Chapter 32
Bone Marrow

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Abstract The bone marrow is the primary site of hematopoiesis. Its dynamic environment can make interpretation of immunohistochemistry problematic. Furthermore, decalcification of the bone can alter antigen expression in the tissue, adding another layer of complexity to immunohistochemistry interpretation. Several variables should be considered during interpretation including processing techniques, antigen distribution, and expression levels. Therefore, this chapter was written taking into consideration these factors. This chapter begins with an extensive immunohistochemical stain list of both frequent and infrequently used markers for bone marrow specimens that includes the cellular location of antigen expression, typical distribution in normal bone marrow, the physiologic function associated with the targeted antigen, and the main use(s) of the marker for bone marrow evaluation. It also provides an overview of immunohistochemical markers, new and old, used most frequently in assessing diseases involving the bone marrow encompassing acute and chronic lymphoid and myeloid leukemias, myeloproliferative neoplasms, myelodysplastic syndromes, mast cell disease, plasma cell dyscrasias, T-and B-cell lymphoproliferative disorders, histiocytic lesions, and metastatic disease.

Frequently Asked Questions

32.1 What is the expression pattern of common immunohistochemical stains in normal bone marrow (Table 32.1)?
32.2 What factors should be considered when developing a bone marrow processing protocol (Table 32.2)?
32.3 How is immunohistochemistry different from flow cytometric immunophenotyping? (Table 32.3)
32.4 What is the distribution and immunophenotype of hematopoietic elements in normal bone marrow (Table 32.4)?
32.5 What is the distribution and immunophenotype of nonhematopoietic elements in normal bone marrow (Table 32.5)?
32.6 How is immunohistochemistry utilized to assess acute leukemia/immature morphology (Table 32.6)?
32.7 How to approach immunohistochemical staining for an acute leukemia (Fig. 32.1)?
32.8 How sensitive are specific immunohistochemical markers for acute leukemia (Table 32.7)?
32.9 What immunohistochemical clues suggest recurrent genetic changes in acute lymphoblastic leukemia (Table 32.8)
32.10 What markers suggest acute myeloid leukemia with genetic changes (Table 32.9)?
32.11 What markers are used to differentiate B-cell acute lymphoblastic leukemia from B-cell non-Hodgkin lymphoma (Table 32.10)?
32.12 What markers are used to differentiate T-cell lymphoblastic leukemia from T-cell non-Hodgkin lymphoma and thymoma (Table 32.11)?
32.13 What immunohistochemical stains are used for bone marrow assessment in patients with cytopenia(s) (Table 32.12)?
32.14 What are the markers for myelodysplastic syndrome (Table 32.13)?
32.15 What are the markers for myeloproliferative disorders (Table 32.14)?
32.16 What are the markers for mast cell disease (Table 32.15)?
32.17 What markers are used for differentiating benign from malignant lymphoid aggregates in bone marrow biopsies (Table 32.16)?
32.18 What are the common immunohistochemical markers for mature B-and T-cell neoplasms (Tables 32.17, 32.18, and 32.19)?
32.19 What markers are useful in hairy cell leukemia (Table 32.20)?

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32.20 What markers are used to differentiate reactive versus malignant plasmacytosis (Table 32.21)?

32.21 What immunohistochemical stains are used for plasma cell myeloma and aggressive plasmacytoid neoplasms (Table 32.22)?

32.22 What markers are used for histiocytic tumors in the bone marrow (Table 32.23)?

32.23 What markers are used for metastatic tumors to the bone marrow (similar to the workup of unknown primary in Chap. 12 (Table 32.24)?

Table 32.1 Summary of distribution, applications, and limitations of useful markers in normal bone marrow

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
2D7	C	Basophils	Component of secretory granules of basophils	Increased expression in myeloid neoplasms (especially CML) [1, 2]
ALK (CD246, Ki-1)	C+N+M	Not expressed	Tyrosine kinase receptor belonging to insulin receptor superfamily, normally silent in lymphocytes, expressed in epithelial tissue	Positive in ALCL and some DLBCLs; correlated with t(2;5) or variant translocations [3]
Annexin A1	C	Granulocytes, macrophages, T cells	Ca^{2+}-dependent phospholipid-binding protein that inhibits NF-κB signal transduction and apoptosis	Nonspecific marker for HCL, myeloid disorders (including AML), and rarely in T ALL, LPL, adenocarcinoma, and melanoma, limited value for disease monitoring [4]
BB1	C	Basophils	Basogranulin is a component of secondary granules of basophils	Increased expression in myeloid neoplasms especially CML [2]
BCL2	M+C	T and B lymphocytes, plasma cells	Protein in mitochondria; inhibits apoptosis	Differentiates FL with (14;18) (q32;q21) from reactive aggregates; positive in other NHLs, AL, LCH and MM with and without translocation [5]
BCL6	N	Granulocytes (weak), monocytes (weak), B lymphocytes in reactive follicles if present	Zinc finger transcription factor, suppresses growth; especially in normal germinal center B/T cells	B-cell malignancies with 3q27 rearrangements/ aberrations (FL, DLBCL, BL, NLPHL), positivity in myeloid cells could make interpretation difficult in lymphoma evaluation [6]
BCL10	C+N	Mature lymphocytes	Lymphocyte maturation, induces apoptosis, and activates NF-κB pathway	Positive in B-cell lymphomas with strong nuclear positivity in LPL and MZL [7, 8]
βF1	M	Rare expression in T cells	T-cell beta chain antigen receptor, positive in αβ T cells	Identifies some unusual CD3-negative T-cell lymphomas
BOB1	N	B lymphocytes (strong) subset of myeloid, erythroid, and megakaryocyte cells (weak)	Coactivator of B-cell transcription factor Oct-2 to stimulate immunoglobulin production and germinal center formation	Overexpressed in B and T-cell lymphomas, MM, some ALs and NLPH "popcorn" cells, lost in HRS cells of CHL [9–11]
BRAF V600E	C	Not expressed	Involved in sending signals inside cells and involved in directing cell growth	Positive in HCL, melanoma, and some carcinomas, including Gl and ovarian primary [12–14]
CAL2	C	Not expressed	Calreticulin (CALR), a gene on chromosome 19, mutant forms are associated with MPNs (ET or PMF)	Positive in megakaryocytes of MPNs with CALR mutation [15, 16]
CCR4	C	Not described	Chemokine receptor of T helper cells, type 2 associated with GATA3 expression	>50% positivity in tumor cells of PTCL associated with worse prognosis if T-bet and CXCR3 negative [17]

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
CD1a	M	None or rare positive dendritic cells	MHC class I-like molecule; associated with β-2-microglobin; used in antigen presentation, thymic T-cell development	Precursor T-cell ALL and Langerhans cell histiocytosis [18, 19]
CD2	M+C	T cells, NK cells	Sheep erythrocyte rosette receptor, LFA-3 (CD58) ligand, adhesion molecule, activates T cells	ALL, AML, T-/ NK-cell neoplasms, and neoplastic mast cells
CD3	M+C+/−G	T cells	Associated with T-cell antigen receptor	Best pan T-cell marker; ALL, T-/NK-cell neoplasms
CD4	M	T cells, monocytes, macrophages, megakaryocytes, and endothelial cells	MHC class II coreceptor and HIV receptor	T-cell neoplasms, BPDC, histiocytic disorders (including HS, HPH)
CD5	M	T cells	Surface glycoprotein that modulates TCR and BCR signaling	Positive in a subset of B-cell malignancies (CLL, MCL, occasionally MZL, LPL, and DLBCL); negative (lost) in T-cell neoplasms
CD7	M	T cells, NK cells, early myeloid precursors	Surface glycoprotein that modulates TCR and BCR signaling	T-cell neoplasms (often lost), acute leukemia
CD8	M	T cells, macrophages, endothelial cells, endosteal lining	MHC class I coreceptor; marker for cytotoxic T cells	T-cell neoplasms (T LGL, T ALL, CD8-positive peripheral T-cell lymphomas)
CD10 (CALLA)	M	Precursor B cells, granulocytes, stromal cells, follicular center cells in reactive follicles if present, endosteal lining cells, adipocytes	Membrane endopeptidase also known at the common lymphocytic leukemia antigen (CALLA) that is expressed in a variety of tissues other than hematopoietic including kidney	Positive in B ALL, NHLs (FL BL, DLBCL, AITL, HCL), and MM, although lost in granulocytes of MDS hard to detect in paraffin tissue biopsy; expressed in metastatic carcinomas (RCC)
CD11b	M	Granulocytes, monocytes, macrophages, NK cells, B lymphocytes subset	Adhesion molecule associated with integrin beta-2 (CD18), CD11b/CD18 heterodimer complex known as Mac-1, LFA-1, or CR3; recognizes iC3b and binds CD54, fibrinogen, factor X and ICAM1	Expressed in B-cell neoplasms (MZL), acute monocytic and myelomonocytic leukemias, dual antibody staining can differentiates recovering marrow promyelocytes (CD117-CD11b+) from malignant APL promyelocytes
CD11c	M	Granulocytes, monocytes, macrophages, NK cells, B lymphocyte subset	Adhesion molecule associated with integrin beta-2 (CD18), CD11b/CD18 heterodimer complex known as Mac-1, LFA-1, or CR3; functions in cell-to-cell interactions	Expressed in B-cell neoplasms (MZL, HCL), unexpressed in monocytes associated with CMML
CD13	M+C	Granulocytes, monocytes, basophils, osteoclasts, endothelial cells	Aminopeptidase N is a type II integral membrane metalloprotease and receptor for coronavirus	Suggests myeloid derivation in AL (CD33 more specific), occasionally positivity in ALL
CD14	M+C	Monocytes, macrophages, Langerhans cells, dendritic cells	Receptor for endotoxin	Although used to identify monocytic lineage, many AMLs with monocytic differentiation lack expression, increased expression in HLH [20–23]

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Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
CD15	M+C	Neutrophils, eosinophils, monocytes, promyelocytes	Carbohydrate adhesion molecule, plays a role in phagocytosis, bactericidal activity, and chemotaxis	Used to evaluated AML and CHL, potentially positive in adenocarcinoma
CD16	M	NK cells, T cells, granulocytes, monocytes, dendritic cells	Fc γ receptor IIIa	Like CD14 lack of expression in monocytic neoplasms is common, in some NK-/T-cell lymphoma
CD19	M	B cells	Coreceptor with CD21; first B-cell antigen expressed	Pan-B-cell marker, utilized mostly in flow cytometry studies, helpful marker to identify immature B cells as it is expressed before CD20
CD20	M+C	B cells	Membrane-spanning 4-domain subfamily A member 1, expressed early in B-cell development and continues to be expressed until downregulation in plasma cells	Routinely used to evaluate for staging of non-Hodgkin lymphoma (note weak expression in CLL), Hodgkin lymphoma, and leukemia, expression parallels PAX-5 and is more specific than CD79a for B cells, targetable therapy available
CD21	M	Follicular dendritic cells within germinal center of reactive lymphoid aggregates	Complement component receptor 2 utilized by EBV to infect B cells, also forms B-cell coreceptor complex	Positive in follicular dendritic cell neoplasms, classification of mature B-cell lymphomas (FL), strong expression in CLL associated with increased prolymphocytes suggesting aggressive disease
CD22	M+G	B lymphocytes	Member of sialic acid-binding Ig-like lectin family, binds sialic acid residues for cell adhesion and signaling	Positive in B-cell lymphomas and leukemias including HCL, aberrant expression in some AMLs, targetable therapy available, expressed in basophils described using flow cytometry [24–26]
CD23	M	B lymphocytes, macrophages, follicular dendritic cells within germinal center of reactive lymphoid follicles	IgE receptor with a role in B-cell activation and growth	Classification of mature B-cell lymphomas (CLL), positive in follicular dendritic network
CD25	M+C	B cells, T cells, macrophages, monocytes, megakaryocytes, adipocytes	IL-2 receptor α chain; transmembrane molecule expressed by activated T cells, B cells, macrophages, and monocytes	Evaluation of HCL, HTVL-1-associated T-cell lymphoma/leukemia, neoplastic mast cells, targetable therapy available
CD26	M	T lymphocytes, NK cells, osteoclasts	Dipeptidyl peptidase-4, multifunctional protein associated with regulating inflammatory response and tumorigenesis, expressed in various hematologic, epithelial, and endothelial cells	Decrease or absent in MF and SS, increased expression in CD30+ ALCL, T-ALL, MM, and CLL [27, 28]
CD27	M	T and B lymphocytes, plasma cells	Member of the TNF-receptor superfamily, required for generation and long-term immunity	Decreased expression in plasma cell neoplasms associated with aggressive disease [29]

