Approach to T- Follicular Helper (TFH) Lymphomas and Mimics

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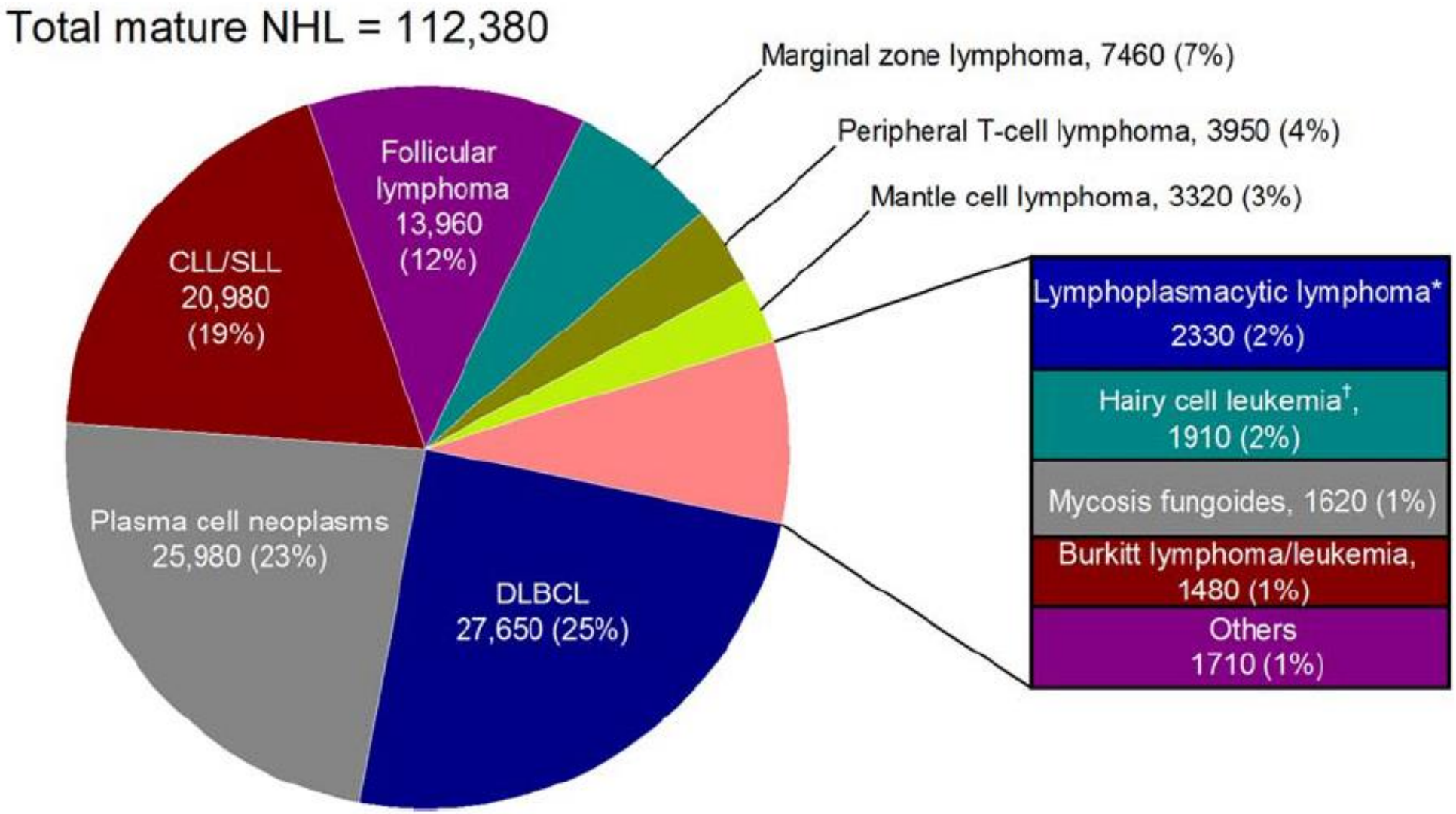
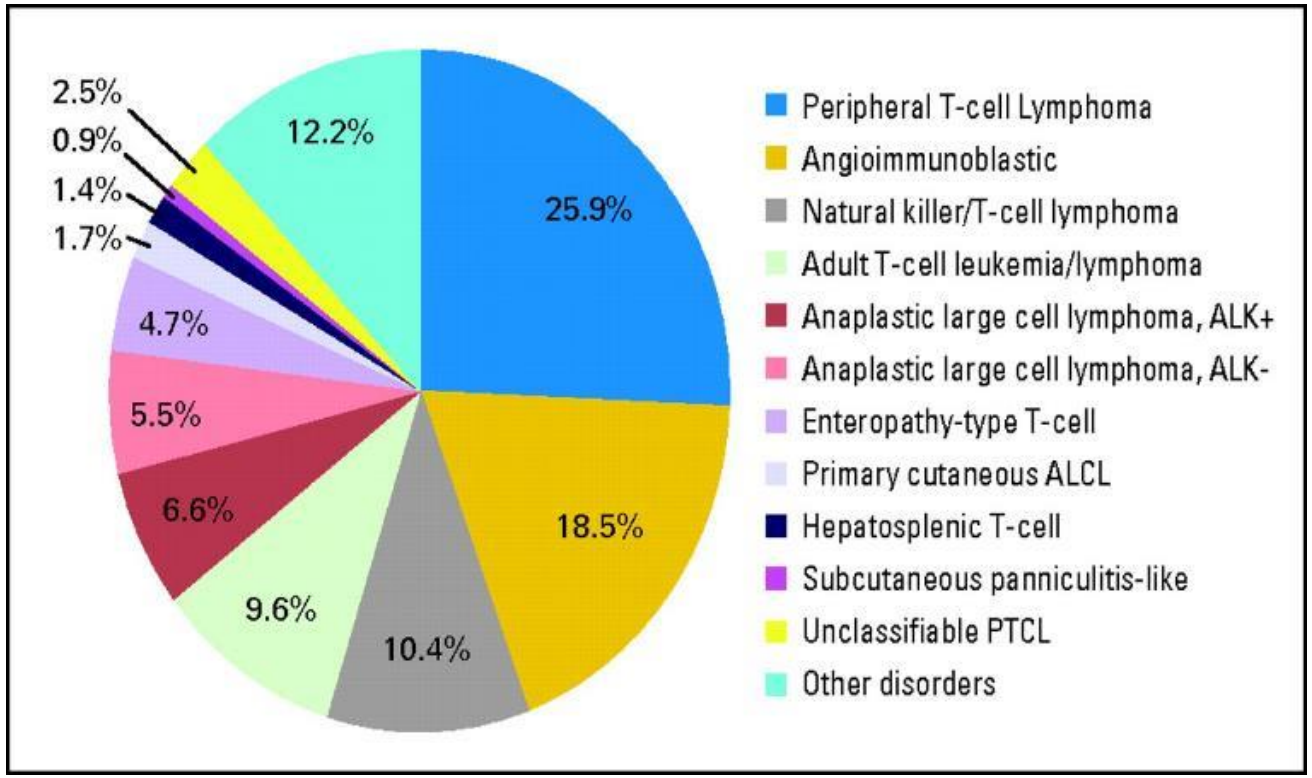
Incidence of T-cell lymphoma

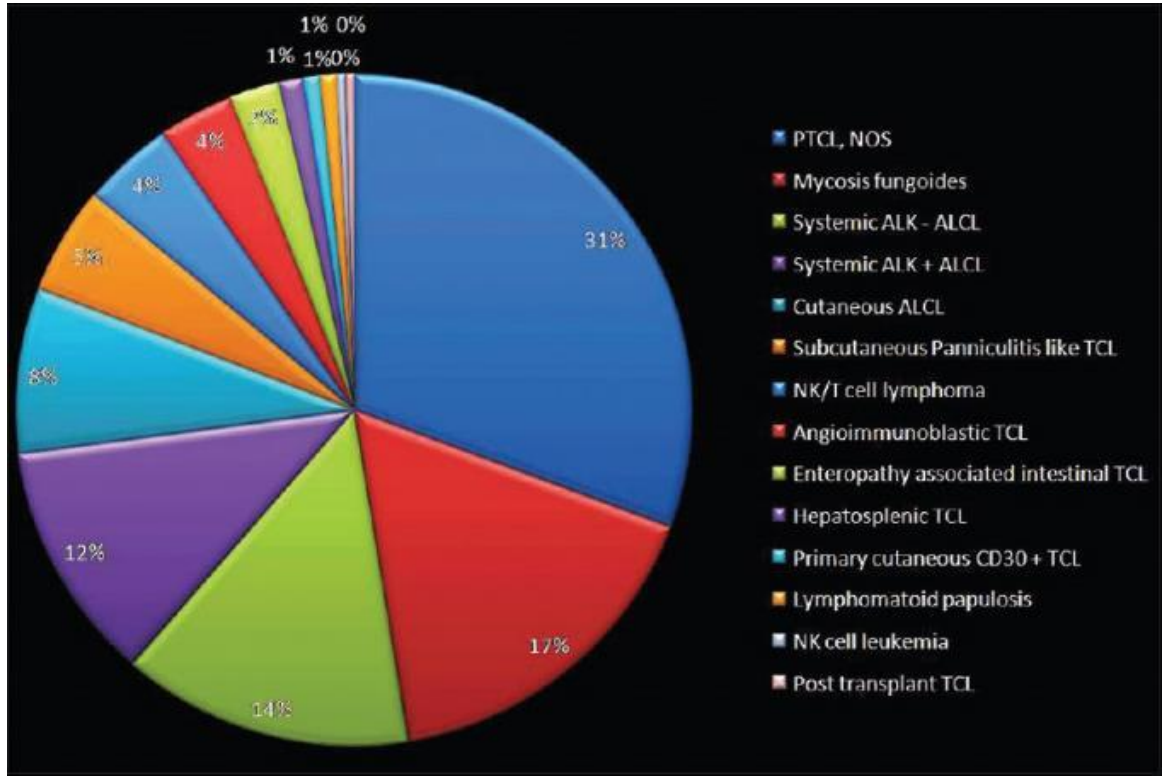


Teras, L.R., et al. CA: A Cancer Journal for Clinicians, 66: 443-459

Frequency of T-cell lymphoma subtypes



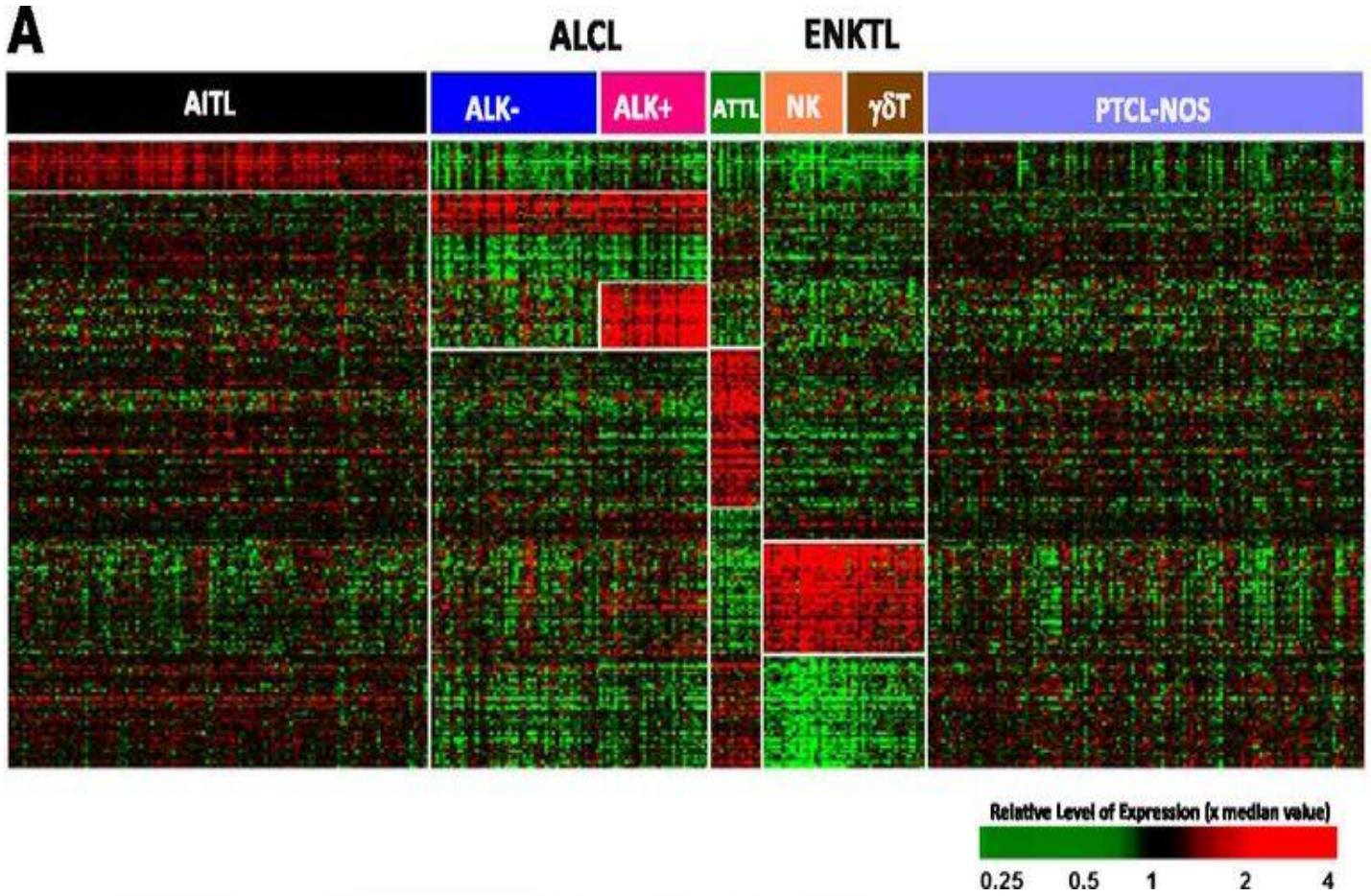
International T-Cell Lymphoma Project JCO 2008;26:4124-4130

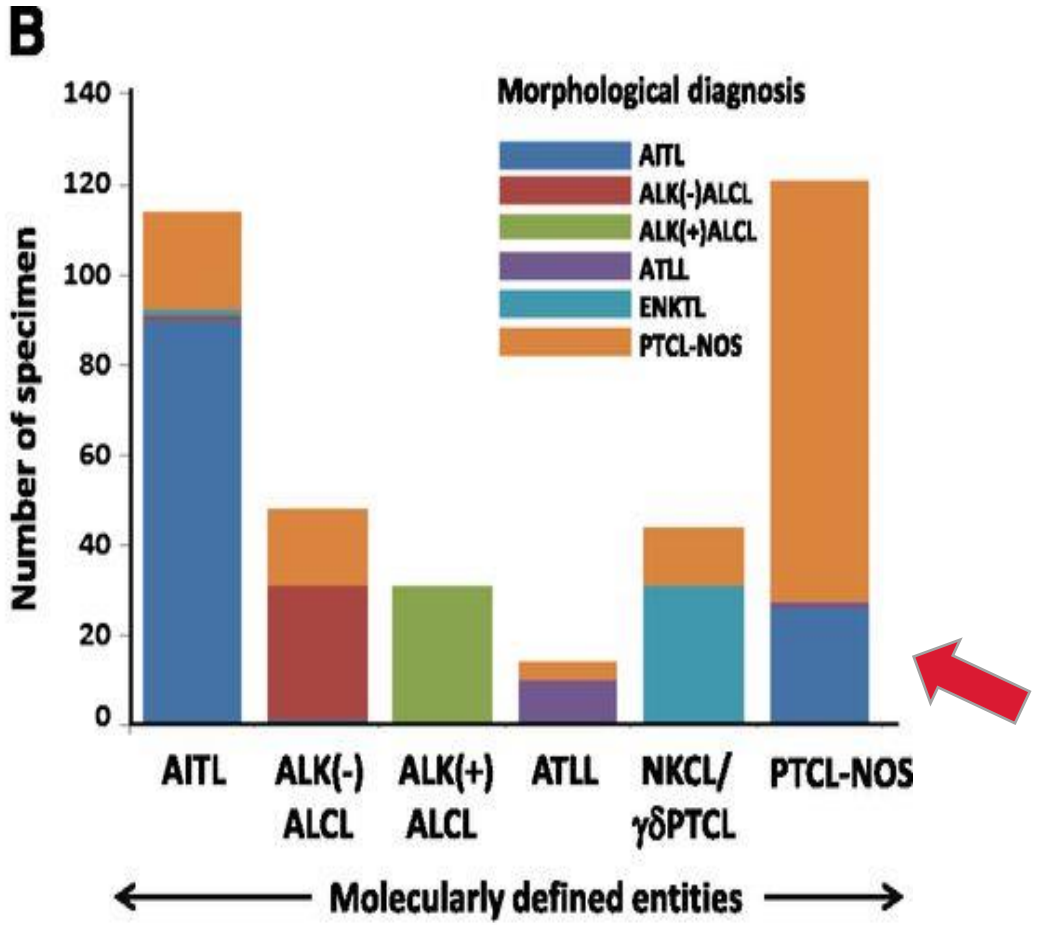


Archana L et al. IJPM, 2018; (61) 2:204-208

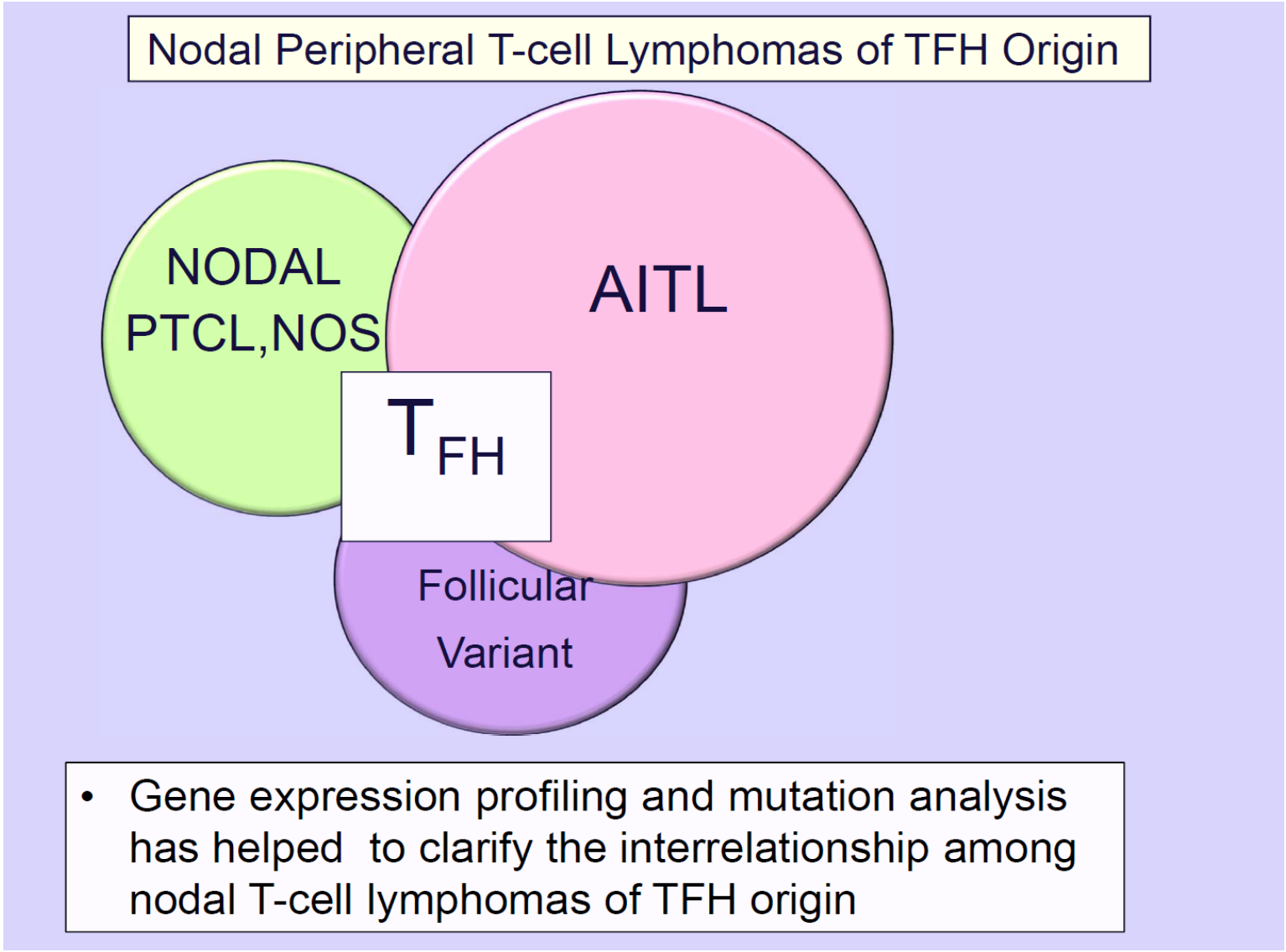
Leukemia lymphoma molecular profiling project: Distinct gene expression profiles of T-cell lymphomas

Iqbal et al., Blood, 2014





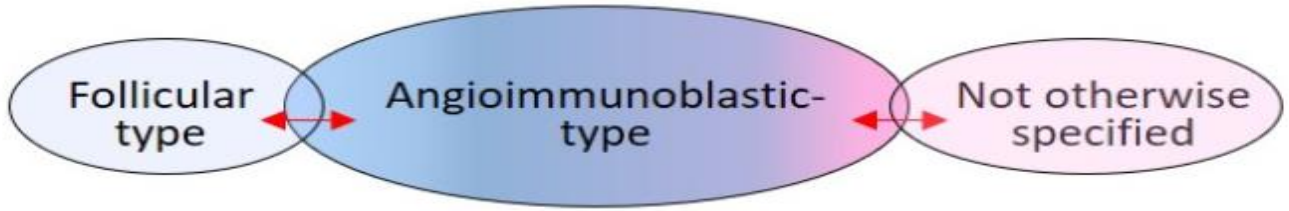
14% of PTCL, NOS had same expression profile as AITL



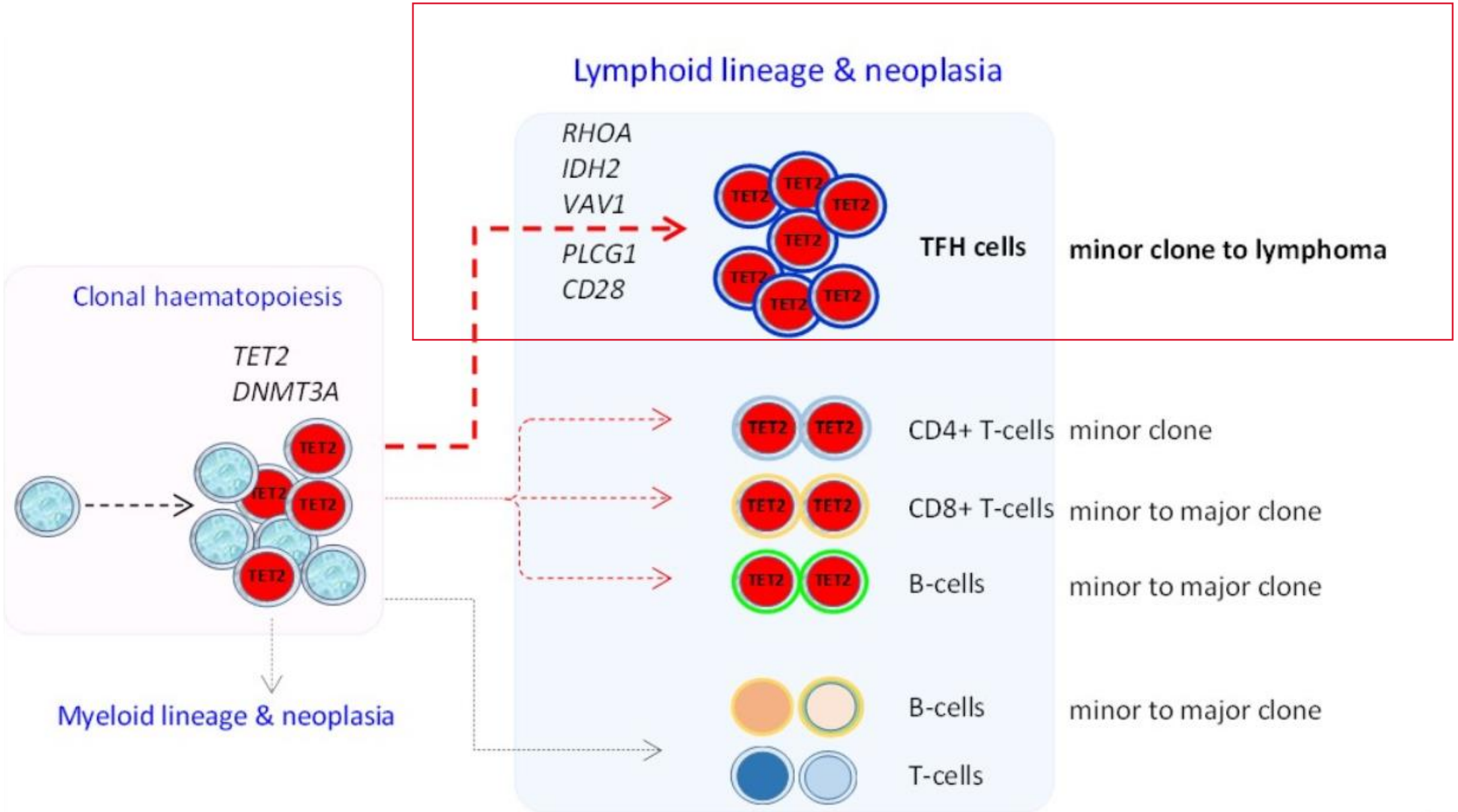
At least 2 or 3 TFH-related antigens should be expressed:

- PD1
- CD10
- BCl6
- CXCL13
- ICOS
- SAP
- CXCR5
- CD57
- HGAL
- CD200

WHO REVISED 4TH EDITION	WHO 5TH EDITION	ICC
Nodal T-follicular helper (TFH) cell lymphoma		
Angioimmunoblastic T-cell lymphoma	Nodal TFH cell lymphoma, angioimmunoblastic-type	Follicular helper T-cell lymphoma, angioimmunoblastic type (Angioimmunoblastic T-cell lymphoma)
Follicular T-cell lymphoma	Nodal TFH cell lymphoma, follicular-type	Follicular helper T-cell lymphoma, follicular type
Nodal peripheral T-cell lymphoma with TFH phenotype	Nodal TFH cell lymphoma, not otherwise specified	Follicular helper T-cell lymphoma, not otherwise specified



Extrafollicular FDC encircling HEV	–	+	–
HEV hyperplasia	–	+	limited
Perifollicular growth pattern	–	+ (patterns 1/2)	–
Intrafollicular growth pattern	+	–	–
Diffuse monomorphic tumour cells	–	tumour cell-rich	typical
Polymorphic infiltrate	–	+	less likely



