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A Pattern-Based and Problem-Oriented Approach to Hematolymphoid Neoplasms:

Practical Bone Marrow Immunohistochemistry

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- **A Pattern-Based Approach to Hematolymphoid Neoplasms**

- Morphology First
- Pattern Recognition
- Focused IHC Selection
- Integrated Diagnosis

Why Bone Marrow IHC is Different

- **Bone Marrow Diagnosis Requires Integration of:**
 - Clinical History
 - CBC Findings
 - Peripheral Blood Smear
 - Bone Marrow Aspirate
 - Core Biopsy Morphology
 - Immunohistochemistry
 - Flow Cytometry
 - Cytogenetics
 - Molecular Studies
- **Key Principle**

IHC answers a diagnostic question; it does not replace morphology.

Diagnostic Workflow

Morphology



Pattern Recognition



Differential Diagnosis



Focused IHC Panel



Ancillary Studies



Integrated Diagnosis

- **Practical Pearl**

Never order stains before generating a differential diagnosis.

Five Fundamental Questions in Bone Marrow Evaluation

- **Ask in Every Case**

- Is cellularity normal?
- Are blasts increased?
- Is there abnormal infiltration?
- Is there abnormal localization of cells?
- Is fibrosis present?

Bone Marrow Pattern Recognition Framework

- **Major Diagnostic Patterns**

- Blast-Rich Marrow
- Hypercellular Marrow
- Lymphoid Infiltration
- Plasma Cell-Rich Marrow
- Eosinophil-Rich Marrow
- Granulomatous Marrow
- Metastatic Involvement
- Hypocellular Marrow
- Fibrotic Marrow

- **Key Message**

Recognize the pattern before selecting stains.

Pattern Recognition Drives Differential Diagnosis

- **Increased Blasts**
 - MDS/MPN/Acute Leukemia
- **Paratrabecular Aggregates**
 - → Follicular Lymphoma
- **Intrasinusoidal Infiltration**
 - HSTCL
 - SMZL
- **Diffuse Fibrosis**
 - PMF
 - MDS-F
 - Hairy Cell Leukemia

- **Key Message**

Pattern recognition narrows the differential diagnosis before IHC.

What IHC Can and Cannot Do

- **IHC Can**

- ✓ Determine lineage
- ✓ Highlight blasts
- ✓ Confirm infiltration patterns
- ✓ Detect subtle marrow involvement

- **IHC Cannot Reliably Determine**

- X Clonality
- X Most molecular abnormalities
- X Disease biology
- X Prognosis by itself

- **Key Message:**

Most diagnostic errors result from overinterpreting immunostains and underinterpreting morphology.

Focused Panel vs Shotgun Approach

Avoid

- CD3
- CD20
- CD34
- CD117
- TdT
- CD138
- CD61
- Cyclin D1
- PAX5
- MPO
- On Every Case

Preferred Approach

Morphology



Differential Diagnosis



Focused IHC Panel



Diagnosis

- **Practical Pearl**

- **A small intelligent panel is better than a large unfocused panel.**

Essential Bone Marrow IHC Markers

Category	Markers
Blasts	CD34, CD117, TdT
Myeloid	MPO, Lysozyme
Megakaryocytes	CD61, CD42b, CD31, CD41
Erythroid	CD71, E-Cadherin, Glycophorin
B Cells	CD20, PAX5, CD79a, CD19
T Cells	CD3
Plasma Cells	CD138, Kappa, Lambda
Mast Cells	Tryptase, CD25, CD117

The Most Important Question Before Ordering IHC

What specific question am I trying to answer?

Question	Useful Markers
Are these blasts?	CD34, CD117, TdT
Myeloid or lymphoid?	MPO, CD3, PAX5
Megakaryocytic?	CD61, CD42b
Erythroid?	CD71, Glycophorin
Plasma cells?	CD138
Mast cells?	Tryptase, CD25, CD117

Order stains to answer a question, not because they are available

Blast-Rich Marrow

- **First Diagnostic Question**
- **Are These Really Blasts?**
- **Mimickers of Blasts**
 - Hematogones
 - Regenerating Precursors
 - Immature Erythroid Cells
 - Mast Cells
 - Small Round Cell Metastases

- **Initial IHC Panel**

- CD34
- CD117
- TdT
- MPO

- **Practical Pearl**

- **Not every immature-looking cell is a blast.**

Diagnostic Approach to Blast-Rich Marrow

Blast-Rich Marrow



CD34 Positive?



Determine Lineage



Myeloid?/Lymphoid?/Megakaryocytic?/
Erythroid?

- **Key Principle**

- **Lineage assignment is the primary goal of IHC in acute leukemias.**

CD34 in Bone Marrow Pathology

- **Why CD34 Matters**

- Blast Identification
- Blast Quantification
- Detection of ALIPs
- Detection of Residual Disease
- Assessment of MDS Progression

- **Limitations**

- Not all AMLs are CD34+
- Not all CD34+ cells are leukemic

- **Practical Pearl**

- **CD34 is a blast marker, not a leukemia marker.**

CD34 Pitfalls

- CD34 positivity = AML **✗** False

- **Reality**

- CD34 highlights:
 - Normal blasts
 - Regenerating precursors
 - Hematogones
 - Leukemic blasts

- **Practical Pearl**

- **CD34 identifies immature cells, not leukemia.**

Not All AMLs Are CD34 Positive

- Acute Promyelocytic Leukemia
- NPM1-mutated AML
- Monocytic AML
- Pure Erythroid Leukemia

Expert Pearl

- **Never exclude AML solely on the basis of CD34 negativity.**

Myeloid or Lymphoid?

- **The Critical Decision Point**
- **Myeloid Lineage**
 - MPO
 - Lysozyme
 - CD68
- **B-Lymphoblastic Lineage**
 - PAX5
 - CD79a
- **T-Lymphoblastic Lineage**
 - CD3
 - CD7
- **Key Message**
 - **Determine lineage before attempting classification.**

Acute Myeloid Leukemia (AML)

- **Typical Findings**

- Increased blasts
- CD34 positive (often)
- CD117 positive
- MPO positive

- **Helpful Markers**

- MPO
- CD34
- CD117
- Lysozyme

- **Practical Pearl**

- **MPO positivity is the strongest evidence of myeloid differentiation.**

AML Pitfall

- **Not All AMLs Express CD34**
- **Frequently CD34 Negative**
 - Acute Promyelocytic Leukemia
 - NPM1-mutated AML
 - Monocytic AML
- **Therefore**
 - CD34 Negative ≠ Not AML
- **Expert Pearl**
 - **Never exclude AML solely because CD34 is negative.**

Acute Lymphoblastic Leukemia (ALL)

- **Typical Findings**

- Diffuse blast infiltration
- TdT positive
- CD34 positive (often)

- **B-ALL**

- PAX5+
- CD79a+

- **T-ALL**

- Cytoplasmic CD3+
- CD7+

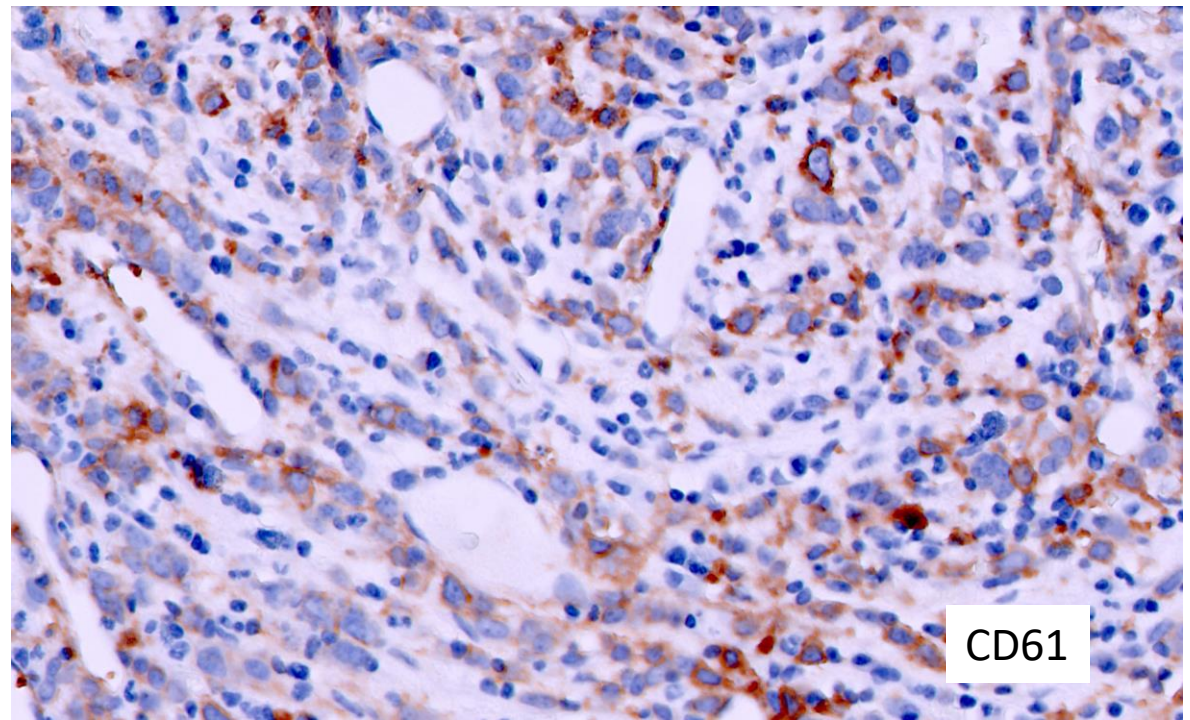
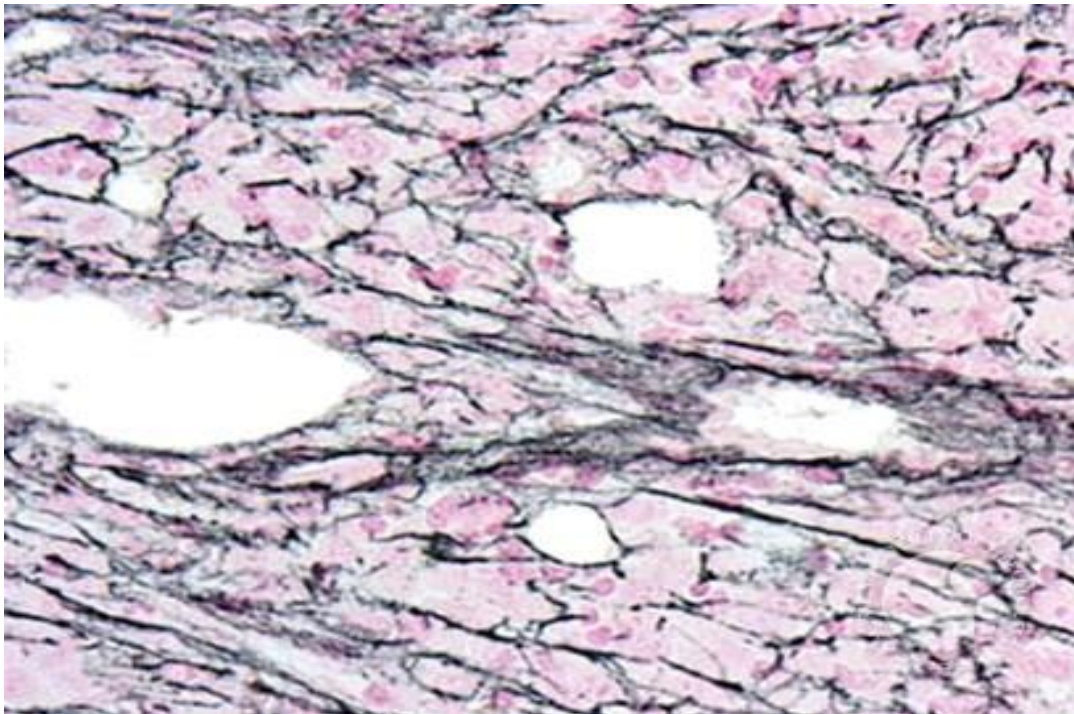
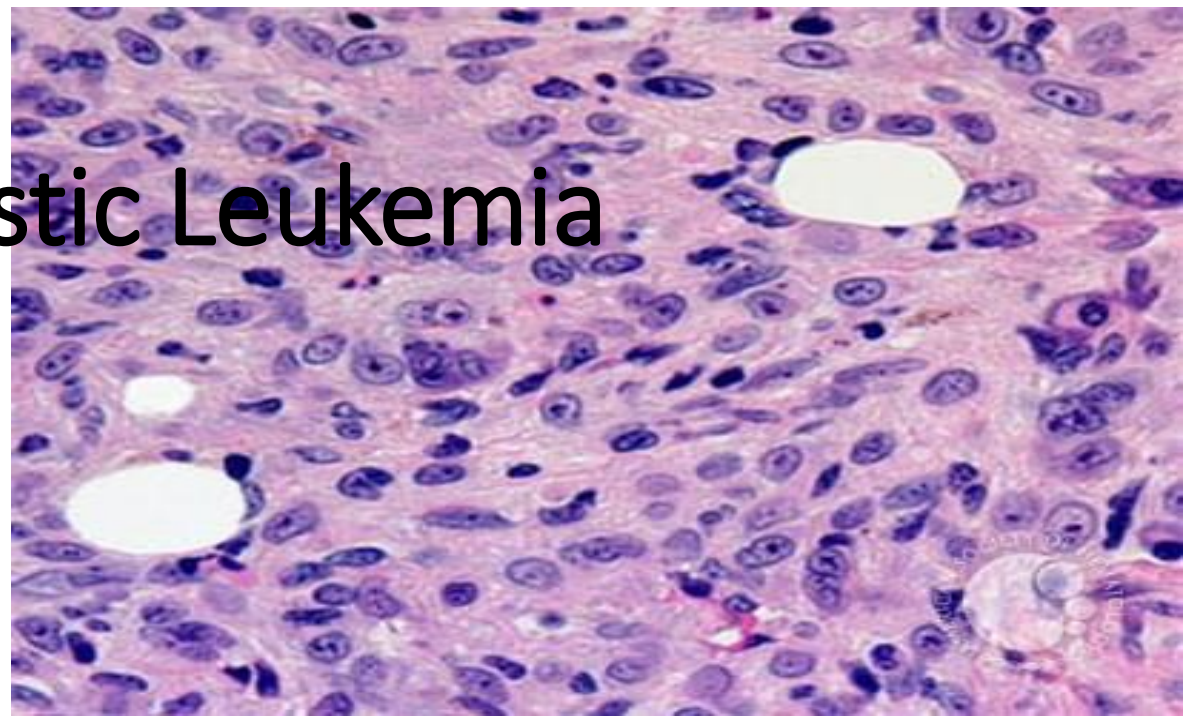
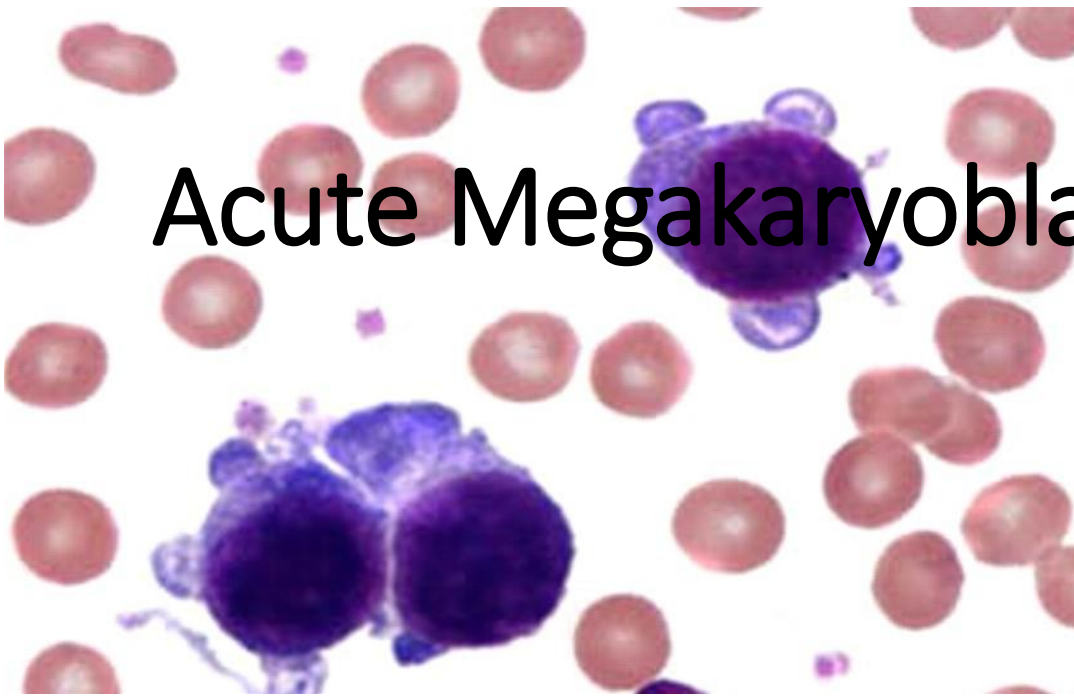
- **Practical Pearl**

