A Practical Approach to the Diagnosis of B-Cell Lymphomas

Robert (Bob) Ohgami, MD, PhD
Professor of Pathology
Chief Medical Director for Research and Development, IOG
University of Utah and ARUP Laboratories

ARUP LABORATORIES

HEALTH UNIVERSITY OF UTAH

Outline

- Introduction
- Body
  - Didactic and case review of B-cell lymphomas
    - Key features and important studies
    - Nuances
- Conclusion
  - Summary
  - Future for B-cell lymphomas

A brief history of lymphoma classification

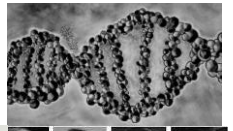
Clinical Morphology
- 1860s: Virchow
- 1920s: Oberling
- 1950s and 196

Clinical Morphology Immunophenotype
- 1974: Lukes a
- 1974: Kiel cla
- 1974: British
- 1978: NCI wo
- 1994: Stein a

Clinical Morphology Immunophenotype Genetics
- 2001: World H
- 2008: World H

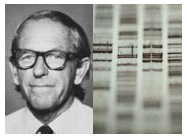
Clinical Morphology Immunophenotype Genetics
- 2017: World H
- 2022: World H
- 2022: ICC

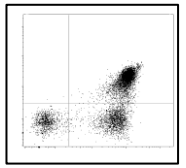
1950s: DNA discovered; Humans have 46 chromosomes

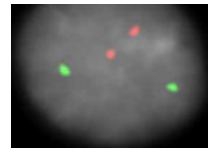


1960s: 1 color flow cytometry; simple IHC

1970s: Sanger sequencing; Q&C and G banding
Improved IHC; Immuno-EM

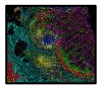






1980s: 4 color flow cytometry; PCR; interphase FISH

1990s: 8 color flow cytometry; CGH



2000s: Mass cytometry; Human genome sequenced; Roche 454

2010s: Imaging mass cytometry; 30+ color flow cytometry, Nanopore, Pacbio "single molecular real-time" sequencer

2020s: Artificial Intelligence and machine learning

Greater importance of Genetics and molecular phenotyping

Norton AJ. Classification of non-Hodgkin's lymphomas. Ballieres Clin Haematol. 1996;9:641–52. doi: 10.1016/s0950-3536(96)80046-1. [PubMed] [CrossRef] [Google Scholar] 5. van Besien K, Schouten H. Follicular lymphoma: A historical review. Leuk Lymphoma. 2007;48:232–43. doi: 10.1080/10428190601059746. [PubMed] [CrossRef] [Google Scholar]
Lukes RJ, Collins RD. A functional approach to the classification of malignant lymphoma. Recent Results Cancer Res. 1974;46:18–30. doi: 10.1007/978-3-642-80829-6_4. [CrossRef] [Google Scholar] 8. Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. Cancer. 1974;34:1488–503. doi: 10.1002/1097-0142(197410)34:8+<1488::AID-CNCR2820340822>3.0.CO;2-
Updated Kiel classification for lymphomas.Stansfeld AG, Diebold J, Noel H, Kapanci Y, Rilke F, Kelényi G, Sundstrom C, Lennert K, van Unnik JA, Mioduszewska O Lancet. 1988 Feb 6; 1(8580):292-3.
Bennett MH, Farrer-Brown G, Henry K, Jaliffe AM, Gerard-Marchant R, Hamlin I, et al. Classification of non-Hodgkin's lymphomas. Lancet. 1974:405–6. doi: 10.1016/S0140-6736(74)91786-3.
National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a working formulation for clinical usage - The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer. 1982;49:2112–35.

What information is necessary to diagnose a B-cell lymphoma?

Information

- Clinical: Age, Gender, Sequelae
- Anatomic: Sites of involvement
- Laboratory data: CBC, LDH, Chem
- Radiology: PET, CT, MRI
- Histomorphology: Architecture of tissue, Neoplastic cell features, Inflammatory response
- Immunophenotype: Flow cytometry, IPOX
- Cytogenetics: Karyotype
- FISH: *MYC, BCL2*
- Molecular: Next generation sequencing, Single gene PCR testing

Broad Pathology Classification of Mature B-cell lymphomas

- **Nodal**
  - Diffuse large B-cell lymphoma, NOS
  - Nodal marginal zone lymphoma
  - Mantle cell lymphoma
  - Follicular lymphoma
  - High grade B-cell lymphomas
  - Nodular lymphocyte predominant HL/BCL
  - T-cell histiocyte rich LBCL
  - SLL
  - ALK+ LBCL
  - KSHV/HHV8 Multicentric CD
  - EBV+ LBCL
  - Primary Mediastinal LBCL
  - Mediastinal Grey Zone Lymphoma
  - HHV8+ germinotropic LPD
  - PTLD/ Lymphoid proliferations/lymphomas associated with immune deficiency and dysregulation
  - Large B-cell lymphoma with *IRF4* rearrangement
  - DLBCL/HGBCL with *MYC* and *BCL2* gene rearrangements
  - HGBCL, NOS
  - HHV8+ DLBCL
- **Cutaneous**
  - Cutaneous MZL
  - Primary cutaneous LBCL, leg type
  - Primary cutaneous FL

- **Extranodal**
  - Extranodal MZL of MALT
  - Lymphomatoid granulomatosis
  - Plasmablastic lymphoma
  - Burkitt lymphoma
  - Duodenal type FL
  - EBV+ MCU
- **Splenic**
  - Hairy cell leukemia
  - Splenic MZL
  - Splenic diffuse red pulp small B-cell lymphoma
  - HCL-V/ Splenic B-cell lymphoma/leukemia with prominent nucleoli
- **Leukemic**
  - Mononclonal B-cell lymphocytosis
  - CLL
  - Leukemic non-nodal MCL
  - Lymphoplasmacytic lymphoma
- **Other**
  - Fibrin associated LBCL
  - Intravascular LBCL
  - DLBCL associated with chronic inflammation
  - Primary effusion lymphoma
  - Fluid overload-associated large B-cell lymphoma/HHV8andEBV negative PEL

LBCL = large B-cell lymphoma; CLL = chronic lymphocytic leukemia; SLL = small lymphocytic lymphoma; MZL = marginal zone lymphoma; HGBCL = high grade B-cell lymphoma
MALT = mucosa associated lymphoid tissue; HL = Hodgkin lymphoma; LPD = lymphoproliferative disorder; CD = Castleman disease; MCU = mucocutaneous ulcer

We cannot cover all

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We will create a foundational framework for diagnoses

So let’s get started!

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How can we handle this?

- We need a starting point
- Anatomic
- Clinical features
- Immunophenotype
- **Cell size**

Cell size

- **Small-medium sized B-cell lymphomas**
- Large in cell size B-cell lymphomas

Small-medium sized B-cell lymphomas

- Follicular lymphomas (FL)
- Marginal zone lymphomas (MZL)
- Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)
- Mantle cell lymphoma (MCL)

There are subtypes and nuances to these groups

Follicular Lymphoma

- In situ follicular neoplasm/neoplasia
- Duodenal type follicular lymphoma
- Primary cutaneous follicle center lymphoma
- Pediatric-type follicular lymphoma
- Testicular follicular lymphoma

Marginal zone lymphoma

- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Primary cutaneous marginal zone lymphoma/LPD
- Nodal marginal zone lymphoma
- Pediatric marginal zone lymphoma
- Splenic marginal zone lymphoma

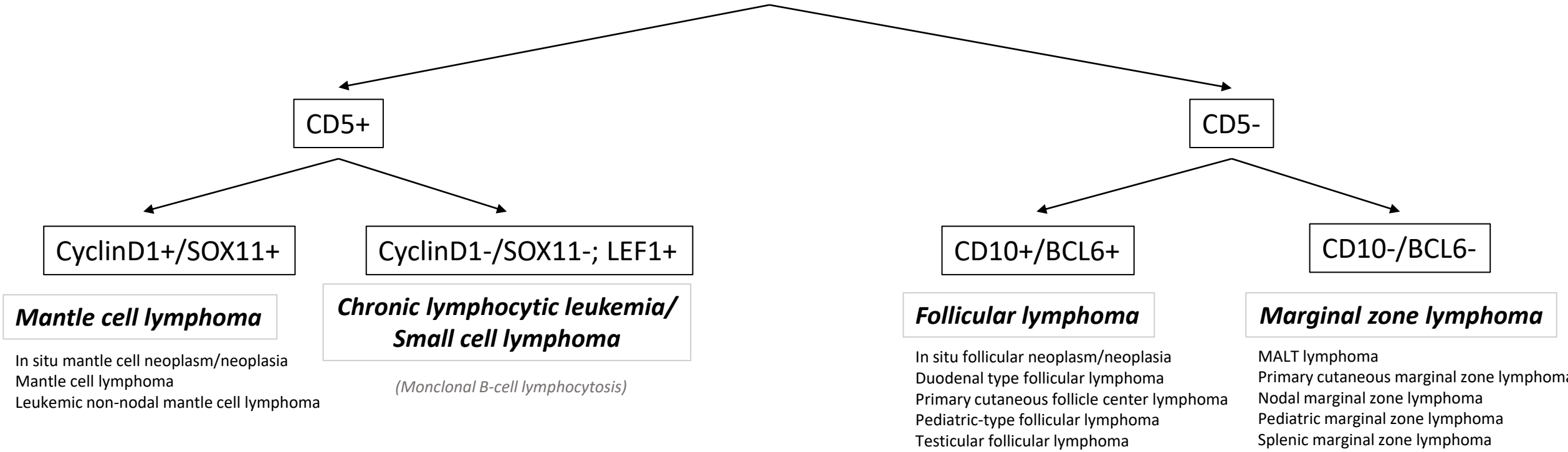
Mantle cell lymphoma

- In situ mantle cell neoplasm/neoplasia
- Mantle cell lymphoma
- Leukemic non-nodal mantle cell lymphoma

Covering each of these

- Not too difficult
- We can use decision trees to help us

Small-medium cell size B-cell lymphoma

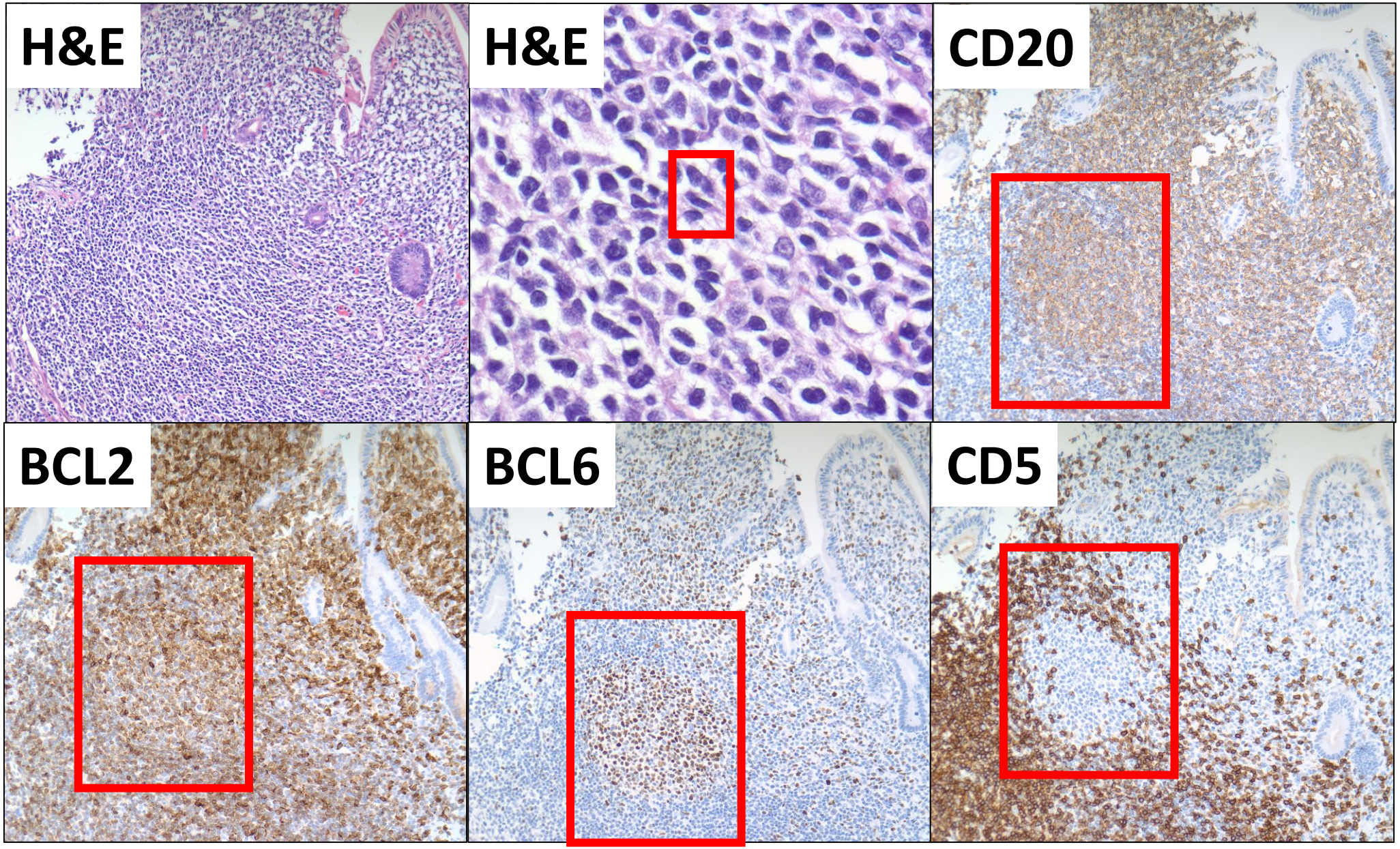


Let’s look at clinical cases to shape this talk

Case 1

- 70 year old
- Some bloating and intestinal discomfort
- GI biopsies performed

In particular a.. .duodenal biopsy



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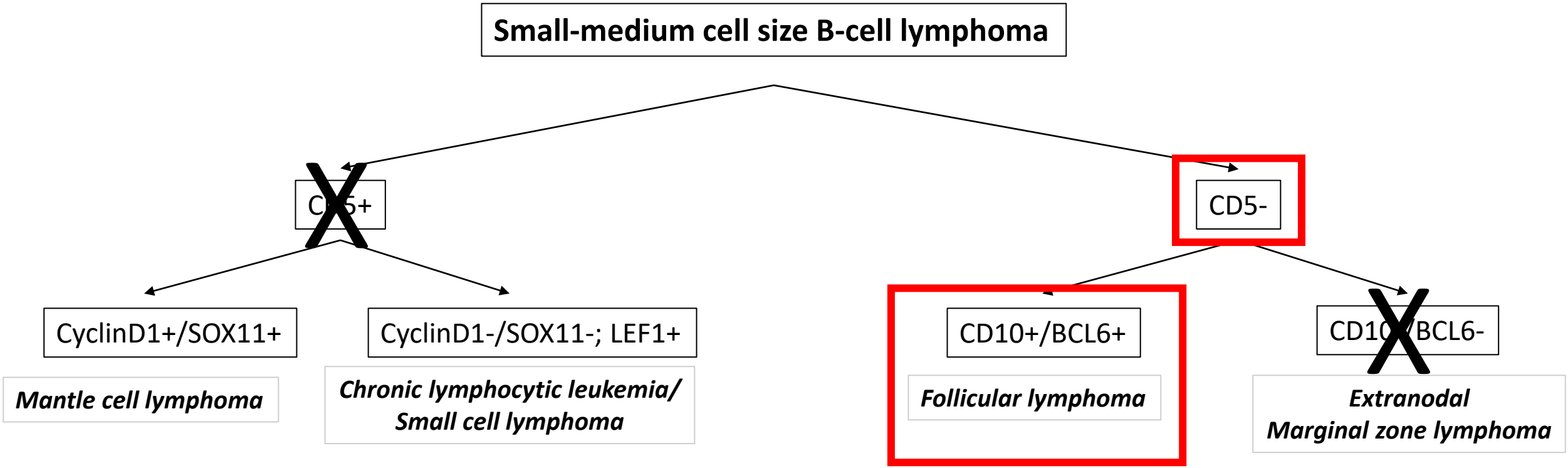


But there is more to this…

- The lesion is in the duodenum…
- No other lymphadenopathy
- This is a “special” follicular lymphoma…
- In the duodenum…

Diagnosis?

Duodenal-type follicular lymphoma

Duodenal-type follicular lymphoma

- Typically adults
- Classically localized to the small intestine (multiple localized polyps may be seen)
- Excellent prognosis (indolent)
- Aggressive features (i.e. extensive infiltration into the muscularis propria or grade 3) are not those of d-FL
- CD20+/CD10+/BCL6+ and **BCL2+**
- Most cases show *BCL2* and/or *BCL6* translocation

“Types” of follicular lymphoma

- Classic/”Adult-type”/”Usual-type” follicular lymphoma
- Pediatric-type follicular lymphoma
- Duodenal-type follicular lymphoma
- Primary cutaneous follicle center lymphoma
- *Large B-cell lymphoma with IRF4 rearrangement*

“Classic” follicular lymphoma

- In adults
- CD20+/CD10+/BCL6+ and **BCL2+**
- This is a systemic lymphoma
- WHO-HAEM5 does not require grading
- ICC
  - Grade 1, 2, 3A, 3B
    - Depends on numbers of centroblasts

Follicular large B-cell lymphoma/ Follicular lymphoma grade 3B

WHO-HAEM5 *ICC*

- Sheets of large B-cells in a follicular pattern
- Usually no *BCL2* translocation
- Molecularly and immunophenotypically distinct from follicular lymphoma
  - Sometimes *MYC* translocation seen
  - Often CD10 negative and MUM1 protein positive

Pediatric-type follicular lymphoma

- In children and young adults
- Localized lymphoma (one site/lymph node involved) usually neck region
- CD20+/CD10+/BCL6+ and **BCL2 protein negative or very dim**
- **NO *BCL2* or *BCL6* gene translocation**
- **Somatic mutations in *IRF8* and *MAPK* pathway**

Primary cutaneous follicle center lymphoma

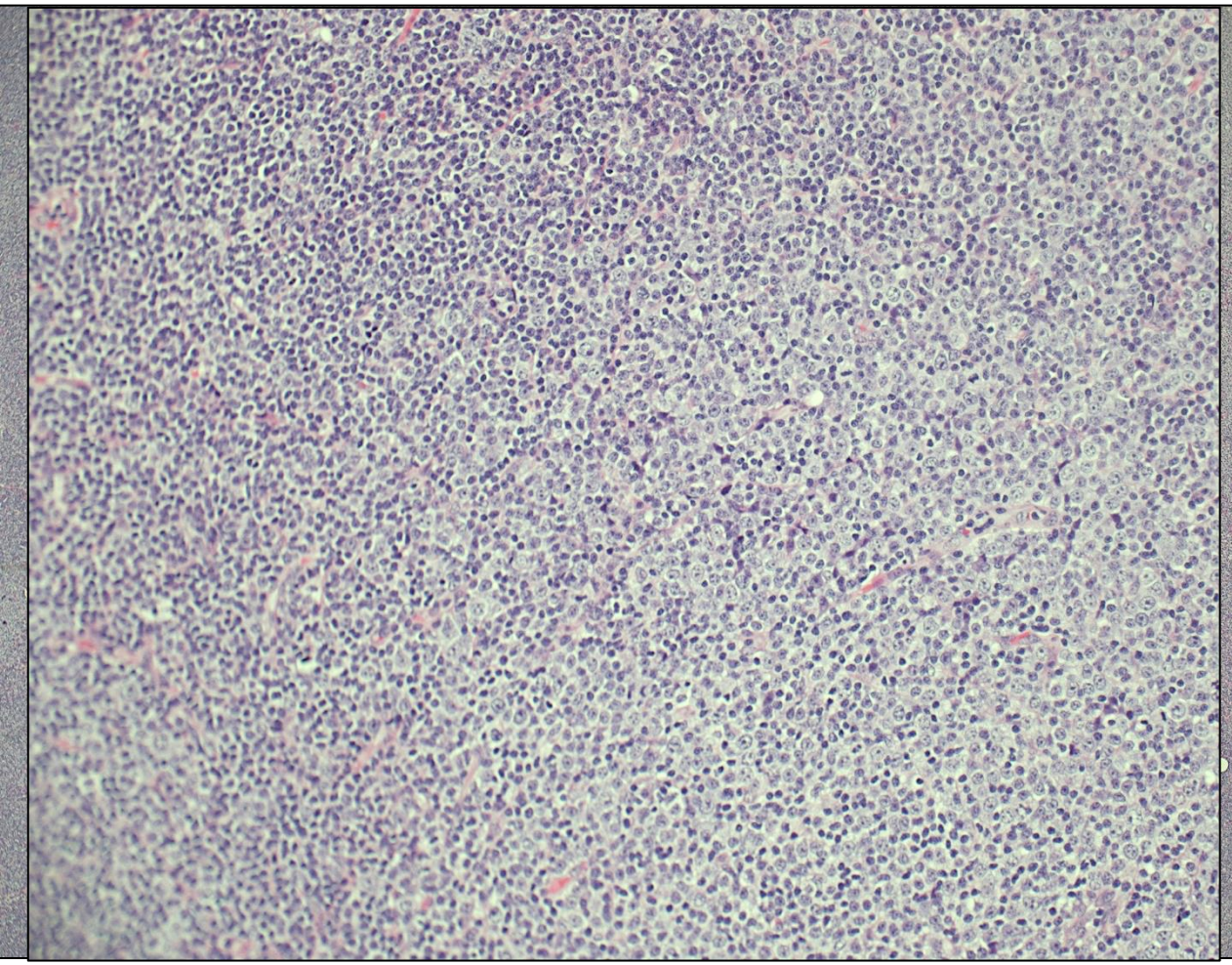
- Usually adults
- In the skin
- CD10+/CD20+/BCL6+ usually **BCL2 protein negative or dim**
- More indolent

