Diagnostic relevance of immunohistochemistry for Bone Marrow diagnosis

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DISCLOSURES

No relevant conflict of interest

# Diagnostic relevance of Immunohistochemistry for Bone Marrow diagnosis

- Immunohistochemistry (IHC) is important component of an integrated approach for bone marrow diagnosis of lymphoid, and hematopoietic neoplasms and follow up disease.

- IHC, in conjunction with flow cytometry (FC), provide essential information about phenotype of hematopoietic/lymphoid proliferations, crucial to establish diagnosis followed by further genetic/molecular characterization for the WHO classification.

- The choice of antibody panels for IHC depends on the clinical history, morphological findings and whether FC analysis has been performed.

- In some circumstances, (i. e. bone marrow fibrosis), IHC is the only technique for immunophenotypic characterization of hematopoietic neoplasms on bone marrow trephine biopsy

- IHC on bone marrow trephine biopsies can be also used in diagnostic workup of metastatic tumor of unknown primary in the bone marrow and characterization of benign marrow conditions.

- IHC is fast and inexpensive technique

Diagnostic relevance of immunohistochemistry (IHC) for bone marrow diagnosis

- PubMed search **bone marrow diagnosis and IHC** in 5/2022 yielded 12,418 results
- BM diagnosis and IHC and lymphoma, 1736 publications
- BM diagnosis and IHC and myeloma, 957 publications
- BM diagnosis and IHC acute leukemia, 1348 publications
- BM diagnosis and IHC and CD34, 892 publications.
- BM diagnosis and IHC and MDS, 400 publications
- BM diagnosis and IHC and MPN, 552 publications
- BM diagnosis and IHC and MDS/MPN 94 publications
- BM diagnosis and IHC and systemic mastocytosis, 105 publications
- BM diagnosis and IHC and metastatic tumor, 753 publications
- BM diagnosis and IHC and infectious organisms , 281 publications

RESULTS BY YEAR

1962   2022
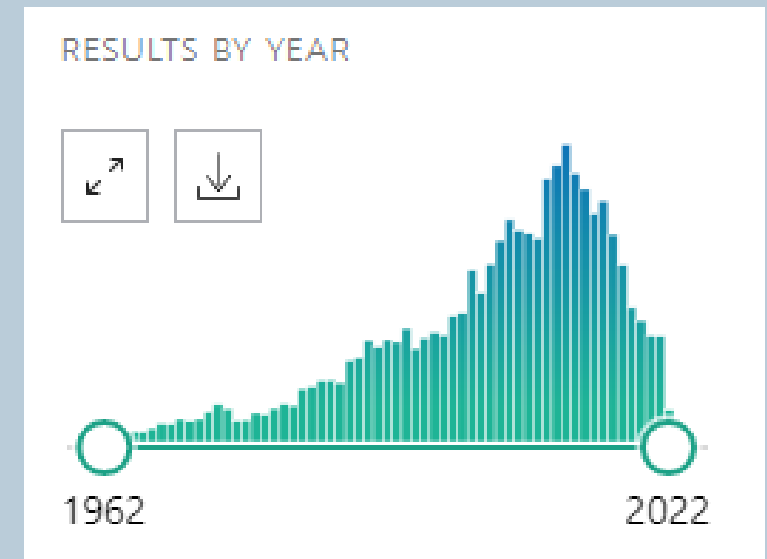
Outlines

- Methodology

- Antibodies/markers commonly used for diagnostic evaluation of bone marrow biopsies by IHC; pattern of expression in normal hematopoietic cells/bone marrow and diagnostic application.

- Antibody panels for specific diagnostic considerations of lymphoid and myeloid neoplasms according to the 2016 WHO classification.

- Diagnostic relevance of immunohistochemistry for diagnosis of metastatic tumors in the bone marrow and reactive conditions.

Methodology

Recommendation for the standardization of BMB IHC procedures published by working group within the International Council for Standardization in Hematology (ICSH)

- **Preanalytical**
  - Ischemic time should be as short as possible. Prevent dehydration by transportation of BM bxs in Hank's solution or physiological saline
  - Selection of fixative and decalcification methods depends on the desirable turnover time and whether a separate aspirate material has been sent for genetic testing.
  - Paraffin embedding is recommended.
  - IHC requires thin sections (2-3 microns thick)

- **Analytical**
  - As each IHC stain is a different test, special standard operating procedure (SOPs) concerning protocol (primary antibody dilution, incubation time, antigen retrieval, detection system, amplification, chromogen and automated stainer platform should be developed and documented.
  - Optimal calibration (analytical sensitivity and specificity) of the particular epitope detection depends on how the results of the IHC test are used and tailored to biologically unique application (e. g. calibration of CD34 for detection of blasts in BM bxs vs detection of vessel in surgical specimen).

- **Post-analytical conditions**
  - Internal positive controls can be used for some antigens present in bone marrow biopsies but cannot fully replace external positive controls. External positive controls used for BM bx should be processed in the same way as BM bxs
  - Negative controls are necessary only in special circumstances, e.g. Avidin-biotin detection systems or in case of BM necrosis.
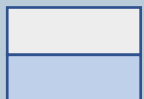
Antibodies/markers commonly used for diagnostic evaluation of bone marrow biopsies by IHC; pattern of expression in normal hematopoietic cells/bone marrow and diagnostic application.

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

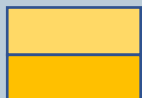| Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell type	Main diagnostic application
ALK-1 (CD246)	Anaplastic lymphoma kinase	No expression	ALK-positive Anaplastic large cell lymphoma (ALCL
Annexin-A1	ANXA, phospholipid binding protein	Myeloid cells, monocytes, macrophages	positive Hairy cell leukemia (HCL), negative in Splenic marginal zone lymphoma (SMZL) and HCL variant
BCL1/ cyclin D1	(B cell lymphoma-1), product of *CCND1* gene, cell cycle regulator that controls transition from G1 to S phase	Endothelial cells, fat cells histiocytes, osteoblasts, dendritic cells.	MCL with t(11;14), plasma cell myeloma with t(11;14), HCL
BCL-2	B-cell leukemia lymphoma-2, blocks apoptosis	Germinal center B cell, weak expression in myeloid cells	Follicular lymphoma with t(14;18)
BCL-6	B-cell lymphoma-6 protein, transcription factor	Geminal center B cell, weak expression in myeloid cells	Germinal center cell derived B-cell lymphoma, some types of peripheral T cell lymphoma
BRAF V600E	Product of mutated *BRAF*	No expression	HCL, Langerhans histiocytosis , BRAF mutated metastatic tumors to BM.

Antibodies used for immunohistochemistry

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
CD1a	Cortical thymocyte antigen, transmembrane glycoprotein, structurally related to MHC proteins	Cortical thymocytes, Langerhans cells , dendritic cells	T-lymphoblastic leukemia/ lymphoma, cortical phenotype, Langerhans histiocytosis (LCH)
CD2	LFA2, sheep red blood cells receptor, main function adhesion and signaling	Thymocytes, T cells, NK cells	T cell (precursor and mature) and NK cell lymphomas
CD3	Protein complex of TCR and T-cell co-receptor (CD3γ, CD3δ, and two CD3ε), involved in T cell activation upon antigen recognition.	T cells; anti- CD3ε antibody used for T cells identification by IHC, will also identify some activated NK cells.	T cell (precursor, mature) and NK cell lymphomas
CD4	Glycoprotein, co-receptor for TCR binding to MHC class II molecule	Thymocyte subset, T-cell subset, monocytes, macrophages, dendritic cells	T cell (precursor and mature) lymphomas
CD5	Leu-1, member of the scavenger receptor cysteine rich superfamily	Thymocyte, T cells, B-cell subset	Classification of small mature B cell lymphomas

Antibodies used for flow cytometry and immunohistochemistry

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name /Function	Expression in normal hematopoietic cell types	Main diagnostic application
CD7	Gp40, member of Ig superfamily, T-lymphocyte interactions during early development	Thymocytes, T-cells, NK cells, small subset of hematopoietic progenitors	T cell (precursor and mature T cell lymphomas, NK cell lymphomas
CD8	T-cell receptor coreceptor binding to MHC class I molecule	Thymocyte subset, T-cell subset, NK-subset	T cell (precursor and mature T cell lymphomas, NK cell lymphomas
CD10	MME, membrane metalloendopeptidase	B-cell precursors, geminal center B-cells, thymocyte subset, neutrophils	Classification of B- and T- cell (precursor and mature cell lymphomas).
CD11c	ITGAX, integrin subunit Alpha X	Dendritic cells, granulocytes, NK cells, B-cell and T-cell subsets, monocytes	Hairy cell leukemia
CD14	LPS R, co-receptor for detection of bacterial liposaccharide	Monocytes, macrophages, Langerhans cells	Classification of myeloid neoplasms with monocytic differentiation.
CD15	FUT4 fucosyltranferase 4	Neutrophils, eosinophils, monocytes	Hodgkin lymphoma, myeloid neoplasms

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
CD19	Member of Ig superfamily, assembles with antigen receptor of B-lymphocytes	B-cells (precursor and mature), reactive plasma cells	B–cell (precursor and mature), plasma cell neoplasms
CD20	MS4A1, Membrane soanning-4 domain A	B cells (mature)	B-cell (precursor and mature) neoplasms
CD21	CD3dR, CR2, EBV-R	B cells in follicular mantles, follicular dendritic cells	Follicular dendritic cell meshworks in lymphomas, follicular dendritic cell sarcoma
CD22	Member of sialic acid-binding immunoglobulin-type lectin family-2 (SIGLEC-2	B cells (precursor and mature)	B-cell (precursor and mature) neoplasms
CD23	FCER2, Fc fragment of IgE receptor 2	B cells, eosinophils, platelets, subset of follicular dendritic cells	Positive in CLL, negative in MCL,
CD25	Interleukin-2 receptor alpha chain	Activated B cells and T cells	HCL, T-cell lymphomas Adult T cell leukemia/lymphoma, systemic mastocytosis

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ AntibodyCD	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
CD30	TNFRSF8, Tumor necrosis factor receptor superfamily member 8	Activated T-cell, B cells, histiocytes	Reed-Sternberg cells, T-cell lymphomas
CD31	Platelet endothelial cell adhesion molecule (PECAM-1)	Platelets, endothelial cells, leukocytes	Vascularity in the bone marrow
CD33	SLIGLEC-3	Granulocytes, monocytes, histiocytes, dendritic cells	Classification of myeloid neoplasms
CD34	Possible adhesion molecule important in early hematopoiesis	Hematopoietic precursors	Diagnosis of acute leukemias, and follow up of residual disease
CD35	CR1, complement receptor 1, C3b/C4b-R	B cells in follicular mantles, follicular dendritic cells	Meshworks of FDCs in lymphomas, follicular dendritic cell sarcoma
CD43	Sialophorin, leukosialin, important in T cell and neutrophils adhesion to endothelium	T cells	T cell and B cell lymphomas, subset of MALT lymphomas, Myeloid sarcoma
CD45	PTPRC, protein tyrosine phosphatase receptor type C	Hematopoietic cells, multiple isoforms from alternative splicing	Diagnosis of hematopoietic and lymphoid neoplasms

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
CD56	Neural cell adhesion molecule-1	NK subset, T cell subset	NK cell lymphomas, plasma cell myeloma
CD57	B3GAT1, beta-1,3 glucuronyltrasferase	NK subset, T cell subset	NK and T cell lymphomas (large T/NK cell granular leukemia (LPD)
CD61	ITGB3, integrin subunit beta 3	Platelets, megakaryocytes	Myeloid neoplasms
CD68R	Helps in homing monocytes, macrophages	Monocytes, macrophages, dendritic cells	Myeloid neoplasms with monocytic differentiation
CD79a	B-cell antigen receptor complex associated protein alpha-chain	B-cell and plasma cells	B cell differentiation
CD103	ITGAE, Integrin subunit Alpha E	B and T cell subsets	B cell lymphoma classification, HCL, vHCL, MZL
CD117	KIT, KIT protooncogene receptor tyrosine kinase	Hematopoietic progenitors, mast cells	Myeloid leukemias, mast cell disorders
CD123	IL3RA, Interleukin 3 receptor subunit alpha	Basophils, plasmacytoid dendritic cells, hematopoietic progenitors	Acute leukemias (myeloid, B-ALL subset), Blastic Plasmacytoid dendritic cell neoplasm

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
CD138	SDC1, Syndecan-1 heparan sulfate proteoglycan	Plasma cells, epithelial and mesenchymal cells	Plasma cell neoplasms, plasmacytic differentiation of B cell lymphomas, metastatic carcinoma to marrow
CD163	Hemoglobin scavenger receptor	Monocytes, histiocytes (tissue macrophages)	Neoplasms with monocytic/histiocytic differentiation
CD200	Ox-membrane glycoprotein	Thymocytes, T-cell and B-cell subsets	Positive in CLL, negative in MCL, aberrant high expression in plasma cell myeloma
CD236R	Glycophorin C, regulates mechanical stability of red cells	Erythroid precursors, red cells	Evaluation of erythropoiesis, normal and neoplastic, erythroleukemia
C-MYC	Plays a role in cell cycle progression, apoptosis and transformation	Some proliferating cells and some stromal cells	Lymphoma classification and prognosis
CK	Cytokeratin (pan-cytokeratin AE1/AE3), epithelial cells	No expression	Metastatic carcinoma to bone marrow
CXCL13	Chemokine (C-X-C motif) ligand 13	Follicular helper T cells	Angioimmunoblastic T cell lymphoma, follicular helper T cell lymphoma