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
CD30	M+C+G	Not expressed, except in eosinophil granules (false positive)	Member of TNF family, CD153 receptor, involved in lymphocyte activation and T-cell-mediated cell death	Positive in CHL, ALCL, some DLBCL, malignant mast cells, mixed germ cell tumor, and embryonal carcinoma; targetable therapy available [30]
CD31	M	Megakaryocytes, platelets, granulocytes, monocytes, endothelial cells	Primary adhesion molecule between endothelial cells and lymphocytes	Use not recommended given nonspecific staining [31]
CD33	M+C	Granulocytes (expression varies), monocytes and macrophages (strong), mast cells	Transmembrane protein present on committed myeloid stem cells that are downregulated with granulocyte maturation and strong in monocytes	Helpful in AL workups with a panel of markers as both AML and ALL can be positive, positive in benign and malignant mast cells, rare expression in plasma cell neoplasms [32]
CD34	C+M+G	Myeloblasts, lymphoblasts, promonocytes, blood vessels, megakaryocytes, stromal cells	Glycophosphoprotein that has a role in cell adhesion and inhibition of hematopoiesis	Primarily used to detect myeloblasts and lymphoblasts; not all neoplastic blasts express CD34 (APL), also used to evaluate angiogenesis and nonhematopoietic neoplasms (GIST, angiosarcoma, SFT) [23, 33]
CD35	M	Follicular dendritic cells, erythrocytes, B- and T-lymphocyte subsets, granulocytes	C3b/C4b receptor; utilized in immune complex uptake and retention especially within lymphoid follicles	Highlights germinal centers uniformly unlike CD21 and CD23, also positive follicular dendritic cell sarcoma [34, 35]
CD38 (VS38)	M	Plasma cells, erythroid/myeloid/lymphoid precursors, monocytes, and osteoclasts	Multifunctional transmembrane protein involved in cell adhesion and calcium regulation	Plasma cell marker, also expressed in B-cell NHLs (including LPL and DLBCL), ALL, and AML; prognostic marker for CLL; targetable therapy available [36–39]
CD42b	M+C	Megakaryocytes, platelets, macrophages	Glycoprotein Ib is a transmembrane protein used to bind vWF	Identifies atypical megakaryocytes in myeloid neoplasms, good sensitivity for megakaryoblasts, positivity in some fungal infections [31, 40, 41]
CD43	M	T cells, granulocytes, monocytes	Sialomucin transmembrane molecule highly expressed on most leukocytes, except resting B cells	Positive in T-cell lymphomas, AL, aberrant expression in mature B-cell lymphomas, mastocytosis, plasma cell disorders, BPDNs, lost in lymphocytes of patients with Wiskott-Aldrich syndrome
CD45 (RA, RB, RC isoforms)	M+C	Most cells negative, positive in blasts, monocytes, lymphocytes, and immature granulocytes	Dominant leukocyte plasma membrane protein tyrosine phosphatase, essential regulator of signal transduction in immune cells; leukocyte common antigen	Used to separate hematopoietic neoplasms from poorly differentiated epithelial and mesenchymal tumors, negative CD45 does not exclude the diagnosis of ALL, some lymphomas, and plasma cell neoplasms

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Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
CD45RO	M+C	Activated lymphocytes, monocytes, blasts, immature granulocytes, plasma cells	Smallest CD45 isoform that has same function as other CD45 isoforms	Same as other CD45 isoforms
CD52 (CDw52)	M	Lymphocytes, monocytes, macrophages	GPI-anchored antigen on T, B cells, monocytes, macrophages associated with complement fixation	Almost universal expression in low-grade B-cell NHL with decreased expression in higher grade lesions, variable expression in T-cell NHL, B ALL, and T ALL, only rare expression in AML; targetable therapy available [42, 43]
CD56 (NCAM)	M	T lymphocytes, NK cells, osteoblasts, endosteal lining cells	Neural cell adhesion molecule (NCAM), membrane glycoprotein belonging to immunoglobulin superfamily, expressed in T-cell subset, NK cells, and neuroendocrine cells	Used most often to differentiate MM from MGUS/reactive plasma cells, also positive in NK-/T-cell lymphomas (T-LGL), BPDCN, myeloid neoplasms (AML, MDS), metastatic diseases (neuroendocrine tumors like neuroblastomas and some sarcomas) [44–47]
CD57	M	No expression or rare NK cells	Glucuronyltransferase gene family protein; marker for NK cells, T-cell subset, and neuroendocrine cells	Positive in some NK-/T-cell lymphomas (especially T-LGL), follicular lymphoma, ALPs, neuroendocrine tumors, and other nonhematopoietic tumors [48–50]
CD61 (Y2/5)	C+M	Megakaryocytes, platelets	Integrin beta-3 encoded by the ITGB3 gene, along with the alpha IIb chain in platelets, participate in cell adhesion as well as cell-surface-mediated signaling	Identify atypical megakaryocytes in myeloid neoplasms, variable staining in megakaryoblasts (CD41b is most sensitive for immature forms), decreased expression if B5 fixative used [40]
CD68	C (granular)	Monocytes, myeloid cells (strongest expression in immature precursors including blasts), megakaryocytes	Lysosomal glycoprotein; marker for mature monocytes, and expressed by dendritic cells, granulocytes, and lymphocytes	Despite low specificity used to suggest monocytic differentiation (PGM1 more specific than KP1 clone, KP1 also stains myeloid sarcoma), increased expression in HLH, 50% of ALL weakly positive in B cells including B-cell NHL, cannot distinguish CML from CMML
CD71	M+C	Erythroid precursors	Transferrin receptor, mediates uptake of transferrin-iron complexes	Detects immature erythroid cells [51]
CD75	M+C	Erythrocytes, B cells	Cell surface sialyltransferase, marker of germinal center	Expressed in B-cell lymphomas including FL, high-grade MALT, HRBCL, and NLPHL
CD79a	M	B cells, megakaryocytes, plasma cells, myeloid precursors	Component of dimeric B-cell receptor that initiates B-cell signaling; sensitive pan-marker for B cells	B and some T-cell lymphomas, plasma cell neoplasms, B ALL, and AML, downregulated in lymphoma associated with immunodeficiencies

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
CD79b	M	B cells	Component of dimeric B-cell receptor that initiates B-cell signaling; later expression then CD79a	Similar to CD79a with decreased or no expression in CLL, HCL, Hodgkin lymphoma, and ALL
CD99	M+C	Blasts, immature granulocytes, plasma cells, and bone marrow lining cells	Transmembrane glycoprotein p30 is a MIC2 gene product expressed to some degree on most tissues	Use limited in bone marrow, but overexpressed in ALL, AML, metastatic Ewing sarcoma, ALK+ ALCL, and some DLBCL
CD103	C+M	T cells, dendritic cells	Mucosal integrin; marker for intestinal intraepithelial lymphocytes	Mainly used for HCL, HCL-v, in other NHLs like ALCL
CD117 (KIT)	C+M	Blasts, granulocytic precursors, mast cells	Type III tyrosine kinase receptor used in cell signal transduction that regulates many cellular activities; found in a variety of normal tissues	Positive in malignant mast cells, AML, and GIST, Ewing sarcoma, germ cell tumors, melanocytic tumors, pulmonary and small cell lung tumors
CD123	M	Plasmacytoid dendritic cells, immature granulocytic precursors	IL3 α-chain receptor marker, involved in proliferation and maturation of B cells	Positive in BPDCN, HCL, BALL, AML with FLT3-ITD mutation, neoplastic mast cells
CD137	C+M	Not expressed	A member of TNF receptor family, regulator of T-cell activation	Expressed in CHL (especially those negative for CD15), follicular dendritic cell neoplasms, large B-cell lymphomas (PBML, DLBCL), T-cell neoplasms (MF, ALK+ALCL, AITL, PTCL) [52, 53]
CD137L	C+M	Rare lymphocytes	Tumor necrosis factor family member, mediates costimulatory and prosurvival signals for T-cell activation and humoral immune response	Used to distinguish reactive lymphoid tissue (negative) from small B-cell lymphomas (positive) including HCL, MCL, FL [52]
CD138	M	Plasma cells	Transmembrane heparin receptor Syndecan-1; marker for plasma cells	Positive in 95% of plasma cell neoplasms, negative in LPL, disrupted staining pattern in fibrotic areas [39, 54, 55]
CD160	M	ND	A mediator through the PI3K/Akt signaling pathway involves cellular activation, survival, and cytokine production	Positive in CLL/SLL and HCL [56, 57]
CD163	C+M	Macrophages and granulocytes	Glycoprotein endocytic scavenger receptor haptoglobin-hemoglobin complex	Positive in histiocytic sarcoma, SHML, and CMML; negative in AML with monocytic differentiation and myeloid sarcoma [57–59]
CD194 (CCR4)	C	Myeloid cells, megakaryocytes, subset of lymphocytes	Chemokine receptor of T helper cells, type 2 associated with GATA3 expression	>50% positivity in tumor cells of PTCL associated with worse prognosis if T-bet and CXCR3 negative [17]

(continued)

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
CD200	M	B cell, T cells, dendritic cells, endothelial cells	Type 1 membrane glycoprotein that is part of Ig superfamily and interacts with CD200R on myeloid/monocyte lineage cells for a suppressive effect on T-cell–mediated immune response, overexpression associated with the ability of tumor cells to escape immune system	Positive in CLL, HCL, B ALL, T ALL, PMBL, MM, ALL, HRS cells of CHL, T cells surrounding LP cells of NLPHL, AITCL, PTCL, DLBCL, neuroendocrine tumors, RCC, and papillary thyroid carcinoma, possibly expressed in AML but studies used flow cytometry [56, 60–65]
CD207	M+C	Not present	Langerin a marker for Langerhans cells of the skin and mucosa is localized in Birbeck granules of cells	Positive in Langerhans cell histiocytosis
CD235a	M	Erythroid precursors	Glycophorin A is a membrane protein of red cells that regulates complement and is a receptor for bacteria, viruses, and *Plasmodium falciparum*	Often negative in erythroblasts, not recommended for erythroleukemia
CD236	M	Erythroblasts	Glycophorin C is a membrane protein of red cells, expressed earlier than CD235a	Can be negative in erythroblasts, caution when using to exclude erythroleukemia
CD278/ICOS	M	Not detected	Inducible T-cell costimulator, member of CD28 and CTLA-4 receptor family	Positive in follicular T helper cells [66]
CD279 (PD-1)	M	Lymphocytes that are interstitial or associated with reactive lymphoid aggregates	Type 1 membrane protein that is part of the CD28 family of coreceptors and regulates T-cell receptor signals	Positive in T-cell lymphomas (AITL, PTCL, ATLL, F, ALCL), CLL, Rosai-Dorfman disease, Castleman disease, NLPHL, FL, and MZL [67]
CD303 (BDCA-2/ CLEC4C)	M+C	Granulocytes and plasmacytoid dendritic cells	A type II c-type lectin. Cross-linking CD303 results in the inhibition of IFN-I production via tyrosine phosphorylation and Src kinase	Positive in normal plasmacytoid dendritic cells and blastoid plasmacytoid dendritic cell neoplasm
Clusterin	M+C+Golgi	Megakaryocytes, dendritic cells	Heterodimeric glycoprotein expressed in a variety of tissues associated with reproduction, lipid transport, complement regulation, apoptosis, and alpha granules of megakaryocytes	Golgi staining characteristic of ALCL, follicular dendritic cell tumor, membranous staining patterns typically seen in other T-cell lymphomas, DLBCL, CHL, and metastatic tumors (including pancreatic adenocarcinoma, prostatic carcinoma, and breast tumors [68, 69]
CXCL13	C+M	Not expressed	Cytokine of CXC chemokine family; markers for follicular center T helper cells	Limited use in AITL since CXCL+ follicular dendritic cells and CD10+ neoplastic cells present in limited number of specimens [70]
CXCR3	C	Not described	Chemokine receptor of T helper cell, type 1, associated with T-bet expression	Greater than 20% expression associated with good prognosis in PTCL [17]