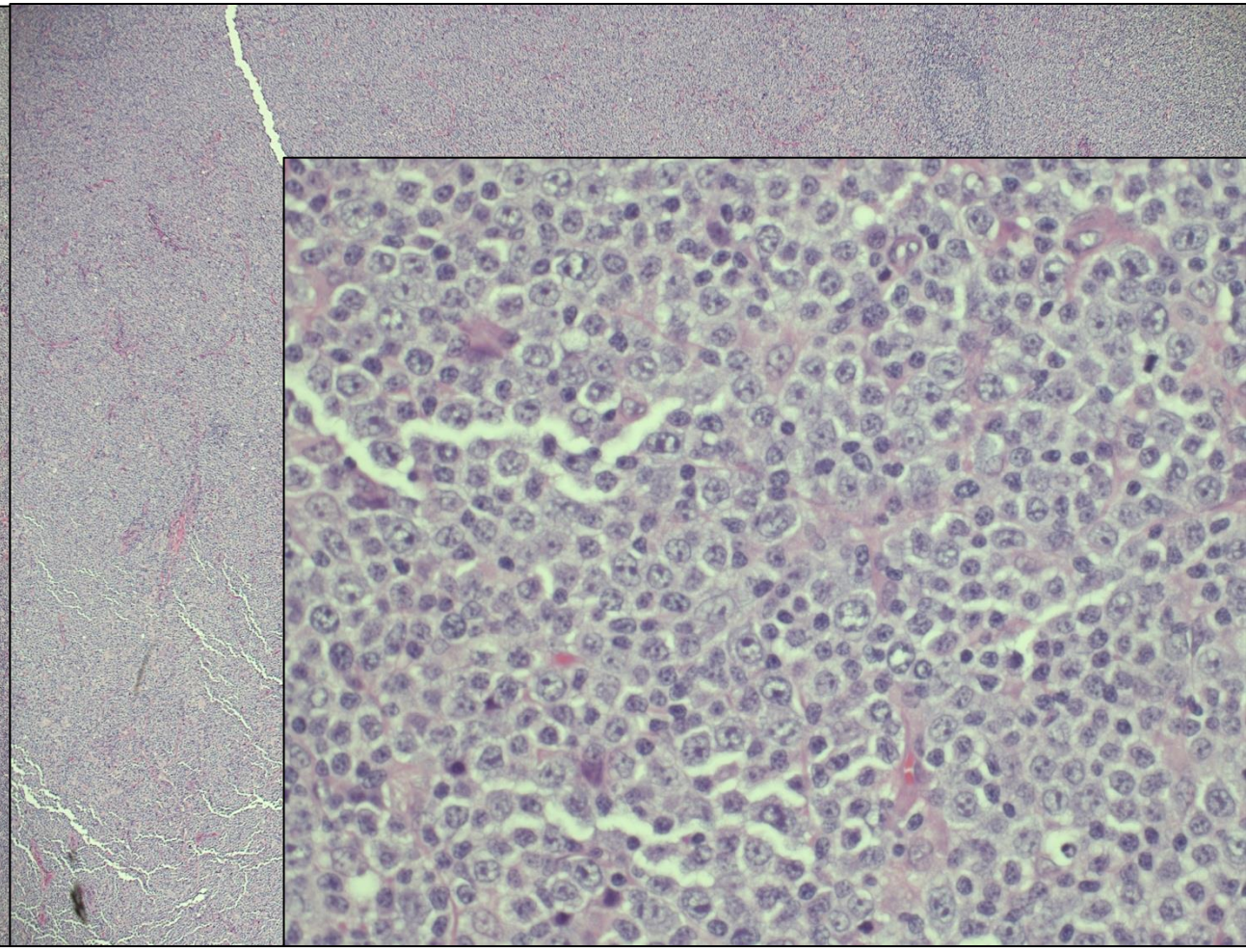
Large B-cell lymphoma with *IRF4* rearrangement

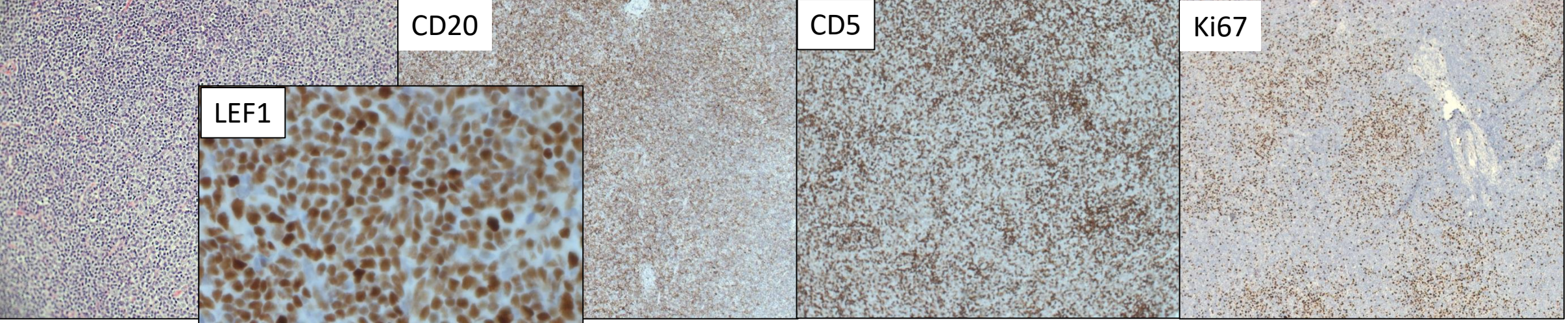
- Kids and younger adults
- Presents at low stage
- Often head and neck region (Waldeyer ring)
- CD20+, CD10+/-, MUM1/IRF4+, BCL6+ protein
- Looks like large B-cell lymphoma or high grade follicular lymphoma
- 100% of cases with *IRF4* translocation

Case 2:

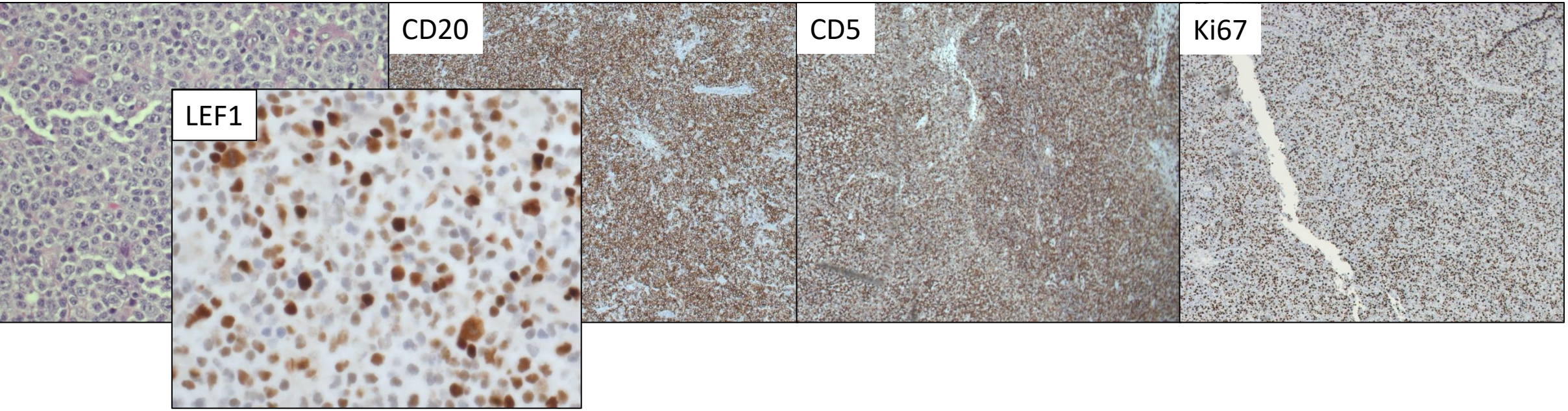
- 67 year old with a history of some lymphoma
- Increasing lymphadenopathy resistant to ibrutinib

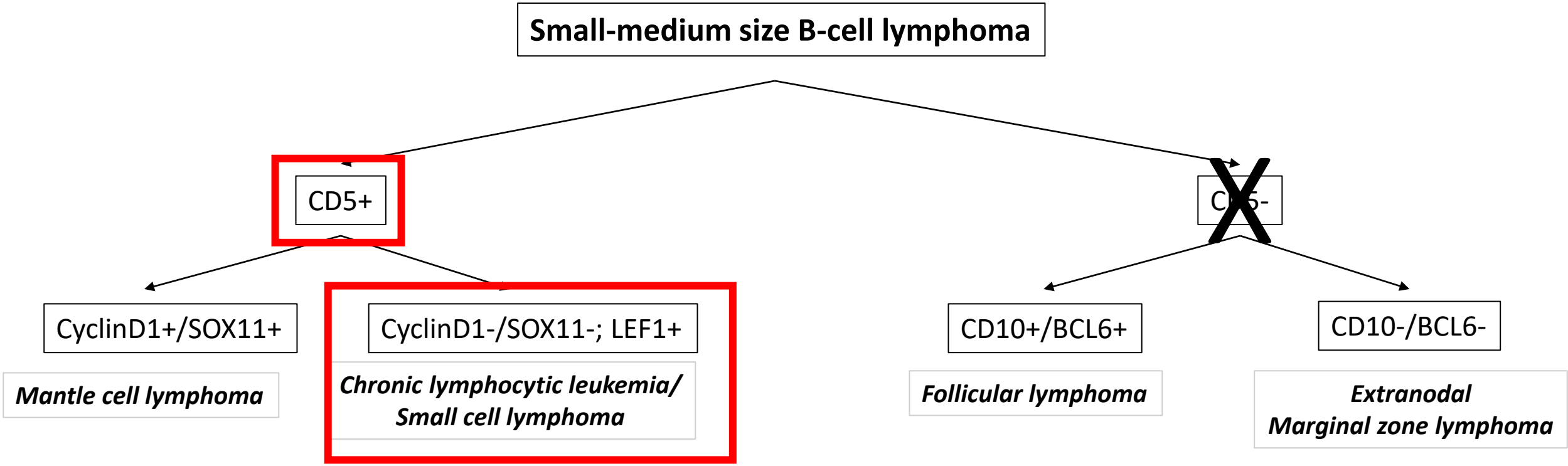






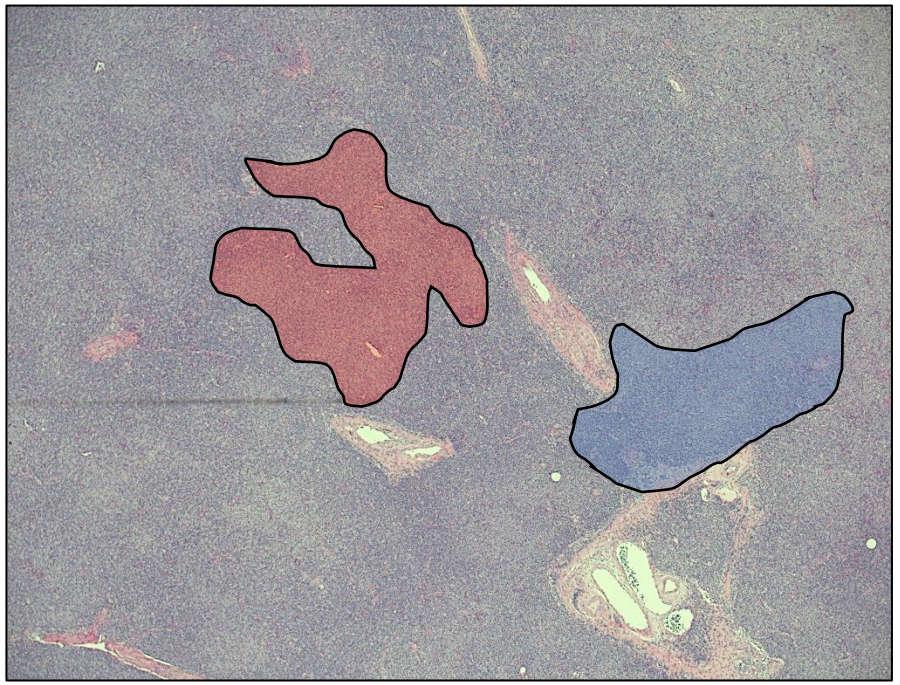
SOX11 and CyclinD1 negative

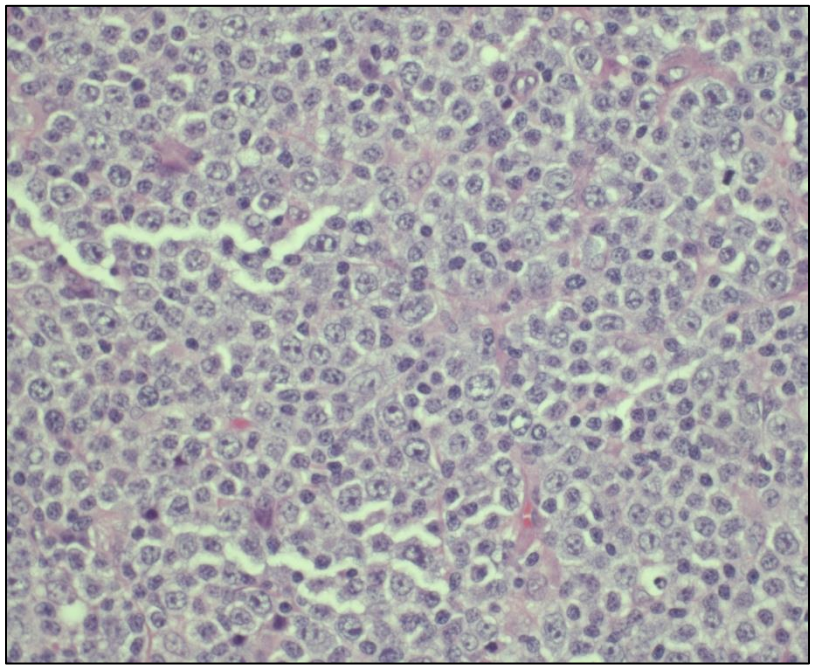




What is the diagnosis?

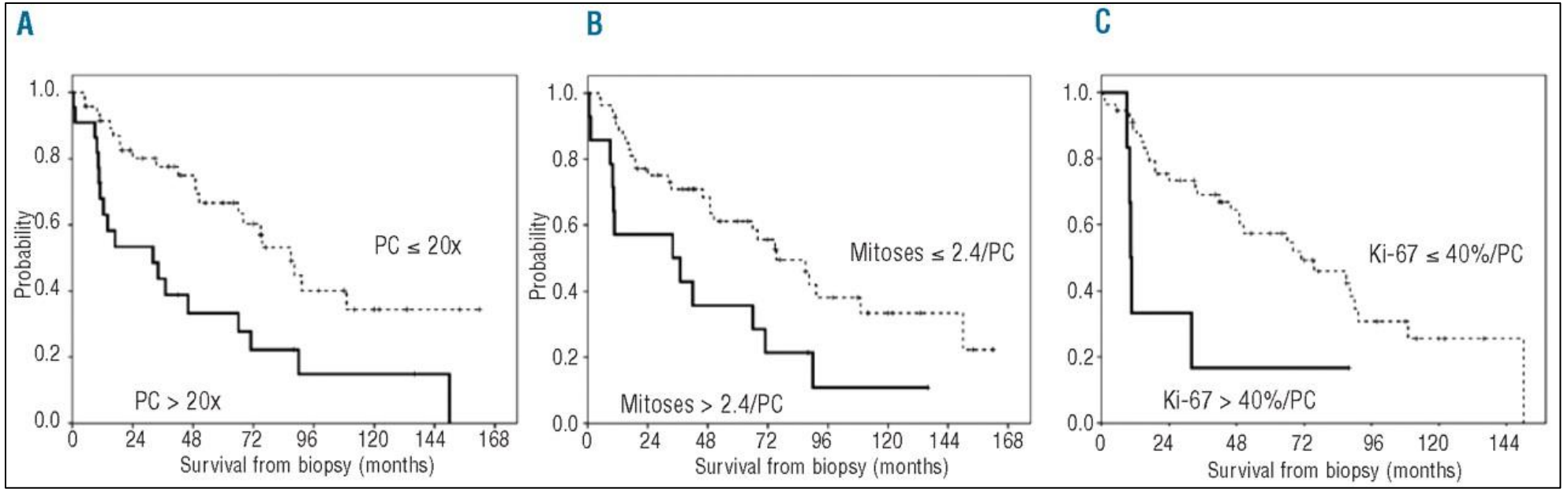
- CLL/SLL
- With enlarged proliferation centers
- And DLBCL





What is the significance of enlarged proliferation centers?

- Accelerated form of CLL/SLL



Giné E, et al., Expanded and highly active proliferation centers identify a histological subtype of chronic lymphocytic leukemia ("accelerated" chronic lymphocytic leukemia) with aggressive clinical behavior. Haematologica. 2010 Sep;95(9):1526-33.

Why isn’t this B-prolymphocytic leukemia?

What about B-cell prolymphocytic leukemia (B-PLL)?

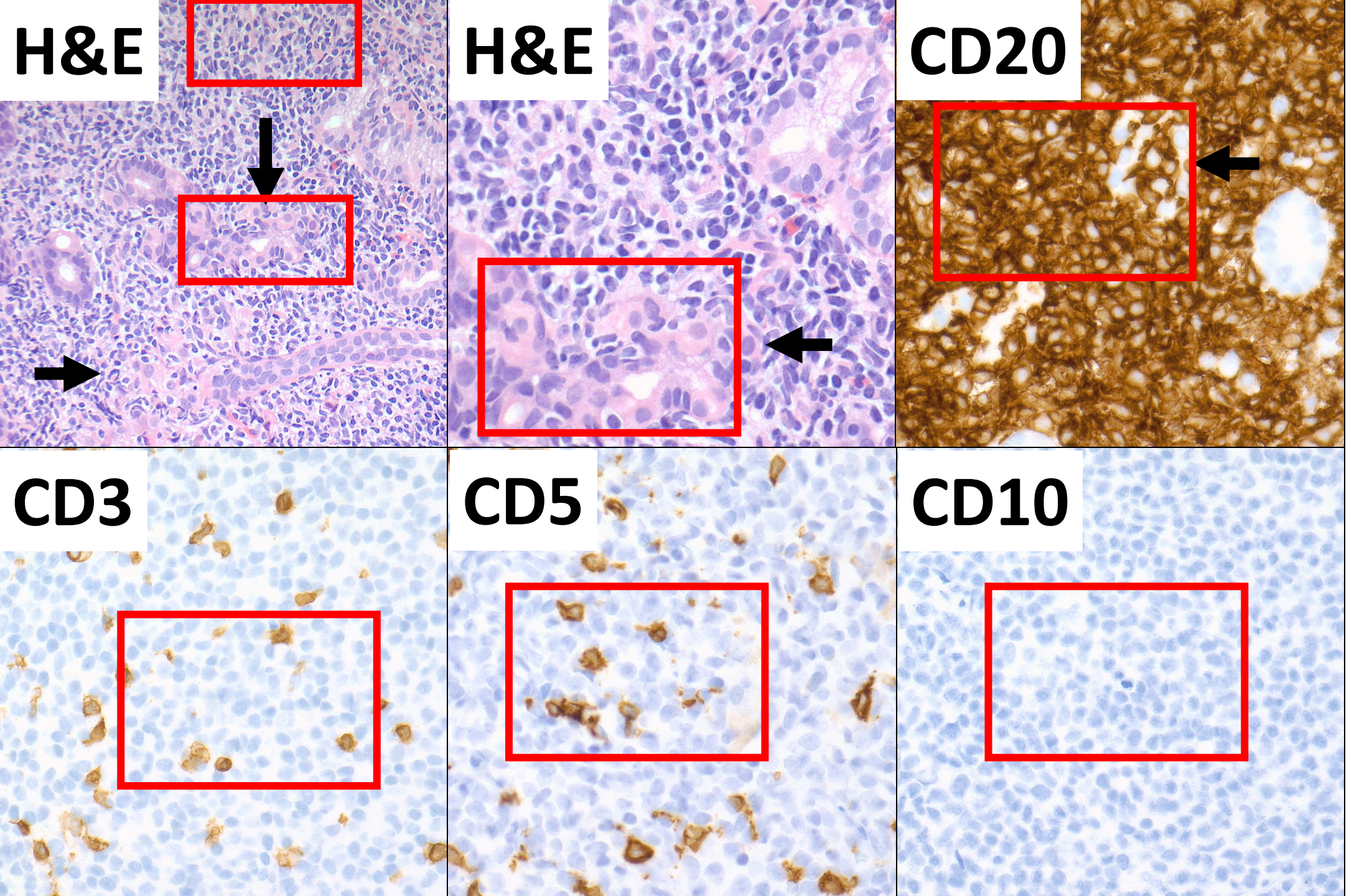
- ICC still has B-PLL as entity
  - No history of CLL/SLL
  - No hairy cell projections
  - No sinusoidal infiltration
  - Complex karyotype (affecting *TP53* and *MYC*)
- WHO-HAEM5 deletes B-PLL as most cases in the past were actually:
  - Leukemic mantle cell lymphoma
  - Prolymphocytic progression of CLL/SLL (>15% prolymphocytes)
  - *Splenic B-cell lymphoma with prominent nucleoli*
    - Hairy cell leukemia variant
    - *(would include cases of ”B-PLL” by ICC)*

