Diagnostic Immunohistochemistry in Hematolymphoid Pathology
A Pattern-Based, Morphology-Driven Approach

PART 2

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Practical Immunohistochemical Approach to T-Cell & NK-Cell Lymphoproliferative Disorders

Learning Objectives

- By the end of this session, participants should be able to:
  - **Recognize major architectural patterns in T-cell lymphomas**
  - Distinguish reactive from neoplastic T-cell proliferations
  - **Apply practical first-line IHC panels**
  - Recognize TFH-derived lymphomas and their pitfalls **Interpret CD30-rich proliferations correctly**
  - Identify key morphologic clues in ALCL and PTCL-NOS
  - **Understand major diagnostic pitfalls in T-cell pathology**
  - Integrate morphology, immunophenotype, and molecular findings in daily sign-out practice

Why Are T-Cell Lymphomas Challenging?

Diagnostic Challenges:

- Rare and heterogeneous neoplasms
- Broad morphologic spectrum
- Significant overlap with reactive conditions
- Frequent aberrant immunophenotypes
- No single marker is fully specific
- Many entities lack a pathognomonic genetic abnormality
- Molecular findings are often supportive rather than diagnostic
- Strong dependence on clinicopathologic correlation

T-cell lymphoma diagnosis is integrative, not marker-based.

Core Diagnostic Philosophy

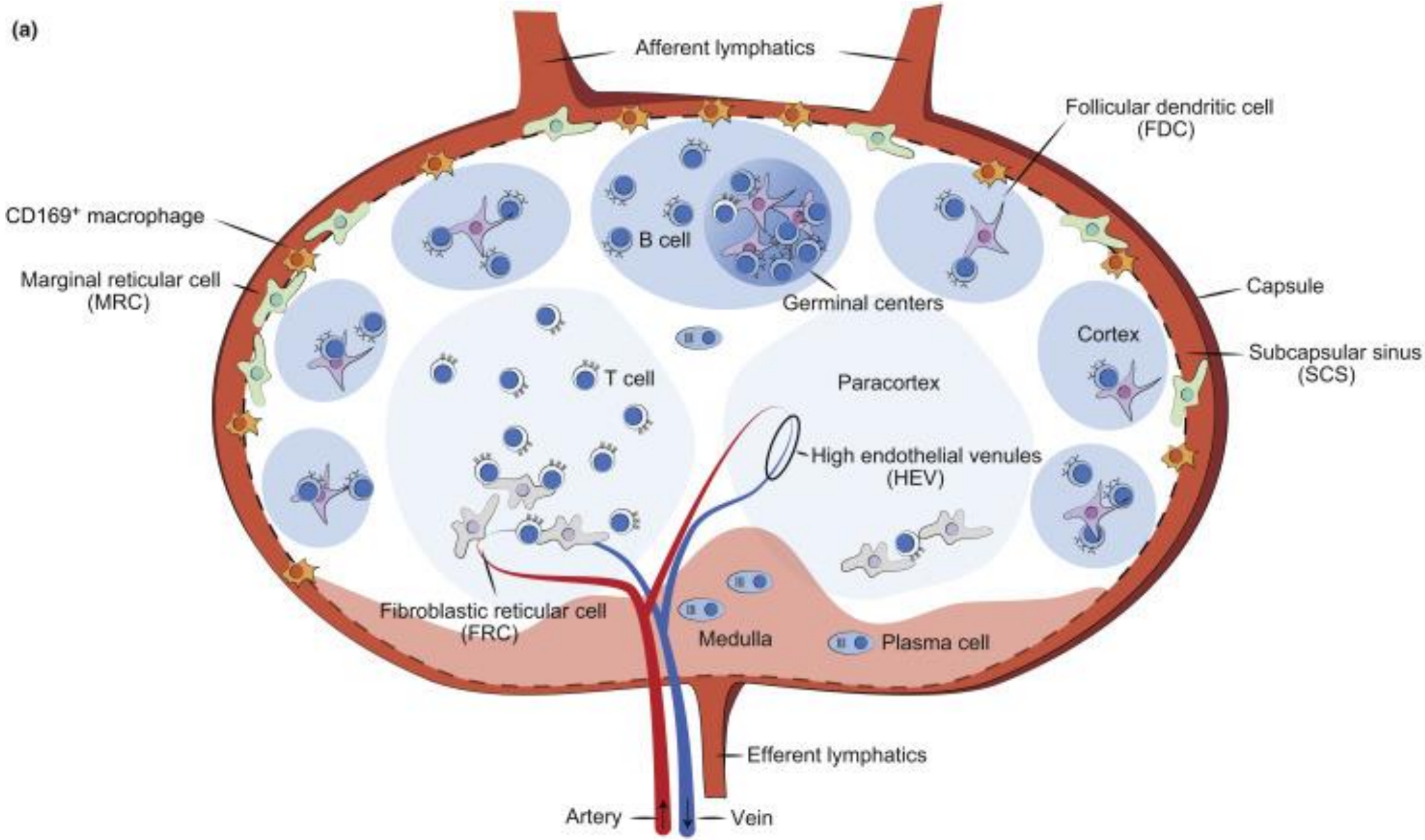
- Architecture → Cytology → Pattern → IHC → Molecular Integration
- An immunostain never means the same thing in every anatomic site.
- Rational marker selection is more valuable than excessively large antibody panels.
- Immunostains should answer a specific diagnostic question:
  - Lineage?
  - TFH phenotype?
  - Cytotoxic phenotype?
  - CD30-rich proliferation?
  - EBV-associated process?

Fundamental Diagnostic Principle

- Paracortical Expansion is a Pattern — Not a Diagnosis
- The paracortex represents the T-cell zone of the lymph node
- Expansion of this compartment may occur in:
  - Reactive immune activation
  - Viral infections
  - Drug reactions
  - Autoimmune disorders
  - T-cell lymphomas
  - EBV-related proliferations
- Morphology determines the initial differential diagnosis
- Immunohistochemistry refines diagnostic probability
- Reactive and neoplastic paracortical processes may closely mimic each other.

Normal Paracortex

- Functional T-Cell Zone of the Lymph Node
  - Located between lymphoid follicles
  - Rich in: T-cells, Dendritic cells & High endothelial venules (HEVs)
  - Site of: Antigen presentation, T-cell activation & Immune regulation
- Normal Immunophenotype:
  - CD3-positive T-cells
  - CD4 usually predominates over CD8
  - Scattered immunoblasts may be present
  - Rare CD30-positive activated cells may be seen


(a)
Afferent lymphatics
Follicular dendritic cell (FDC)
CD169⁺ macrophage
B cell
Marginal reticular cell (MRC)
Germinal centers
Capsule
Cortex
Subcapsular sinus (SCS)
T cell
Paracortex
High endothelial venules (HEV)
Fibroblastic reticular cell (FRC)
Medulla
Plasma cell
Efferent lymphatics
Artery
Vein

Morphologic Features of Paracortical Expansion

- Key Low-Power Findings
  - Expansion of interfollicular regions
  - Increased cellularity within the T-cell zone
  - Variable preservation of nodal architecture
  - Compression or attenuation of follicles
  - Prominent vascular proliferation may be present

Important Diagnostic Question

- Is the infiltrate:
    - Polymorphous and reactive?

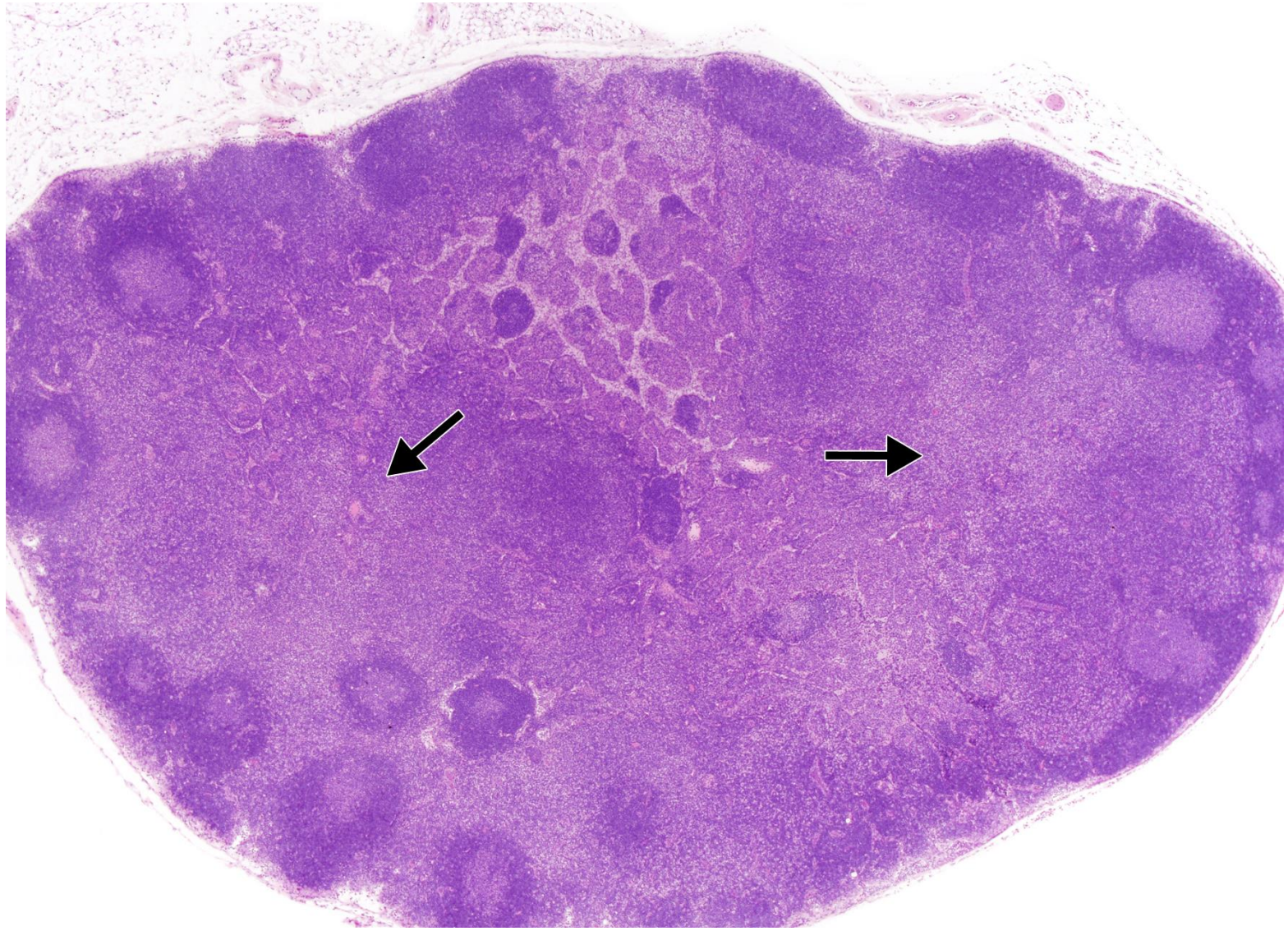
      OR
    - Monotonous and neoplastic?

Reactive Paracortical Hyperplasia

- Typical Morphologic Features
  - Preserved overall nodal architecture
  - Expanded paracortex with mixed inflammatory cells
  - Variable vascular prominence
  - Polymorphous cellular population:
    - Small lymphocytes+ Immunoblasts+ Plasma cells+ Histiocytes+ Eosinophils (variable)

Preserved follicles and polymorphous cellularity favor a reactive process.

