

16 A Rapid Guide to Immunostains

Table 1 Site and System Specific Markers in Carcinomas

Marker	Main tumor	Other cacinomas positive
Specific		
α amylase	Acinic cell carcinoma (salivary and pancreas)	
CAIX	Renal clear cell (nearly 100%)	
hCG	Trophoblastic epithelium	
Thyroglobulin	Thyroid (not anaplastic and medullary)	
Trypsin	Acinar pancreatic carcinoma	
Uroplakin III	Urothelial (57%)	
Multispecific		
Beta catenin	Pancreatic solid pseudopapillary tumor	Colon cancer
CDX2	Colon (>90%)	Intestinal differentiation
c-Kit	Thymic carcinoma (>90%)	Chromophobe RCC >90%, renal oncocytoma and adenoid cystic (50-90%)
DOG1	Salivary acinic cell tumor (>90%)	Salivary polymorphous low grade , adenoid cystic (50-90%)
GATA3	Breast (90%)	Urothelial (86%), salivary duct (50-90%)
GCDFP-15	Breast (60%)	Salivary and sweat gland
HepPar-1	Hepatocellular carcinoma (90%)	Hepatoid carcinoma, stomach signet ring
Mammaglobin	Breast (85%)	Endometrioid (40%), salivary and sweat gland
Napsin A	Lung adenocarcinoma (80%)	Some papillary RCC
PAP	Prostate (95%)	Carcinoid, bladder
PSA	Prostate (80%)	Salivary, bladder
PAX2	Renal clear cell (80%)	Mullerian carcinoma (30%)
PAX8	Pan renal	Thyroid, pan mullerian
P40	Squamous carcinoma (nearly 100%)	Urothelial carcinoma
pCEA	Hepatocellular carcinoma (50%)	Hepatoid carcinoma
RCC	Renal clear cell (84%)	Breast (33%), adrenocortical (22%), colon (37%)
TTF-1	Thyroid (except anaplastic)	Lung adenocarcinoma (70%), small cell carcinoma (>90%)
WT1	Ovarian, serous (90%)	Mesothelioma (90%)

N.B. Reactivity rate shown in brackets. Abbreviations: CAIX carbonic anhydrase IX, hCG human chorionic gonadotropin, RCC renal cell carcinoma, DOG1 discovered on GIST 1, GATA3 (GATA binding protein 3 to DNA sequence [A/T]), GCDFP-15 gross cystic disease fluid protein, HepPar-1 hepatocyte paraffin 1, PAP prostate acid phosphatase, PSA prostate specific antigen, PAX2 paired box gene 2, PAX8 paired box gene 8, pCEA polyclonal carcinoembryonic antigen, TTF-1 thyroid transcription factor 1, WT1 wilms tumor gene.

Table 2 Immunophenotypic Expression of Neuroectodermal/ Neuroendocrine Tumors

Tumor	Marker
Intracranial (central)	
Gliomas	GFAP, ependymoma well differentiated is EMA +
Meningioma	EMA, PR (50%), fibrous (S-100 in 80%), secretory (CK in >50%)
Neuronal	NEP, SYN, NeuN1
PNET(medulloblastoma)	CD56 (>90%), SYN (50-90%)
Choroid plexus tumors	CK, Podoplanin (D2-40)
Extracranial (peripheral)	
Epithelial	
Melanoma	S-100, HMB-45, Melan-A (all >90%)
Endocrine pancreas	CHR (>90%), CK (>90%), hormones
Carcinoid	CHR (>90%), CK (>90%)
Small cell carcinoma	CHR (>90%), CK (>90%)
Merkel tumor	CK20 (perinuclear, >90%), polyoma virus (>90%), CHR (50-90%)
Neural	
Shwann cell (MPNST)	Myelin basic protein (>90%), S-100 (50-90%), SOX10 (10-50%)
Granular cell tumor	S-100 (>90%), SOX10 (>90%)
Paraganglioma	CHR (50-90%), S-100 (sustentacular cells)
Neuroblastoma	SYN, S-100 (50-90%)
Ewing/PNET	CD99 (>90%), Fli-1 (50-90%)

Abbreviations: GFAP glial fibrillary astrocytic protein, EMA epithelial membrane antigen, PR progesterone receptor, CK cytokeratin, NEP neurofilament, SYN synaptophysin, NeuN1 neuronal nuclei 1, HMB-45 human melanoma black, CHR chromogranin, SOX10 SRY-related HMG-box 10.

