Penn Medicine

Multi-Institutional Hematopathology Interesting Case Conference

09.24.2025

Giby V. George, MD
Fellow, Hematopathology

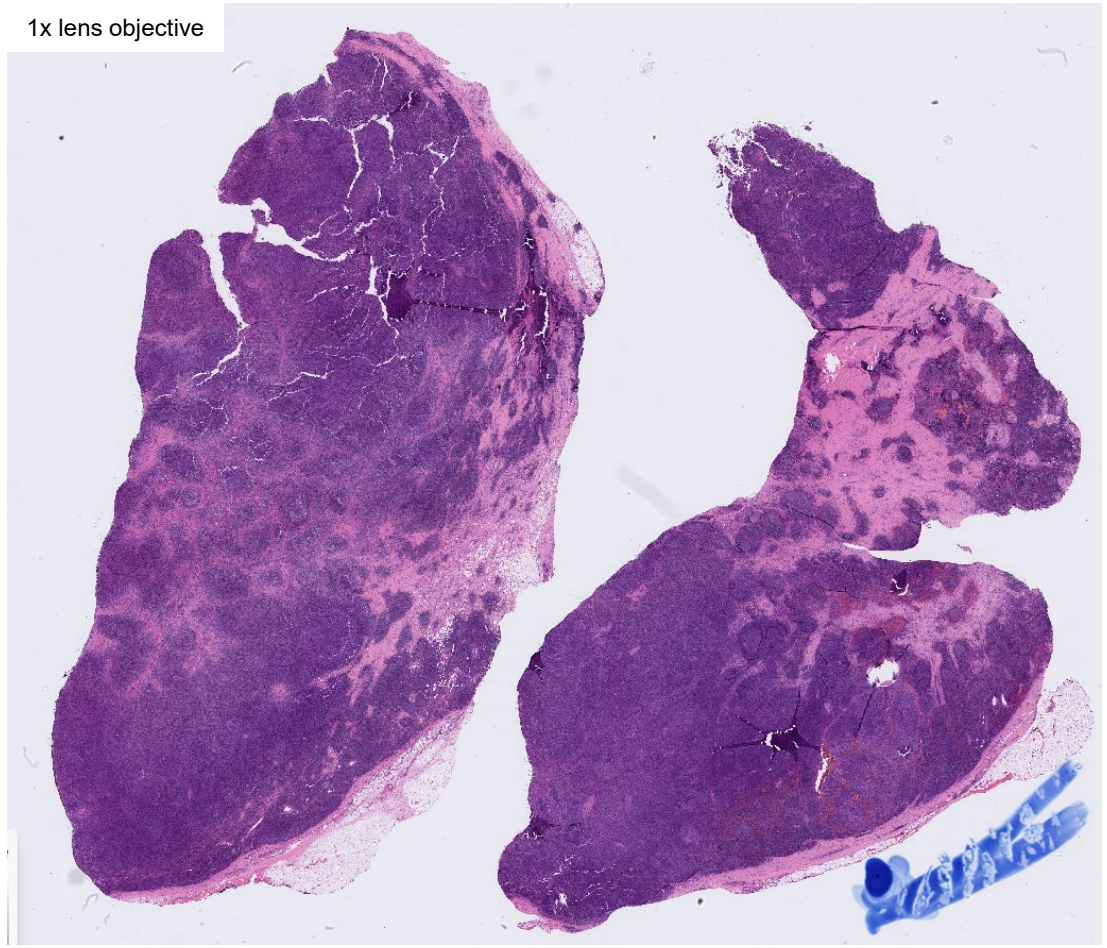
Outline

- Brief clinical history
- Histomorphology
- Immunophenotype
- Final diagnosis
- Genomic studies
- Case discussion and review of the literature

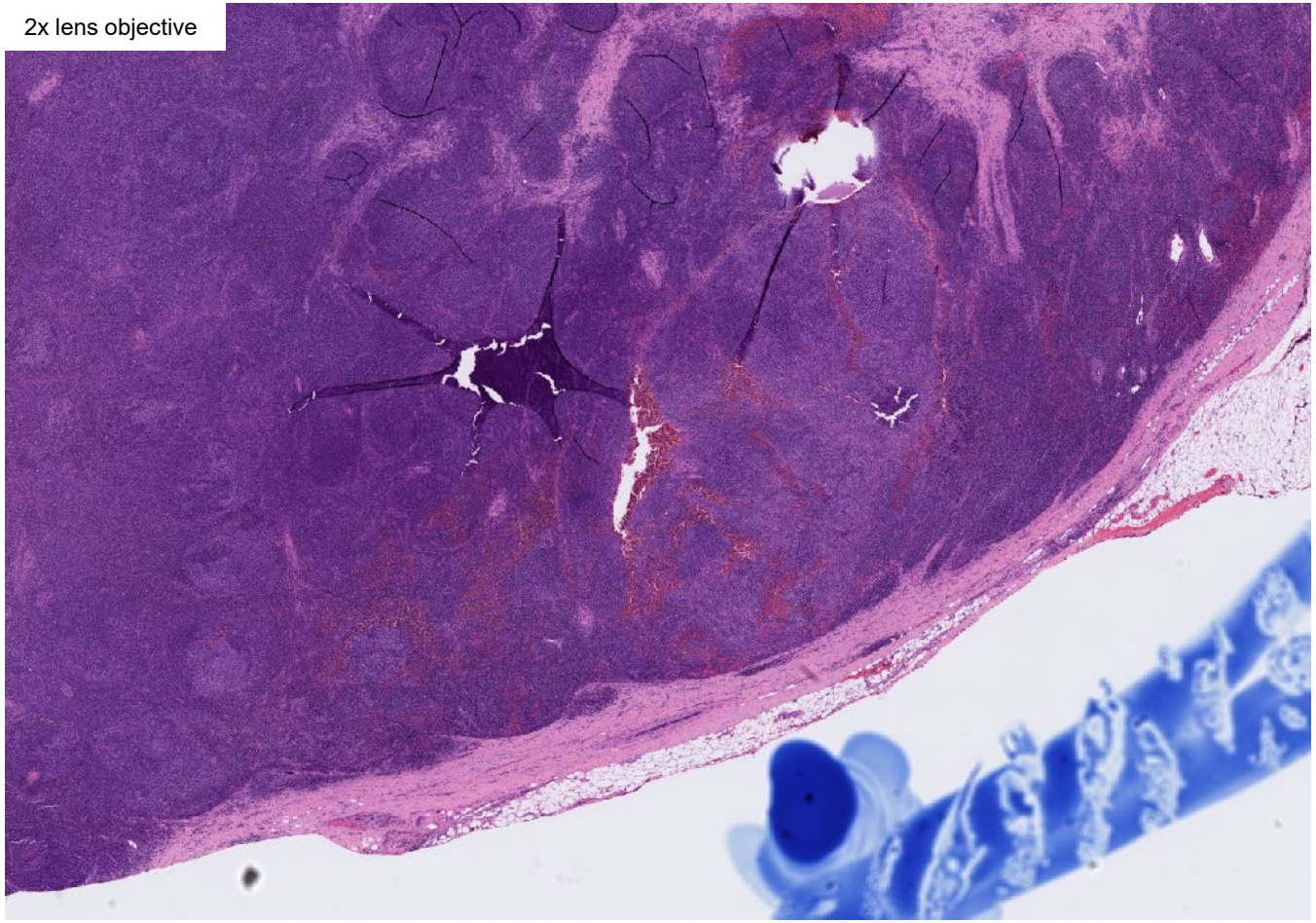
Brief Clinical History

- 61 year-old-female
- No relevant past medical or surgical history
- Isolated left inguinal lymph node enlargement