Chronic lymphocytic leukemia/small lymphocytic lymphoma

- Immunophenotype: CD19, CD20, CD5, CD23
- Molecular: *IGHV* and *TP53*; *NOTCH1, SF3B1*, *BIRC3*
- Cytogenetics: Complex karyotype
- WHO-HAEM5 has Prolymphocytic progression of CLL/SLL (>15% prolymphocytes in BM or PB)
- Accelerated CLL
- Richter's transformation
- Richter-like transformation (after Ibrutinub interruption)
  - Just re-start Ibrutinib and large cells go away

Case 3

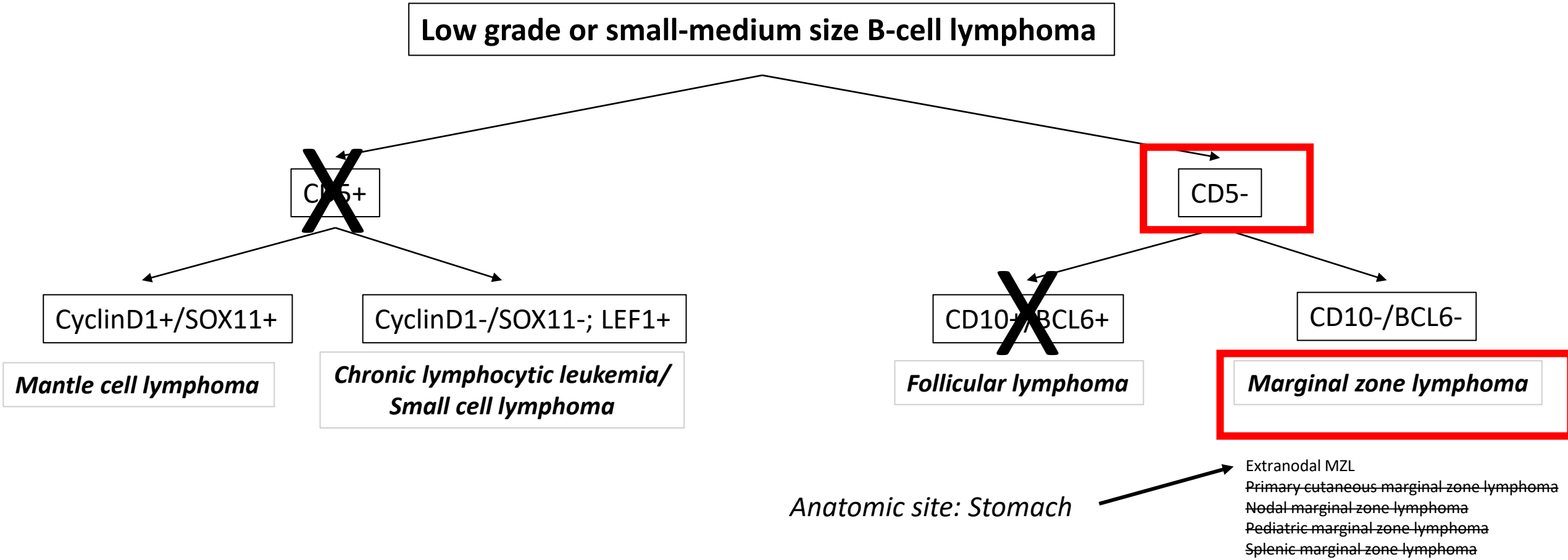
- 63 year old male
- Complains of some mild indigestion
- Stomach biopsy performed


H&E
H&E
CD20
CD3
CD5
CD10

What is this?

One way to approach the diagnosis…

Use algorithms



Extranodal marginal zone lymphoma

Extranodal marginal zone lymphoma (ENMZL)

- In stomach ***Helicobacter Pylori*** infections (30-90% of cases)
- ENMZL show **monocytoid appearance**, with **cleared cytoplasm**, and **lymphoepithelial lesions** (destructive invasion of B-cells into glands)
- Some cases have **amyloid deposition**; this is not ~~primary amyloidosis~~
- The immunophenotype is **CD20 positive**; **no CD5 and CD10**.
- CD43+ can help determe that the infiltrate is neoplastic.
- A subset of cases may be positive for **t(11;18)(q21;q21)** (may be more resistant to antibiotic therapy)
- Trisomies of chromosome 3 or 18 can be seen

Differential diagnosis and pointers

- Mantle cell lymphoma, follicular lymphoma, reactive infiltrate
- **Cyclin D1 and SOX11 are always negative** (rules out mantle cell lymphoma)
- **MNDA and CD43 may be positive** in a subset of cases (argues against follicular lymphoma)
- **BCL2 is often positive** and **kappa and lambda IHC may show monotypia** (rules out reactive infiltrate)
- **You don’t get atypical reactive marginal zone hyperplasia** in the stomach
- You can get atypical reactive marginal zone hyperplasia in other areas of the gut

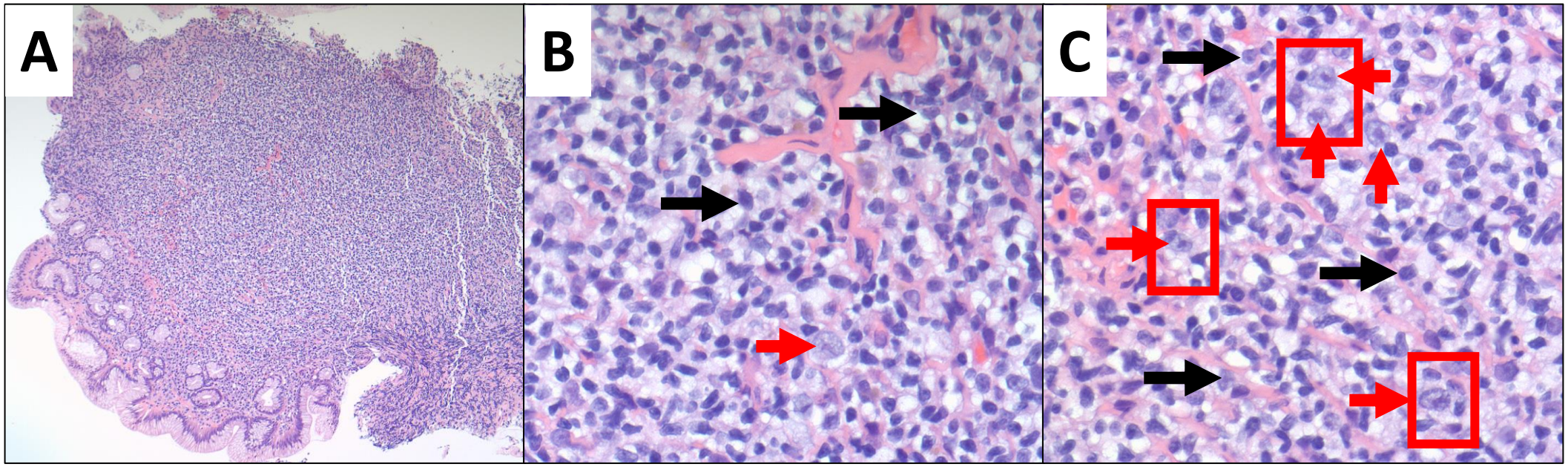
Extranodal marginal zone lymphoma (ENMZL) vs reactive marginal zone hyperplasia (rMZH)

	ENMZL	rMZH
Morphology	Small-medium size lymphocytes with cleared cytoplasm/monocytoid in a diffuse or marginal zone pattern. Lymphoepithelial lesions and/or follicular colonization may be present.	Small-medium size lymphocytes with cleared cytoplasm/monocytoid in a marginal zone pattern. Lymphoepithelial lesions and/or follicular colonization are rare.
Immunophenotype	CD20+, CD43+/-, MNDA+/-, Bcl-2+/-	CD20+, CD43+/-, MNDA+/-, Bcl-2+
B-cell light chain restriction (by immunostains)	Restricted light chain expression expected.	May show restricted light chain expression
B-cell clonality (by DNA sequencing)	Clonal	Non-clonal

But as I said…

- You can get atypical reactive marginal zone hyperplasia in some areas of the gut
- **You don’t get atypical reactive marginal zone hyperplasia** in the stomach

Then you look in another area of the biopsy…



Larger cells are seen…

What do you do when you have increased large cells?

- If you see “sheets” then it is diffuse large B-cell lymphoma
- However, cases with fewer than 20% large cells are NOT LBCL
- When you see sheets of large B-cells outside follicles = LBCL
- With >20% large cells, literature supports these cases behave clinically more aggressively than typical ENMZL
- Ki67, p53, c-myc immunostains may be useful to diagnose large cell transformation in ENMZL.

May reflect underlying genetic abnormalities

Transformations of indolent B-cell lymphomas

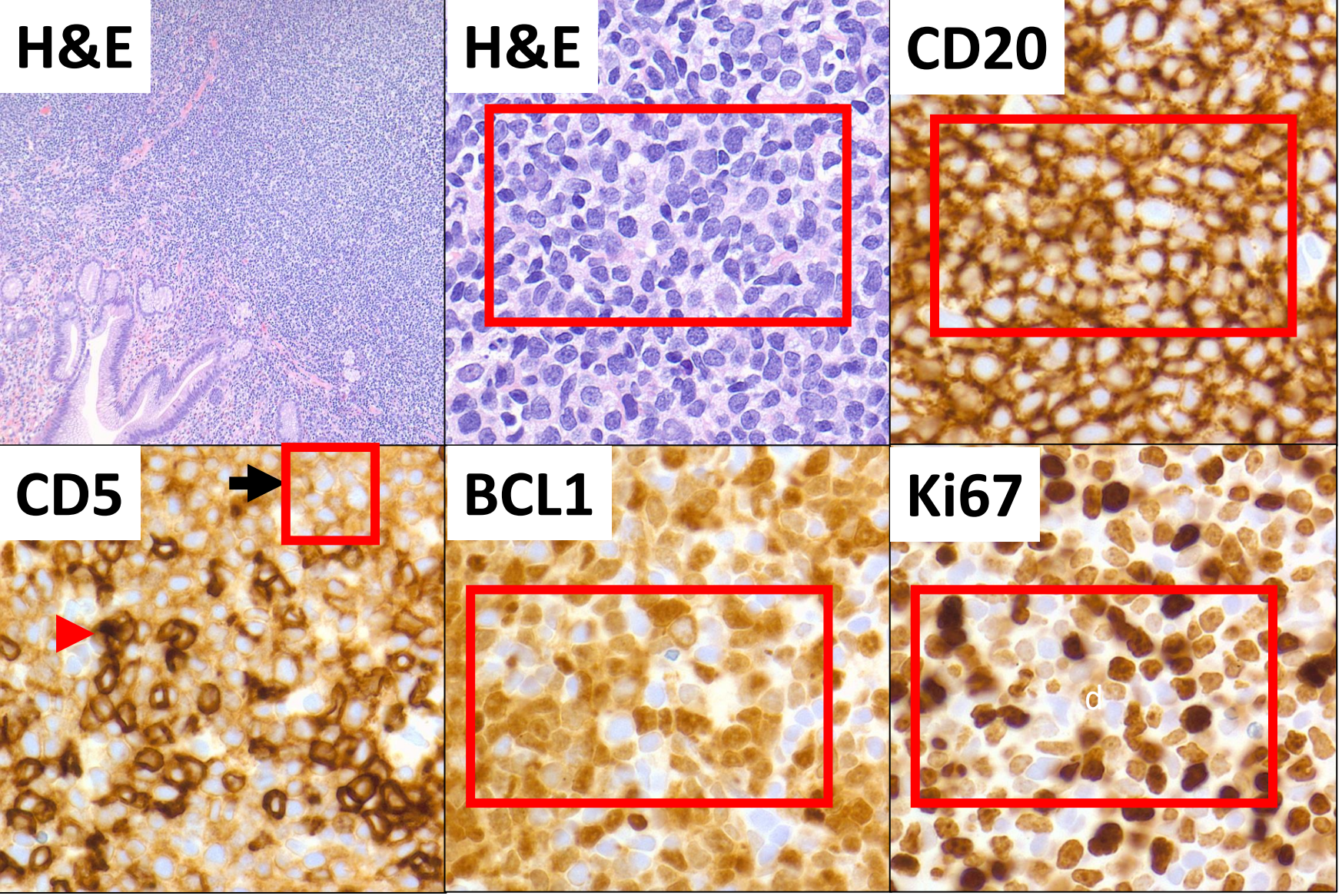
WHO-HAEM5

- New entity in WHO-HAEM5
- What is this?
  - When you see DLBCL in the setting of low-grade lymphoma (i.e. CLL/SLL, FL, MZL)
- <u>DLBCL MUST be clonally related to the low-grade lymphoma</u>
- Signout: Large B-cell lymphoma transformed from ENMZL

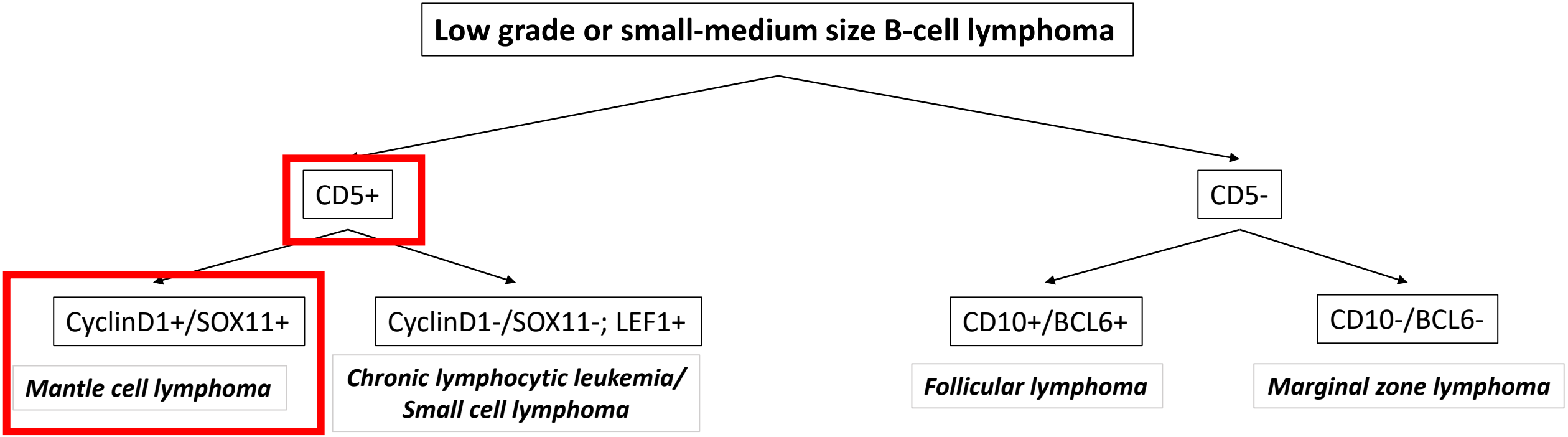
In CLL/SLL 22% of cases with DLBCL; the DLBCL is NOT related to the CLL/SLL
Subset of MZL with DLCBL; the DLBCL may not always be related to MZL though typically it is

Case 4

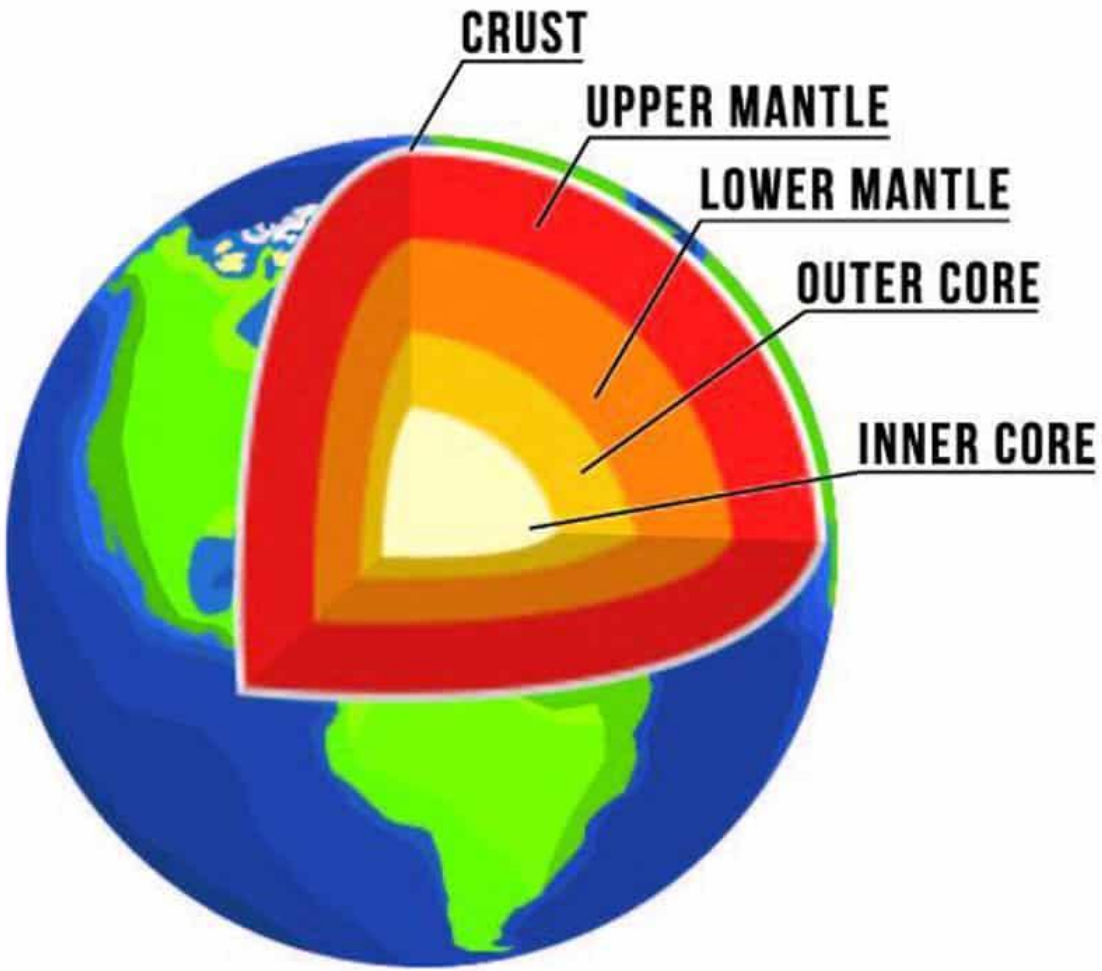
- 51 year old with polyps discovered along the small intestine during routine GI biopsy

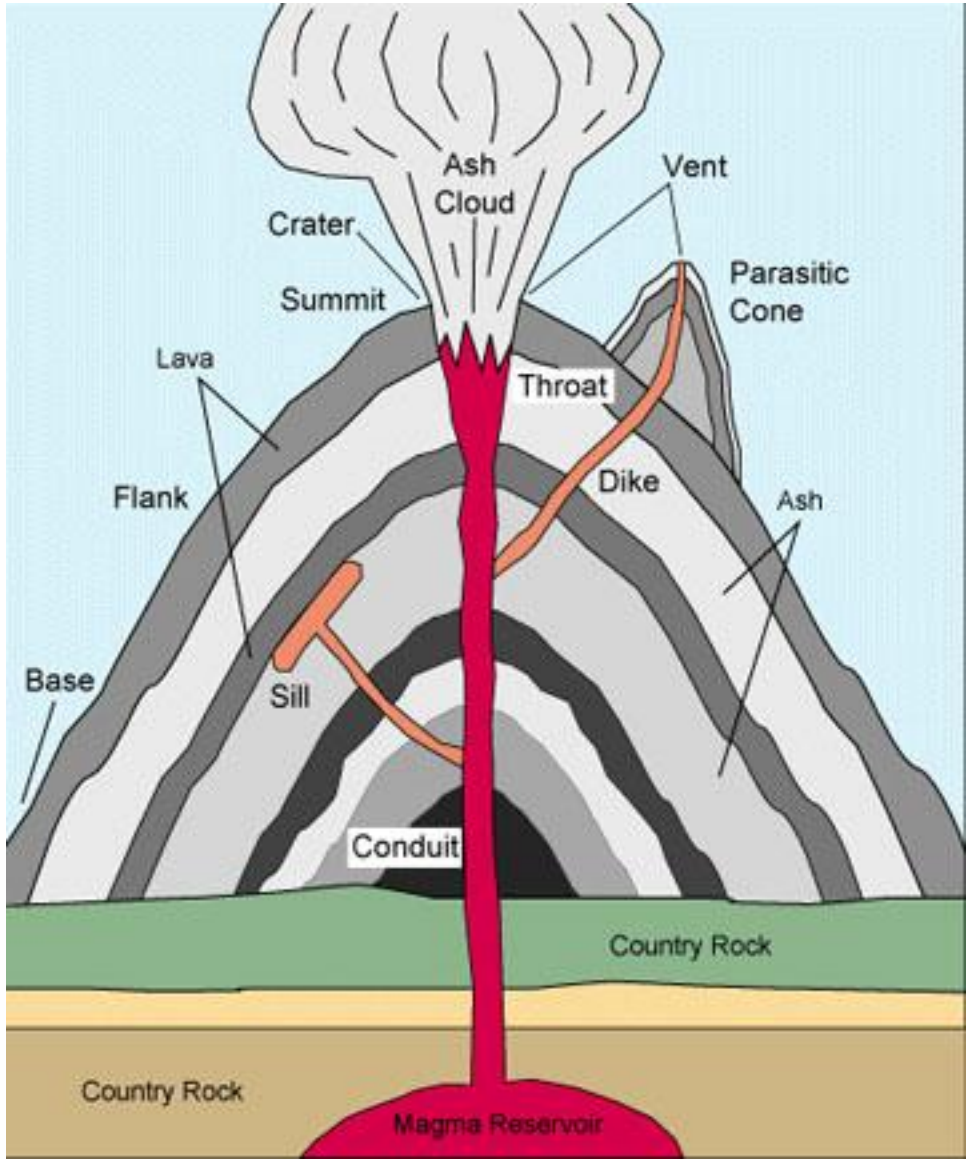


What is this?



