), and/or the number of cells immunoreactive (e.g., focal versus diffuse).
- Integration of the results into the final diagnosis, specifying whether they confirm or support a diagnosis, make one diagnosis more likely than others, or exclude one or more diagnoses.

ELECTRON MICROSCOPY

EM continues to have an important role in surgical pathology.[15]

Indications for EM studies

- Diagnostic renal biopsies for glomerular disease
- Adenocarcinoma versus mesothelioma (see Table 7-29)
- Difficult to classify tumors (Table 7-31)
- Nerve (e.g., toxic or drug-induced neuropathy) and muscle biopsies (e.g., inclusion body or nemaline myopathy)
- Bullous skin diseases (e.g., epidermolysis bullosa)
- Ciliary dysmorphology (primary ciliary dyskinesia or Kartagener's syndrome)
- Endomyocardial biopsies (e.g., Adriamycin toxicity, amyloid, nemaline)
- Liver biopsies for microvesicular fat in acute fatty liver of pregnancy
- Small bowel biopsies to look for pathogens (e.g., Whipple's disease)
- Congenital, inherited, and metabolic diseases (e.g., ceroid lipofuscinoses)
- Prion diseases.

Method

Ultrastructural details of tissues are rapidly lost; therefore fresh tissue must be fixed rapidly and well for EM. Tissues are usually fixed in special fixatives for EM to preserve lipids and glycogen (e.g., 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4).

1. Place a small fragment of tissue in a drop of fixative on a cutting surface.
2. Cut the tissue into multiple tiny fragments, each no greater than 0.1 cm in any dimension.
3. Place the tissue into the vial of fixative. Shake the vial to make sure all the tissue fragments are covered by fixative.

Note. If tissue from a small biopsy is found to be nondiagnostic on H&E, any tissue saved for EM should be retrieved for examination by light microscopy.

Results

A separate EM report is usually issued. The results should be incorporated into the final diagnosis.

SNAP-FROZEN TISSUE

Frozen tissue is useful for immunoperoxidase staining (some antibodies only detect antigens in frozen tissue), enzyme studies (muscle biopsies), and to save tissue for DNA or RNA studies.

Indications

Frozen tissue is useful for all specimens in which there is a question of a lymphoproliferative disorder, sarcomas, unusual tumors, and muscle biopsies.

Methods

Small (approximately $0.5 \times 0.5 \times 0.3$ cm^3) portions of tissue are placed in a clean specimen container moistened with a small amount of normal saline until they can be frozen.

Table 7-31 Electron microscopic features of poorly differentiated tumors

TUMOR	ULTRASTRUCTURE	ADDITIONAL TESTS	COMMENTS
Carcinoma	Well-developed desmosomes (pentalayered with a dense central line in the intracellular space) with intermediate filament attachment Tonofilaments and bundles of filaments (keratin) Adenocarcinomas: Intercellular lumina (but also present in vascular tumors) Microvilli Intracellular lumina (mucin vacuoles in signet ring cells) Squamous cell carcinomas Numerous intermediate filaments (keratin) and desmosomes	IHC: Cytokeratins are present in almost all carcinomas if broad-spectrum antibodies are used EMA is present in almost all carcinomas, but is less specific and sensitive Additional markers can be used to identify specific carcinomas	Other tumors can also be keratin positive and have desmosomes, filaments, and cytokeratin (mesothelioma, meningioma, synovial sarcoma, and epithelioid sarcoma)
Melanoma	Melanosomes in various stages of development, indicative of a melanin-forming cell type Abnormal pleomorphic melanosomes may be present in melanomas Desmoplastic melanomas lack melanosomes	IHC: S100, HMB-45, MART-1 HMB-45 and MART-1 may be absent in non-epithelioid melanomas The HMB-45 epitope (gp100) is present in immature melanosomes or premelanosomes, but is not specific to these structures	Melanosomes are also seen in clear cell sarcoma, pigmented schwannomas, PEComa, and other rare tumors. Mature forms can be taken up by melanophages, keratinocytes, and carcinomas
Lymphoma	No specific features are present. The cells lack cellular junctions and there is a paucity of cytoplasmic organelles	IHC: LCA	LCA may be absent in 30% of anaplastic (ALK1) lymphomas. These tumors can be EMA (+) but are keratin (–)
Sarcoma	Some types have specific diagnostic features of cell type (e.g., neural, smooth muscle, striated muscle) No well developed desmosomes	IHC: May be helpful for identifying specific types	Keratin negative except for synovial sarcoma and epithelioid sarcoma (or rarely in other types)

Protocol for freezing tissue

Equipment needed:

- Flask
- Labeled self-seal bag
- Pyrex beaker
- Liquid nitrogen
- Tin foil
- Isopentane (2-methylbutane).

Technique

1. Fill a flask half full with liquid nitrogen. Always wear protective gloves and a face shield. Dry ice can also be used. The liquid nitrogen is kept in a canister in the Reproductive Endocrinology Laboratory. Gloves and a face shield are stored nearby.
2. Work in a safety cabinet and wear surgical gloves and goggles. Label a freezer bag with the following:
   - Date
   - Patient's name in full
   - Histology reference number
   - Diagnosis/type of tissue.
3. Place 2 cm ($^3/_4$ inch) of isopentane (2-methylbutane) into a glass beaker. *Lower it gently* into the flask containing liquid nitrogen. The isopentane is ready to use when the liquid base is frozen solid (white particles will appear) and the remaining liquid is viscous. Remove the beaker from the flask carefully.
4. Using the forceps, drop small pieces of tissue directly into the isopentane. Freeze the tissue for approximately 30 seconds.
5. Remove the tissue with the forceps and quickly wrap in aluminum foil. Place the tissue in a labeled freezer bag and place in the flask containing the liquid nitrogen.
6. Transfer the specimen bag from the flask to a –20°C freezer.

Table 7-32 Cells, tumors, and structures with characteristic findings by electron microscopy

TUMOR	EM FINDINGS	CORRELATIONS AND OTHER DIAGNOSTIC TESTS
Alveolar soft part sarcoma	Rhomboid, rod-shaped, or spiculated crystals in a regular lattice pattern	The characteristic cytoplasmic crystals are composed of monocarboxylate transporter 1 (MCT1) and its chaperone CD147. These proteins are found in many other cell types and are not specific for this tumor Cytogenetics: t(X;17) creates a ASPL–TFE3 fusion protein IHC: TFE3 positive (as well as rare pediatric renal tumors with the same translocation). Immunoreactivity is not present in other tumors or normal tissues Histo: The crystals are PAS with diastase positive
Amyloid	Non-branching fibrils, 7.5 to 10 nm in width and up to 1 μm in length	May be present associated with plasma cell tumors, medullary carcinoma of the thyroid, Alzheimer's disease, or as an isolated finding (primary amyloidosis) IHC: Can be used to identify specific types of amyloid (e.g., lambda or kappa chains, β2-microglobulin, calcitonin, tau)
Bronchioloalveolar carcinoma of the lung (BAL)	Lamellar (surfactant) "myelin-like" granules in the supranuclear cytoplasm (typical of type II pneumocytes) Clara-like electron-dense granules in supranuclear cytoplasm. Intranuclear inclusions comprised of parallel microtubular arrays These features can also be seen in metastatic BAL	Cytogenetics: These carcinomas are less likely to be associated with smoking and have fewer cytogenetic changes Bronchioloalveolar carcinomas or adenocarcinomas with features of BAL are more likely to respond to Iressa (38%) as compared to other lung carcinomas (14%) due to specific mutations in EGFR Mucinous BAL has intranuclear inclusions but generally lacks the other EM features
Chordoma	Desmosomes, large vacuoles, glycogen, dilated ER, cytoplasmic invaginations, and intermediate filaments The physaliphorous (having bubbles or vacuoles) appearance is due to dilated ER, glycogen, and cytoplasmic invaginations	IHC: keratin (corresponds to intermediate filaments), EMA, S100
Clear cell sarcoma	Melanosomes in various stages of development Glycogen (resulting in clear cytoplasm)	Cytogenetics: t(12;22) EWS–ATF1 fusion protein IHC: S100, HMB-45
Dense core granules	Dense core granules (vesicle bound by a single membrane with a dense center—60 to 300 nm), cytoplasmic organelles involved in regulated exocytosis of cell products Examples: Pancreatic beta cells (insulin): angular crystalline inclusions Pheochromocytoma (epinephrine and norepinephrine): large, pleomorphic, often clear or only partially filled Carcinoid: Foregut—small, round Midgut—larger, pleomorphic Hindgut—mixed	Found in tumors of neuronal or neuroendocrine origin Vesicles are comprised of granins (predominantly chromogranin A, chromogranin B, and secretogranin II) and various peptide hormones and transmitters, ATP, and biogenic amines IHC: chromogranin A (most specific). Specific products of tumors can also be detected *Note:* Prostate cancers and breast cancers can also show strong chromogranin positivity and can be mistaken for neuroendocrine tumors, particularly at metastatic sites
Desmoplastic small round cell tumor	Numerous desmosomes and tight junctions, numerous cell processes, large number of organelles (mitochondria and RER), microfilaments, small neurosecretory granules	Cytogenetics: t(11;22) EWS–WT1 fusion protein IHC: keratin, desmin, WT1, actin, EMA, NSE