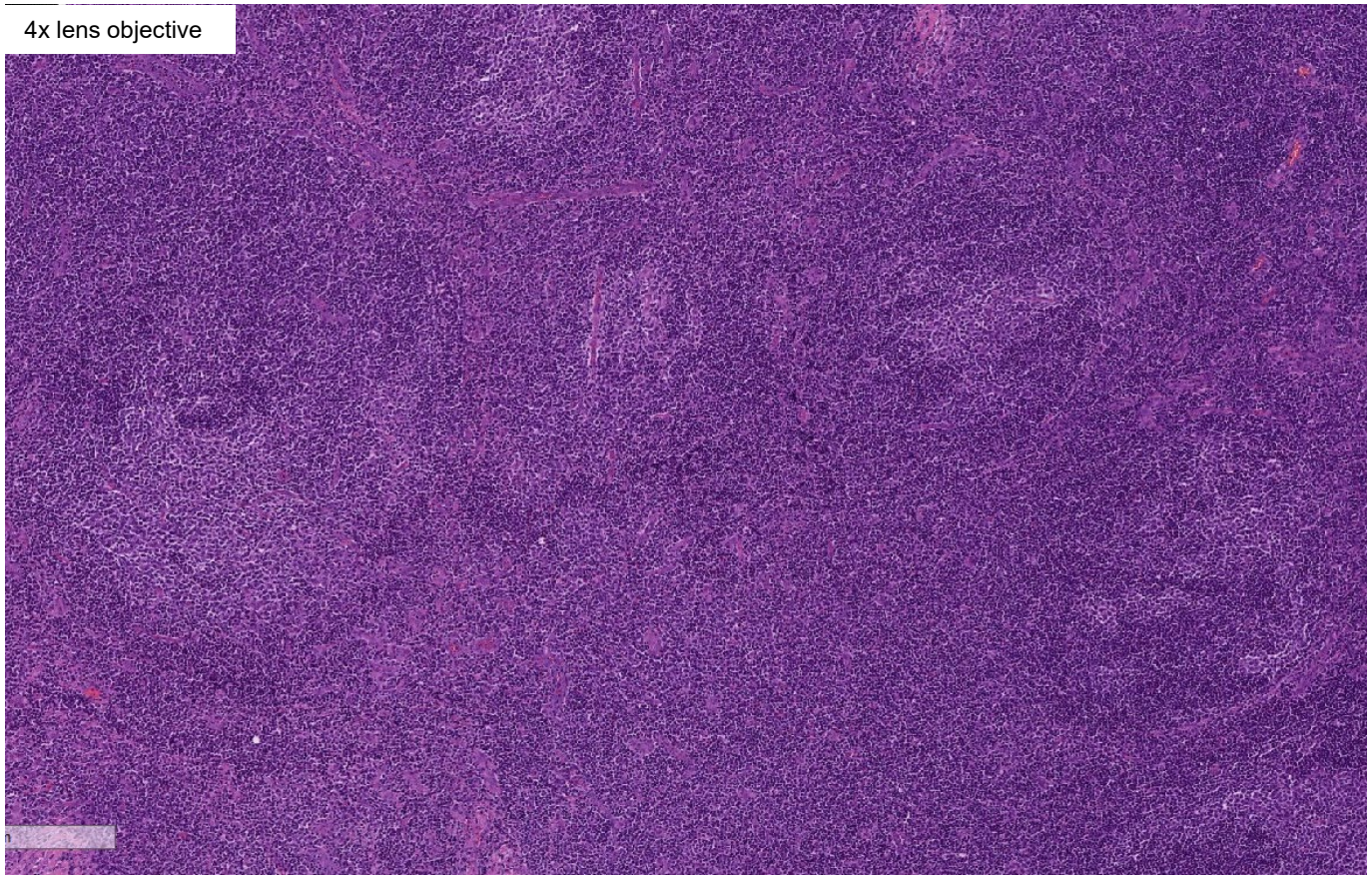
1x lens objective



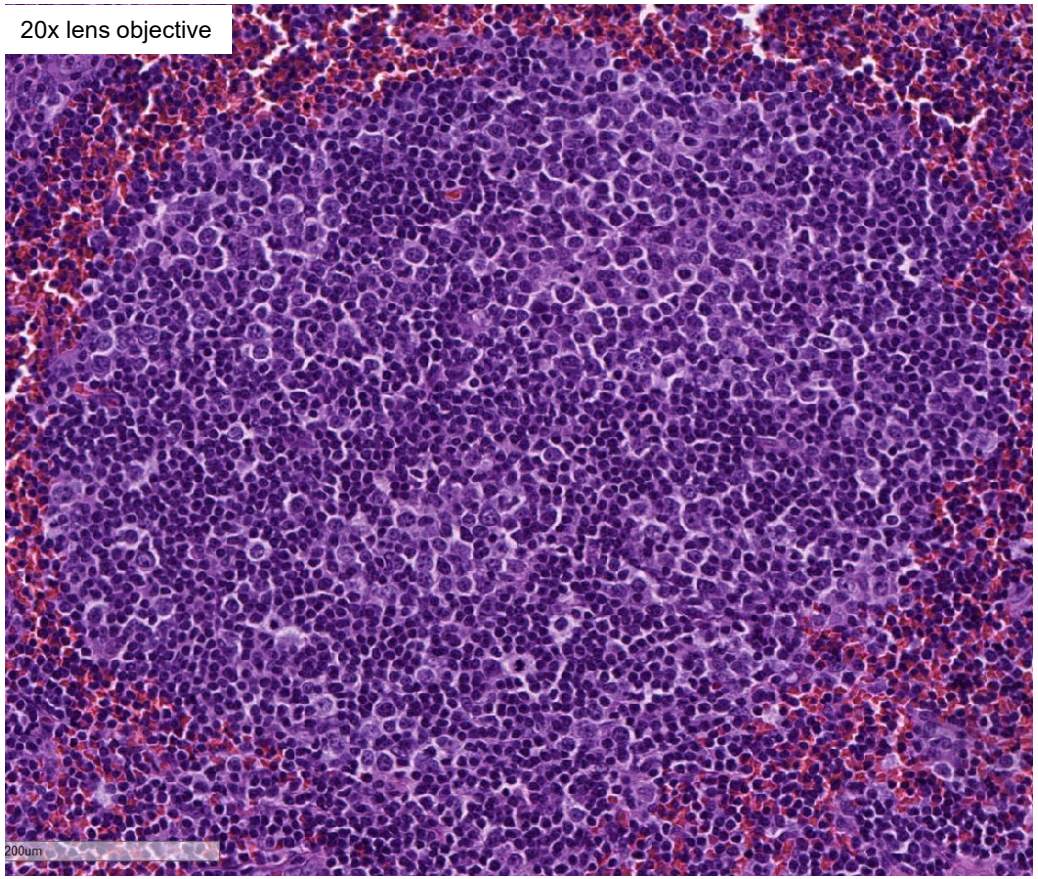
2x lens objective



4x lens objective

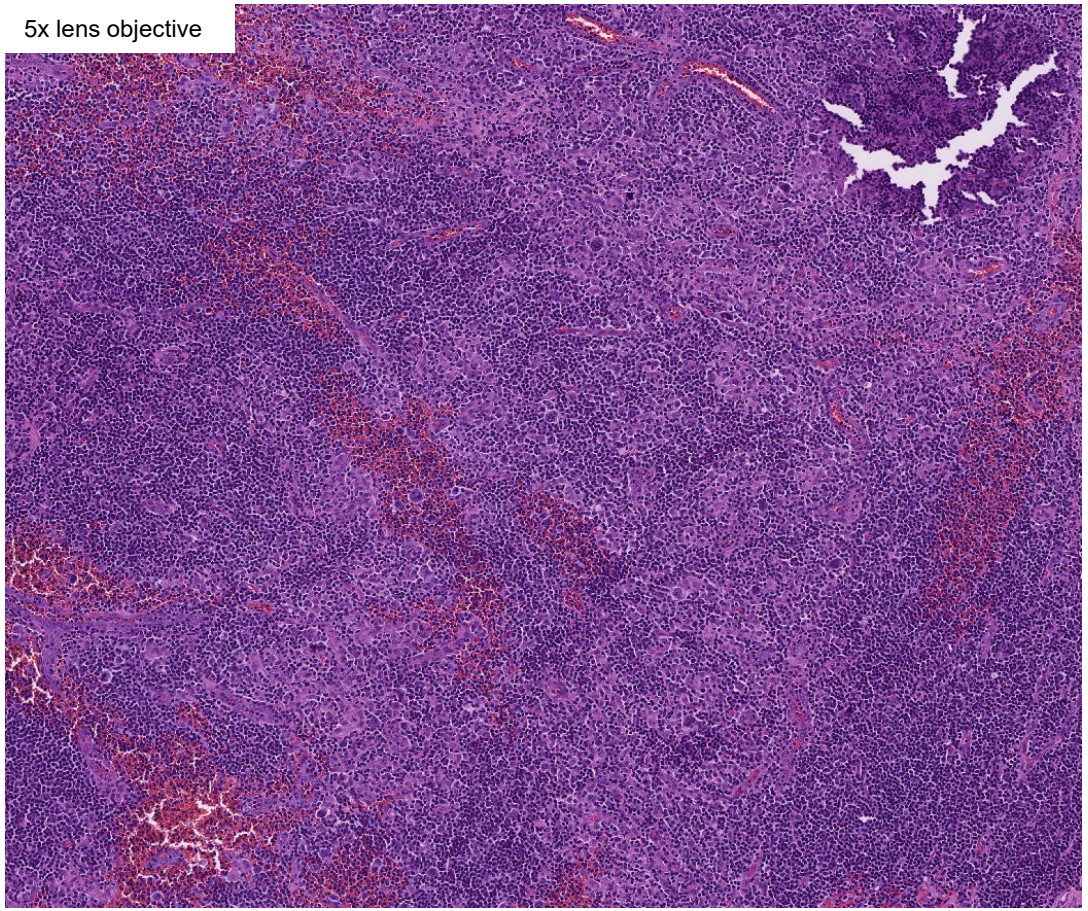


20x lens objective

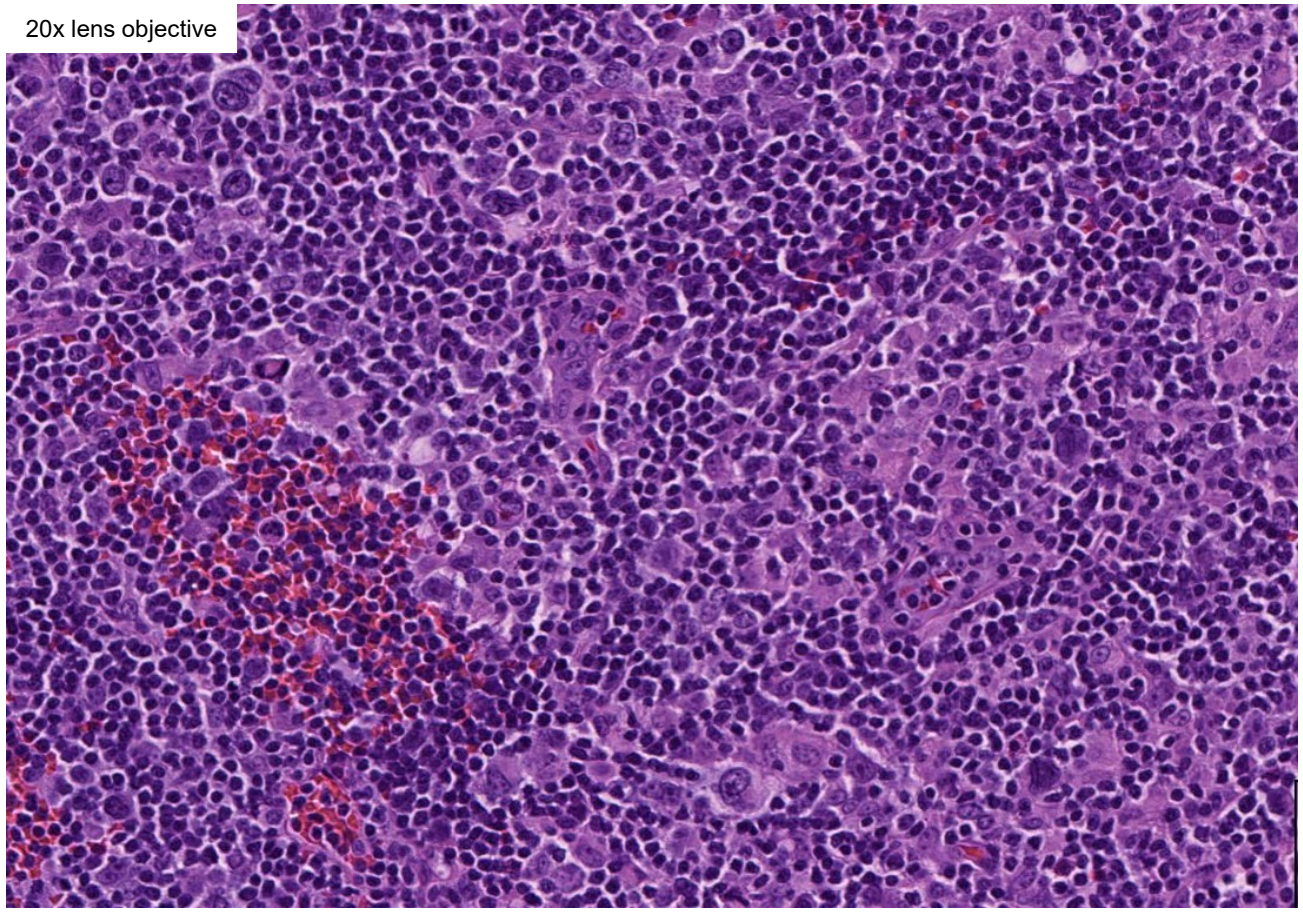


Penn Medicine

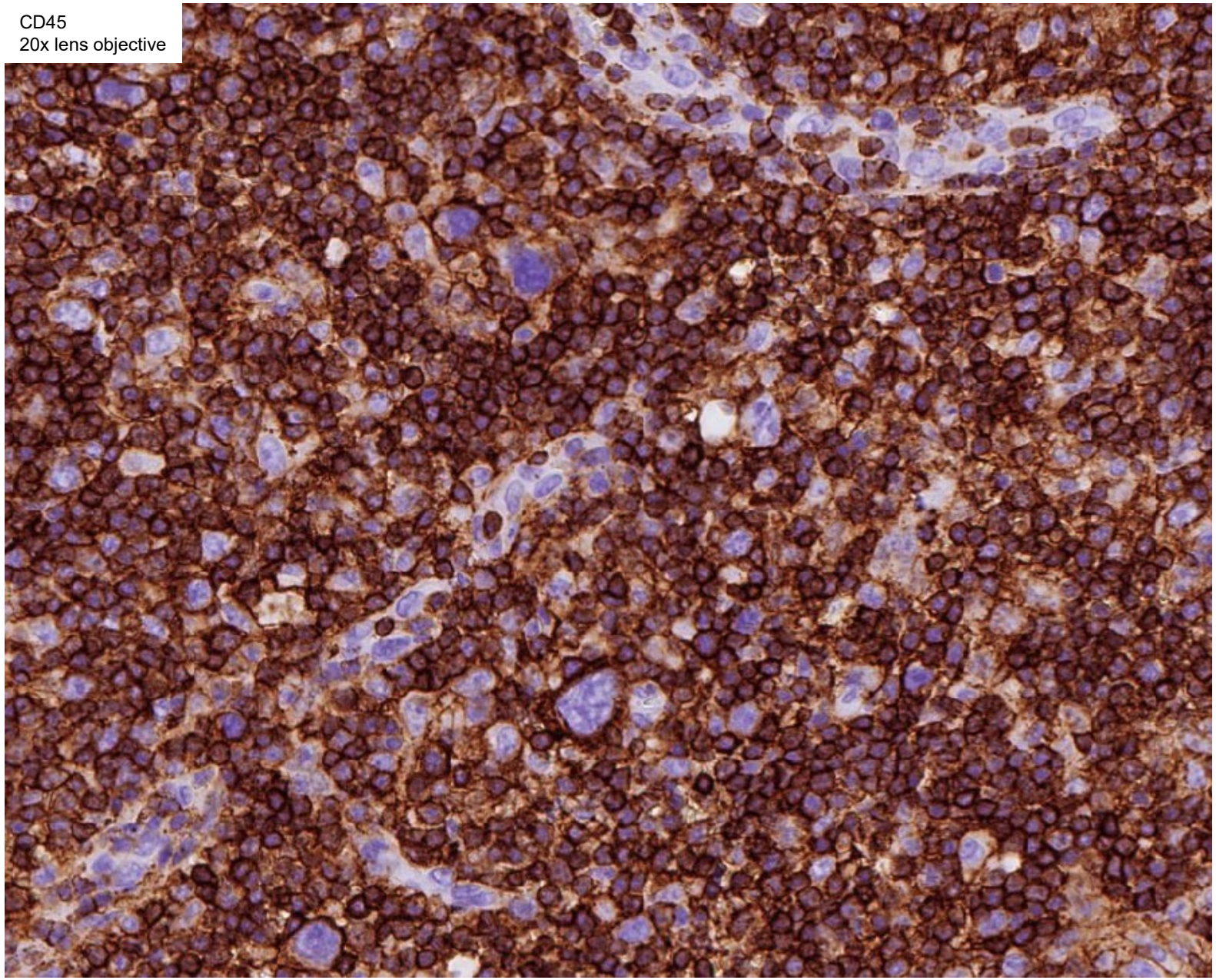
5x lens objective



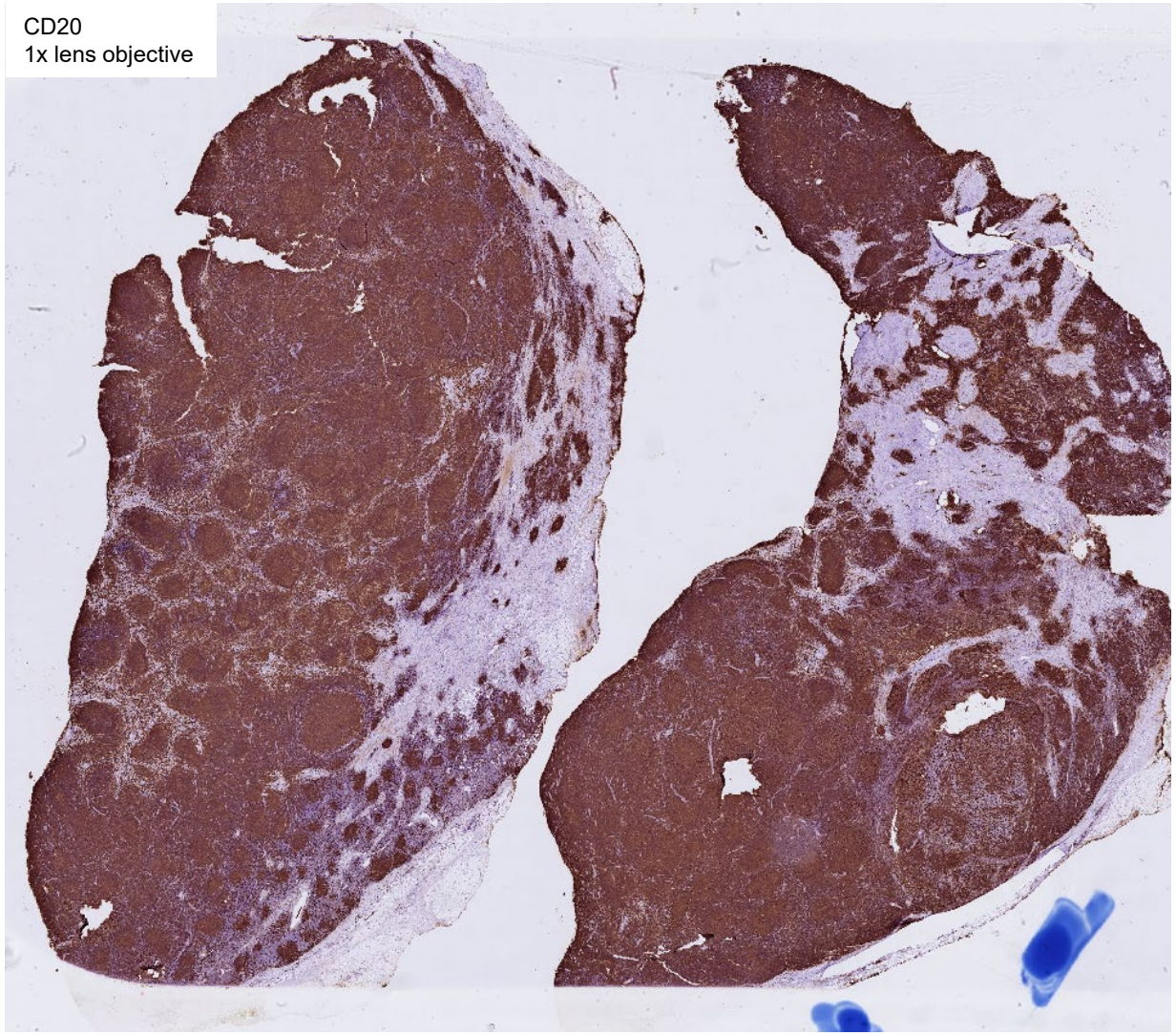
20x lens objective



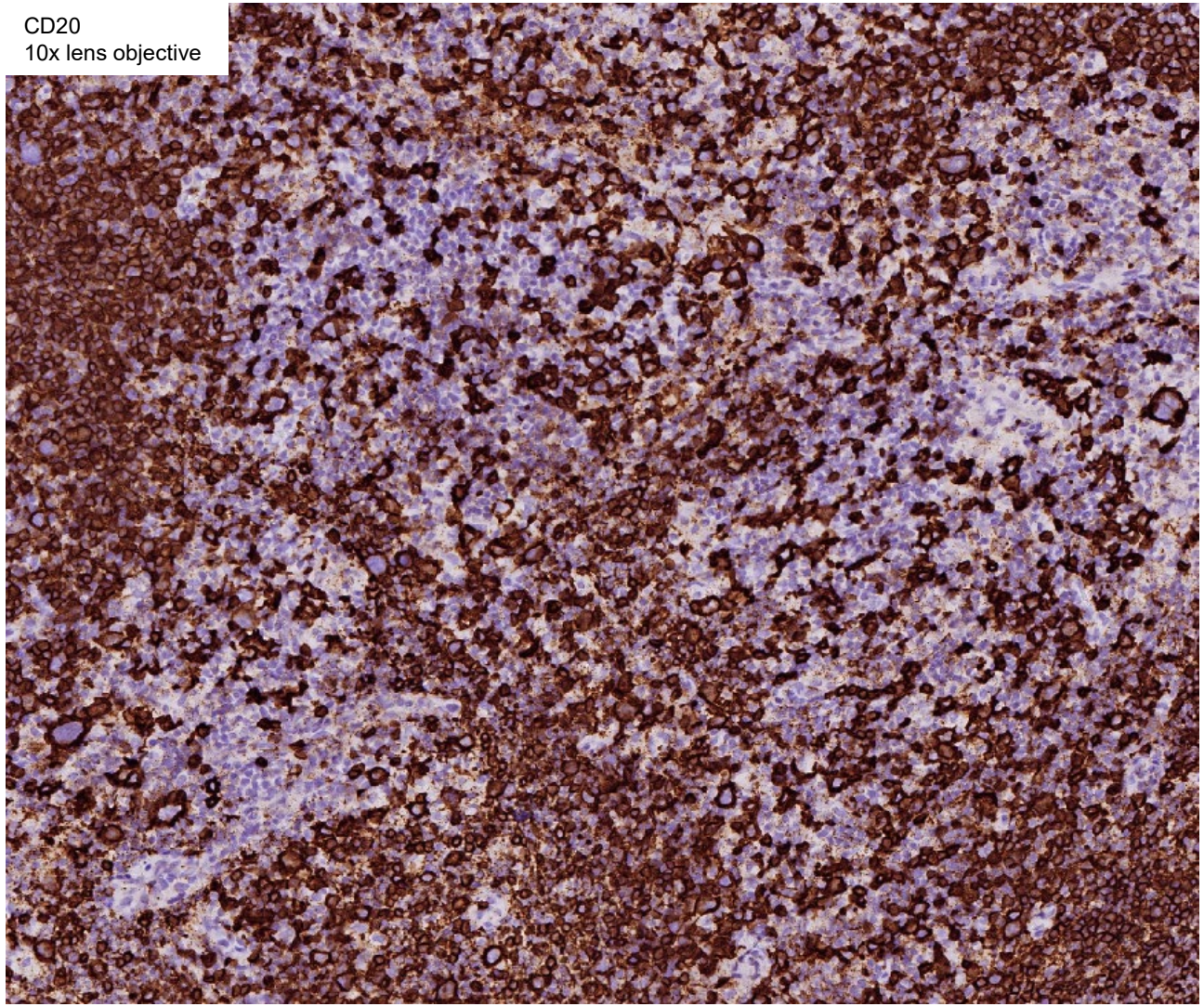
CD45
20x lens objective



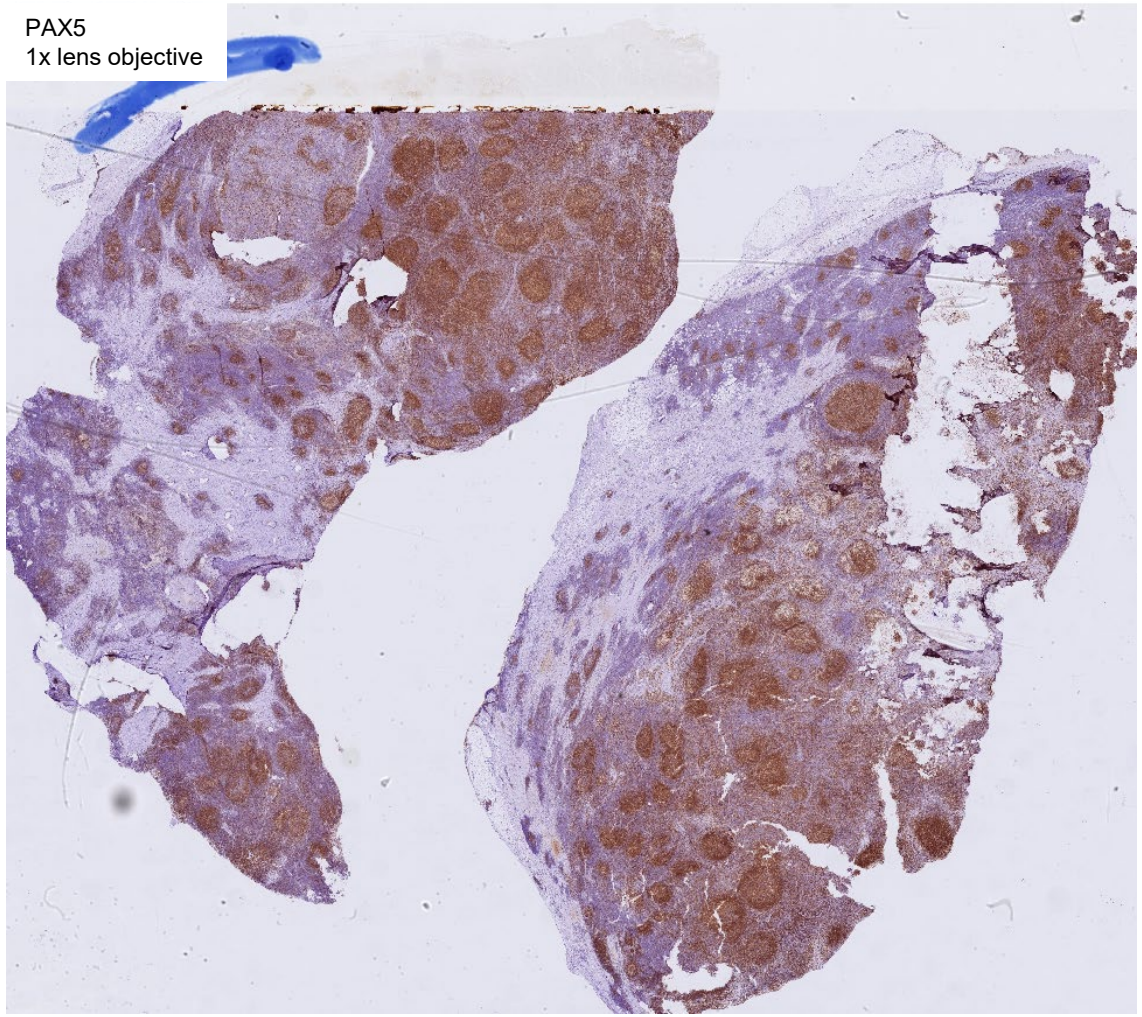
CD20
1x lens objective



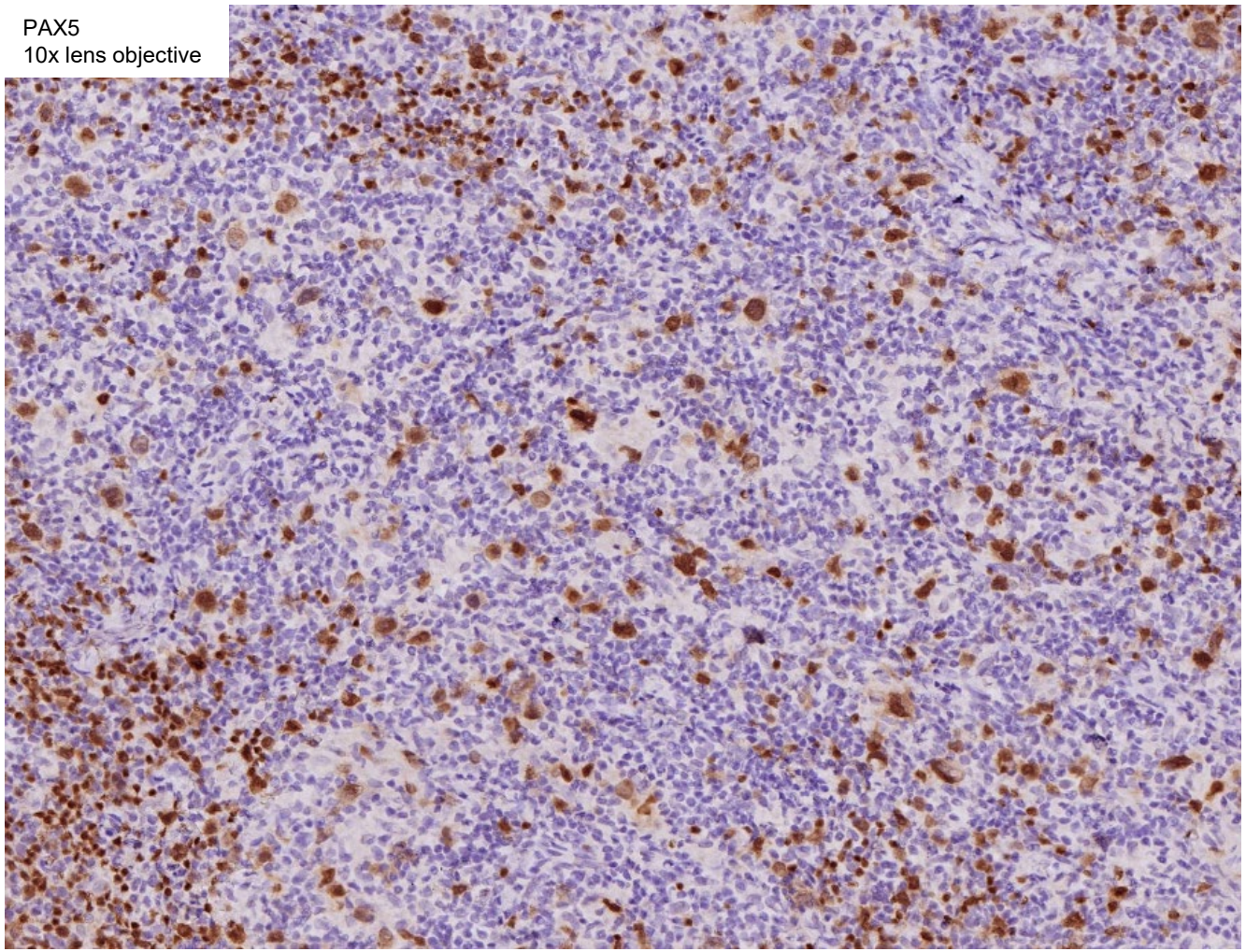
CD20
10x lens objective



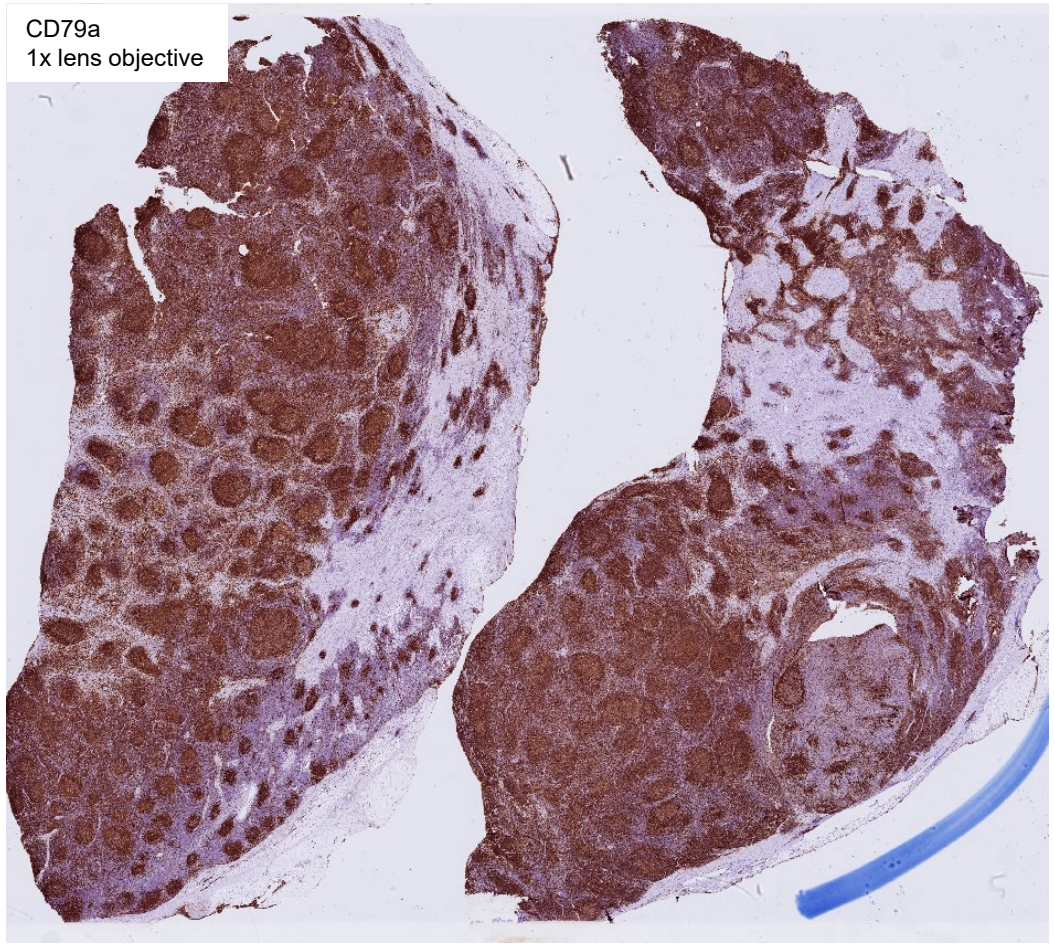
PAX5
1x lens objective



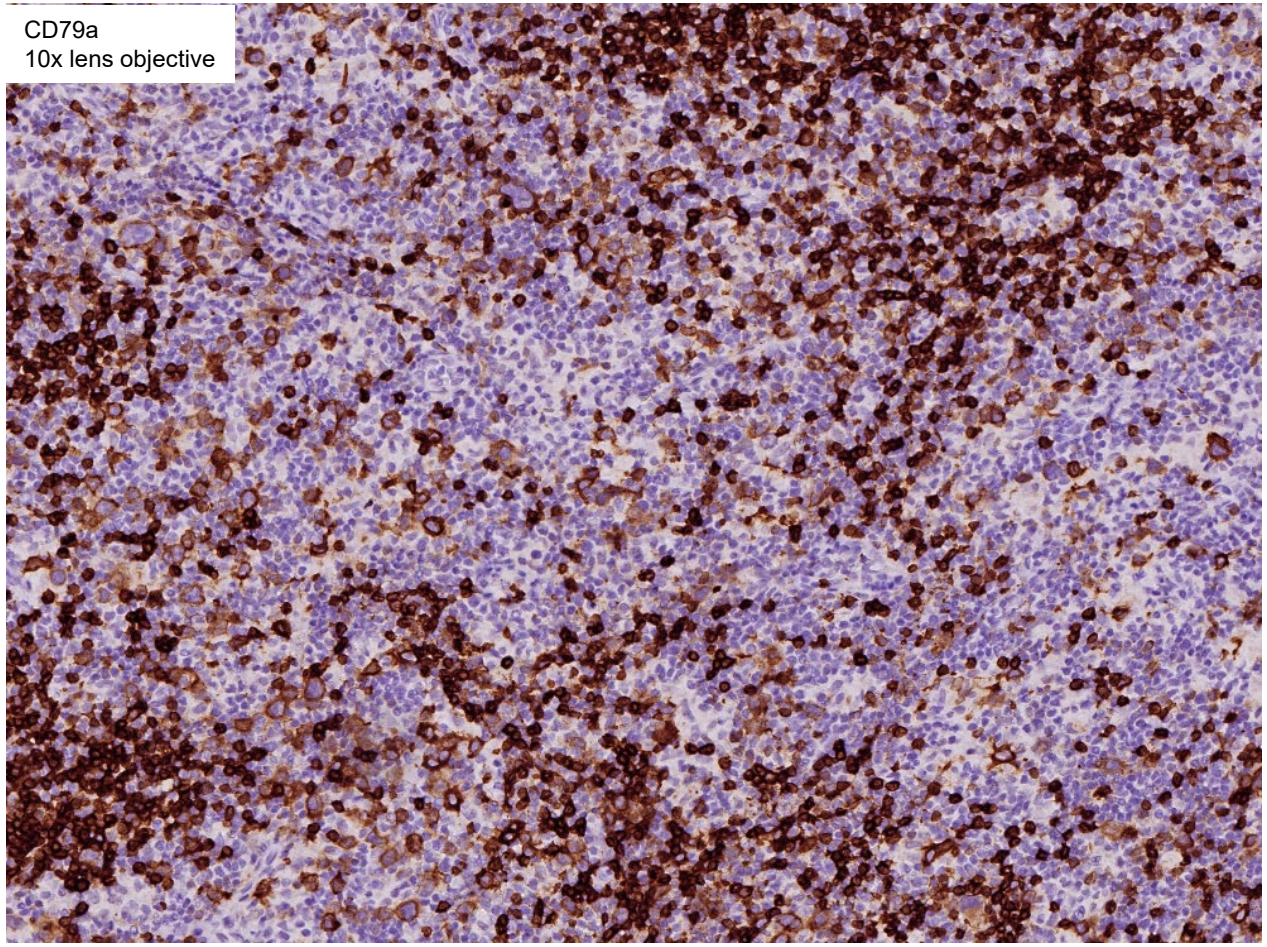
PAX5
10x lens objective



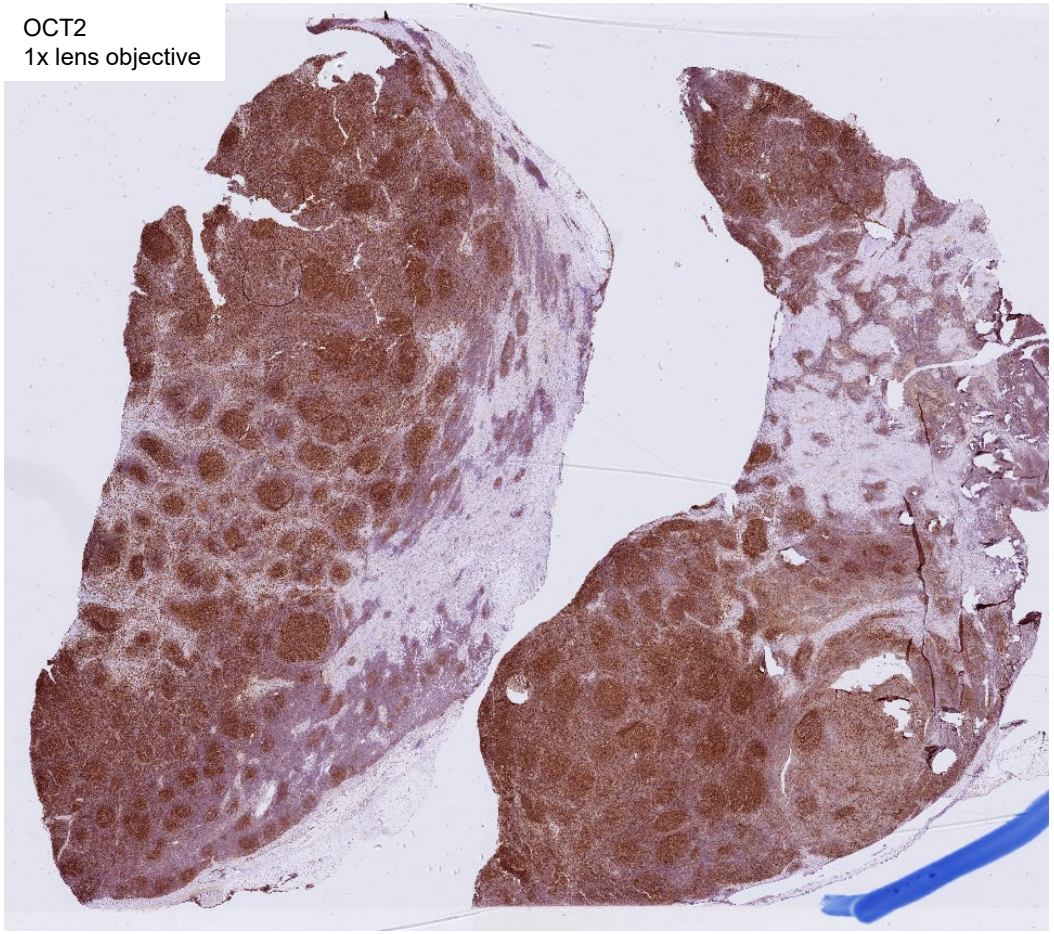
CD79a
1x lens objective



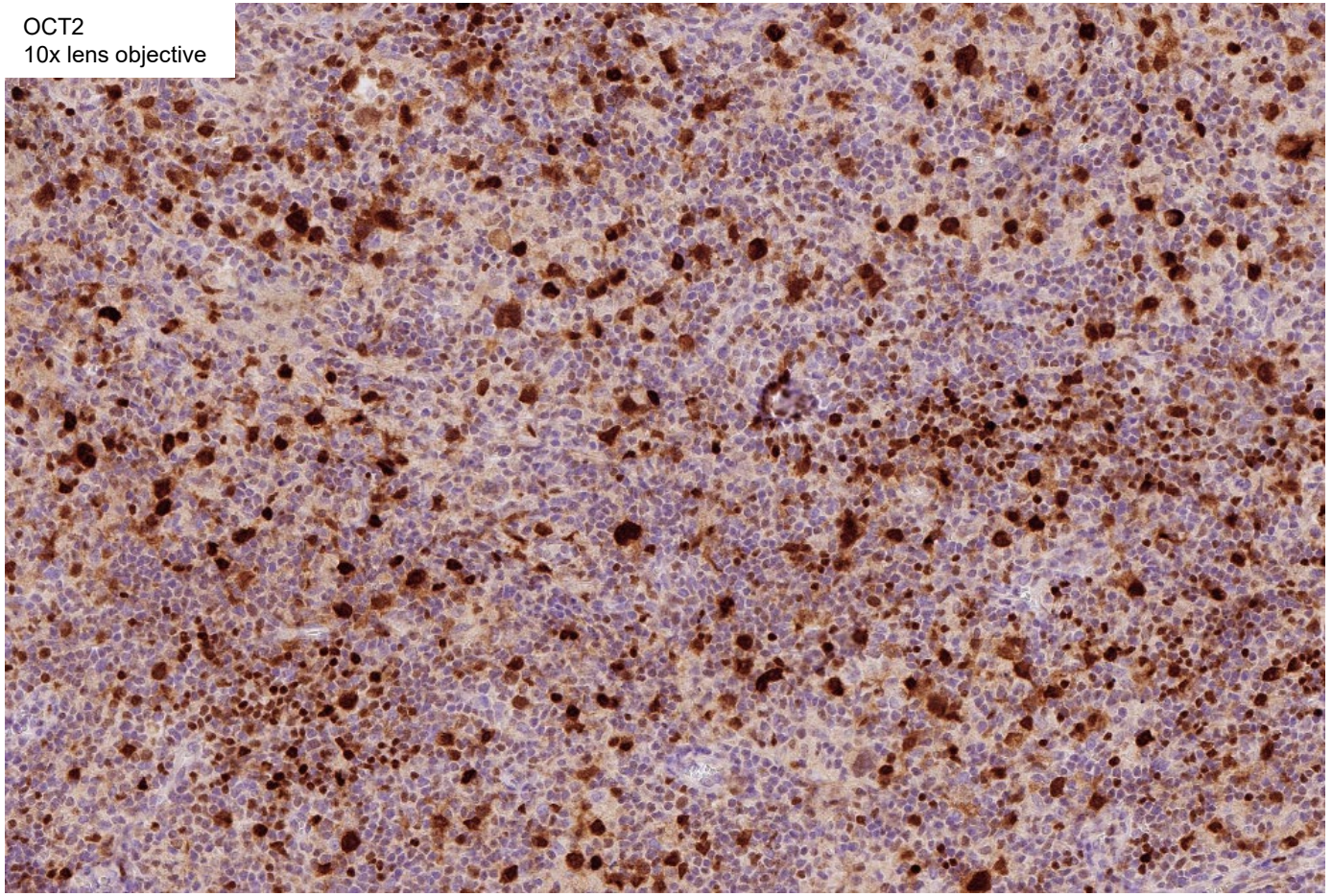
CD79a
10x lens objective



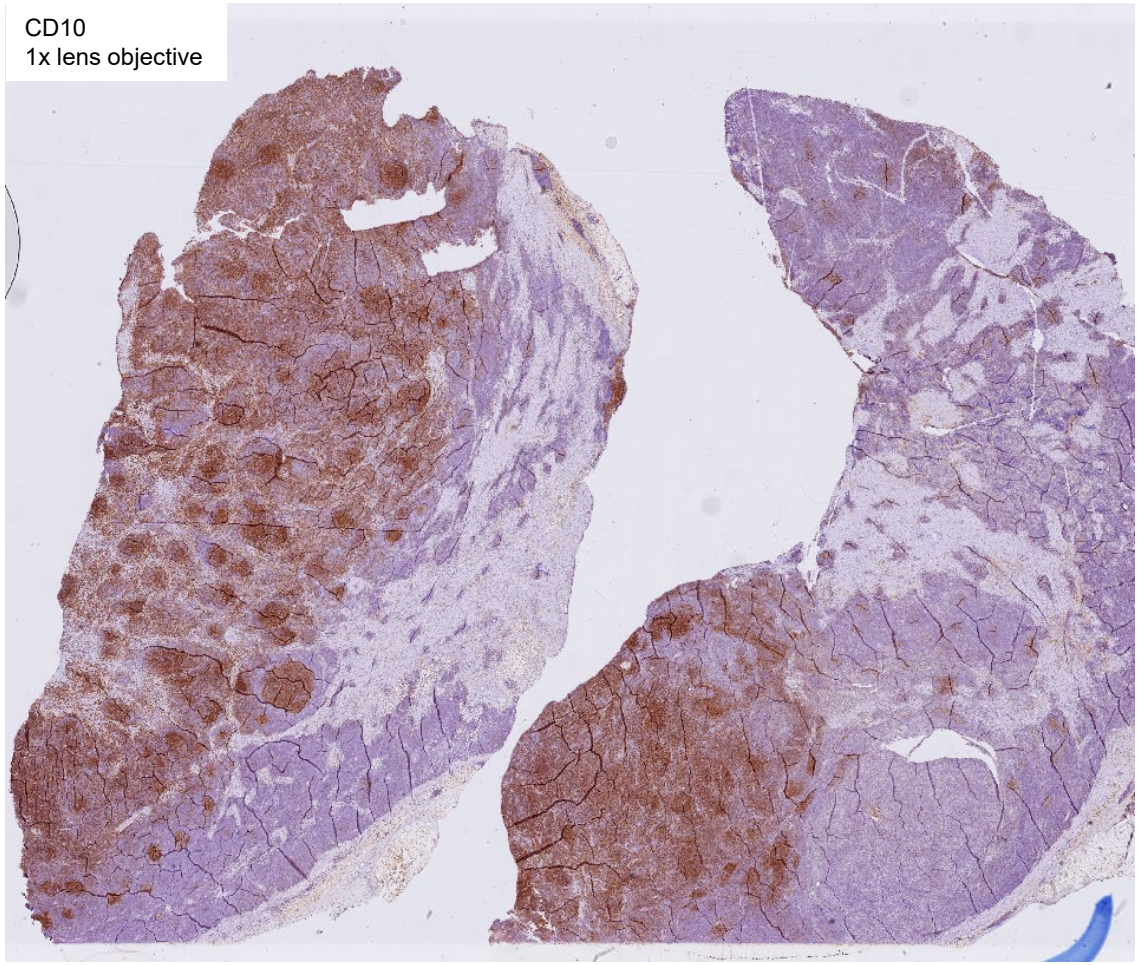
OCT2
1x lens objective



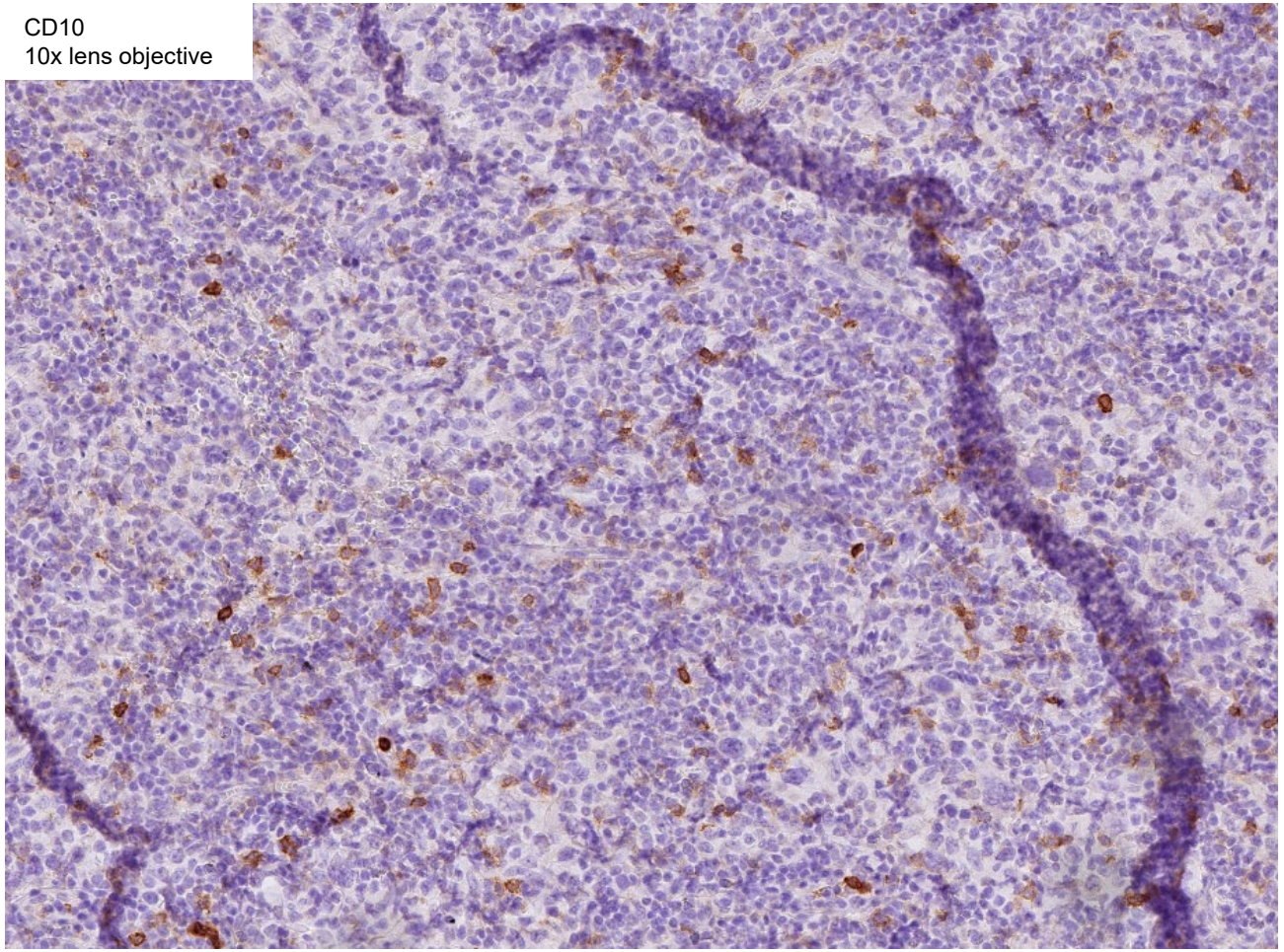
OCT2
10x lens objective



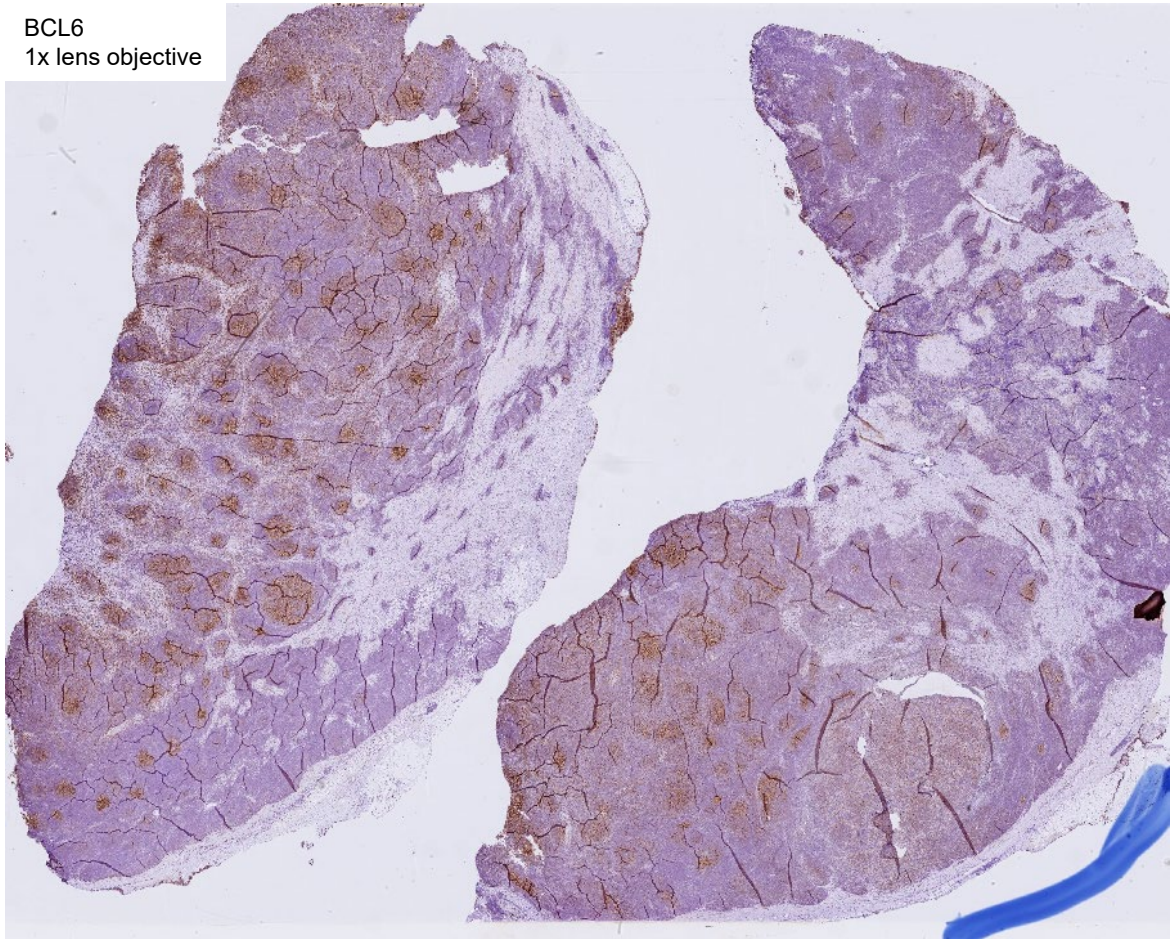
CD10
1x lens objective



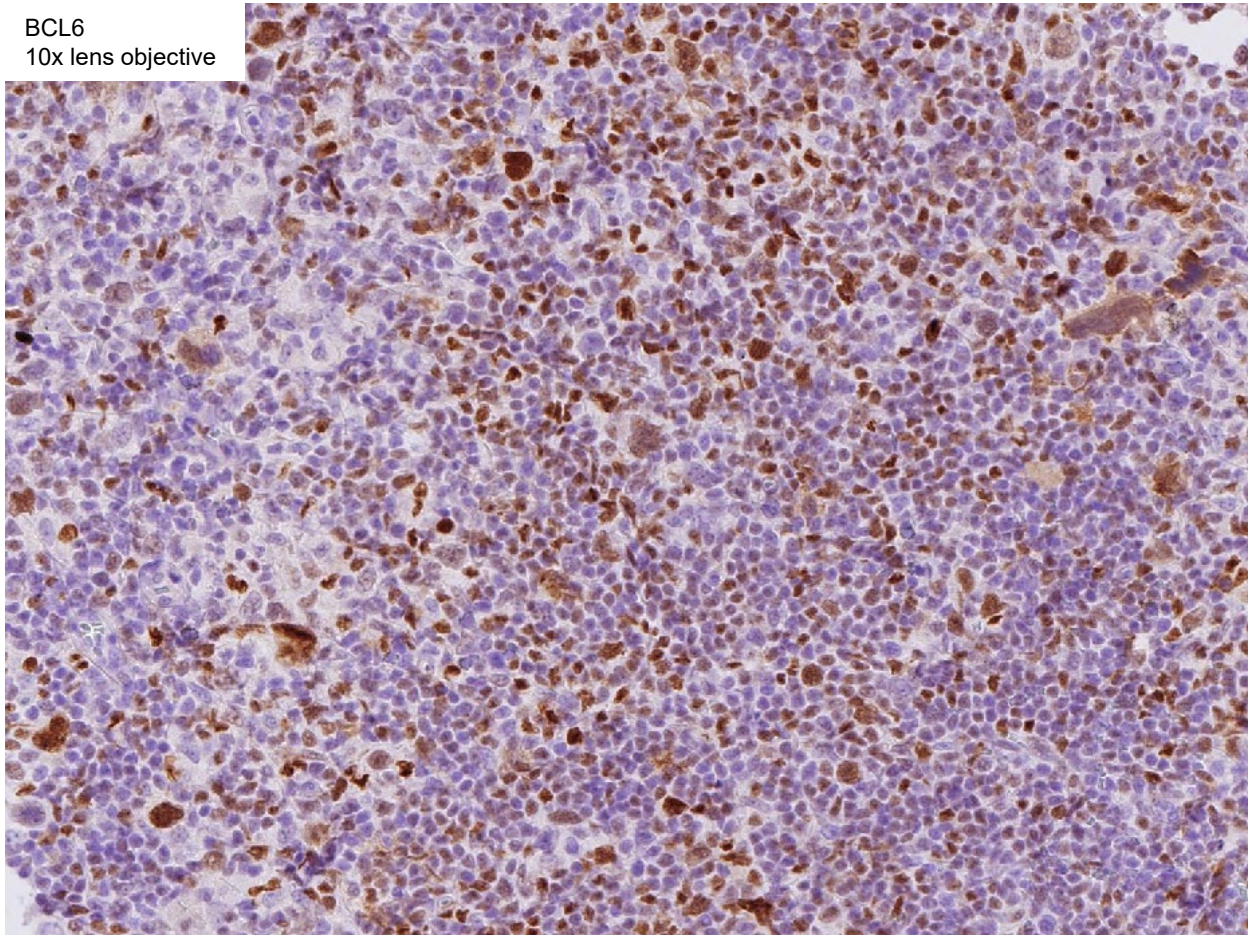
CD10
10x lens objective