Reactive Paracortical Hyperplasia

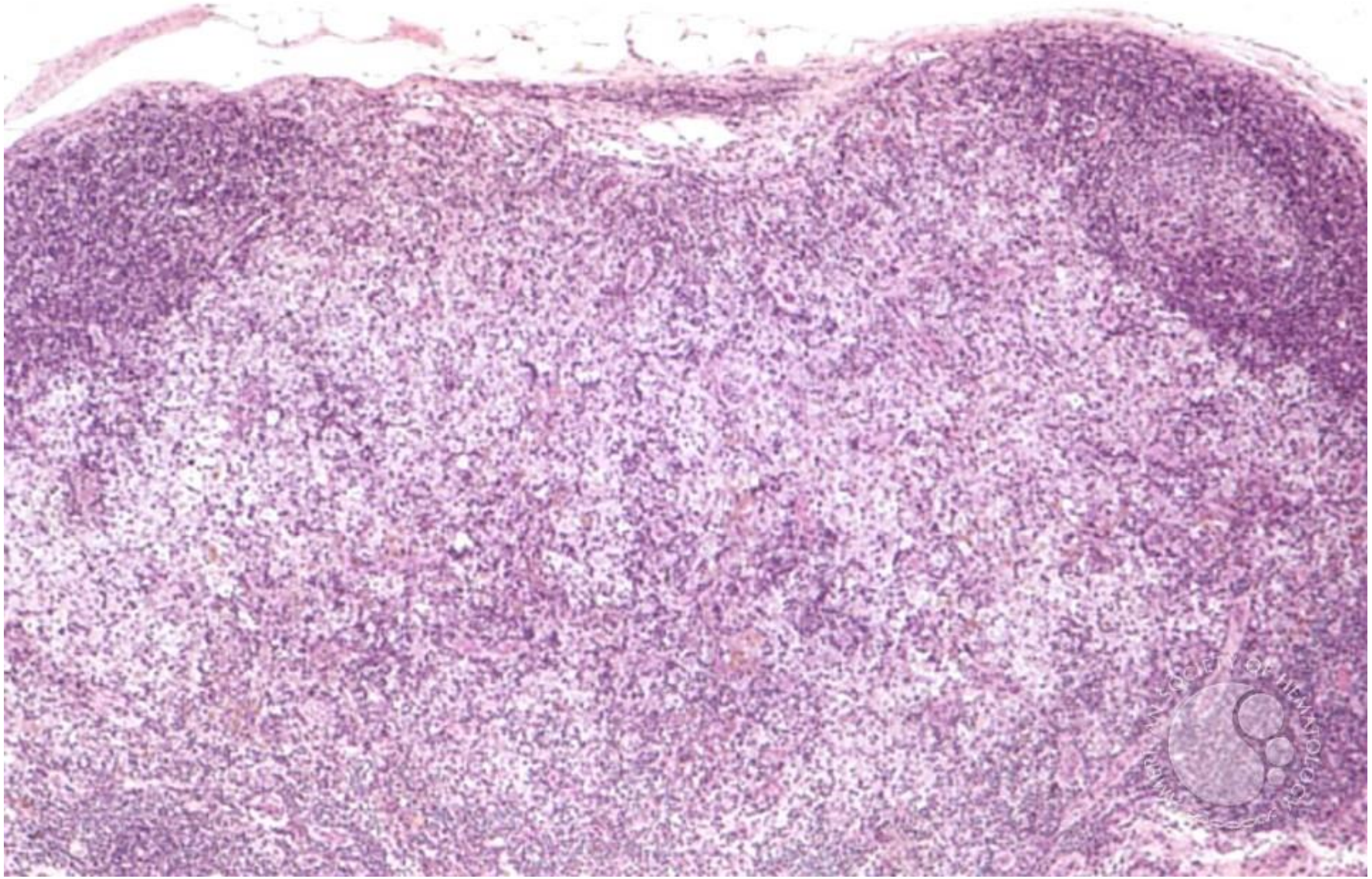


Causes of Reactive Paracortical Expansion

- Common Reactive Settings
  - Viral Infections: EBV, CMV, HIV
  - Immune Stimulation
    - Drug reactions
    - Autoimmune disorders
    - Post-vaccination lymphadenopathy
  - Other Conditions
    - Dermatopathic lymphadenitis
    - Systemic inflammatory disorders

Clinical correlation is essential in reactive paracortical patterns.

Dermatopathic lymphadenitis



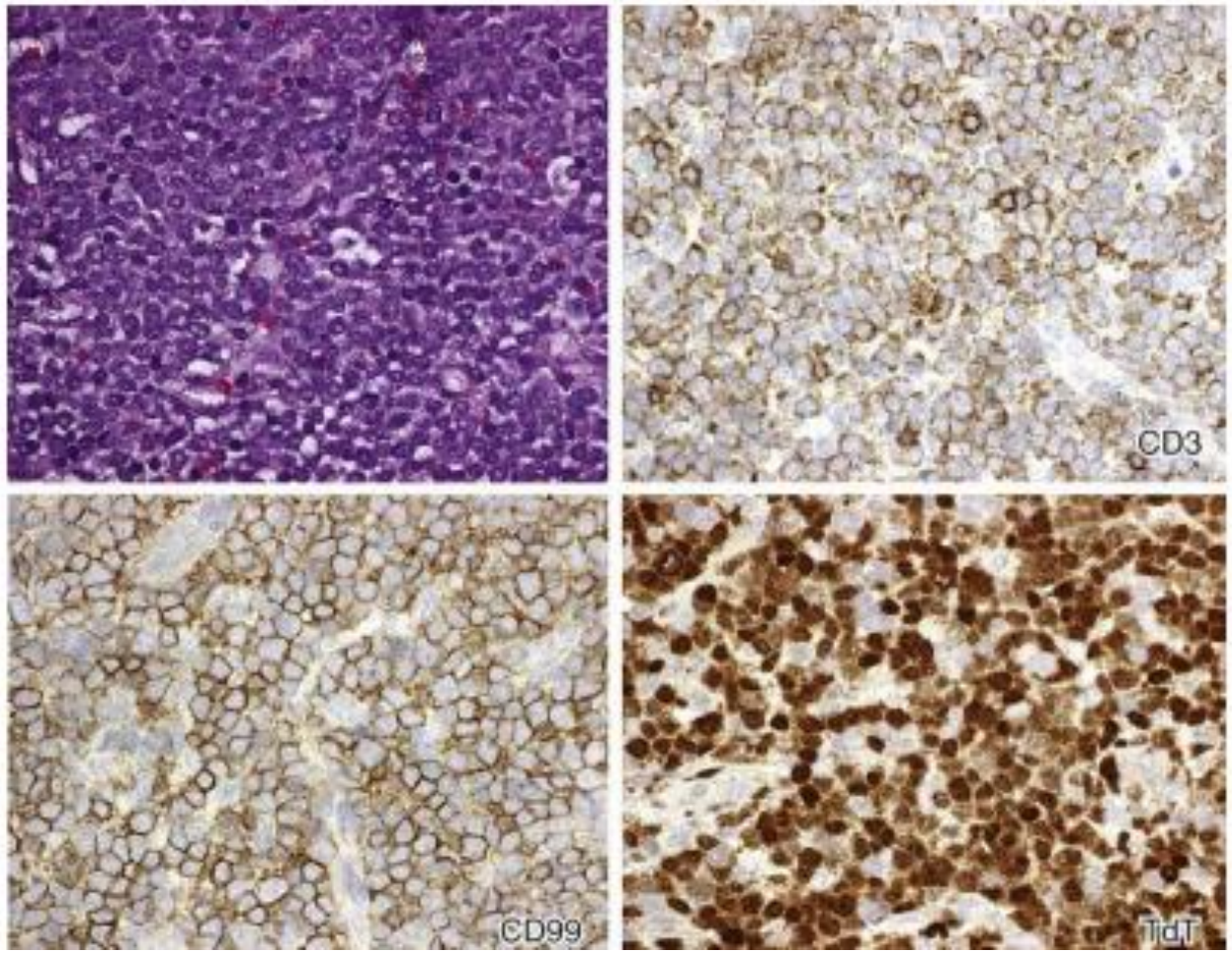
Practical Diagnostic Workflow

- **Step 1 — Blastic or Mature?**
- Blastic | Mature
- Monomorphic | Pleomorphic/polymorphous
- **Step 2 — Nodal or Extranodal?**
- **Step 3 — Identify the Architectural Pattern**
- **Step 4 — Apply Targeted Immunohistochemistry**
- **Step 5 — Integrate Molecular Findings**

Monomorphic = one cell type: T-LBL, T-PLL, MEITL
Pleomorphic = variable tumor cells: PTCL-NOS, ALCL
Polymorphous = mixed cell populations: AITL

Pattern recognition narrows the differential before immunostains do.

Lymphoblastic T-cell lymphoma/leukaemia



Mediastinal mass shows malignant lymphoblasts with dispersed chromatin. The tumor expresses CD3, CD99, and terminal deoxynucleotidyl transferase (TdT) as indicated.

WHO/ICC 2022 — Major Mature T/NK-Cell Neoplasms

Nodal

- Nodal TFH-cell lymphoma
- PTCL-NOS
- ALCL (ALK+ / ALK−)

Extranodal

- ENKTL
- Intestinal T-cell lymphomas
- Hepatosplenic T-cell lymphoma

Leukemic / Disseminated

- T-PLL
- ATLL
- T-LGL leukemia
- Sézary syndrome

- **PTCL-NOS remains a diagnosis of exclusion.**

T/NK Lymphoma: Architecture (Pattern) → Major Differential

Pattern (Architecture)	Major Differential Diagnosis	Key clue
Paracortical expansion	TFH lymphoma vs Reactive	Architecture often more specific than single markers
Diffuse pleomorphic infiltrate	PTCL-NOS	Diffuse atypical pleomorphic T-cells
Sinusoidal growth	ALCL	Sinus-filling large anaplastic cells
Angiocentric / necrotic	ENKTL	Angioinvasion/angiodestruction + necrosis
Epidermotropic	Mycosis fungoides	Epidermotropism ± Pautrier microabscess
Leukemic pattern	T-PLL / ATLL	Predominant blood/Marrow involvement
Intrasinusoidal BM infiltration	HSTCL	Classic intrasinusoidal BM pattern

Diagnostic Approach to T-Cell Lymphomas

Stepwise approach to nodal T-cell/NK-cell infiltrates,

Step 1 — Evaluate Morphology

- Paracortical or diffuse or sinusoidal pattern?
- Pleomorphic/polymorphous or monotonous infiltrate?
- Vascular proliferation?
- Eosinophils/plasma cells present?
- Large atypical cells present?

- Step 2 — Establish T-cell Lineage
  - CD20,CD3,CD2, CD5 & CD7
- Step 3 — Subclassify
  - TFH phenotype?
  - Cytotoxic phenotype?
  - CD30-rich proliferation?
  - ALK-positive disease?

T-cell lymphoma diagnosis is integration-driven, not marker-driven.

Initial IHC Approach in Paracortical Expansion

- **Key Diagnostic Questions**
  - Is the process T-cell or B-cell predominant?
  - Is there aberrant antigen loss?
  - Are activated large cells present?
  - Is EBV involved?

Always interpret immunostains in architectural context.