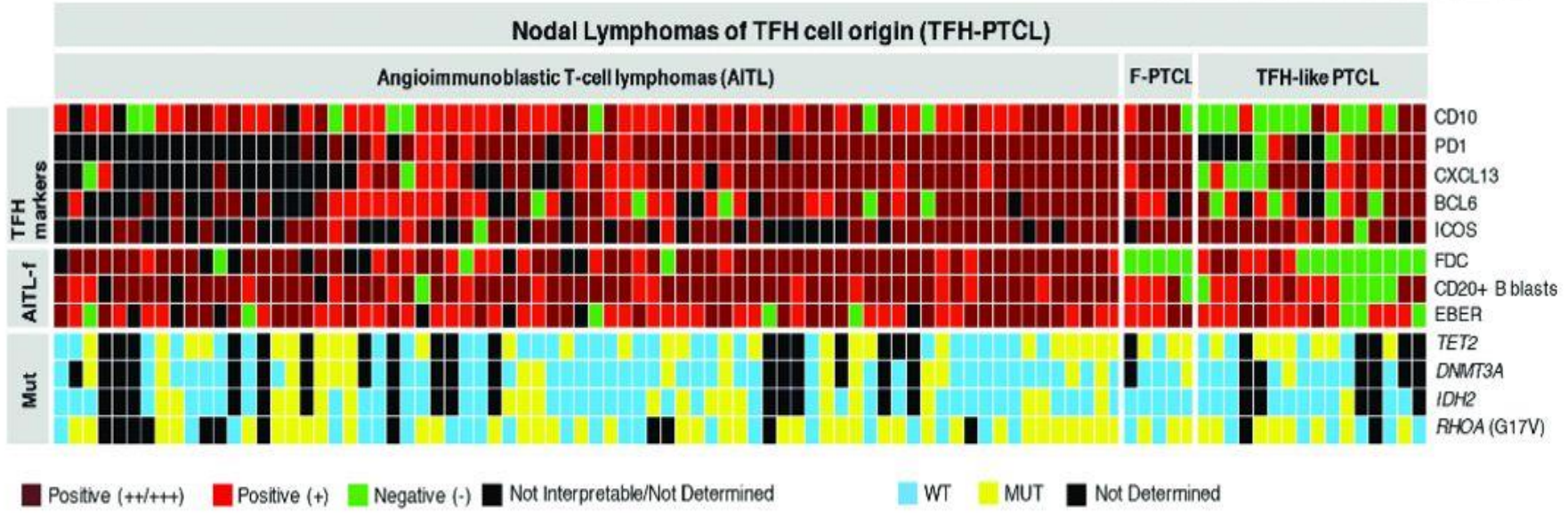
WHO online

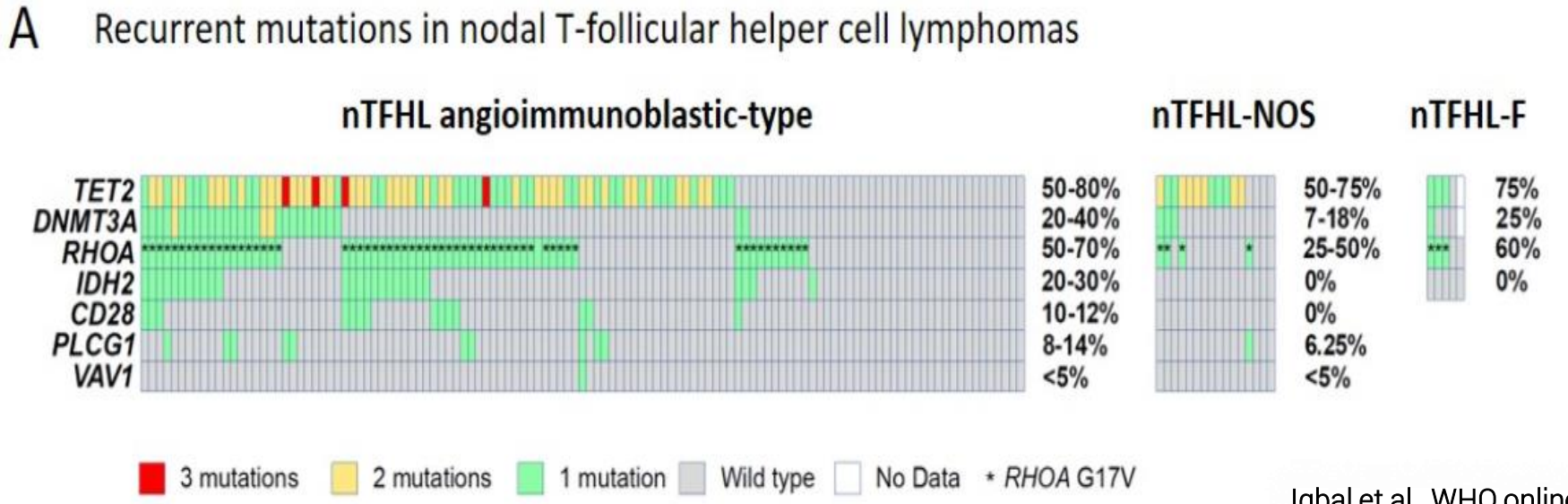
Dobay et al., Haematologica, 2017

-Early mutations (TET2 and DNMT3A) and RHOA mutations higher frequency

-IDH2 mutation-relatively specific for AITL (large clear cell morphology)

-Fewer TFH IHC markers expressed in TFH, NOS





Iqbal et al., WHO online

Nodal T-cell lymphomas of T-follicular helper cell (TFH) origin

- Postulated normal counterpart: CD4+ T-follicular helper T cells (effector T-cells)
- Three clinicopathologic subtypes
  - » Angioimmunoblastic T-cell lymphoma (AITL) type
  - » Follicular type
  - » NOS
- Cutaneous T-cell lymphomas with TFH origin not included
- One subtype can relapse/progress to another, suggesting a biological continuum

CASE

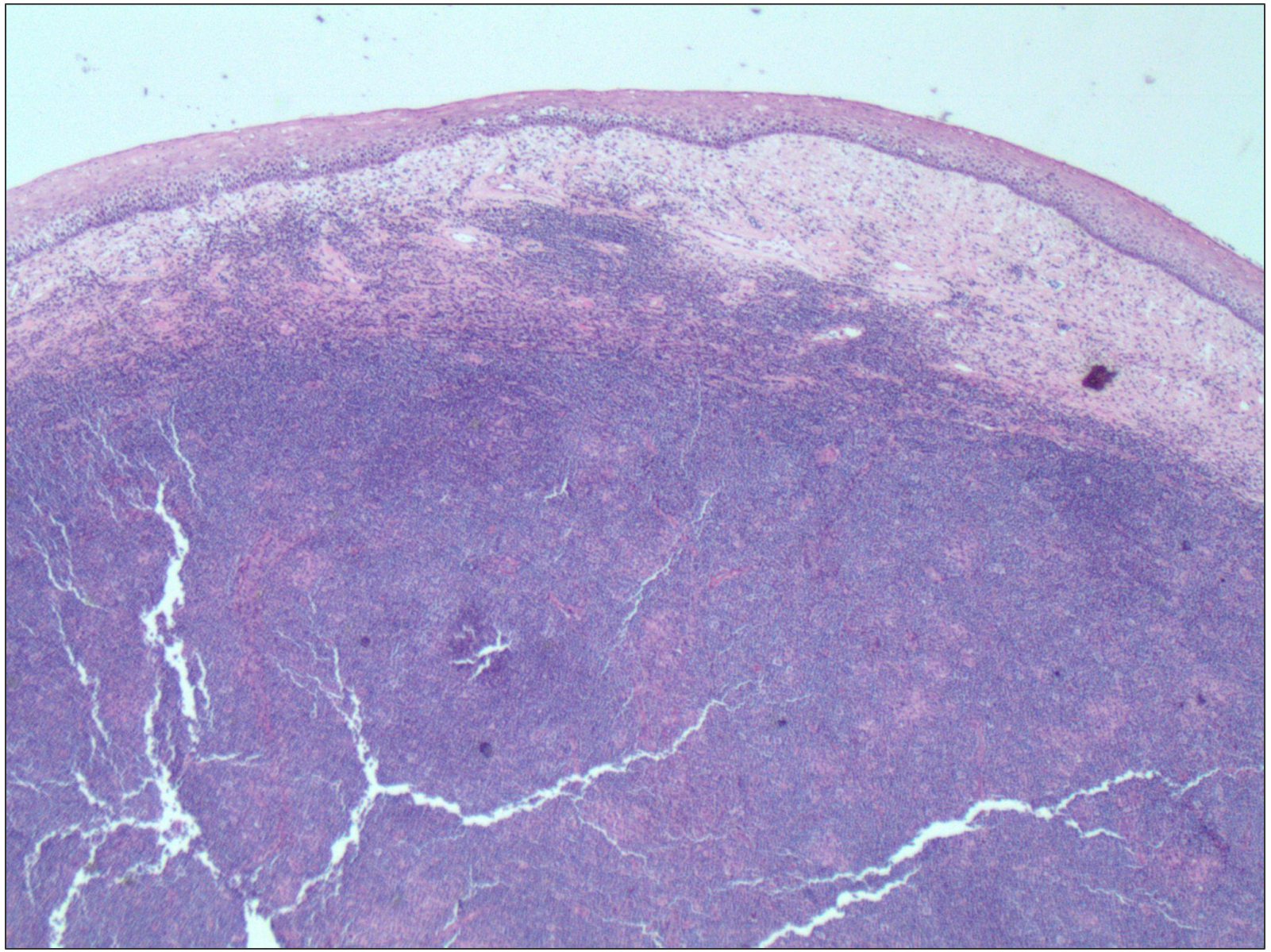
Patient History

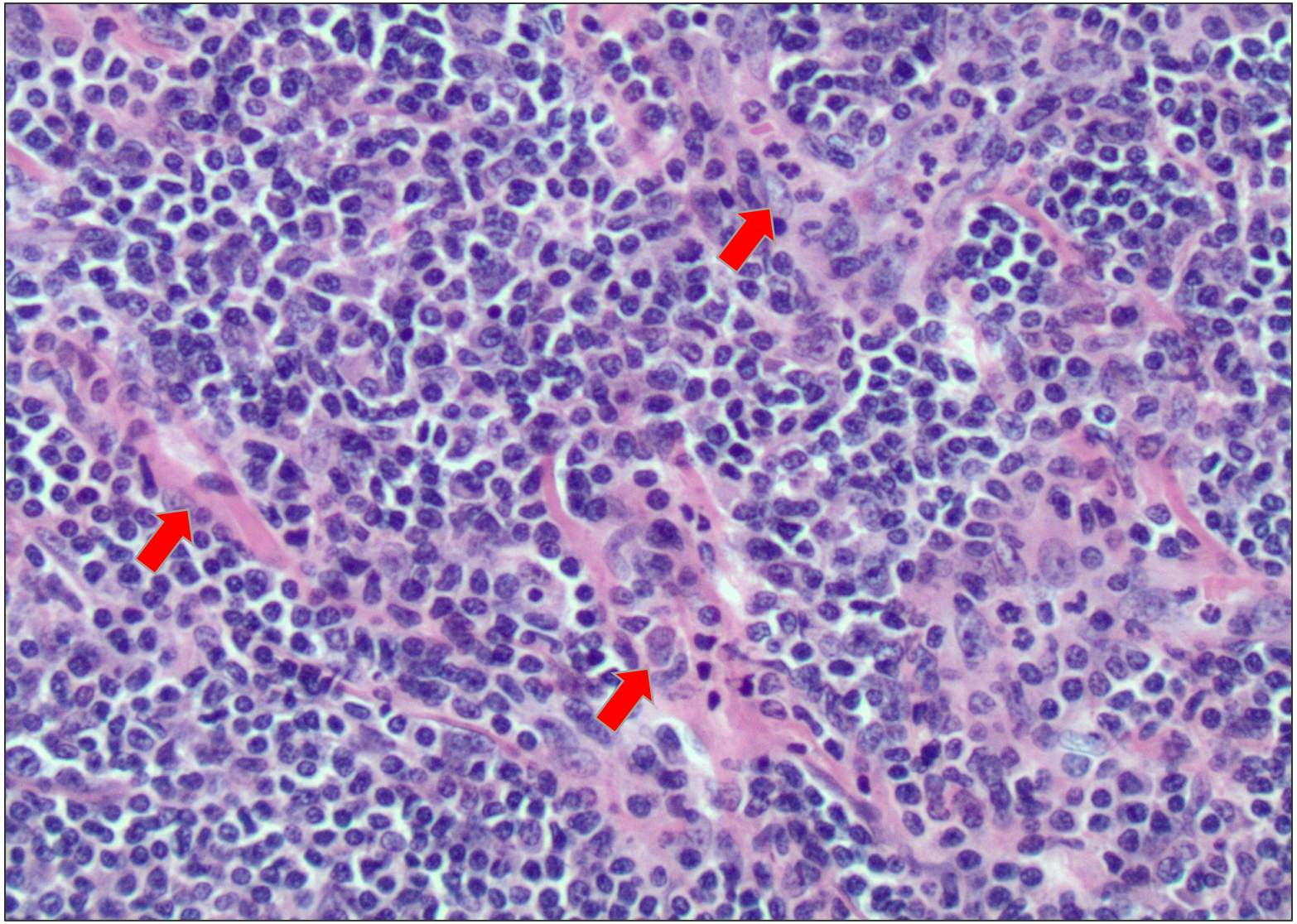
- A 58-year-old male with bilateral neck masses for 7 months, 10 lb weight loss and occasional night sweats
- Physical examination revealed confluent bilateral neck masses
- CT Neck:

  1) 2.4 x 2.5 x 3.8 cm exophytic oropharyngeal mass

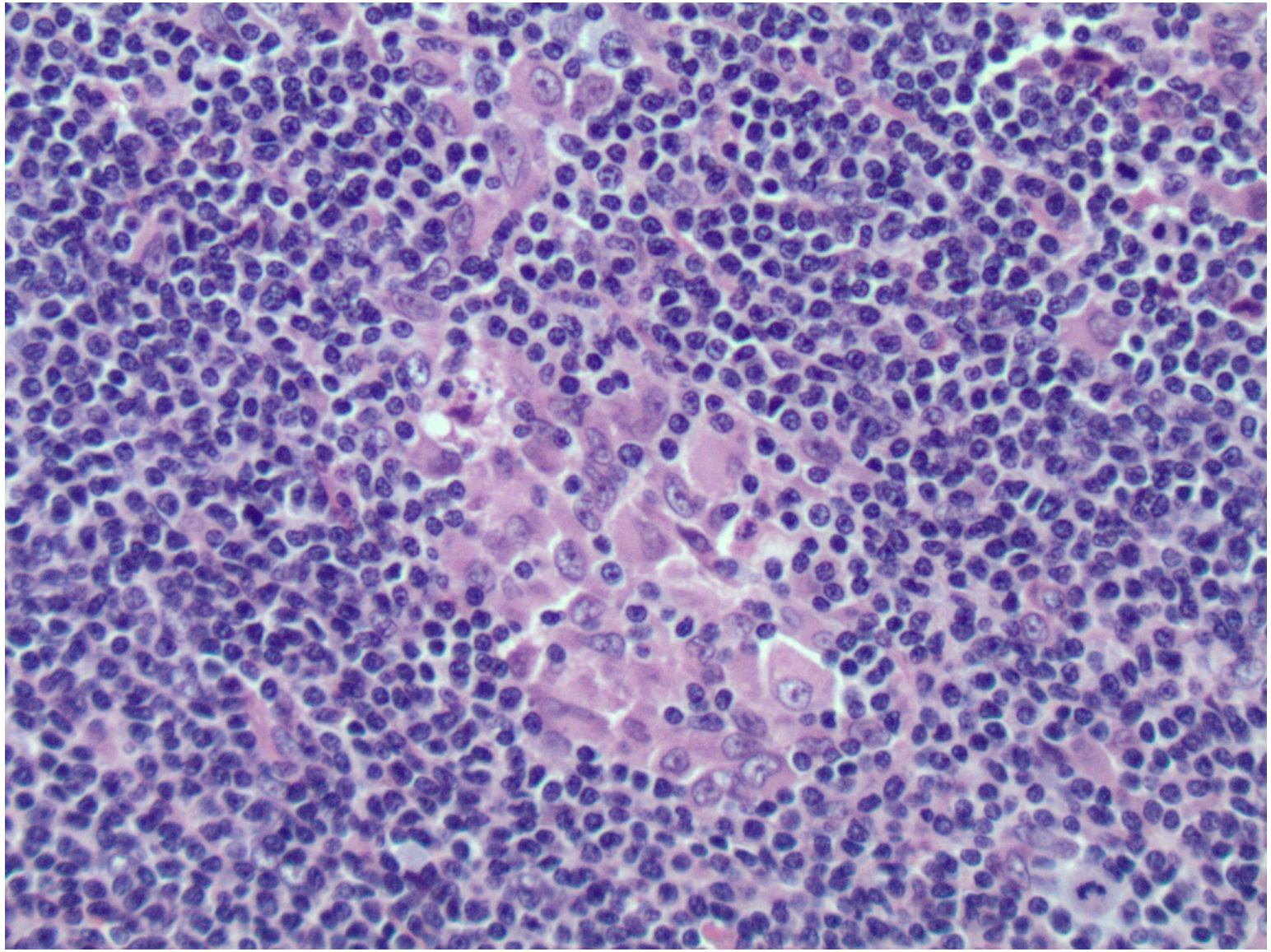
  2) Multiple enlarged bilateral cervical lymph nodes largest measuring 4 cm on the right side
- Clinical concern of a squamous cell carcinoma with metastasis, and a biopsy of the oropharyngeal mass and cervical lymph node was done

BASE OF TONGUE BIOPSY

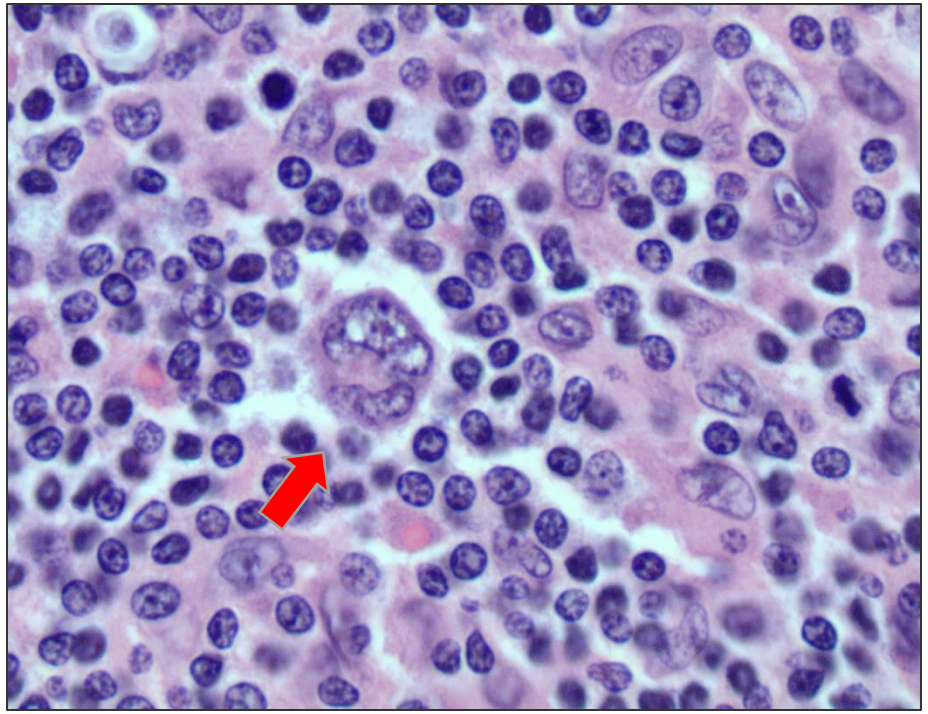




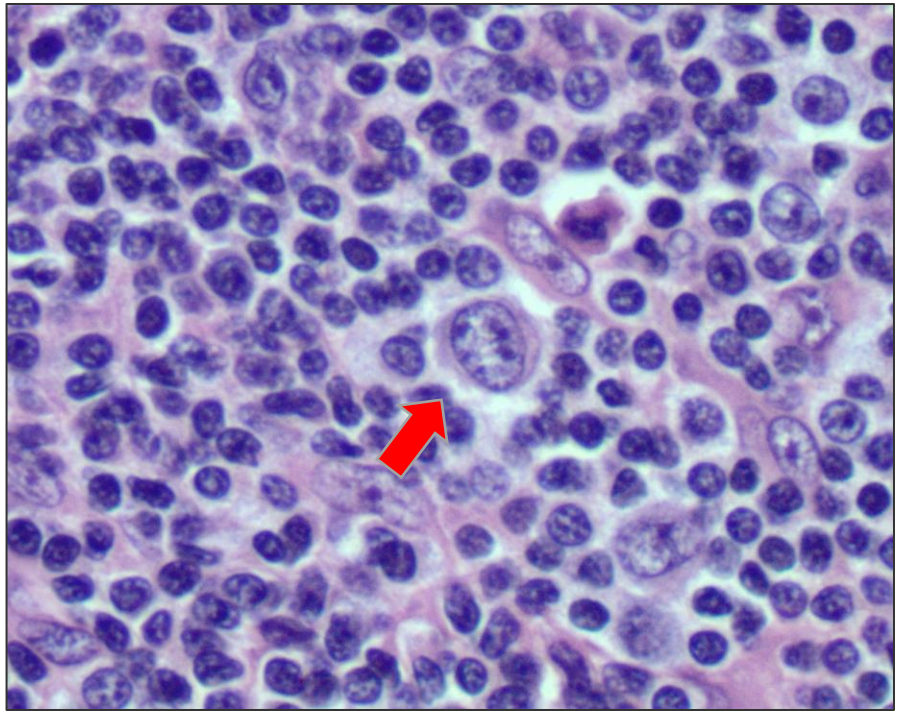
- Proliferation of high endothelial venules
- Background of small lymphocytes and plasma cells