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
E-cadherin	Epithelial cadherin	Immature Erythroid precursors	MDS, erythroleukemia
EBER /ISH	Epstein Barr Virus (EBV) encoded RNA , detected by in situ hybridization	No expression or only very rare EBER+ B cells	Extranodal NK/T cell lymphoma, nasal type, B and T cell LPD associated immunodeficiency / immunosuppression, PTLD. HL, BL, EBV+ DLBCL
FVIII/vWF	Von Willebrand factor	Megakaryocytes, platelets, endothelial cells	Detection of atypical/dysplastic megakaryocytes, megakaryocytic differentiation of leukemia
Granz B	Granzyme B	NK cells, activated cytotoxic T cells	T-and NK- cell neoplasms
HGB A	Hemoglobin A	Nucleated erythroid precursors and red cells	Evaluation of M:E ratio, erythroleukemia
HGAL	Human germinal center-associated lymphoma, germinal center B-cell expressed transcript 2	Normal geminal centers B cells Marker of T follicular helper cells	Follicular lymphoma, diffuse large B cell lymphoma of GCB cell of origin, marker of follicular T helper cells and aid in diagnosis of lymphoma of TFH derivation

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

| Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
HHV-8/KSHV	Human Herpes Virus -8 Kaposi sarcoma Herpes Virus (LANA OFR 73)	No expression	The presence of HHV-8+ cells within the marrow implies dissemination of the virus in HHV8+ MCD, Primary effusion Lymphoma- infrequent BM involvement
ICOS	Inducible T cell costimulator	Follicular helper T cells	Angioimmunoblastic T cell lymphoma, follicular helper T cell lymphoma
KI67	Marker of proliferation expressed form G1 to M phase of the cell cycle	Proliferating cells in the bone marrow	Proliferating fraction of tumor infiltrates
LEF1	Lymphoid enhancer binding factor-1	B cell precursors, T cells	Overexpressed in B-CLL
LMO2	LIM domain only protein-2	Germinal center B cells, B cell precursors, T cells	Follicular lymphoma, ALL
Lysozyme	LZ, also called muramidase	Mature monocytes and neutrophils	Myelomonocytic leukemias
MPO	Myeloperoxidase	Myeloid cells	Myeloid cell differentiation

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
MUM1/IRF4	Multiple myeloma oncogene-1 /Interferon regulatory factor-4	Normal plasma cells	Plasma cell myeloma, subtyping of diffuse large B cell lymphoma, some peripheral T cell lymphomas
NPM1	Nucleophosmin	Ubiquitously expressed in nuclei	Cytoplasmic expression in AML with mutated *NPM1*
Parvovirus B19	Viral capsid protein of parvovirus 19	No expression	Suspected parvovirus infection with maturation block of erythropoiesis
PAX5	Paired box-5 transcription factor	Normal B cells from precursors to mature B cells, downregulated in plasma cells	B-ALL, and mature B cell lymphoma, aberrant expression in some AML, weak expression in Reed -Sternberg cells, lack of expression in myeloma.
PD-1 (CD279)	Programmed (cell) death-1	T-cell subsets	T-cell lymphoma
PD-L1 (CD274)	Programmed (cell) death ligand-1	Macrophages, activated lymphocytes	Hodgkin lymphoma, aggressive EBV+ DKBCL, EBV-negative DLBCL
Perforin	Pore forming cytolytic protein	NK cells and activated cytotoxic T cells	T-and NK-cell neoplasms

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
P53	Product of tumor suppressor gene *TP53*. Apoptosis and cell cycle regulation	No expression	Malignancies with TP53 mutation
S100	Calcium modulating protein	Dendritic cells	Histiocytic proliferations, Rosai Dorfman Disease, melanoma metastases to BM
SOX11	Transcription Factor	No expression	Mantle cell lymphoma, Burkitt lymphoma
TIA-1	T-cell intracellular antigen-1	Cytotoxic cells, NK cells, monocytes	T-cell and NK cell neoplasms
T-BET/ TBX21	T-box–transcription factor. Master regulator of the T–helper (Th)1 response	CD4+T cells committed to Th1 T cell development	HCL cells, diagnosis of minimal BM involvement by HCL, HL, PTCL-TBX21
TdT	Terminal deoxynucleotidyl transferase	Normal B cell precursors, Thymocytes	B and T cell precursors lymphoblastic leukemias, some AML, double hit lymphomas

Antibodies commonly used for diagnostic evaluation of BM biopsies by IHC

Antigen/ Antibody	Name/function	Expression in normal hematopoietic cell types	Main diagnostic application
TCRβ (βF1)	Beta chain of TCR $\alpha\beta$ heterodimer (bF1)	TCR $\alpha\beta$+T cells, the majority of T cells	classification of T cell lymphomas involving BM
TCRδ	Delta chains of TCR $\gamma\delta$ heterodimer T cells	TCR $\gamma\delta$ T cells, minor population in normal BM	Classification of T cell lymphomas involving BM
Tryptase	Mast cell tryptase	Normal mast cells	Mastocytosis
GATA 3	Transcription Factor, a member of GATA family zinc finger TFs, critical for the embryonic development of various tissues, regulates hematopoietic stem cell maintenance and cell cycle entry, required for early T lineage progenitor development	T cells.	PTCL-GATA3

Examples of expression in normal bone marrow of commonly used lymphoid and hematopoietic markers for bone marrow evaluation by IHC
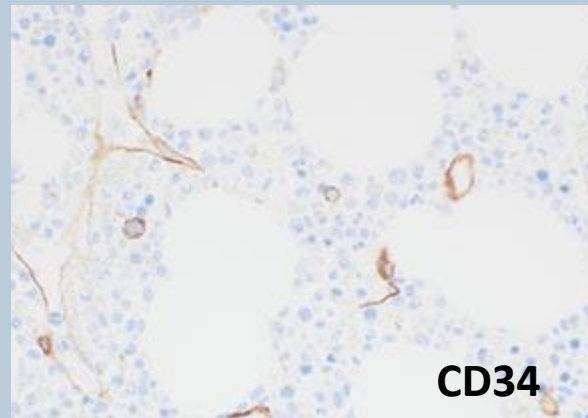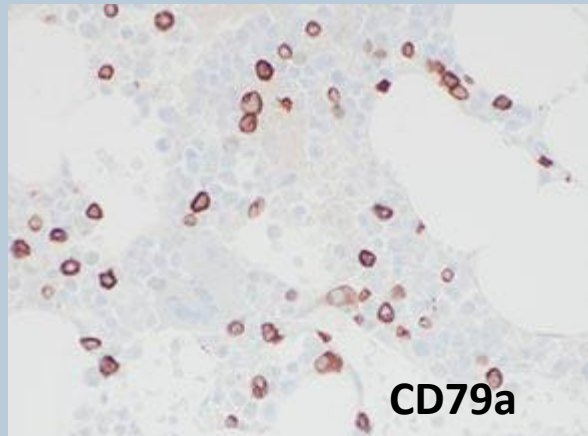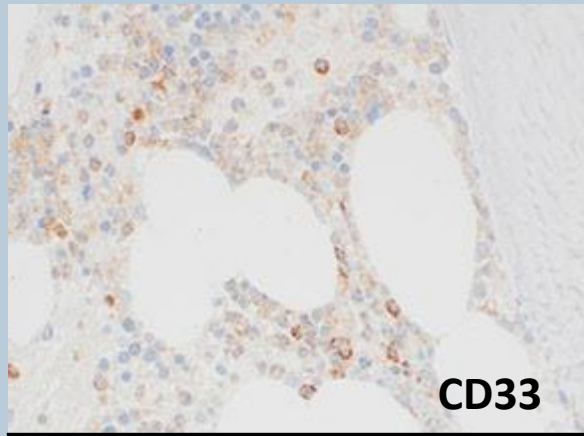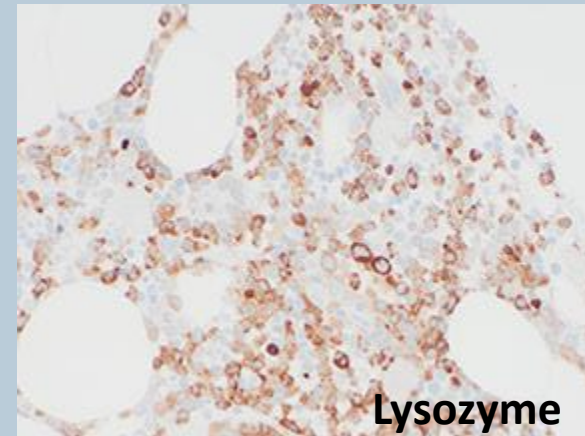

CD4
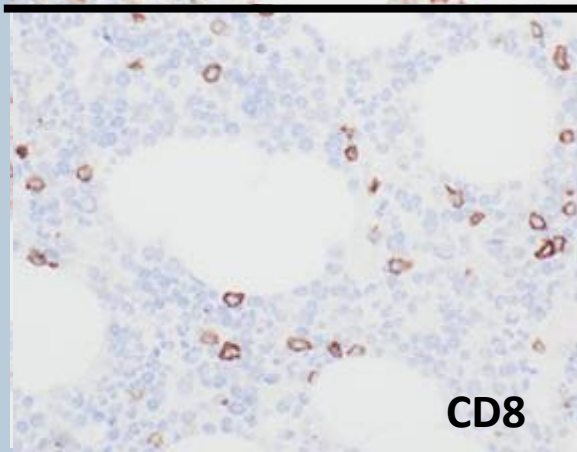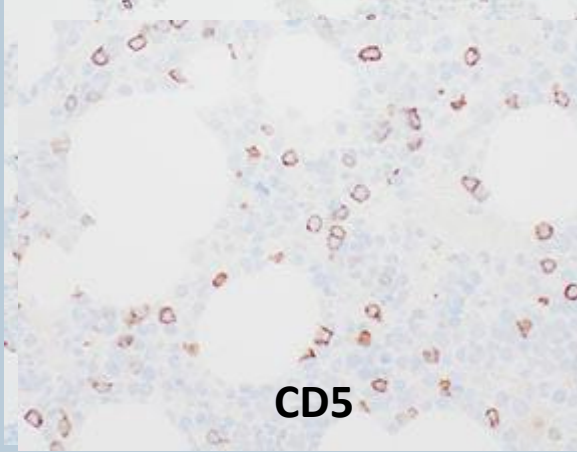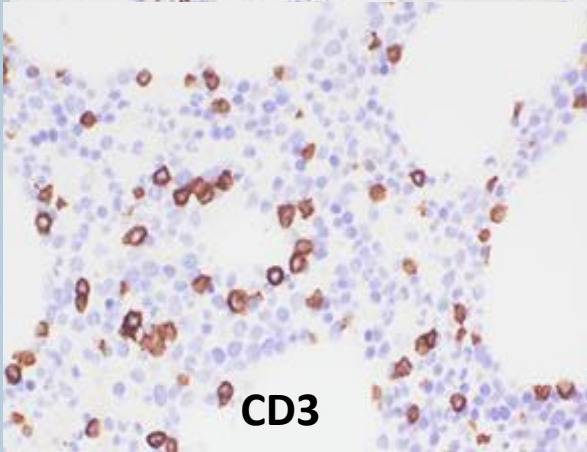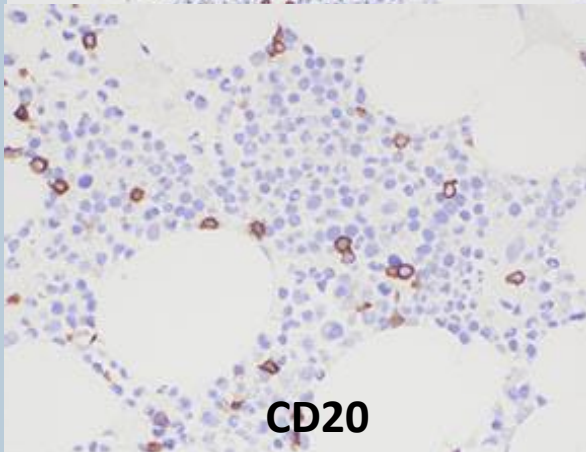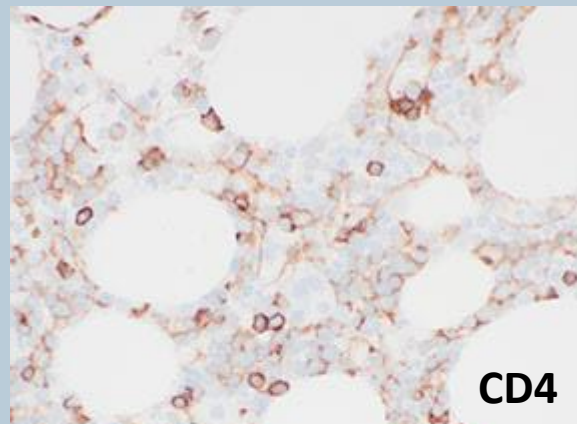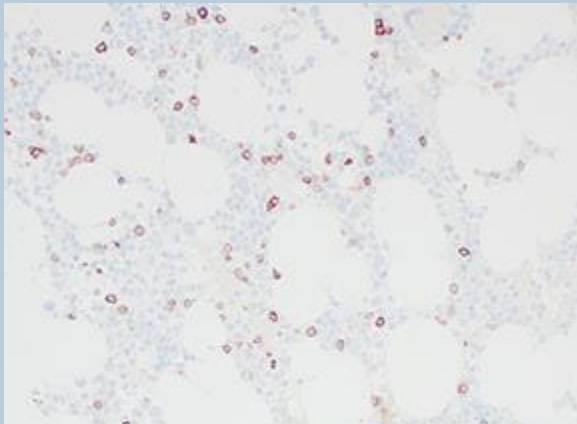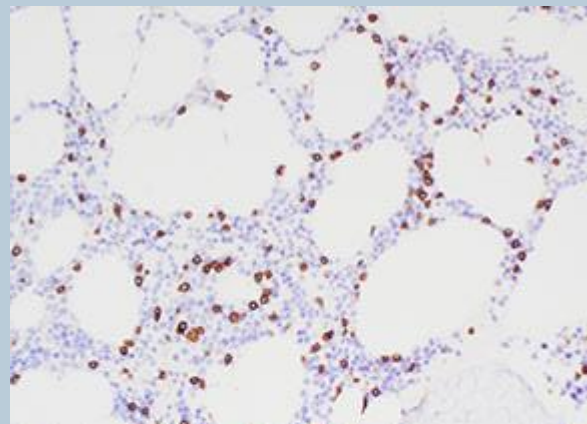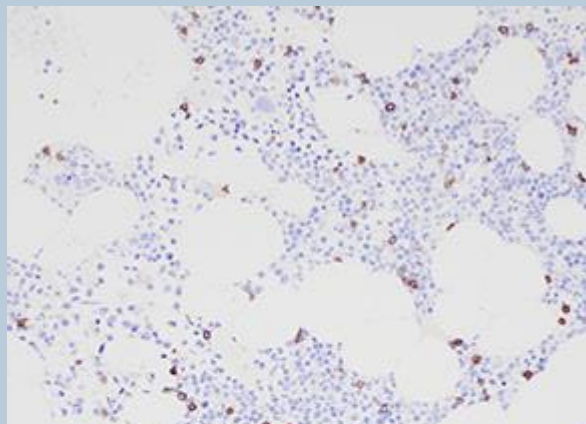
CD20