Essential IHC Panel of T cell lymphoma

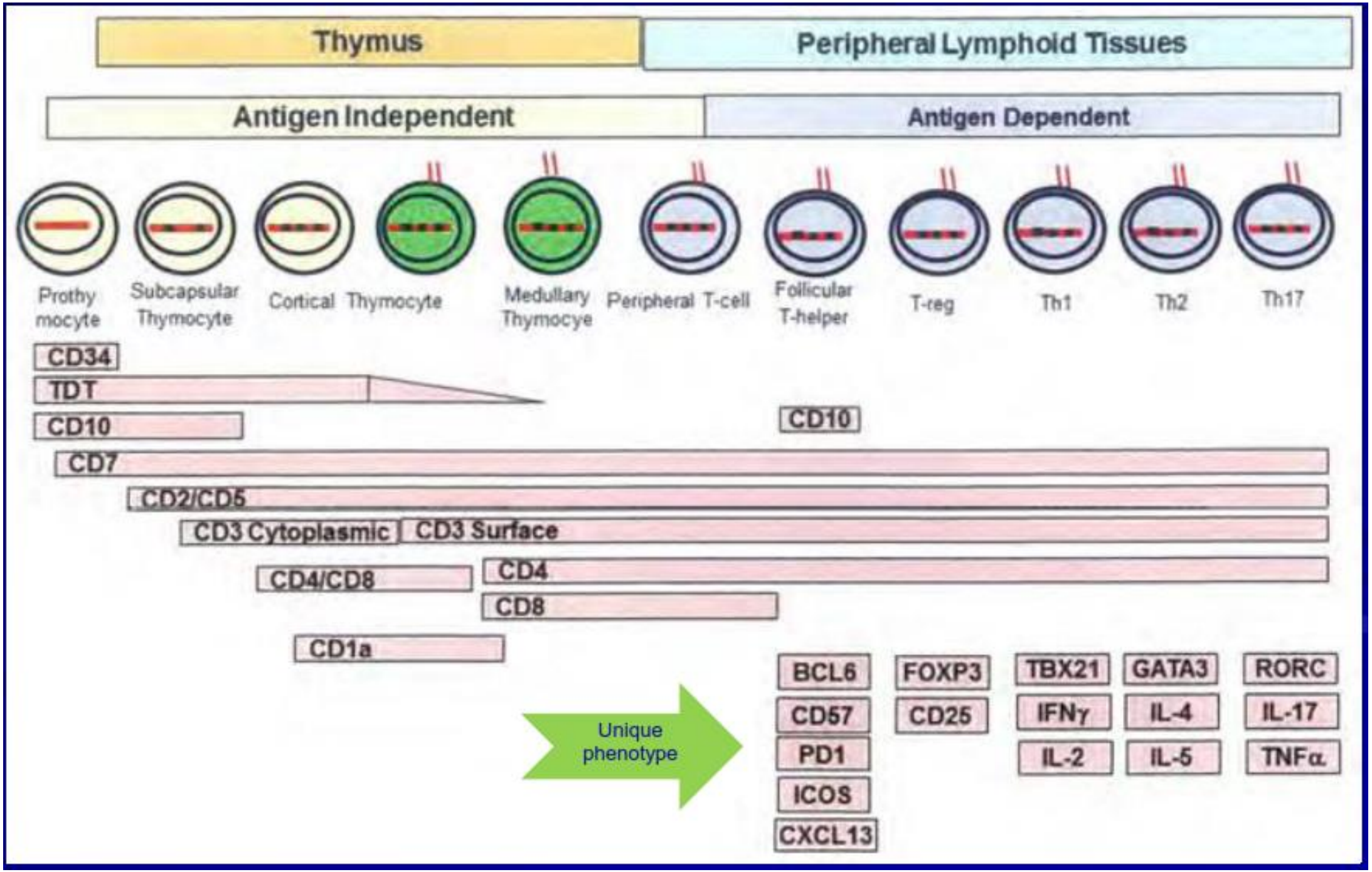
Marker	Diagnostic Utility
CD3	T-cell lineage
CD20 / PAX5	Exclude B-cell lymphoma
CD4 / CD8	T-cell subset
CD30	Activated cells / ALCL
ALK	ALCL subclassification
PD1 / ICOS / CXCL13	TFH phenotype
CD21 / CD23	FDC meshwork
EBER	EBV-associated lesions
TIA1 / Granzyme B / Perforin	Cytotoxic phenotype
TdT	Immaturity
Ki67	Proliferation index

Pan–T-Cell Markers

Diagnostic Utility：

- CD3:
  - Most specific T-cell lineage marker
  - Cytoplasmic or membranous staining
- CD2:
  - Sensitive but less specific
- CD5:
  - Frequently lost in T-cell lymphomas
- CD7:
  - Most commonly lost T-cell marker

Loss of pan–T-cell markers supports aberrancy but is not entirely specific for lymphoma


Thymus
Peripheral Lymphoid Tissues
Antigen Independent
Antigen Dependent
Prothy mocyte
Subcapsular Thymocyte
Cortical Thymocyte
Medullary Thymocye
Peripheral T-cell
Follicular T-helper
T-reg
Th1
Th2
Th17
CD34
TDT
CD10
CD10
CD7
CD2/CD5
CD3 Cytoplasmic
CD3 Surface
CD4/CD8
CD4
CD8
CD1a
Unique phenotype
BCL6
CD57
PD1
ICOS
CXCL13
FOXP3
CD25
TBX21
IFNγ
IL-2
GATA3
IL-4
IL-5
RORC
IL-17
TNFα

Aberrant T-Cell Phenotype

- Clues Suggesting T-Cell Lymphoma
  - Loss of pan–T-cell antigens: CD5 loss, CD7 loss, CD2 loss (less common)
  - Marked CD4 or CD8 predominance
  - Monotonous cytology
  - Diffuse architectural effacement

Aberrant phenotype supports lymphoma but is not entirely specific

Non-Malignant Causes of Pan-T Marker Loss

- **Severe Inflammation/Infection:** Viral triggers (e.g., EBV/Infectious Mononucleosis) can cause transient down-regulation of **CD7**.
- **Chronic Dermatoses:** Benign inflammatory skin conditions may show loss of T-cell markers.
- **Aging (Immunosenescence):** Natural expansion of healthy **CD7-negative** T-cell populations in the elderly.
- **Autoimmune Disorders:** Certain rheumatologic conditions can harbor “aberrant-looking” reactive T-cells.

CD4 and CD8 Patterns; Utility in T-Cell Proliferations

- Reactive Lymph Nodes:
  - Mixed CD4 and CD8 population
  - Usually CD4 predominant
- Suspicious Patterns:
  - Marked CD4 predominance
  - Marked CD8 predominance
  - Double-negative population
  - Double-positive population
- Common Associations
  - TFH lymphomas → usually CD4-positive
  - Cytotoxic T-cell lymphomas → often CD8-positive

Extreme polarization may suggest neoplasia.

CD30 in Reactive Conditions

- Important Interpretive Pitfall
  - CD30 is an activation marker
  - Reactive immunoblasts may show strong CD30 positivity
  - CD30 positivity alone does NOT establish lymphoma
- Reactive CD30-Positive Conditions
  - Viral lymphadenitis
  - EBV-related proliferations
  - Drug reactions
  - Reactive immunoblastic proliferations

Distribution and morphology are more important than isolated positivity.

CD30 Positivity — Major Diagnostic Pitfall

- **Reactive Pattern**
  - Scattered positive cells
  - Variable intensity
  - Polymorphous background
- **Suspicious Pattern**
  - Diffuse strong staining
  - Uniform intensity
  - Cohesive growth

Major Diagnostic Question

Is this:

Reactive CD30-positive immunoblastic proliferation?
OR
ALCL? OR Hodgkin lymphoma? OR PTCL-NOS with CD30 expression OR Large B cell lymphoma?

Nodal TFH Lymphomas; WHO/ICC Concept Update

- Angioimmunoblastic T-cell lymphoma (AITL) now belongs to Nodal T-Follicular Helper (TFH) Cell Lymphomas
- Major Categories
  - Nodal TFH lymphoma, angioimmunoblastic type
  - Nodal TFH lymphoma, follicular type
  - Nodal TFH lymphoma, NOS
- Neoplastic cells derive from T-follicular helper cells of the germinal center microenvironment.

Practical Approach to Nodal T-Cell Lymphomas

Question 1

- **Is there a TFH phenotype?**
- Look for:
  - PD1
  - ICOS
  - CXCL13
  - CD10
  - BCL6

Question 2

- **Is there diffuse strong CD30 expression?**
- Think:
  - ALCL
  - Hodgkin-like proliferations

Question 3

Is there a cytotoxic phenotype?

Think:

- ENKTL
- Cytotoxic PTCL
- Aggressive NK-cell leukemia

Question 4

Does morphology fit PTCL-NOS?

Only after excluding defined entities.

Morphology of TFH Lymphomas

- Characteristic Histologic Features:
  - Partial or diffuse nodal effacement
  - Marked paracortical expansion
  - Polymorphous inflammatory background
  - Prominent arborizing high endothelial venules (HEVs)
  - Expanded follicular dendritic cell (FDC) meshworks
  - Plasma cells and eosinophils are commonly present
  - EBV-positive B-immunoblasts are frequently identified in the background.

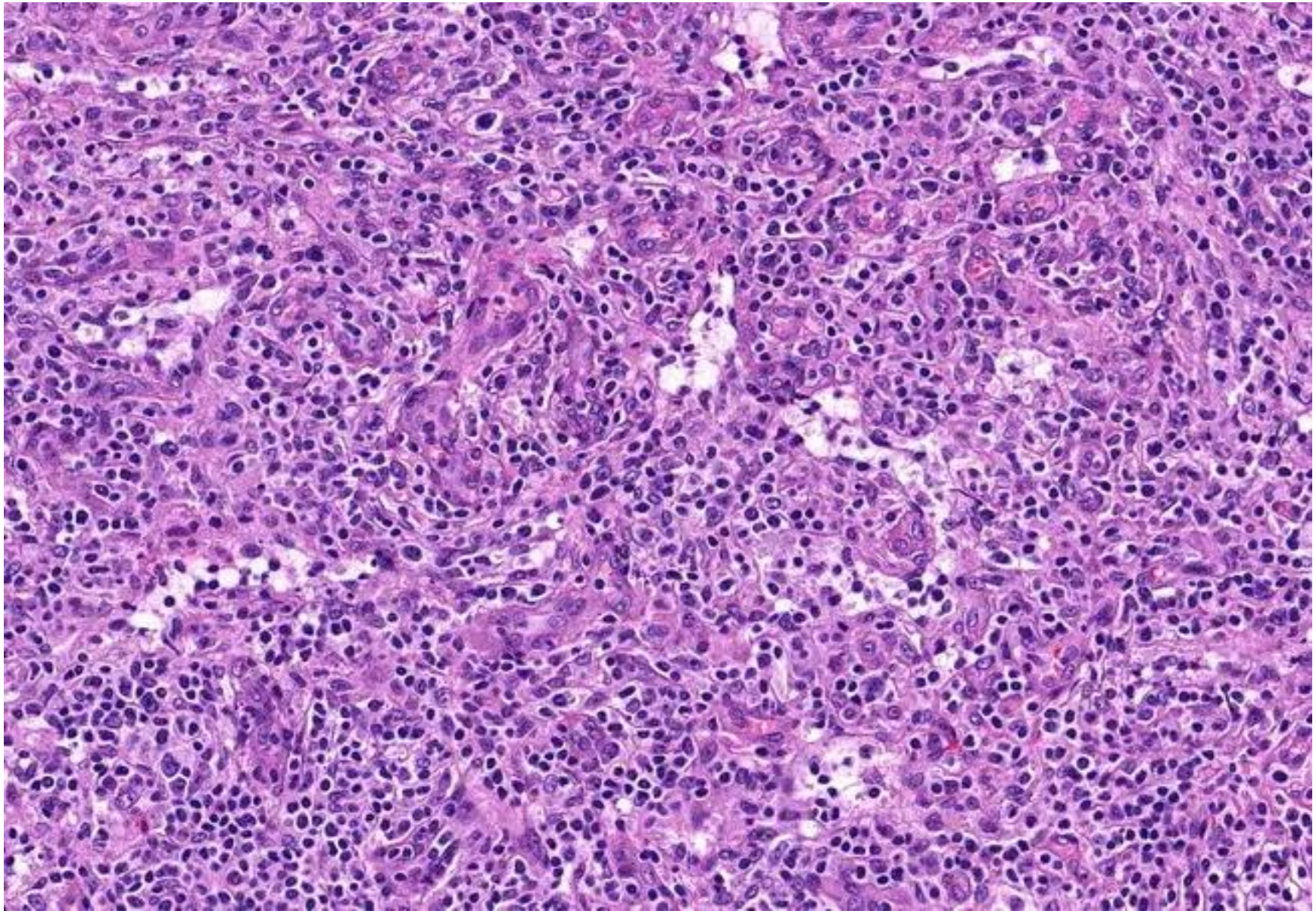
Cytologic Features in TFH Lymphomas

- Neoplastic T-Cells
  - Small-to-medium atypical lymphoid cells
  - Irregular nuclear contours
  - Pale or clear cytoplasm may be present
  - Variable cytologic atypia
- Background Population
  - Immunoblasts
  - Plasma cells
  - Histiocytes
  - Eosinophils

The reactive background may obscure the neoplastic T-cell population.