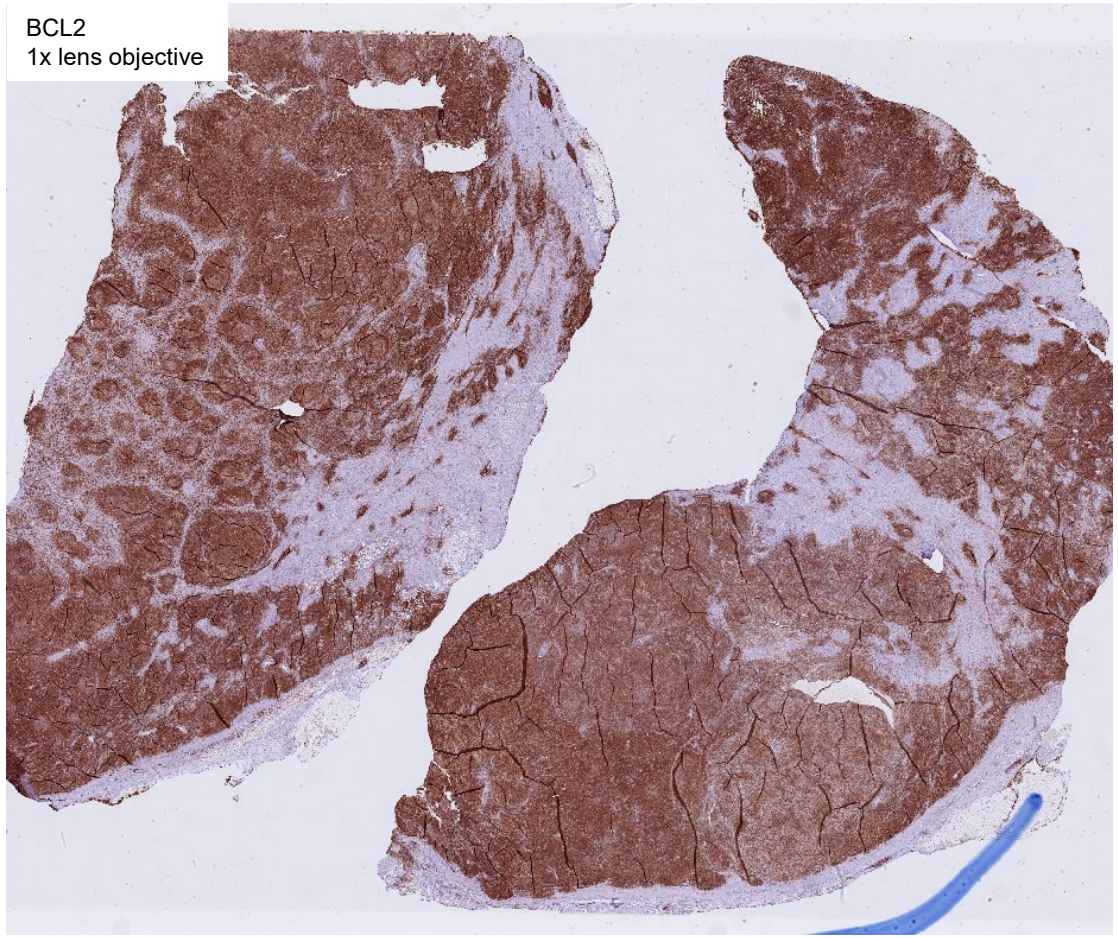
BCL6
1x lens objective



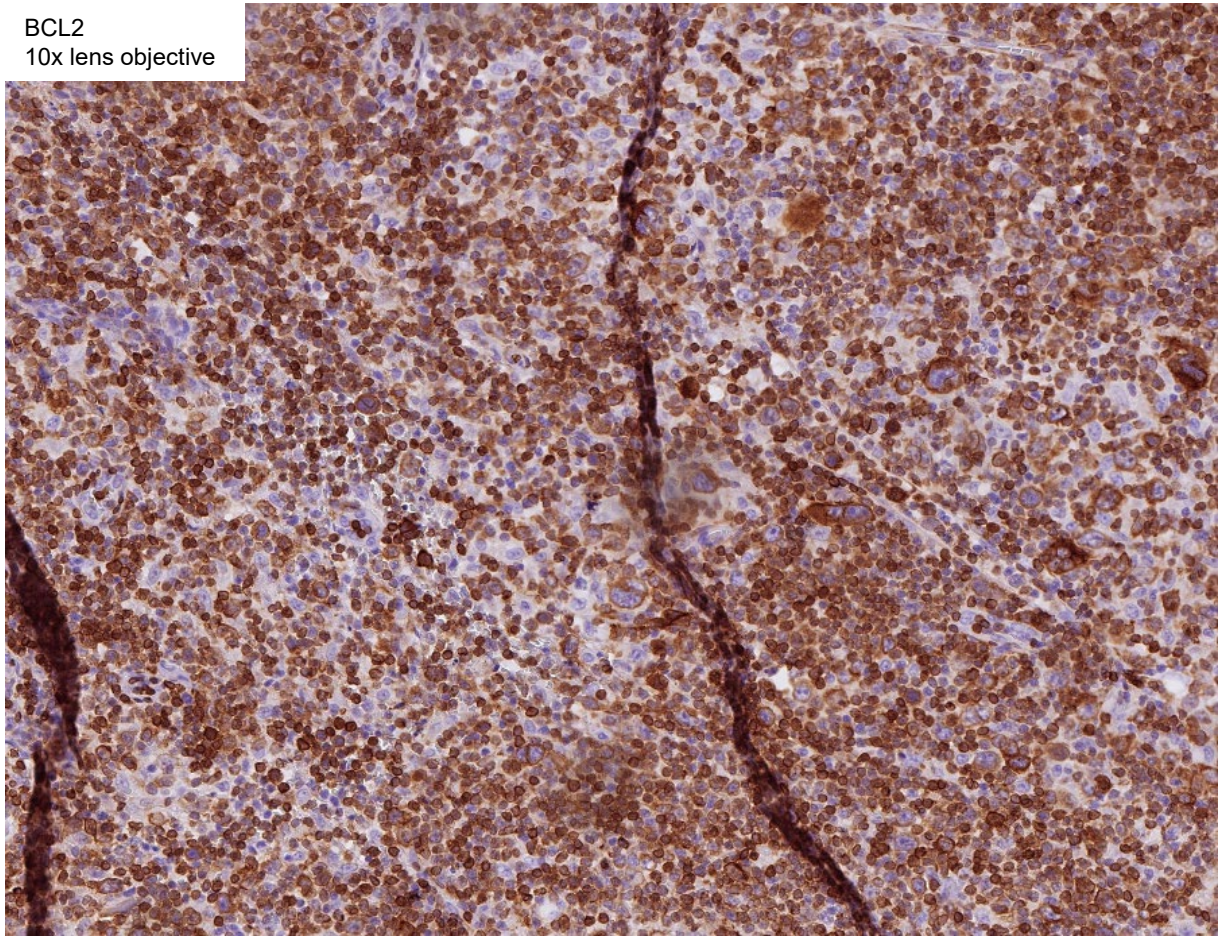
BCL6
10x lens objective



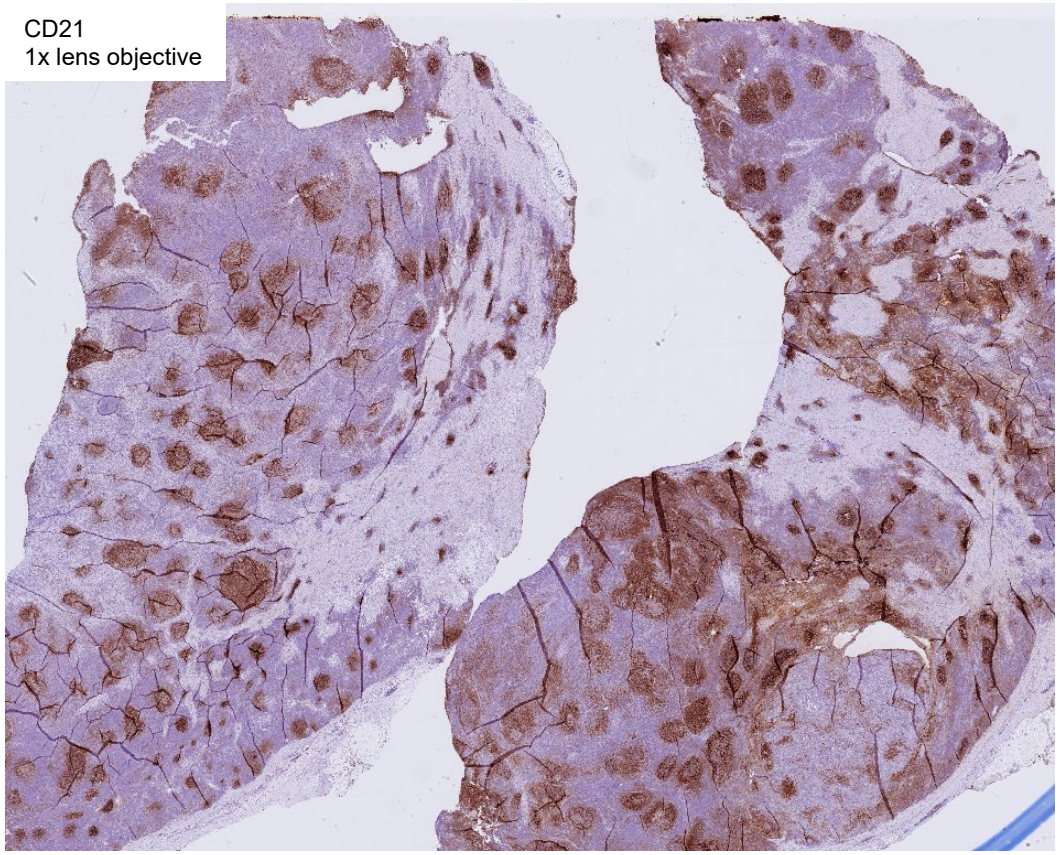
BCL2
1x lens objective



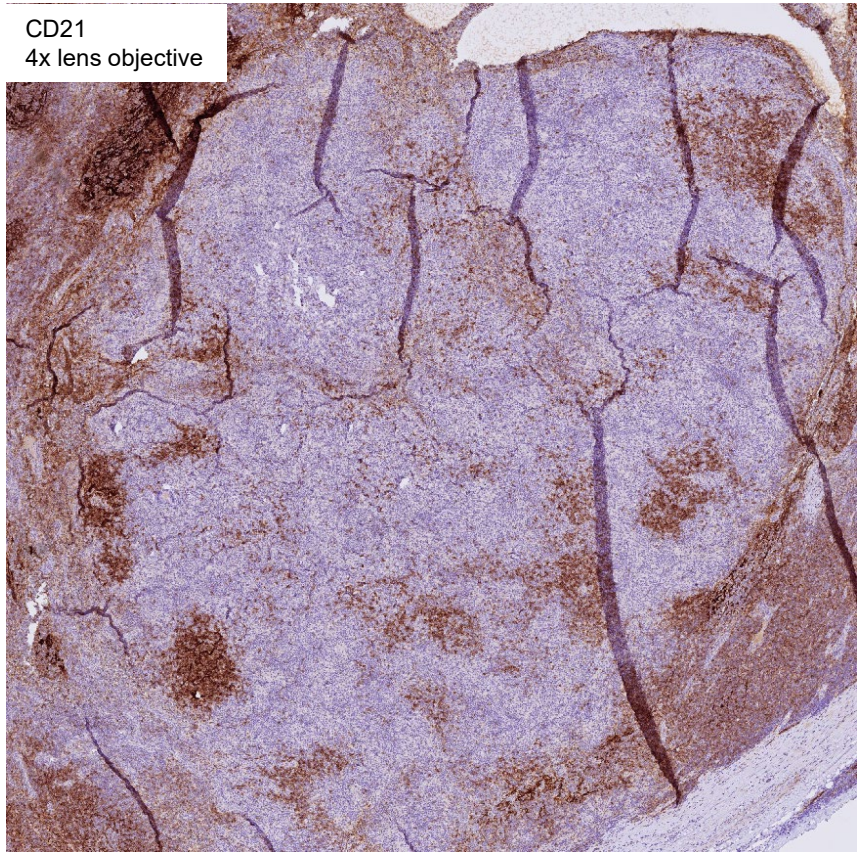
BCL2
10x lens objective



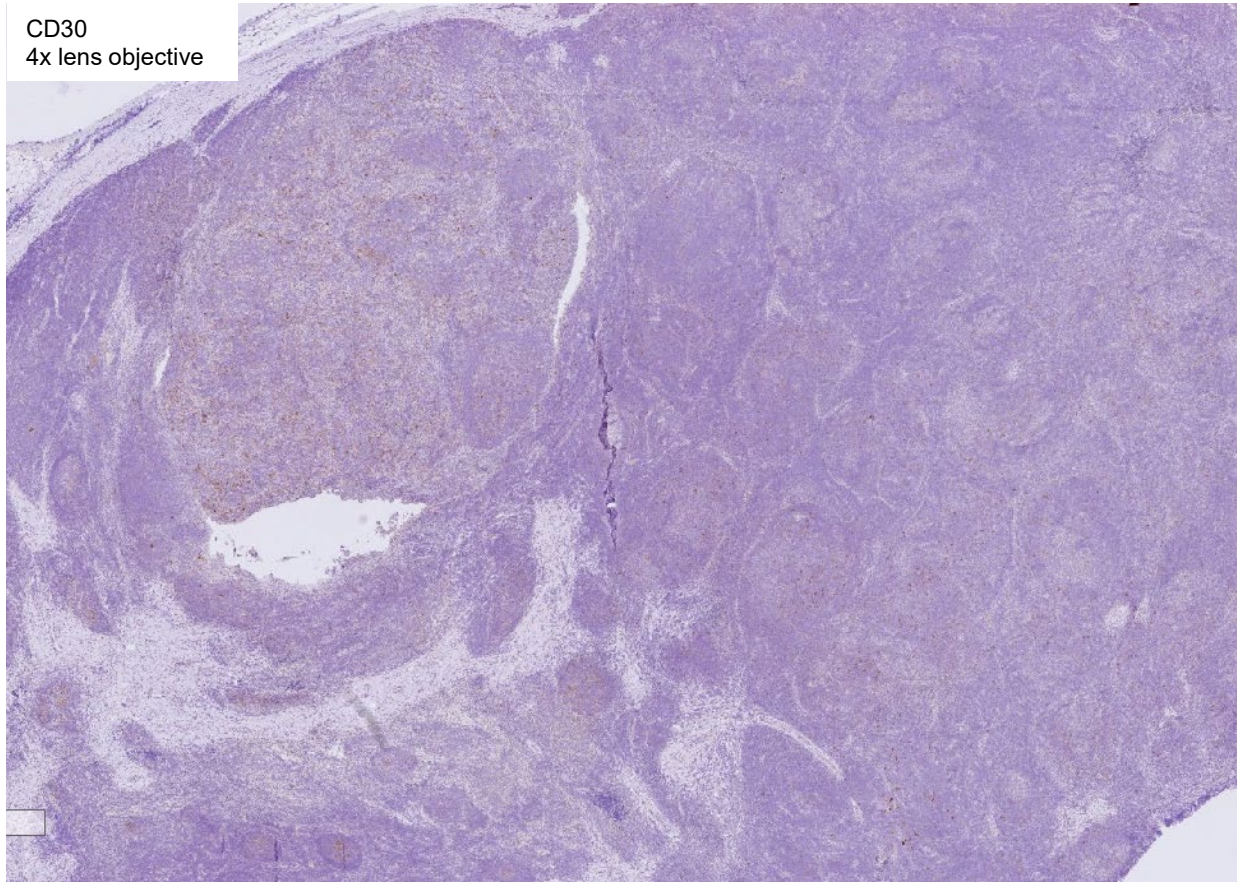
CD21
1x lens objective



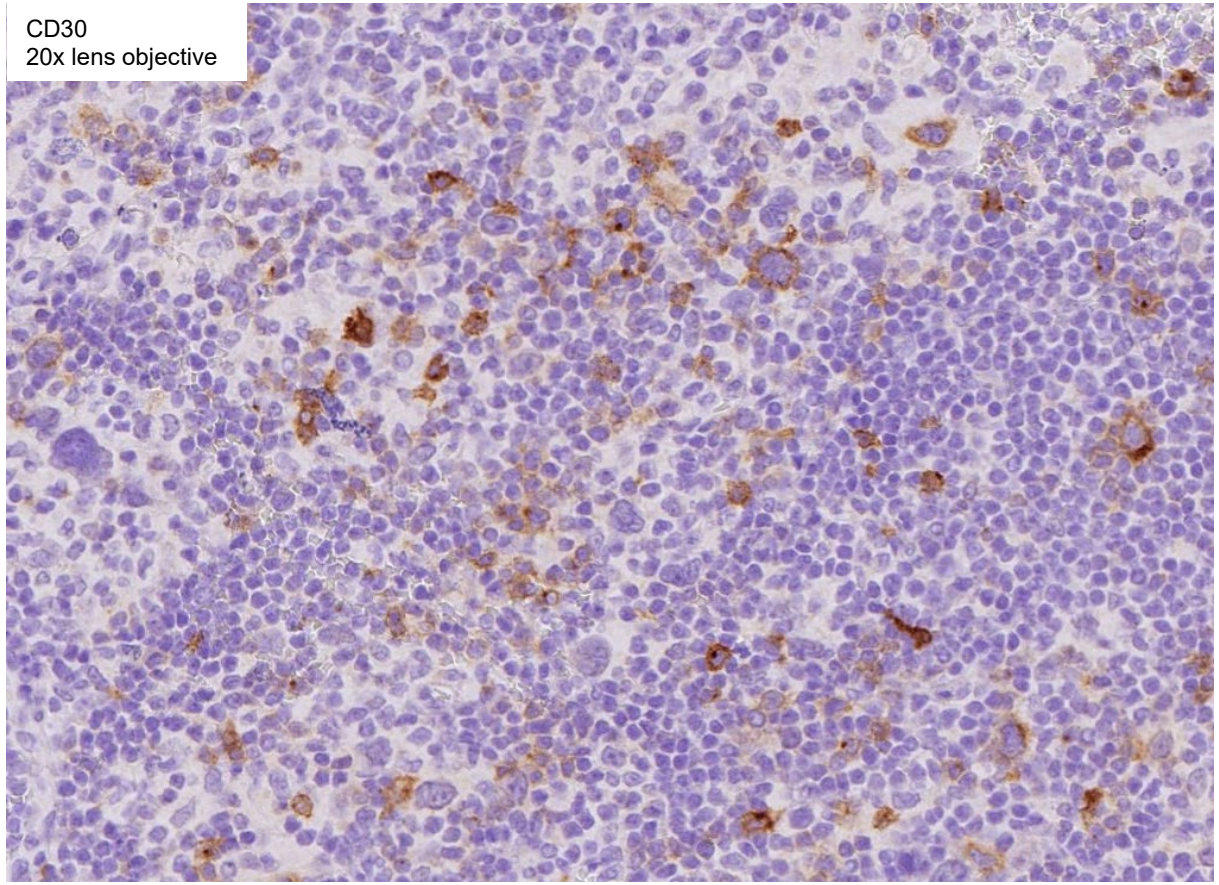
CD21
4x lens objective

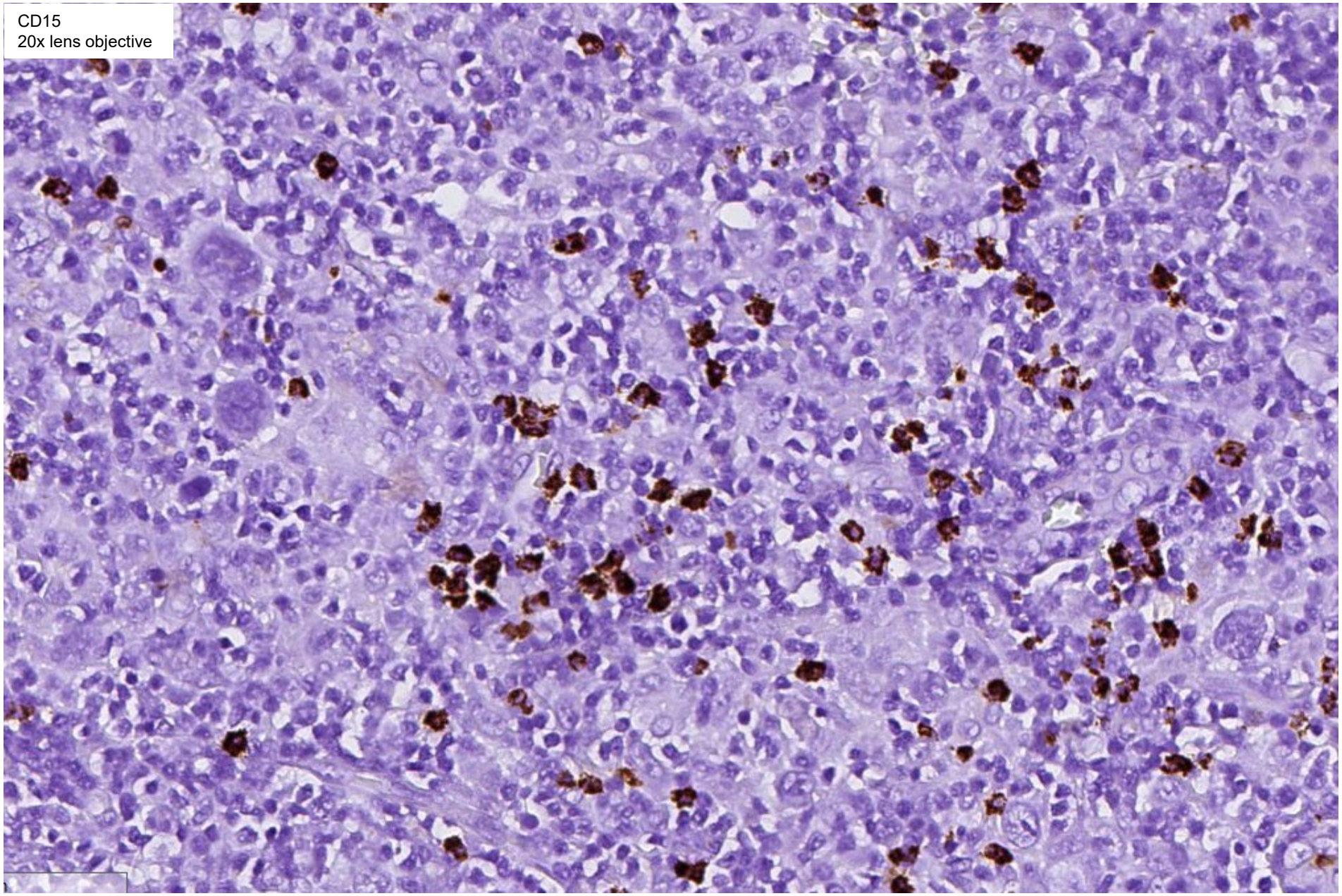


CD30
4x lens objective

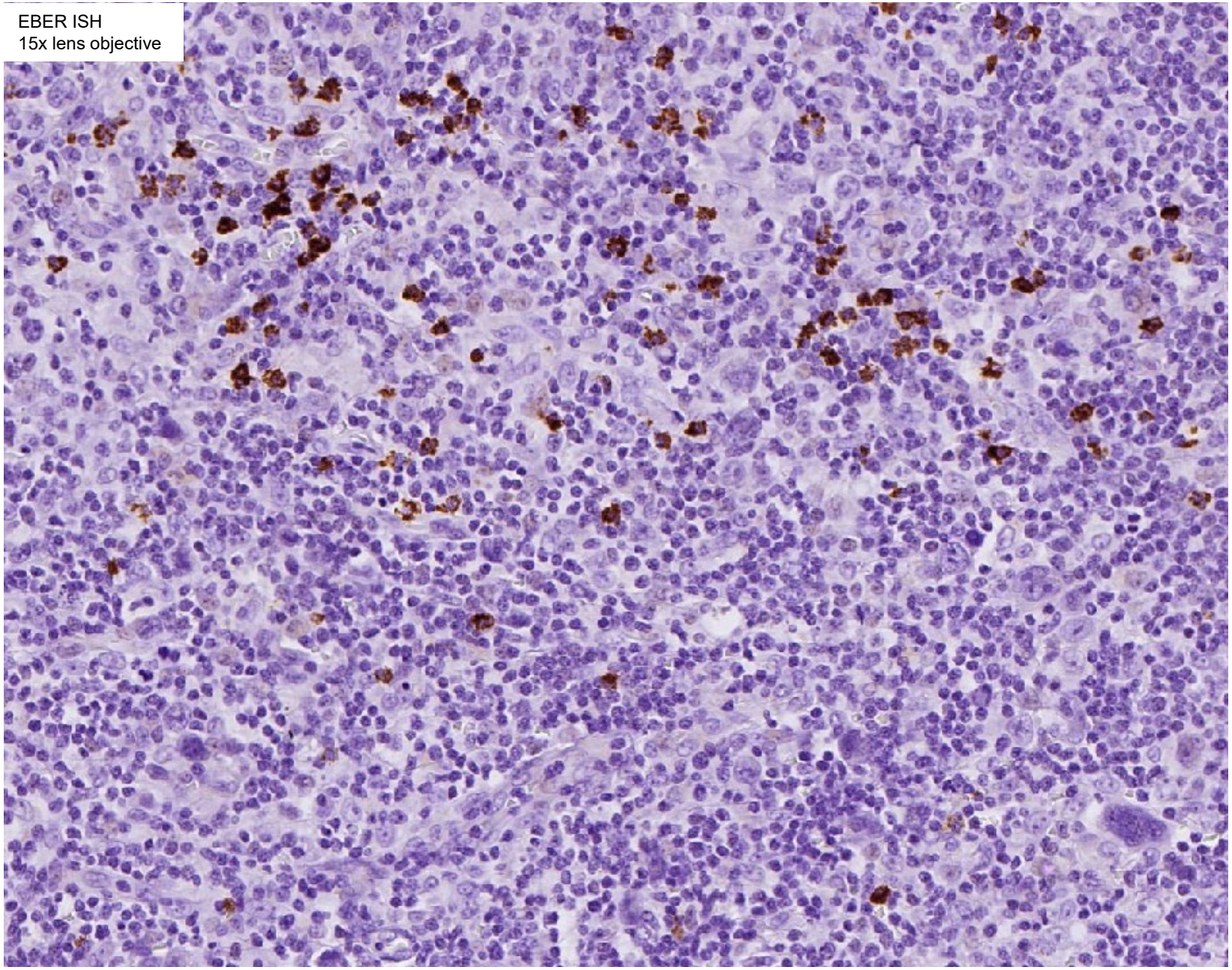


CD30
20x lens objective



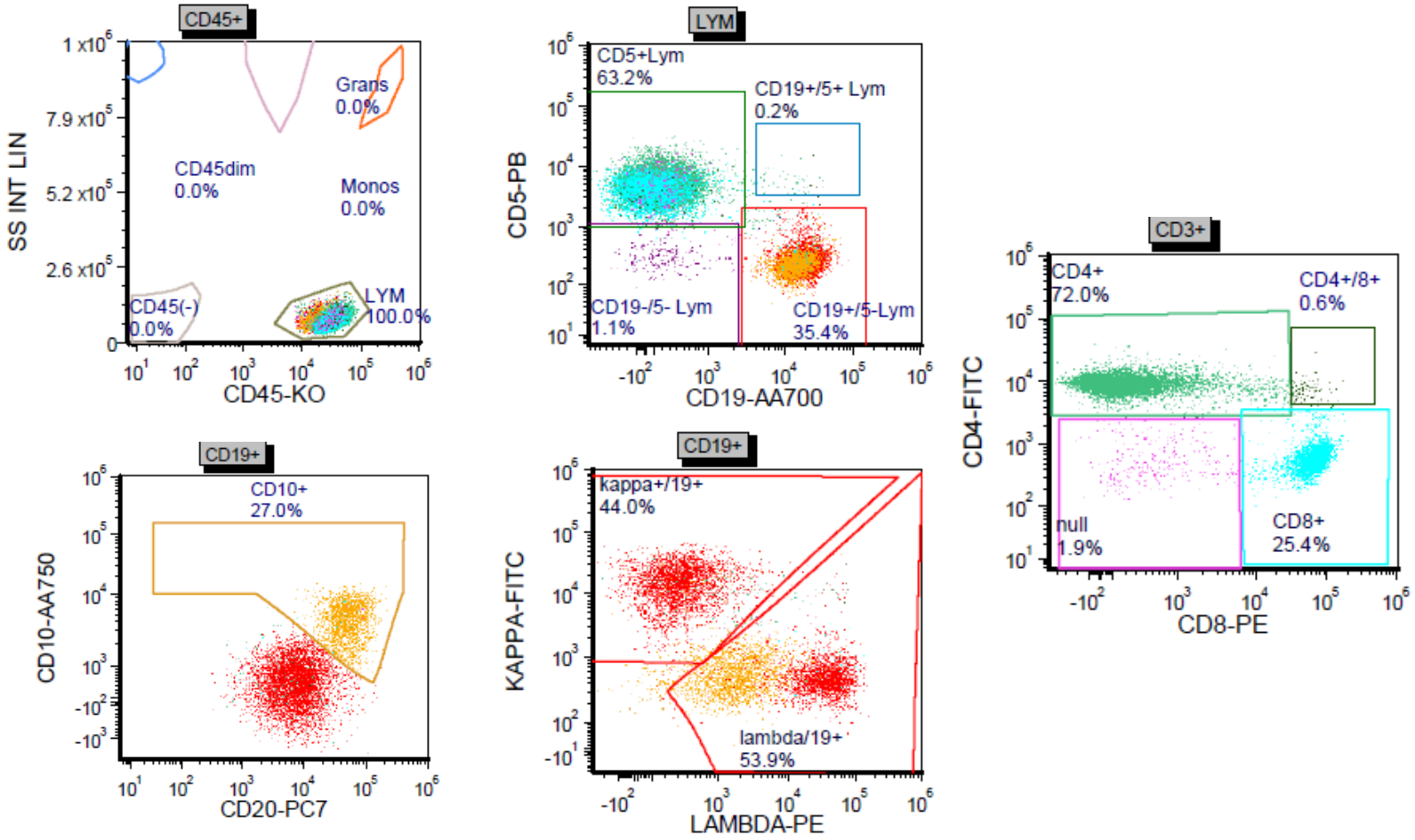

CD15
20x lens objective

EBER ISH
15x lens objective



Additional Immunostains

Flow Cytometry



- Aberrant B-cell population with the following immunophenotype: CD19+ CD20+ CD10+ lambda-restricted
- CD4:CD8 ratio of 2.8

Penn Medicine

Diagnosis

Lymph node, left inguinal, excisional biopsy:

- **Follicular lymphoma, WHO grade 1-2 of 3, follicular pattern**
- **Atypical large B-cell infiltrate, see note and microscopic description.**

