Markers and Immunoprofile of Lymphoid Neoplasms

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M. S. A. Tuffaha et al., *Immunohistochemistry in Tumor Diagnostics*,
https://doi.org/10.1007/978-3-031-45024-2_16

The lymphoid tissue is a microenvironment composed of B-, T-, and NK-lymphocytes in different maturation and differentiation stages, plasma cells, macrophages, dendritic cells, reticular cells, granulocytes, stromal cells, and capillaries. All of these components must be considered for the interpretation of lymphoproliferative neoplasms. For the initial diagnosis, screening markers can be helpful. Additional markers for specific lymphoma types must be used to precise the diagnosis. Markers listed in different parts of this chapter are essentially used for orientation. The final diagnosis must be made taking into consideration the clinical data, histomorphology, and immunophenotype, including immunohistochemistry and flow cytometry, in addition to molecular genetic analysis if necessary. The fifth revision of the World Health Organization classification of hematolymphoid neoplasms was considered in this chapter [1].

16.1 Screening Markers for Lymphoid Neoplasms

CD45 (LCA), B-cell markers, T-cell markers, TdT, CD34, and Ki-67 [2–4].

16.1.1 CD45

CD45 (LCA)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Lymphoma/ leukemia	Granulocytic sarcoma, histiocytic sarcoma, dendrocytoma, interdigitating dendritic cell sarcoma, giant cell tumor of tendon sheet	Mature and immature hematopoietic cells (including B- and T-lymphocytes, monocytes, macrophages, plasma cells, and mast cells), dendritic cells, osteoclasts, medullary thymocytes, fibrocytes
Positive control: appendix		

Diagnostic Approach CD45, also known as leukocyte-common antigen (LCA), is a family of high molecular mass integral membrane glycoprotein molecules expressed on all mature and immature hematopoietic cells except mature red cells and their immediate progenitors, megakaryocytes and platelets.

Diagnostic Pitfalls CD45 is a specific marker for hematopoietic and lymphatic tumors; nonetheless, less than 3% of B-cell lymphoma, about 10% of T-cell lymphoma, and about 30% of precursor B- and T-lymphoblastic lymphomas (ALL) lack the expression of CD45. Most representative examples of CD45 negative lymphomas are ALK-positive large B-cell lymphoma, anaplastic large-cell lymphoma, and plasmablastic lymphoma. In suspicious cases, the use of other lymphoid markers is required. Membranous CD45 expression is reported in sporadic cases of undifferentiated, neuroendocrine, and small-cell carcinomas. Necrotic carcinomas can also imitate a membranous LCA positivity, which also holds true for other immunohistochemical markers, as, in general, necrosis may display a false positivity.

16.1.2 Terminal Deoxynucleotidyl Transferase

Terminal deoxynucleotidyl transferase (TdT)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B- and T-ALL	AML, CML, Merkel cell carcinoma	Pro- and pre-B-lymphocytes, prothymocytes, subcapsular and cortical thymocytes
Positive control: ALL		

Diagnostic Approach Terminal deoxynucleotidyl transferase (TdT) is a DNA nuclear polymerase encoded on chromosome 10, catalyzing the template-independent polymerization of deoxynucleotidyl triphosphates to double-stranded gene segment DNA. TdT is mainly expressed in precursors of B- and T-lymphocytes, including prothymocytes and thymocytes. The expression of TdT is specific for precursor cell lymphomas of T- and B-cell origin, namely, acute lymphoblastic leukemia. In the normal bone marrow, 1–2% of nucleated cells are positive for TdT, and most are B-cell precursors. In the normal thymus, different percentages of cortical T-cells are TDT positive depending on their maturation stage.

Diagnostic Pitfalls It is essential to consider that TdT may be positive in some types of acute myeloid leukemia, especially minimally differentiated AML (M0) and AML with t(6;9) in addition to blast crisis of chronic myeloid leukemia (CML) and myeloid sarcoma. The expression of TdT is also characteristic for the immature T-lymphocytes associated with the thymoma types A, B, and AB but not thymic carcinoma.

The expression of TdT is also reported in a large percentage of Merkel cell carcinoma, which may also be positive for PAX-5 [5, 6].

CD5 and CD10 are further markers important for the diagnosis and classification of lymphomas. Both do not have lineage specificity and may be expressed in both B- and T-cell lymphomas in addition to other nonlymphoid neoplasms, mainly epithelial tumors.

16.1.3 CD10

CD10 (CALLA)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Burkitt lymphoma – Acute lymphoblastic lymphoma/leukemia – Angioimmunoblastic T-cell lymphoma – Endometrial stromal tumors – Clear cell renal cell carcinoma – Hepatocellular carcinoma	Follicular lymphoma; plasma cell neoplasms; transitional cell carcinoma; various adenocarcinomas including pulmonary, colorectal, and prostatic adenocarcinomas; melanoma; placental site trophoblastic tumor; choriocarcinoma; myofibroblastoma; mesothelioma; rhabdomyosarcoma; osteosarcoma; leiomyosarcoma; Ewing's sarcoma; solitary fibrous tumor; atypical fibroxanthoma	Pro-B- and pre-B-cells, germinal center B-cells, prothymocytes, subcapsular thymocytes and follicular T-helper cells, granulocytes, adrenal cortex, endometrial stroma cells, hepatocytes and bile duct canaliculi, glomerular epithelial cells and cells of proximal renal tubules, epithelium of seminal vesicles, endothelial cells, myoepithelial cells, fibroblasts, brain tissue, choroid plexus, fetal intestinal epithelium, mesonephric remnants
Positive control: appendix/tonsil		

Diagnostic Approach CD10 (neprilysin) is a zinc-dependent cell membrane metalloprotease involved in the post-secretory processing of neuropeptides and *vasoactive peptides*. Despite the name of CD10 as the common acute lymphoblastic leukemia antigen (CALLA), CD10 is not a cell line- or tumor-specific marker as it is expressed in a long list of tissue and tumor types of lymphoid (B- and T-cells), myelogenous, epithelial/myoepithelial, and mesenchymal origin mentioned in the above table [7, 8]. CD10 is a maturation marker of granulocytes and, together with CD15, labels the blasts of low-risk MDS.

In diagnostic immunohistochemistry, CD10 must be used in a panel with other tissue- and cell-specific markers [2]. The expression pattern of CD10 (membranous or cytoplasmic) is highly variable, depending not only on the tumor type but also on differentiation grade, as the cytoplasmic stain is usually seen in poorly differentiated carcinomas.

16.1.4 CD5

CD5		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mantle cell lymphoma – B-CLL	T-ALL, T-cell lymphoma, prolymphocytic leukemia, adenocarcinomas of different origins, atypical thymoma and thymic carcinoma	T-cells, a subset of B-cells of the mantle zone of the spleen and lymph nodes
Positive control: appendix/tonsil		

Diagnostic Approach CD5 (lymphocyte antigen T1, Leu-1) is a glycoprotein receptor encoded on chromosome 11. The expression of CD5 begins at the prothymocyte stage and persists in the majority of T-lymphocytes. CD5 labels the majority of T-cell lymphomas, including T-ALL, adult and peripheral T-cell lymphoma, mycosis fungoides, and T-cell large granular lymphocytic leukemia. The expression of CD5 is not restricted to T-lymphocytes but is also found in a small subset of adult B-lymphocytes, including mantle zone lymphocytes, in addition to more than 50% of fetal B-lymphocytes and lymphomas of B-cell origin, mainly mantle cell lymphoma and B-CLL (Figs. 16.1 and 16.2).

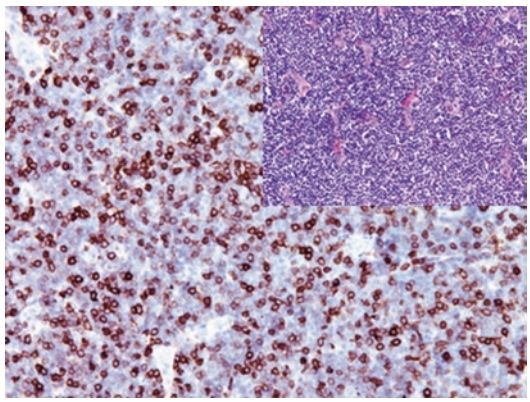


Fig. 16.1 Weak to moderate CD5 expression in the cells of B-CLL. T-lymphocytes with strong membranous CD5 expression

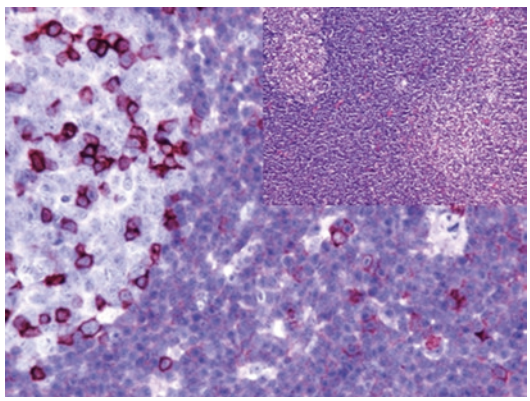


Fig. 16.2 Cells of mantle cell lymphoma showing moderate membranous CD5 expression. Associated T-lymphocytes with strong CD5 expression

Diagnostic Pitfalls The expression of CD5 is not limited to lymphoid tissue but is found in adenocarcinomas of different origins, renal cell carcinoma, and adrenocortical carcinoma in addition to squamous cell carcinoma. Furthermore, CD5 is a diagnostic marker for atypical thymoma and thymic carcinoma (see Chap. 4). A focal weak CD5 expression can also be found in mesothelioma, transitional carcinoma, squamous cell carcinoma, and adenocarcinomas of different origins [9].

16.1.5 CD34

CD34 is a cell surface adhesion glycoprotein and a marker for endothelial and stem cells listed with the markers of vascular tumors (Chap. 25). CD34 is an important marker for the diagnosis of lymphoid and hematopoietic neoplasia. Besides CD117, CD34 is also expressed on the precursors of B- and T-lymphocytes, myeloblasts, and mast cell progenitors. CD34 is expressed on different percentages of B-ALL, T-ALL, and AML blasts and is helpful for the diagnosis of MDS.

16.1.6 Ki-67

Ki-67 is a nonhistone nuclear protein in humans encoded by the MKI67 gene on chromosome 10q26.2, involved in the early steps of polymerase I-dependent ribosomal RNA synthesis and DNA replication and expressed in the active cell cycle. The expression of Ki-67 begins in the G_1 phase and persists during the active phases of the cell cycle throughout the S, G_2, and M phases, whereas the peak of the Ki-67 expression appears in the early M phase. Ki-67 is rapidly catabolized at the end of the M phase with a half-life of 1–1.5 h and is undetectable in the G_0 phase or in the initial stage of the G_1 phase. Cells during the DNA repair also lack the Ki-67 expression.

The expression of Ki-67 strongly correlates with the intensity of cell proliferation and tumor grade. In routine histopathology, Ki-67 is an important marker for the assessment of cell pro-

liferation. The Ki-67 index is an important criterion for tumor diagnosis (benign, borderline, malignant, low- or high-grade tumor). Furthermore, it is a helpful marker to differentiate between atrophy or thermal alterations and dysplasia. Few tumors show a Ki-67 index of nearly 100%, which can be used as a diagnostic clue; most representative examples are small-cell lung carcinoma, Burkitt lymphoma, and plasmablastic lymphoma (Fig. 16.4). In routine hematopathology, the Ki-67 index is an important parameter to classify low- and high-malignant lymphomas (Fig. 16.3). Additionally, the Ki-67 index is a well-known prognostic marker correlating with the biological behavior of tumors such as breast carcinoma and neuroendocrine tumors. Nonetheless, it is a challenge to standardize Ki-67 staining and to establish a robust and reliable Ki-67 evaluation, which tends to show considerable interlaboratory variability.

Noteworthy is the aberrant membranous expression of Ki-67 characteristic for sclerosing pneumocytoma and hyalinizing trabecular tumor of the thyroid.

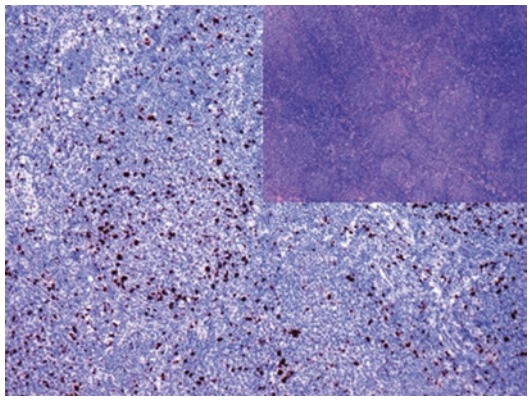


Fig. 16.3 Characteristic low proliferation index (Ki-67) in neoplastic follicles of follicular lymphoma grade 1–2

16.2 Markers and Immunoprofile of B-Cell Neoplasms

16.2.1 B-Lineage-Specific Markers

CD10, CD19, CD20, CD79a, PAX-5.

16.2.2 Markers for Specific Lymphoma Types

CD5, CD23, CD34, LEF-1, bcl-2, Bcl-6, LMO2, HGAL, cyclin D1, SOX11, ARTA1, TRAP, HHV-8, and TdT [2–4, 10].

16.2.3 Therapy-Related Markers

CD19, CD20, CD30, p53, Ki-67.

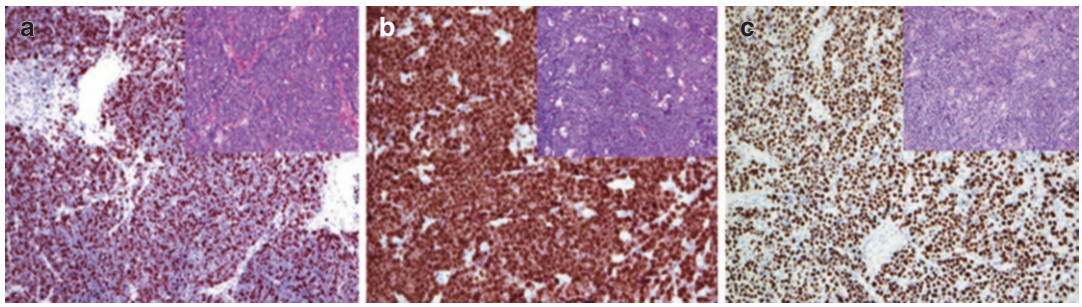


Fig. 16.4 Three tumor types with high Ki-67 index (~100%). (**a**) Small-cell carcinoma, (**b**) Burkett's lymphoma, (**c**) plasmablastic lymphoma

16.2.4 CD19

CD19 (B4)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-cell lymphoma/leukemia	Myeloblasts in (M0) AML with t(8;21), blast phase of CML	B-cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD19 (β-integrin) is a single-chain glycoprotein and a member of the immunoglobulin family encoded on chromosome 16. CD19 is an early naïve B-lymphocyte antigen, which remains through the B-lymphocyte differentiation stages and disappears in the plasma cell stage. The CD19 expression is also found on the surface of follicular dendritic cells. CD19 is an excellent B-lymphocyte marker, and antibodies to CD19 are available for both flow cytometry and paraffin histology [11]. CD19 is negative in ALK + large B-cell lymphoma, primary effusion lymphoma, and plasma cell neoplasia.

16.2.5 CD20

CD20 (B1 antigen)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-cell lymphoma/leukemia	Epithelial cells of thymomas type A and AB	B-cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD20 is a transmembrane non-glycosylated phosphoprotein encoded by the MS4A1 gene on chromosome 11, acting as a receptor during B-cell activation and differentiation. CD20 appears on the B-cells after CD19 and CD10 in the naïve B-lymphocytes and remains until the late stages of B-lymphocyte differentiation but disappears in the plasma cell stage. Characteristic for CD20 is the membranous expression pattern, whereas the cytoplasmic or nucleolar expression patterns are nonspecific.

Diagnostic Pitfalls CD20 is a pan B-lymphocyte marker, but some types of B-cell lymphomas may be CD20 negative or show a very weak expression level; consequently, in doubtful cases, it is important to use two B-cell markers to ensure or exclude the B-cell origin of the neoplasm. Optimal combinations are CD20/CD19 and CD20/PAX-5 or CD20/CD79. Few B-cell lymphoma types are negative for CD20, such as plasmablastic lymphoma, ALK + large B-cell lymphoma, and primary effusion lymphoma. Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma are usually negative for CD20 but often express the nuclear B-cell marker PAX5 (see below). Generally, the expression of CD20 is restricted to B-lymphocytes; nevertheless, CD20 expression is reported in rare cases of peripheral T-cell lymphoma.