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
Cyclin D1 (BCL1)	N	Endothelium	Central role in cell cycle by regulating cyclin-dependent kinases in G1 cell cycle as the cell prepares to initiate DNA synthesis	Detects B-cell malignancies with t(11;14)(q13;q32) translocation most notably MCL, occasionally positivity in B-PLL, HCL, plasma cell neoplasms, and DLBCL [71, 72]
Cyclin D2	C	Lymphocytes, plasma cells	Central role in cell cycle by regulating cyclin-dependent kinases in G1 cell cycle as the cell prepares to initiate DNA synthesis	Positive in DLBCL, FL, MZL, MCL, CLL, BL, BALL, PTCL AITL, ALCL, CHL, and AML
Cyclin D3	C+N	Lymphocytes, plasma cells, megakaryocytes	Central role in cell cycle by regulating cyclin-dependent kinases in G1 cell cycle as the cell prepares to initiate DNA synthesis	Positive in DLBCL, FL, MZL, MCL, CLL, BL, BALL, PTCL AITL, ALCL, CHL, and AML
DBA44	C+M	Lymphocytes (membranous)	Immunoglobulin M heavy chain subclass	Cytoplasmic projection noted in HCL (>95% +), some B-cell lymphomas (LPL, CLL), rare MF
DcR3	C	Not expressed	Member of the tumor necrosis factor receptor superfamily that prevents cytokine responses	Positive in aggressive DLBCL and metastatic tumors [73]
BZLF-1	N	Not detected	A EBV protein that indicates transition from latent to lytic phase	Detects EBV but is typically negative in HL and nasopharyngeal carcinoma
EBI3	C+M	Not detected	A member of the hematopoietin receptor family related to IL-12 p40 homologue and induced by EBV	Positive in c-Myc negative lymphomas with an inverse correlation between the EBI3 expression and c-MYC translocation, positive in HRS cells, HL, ATL [74, 75]
EBER	N	Not detected	EBV-encoded RNA, EBER1 and EBER2, expressed in latently infected cells	Most sensitive marker for EBV-related malignancies including lymphomas, carcinomas, and sarcoma
EBNA-1/EBNA-2	N	Not detected	EBV-associated protein expressed in latently infected cells	Detects EBV but is typically negative in HL and nasopharyngeal carcinoma
LMP-1	C+M (granular)	Granules of eosinophils and plasma cells	EBV-associated protein expressed in latently infected cells	Detects EBV as effectively as EBER in CHL, PTLD, and infectious mononucleosis falsely negative in other lymphomas (BL), decalcification, poor fixation effects retrieval, nonspecific staining at edge of tissue [76–79]
E-cadherin	M	Erythroblasts	Epithelial calcium-dependent adhesion protein, transmembrane protein expressed primarily in epithelial cells to establish and maintain cell-to-cell adhesion, also expressed in erythroid precursors	Can be combine with CD117 to determine erythroblasts (often the only specific erythroid marker positive) [80]

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Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
EMA (MUC-1)	C+M	Weak expression in monocytes, granulocytes	Episialin-glycoprotein	Positive in some ALCL, NLPHL, plasma cell neoplasm, carcinoma, AML
Fascin	C	Endothelial cells, osteoclasts, dendritic cells	Actin bundling protein that creates microfilament bundles used for cell mobility; marker for follicular dendritic cells, histiocytes, and EBV-infected immunoblasts	Positive in HRS cells of CHL, diffuse positivity in numerous spindle cell neoplasms limits its use, T- and B-cell lymphomas including ALCL, carcinoma, expression varies and maybe related to technical processing [5, 81, 82]
FLIP	C	Mononuclear cells (granulocytes, erythrocytes, monocytes), megakaryocytes	Fas-associated death domain-like interleukin-1β-converting enzyme inhibitory protein, negatively modulates apoptosis	Worse prognostic marker for NHL, use in bone marrow limited given expression in hematopoietic tissue which varies with antibody [83–85]
FOXP1	N	Mononuclear cells (granulocytes and erythrocytes)	Forkhead Box P1 is a transcription factor important for cell growth and differentiation	Overexpression in activated B-cell DLBCL, AML, MDS, ALL, and malignant plasma cells, clinical utility limited [85–90]
GATA3	N		Transcription factor of T helper cell type 2 T_H2 that regulates cell differentiation by cytokine secretion and inhibition of IFN-γ through repression of IL-12 signaling	>50% positivity in tumor PTCL associated with worse prognosis in PTCL if T-bet and CXCR3 negative [17]
GCET1	C	Not expressed	A serine protease inhibitor and specifically expressed in GC B cells	Positive in GC lymphocytes, FL, BL, DLBCL, THRBL, NLPHL [91]
Granzyme A, B, M	C (granular)	Rare cytotoxic T cells	Neutral serine proteases inside granules of T cells and NK cells	Positive in some T-cell lymphomas (T-LGL, ALCL) [50, 92]
Hemoglobin A	C	All stages of RBCs	Tetrad of globin chains used to transport oxygen to tissue	Use is limited since expressed in late-stage erythrocytes
HGAL	C	Not expressed	GC-specific gene involved in negative regulation of lymphocyte and lymphoma cell motility	Positive in B-cell lymphomas especially those with GC derivation (FL, BL, DLBCL, PMBL); however, it is not a specific or sensitive as Han Criteria for GC-derived lymphomas, positive in Hodgkin and LP cells [93]
HHV8	C	Not expressed	Kaposi's sarcoma-associated herpesvirus, formal name—human herpesvirus type 8	Positive in PEL, large B-cell lymphoma arising in HHV8 associated MCD [94]
HLA-DR	C+M	Myeloid precursors, B and T lymphocytes, monocytes	Class II human leukocyte antigens are used for antigen presentation	Negative in APL
Immunoglobulins heavy chains (IgA, IgD, IgG, IgM)	C+M	Plasma cells and B lymphocytes	Antibody proteins synthesized by B cells and plasma cells	Used to detect monoclonal lymphoid (IgM) and plasma cell (IgG) populations; however, artifactual membranous staining is a common limiting interpretation

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
Immunoglobulins—light chains (ƙ/ƛ)	M or C	Plasma cells and B lymphocytes	Antibody proteins synthesized by B cells and plasma cells	Used to detect monoclonal populations; however, artifactual membranous staining is a common limiting IHC interpretation, in situ hybridization more common used
IMP3/KOC	C	Not described	Insulin-like growth factor II mRNA-binding protein mediates organogenesis, mRNA trafficking, and cell growth	Positive in reactive germinal centers; however, numerous hematologic malignancies show positivity that are not necessarily of GC origin
IRTA1	M+C	Not expressed	Member family of IRTA1-IRTA5 cell surface receptors involved in B-cell-mediated immune responses, intercellular communication, cell adhesion, and cell migration	May be helpful in distinguishing MZL (positive) from LPL (negative) with IHC performing better than ISH, also positive in splenic MZL, rare FL, some DLBCL, and ALL, studies not performed on bone marrow specimens
KLF4	N	Monocytes, neutrophils, and endothelial cells	Transcription factor important in cellular differentiation and proliferation, especially epithelial cells of GI tract, reproductive tract, lung, and vascular endothelium; role in monocyte differentiation	Positive in disease with mature and immature monocytes, including AML with and without monocytic differentiation and CMML [21]
LAT (linker for activation of T-cells)	C+M	T cell, NK cells, megakaryocytes, mast cells	Involved in T-cell activation and platelet aggregation	Identify atypical megakaryocytes, megakaryoblasts, and mast cells [95, 96]
LEF1	N	Lymphocytes, variable weak staining in rare myeloid precursors	Transcription factor of lymphoid cells, involved in Wnt/β-catenin pathway and early lymphocyte and myeloid development	Positive CLL, BL, T cell lymphomas, T and B ALL, downregulation in myeloid neoplasms associated with disease progression and high expression in myeloid neoplasms favorable [97–99]
LMO2	N	Immature erythroid and myeloid precursors, megakaryocytes, endothelium	LIM Domain Only 2 contains transcription factors that play an important role in angiogenesis and erythropoiesis	Positive in follicular-derived tumors (FL, BL, DLBCL, PMBCL), LP cell of NLPHL, T ALL, B ALL, AML and NK lymphomas, decalcification may weaken expression [100]
Lysozyme	C	Granulocytes (weak), monocytes	Peroxidase molecule with bactericidal activity	Used mainly with CD14 and CD68 to suggest monocytic differentiation
Mast cell tryptase	C	Mast cells, basophils	Serine protease; marker of mast cells	Used to detect mast neoplasms
MAL	M+C	Not described	Membrane protein associated with lipid raft organization during T-cell activation and important in apical transport in epithelial cells	Used mainly to differentiate PMBL (positive) from DLBCL (negative), occasionally expressed in CHL and mediastinal gray zone lymphoma, use in bone marrow not well described [101]

(continued)

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
MATK	N+C	Hematopoietic cells including megakaryocytes	MATK gene produces enzyme involved in signal transduction of hematopoietic cells and plays an inhibitory role in T-cell proliferation via inactivating Src family kinases	Expressed in several B-and T-cell lymphomas (cytoplasmic) and nuclear expression unique for type II enteropathy associated T-cell lymphoma [102]
MET	M	Not described	Receptor for hepatocyte growth factor and essential for embryonic development and wound healing	Normally expressed by cells of epithelial origin, positive in some HRS cells of CHL but limited utility in bone marrow
MIB-1(Ki67)	N	Erythroid: 75–95% Myeloid: 15–80% Megakaryocytes: 10–20%	Nuclear proliferation antigen expressed during G1, S, G2, and M phases	Limited value in bone marrow given baseline rates, may help with detection of low-grade lymphomas which have a low proliferation rate [101]
MNDA	N	Myeloid cells, monocytes	Myeloid nuclear differentiation antigen: A member of the interferon (IFN)-regulated 200 family to mediate protein-protein interactions	Positive in myelomonocytic cells, most of MZL, some of CLL/SLL, MCL, LPL, HCL, and DLBCL [103–105]
MUM1/IRF1	N	Plasma cells	Belongs to interferon regulatory factor (IRF) family, important roles include resistance against viral infections and cell proliferation a	Nonspecific marker expressed in various lymphoproliferative disorders and used for subtyping DLBCL, where its expression in the absence of CD10 indicates activated be cell type plasma cell neoplasm [106, 107]
c-MYC	N	Immature hematopoietic cells (<20% of total cellularity)	Protein of an oncogene *c-MYC*	Increased expression in numerous lymphomas and leukemias, BL classically with >90% positive while other lymphomas show lower expression, important for DLBCL subtyping and double expressor status (BCL-2 and Myc) but expression does not correspond to gene rearrangements
MYD88	C	High expression in hematopoietic elements	Myeloid differentiation primary response gene 88, adapter protein in Toll-like receptor, IL-1 receptor and IL-18 receptor pathways to activate the transcription factor NF-κB	Positive in LPL/WM, however strong reactivity in bone marrow elements limits utility in bone marrow
Myeloperoxidase (MPO)	C	Granulocytes	Peroxidase molecule with bactericidal activity	Lineage-specific marker for myeloid differentiation; however, B-ALL can be MPO positive (weaker) especially with polyclonal antibodies