NOTE: In summary, most of the lymph node is involved by classic follicular lymphoma. However, a separate and sharply demarcated focus of scattered large pleomorphic cells is present in a polymorphous lymphohistiocytic background with scattered eosinophils. Although some features resembled classic Hodgkin lymphoma, these large cells are entirely negative for CD30 and CD15, show variable expression of CD45, and retain a complete B cell immunoprofile. The large number of small B cells in the background and CD4+ T cell dominance are not consistent with T-cell/histiocyte rich large cell lymphoma. This may represent early large cell transformation of the underlying follicular lymphoma or a distinct evolving lymphoproliferative disorder that is difficult to precisely classify. Molecular, FISH, and gene sequencing studies will be performed to compare the mutational profile of this large cell infiltrate to the follicular lymphoma, and these results will be integrated in an addendum.

Genomic Studies

FISH:

- Negative for *BCL2* and *MYC* gene rearrangements
- Negative for *TNFRSF4* (1p36) alteration

Molecular:

- Positive for an identical *IGH* gene rearrangement pattern in areas of classic follicular lymphoma and the focus with scattered large B cells, confirming a clonal relationship.

Genomic Studies Cont'd

Next-generation sequencing:

Tier 2 Variants:

CREBBP p.L1499P, c.4496T>C, NM_004380.3 Missense variant (VAF: 18%)

STAT6 p.E377K, c.1129G>A, NM_003153.5 Missense variant (VAF: 16%)

TP53 p.R248Q, c.743G>A, NM_000546.6 Missense variant (VAF: 10%)

- The large cell area showed a profile consistent with the EZB/C3 cluster of GCB-like diffuse large B cell lymphoma, suggesting that this focus represents early large cell transformation of follicular lymphoma

Follicular Lymphoma and Transformation

- Large B-cell lymphoma (most commonly DLBCL)
- B-lymphoblastic leukemia/lymphoma
- Classic Hodgkin lymphoma
- Histiocytic or dendritic cell sarcoma (transdifferentiation)

Follicular Lymphoma Genetics

- t(14;18)(q32;q21) *IGH::BCL2* translocation leading to the overexpression of BCL2 in 85-90% of cases
  - Rarely, t(2;18)(p12;q21) or t(18;22)(q21;q11)

- Frequent copy-number alterations include chromosomal gains of 18, X, 1q, 2p, and 7, and less commonly gains of 17q, 6p, 8q, 12q as well as deletions of 6q and 1p and less frequently deletions of 10q and 9p.