Table 7-32 Cells, tumors, and structures with characteristic findings by electron microscopy—*cont'd*

TUMOR	EM FINDINGS	CORRELATIONS AND OTHER DIAGNOSTIC TESTS
Endothelial cells	Weibel–Palade bodies (cigar-shaped membrane bound structures filled with tubules in parallel arrays) Intracytoplasmic lumina may be present in normal cells and in epithelioid vascular neoplasms	Weibel–Palade bodies are frequently absent in tumors arising from endothelial cells (e.g., angiosarcomas). IHC markers are more sensitive to detect endothelial derivation The membranes are formed by P-selectin and the tubules contain FVIII IHC: Vascular markers (CD34, CD31, FVIII)
Ewing's sarcoma (PNET)	Homogeneous cell population characterized by the lack of specialized features, large pools of glycogen, no organelles, no extracellular matrix, variable numbers of neurosecretory granules and cell processes	Cytogenetics: t(11;22) EWS–FLI1 fusion protein (and other less common variants) IHC: CD99. FLI1 is also present, but is less specific Histo: PAS +/– diastase can detect glycogen, but is not currently used for diagnosis
Granular cell tumor	Numerous lysosomes (filled with tubular, vesicular, and amorphous material), phagosomes, and granules (correlating with the "granular" cytoplasm), reduplicated basal lamina surrounding groups of cells	IHC: S100, inhibin, CD68, calretinin
Langerhans cell histiocytosis	Birbeck granules (rod or tennis racket shaped) structures of variable length with a central periodically striated lamella	May serve as a reservoir for Langerin (a transmembrane type II Ca^{2+}-dependent lectin) and CD1a in the endosomal recycling compartment IHC: CD1a, Langerin, S100
Mast cells	Lamellar or scroll-like membrane pattern, granules of variable size	IHC: CD117 (c-kit), mast cell tryptase
Medullary carcinoma of the thyroid	Numerous neurosecretory granules (calcitonin) associated with stromal amyloid (calcitonin)	Cytogenetics: mutations in the *RET* gene (sporadic and germline) IHC: Calcitonin (in tumor cells and amyloid), chromogranin
Merkel cell carcinoma	Neurosecretory granules in processes or along cell membranes (subplasmalemmal)	IHC: chromogranin, NSE, cytokeratin 20
Mesothelioma	Elongated, serpiginous, and branched microvilli (generally 10 to 16 length: 1 width) apical without a glycocalyx or actin rootlets	Cytogenetics: Characteristic chromosome deletions and loss of 9 and 22 IHC: Calretinin, WT1
Neuroblastoma	Cellular processes with microtubules (neuropil), dense core granules, Homer–Wright rosettes (the center is comprised of a tangle of cell processes), synaptic vesicles, no glycogen	Cytogenetics: Changes are linked to prognosis IHC: chromogranin, NSE, NFP, synaptophysin
Oncocytoma	Numerous mitochondria packed in the cytoplasm (correlating with the granular appearance of the cytoplasm). In contrast, chromophobe renal cell carcinoma has fewer mitochondria and more microvesicles	Cytogenetics: Monosomy with loss of X or Y, 11q13. Chromophobe carcinomas have different cytogenetic changes IHC: RCC is negative in oncocytomas but positive in 45–50% of chromophobe renal cell carcinomas
Perineurioma	Long cell processes wrapping around adjacent cells	IHC: Claudin-1 (a component of tight junctions), EMA
Rhabdoid tumor of the kidney	Large paranuclear whorls of intermediate filaments (corresponding to cytokeratin and vimentin) and occasional tonofilaments	Cytogenetics: hSNF5/INI1 deletions and mutations IHC: Cytokeratin, vimentin

Table 7-32 Cells, tumors, and structures with characteristic findings by electron microscopy—*cont'd*

TUMOR	EM FINDINGS	CORRELATIONS AND OTHER DIAGNOSTIC TESTS
Rhabdomyosarcoma	Parallel thick (12 to 15 cm) and thin (6 to 8 nm) myosin-actin filaments, Z bands, filament ribosomal complexes Spider cells may be seen in cardiac tumors (clear cytoplasm divided by cytoplasmic processes and cross striations formed by leptofibrils)	Cytogenetics: Characteristic changes in alveolar and embryonal types IHC: Muscle markers (HHF-35, desmin, myf4)
Schwannoma	Basal lamina prominent, often reduplicated. Luse bodies (long spacing collagen, extracellular), myelin figures, long cell processes wrapping around collagen, may rarely have melanosomes (melanotic schwannoma)	Cytogenetics: Deletion of 2q (NF2 inactivation) IHC: S100

For additional information see also Tables 7-8, 7-9, 7-15, 7-33.

7. Discard the isopentane in a waste bottle. Liquid nitrogen can be allowed to evaporate.
8. Sterilize the beaker and forceps with 10% formalin.
9. Dispose of contaminated sharps and specimen containers in appropriate impervious biohazard containers. Wash your hands after removal of gloves.

If there is insufficient tissue for snap freezing, a frozen section from the OR Consultation Room may be saved frozen for potential studies. Many cryostats undergo an automatic defrost cycle and tissue left as a block in the cryostat will thaw and refreeze. Thus tissue to be saved frozen should be transferred to a freezer.

Results

The results of immunoperoxidase studies on frozen tissue are usually incorporated into the surgical pathology report.

IMMUNOFLUORESCENCE

Like immunoperoxidase studies, immunofluorescence detects antigens in tissues. However, because amplification of the signal is not used, it is better suited for precise localization of antigen/antibody complexes in tissues or for determining the deposition pattern of immune complexes (e.g., linear versus granular). Thus, it is most useful for the investigation of diseases related to immune complex deposition such as glomerular diseases and bullous diseases of the skin.

Tissue for immunofluorescence may be snap frozen (see instructions above) or stored in special fixatives for IF. If the specimen is not frozen, special care must be taken to ensure that the biopsy is kept moist in a sealed container.

Direct IF uses antibodies to detect antigens in the patient's *tissues*.

Indirect IF uses control tissues to detect antibodies (e.g., anti-BM) in the patient's *serum*.

Indications

Biopsies of some skin diseases (e.g., lupus, pemphigus, pemphigoid, and dermatitis herpetiformis), all diagnostic non-transplant renal biopsies, some transplant renal biopsies, and the evaluation of vasculitis in nerve biopsies.

Method

Tissue must be submitted fresh.

Results

The results of the examination are usually incorporated into the surgical pathology report.

Immunofluorescence of skin lesions

SLE (lupus band test). There is linear or granular staining along the dermal–epidermal junction for multiple immunoreactants (most commonly IgG and less often IgM or C3) in approximately 80% of cases. The specificity increases with the number of positive immunoreactants. Uninvolved sun-exposed skin shows positivity in most patients with active systemic lupus. Uninvolved skin in patients with discoid lupus is usually negative for this test.

Herpes gestationis. Perilesional skin shows linear basement membrane zone C3 and sometimes IgG.

Dermatitis herpetiformis. Granular IgA is seen at the tips of dermal papillae of uninvolved skin.

Pemphigus. IgG and C3 between epidermal cells create a net-like pattern. In pemphigus vulgaris, a split just above the basal cell layer creates a "tombstone" appearance to the row of basal cells at the base of the

vesicle. In pemphigus foliaceus and related disorders, the split occurs near the granular cell layer.

Pemphigoid. Ig and C3 are present along the basement membrane but not between cells. Indirect IF reveals an anti-BM antibody.

MOLECULAR GENETIC PATHOLOGY

Molecular genetic pathology is the newest subspeciality in pathology with board certification. Molecular diagnostics incorporates many types of techniques for the investigation of genetic alterations in cells and viruses (e.g., Southern blotting, PCR analysis, FISH). It has applications in three main areas:

1. Inherited diseases:
   - Identification of inherited diseases (e.g., cystic fibrosis, hemochromatosis, factor V Leiden, prothrombin 20210A, fragile X syndrome)
   - Identification of genes conferring susceptibility to diseases (e.g., *BRCA1*).
2. Infectious diseases:
   - Detection of organisms
   - Identification of specific organisms
   - Quantitation of viral infection (e.g., HIV viral load).
3. Cancer
   - Identification of specific genetic alterations associated with tumors (Table 7-33)
   - Identification of clonality in hematolymphoid proliferations (Table 7-34)
   - Detection of minimal residual disease after treatment.