Epithelioid histiocytes and granulomas

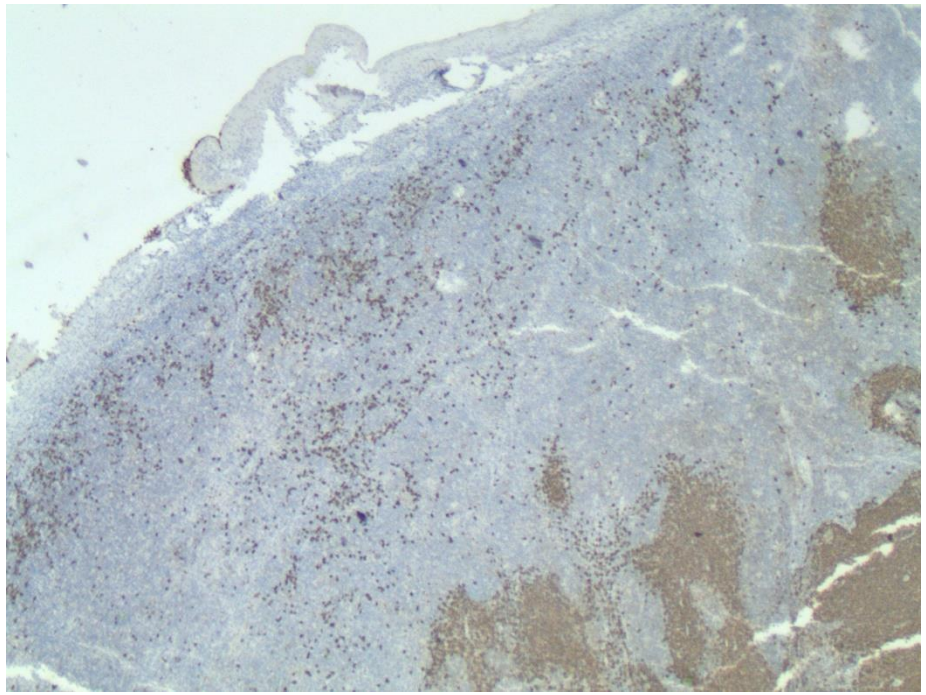


Hodgkin (Reed-Sternberg) like cells

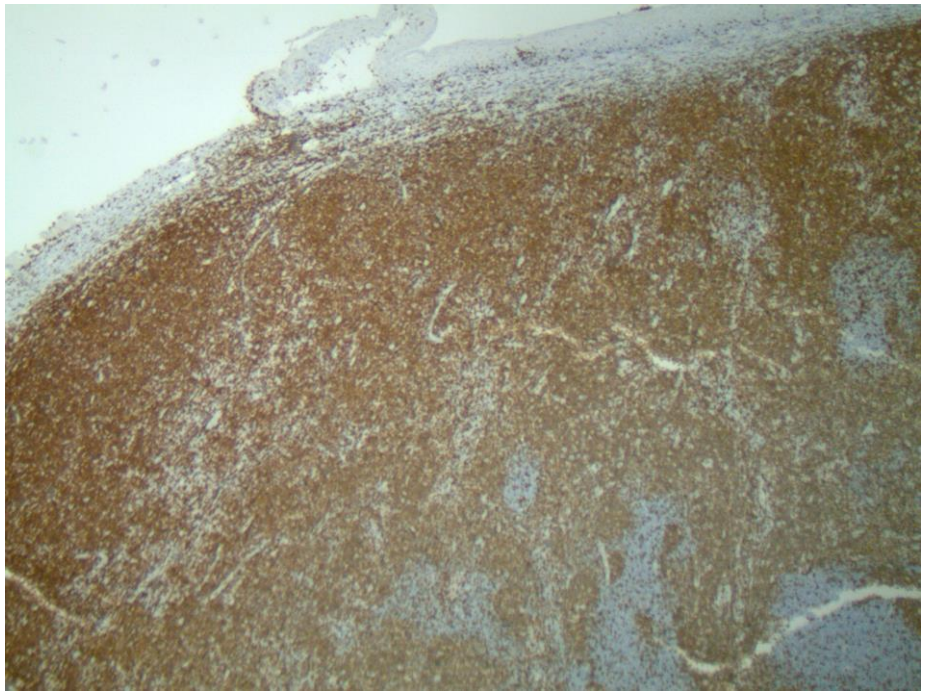


Centroblastic and Immunoblastic cells

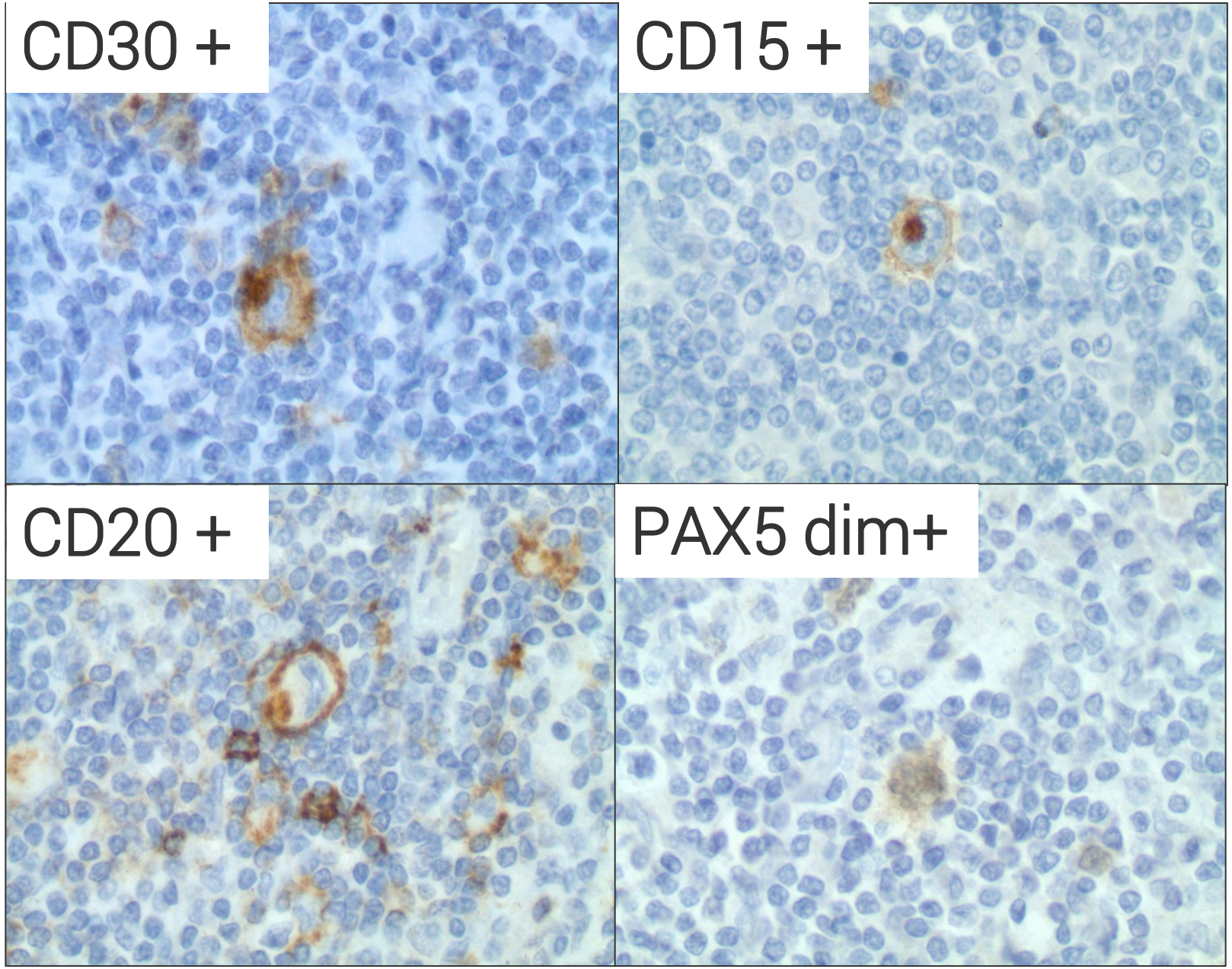
CD20, 2x

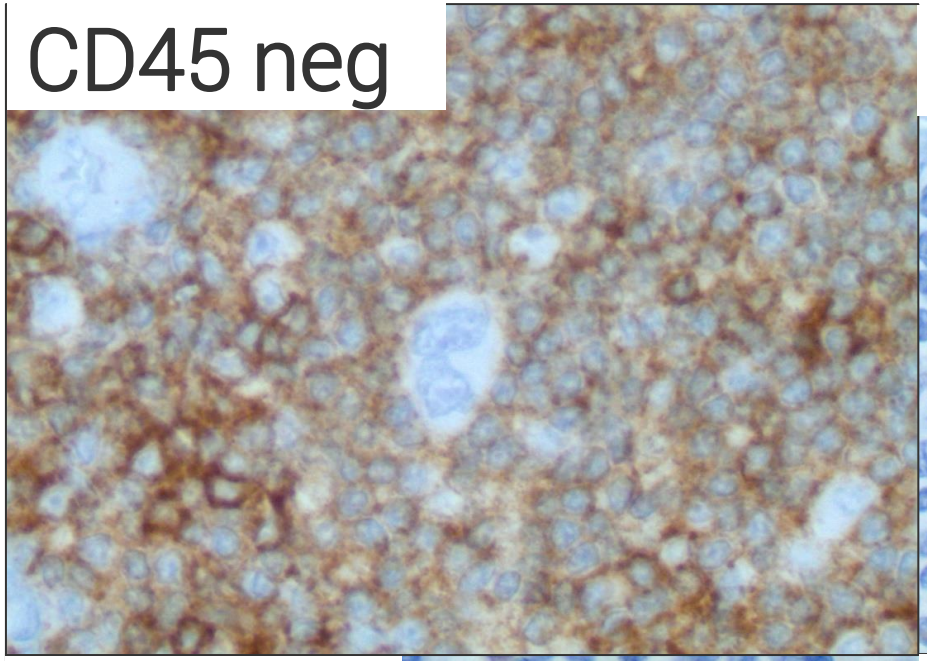


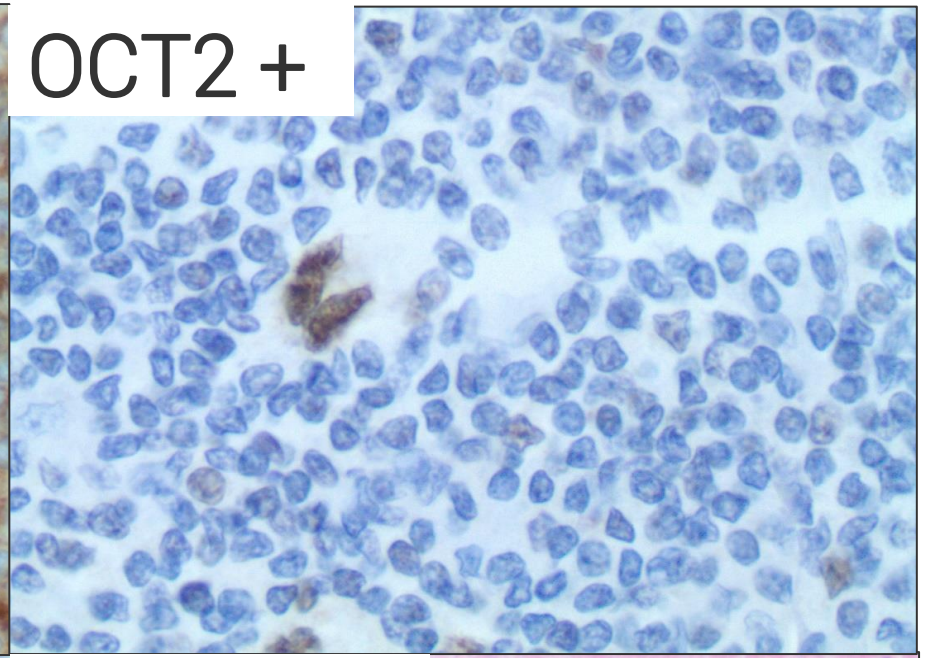
CD3, 2x

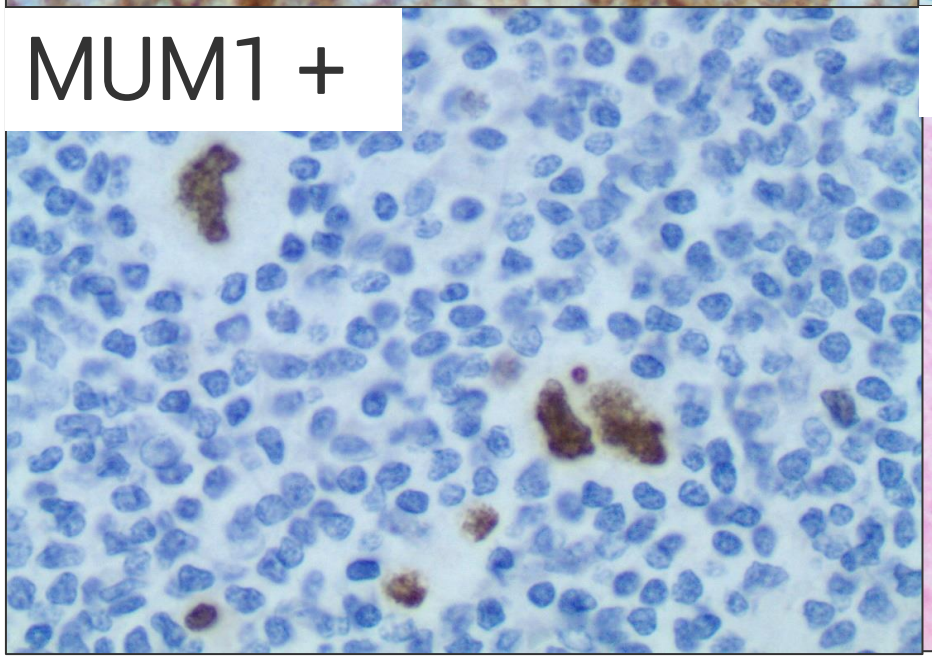


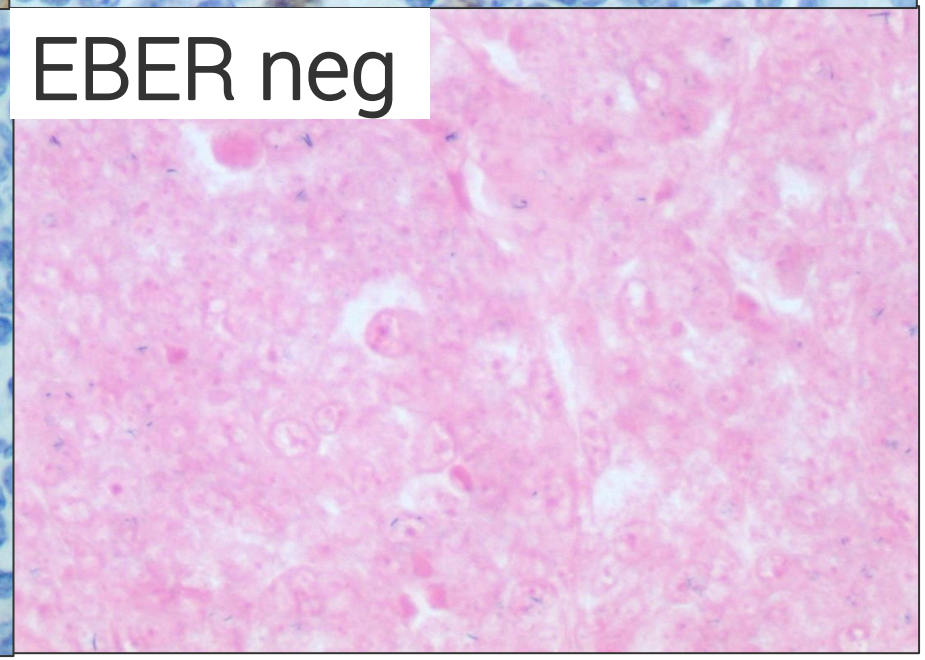
CD20 positive follicles are pushed aside and compressed by the expanded T cell population


CD30 +
CD15 +
CD20 +
PAX5 dim+

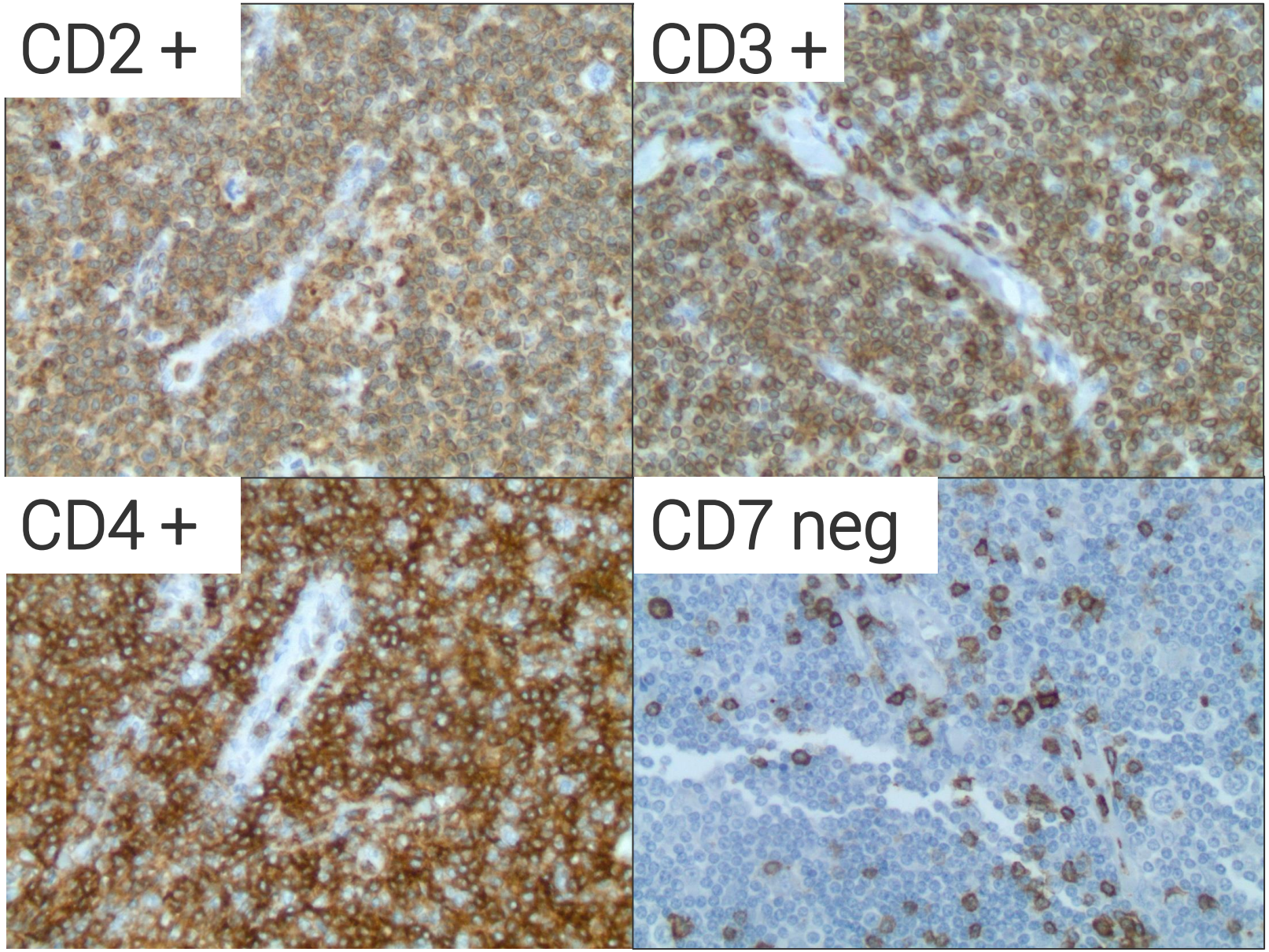

CD45 neg

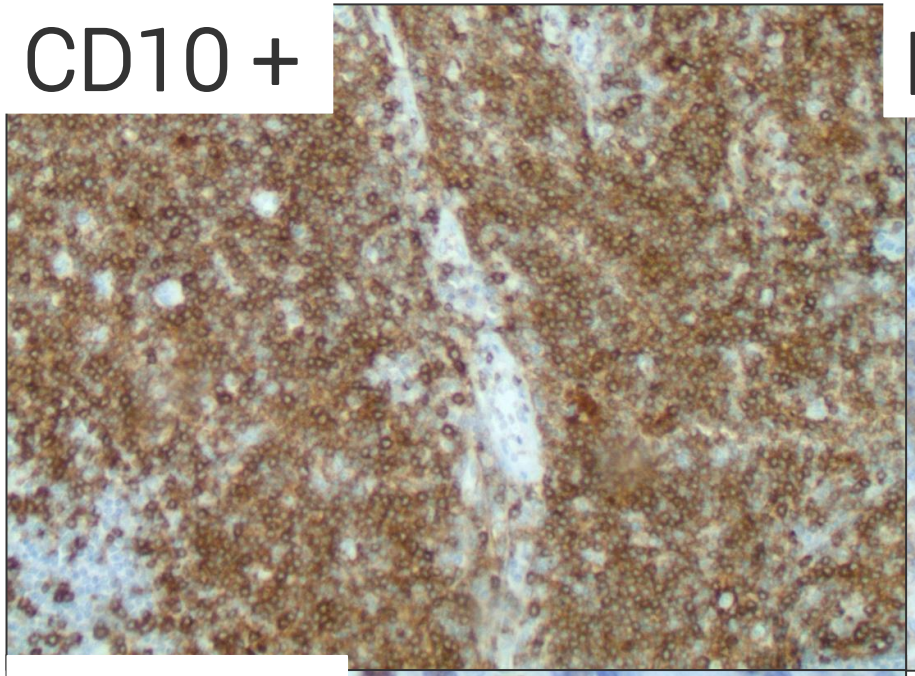

OCT2 +

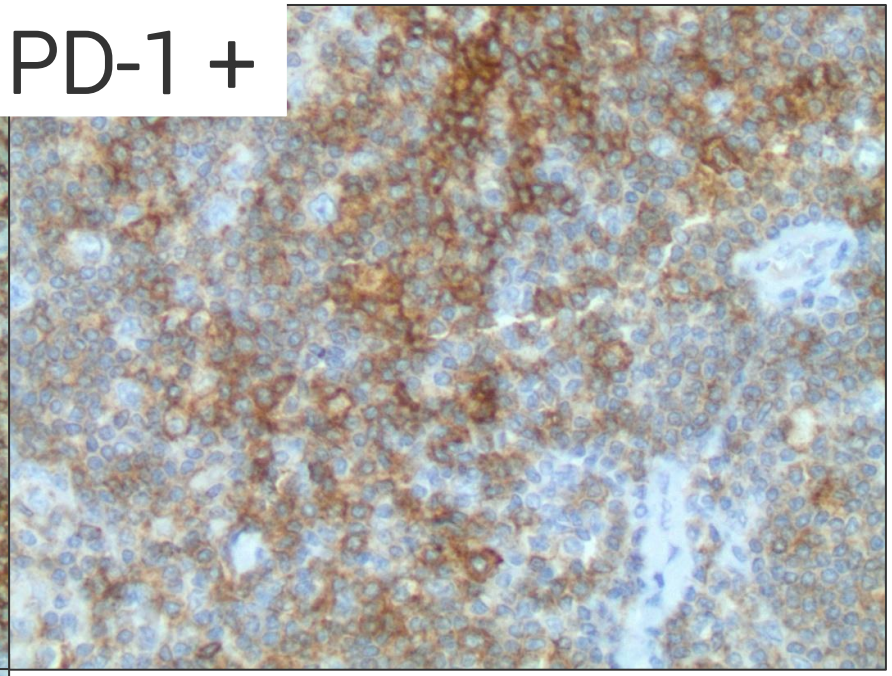

MUM1 +

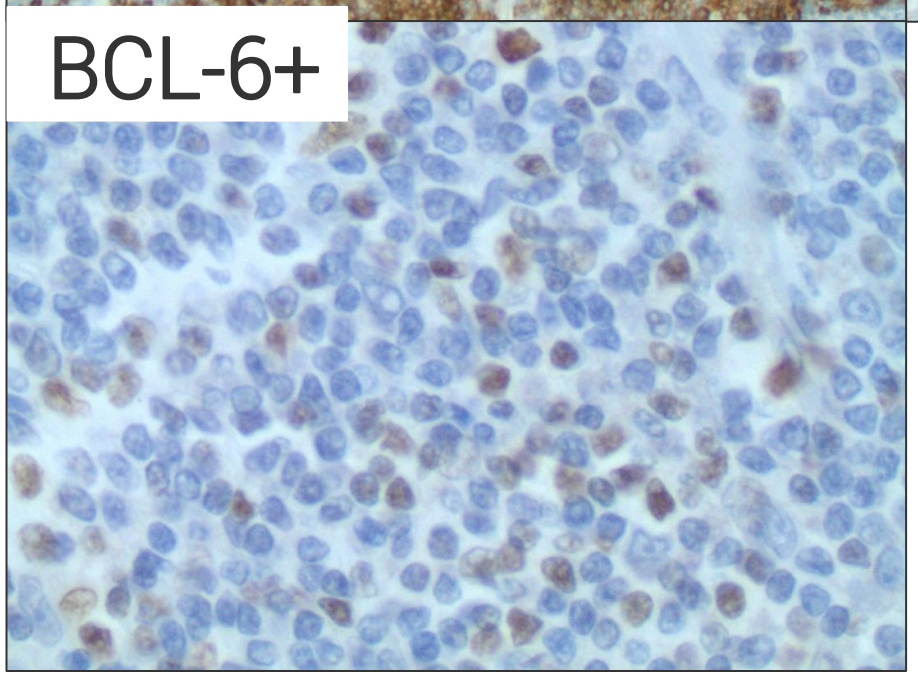

EBER neg

Classic Hodgkin Lymphoma???

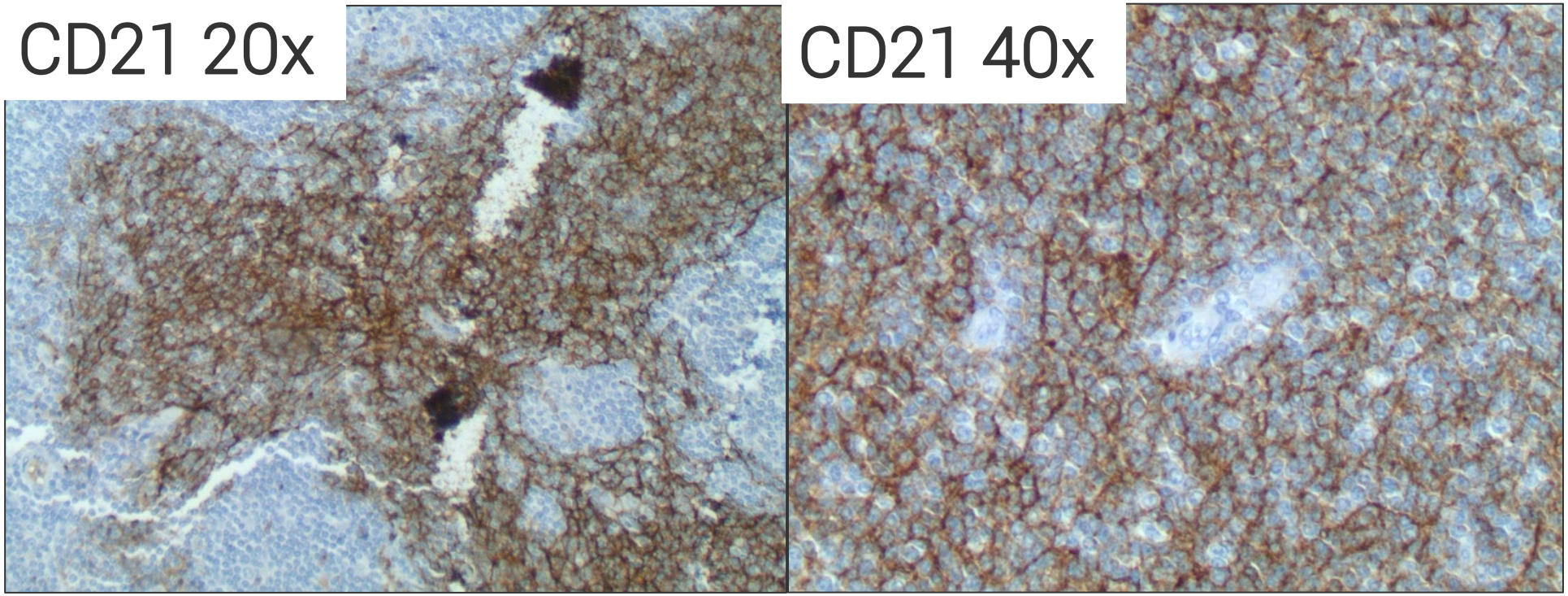

CD2 +
CD3 +
CD4 +
CD7 neg







Follicular helper T-cell markers

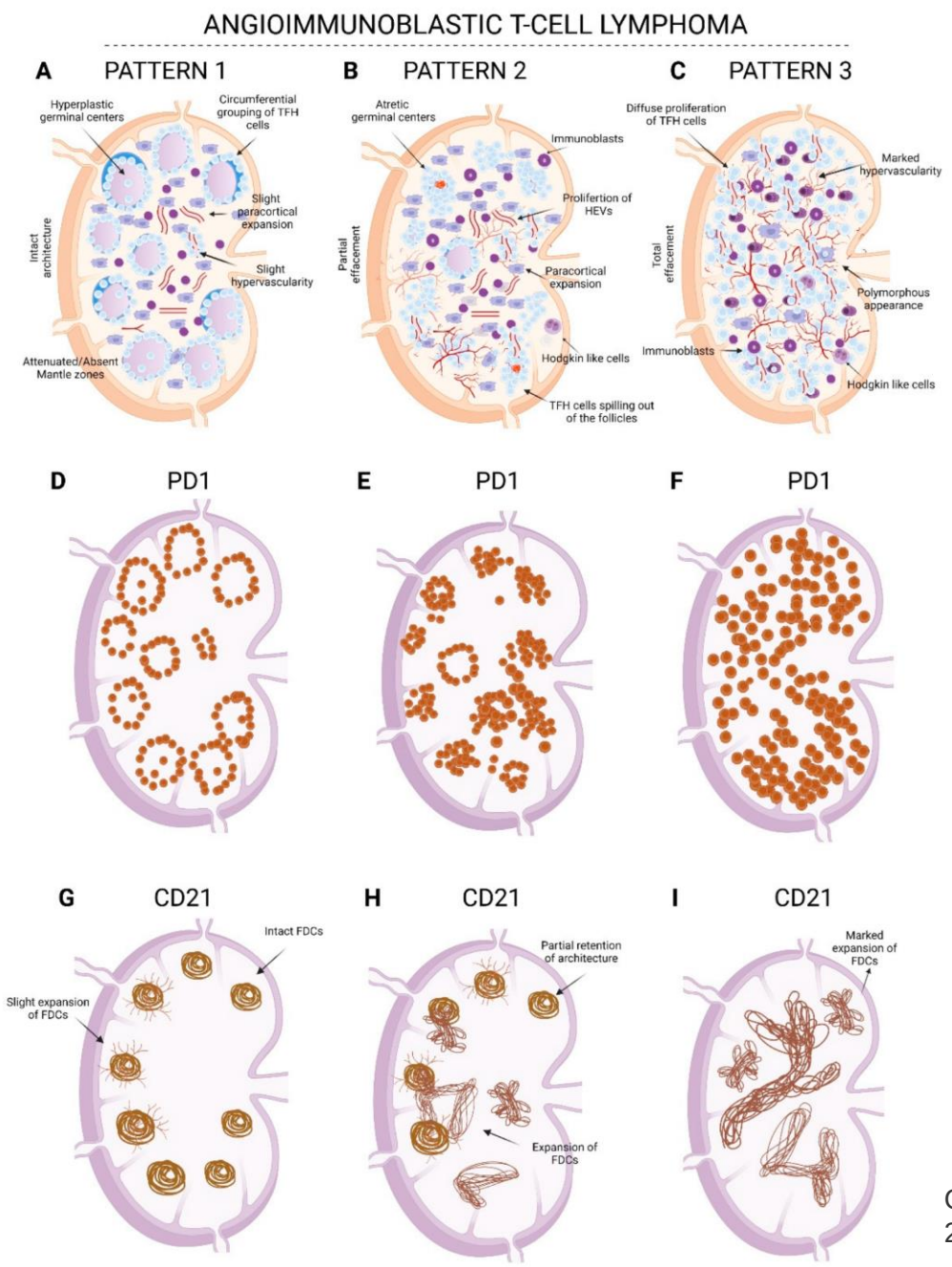


CD21 demonstrates expansion of follicular dendritic meshworks around blood vessels

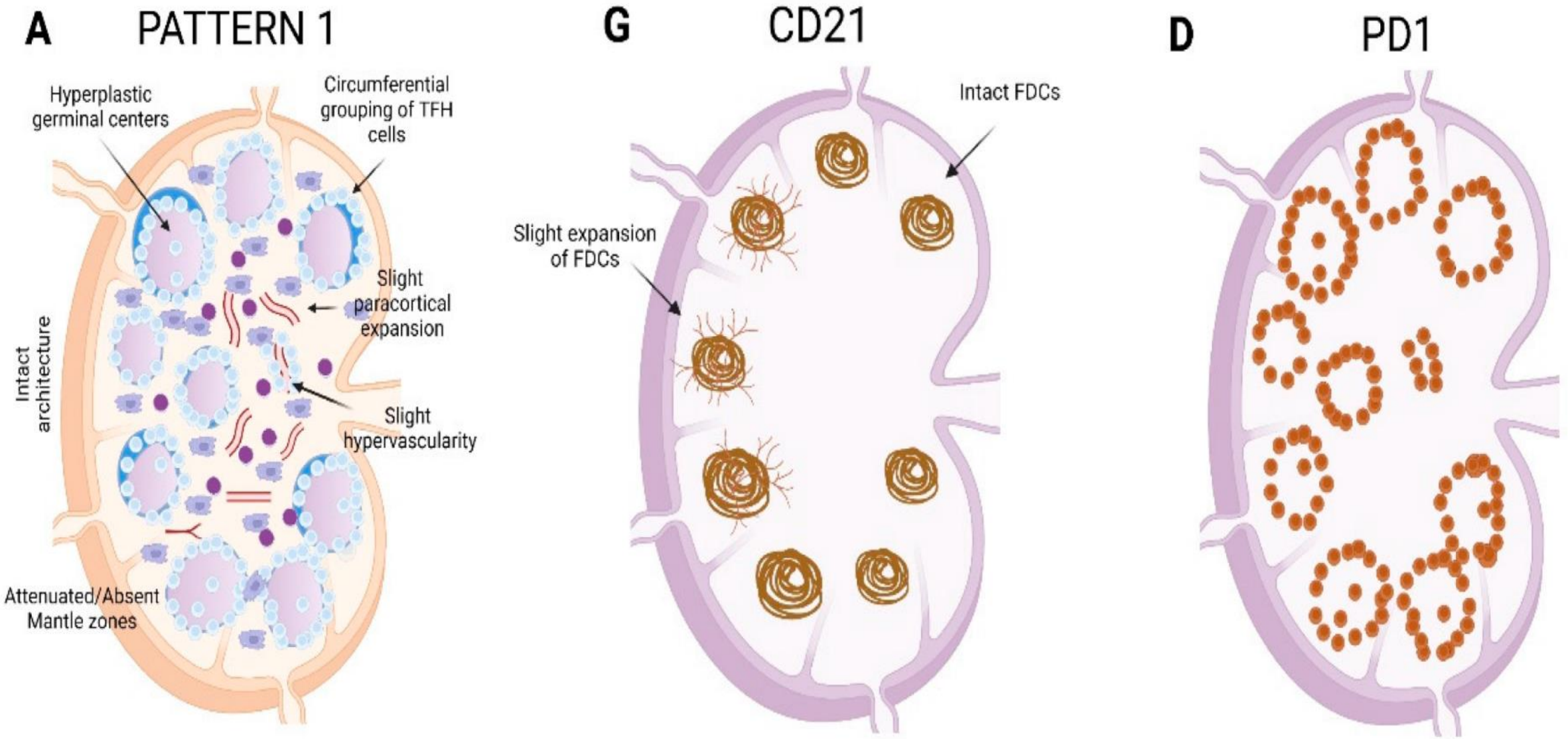
Angioimmunoblastic T-cell lymphoma???