CD20 expression is also characteristic for rare epithelial tumors, found on the neoplastic epithelial cells of thymomas type A and AB, whereas thymomas type B1, B2, B3, and C and in normal thymic epithelium lack the expression of CD20. The CD20-positive thymic cells are negative for all other B-cell markers (Fig. 16.5). Aberrant CD20 expression is also reported in a small subset of thyroid carcinoma, mainly papillary thyroid carcinoma [12].

A diagnostic pitfall is the interpretation of CD20 stain in tissue or bone marrow samples after targeted anti-CD20 immunotherapy (rituximab) exhibiting the loss of CD20-positive

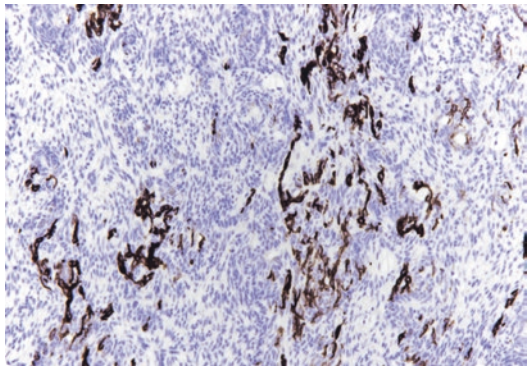


Fig. 16.5 CD20 expression on the epithelial cells of type A thymoma

B-lymphocytes, which also may be associated with the loss of CD19. In such biopsies, the absence of CD20-positive lymphocytes does not exclude the presence of lymphoma cells and other B-cell markers, such as PAX5 and CD79a, can be helpful in detecting lymphoma cells.

16.2.6 CD22

CD22 (sialic acid binding Ig-like lectin 2, Siglec-2) is a type I transmembrane glycoprotein composed of two α- and β-chains that acts as a mediator in B-cell–B-cell interaction. CD22 is expressed in the cytoplasm of early B-lymphocytes after CD19, followed by the membranous expression on mature B-lymphocytes, and disappears in plasma cells. CD22 is also expressed on basophils and mast cells. CD22 is a marker for B-cell lymphomas.

16.2.7 CD23

CD23 (low affinity IgE receptor)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-CLL – Follicular dendritic cell tumors	Mediastinal large B-cell lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, DLBCL, Reed–Sternberg cells	Follicular dendritic cells, EBV transformed lymphoblasts, monocytes, platelets
Positive control: appendix/tonsil		

Diagnostic Approach CD23, also known as low affinity IgE receptor, is a type II transmembrane glycoprotein involved in the regulation of IgE response. CD23 has two forms, a and b, with different amino acid sequences. Type a is involved in the differentiation of B-cells and expressed on mature B-cells, and type b plays a role in the regulation of allergic reactions and is expressed on B- and T-cells, activated macrophages, and eosinophils. CD23 is also a good marker for follicular dendritic cells. It is important to mention that the expression of CD23 is activated by EBV infection. CD23 is an important marker used to discriminate B-CLL (strongly positive) from other lymphoma types with similar morphology (Fig. 16.6), while it is negative in t(14;19) associated B-CLL. CD23 also labels mediastinal large B-cell lymphoma, lymphoplasmacytic lymphoma, and a small subset of multiple myeloma in addition to Reed–Sternberg cells in Hodgkin lymphoma. It is also an important marker for follicular dendritic cell tumors (see Chap. 19).

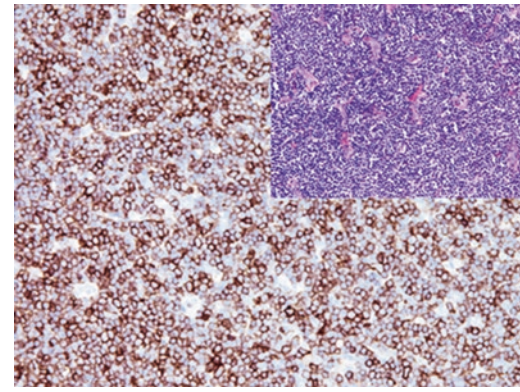


Fig. 16.6 B-CLL with strong membranous CD23 expression on neoplastic cells

16.2.8 CD79a

CD79a		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-cell leukemia/ lymphomas	Acute promyelocytic leukemia (FAB-M3), multiple myeloma, T-ALL	B-cells, a small population of CD3+ T-cells, a subset of megakaryocytes and endothelial cells
Positive control: appendix/tonsil		

Diagnostic Approach CD79a is a disulfide-linked heterodimer associated with the membrane-bound immunoglobulin receptor complex. CD79a appears in the pre-B-lymphocyte stage before the IgH chain rearrangement and persists until the plasma cell development, rendering the majority of normal and neoplastic plasma cells positive for CD79a. CD79a exhibits a membranous expression pattern, but plasma cells may also show a cytoplasmic stain pattern. The expression of CD79a is independent of the expression of CD20 and remains positive after the anti-CD20 immunotherapy.

Diagnostic Pitfalls CD79a is less reliable than CD20 for the diagnosis of B-cell lymphoma, as it is positive in a small fraction of T-ALL, AML (FAB-M3), and the majority of plasma cell neoplasms.

16.2.9 PAX-5

PAX-5 (B-cell-specific activator protein, BSAP)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-cell lymphoma/leukemia – Reed–Sternberg cells of classic Hodgkin lymphoma	AML with t(8;21), Merkel cell carcinoma, small-cell carcinoma, alveolar rhabdomyosarcoma, Wilms tumor, glioblastoma and neuroblastoma, mesonephric and Müllerian tumors	Pre-B- to mature B-cells
Positive control: appendix/tonsil		

Diagnostic Approach PAX-5 (also known as B-cell activator protein, BSAP) is a PAX (paired box) family member that includes nine transcription factors involved in tissue and organ differentiation. PAX-5 is a B-cell-specific transcription factor encoded by the gene located at chromosome 9p13 and expressed in the early pro-B, pre-B, and naïve stages of B-cell development until the mature B-cells [13]. Plasma cells, T-lymphocytes, and macrophages constantly lack PAX-5 expression. PAX-5 is one of the best markers of B-cell lymphomas (Fig. 16.7). It is also expressed in the L&H cells of nodular lymphocyte predominance Hodgkin lymphoma and in the majority of Hodgkin cells in classic Hodgkin lymphoma.

The PAX-5 gene is a partner of the t(9;14)(p13;q32) translocation associated with the plasmacytoid subtype of small lymphocytic lymphoma.

Diagnostic Pitfalls PAX-5 can be positive in some tumors resembling lymphoma, such as Merkel cell carcinoma, small-cell carcinoma,

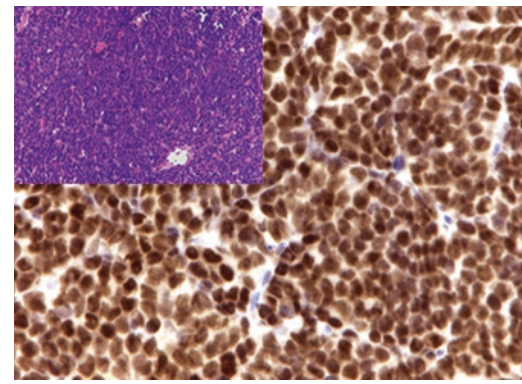


Fig. 16.7 Strong nuclear PAX-5 expression in the cells of diffuse large B-cell lymphoma

atypical carcinoid, and also rarely in acute lymphoblastic lymphoma of T-cell origin [14, 15]. PAX-5 maybe also expressed in acute myeloid leukemia, mainly the type associated with the t(8;21)(q22;q22) translocation. PAX-5 positivity is reported in rare cases of breast, endometrial, and transitional carcinomas in addition to alveolar rhabdomyosarcoma, but it is constantly negative in embryonal-type rhabdomyosarcoma [16, 17].

16.2.10 Cyclin D1

Cyclin D1 (bcl-1)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mantle cell lymphoma	Inflammatory pseudotumor (myofibroblastic tumor), hairy cell leukemia, multiple myeloma, parathyroid adenoma/carcinoma, pulmonary adenocarcinoma, breast and prostate carcinoma, transitional cell carcinoma, solid pseudopapillary neoplasm of the pancreas	Cells in the G_1 phase of the cell cycle, histiocytes, endothelial cells
Positive control: mantle cell lymphoma		

Diagnostic Approach Cyclin D1 (also known as bcl-1) is a cell cycle protein encoded on chromosome 11q13 and involved in the regulation of cyclin-dependent kinases of the first gap phase (G_1) of the cell cycle. The expression of cyclin D1 is not restricted to lymphoid neoplasms and is found in a number of nonlymphoid epithelial and mesenchymal tumors. The cyclin D1 overexpression—caused by the t(11;14) translocation associated with mantle cell lymphoma—makes it a characteristic marker for this lymphoma type (Fig. 16.8). In routine immunohistochemistry, cyclin D1 is usually used in combination with CD5, Sox-11, and other B-cell markers [2, 18].

A subset of multiple myeloma that also harbors the t(11;14) translocation is positive for cyclin D1; this myeloma type is usually associated with a favorable prognosis.

Diagnostic Pitfalls Other lymphoma types exhibiting similar morphology, such as hairy cell leukemia and B-CLL, may also be positive for cyclin D1; however, the staining intensity is much less than mantle cell lymphoma [19]. A small subset of mantle cell lymphoma lacks the expression of cyclin D1; this subset is usually positive for Sox-11, which is to consider in the differential diagnosis. Cyclin D1 is also expressed in some carcinoma types, such as adenocarcinomas of the breast and prostate, besides some mesenchymal tumors, such as inflammatory myofibroblastic tumor.

16.2.11 Sox-11

Sox-11		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mantle cell lymphoma	Hairy cell leukemia, Burkitt lymphoma, T- and B-ALL, prolymphocytic leukemia, ovarian carcinoma, solid pseudopapillary neoplasm of the pancreas	Immature neurons
Positive control: mantle cell lymphoma		

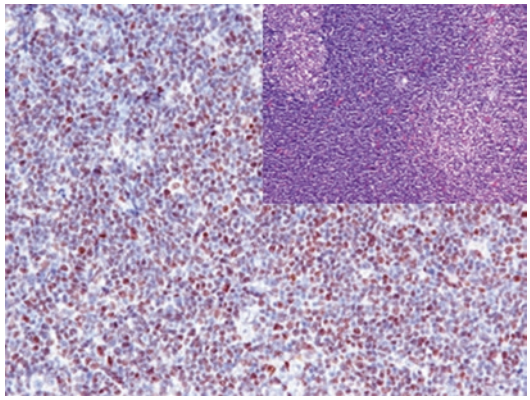


Fig. 16.8 Mantle cell lymphoma showing strong nuclear cyclin D1 expression in neoplastic lymphocytes

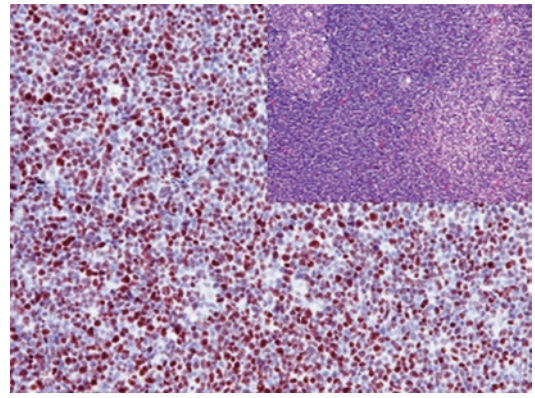


Fig. 16.9 Mantle cell lymphoma with strong nuclear Sox-11 expression in neoplastic lymphocytes

Diagnostic Approach Sox-11 is a member of the Sox family of transcription factors (**s**ex-determining region Y-b**ox 11**), a transcription factor involved in embryogenesis and development of the central nervous system. SOX-11 also takes part in the regulation of PAX-5 transcription.

Sox-11 strongly stains both cyclin D1 positive and negative mantle cell lymphomas (Fig. 16.9) in addition to other lymphoma types, including hairy cell leukemia, Burkitt lymphoma, and B- and T-ALL [20–22]. Sox-11 is constantly negative in B-CLL, follicular lymphoma splenic marginal zone lymphoma, diffuse large B-cell lymphoma, and multiple myeloma.

In epithelial tumors, the expression of Sox-11 is found in pulmonary neuroendocrine carcinomas (Fig. 16.10) and in a subset of ovarian carcinomas; the latter later are generally associated with a good prognosis [23].

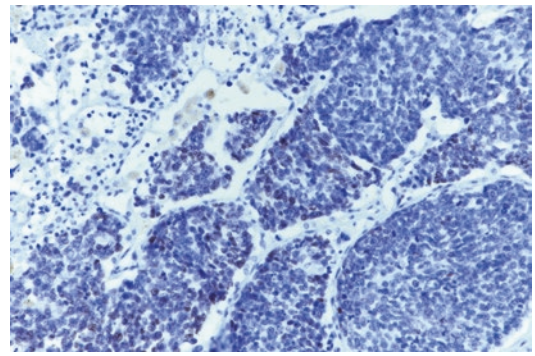


Fig. 16.10 Pulmonary neuroendocrine carcinoma with focal nuclear Sox-11 expression

16.2.12 bcl-2

bcl-2		
Expression pattern: cytoplasmic (mitochondrial membrane)		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Follicular lymphoma – Lymphoid tumors: the majority of B-cell lymphomas, a subset of T-cell lymphoma	– Epithelial tumors: carcinoma of the breast, nasopharyngeal carcinoma, basal cell carcinoma – Neuroendocrine tumors: adrenocortical tumors, thyroid carcinoma – Mesenchymal tumors: solitary fibrous tumor, synovial sarcoma, hemangiosarcoma, neurofibroma, schwannoma, dermatofibrosarcoma protuberans, spindle cell lipoma, rhabdomyosarcoma	Small B-lymphocytes in primary follicles and in the mantle and marginal zones, a subset of T-lymphocytes, medullary cells in the thymus and adrenal cortex, basal keratinocytes of the epidermis
Positive control: appendix/tonsil		

Diagnostic Approach bcl-2 (**B-c**ell **l**ymphoma **2** protein) is a family of regulator proteins involved in the regulation of programmed cell death divided into two main groups: the bcl-2 group as an antiapoptotic and proapoptotic group (effectors and activators). The bcl-2 proteins are encoded by the bcl-2 gene on chromosome 18q21. The bcl-2 gene is transcribed into three mRNA variants, translated into two homologous integral cell and mitochondrial membrane proteins.

The t(14;18)(q32;q21) translocation characteristic for 90% follicular lymphoma juxtaposes the bcl-2 gene to the Ig heavy-chain gene resulting the deregulation of the bcl-2 gene and the overexpression of the bcl-2 protein giving a survival advantage for lymphoma cells. One of the main diagnostic benefits of bcl-2 is to distinguish between reactive lymph nodes with follicular hyperplasia exhibiting bcl-2 negative germinal centers and associated with high proliferative activity and grade 1 follicular lymphoma with bcl-2 positive neoplastic follicular B-cells and usually with low proliferative activity (Fig. 16.11) [2]. Generally, all grade 1 follicular lymphomas are positive for bcl-2, and about 85% of grade 2 and up to 75% of grade 3 are positive for bcl-2. To consider are the bcl-2 negative follicular lymphoma types such as pediatric type follicular lymphoma.

The expression of bcl-2 is not specific for follicular lymphoma but found in the majority of B-cell lymphomas and in a subset of T-cell lymphomas.

The expression of bcl-2 is also found in a large number of epithelial, neuroendocrine, and mesenchymal tumors [2].