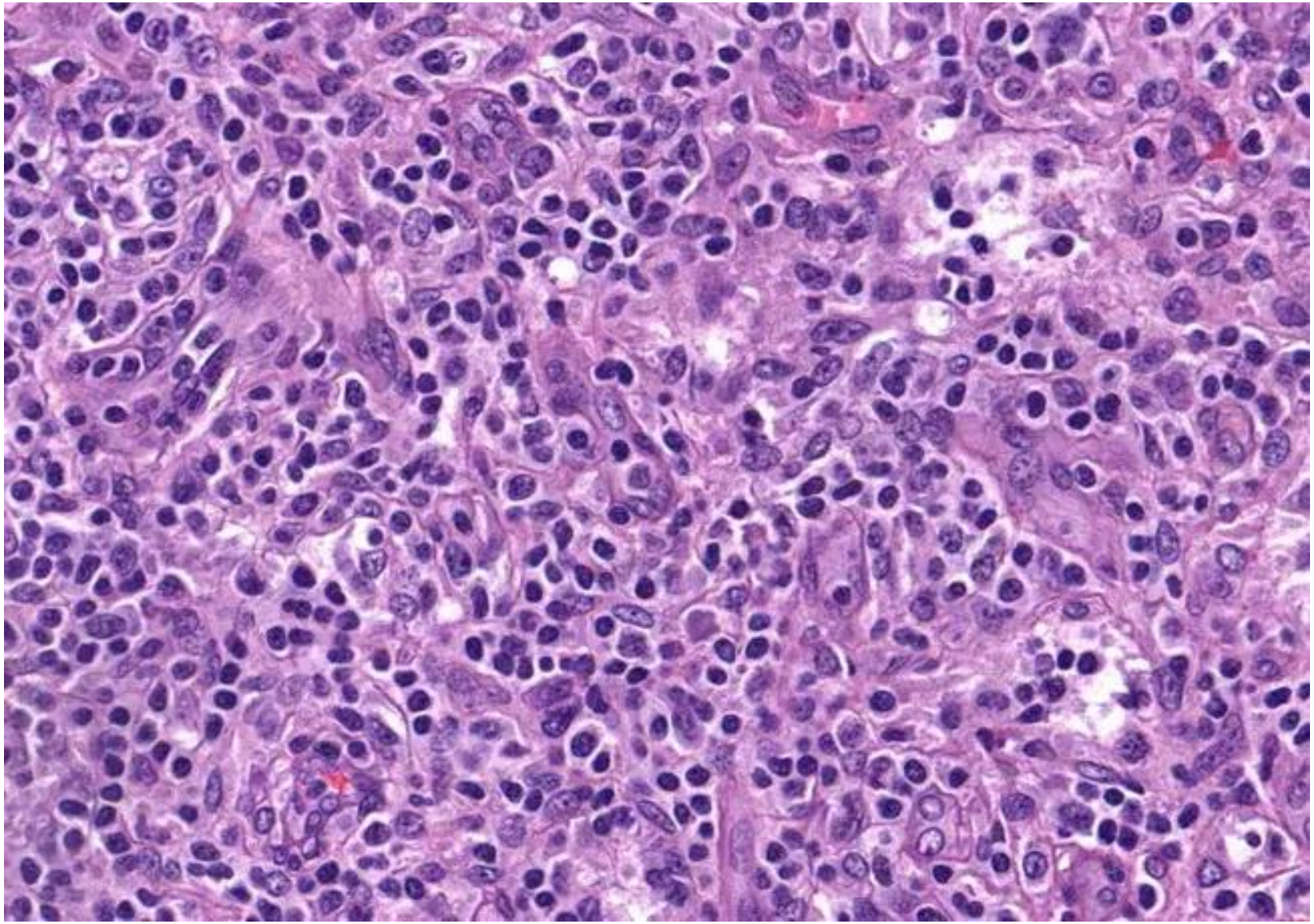
Morphology of TFH Lymphomas

Branching high endothelial venules



Morphology of TFH Lymphomas

Higher magnification view showing a few **high endothelial venules** surrounded by a **polymorphic infiltrate** consisting of small to medium-sized lymphocytes (neoplastic cells) admixed with neutrophils, plasma cells, dendritic cells, and histiocytes.



Histologic Patterns of AITL

- **Pattern 1 — Early AITL**
  - Hyperplastic follicles
  - Expanded paracortex
  - Preserved partial architecture
- **Pattern 2 — Classic AITL**
  - Burned-out germinal centers
  - Prominent HEVs
  - Expanded FDC meshworks
- **Pattern 3 — Diffuse Pattern**
  - Complete architectural effacement
  - Marked polymorphous infiltrate
  - Difficult distinction from PTCL-NOS

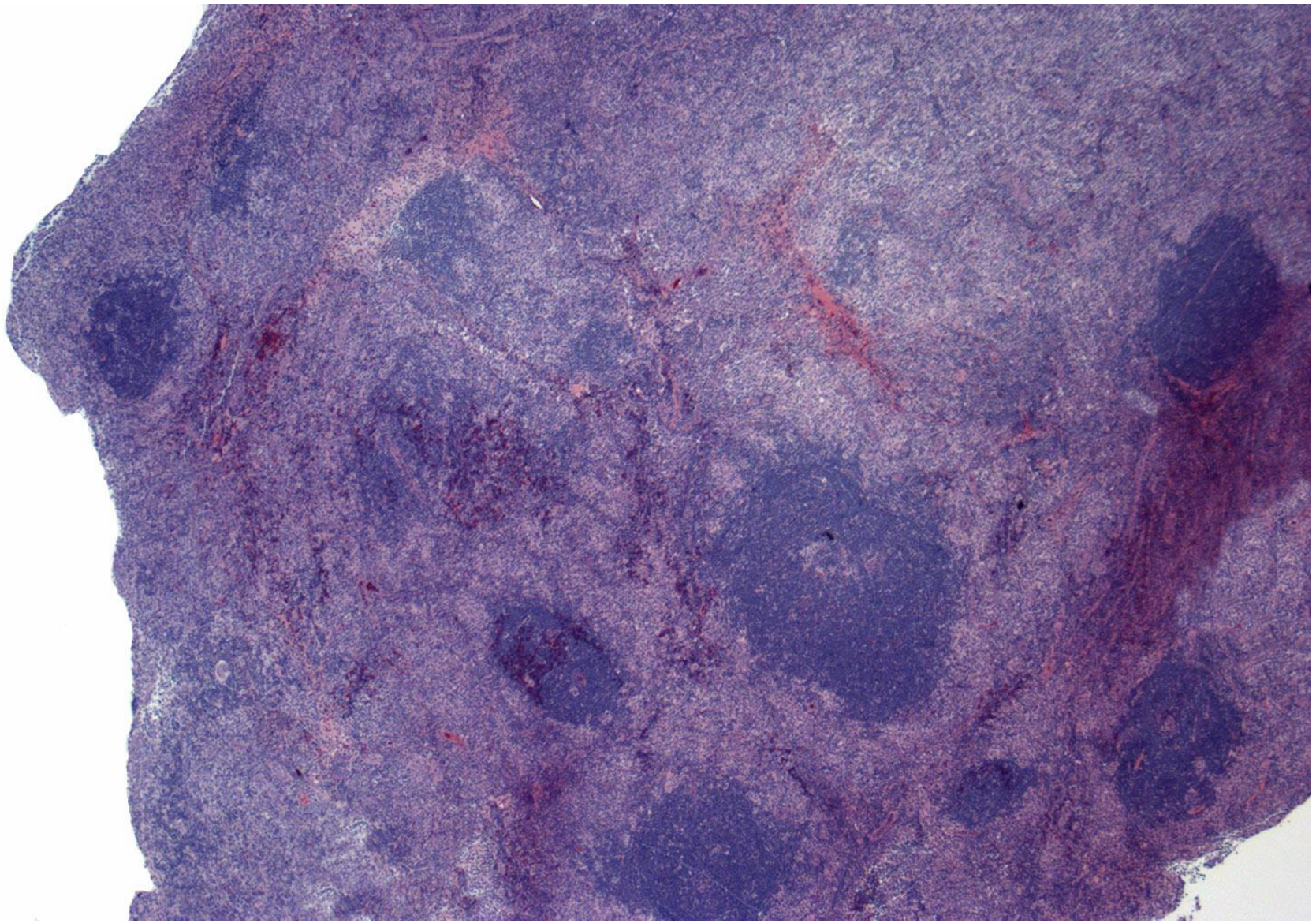
AITL morphology exists on a spectrum.

Pattern 1 — Early AITL

Hyperplastic follicles

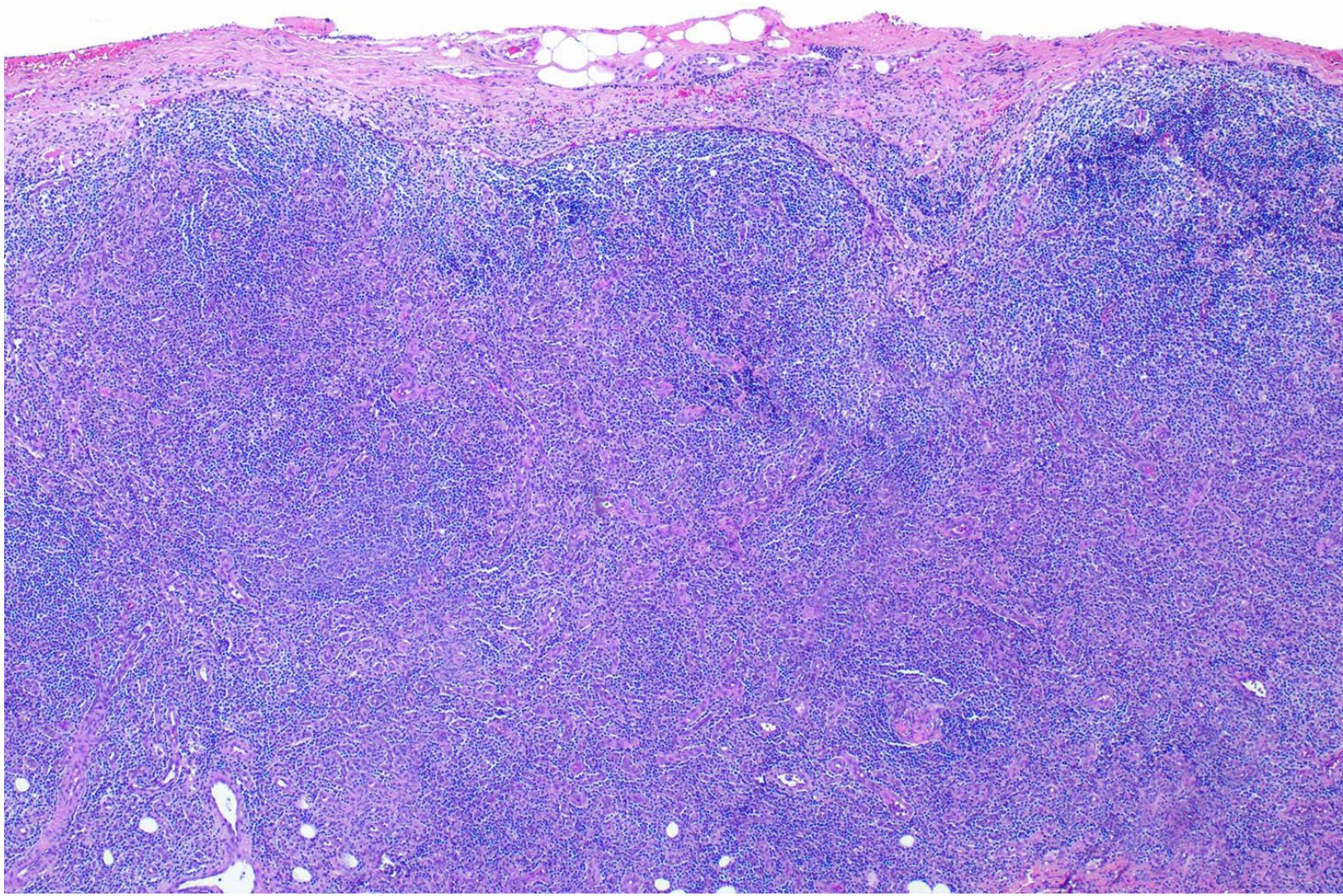
Expanded paracortex

Preserved partial architecture



Pattern 3 AITL — Diffuse Pattern

Complete architectural effacement
Marked polymorphous infiltrate
Difficult distinction from PTCL-NOS