CD3

CD5

CD8

Expression in normal bone marrow of commonly used lymphoid and hematopoietic markers for bone marrow evaluation by IHC.
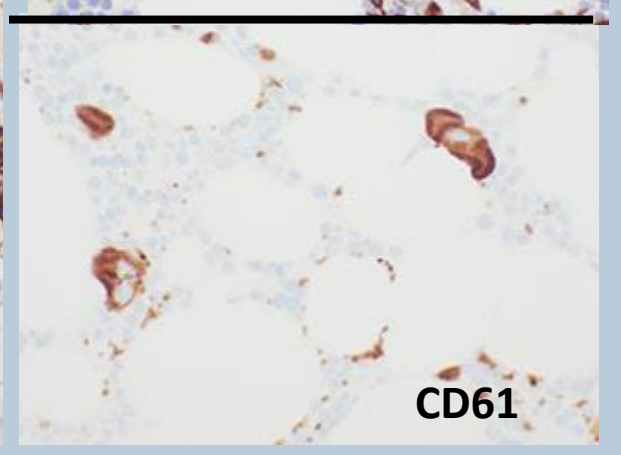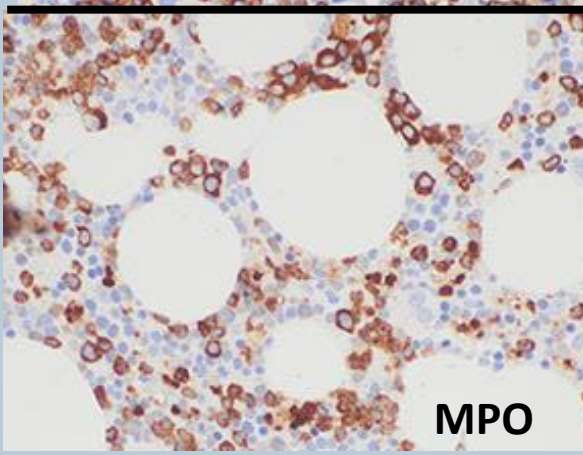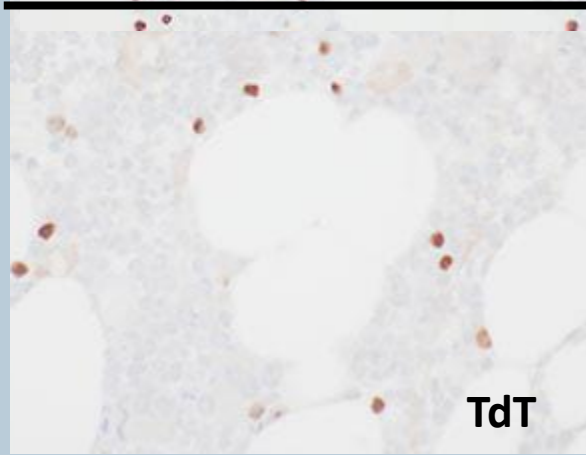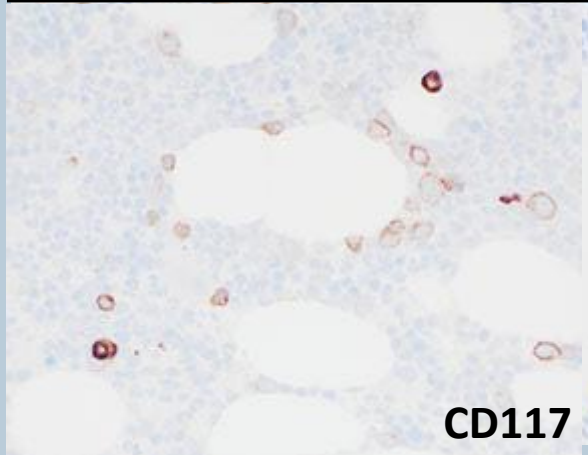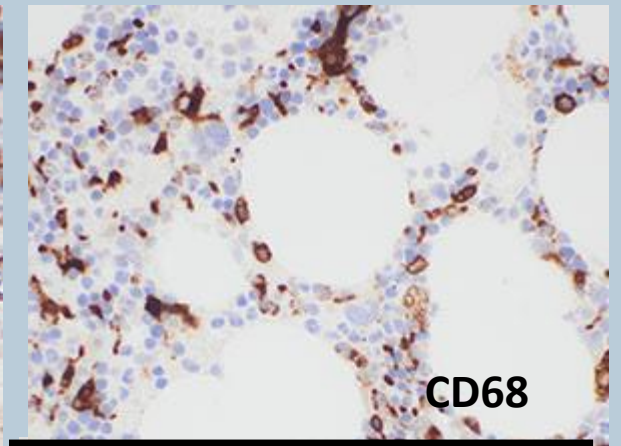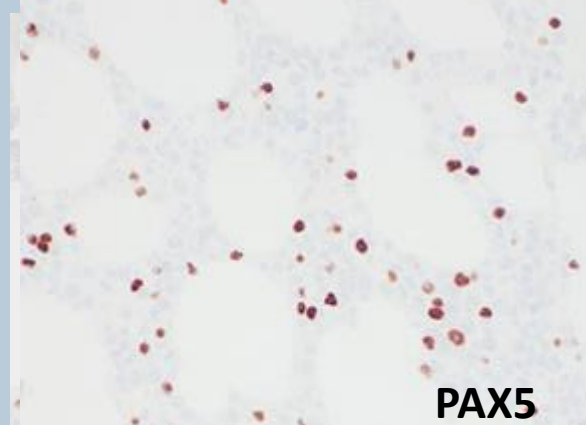
Examples of expression in normal bone marrow of commonly used lymphoid and hematopoietic markers for bone marrow evaluation by IHC
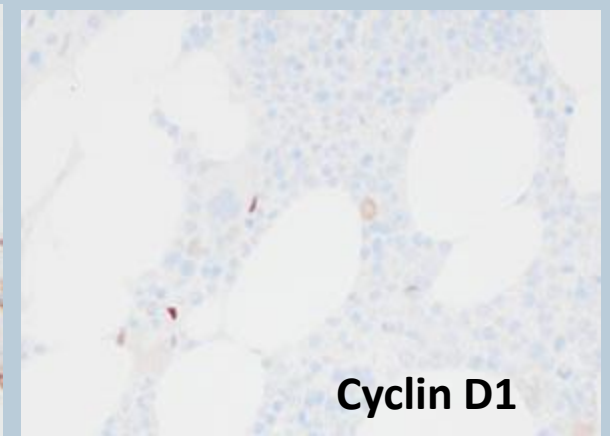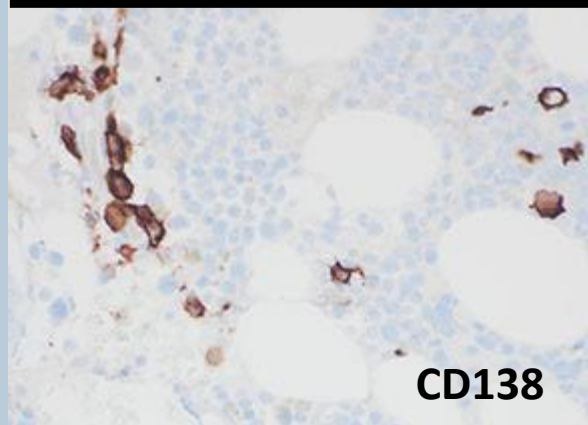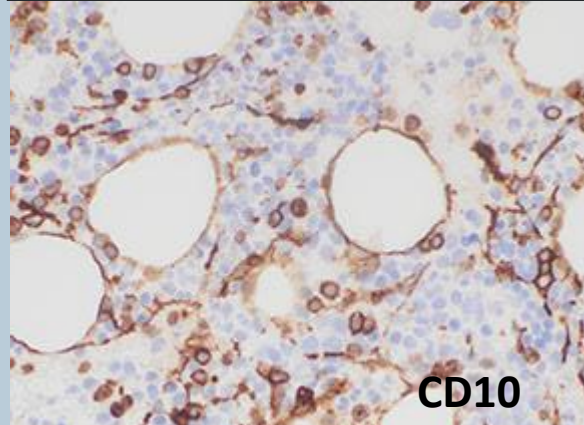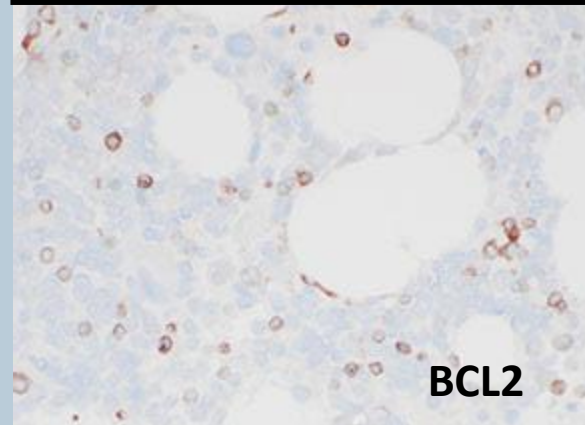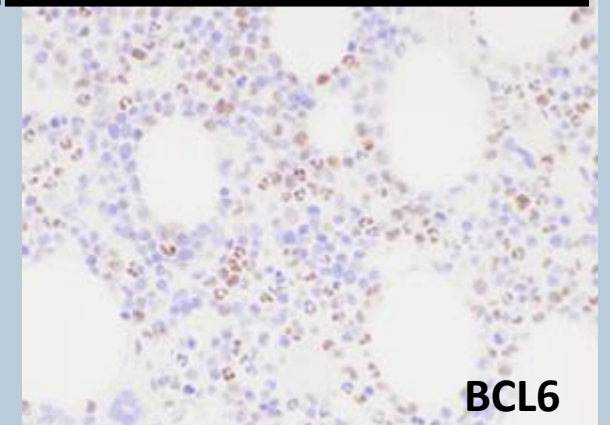
Antibody panels for specific diagnostic considerations of lymphoid and myeloid neoplasms according to the 2016 WHO classification

IHC markers useful for differential diagnosis of major B cell lymphoproliferative disorders in bone marrow trephine

WHO 2016 category	CD5	CD10	CD11c	CD19	CD20	CD22	CD23	CD25	CD103	CD123	CD200	PAX5	CD79a	CD138	CD30	Anne-xin 1	LEF1	BCL1	Bcl2	BCL6	MUM1/IRF4	C-MYC
CLL	+	-	±	+	+w	-/r+	+/r-	+/-	-	-	+	+	+	-	-	-	+	-	+	-	±	-
HCL	-/r+	-/r+	+s	+	+s	+	-/r+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	-
HCLv	-	-/r+	±	+	+	+	-	-	+/r-	-/r+	+	+	+	-	-	-	-	-	+	-	±	-
LPL	-/r+	-	-	+	+/r-	+	-/r+	-	-	-	-	+	+	±	-	-	-	-	+	-	-/r+	-
SMZL	-	-	+	+	+	+	-	±	-/r+	-	+	+	+	-/r+	-	-	-	-	+	-	-/r+	-
MCL	+	-	-	+	+	+	-/r+	-	-	-	-	+	+	-	-	-	-	+/r-	+	-	-	-
FL	-	+	-	+	+	+	±	-	-	-	±	+	+	-/r+	-/r+	-	-/r+	-	+/r-	+	±	-
DLBCL	±	±	±	±	±	+	±	±	±	-	±	+/r-	+/r-	±	±	-	-/r+	-	+/r-	±	±	±
BL	-	+	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+