- TdT identifies immaturity but does not determine lineage.
- 30% AML cases also express TdT especially in immature AML such as AML-M0 and AML-M1.

Acute Megakaryoblastic Leukemia

- **AML with Megakaryocytic Differentiation**
- **Morphologic Clues**
 - Fibrotic marrow
 - Dry tap
 - Dysplastic megakaryocytes
- **Useful Markers**
 - CD61
 - CD42b
 - CD41
 - CD31
- **Key Point**
 - MPO may be negative
- **Practical Pearl**
 - **Always consider AMKL in a fibrotic marrow with increased blasts.**

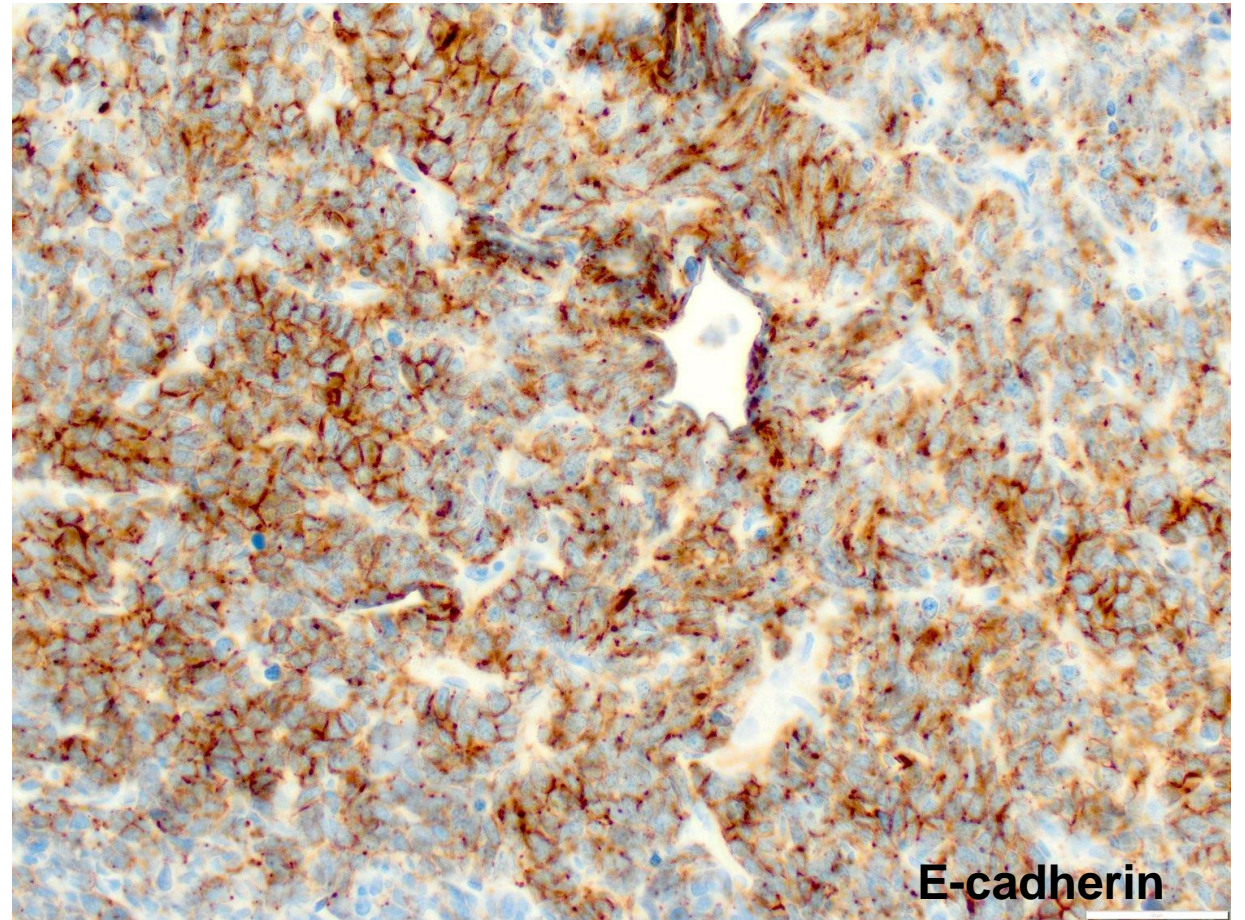
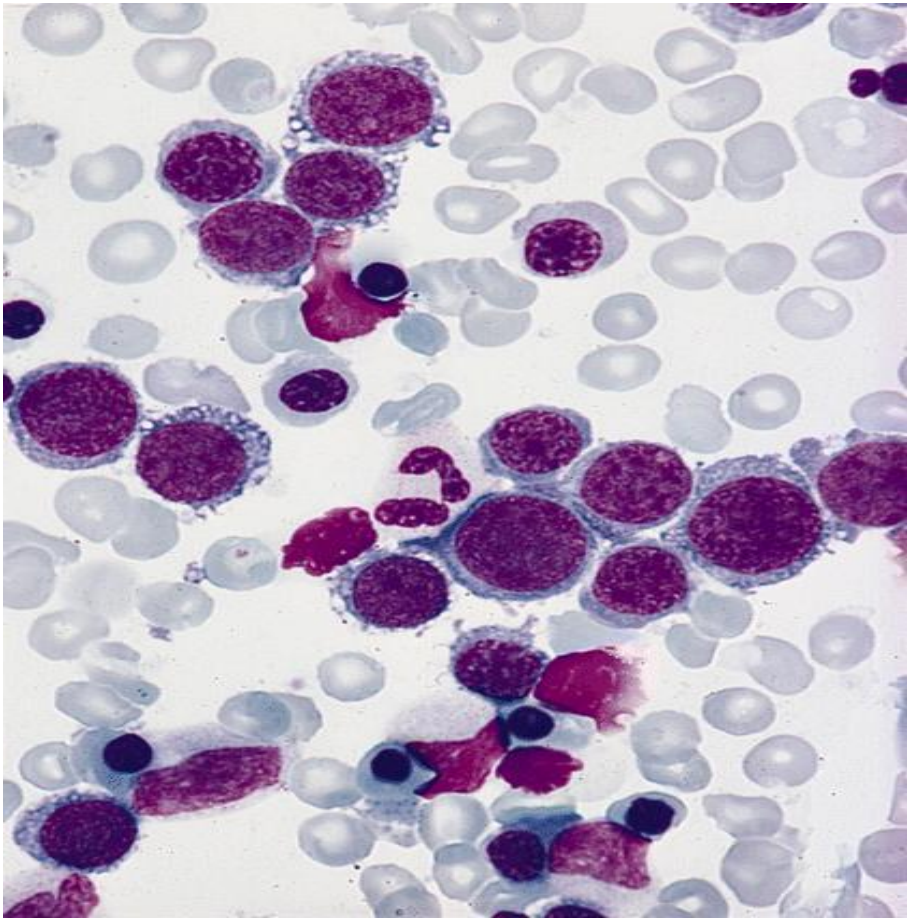
Acute Megakaryoblastic Leukemia



Pure Erythroid Leukemia

- **Rare but Important**
- Erythroid precursors $\geq 80\%$ and proerythroblasts $\geq 30\%$ of the bone marrow cells
- **Morphology**
 - Sheets of erythroblasts
 - Marked atypia
 - Hypercellular marrow
- **Helpful Markers**
 - CD71
 - Glycophorin A
 - E-Cadherin
- **Usually Negative**
 - MPO
 - CD61
- **Practical Pearl**
 - **CD71 highlights erythroid differentiation but is not entirely specific.**

Pure Erythroid Leukemia



Acute Leukemia Lineage Assignment

Diagnosis	Key Markers
AML	MPO, CD117, CD68,CD163
B-ALL	CD20, PAX5, CD79a
T-ALL	CD7, CD3,CD2
AMKL	CD61, CD42b,CD31
Pure Erythroid Leukemia	CD71, Glycophorin

Key Message

First establish lineage, then classify the leukemia.