- Frequent mutations in epigenetic regulators and chromatin remodeling genes
  - *KMT2D/MLL2* (90% of cases), *CREBBP* (33-75%), *EZH2* (25%) and *EP300*
  - Other genes: *ARID1A*, *MEF2B*, *KMT2C, BCL2, TNFRSF14, STAT6, CARD11, FOXO1, NOCTH1/2, and TNFAIP3*

Mediastinal Gray Zone Lymphoma (MGZL)

- Previously, termed “B-cell lymphoma unclassifiable, with features intermediate between DLBCL and CHL (gray zone lymphoma)” in the WHO 2008 edition.
- This entity represents an intermediate between primary mediastinal B-cell lymphoma and classic Hodgkin lymphoma, nodular sclerosis subtype.
- According to the ICC, extra-mediastinal cases with overlapping morphology and immunophenotype are more similar to conventional DLBCL or EBV-positive DLBCL, NOS.
- According to the WHO, rare cases without anterior mediastinal involvement occur may be referred to as grey zone lymphoma with primary extramediastinal presentation (PEMGZL).

MGZL Histopathology and Criteria

- MGZL shows asynchrony between morphology and immunophenotype, which may present in two ways:
  - Some cases show the morphology of NSCHL but with a preserved B-cell program
  - Others show a diffuse proliferation of large cells, which in addition to B-cell marker expression show strong and homogeneous CD30 positivity and often CD15 positivity

Essential and desirable diagnostic criteria

Essential for CHL-like MGZL: confluent growth of pleomorphic cells within a variably abundant microenvironment and dense fibrotic stroma; uniform strong expression of CD20, PAX5, and at least one additional B-cell marker (CD19, CD79a, BOB1, OCT2); positive expression of CD30, with varying intensity.

Essential for PMBCL-like MGZL: monomorphic sheets of medium-sized to large neoplastic cells within a variably dense fibrotic stroma; strong and uniform positive expression of CD30 and partial or complete loss of B-cell markers, or strong CD15 expression.

Desirable for both: because the complex histological features are often not reliably identifiable in core needle biopsies, a larger biopsy is strongly preferred for the diagnosis; absence of EBV.

MGZL Genetics

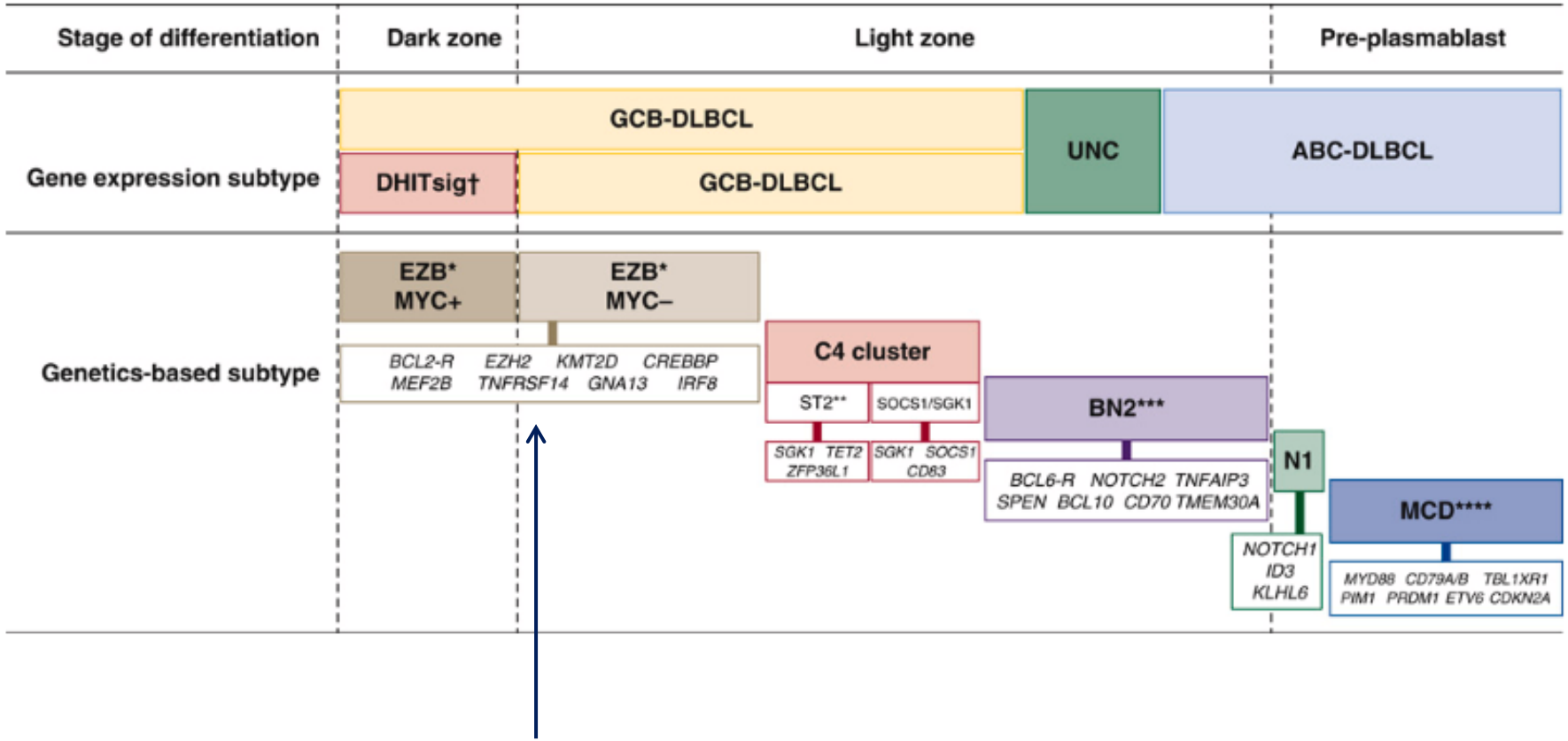