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
NPM1	C (MT) N(WT)	Normal hematopoietic element nuclear staining only	B23 protein that resides primarily nucleus, but shuttles continuously between the nucleus and the cytoplasm	Expressed in AML, APL, and ALCL with t(2;5), can be used for disease monitoring, 3 antibody types: combined monoclonal anti-NPM1 wt+mt, most reliable [108–110]
Oct2	N	Rare lymphocytes	POU family of transcription factor required for immunoglobulin and germinal center formation with co-activator BOB.1	Overexpressed in B-and T-cell lymphomas, MM, some ALs and NLPH "popcorn" cells, lost in HRS cells of CHL
P53	N	Occasional mononuclear cells with weak to moderate (1–2+) nuclear staining	Surrogate for mutation of tumor suppressor geneTP53 mutation which is responsible for a number of cellular activities including cellular growth arrest, apoptosis, DNA repairs, and angiogenesis	Biomarker in hematologic neoplasms, >5% of tumor cells should be positive, possibly associated with favorable prognosis, if negative it does not exclude mutation, PCR is more sensitive, epithelial cells positive [111, 112]
P63	N	Not detected	Surrogate for TP63 mutation responsible for cell cycle arrest, apoptosis in response to DNA damage, ectoderm development, maternal reproduction, and metabolism	Biomarker in hematologic neoplasms, associated with TP53 mutation and unfavorable prognosis, if negative it does not exclude mutation, PCR is more sensitive, epithelial cells positive [113]
Parvovirus B19	N	Not expressed	Virus that infects erythroid precursors, hepatocytes, and other cells and only replicates in erythroid precursors	Useful for differentiating erythrocytopenia from MDS, and bone marrow failure
PAX5/BSAP	N	B lymphocytes	B-cell-specific activator protein expressed in mature B cells and some early B cell progenitors, it is lost in plasma cells	Used for B-cell lineage; however, B-ALL may be negative and some AML (especially t(8;21) may express, used after rituximab therapy to detect residual disease, can separate Hodgkin cells from megakaryocytes
PD1 (CD279)	M+C	Rare T lymphocytes	Programmed death 1, a member of the extended CD28/CTLA-4 family of T-cell regulators, PD-1 and its ligands negatively regulate immune responses	Nonspecific marker positive in several hematologic malignancies, particularly used for CD3+/PD-1+ T cells ringing LP cells in NLPHL and AITL, positivity may suggest the potential use of checkpoint inhibitors see PDL1 [67, 114]
PDL1 (CD274)	C+M	Macrophages	Cell surface glycoprotein that regulates the cellular immune response and serves as a targetable immune checkpoint molecule	If expressed on tumor cells can be utilized with background PD1 positive T cells to induce immune attack on tumor [115, 116]
PDL2 (CD273)	C+M	Not detected	Cell surface glycoprotein that regulates the cellular immune response similar to PDL1 and serves as a targetable immune checkpoint molecule	If expressed on tumor cells can be utilized with background PD1 positive T cells to induce immune attack on tumor [115]

(continued)

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
Perforin	C	Not detected	One of the cytotoxic granules in T cells and NK cells	Positive in some T-cell and NK-cell lymphomas
PKC-β	C+M	Not described	Protein kinase C is a family of enzymes involved in controlling other protein functions through phosphorylation	Biomarker in DLBCL associated with inferior prognosis [117]
Podoplanin (D2-40)	M	Lymphatic vessels located in periosteum if present	Type-1-heavily O-glycosylated glycoprotein present in lymphatic cells and renal podocytes	Used to detect follicular dendritic cell networks and tumors, positive in osteogenic and chondrogenic tumors [118–120]
PRDM1 (BLIMP1)	C+M	Plasma cells	Human homologue of Blimp-1 is a zinc finger containing transcriptional factor affecting terminal differentiation of B cells to plasma cells	Expressed in reactive and neoplastic plasma cells, as well as B and T lymphomas (including, LPL, plasmablastic DLBCL, PTCL ALCL) and rare CHL [121–123]
PU.1	N	Granulocytes including early precursors, hematogones, monocytes, mast cells, and osteoclasts	Ets transcription factor essential for early development of B-lymphoid cell and myeloid precursors	Utility limited in bone marrow positive in multiple lineages (including T ALL) and differentiation [124]
REL	C+M (wild type) N (mt)	Scattered positivity in mononuclear cells, not well described	Proto-oncogene protein encoded by the *REL* gene is a member of the NF-κB family of transcription factors and plays role in cell growth, differentiation, and survival	Amplified or aberrant expression in nucleus in several B-cell lymphomas, including DLBCL, PMBL, HL, clinical use limited
S100	N+C	Stroma cells and dendritic cells	Low molecular weight protein, present in cells derived from the neural crest	Positive in Langerhans cell tumor, interdigitating dendritic cell sarcoma, Rosai-Dorfman disease
Serglycin	C+M+perinuclear	Myeloblasts, promyelocytes, myelocytes, megakaryocytes, endothelial cells	Hematopoietic proteoglycan core protein or secretory granule proteoglycan core protein	Perinuclear expression suggests AML as it is negative in ALL and mature lymphoma [125]
SOX11	N	Granulocytes, megakaryocytes	Neural transcription factor, expressed during fetal life, has several important roles during organogenesis	Used mainly in MCL, especially the blastoid variant negative cyclin D1, positivity also noted in some HCL, BL, ALL, and epithelial tumors, neuroectodermal tumors (Ewing, neuroblastoma) [126–129]
Spectrin	C+M	Early erythroblasts (cytoplasmic), late erythroblasts(membranous)	Cytoskeleton component seen early in erythroid differentiation	Erythroblast identification [130]
Stabilin-1	C	Sinusoidal endothelial cells	Transmembrane receptor protein that may function in angiogenesis, lymphocyte homing, cell adhesion, or receptor scavenging	May home and adhere metastatic tumor cells
STAT3	N (mt) C (wt)	Nuclear staining not present, but cytoplasmic variably expressed in hematopoietic elements	Cytoplasmic transcription factor that mediates expression of a variety of genes playing key role in growth and apoptosis	Mutant forms expressed in nuclei of lymphomas and can be used to show clonal population especially in ALK-ALCL and TLGL [131, 132]

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
Stathmin (STMN1)	C	Myeloid and erythroid precursors, megakaryocytes	Regulatory protein of microtubule dynamics and is associated with erythroid and megakaryocytic differentiation in bone marrow	Increased expression in high risk MDS, MDS with increased blasts, FL and some T-cell lymphomas [133, 134]
Survivin	N	Plasma cells, megakaryocytes	Inhibitor of apoptosis that acts to inhibit terminal effectors capases	Positive in aggressive DLBCL, HL, T-cell lymphomas; however, staining not reliable in bone marrow [73, 135]
T-bet/TBX21	N	Rare reactive T cells	T-box transcription factor, utilized in Th1 T-cell development and in B cell for immunoglobulin isotype switching	Expressed in B- and T-cell lymphomas including CHL, can be used in ALL to suggest B cell, >20% positivity in PTCL associated with good prognosis [17, 136–139]
TCL1	N+C	Lymphoid precursors	Proto-oncogene located on chromosome 14q32	Positive in 90% of TPLL, blastic plasmacytoid cell neoplasm, T ALL, and B-cell lymphomas (CLL, MCL, BL, FL, DLBCL) [140, 141]
TCR beta F1	M+C	Rare alpha beta cells	Member of immunoglobin super family and component of CD3/TCR complex	Used to assess clonality in T-cell lymphomas [142, 143]
TCRδ1	M	Rare gamma delta T cells	Member of immunoglobin super family and component of CD3/TCR complex	Positive in PCGD-TCL, most HSTLs [143]
TdT	N	Lymphoid precursors s	Terminal deoxynucleotidyl-transferase; DNA polymerase	Positive in B ALL, T ALL, some AMLs, increased numbers seen in reactive conditions, but >5 cell clusters suggest malignant process [144]
TIA-1	C	Monocytes, granulocytes, activated T and NK cells	1 of 3 cytotoxic molecules (TIA-1, perforin, granzyme B) stored in cytoplasmic granules of T and NK cells, unlike other cytotoxic molecules expressed regardless of activation status	Cytotoxic molecules expressed in T-cell and NK-cell lymphomas (T-LGL, HSTCL, TPLL usually only express TIA-1), interpretation could be difficult since expressed by many cells
TNFAIP2	M	Not described	A protein upregulated in response to TNF signaling that may play a role in myelopoiesis	Positive in follicular dendritic cells, interdigitating dendritic cells, macrophages, HRS cells of CHL, LP cells of NLPHL and PMBL [145]
TRAF1		Not described	signaling molecule and NFκB target gene, mediates anti-apoptotic signals from TNF receptors	c-rel and TRAF1 expressed in 80% of CHL and 50% of PMBL, only rarely in DLBCL-NOS (100,101)
TRAP	M	Osteoclasts, macrophages	Tartrate-resistant alkaline phosphatase, glycosylated monomeric metalloenzyme	Positive in HCL, ENMZL, SMZL Gaucher's disease, and bone diseases [4]
Ulex europaeus, agglutinin-1	C+M (granular)	Megakaryocytes, vascular endothelium	Lectin from gorse seed	Detects megakaryocytes in normal marrow, MDS, and CMPD [146–148]

(continued)

Table 32.1 (continued)

Antibody (other names)	Cellular staining pattern	Distribution pattern in normal bone marrow	Targeted antigen function	Key applications and pitfalls
Von Willebrand Factor (VWF)	C	Megakaryocytes, endothelium	Large, multimeric glycoprotein found in platelet α-granules and endothelial cell cytoplasm that is essential for hemostasis	Detects normal/abnormal megakaryocytes in MDS, with decrease sensitivity in megakaryoblasts, high background staining noted [31, 40, 146]
VEGF	C	Myeloblasts, promyelocytes, myelocytes, megakaryocytes, lymphocytes, plasma cells	Essential mediator of leukemia-dependent angiogenesis	Helpful in defining immature myeloid cells, increased expression in hematolymphoid neoplasm, and may have prognostic and treatment implications [149]
Vimentin	C+M	Stroma, immature precursors of all lineages, mature monocytes, granulocytes, T cells	Intermediate filament protein	Negative staining most useful, negative in AML-MK differentiation, TCHRL, NLPHL
XBP1	C	Not described	Transcription factor that regulates MHC-II to differentiate plasma cells and eosinophils, as well as control angiogenesis and stress response	May aid in PBL diagnosis along with BLIMP1 [123, 150]
ZAP70	M	Precursor B cell, mature T cells	Zeta-chain-associated protein kinase 70, member of protein-tyrosine kinase family, expressed normally in T cells and NK cells playing critical role in T-cell signaling	Prognostic marker for B-CLL, expression is not limited to CLL and is found in other NHLs and B ALL, weaker staining noted in bone marrow specimens