Table 3 Immunophenotypic Expression of Germ Cell Tumors

Germ cell tumors	SALL4 (100%), PLAP
Seminoma	CD117 (membranous, 90%), D2-40
Embryonal carcinoma	CK (80%), CD30 (90%), SOX2 (100%)
Yolk sac tumor	AFP, glypican-3
Choriocarcinoma	Beta hCG, CD10 (>90%), EMA (50%)
Teratoma	Vimentin, CK, EMA

N.B. Reactivity rate shown in brackets. Abbreviations: SALL sal-like 4, PLAP placental alkaline phosphatase, CK cytokeratin, SOX2 sex determining region of Y chromosome-related high mobility group box2, AFP alpha fetoprotein, HCG human chorionic gonadotropin, EMA epithelial membrane

Table 4 Immunophenotypic Expression of Malignant Mesenchymal Tumors

Tumor	Markers
Fibrous and fibrohistiocytic	
Fibromatosis	Beta catenin (70-90%)
Fibrosarcoma	Marker negativity
Inflammatory myofibroblastic tumor	ALK (50%)
Low-grade fibromyxoid sarcoma	MUC4 (nearly all)
Sclerosing epithelioid fibrosarcoma	MUC4 (70%)
Solitary fibrous tumor	CD34 (90%), CD99 (90%), bcl2 (90%), STAT6 (consistent), GRIA2 (majority)
Dermatofibrosarcoma protuberans	CD34 (75%)
Malignant fibrous histiocytoma	CD68 (50-90%)
Lipomatous tumors	S-100, MDM2, CDK4(95-100% of atypical lipomatous & dedifferentiated)
Rhabdomyosarcoma	Desmin, myoD1, myogenin
Smooth muscle tumor	SMA (90%), desmin (70%)
Vascular tumor	ERG, CD31, CD34
Gastrointestinal stromal tumor	c-Kit (90%), DOG1(75-100%)
Mesothelioma	Calretinin (90%), CK5/6 (90%)
Bone tumors	
Chondrosarcoma	S-100 (consistent in well differentiated)
Chordoma	Brachyury (consistent), CK, S-100 (80%)
Giant cell tumors, stromal cells	p63
Osteosarcoma	SATB2 (consistent), MDM2 &CDK4 (67% in low grade, 12% in high grade), osteocalcin (70%, practical difficulty)
Tenosynovial giant cells	CD68 and LCA (in multinucleated giant cells)
Tumors of Uncertain Differentiation	
Alveolar soft part sarcoma	TFE3 (up to 100%)
Desmoplastic small round cell tumor	CK (90%), desmin (90%), WT1(N-terminus, 90%)
Epithelioid sarcoma	CK, EMA, INI loss in 95%, CD34 (50%)
Extrarenal rhabdoid tumor	CK, INI loss in 85%,
Glomus tumor, spindle cells	SMA
Parachordoma	CK, S-100
PEComa	Actin (90%), HMB45 (90%), Melan A (60%)
Synovial sarcoma	TLE1(80-90%), CK, Bcl-2 (90%), CD99 (50%)
Undifferentiated sarcoma	Vimentin
Carcinosarcoma	Vimentin, desmin, CK

NB. Reactivity rate shown in brackets. Abbreviations: ALK anaplastic lymphoma kinase, MUC4 mucin 4, STAT6 signal transducer and activator of transcription 6, GRIA2 glutamate ionotropic receptor AMPA type subunit 2, MDM2 murine double minute 2 homolog, CDK4 cyclin dependent kinase 4, SMA smooth muscle actin, ERG v-ets avian erythroblastosis virus e26 oncogene homolog, DOG1 discovered on GIST 1, SATB2 special AT-rich sequence-binding protein 2, TFE3 transcription factor E3, CK cytokeratin, WT1 wilms tumor1, EMA epithelial membrane antigen, SMARCB1 (known as INI) SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1, HMB45 human melanoma black, TLE1 transducin-like enhancer of split 1.