Mantle cell lymphoma





Mantle cell lymphoma

- Mantle cell lymphoma may present anywhere in the gastrointestinal tract.
- In the colon, mantle cell lymphoma may present as an intestinal polyposis (so can follicular lymphoma or marginal zone lymphoma)
- Cyclin D1 protein expression and the identification of t(11;14)(q13;q32) are diagnostic of this entity
- A Ki67 index must be evaluated to assess for prognosis
- *TP53* mutations are important for appropriate treatment
- Mantle cell lymphoma is **not a low grade lymphoma**, it typically proliferates more aggressively.

Mantle cell lymphoma

- In situ mantle cell neoplasm/neoplasia
  - Incidental finding
  - Slightly expanded mantle zone with cyclinD1 positive B-cells
  - Shows t(11;14)(q13;q32)
  - Indolent
- Leukemia non-nodal mantle cell lymphoma
  - In blood and bone marrow and spleen
  - Shows t(11;14)(q13;q32)
  - Often SOX11 negative but cyclinD1 positive; low Ki67, CD5 may be dimmer
  - May have some CD200 and CD23 expression
  - Clinically more indolent

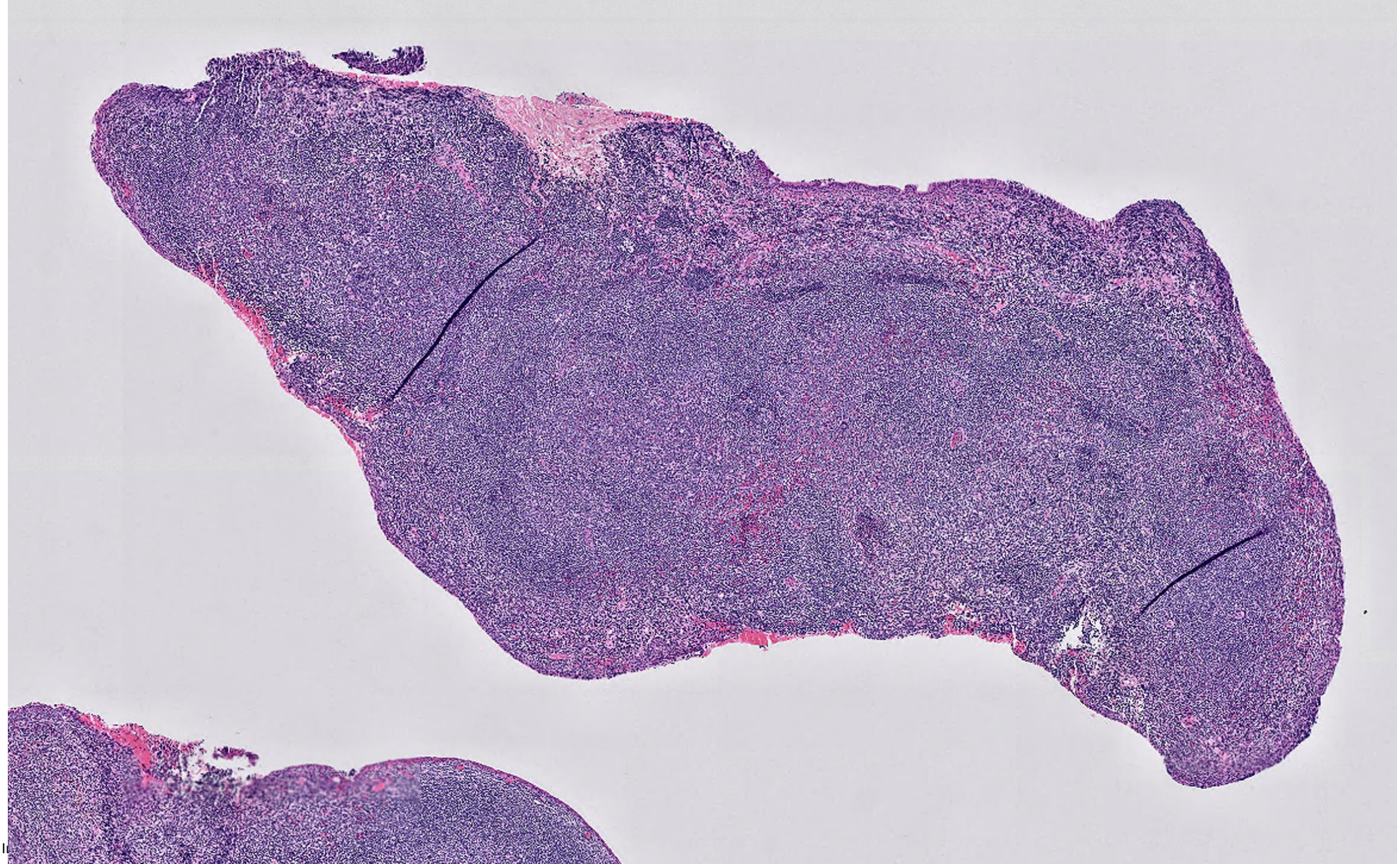
Some like tables

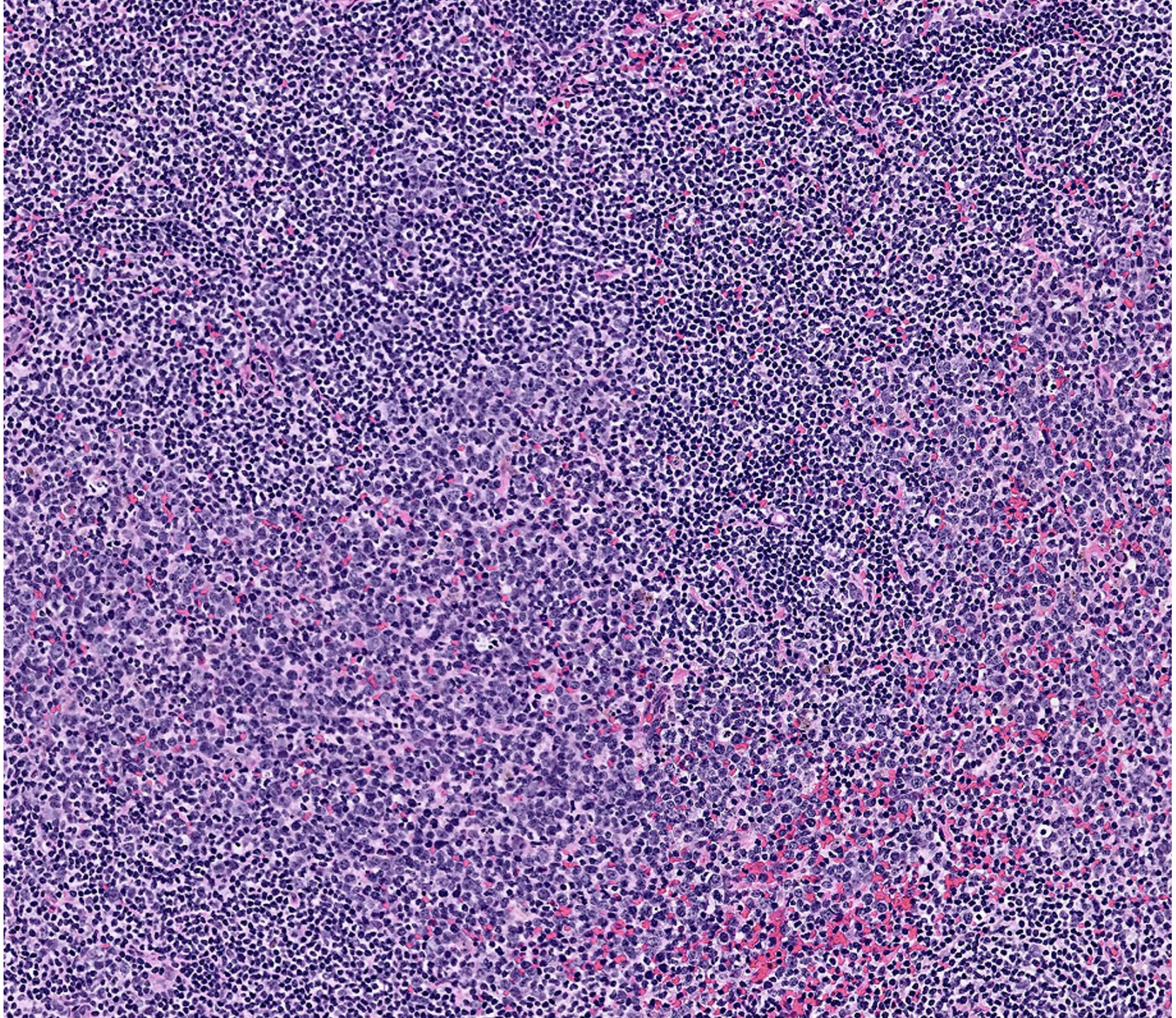
Small-medium size B-cell lymphomas

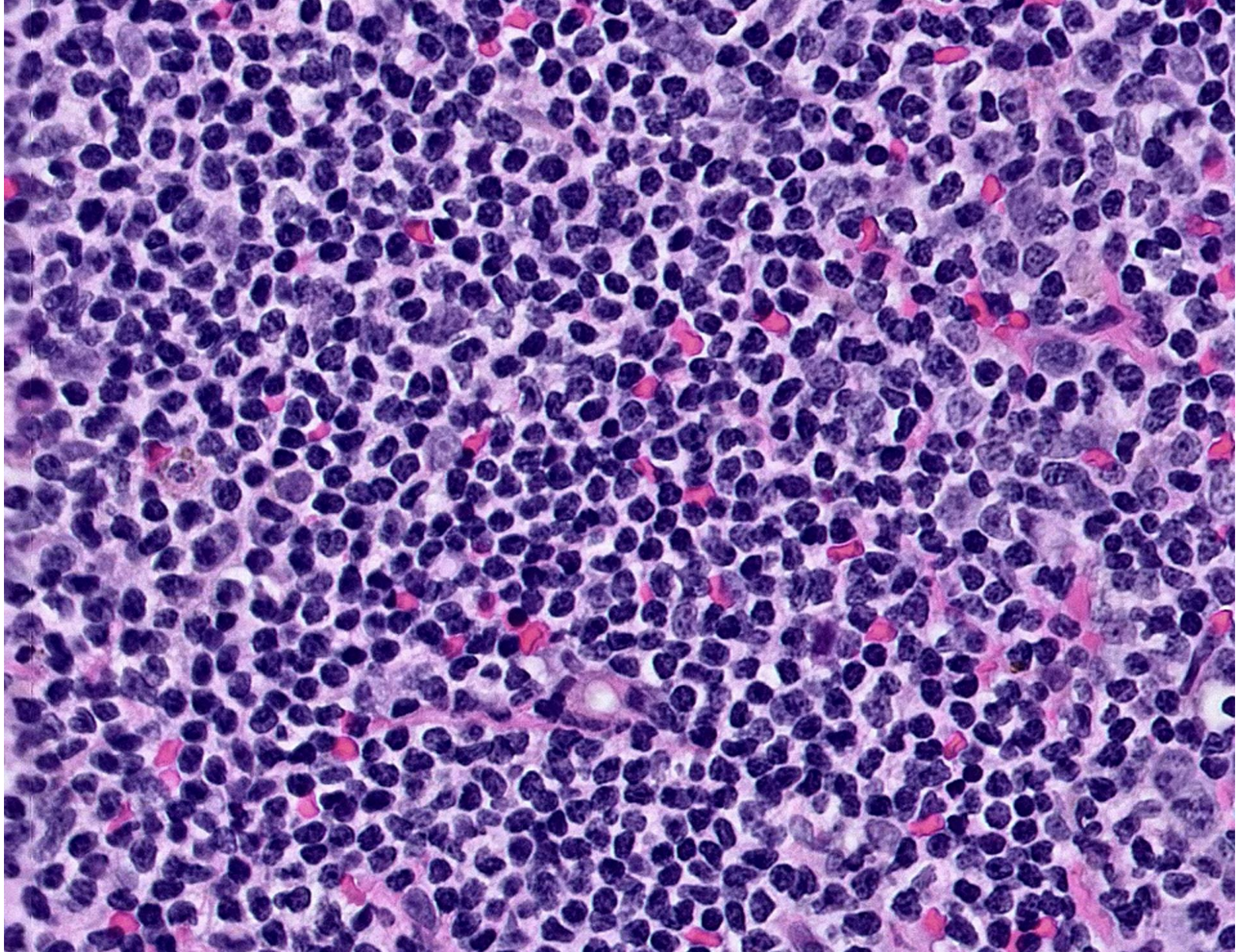
Entity	Morphology	Immunophenotype	Genetics
ENMZL	Monotonous infiltrate of small cells with cleared cytoplasm or monocytoid appearance, may show plasmacytic differentiation.	CD20+, CD5-, CD10-, BCL6-, Cyclin D1- ,CD43+/-, MNDA+/-	May show t(11;18)(q21;q21);BIRC3/MALT1, trisomy 3 or 18
CLL/SLL	Monotonous infiltrate of small cells with blocky chromatin. Proliferation centers may be seen.	CD20+, CD5+, CD10-, BCL6-, Cyclin D1-, CD43+/-, MNDA+/-, LEF1+	May show deletions of 11q, 13q, 17p, and trisomy 12
FL	Back to back follicles without polarization and no tingible body macrophages. Centrocytic cells and centroblastic cells in varying proportions depending on grading (1-3A/B)	CD20+, CD5-, CD10+, BCL6+, Cyclin D1-, CD43-, MNDA-	t(14;18)(q32;q21); IGH/BCL2 or less frequently t(3;14)(q27;q32); IGH/BCL6. The presence of either of these translocations in the setting of small B-cells is diagnostic for FL.
MCL	Vague nodules or diffuse effacement by monotonous small-medium size cells with irregular indented nuclei.	CD20+, CD5+, Cyclin D1+, SOX11+, CD10-, BCL6-	t(11;14)(q13;q32); IGH/CCND1. The presence of this translocation is diagnostic for MCL.

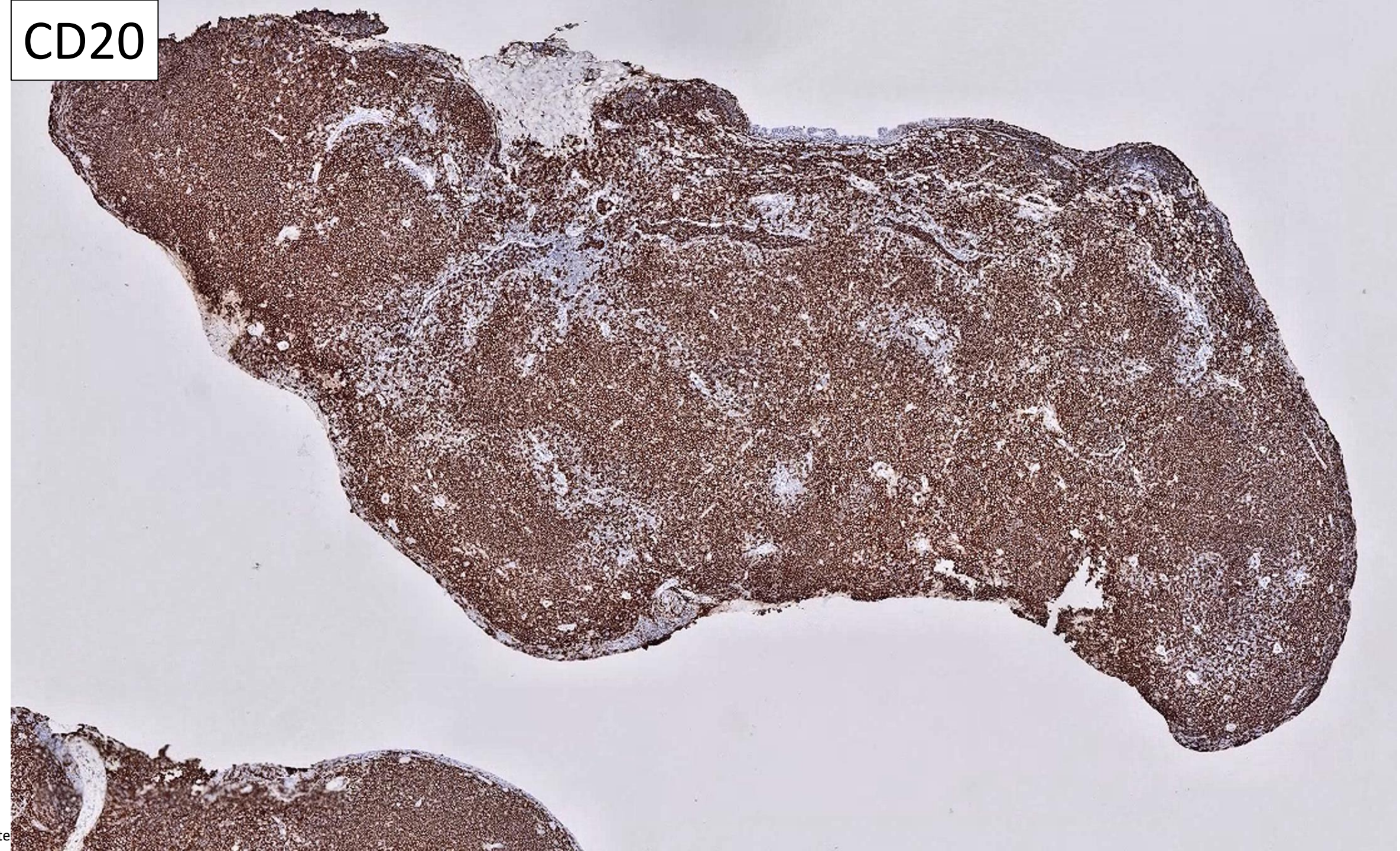
Case 5

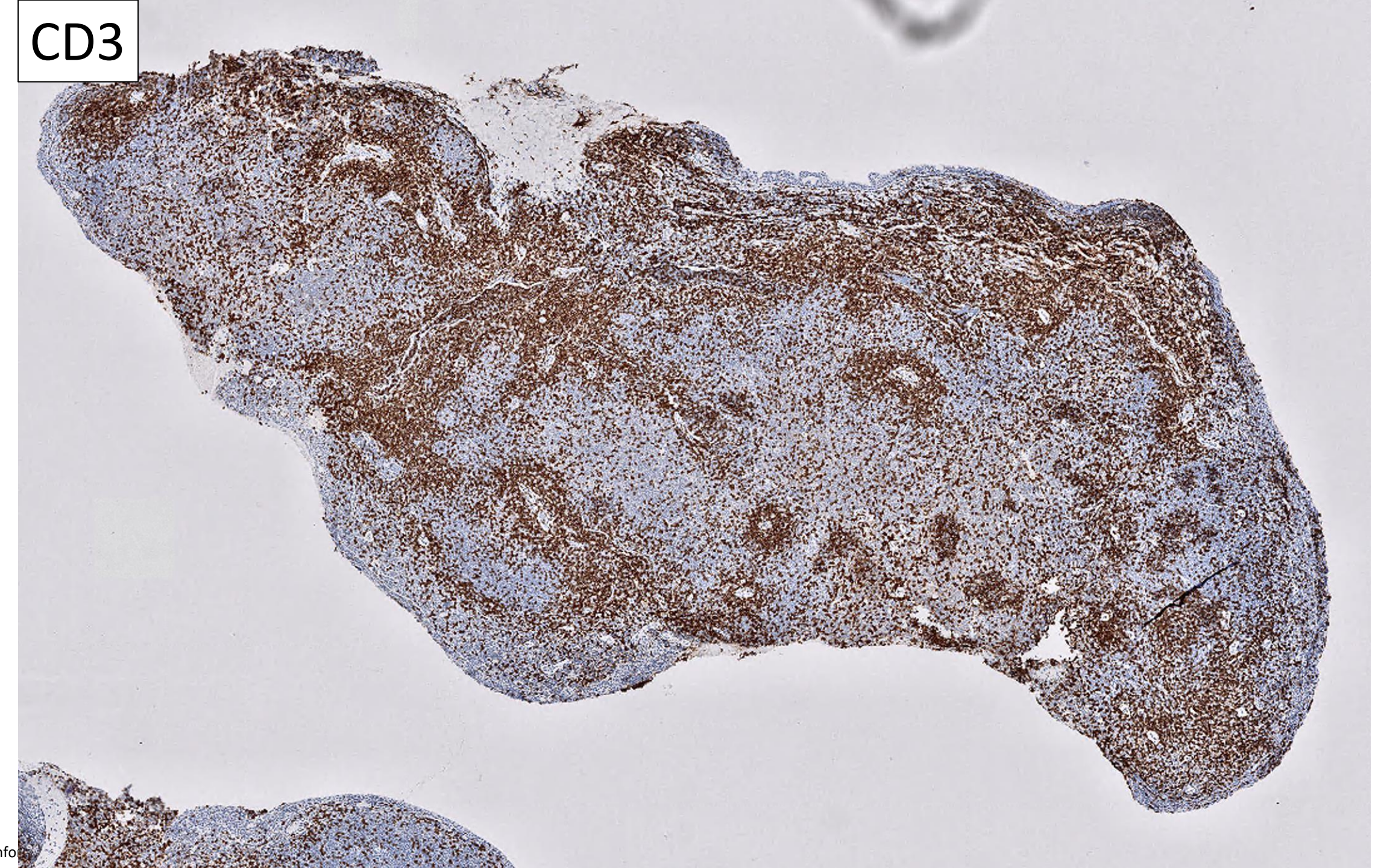
- 64 year old with lesions of the conjunctiva suspicious for lymphoma