MDS with Increased Blasts

- **One of the Most Important Uses of CD34**
- **Why Use CD34?**
 - Blast Enumeration
 - Blast Clustering
 - ALIP Detection
- **Findings**
 - Increased CD34+ cells
 - Abnormal Distribution
 - Blast Aggregates
- **Practical Pearl**
 - **CD34 often reveals more blasts than are appreciated on H&E sections.**

Erythroid Series



Internuclear Bridges



Multi-nucleation



Karyorrhexis



Nuclear Budding



Ring Sideroblast

Granulocytic Series



Pseudo-Pelger-Huet/ Hypolobulated



Large Agranular Neutrophil



Coalescent Granules

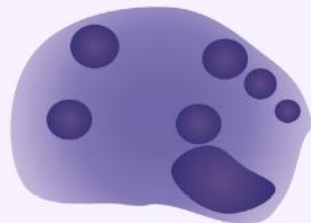


Hyper-Segmentation



Auer Rod

Megakaryocytic Series



Multi-Nucleation



Hypo-Lobulation

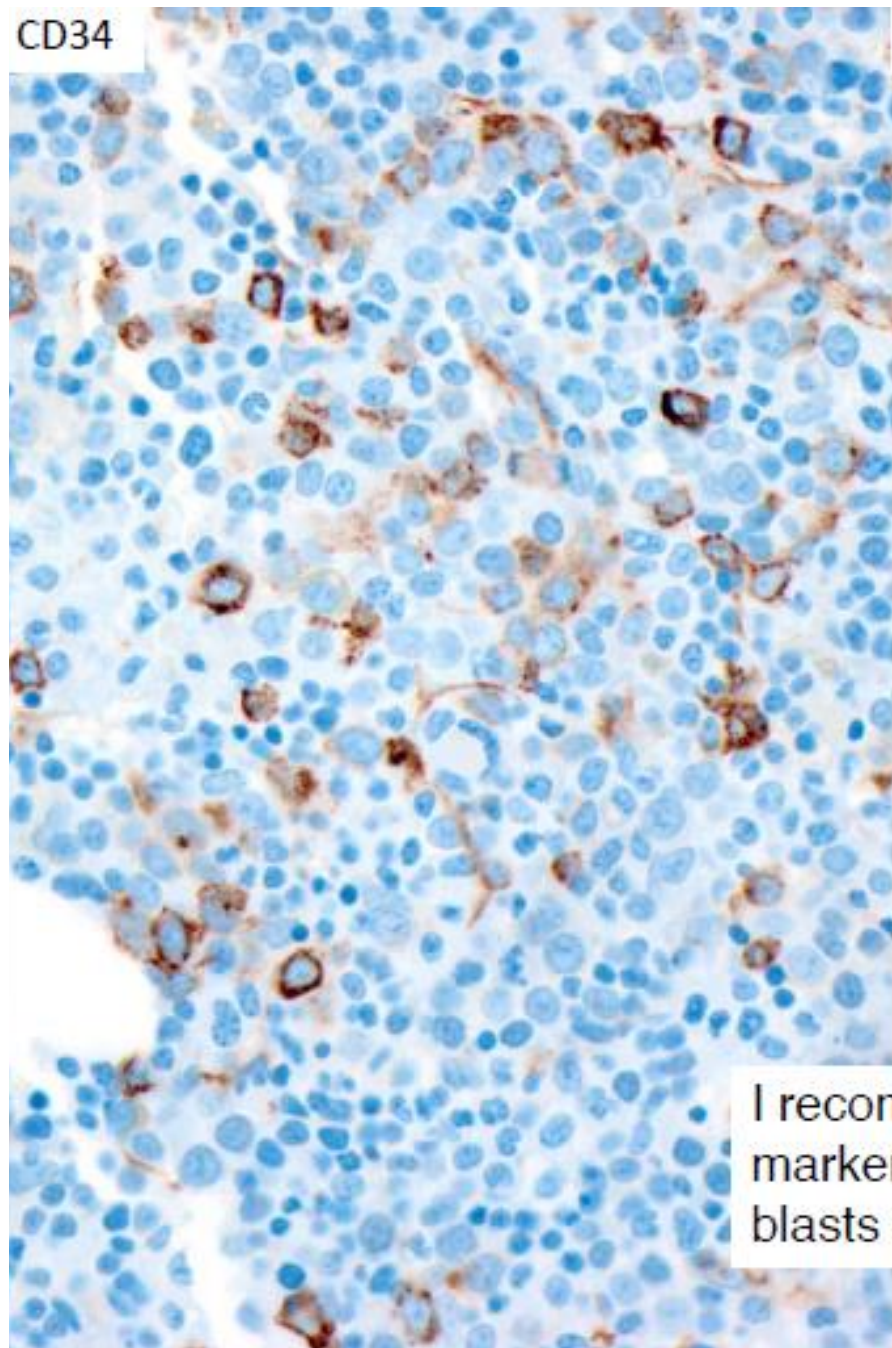


Mononuclear Micromegakaryocytes

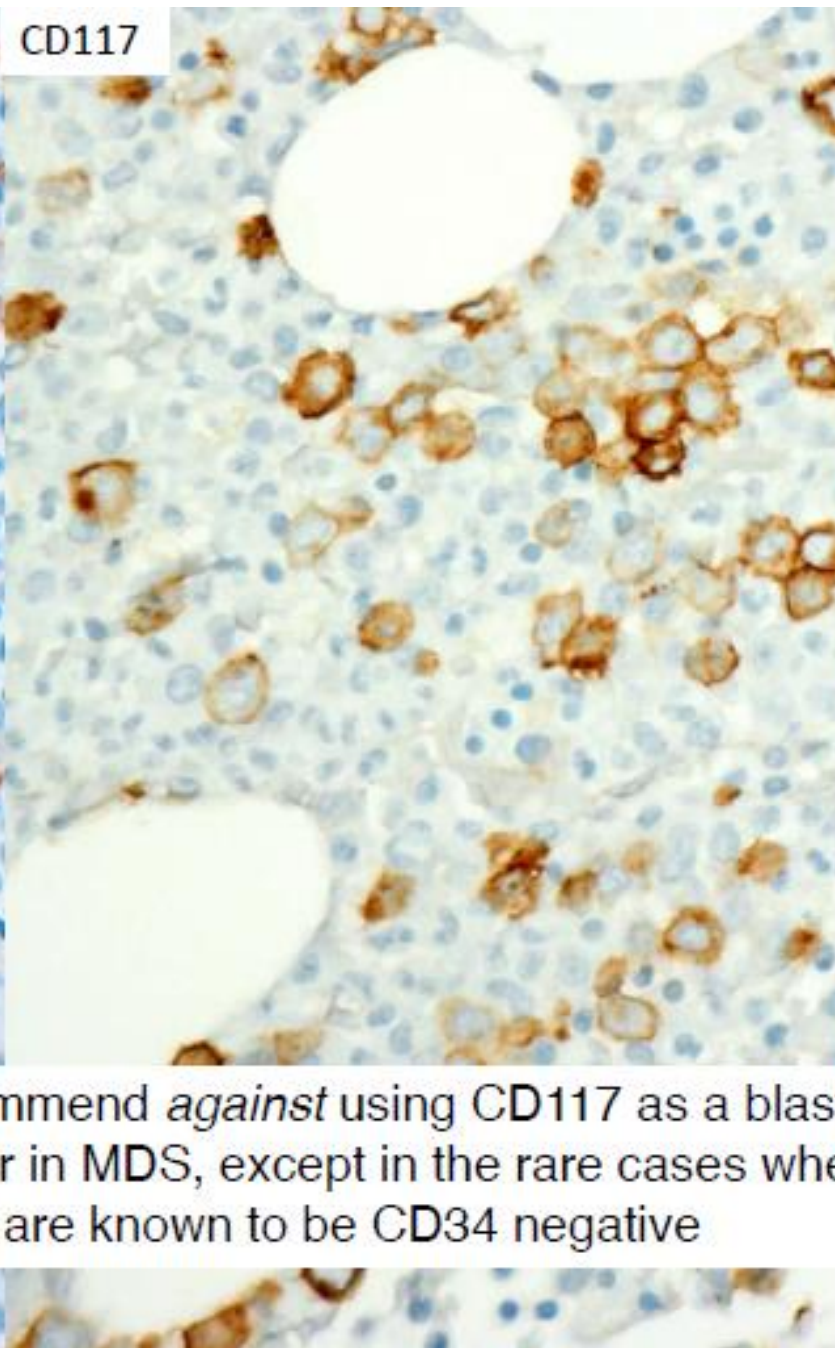


Widely Separated Nuclear Lobes/ Pawn Ball Mega

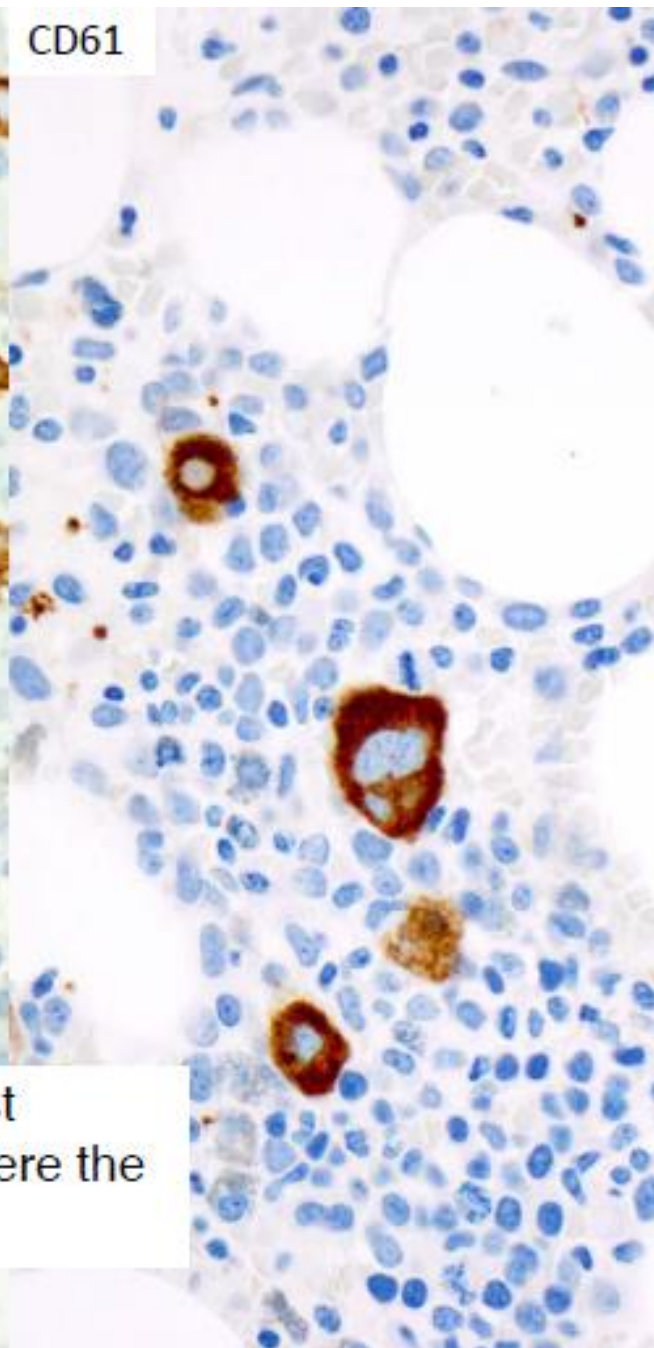
CD34



CD117



CD61



I recommend *against* using CD117 as a blast marker in MDS, except in the rare cases where the blasts are known to be CD34 negative

Blast counting in MDS: Immunohistochemistry

- Unlike some other myeloid neoplasms, the blasts in MDS are nearly always (>90%) CD34+, facilitating their detection by IHC
- When should CD34 staining be done on the biopsy?
 - In cases of poor aspirate material
 - In cases with increased marrow fibrosis
 - In other cases when blasts may be difficult to identify
 - In all cases?
- I recommend in using the *higher blast count* (aspirate or biopsy) to establish the disease classification in MDS

Table 2 Comparison of blast percentage by CM and CD34 IHC in BM biopsies

	Percentage of CD34+ cells by IHC			Total
	< 5%	≥ 5–< 10%	≥ 10–< 20%	
BM blasts %				
< 5%	72 (55%)	9 (7%)	3 (2%)	84 (64%)
≥ 5–< 10%	9 (7%)	10 (8%)	11 (8%)	30 (23%)
≥ 10–< 20%	3 (2%)	2 (2%)	13 (10%)	18 (14%)
Total	84 (64%)	21 (16%)	27 (21%)	132 (100%)

“In cases with increased blast percentages in BM histology, final MDS classification should be based on the higher blast count even if the percentage of blasts by [aspirate count] is below the 5% threshold.”