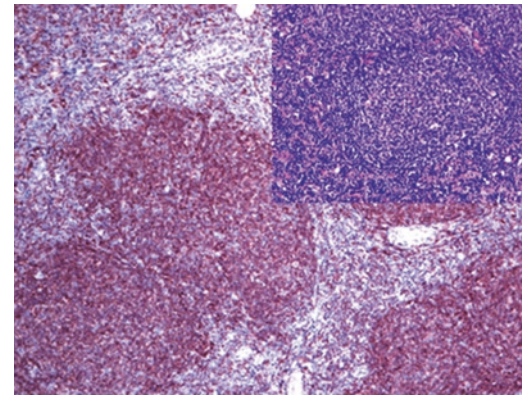


Fig. 16.11 Follicular lymphoma with strong diffuse bcl-2 expression in neoplastic follicles

Diagnostic Pitfalls Ten to 15% of grade 1–2 follicular lymphomas lack the expression of bcl-2 detected by immunohistochemistry. This phenomenon is also found in up to 70% of grade 3a and 3b follicular lymphomas. It can be either due to mutations within the bcl-2 gene producing mutated bcl-2 proteins not recognized by the standard antibodies or due to other equivalent mutations causing the upregulation of the bcl-2 expression.

In lymph nodes, the expression of bcl-2 is found in the B-cells of primary follicles, which may be misdiagnosed as the manifestation of grade 1 follicular lymphoma. Finally, different antibody clones to the bcl-2 molecules may show different stain results. In doubtful cases, it is recommended to repeat the immunohistochemical stain using another antibody clone. Finally, the molecular detection of the t(14;18) translocation or other equivalent genetic anomalies is also helpful for further characterization of the lymphoma types.

16.2.13 bcl-6

bcl-6		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Follicular lymphoma (inter- and intrafollicular cells) – Anaplastic CD30+ large-cell lymphoma (ALK+/−)	Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, L&H cells in nodular lymphocyte predominance Hodgkin lymphoma, angioimmunoblastic lymphoma, T-ALL	Germinal centers of lymph nodes, a subset of intrafollicular CD4+ T-lymphocytes (TFH)
Positive control: appendix/tonsil		

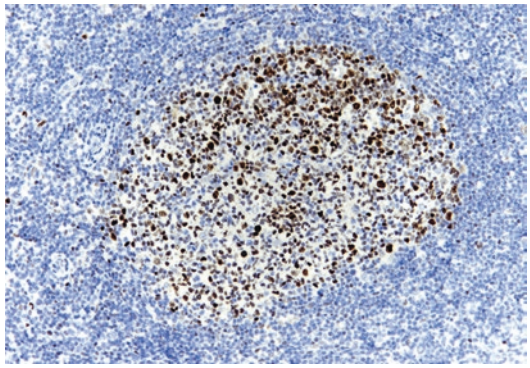


Fig. 16.12 bcl-6 expression in intrafollicular neoplastic cells of follicular lymphoma

Diagnostic Approach bcl-6 (**B-c**ell **l**ymphoma **6** protein) is a sequence-specific transcriptional repressor protein involved in the regulation of B-cell differentiation. Bcl-6 is normally expressed in nonneoplastic germinal center B-lymphocytes with a high proliferation rate and active somatic mutations. Furthermore, bcl-6 is a master transcription factor essential for the transformation of naïve CD4+ T helper cells into follicular helper cells (TFH cells).

bcl-6 is a marker for lymphomas of germinal center origin such as follicular lymphoma (intra- and interfollicular cells) (Fig. 16.12), Burkett's lymphoma, mediastinal large B-cell lymphoma, majority of Hodgkin cells, and nodular lymphocyte predominance Hodgkin lymphoma [2]. The bcl-6 gene is found to be translocated or hypermutated in ~40% of diffuse large B-cell lymphoma and ~15% of follicular lymphoma, causing the overexpression of the bcl-6 protein [24]. It is to consider that the immunohistochemical expression of bcl-6 is not a surrogate marker for mutations or rearrangements within the bcl-6 gene.

The expression of bcl-6 is also characteristic for some NK-cell/T-cell lymphoma types, such as angioimmunoblastic lymphoma and T-ALL. Mantle cell lymphoma, marginal zone lymphoma, and ALL are constantly bcl-6 negative.

16.2.14 bcl-10

bcl-10 (also known as **B-c**ell **l**ymphoma/leukemia **10**) is an apoptotic regulatory nuclear protein encoded on chromosome 1, involved in antigen-receptor-mediated lymphocyte activation through the NF-Kappa B pathway. Bcl-10 is expressed in the germinal center and marginal zone B-lymphocytes and is also weakly expressed in mantle zone B-lymphocytes beside a subset of T-lymphocytes. Bcl-10 labels different B-cell lymphoma types, including follicular lymphoma and extranodal marginal zone lymphoma of MALT type and weakly also mantle cell lymphoma. MALT lymphomas bearing the t(1;14)(p22;q32) translocation show a strong bcl-10 expression due to truncation of the bcl-10 gene and loss of the apoptotic activity of the encoded protein, while MALT lymphomas lacking this translocation and associated with other translocations show less expression intensity [25].

In the exocrine pancreas, bcl-10 is a marker for acinar cell differentiation and acinic cell carcinomas.

16.2.15 CD11c

CD11c		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hairy cell leukemia	AML (M4 and M5), follicular lymphoma, Langerhans cell histiocytosis, lymphoplasmacytic lymphoma, B-CLL, splenic lymphoma, NK-cell lymphoma	Myeloid hematopoietic cells, granulocytes, macrophages, NK-cells, dendritic cells, a subset of activated T-lymphocytes, histiocytes
Positive control: appendix/tonsil		

Diagnostic Approach CD11c: (also known as integrin alpha X, CR4, LeuM5) is an integrin glycoprotein composed of alpha and beta chains involved in the adhesion and chemotaxis of monocytes, primarily expressed on myeloid hematopoietic cells. CD11c is a marker for different lymphoid and myeloid neoplasms. It is strongly expressed in hairy cell leukemia and natural killer cell lymphoma (Fig. 16.13). CD11c is also found in about 50% of AML (M4 and M5) and in some cases of follicular lymphoma, Langerhans cell histiocytosis, lymphoplasmacytic lymphoma, splenic lymphoma with villous lymphocytes, and B-CLL. The expression of CD11c on cells of B-CLL is usually associated with a good prognosis.

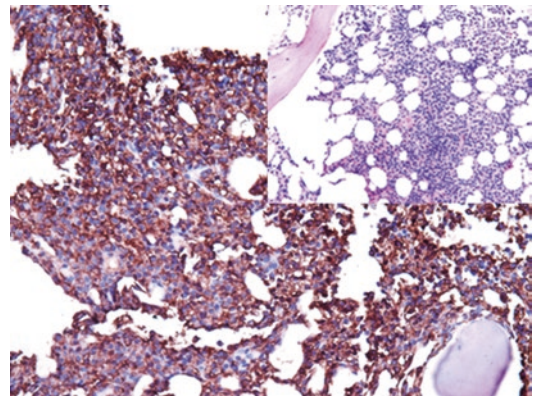


Fig. 16.13 Bone marrow infiltrated by hairy cell leukemia, neoplastic lymphocytes with strong CD11c expression

16.2.16 Tartrate-Resistant Acid Phosphatase (TRAP)

Tartrate-resistant acid phosphatase (TRAP)		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hairy cell leukemia – Osteoclastoma (giant cell tumor)	Mantle cell lymphoma, mediastinal B-cell lymphoma, splenic marginal cell lymphoma	Osteoclasts, macrophages, lymphocytes of the marginal zone, neurons, decidual cells, prostatic glands, red blood cells
Positive control: osteoclasts, hairy cell leukemia		

Diagnostic Approach Tartrate-resistant acid phosphatase (TRAP; also called acid phosphatase 5) is a glycosylated monomeric iron-binding metalloprotein enzyme with high activity toward phosphoproteins, ATP, and 4-nitrophenyl phosphate, normally found in different tissue types, and is highly expressed in osteoclasts and macrophages.

TRAP is a specific marker for hairy cell leukemia but should be combined with other markers such as CD11c and DBA 44 (Fig. 16.14).

Diagnostic Pitfalls Another lymphoma type, such as marginal zone B-cell lymphoma, may reveal weak TRAP positivity. TRAP is also expressed in bone marrow macrophages [26].

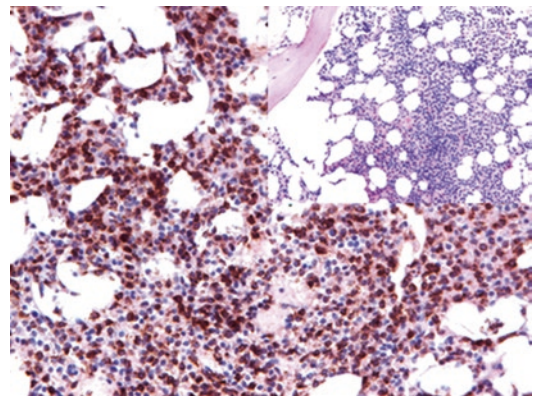


Fig. 16.14 Bone marrow trephine biopsy infiltrated by cells of hairy cell leukemia exhibiting strong cytoplasmic TRAP expression in neoplastic lymphocytes

16.2.17 Immunoglobulin Superfamily Receptor Translocation-1

IRTA-1 (CD307d, also called FCRL4) is the fourth member of the immune receptor translocation-associated protein family (IRTA-1-5) clustered as CD307. IRTA-1 is a cell surface receptor involved in the lymphogenesis of B-lymphocytes in addition to intercellular communication. IRTA-1 is positive in the B-cells of the marginal zone. IRTA-2 is also positive in the B-cells of the marginal zone and centrocytes. IRTA-3 is positive in the germinal centers. IRTA-4 and IRTA-5 are expressed in the mantle zone.

IRTA-1 is a helpful marker to discriminate between marginal zone lymphoma and other lymphoma types as it is expressed in more than 90% of extranodal marginal zone lymphoma, including MALT lymphoma, and in about 75% of nodal marginal zone lymphoma but negative in splenic marginal zone lymphoma (Fig. 16.15). B-CLL and mantle cell lymphoma may be also positive for IRTA-1 but can be distinguished from marginal cell lymphoma by other specific markers for both lymphoma types, including LEF-1 and cyclin D1 and SOX-11. Other lymphoma types, including follicular lymphoma, Burkitt lymphoma, hairy cell leukemia, and plasma cell neoplasms, also lack the expression of IRTA-1 [27, 28]. IRTA-1 cannot distinguish between reactive and neoplastic marginal zone lymphocytes.

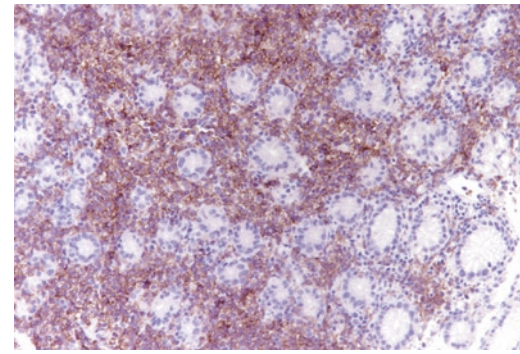


Fig. 16.15 IRTA-1 labels neoplastic lymphocytes of extranodal marginal zone lymphoma (MALT lymphoma)

16.2.18 LIM Only Transcription Factor 2

LIM only transcription factor 2 (LMO2)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Follicular lymphoma – Mediastinal large B-cell lymphoma – Burkitt lymphoma	Subset of B- and T-ALL and AML, endothelial tumors. GIST, myoepithelial tumors, juvenile xanthogranuloma	Germinal centers of lymph nodes, hematopoietic precursors, endothelium, breast myoepithelial cells, basal cells of prostatic gland, endometrial glands in the secretory phase
Positive control: tonsil/lymph node		

LMO2 (also known as TTG2 or RBTN2) is a transcription factor regulating the yolk sac angiogenesis and erythropoiesis, normally expressed in erythroid and myeloid precursors as well as megakaryocytes and endothelial cells. The LMO2 protein is expressed in pro- and pre-B-lymphocytes in addition to germinal center B-lymphocytes. LMO2 is a marker for several lymphoma types derived from germinal center cells. It is expressed in up to 70% of all grades of follicular lymphoma, mediastinal large B-cell lymphoma, Burkitt lymphoma and diffuse large B-cell lymphoma, and B- and T-ALL. CLL, mantle cell lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, and peripheral T-cell lymphomas usually lack the expression of LMO2. LMO2 is expressed in lymphocyte-predominant Hodgkin lymphoma but not in classical Hodgkin lymphoma. Furthermore, LMO2 labels the myeloid blasts of acute myeloid leukemia [29, 30]. In addition to lymphoid and hematopoietic neoplasms, LMO2 labels normal blood and lymph vessel endothelium and the majority of benign and malignant endothelial tumors [31].

16.2.19 Human Germinal Center Associated Lymphoma

HGAL, also known as germinal center B-cell expressed transcript 2 (GCET-2), is exclusively expressed in the cytoplasm and on the membrane of germinal center B-lymphocytes and especially accentuated in the proliferating cells within the dark zone of germinal centers. HGAL is involved in the regulation of lymphocyte motility. Lymphocytes within the mantle and marginal zones and interfollicular and paracortical regions lack the expression of HGAL. HGAL is a marker for B-cell lymphomas derived from germinal center lymphocytes and expressed in 100% of Burkitt lymphoma, more than 90% of follicular lymphomas and mediastinal lymphoma, and about 70% of diffuse large B-cell lymphoma. The expression of HGLA is reported in less than 5% of marginal zone lymphoma, whereas mantle cell lymphoma and B-CLL are completely negative for HGAL [32, 33].

16.2.20 Lymphoid Enhancer Binding Factor

Lymphoid enhancer binding factor (LEF-1)		
Expression pattern: nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– B-CLL – Sinonasal glomangiopericytoma – Basal cell adenoma/ adenocarcinoma of salivary gland	Diffuse large B-cell lymphoma, ALK-negative anaplastic large-cell lymphoma, solid pseudopapillary neoplasm of the pancreas, pancreatoblastoma, invasive micropapillary breast carcinoma, papillary thyroid carcinoma (cribriform morular variant), cutaneous basal cell carcinoma	T-lymphocytes, hair follicles
Positive control: tonsil		

LEF-1 is a nuclear protein and a member of the T-cell-specific factor family that binds to the T-cell receptor playing a role in the regulation of cell proliferation and lymphopoieses and differentiation of respiratory submucosal glands. LEF-1 is normally expressed in pre-B- and T-lymphocytes but not in mature B-cells. LEF-1 labels different types of T-cell lymphomas. In B-cell lymphomas, LEF-1 labels the neoplastic small lymphocytes of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (Fig. 16.16), whereas other low-grade B-cell lymphomas, including mantle cell lymphoma, marginal zone lymphoma, and follicular lymphoma, lack the expression of LEF-1 [34]. The LEF-1 expression is found in

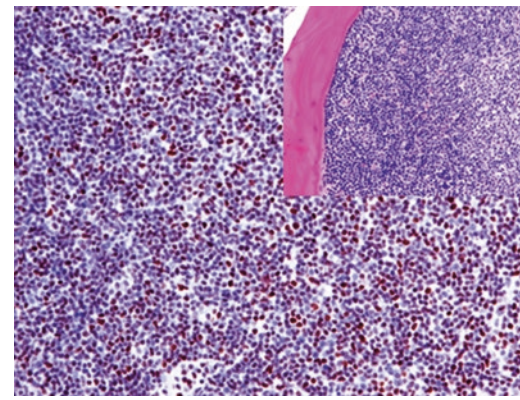


Fig. 16.16 Bone marrow, nuclear LEF-1 expression in CLL neoplastic cells

about one-third of diffuse large B-cell lymphoma and specific for ALK-negative anaplastic large-cell lymphoma with the DUSP22 rearrangement. LEF-1 is not a specific lymphoma marker as it is also expressed in different carcinoma types, such as colorectal adenocarcinoma [35]. Furthermore, the nuclear expression of LEF-1 is also characteristic for sinonasal glomangiopericytoma (see Chap. 3, Fig. 3.10) and solid pseudopapillary neoplasm of the pancreas in addition to invasive micropapillary carcinoma of the breast [36, 37].

In salivary gland tumors, LEF-1 is positive in most basal cell adenomas of the salivary glands, whereas adenoid cystic carcinoma and acinic cell carcinoma usually lack the expression of LEF-1.

16.2.21 Annexin A1

Annexin A1 (Lipocortin) is a member of calcium-dependent phospholipid binding proteins located on the cell membrane and in the cytoplasm, and involved in the regulation of inflammatory reaction and phagocytosis. Annexin A1 is highly upregulated in hairy cell leukemia and used as a specific marker for this lymphoma type. In nonlymphoid neoplasia, annexin A1 is highly expressed in cholangiocarcinoma. In renal tumors, the expression of Annexin A1 is an indicator of the response to TKI.