TFH Markers, Core TFH Immunophenotype

The “Golden Rule” for TFH Phenotype

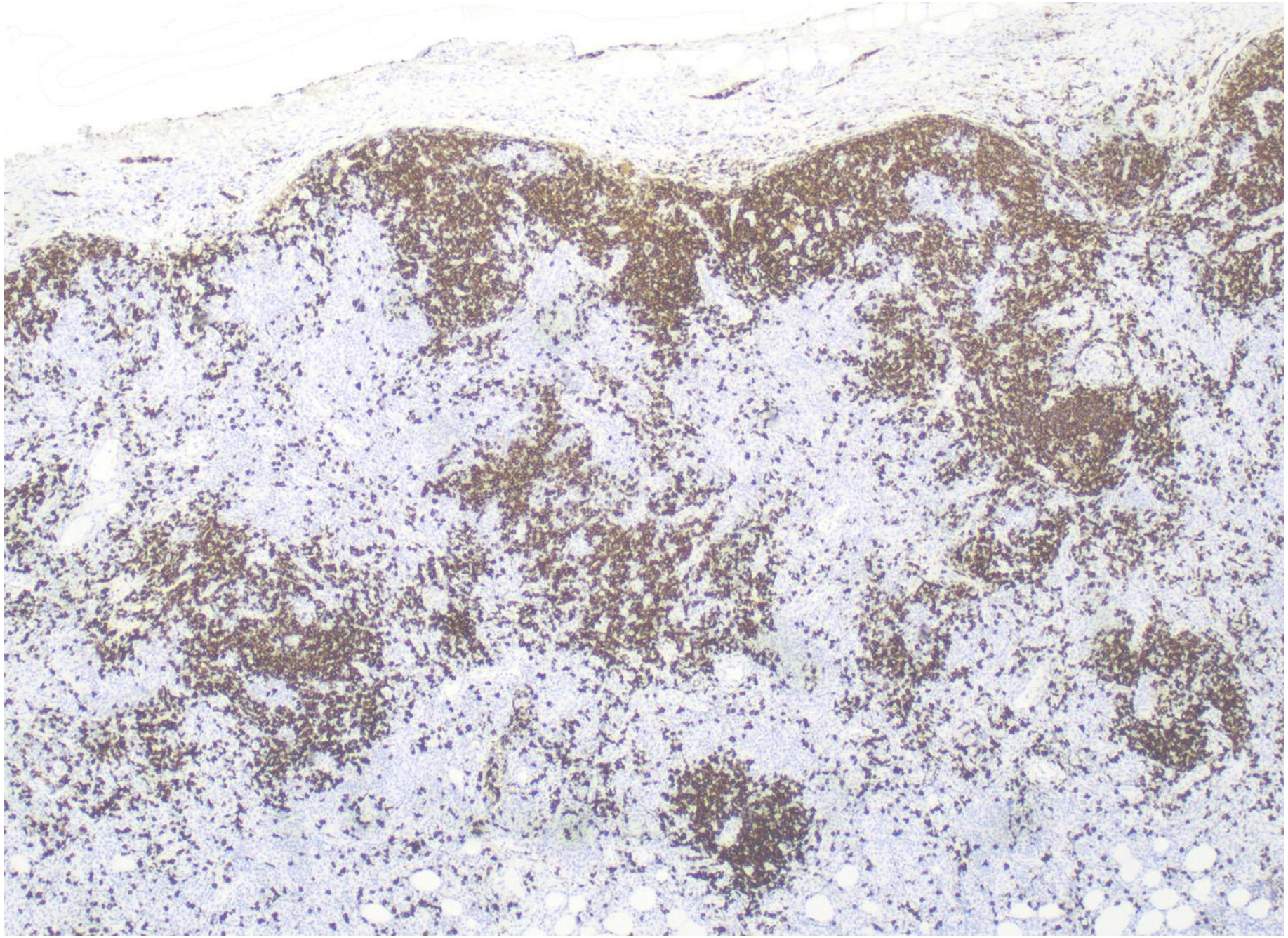
Neoplastic T-cells must express **at least 2 (preferably ≥3)** of the following TFH-associated markers:

- **PD-1 (CD279):** High sensitivity; lacks specificity (expressed in reactive T-cells).
- **ICOS:** High sensitivity; early activation marker.
- **CXCL13:** High specificity; shows characteristic **punctate cytoplasmic** staining.
- **CD10: Highest diagnostic weight.** Not expressed by normal paracortical T-cells.
- **BCL6:** Nuclear marker; also highlights background Germinal Centers.

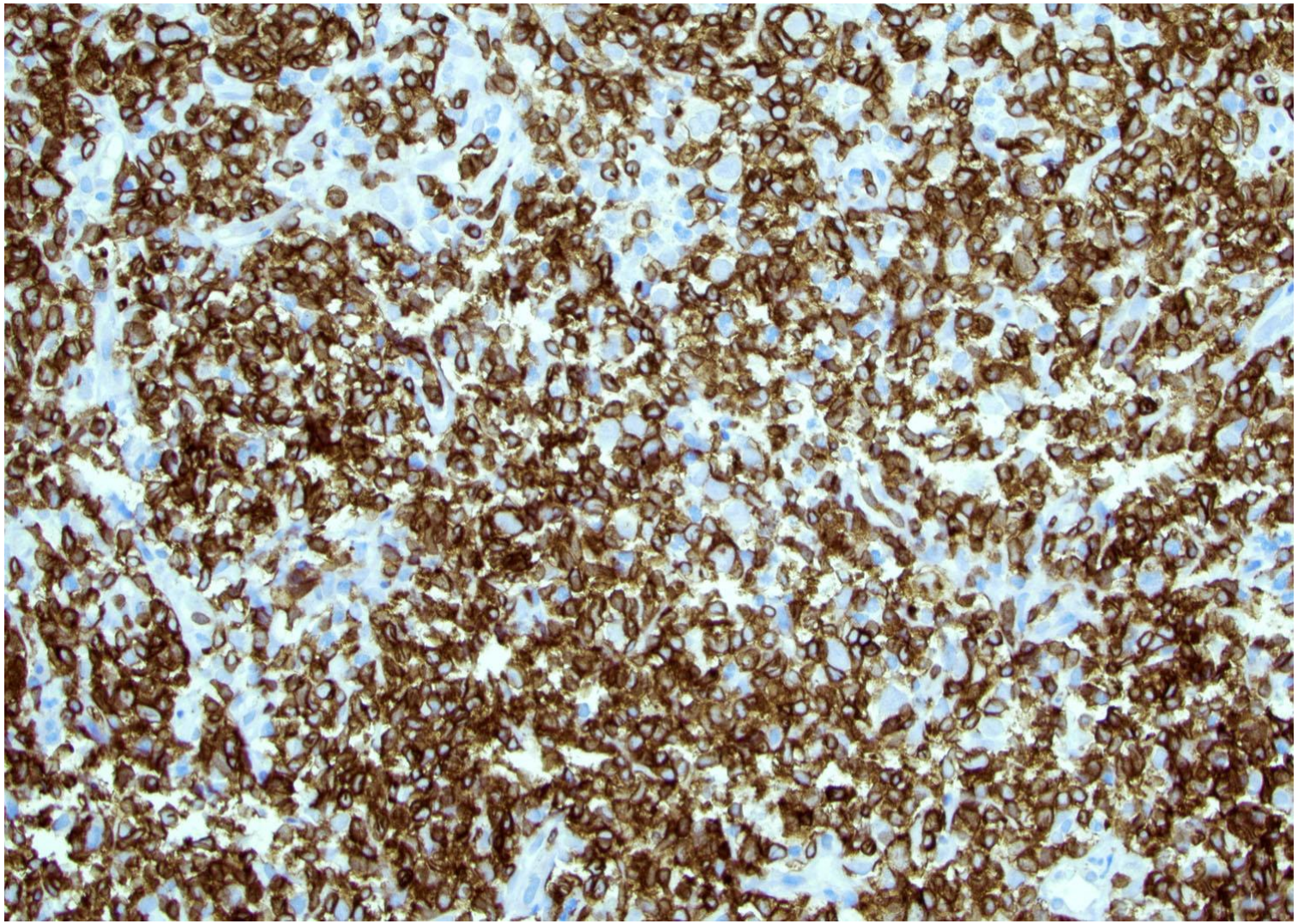
- Typical Phenotype
  - CD4-positive T-cells
  - Strong PD1 expression
  - Variable expression of additional TFH markers

Expression of multiple TFH markers is more convincing than isolated positivity.

Immunohistochemistry for CD20 shows displaced B cells in subcapsular areas as well as remnants of lymphoid follicles. Compared with other T cell lymphomas, B cells are more abundant in AITL



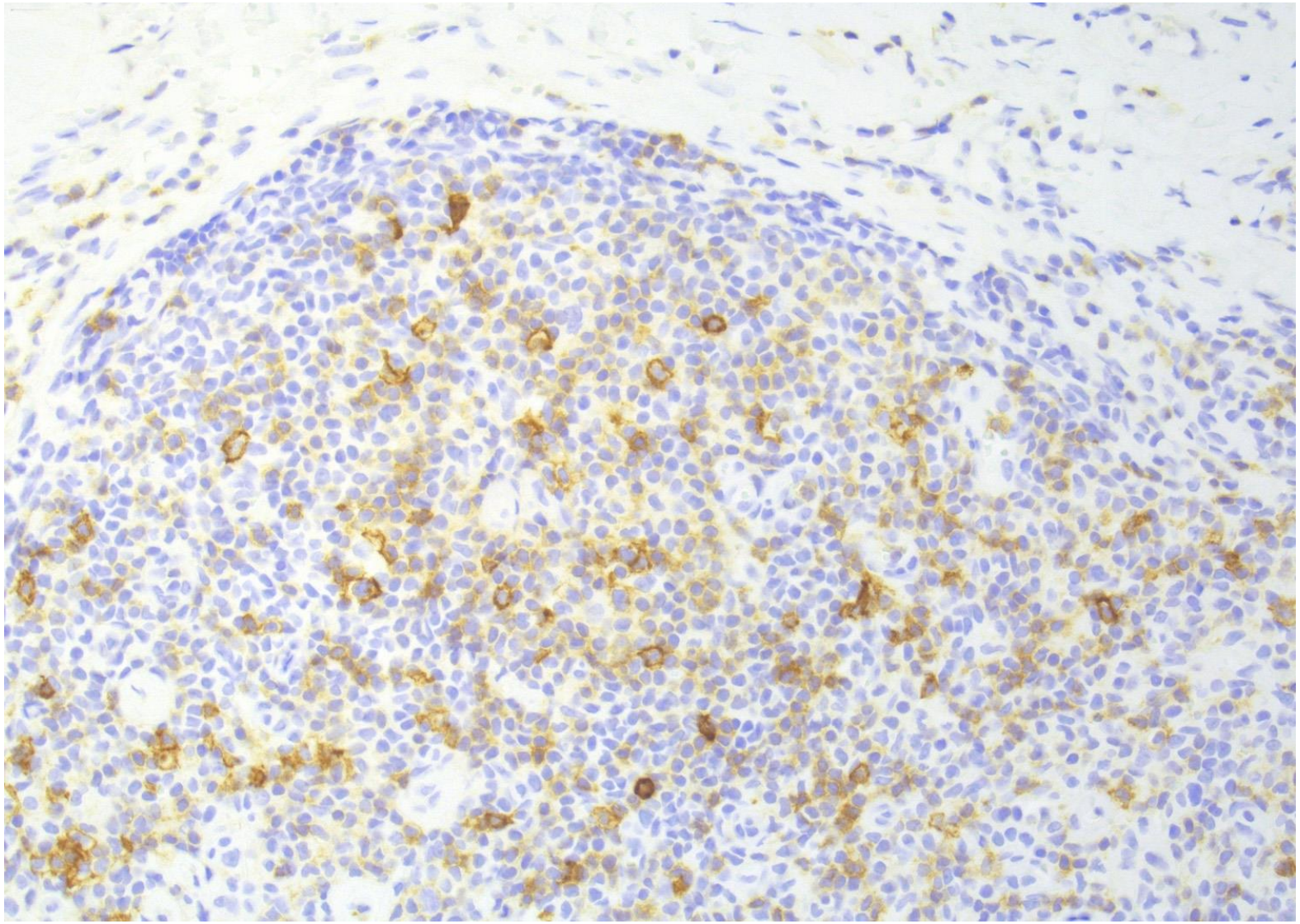
Immunohistochemistry for CD3 shows diffuse and strong positivity in both small and intermediate size lymphoma cells in AITL.



PD1 Interpretation and Pitfalls

- Biology
  - PD1 is a normal marker of reactive TFH cells
  - Reactive germinal centers normally contain PD1-positive T-cells
- Suspicious Pattern
  - Expanded extrafollicular PD1-positive T-cells
  - Diffuse paracortical PD1 expansion
- Important Pitfall
  - PD1 positivity alone does NOT establish TFH lymphoma
  - Morphology and distribution pattern remain critical.