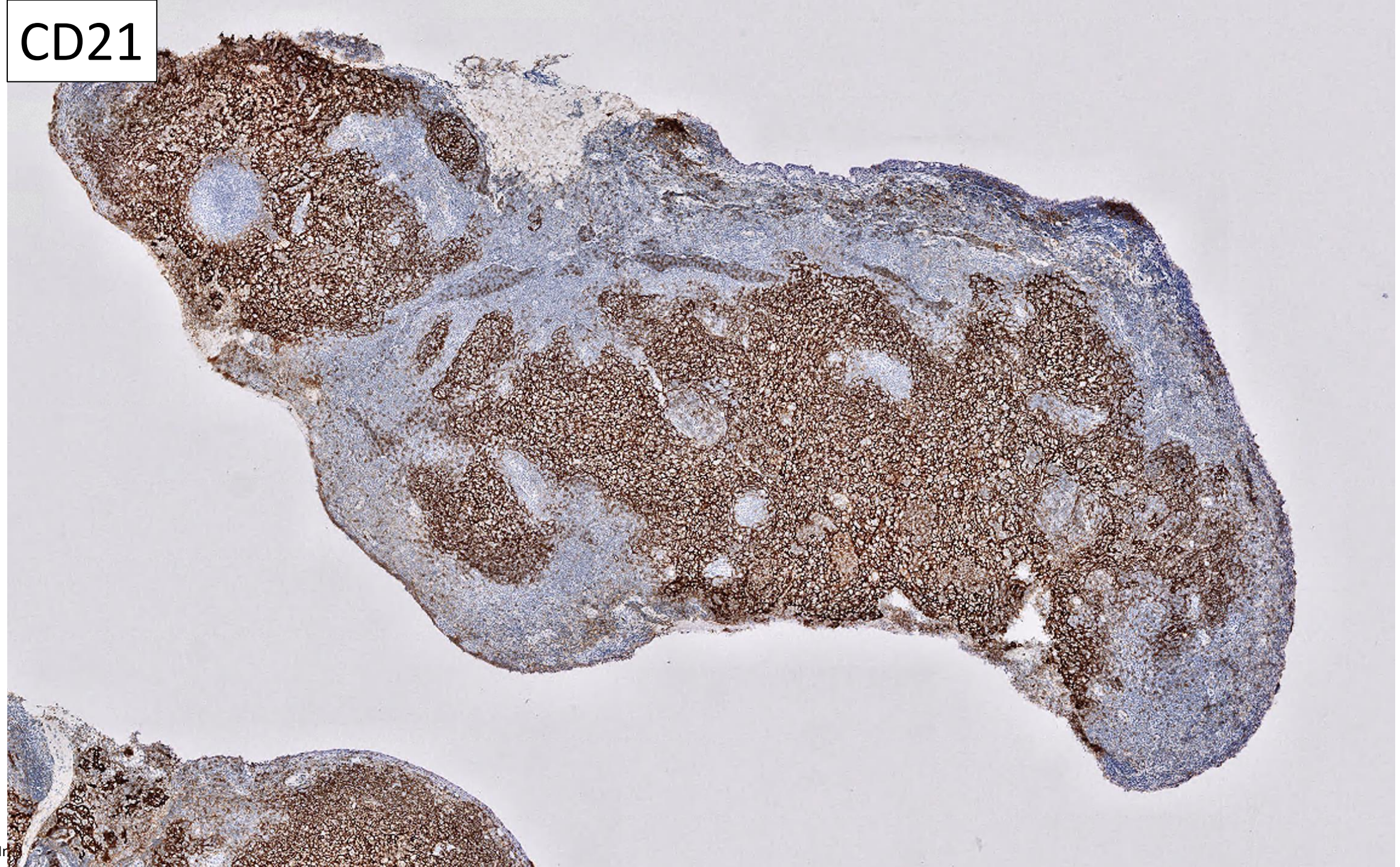


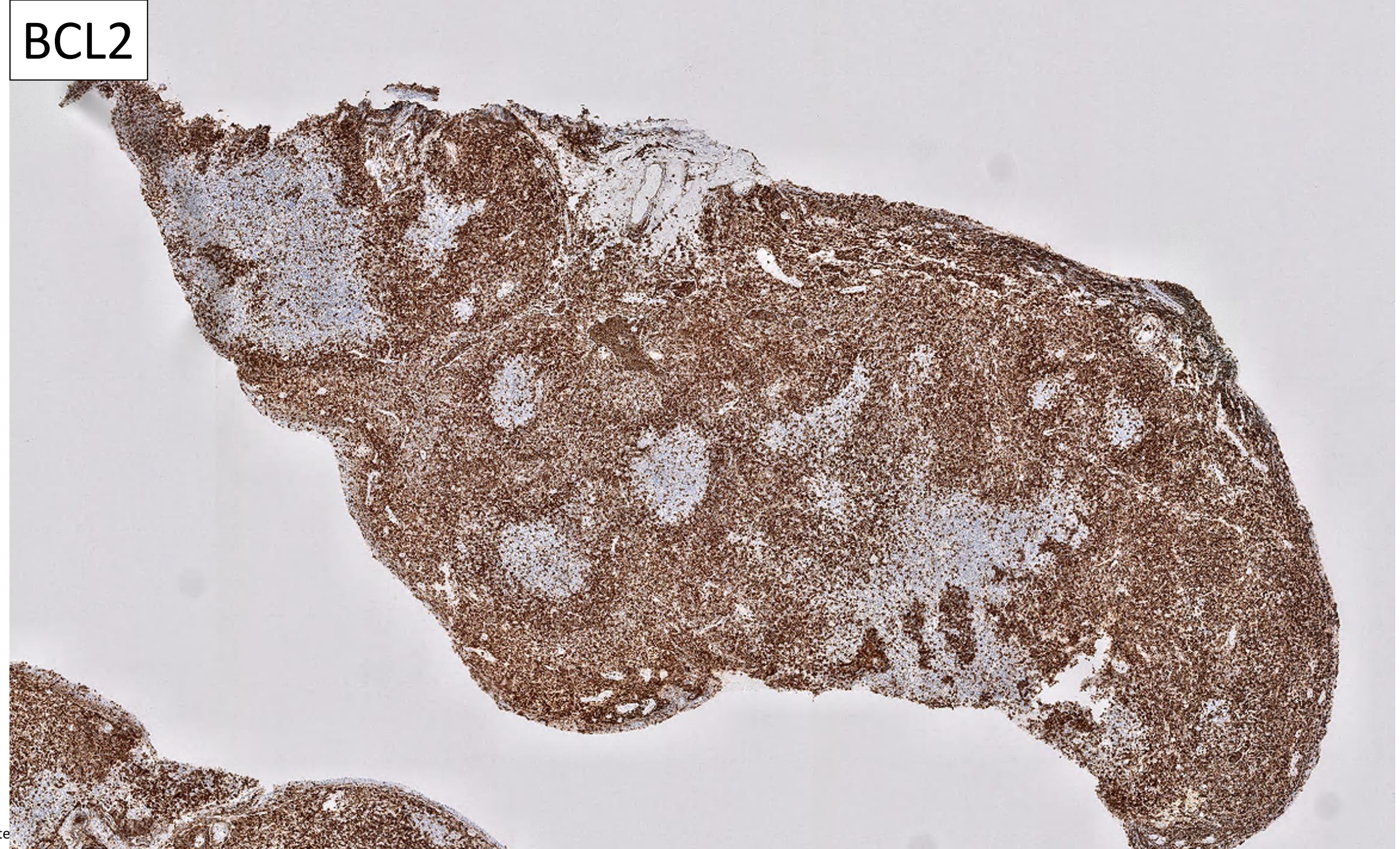


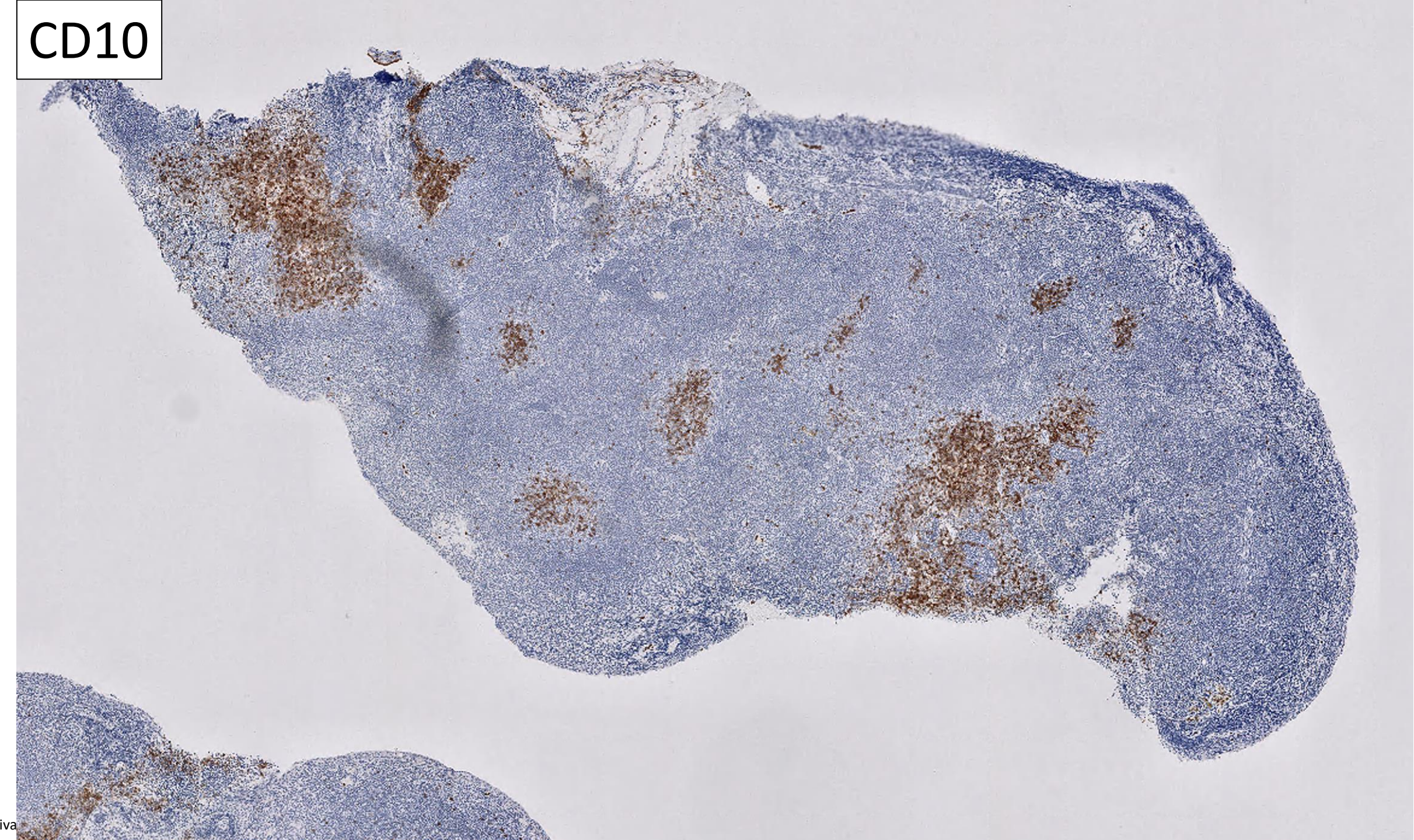






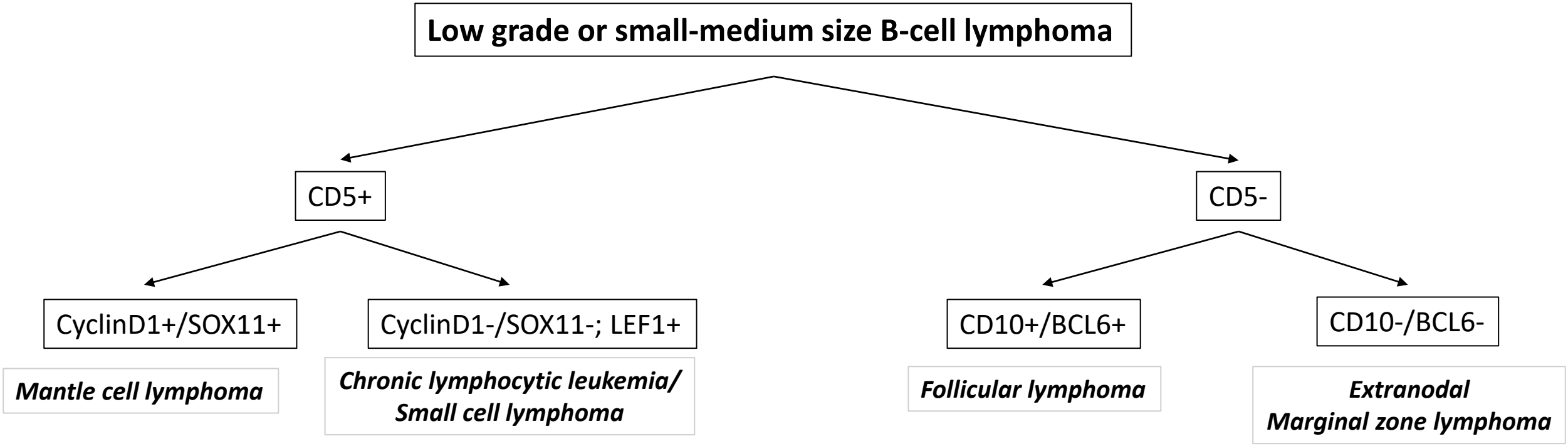






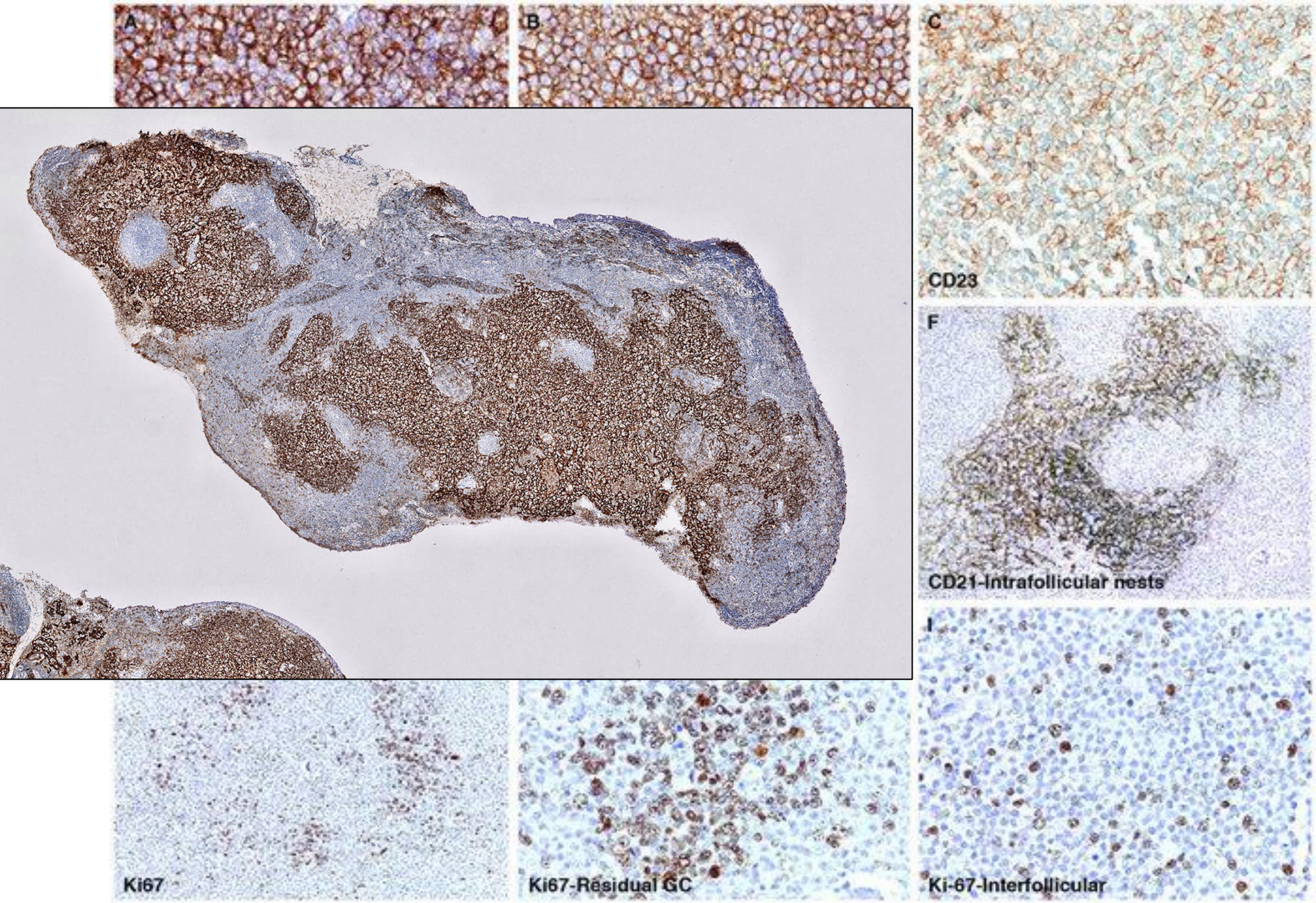
Other negative markers

- CD5 and BCL1/CyclinD1 and SOX11 negative



Diagnosis?

Marginal zone lymphoma



Salama ME, Lossos IS, Warnke RA, Natkunam Y. Immunoarchitectural patterns in nodal marginal zone B-cell lymphoma: a study of 51 cases. Am J Clin Pathol. 2009 Jul;132(1):39-49.

However… molecular was done on this case

Next generation sequencing

- *MYD88* and *TBL1XR1* mutations seen!
- What does this mean? Is this lymphoplasmacytic lymphoma?
- We often think of *MYD88* in lymphoplasmacytic lymphoma or diffuse large B-cell lymphoma
- But these mutations are all consistent with MZL of the ocular adnexa (conjunctiva)
- Combinations of these mutations = MZL in this location

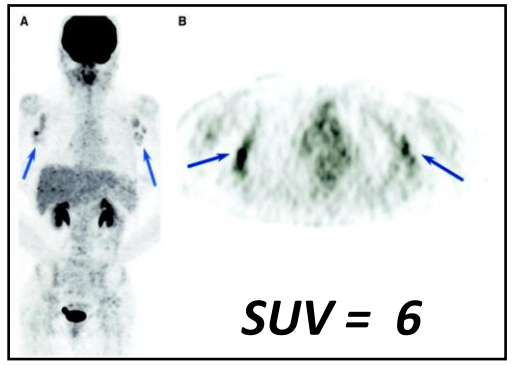
Case 6

- 24 year old with diffuse lymphadenopathy
- Given dose of steroids
- Elevated LDH, **PET shows SUV of 16** in lymph nodes

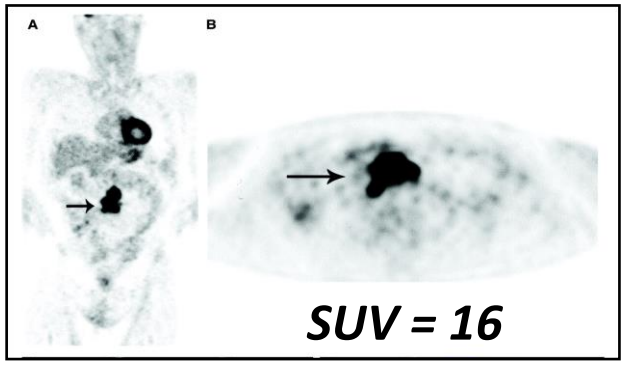
Why focus on SUV?

PET, SUV and aggressive lymphomas

- SUV >10 typically is an aggressive lymphoma



Low grade follicular lymphoma



Diffuse large B-cell lymphoma

1. Yuasa et al., Clinical Significance of Uptake Value on F18-FDG PET/CT and Histological Grade in 164 Patients with Follicular Lymphoma Including Transformation - a Single Center Retrospective Study. Blood (2019) 134 (Supplement_1): 1529.
2. Gallamini A, Borra A. FDG-PET Scan: a new Paradigm for Follicular Lymphoma Management. *Mediterr J Hematol Infect Dis*. 2017;9(1):e2017029. Published 2017 Apr 15. doi:10.4084/MJHID.2017.029
3. Schöder et al., Intensity of ^{18}Fluorodeoxyglucose Uptake in Positron Emission Tomography Distinguishes Between Indolent and Aggressive Non-Hodgkin's Lymphoma. Journal of Clinical Oncology 2005 23:21, 4643-4651
4. Ngeow JYY, Quek RHH, Ng DCE, et al. High SUV uptake on FDG-PET/CT predicts for an aggressive B-cell lymphoma in a prospective study of primary FDG-PET/CT staging in lymphoma. *Ann Oncol*. 2009;20(9):1543-1547.