16.2.22 c-myc

c-myc is a member of the myc family composed of three related transcription factors c-myc, l-myc, and n-myc encoded on chromosomes 8, 1, and 2, respectively. The product of the c-myc gene is a nuclear phosphoprotein and a transcription factor involved in the regulation of different stages of the cell cycle, including growth, proliferation, differentiation, and apoptosis. The c-myc gene is one of the most common mutated genes in human malignancies. In routine immunohistochemistry, the overexpression of c-myc in more than 40% of tumor cells correlates with the presence of an activating mutation. In ~90% of Burkitt lymphoma, the expression of c-myc is activated by one of the specific translocations: t(8;14)(q24;q32) or t(8;22)(q24;q11) (Fig. 16.17). Eight to 14% of diffuse large B-cell lymphoma is also associated with a c-myc activating translocation. High-grade B-cell lymphomas associated with c-myc, bcl-2, and/or bcl-6 gene rearrangements, so-called double or triple hit lymphomas, usually have a poor prognosis.

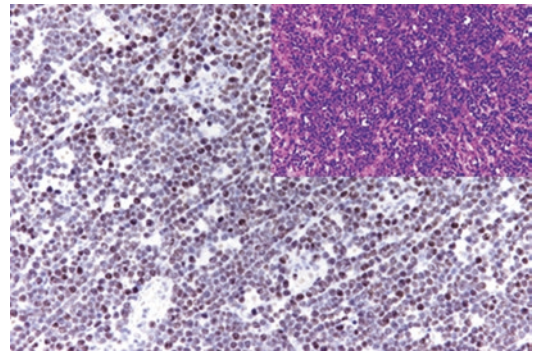


Fig. 16.17 Burkitt lymphoma with nuclear c-myc expression in neoplastic lymphocytes

16.2.23 FOXP1

FOXP1 (Forkhead box protein 1) is a member of the forkhead box family of transcription factors. FOXP1 is expressed in nonneoplastic activated B-lymphocytes and overexpressed in the nongerminal center (ABC) type diffuse large B-cell lymphomas (DLBCL).

Evolution of immunoprofile of nonneoplastic B-lymphocytes	
Cell type	Immunoprofile
Pluripotent stem cell	CD117, CDw123, CD243, CDw338, HLA-DR (CD74)
Lymphoid stem cell	CD10, CD34, CD38, CD117, CD124, CD127, CD228, TdT, HLA-DR (CD74)
Pro-B-cell	CD19, cCD22, CD24, CD34, CD38, CD72, cCD79a, CD79b, CD124, CD127, PAX-5, TdT, HLA-DR
Early B-cell	CD10, CD19, CD20, CD21, CD22, CD24, CD34, CD72, cCD79a, CD79b, CD124, PAX-5, TdT, HLA-DR
Pre-B-cell	CD9, CD19, CD20, CD21, CD22, CD24, CD38, CD40, CD72, CD74, cCD79, CD124, PAX-5, TdT, HLA-DR
Naïve B-cell	CD19, CD20, CD21, CD22, CD24, CD23, CD35, CD40, sCD79, CD124, PAX-5, s-IgM, s-IgD
Follicle center B-cell	CD10, CD19, CD20, CD21, CD22, CD38, CD79a, PAX-5, HLA-DR (CD74), sIgM, sIgG, s-IgA, bcl-6
Immunoblast	CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD72, CD 74, CD79a, CD139, CD275, CD316, CD317, HLA-DR, s-IgM, s-IgG, s-IgA, bcl-6
Marginal zone B-cell	CD1c, CD19, CD20, CD21, CD27
Lymphoplasmacytoid cell	CD19, CD20, CD38, CD79a, CD79b, CD275, CD316, CD317
Plasmablast	CD27, CD38
Plasma cell	CD38, CD79a, CD126, CD138, CD269, CD275, CD316, CD317, CD317, cIg, IRF4/MUM-1

Immunoprofile of B-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
B-lymphoblastic leukemia/lymphoma	**CD19**, CD79a, **TdT**, **PAX-5**, HLA-DR (CD74), ERG, cIgM Proliferation index (Ki-67): 50–90%	**CD10**[a], CD22, CD34, CD24[a], CD45, CD99, **CD34**[j], FLI-1, LMO2	CD20, CD13	
	Flow cytometry			
	CD19, cCD22, CD38, cCD79a, HLA-DR	CD9[i], CD10[a], sCD22, CD24[a], CD34[j], TdT	CD13, CD20, CD45	MPO
Monoclonal B-cell lymphocytosis	CLL type: see B-CLL immunoprofile (B-cell accounts in peripheral blood <5 × 10^9/L with B-CLL phenotype with no signs of lymph node involvement) Non-CLL phenotype: CD5 and CD23 (−/+)			
	Flow cytometry			
	– CD5 and CD23 on >20% of CD19+ lymphocytes – Kappa-lambda ratio of >25% of mature B-lymphocytes			
B-cell chronic lymphocytic lymphoma (B-CLL)/small lymphocytic lymphoma (B-SLL)	**CD5**[h], CD19, **CD20**, CD22, **CD23**[h], CD74, CD79a, **CD160**, **CD200**, **LEF-1**, ROR-1, PAX-5, p27, bcl-2, sIgM Proliferation index (Ki-67): ~5%	CD22, CD43, MUM-1, sIgD	CD11c, CD38[b] ZAP-70[b], DBA44	CD10, SOX-11, bcl-6
	Flow cytometry			
	CD5[h], CD11cweak, CD19, CD23, CD25, CD43, CD200, sIgweak, ROR-1 κ-/λ-light-chain restriction	CD11c, CD20weak, CD22weak, CD81	CD38, CD49d[b], CD79a	CD3, CD10, CD25, CD79b, CD81, CD103, FMC7

B-cell prolymphocytic leukemia	**CD19**, **CD20**, CD22, CD25, CD27, CD74, CD79a, PAX-5, bcl-2	sIgM, sIgD	CD23	CD5, CD10, CD23, CD43, CD138, cyclin D1
	Flow cytometry			
	CD19, CD20, CD22, CD25, CD79a, FMC7		CD5, CD23, CD38	CD10
Lymphoplasmacytic lymphoma	Small B-lymphocytes: **CD19**, **CD20**, CD22, CD43, CD74, CD79a, **CD200**, PAX-5, IgM Plasmacytoid cells and plasma cells: CD19, CD38, CD79a, CD138, MUM-1 Proliferation index (Ki-67): ~5–10%	Bcl-2, MYD88	CD5, CD23,	Lymphocytes: CD3, CD10, CD103, cyclin D1 Plasma cells: CD56
	Flow cytometry			
	In B-cells: CD19, CD20, CD200 In plasma cells: CD19, CD38, CD138, cIgM	CD11c, CD22, CD43, CD25, FMC7	CD5, CD23,	CD3, CD10, CD56, CD103
Mantle cell lymphoma/in situ mantle cell neoplasm	**CD5**, CD19, CD20, CD22, CD37, CD43, CD74, CD79a, sIgM, sIgD, **cyclin D1**, **SOX-11**, PAX-5, FMC-7 Proliferation index (Ki-67): 5–50%	Bcl-2	MUM-1	CD10, CD11c, CD23, CD138, bcl-6
	Flow cytometry			
	CD5, CD19, CD22, CD79a, CD79b, FCM-7, bcl-2	CD11c, CD43, SOX-11		CD3, CD10, CD11c, CD23, CD103, CD138, CD200
Follicular lymphoma/in situ follicular neoplasia/ duodenal type follicular lymphoma	CD19, **CD20**, CD22, CD74, CD79a, PAX-5, **HGAL**, sIg, bcl-2 Nodular meshwork of follicular dendritic cells positive for CD21 and CD23 (see Fig. 19.3) **Proliferation index (Ki-67) in bcl-2 positive neoplastic follicles (low grade): <15%** **Proliferation index (Ki-67) in bcl-2 negative reactive follicles: >60%**	CD10, **bcl-6**, **LMO2**, **HGAL** κ/λ light-chain restriction		CD5, CD23, CD43, SOX-11, cyclin D1
	Flow cytometry			
	CD19, CD20, CD22, CD79a, CD10, sIg			CD3, CD5, CD11c, CD43, CD103, CD200
Pediatric type follicular lymphoma	CD19, CD20, CD22, CD74, CD79a, PAX-5, CD10, **HGAL**, **LMO2**, sIg Proliferation index (Ki-67): >30%	**Bcl-6**, CD43		MUM-1, **bcl-2**[c]

Primary cutaneous follicle center lymphoma	CD20, PAX-5, **bcl-6**		CD10, CD30, CD23, bcl-2	CD3, CD5, CD43, MUM-1, bcl-2, cyclin D1
Nodal marginal zone B-cell lymphoma	CD19, **CD20**, CD21, CD22, CD35, CD74, CD79a, **IRTA-1**, MNDA, PAX-5, sIgM	sIgA, sIgG, CD43, CD11c, bcl-2	CD38, MUM-1, TRAP	CD3, CD5, CD10, CD21, CD 23, CD138, bcl-6, SOX-11, sIgD, cyclin D1
	Flow cytometry			
	CD1d, CD19, CD22, CD74, CD79a, sIg	CD43		CD5, CD10, CD21, CD23, Cd43, CD103, CD200, bcl-6
Extranodal marginal zone B-cell lymphoma of MALT type	CD19, **CD20**, CD21, CD22, CD35, CD74, CD79a, PAX-5, sIgM, bcl-2, **IRTA-1**	CD11c, CD43, MUM-1, bcl-10, sIgD, sIgA, sIgG	CD43	CD3, CD5, CD10, CD23, SOX-11, cyclin D1, bcl-6
	Flow cytometry			
	CD19, CD21, CD35, FMC7, IgM	CD11c	CD23, IgA, IgG	CD3, CD5, CD10, CD25, CD103, IgD
Splenic marginal zone B-cell lymphoma	CD19, **CD20**, CD21, CD22, CD35, CD74, CD79a, PAX-5, bcl-2, sIgM, sIgD Proliferation index (Ki-67): <5%	sIgA, CD11c, DBA44	CD23, CD25, CD43, CD103, sIgG	CD3, CD5, CD10, CD43, CD103, bcl-6, **cyclin D1**, annexin A1, IRTA-1
	Flow cytometry			
	CD19, CD11c, CD20, CD22, CD200, FCM-7, IgM, IgD		CD11c, CD23, CD25, CD103	CD3, CD5, CD10, CD38, CD43, CD103, CD123
Splenic diffuse red pulp small B-cell lymphoma	CD20, CD19, CD79a, DBA44, PAX-5	Cyclin D3	CD103	CD3, CD5, CD10, CD21, CD23, CD25, CD38, CD43, cyclin D1, annexin A1
	Flow cytometry			
	CD11c, CD1d, CD19, CD20, CD22, CD25, CD103, CD123, CD180, CD200, FCM-7, sIg		CD103	CD3, CD5, CD10, CD21, CD23, CD25
Hairy cell leukemia	**CD11c**, CD19, **CD20**, CD22, **CD25**, CD74, CD79a, **CD103**, CD123, **annexin A1**, **TRAP**, **DBA.44 (CD76)**, **BRAF-v600E**, PAX-5, bcl-2, sIgM Proliferation index (Ki-67): <5%	CD23, CD68 (cytoplasmic dots), PCA-1, HC1, HC2, cyclin D1	CD5	**CD10**, **CD23**, **CD43**, bcl-6
	Flow cytometry			
	CD11c, CD1d, CD19, CD20, CD22, CD25, CD103, CD123, CD200, FCM-7, sIg		CD23weak	CD3, CD5, CD10, CD27, CD43, CD180
Diffuse large B-cell lymphoma (DLBCL) – Germinal center-cell type (GCB)[d] – Activated B-cell type (ABC)[d]	CD19, **CD20**, CD22, CD74, CD79a, CD45, PAX-5, bcl-2 Proliferation index (Ki-67): >40%	Bcl-6, FOXP1[l]	CD5, CD10, CD30, Fascin, p63, MUM-1[e]. Islet-1[k]	CD3, CD15, CD200

T-cell/histiocyte-rich variant of diffuse large B-cell lymphoma	Neoplastic cells: CD19, **CD20**, CD22, CD74, CD79a, CD45, PAX-5, bcl-6, BOB 1, OCT-2 Nonneoplastic microenvironment cells (>80% of cell population): lymphocytes positive for CD3, CD8, and cytotoxic molecules and histiocytes positive for CD68 and CD163		CD30, bcl-2, EMA	CD3, CD5, CD10, CD15, bcl-2, PU 1
Mediastinal (thymic) large B-cell lymphoma	CD19, **CD20**, CD45, CD74, CD79a, CD200, PAX-5, Oct-2, STAT-6	**CD23**, MUM-1, **CD30**, **HGAL**, **LMO2**, bcl-2, bcl-6, PD-L1, p16, p63	CD10	CD3, CD5, CD15, CD21
	Flow cytometry			
	CD19, CD22, CD79a, CD200	CD23, CD30, HLA-DR		CD3, CD5, sIg
ALK-positive large B-cell lymphoma	**ALK**, EMA, CD38, CD138, VS38c, MUM-1 κ-/λ-light-chain restriction Proliferation index (Ki-67): >90%		CD4, CD10, CD38, CD45, CD43, CD79a, EMA, Pan-CK	CD3, CD19, CD20, CD22, CD30, PAX-5
Large B-cell lymphoma with IRF-4 rearrangement	CD19, CD20, CD22, **MUM-1**, **bcl-6**	CD10, bcl-2	CD5	
Fibrin-associated large B-cell lymphoma	CD19, CD22, CD79a, PAX-5 Proliferation index (Ki-67): >90%	CD30, bcl-2, bcl-6		CD10
Primary cutaneous diffuse large B-cell lymphoma, leg type	CD19, CD20, CD79a, PAX-5, bcl-2, MUM-1 Proliferation index (Ki-67): >40%	Bcl-6, P63		CD10
KSHV-/HHV8-positive diffuse large B-cell lymphoma	**HHV-8**, CD19	CD30	CD20, CD38	CD79a, CD138
Intravascular large B-cell lymphoma	CD19, CD20, CD79a, PAX-5, MUM-1	Prostatic acid phosphatase, Bcl-2	CD5, CD10, bcl-6	CD3, CD23, cyclin D1
Primary effusion lymphoma	CD45, CD30, **CD79a**, CD38, CD138, VS38c, **HHV-8**, MUM-1	**PAX-5**, EMA, EBV	CD4, CD7	CD10, CD19, CD20, CD43, PAX-5, bcl-6
	Flow cytometry			
	CD138, CD71		CD20, CD23	CD10, CD19, CD22, FMC7
Burkitt lymphoma	**CD10**, CD19, **CD20**, CD22, CD38, CD74, CD79a, PAX-5, sIgM, **c-myc**, **HGAL**, CD43, Oct-2, p53 Proliferation index (Ki-67): >95%	Bcl-6, EBV, LMO2, SOX11, CD43, **adipophilin**[g]	MUM-1	CD5, CD23, TdT, bcl-2, cyclin D1
	Flow cytometry			
	CD10, CD19, CD20, CD22, CD38, CD71, CD77, sIgM, HLA-DR, FMC7			CD5, CD23, CD34, CD44, TdT
High-grade B-cell lymphoma with 11q aberrations (Burkitt-like lymphoma with 11q aberration)	CD19, CD20, CD22, CD38, CD74, CD79a, PAX-5, MUM-1 Proliferation index (Ki-67): >95%	CD10, CD43, bcl-6, LMO-2, sIgG, IgM	CD56	**c-myc**, bcl-2

EBV-positive DLBCL	**EBV**[f], CD19, **CD30**, MUM-1, PAX-5	CD20, CD15, bcl-2		CD10
Fibrin-associated large B-cell lymphoma	CD19, CD20, CD79a, PAX-5, MUM-1 Proliferation index (Ki-67): >90%	CD30, bcl-2, bcl-6	CD3, CD4, CD43	CD10
Fluid overload-associated large B-cell lymphoma	CD19, CD79a, PAX-5,	CD20	CD30, CD138	LMO-2
Primary large B-cell lymphoma of immune-privileged sites	CD19, CD20, CD79a, PAX-5, bcl-2, bcl-6, MUM-1 Proliferation index (Ki-67): >80%			
EBV-positive mucocutaneous ulcer	Large B-cells: EBV(f), CD19, MUM-1, PAX-5 T-cells: CD3, CD8	CD30		
Lymphomatoid granulomatosis	EBV, CD19, **CD20**, CD79a, PAX-5	CD30		CD15