Molecular genetic studies are especially helpful for difficult-to-classify hematolymphoid proliferations because of the frequent and characteristic rearrangements that occur in many of these disorders. Unlike cytogenetics, the cells need not be viable; however, it is preferable that the nucleic acids are relatively intact. Southern blot and RNA based PCR (RT-PCR) assays are best performed on fresh or frozen tissues. Formalin-fixed, paraffin-embedded tissue is amenable to DNA-based PCR assays. Some fixatives (e.g., Bouin's) cause extensive breakage of DNA and may preclude genetic analysis of the tissue.

Indications

- B-cell proliferations: clonal rearrangements of the immunoglobulin heavy and light chain genes; specific translocations (see Table 7-34).
- T-cell proliferations: rearrangements of the γ and β T-cell receptor genes.
- Leukemias (see Table 7-34).
- Post-transplant lymphoproliferative disorders: clonal populations of EBV-infected cells.
- Oligodendrogliomas: PCR-based LOH analysis for 1p/19q deletions.

Method of submitting tissue

Fresh or frozen tissue (e.g., snap-frozen tissue) as well as fluids may be used. Cytologic preparations can be used for FISH.

Results

The results are usually either reported separately or incorporated into the surgical pathology report.

CYTOGENETICS

Cytogenetic studies have been demonstrated to be useful in several areas important to pathology:

Tumor classification, particularly sarcomas, lymphomas, brain tumors, and other unusual tumors (Ewing's sarcoma, synovial sarcoma).

Benign versus malignant lesions, for example:
- Reactive mesothelial cells versus mesothelioma
- Lipoma versus liposarcoma.

Prognosis, for example in neuroblastoma and multiple myeloma.

Research: Translocations are common to many tumors and usually identify genes important to the pathogenesis of the tumor.

Cells may be cultured to perform complete karyotype analysis or tissues can be analyzed for specific chromosomal alterations by fluorescence in situ hybridization (FISH). FISH studies can be performed on cultured cells, cytology preparations, and fixed and embedded tissues.

Indications

Cytogenetic studies are indicated for soft tissue tumors, mesotheliomas (tissue or pleural fluid), unusual tumors, poorly differentiated tumors, all subcutaneous lipomas larger than 5 cm, all subfascial lipomas (for karyotype), and oligodendrogliomas (for FISH).

Method for submitting tissue

Tissue for karyotyping must be fresh, viable, and relatively sterile. However, tissue may be submitted even if it has not been handled under strictly sterile conditions (contamination is not usually a problem). If specimens are to be held overnight, the tissue should be minced (into 1-mm cubes) in a sterile specimen container, covered with culture medium, and held overnight in the refrigerator. Fluids may also be submitted for analysis (especially pleural effusions with a suspicion of mesothelioma).

Results

The results of the cytogenetic analysis should be incorporated into the final diagnosis.

Table 7-33 Common cytogenetic and genetic changes in solid tumors of diagnostic or therapeutic significance

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Adenoid cystic carcinomas	6q translocations and deletions	>50%		
Adrenal cortical carcinomas		2p16 loss 17p13 LOH 11p15 LOH with duplication of the active paternal allele leading to IGF-II overexpression	>90% 85% 85%	This area is close to the region associated with Carney complex type 2 These changes are less common in localized tumors (25–35%) but, if present, such tumors are more likely to metastasize. The 11p15 imprinted region is also involved in Beckwith–Wiedemann syndrome
Alveolar soft part sarcoma	der(X)(X;17)(p11;q25)	*ASPL–TFE3* fusion	>90%	TFE3 can be detected by IHC. This translocation is also present in rare papillary-like renal tumors in young adults (see "Renal tumors" below)
Aneurysmal bone cyst	t(16;17)(q22;p13)	*CDH11–USP6* fusion	>50%	
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	*FUS–ATF* fusion		
Breast carcinoma		HER-2/neu amplification *BRCA1* and *BRCA2* germline mutations	20–30% <5%	Detected by FISH (gene amplification) or IHC (protein overexpression). Positive carcinomas are more likely to respond to Herceptin Patients are more likely to be young and have multiple carcinomas. *BRCA1* carcinomas are frequently high grade, have "medullary" features, and lack ER, PR, HER-2. *BRCA2* carcinomas have no specific pathologic features
Carcinoma of the upper aerodigestive tract in children	t(15;19)(q13;p13.2)	*BRD4–NUT* fusion		Patients with this translocation have a poor prognosis
Chondromyxoid fibroma	Deletion of 6q		>75%	
Clear cell sarcoma	t(12;22)(q13;q12)	*EWS–ATF1* fusion	>75%	
Colon carcinoma		*hMLH1* and *hMSH2* mutations *EGFR (HER1)* overexpression	15% of sporadic carcinomas 82% of all carcinomas	95% of HNPCC patients have germline mutations in these genes. Absence can be detected by IHC or by PCR assays for microsatellite instability. Mutations are correlated with characteristic clinical, pathologic, and treatment response features Approximately 23% of patients treated with cetuximab[b] and chemotherapy respond. IHC for EGFR may be used to select eligible patients

Table 7-33 Common cytogenetic and genetic changes in solid tumors of diagnostic or therapeutic significance—*cont'd*

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Colon carcinoma (Continued)		*APC* mutations	80% of all carcinomas	Also present as a germline mutation in familial adenomatous polyposis syndrome
		LKB1/STK11 LOH	~15%	Germline mutations occur in some cases of Peutz–Jeghers syndrome. Mutations appear to be rare in sporadic colon carcinoma but LOH is observed in some
		DPC4 (*Smad4* or *MADH4*) mutations (18q21.1)	10–20%	Germline mutations occur in some cases of juvenile polyposis syndrome. Mutations in sporadic carcinomas are uncommon
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWS–WT1* fusion	>75%	WT1 can be detected by IHC
Dermatofibrosarcoma protuberans	t(17;22)(q21;q13) resulting in a ring chromosome	*COL1A1–PDGFB* fusion	>75%	The same translocation is present in giant cell fibroblastoma, but without formation of a ring chromosome
Endometrial stromal tumor	t(7;17)(p15;q21)	*JAZF1–JJAZ1* fusion	30%	
Ewing's sarcoma (PNET)	t(11;22)(q24;q12) t(21;22)(q22;q12) t(2;22)(q33;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) inv(22)(q12)(q12)	*EWS–FLI1* fusion *EWS–ERG* fusion *EWS–FEV* fusion *EWS–ETV1* fusion *EWS–E1AF* fusion *EWS–ZSG* fusion	>80% 5–10% <5% <5% <5% <5%	FLI1 can be detected by IHC but is not specific for Ewing's
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21)	*EWS–NR4A3* fusion *TAF2N–NR4A3* fusion *TCF12–NR4A3* fusion	>75% <10% <10%	
Fibromatosis (desmoid)	Trisomies of 8 and 20 Deletion of 5q	 APC inactivation	30% 10%	
Fibromyxoid sarcoma, low grade	t(7;16)(q33;p11.2)	*FUS–BBF2H7* fusion	Unknown	
Fibrosarcoma, infantile	t(12;15)(p13;q26) Trisomies 8, 11, 17, 20	*ETV6–NTRK3* fusion	>75% >75%	The same translocation is seen in cellular mesoblastic nephroma
Gastrointestinal stromal tumor	Monosomies 14 and 22 Deletion of 1p	 *KIT* or *PDGFRA* mutation	> 75% >25% >90%	CD117 (KIT) is detected by IHC and is useful for diagnosis. Gleevec[c] is effective against tumors with activating mutations in either gene
Germ cell tumors	Isochromosome 12p	 *KIT* mutations	>80–90% 25–70%	Includes all histologic subtypes Seminomas
Giant cell tumor	Telomeric changes		>50%	

Table 7-33 Common cytogenetic and genetic changes in solid tumors of diagnostic or therapeutic significance—*cont'd*