Blast counting in MDS

Accurate blast counting is critical!

- *First choice*: Well-stained aspirate smear
- *Second choice*: Good touch preparation
- *Third choice*: Bone marrow core biopsy

Flow cytometry usually correlates with aspirate smear blast count, but. . .

- Cells may be lost in processing
- Peripheral blood contamination may affect count
- Aspirate blast count remains the 'gold standard'

Abnormal Localization of Immature Precursors

- **Definition ALIPs**

- Clusters of immature precursors located away from normal endosteal niches

- **Significance**

- Supports MDS
- Associated with disease progression
- Associated with higher-risk disease

- **Best Marker**

- CD34

- **Expert Pearl**

- **ALIPs are among the strongest histologic clues to MDS.**

≥3 ALIP foci per biopsy section

Each focus contains ≥3 immature precursors

Located away from the endosteal/paratrabecular region, in the intertrabecular marrow.

ALIPs Pitfalls

- **Abnormal Localization of Immature Precursors helpful in MDS**

BUT

- **Beware**
 - Regenerating marrow
 - Post-therapy marrow
 - Growth factor effect

Expert Pearl

- **ALIPs support MDS but are not diagnostic in isolation.**

AML vs High-Grade MDS

A Common Diagnostic Challenge: Helpful Features

Feature	AML	MDS-IB
Blasts	Higher	Lower
CD34 Clusters	Common	Common
Dysplasia	Variable	Prominent
ALIPs	Possible	Common

Key Message

IHC supports the diagnosis but cannot replace blast counting and morphology.

TP53-Mutated Myeloid Neoplasms

- **Why p53 Matters**

- Associated With:

- High-risk MDS
- AML
- Complex Karyotype
- Poor Prognosis

- **Abnormal Patterns**

- **Overexpression Pattern**

- Strong nuclear staining

- **Null Pattern**

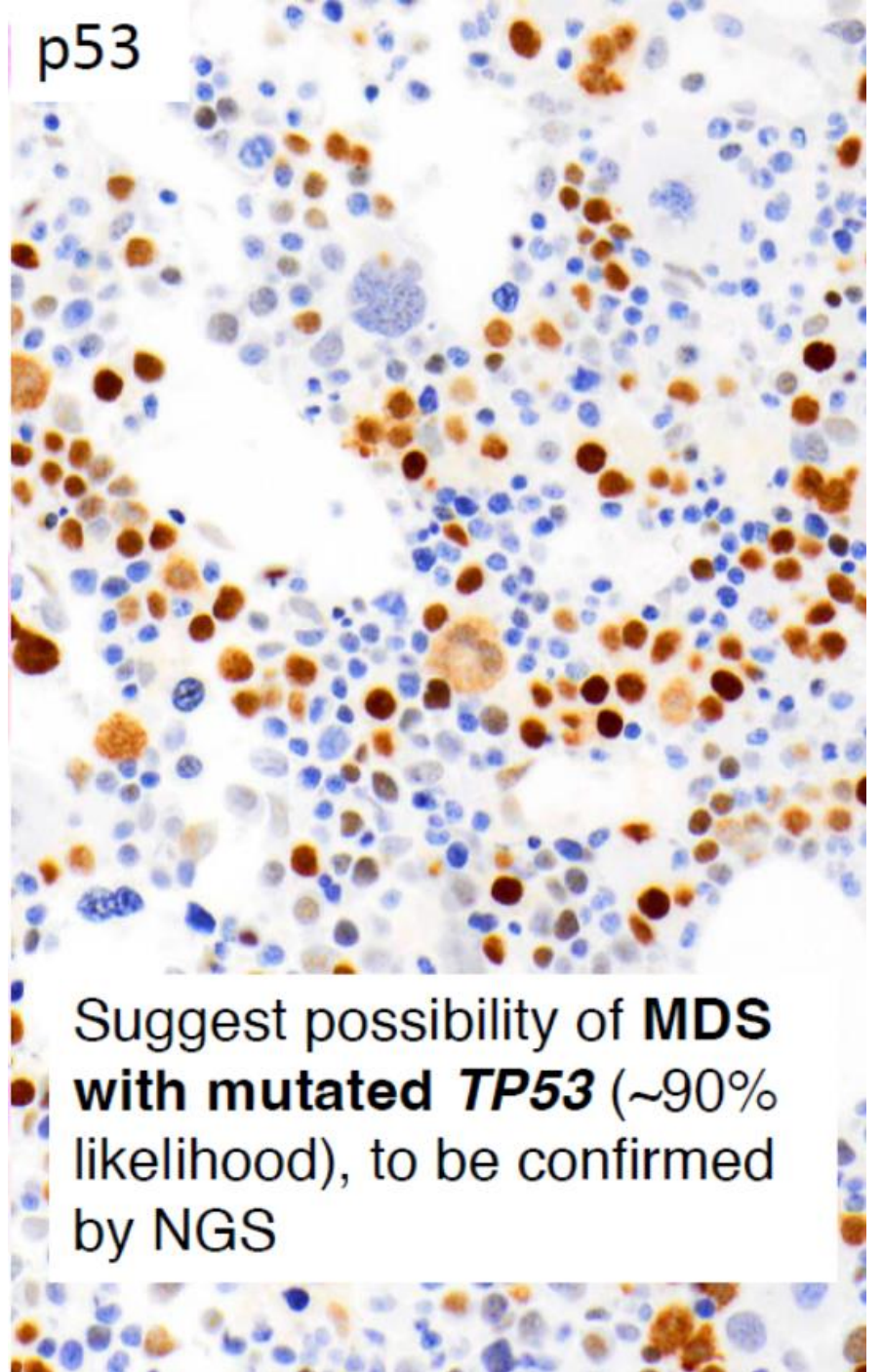
- Complete absence of staining

- **Practical Pearl**

- **Both overexpression and null patterns may indicate TP53 alteration.**

p53 IHC as a surrogate for TP53 mutation

Pattern	Interpretation
Scattered weak staining (<1-2%)	Wild-type pattern
≥10% strong (3+) nuclear staining	Aberrant overexpression pattern, highly specific for TP53 mutation
Complete absence (null pattern) with positive internal controls	Aberrant pattern, often truncating TP53 mutation



Hematogones vs B-ALL; A Classic Pitfall

- **Hematogones**

- Benign B-cell precursors
- Regenerating marrow
- Post-chemotherapy

- **B-ALL**

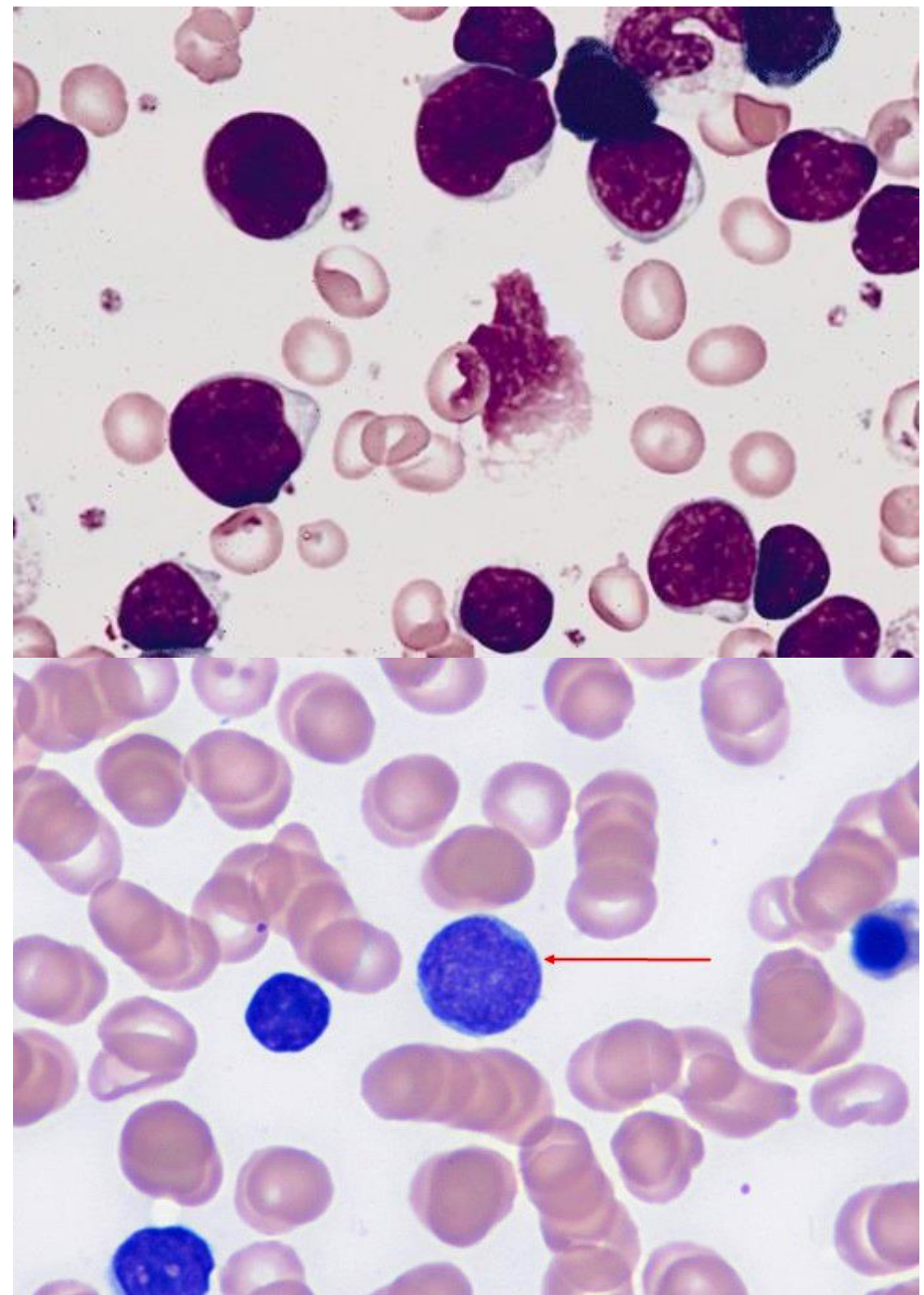
- Monotonous blast population
- Diffuse infiltration
- Marrow replacement

- **Practical Pearl**

- **Flow cytometry is usually superior to IHC for this distinction.**

Hematogones

- Hematogones are benign B cell precursors and their degree of maturation can be classified by flow cytometry into 3 stages (1, 2 and 3)
- Morphologically, they contain scant cytoplasm and often have round, indented nuclei with absent or inconspicuous nucleoli; the presence of nucleoli generally indicates immaturity
- Increased numbers of hematogones may mimic B lymphoblastic leukemia (B ALL) due to the morphologic and immunophenotypic similarities



Blast-Rich Marrow: Essential Panel

- **Initial Panel**

- CD34
- CD117
- MPO
- TdT

- **If Megakaryocytic Differentiation Suspected**

- CD61
- CD42b

- **If Erythroid Differentiation Suspected**

- CD71
- Glycophorin
- E-Cadherin

- **If TP53 Disease Suspected**

- p53

- **Final Pearl**

- **A focused panel answers the diagnostic question more effectively than a large screening panel.**

Hypercellular Marrow Pattern

- **First Diagnostic Question**
- **Why is the marrow hypercellular?**
- **Major Categories**
 - Myeloproliferative Neoplasms (MPN)
 - Myelodysplastic Syndromes (MDS)
 - MDS/MPN Overlap Neoplasms
 - Acute Leukemia
 - Reactive Myeloid Hyperplasia
- **Key Principle**
 - Determine which hematopoietic lineage is expanded.

Hypercellular Marrow: Diagnostic Framework

Increased Blasts?



MDS-IB
AML

Granulocytic Expansion?



CML
Reactive Myeloid
Hyperplasia

Megakaryocytic Expansion?



ET
PMF
Pre-PMF

Erythroid Expansion?



PV
AML-M6
Megaloblastic anemia
Hemolysis

- **Key Message**
 - Identify the dominant proliferating compartment.