[a]Negative in ALL with 11q23 translocation
[b]The expression of CD38, CD49d, or ZAP70 in B-CLL correlates with a worse prognosis
[c]Pediatric type follicular lymphoma lacks the t(14;18) translocation
[d]See the modified Hans Algorithm 16.1 and table below [38]
[e]Positive in ABC (activated B-cell-like) subtype of DLCBL
[f]EBV antigens: EBER, LMP1, EBNA2
[g]Due to the presence of intracytoplasmic lipid vacuoles in cells of Burkitt lymphoma (see Fig. 16.18)
[h]Atypical CLL may be negative for CD5/CD23 and strong CD20/FMC7 expression
[i]CD9 negative in precursor B-cell ALL with t(12;21)
[j]CD34 negative in precursor B-cell ALL with t(0;22)
[k]See Fig. 16.19
[l]Expressed only in non-GCB (ABC) type

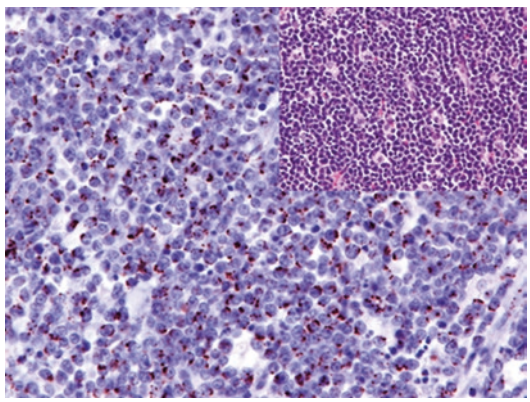


Fig. 16.18 Intracytoplasmic adipophilin expression in the cells of Burkitt lymphoma

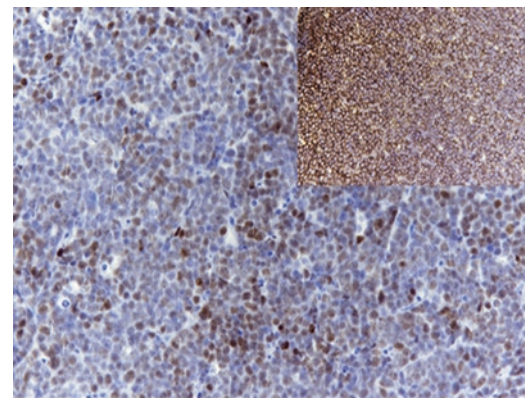


Fig. 16.19 Diffuse large B-cell lymphoma with strong membranous CD20 expression (upper left picture). Lymphoma cells also exhibit a marked nuclear Islet-1 expression

16.3 Markers and Immunoprofile of Plasma Cell Neoplasms

16.3.1 Immunohistochemical Markers for Plasma Cell Neoplasms

CD20, CD38, CD56, CD138, VS38c, CD79a, MUM-1, κ and λ light chains.

16.3.2 CD38

CD38		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell neoplasms – Plasmablastic lymphoma	Pre-T-ALL, B-ALL, primary effusion lymphoma, subtypes of B-cell lymphoma, AML	Plasma cells, erythroid and myeloid precursors, early B- and T-cells, NK-cells, pancreatic islets, normal epithelium of prostate, astrocytes and pyramidal neurons
Positive control: appendix		

Diagnostic Approach CD38 (also known as ADP-ribosyl cyclase) is a transmembrane glycoprotein involved in signal transmission and regulation of intracytoplasmic calcium concentration. CD38 is expressed in most CD34 positive pluripotent stem cells and in different maturation stages of B- and T-lymphocytes, plasma cells, and myeloid cells [18]. In B-cells, the expression is found in germinal center B-cells and memory B-cells in the marginal zone. CD38 is commonly used in diagnostic panels for multiple myeloma. CD38 may also be expressed on a subset of B-CLL cells and is considered an adverse prognostic factor. CD38 is a target for specific therapeutic antibodies used for the treatment of multiple myeloma.

Diagnostic Pitfalls CD38 has a broad expression spectrum and is found in different hematopoietic and non-hematopoietic cells; accordingly, the CD38 expression does not prove the plasma cell origin, and the plasma cell nature must be confirmed by other more specific markers.

16.3.3 CD138

CD138 (Syndecan-1)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell tumors (myeloma, plasmacytoma)	Primary effusion lymphoma, different carcinoma types including thyroid, breast, lung, head and neck, urothelium, prostate, and liver, neuroendocrine tumors, thymoma, tumors of the adrenal cortex, keratoacanthoma, malignant melanoma, osteoid forming tumors	B-cell precursors, plasma cells, stratified squamous epithelium, hepatocytes
Positive control: tonsil/squamous epithelium		

Diagnostic Approach CD138 (syndecan-1) is a transmembrane antigen and one of the four members of the syndecan family. CD138 is expressed in different maturation stages of B-lymphocytes but lost at the pre-B stage. CD138 is strongly expressed in plasma cells in addition to different types of epithelial and mesenchymal cells and binds to various growth factors and extracellular matrix proteins regulating cell differentiation and cell adhesion.

Diagnostic Pitfalls CD138 is widely used as a marker for plasma cells and plasma cell neoplasms (Fig. 16.20); however, the expression of CD138 is found in a large number of epithelial tumors and some mesenchymal tumors. Among the epithelial tumors, CD138 is found in squamous cell carcinoma and adenocarcinomas of different origins, including pulmonary and prostatic adenocarcinomas, which makes it necessary to consider these carcinomas in the differential diagnosis [39]. A particular pitfall is the plasmacytoid urothelial carcinoma, which is often strongly positive for CD138 and can be mistaken for a plasmacytoma. To differentiate between epithelial and plasma cell tumors, it is recommended to run a parallel reaction with a pan-cytokeratin marker but not EMA, as EMA may also be positive in plasma cell disorders as well [9]. The cytoplasmic expression of κ or λ light chains in the plasma cells is also essential to confirm the diagnosis of plasma cell neoplasia and determine the clonality of the plasma cell population. CD138 is also expressed in other mesenchymal tumors such as alveolar soft part sarcoma, synovial sarcoma, and schwannoma, in addition to malignant melanoma and bone-forming tumors, including osteosarcoma [40].

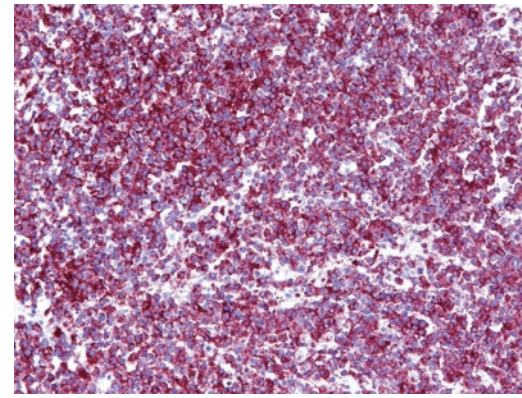
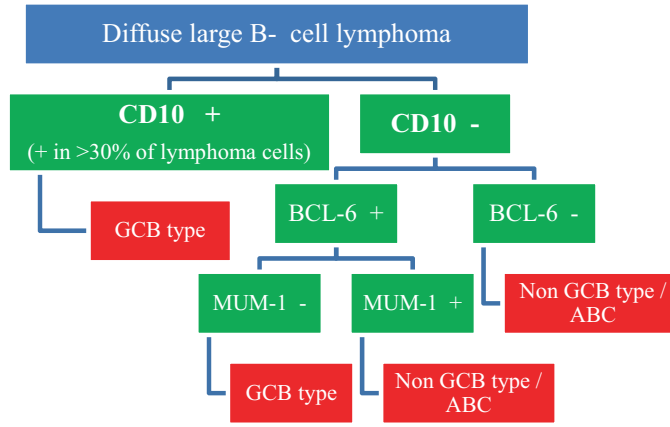


Fig. 16.20 Multiple myeloma with strong membranous CD138 expression

16.3.4 Multiple Myeloma Oncogene 1/IRF4

Multiple myeloma oncogene 1/IRF4 (MUM-1)		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell neoplasms – Plasmablastic lymphoma – Diffuse large B-cell lymphoma ABC type – Anaplastic CD30+ large-cell lymphoma ALK +/− – Large B-cell lymphoma with IRF-4 rearrangement – Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma	CLL, marginal zone lymphoma, intravascular large B-cell lymphoma, primary mediastinal large-cell lymphoma, angioimmunoblastic T-/NK-cell lymphoma, CNS lymphoma, malignant melanoma	B-cells (centrocytes), plasma cells, T-follicular helper (TFH) cells
Positive control: appendix		

Diagnostic Approach **Mu**ltiple **m**yeloma **1** protein (MUM-1, also known as the interferon regulatory factor 4), is a lymphocyte-specific transcriptional activator expressed in the final differentiation stage of intra-germinal center B-lymphocytes. MUM-1 also plays a role in the differentiation of plasma cells, T-lymphocytes, myeloid cells, and dendritic follicular cells. MUM-1 is also a marker for post-germinal center B-cells (late centrocytes), memory B-cells in the marginal zone, and nongerminal/activated B-cell phenotype lymphomas (see modified Hans Algorithm 16.1). MUM-1 is also an essential marker for plasma cells and plasma cell neo-



Algorithm 16.1 Modified Hans algorithm of DLBCL [38]. *GCB* germinal center B-cell type, *ABC* activated B-cell type

- GCB: Germinal center B-cell type
- ABC: Activated B-cell type

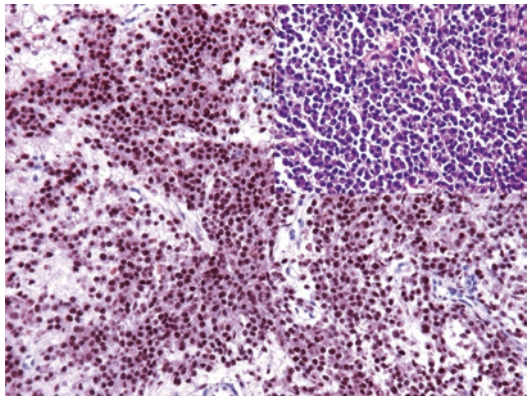


Fig. 16.21 Strong nuclear MUM-I expression in multiple myeloma cells

plasm (Fig. 16.21). Furthermore, MUM-1 is expressed in a subset of T-cells (TFH) and related lymphoma types. The expression of MUM-1 is activated in EBV-infected lymphocytes, which is also a diagnostic marker for Hodgkin cells in classic Hodgkin lymphoma. MUM-1 is usually negative in the cells of nodular lymphocyte-predominant Hodgkin lymphoma. Bcl-6 positive B-cells usually lack the expression of MUM-1.

Diagnostic Pitfalls The expression of MUM-1 is not limited to plasma cell neoplasm or B-cell lymphomas. Weak MUM-1 expression can be noted in some types of T-/NK-cell lymphomas, namely, those originating from follicular helper T-cells such as angioimmunoblastic T-cell lymphoma. MUM-1 stains also the majority of anaplastic CD30-positive large-cell lymphomas, both ALK+ and ALK−. MUM-1 stains also a subset of malignant melanoma, which can also be positive for other plasma cell markers such as CD138 and VS38c. Because of the multilineage expression of the MUM-1 protein, the immunostaining must be carefully interpreted in combination with other more specific antibodies to exclude other possible differential diagnoses [41, 42].

16.3.5 VS38c

VS38c (plasma cell marker)		
Expression pattern: cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Plasma cell neoplasms – Lymphoma with plasmacytic differentiation	Rare carcinoma types of different origins, malignant melanoma, clear cell sarcoma of soft tissue, neuroendocrine tumors, osteosarcoma	Plasma cells and plasmablasts, B-immunoblasts, epithelial cells (mucous glands, pancreatic epithelium, secretory breast cells, thyroid follicles), melanocytes, osteoblasts
Positive control: appendix		

Diagnostic Approach VS38c (rough endoplasmic reticulum-associated antigen, also known as cytoskeleton-linking membrane protein 63) is a sensitive screening marker for plasma cells and cells with plasmacytoid differentiation. VS38c is expressed on the endoplasmic reticulum in the cell cytoplasm. The expression of VS38c is found in plasma cells, plasmablasts, lymphoplasmacytoid cells, and B-immunoblasts and related neoplasms.

Diagnostic Pitfalls Despite the specificity and high sensitivity of VS38c to normal and neoplastic plasma cells, it is always important to keep in mind that other tumor types, such as melanocytic and neuroendocrine tumors, may be also positive for this marker [43]. Paratrabecular osteoblasts in trephine biopsies are also positive for VS38c. VS38c is also a sensitive but less specific marker for osteosarcoma.

16.3.6 Kappa and Lambda Light Chains

Each molecule of the five major classes of immunoglobulins consists of a combination of two identical heavy-chain molecules and two identical light-chain molecules. The light-chain molecules are divided into two classes: kappa and lambda light chains; on the other hand, each B-lymphocyte or plasma cell is able to produce either kappa or lambda light chain. In a polyclonal lymphocyte or plasma cell population, the kappa to lambda ratio is approximately 2:1. The clonal restriction of one of both chains indicates a monoclonal/neoplastic nature of this lymphocyte or plasma cell population. In routine histopathology, the expression of the light chains can be indicated either by conventional immunohistochemistry or in situ hybridization.

Immunoprofile of plasma cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Plasma cell myeloma/ plasmacytoma – Monoclonal gammopathy of undetermined significance (MGUS) – Heavy-chain disease – Plasma cell myeloma – Solitary plasmacytoma of the bone – Extraosseous plasmacytoma – Monoclonal immunoglobulin deposition disease	**CD38**, **VS38c**, **CD138**, CD229, CD319, PCA-1, MUM-1, vimentin **κ or λ Ig light-chain restriction** Proliferation index (Ki-67): ~50–60%	CD43, CD56, CD79a	CD45, EMA, cyclin D1, CD20, CD31, CD33, CD117, steroid hormone receptors (ER)	CD19, CD22, PAX-5, E-cadherin
	Flow cytometry			
	CD38, CD138, CD27, CD29, CD44, CD54, CD86, CD126 **κ or λ Ig light-chain restriction**	CD56	CD31, CD117, CD200	CD19, CD45

16.4 Markers and Immunoprofile of T-Cell Neoplasms

16.4.1 Immunohistochemical Markers for T-Cell Lineage and T-Cell Lymphoma

CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD34, CD43, TdT, ALK, TCL-1, LEF-1, ICOS, TCR, CXCL13, PD-1 [2, 10, 44].

16.4.2 CD2

CD2 (LFA-2)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– T-cell lymphoma	Neoplastic mast cells (mastocytosis)	Thymocytes, mature peripheral T-cells, NK-cells
Positive control: appendix/tonsil		

Diagnostic Approach CD2 is a transmembrane glycoprotein (E rosette receptor) encoded on chromosome 1 and appears in the early stages of T-cell development at the prothymocyte stage. CD2 is the ligand for CD59 and mediates the adhesion between T-lymphocytes and other cells, binding to CD48 and CD58 (LFA3) surface proteins expressed on the antigen-presenting cells, and plays an important role in the activation of memory T-lymphocytes. CD2 is an excellent marker for T-lymphocytes and NK-cells and labels T-cell lymphomas and the majority of NK-cell neoplasms. CD2 is negative in B-lymphocytes with the exception of a small subset of thymic B-cells but negative in all B-cell lymphomas. CD2 is negative in normal mast cells, and the expression of CD2 in mast cells is considered a criterion of malignancy (see Chap. 18).

16.4.3 CD3

CD3		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– T-cell lymphomas	NK-lymphoma (cytoplasmic stain)	Thymocytes, peripheral mature T-cells, activated NK-cells, Purkinje cells of the cerebellum
Positive control: appendix/tonsil		

Diagnostic Approach CD3 is a complex structure composed of five polypeptide chains (γ, δ, ε, ζ, and η) forming three dimers. In early embryogenesis, CD3 is expressed in the cytoplasm of the prothymocytes and persists through all differentiation stages of T-lymphocytes until mature cells. CD3 builds a complex with the T-cell receptor on the membrane of T-lymphocytes responsible for recognizing antigens, leading to the activation of both T-cytotoxic and T-helper immune response. CD3 is the most commonly used pan-T-cell marker expressed in the vast majority of T-cell lymphomas. CD3 labels also a subset of the NK-lymphomas, usually exhibiting a cytoplasmic stain pattern using CD3ε specific antibodies.