Case 6

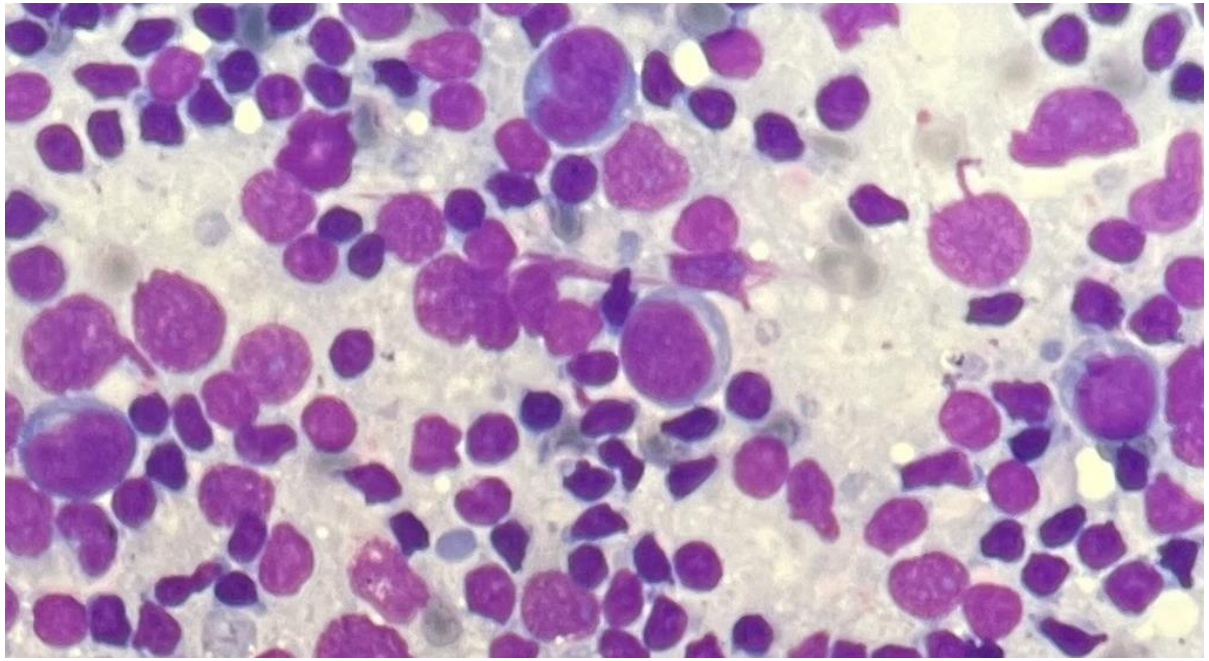
- 24 year old with diffuse lymphadenopathy
- Given dose of steroids
- Elevated LDH, PET shows SUV of 16 in lymph nodes
- Clinical suspicion for an aggressive lymphoma

What do we know about aggressive lymphomas?

Large B-cell lymphoma features

- ~150,000 new cases/year.
- Risk factors: genetic features, immune dysregulation, viral, environmental, occupational exposure
- De novo or transformation
- 50% of patients are low stage (I/II).
- Present with rapidly enlarging lymph nodes or mass(es)
- 30-40% extranodal sites involved: GI tract, head and neck, bone, liver, kidney and adrenal gland.
- 20% have bone marrow involvement.

Patient gets an FNA



What is role of FNA in diagnosis of large B-cell lymphoma?

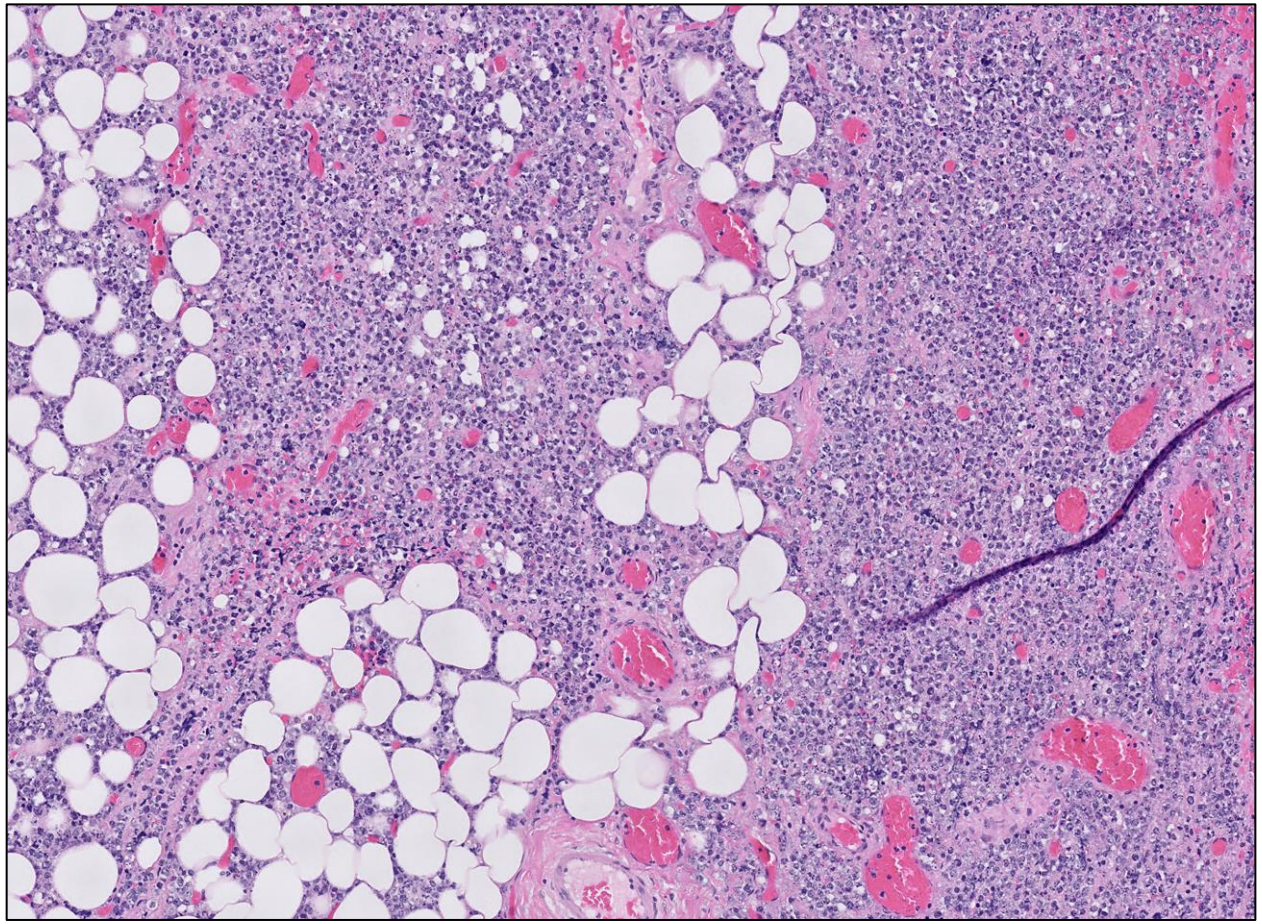
- WHO-HAEM5 recommends tissue biopsy for definitive diagnosis.

Balassanian R, Wool GD, Ono JC, et al. A superior method for cell block preparation for fine-needle aspiration biopsies. Cancer Cytopathol. 2016

FNA diagnosis

- Suspicious for lymphoma

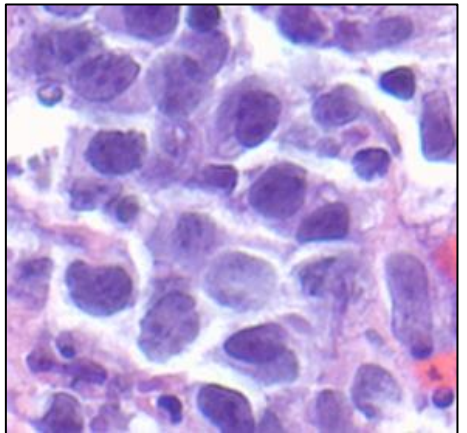
Lymph node biopsy



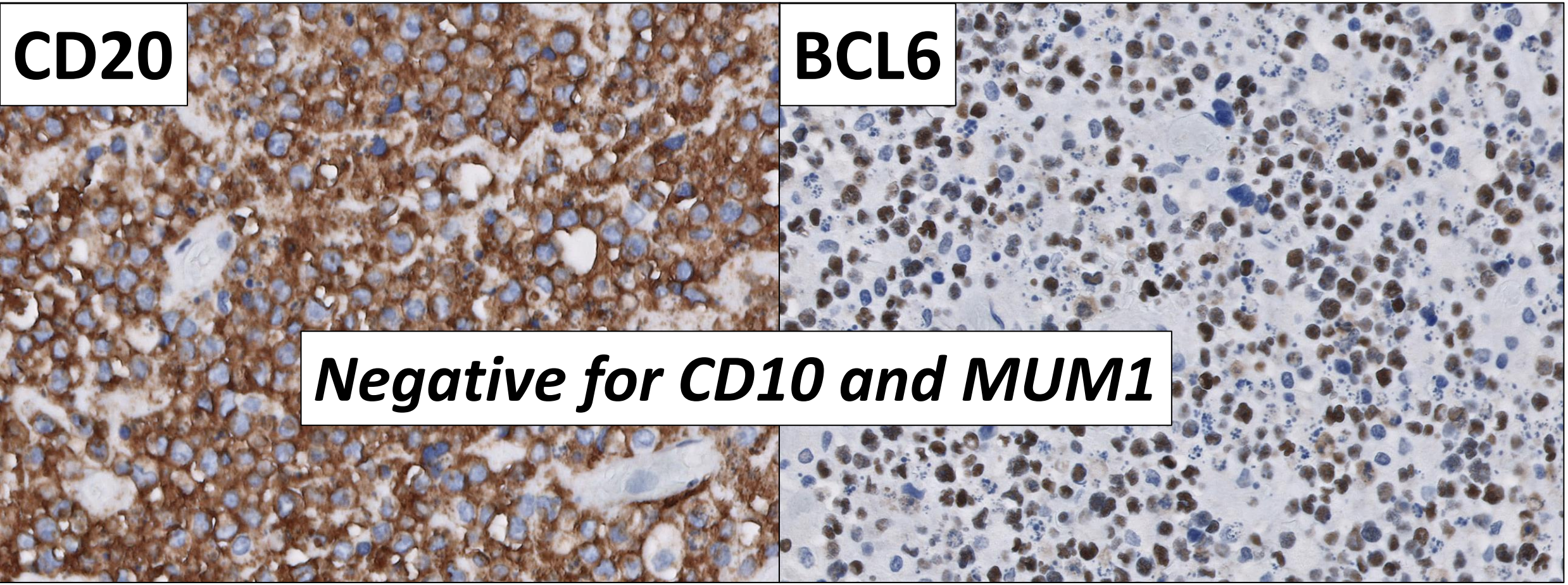
- Large cells
- Sheets
- Centroblastic
- Mitotic figures

Morphology of large B-cell lymphomas

- **Centroblastic:** ~ 80% of cases. Large cells, round to oval nuclei, typically several small nucleoli.
- **Immunoblastic:** ~10% of cases. Large cells, single prominent, centrally located nucleolus. Poor prognosis, often associated with IGH-MYC gene rearrangements.
- **Anaplastic:** 5% of cases. Very large cells, bizarre nuclei, often cohesive tumor-like growth, may have sinusoidal involvement. Often TP53 mutations and CD30 expression.

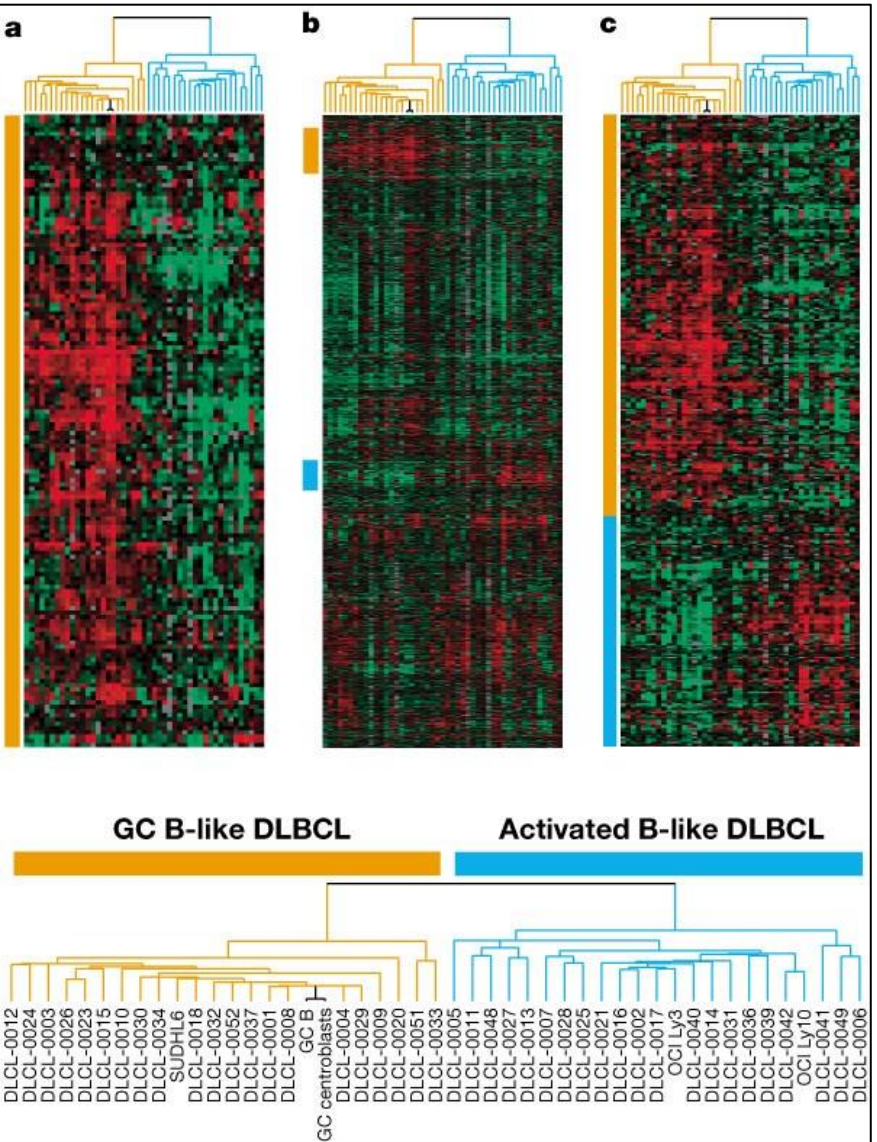


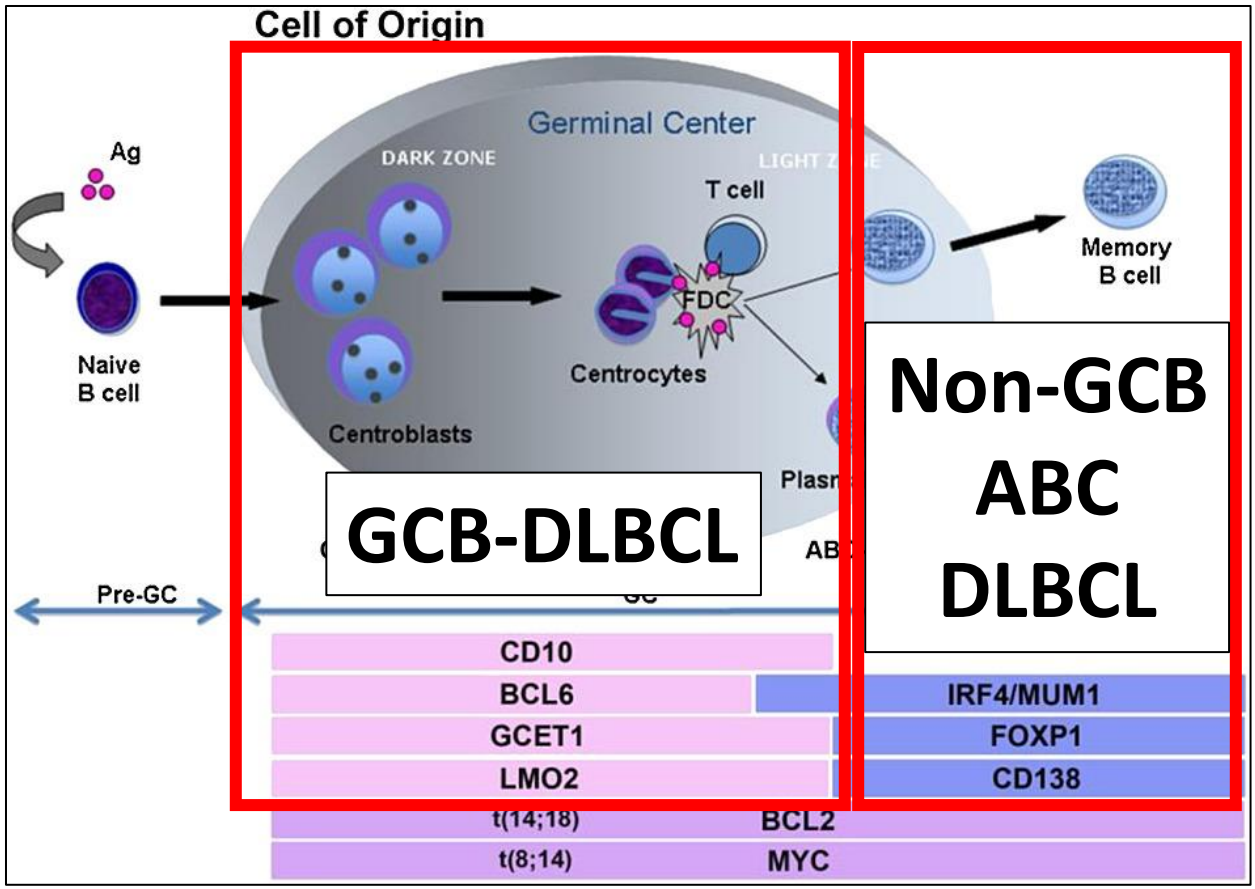
Immunohistochemistry



Is this important?

Diffuse large B-cell lymphoma, cell of origin

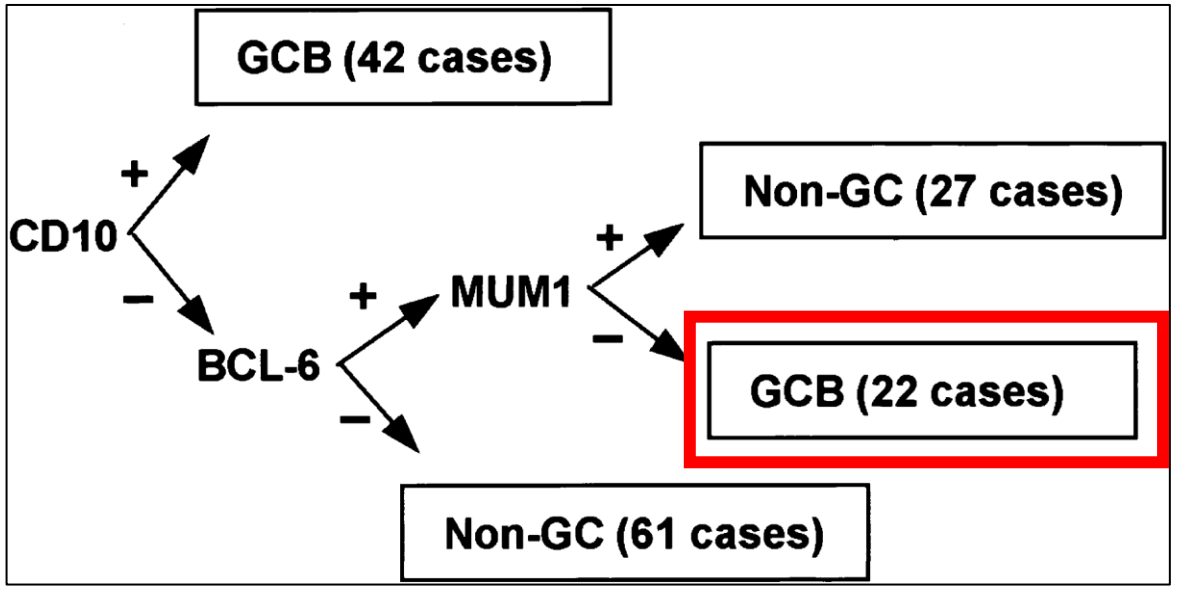


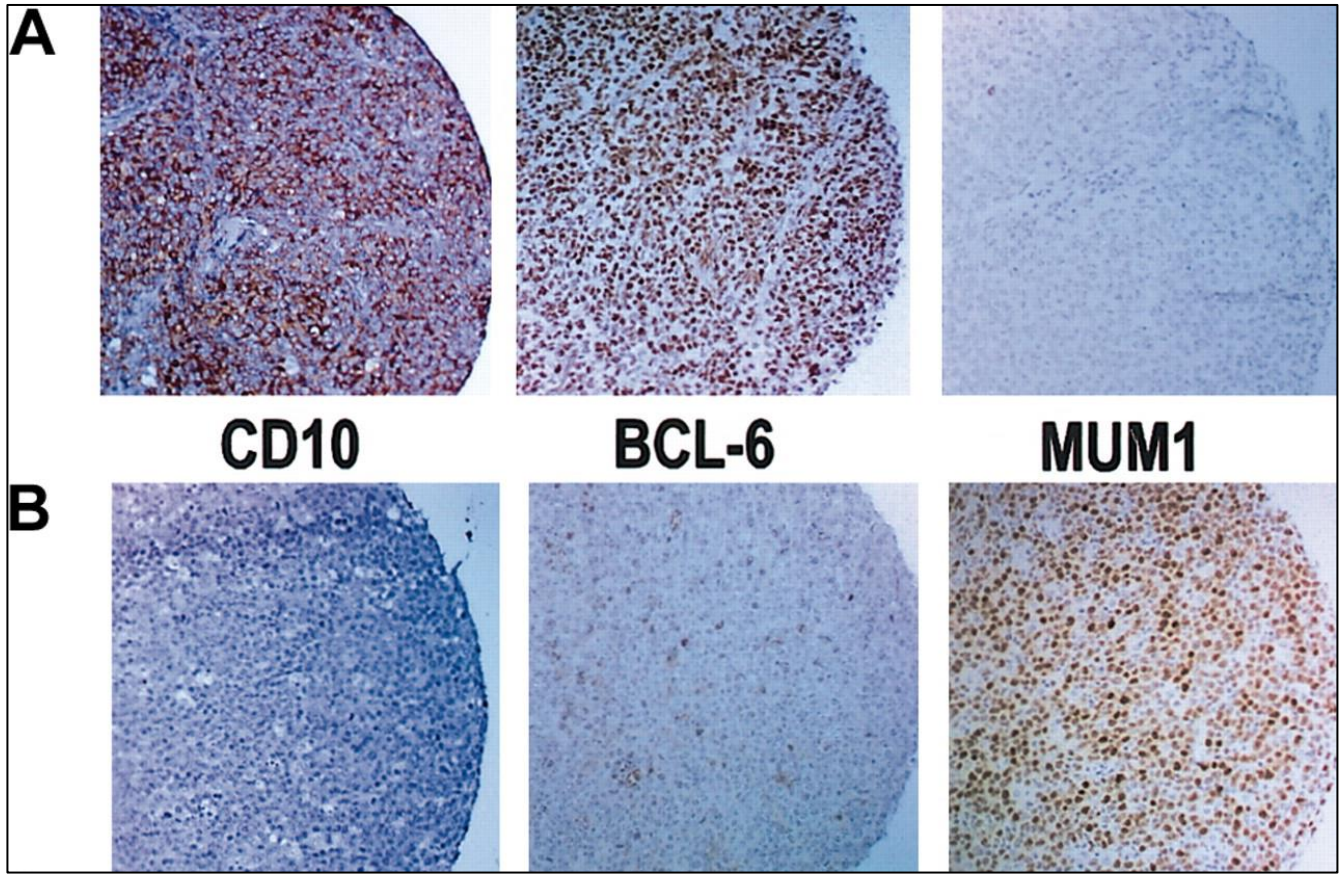


Quintanilla-Martinez, Hematological Oncology 2015

Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403(6769):503-511. doi:10.1038/35000501