- Common findings include amplifications in 2p16.1 (REL/BCL11A locus), alterations affecting the JAK2/PDL2 locus at 9p24.1, and rearrangement of the CIITA locus at 16p13.13.3

- By next-generation sequencing, the following may be seen:
  - Mutations in *SOCS1, B2M, TNFAIP3, GNA13, LRRN3*, and *NFKBIA*
  - JAK-STAT and NF-κB pathway mutations (similar to CHL)

  - Notably, the usual alterations commonly seen in DLBCL are absent (GCB-DLBCL - *KMT2D*, *CREBBP*, and *BCL2*), with an absence of translocations involving *BCL2*, *BCL6*, and *MYC*).

DLBCL LymphGen Algorithm

- Based on genetic profiling studies by Chapuy et al, Schmitz et al, Wright et al
- Seven subtypes
- EZB (Cluster C3/BCL2) subtype (GCB-DLBCL):
  - *BCL2* rearrangements
  - Mutations in *EZH2* along with other chromatin modifying genes (*CREBBP*, *TNFRSF14*, PTEN)
  - Shares mutations with FL
  - Further subdivided into EZB-MYC positive and EZB-MYC negative.



Penn Medicine

Conclusions

- In combination with the molecular findings, the sharply demarcated focus represents a transformation of the low-grade follicular lymphoma and may best be classified as a large B-cell lymphoma given the genetic findings.

Thank you!
Questions?