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Giant cell tumor, diffuse type (PVNS)	Trisomies 5 and 7 t(1;2)		>25%	
Hepatoblastoma	Trisomies 2q and 20		>75%	
Hibernoma	11q13 rearrangement		>50%	
Inflammatory myofibroblastic tumor	2p23 rearrangement	*ALK* fusion with multiple partners	50%	ALK can be detected by IHC in one third of cases
Leiomyoma, Uterine	t(12;14)(q15;q24) deletion of 7q	*HMGA2* rearrangement	40%	Uterine leiomyosarcomas have more complex karyotypes
Lipoblastoma	8q12 rearrangement polysomy 8	*PLAG1* oncogene	>80%	
Lipoma Typical Spindle cell or pleomorphic Chondroid	 12q15 rearrangement 6p21 rearrangement Deletion of 13q or 16q t(11;16)(q13;p12-13)	 *HMGA2* rearrangement *HMGA1* rearrangement	 60% >75%	
Liposarcoma Well-differentiated Myxoid/round cell Pleomorphic	 Ring form of chrom 12q1,5/giant markers t(12;16)(q13;p11) t(12;22)(q13;q12) Complex	 *HMGA2, MDM2* amplification *FUS–CHOP* fusion *EWS–CHOP* fusion	 >75% >75% <5% 90%	
Lung adenocarcinomas that respond to gefitinib (most have features of bronchioloalveolar carcinoma)	Fewer changes than seen in carcinomas associated with smoking	*EGFR*—small deletions or amino acid substitutions	10–20% of all lung carcinomas	Mutations predict response to the tyrosine kinase inhibitor gefitinib (Iressa)[d] 40–80% of lung carcinomas show *EGFR* overexpression by IHC, but only carcinomas with specific mutations respond to gefitinib
Medulloblastoma	Isochromosome 17q		>25%	
Meningioma	Monosomy 22 1p deletion		90% 25%	
Mesothelioma	Deletion of 1p Deletion of 9p Deletion of 22q Deletions of 3p and 6q	? *BCL10* inactivation p15, p16, and p19 inactivation *NF2* inactivation	>50% >75% >50% >50%	Cytogenetic changes are less complex than those seen in carcinomas. Cytogenetic analysis of cytologic specimens (e.g., pleural fluid) can be of value if larger biopsies are not available
Mucoepidermoid carcinoma	t(11;19)(q21;p13)	*MECT1–MAML2* fusion	>50%	
Neuroblastoma	 Hyperdiploid, no 1p deletion 1p deletion Double minute chromosomes	 N-*myc* amplification	 40% 40% >25%	 Good prognosis Poor prognosis

Table 7-33 Common cytogenetic and genetic changes in solid tumors of diagnostic or therapeutic significance—*cont'd*

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Oligodendroglioma	Deletion of 1p36 and 19q13.3 9p21 deletion	 *CDKN2A* (p16) deletion	50%	Useful for diagnosis and to predict response to radiation and/or chemotherapy Occurs in some anaplastic oligodendrogliomas. Poor prognostic factor
Osteochondroma	Deletion of 8q	*EXT1* inactivation	>25%	
Osteosarcoma Low grade High grade	 Ring chromosomes Complex	 RB and P53 inactivation	 >50% >80%	
Pheochromocytoma Sporadic (70%) Hereditary (30%)		Losses on 1p Germline mutations in *RET, VHL, NF1, SDHB, SDHD, MEN2A, MEN2B*	>80% >90% of hereditary cases	Patients are more likely to be young (<50), have multiple tumors, and have a family history of pheochromocytoma, paraganglioma, or medullary carcinoma of the thyroid
Pleomorphic adenoma (salivary)	8q12 rearrangement 12q15 rearrangement	*PLAG1* fusion genes *HMGIC* oncogenes	>50% <20%	
Renal tumors Clear cell carcinoma Papillary carcinoma: adult	 Deletion of 3p Trisomies 3, 7, 12, 16, 17, and 20	 *KIT* mutations	 >90% >90% >90%	 CD117 (c-kit) present by IHC in cytoplasm and is associated with activating mutations
"Papillary-like" carcinoma: young adults	t(X;1)(p11.2;q21) t(X;1)(p11.2;p34) inv(X)(p11.2q12) t(X;17)(p11.2;q25.3) t(6;11)(p21.1;q12)	*PRCC–TFE3* fusion *TFE3–PSF* fusion *TFE3–NonO* fusion *RCC17(ASPL)–TFE3 fusion* *TFEB-Alpha* fusion		The majority of these carcinomas are associated with fusion proteins involving TFE3 or TFEB. The *ASPL–TFE3* fusion is also present in alveolar soft part sarcoma
Oncocytoma	–1, –X or –Y 11q13 rearrangement		>25% >25%	
Chromophobe carcinoma	Monosomies 1, 2, 3, 6, 10, 13, 17, and 21		>75%	CD117 (c-kit) is present by IHC on membranes, but activating mutations have not been detected
Retinoblastoma	13q14 deletion Isochromosome 6p	*RB1* inactivation	>75% 25%	40% of cases are due to germline mutations in *RB1*
Rhabdoid tumor of the kidney and Atypical teratoid/ rhabdoid tumor (AT/RT)	Normal karyotype Monosomy 22	*hSNF5/INI1* (22q11.2) deletions and mutations *hSNF5/INI1* deletions and mutations	>90%	Infants and children with both tumors have a germline mutation in *INI1* (rhabdoid predisposition syndrome) Choroid plexus carcinomas are also associated with non-function of this gene (70%)

Table 7-33 Common cytogenetic and genetic changes in solid tumors of diagnostic or therapeutic significance—*cont'd*

TUMOR TYPE	CHARACTERISTIC CYTOGENETIC CHANGES	GENETIC CHANGES	FREQUENCY	COMMENTS
Rhabdomyosarcoma				
Alveolar	t(2;13)(q35;q14)	*PAX3–FKHR* fusion	>75%	Poor 4-year survival if metastatic (8%)
	t(1;13)(p36;q14), double minutes	*PAX7–FKHR* fusion	10–20%	Better 4-year survival if metastatic (75%)
Embryonal	Trisomies 2q, 8, and 20		>75%	
		LOH 11p15	>75%	
Schwannoma and perineurioma	Deletion of 22q	*NF2* inactivation	>80%	5% of cases of vestibular schwannomas are associated with neurofibromatosis type 2 (germline *NF2* mutations)
Synovial sarcoma				
Monophasic	t(X;18)(p11;q11)	*SYT–SSX1*/*SYT–SSX2* fusion	>90%	
Biphasic	t(X;18)(p11;q11)	*SYT–SS1* fusion	>90%	
Thyroid carcinoma				
Papillary	10q11 rearrangement	*RET* fusion oncogenes	>30%	
	1q21 rearrangement	*NTRK1* fusion oncogenes	>10%	
		BRAF oncogenes	30%	
Follicular	t(2;3)(q13;p25)	*PAX8–PPARG* fusion	>40%	
Medullary				
Sporadic (75%)		*RET* activating mutations	>90%	
Hereditary (25%)		Germline *RET, MEN2A*, or *MEN2B* mutations	>90%	Indication for screening for pheochromocytoma and screening family members
Wilms' tumor, pediatric	Deletion 11p13	*WT1* inactivation	25%	Germline mutations occur in several syndromes. WT1 mutations also occur in sporadic tumors
	Trisomy 12		40%	

[a] Trastuzumab (Herceptin) is a monoclonal antibody directed against the HER-2/neu receptor. Patients are selected for treatment by testing carcinomas with IHC or FISH.

[b] Cetuximab (C225, Erbitux ™) is a monoclonal antibody directed against the EGFR receptor. A test has been approved by the FDA for the determination of EGFR (DakoCyomation, EGFR PharmDX). This test is not used for lung carcinomas (see note "d" below).

[c] Imatinib mesylate (STI571, Gleevec™, Glivec™) is a small molecule tyrosine kinase inhibitor that may be used for the treatment of tumors overexpressing tyrosine kinases:

Bcr–Abl tyrosine kinase: CML, ALL (Ph+)
KIT tyrosine kinase: GIST, systemic mastocytosis, some types of AML
PDGFR kinase: CMML, chronic eosinophilic leukemia, rare cases of GIST.

The KIT protein (CD117) is encoded by the c-*KIT* proto-oncogene and is a transmembrane receptor protein with tyrosine kinase activity. Mutations may render KIT independent of its ligand, SCF (stem cell factor). Mutated proteins may or may not respond to therapy with imatinib. Wild-type KIT and KIT with mutations in the juxtamembrane domain (the intracellular segment between the transmembrane and tyrosine kinase domains) are found in GISTs and are sensitive to imatinib. Other tumor types are associated with mutations in the enzymatic domain and the altered protein is generally not sensitive to imatinib. Overexpression of the protein is detected by IHC.

[d] Gefitinib (Iressa) is a tyrosine kinase inhibitor effective against a small subset of lung adenocarcinomas with specific activating mutations in EGFR. IHC for EGFR is not helpful for identifying carcinomas likely to respond to treatment.

For additional information on specific genes, see Online Mendelian Inheritance in Man (OMIM; www.ncbi.nlm.nih.gov).