AITL angioimmunoblastic T-cell lymphoma; *ALCL* anaplastic large cell lymphoma; *ALK* anaplastic lymphoma kinase; *ALL* acute lymphoblastic leukemia; *AML* acute myeloid leukemia; *APL* acute promyelocytic leukemia; *BCR* B-cell receptor; *BL* Burkitt lymphoma; *BPDC* blastic plasmacytoid dendritic cell neoplasm; *B-PLL* B-cell prolymphocytic leukemia; *C* cytoplasmic staining; *CALLA* common acute lymphocytic leukemia antigen; *CD* cluster of differentiation; *CHL* classical Hodgkin lymphoma; *CLL* chronic lymphocytic leukemia; *CR3* complement receptor 3 *DcR3* decoy receptor 3; *DLBCL* diffuse large B-cell lymphoma; *DLBCL/BL* B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma; *EATL* enteropathy-associated T-cell lymphoma; *EBV* Epstein-Barr virus; *EBI3* Epstein-Barr virus-induced gene 3; *EC* embryonal carcinoma; *EMA* epithelial membrane antigen; *ET* essential thrombocytosis; *FL* follicular lymphoma; *G* Golgi accentuation pattern; *GC* germinal center; *GI* gastrointestinal; *GIST* gastrointestinal stromal tumor; *GPI* glycosylphosphatidylinositol; *HCC* hepatocellular carcinoma; *HCD* heavy chain disease; *HCL* hairy cell leukemia; *HGAL* human germinal center–associated lymphoma; *HIV* human immunodeficiency virus; *HLH* hemophagocytic lymphohistiocytosis; *HRS cell* Hodgkin and Reed-Sternberg cell; *HS* histiocytic sarcoma; *HSTL* hepatosplenic T-cell lymphoma; *ICOS* inducible T-cell costimulator; *IgH* immunoglobulin heavy chains; *IgL* immunoglobulin light chain; *IMP3* insulin-like growth factor II mRNA-binding protein 3; *IRTA1* immunoglobulin superfamily receptor translocation-associated 1; *KLF4* Krüppel-like factor 4; *KIR* killer-cell immunoglobulin-like receptors; *LFA-1* lymphocyte function-associated antigen 1; *LEF1* lymphoid enhancer-binding factor-1; *LHCDD* light and heavy chain deposition disease; *LOM2* LIM domain only 2; *LP cell* lymphocyte predominant cell; *LPL* lymphoplasmacytic lymphoma; *Mac-1* macrophage-1 antigen; *MAL* myelin and lymphocyte protein; *MATK* megakaryocyte-associated tyrosine kinase; *MCD* multicentric Castleman disease; *MCL* mantle cell lymphoma; *MDS* myelodysplastic syndrome; *M* membranous staining; *MF* mycosis fungoides; *MHC* major histocompatibility complex; *MM* multiple myeloma; *MNDA* myeloid cell nuclear differentiation antigen; *MPN* myeloproliferative neoplasm; *MST1R* macrophage-stimulating 1 receptor (also known as CD136); *MT* mutant; *MYD88* myeloid differentiation primary response gene 88; *MZL* marginal zone lymphoma; *NHL* non-Hodgkin lymphoma; *NK* nature killer cell; *PCGD-TCL* primary cutaneous gamma delta T-cell lymphoma; *PD-1* programmed cell death protein 1; *PD-L1 and –L2* programmed death ligand 1 and 2; *PMBL* primary mediastinal (thymic) large B-cell lymphoma; *PMF* primary myelofibrosis; *PNET* primitive neuroectodermal tumor; *PTL* peripheral T-cell lymphoma; *RCC* renal cell carcinoma; *RNA* ribonucleic acid; *RS cell* Reed-Sternberg cell; *SIGLEC* sialic-acid-binding immunoglobulin-like lectins; *SFT* solitary fibrosis tumor; *SMZL* splenic marginal zone lymphoma; *SPTCL* subcutaneous panniculitis-like T-cell lymphoma; *SS* Sezary syndrome; *STAT3* signal transducer and activator of transcription 3; *TCL1* T-cell leukemia/lymphoma 1; *TCR* T-cell receptor; *Th* helper T cells; *TNF* tumor necrosis factor; *T-LGL* T-cell large granular lymphocytic leukemia; *TNFAIP2 TNF*-alpha inducible protein-2; *TPL* T-cell prolymphocytic leukemia; *TRAF1* TNF receptor-associated factor 1; *TRAP* tartrate-resistant acid phosphatase; *VEGF* vascular endothelial growth factor; *VWF* von Willebrand Factor; *WM* Waldenstrom macroglobulinemia; *WT* wild type; *XBP1* X-box-binding protein 1

32.2 What factors should be considered when developing a bone marrow processing protocol?

- Preanalytical handling of bone marrow specimens is different from tissue specimens and affects antigen retrieval.
- Optimal histologic processing of the bone marrow biopsy requires proper fixation and either decalcification of the bone biopsy or tungsten-carbide knives for sectioning. Decalcification remains the most widely utilized method despite its adverse consequences to epitope and nucleic acids. A general rule when using decalcification is, "the faster the biopsy is decalcified the faster the epitopes and nucleic acids are destroyed."
- Numerous processing protocols exist and use different fixatives and decalcification solutions (Table 32.2) and each has its own unique advantages and disadvantage. The Geisinger protocol uses B-plus fixative for a minimum of 3 hours and Decalcifier B for 30 minutes to allow for next-day interpretation.
- Immunohistochemical protocols may not work on tissue from an outside institution.

Table 32.2 Different protocols for bone marrow fixation and decalcification

Turnaround time[a]	Fixative	Fixative time	Decalcification	Decal time	Comments
Very short	Acetic acid-zinc-formalin (AZF)	2–72 h	Shandon™ TBD-1™ Decalcifier	30–40 min	Excellent morphology Good IHC Suboptimal nucleic acid preservation
	B-plus fixative	4–6 h	RDO GOLD™ or Decalcifier B™ (HCL-based)	30–60 min	Good morphology and IHC Timing of decalcifier important Poor nucleic acid preservation
	B5 fixative	4–24 h	Surgipath Decalcifier (formic acid-based)	60–90 min	Excellent morphology Good IHC B5 contains mercuric chloride Timing important Fair nucleic acid preservation
	Bouin fixative	4–12 h	–	–	Not recommended for IHC Poor nuclei acid preservation Contains picric acid
	AZF	6–8 h	Gooding and Stewart's	6 h	Hammersmith protocol Excellent morphology Good IHC Good nucleic acid preservation
	Lowy formalin mercuric chloride acid solution	20 h	–	–	1-step fixation/decalcification procedure Contains mercuric chloride Many antigens well preserved
	Schäfer's fixative	6–10 h	14% EDTA	16–24 h	Allows histochemical detection of tartrate-resistant acid phosphatase and naphthol AS-D chloroacetate esterase and specific platelet esterase Good IHC
Lengthy	10% buffered formalin	8–72 h	14% EDTA	16–24 h	Preferred protocol Excellent morphology and IHC Good nucleic acid preservation

h hours; *min* minutes
References: [151–154]
[a]Faster turnaround times achieved by warming decalcifiers to 37 °C and/or utilizing stir bars

32.3 How is immunohistochemistry different from flow cytometric immunophenotyping (Table 32.3)?

- Bone marrow immunophenotyping is primarily performed using flow cytometry and fresh bone marrow aspirate or disaggregated cells from a fresh tissue biopsy.
- Peripheral blood containing abnormal cells can be used. Caution is advised as different phenotypes can be observed between the blood and bone marrow specimens, especially in immature cell populations. When peripheral blasts are ≥30%, the phenotype is usually considered equivalent to bone marrow phenotype and further workup on the bone marrow is not typically required.
- Instances when IHC should be utilized include the following:
  - Flow cytometry phenotype is ambiguous/undifferentiated or insufficient for lineage subtyping.
  - Abnormal cells are unevenly distributed.
  - Bone marrow morphology does not match flow cytometry involvement and/or phenotype.
  - Antigen of interest is only available by immunohistochemistry.

Table 32.3 Comparison of immunophenotyping techniques

	Immunohistochemistry	Flow cytometry
Specimen type	Fixed tissue Limited material (dry tap/hypocellular)	Fresh aspirate/tissue
Turnaround time	Longer (days)	Shorter (hours)
Antigen(s) per study	Single or dual	Multiparametric
Assessment	Architecture & cytologic Subjective interpretation Semiquantitative results	Maturational pattern Less subjective interpretation Quantitative
Sensitivity	Better if nonviable cells present, unevenly distributed cell populations, or specimen hypocellular	Overall more sensitive, MRD monitoring possible
Antibody selection	Typically limited ALK, Annexin A, CD30, Cyclin D1, cytokeratin, EBV, MYC, NPM1, P53	More extensive panels Surface κ/λ light chains, CD41, CD94
Preferred study based on disease	ALK(+) ALCL, CHL, DLBCL, mastocytosis, MPN, metastatic tumor, plasma cell neoplasms	Leukemia, low-grade lymphomas, MDS, monocytoid neoplasms, NK-cell neoplasms

ALK anaplastic lymphoma kinase; *ALCL* anaplastic large cell lymphoma; *CHL* classical Hodgkin lymphoma; *DLBCL* diffuse large B-cell lymphoma; MDS myelodysplastic syndrome, *MPN* myeloproliferative neoplasm; *NK* natural killer cell

32.4 What is the distribution and immunophenotype of normal hematopoietic elements in bone marrow?

- Bone marrow biopsies are small in size (1–2 cm ideally) yet typically represent overall marrow cellularity and distribution of hematopoietic elements.
- Immunohistochemistry can help assess cellular distribution and enumeration, but if abnormal cell population is admixed normal hematopoietic elements, it could also be a potential pitfall if positive staining is of normal cells is misinterpreted.
- Table 32.4 describe normal bone marrow elements and key immunohistochemical features.

Table 32.4 Common IHC stains used to identify hematopoietic elements

Hematopoietic element	Immunohistochemical stains	BM distribution and enumeration
Immature blasts (0–3%)	CD34, CD99, CD117, TdT, CD45(dim)	Adjacent to bone trabeculae and adventitial of arterioles, <5% of cellularity
Granulocytes[a] (40–65%) Promyelocytes Myelocytes Metamyelocytes Bands/neutrophils	CD13, CD15, CD33, CD117, MPO, lysozyme	Maturing granulocytes radiate from center of intertrabecular, majority of cells (M:E ratio = 3:1)
Eosinophils (1–5%)	CD13, CD15, CD33 Granules can show nonspecific staining of other markers	Individually intertrabecular cells
Basophils (0–1%)	2D7, BB1, CD33, CD123	Individually intertrabecular cells, cannot be seen on H&E
Erythrocytes (15–25%)	Erythroblasts: CD117, CD236 (GPC), E-cadherin, Spectrin (C) Late precursors: CD71, HGB-A, Spectrin (M), CD235a (GPA)	Intertrabecular islands
Megakaryocytes (<1%)	CD31, CD34, CD42b, CD61, LAT, UEA-1, VWF Numerous markers display weak cytoplasmic staining	Paratrabecular large multinucleated cells with abundant cytoplasm found individually or in loose clusters of 2–5 cells
Lymphocytes (10–15%) T lymphocytes[a] B lymphocytes[a] Natural killer (NK) cells	CD2, CD3, CD4, CD5, CD8 CD19, CD20, CD22, CD79a, PAX-5 CD2, CD7, CD8, CD56, CD57, TIA-1, granzyme-B	Usually scattered individual cells T cells > B cells > NK cells, small interstitial lymphoid aggregates present in elderly
Plasma cells (0–1%)	CD38, CD138, ƙ/ƛ light chains, CD20(v), CD79a(v)	Individually scattered cells, small cell clusters adjacent to blood vessels
Monocytes/histiocytes (<1%)	CD4, CD14, CD15, CD16, CD64, CD163, KLF4, lysozyme	Inconspicuously located in marrow
Mast cells (<1%)	CD117, tryptase	Periphery of lymphoid follicles and small arteries
Dendritic cells (<1%)	Langerhans: S-100, CD1a CD16 Follicular dendritic: CD21, CD23, CD35 Plasmacytoid dendritic: CD68, CD123	Rare in bone marrow
Osteoclasts (<1%)	CD26, CD38	Large multinucleated cells adjacent to bone