CLL-chronic lymphocytic leukemia, HCL-hairy cell leukemia, HCLv- HCL variant, LPL-lymphoplasmacytic lymphoma, SMZL-splenic marginal zone lymphoma, MCL-mantle cell lymphoma, FL-follicular lymphoma, DLBCL-diffuse large B –cell lymphoma, BL-Burkitt lymphoma

+=mostly positive, - =mostly negative, ± = variable expression, r+=rarely positive, -r =rarely negative, s = strong, w =weak expression
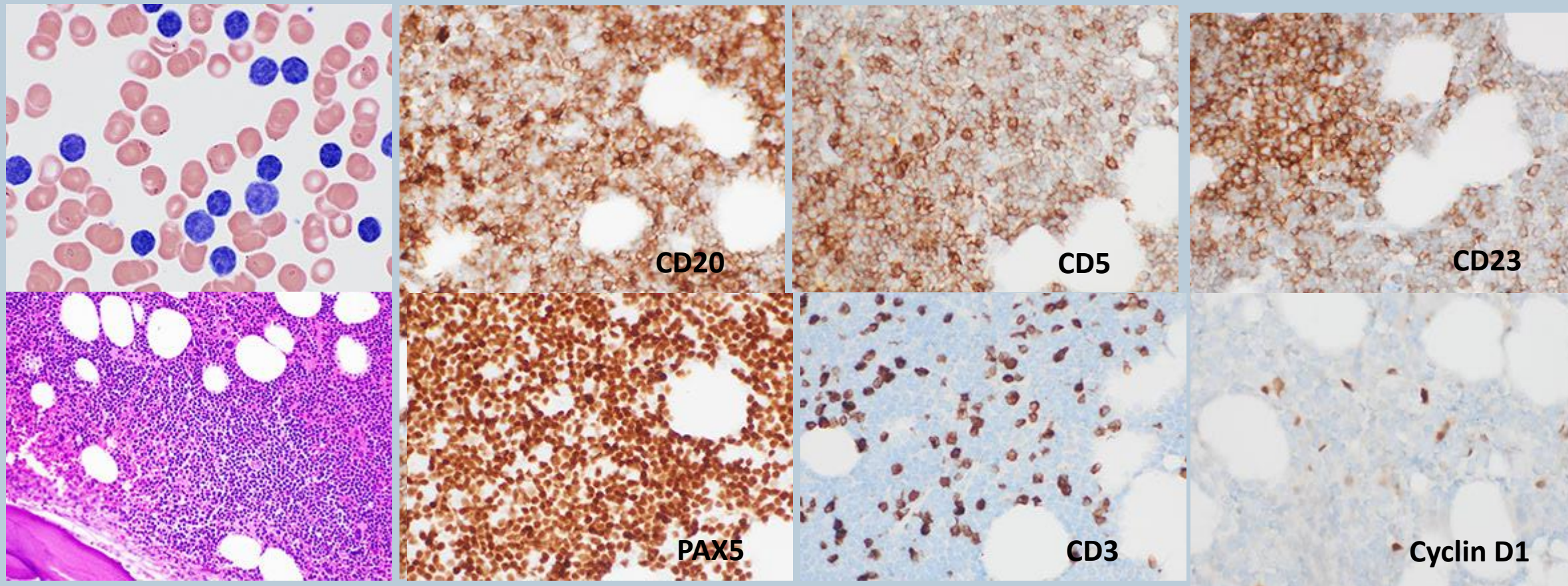
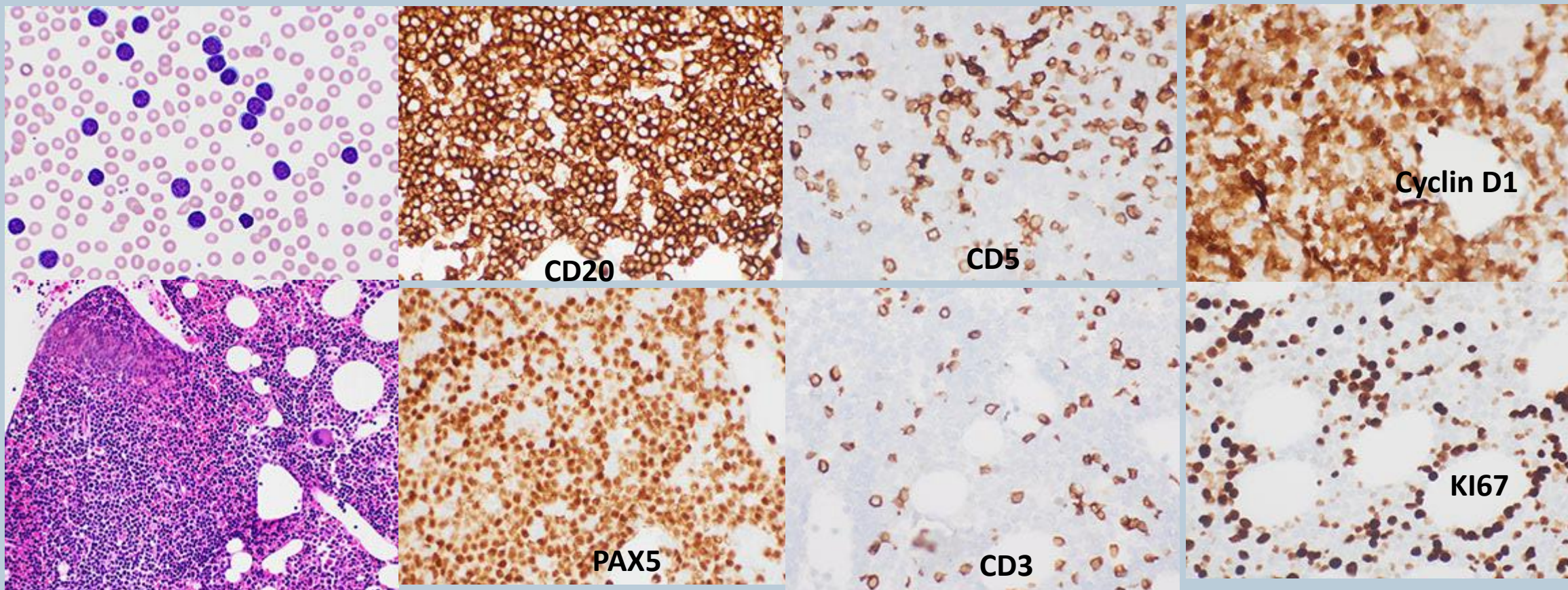# Example of IHC utility for bone marrow diagnosis of CLL/SLL.

Phonotype: **CD20+, PAX5+, CD5+(weak), CD23**+, CD3-, cyclinD1-

75 year old man with WBC 234 k/ul with lymphocytosis 217.68 K/ul

Example of IHC utility for bone marrow diagnosis of Mantle cell lymphoma

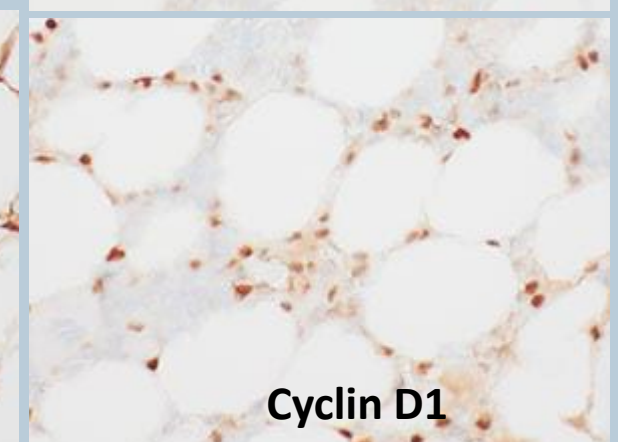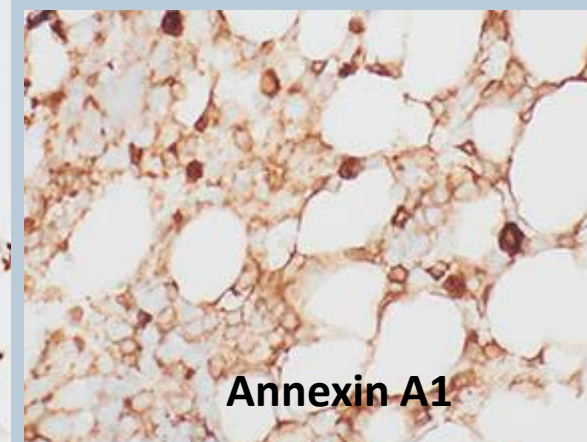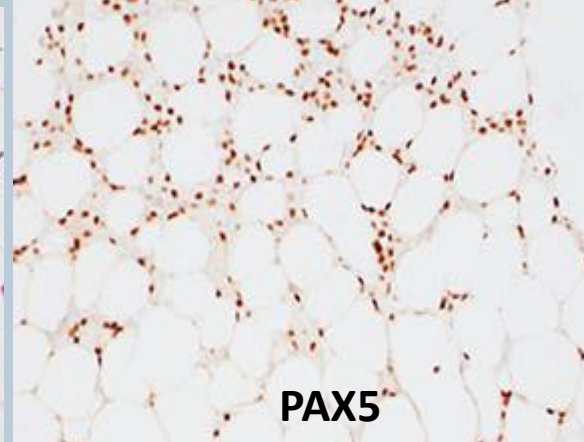**Phonotype**: **CD20+, PAX5+, CD5-, Cyclin D1+,** CD3-

76 year old man with diagnosis of mantle cell lymphoma. Bone marrow biopsy is performed for staging

FISH studies on PB detected deletion of 17p (in 85.5% of cells)
and *IgH/CCND1* rearrangement in 79.5% of cells.

Example of IHC utility for bone marrow diagnosis of Hairy cell leukemia

Phonotype: **CD20+, PAX5+, Annexin-A1+, CD123+, cyclin D1+, CD5--**
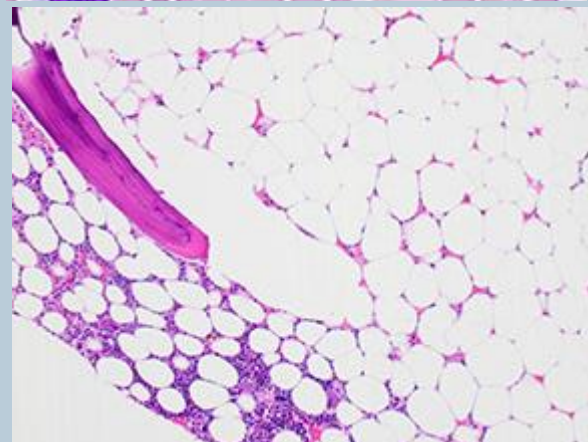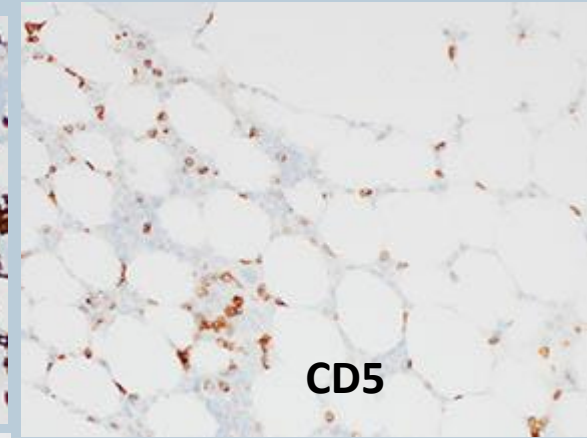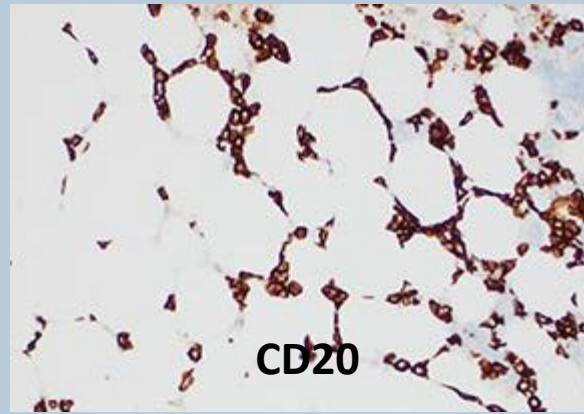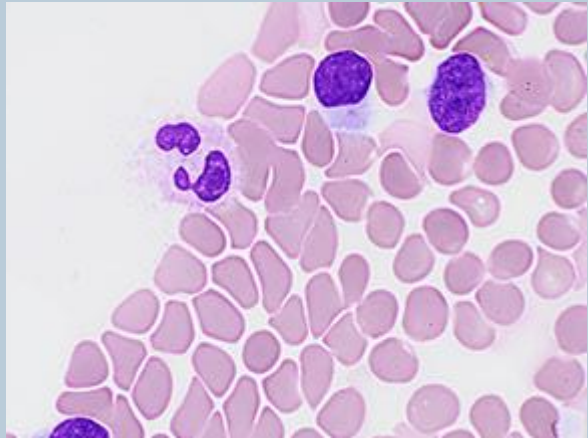
73 year old man with pancytopenia

Example of IHC utility for bone marrow diagnosis of Follicular lymphoma
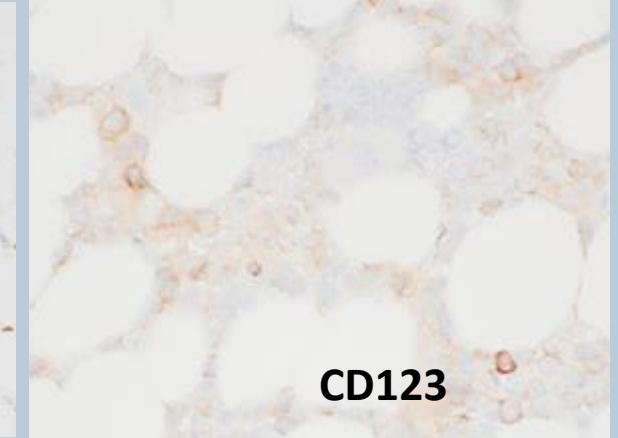
Phonotype: **CD20+, CD10+, BCL6+, BCL2+, CD5-,** cyclin D1-

7o year old woman diagnosed with low grade follicular lymphoma.
Bone marrow biopsy is performed to evaluate the marrow status.