Role of IHC in Myeloproliferative Neoplasms

- **Most Useful Markers**

- CD61
- CD42b
- MPO
- CD71
- CD34

- **Questions Answered by IHC**

- ✓ Which lineage is expanded?
- ✓ Are blasts increased?
- ✓ Are megakaryocytes abnormal?
- ✓ Is fibrosis-associated disease present?

- **Expert Pearl**

- **In MPNs, morphology remains more important than immunophenotype.**

Polycythemia Vera (PV)

- **What Does IHC Add?**

- **Typical Findings**

- Hypercellular marrow
- Panmyelosis
- Trilineage proliferation

- **Helpful Markers**

- MPO
- CD71
- CD61

- **Findings**

- MPO → Granulocytic expansion
- CD71 → Erythroid expansion
- CD61 → Megakaryocytic proliferation

Practical Pearl

CD71 beautifully highlights erythroid hyperplasia in PV.

Essential Thrombocythemia (ET)

- **Characteristic Findings**

- Increased megakaryocytes
- Large hyperlobated nuclei
- Loose distribution

- **Useful Markers**

- CD61
- CD42b

- **Findings**

- Increased megakaryocyte number
- Preserved maturation
- Minimal clustering

- **Key Message**

- **ET is primarily a morphologic diagnosis.**

Prefibrotic Primary Myelofibrosis

The Great Mimicker of ET

- **Morphologic Features**

- Hypercellular marrow
- Dense megakaryocytic proliferation
- Nuclear atypia
- Tight clustering

- **Useful Markers**

- CD61
- CD42b
- CD34

Practical Pearl

IHC highlights abnormal megakaryocytes, but diagnosis depends on morphology.

ET versus Prefibrotic PMF

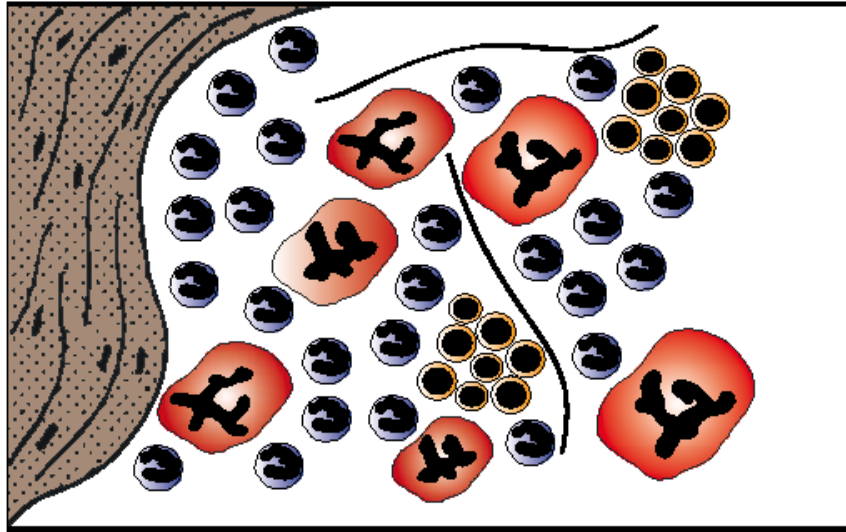
Feature	ET	Pre-PMF
Cellularity	Mild ↑	Marked ↑
Megakaryocyte Clusters	Loose	Dense
Nuclear Atypia	Mild	Marked
Granulocytic Expansion	Minimal	Common
CD34 Increase	Rare	More Frequent

Expert Pearl

The most difficult MPN diagnosis is distinguishing ET from pre-PMF.

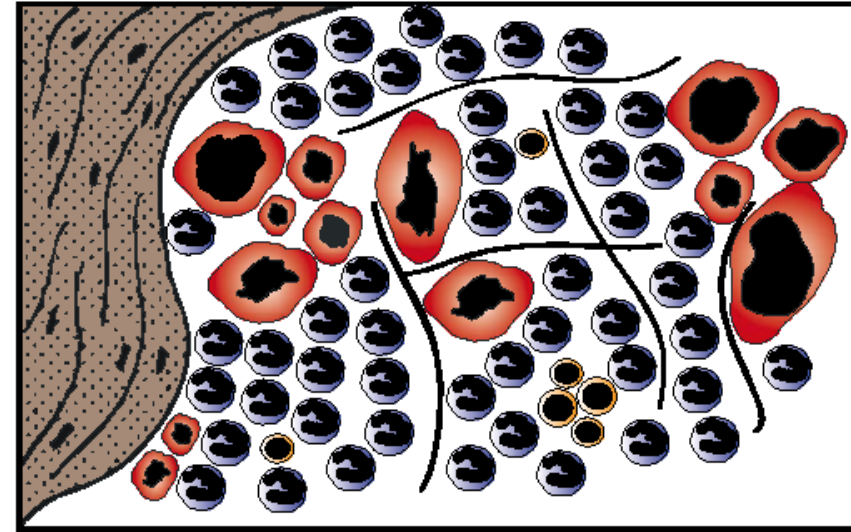
ET

- no or only slight increase in age-matched cellularity
- no significant increase in granulo- and erythropoiesis
- prominent large to giant mature megakaryocytes with hyperlobulated or deeply folded nuclei, dispersed or loosely clustered in the marrow space
- no or very rarely minor increase in reticulin fibers



PMF (early-prefibrotic stage)

- marked increase in age-matched cellularity
- pronounced proliferation of granulopoiesis and reduction of erythroid precursors
- dense or loose clustering and frequent endosteal translocation of medium sized to giant megakaryocytes showing hyperchromatic, hypolobulated, bulbous, or irregularly folded nuclei and an aberrant nuclear/cytoplasmic ratio
- no or no significant increase in reticulin fibers



● Megakaryopoiesis; ● Granulopoiesis; ● Erythropoiesis; ʌ Reticulin fibers

CD34 in PMF

- **Why Use CD34?**

- Blast quantification
- Detection of accelerated phase
- Detection of leukemic transformation

- **Concerning Findings**

- Increased CD34+ cells
- CD34+ clusters
- Diffuse blast expansion

- **Key Message**

- **CD34 is critical for recognizing progression of PMF.**

Chronic Myeloid Leukemia (CML)

- **Typical Morphology**

- Marked hypercellularity
- Granulocytic hyperplasia
- Small hypolobated megakaryocytes

- **Helpful Markers**

- CD61
- CD34

- **Practical Pearl**

- **Diagnosis of CML is molecular, not immunohistochemical.**

MDS/MPN Overlap Neoplasms

- **Examples**

- CMML
- MDS/MPN with Neutrophilia
- MDS/MPN NOS

- **Role of IHC**

- Blast assessment
- Monocytic expansion assessment
- Fibrosis evaluation

- **Useful Markers**

- CD34
- CD68
- Lysozyme

- **Key Message**

- **IHC plays a supportive rather than primary role in MDS/MPN diagnosis.**

Hypercellular Marrow Pattern: Practical Panel

- **Suspected MPN**

- CD61
- CD42b
- CD34

- **Suspected MDS**

- CD34
- CD117
- p53

- **Suspected CMML**

- CD34
- CD68
- Lysozyme

- **Suspected CML**

- MPO
- CD61
- CD34

Expert Pearls in Myeloid Neoplasms

- **Pearl #1**

- Morphology is more important than IHC in MPNs.

- **Pearl #2**

- CD34 is the most valuable stain in MDS.

- **Pearl #3**

- CD61 is indispensable for evaluating megakaryocytic disorders.

- **Pearl #4**

- p53 is a surrogate marker, not a mutation test.

- **Pearl #5**

- Never diagnose CML by IHC alone.

Lymphoid Infiltrates in Bone Marrow

- **What is the pattern of involvement?**

- **Major Patterns**

- Interstitial
- Nodular
- Paratrabecular
- Diffuse
- Intrasinusoidal

Key Principle

- **The infiltration pattern often narrows the differential diagnosis before IHC.**

Diagnostic Approach to Lymphoid Infiltrates

Lymphoid Infiltrate



Pattern Recognition



B-cell or T-cell?



Small Cell or Large Cell?



Focused IHC Panel



Classification

- **Initial Screening Panel**

- CD20

- PAX5

- CD3

Practical Pearl

- **Start with lineage assignment before subtype classification.**

Interstitial Pattern

Common Causes

- CLL/SLL
- Lymphoplasmacytic Lymphoma
- T-LGL Leukemia
- Early Marrow Involvement by Lymphoma

• Helpful Markers

- CD20
- CD3
- CD5
- CD23

Practical Pearl

- **CLL commonly shows an interstitial or mixed interstitial-nodular pattern.**

CLL/SLL in Bone Marrow

- **Common Patterns**

- Interstitial
- Nodular
- Diffuse

- **Characteristic Phenotype**

- CD20 (dim)
- CD5+
- CD23+
- PAX5+

- **Diagnostic Utility of IHC**

- ✓ Confirmation of marrow involvement
- ✓ Assessment of infiltration pattern

- **Expert Pearl**

- Flow cytometry is usually more informative than IHC in CLL.

Nodular Lymphoid Aggregates: Reactive or Neoplastic?

- **Reactive Aggregates**

- Small
- Well-circumscribed
- Non-paratrabeular
- Few aggregates
- Mixed B and T cells
- Germinal centers may be present

- **Neoplastic Aggregates**

- Large & Infiltrative
- Monotonous & paratrabeular
- B-cell predominant
- Multiple aggregates

- **Useful Markers**

- CD20
- CD3

- **Practical Pearl**

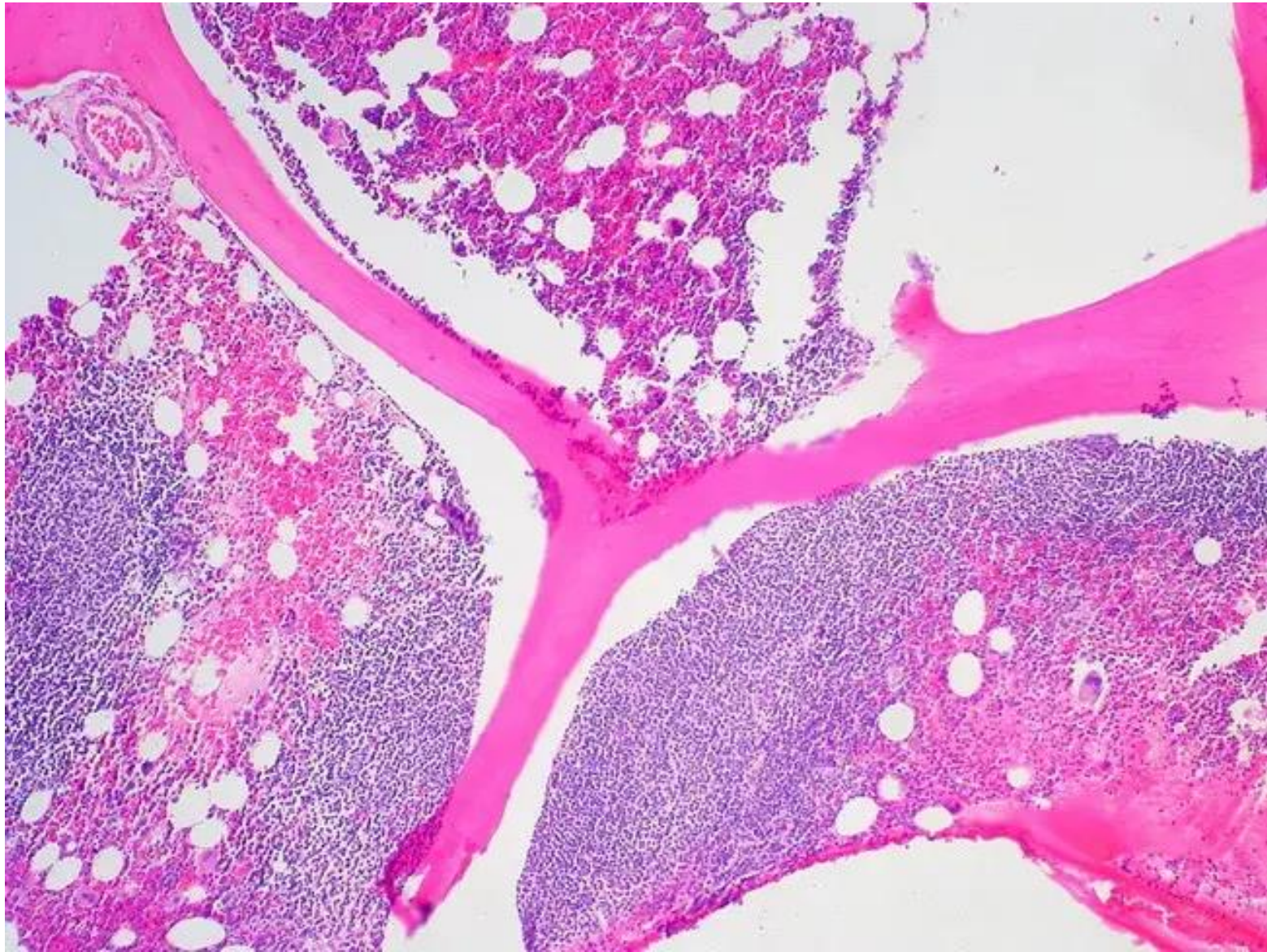
- **Mixed B- and T-cell populations favor a reactive aggregate.**