GLY A glycophorin A; *GLY C* glycophorin C; *HGB* hemoglobin; *M* membranous, *M:E* myeloid to erythroid, *MPO* myeloperoxidase
[a]Lineage-specific markers: Granulocytes—MPO; B lymphocytes—CD19, CD22, CD79a, PAX-5; T lymphocytes—CD3

32.5 What is the distribution and immunophenotype of nonhematopoietic elements in normal bone marrow (Table 32.5)?

Table 32.5 Immunohistochemical staining of nonhematopoietic elements in normal bone marrow

Nonhematopoietic elements	Immunohistochemical stains	BM distribution and enumeration
Adipocytes	CD10, CD25	Largest interstitial cells that increase in amount with age
Connective tissue, fibroblasts, myofibroblasts	CD10, Cyclin D1	Delicate interstitial network
Endothelium	CD13, CD31, CD34	Flattened delicate cellular channels
Trabeculae-associated cells (osteoblasts, endosteal lining cells)	CD56	Mononucleated cells adjacent to bone

32.6 How is immunohistochemistry utilized to assess acute leukemia/immature morphology?

- If immunohistochemistry is utilized for initial diagnosis and classification recommend a judicious panel based on morphologic findings (Table 32.6).
- Misclassification is more likely if only a few stains are utilized. For example, myeloperoxidase (MPO) a myeloid lineage-specific marker, can be observed in B-cell acute lymphoblastic leukemia (especially if the polyclonal antibody is utilized). If only MPO is used, misclassification is possible.
- Olsen et al. proposed a stepwise approach for working up acute leukemia (Fig. 32.1), which minimizes cost and unexpected findings associated with larger immunohistochemical panels. A panel is recommended over individual lineage-specific markers since many antigens are aberrantly expressed in various leukemias (Table 32.7).
- Immunohistochemical staining patterns also may suggest various cytogenetic and molecular findings (Tables 32.8 and 32.9). However, it should not be solely utilized for genetic subtyping, and if tissue is negative, it does not exclude those abnormalities as processing may have affect antigen retrieval.
- Differential diagnosis of immature cells should include lymphomas with blastoid morphology (mantle cell lymphoma, high-grade B-cell lymphoma) and blastic plasmacytoid dendritic cell tumor (Tables 32.10 and 32.11).

Table 32.6 Assessment of acute leukemia by morphology and immunohistochemistry

Lineage	Nuclear features/ chromatin pattern	Cytoplasm features/granules	Key IHC markers (positive unless noted)
Lymphoid B cell T cell	Round and smooth, Finely dispersed	Scant, blue gray, Rare granules	TdT, HLA-DR, CD45(dim), CD99[a] B cell: CD19[b], CD10, CD22, CD79a, PAX-5 T cell: CD1a, CD2[b], CD3, CD4/8, CD5, CD7, CD99
Myeloid	Round-reniform, Coarse vesicular	Pink-blue, +/– Granules +/– Auer rods	MPO[b], CD13, CD33, CD34, CD45 (dim), CD117, HLA-DR
Promyelocytic	Reniform-bilobed Dispersed chromatin	Pink-blue, ++ Granules ++ Auer rods[e]	CD34(–), HLA-DR(–), CD45 (dim), CD13, CD15, CD2, E
Monocytic	Bilobed-indented Fine to coarse	Blue-gray, +/– fine granules	CD34(–), HLA-DR, CD14, CD45(dim), CD64, CD68[c], CD163, KLF4
Erythroid	Round-oval Coarse and dense	Vacuoles None to rare	CD34(–), HLA-DR, CD13, CD33, CD45 (dim), CD117, CD235, E-cadherin, Glycophorin A, HBG-A, LMO2
Megakaryocytic	Large, multilobed Coarse and dense	Pseudopods None to rare	CD34(–), HLA-DR(–), CD117, CD31, CD42b, CD61, LAT, VWF, Ulex-E, vimentin (–)[d]

[a]Also positive in lymphomas and Ewing's sarcoma
[b]Lineage-specific markers
[c]Recommend PGM1 clone which is more specific for monocytes
[d] Vimentin positive in most AMLs but is negative in megakaryocytic leukemias
[e]Microgranular variant typically does not have Auer rods

32.7 How to approach immunohistochemical staining for an acute leukemia?

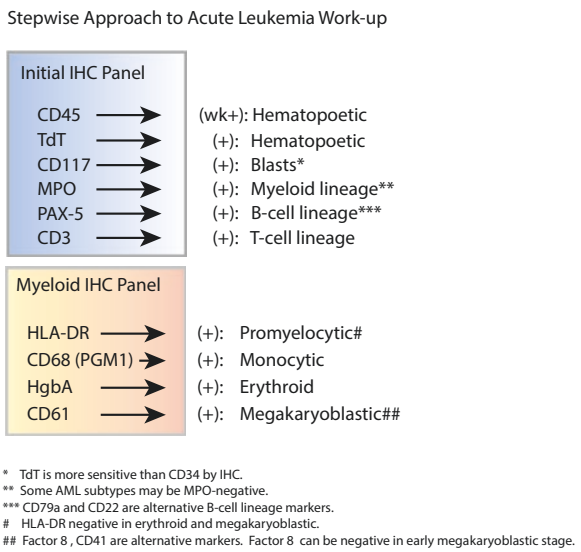

Fig. 32.1 Stepwise approach to IHC acute leukemia workup

32.8 How sensitive are specific immunohistochemical markers for acute leukemia?

Table 32.7 Immunohistochemical stains and percent positivity in acute leukemias

Antigens	B–ALL	T–ALL	AML–MD	AML–Myeloid	APL	AML–MM	AML–Mono	AML–E	AML–Mega	BPDC
CD45(w)	+	+	+	+	+	+	+	–/+	–	+
TDT	95	99	20	–/+	–	–	–	–	–	–/+
CD34	85–90	75	ƛ	ƛ	ƛ	ƛ	ƛ	ƛ	ƛ	–
HLA-DR	95	20–Oct	+	+	–/+	+	+	–/+	–/+	+
CD1a	–	20–30	–	–	–	–	–	–	–	–
CD2	–	90–95	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	–/+
CD3	–	99	–	–	–	–	–	–	–	–
CD4/CD8	–	ȣ	–	–	–	‖	‖	–	–	CD4+
CD5	–	95	ǂ	ǂ	ǂ	ǂ	ǂ	–	–	–/+
CD7	–	>95	ǂ	ǂ	ǂ	ǂ	ǂ	–	–/+	–/+
PAX-5	>95	–	–	–/+[h]	–	–/+[h]	–	–	–	–
CD10	85	20–Oct	–	–	–	–	–	–	–	–
CD19	>95	–	–	–	–	–	–	–	–	–
CD20	40(w)	–	–	–	–	–	–	–	–	–
CD22	90	–	–	–	–	–	–	–	–	–
CD79a	80	<5	0–33¥	0–33¥	0–90¥	0–33¥	0–33¥	–	0–50¥	–
CD43	80	>95	+	+	+	+	+	+	+	+/–
MPO	m	–	–/+	+/–	–/+	+	–	–	–	–
CD13	–	–	+/–	+	+	+/–	–/+	–	+/–	–
CD14	–	–	–	–	–	+	+/–	–	–	–
CD15	20–40	20	–	+	–	+/–	+/–	–/+	–/+	–
CD33	–/+	–	+/–	+	+/–	–/+	+/–	–	+	–/+
CD117	–	<1	+	+/–	+	+/–	–	+/–	+/–	–/+
CD14	–	–	–	–	–	–/+	–/+	–	–	–
CD64	–	–	–	–	–	+	+	–	–	–
CD68	–/+	–	+/–	+/–	–/+ ɤ	+	+/–	–	–	+
CD163	–	–		–	–	+/–	+/–	–	–	+
KLF4	ND	ND	–	–	–	+/–	+	–	–	ND
Lysozyme	–	–	–	+/–	ND	+	+/–	–	–	–
CD42b	–	–	–	–	–	–	–	–	+/–*	–
CD61	–	–	–	–	–	–	–	–	+/–	–
LAT	–	–	–	–	–	–	–	–	–/+	–
VWF	–	–	–	–	–	–	–	–	–/+	–
HGB-A	–	–	–	–	–	–	–	–/+	–	–
CD236 (GPC)	–	–	–	–	–	–	–	+/–	–	–
E-cadherin	–	–	–	–	–	–	–	+/–	–	–
CD56	ł	<5	Ł	Ł	Ł	Ł	Ł	ł	Ł	+
CD123	+/–	ND	–/+	–/+	–/+	–/+	–/+	–/+	–/+	+
CD303	–	–	–	–	–	–	–	–	–	+

AML-MD Acute myeloid leukemia – minimally differentiated; *AML-myeloid* Acute myeloid leukemia – myeloid differentiated; *APL* acute promyelocytic leukemia; *AML-MM* acute myeloid leukemia myelomonocytic leukemia; *AML-Mono* acute myeloid leukemia with monocytic differentiation; *AML-E* acute myeloid leukemia with erythroid differentiation; *AML-Meg* acute myeloid leukemia with megakaryocytic differentiation; *B-ALL* B-cell acute lymphoblastic leukemia; *T-ALL* T-cell acute lymphoblastic leukemia; *MPO* myeloperoxidase; *TDT* terminal deoxynucleotide transferase; *HGB-A* hemoglobin A; *GPA* glycophorin A; (*w*) weak

[a] TCD34 is positive in 50% of AML

[b]CD2, CD5, and/or CD7 are expressed in 5–10% of AMLs. CD2 is often expressed in APL

[c]T-ALLs are CD4+/CD8+ or CD4−/CD8−

[d]CD4 expression suggests monocytic differentiation

[e] Associated with AML with t(8;21)

[f]CD56 is expressed in 20% of AMLs (majority of cases having monocytic differentiation), rare B-ALL express CD56

[g]CD79a expression in AML/APL only associated with clones HM47/A9 (Novocastra, Newcastle Upon Tyne, England), HM57 (DAKO, Carpinteria, CA), and 11D10 (Novocastra). CD79a negative with clones 11E3 (Novocastra), and JCB117 (DAKO)

[h] MPO expression with typical B-cell lymphoblast phenotype should be diagnosis as B-ALL

32.9 What immunohistochemical clues suggest recurrent genetic changes in acute lymphoblastic leukemia?

Table 32.8 IHC clues suggesting recurring genetics changes in acute lymphoblastic leukemia

ALL cytogenetic subtypes	Immunohistochemical stains
B-ALL	
t(9;22); *BCR-ABL*	CD13, CD33, CD25
t(v;11q23.3); *KMT2A-rearranged*	CD10(−), CD15, CD22(w)
t(12;21); *ETV-RUNX1*	CD13+, CD33
Hyperdiploidy	None
Hypodiploidy	None
t(5;14); *IGH/IL3*	None
t(1;19); *TCF-PBX1*	CD34(−)
BCR-ABL1-like	None
iAMP21	None
T-ALL	
ETP	CD117, HLA-DR, CD13, CD33, CD11b

32.10 What markers suggest an acute myeloid leukemia (AML) with genetic changes?

Table 32.9 Immunohistochemistry in acute myeloid leukemia (AML) with genetic changes

AML genetic change	Immunohistochemical stains
t(8;21)	CD34, HLA-DR, CD19(w), CD56, PAX-5, CD79a
t(16;16)/inv(16)	CD34, HLA-DR, CD13, CD33, CD2, CD4, CD11b, CD11c, CD64
t(15;17); *PML-RARA*	CD13, CD33, CD2, CD56, CD117, CD34(−), HLA-DR(−)
t(9;11); *KMT2A-MLLT3*	CD4, CD7, CD56
t(6;9); *DEK-NUP214*	CD34, CD117, HLA-DR, TdT
t(3;3); *GATA2, MECOM/EVI1*	CD34, CD117, HLA-DR, CD7, CD42b(subset), CD56(subset)
t(1;22); *RBM15-MKL1*	CD42b, CD56, CD34(−), HLA-DR(−)
t(9;22); *BCR-ABL1*	CD34, CD117, CD13, CD33, CD7, CD19, TdT
FLT3-ITD	CD123
NPM1	CD13, CD33(bright), CD123 CD14, CD64, NPM1
CEBPA	CD34, HLA-DR, CD7[a], CD13, CD15[a], CD33
RUNX1	CD34, HLA-DR, CD13, CD14, CD64, MPO
Therapy related	CD34, HLA-DR, CD13, MPO, CD7, CD56, TP53
MDS-related changes	Variable

[a]Associated with biallelic CEBPA

32.11 What markers are used to differentiate B-cell acute lymphoblastic leukemia (B-ALL) from B-cell non-Hodgkin lymphoma (B-NHL)?