IHC antibodies panels by diagnosis of bone marrow involvement by Hodgkin lymphoma.

- Bone marrow involvement by Hodgkin lymphoma is rare (~10% incidence), and consistent with stage IV disease. Examination of bone marrow biopsy in patients with Hodgkin lymphoma is important for disease staging.
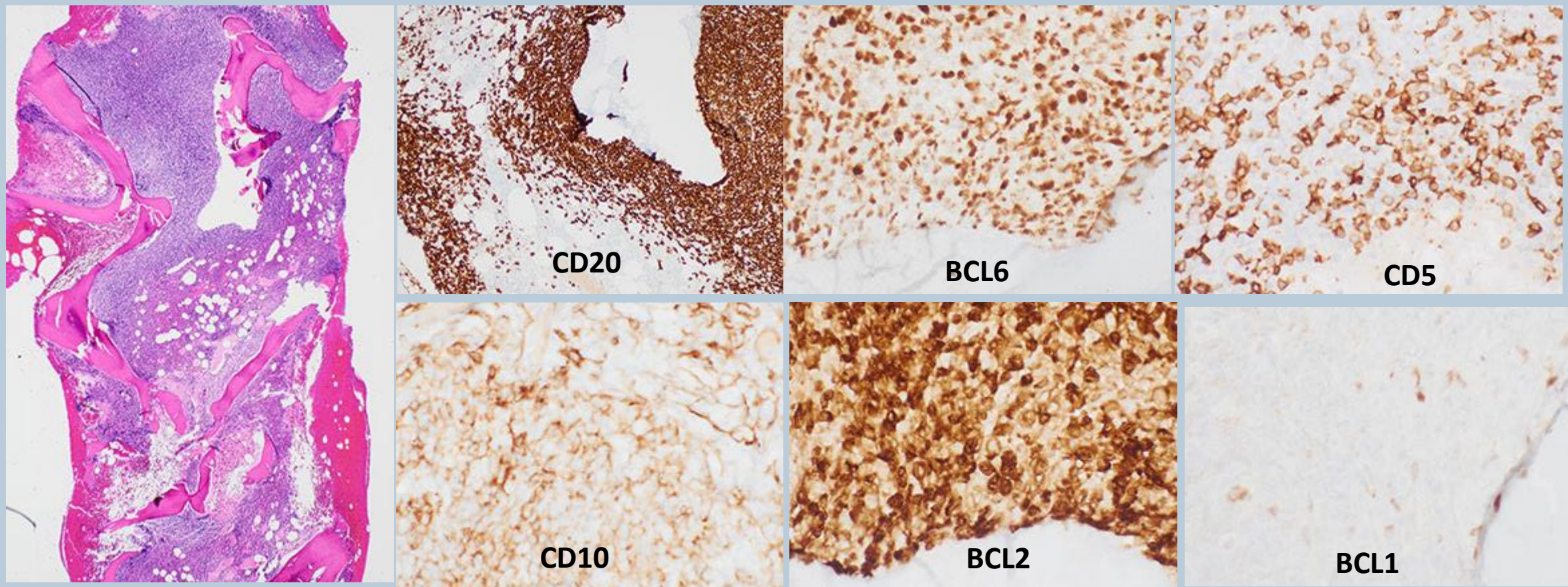- Isolated bone marrow involvement by cHL without nodal or other extranodal manifestation is extremely rare (0.25%), usually occurring in HIV+ patients, and is typically EBV associated.

WHO 2016 category	CD 30	EMA	PAX 5	CD 15	CD 45	CD 20	CD 79a	OCT 2	BOB 1	MUM 1	BCL 6	EBER / ISH	CD3	CD 68	CD 57	PD 1	IgD	CD 21
NLPHL	-	+	+S	-	+	+	+	+	+	+	+	-/+	+ T cells	+ Histio-cytes	+ Trosettes	+ Trosettes	+ B cells	+ FDCs
C HL	+	-	+w	±	-/s+	±	±	±	±	+	+	±	+ T cells	+ Histio cytes	-/+	-/+	-	-/+

XV EBMWG **2022**
LUND SWEDEN
MAY 28th - 31st

Example of IHC utility for bone marrow diagnosis of involvement by Hodgkin lymphoma

Primary BM c HL with intense fibrosis and inflammatory background
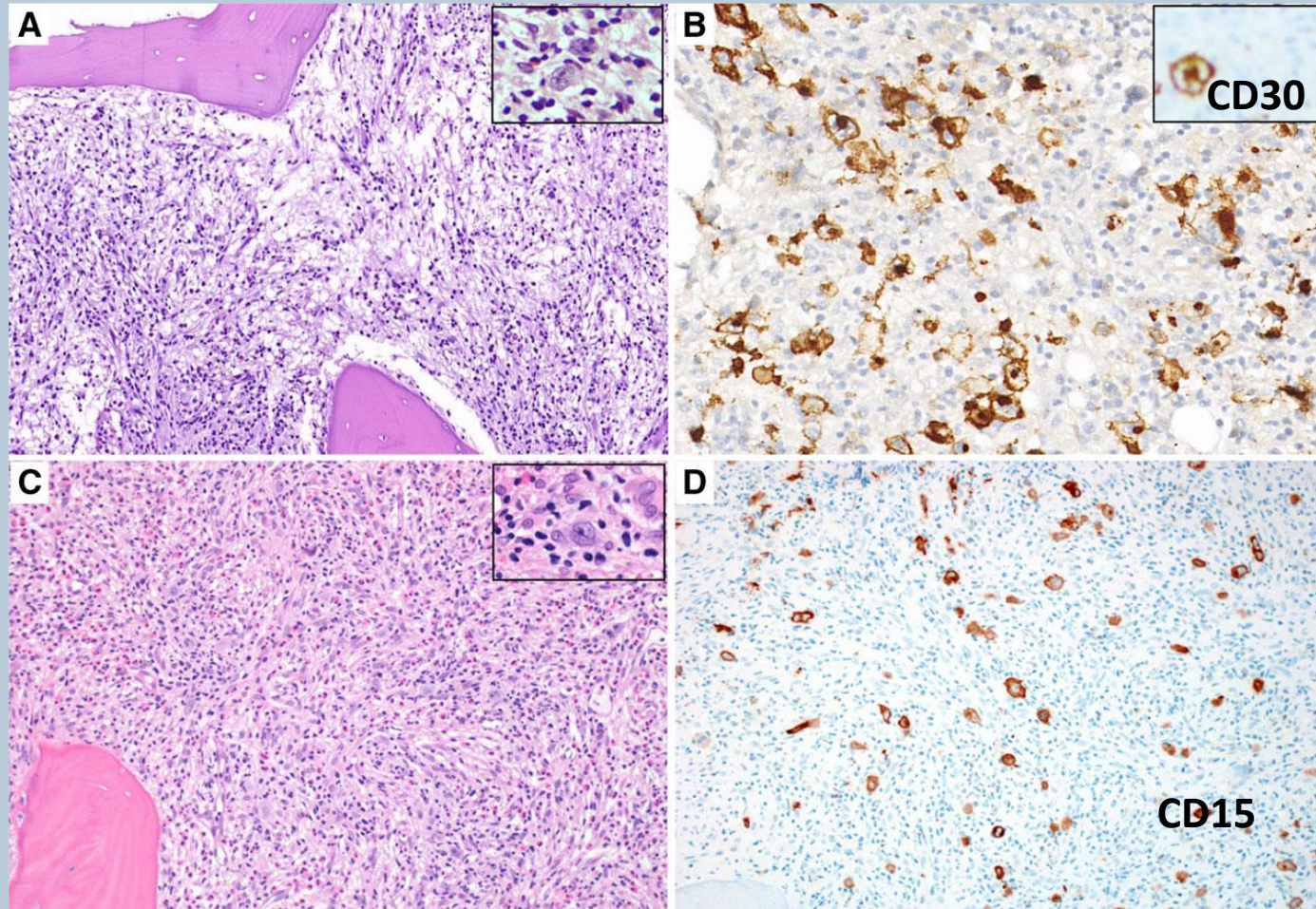
Laurent C, Arber DA, Johnston P et al. Diagnosis of classic Hodgkin lymphoma on bone marrow biopsy. Histopathology. 2020; 76:934-941

Bone marrow involvement in peripheral T-cell lymphomas

Primary presentation	Lymphoma type	Histological BM involvement %
Leukemic/disseminated	**T-cell prolymphocytic leukemia (T-PLL)**	**>95**
	T-cell large granular lymphocytic leukemia (T-LGL)	**>95**
	Adult T-cell leukemia/lymphoma (ATLL)	**60-70**
	Aggressive NK-cell leukemia	**>95**
Nodal	**Angioimmunoblastic T cell lymphoma**	**60-70**
	ALCL	15-25
	PTCL, unspecified	**20-40**
Extranodal	Mycosis fungoides	<5
	Primary cutaneous ALCL	<5
	Subcutaneous panniculitis –like TCL	<5
	Enteropathy-type TCL	<5
	Hepatosplenic TCL	**>95**
	Extranodal NK/TCL, nasal type	<5

Dogan A, Morice W. Bone marrow histopathology in peripheral T cell lymphoma. BJH 2004; 127:14-154

IHC antibodies panels useful for differential diagnosis of major T cell lymphoproliferative disorders in bone marrow trephine biopsies

WHO 2016 category	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD16	CD25	CD56	CD57	TCR αβ	TCR γδ	CD30	TiA1	Granz B	Perf	ALK1	PD1	BCL6	CXCL13	ICOS	TCL1
T-PLL	+	+	±	+	+	-/r+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
ATLL	+	+	+	+	-/r+	-	-	-	+	-	-	+	-	r+	-	-	-	-	-	-	-	-	-
SS	+/r-	+	+	+	-/r+	-	-	-	-/r+	-	-	+	-	-	-	-	-	-	+	-	-	+	-
AILT	+	+	+	+/r-	±	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	-
ALCL	±	±	+/-	-	-	-/+	-	+	-	+	-	-	-	+	+	+	+	+s	-	+	-	-	-
LGL	+/r-	+/r-	-	-/r+	±	+	-	±	-	-/r+	+	+	+s	-	+	+	+	-	-	-	-	-	-
CNKLPD	+	-	-	-	±	±	-	±	-	±	±	-	-	-	+	+	+	-	-	-	-	-	-
ANKL EBV+	+/r-	+*	-	-	-/r+	-/r+	-	+	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-
HSTCL	+	+	-	-/r+	+/r-	-/r+	-	-/r+	-	±	-/r+	+r	+	-	+	-	-	-	-	-	-	-	-
PTCL	+/r-	+	±	±	±	±	-	-	-/r+	-/r+	-	+	-	±	-/+	-/+	-/+	-	-/+	-	-	-	-

T-PLL:T-cell prolymphocytic Leukemia; ATLL: Adult T-cell leukemia/lymphoma; SS: Sezary syndrome AILT: Angioimmunoblastic T cell lymphoma; ALCL: Anaplastic large cell lymphoma; LGL: T-cell Large granular lymphocyte leukemia, CNKLPD: Chronic natural killer lymphoproliferative disorder, ANKL: Aggressive natural killer cell leukemia; HSTCL: Hepatosplenic T-cell lymphoma, PTCL: Peripheral T cell lymphoma

+=mostly positive, - =mostly negative, ± = variable expression, r+=rarely positive, r- =rarely negative; s=subset; * CD3 epsilon.