Angioimmunoblastic T-cell lymphoma

- Most common type of node-based T-cell lymphoma (30-40% of non-cutaneous T-cell lymphomas)
- A unique clinicopathologic entity:
  - » Clinical manifestations (lymphadenopathy, hypergammaglobulinemia, skin rashes, autoimmune, pleural effusion etc.) +
  - » Polymorphous proliferation in lymph nodes +
  - » Proliferation of high endothelial venules and FDC meshworks +/-
  - » EBV+ B-cells (dropped in WHO 5th edition)
- BM, skin
  - » Lymphoid aggregates with similar morphology

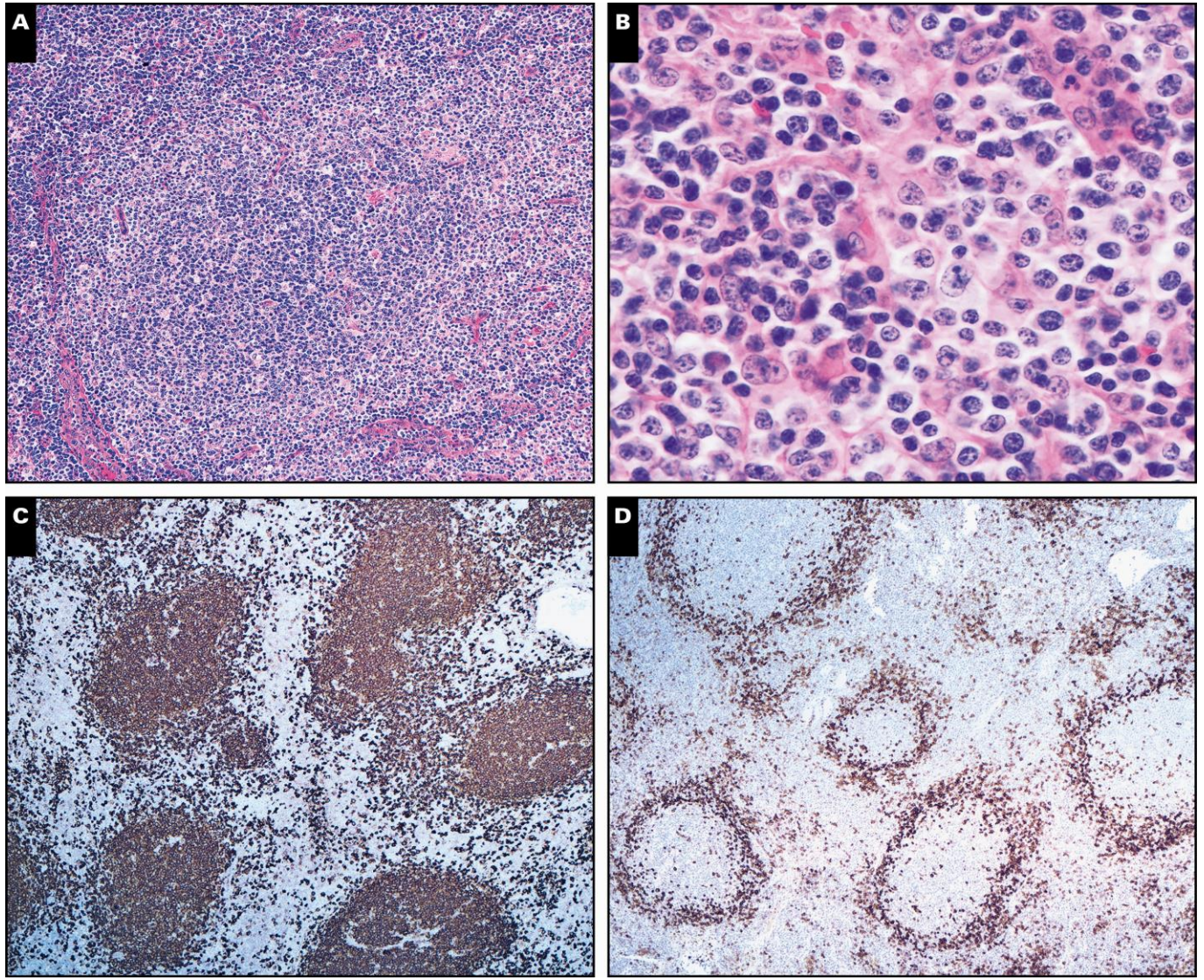


Ganapathi K, Karner K and Menon MP et al. Hemato, 2022



PATTERN 1 AITL

Germinal Center



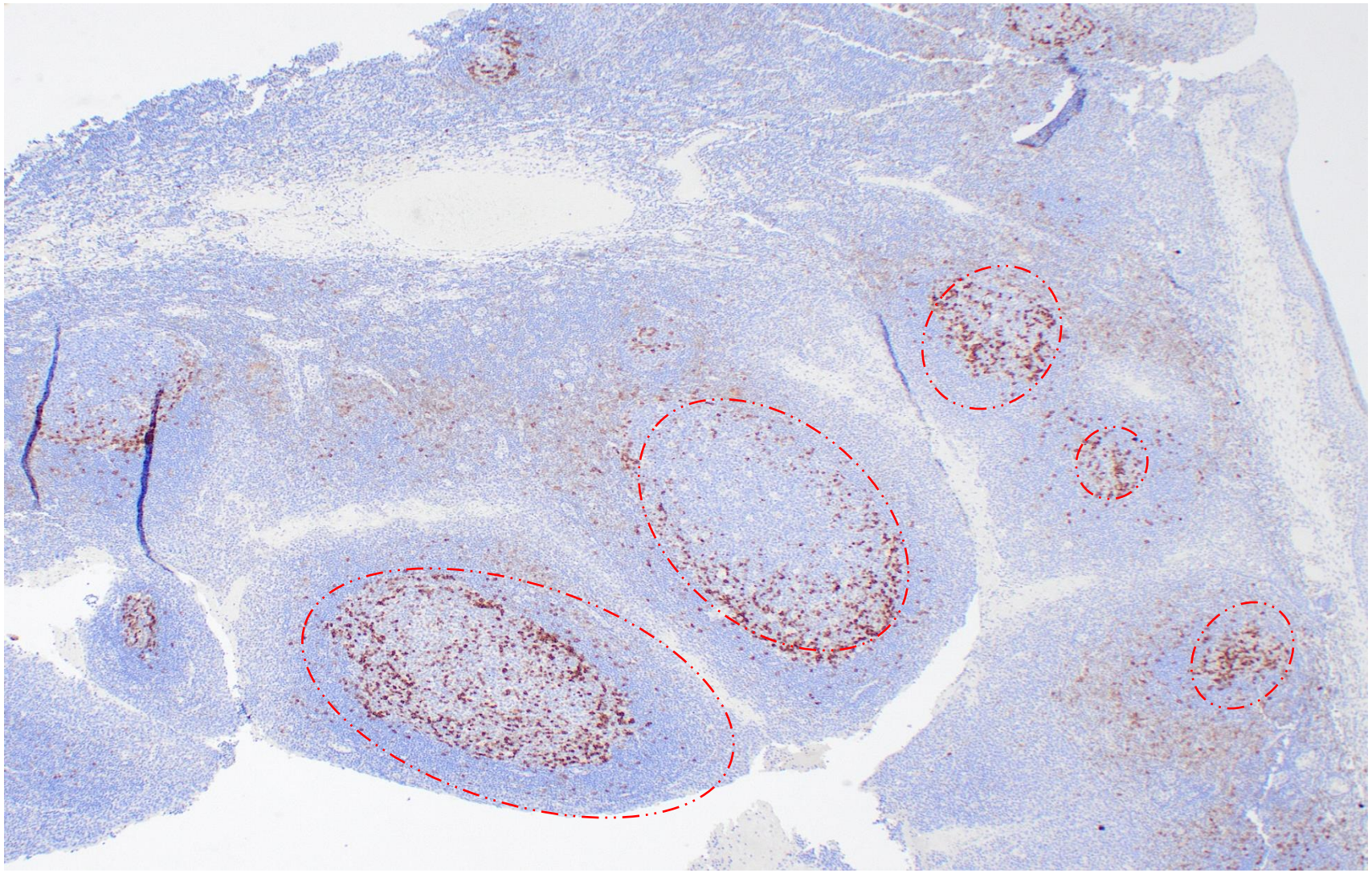
HEVs surrounding GC

CD20

PD-1

Am J Clin Pathol, Volume 156, Issue 1, July 2021, Pages 1–14, https://doi.org/10.1093/ajcp/aqab090

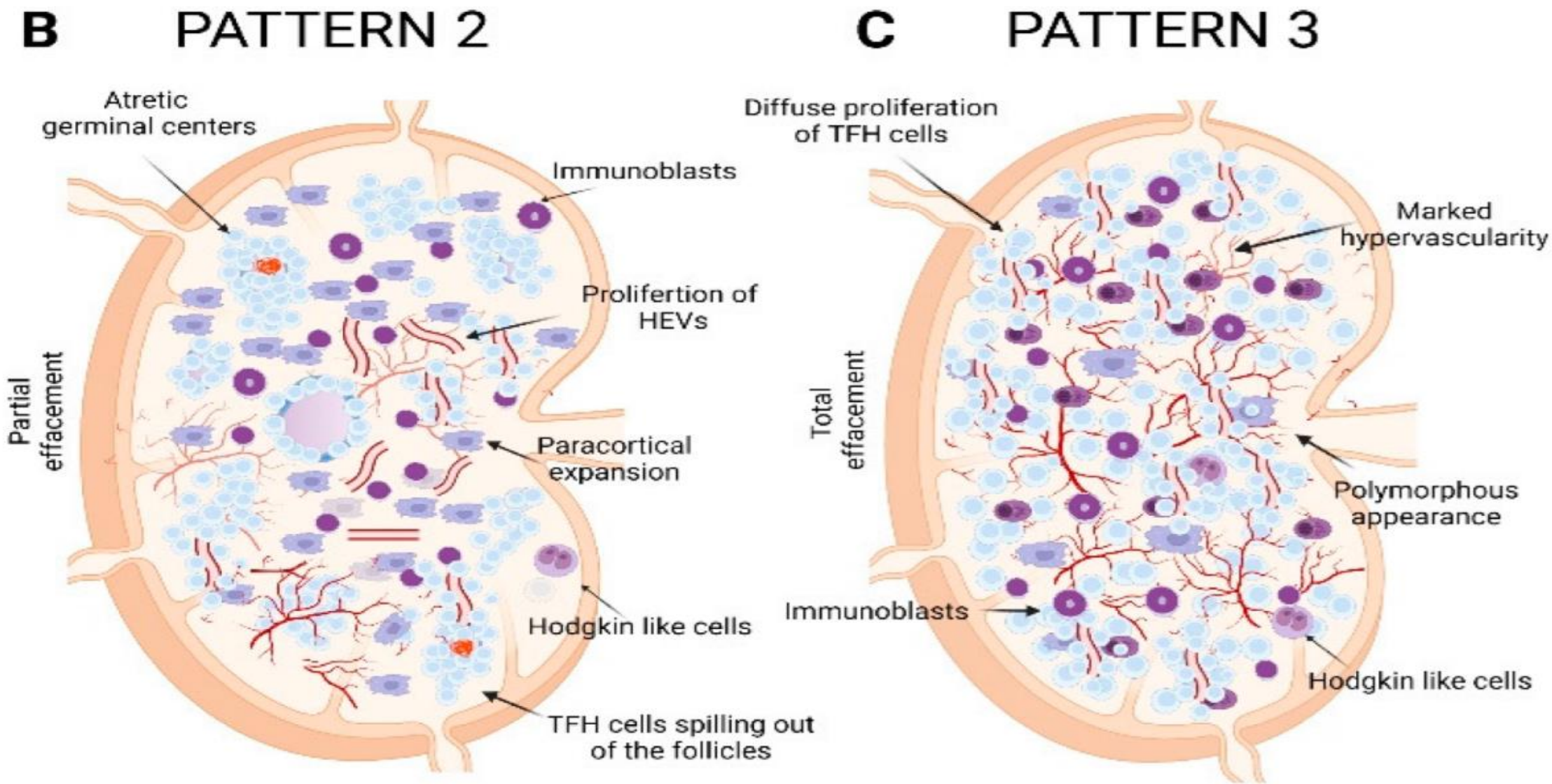
REACTIVE GERMINAL CENTER



PD-1

- **T-Follicular helper cells (TFH)**
- Appear uniformly distributed or polarized within germinal-center
-

REACTIVE LYMPHOID HYPERPLASIA	PATTERN 1 AITL
• No definite cytologic atypia	• Atypical clear cells around GC or around HEVs
• Strong CD10 or PD1-positive cells are confined to the germinal centers	• Strong CD10 or PD1-positive cells surround GC and some HEVs
• No FDC meshwork outside germinal centers	• Slight FDC expansion into paracortex
• No marked hypervascularity outside the GC	• Slight increase in hypervascularity of paracortex
• No T-cell clonality	• Abnormal immunophenotype or T-cell clonality



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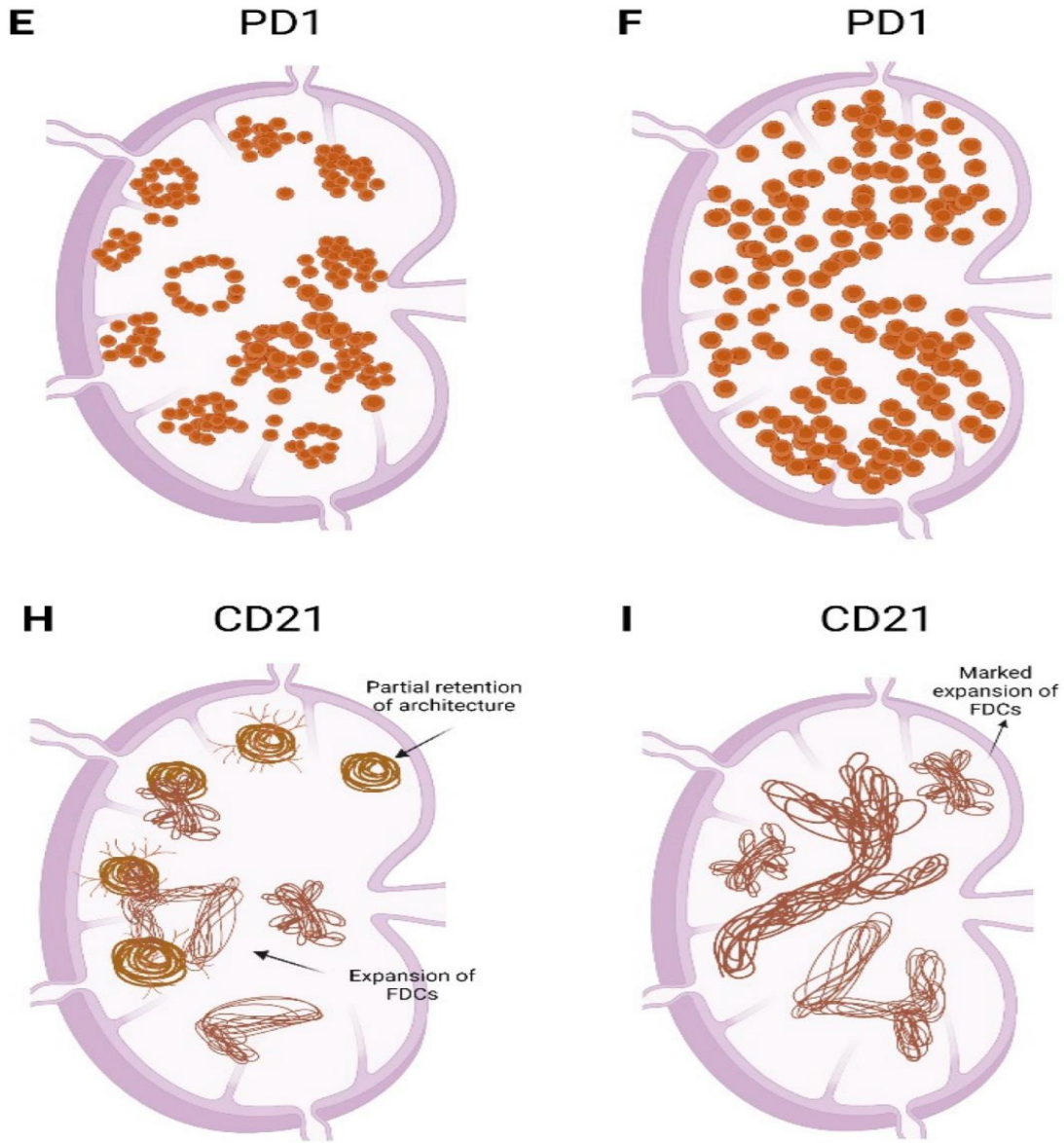
Pattern 2