Positivity for the checkpoint molecule PD-1 in scattered lymphoma cells supports a T follicular helper (TFH) phenotype.

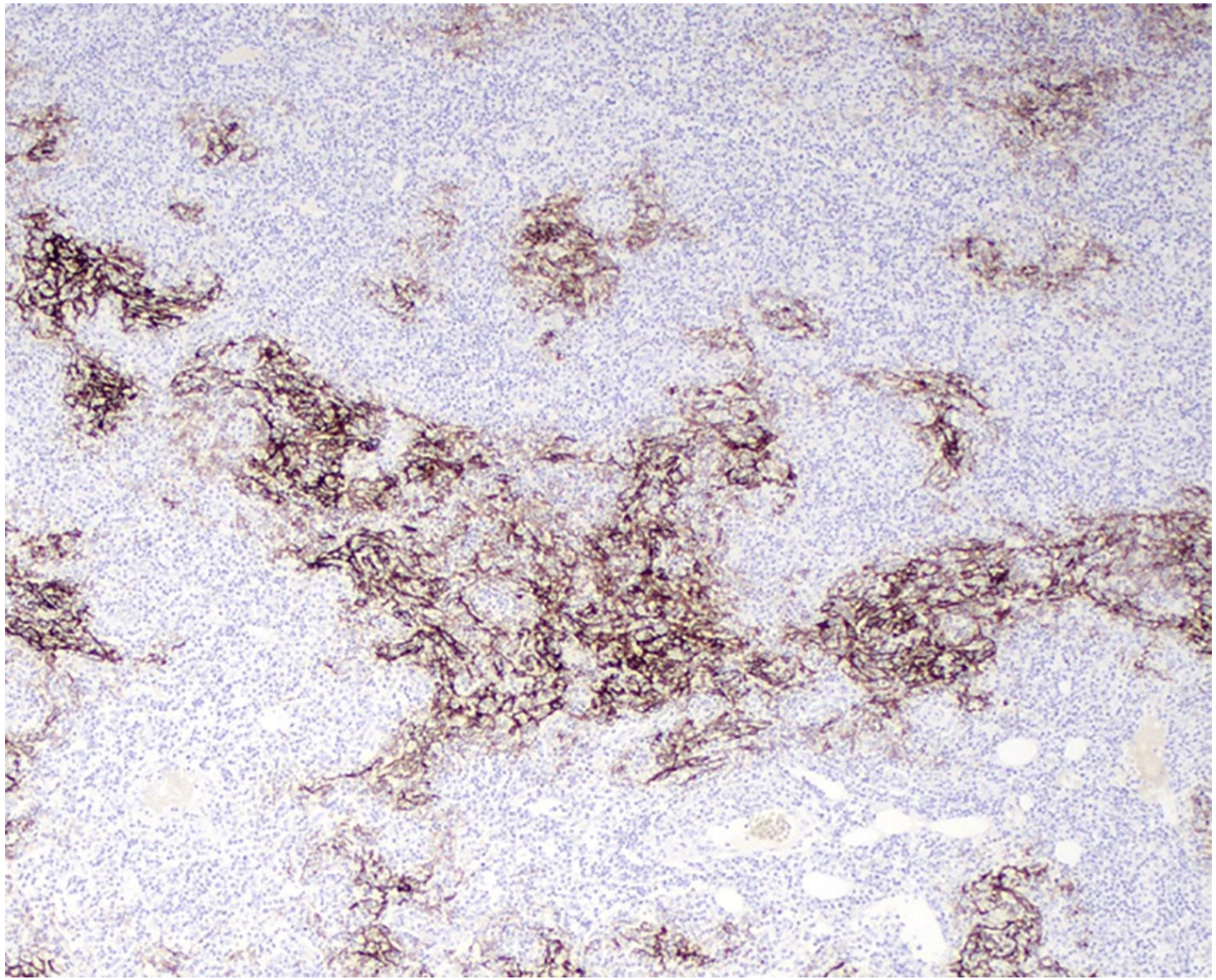


Follicular Dendritic Cell Expansion

Important Diagnostic Clue in TFH Lymphomas:

- Expanded FDC meshworks outside follicles strongly support TFH lymphoma.
- Markers:
  - CD21
  - CD23
  - CD35
- Characteristic Pattern
  - Expanded FDC meshworks
  - Irregular or disrupted architecture
  - Extrafollicular extension

Immunohistochemistry for CD21 highlights follicular dendritic cells (FDCs) meshworks that are expanded and distorted around vessels and admixed with lymphoma cells.



EBER in TFH Lymphomas

Role of EBV

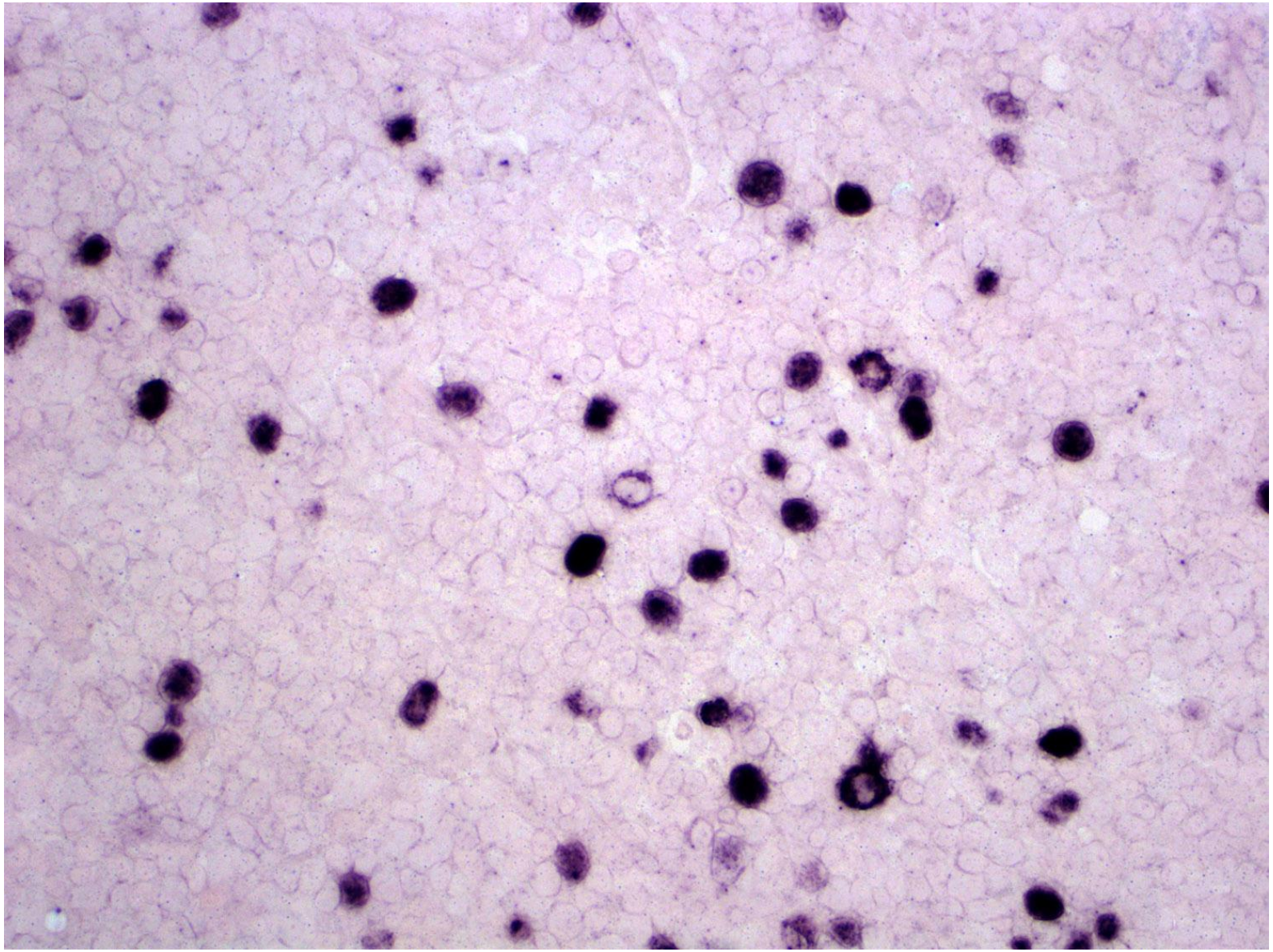
- EBV-positive B-immunoblasts are commonly present
- The neoplastic T-cells are usually EBV-negative

Diagnostic Pitfall

- EBV-positive immunoblasts may mimic:
  - Classical Hodgkin lymphoma
  - EBV-positive diffuse large B-cell lymphoma

Interpretation requires correlation with morphology and lineage markers.

Epstein-Barr virus encoded small RNAs (EBER) in situ hybridization shows positivity in scattered large cells



Practical IHC Panel for TFH Lymphomas

- Suggested Diagnostic Panel:
  - CD3, CD4,CD5, CD7, PD1, ICOS, CXCL13, CD10, BCL6, CD21 / CD23, CD20, EBER, Ki67
- Diagnostic Goals
  - Identify aberrant T-cell phenotype
  - Demonstrate TFH differentiation
  - Evaluate FDC meshworks
  - Detect EBV-positive B-immunoblasts

Differential Diagnosis of AITL

Entity	Key Distinguishing Features
Reactive hyperplasia	Preserved architecture, no atypical TFH cells
Classical Hodgkin lymphoma	No TFH network or FDC expansion
PTCL-NOS	Lacks TFH phenotype
T-cell rich large B-cell lymphoma	B-cell driven process
Viral lymphadenitis	Reactive immunoblasts without TFH abnormalities

Clonality Important Concept

- Clonal TCR rearrangement is supportive, NOT diagnostic.
- **Clonality May Be Seen In:**
  - Chronic inflammation
  - Autoimmune disease
  - Viral infections
  - Reactive proliferations
- **Diagnostic Integration Required**
  - Morphology
  - Architecture
  - Immunophenotype
  - Clinical findings
- **Clonality does not equal malignancy.**

Anaplastic Large Cell Lymphoma (ALCL)

- **WHO/ICC Categories**
  - ALK-positive ALCL
  - ALK-negative ALCL
  - Breast implant-associated ALCL
  - Primary cutaneous ALCL
- **Shared Features**
  - Strong diffuse CD30 expression
  - Large pleomorphic cells
  - Hallmark cells
  - Cohesive growth pattern

Anaplastic Large Cell Lymphoma (ALCL)