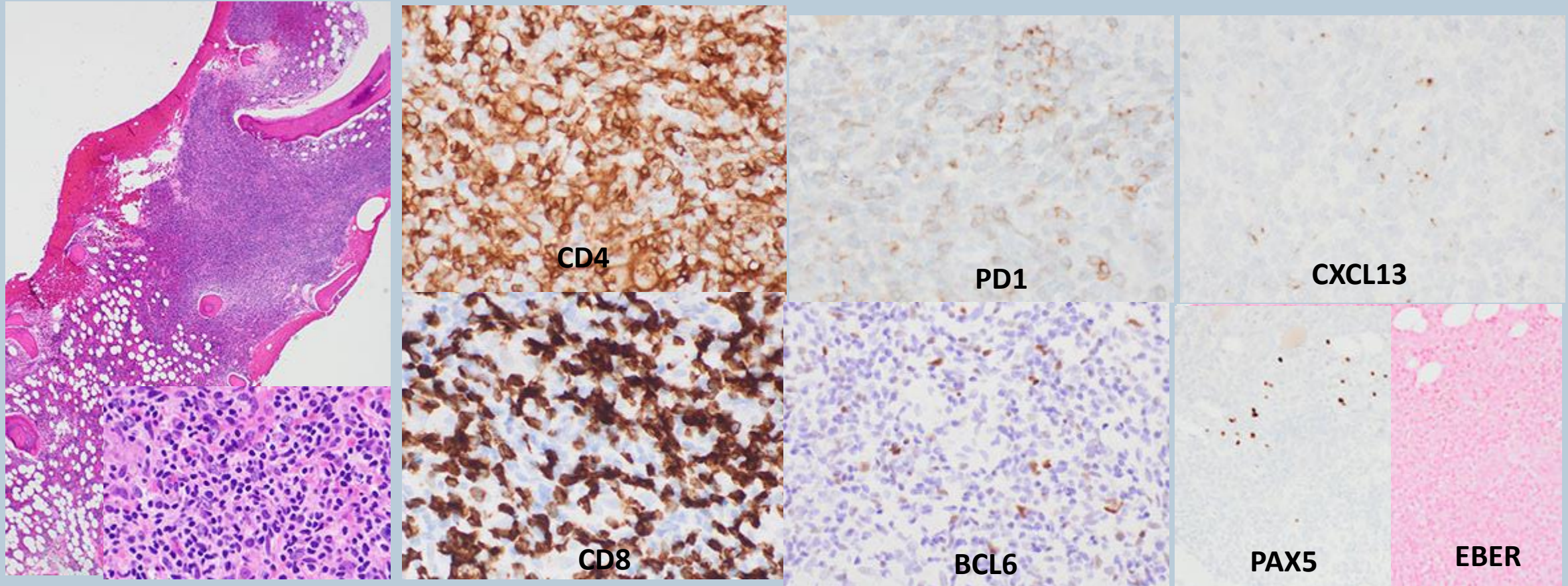# Example of IHC utility for bone marrow diagnosis of Angioimmunoblastic T cell lymphoma

Phenotype: **CD4+, PD1+, vCD10+, vBCL6+, vCXCL13+, EBER-,** CD8-

70 years old woman with diffuse lymphadenopathy and diagnosis of AILT two years ago on pralatrexate. Bone marrow biopsy is performed to evaluate the current marrow status.

WHO 2016 category	CD34	CD117	CD123	TdT	CD19	CD22	CD79a	PaX5	CD10	CD20	CD25	CD7	CD2	CD3*	CD5	CD4	CD8	CD1a	MPO	CD33	CD68	CD163	lyz	CD56	NPM1	P53
B-ALL	±	-	-/+	+	+	+	+	+	±	±	S+	-	-	-	-	-	-	-	S+	S+	-	-	-	-	-	-
T-ALL	±	S+	-	+	-	-	S+	-	S+	-	-	+	±	+	±	±	±	±	-	S+	-	-	-	-	-	-
AML	±	±	±	±	±	r+	S+	S+	S+	-	S+	S+	-	-	r+	±	-	-	±	±	±	±	±	S+	S+	S+

S=subset, ± =variable expression, r-= rarely positive

- In most cases of acute leukemias, FC analysis is performed up front and IHC can be limited.
- If FC was not performed, IHC is useful in immunophenotypic characterization of acute leukemia and establishing diagnosis.
- If the expression of intracytoplasmic markers necessary for diagnosis of mixed phenotype leukemia (MPAL) according to the WHO criteria has not been performed, IHC for MPO, PAX5, CD22, CD79a, lysozyme will be helpful to exclude or confirm MPAL.
- P53 expression in AML is useful tool in identifying AML-MRC that are TP53 mutated and have complex karyotype and poor prognosis

Example of IHC utility for bone marrow diagnosis of B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KTM2A*-rearranged

Phenotype: vCD34+, PAX5+, CD19+, CD10-, CD15+/-, CD33+, MPO-, CD3-.-
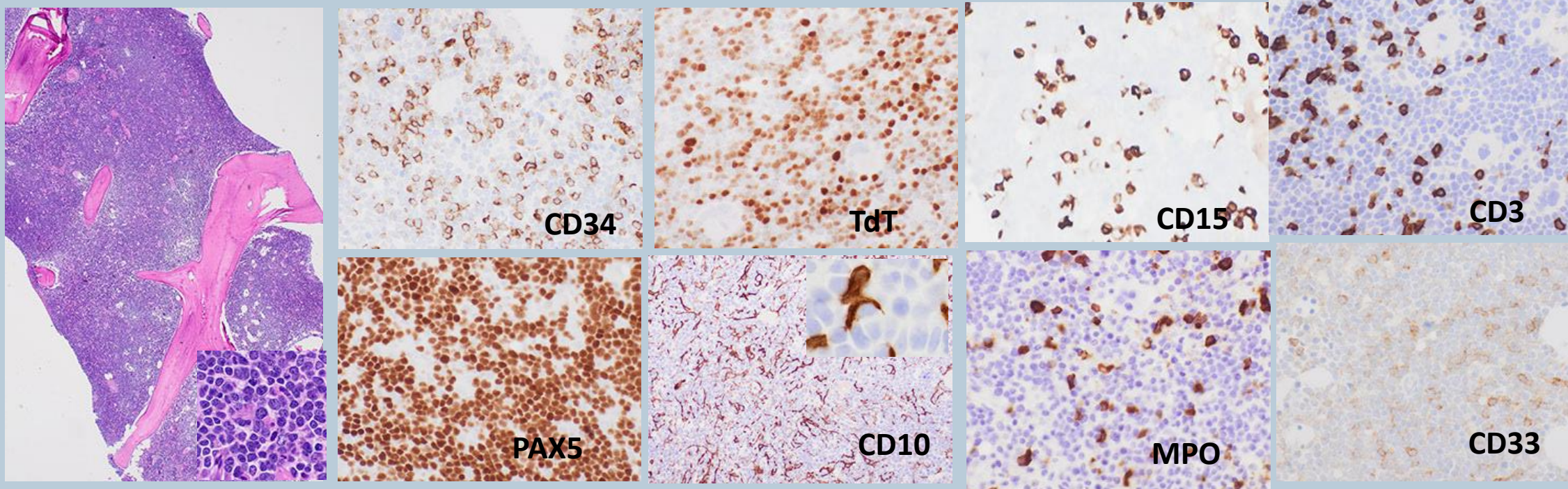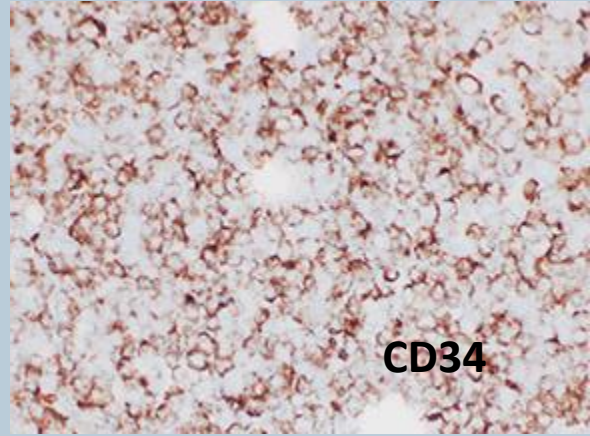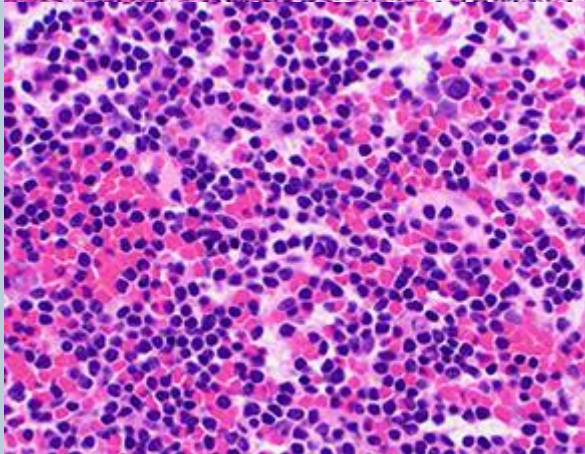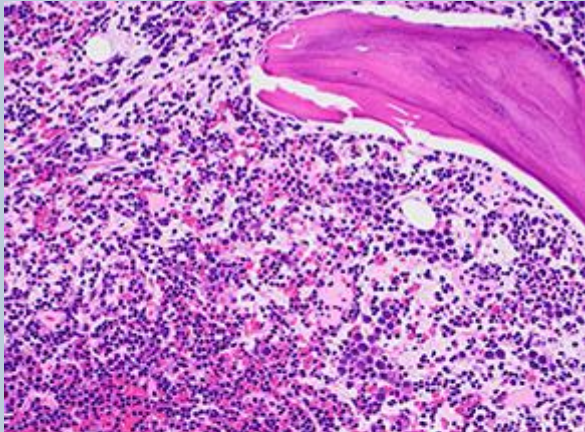

33 years old man with leukopenia with occasional circulating blasts, fevers, lightheadedness and dyspnea admitted for diagnostic work up.

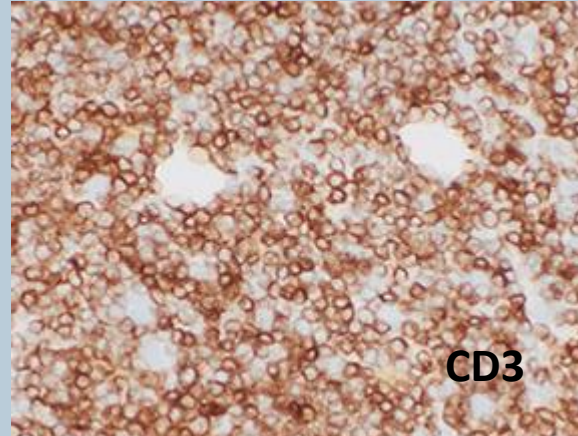FISH analysis showed t(v;11q23.3);*KMT2A* rearrangement

Example of IHC utility for bone marrow diagnosis of T-lymphoblastic leukemia/lymphoma
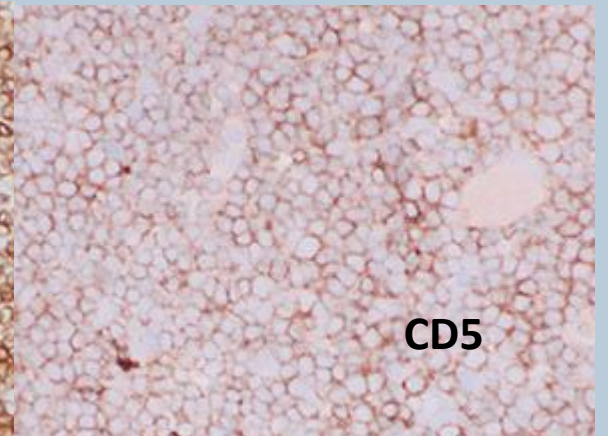
Phenotype: CD34+, CD7+strong, cyt.CD3+, CD2-, CD5+(weak), v CD1a+, CD4-, CD8-,
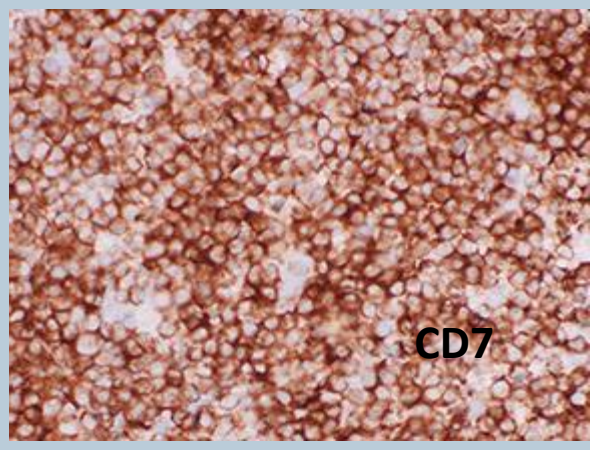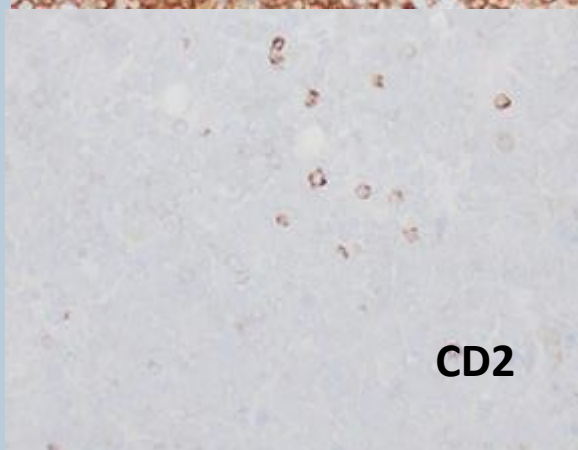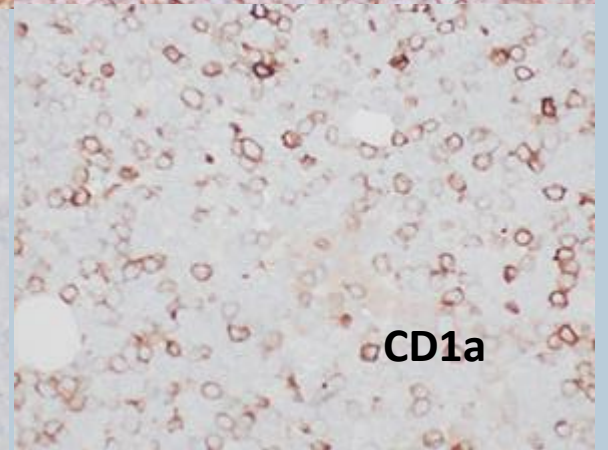
75 years old woman with clinical history of breast cancer , status post bilateral mastectomies and adjuvant chemotherapy and radiation found to have leukocytosis with circulating blasts. Flow cytometry of peripheral blood revealed 80% blasts positive for Cd13 and Cd33 as well as aberrant CD7 expression.