Pattern 3

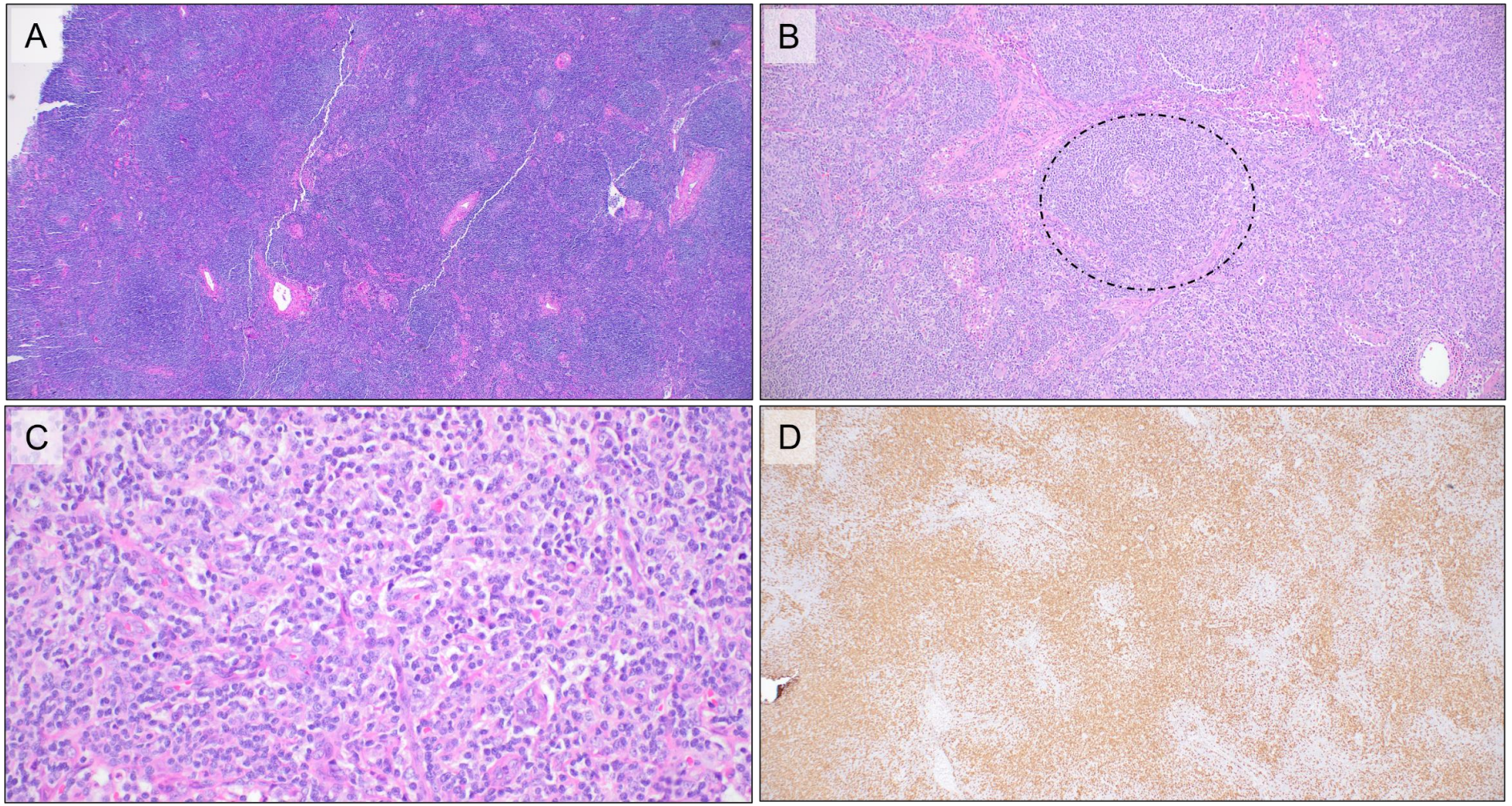


AITL Patterns

Ganapathi K, Karner K and Menon MP et al. Hemato, 2022

Partially retained architecture

Atretic germinal centers



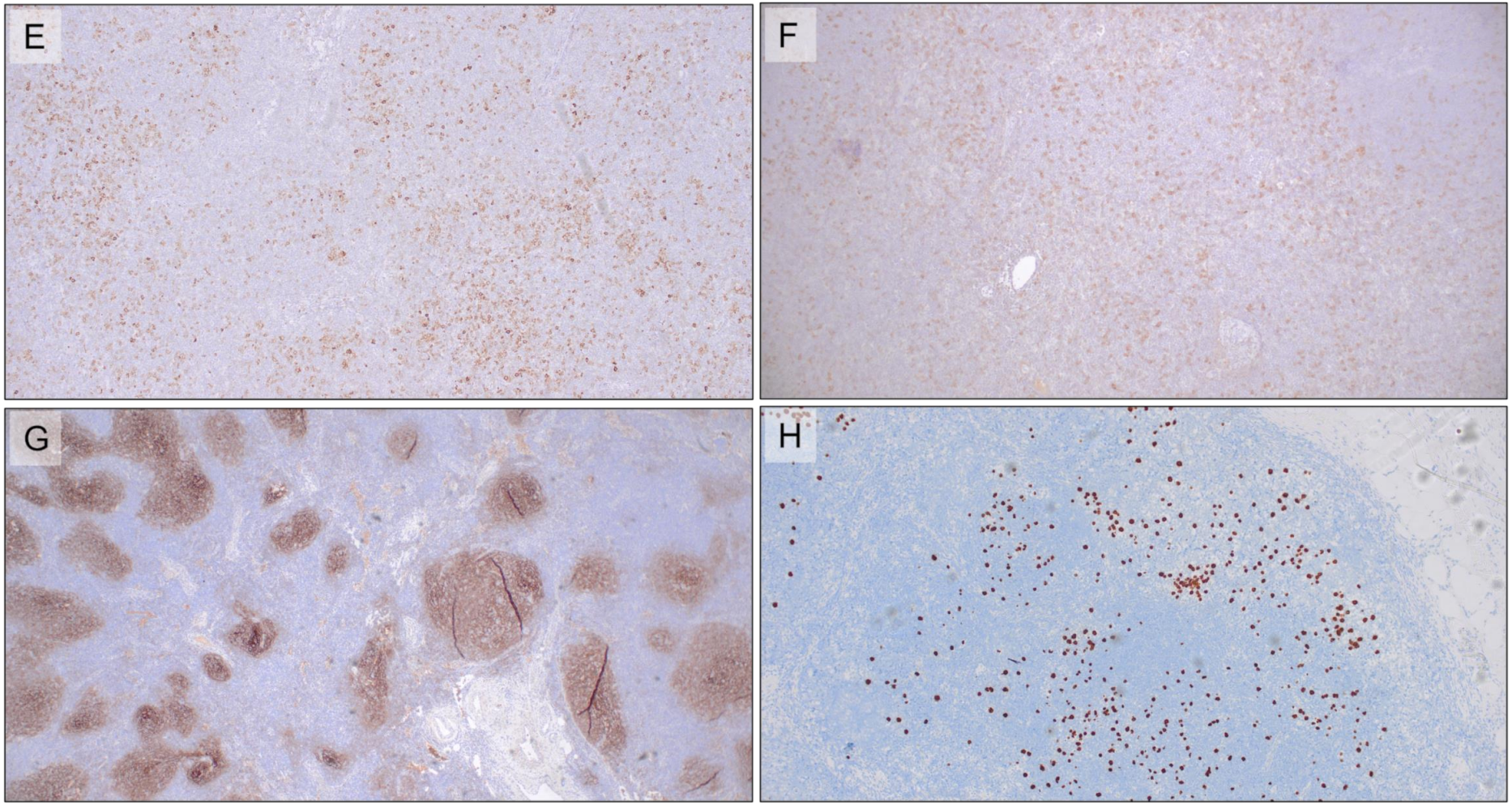
Some polymorphous areas

CD3

AITL Pattern 2

PD-1

CD10



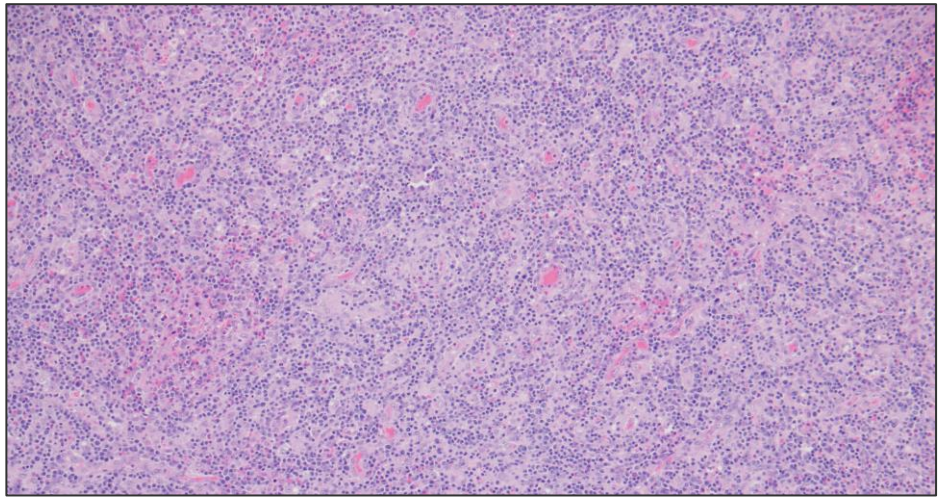
CD20

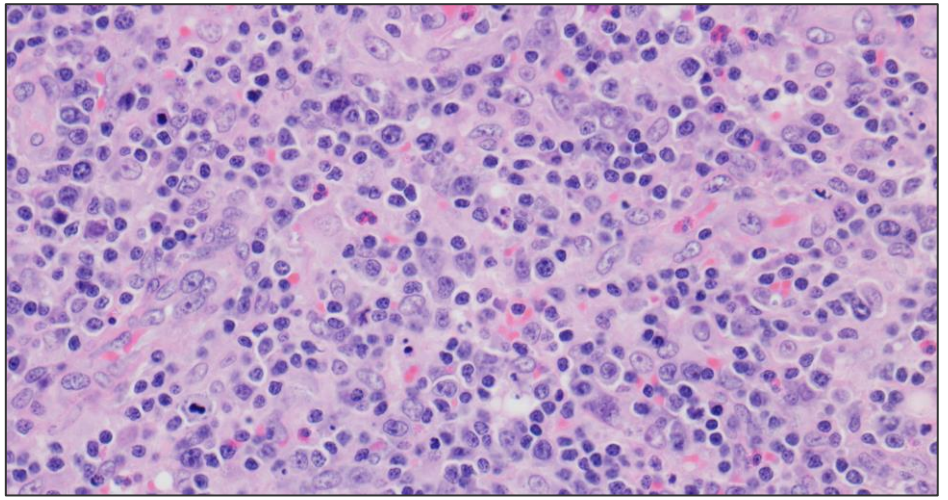
EBV

AITL Pattern 2

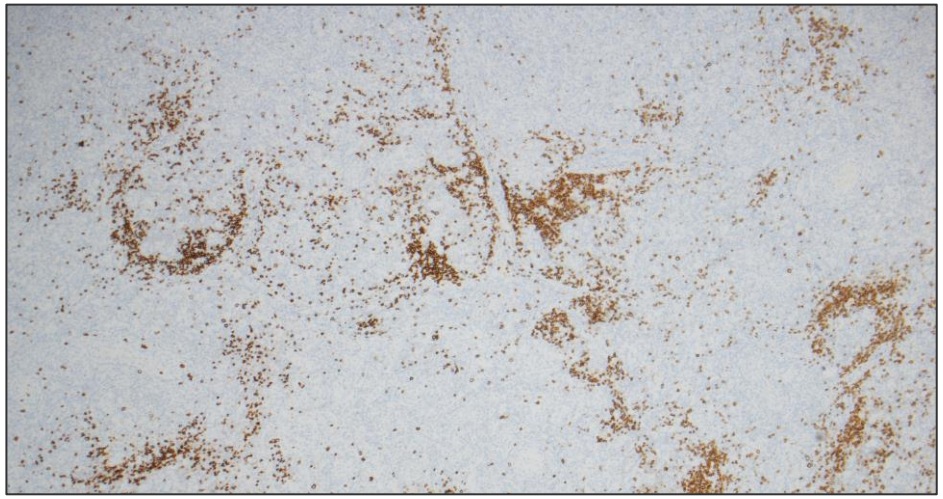
Totally effaced architecture

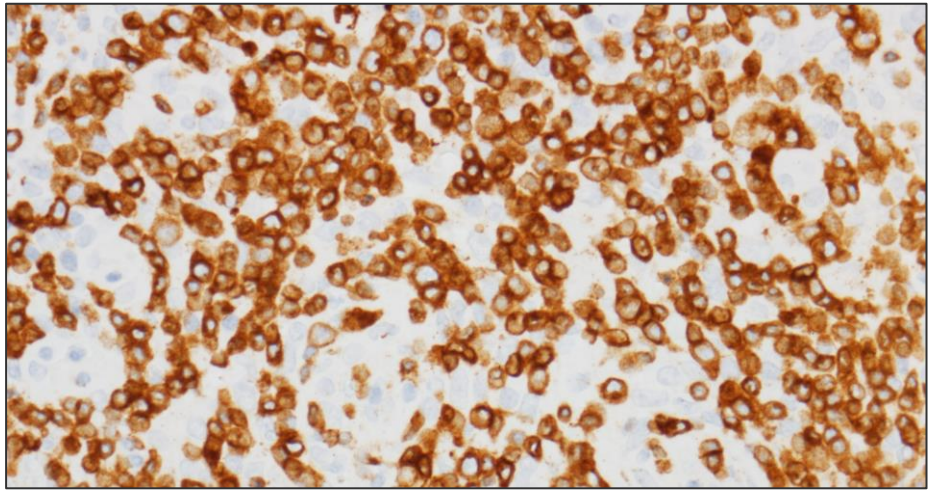
Polymorphous infiltrate





CD20

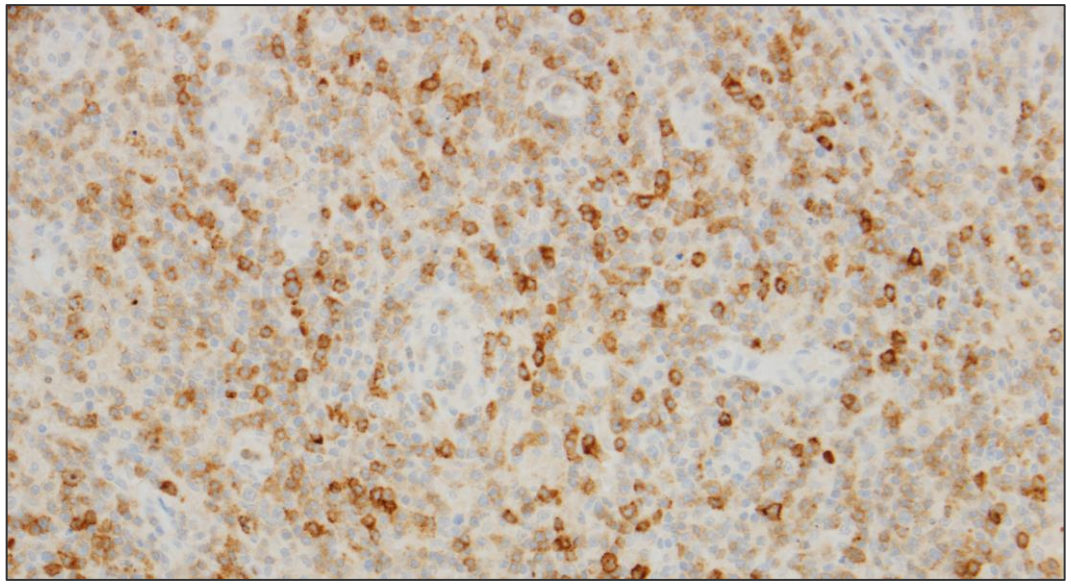


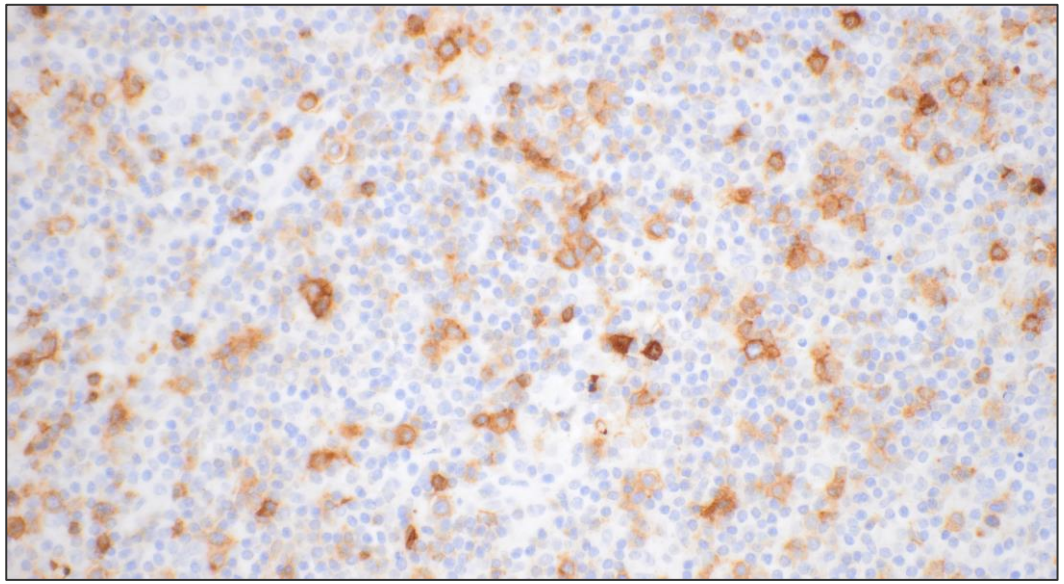


CD3

AITL Pattern 3

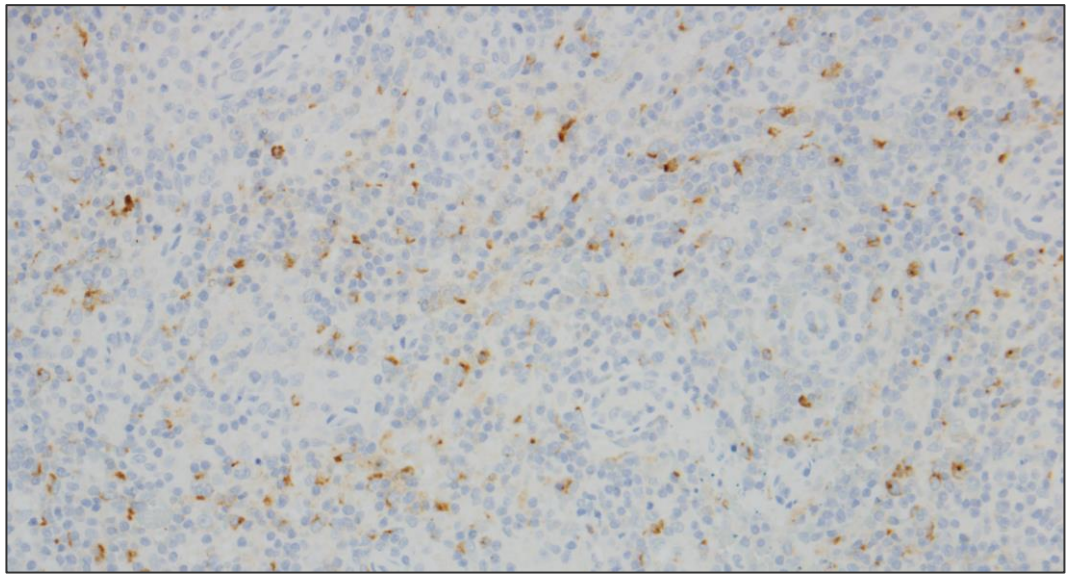
PD1

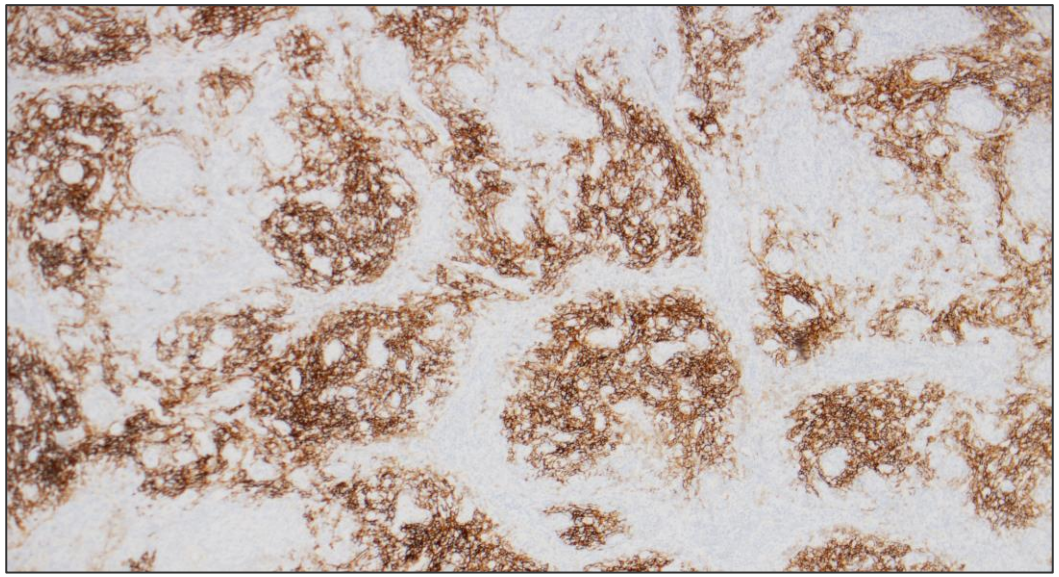




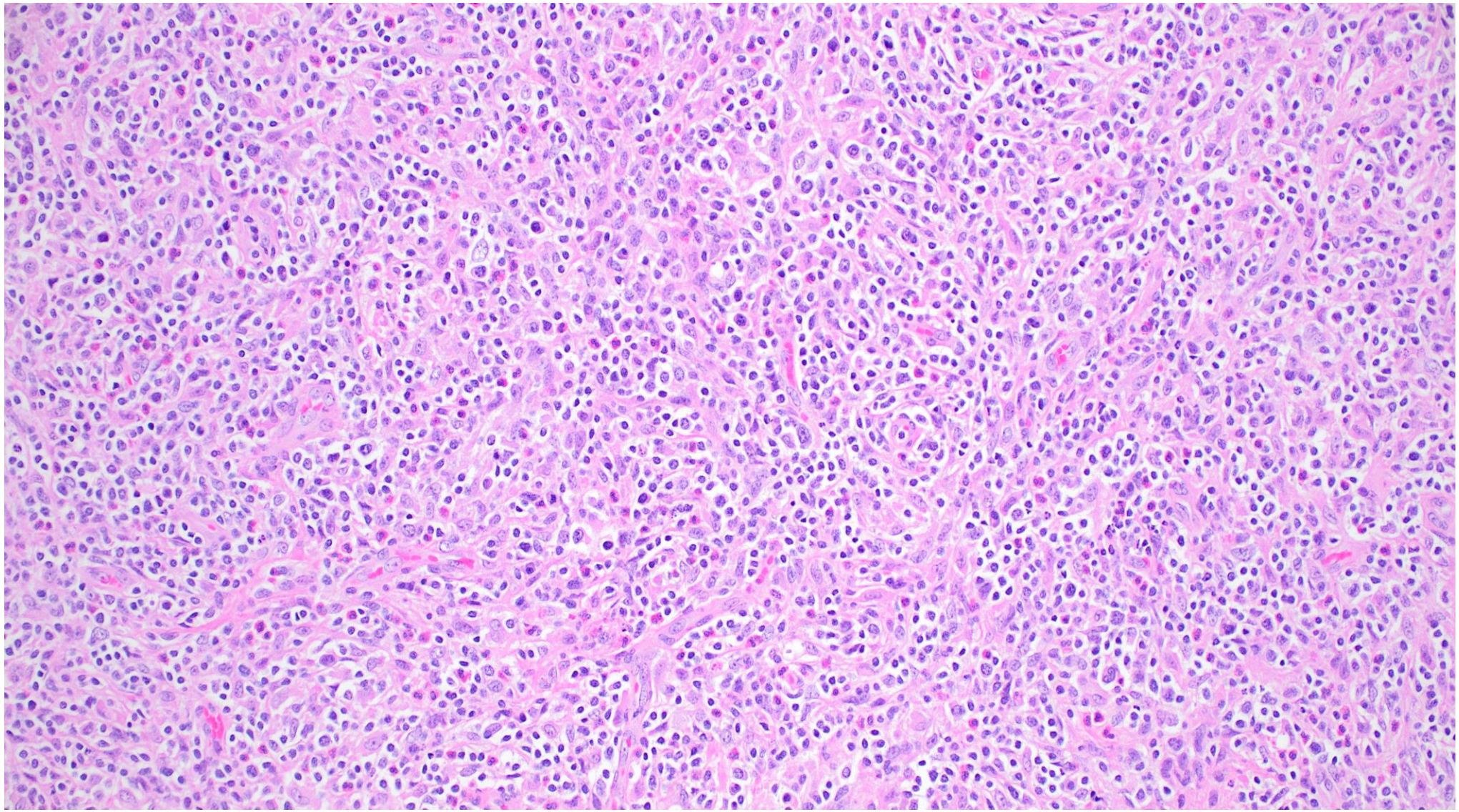
CD10

CXCL13



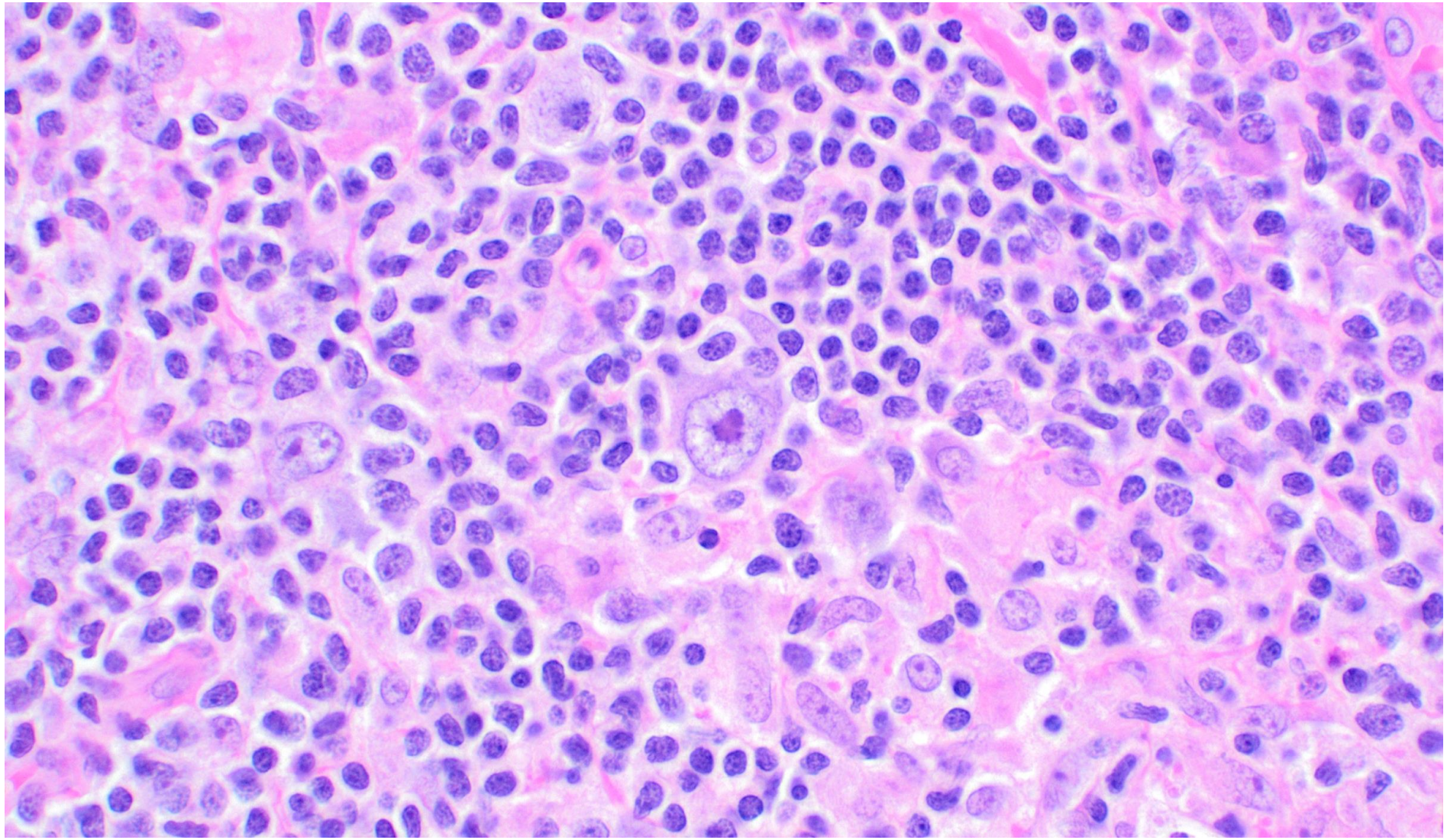


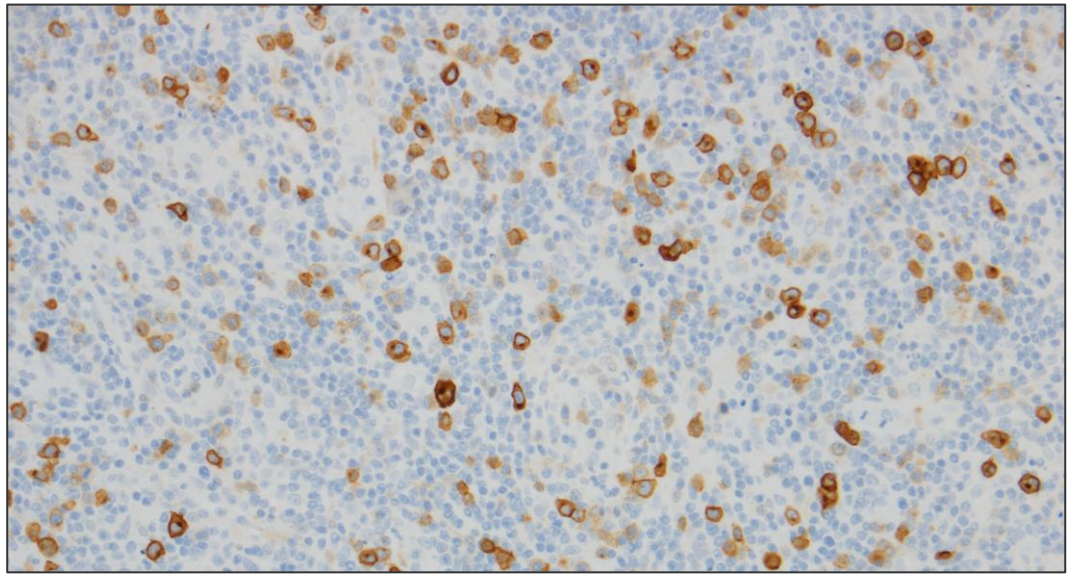
CD21



Proliferation of High endothelial venules

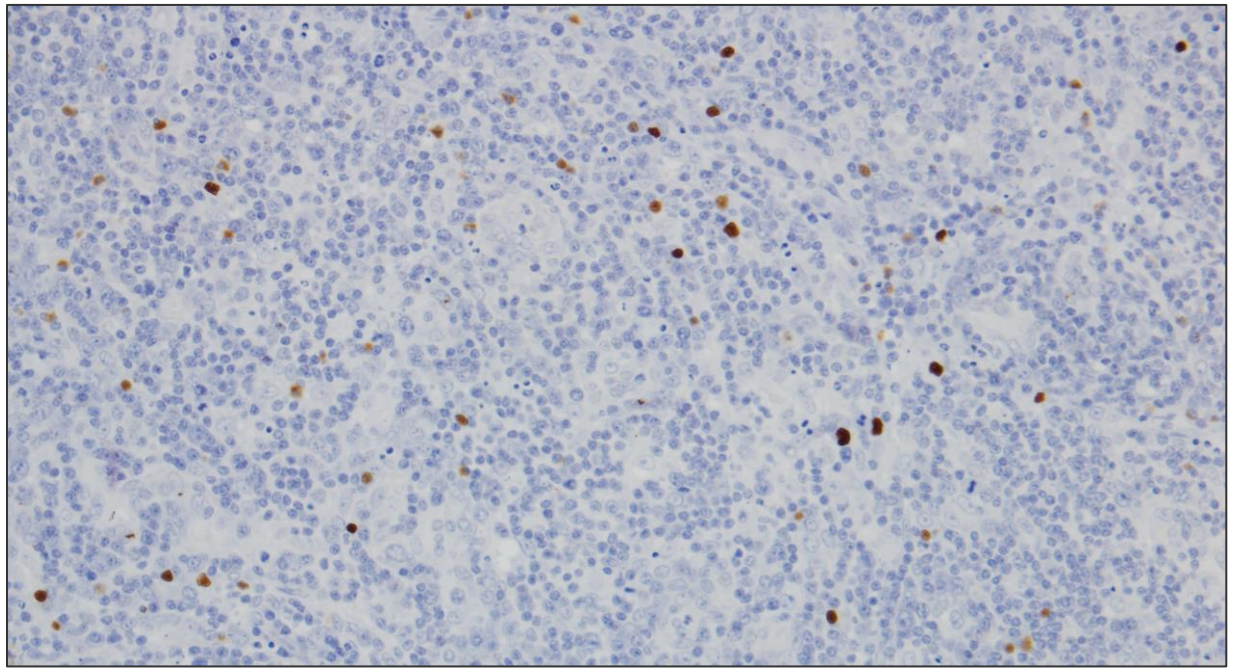
Hodgkin/Reed-Sternberg like cells





CD30

EBER



AITL – Immunophenotype

- Positive
  - » Pan T-cell antigens; CD2, CD3, CD5 (CD7 loss common)
  - » By definition all cases are CD4+ (Rarely CD4 neg)
  - » TFH markers - CD10, BCL6, CXCL13, ICOS, PD1 (ideally 3 but at least 2 with strong expression)
    - PD1 and ICOS - most sensitive
    - CD10 and CXCL13 - most specific
  - » EBV - almost always positive in the B-immunoblasts and H/RS-like cells
  - » CD21/CD23 - expanded FDC meshworks
- Negative
  - » CD8, CD56, cytotoxic markers

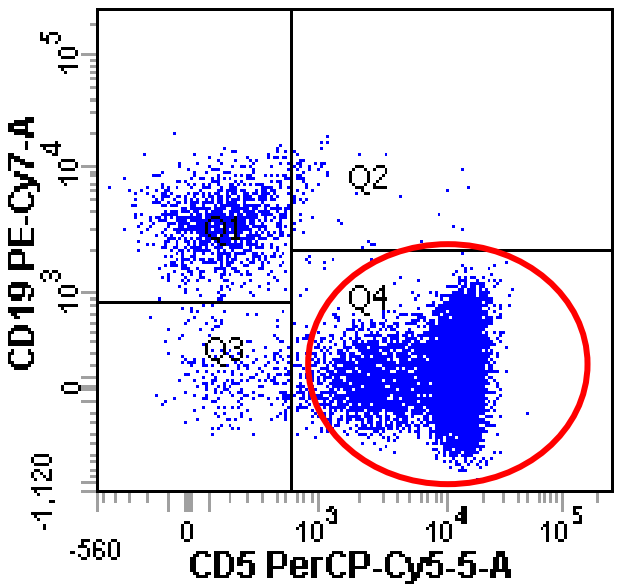
AITL - Genetics

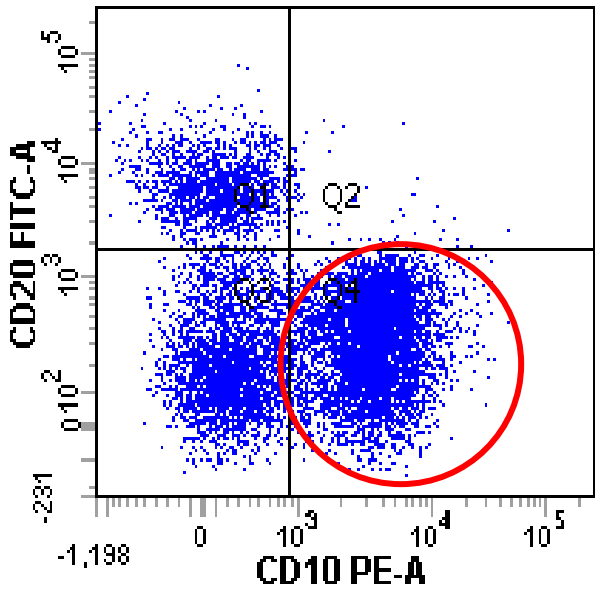
- Postulated COO
  - » Mature CD4+ T_{FH} cell