16.4.4 CD4

CD4		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Mycosis fungoides – T-cell lymphomas	Histiocytic neoplasms, acute myeloid leukemia, tumors of the parathyroid gland	Thymocytes, T-helper/inducer cells, macrophages, granulocytes, Langerhans cells, parathyroid chief cells, dendritic cells, hepatic sinusoidal cells
Positive control: appendix/tonsil		

Diagnostic Approach CD4 is a transmembrane glycoprotein and a member of the immunoglobulin family expressed on the surface of different types of T-lymphocytes, including Th1, Th2, Th9, Th17, Th21, TFH, and Treg lymphocytes in addition to the majority of thymocytes and a subset of monocytes, macrophages, and dendritic cells. CD4 is a marker of lymphomas originating from these cells, which include the majority of peripheral T-cell lymphomas and cutaneous lymphomas, mainly mycosis fungoides and other histiocytic and myeloid neoplasms (See Chap. 19). T-lymphocytes with TCRγδ and tumors originating from these cells are usually negative for CD4.

Diagnostic Pitfalls CD4 can also be expressed on different hematopoietic precursors, including erythroid and myeloid precursors, in addition to megakaryocytes. In immunohistochemistry and flow cytometry, CD4 is used in a panel with CD3 and CD8 and CD19. CD4 can also be positive in subtypes of acute myeloid leukemia, namely, AML with monocytic differentiation and histiocytic neoplasms (Fig. 16.22).

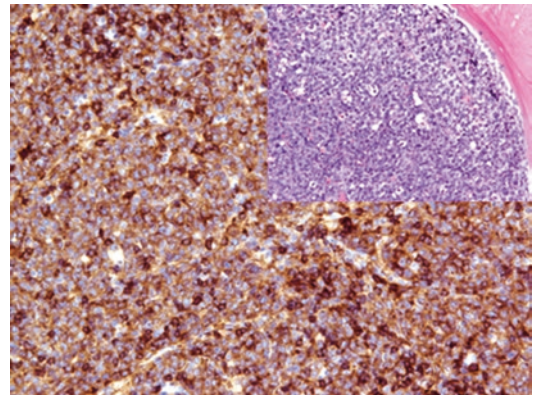


Fig. 16.22 Diffuse CD4 expression in myeloid blasts of AML (M5)

16.4.5 CD7

CD7		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– T-ALL and T-cell lymphomas	CML, AML, immature myelomonocytic neoplasms, cholangiocarcinoma, pancreas carcinoma	Thymocytes, mature T-cells and NK-cells, pre-B-cells, monocytes, early myeloid cells
Positive control: appendix/tonsil		

Diagnostic Approach CD7 is a membranous glycoprotein and a member of the immunoglobulin family involved in T-cell/B-cell interaction and activation of cytokine production. CD7 is expressed in early T-lymphocytes, thymocytes, NK-cells, and a subset of myeloid cells. The expression of CD7 persists in the majority of mature T-lymphocytes and in T-cell/NK-lymphomas derived from these cells, whereas the cells of adult T-cell lymphoma/leukemia and the cells of Sézary syndrome and mycosis fungoides usually lack the expression of CD7. Together with CD34 and CD117, CD7 labels the blasts of high-risk MDS.

Diagnostic Pitfalls CD7 is expressed in a subset of AML, mainly M4/5, in addition to CML. CD7 can also be positive in some carcinoma types, such as pancreatic and bile duct carcinomas [9].

16.4.6 CD8

CD8		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Subcutaneous panniculitis-like T-cell lymphoma – T-cell large granular lymphocytic leukemia	Monomorphic epitheliotropic intestinal T-cell lymphoma, primary cutaneous aggressive epidermotropic cytotoxic T-cell lymphoma, T-PLL, CLL, mantle cell lymphoma	Suppressor/cytotoxic T-cells and subset of NK-cells
Positive control: appendix/tonsil		

Diagnostic Approach CD8 is a transmembrane disulfide-linked heterodimeric glycoprotein composed of either α- and β-chain or two α-chains functioning as a co-receptor for the T-cell receptor playing a role in the T-cell signaling cascade. CD8 is expressed in the suppressor/cytotoxic T-lymphocytes in addition to a subset of NK-cells. CD8 is a marker of many types of T-/NK-cell lymphomas (Fig. 16.23).

Diagnostic Pitfalls CD8 is expressed in a small subset of B-cell lymphomas and should generally be a part of a panel with CD3, CD4, and CD20 [9]. The expansion of CD8-positive T-cell population is noted in lymph nodes associated with acute infectious mononucleosis.

16.4.7 CD30

CD30 (Ki-1) is a transmembrane receptor participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed in activated B-, T-, and NK-cells. In addition to Hodgkin lymphoma and some other lymphoma types, CD30 is a diagnostic marker for anaplastic large-cell lymphoma (Fig. 16.24). CD30 is listed in detail with the markers of Hodgkin lymphoma.

16.4.8 CD43

CD43		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– T-/NK-cell lymphomas	B-ALL, Burkitt lymphoma, mantle cell lymphoma, marginal zone lymphoma, granulocytic (myeloid) sarcoma, adenoid cystic carcinoma	Activated B-cells, T-cells, NK-cells, plasma cells, granulocytes, megakaryocytes, cutaneous mast cells
Positive control: appendix/tonsil		

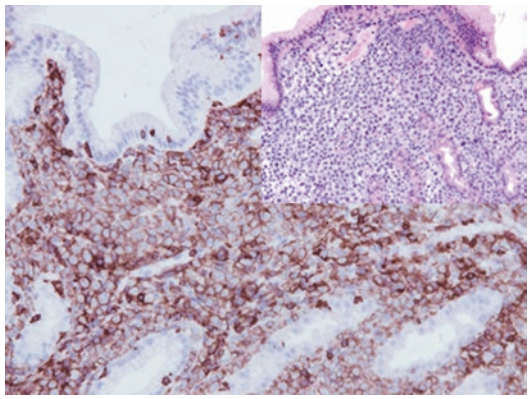


Fig. 16.23 Diffuse CD8 expression in cells of enteropathy-type T-cell lymphoma (type II)

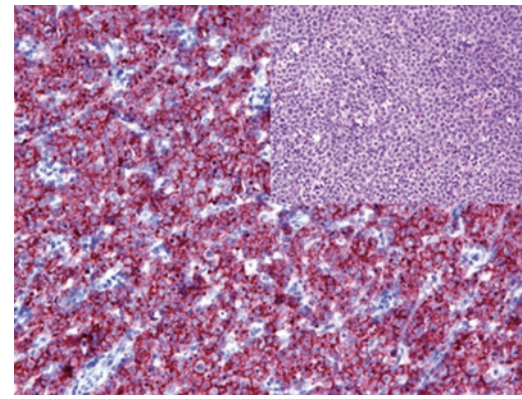


Fig. 16.24 Diffuse CD30 expression in anaplastic large-cell lymphoma

Diagnostic Approach CD43 (also known as Sialophorin or Leukosialin) is a sialoglycoprotein encoded on chromosome 16 functioning as an antiadhesion molecule. CD43 is expressed on the membrane and in the cytoplasm of the T-/NK-lymphocytes, different cells of the myeloid lineage, plasma cells, and neoplasms originating from these cells. CD43 is expressed in the majority of T-/NK-cell lymphomas, including T-ALL and a subset of B-cell lymphomas.

Noteworthy is the so-called "CD43-only pattern" characteristic for some rare tumors that express only CD43 in addition to vimentin. The CD43-only immunophenotype is characteristic for a subset of the following neoplasms, which is to be considered in the differential diagnosis:

- Myeloid sarcoma and subsets of AML
- Anaplastic large-cell lymphoma and NK tumors
- Plasma cell neoplasms
- Langerhans cell histiocytosis

Diagnostic Pitfalls The expression of CD43 correlates with the expression of CD5 and is not restricted to T-cell lymphomas but also found in many types of B-cell lymphoma/leukemia, including B-ALL and a subset of B-CLL and SLL, Burkitt lymphoma, mantle cell lymphoma, and nodal/extranodal marginal zone lymphoma in addition to diffuse large B-cell lymphoma [2]. Since normal B-lymphocytes lack the expression of CD43, CD43 positive B-lymphocytes are assumed to be neoplastic. Generally, CD43 must be used in a panel with other, more specific lymphoma markers. Adenoid cystic carcinoma is one of the rare non-hematopoietic tumors that express CD43.

16.4.9 CD103

CD103 is the alpha E integrin subunit of the heterodimeric αEβ7 integrin (also known as antihuman mucosal lymphocyte 1 antigen). CD103 is expressed in different types of T-lymphocytes mainly intestinal and intraepithelial CD8+ T-lymphocytes and mucosa-associated T-lymphocytes, cytotoxic and activated T-lymphocytes in addition to dendritic cells, and a small subset of B-lymphocytes. CD103 is a marker for enteropathy-associated T-cell lymphoma and monomorphic epitheliotropic intestinal T-cell lymphoma in addition to hairy cell leukemia.

16.4.10 Anaplastic Lymphoma Kinase

Anaplastic lymphoma kinase (ALK, CD246, p80)		
Expression pattern: cytoplasmic/nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Anaplastic large-cell lymphoma – Inflammatory myofibroblastic tumor – ALK-positive large B-cell lymphoma – ALK rearrangement renal cell carcinoma – Therapy-related biomarker in pulmonary non-small-cell carcinoma	ALK-positive histiocytosis, malignant peripheral nerve sheath tumor, rhabdomyosarcoma, neuroblastoma, glioblastoma, Ewing's sarcoma/PNET, leiomyosarcoma, thyroid carcinoma, salivary gland carcinoma (intraductal and ductal carcinoma, myoepithelial carcinoma, secretory carcinoma)	Glial cells, neurons, endothelial cells, pericytes, T-lymphocytes
Positive control: anaplastic lymphoma/brain tissue/appendicular ganglion cells		

Diagnostic Approach Anaplastic lymphoma kinase (ALK) is a membrane-associated kinase encoded on chromosome 2p23 and clustered as CD246. ALK is expressed during embryogenesis, plays an important role in the differentiation of the nervous system, and remains positive in glial cells. In normal tissue, ALK is only detected in glial cells, neurons, endothelium, and pericytes. Other tissue types, including lymphoid tissue, usually lack the expression of ALK. The ALK expression is found in various tumor types due to the activation of the ALK gene transcription caused by the stimulation by a promotor of another gene due to different translocations or

gene rearrangements [45]. The t(2;5)(p23;q35) translocation is the most common genetic anomaly characteristic for ALK-positive anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor [46]. The nucleophosmin (NPM) gene located on chromosome 5q35 is a housekeeping gene encoding a nuclear phosphoprotein which is the fusion partner of the ALK gene in this translocation generating the active NPM-ALK fusion gene, which in turn encodes a chimeric tyrosine kinase composed of the entire cytoplasmic ALK domain and a part of the NPM protein (known as p80). The unregulated expression of the NPM-ALK fusion protein causes the dysregulation of the tyrosine kinase regions in tumor cells. t(1;2)(q25;p23), inv. 2(p23;q35), t(2;3)(p23;q12.2), t(2;13)(p23;q34), t(2;17)(p23;q25), t(2;19)(p23;p13.1), t(2;22)(p23;q11.2), and t(X;2)(q11–12;p23) are further but less common genetic abnormalities associated with anaplastic large-cell lymphoma and other solid tumors.

The ALK molecule is the target for specific kinase inhibitors used to treat ALK-positive tumors, including pulmonary adenocarcinoma and ALK-positive anaplastic large-cell lymphoma. The immunohistochemical detection of ALK in tumor cells is a surrogate for a possible ALK gene rearrangement, which can be later confirmed by one of the molecular methods or FISH (see Chap. 3).

Diagnostic Pitfalls A strong ALK expression is also characteristic for ALK-positive large B-cell lymphoma. This rare lymphoma type lacks the t(2;5) translocation and is consistently CD30 negative (Fig. 16.25).

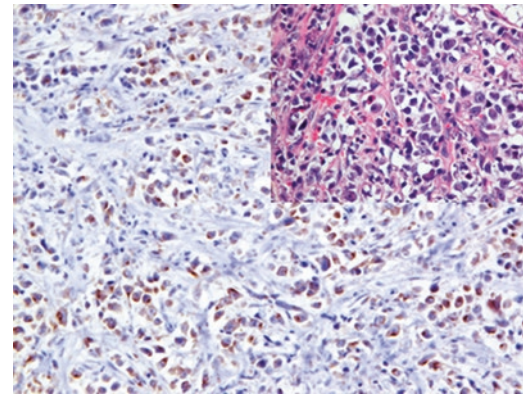


Fig. 16.25 Anaplastic large-cell lymphoma exhibiting ALK expression in lymphoma cells

16.4.11 T-Cell Leukemia Protein 1 (TCL-1)

T-**c**ell **l**eukemia protein **1** (TCL-1) is an oncoprotein encoded on chromosome 14q32.1 functioning as AKT kinase (an isoform of protein kinase B) coactivator involved in survival pathways by inhibiting the apoptotic cascades. TCL-1 is normally expressed in the early embryogenesis of lymphocytes in addition to nonneoplastic B-cells of the mantle zone, plasmacytoid dendritic cells, and testicular germ cells. TCL-1 is overexpressed in T-cell prolymphocytic leukemia as a result of the t(14;14)(q11;q32) rearrangement specific for this leukemia type, typically exhibiting a strong nuclear expression pattern. Other T-cell lymphoma types usually lack TCL-1 positivity. TCL-1 is a marker for plasmacytoid dendritic cell neoplasm but is negative in other histiocytic and myelomonocytic neoplasms. TCL-1 is expressed in different lymphoma types of B-cell origin, and a strong expression is found in Burkitt lymphoma. Follicular lymphoma, mantle cell lymphoma, CLL, hairy cell leukemia, and diffuse large-cell lymphoma show weak to moderate expression intensity, whereas marginal zone lymphoma, CD30+ anaplastic lymphoma, and plasma cell tumors are constantly negative for TCL-1.

The expression of TCL-1 is also one of the specific markers for testicular intratubular germ cell neoplasms (IGCN), seminoma, and ovarian dysgerminoma. TCL-1 is not a marker for other germ cell tumors.

16.4.12 Programmed Cell Death Protein 1 (PD-1)

Programmed cell death protein 1 (PD-1, clustered as CD279) is a type I membrane protein encoded by the PDCD1 gene on chromosome 2q37.3 and a member of the CD28/CTLA-4

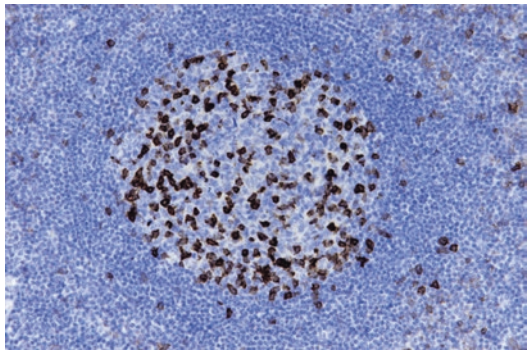


Fig. 16.26 Strong PD-1 expression in follicular helper T-lymphocytes and few peripheral T-lymphocytes

family of receptors. PD-1 binds to its two ligands PD-L1 and PD-L2, which are involved in the regulation of the immune response (see Chap. 31). The PD-1 expression is found on CD4+ follicular helper T-lymphocytes and activated T-lymphocytes in addition to a small subset of B-lymphocytes and myeloid cells (Fig. 16.26).

In routine immunohistochemistry, PD-1 is a marker for angioimmunoblastic T-cell lymphoma. PD-1 is also expressed in a subset of NOS peripheral T-cell lymphoma in addition to a subset of ALK + and ALK − anaplastic large-cell lymphoma.

PD-1 is a helpful marker for the diagnosis of both classic Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma as it is strongly expressed on the T-lymphocytes surrounding the Hodgkin cells.

Both PD-1 and PD-L1 are the targets for different specific checkpoint inhibitors used in tumor therapy (see Chap. 31).