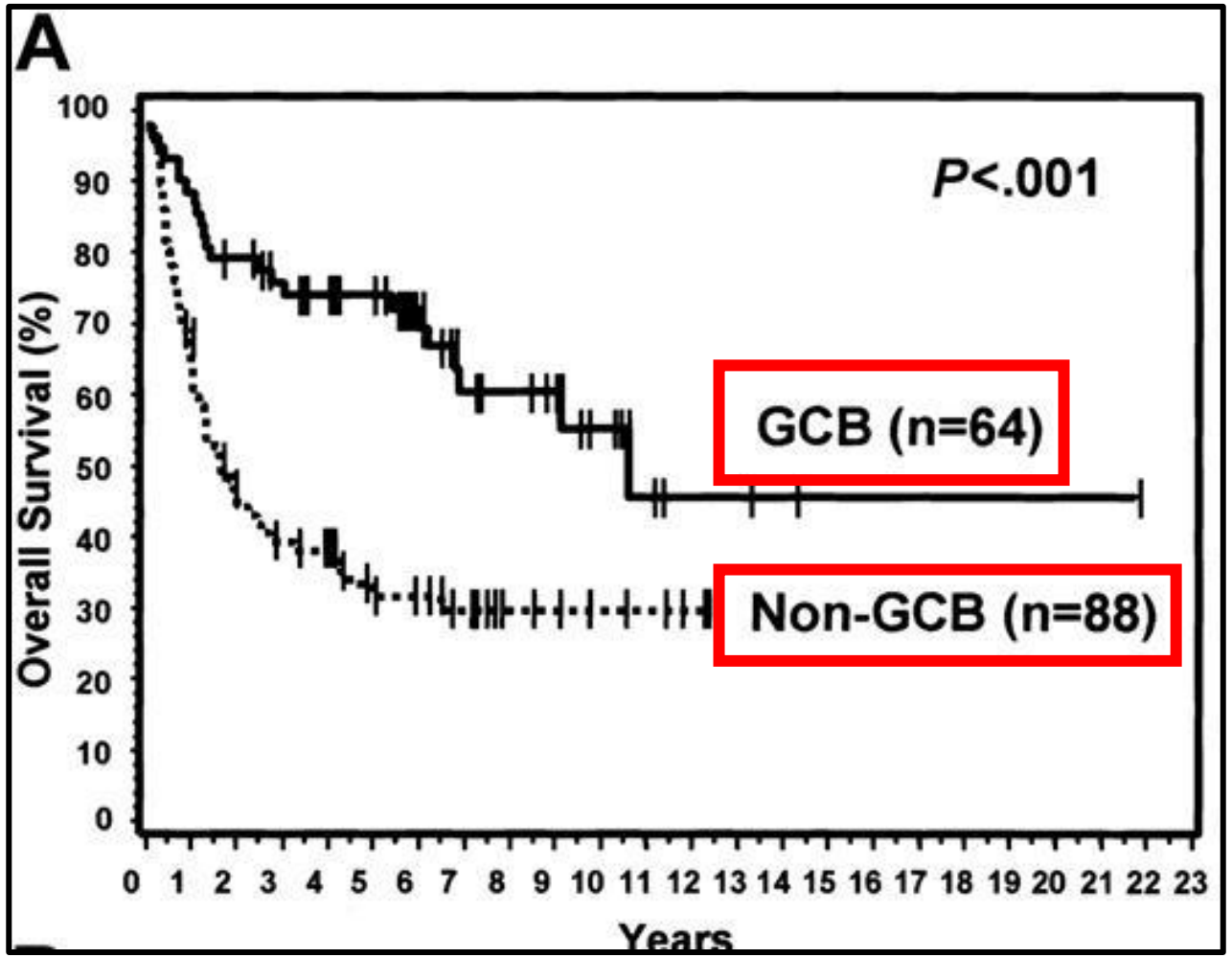
Cell of origin (COO), Hans classifier





Christine P. Hans,Dennis D. Weisenburger,Timothy C. Greiner,Randy D. Gascoyne,Jan Delabie,German Ott,H. Konrad Müller-Hermelink,Elias Campo,Rita M. Braziel,Elaine S. Jaffe,Zenggang Pan,Pedro Farinha,Lynette M. Smith,Brunangelo Falini,Alison H. Banham,Andreas Rosenwald,Louis M. Staudt,Joseph M. Connors,James O. Armitage,Wing C. Chan, Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray, Blood, 2004,

Germinal center B-cell (GCB) vs non-GCB



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But in recent years… WHO-HAEM5 and ICC

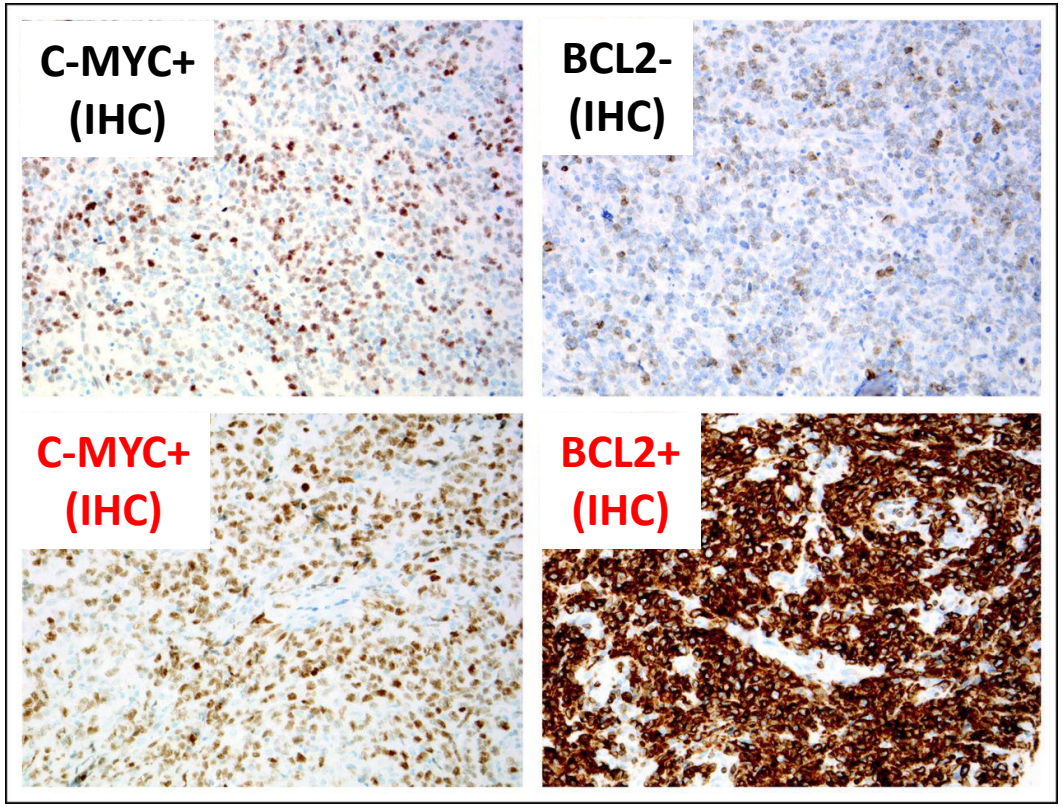
- Cell of origin classification may not be useful anymore
- Though cell of origin classification is still recommended

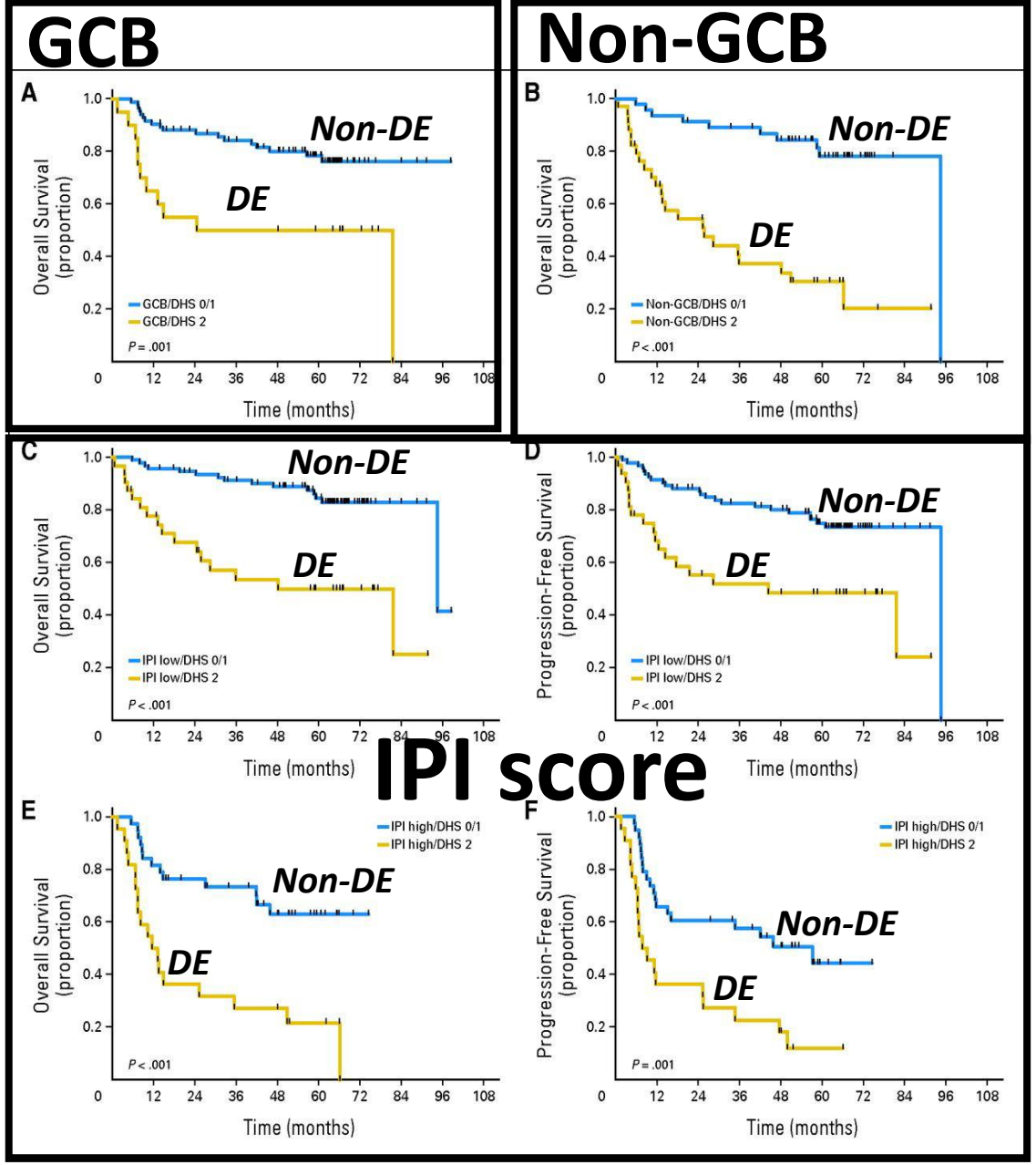
Negative for

- CD10
- MUM1

- **BCL2**
- **C-MYC**

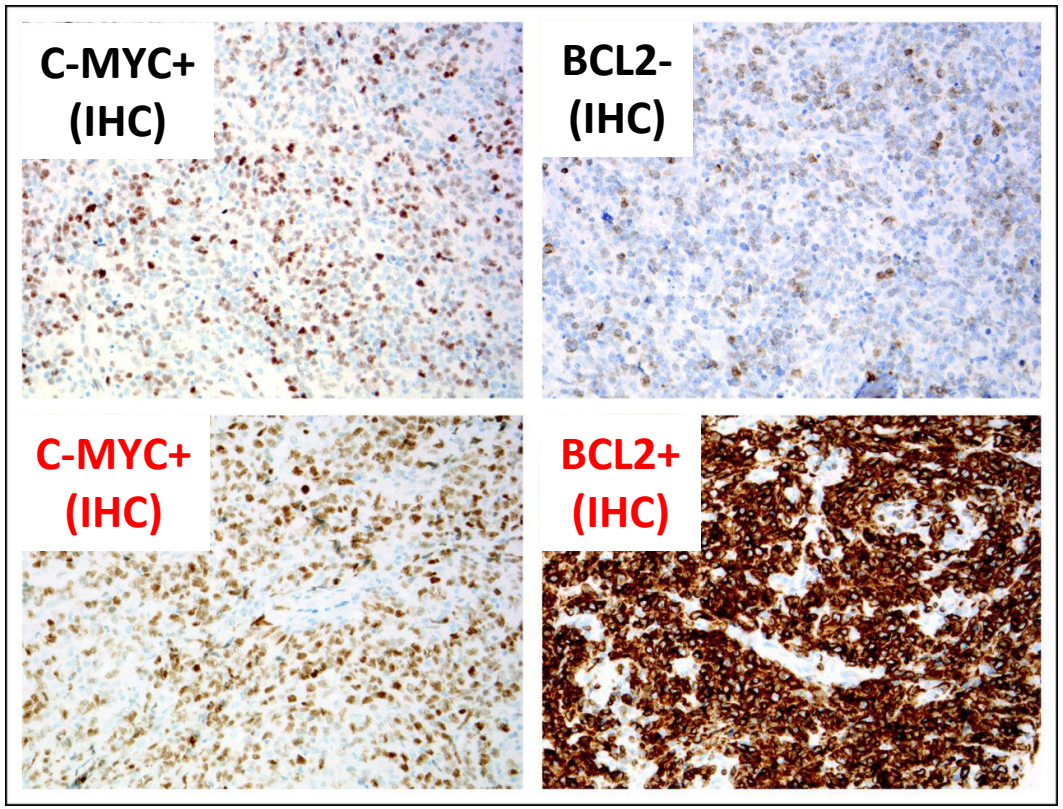
Double-Expressor (DE)





1.Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol. 2012;30(28):3460-3467.
2.Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol. 2012;30(28):3452-3459. doi:10.1200/JCO.2011.41.0985
3.Staiger AM, Ziepert M, Horn H, et al. Clinical Impact of the Cell-of-Origin Classification and the MYC/ BCL2 Dual Expresser Status in Diffuse Large B-Cell Lymphoma Treated Within Prospective Clinical Trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group. J Clin Oncol. 2017;35(22):2515-2526.
4.Horn H, Ziepert M, Becher C, et al. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood. 2013;121(12):2253-2263. doi:10.1182/blood-2012-06-435842

Double-Expressor (DE)



→

<u>In 2022</u>

Is this prognostically Important?

…maybe not…

1.Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol. 2012;30(28):3460-3467.
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3.Staiger AM, Ziepert M, Horn H, et al. Clinical Impact of the Cell-of-Origin Classification and the MYC/ BCL2 Dual Expresser Status in Diffuse Large B-Cell Lymphoma Treated Within Prospective Clinical Trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group. J Clin Oncol. 2017;35(22):2515-2526.
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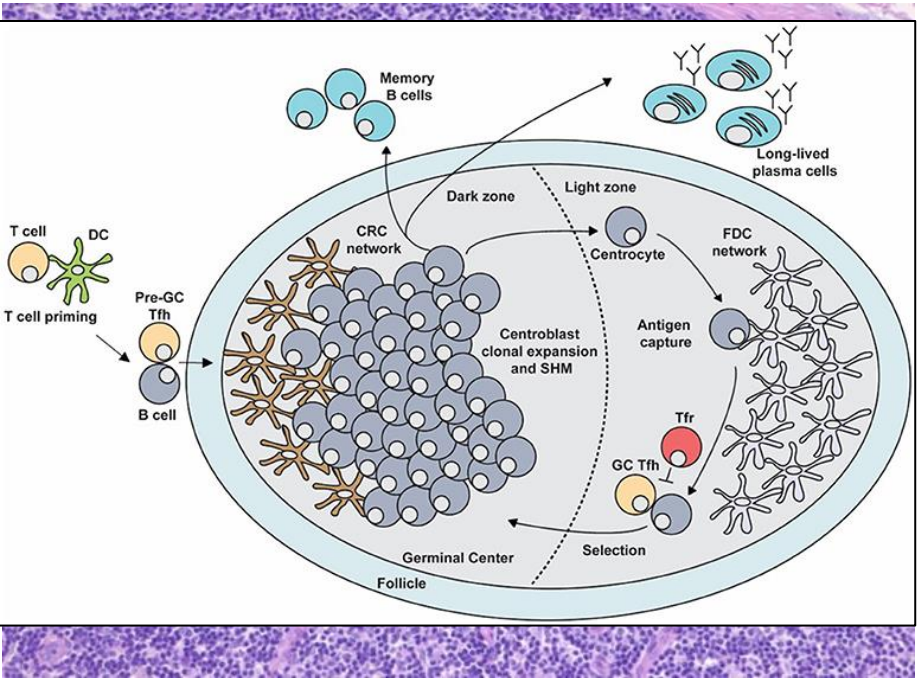
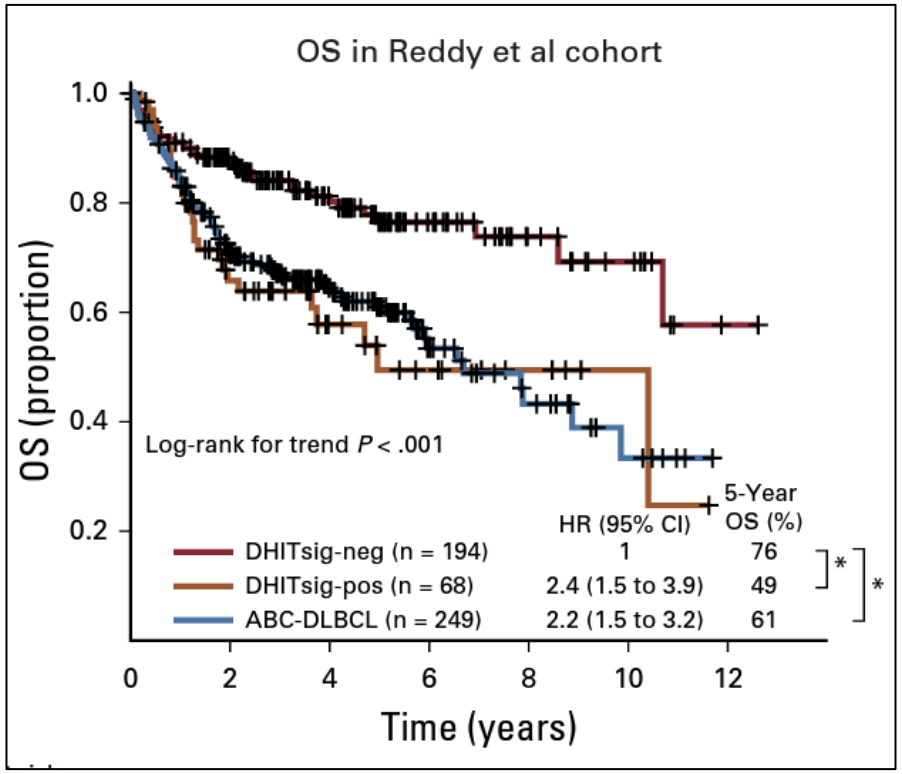
Double-Hit Signature positive (DHTsig+)/Molecular high grade (MHG)

- (DHTsig+)/ molecular high grade (MHG) signature, (dark zone expression)
- Poor prognosis to standard treatment



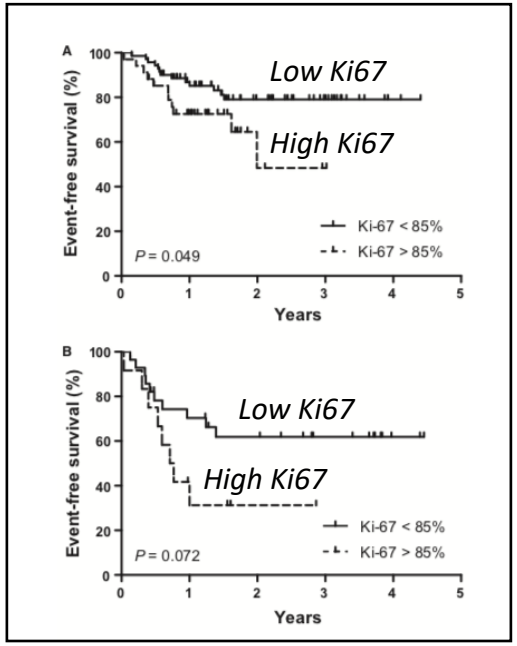


van den Brand, M. (2020). Lymph Node. In: Molina, T.J. (eds) Hematopathology. Encyclopedia of Pathology. Springer, Cham.