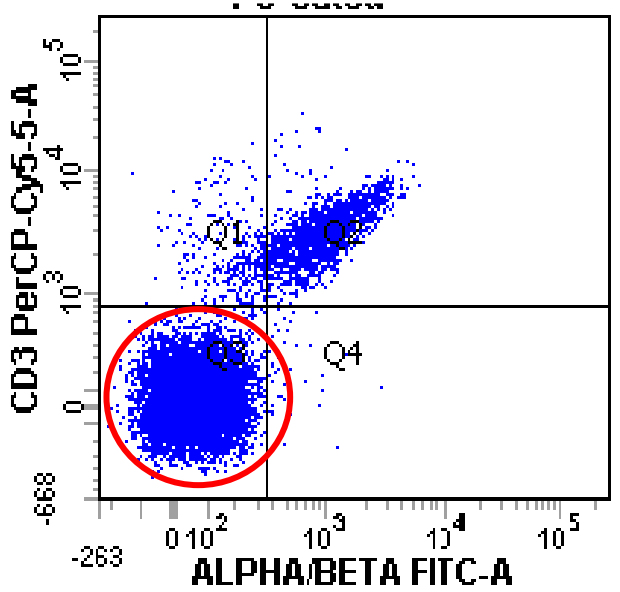
- Karyotype
  - » Gains of 3, 5, X, 18, 19 and loss of 7 - frequent
  - » CGH – 22q, 19, 11p11 gains, 13q losses

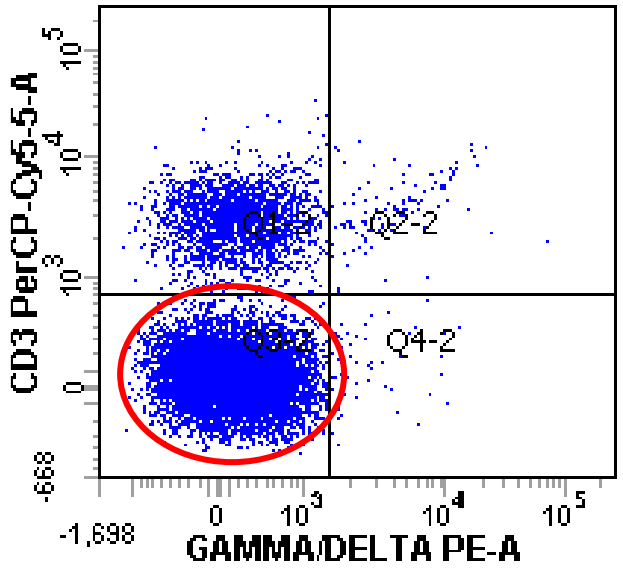
- Mutations
  - » RHOA G17V (50-70%); IDH2 R172 (20-30%), TET2 (50-80%); DNMT3A (20-40%); CD28, FYN kinase (5-10%)

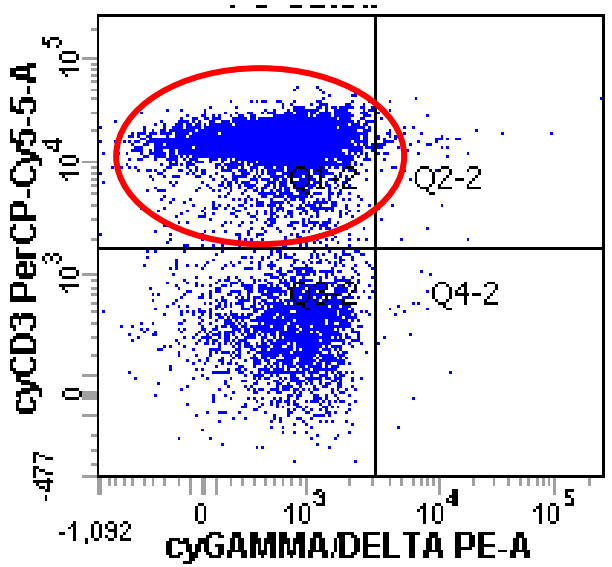
Back to our case…….

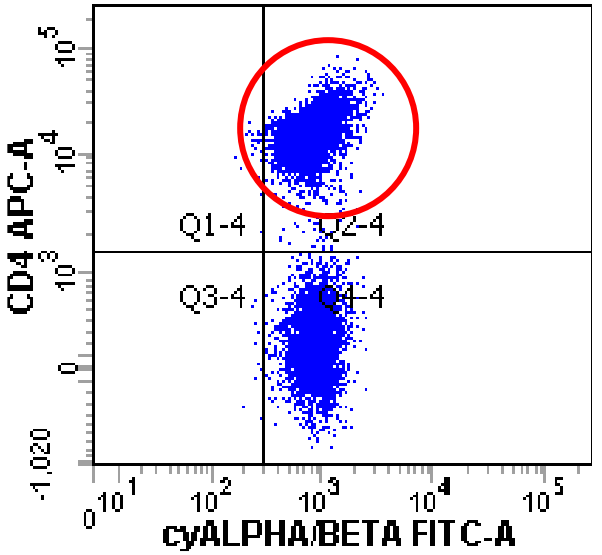

CD19 PE-Cy7-A
CD5 PerCP-Cy5-5-A
Q1
Q2
Q3
Q4


CD20 FITC-A
CD10 PE-A
Q1
Q2
Q3
Q4


CD3 PerCP-Cy5-5-A
ALPHA/BETA FITC-A
Q1
Q2
Q3
Q4


CD3 PerCP-Cy5-5-A
GAMMA/DELTA PE-A
Q1-2
Q2-2
Q3-2
Q4-2


cyCD3 PerCP-Cy5-5-A
cyGAMMA/DELTA PE-A
Q1-2
Q2-2
Q3-2
Q4-2


CD4 APC-A
cyALPHA/BETA FITC-A
Q1-4
Q2-4
Q3-4
Q4-4

PHENOTYPE OF HODGKIN/REED-STERNBERG LIKE CELLS (HRS)

Positive

CD20
PAX5 (dim)
CD79a
OCT2
CD30
CD15
MUM1

Negative

T-cell markers
EBER
TIA-1
Peforin
Granzyme-B

PHENOTYPE OF HODGKIN/REED-STERNBERG LIKE CELLS (HRS)

Positive

CD20
PAX5 (dim)
CD79a
OCT2
CD30
CD15
MUM1

Negative

T-cell markers
EBER
TIA-1
Peforin
Granzyme-B

PHENOTYPE OF T-CELLS

Positive

cyCD3
CD4
cyTCR beta
CD2
CD5 (abnormally bright)
CD10 (dim)

Negative

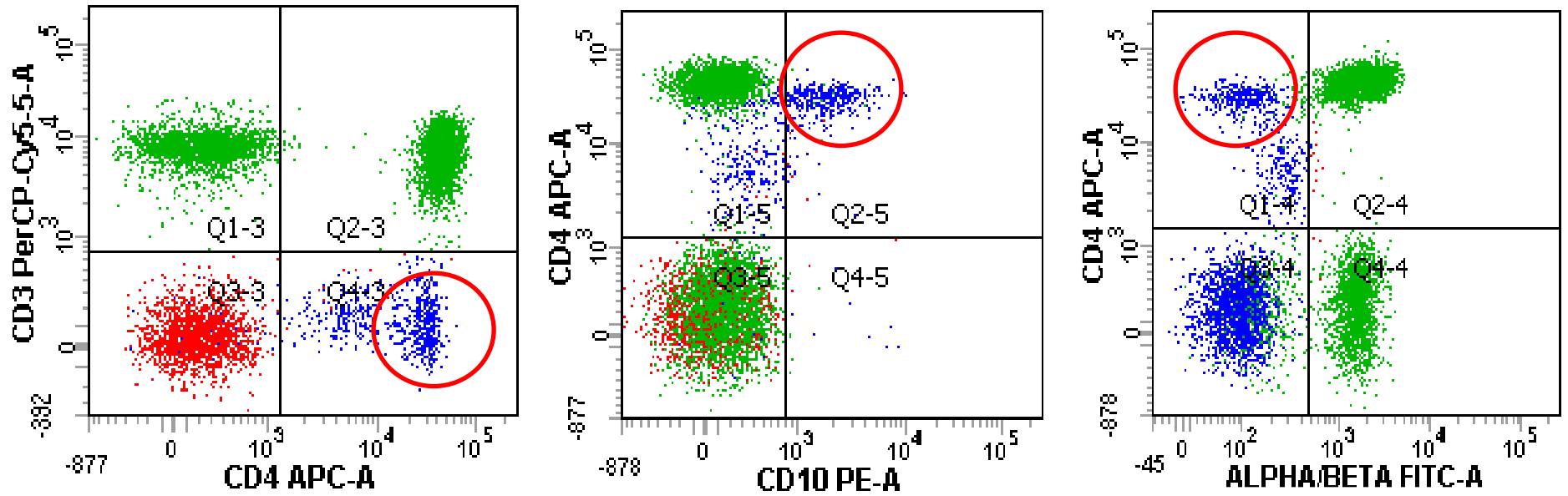
Surface CD3
CD7
CD8
TCR beta surface
TCR gamma surface
TCR gamma cyto
CD56 and CD16
CD57
TIA, Perforin and Granzyme
CD19 and CD20

PCR for T-cell gene rearrangement - Monoclonal T-cell rearrangement

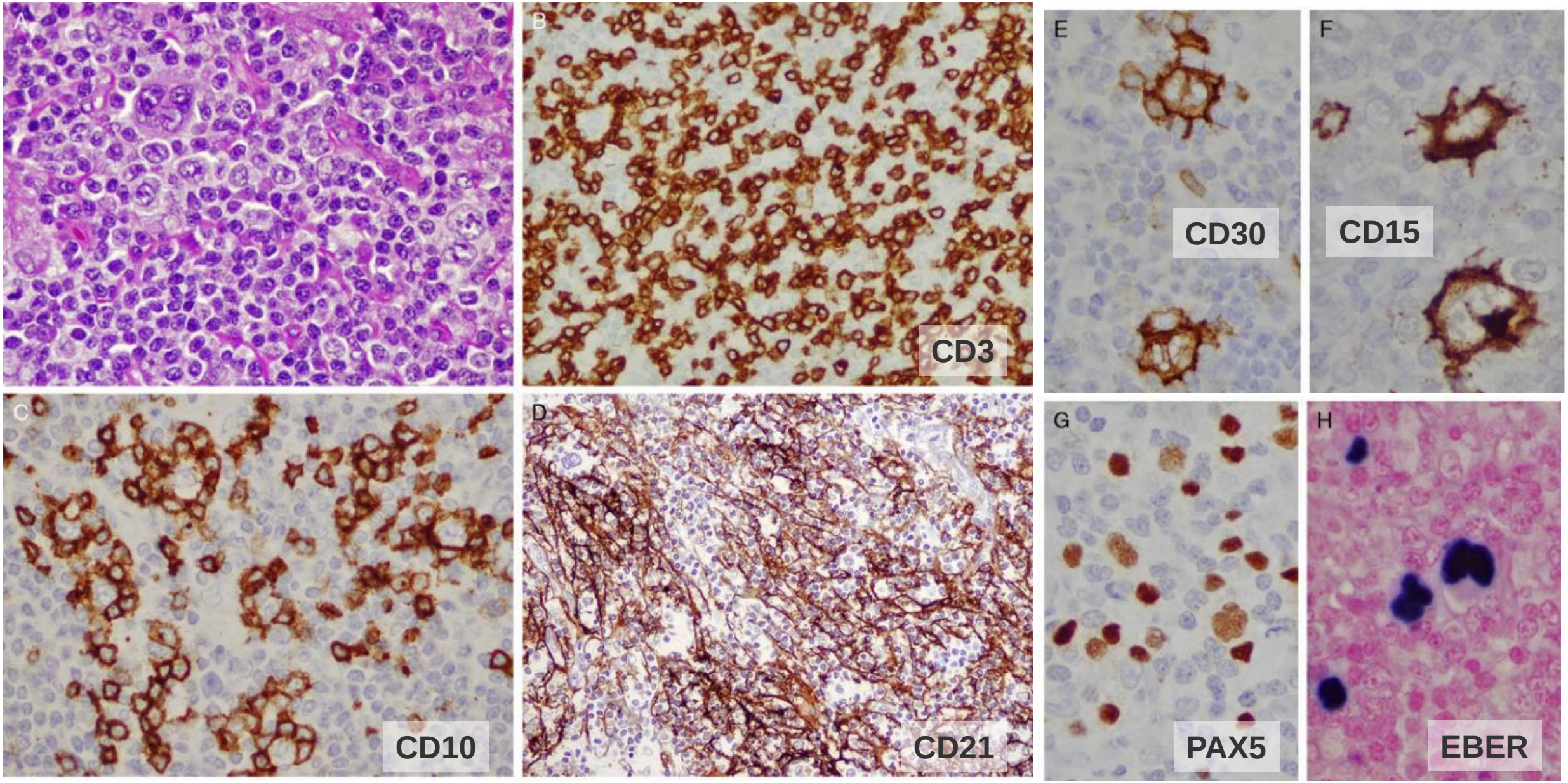
FINAL DIAGNOSIS

Angioimmunoblastic T-cell Lymphoma (AITL)

BONE MARROW BIOPSY



Bone marrow biopsy demonstrated minor involvement by AITL



Nicolae A et al. Am J Surg Pathol. 2013 Jun;37(6):816-26

Hodgkin/Reed-Sternberg (HRS) like cells in T-cell lymphomas

- EBV+ HRS like cells can be seen in various T-cell lymphomas including AITL, PTCL and Adult T-cell leukemia/lymphoma (1,2)
- EBV negative HRS like cell type is much less common (approximately 8% of all T-cell lymphomas with HRS like cells) (5/57 cases) (2)
- EBV neg type almost exclusively in T-cell lymphoma of follicular helper type (TFH type) (2)
- No statistical difference in prognosis (EBV+ vs. negative)

1. Quantanilla-Martinez et al., AJSP, 1989, 2. Nicolae et al., AJSP, 2013,

Hodgkin lymphoma vs. T-cell lymphoma

- AITL could be easily misdiagnosed as Classic Hodgkin Lymphoma (CHL), mixed cellularity type

- PTCL, follicular type could be easily misdiagnosed as nodular lymphocyte predominant Hodgkin lymphoma or lymphocyte rich CHL, nodular type.

Features favoring T-cell lymphoma OVER Hodgkin lymphoma

- Cytologic atypia of background T-cells
- Lack of typical inflammatory milieu (small lymphocytes, eosinophils, histiocytes etc.) seen in CHL *(caveat is clusters of histiocytes or eosinophils can also be seen in T-cell lymphomas AND lymphocyte rich CHL typically lacks the inflammatory milieu)*
- Expansion of FDC meshworks beyond B-cell areas
- Clusters and sheets of TfH cells (CD10, PD1, BCL6, ICOS, CXCL13 etc.) (i.e. beyond just PD-1 positive rosettes!!)
- Especially look for CD10+ rosettes (not seen in Hodgkin lymphoma)

Features favoring T-cell lymphoma OVER Hodgkin lymphoma

- Immunophenotypic aberrancy or loss of T-cell markers
- Retention of B-cell program in HRS like cells (Positive for CD20, OCT2, BOB1, CD79a and PAX5 *(caveat; these cells can express both CD30 and CD15)*
- PCR for T-cell gene rearrangement: clonal rearrangement in T-cell lymphomas
- Look for characteristic RHOA or IDH2 mutations
- Flow cytometry : Look for CD3 dim/neg, CD4+ T cell population; minimal flow panel consisting of CD3, CD4, CD5, CD10 and CD14 is recommended *(Alikhan et al., Mod. Path, 2016 and Serke et al., Cytometry, 2000)*

Follicular Helper T-cell lymphoma, Follicular type (ICC)

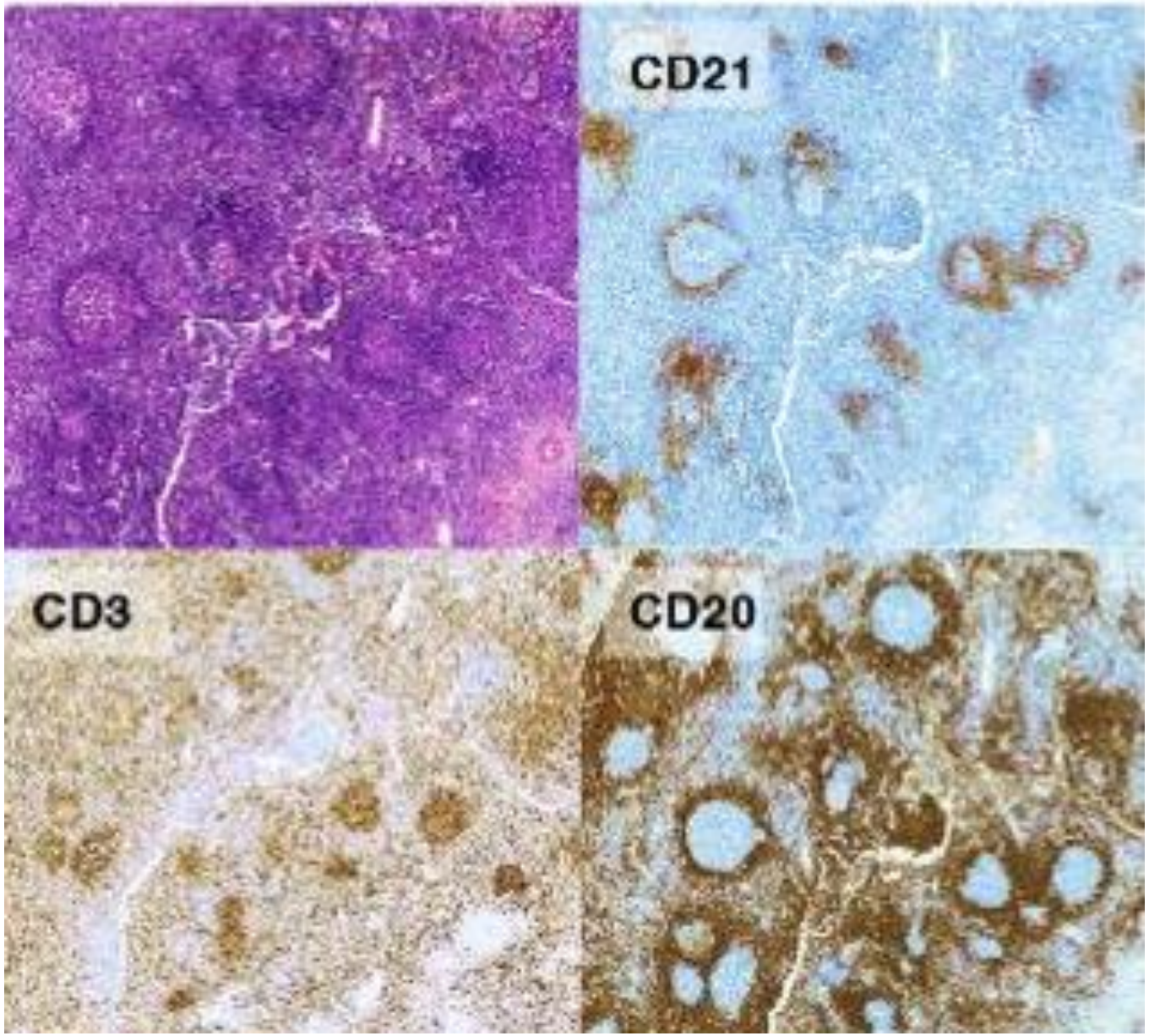
Nodal T-Follicular Helper lymphoma, Follicular type (WHO 5)

TFH lymphoma, follicular type

- 1.8-2.6% of non-cutaneous peripheral T-cell lymphomas
- Clinical features similar to AITL
- Two morphologic subtypes

  1) Follicular lymphoma type

  2) PTGC type
- Lack of HEV hyperplasia, polymorphous infiltrate, and FDC meshwork expansion beyond nodules
- CAVEAT! Pure TFH, follicular type is RARE; occurs in combination with AITL
- Similar immunophenotype to AITL
- Similar mutational profile and genetics to AITL; t(5;9)(q33;q22)/*ITK::SYK in a subset*



Dobay et al, Hematologica, 2017

TFH lymphoma, follicular lymphoma like

The main differential based on morphology is Follicular (B-cell) lymphoma!

Immunostains should reveal that the follicles are composed of T-cells

The American journal of surgical pathology

Author Manuscript HHS Public Access

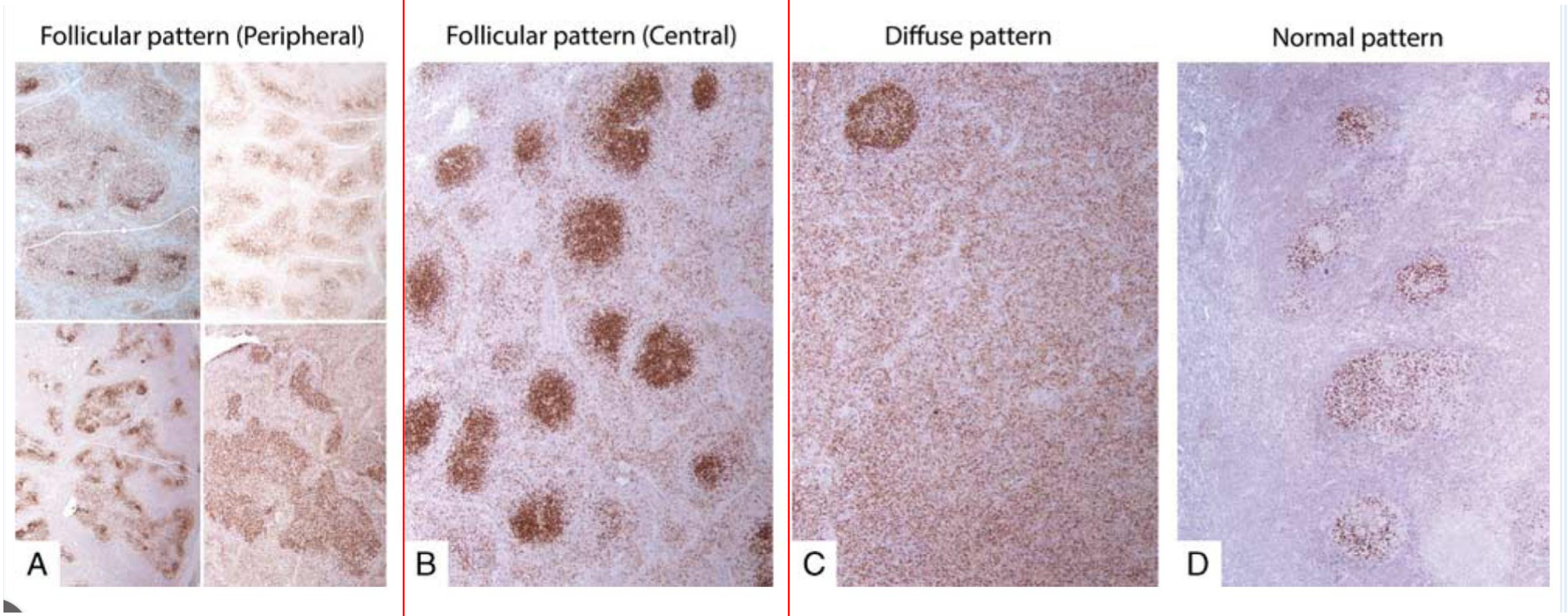
Expansion of PD1-positive T Cells in Nodal Marginal Zone Lymphoma

A Potential Diagnostic Pitfall

Caoimhe Egan, MB, BCh, BAO, Camille Laurent, MD, PhD, [...], and

Elaine S. Jaffe, MD

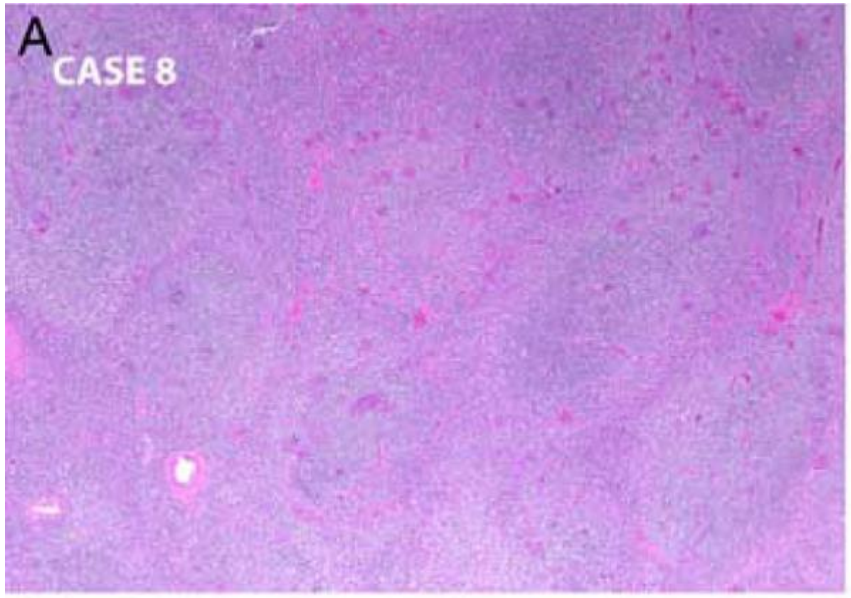
PD-1 PATTERNS IN NODAL MARGINAL ZONE LYMPHOMA

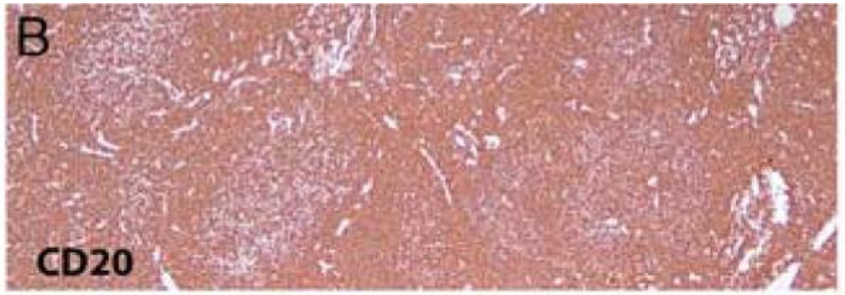


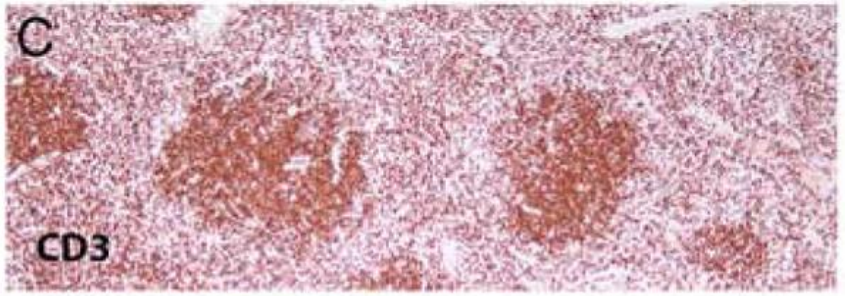
33% 18% 8% 33%

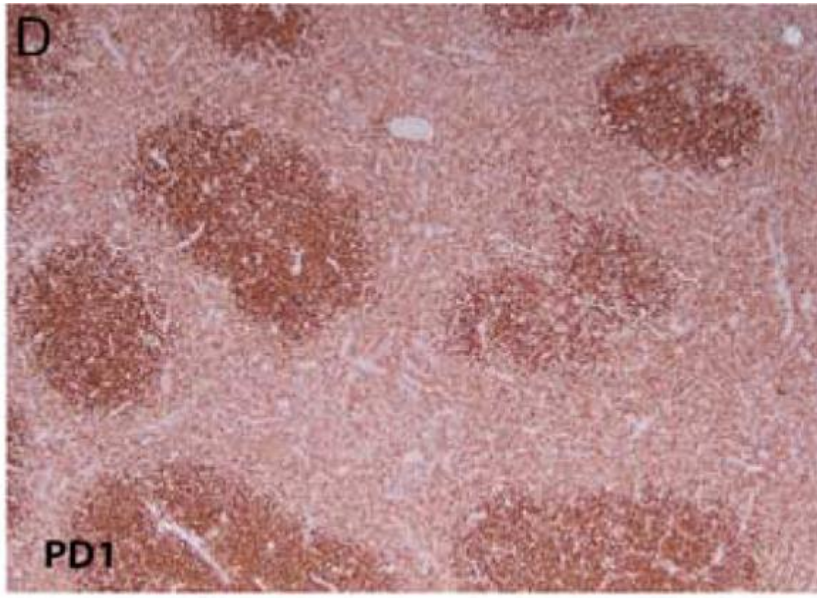
PD-1 PATTERNS IN NODAL MARGINAL ZONE LYMPHOMA