16.4.13 T-Cell Receptor (TCR)

The T-cell receptor (TCR) is a molecule that belongs to the immunoglobulin (Ig) superfamily, expressed on the membrane of T-lymphocytes, responsible for identifying the antigens bound to the major histocompatibility complex (MHC) molecules. Each TCR is composed of two different protein chains, whereas 95% of T-lymphocytes consist of alpha and beta chains (TCRα and TCRβ) and 5% are composed of gamma and delta chains (TCRγ and TCRδ; γ/δ lymphocytes). In routine immunohistochemistry, the expression of the TCR on lymphocytes confirms the T-cell lineage of these cells, and antibodies to the chains mentioned above can be helpful in classifying the T-cell lymphomas. NK cells and NK-cell lymphomas lack the expression of the TCR.

16.4.14 ICOS

ICOS (**i**nducible T-cell **co**-**s**timulator, clustered as CD278) is a member of the CD28 family that regulates the T-cell activity and immune responses and plays a role in the regulation of T-follicular helper cells. The ICOS molecule contains an extracellular, a transmembrane, and an intracellular domain and is primarily expressed on activated CD4+ and CD8+ T-cells. ICOS is a sensitive marker for T-cell lymphomas of follicular helper T-cell origin, mainly angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphomas with T-follicular helper phenotype.

16.4.15 CXCL13 (CXC Motif Chemokine Ligand 13)

CXCL13 is a member of the chemokine family listed in Chap. 20. CXCL13 is strongly expressed on follicular helper CD4+ T-lymphocytes and follicular dendritic cells. CXCL13 is a marker for angioimmunoblastic T-cell lymphoma.

16.5 Markers and Immunoprofile of NK-Cell Neoplasms

16.5.1 Immunohistochemical Markers for NK-Cell Lymphoma

CD2, CD3, CD56, cytotoxic molecules (TIA-1, granzyme B, perforin), and EMA [2, 10].

16.5.2 CD56

CD56 (N-CAM; NKH1)		
Expression pattern: membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– NK-cell lymphomas – Multiple myeloma – Acute and chronic myeloid leukemia – Neuroendocrine tumors (large-cell neuroendocrine carcinoma, small-cell carcinoma, carcinoid and Merkel cell carcinoma) – Pheochromocytoma – Neuroblastoma – Ovarian sex cord-stromal tumors	Synovial sarcoma, embryonal and alveolar rhabdomyosarcoma, angiosarcoma, solitary fibrous tumor, chordoma, epithelioid sarcoma, leiomyoma and leiomyosarcoma, Ewing's sarcoma/PNET, medulloblastoma, schwannoma and neurogenic sarcoma, astrocytomas, ependymoma, meningioma, retinoblastoma, paraganglioma, melanoma, mesothelioma, bile duct adenoma	NK-cells, activated T-cells, a subset of monocytes, cerebellum and brain cortex, neuromuscular junctions, neurons, intestinal ganglion cells, neuroendocrine tissue, thyroid follicular cells, hepatocytes, epithelium of renal tubules, osteoblasts
Positive control: brain tissue/intestinal ganglion cells		

Diagnostic Approach CD56 (neural cell adhesion molecule, N-CAM) is a transmembrane adhesion molecule and a member of the Ig superfamily involved in the development of neural cells and differentiation of neural tissue. Normally, CD56 is expressed on the membrane of neuroectodermal cells, NK-cells, activated T-cells, myoblasts, and skeletal muscle. CD56 is an important marker for NK-cell lymphoma and a helpful marker for the diagnosis of pulmonary and extrapulmonary small-cell carcinomas. CD56 is also a sensitive but less specific marker for ovarian sex cord-stromal tumors.

Diagnostic Pitfalls CD56 is an unspecific marker with a very wide expression spectrum. It is found in a small subset of CD4 and CD8-positive T-cells and plasma cells. CD56 is also expressed on multiple myeloma cells, whereas CD56-negative myelomas are found to have a poor prognosis. CD56 may also be expressed on other tumors with similar morphology, such as embryonal rhabdomyosarcoma, neuroblastoma, malignant melanoma, neurogenic sarcoma, and synovial sarcoma, which is to consider in the differential diagnosis [9, 47].

Granular cell tumor, neurofibroma, solitary fibrous tumor, and angiosarcoma lack the expression of CD56.

16.5.3 Cytotoxic Molecules (Granzyme B, Perforin, and TIA-1)

Cytotoxic molecules are a heterogeneous group of intracytoplasmic cytotoxic molecules found in the T-lymphocytes and natural killer (NK) cells. Antibodies to the cytotoxic molecules are important markers for the diagnosis of T-cell and NK lymphomas. Perforin, granzyme B, and TIA-1 are the most commonly used cytotoxic molecules in routine immunohistochemistry.

16.5.4 Perforin

Perforin (complement 9 related protein, also known as cytolysin) is a cytolytic pore-forming protein found in the granules of cytotoxic T-lymphocytes and natural killer cells. It is able to

perforate a pore in the membrane of targeted cells to enable granzyme to enter the targeted cells.

16.5.5 Granzyme B

Granzyme B is a serine protease stored in specialized lytic granules of cytotoxic T-lymphocytes and natural killer cells together with perforin. Granzyme B seems to enter the target cell through a perforin-caused transmembrane pore to induce DNA fragmentation, initiating apoptosis of targeted cells.

16.5.6 TIA-1

TIA-1 (**T**-cell restricted **i**ntracellular **a**ntigen-**1**, also known as nucleolysin) is a cytotoxic granule-associated protein expressed in NK-cells and cytotoxic T-lymphocytes. TIA-1 has nucleolytic activity against targeted cells, initiating apoptosis. TIA-1 is a marker for NK-cell lymphomas and is also used to label tumor-infiltrating lymphocytes. The expression of TIA-1 is also described in cutaneous mast cells.

Evolution of immunoprofile of nonneoplastic T-lymphocytes	
Cell type	Immunoprofile
Prothymocyte	CD2, cCD3, CD7, TdT, LMO2
Stage I thymocyte (subcapsular)	CD2, cCD3, CD5, CD7, CD10, CD34, TdT
Stage II thymocyte (cortical)	CD1a, CD2, cCD3, CD4, CD5, CD7, CD8, CD38, CD52, CD165, CD200, TdT
Stage II thymocyte (medullary) T-helper/inducer cells	CD2, cCD3, CD4, CD5, CD7, CD27, CD28, CD48, CD52, CD69, CD121a, CD127, CD155, CD165, CD200
Stage II thymocyte (medullary) T-suppressor and cytotoxic cells	CD2, cCD3, CD5, CD7, CD8, CD27, CD28, CD48, CD52, CD69, CD121a, CD127, CD155, CD165, CD200
Follicular T-helper	CD2, CD3, CD4, CD5, CD7, CD10, CD57, bcl-6, PD-1
Natural killer cell (NK cell/null cell/LGL)	CD11b, CD11c, CD16, CD48, CD56, CD57, CD69, CD94, CD122, CD158, CD159, CD161, CD200, CD226, CD224, CD247

Immunoprofile of T-cell and NK-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
T-lymphoblastic leukemia/ lymphoma: subtypes see below (a–d)	**CD2**, **TdT**, CD7, ERG Proliferation index (Ki-67): 40–80%	cCD3, CD10, CD4, CD5, CD8, CD33, CD34, CD99 Fli-1, LMO2	CD1a, CD13, CD15	PAX-5, CD19, CD20, MPO
	Flow cytometry:			
	CD7, CD38, TdT	cCD3, CD5, CD34	CD1a, CD11b, CD13, CD33	CD19, MPO
(a) Pro-T-cell precursor lymphoblastic leukemia	cCD3, CD7	CD34, CD2,	CD4, CD5, CD33, CD56, CD117, HLA-DR	CD1a, CD8, MPO
	Flow cytometry			
	CD7		CD5, CD8	CD1a, mCD3, CD4
(b) Pre-T-cell ALL	cCD3, CD34, TdT			**CD4**, **CD8**
(c) Cortical T-cell ALL	**CD1a**, cCD3, CD4, CD10, TdT	CD8	CD2	
(d) Medullary T-cell ALL	sCD3, CD4/CD8			CD1a

T-cell prolymphocytic leukemia	CD2, cCD3, CD5, CD7, CD43, **TCL-1**	sCD3, CD4	CD8, CD38	CD1a, CD10, CD25, CD28, CD30, CD56, TdT, cytotoxic molecules
	Flow cytometry			
	CD2, cCD3, CD4, CD7, CD8, CD43, CD52, TCL1	sCD3, CD26	CD52, CD38	CD1a, CD16, CD19, CD30, CD56, HLA-DR
NK-lymphoblastic leukemia/ lymphoma	CD34, **CD56**	TdT, CD2, cCD3, CD5, CD94	CD33, CD117	CD1a, mCD3, CD4, CD8, CD19, CD20, MPO
T-cell large granular lymphocytic leukemia	CD2, **CD3**, CD8 (in the common type), CD16, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD38, CD57 (in the common and NK-cell types), TCRβ	CD56, CD4 (+ in rare types)	CD5, CD7, CD10, CD25
	Flow cytometry			
	CD3; CD5; CD7; CD8; CD16; CD57; CD122; CD158a, b, e; CD329			CD4, CD19
NK-large granular lymphocytic leukemia	CD2, CD8 (in the common type), CD16, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD3, CD56		
	Flow cytometry			
	CD5; CD7; CD8; CD16; CD56; CD57; CD122; CD158a, b, e; CD329	cCD3, CD16		sCD3, CD4, CD19
Adult T-cell lymphoma/ leukemia (HTLV1+)	CD2, **CD3**, CD5, CD62, HLA DR	**CD4**, **CD25**, MUM-1	CD15, CD30, CD56	CD7, CD8, ALK
	Flow cytometry			
	CD2, CD4, CD5, CD25, CD27, CD52	CD3		CD1a, CD7, CD8, CD10, CD19, CD26
Aggressive NK-cell leukemia	CD2, cCD3ε, CD30 (only in large transformed cells), CD56, cytotoxic molecules (TIA-1, granzyme B), EBV	CD7, CD16, EMA	CD8, CD16	sCD3, CD4, CD5, CD8, CD57
	Flow cytometry			
	CD2, cCD3ε, CD7, CD56, CD16, CD29, CD43, CD54, CD122, CD161, HLA-DR		CD30	mCD3, CD4, CD5, CD8, CD19, CD57
Indolent T-cell lymphoproliferative disorder of the GI tract	CD2, CD3, CD8	CD5, CD7, TIA-1		CD4, CD30, CD56
Enteropathy-associated T-cell lymphoma	CD2, **cCD3**, CD7, CD103	CD30, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD8	CD4, CD5, CD56
	Flow cytometry			
	cCD3, CD30, CD103, TCRγβ, TCRγδ	CD2	CD4	CD4, CD8, CD56

Monomorphic epitheliotropic intestinal T-cell lymphoma	CD2, CD3, CD7, **CD8**, **CD56**, TIA-1		CD20	CD4, CD5
	Flow cytometry			
	CD2, cCD3, CD7, CD8, CD56, CD103, TCRγβ, TCRγδ			CD30
Intestinal T-cell lymphoma NOS	CD3	Cytotoxic molecules (TIA-1, perforin)	CD30	CD56
EBV-positive nodal T- and NK-cell lymphoma	CD2, CD3, cytotoxic molecules (TIA-1, perforin, granzyme B), EBV	CD8, CD56	CD5, CD5	
Extranodal NK-/T-cell lymphoma (extranodal NK/T-cell lymphoma, nasal type)	**CD2**, **CD3ε**, CD43, **CD56**, CD94, **cytotoxic molecules** (TIA-1, perforin, granzyme B), EBV		CD4, CD7	**CD3**, CD5, CD8, CD57, CD161, TdT
	Flow cytometry			
	CD2, cCD3ε, CD25, CD26, CD38, CD56, CD94, HLA-DR			mCD3, CD4, CD5, CD7, CD8, CD16, CD57
Hepatosplenic γδ T-cell lymphoma	CD2, **CD3**, CD43, CD45RO, **TIA-1**	CD2, CD7, CD56	CD16, CD11c, CD11b, granzyme	CD4, CD5, CD8, CD30, CD57, perforin
	Flow cytometry			
	CD2, CD3, CD7, CD16, CD56, TCRγδ	CD56, TCRαβ	CD8, CD16	CD4, CD5, CD19, CD25, CD57
ALK-positive anaplastic large-cell lymphoma	**ALK**, **CD30**, CD4, CD43, MUM-1, clusterin[d], **cytotoxic molecules** (TIA-1, perforin, granzyme B)	CD2, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, Fascin, bcl-6	CD8, CD20, CD28, PAX-5
ALK-negative anaplastic large-cell lymphoma	**CD30**, clusterin[d], CD43, MUM-1, **cytotoxic molecules** (TIA-1, perforin, granzyme B)	CD2, CD4, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, Fascin, bcl-6	**ALK**, CD8, CD20, CD28, PAX-5, bcl-2
Breast implant-associated anaplastic large-cell lymphoma	CD4, CD43, **CD30**, bcl-2 Proliferation index (Ki-67): >80%	CD2, CD3, CD5, EMA, clusterin, cytotoxic molecules (TIA-1, perforin, granzyme B), EMA	CD15	CD10, ALK
	Flow cytometry			
	CD2, CD4, CD25, CD30, CD38	CD13, CD33, TIA-1	CD15	
Nodal TFH-cell lymphoma, angioimmunoblastic type	CD2, **CD3**, CD4, CD5, CD7, **CD10**, CD28, **PD-1** (**CD279**), **ICOS**[g] (**CD278**), **CXCL13**[c] Expanded CD21, CD23, and CD35 positive meshwork of follicular dendritic cells	Bcl-6, CD38	CD8, CD30 EBV+ B-cell blasts	CD15
	Flow cytometry			
	CD3, CD4, CD5, CD10, CXCR5			CD8, CD20

Nodal TFH-cell lymphoma, follicular type	CD3, CD4, **CD10**, **PD1** (**CD279**), **bcl-6**, **CXCL13**[c]			
	Flow cytometry			
	CD4, CD10, CD57, CD200, CD278, CXCL13, CXCR5			
Nodal TFH cell lymphoma, NOS	CD3, CD4, CD10, PD1 (CD279), bcl-6, CXCL13[c]			
Peripheral T-cell lymphoma (NOS)	CD2, **CD3**, CD4	CD4, CD7, CD5, GATA-3	CD8, CD25, CD30, CD134	**ALK**, TdT, CD1a, CD10, CD15[a], CD19, CD20[b], bcl-6
	Flow cytometry			
	CD2, CD3, CD4,	CD5, CD7	CD8	CD19
Hydroa vacciniforme lymphoproliferative disorder (Hydroa vacciniforme-like T-cell lymphoma)	CD2, CD3, CD8, EBV	Cytotoxic molecules (TIA-1, perforin, granzyme B)	CD4	
Systemic EBV-positive T-cell lymphoma of childhood				
Primary cutaneous T-cell lymphomas				
Primary cutaneous CD4 positive small/medium T-cell lymphoproliferative disorder	CD2, CD3, **CD4**, CD38, bcl-6	PD-1	CD10	CD8, CD30
	Flow cytometry			
	CD4, CD38, CXCL13			CD10, CD30
Primary cutaneous acral CD8-positive lymphoma	CD2, CD8	CD3, CD5, CD7, cytotoxic molecule TIA-1	CD4	CD30, CD56, PD-1, EBV
	Flow cytometry			
	CD2, CD3, CD5, CD7, CD8, TIA-1			CD30, CD56
Mycosis fungoides/Sézary syndrome	CD2, **CD3**, CD4, CD5, TRβ Proliferation index (Ki-67): <5%		CD25	CD1a, CD7, CD8, CD10
	Flow cytometry			
	CD3, CD4, CD5			CD7, CD8, CD19, CD26
Primary cutaneous CD30-positive T-cell lymphoproliferative disorder: lymphomatoid papulosis	**CD30**[d,e], CD4, CD25	CD45, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD2, CD3, CD5, CD7, CD56	Clusterin, CD8[f], CD15, EMA, CD246 (ALK, p80), PAX-5, EBV
Primary cutaneous anaplastic large-cell lymphoma: primary cutaneous anaplastic large-cell lymphoma	CD4	CD2, CD3, CD5, CD7, CD30	CD15	
Subcutaneous panniculitis-like T-cell lymphoma	CD2, CD3, CD7, CD8, CD43, CD45, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD7, CD25	CD30	CD4, CD30, CD56
	Flow cytometry			
	CD3, CD5, CD7, CD8, TIA-1			CD4, CD56

Primary cutaneous gamma delta T-cell lymphoma	CD2, CD3, cytotoxic molecules (TIA-1, perforin, granzyme B), TCRγ, TCRδ	CD7, CD56	CD8	CD1a, CD4, CD5, CD57
	Flow cytometry			
	CD3, CD56, perforin, TIA-1, granzyme B			CD4, CD5, CD7, CD8
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma	CD3, **CD8**, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD7,	CD2, CD15, CD30	CD4, TCRγ, TCRδ
	Flow cytometry			
	CD3, CD8	CD7		CD2, CD4, CD5, CD56
Primary cutaneous peripheral T-cell lymphoma, NOS	CD2, CD3	CD4	CD8	

[a]CD15 may be expressed in large cells of peripheral T-cell lymphoma
[b]B-cell antigens may be expressed in very rare cases (<5%) of peripheral T-cell lymphoma
[c]CXCL13: CXC motif chemokine ligand 13; see Chap. 19 [48]
[d]Golgi stain pattern
[e]CD30 positive only in RS-like cells of type A lymphomatoid papulosis
[f]CD8 positive in type D lymphomatoid papulosis
[g]ICOS: inducible co-stimulator (CD278)

16.6 Markers and Immunoprofile of Hodgkin Lymphoma

Classical Hodgkin lymphoma is a malignant proliferation of lymphocytes originating from germinal center B-lymphocytes. Classical Hodgkin lymphoma includes four subtypes composed of neoplastic mononucleated Hodgkin cells and multinucleated Reed–Sternberg cells in a unique nonneoplastic microenvironment composed of different lymphocytes and inflammatory cells, while the malignant cells represent less than 2% of the total cell population. The Hodgkin cells have a characteristic immunoprofile diagnostic for these cells and are typically labeled by CD15, CD30, MUM-1, STAT-6, PAX-5, IMP3, PD-L1, and J-chain but are usually negative for CD45 and CD20. The surrounding T-cells show a PD-1 positivity.