Example of IHC utility for bone marrow diagnosis of T-lymphoblastic leukemia/lymphoma

Phenotype: CD4-, CD8-, TdT+, PAX5-, CD79a+, CD33+weak, MPO-, lysozyme-.
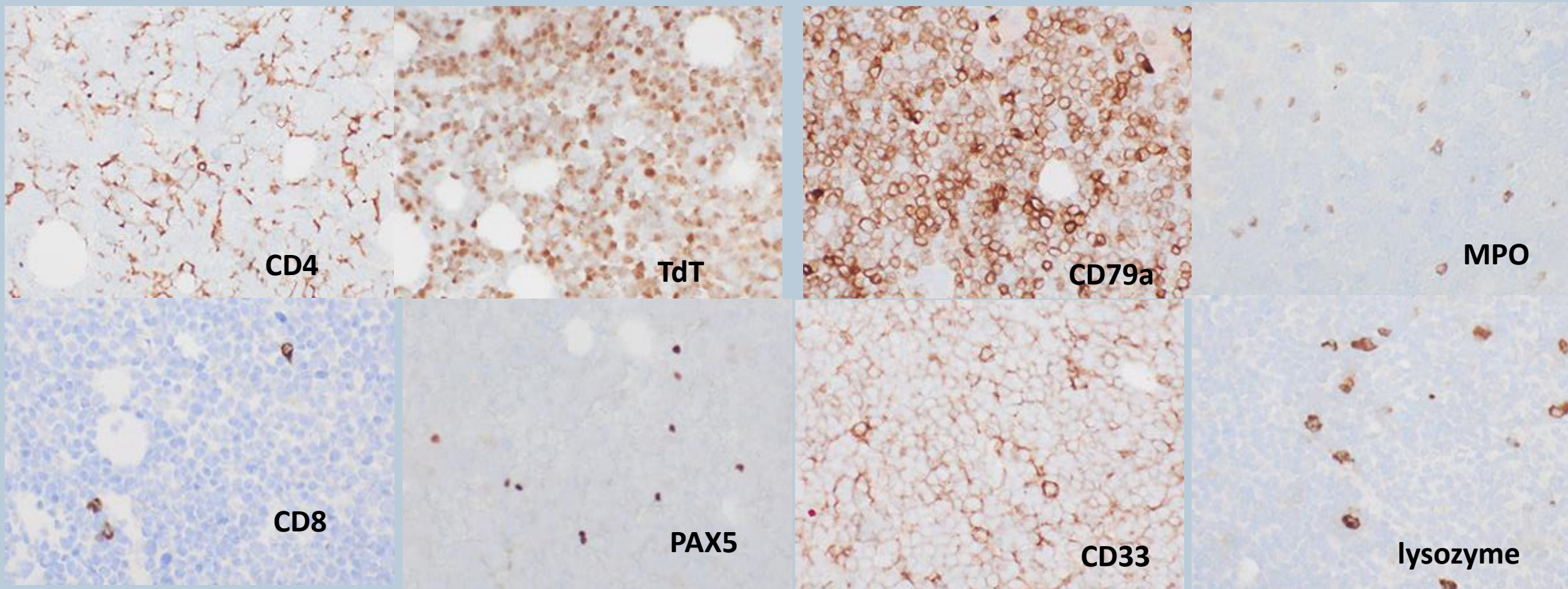
IHC markers for bone marrow evaluation of myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN) , and MDS/MPN, and systemic mastocytosis

WHO 2016 category	Immature compartment		Erythropoiesis				Grans	Megakaryocytes			Monocytes				Mast cells			
	CD34	CD117	GycoC	HGBA	ECad	Spectrin	MPO	CD61	vWF	CD42	CD14	Lyz	CD68	CD163	MCT	CD25	CD2	P53
MDS	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	-	-	±
MPN	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	-	-	
MDS/MPN	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	+s	-	-	
MDS+/- AHN		+													+	+	+/-	

+s=subset positive

- The presence of CD34 in clusters confers worse prognosis in MDS patients.
- IHC for CD34 is especially useful in cases with fibrosis for evaluation of blast count.
- CD117 IHC is useful in patients with CD34(-) blast cell population, however assessment is more difficult as CD117+ cells include immature myeloid and erythroid precursors and mast cells.
- IHC for P53 may be useful in evaluation of high risk MDS, and progression of MDS with del 5q on lenalidomide therapy
- CD20 and CD3 may add to exclude lymphoma or prove reactive character of lymphoid infiltrate.

Example of IHC utility for bone marrow evaluation in MDS/MPN

CMML-1

CD33

Lysozyme

CD14

CD34

CD61
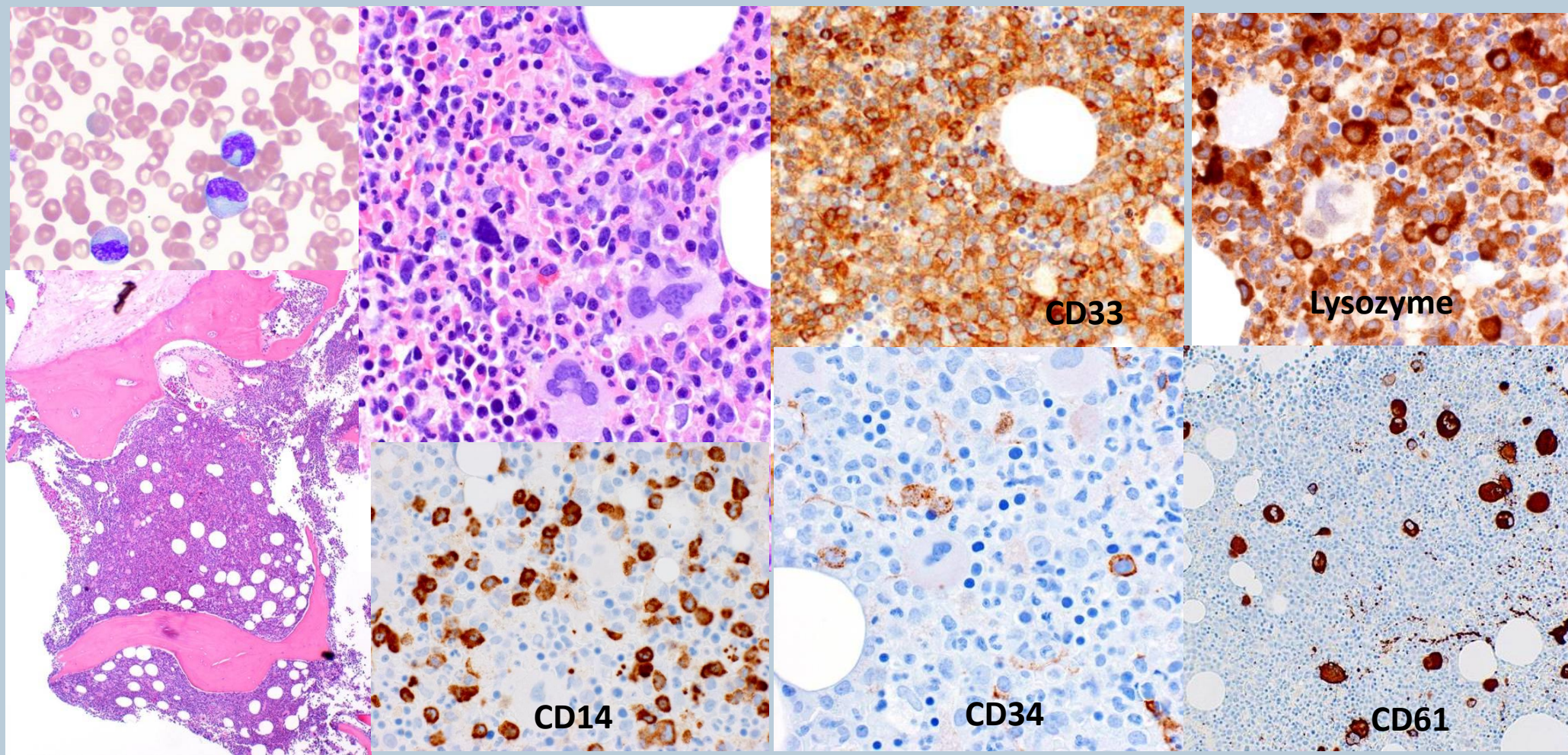
Example of IHC utility for bone marrow evaluation in MDS/MPN

CMML-2
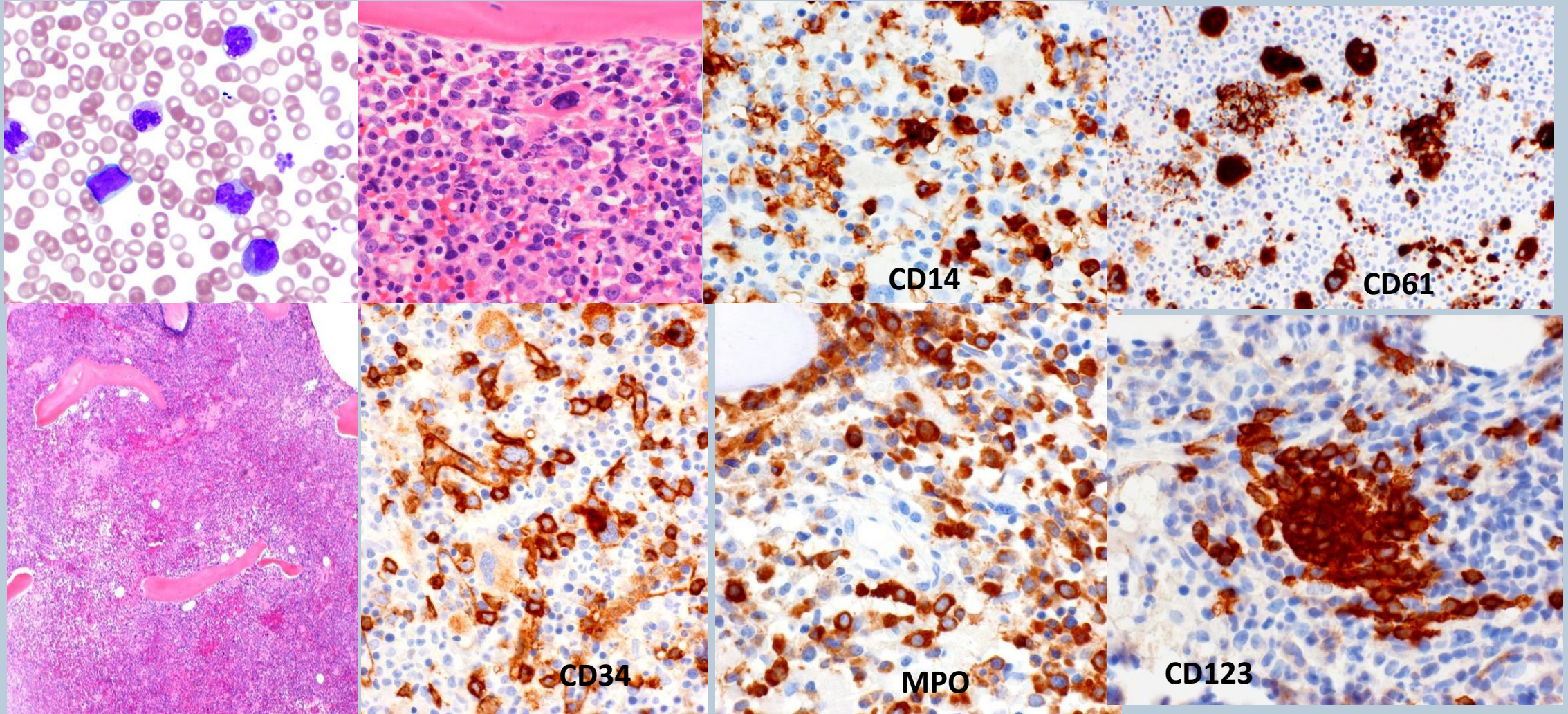
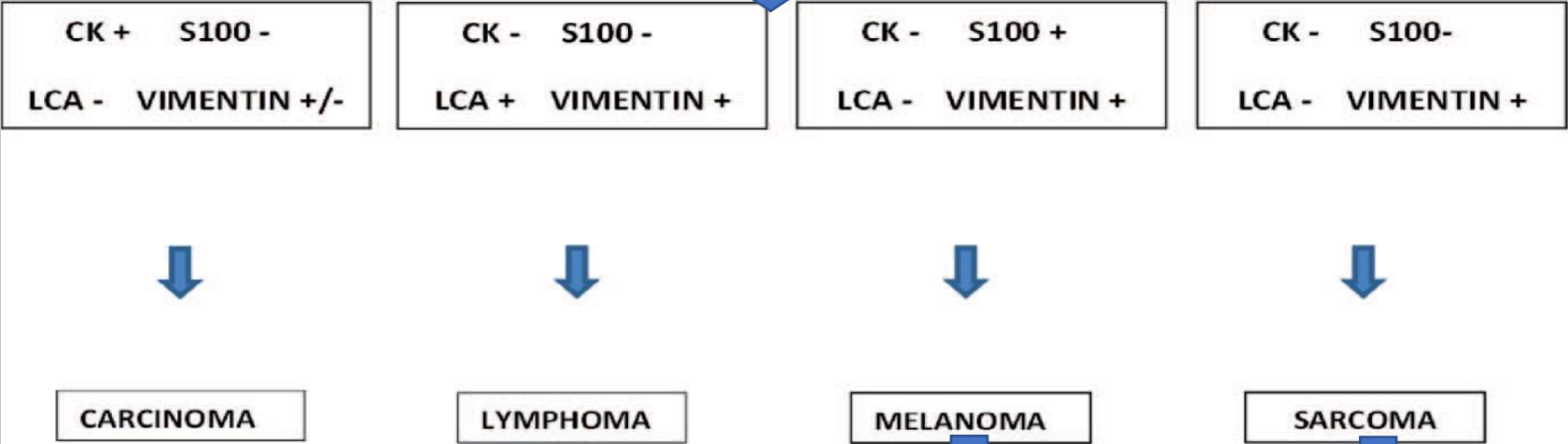