Table 32.10 Immunohistochemistry to differentiate B-cell acute lymphoblastic leukemia (B ALL) from B-cell non-Hodgkin lymphoma (B-NHL)

Markers	B ALL/B LBL	B NHL
CD45	+ (w)	++
CD34	+/−	−
CD117	−	−
TdT	+	−
CD10	+/−[b]	+/−[b]
CD20	−[c]	++
CD22	−/+	+
CD79a	+	+
PAX-5	+	+
BCL-6	−[d]	+/−
Surface light chains	−[c]	+

[a]*LCA* leukocyte common antigen; *PAX-5* paired box gene 5; *TdT* terminal deoxynucleotidyl transferase; *BCL-6* B cell lymphoma 6

[b]Early precursor B ALL can be CD10(−). CD10 expression is seen in B NHL of germinal center cell origin (follicular lymphoma, diffuse large B-cell lymphoma, and Burkitt lymphoma)

[c]Occasional cases of B ALL may have CD20 and/or surface light chain expression

[d] BCL-6 is positive in B NHL of germinal center cell origin ((follicular lymphoma, diffuse large B-cell lymphoma, and Burkitt lymphoma)

32.12 What markers are used to differentiate T-cell lymphoblastic leukemia from T-cell non-Hodgkin lymphoma and thymoma?

Table 32.11 Immunohistochemistry to distinguish T-cell lymphoblastic leukemia (T ALL) from T-cell non-Hodgkin lymphoma (T-NHL) and thymoma

Markers	T ALL/T LBL	T NHL	Thymoma [h]
CD45RB (LCA)	+ (weak)	++	+ (v)
CD34	+ [b]	–	+/–
CD117	–	–	–
TdT	+	–	+/–
CD1a	+	–	+/–
CD2	+/– [c]	+/– [c]	+
CD3	+ (c > m)	+/–	+ (c,m)
CD4/CD8	+/– [d]	–/+[d]	+/–
CD5	+/– [c]	+/– [c]	+
CD7	+/– [c]	+/– [c]	+
CD10	+/–	+/–	+
AE1/AE3	–	–	+/–
EMA	–	+/–	+/–

[a]*LCA* leukocyte common antigen; *TdT* terminal deoxynucleotidyl transferase; *EMA* embryonic membrane antigen; *c* cytoplasmic, *m* membranous

[b]Expression of TdT, CD34, and/or CD1a with double positivity or negativity for CD4 and CD8 in bone marrow favor T ALL; however, the differential diagnosis with metastatic lymphocyte rich thymoma should be entertained especially if there is a mediastinal mass as the phenotypical distinction of cortical thymocytes and T ALL/LBL can be difficult. Epithelial markers (AE1/AE3 and EMA) and flow cytometric analysis can help differentiate. T ALL will lack cytokeratin markers and form discrete population on flow cytometry while thymomas may show cytokeratin expression with variable expression (smear pattern) of hematologic markers

[c]T ALL and T NHL can variably express CD2, CD5, and CD7, and these markers are not reliable in distinguishing T ALL from T NHL

[d] Coexpression of CD4 and CD8 (double positive) or absence of expression of CD4 and CD8 (double negative) favor T ALL, but some T NHL are double negative (γ/δ T NHL) or double positive (T-cell prolymphocytic leukemia and T-cell leukemia/lymphoma). A helpful feature is T NHLs are negative for immature markers like TdT

32.13. What immunohistochemical stains are used for bone marrow assessment in patients with cytopenia(s)?

Table 32.12 Immunohistochemical stains used for bone marrow assessment in patients with cytopenia(s)?

Disease	Immunohistochemistry	Morphologic features
Myelodysplastic syndrome (MDS)	CD34, CD117, CD42b, CD61, VWF, ULEX-E, LAT MPO, CD56(+) HGB-A, Spectrin,	Blasts increased >5% of cellularity and/or ALIP Increased megakaryocytes, small size, hypolobated clustering, paratrabecular Increased cellularity including dyspoietic granulocytes and loss of erythrocyte colony formation
Acute panmyelosis with myelofibrosis	CD34, CD117, MPO, CD33, GPA/GPC, HGB-A, Spectrin CD42b, CD61, VWF, LAT	Increased myeloblasts, immature myeloid and erythroid precursors, and megakaryocytes Small dysplastic megakaryocytes negative for CD42b Fibrotic marrow
Aplastic anemia	CD34, CD117, MPO, CD42b/ CD61, CD2, CD3, CD20, CD25, CD138, ƙ/ƛ light chains, MCT	Blasts <5% of cellularity Decreased cellularity with decreased hematopoietic elements and increased polyclonal lymphocytes, plasma cells, histocytes, and reactive mast cells (CD2-CD25-CD117+MCT+)
Hairy cell leukemia	CD20, CD25, CD103, CD123, TRAP, Annexin-A, BRAF	Clonal monomorphic intertrabecular lymphocytes Increased fibrosis
Hypocellular AML	CD34, CD117, CD42b/CD61, CD56, CD235/HGB-A	Decreased cellularity and hematopoietic elements Blasts >20% of cellularity
Large granular lymphocytic leukemia	CD3, CD4, CD5, CD7, CD8, CD20, CD56, CD57, TIA-1, granzyme B	Subtle T-cell lymphocytosis with coexpression of CD57 Inversion of myeloid maturation
Infection	HGB-A, Parvovirus B19	Erythrocytopenia

ALIP abnormal localization of immature precursors >2 clusters (minimum 3 blasts/cluster)

32.14 What are the marker for myelodysplastic syndrome?

Table 32.13 Markers for myelodysplastic syndrome (MDS)

Antibodies		Localization
CD14	Positive in monocytes, macrophages, follicular dendritic cells, monocytic leukemias	M
CD15	Highlights granulocytes	C, M
CD16	Highlights granulocytes	M
CD34	Highlights blasts	C, M
CD56	Positive in monocytes, NK cells, myeloma, dendritic cells, and osteoblasts	M
CD61 (42b)	+ in megakaryocytes and megakaryoblasts	C and M
CD68	Highlights monocytes, myeloid cells, and macrophages	C (granular)
CD117	Highlights blasts	M
HgbA	Useful in staining erythroblasts and nucleated RBCs	C
Mast cell tryptase	+ in mast cells	C
Others: MPO	Not too useful since all myeloid cells will stain, useful in differentiating myeloid from lymphoid lines	C
Parvovirus B19	Useful in the differential diagnosis of erythrocytopenia with abnormal nucleated RBCs	N

C cytoplasmic; *M* membrane; *N* nuclear
Note: IHC can be useful in determining the distribution of blasts and immature cells in MDS or myeloproliferative neoplasms (MPN)
"Normal" blasts are usually found around the bony trabecula. They are abnormal if found in the marrow cavity

32.15 What immunohistochemical stains are used for the evaluation of common myeloproliferative neoplasms and chronic myelomonocytic leukemia?

- The most common myeloproliferative neoplasms (MPNs) are chronic myelogenous leukemia (CML), polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocytosis (ET) and these disorders are assessed by morphology predominately.
- Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative disorder also predominately assessed by morphology.
- The common MPNs and CMML have characteristic features that overlap with each other.
- Immunohistochemistry can help highlight morphologic features when needed.
- Cytogenetic and molecular mutational studies are used to categorize these neoplasms and surrogate immunohistochemical markers are limited.

Table 32.14 Markers for common myeloproliferative neoplasms (chronic myelogenous leukemia, polycythemia vera, primary myelofibrosis, essential thrombocytosis) and chronic myelomonocytic leukemia

	Antibodies	Localization
Blast enumeration	CD34, CD117, TdT, CD14, CD68, CD163, KLF4	*MPNs:* >10% blasts → accelerated phase *CMML:* >5% blasts→CMML-1 >10% blasts→CMML-2
Myelopoiesis evaluation	MPO, CD13, CD33 CD2, CD56	*CML:* Increased number (+) left shifted *PV:* Increased number (−) left shift *PMF:* Increased (+/−) left shift (profibrotic only) *ET:* Abnormalities uncommon *CMML:* Increased number (+) dyspoiesis (aberrant CD56, CD2 in myeloid precursors)
Erythropoiesis evaluation	HGB A, GPA, GPC, Spectrin	*CML:* Decreased quantity (−) left shift *PV:* Increased quantity (+) left shifted *PMF:* Abnormalities uncommon *ET:* Abnormalities uncommon *CMML:* Increased number

(continued)

Table 32.14 (continued)

	Antibodies	Localization
Megakaryocyte evaluation	CD34, CD42b, CD61, LAT, ULEX-E, VWF	*CML:* Quantity varies, small sized, hypolobated (dwarf) *PV:* Increased quantity, medium to large size, loose small-size clusters, naked to hyperlobated nuclei *PMF:* Increased quantity, dense large-sized clusters, hypolobated bizarre/naked nuclei, endosteal location *ET*: Increased quantity, few loose clusters, staghorn-nuclei *CMML:* Quantity varies, small size, hypolobated
Vascular proliferation	CD31, CD34	*CML:* Abnormalities uncommon *PV:* Dilated and filled with RBCs *PMF:* Increased number without dilation *ET:* Abnormalities uncommon *CMML:* Abnormalities uncommon
Basophilia	2D7+, BB1	Increased in MPNs compared to reactive conditions, especially in CML
Monocytosis	CD14, CD16, CD68, CD123, KFL4	Increased in CMML and some MPNs,
Plasmacytoid Dendritic cell nodules	CD4, CD56, CD68, CD123, CD303, BCL11A, granzyme B	Increased in CMML and positive for CD4, CD68, CD123, CD303, BCL11A, granzyme and negative for CD56 (differentiating it from BPDCN)
Fibrotic areas and mast cells	CD2, CD25, CD117, Mast cell tryptase	Use if paratrabecular or perivascular fibrosis (+/−) eosinophilia to evaluate for clonal mast cells (positive for all markers) versus reactive mast cells (lack CD2 and CD25)
Genetic markers	CAL2	Surrogate marker for calretinin mutation positive in ET and PMF

CML chronic myelogenous leukemia; *CMML* chronic myelomonocytic leukemia, *ET* essential thrombocytosis; *PMF* primary myelofibrosis, *PV* polycythemia vera

32.16 What are the markers for mast cell disease?

Table 32.15 Markers for mast cell disease

Antibody	Benign mast cells	Malignant mast cells	Localization
CD2	–	+	M
CD25	–	+	C and M
CD117	+	+	M
Mast cell tryptase	+	+	C

C cytoplasmic; *M* membrane; *N* nuclear

Note: 1. Mast cell disease in the marrow can present as "myelofibrosis." Mast cell tryptase and CD117 can be used to determine if it is myelofibrosis or mast cell disease presenting as a fibrotic marrow

2. An increase in marrow mast cells should be viewed with caution since multiple myeloma, lymphoproliferative disorders, and reactive pictures can also have an increase in mast cells

32.17 What markers are used for differentiating benign from malignant lymphoid aggregates in marrow biopsies?

Table 32.16 Markers for differentiating benign from malignant lymphoid aggregates in bone marrow biopsies