Table 7-34 Common cytogenetic changes in lymphomas and leukemias

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
Chronic leukemias and mastocytosis				
CML (Ph^1)	t(9;22)(q34;q11.2)	*BCR–ABL* fusion (usually p210, but also p190 and p230 fusion proteins)	90–95%	Philadelphia chromosome. Also present in 5% of children and 15–30% of adults with ALL and 2% of patients with AML Treated with the ABL tyrosine kinase inhibitor imatinib (Gleevec)[a]
	Other variants or cryptic translocations	*BCR–ABL* fusion (usually p210, but also p190 and p230 fusion proteins)	5–10%	
CML, accelerated phase or blast phase	Additional changes: extra Ph, +8, or i(17)(q10)		80%	May be myeloid (70%) or lymphoid (30%)
Chronic myelomonocytic leukemia with eosinophilia	t(5;12)(q33;p13)	*ETV6*(also called *TEL*) *–PDGRFbeta* fusion	Rare	Excellent response to imatinib[a]
Chronic eosinophilic leukemia/hyper-eosinophilic syndrome	Cryptic del(4)(q12) – interstitial 800 kb deletion	*FIP1L1–PDGFRalpha* fusion	~50%	The fusion protein is an activated tyrosine kinase. Excellent response with the tyrosine kinase inhibitor imatinib[a]
	t(1;5)(q23;q33)	*myomegalin–PDGFRbeta* fusion protein	? Rare	May be more common in infants and women. Excellent response to imatinib[a]
Mastocytosis		c-*KIT* point mutations (Asp816Val)	100%	CD117 (c-kit) is detected by IHC in normal and abnormal mast cells. The most common mutations do not result in proteins sensitive to imatinib
	Cryptic del(4)(q12) – interstitial 800 kb deletion	*FIP1L1–PDGFRalpha* fusion	~60% of patients with eosinophilia	Found in mastocytosis with associated eosinophilia. These patients do not have the typical c-KIT mutation. Excellent response to treatment with imatinib[a]
Acute myeloid leukemia				
AML	Normal karyotype	*FLT3* (13q12) internal tandem duplications (ITD, 20%) or point mutations (7%)	20% 20–30% of AML with normal karyotype	More common in monocytic AML (M5), less common in myeloblastic leukemia with maturation (M2) or erythroleukemia (M6). Less common in AML with cytogenetic changes (10%). Poor prognostic factor Results in an activated tyrosine kinase. Current trials are evaluating response to a kinase inhibitor, PKC412.
AML (M1, M2, or M4)	t(6;9)(p23;q34)	*DEK–CAN* fusion	1% of all AML	Poor prognosis
		FLT3 ITDs	90% of this AML type	

Table 7-34 Common cytogenetic changes in lymphomas and leukemias—*cont'd*

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
Acute myeloid leukemia				
AML with t(8;21) (M2)	t(8;21)(q22;q22)	*AML1–ETO* fusion	5–12% of AML	30% of cases of AML with karyotypic abnormalities and maturation. Maturation in neutrophilic lineage. Usually younger patients, good prognosis
		c-*KIT* mutations	~50% of this AML type	Response to imatinib[a] untested
Acute promyelocytic leukemia (M3, M3v.)	t(15;17)(q22;q11-12) t(11;17)(q23;p21) t(5;17)(q34;q12) t(11;17)(p13;q21)	*PML–RARα* fusion *PLZF–RARα* fusion *NPM1-RARα* fusion *NUMA–RARα* fusion	5–8% of AML (95–100% of APML)	Abnormal promyelocytes predominate. Usually occurs in adults in mid-life. Treatment with all *trans*-retinoic acid acts to differentiate the cells. Favorable prognosis
		FLT3 ITDs	32% of APML	
AML with inv(16) or t(16;16)	inv (16)(p13)(q22) t(16;16)(p13;q22) del(16q) Other rare variants or cryptic translocations	*CBFbeta–MYH11* fusion	10–12% of AML (100% of M4EO)	Monocytic and granulocytic differentiation and abnormal eosinophils in the marrow. Usually younger patients. Favorable prognosis
		c-*KIT* mutations	~50% of this AML type	Response to imatinib[a] untested
AML with 11q23 abnormalities	11q23 abnormalities	*MLL* fusion with numerous different partners	5–6% of AML	Usually associated with monocytic features. Occurs in infants and in patients after therapy with topoisomerase II inhibitors. Intermediate prognosis
AML and MDS, therapy related	5q–/7q–/12p–/20q– t(9;11), t(11;19), t(6;11) Other less common changes	*MLL* balanced translocations		Occurs after alkylating agents and/or radiation, usually 5 to 6 years after treatment. Poor prognosis Occurs after DNA-topoisomerase II inhibitors, usually 3 years after treatment. Long-term prognosis unknown
B Cell				
Precursor B-lympho-blastic leukemia/ lymphoblastic lymphoma (ALL)	t(9;22)(q34;q11.2)	*BCR–ABL* fusion (usually p190 (esp. in children), but also p210 protein)	5% of childhood ALL 20–25% of adult ALL	Philadelphia chromosome Poor prognosis
	t with 11q23	*MLL* rearrangements		Poor prognosis. Usually infants
	t(12;21)(p13;q22)	*TEL–AML1* fusion	>50% of childhood ALL or hyperdiploid	Good prognosis. This translocation is not detected by standard cytogenetics
	t(1;19)(q23;p13.3)	*PBX1–E2A* fusion	5–6%	Pre-B-ALL; most common translocation in childhood. Unfavorable but modified by therapy

Table 7-34 Common cytogenetic changes in lymphomas and leukemias—*cont'd*

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
B Cell				
	Hypodiploid Hyperdiploid >50 t(5;14)(q31;q32) t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11) t(17;19)(q21;p13) t(4;11)(q21;q23)	 *IL3–IGH* fusion *MYC–IGH* fusion *IGK–MYC* fusion *MYC–IGL* fusion *HLF–E2A* fusion *MLL–AF4* fusion		Poor prognosis Good prognosis (= DNA Index 1.16 to 1.6) Poor prognosis Good prognosis Good prognosis Good prognosis Poor prognosis Poor prognosis
ALL, therapy related				Similar to therapy related AML
Small lymphocytic lymphoma/CLL	Trisomy 12	 del(11q22-23)—ATR del(13q14) —DBS319 17p — p53 I_gV_H not mutated I_gV_H (mutated, >2% difference in nucleotide sequence)	16% 18% 55% 7% 40–50% 50–60%	Usually do not have I_gV_H mutations. Aggressive clinical course Poor prognosis Detected by FISH Usually do have I_gV_H mutations Long-term survival Detected by FISH Worse prognosis Detected by FISH Worse prognosis (<8 year median survival) Better prognosis (median survival >24 years)
Lymphoplasmacytic lymphoma/ Waldenström's macroglobulinemia	t(9;14)(p13;q32)	*PAX-5–IGH* fusion	50%	This rearrangement may be less common in cases associated with Waldenström's macroglobulinemia or if node-based
Mantle cell lymphoma	t(11;14)(q13;q32)	*CCND1–IGH* fusion *ATM* point mutations	>95%	Overexpression of cyclin D1 detected by IHC
Marginal zone lymphoma (MALT)	+3 t(1;14)(p21;q32) t(11;18)(q21;q21) t(11;14)(q21;q32)	 *BCL-10–IGH* fusion *API2–MALT1* fusion *MALT1–IGH* fusion	60% 25–50%	
Follicular lymphoma	t(14;18)(q32;q21) t(2;18)(p12;q21)	*IGH–BCL-2* fusion *IGK–BCL-2* fusion	70–95% Rare	
Burkitt's lymphoma and Burkitt-like lymphoma	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	*MYC–IGH* fusion *MYC–IGK* fusion *MYC–IGL* fusion	85% Rare Rare	
Mediastinal (thymic) large B-cell lymphoma	9p+	*REL* amplification		
Diffuse large B-cell lymphoma	t(3q27;v) t(14;18)(q32;q21)	*BCL6* translocations with many partners *BCL2–IGH* fusion	30% 20–30%	BCL6 is detected by IHC in most cases, BCL2 in some cases
Hairy cell leukemia				No consistent changes
Primary effusion lymphoma				No consistent changes