Paratrabecular Pattern

- **Think First Of** Follicular Lymphoma
- **Useful Markers**
- CD20
- CD10
- BCL6
- BCL2

Expert Pearl

- **Paratrabecular lymphoid aggregates are highly suggestive of Follicular Lymphoma.**



Bone marrow involvement in follicular lymphoma (FL) consists of **large paratrabeccular lymphoid aggregates**

Follicular Lymphoma

Typical Marrow Findings

- Paratrabecular aggregates
- Multiple foci
- Monotonous B cells

Helpful Markers

- CD20
- CD10
- BCL6
- BCL2

Practical Pearl

- **The pattern is often more informative than the immunophenotype.**

Mantle Cell Lymphoma

- **Common Patterns**

- Nodular
- Diffuse
- Interstitial

- **Characteristic Phenotype**

- CD20+
- CD5+
- Cyclin D1+
- SOX11+

- **Practical Pearl**

- **Always consider Mantle Cell Lymphoma in a CD5-positive B-cell infiltrate.**

Diffuse Marrow Infiltration

Differential Diagnosis

- DLBCL
- Burkitt Lymphoma
- Blastoid Mantle Cell Lymphoma
- ATLL

Useful Markers

- CD20
- PAX5
- CD3
- Ki-67
- **Key Message**
- **Diffuse replacement usually indicates aggressive disease.**

Intrasinusoidal Pattern

Major Diagnoses

- Hepatosplenic T-cell Lymphoma
- Splenic Marginal Zone Lymphoma
- T-LGL Leukemia
- Hairy Cell Leukemia Variant

• Useful Markers

- CD3
- CD20

Expert Pearl

- **Whenever you see an intrasinusoidal pattern, actively exclude HSTCL.**

Hepatosplenic T-Cell Lymphoma (HSTCL)

Characteristic Findings

- Intrasinusoidal infiltration
- Cytopenias
- Hepatosplenomegaly
- Minimal lymphadenopathy

Phenotype

- CD3+
- CD56+
- TCR $\gamma\delta$ (often)
- CD4–
- CD8– or CD8+

Practical Pearl

- **Pattern recognition is often the key to diagnosis.**

T-LGL Leukemia

- **Bone Marrow Findings**

- Interstitial infiltration
- Intrasinusoidal infiltration
- Often subtle involvement

- **Helpful Markers**

- CD3
- CD8
- TIA1
- Granzyme B

- **Practical Pearl**

- **The marrow infiltrate may be surprisingly inconspicuous.**

Hairy Cell Leukemia

Typical Findings

- Interstitial infiltration
- Diffuse infiltration
- Fibrosis

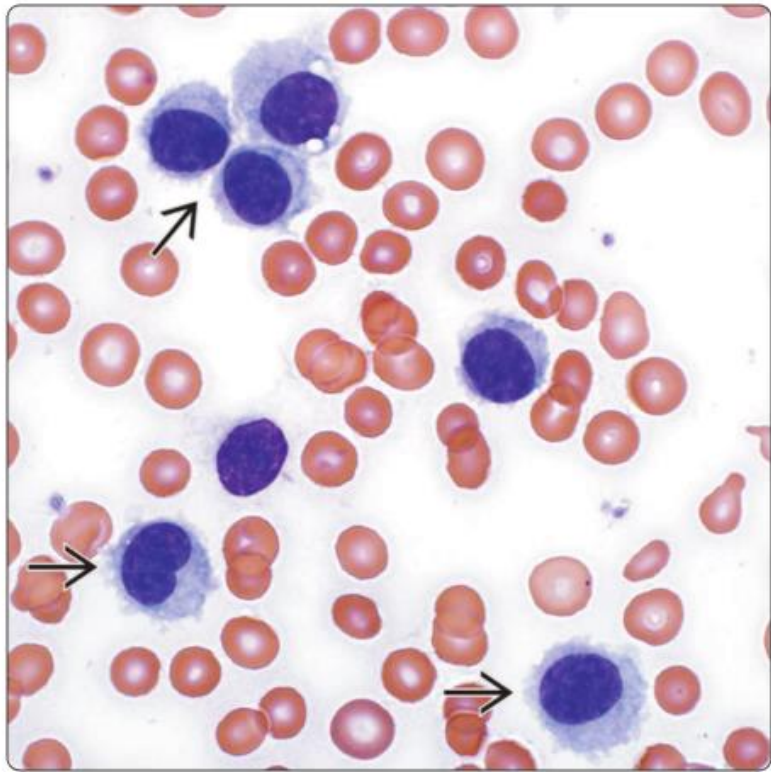
• Useful Markers

- Annexin A1
- CD20
- DBA44

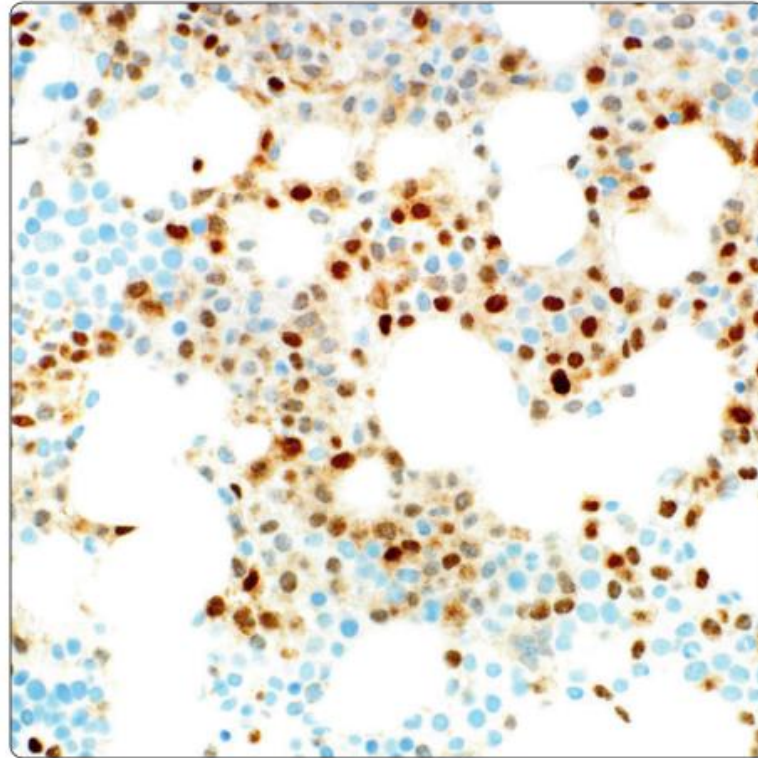
Key Message

- **Annexin A1 remains one of the most specific markers for classic Hairy Cell Leukemia.**

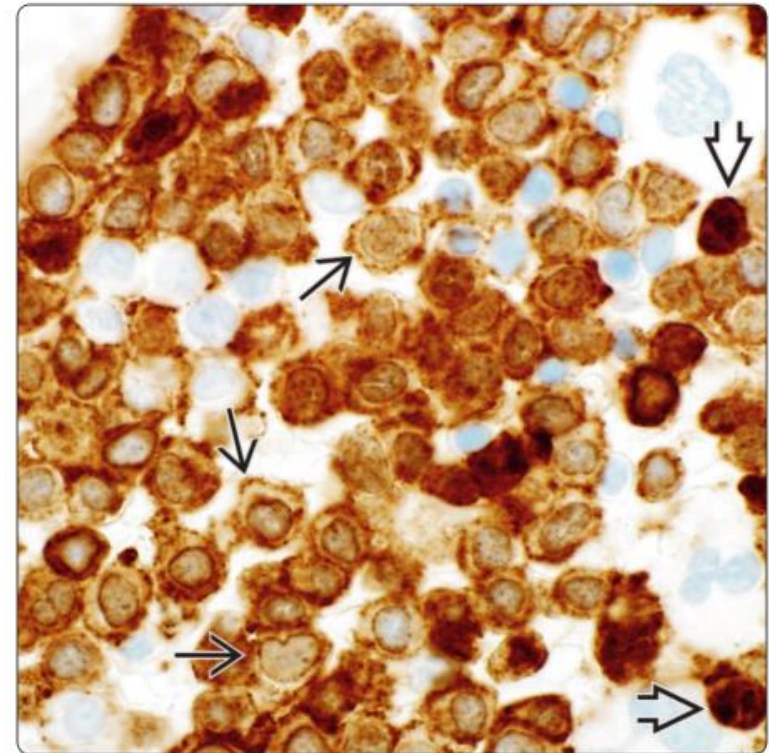
Hairy Cell Leukemia



Variable and Weak Cyclin-D1 Positivity in Hairy Cell Leukemia



Annexin-A1 in Hairy Cell Leukemia



Lymphoplasmacytic Lymphoma (LPL)