MARGINAL ZONE LYMPHOMA (CENTRAL FOLLICULAR PATTERN)



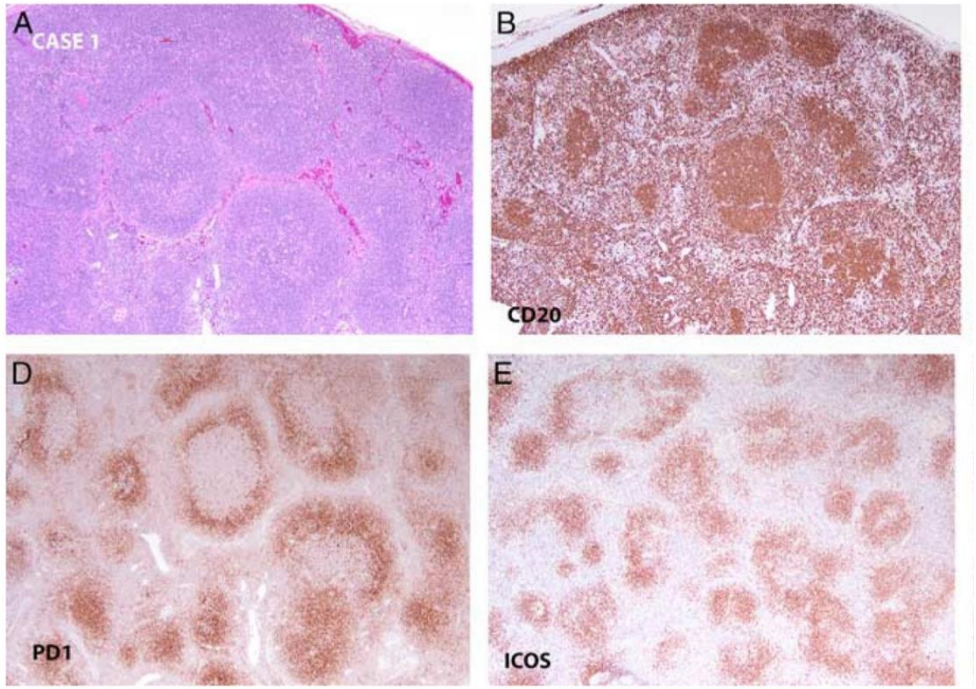




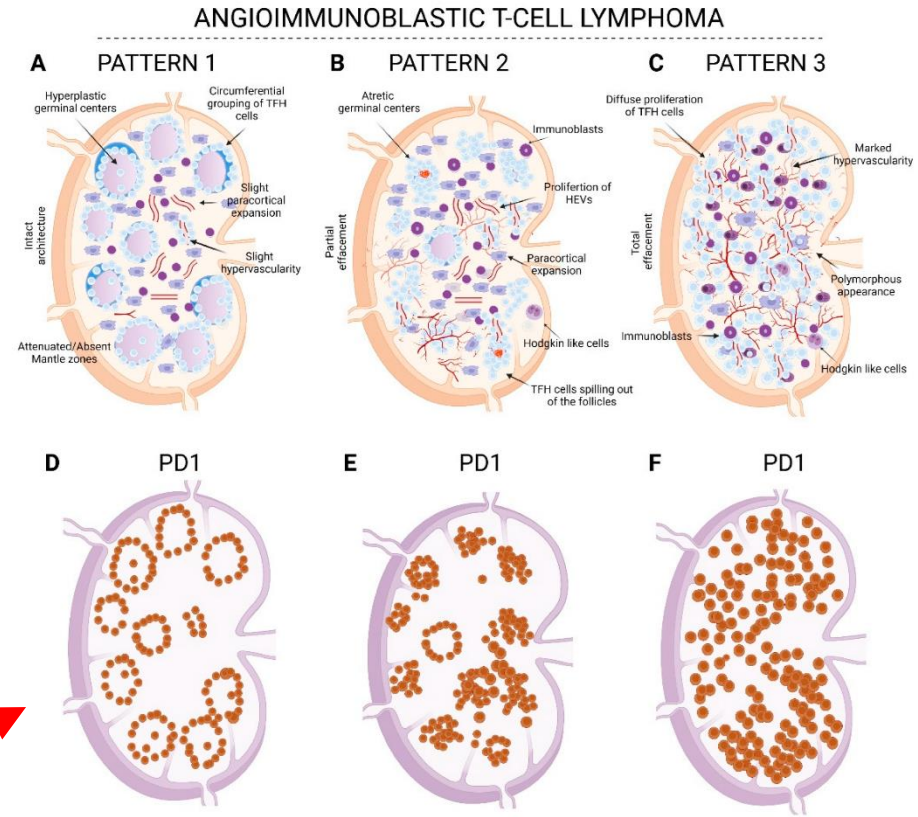


PD-1 PATTERNS IN NODAL MARGINAL ZONE LYMPHOMA

MARGINAL ZONE LYMPHOMA, Peripheral follicular pattern



Egan et al, AJSP, 2020



Ganapathi, Karner, Menon, 2022

MARGINAL ZONE LYMPHOMA	TFH LYMPHOMA
Increased interfollicular B-cells	Not seen
T-cells lack cytologic atypia	Cytologic atypia in T-cells
B-cells: monotypic (flow) T-cells: lack immunophenotypic aberrancies	T-cells: immunophenotypic aberrancies (e.g. flow surface CD3 dim/neg, CD4+, CD10+ etc.)
Clonal IgH rearrangement Polyclonal TRG	Clonal TRG Caveat: Clonal IgH rearrangement or monotypic B/plasma cells can be seen

Can NGS studies help?

LETTER TO THE EDITOR

Mutational Analysis Reinforces the Diagnosis of Nodal Marginal Zone Lymphoma With Robust PD1-positive T-Cell Hyperplasia

Hurwitz, Stephanie N. MD, PhD*; Caponetti, Gabriel C. MD*; Smith, Lauren MD†; Qualtieri, Julianne MD*; Morrissette, Jennifer J.D. PhD*; Lee, Won Sok MD*; Frank, Dale M. MD*; Bagg, Adam MD*

Author Information

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doi: 10.1097/PAS.0000000000001515

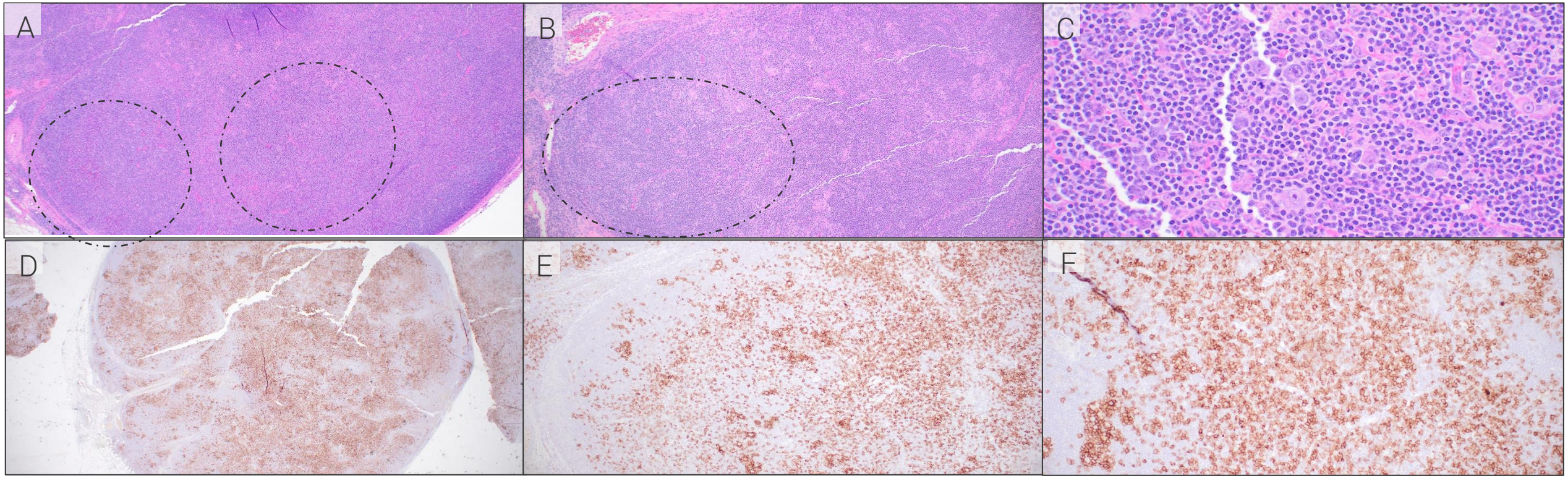
- MZL-associated mutations: *NOTCH2, KLF2, TNAIFP3, TP53, EZH2 etc.*
- *Lack of TFH-lymphoma associated mutations:* *RHOA, IDH2, TET2 etc.*

TFH LYMPHOMA, FOLLICULAR VARIANT (PTGC like)

Nodules

Nodules

Immunoblastic, HRS and LP like cells

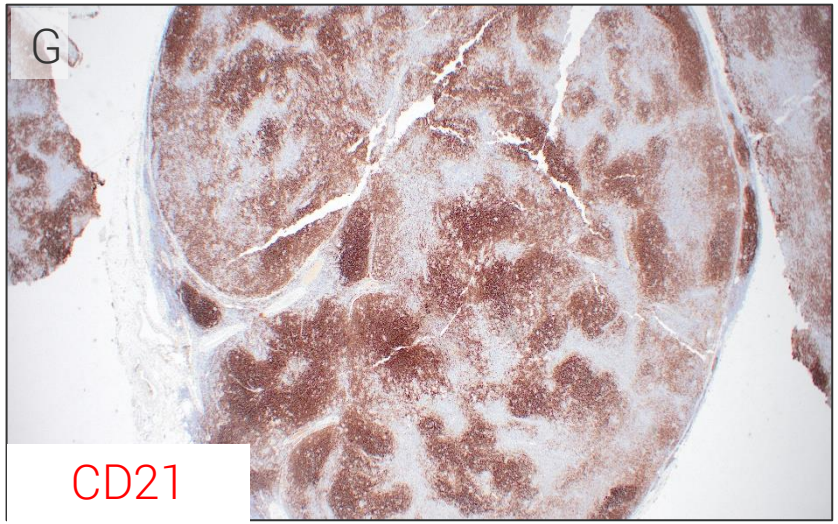


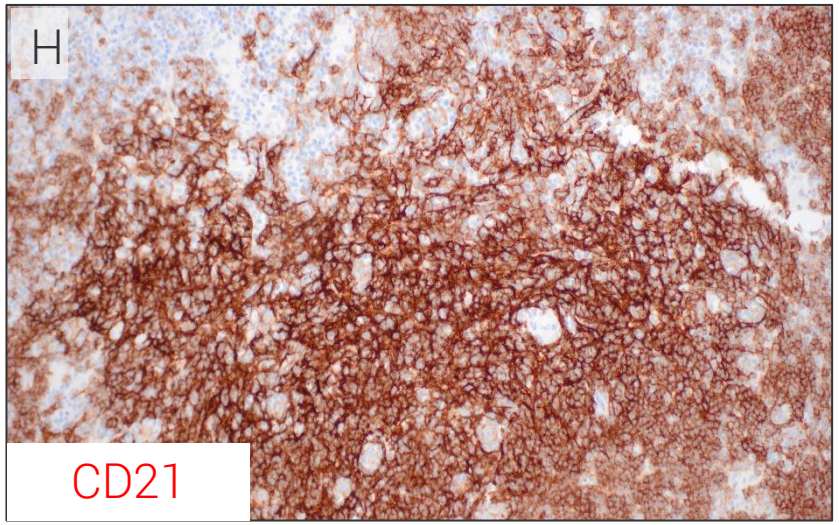
PD-1

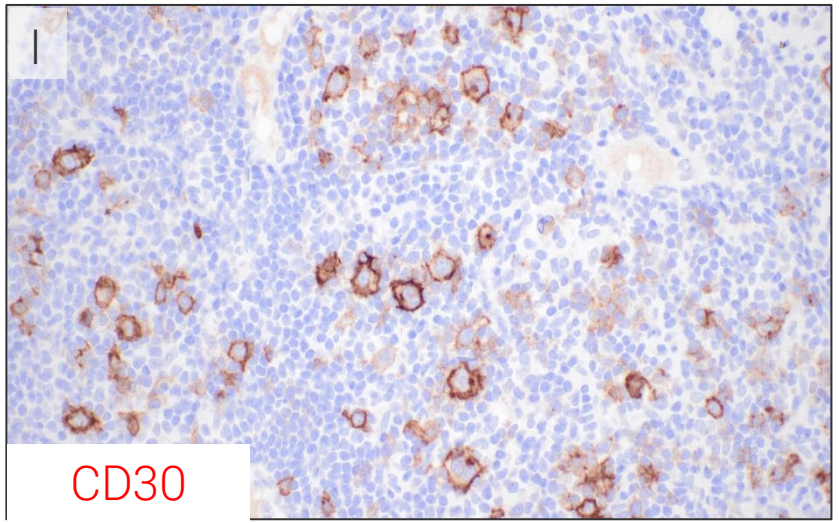
PD-1 rosettes

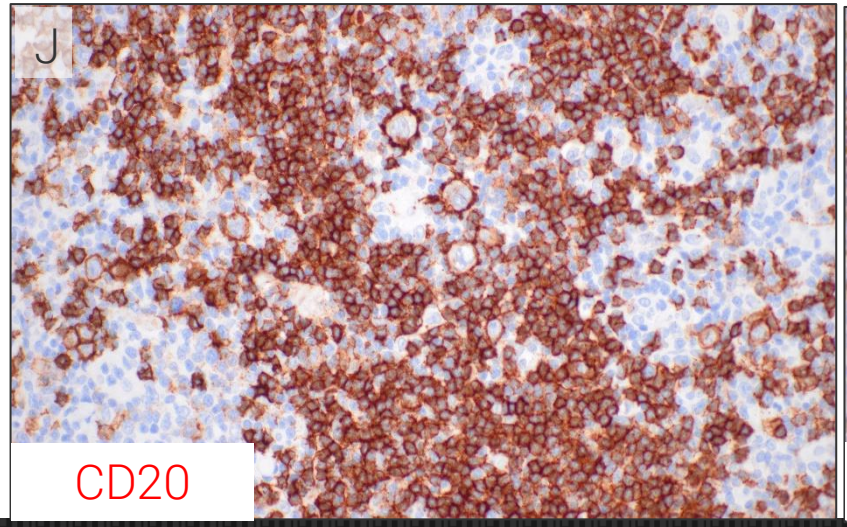
PD-1 marked increased between the nodules

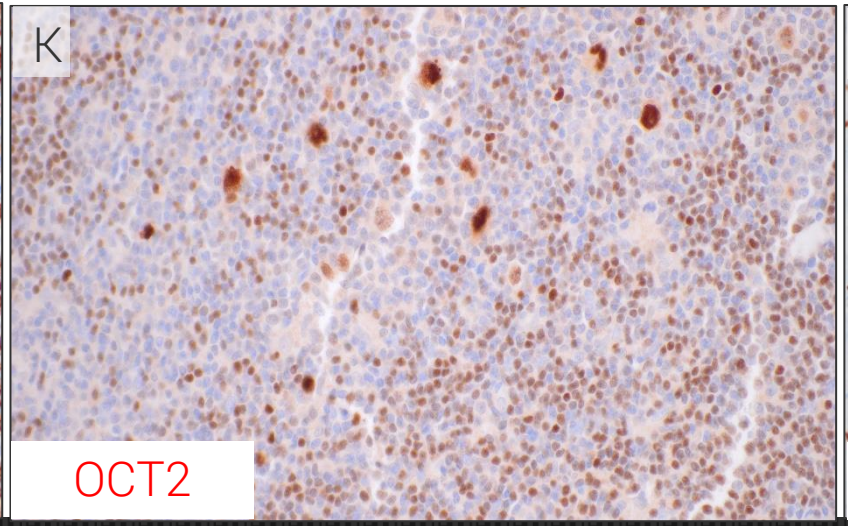
TFH LYMPHOMA, FOLLICULAR VARIANT
(PTGC like)

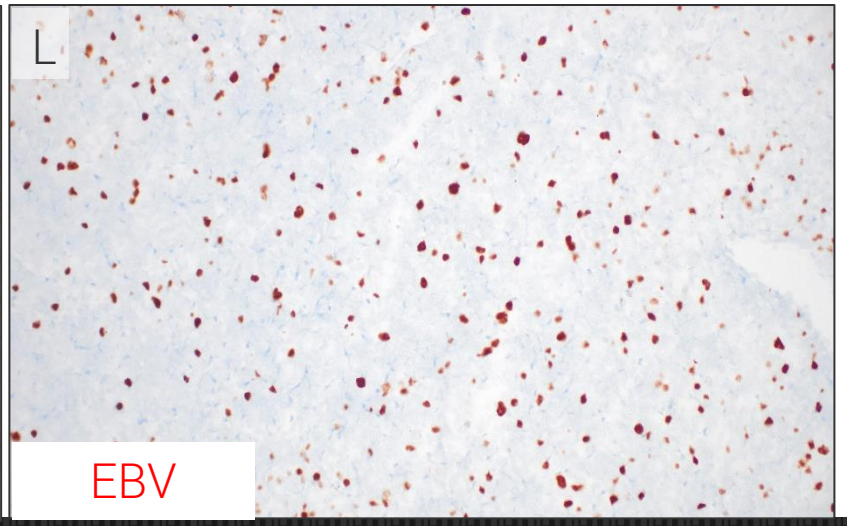












TFH LYMPHOMA, FOLLICULAR TYPE

VS.

PROGRESSIVE TRANSFORMATION OF GERMINAL CENTER

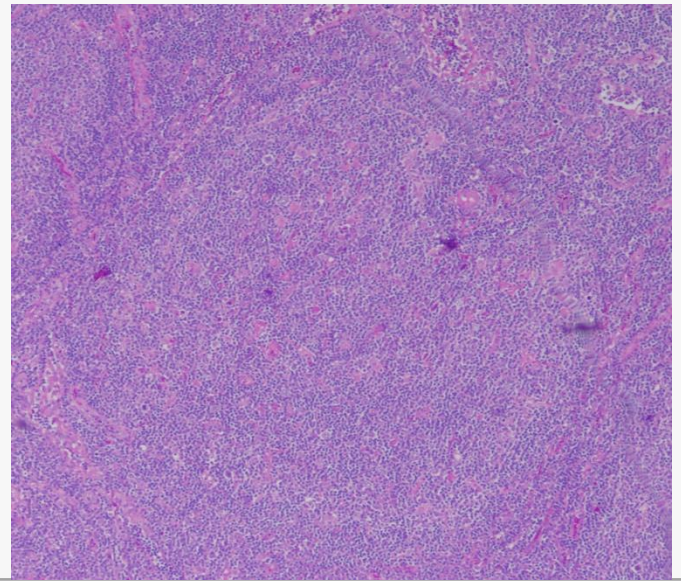
VS.

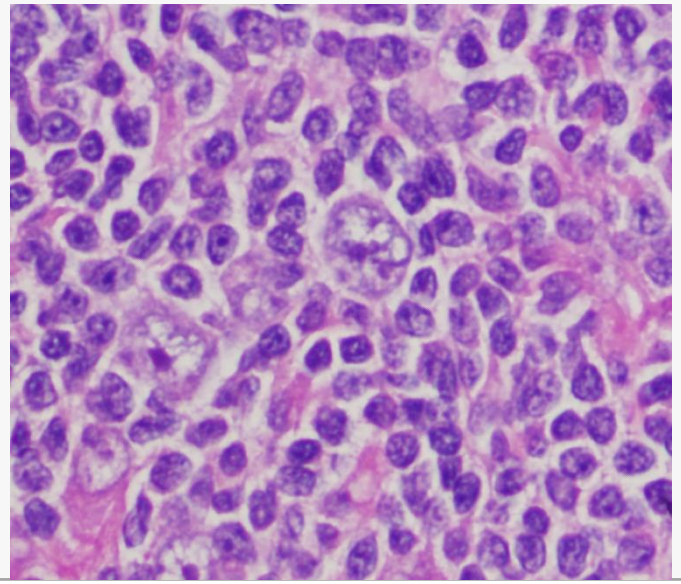
NODULAR LYMPHOCYTE PREDOMINANT B-CELL LYMPHOMA

VS.

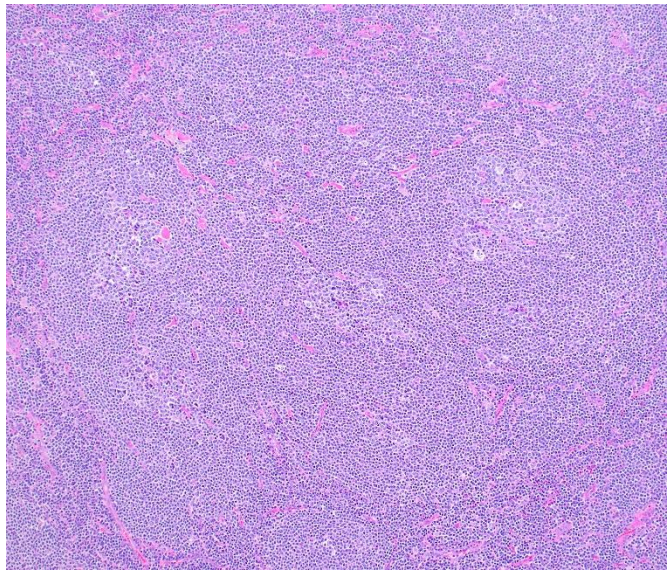
LYMPHOCYTE RICH CLASSIC HODGKIN LYMPHOMA

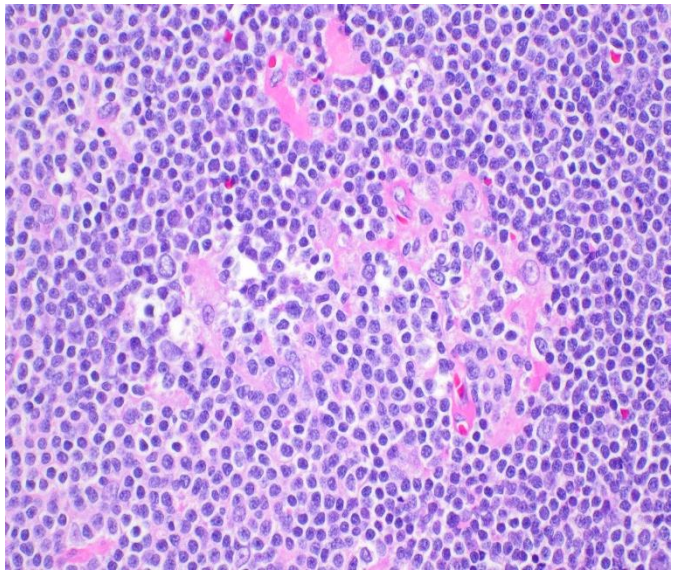
NLPHL VS. PTGC





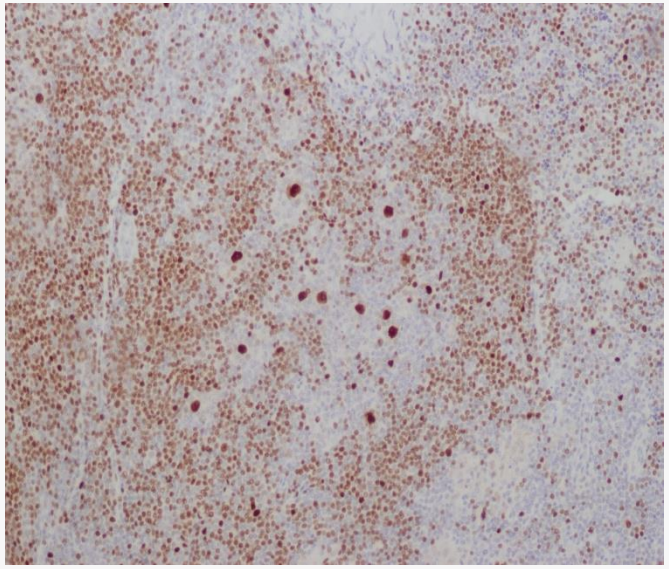
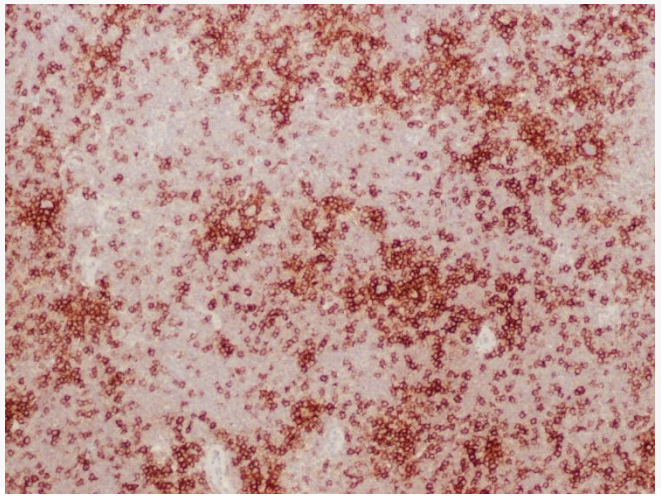
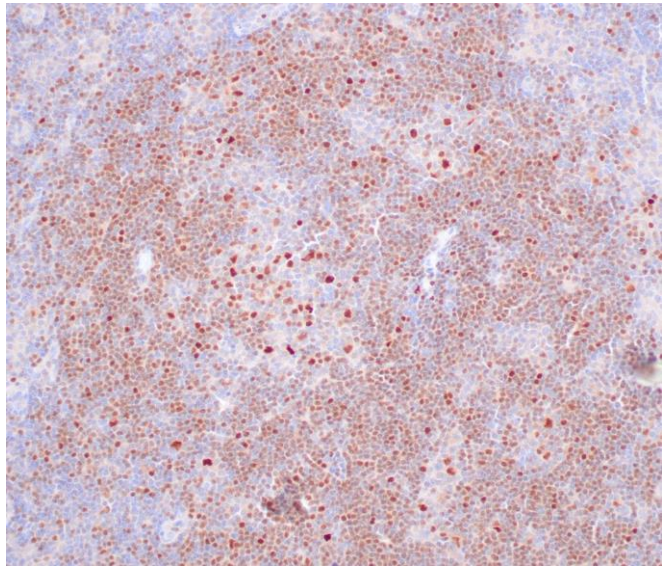
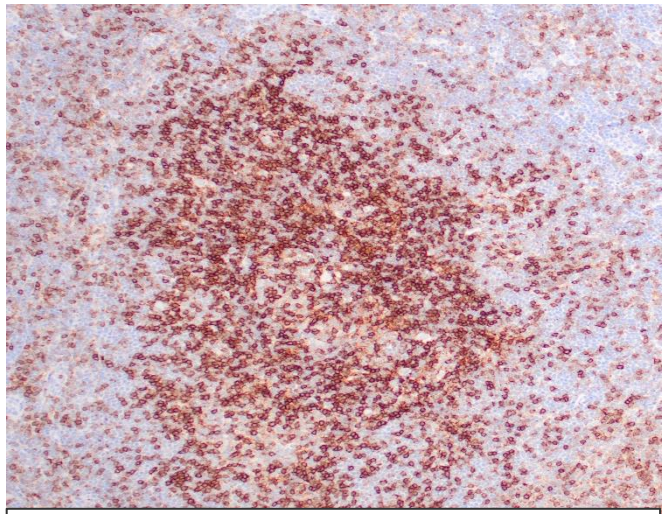
NLPHL





PTGC

NO Popcorn/LP cells

	OCT2	PD-1	
		 PD-1 rosettes	NLPHL
		 Increased PD-1, but no rosettes	PTGC

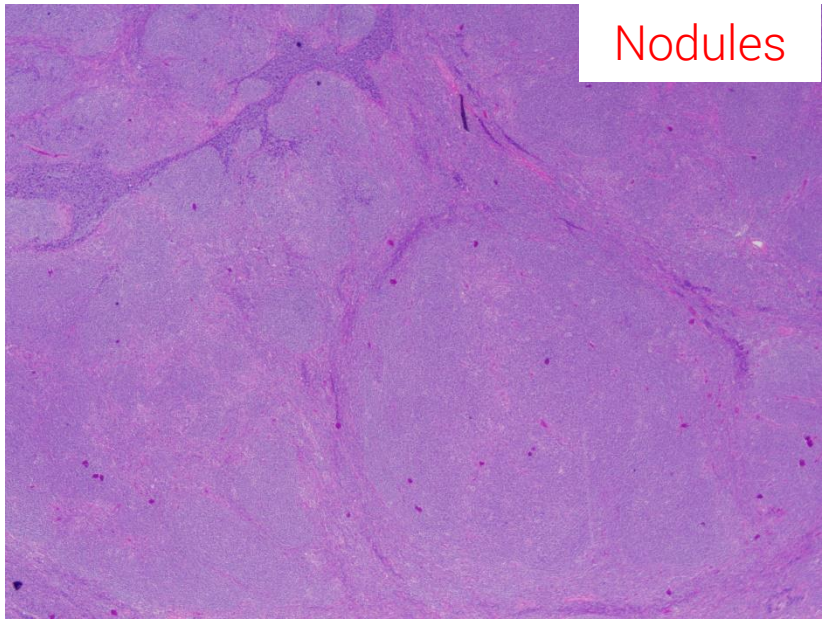
HOW ARE PD-1 POSITIVE T-CELL ROSETTES HELPFUL?