Marker	Localization	Benign distribution	Malignant
Bcl2	N	T cells and B cells, negative in germinal center of follicle and diffusely positive in primary follicles	Positive in germinal center
CD3	M	T-cell marker, T cells > B cells, T cells mainly outer rim of secondary follicle	Either absent or diffusely positive
CD5	M	T cells > B cells, T cells mainly outer rim of secondary follicle	Aberrant coexpression on B cells
CD10	M	Germinal center B cells	Can be negative in germinal centers within bone marrow, consider IHC for follicular dendritic cells (CD21/CD23) or other studies to exclude malignancy
CD20	M	B-cell marker, B cells < T cells, B cells mainly in center of secondary follicle	Either absent or diffusely positive
PAX-5	N	B-cell marker, B cells < T cells, B cells mainly in center of secondary follicle	Either absent or diffusely positive
CD137L	C+M	Strong expression in B cells of primary follicles, weak expression in germinal center of secondary follicle	Diffuse positivity and/or strong staining in germinal center, only good for B-cell neoplasms
Ki-67 (MIB1)	N	Proliferation marker, usually moderate to high with polarization in reactive germinal center	Low proliferation and/or lack of polarization

C cytoplasmic; *M* membrane; *N* nuclear
Note: Elderly females frequently have lymphoid aggregates of no consequence in the bone marrow
Often in bone marrow biopsies, the sample is limited so there may not be enough tissue to run a panel. Shallow sections (don't cut into the block) are needed. CD3 and CD20 are recommended to start with
Malignant: Usually paratrabecular except CLL uniform pattern. Close to surface of bone predominance of B or T cells
Benign: Central or perivascular. May have partial germinal centers. Deep in marrow. Mixed T- and B-cell infiltrate

32.18 What are the common immunohistochemical markers for mature B-and T-cell neoplasms?

- Bone marrow examination for lymphoma is performed in several different clinical scenarios:
  - Establish a primary diagnosis
  - Extramedullary lymphoma staging workup
  - Leukemic manifestation of lymphoma
- Immunohistochemical stains may not work as well on decalcified tissue creating a potential pitfall, especially when establishing a new diagnosis.
- Several tables show immunohistochemical stains for lymphomas that most commonly involve the bone marrow (Table 32.17).
- More extensive immunohistochemical evaluations for lymphomas are available in Chap. 31.

Table 32.17 Immunophenotype of small cell B-cell lymphomas

	CLL	PLL	MCL	FL	LPL	MZL	HCL	
CD19	+++	+	+++	+++	++	+++	+++	
CD20	+++	+++	+++	+++	++	+++	+++	
CD79a	–/+	+	+	+	++	+	+	
PAX5	++	++	++	++	++	++	++	
CD5	+	V	+	–	–/+	–/+	–	
CD10	–	–	–	+	–	–/+	–	
CD21	–/+	–/+	–	+	–	–	–	
CD22	–/(w)	+	+	+	+	+	+	
CD23	+	–/+	–	+	–	–	–	
CD138	–	–	–	–	V[a]	–	–	
CD160	++	–	–	–	–	–	++	
CD200	+++	+/–	–	W	W	+	+++	
Cyclin D1	–	–	+/–	–	–	–	+s	
BCL2	+/–	ND	+/–	+	ND	+/–	+	
BCL6	–	ND	–	+	–	–	–	
BCL10	ND	ND	+ (c)	+ (c)	+ (n,c)	+ (n,c)	ND	
BRAF	–	–	–	–	–	–	+	
IRTA	–	–	–	/+	–	+	–	
LEF1	++	–	–	–/+	–	–	–	
MUM-1	+[c]	ND	–	–	+/–	–/+	ND	
Sox 11[b]	–	–	++	–	–	+	–/+	
sIg	+/–	+++	++	+++	+++	+++	+++	+++

References: [155–157]
[a]Plasmacytic component
[b]Sox11+ in granulocytes
[c]Positivity in proliferation centers

Table 32.18 Immunohistochemistry of large B-cell lymphomas frequently involving bone marrow

	CHL	THRLBCL	DLBCL	BL
CD45	+	+	+	+
PAX-5	+(w)	+	+	+
CD20	−/+	+	+	+
CD30	+	−/+	−/+	−
CD15	+/−	−	−	−
CD10	ND	−	+/−	+
BCL-2	ND	+/−	+/−	−
BCL-6	ND	+	+/−	+
EMA	−	+	−/+	
EBER	+/−	−	−/+	+/−
BOB.1	−	+	+	ND
MYC	−	ND	+/−	+
MUM-1	+	ND	+/−	+/−
OCT-2	−	+	+	ND
MIB-1	+	+	++	+++

BL Burkitt lymphoma; *DLBCL* Diffuse large B-cell lymphoma; *CHL* classical Hodgkin lymphoma; *TRLBCL T*-cell histiocyte-rich B-cell lymphoma; *ND* not described; *w* weak

Table 32.19 Immunohistochemistry for T-cell lymphomas frequently involving bone marrow

T-NHL	Immunophenotype of neoplastic cells
AITL	Neoplastic cells: CD2, CD3, CD5, CD7, CD4 > CD8, ICOS, CXCL13, PD-1 FDC networks: CD21/CD23 Admixed B cells: CD20, EBV
ALCL	CD30, EMA, ALK-1, variable T-cell marker expression (CD2+, CD4+ usually), CD137, PD-1
ATLL	CD2, CD3, CD5, CD4, CD25, CD30+/−, PD-1
HSTL	CD2, CD3, TIA-1, granzyme B Double negative T cells (CD4-CD8-)
MF/SS	CD2, CD3, CD4, CD5, CD137 Negative: CD7, CD25, CD26
T-PLL	CD2, CD3, CD5, CD7, CD4+/CD8- or CD4+CD8+ (most common), TCL-1
PTCL	T cells: CD2, CD3, CD5, CD4 > CD8, EBV, CD137, CD30(v) Cytotoxic T-cell subset: CD8 > CD4, (+/−)TIA-1, granzyme B, perforin, CD56, EBV

AITL angioimmunoblastic T-cell lymphoma; *ALCL* anaplastic large cell lymphoma; *ANKL* aggressive natural killer cell leukemia, *ATLL* adult T-cell leukemia/lymphoma; *HSTL* hepatosplenic T-cell lymphoma; *MF/SS* mycosis fungoides/Sezary syndrome; *T-PLL* T-cell prolymphocytic leukemia

32.19 What markers are useful in hairy cell leukemia?

Table 32.20 Useful markers in hairy cell leukemia

	HCL	HCL-v	SMZL	Other positive cells/diseases/potential pitfalls
CD5	−	−	−/+	T cells and B-NHL (CLL, MCL)
CD11c	+	+	+/−	Other B-NHL (CLL)
CD22	+	+	+	Most B-NHL
CD23	−	+	−	Other B-NHL (CLL, FL)
CD25	+	−	−/+	CLL
CD52	+	+	+	Other B-NHL
CD103	+	+/−	−	
CD123	+	−/+	−/+	Other B-NHL (CLL, MCL), AML, ALL, MCD
CD200	+	+	−	Other B-NHL, MM, and ALL
Annexin A1	+	−	−	Background granulocytes
DBA.44	+	+	+/−	Other B-NHL (LPL, MCL)
BRAFV600E	+/−	−	−	Decalcification may affect staining
T-bet	+	ND	+	CLL
TIA-1	+	−	−	T-LGL
TRAP	+	−	−	Other B-NHL and rare AML
SOX-11	−/+	ND	+	Other B-NHL (MCL)
Cyclin D1	+/−	−	−	Other B-NHL and MM

HCL hairy cell leukemia, *HCL-v* hairy cell leukemia-variant, *SMZL* splenic marginal zone lymphoma, *ALL* acute lymphoblastic lymphoma, *B-NHL* B-cell non-Hodgkin lymphoma, *CLL* chronic lymphocytic leukemia, *FL* follicular lymphoma, *LPL* lymphoplasmacytic lymphoma, *MCL* mantle cell lymphoma, *MM* multiple myeloma, *ND* not described

32.20 What markers are used to differentiate reactive versus malignant plasmacytosis?

Table 32.21 Immunohistochemistry in reactive versus malignant plasmacytosis

	Reactive plasma cells	Plasma cell myeloma
CD45	+	−/+
CD19	+	−
CD20	−	−/+
CD38	+ (bright)	+ (dim)
CD56	−	+/−
CD79a	+	+
CD117	−	+/−
CD138	+	+(bright)
CD200	−	+/−
Cyclin D1	−	+/−
κ/λ ish	κ > λ (1–2:1 ratio)	κ or λ restricted

Note: Often plasma cell dyscrasias and lymphomas have an increase in mast cells, offering a clue to the underlying process

32.21 What immunohistochemical stains are used for plasma cell myeloma and aggressive plasmacytoid neoplasms?

Table 32.22 Immunohistochemical stains for plasma cell myeloma and aggressive plasmacytoid neoplasms

	Plasma cell myeloma	Plasmablastic lymphoma	Diffuse large B-cell lymphoma, NOS	ALK (+) large B-cell lymphoma
ALK-1	–	–		+
Bcl-2	+	–/+	+/–	ND
BCL-6	–	–/+(w)	+/–	ND
BLIMP1	+	+	–	+
CD4	–/+	–/+	–	+
CD10	–/+	+/–	+/–	ND
CD20	–	–	++	–/+
CD30	–	–	–/+	–/+
CD38	+	+	+	+
CD45	–/+	+/–	++	+(wk)
CD56	+/–	–/+	–	–
CD79a	+	–/+	++	–/+
CD117	+/–	–	–	–
CD138	+	+	–	+
Cyclin D1	+/–	–	–	–
EBER	–	+	–/+	–
EMA	–	+	–	++
HHV-8	–	–	–	–
κ/λ ish	κ or λ restricted	(v)	(v)	κ or λ restricted
Ki-67	+/++	+++	++	+++
MUM-1	+	+	+	+
PAX-5	–	–/w	+	–/+
XBP1	+	+	–	+

κ/λ ish kappa/lambda in situ hybridization; *ND* not described, *v* variable, *w* weak

32.22 What markers are used for histiocytic tumors in the bone marrow?

Table 32.23 Immunohistochemistry of common histiocytic lesions involving bone marrow

Marker	Langerhans cell histiocytosis/sarcoma	Erdheim-Chester disease	Histiocytic sarcoma
CD1a	++	–	–
CD4	–	+(v)	
CD21	–	+(v)	–
CD68	+	+	+
CD163	–	+	+
Fascin	–	+	–
Factor XIIIa	–	+	–
Langerin	++	–	–
Lysozyme	+/–	–	+
MPO	–	–	–
S-100	++	–+	–/+(w)
BRAF	–	+	–
PDL1	+	–	–

v variable; *w* weak

32.23 What markers are used for metastatic tumors to the bone marrow (similar to workup of unknown primary in Chap. 12)?

Table 32.24 Markers for metastatic tumors to the bone marrow

Antibodies	Literature
CK A1, A3	Stains cytokeratin
CK7/CK20	See chart below
CK7/CK20 positive	Transitional cell, pancreatic and ovarian mucinous carcinomas
CK7 positive / CK20 negative	Breast, lung (non-small cell) ovarian serous, endometrial carcinomas and mesothelioma (epithelial) and thymoma
CK7 negative / CK20 positive	Colorectal carcinoma
CK7/CK20 negative	Hepatocellular, renal cell, prostatic adenocarcinoma; squamous cell and small cell [neuroendocrine (NE)] carcinomas
HMB45	Useful in melanoma
MelanA	Useful in melanoma
S100	Useful in tumors with neural origin or differentiation
SMA	Stains smooth muscle
Synaptophysin	Neuroendocrine marker
Vimentin	Positive in sarcomas, AML

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