- **Typical Findings**

- Interstitial infiltration
- Plasma cells
- Mast cell increase

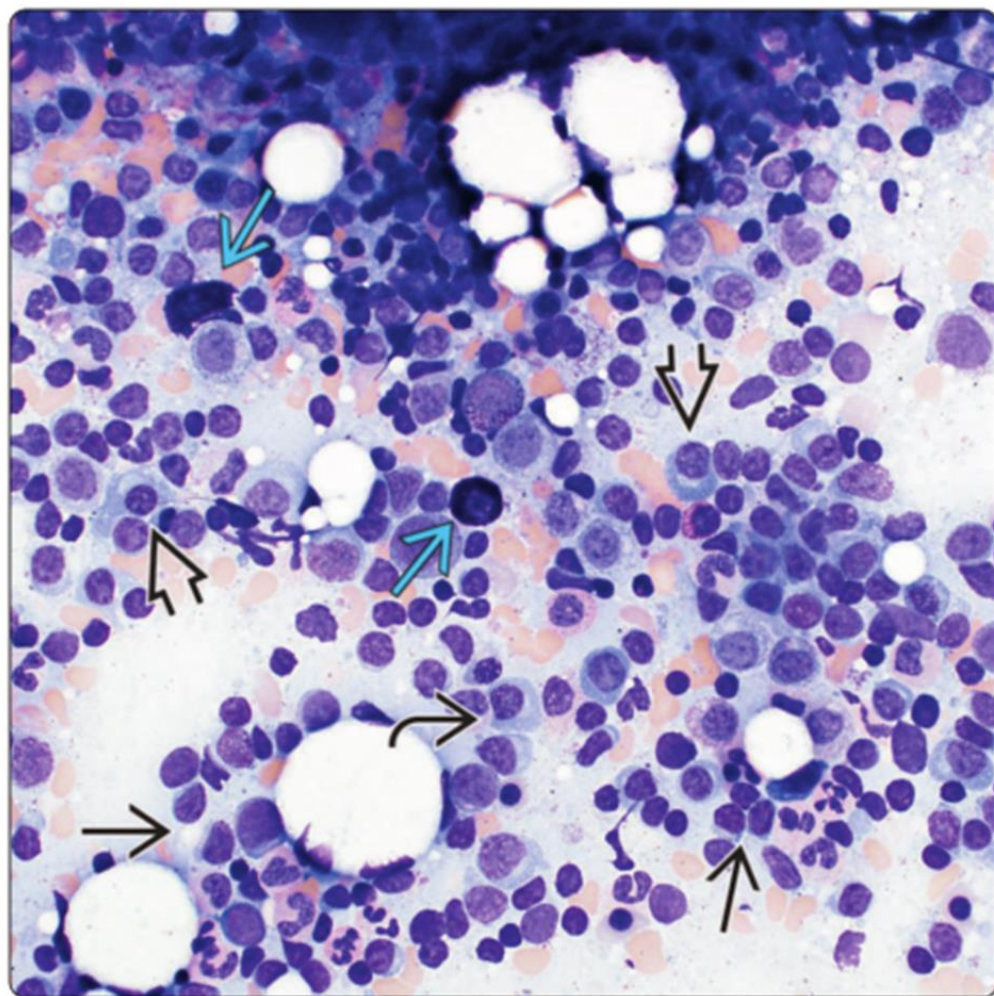
- **Helpful Markers**

- CD20
- CD138
- Kappa/Lambda

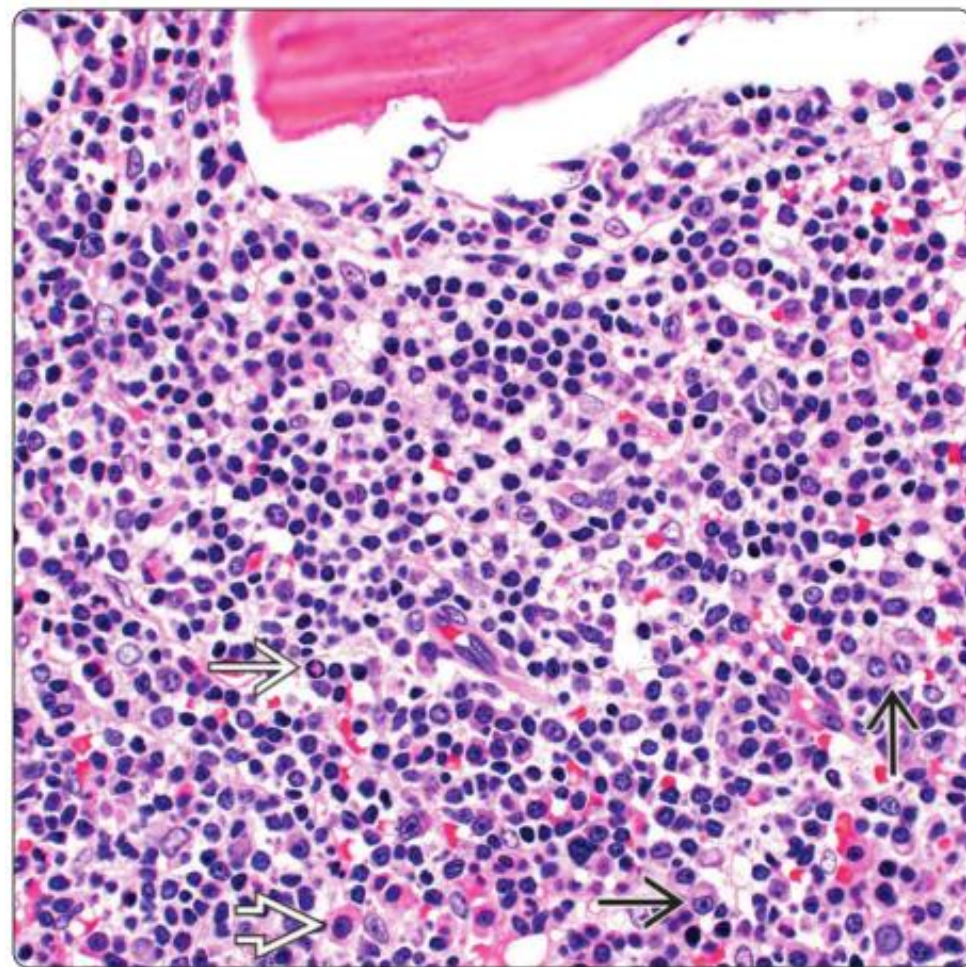
- **Practical Pearl**

- **Increased mast cells may provide an important clue to LPL.**

Lymphoplasmacytic Lymphoma



Lymphoplasmacytic Lymphoma



Bone Marrow Involvement by Aggressive B-Cell Lymphomas

Common Entities

- DLBCL
- Burkitt Lymphoma
- High-Grade B-cell Lymphoma

Useful Markers

- CD20
- PAX5
- CD10
- BCL6
- MUM1
- Ki-67

Practical Bone Marrow Lymphoma Panel

- **Initial Panel**

- CD20
- PAX5
- CD3

- **If B-cell Lymphoma Suspected**

- CD5
- CD10
- BCL6
- Cyclin D1

- **If T-cell Lymphoma Suspected**

- CD4
- CD8
- TIA1
- Granzyme B

- **Key Principle**

- **Expand the panel only after lineage assignment.**

Expert Pearls in Bone Marrow Lymphoma Diagnosis

Pearl #1

- Pattern recognition precedes immunophenotyping.

Pearl #2

- Paratrabecular aggregates suggest Follicular Lymphoma.

Pearl #3

- Intrasinusoidal infiltration suggests HSTCL until proven otherwise.

Pearl #4

- Reactive aggregates contain mixed B and T cells.

Pearl #5

- Flow cytometry frequently provides more information than IHC for small B-cell neoplasms.

Hematologic and Peripheral Blood/Bone Marrow Features of Mature B-Cell Leukemias

Neoplasm	Key Peripheral Blood Features	Key Bone Marrow Features
Chronic lymphocytic leukemia/small lymphocytic lymphoma	Lymphocytosis with fragile (smudge) cells	Nodular pattern; can be diffuse; may see proliferation foci
B-prolymphocytic leukemia	Marked lymphocytosis, cytopenias	Diffuse infiltrate; single prominent nucleoli
Splenic marginal zone lymphoma	May see mild lymphocytosis	Sinusoidal, interstitial and nodular infiltrates with occasional associated benign germinal centers
Hairy cell leukemia	Pancytopenia; rare hairy cells	Interstitial/diffuse tumor cells (+) for annexin-A1, TRAP, CD123, and BRAF by IHC; fried-egg appearance; <i>BRAFV600E</i> mutation (+)
Follicular lymphoma	Occasional involvement	Paratrabecular; centrocyte cytology
Mantle cell lymphoma	Occasional involvement	Paratrabecular/diffuse; variable cytology; <i>CCND1-IGH</i> fusion
Lymphoplasmacytic lymphoma	Occasional involvement; rouleaux	Paratrabecular and diffuse involvement; lymphoid and plasmacytic infiltrate with admixed mast cells; <i>MYD88 L265P</i> mutation (+)
Burkitt lymphoma	May involve peripheral blood, cytopenias, leukoerythroblastosis	Focal/diffuse; starry-sky appearance; <i>MYC</i> rearrangement
Diffuse large B-cell lymphoma	Rare in peripheral blood, leukoerythroblastosis	Focal/diffuse

Characteristic Immunophenotypic Profiles of Selected Mature B-Cell Leukemias/Lymphomas

Neoplasm	CD20	CD5	CD10	sIg	CD25	CD103	Other
Chronic lymphocytic leukemia	dim (+)	(+)	(-)	dim (+)	(-)	(-)	CD23(+), CD200(+)
Mantle cell lymphoma	(+)	(+)*	(-)**	(+)	(-)	(-)	Cyclin-D1 uniformly (+); <i>CCND1-IGH</i> fusion
Follicular lymphoma	(+)	(-)	(+)*	(+)*	(-)	(-)	<i>IGH-BCL2</i> fusion
Burkitt lymphoma	(+)	(-)	(+)	(+)	(-)	(-)	<i>MYC</i> -rearranged
Hairy cell leukemia	bright (+)	(-)	(-)**	bright (+)	(+)	(+)	Annexin-A1(+), <i>BRAFV600E</i> mutation (+); cyclin-D1 variably (+)
Splenic marginal zone lymphoma	(+)	(-)**	(-)	(+)	(-)	Rare (+)	Sinusoidal bone marrow pattern; may have associated clonal plasma cell population
Lymphoplasmacytic lymphoma	(+)	(-)**	(-)	(+)	(-)	(-)	<i>MYD88</i> L265P mutation (+), associated clonal plasma cells CD19(+), CD45(+); typically IgM paraprotein

*Occasionally negative; **occasionally positive.

PLASMA CELL DISORDERS AND MAST CELL NEOPLASMS

Plasma Cell-Rich Marrow

Are These Reactive or Neoplastic Plasma Cells?

• Major Differential Diagnoses

- Reactive Plasmacytosis
- MGUS
- Plasma Cell Myeloma
- Plasma Cell Leukemia
- Lymphoplasmacytic Lymphoma

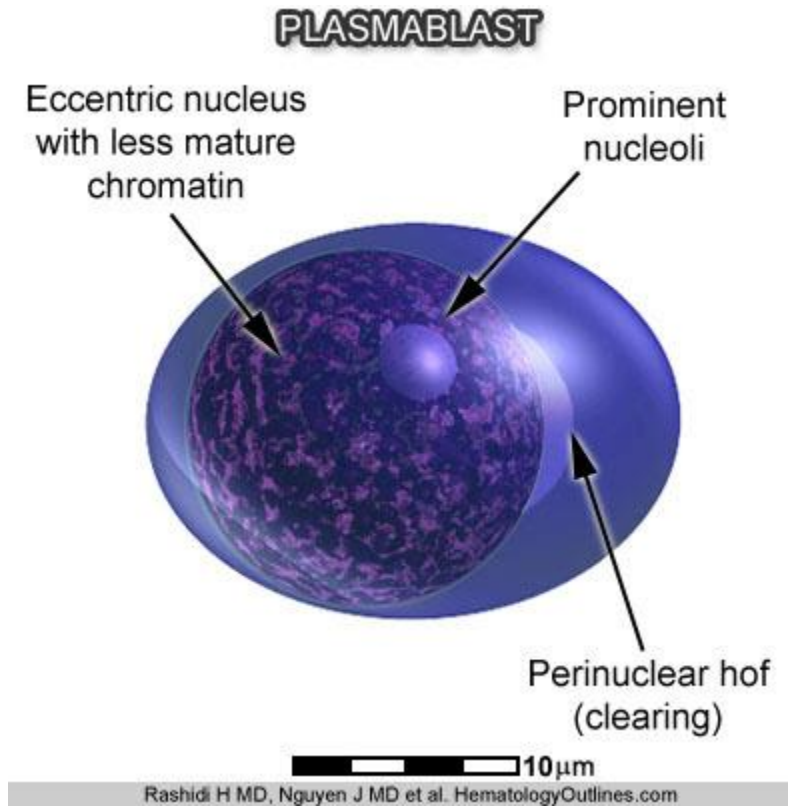
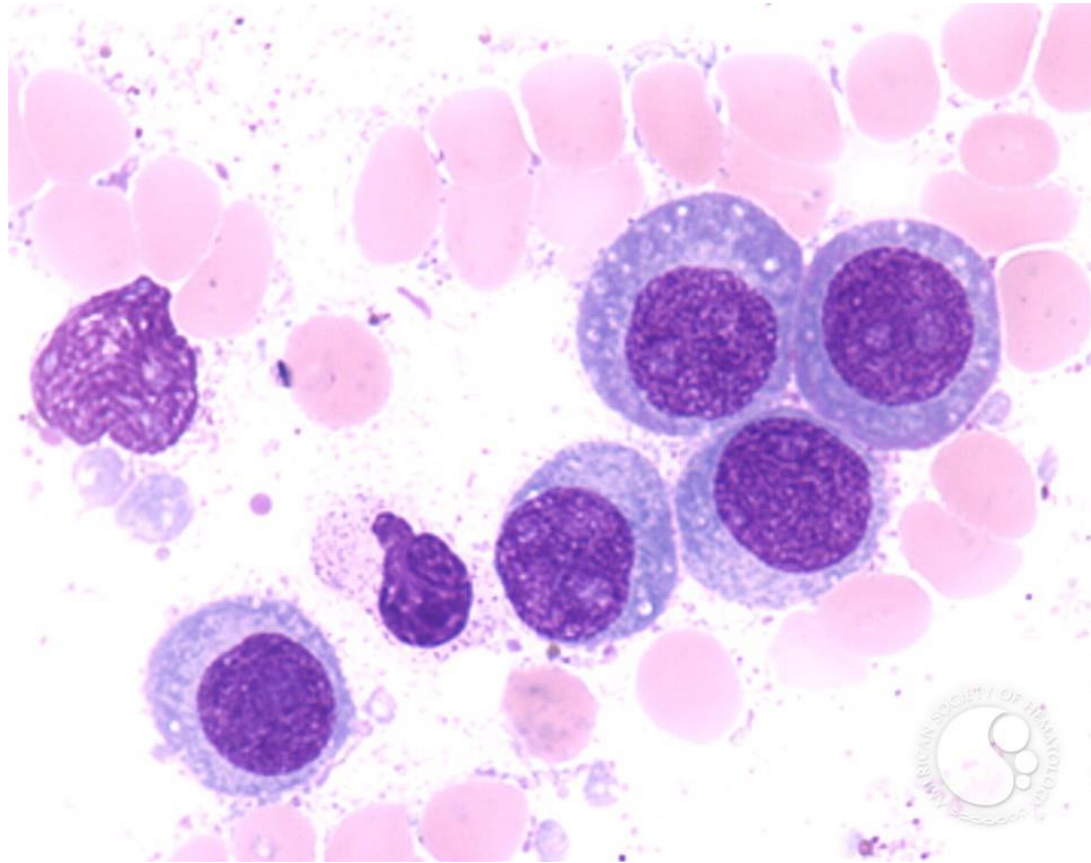
Initial IHC Panel