Nodular lymphocyte-predominant Hodgkin lymphoma is another lymphoma type distinct from classical Hodgkin lymphoma, composed of large centroblasts with multilobulated nuclei (LP, popcorn cells) in a microenvironment exhibiting nodular or diffuse appearance composed of small lymphocytes and histiocytes. The LP cells are typically positive for CD45, CD20, bcl-6, and IMP-3 but negative for CD15, CD30, and bcl-2. The LP cells are usually surrounded by rosettes of CD3- and CD57-positive T-lymphocytes.

16.6.1 Diagnostic Antibody Panel for Classical Hodgkin Lymphoma

CD15, CD30, MUM-1, IMP3, PAX-5, STAT-6, PD-L1, Fascin, J-chain [49–51].

16.6.2 Diagnostic Antibody Panel for Nodular Lymphocyte-Predominant Hodgkin Lymphoma

CD19, CD20, PAX-5, J-chain, BOB.1, Oct-2, and EMA [49].

16.6.3 CD15

CD15		
Expression pattern: membranous/cytoplasmic and juxtanuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hodgkin lymphoma (Reed–Sternberg cells) – Myeloid leukemia/myeloid sarcoma	Adenocarcinoma, sweat and sebaceous gland tumors, thymoma, ovarian carcinoma, renal cell carcinoma, thyroid carcinoma, peripheral T-cell lymphoma, ALCL	Granulocytes and precursors (neutrophils and eosinophils), monocytes, activated B- and T-cells, proximal tubules of the kidney, intestinal Paneth cells
Positive control: appendix		

Diagnostic Approach CD15 (X hapten) is a cell surface granulocyte-associated glycoprotein involved in the regulation of neutrophil functions. CD15 is commonly used as a marker for normal and neoplastic myeloid cells and monocytes but is frequently lost in cells of AML. In combination with CD30, CD15 is a marker for Reed–Sternberg cells in classical Hodgkin lymphoma found in 75–85% of the cases (Fig. 16.27).

CD15 is also expressed on different carcinoma types but is usually negative in mesothelioma. Carcinomas positive for CD15 are reported to have a worse prognosis.

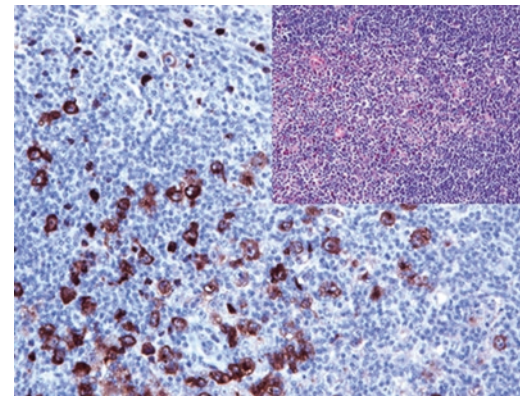


Fig. 16.27 Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma with strong CD15 expression

Diagnostic Pitfalls Since CD15 is expressed in different hematopoietic and non-hematopoietic neoplasms, including adenocarcinomas, it is important to consider possible differential diagnoses and support the final diagnosis by other, more specific antibodies.

16.6.4 CD30

CD30		
Expression pattern: membranous/cytoplasmic paranuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Reed–Sternberg cells in classic Hodgkin lymphoma – Systemic and cutaneous anaplastic large-cell lymphoma – Primary mediastinal large B-cell lymphoma	Embryonal carcinoma, systemic mastocytosis, NK-/T-cell lymphoma, nasopharyngeal carcinoma, pancreatic adenocarcinoma melanoma, angiosarcoma, mesothelioma	Granulocytes, monocytes, activated B-, T-(immunoblasts), and NK-cells, a small subset of plasma cells, exocrine pancreas glands, Purkinje cells of the cerebellum, cortical neurons, decidual cells
Positive control: embryonal carcinoma		

Diagnostic Approach CD30 (Ki-1)—also known as lymphocyte activation antigen—is a transmembrane glycoprotein receptor with intracellular, transmembrane, and extracellular domains. CD30 is a member of the tumor necrosis factor superfamily (TNFRSF8), participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed on activated B-, T-, and NK-cells but absent or minimally expressed in resting lymphocytes. CD30L and CD153 are the ligands that bind to the CD30 molecule and are expressed by histiocytes, granulocytes, and activated lymphocytes.

One of the major utilities of CD30 in routine immunohistochemistry is to highlight Hodgkin cells and multinucleated Reed–Sternberg cells in different types of classical Hodgkin lymphoma (Fig. 16.28). CD30 is also a diagnostic marker for anaplastic large-cell lymphoma and primary mediastinal large B-cell lymphoma, as well as high-malignant types of systemic mastocytosis [52].

The expression of CD30 is not restricted to lymphoid tissue and lymphoid neoplasms but is also found in other different epithelial and mesenchymal tumors. CD30 is a useful marker for the diagnosis of embryonal carcinoma. CD30 labels other carcinoma types, such as nasopharyngeal carcinoma and pancreatic adenocarcinoma. In mesenchymal tumors, CD30 labels about 30% of angiosarcoma [53].

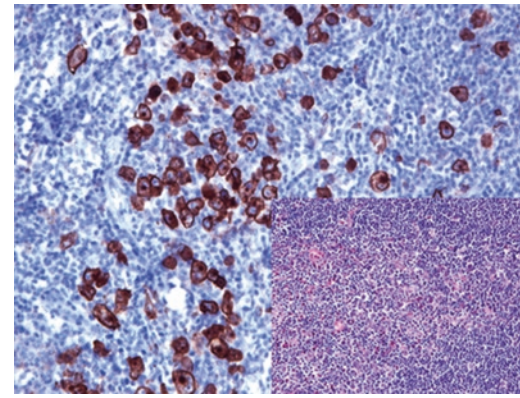


Fig. 16.28 Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma with strong CD30 expression

CD30 is the therapeutic target for specific antibodies used to treat classical Hodgkin lymphoma, anaplastic large-cell lymphoma, peripheral T-cell lymphoma NOS, and systemic mastocytosis.

Diagnostic Pitfalls CD30-positive cells may be found in different T- and B-lymphoma types. CD30 stains also nonneoplastic activated T- and B-immunoblasts in reactive lymph nodes, spleen, thymus, and tonsil in addition to lymphocytes carrying EBV, HIV, or other oncogenic viral genomes; consequently, not all CD30-positive cells are Hodgkin cells.

16.6.5 Fascin

Fascin (actin bundling protein; p55)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Reed–Sternberg cells in classic Hodgkin lymphoma – Anaplastic large-cell lymphoma – Follicular and interdigitating dendritic cell tumors	Adenocarcinomas of the breast, colon, biliary tract, pancreas, lung, ovary, and skin; papillary transitional cell carcinoma of the bladder; juvenile xanthogranuloma; diffuse large B-cell lymphoma; synovial sarcoma	Interdigitating and follicular dendritic cells, endothelial cells, EBV-infected B-lymphocytes
Positive control: lymph node		

Diagnostic Approach Fascin is an Actin binding protein involved in cell adhesion and motility. It is normally expressed in interdigitating and follicular dendritic cells and variably in endothelial cells but constantly negative in lymphocytes, plasma cells, and myeloid cells. Fascin is a good marker for Reed–Sternberg cells in classical Hodgkin lymphoma. It is also expressed on the membrane of anaplastic large-cell lymphoma and subtypes of diffuse large B-cell lymphoma.

Fascin is constantly negative in the normal epithelium but positive in many types of trans-

formed or neoplastic epithelium [54]. This phenomenon may be used for the differentiation between hyperplastic and neoplastic urothelium.

Diagnostic Pitfalls Because of the wide expression spectrum of Fascin, many differential diagnoses must be considered in the interpretation of the Fascin immunostaining. In addition to Reed–Sternberg cells, Fascin positive cells in lymph nodes may be activated B-lymphocytes, cells of diffuse large B-cell lymphoma, or even disseminated cells of metastatic adenocarcinoma.

16.6.6 Insulin Like Growth Factor II mRNA-Binding Protein 3 (IMP3)

IMP3 is a cytoplasmic protein mediating RNA trafficking and cell growth, highly expressed in early embryogenesis. Benign adult tissue usually lacks the expression of IMP3 with the exception of fibroblasts, a subset of lymphocytes (mainly germinal center lymphocytes), ovarian and testicular tissue, placenta, and brain. IMP3 is expressed in different premalignant and malignant lesions and in different carcinoma types, including pulmonary carcinoma, esophageal and pancreatic carcinoma, cervical and endometrial carcinoma, transitional cell carcinoma, renal cell carcinoma, and neuroendocrine carcinoma.

In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. It is a useful marker to discriminate between pancreatic adenocarcinoma positive for IMP3 and inflammatory pancreas lesions usually negative for IMP3 (see Chap. 8). IMP3 selectively stains Hodgkin and Reed–Sternberg cells in both classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma (Figs. 16.29 and 16.30).

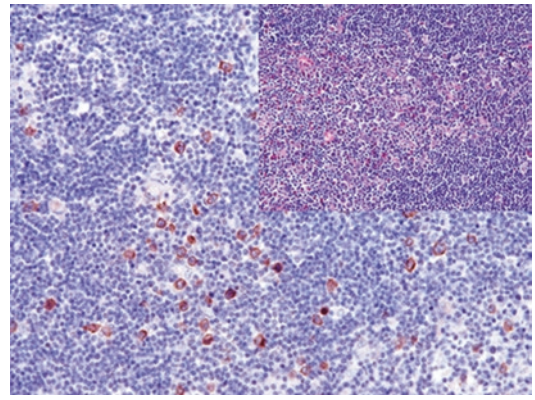


Fig. 16.29 IMP3 selectively labels the Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma

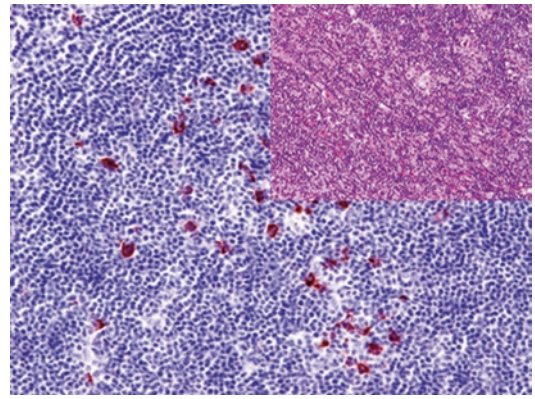


Fig. 16.30 Nodular lymphocyte-predominant Hodgkin lymphoma. IMP3 selectively labels the Hodgkin cells

Diagnostic Pitfalls IMP3 may be positive in other extrafollicular blasts and must be used with other more specific markers to label Hodgkin cells.

16.6.7 STAT-6

STAT-6 is a member of the STAT family of cytoplasmic transcription factors listed in Chap. 23. STAT-6 also labels the nuclei of Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma but is negative in nodular lymphocyte-predominant Hodgkin lymphoma [55].

In routine immunohistochemistry, STAT-6 has a specific nuclear expression pattern characteristic for different tumors, including solitary fibrous tumor and HRS cells of classical Hodgkin lymphoma.

Diagnostic Pitfalls A nonspecific cytoplasmic staining pattern found in different mesenchymal, histiocytic, and lymphoid cells (Fig. 16.31).

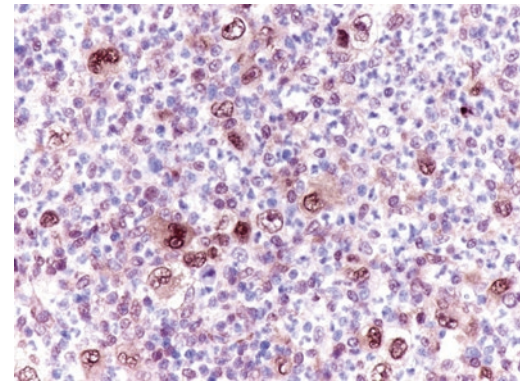


Fig. 16.31 STAT-6 highlighting the nuclei of Hodgkin and Reed–Sternberg cells in classical Hodgkin lymphoma. Lymphocytes and histiocytes in the background exhibit a nonspecific cytoplasmic expression pattern

Immunoprofile of Hodgkin lymphoma				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Classical Hodgkin lymphoma (Hodgkin and Reed–Sternberg cells[a]) in classical subtypes – Nodular sclerosis – Lymphocyte rich classic – Mixed cellularity – Lymphocyte-depleted – Unclassifiable	**CD30**, **PD-L1**, **PAX-5**, **MUM-1**, IMP3, Fascin	**CD15**, STAT-6, CD25, CE40, CD83, CD123, CD138, CD200, HLA-DR, p53 EBV (LMP1)	LMO2, HGAL, CD20, CD79	CD45, CD43, Oct-2, BOB.1, J-chain, PU.1, EMA, bcl-6, CD22, ALK
Main background cells in classical Hodgkin lymphoma	Nodular sclerosis: CD3 and CD4 positive T lymphocytes, macrophages, eosinophils, neutrophils, fibroblasts, plasma cells Mixed cellularity: lymphocytes, plasma cells, eosinophils, histiocytes Lymphocyte-depleted: fibroblasts Lymphocyte rich: mainly B lymphocytes, histiocytes, loose CD21 positive follicular dendritic cell meshwork			
Nodular lymphocyte-predominant Hodgkin lymphoma Lymphocyte-predominant cells (LP) or popcorn cells[a,b]	CD19, CD20, CD22, CD45, CD86, bcl-6, **Oct-2**, HGAL, PAX5, BOB.1, **J-chain**, IMP3	CD75, CD79a, CD40, PU.1, T-bet, **EMA**	MUM-1	CD10, CD15, CD30, CD138, CD200, bcl-2, p53, Fascin, ALK (p80), EBV, STST-6
Main background cells in nodular lymphocyte-predominant Hodgkin lymphoma	Small B-lymphocytes, T-lymphocytes LP cells surrounded by rosettes of CD3+, CD4+, CD57+, and PD-1+ activated lymphocytes			

[a]Usually, without IgH or TCR gene rearrangements
[b]Also known as lymphocytic/histiocytic Reed–Sternberg cells (L&H cells)

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