Table 7-34 Common cytogenetic changes in lymphomas and leukemias—*cont'd*

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
B Cell				
Plasmacytoma/myeloma	t(11;14)(q13;q32) t(6;14)(p21;q32) t(4;14)(p16;q32) t(14;16)(q32;q23) Monosomy 13/13q–	*CCND1–IGH* fusion *CCND3–IGH* fusion *FGF23–IGH* fusion *IGH–MAF* fusion	 15–40%	Best prognosis Adverse prognosis Adverse prognosis
T Cell				
Precursor lymphoblastic leukemia/lymphoblastic lymphoma	Translocations involving *TCR* alpha, beta, delta, and gamma and partner genes *MYC, TAL1, RBTN1, RBTN2, HOX11*, and LCK del(1) t(1;14) t(5;14) del(9p)	 *Tal1* (small deletion) *Tal1–TCRdelta* fusion *HOX11L2–TCRdelta* fusion *CDKN2A* deletion	30% 30% 25% >30%	 Adolescents Adolescents Young children
T-cell prolymphocytic leukemia	inv(14)(q11)(q32) t(14;14)(q11;q32) t(7;14)(q35;q32.1) chrom 8 abnormalities	*TCRα/β–TCL1* & *TCL1b* fusion *TCRα/β–TCL1* & *TCL1b* fusion *TCRβ–TCL 1A* fusion	80% 10% 70–80%	
Adult T-cell lymphoma/leukemia				No consistent changes
Mycosis fungoides and Sézary syndrome				No consistent changes
Peripheral T-cell lymphoma, NOS				No consistent changes
Hepatosplenic T-cell lymphoma	i(7q)(q10)		100%	
Panniculitis-like T-cell lymphoma				No consistent changes
Angioimmunoblastic lymphoma	Trisomy 3, trisomy 5, + X			
Enteropathy-type T-cell lymphoma				No consistent changes.
Anaplastic large cell lymphoma (CD30+)	t(2;5)(p23;q35) 2p23 rearrangements	*NPM1–ALK* fusion protein (p80) *ALK* fusion with other partners	70–80%	ALK detected by IHC in nucleus, nucleolus, and cytoplasm ALK detected by IHC in cytoplasm

Table 7-34 Common cytogenetic changes in lymphomas and leukemias—*cont'd*

TUMOR TYPE	CYTOGENETIC CHANGES	MOLECULAR EVENTS	FREQUENCY	COMMENTS
T Cell				
Extranodal NK/T-cell lymphoma, nasal type				No consistent changes.
Blastic NK-cell lymphoma				No consistent changes.

[a] Imatinib mesylate (STI571, Gleevec™, Glivec™) is a small molecule tyrosine kinase inhibitor that may be used for the treatment of tumors overexpressing tyrosine kinases:

Bcr-Abl tyrosine kinase: CML, ALL (Ph+)
KIT tyrosine kinase: GIST, systemic mastocytosis, some types of AML
PDGFR kinase: CMML, chronic eosinophilic leukemia, rare cases of GIST

The KIT protein is encoded by the c-*KIT* proto-oncogene and is a transmembrane receptor protein with tyrosine kinase activity. Mutations may render KIT independent of its ligand, SCF (stem cell factor). Mutated proteins may or may not respond to therapy with imatinib. Wild-type KIT and KIT with mutations in the juxtamembrane domain (the intracellular segment between the transmembrane and tyrosine kinase domains) are found in GISTs and are sensitive to imatinib. Other tumor types are associated with mutations in the enzymatic domain and the altered protein is generally not sensitive to imatinib.

For additional information on specific genes, see Online Mendelian Inheritance in Man (OMIM; www.ncbi.nlm.nih.gov).

Tumors and diseases associated with germline mutations

The following features are suggestive of a hereditary susceptibility to cancer:

- Two or more close relatives on the same side of the family with cancer
- Evidence of autosomal dominant transmission
- Early development of cancer in the patient and relatives (in general, under 50 years of age)
- Multiple primary cancers
- Multiple types of cancers
- Unusual pathologic features of tumors (Table 7-35)
- A constellation of tumors suggestive of a specific syndrome (Table 7-36).

Pathologists can aid in the detection of hereditary carcinomas by being aware of the types and pathologic characteristics of carcinomas associated with these syndromes. Patients with germline mutations are important to identify in order to:

- Screen patients for other common tumors or other components of the disease
- Consider prophylactic surgery or preventive interventions
- Offer screening to family members at risk and genetic counseling.

Although the sporadic forms of cancers in general far outnumber cases associated with germline mutations, in some cases the appearance or site of a carcinoma is highly suggestive of a known syndrome and further investigation may be warranted.

Table 7-35 Pathologic features of tumors and diseases suggestive of a germline mutation

TYPE OF TUMOR	PERCENTAGE OF CASES RELATED TO KNOWN GERMLINE MUTATIONS	SYNDROMES/GENES INVOLVED	CLUES FOR THE PATHOLOGIST
Adrenocortical carcinoma in children	50–100%	Li–Fraumeni, Beckwith–Wiedemann, MEN1	Unusual occurrence in a child
Angiomyolipoma of kidney	20%	Tuberous sclerosis	Patients may be screened for other features of tuberous sclerosis
Basal cell carcinoma	Rare if solitary	Nevoid basal cell carcinoma syndrome	Risk of a mutation is increased if multiple or if tumor occurs at <30 years of age
Breast cancer, poorly differentiated, ER negative[a]	>25% if <35 years old, <10% if >35 years old	*BRCA1*	BRCA1 cancers are more likely to have "medullary" features, and be ER– PR– HER-2/neu–. *BRCA1* mutation more likely if patient has a family history or has bilateral cancer
Breast cancer, male	4–14%	*BRCA2*	Cancers are of no specific type
Colorectal carcinoma, poorly differentiated, mucinous, or with prominent lymphocytic infiltrate	~10–15% overall, ~80% if patient is <40	*HNPCC*	*HNPCC* carcinomas are more likely right-sided (two thirds), poorly differentiated ("medullary"), mucinous, signet ring, lymphocytic infiltrate. IHC for MSH2 and MLH1 can be used to detect many, but not all, cases, but MLH1 may also be absent in sporadic cases
GI neuroendocrine tumors: Somatostatinoma PPoma Non-functioning Gastrinoma Glucagonoma VIPoma Insulinoma Carcinoid	 45% 18–44% 18–44% 20–25% 1–20% 6% 4–5% Rare	*MEN1* mutations	*MEN1* mutations are also found in 15–70% of sporadic neuroendocrine tumors
Hirschsprung's disease	20–40%	MEN2A (*RET* mutations in codons 609, 618, 620)	
Juvenile (hamartomatous) polyps	Rare if solitary	Juvenile polyposis syndrome (JPS)	Suspect JPS if there are >5 polyps, if present throughout the GI tract, or if there is a family history of juvenile polyps
Medullary carcinoma of the thyroid	25%	MEN2A, MEN2B, Familial medullary carcinoma (*RET* mutations)	May be multiple and associated with C-cell hyperplasia Cancers in occur in children in MEN2B and in young adults in MEN2A
Medulloblastoma	Rare (?)	Nevoid basal cell carcinoma syndrome	If <3 years of age or of desmoplastic type, risk of mutation is increased
Myxoma, cardiac	<5%	Carney complex	Increased likelihood if multiple, right sided, and/or recurrent and in young patients (<30)
Neurofibromas	~10% if solitary but > 90% if plexiform	Neurofibromatosis type 1	Increased risk if there are ≥2 neurofibromas or one plexiform neurofibroma

Table 7-35 Pathologic features of tumors and diseases suggestive of a germline mutation—*cont'd*

TYPE OF TUMOR	PERCENTAGE OF CASES RELATED TO KNOWN GERMLINE MUTATIONS	SYNDROMES/GENES INVOLVED	CLUES FOR THE PATHOLOGIST
Ovarian carcinoma	Rare	*BRCA1, BRCA2*	Increased risk if there is a history of breast cancer *BRCA1*-associated carcinomas are more likely to be serous in type
Pheochromocytoma	30% of all cases, 59% if patient is <18, 84% if bilateral	MEN2A, MEN2B, VHL, isolated familial pheochromocytoma	Multiple tumors, hyperplasia of the medulla
Primary pigmented nodular adrenocortical disease	>90%	Carney complex	May present with Cushing's syndrome Most are associated with germline mutations, but patients may not have other manifestations of the Carney complex
Retinoblastoma	40% of all cases, 100% if bilateral or with a positive family history	*RB* mutations (13q14.1-q14.2)	
Rhabdomyoma of heart in infants	50%	Tuberous sclerosis	
Sarcoma, children	7–33%	Li–Fraumeni, basal cell nevus syndrome, neurofibromatosis type 1, pleuropulmonary blastoma syndrome	
Sebaceous carcinoma	~10% if ocular, 40% if above the chin, 80% if elsewhere	HNPCC	Increased likelihood if the tumor has cystic degeneration or features of keratoacanthoma Usually due to germline *MSH2* mutations
Schwannoma, psammomatous melanotic	>50%	Carney complex	Higher likelihood if patient is young (<30 years) and/or multiple tumors present
Schwannoma, vestibular	5%	Neurofibromatosis type 2	Risk is increased if the patient is <30 or if there is bilateral involvement Sporadic cases almost all have somatic *NF2* mutations
Sertoli cell tumor, large-cell calcifying	25–35%	Carney complex, Peutz–Jeghers	Most are bilateral and multifocal in young patients. Rarely malignant
Trichilemmoma, facial, multiple	~80%	*PTEN*	Sporadic tumors also have loss of PTEN, which can be shown by IHC.
Wilms' tumor	10–15%	Germline mutations in *WT1* (11p13)	Nephrogenic rests are present and may be extensive 5–10% of cases associated with germline mutations are multicentric or bilateral Associated with WAGR syndrome (Wilms' tumor, aniridia, GU anomalies, mental retardation) and Denys–Drash syndrome

[a] See reference 17 for additional information relating pathologic characteristics to risk of a *BRCA1* mutation.