Table 5 Diagnostic Approach of Lymphoma and Histiocytic Tumors According to the Pattern, Cell Size and Immunophenotype

Pattern	Cell size	Diagnosis	Immunophenotype
Nodular	Variable	Follicular lymphoma	CD20+, bcl2+ (50-90%), bcl6+, CD10+(-ve in high grade), Mum1+ (-ve in low grade), CD23-, CD5-
		Hodgkin, nodular sclerosis	CD15& CD30+ in 80%, Mum1+, weak CD20 in 30%, EMA-
		Nodular lymphocyte predominance Hodgkin	CD20+, EMA+, CD3-, CD30-, CD15-
		Follicular dendritic cell sarcoma	CD21+, S-100-, CD1a-, CD68-
		Nodal marginal zone lymphoma	CD20+, CD43+ (50%), bcl2+, CD5-, CD23-, CD10-, bcl6-, cyclinD1-
		Mantle cell lymphoma	CD20+, CD5+, cyclinD1+, SOX11+ (specific), CD23-, CD10-
Diffuse	Small	Small lymphocytic lymphoma	CD20+, CD23+, CD5+, CD3-
		Mantle cell lymphoma	CD20+, CD5+, cyclinD1+, SOX11+ (specific), CD23-, CD10-
		Marginal zone lymphoma	CD20+, CD43+ (50%), CD5-, CD23-, cyclinD1-
		Lymphoplasmacytic lymphoma	CD20+, IgM+, CD5-, CD23-, CD10-
		Plasma cell myeloma	CD38+, CD138+, CD56+, CD19-
	Intermediate	Burkitt lymphoma	CD20+, CD10+, bcl6+, Ki (90-100%), bcl2-, TdT-
		Lymphoblastic lymphoma	B phenotype: TdT, Pax5& CD79a+ T phenotype: TdT, CD3& CD5+, CD4/CD8 often double positive
	Large	Diffuse large B-cell lymphoma	CD20+, Pax5+, Oct2+, CD3-, (variable expression of CD10, bcl2, bcl6 & Mum1)
		Anaplastic large cell lymphoma	CD30+, CD43+, ALK+ (60-85%), CD45&CD45RO variable, CD3 often -ve, CD15-
		Peripheral T-cell lymphoma	CD3+, CD20-
		Hodgkin, lymphocyte depletion	CD15& CD30+ in 80%, Mum1+, weak CD20 in 30%, EMA-
	Mixed	Hodgkin, mixed cellularity	CD15& CD30+ in 80%, Mum1+, weak CD20 in 30%, EMA-
		Peripheral T-cell	CD3+, CD20-
		T-cell rich B-cell	CD20+, CD3 (large cell-, small cell+)
		Histiocytic sarcoma	CD68+, CD21-

N.B. In small cell lymphoma, the size is equal to that of normal lymphocyte, but in large cell lymphoma, it is more than the double size of normal lymphocyte.

Valuable Remarks

- (1) Ki-67 is useful top grade both nodular and diffuse lymphoma and to help to avoid underdiagnosis of mantle cell and Burkitt types (Table 9).
- (2) P53 is a useful prognostic marker for DLBCL, MALT and follicular lymphomas.

Precautionary remarks

- (1) Cyclin D1 and ALK are not specific markers.
- (2) Marker expression may disagree with gene translocation.
- (3) CD34 and CD117 may be negative in 25% of cases, hence missing the diagnosis of a blast leukemic crisis

Table 6 Work Up for Undifferentiated/Unclassified Tumor in Adults with Single Expression (Also Applicable for Metastasis with Unknown Primary)

Primary panel/ lineage	Pan CK	Vi- mentin	LCA	S-100	Additional markers
Carcinoma	+ve				CK7&20, site specific markers
Sarcoma		+ve			Desmin, CD34, SMA, etc.
Lymphoma		+ve	+ve		CD20, CD3, etc.
Melanoma		+ve		+ve	HMB45, melan A
Plasma cell myeloma		+ve			CD138

In case of co-expression of initial markers, the work up is as follows:

- (1) CK & vimentin: Carcinosarcoma, synovial sarcoma (TLE1, CK7), epithelioid sarcoma (CD34, INI-), epithelioid angiosarcoma (ERG,CD31), mesothelioma (calretinin, mesothelin).
- (2) CK & S-100: Neuroendocrine carcinoma (CD56), myoepithelial tumors (p63).
- (3) Vimentin & S-100: Neuroectodermal tumors, histiocytosis (Langerhans histiocytosis: CD1a, langerin. Non langerhans: CD68, CD163, CD4. Follicular dendritic: CD21, CD23, CD35. Interdigitating dendritic: negative for follicular dendritic markers)

Table 7 Work Up for Undifferentiated/Unclassified Tumor in Childhood (Also Applicable for Metastasis with Unknown Primary)