- CD138
- Kappa
- Lambda

Key Principle

- **Demonstration of clonality is the primary goal of IHC.**

Immature plasma cells



CD138 in Bone Marrow Pathology

Why CD138 Matters

- ✓ Highlights plasma cells
- ✓ Quantifies plasma cell burden
- ✓ Detects subtle infiltration
- ✓ Assesses distribution pattern

Limitations

- ✗ Does not determine clonality
- ✗ Does not distinguish reactive from neoplastic plasma cells

Practical Pearl

- **CD138 is a plasma cell marker, not a myeloma marker.**

Plasma Cell Distribution Patterns

Reactive Plasmacytosis

- Scattered cells
- Perivascular distribution
- No clustering

Plasma Cell Neoplasm

- Clusters
- Sheets
- Paratrabecular aggregates

Practical Pearl

- **Large plasma cell aggregates strongly favor neoplasia.**

Kappa and Lambda Light Chains

Reactive Plasma Cells

- Mixed Kappa
- Mixed Lambda

Plasma Cell Neoplasm

- Kappa Restricted
- or
- Lambda Restricted

Key Message

- **Light chain restriction is one of the strongest indicators of plasma cell neoplasia.**

Plasma Cell Myeloma

Bone Marrow Findings

- Increased plasma cells
- Clusters or sheets
- Monotypic light chain expression

Helpful Markers

- CD138
- Kappa
- Lambda

Optional Markers

- Cyclin D1
- CD56: positivity favors neoplastic plasma cells.

Practical Pearl

- **Pattern of infiltration is often more informative than percentage alone.**

Plasma Cell Myeloma Patterns

- Interstitial
 - Nodular
 - Paratrabecular
 - Diffuse
 - Packed Marrow
- **Key Message**
 - **Multiple patterns may coexist within the same biopsy.**

MGUS vs Myeloma

Feature	MGUS	Myeloma
Plasma Cells	<10%	≥10%
End Organ Damage	Absent	Present
Clonality	Present	Present
CD138	Positive	Positive

Key Message

IHC cannot distinguish MGUS from myeloma without clinical correlation.

MGUS

✓ Serum monoclonal protein < 3 g/dL

Plasma Cell Myeloma Pitfalls

Pitfall #1

- Reactive plasmacytosis may appear extensive

Pitfall #2

- Clonality may be difficult after therapy

Pitfall #3

- Decalcification may reduce light-chain staining

Expert Pearl

- Always interpret Kappa/Lambda together with morphology and clinical data.

Mast Cell-Rich Marrow

• Normal Mast Cells

- Round to oval cells
- Abundant metachromatic granules
- Centrally located nucleus
- Usually scattered individually in the marrow
- CD117 (KIT): Positive
- Tryptase: Positive
- CD25: Negative
- CD30: Negative
- CD2: Negative

• Neoplastic Mast Cells

- Often spindle-shaped (atypical morphology)
- May be hypogranular
- Irregular nuclear contours
- Frequently form clusters or aggregates
- CD117 (KIT): Positive
- Tryptase: Positive
- CD25: Positive
- CD30: Positive (WHO 2022)
- CD2: May be positive

Systemic Mastocytosis

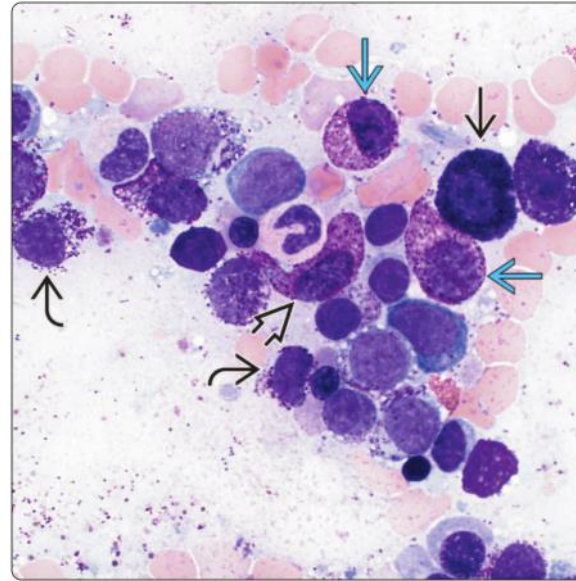
- **Clues**

- Unexplained eosinophilia
- Fibrotic marrow
- Dense aggregates
- Atypical spindle-shaped cells
- **CD25 expression supports neoplastic mast cell proliferation**

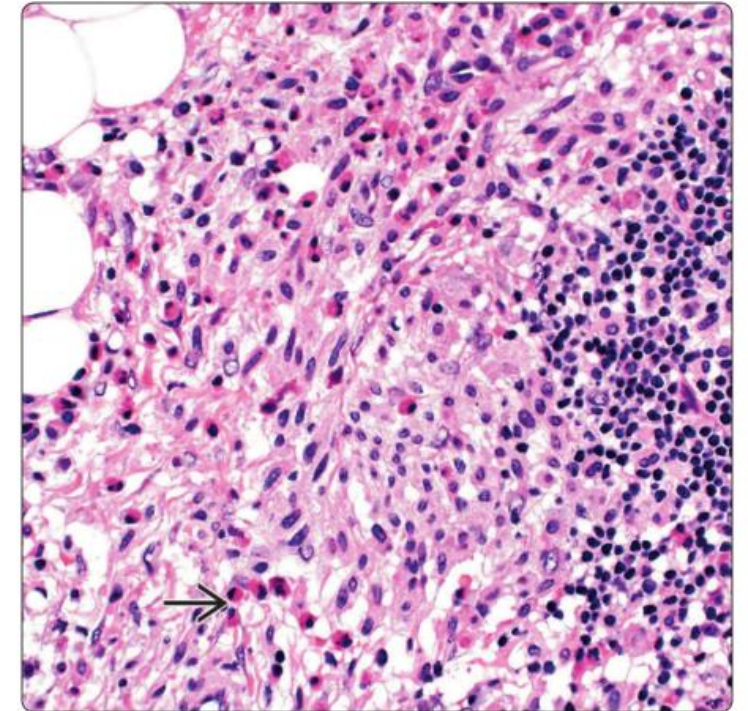
- **Expert Pearl**

- Always consider systemic mastocytosis in eosinophil-rich marrows.

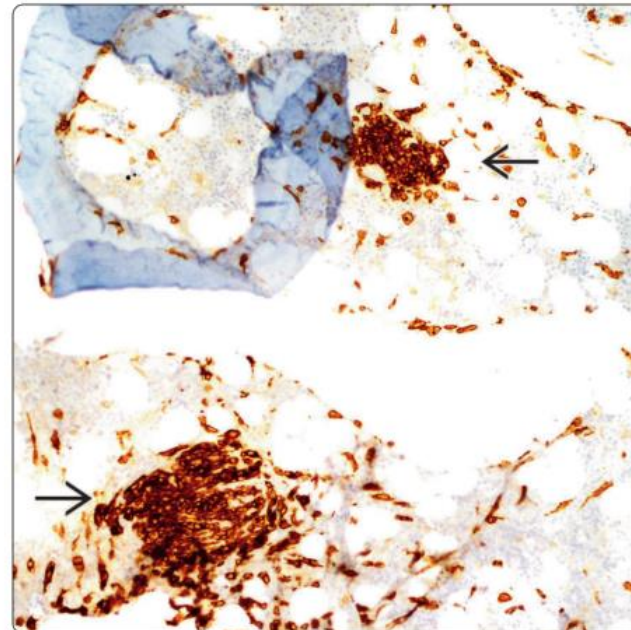
Atypical Mast Cells



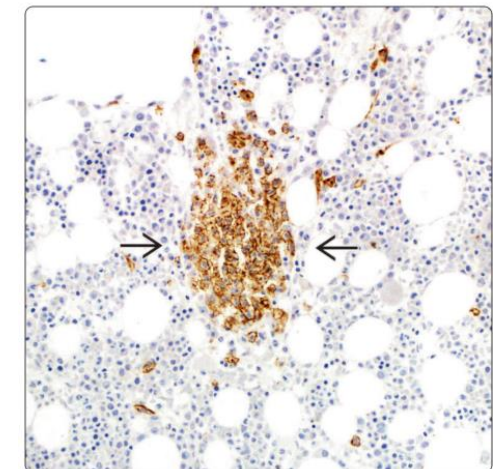
Mast Cell Aggregate



CD117 Highlights Mast Cell Infiltrates



Aberrant CD25 on Mast Cells



Mastocytosis Pitfalls

Pitfall #1

- CD117 also stains myeloid precursors

Pitfall #2

- Reactive mast cells may increase in many disorders

Pitfall #3

- Small mast cell aggregates can be overlooked

Expert Pearl

- **CD25 is often the most useful discriminator between reactive and neoplastic mast cells.**

Common Causes of Diagnostic Error

- **Why Do We Misdiagnose Bone Marrow Biopsies?**
- **Major Reasons**
 - Overreliance on IHC
 - Underappreciation of morphology
 - Ignoring infiltration patterns
 - Misinterpretation of CD34
 - Inadequate clinical correlation
 - Failure to recognize artifacts
- **Expert Pearl**
 - **Most marrow diagnostic errors originate from morphology, not immunohistochemistry.**

Decalcification Artifacts

- **A Major Source of Error**

- **Affected Markers**

- Ki-67
- TdT
- Cyclin D1
- Light chains

- **Consequences**

- Weak staining
- False negative results

- **Practical Pearl**

- **Always consider decalcification when staining is unexpectedly weak.**

The Diagnostic Landscape Has Changed

- **Traditional Era**

Morphology

IHC



Diagnosis

- **Modern Era**

Morphology

IHC

Flow Cytometry

Cytogenetics

NGS



Integrated Diagnosis

Expert Pearl

Many modern hematologic neoplasms are ultimately defined by molecular abnormalities rather than immunophenotype

Key Message

IHC is no longer the endpoint of diagnosis; it is one component of an integrated diagnostic approach.

When Flow Cytometry Is Better Than IHC

- **Common Scenarios**

- **CLL/SLL**
- **T-LGL Leukemia**
- **Hematogones vs B-ALL**
- **MRD Detection**

- **Practical Pearl**

- **Whenever precise immunophenotypic characterization is required, flow cytometry usually outperforms IHC.**

When IHC Is Better Than Flow Cytometry

- **Common Scenarios**

- **Assessment of Marrow Architecture**
- **Detection of ALIPs**
- **Evaluation of Fibrotic Marrow**
- **Detection of Small Focal Infiltrates**
- **Assessment of Infiltration Pattern**

- **Key Message**

- **Flow cytometry characterizes cells; IHC localizes cells.**

Bone Marrow Evaluation After Therapy

Why It Is Challenging

- **Post-Chemotherapy**

- Regenerating precursors
- Increased hematogones
- Hypocellularity

- **Post-HMA Therapy**

- HMA = Hypomethylating Agent :
Azacitidine Decitabine
- Persistent dysplasia
- Blast reduction

- **Post-Transplant**

- Mixed cellular populations
- Regeneration

Expert Pearl

Post-treatment marrows are among the most difficult specimens in hematopathology.

Minimal Residual Disease (MRD)

Can IHC Detect MRD?

- **Strengths**
- ✓ Detection of focal residual disease
- ✓ Architectural assessment

Limitations

- ✗ Low sensitivity
- ✗ Inferior to flow cytometry
- ✗ Inferior to molecular assays

Key Message

- **IHC is useful for residual disease assessment but should not be considered a primary MRD technique.**

Take-Home Messages

1. Morphology remains the foundation.
2. Recognize the pattern before ordering stains.
3. Use focused, question-driven panels.
4. Use Cost-Effective panels.
5. CD34 is the most useful marrow screening stain.
6. Pattern recognition often narrows the diagnosis before IHC.
7. Flow cytometry and IHC are complementary.
8. Modern hematopathology requires molecular integration.
9. Interpret stains in clinical context.
10. Avoid shotgun panels.



Thank You!