Table 7-36 Hereditary syndromes associated with multiple tumors

SYNDROME	GERMLINE MUTATIONS	TUMORS (% OF PATIENTS DEVELOPING TUMOR)	COMMENTS
Beckwith–Wiedemann syndrome	11p15 abnormalities (loss of methylation, uniparental disomy, mutations in *CDKN1C*)	Wilms' tumor, neuroblastoma, hepatoblastoma, adrenocortical carcinoma, rhabdomyosarcoma	Macrosomia, macroglossia, visceromegaly, ear creases and pits, omphalocele, hypoglycemia
BRCA1 and 2	*BRCA1* (17q21), *BRCA2* (13q12.3)	Breast (85%), ovary (BRCA1 63%, BRCA2 27%), prostate carcinoma, others	BRCA1 breast cancers are more often poorly differentiated, have medullary features, are ER– PR– HER-2/neu–, and have p53 mutations. Ovarian carcinomas are generally serous (90%), high grade, and bilateral. BRCA2 cancers do not have specific pathologic features
Carney complex	Type 1 (CNC1): PRKAR1A (17q23-24) Type 2 (CNC2): locus at 2p16	Myxomas (cardiac, cutaneous, breast), primary pigmented nodular adrenocortical disease (25%), large-cell calcifying Sertoli cell tumors (>90% males), multiple thyroid nodules or carcinoma (75%), growth hormone producing pituitary adenoma (10%), psammomatous melanotic schwannoma (10%), breast duct adenomas, osteochondromyxoma of bone Pigmented skin lesions (lentigos, blue nevi (especially epithelioid blue nevus), cafe-au-lait spots)	
Carney triad	Unknown	Gastric gastrointestinal stromal tumor, pulmonary chondroma, extra-adrenal paraganglioma Also esophageal leiomyomas and adrenocortical tumors	Most patients are young and female. Only 22% have all three tumors. Most family members are not affected
Familial adenomatous polyposis (FAP; including Gardner syndrome and Turcot syndrome)	*APC* (5q21-22)	Colorectal carcinoma, upper GI carcinoma, desmoid, osteoma, thyroid, brain (one third to two thirds are medulloblastomas—Turcot syndrome)	
Familial medullary thyroid carcinoma	*RET* mutations in codons 10, 11, 13, 14 (10q11.2)	Medullary thyroid carcinoma	Cancers usually occur in adults
Hereditary diffuse gastric cancer syndrome	*CDH1* (e-cadherin) (16q22.1)	Signet ring cell carcinoma of the stomach (67% men, 83% women), lobular carcinoma of the breast (39% women)	50% of sporadic signet ring cell carcinomas have *CDH1* somatic mutations and all show loss of e-cadherin by IHC
Hereditary non-polyposis syndrome	Mismatch repair genes: *MSH2* (2p22-p21) (40%), *MLH1* (3p21.3) (40%), *MSH6* (2p16) (5–7%), *PMS2* (7p22) (rare)	Colon carcinoma (80%), endometrial carcinoma (20–60%), ovarian carcinoma (9–12%), stomach carcinoma (11–19%), hepatobiliary tumors (2–7%), transitional cell carcinoma (4–5%, esp. ureter and renal pelvis), small bowel tumors (1–4%), lymphoma (rare) Sebaceous skin tumors, adenomas, epitheliomas, carcinoma, keratoacanthomas (Muir–Torre, usually *MSH2*)	Colon carcinomas are more likely (overall, 66%) to be on the right side, poorly differentiated ("medullary"), mucinous, signet ring, or undifferentiated, with a prominent lymphocytic infiltrate IHC can be used to detect the absence of *MSH2* (usually

Table 7-36 Hereditary syndromes associated with multiple tumors—*cont'd*

SYNDROME	GERMLINE MUTATIONS	TUMORS (% OF PATIENTS DEVELOPING TUMOR)	COMMENTS
			due to germline mutations) and *MLH1* (can be due to germline mutations, epigenetic changes, or less commonly, somatic mutations) in many patients MSI testing is also used
Juvenile polyposis syndrome	*MADH4* (or *SMAD4*) (18q21.10) (15%) or *BMPR1A* (10q22.3) (25%)	Hamartomatous (juvenile) polyps, GI carcinomas	
Li–Fraumeni	p53 (17p13.1), rarely *CHEK2* (22q12.1)	Sarcomas, breast cancer, leukemia, osteosarcomas, brain tumors, adrenocortical carcinoma, others	
MEN1	*MEN1* (11q13)	Pituitary adenoma, pancreatic islet cell tumors, parathyroid adenomas, adrenocortical tumors, carcinoids, lipomas	*MEN1* mutations also occur in 15–70% of sporadic neuroendocrine tumors
MEN2A	*RET* exon 10 and 11 missense mutations (10q11.2)	Medullary thyroid carcinoma (95%), hyperplasia of the parathyroids (15–30%), pheochromocytoma (50%), ganglioneuromatosis of GI tract Subsets of patients have Hirschsprung's disease or cutaneous lichen amyloidosus	Specific mutations correlate with age at development of medullary thyroid carcinoma
MEN2B	*RET* missense mutation in exon 16 (10q11.2)	Medullary thyroid carcinoma (100%), pheochromocytoma (50%) Mucosal neuromas of lips and tongue	Marfanoid habitus, distinctive facies
Nevoid basal cell carcinoma syndrome (Gorlin syndrome)	*PTCH* (9q22.3)	Basal cell carcinomas (90%), odontogenic keratocysts (90%), cardiac or ovarian fibromas (20%), medulloblastoma in childhood (5%)	Macrocephaly, skeletal anomalies, palmar or plantar pits, calcification of falx (90%)
Neurofibromatosis type 1	*NF1* (17q11.2)	Neurofibromas (esp. plexiform) (100%), optic gliomas, adrenal ganglioneuromas, pheochromocytoma (0.1–6%), MPNST (10%), leukemia, ganglioneuromatosis of the GI tract	Café-au-lait macules (95%), iris hamartomas (Lisch nodules), axillary freckling
Neurofibromatosis type 2	*NF2* (22q12.2)	Bilateral vestibular schwannomas (100%, 40% have lobular pattern), schwannomas of other nerves, meningiomas (50%, often fibroblastic)	
Peutz–Jeghers (hamartomatous polyp) syndrome	*LKB1/STK11* (19p13.3)	Colon, breast, stomach, pancreas, small bowel, thyroid, lung, uterus, sex cord stromal tumors, calcifying Sertoli cell tumors Hamartomatous polyps of GI tract	Perioral pigmentation
Pheochromocytoma or paraganglioma, familial	*SDHB* (1p36.1-p35), *SDHD* (11q23) *SDHC* (1q21) (paraganglioma)	Pheochromocytoma, paraganglioma	Patients are more commonly young (<40), with multifocal adrenal tumors, or extra-adrenal disease *SDHD* is imprinted and only confers susceptibility after paternal transmission

Table 7-36 Hereditary syndromes associated with multiple tumors—*cont'd*

SYNDROME	GERMLINE MUTATIONS	TUMORS (% OF PATIENTS DEVELOPING TUMOR)	COMMENTS
PTEN hamartoma syndrome (including 80% of Cowden's syndrome, 50–60% of Bannayan–Riley–Ruvalcaba syndrome)	*PTEN* (10q23.31)	Breast cancer (25 to 50%), thyroid carcinoma (10%,esp. follicular), endometrial carcinoma (5–10%), hamartomatous polyps of GI tract Multiple facial trichilemmomas, acral keratosis, oral papillomatous lesions, mucosal lesions	Macrocephaly (megalencephaly, 97th percentile), Lhermitte–Duclos disease
Tuberous sclerosis	*TSC1* (9q34), *TSC2* (16p13.3)	Subependymal glial nodules (90%), cortical or subcortical tubers (70%), angiomyolipoma of kidney (70%), lymphangiomyomatosis of lung (1–6%), rhabdomyoma of heart (47–67%) Skin lesions (100%, including myomelanotic macules, multiple facial angiofibromas, shagreen patch, fibrous facial plaque, ungual fibroma)	Seizures (80%), developmental delay or retardation (50%)
Von Hippel–Lindau (VHL)	*VHL* (3p26-p25)	Hemangioblastomas (retinal, cerebellar, spinal cord) (80%), renal cell carcinoma (40%), renal cysts, pancreatic cysts, Pheochromocytoma, endolymphatic sac tumors (10%), epididymal cystadenomas	

For additional information on most syndromes, see http://www.genetests.org/ and Online Mendelian Inheritance in Man (OMIM; www.ncbi.nlm.nih.gov).