Primary panel/ lineage	Pan CK	LCA	CD99	Desmin	S-100	Additional markers
Ewing sarcoma/PNET			+ve			Fli-1
Lymphoblastic lymphoma		+/-ve	+ve			CD3, CD20, TdT
Rhabdomyosarcoma				+ve		Myogenin, MyoD1
Wilms tumor, blastema predominant	+ve					WT1
Neuroblastoma					+ve	Synaptophysin
Desmoplastic small round cell tumor	+ve		+ve	+ve		WT1
Germ cell tumor						SALL4, CD30, EMA

Table 8 The Nonspecificity of Immunostains

Marker	Main tumor	Other tumors positive
ALK	Anaplastic large cell lymphoma (70%)	Inflammatory myofibroblastic tumor (60%), lung adenocarcinoma
CD34	Vascular tumors	Lymphoblastic lymphoma, many soft tissue tumors
CD56	Small cell carcinoma	NK/T cell lymphoma, neoplastic plasma cells
CD99	Ewing sarcoma	Lymphoblastic lymphoma, synovial sarcoma, sex cord stromal tumors
CD138	Plasma cell myeloma	Squamous cell carcinoma
c-Kit	GIST	Seminoma, blasts in leukemia, thymic carcinoma
Cyclin D1	Mantle cell lymphoma	Undifferentiated uterine sarcoma
D2-40	Lymphatic tumors	Mesothelioma, skin adnexal tumors, germ cell tumors
GATA3	Breast and urothelial carcinoma	Adnexal and squamous carcinoma, mesothelioma
Glypican 3	Hepatocellular carcinoma	Melanoma, yolk sac tumor, ovarian carcinoma
HMB45	Melanoma	PEComas, adrenal cortical, sex cord stromal, angiomyolipoma
Mammaglobin	Breast	Adnexal tumors
Napsin A	Lung carcinoma	Ovarian clear cell carcinoma, renal cell carcinoma
P63, P40	Squamous carcinoma	Urothelial carcinoma
PAX8	Pan renal and mullerian	Thyroid
SOX10	Melanoma	Nerve sheath tumors, clear cell sarcoma
TTF1	Lung and thyroid	Small cell carcinoma
WT1	Wilms tumor	Ovarian serous, mesothelioma

Table 9 Ki-67 Scale for Assessment of Tumor Behavior Based on Established Reported Values)

Rate (%)	Epithelial	Mesenchymal	Lymphoma	Neuroectodermal/ neuroendocrine
90			Burkitt	Small cell carcinoma
80			Lymphoblastic	
70	Serous carcinoma			
50		Rhabdomyosarcoma	Multiple myeloma	
40	Endometrioid adenocarcinoma		Diffuse large, mantle cell	Large cell neuroendocrine carcinoma
20	Ovarian serous carcinoma high grade, breast high risk, adenoid cystic carcinoma	Malignant phyllodes	Low grade NHL	GIT neuroendocrine tumor grade 3
15	Serous tubal intraepithelial carcinoma	Uterine leiomyosarcoma		Glioblastoma
10	Adrenal carcinoma, myoepithelial carcinoma, adenomatous polyps		Lymphoplasmacytic plasmacytoma	Anaplastic astrocytoma, meningioma grade 3
5	Adrenal adenoma	Uterine leiomyoma, borderline phyllodes	Small lymphocytic, splenic marginal, hairy cell, mycosis fungoides	Diffuse astrocytoma, pulmonary grade 1 neuroendocrine tumor
2	Hyperplastic colonic polyps	Fibromatosis, benign phyllodes		Pilocytic astrocytoma, GIT well differentiated neuroendocrine tumor

Valuable Ki-67 Remarks

In some lesions, the distribution of immunoeexpression is more diagnostic than Ki-67 rate. Examples (1) Polar expression of Ki-67 in reactive germinal centers (2) Superficial expression in benign nevus and benign neurofibroma (3) Basal location of expression in squamous dysplasia

Precautionary Ki-67 Remarks

(1) Ki-67 rate is high in inflammatory reactions (T-lymphocytes, histiocytes and mast cells), (2) High Ki-67 rate in squamous epithelium after viral infection (HPV) (3) Some benign tumors (e.g. cellular neurofibroma) may exhibit high Ki-67 rate (>20%), whereas MPNST may show low expression (4) Ki-67 is of no diagnostic value of tumor behavior in thyroid tumors and paraganglioma.

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