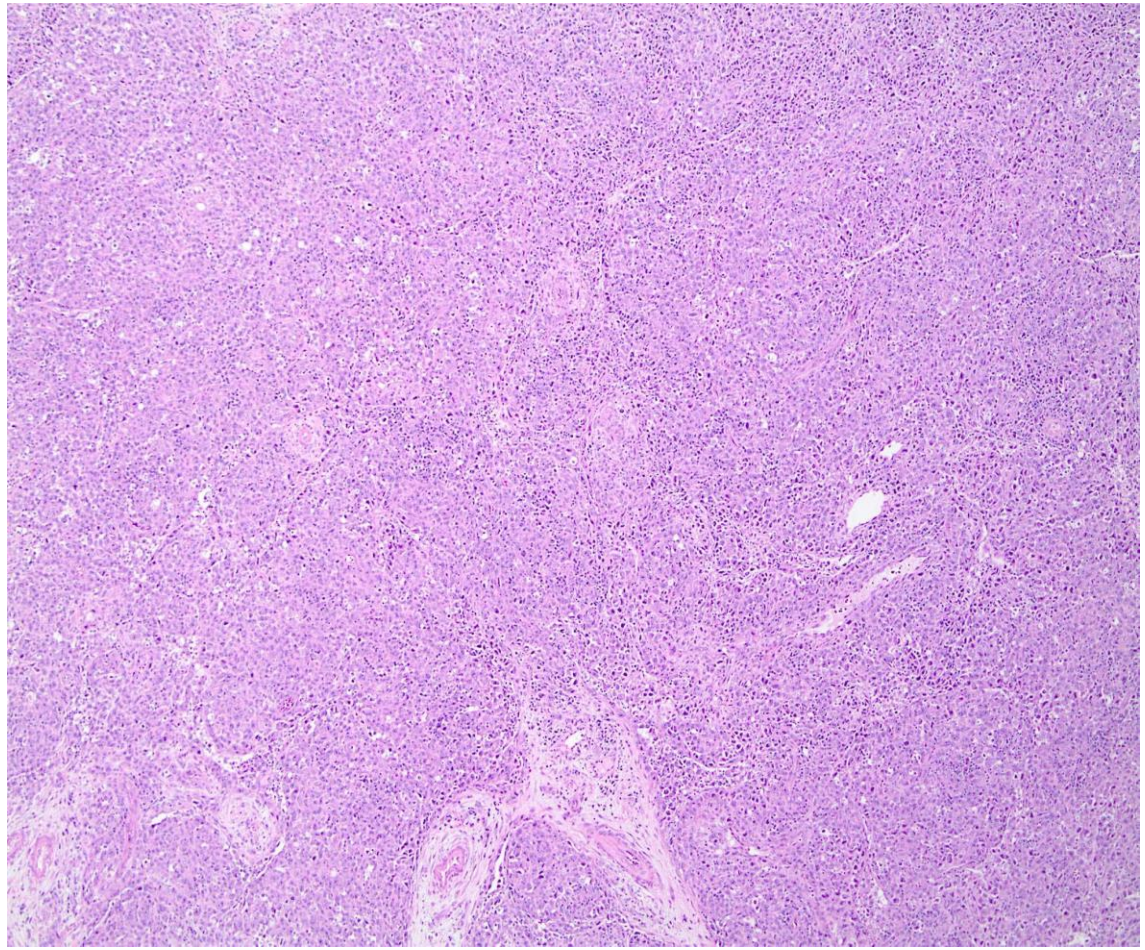
- Morphology:
  - Paracortical/Diffuse/ sinusoidal growth pattern
  - Large pleomorphic cells
  - Abundant cytoplasm
  - Horseshoe-shaped or kidney-shaped nuclei
  - “Hallmark cells”: Recognition of hallmark cells strongly supports ALCL in the appropriate immunophenotypic setting.
- Background: Inflammatory infiltrate is common

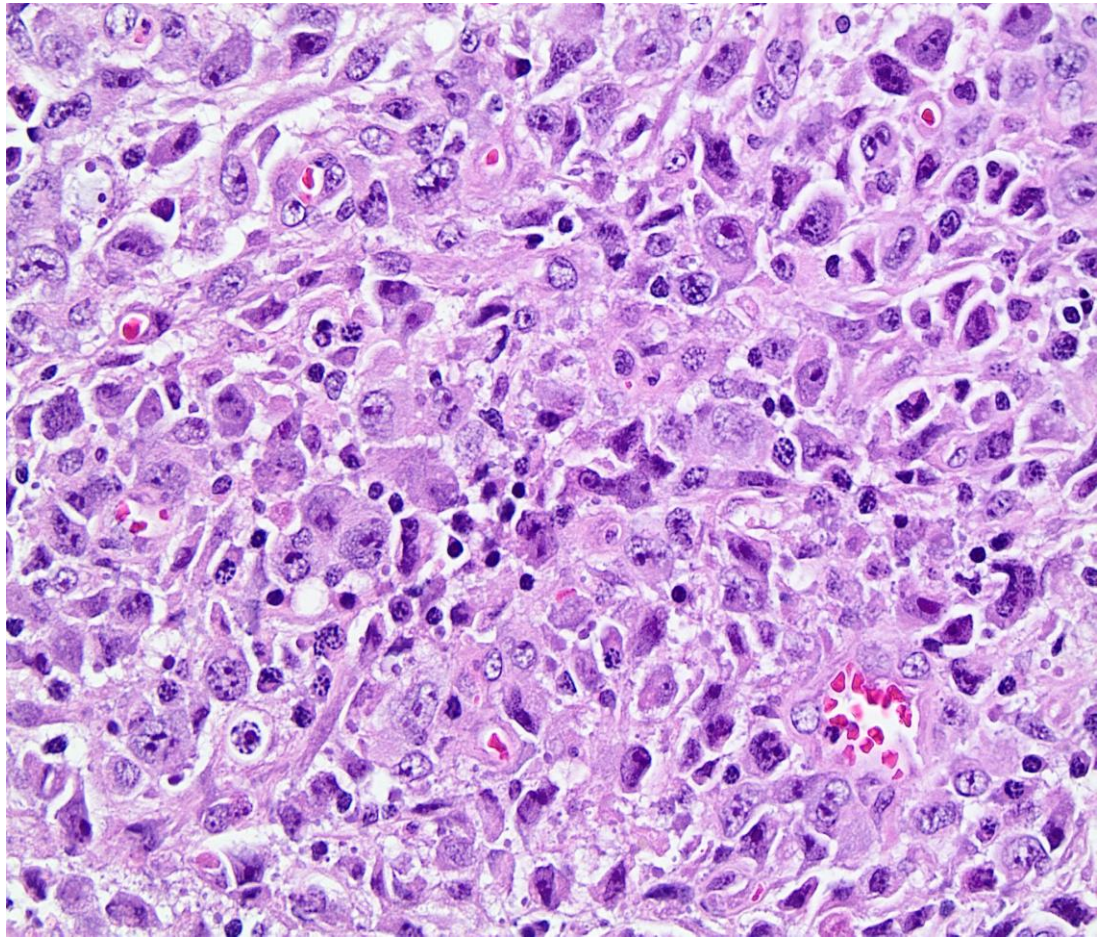
Sinusoidal Growth Pattern in ALCL

- **Characteristic Finding**
  - Tumor cells preferentially involve:
    - Lymph node sinuses
    - Perinodal tissues
- **Major Pitfall**
  - May mimic:
    - Metastatic carcinoma
    - Melanoma

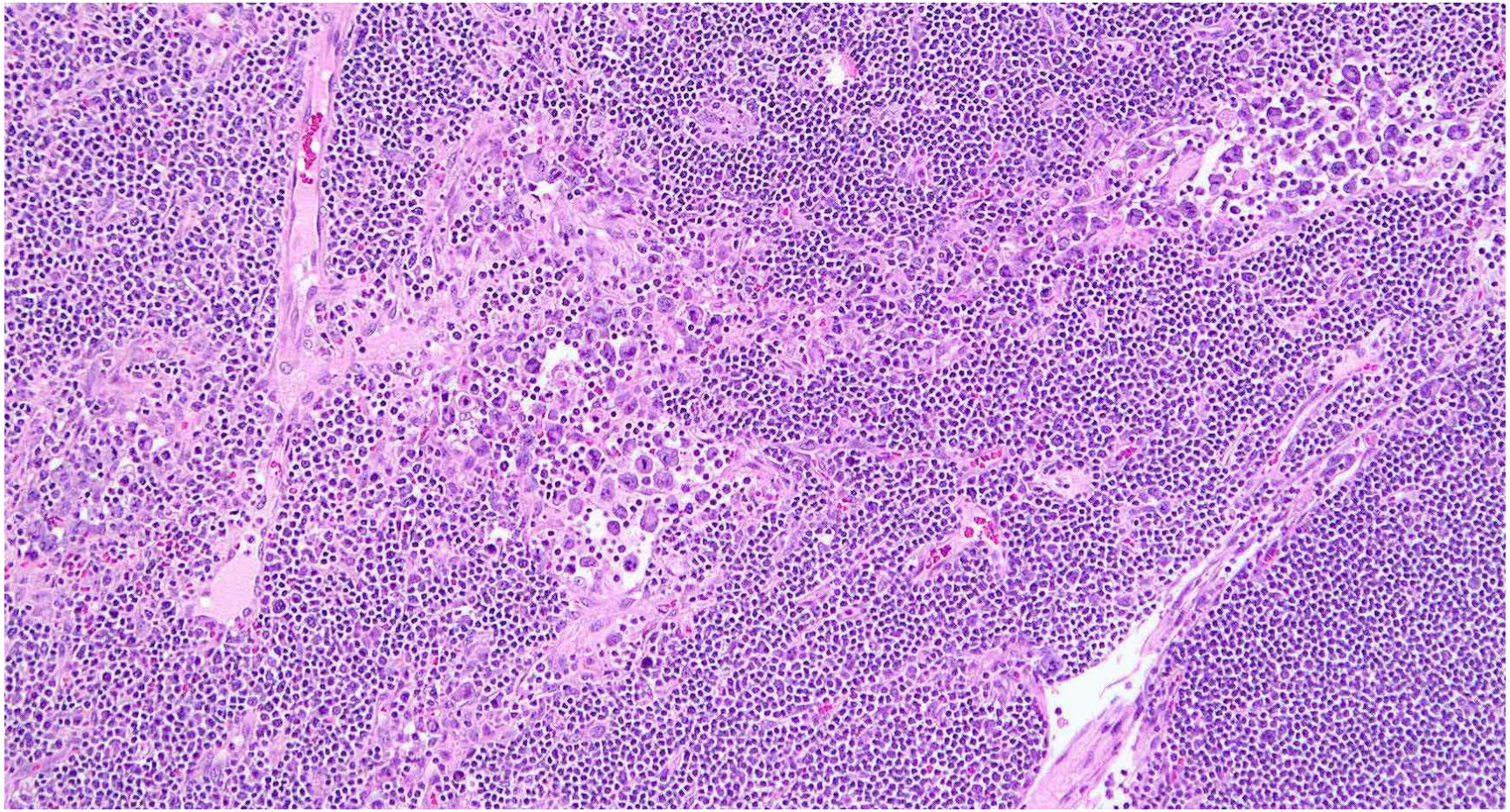
Sinusoidal growth is one of the most helpful low-power clues to ALCL.

Anaplastic Large Cell Lymphoma (ALCL)