ANALYTICAL CYTOLOGY (FLOW CYTOMETRY)

Flow cytometers analyze populations of thousands of disaggregated cells as they pass by stationary detectors. Cell size and cytoplasmic granularity can be measured as well as DNA content and the presence or absence of immunohistochemical markers added to the cell suspension. Newer techniques can analyze three or more features simultaneously to divide cells into unique populations. DNA content can be used to determine the number of cells in S-phase (a measure of proliferation—S-phase fraction). Because cells are not visualized by this technique, it is important to be sure that only lesional tissue is submitted.

Indications for ploidy and S-phase analysis

- Hydatidiform moles: complete (diploid), partial (triploid).
- Some carcinomas: DNA ploidy and S-phase fraction have been reported to be of prognostic significance for some carcinomas (e.g., colon, breast, and prostate) but the analysis is not routinely performed at all institutions or used by all oncologists.

Indications for cell surface marker analysis

- Lymphomas.
- Leukemias.

Method for submitting tissue

Single cell suspensions are necessary for analysis. For fresh tissues, cells must be viable. Fresh tissue (approximately 0.3 to 0.5 cm^3) is placed in a specimen container and kept moist with HBSS. Tissues can be held overnight in the refrigerator.

Formalin-fixed paraffin-embedded sections may also be used for DNA ploidy analysis by the Hedley method, although the results are not as satisfactory due to nuclear fragmentation.

Results

The results are usually incorporated into the final surgical pathology report.

CYTOLOGIC PREPARATIONS FROM SURGICAL SPECIMENS

Cytologic preparations of surgical specimens often provide additional information.

Intraoperative diagnosis. Touch preps or smears are especially valuable for:

- Infectious cases (to avoid contamination of the cryostat and aerosolization of infectious agents)
- Neuropathology cases, for diagnosis and for the performance of cytogenetic (FISH) analysis

- Tumors (for excellent cytologic detail, especially lymphomas and papillary carcinomas of the thyroid).

Special stains. Stains for microorganisms can be performed the same day on cytologic smears of specimens from critically ill patients. Do not submit air dried smears of infectious cases for staining as the unfixed material may constitute a hazard to laboratory personnel.

Fat is dissolved during routine processing, but can be demonstrated with fat stains on air dried slides.

Genetic studies (FISH). Nuclei are intact in touch preparations, unlike tissue sections in which the only partial nuclei are present. This feature makes touch preparations superior for techniques such as FISH and image analysis.

It is always a useful exercise to look at cytology preparations and the corresponding surgical specimen to learn the comparative morphology of these techniques.

SPECIMEN RADIOGRAPHY

Specimen radiographs are often preferred to patient radiographs:

- A permanent record of the radiograph can be kept with the case.
- A radiograph of the specimen may reveal more details of the underlying process (e.g., fewer structures may be present to complicate the appearance).
- A significant time interval may have elapsed between the patient radiograph and the surgical excision.
- The radiograph often indicates sites that are important to examine histologically (tumor invasion into a rib or microcalcifications in a breast biopsy).
- The specimen radiograph can confirm that the clinical lesion was removed.

Indications

- Tumors of bone and cartilage.
- Tumors invading into bone.
- Avascular necrosis.
- All bioprosthetic heart valves (to document the degree of calcification).
- Breast biopsies or mastectomies performed for mammographic lesions that cannot be located grossly. Paraffin blocks of breast tissue can be radiographed if microcalcifications were seen by specimen radiography but not in histologic sections and were not identified prior to processing.

Calcifications can dissolve in formalin over several days. If the demonstration of calcifications is important (e.g., mammographically detected calcifications) it is preferable to process the tissue within 1 to 2 days. If processing is to be delayed, the tissue can be stored in ethanol.

Method

Radiographic equipment is available in radiology departments and in some pathology departments. The specimen may be placed on a piece of wax paper (to keep the surfaces clean) lying on the film. Specimens can be radiographed after decalcification (not all calcium is removed) but best results are obtained on fresh undecalcified specimens. Lungs should not be inflated prior to radiography.

If the specimen is small, two exposures at different settings or at different angles may be useful. Lead sheets can be used to allow two exposures on one piece of film.

If the film is too dark (overexposed), the exposure is too high and a lower setting should be tried. If the film is too light (i.e., unexposed) the exposure is too low and a higher setting is indicated.

Special injection techniques with radiocontrast media are available for unusual specimens such as a recipient lung with pulmonary hypertension or vascular ectasia of the bowel.

Octreotide and sentinel nodes. Labeled compounds are sometimes used to localize certain types of tumors (generally neuroendocrine) or sentinel lymph nodes. The patient is injected with the isotope prior to surgery and the surgeon uses a handheld probe to identify the labeled tissue. The amount of radioactivity in the tissue is small; generally it does not pose a hazard to pathologists handling the tissue and does not need special disposal methods. However, each pathology department should consult with the radiation safety department to ensure appropriate handling of such tissues. In some cases, if a gross lesion is not present corresponding to the area of octreotide uptake, specimens can be imaged using a gamma camera.

Results

The radiographs are documented in the gross description and any information gained from the radiograph is incorporated into the surgical pathology report.

TISSUE FOR RESEARCH: TUMOR BANK

The pathology department is a unique resource for researchers who need human tissues. The pathologist plays a key role as patient advocate and diagnostician in order to provide appropriate human tissues for biologic research. Most hospitals have a policy that allows the release of tissue for research *if it would otherwise be discarded*. Therefore, tissue is never given away for research until all necessary tissue has been taken for diagnosis. Tissue from primary diagnostic breast biopsies and open lung biopsies without gross lesions should not be given away. It is in the best interest of the patient that a pathologist evaluates the specimen rather than have tissue given away by a nonpathologist who is not aware of what is needed for diagnosis.

Indications

By request of researchers who have obtained permission from the hospital Human Studies Committee.

Method

Adequate information must be provided by the clinician to allow the pathologist to determine how much of the tissue is needed for diagnostic purposes. Research laboratories should provide containers for the transport of specimens. The name of the laboratory, the type of tissue, and the amount of tissue allocated for research must be carefully documented. Tissue should never be given away if there is any question as to the need for the tissue for diagnostic purposes. In some cases it may be preferable (or possibly required) to withhold the name or other identifiers of the patient for medical confidentiality.

MICROBIOLOGIC CULTURE AND SMEARS

The investigation of infectious disease by culture is complementary to its investigation by histologic sections (Table 7-37).

Table 7-37 Identification of infectious diseases

CULTURE	HISTOLOGIC SECTIONS
Can be performed on aspirates, swabs, fluids, or tissues	Requires surgical excision of tissues
Cultures amplify the number of organisms present, allowing them to be recognized	Organisms may be rare, or not seen in tissue sections
The specific organism can be identified and tested for drug susceptibility	Categories of organisms can be recognized but specific identification may not be possible
Some organisms cannot be cultured	Many organisms can be identified that will not grow in culture or that require long culture times (e.g., *Mycobacterium tuberculosis*)
It may be difficult to exclude contamination for a positive culture	Morphologic evidence of an inflammatory response provides evidence for a clinical infection. The location of the infection may be of diagnostic importance (e.g., cellulitis versus necrotizing fasciitis or superficial colonization of devitalized tissue versus deep infections involving viable tissues)

Indications

- Suspected infectious processes.
- Suspected sarcoid to exclude an infectious process.

Method

Tissue is kept as sterile as possible. Suture removal kits are a convenient source of sterile scissors and forceps. Serially section the specimen to determine whether there are focal lesions. Place representative sections in a sterile specimen container. Label with the patient's name and unit number, patient's physician, type of specimen, collection date, and time of collection (required for JCAHO accreditation).

Three different types of culture are often requested (requiring three different requisition forms):

1. Routine culture. The usual request for routine specimens would be:

- Bacteria (only includes aerobic culture)
- Mycobacteria
- Fungal.

Other organisms require special culture techniques and must be specifically requested:

- Anaerobic bacteria
- *Salmonella, Shigella*, and *Campylobacter*
- *Nocardia*
- *Neisseria gonorrhoeae*
- *Brucella*
- *Legionella*
- *Francisella tularensis*
- *Helicobacter*.

2. Viral culture. CMV, varicella zoster, adenovirus, and herpes simplex are most commonly requested. Cultures for influenza A and B, Respiratory synctial virus, and parainfluenza require special techniques.

3. Mycoplasma. Usually requires special cultures. Occasionally, mycoplasma can be detected on anaerobic cultures, but this is not the optimal means for identifying this organism.

Results

The results are generally reported by the microbiology laboratory. It is helpful to correlate the results with the pathologic findings, when possible.

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