CD14

CD61

CD34

MPO

CD123

Diagnostic relevance of immunohistochemistry for diagnosis of metastatic tumors in the bone marrow and reactive conditions.

Diagnostic work up of metastasis of unknown primary to the bone marrow

Review the clinical information (age, sex, imaging , laboratory data)
together with histomorphology of the metastatic tumor

↓

Order cytokeratin (CKAE1/AE3 and CKCAM) , S100, CD45(LCA) and Vimentin

CK + S100 -	CK - S100 -	CK - S100 +	CK - S100-
LCA - VIMENTIN +/-	LCA + VIMENTIN +	LCA - VIMENTIN +	LCA - VIMENTIN +

↓

CARCINOMA	LYMPHOMA	MELANOMA	SARCOMA

↓

CARCINOMA
Order CK7, CK20 and dependent on morphology results CK5, TTF1, GATA3, CD56, synaptophysin Chromogranin A, ER PSA, PSAP, MUC1, CD10, CEA, CDX2, P63, PAX8 for differentiation of epithelia tumors

LYMPHOMA
Continue with Lymphoma work up

MELANOMA
SOX10 , HMB45, MITF, MART-1

Lipogenic tumors MDM2 and CDK4

Neurogenic tumors: TLE

SARCOMA
CD34, CD31, Erg, Fli-1, CD117, DOG1, Desmin, MUC4, ALK-1, SMA, MYoD1, myogenin, CD10, ER, TFE3

Small round cell tumor CD99, NSE, WT1

Metastatic breast carcinoma to BM
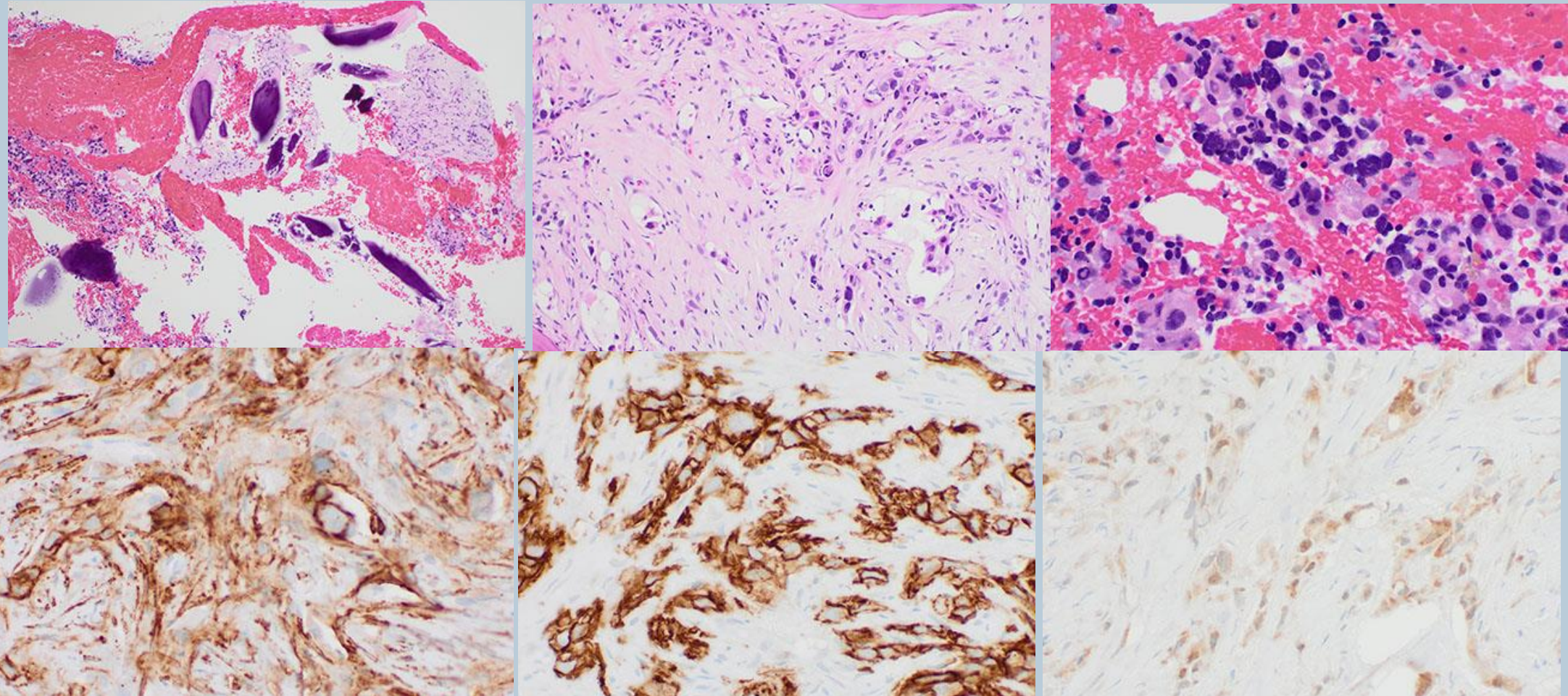
CD138

E-Cadherin

GATA-3

IHC utility for evaluation of reactive changes in bone marrow

- Evaluation of lymphoid aggregates, which are common especially in the elderly and autoimmune diseases, and distinction between reactive (benign) and neoplastic aggregates.-
  - Reactive aggregates are small, with well defined borders, distributed randomly in bone marrow, have polymorphic cellular composition, some may contain germinal centers. By IHC benign lymphoid aggregates show a polytypic staining pattern with mixed B and T cells , and polytypic plasma cells. Ambiguous cases may require molecular analysis of clonality

- Evaluation of increased number of hematogones in regenerative marrow , post chemotherapy or after allogeneic hematopoietic stem cell transplant.
  - In contrast to B-ALLL/LBL, hematogones show a range of maturation with variable expression of TdT, CD34, CD10, CD20, and CD79A . Usually mature forms lacking TdT expression predominate.

- Identification of infectious agents, especially in immunosuppressed patients.
  - Parvovirus B19 infection, which causes aplastic crisis and arrest in maturation of erythroid precursors.
  - EBV infection/reactivation.
  - Evaluation of HLH
  - Evaluation of marrow granulomas

Conclusion

- Immunohistochemistry on bone marrow trephine biopsies is a valuable complementary technique for bone marrow diagnosis of lymphoid and hematopoietic neoplasms and diagnostic work up of metastatic tumors in the bone marrow and reactive bone marrow changes.

- The results of IHC evaluation should be a part of integrated diagnostic BM report.

- Utilization of immunohistochemistry for bone marrow diagnosis is increasing with growing numbers of available antibodies/tumor markers reactive in paraffin tissue sections.

- Assurance of optimal performance of IHC for bone marrow diagnosis requires adherence to recommendations for the standardization of BMB IHC procedures published by Working Group within the International Council for Standardization in Hematology (ICSH).

References

1. Torlakovic EE, Naresh K, Kremer M et al. Call for a European programme in external quality assurance for bone marrow immunohistochemistry; report of a European Bone Marrow Working Group pilot study. J Clin Pathol; 2009;62(6):547-51.

2. Torlakovic EE, Brynes RK, Hyjek E et al. ICSH guidelines for the standardization of bone marrow immunohistochemistry. Int Jnl Lab Hem 2015, 37:431-449.

3. Torlakovic EE, Nielsen S, Francis G et al. Standardization of positive controls in diagnostic immunohistochemistry: recommendation from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol.2015;2391):1-18.

4. Torlakovic EE, Francis G, Garratt J. et al. Standardization of negative controls in diagnostic immunohistochemistry: recommendation from the International ad hoc expert panel. Appl Immunohistochem Mol Morphol 2014; 22(4): 241-52.

5. Engel P, Boumsell L, Balderas R et al. CD nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a driving force in Immunology. J Immunol 2015, 195:4555-4563

6. Porwit A, Ehinger M. Immunohistochemistry and Flow cytometry in Bone Marrow Haematopathology, in Diagnostic Bone Marrow Haematopathology, Van de Walt J, Orazi A, Arber D eds. Cambrige University Press 2021, pp 340-361.

7. Torlakovic EE, Naresh K, Brunning RD. Bone marrow Immunohistochemistry. American Society for Clinical Pathology Press, Chicago. 2009.

8. WHO classification of tumours of Haematopoietic and Lymphoid tissues. Swerdlow SH et al eds. IARC Press. Lyon 2017

9. Kremer M, Quintanilla-Martinez L, Nahrig J et al. Immunohistochemistry in bone marrow pathology: a useful adjunct for morphologic diagnosis. Virchows Arch (200) 447: 920-937.

10. Dogan A and Morice WG. Bone marrow histopathology in peripheral T-cell lymphomas. BJH 004; 127: 140-154

11. Fallini B, Nicoletti I, Bolli N et al. Translocations and mutations involving the nucleophosmin (NPM1) gene in lymphomas and leukemias. Haematologica 2007; 92: (4):519-32.

12. Fernandez –Pol S, Ma L, Ohgami RS, Arber DA. Immunohistochemistry for p53 is a useful tool to identity cases of acute myeloid leukemia with myelodysplasia-related changes that are TP53 mutated , have complex karyotype, and have pood prognosis. Mod Pathol.2017; 30(3):382-92.

13. Valent P, Orazi A, Busche G et al. Standards and impact of hematopathology in myelodysplastic syndromes (MDS). Oncotarget. 2010; 1(7):483-96

14. Orazi A. Histopathology in the diagnosis and classification of acute myeloid leukemia, myelodysplastic syndromes and myelodysplastic/myeloproliferative diseases. Pathobiology.2007; 74(2):97-114.

15. Della Porta MG, Malcovati L, Boveri E et al. Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. J Clin Oncol 2009; 27(5):754-62. Naresh KN, Lampert IA. CD117 expression as an aid to identify immature myeloid cells and foci of ALIP in bone marrow trephines. Am J Hematol 2006; 81(1):79.

16. Saft L. Karimi M, Ghaderi M et al. p53 protein expression independently predicts outcome in patients with lower risk myelodysplastic syndromes with del(5q). Haematologica 2014; 99(6):1041-9.

17. Loghavi S, Al-Ibraheemi A, Zuo Z et al. TP53 overexpression is an independent adverse prognostic factor in de novo myelodysplastic syndrome s with fibrosis. Br J Haematol 2015; 171(1):91-9.

18. Kandukuri S, Lin F, Gui L et al. Application of immunohistochemistry in undifferentiated neoplasms. Arch Pathol Lab Med 2017;141:1014-1032.

19. Rimsza LM. Larson RS, Winter SS et al. benign hematogone-rich lymphoid proliferations can be distinguished from B-lineage acute lymphoblastic leukemia by integration of morphology, immunophenotype, adhesion molecule expression and architectural features. Am J Clin Pathol 2000;114:66-75.

20. Laurent C, Arber DA, Johnson P et al. Diagnosis of Hodgkin lymphoma on bone marrow biopsy. Commentary. Histopathology.202; 76:934-941.