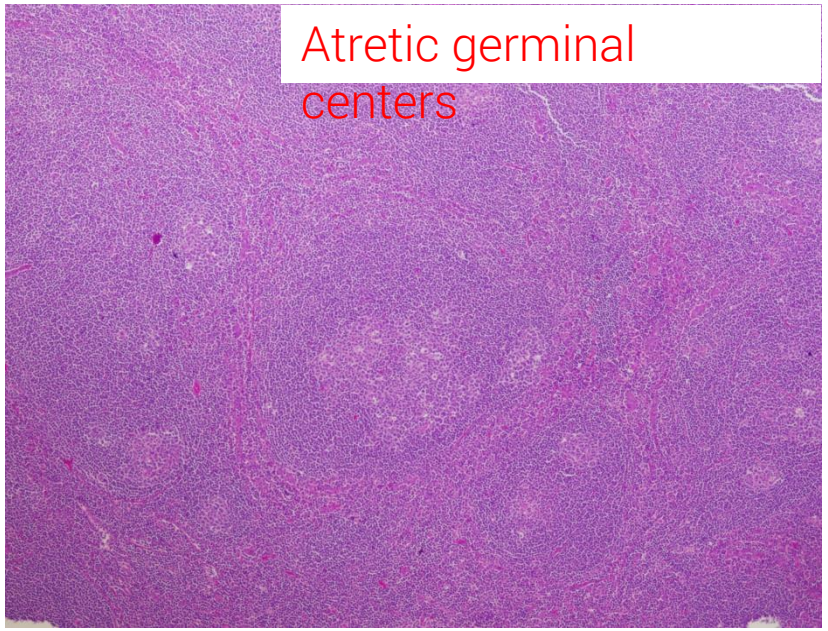
PD-1 rosettes

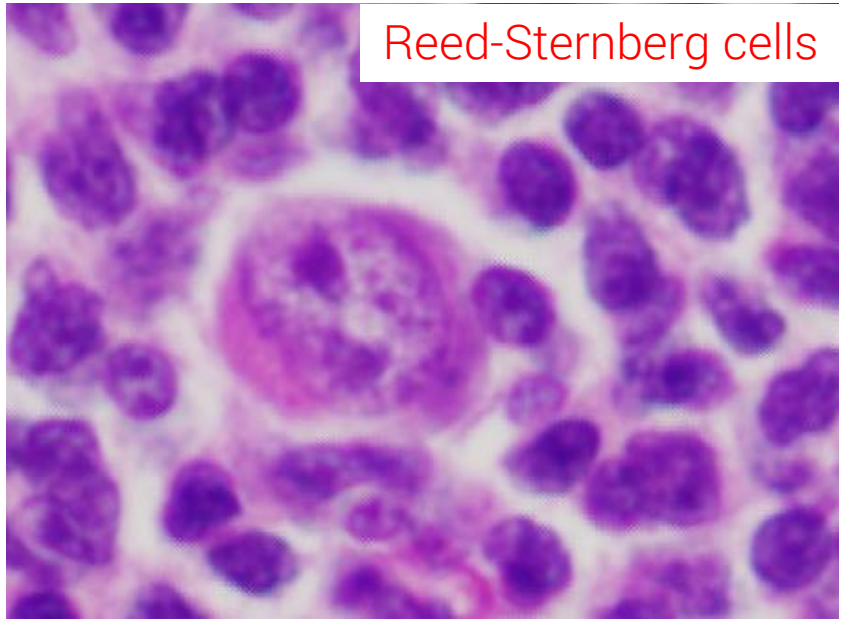
- Nodular lymphocyte predominant (Hodgkin) lymphoma
- Lymphocyte-rich classic Hodgkin lymphoma
- Follicular helper T-cell lymphoma
- Viral lymphadenitis especially Infectious mononucleosis

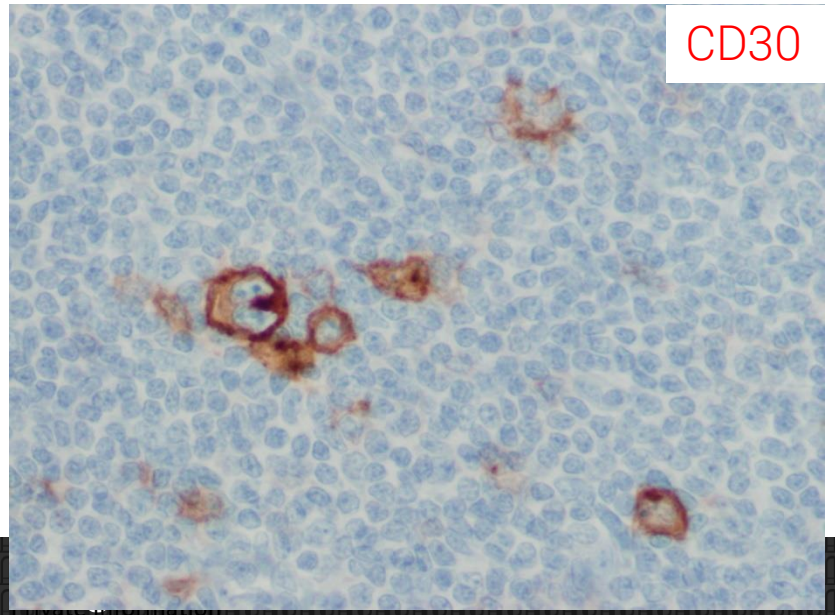
PD-1 rosettes typically absent in PTGC

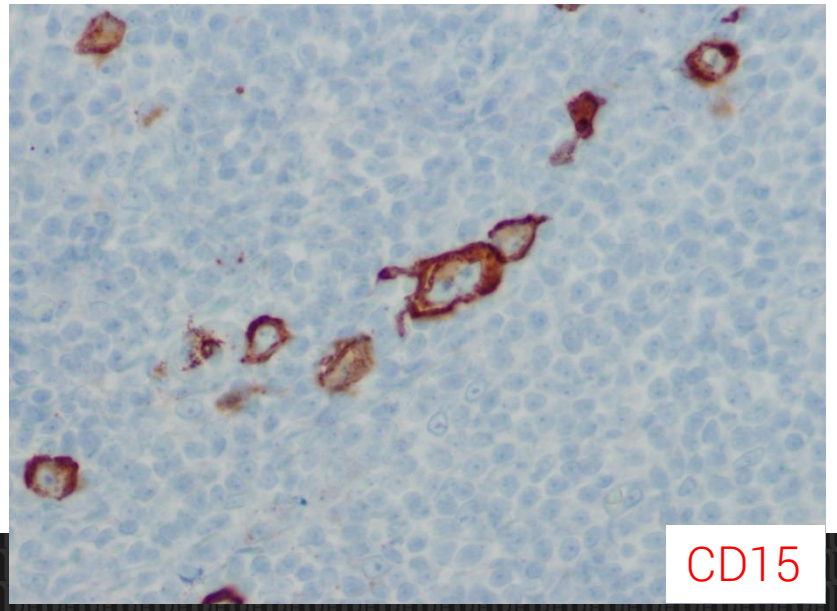
Lymphocyte-rich classic Hodgkin Lymphoma (LRCHL)

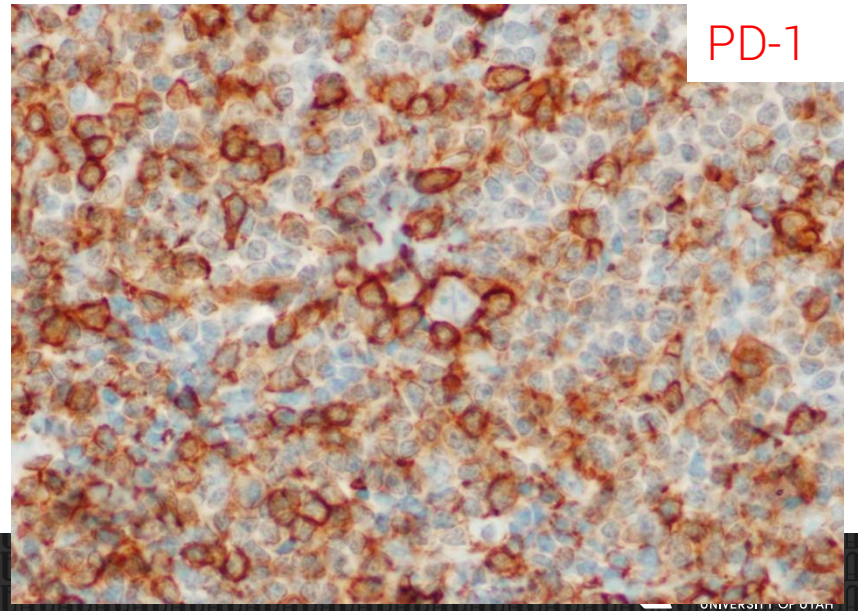












TFH LYMPHOMA, FOLLICULAR VARIANT

- Follicular lymphoma like pattern or PTGC-NLPHL like pattern
- Features favoring Follicular T-cell lymphoma over LRCH and NLPHL:
  - **Clinical**: Typical clinical features (diffuse lymphadenopathy, hepatosplenomegaly, skin rashes, B-symptoms etc.)
  - **Age**: middle age and older patients

FEATURES FAVORING FOLLICULAR T-CELL LYMPHOMA OVER NLPHL AND LRCHL

- Cytologic atypia of background T cells
- Immunophenotypic T-cell aberrancies
- Demonstrable TFH phenotype (PD-1, ICOS, CXCL13, BCL-6 and CD10) (2 out of 3 or 3 out of 5 markers)
- Clusters and sheets of TfH cells (CD10, PD1, BCL6, ICOS, CXCL13 etc.) (i.e. beyond just PD-1 positive rosettes!!)
- CD10 + T-cell rosettes

FEATURES FAVORING FOLLICULAR T-CELL LYMPHOMA OVER NLPHL AND LRCHL

- **HRS-like and LP-like cells**: CD30 pos +/- CD15
- **HRS and LP-like cells: retain** B-cell phenotype in most cases (CD20, OCT2, BOB1, CD79a, and PAX5) *(caveat; these cells can downregulate CD20 and PAX5)*
- ***HRS and LP like cells:*** *EBV+, sometimes EBV neg*
- CD30 staining in neoplastic T-cells (helpful feature)

- Flow cytometry : Look for CD3 dim/neg, CD4+ T cell population; minimal flow panel consisting of CD3, CD4, CD5, CD10 and CD14 is recommended

(Alikhan et al., Mod. Path, 2016 and Serke et al., Cytometry, 2000)

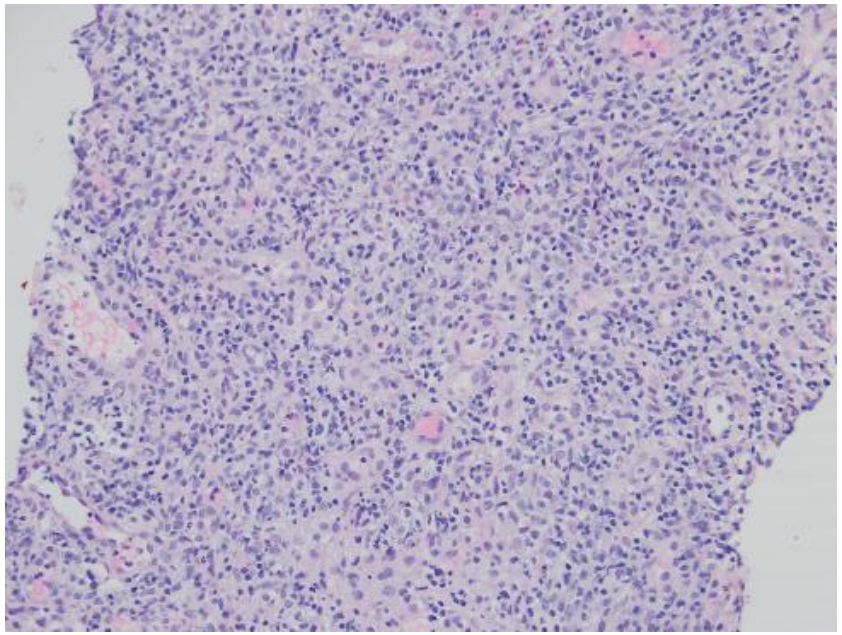
- PCR: Clonal T-cell rearrangement
- CYTOGENETICS/FISH: t(5;9) (*ITK-SYK*) (20% cases)
- NGS: RHOA (Gly17Val), TET2, DNMT3A, IDH2 (AITL)

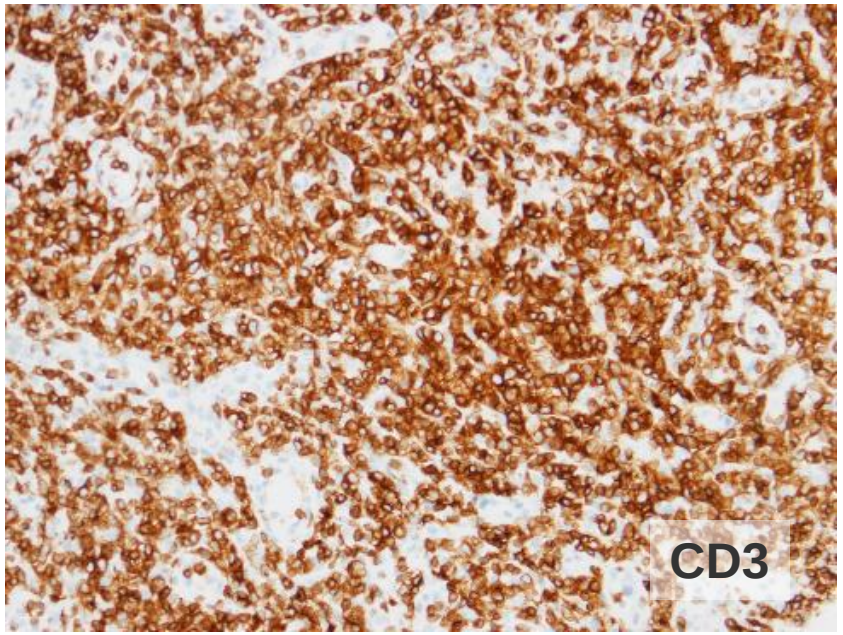
Follicular Helper T-cell lymphoma, NOS (ICC)

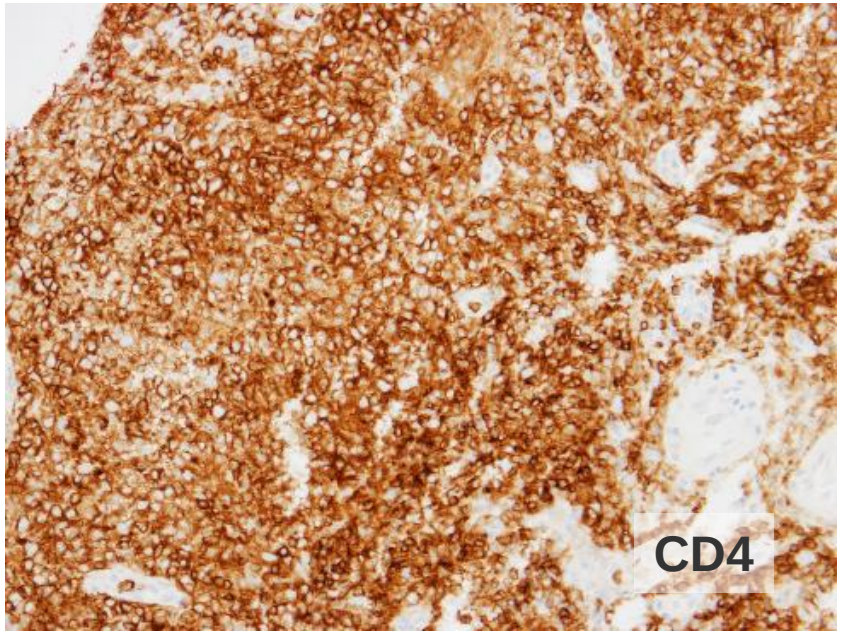
Nodal T-Follicular Helper lymphoma, NOS (WHO 5)

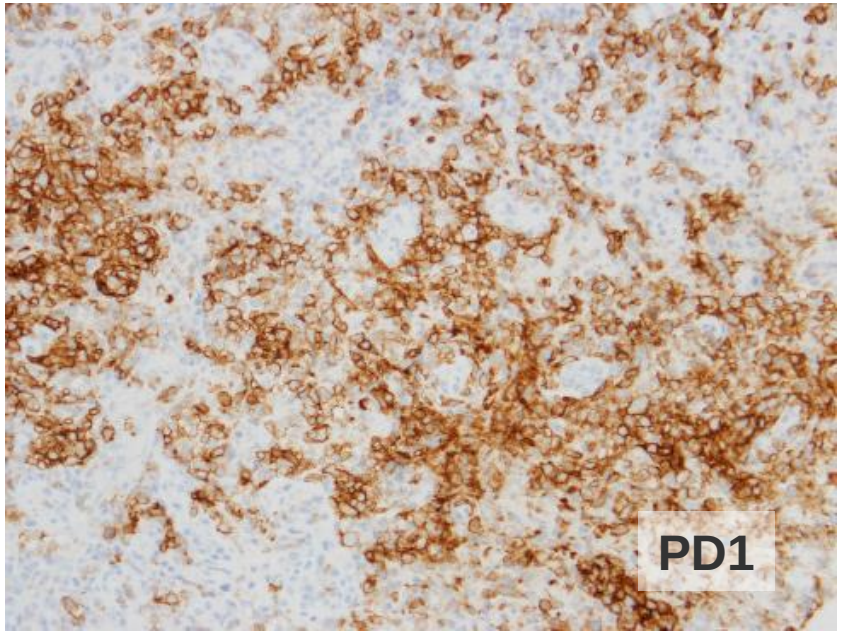
Follicular T-cell lymphoma, NOS

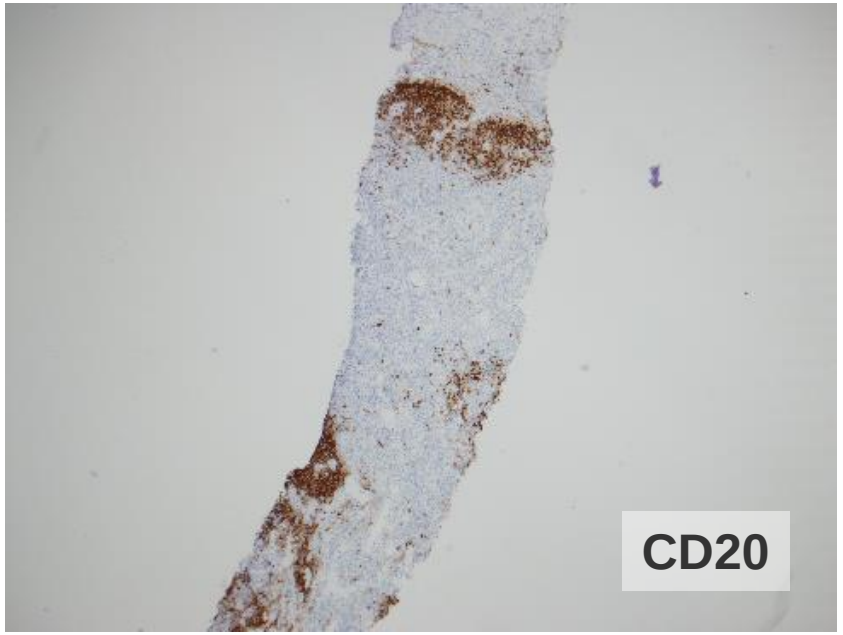
- Diagnosis assigned to nodal T-cell lymphomas of TFH origin lacking the clinicopathologic features of AITL or Follicular variant
- Morphologically closer to PTCL (effaced or T-zone variants), NOS but express TFH markers
- No vascular proliferation or expanded FDC meshworks
- Comprise a significant subset of PTCL, NOS (~ 30%)
- Good practice to perform TFH markers in suspected PTCL, NOS to identify these cases
- They can relapse as AITL and conversely AITL can relapse as PTCL-TFH
- Show similar mutational spectrum and gene expression pattern as AITL

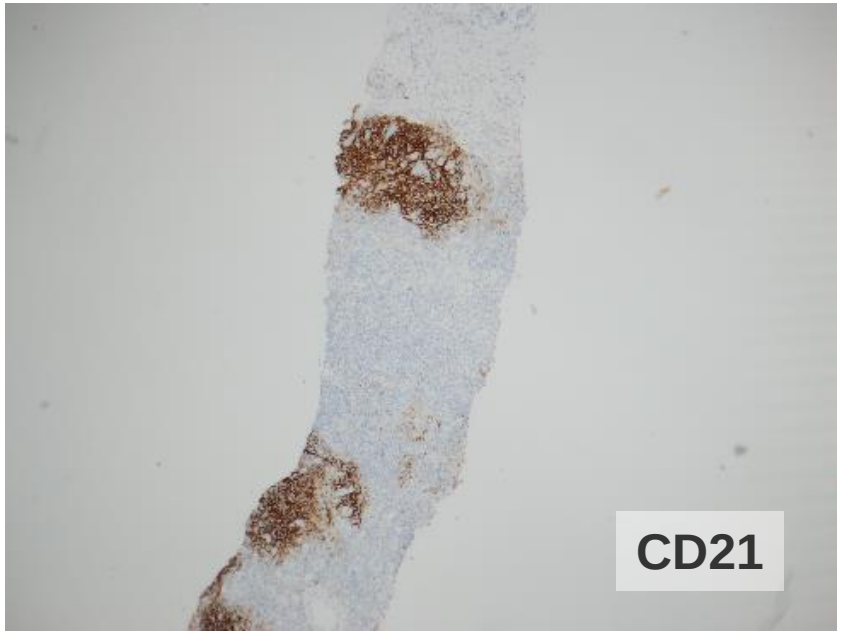



CD3


CD4


PD1


CD20


CD21

Reactive lymphadenopathies with ABNORMAL PD-1 pattern

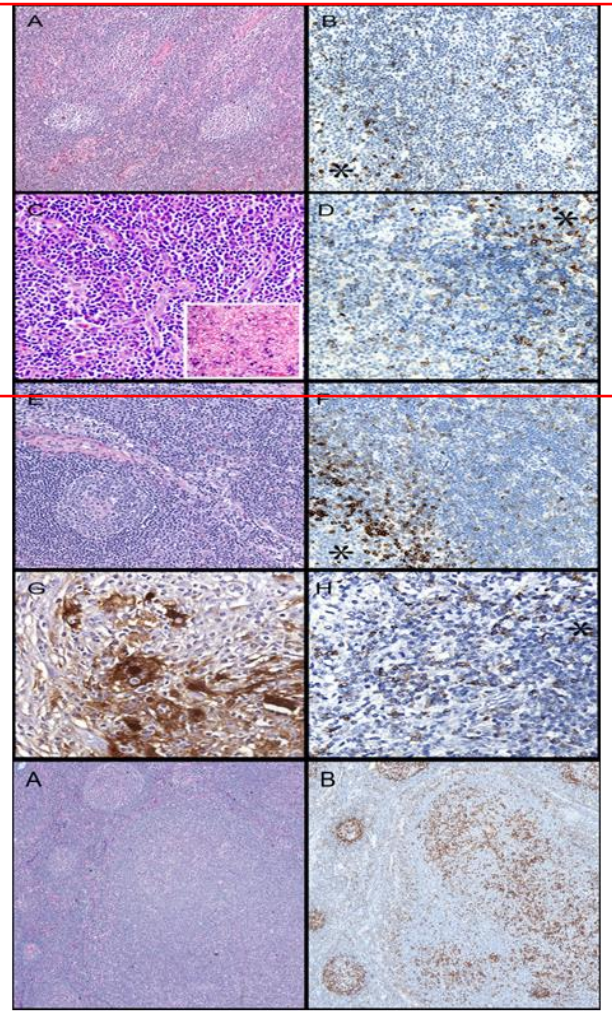
Atypical paracortical hyperplasia

Infectious mononucleosis

HIV lymphadenopathy

Rosai-Dorfman disease

Progressive transformation of germinal centers (PTGC)



PD-1

Strong PD-1 positive cells mostly restricted to germinal centers or mantle zone

Increased PD-1 positive outside of the follicles

Krishnan et al, AJSP, 2010

Expansion of reactive TFH cells

- Various reactive lymphadenopathies
- Classic Hodgkin Lymphoma
- Nodular Lymphocyte Predominant Hodgkin Lymphoma
- Marginal zone lymphoma
- Follicular Lymphoma

CONCLUSIONS

- TFH lymphomas constitute one of the largest subtypes of nodal T-cell lymphomas
- Broad morphologic spectrum with a wide range of tumor cell content
- Potential to be misdiagnosed and a source of difficulty for pathologists
- A combined clinical, morphologic, immunophenotypic, and molecular/genetic approach is needed to arrive at a correct diagnosis