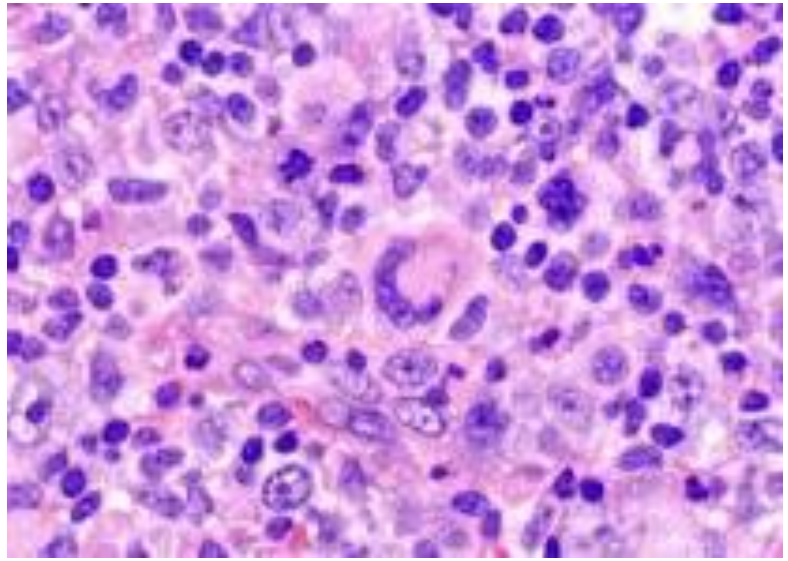


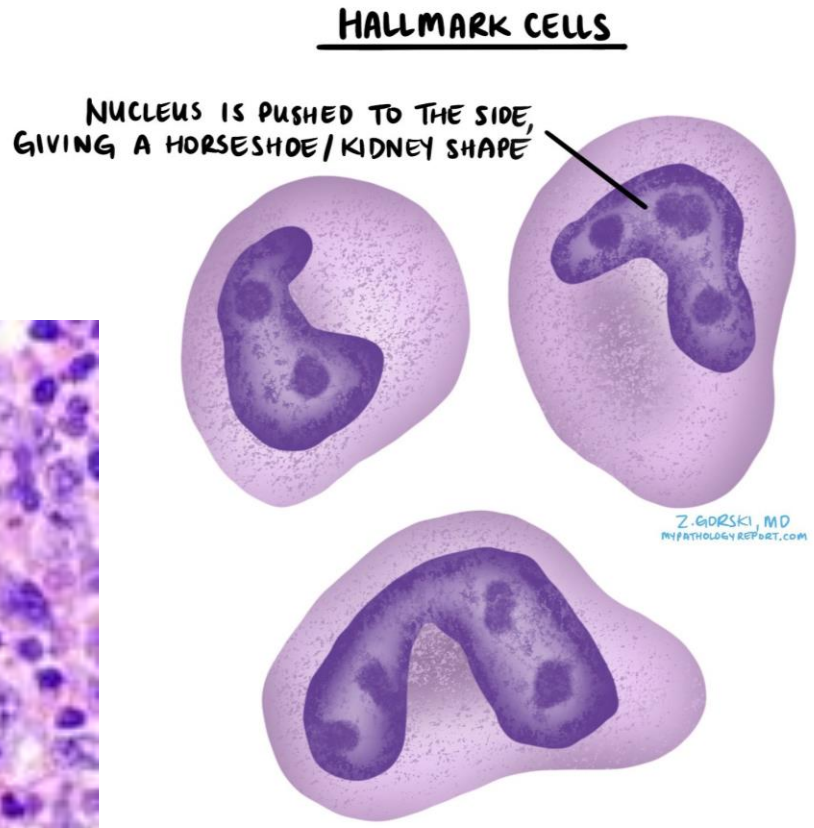
Sinusoidal Growth Pattern in ALCL



Hallmark Cells — The Morphologic Signature of ALCL

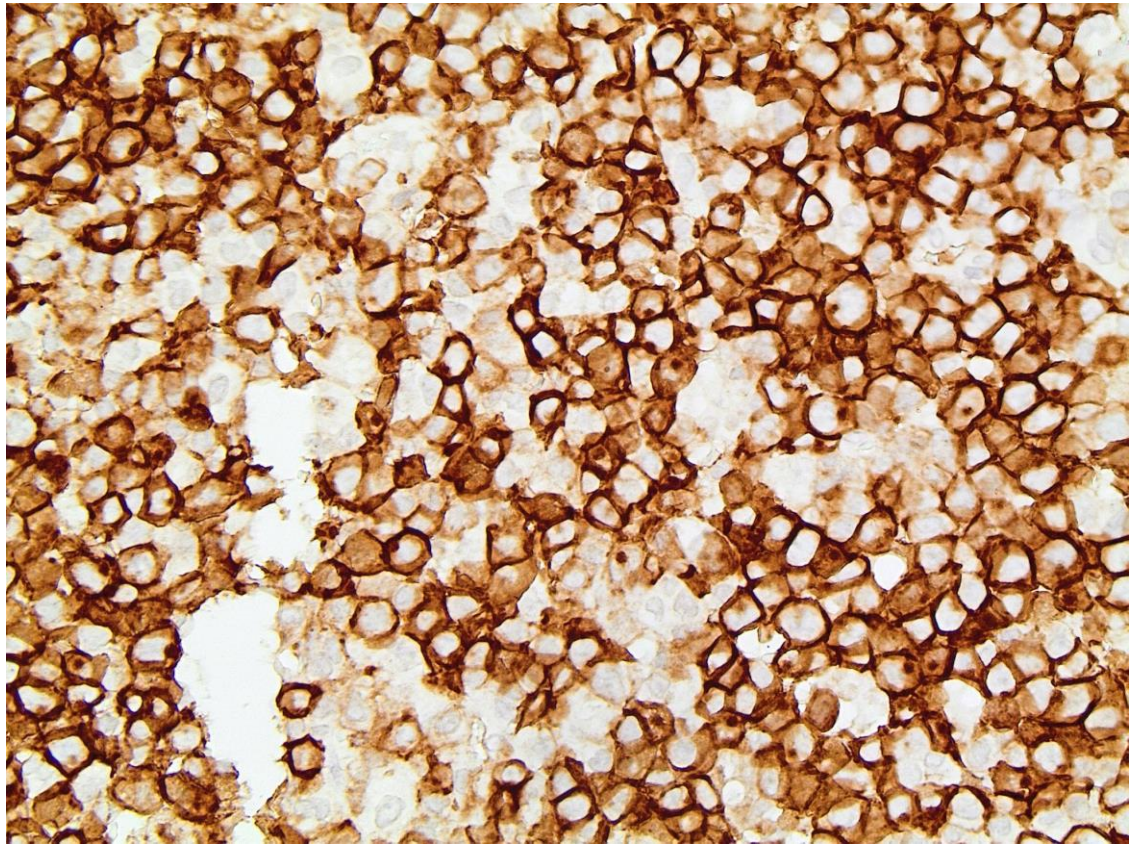
- **Hallmark Cells in ALCL:**
  - Eccentric horseshoe-shaped nuclei
  - Paranuclear eosinophilic region (Golgi zone)
  - Abundant cytoplasm





CD30 Pattern in ALCL

- **CD30 Pattern in ALCL:** Strong, uniform, and diffuse (membrane + Golgi) staining in >75-90% of cells.
- PTCL with CD30 expression: CD30 positivity must always be interpreted in morphologic and lineage context.



The neoplastic cells are diffusely and strongly positive for CD30

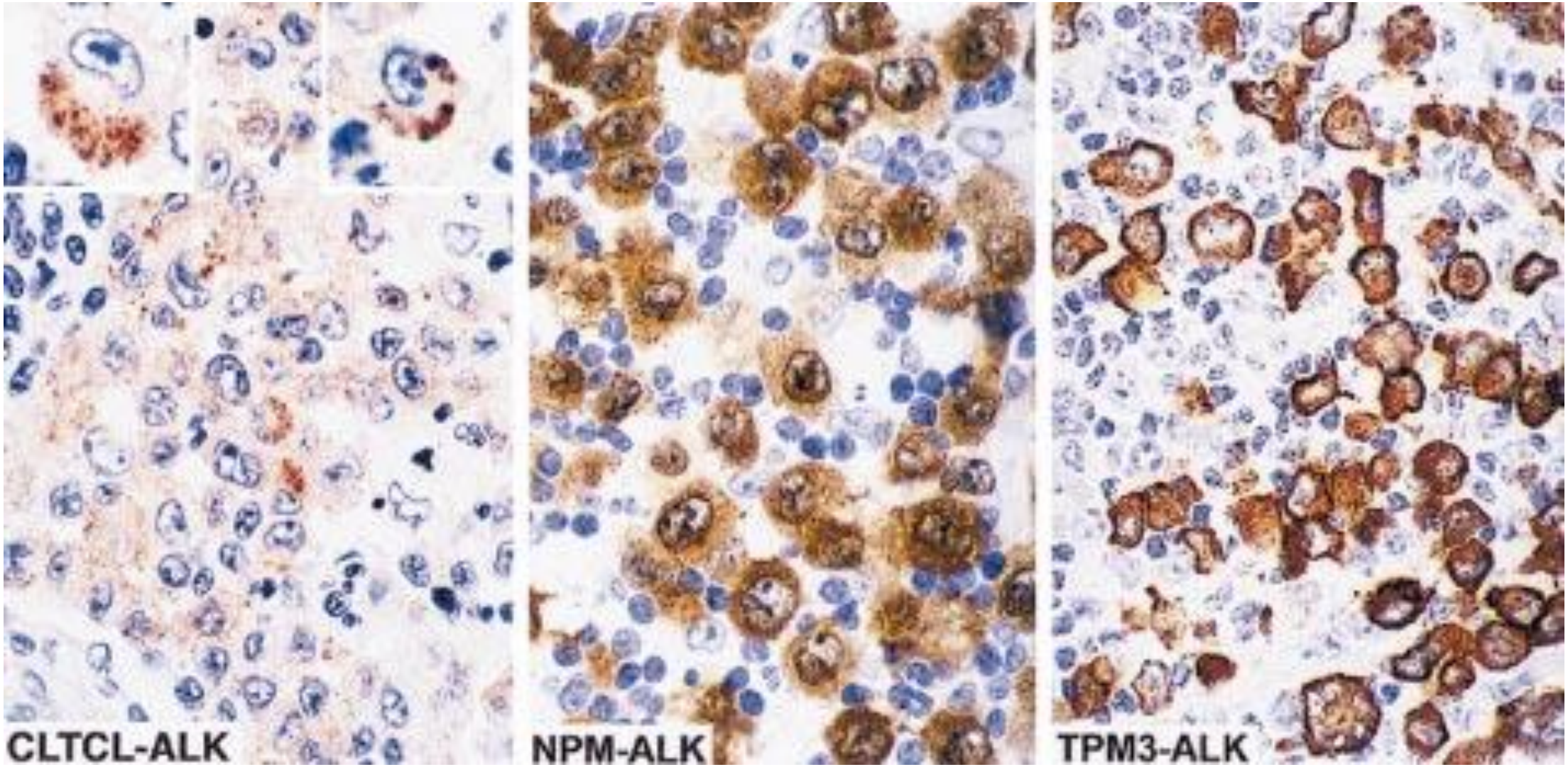
ALK Immunostaining Patterns

ALK Pattern	Possible Fusion Partner
Nuclear + cytoplasmic (70%-80%)	NPM1::ALK t(2;5)(p23;q35)
Cytoplasmic only 10% to 20%	TPM3::ALK t(1;2)(q25;p23)
Membranous	MSN::ALK t(X;2)(q11–12;p23)
Granular cytoplasmic	CLTC::ALK t(2;17)(p23;q23)

CLTC = **clathrin heavy chain** MSN = **moesin** NPM1 = **nucleophosmin 1** ALK = **anaplastic lymphoma kinase**

TPM3 = **tropomyosin 3**

ALK staining pattern may predict the underlying fusion partner.


CLTCL-ALK
NPM-ALK
TPM3-ALK

Immunophenotype of ALCL; Typical Marker Profile

- CD30: Strong diffuse positivity; ALCL is a CD30-rich T-cell lymphoma
- ALK:
  - Positive in ALK-positive ALCL
  - Negative in ALK-negative ALCL
- Variable T-cell marker expression
  - EMA often positive
  - Cytotoxic markers frequently positive
  - Loss of pan–T-cell antigens is common.

Common Immunophenotype of Anaplastic Large Cell Lymphoma (ALCL)

Marker	Typical Result
CD30	Strong, diffuse positive
ALK	Positive in ALK+ ALCL
CD4	Commonly positive
CD8	Usually negative
CD3	Often negative or weak
CD5	Often negative
EMA	Often positive
TIA-1 / Granzyme B / Perforin	Frequently positive

ALK-Positive vs ALK-Negative ALCL

Feature	**ALK+ ALCL**	**ALK– ALCL**
Age group	Younger patients	Older adults
Prognosis	Better	Less favorable
ALK staining	Positive	Negative
Morphology	Similar	Similar
CD30	Strong diffuse	Strong diffuse

Morphologic Variants of ALCL

Variant	Key Features
Classic	Hallmark cells
Small cell variant	Small atypical cells, weaker CD30
Hodgkin-like	RS-like cells
Lymphohistiocytic	Heavy inflammatory background
Sarcomatoid	Spindle-cell morphology
Signet-ring	Cytoplasmic vacuoles

Important Concept
ALCL has one of the broadest morphologic spectra among T-cell lymphomas.

Genetics of ALK-Negative ALCL

- **Important Rearrangements**
  - DUSP22 rearrangement
  - TP63 rearrangement

Alteration	**Clinical Implication**
DUSP22 rearrangement	More favorable prognosis
TP63 rearrangement	Aggressive behavior

Peripheral T-Cell Lymphoma, NOS

- Definition:
  - A mature T-cell lymphoma that does not fulfill criteria for a more specific entity.
- Morphologic Features
  - Diffuse or paracortical infiltrate
  - Architectural effacement
  - Variable cytologic atypia
  - Often monotonous atypical T-cells
- PTCL-NOS is largely a diagnosis of exclusion.

Immunophenotype of PTCL-NOS

- Common Findings
  - CD3-positive
  - CD4-positive more common than CD8-positive
  - Frequent loss of CD5 & CD7
- Variable Expression
  - CD30
  - Cytotoxic markers
  - TFH markers (usually absent or incomplete)
- Aberrant loss of pan–T-cell markers is common.

PTCL-NOS: Diagnostic Pitfalls

- Major Differential Diagnoses
  - Reactive paracortical hyperplasia
  - TFH lymphoma
  - ALCL
  - Hodgkin-like proliferations
- Important Diagnostic Principle
  - Before diagnosing PTCL-NOS, exclude TFH lymphoma, ALCL & EBV-related proliferations
  - PTCL-NOS should not become a “wastebasket diagnosis.”


thank you