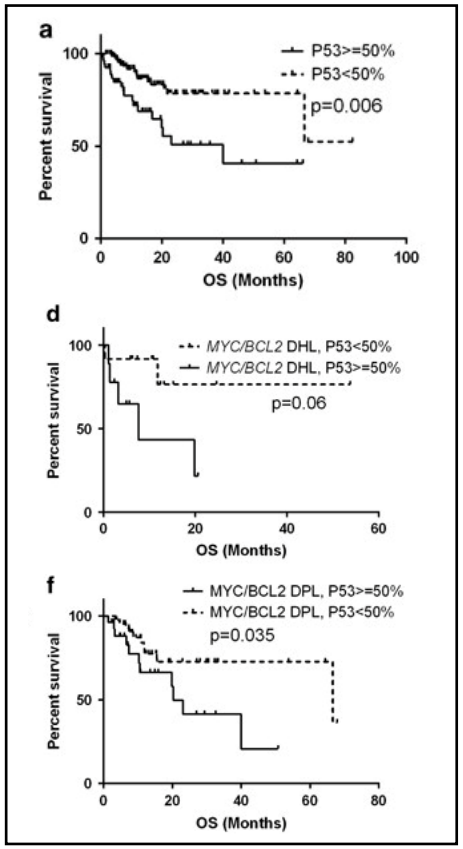
Ennishi D, Jiang A, Boyle M, et al. Double-Hit Gene Expression Signature Defines a Distinct Subgroup of Germinal Center B-Cell-Like Diffuse Large B-Cell Lymphoma. *J Clin Oncol*. 2019;37(3):190-201.

Other stains

- Ki67: low (30%)
  - Low is Better survival



- p53: was low and considered negative (<10%)
  - Low is better survival



1.Yoon DH, Choi DR, Ahn HJ, et al. Ki-67 expression as a prognostic factor in diffuse large B-cell lymphoma patients treated with rituximab plus CHOP. *Eur J Haematol*. 2010;85(2):149-157. doi:10.1111/j.1600-0609.2010.01467.x
2.Wang X, et al. P53 expression correlates with poorer survival and augments the negative prognostic effect of MYC rearrangement, expression or concurrent MYC/BCL2 expression in diffuse large B-cell lymphoma. *Mod Pathol* 30, 194–203 (2017).
Private Information

Other stains

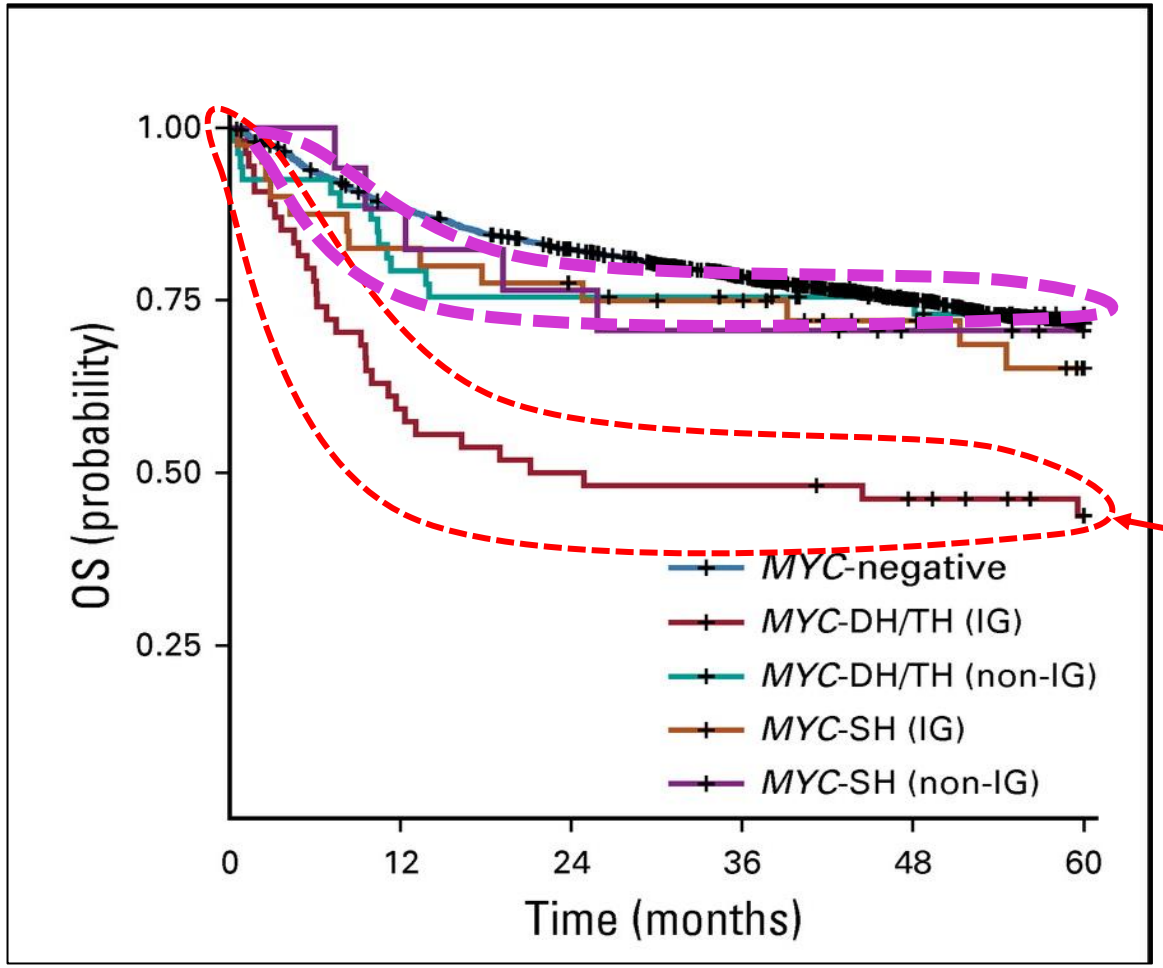
- ***Negative for CD3***
  - Rare cases may show aberrant CD3 expression. Typically expression is cytoplasmic and dim.
- ***Negative for TdT***
  - Almost all cases lack TdT. Expression of partial TdT can be seen in mature LBCL with MYC and BCL2 gene rearrangements.
- ***Negative for CD30***
  - Relevant in the context of targeted therapies (Brentuximab)

FISH

- No *MYC*, *BCL2* or *BCL6* translocation

Why do we care about *MYC*, *BCL2* and *BCL6*?

MYC, *BCL2* and *BCL6* rearrangements



Immunoglobulin partner for prognostic impact

MYC with *BCL2* and/or *BCL6* gene rearrangement (IG partner)

1. *Lauren C. Chong, Susana Ben-Neriah, Graham W. Slack, Ciara Freeman, Daisuke Ennishi, Anja Mottok, Brett Collinge, Pau Abrisqueta, Pedro Farinha, Merrill Boyle, Barbara Meissner, Robert Kridel, Alina S. Gerrie, Diego Villa, Kerry J. Savage, Laurie H. Sehn, Reiner Siebert, Ryan D. Morin, Randy D. Gascoyne, Marco A. Marra, Joseph M. Connors, Andrew J. Mungall, Christian Steidl, David W. Scott; High-resolution architecture and partner genes of MYC rearrangements in lymphoma with DLBCL morphology. Blood Adv 2018; 2 (20): 2755–2765.*
2. Clipson A, Barrans S, Zeng N, et al. The prognosis of *MYC* translocation positive diffuse large B-cell lymphoma depends on the second hit. *J Pathol Clin Res*. 2015;1(3):125-133. Published 2015 Mar 30. doi:10.1002/cjp2.10
3. Ye Q, Xu-Monette ZY, Tzankov A, et al. Prognostic impact of concurrent MYC and BCL6 rearrangements and expression in de novo diffuse large B-cell lymphoma. *Oncotarget*. 2016;7(3):2401-2416. doi:10.18632/oncotarget.6262
4. Rosenwald A, Bens S, Advani R, et al. **Prognostic Significance of *MYC*Rearrangement and Translocation Partner in Diffuse Large B-Cell Lymphoma: A Study by the Lunenburg Lymphoma Biomarker Consortium. *J Clin Oncol*. 2019;37(35):3359-3368.**

Are all double-hit lymphomas equal?

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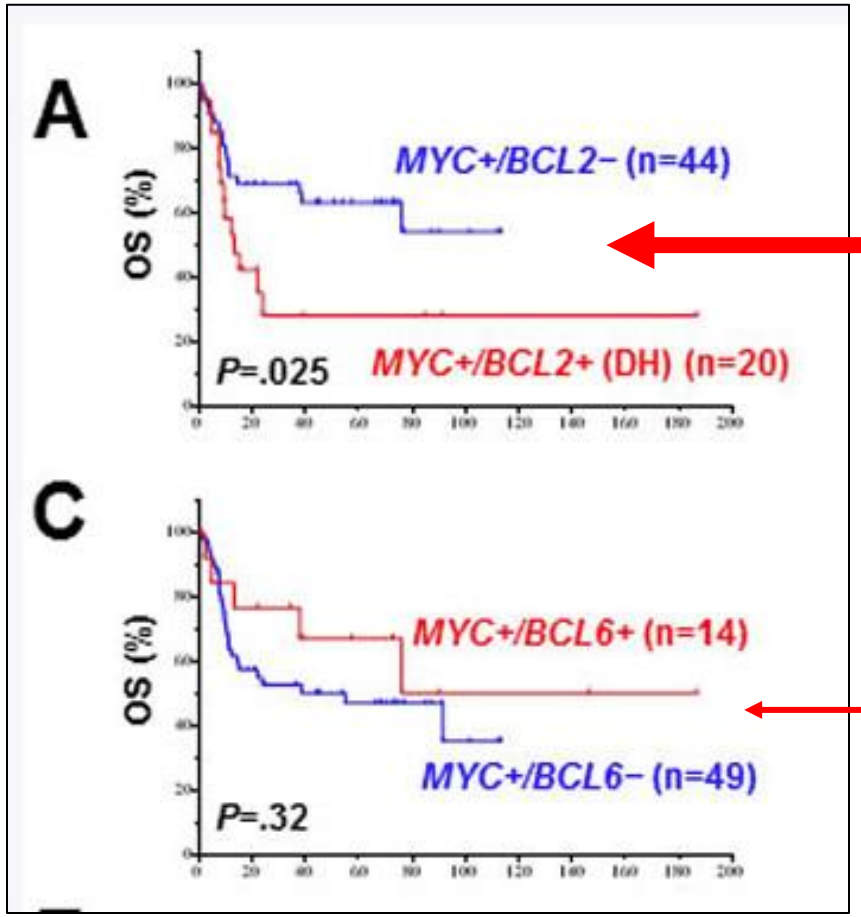
Is *MYC* and *BCL6* the same as *MYC* and *BCL2?*



MYC with *BCL2*:
Double hit lymphoma

NO prognostic value of *MYC* with ***BCL6***:
Double hit lymphoma

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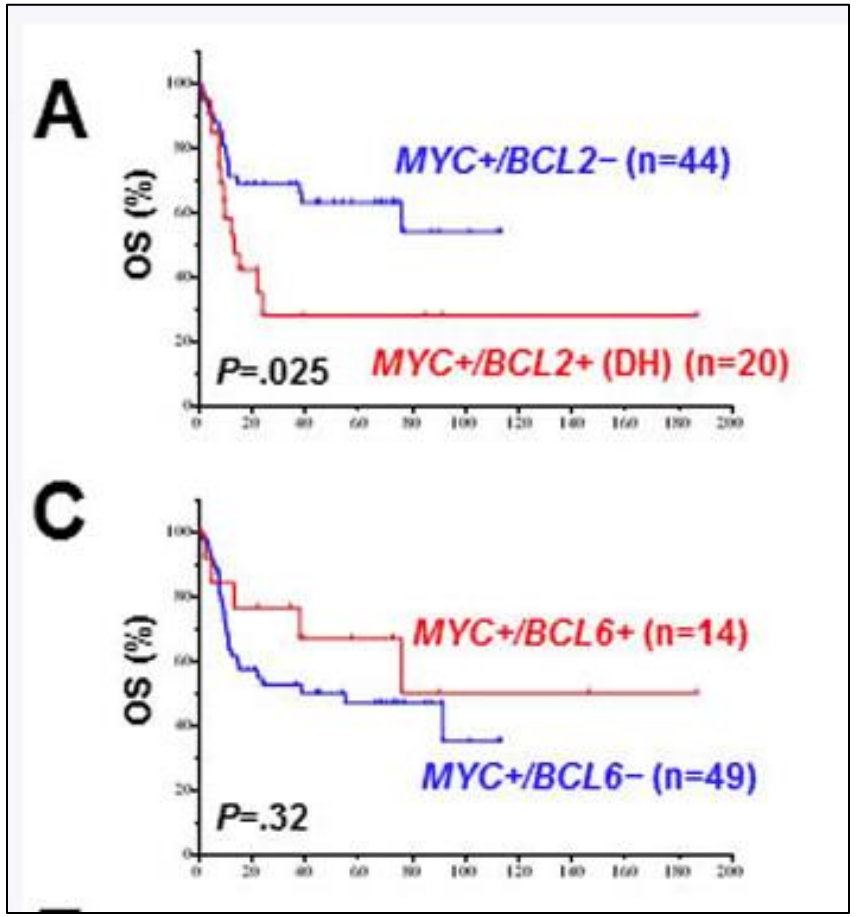

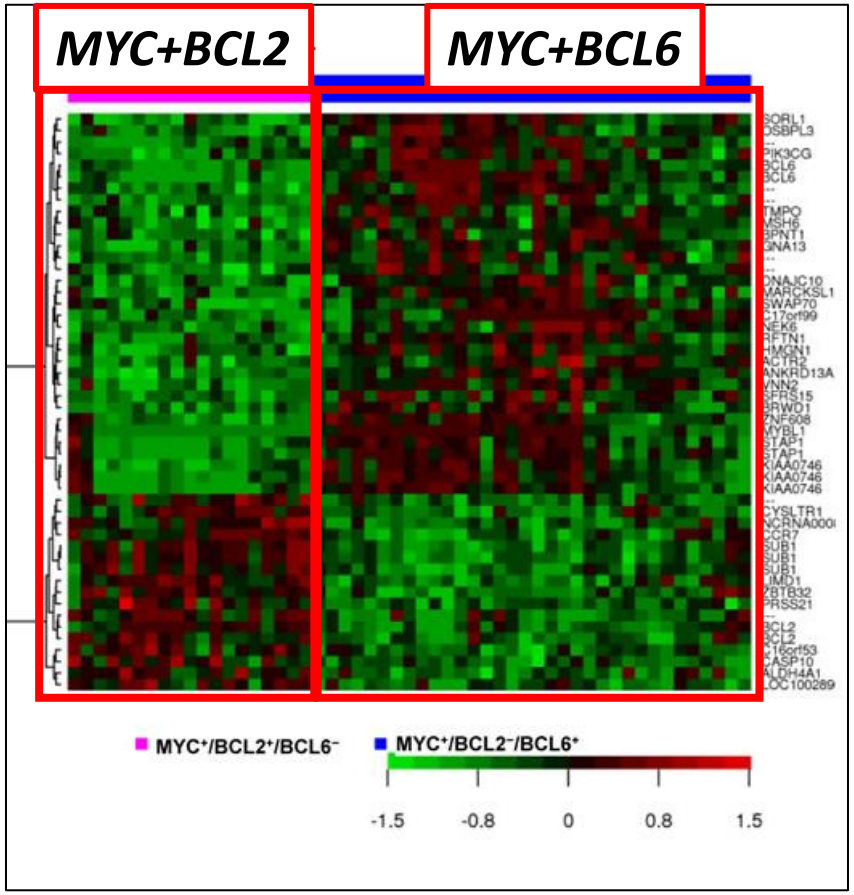

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Diffuse large/High-grade B-cell lymphoma with *MYC* and *BCL2* gene rearrangements

- Aggressive lymphoma in elderly (7th decade of life)
- Stage by IPI
- Mutations in *BCL2, KMT2D, CREBBP, EZH2*, *TNFRSF14* (FL type genes) and *MYC*

Back to our case…

Next generation sequencing identifies

- Mutations in:
  - SOCS1 p.C178*, SOCS1 p.L84fs
  - GNA13 p.Q27*
  - BTG1 p.Q45*, BTG1 p.S65P
  - STAT6 p.N421S, STAT6 p.N417Y

What do we do with these results?

How do we interpret these results?

- There have been multiple DLBCL molecular subtyping studies in the last <5 years
- Lacy et al., Wright et al., Chapuy et al., Schmitz et al., others

1. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes [published correction appears in Nat Med. 2018 Aug;24(8):1292] [published correction appears in Nat Med. 2018 Aug;24(8):1290-1291]. Nat Med. 2018;24(5):679-690.

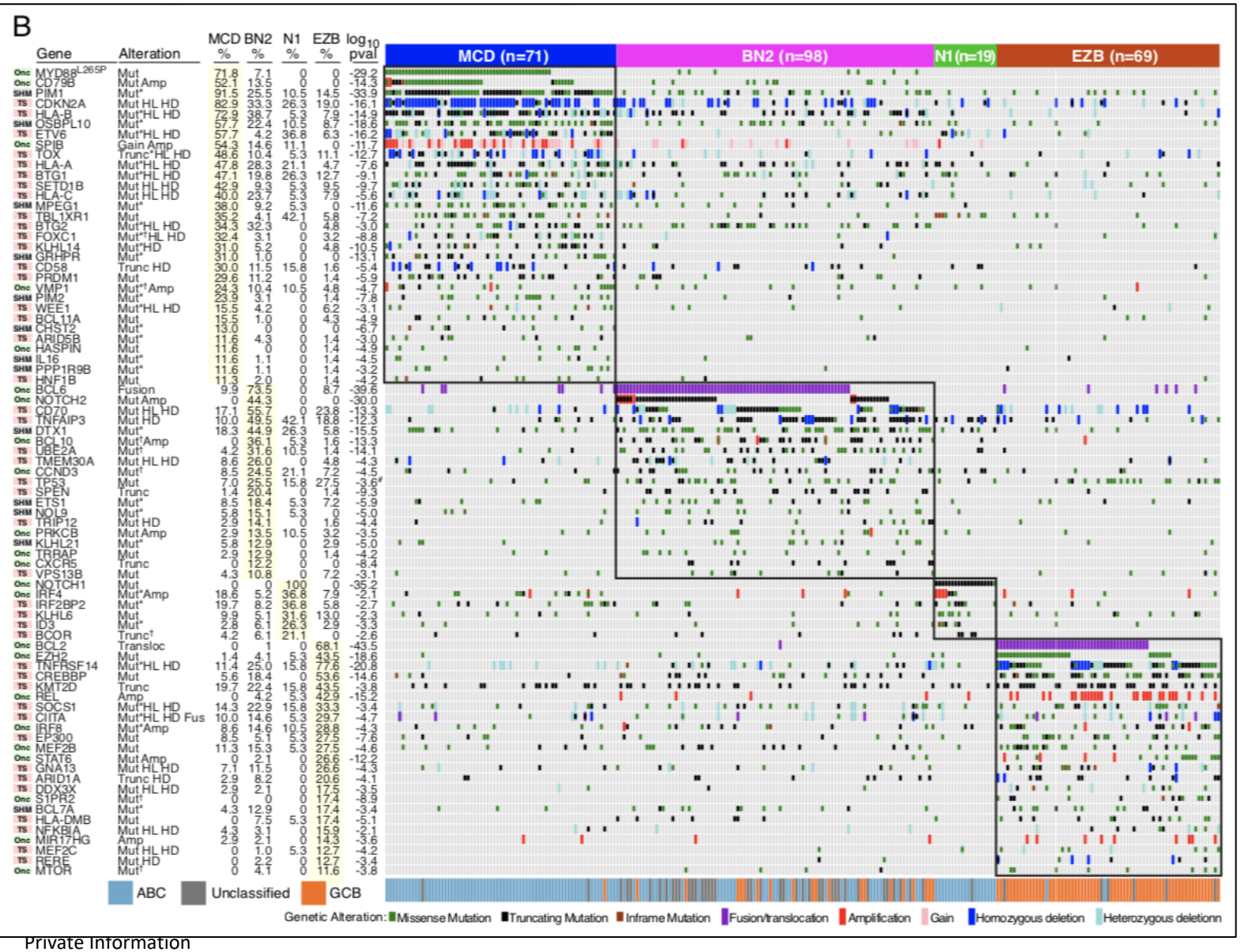
2. Wright, George W et al. "A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications." Cancer cell vol. 37,4 (2020): 551-568.e14.

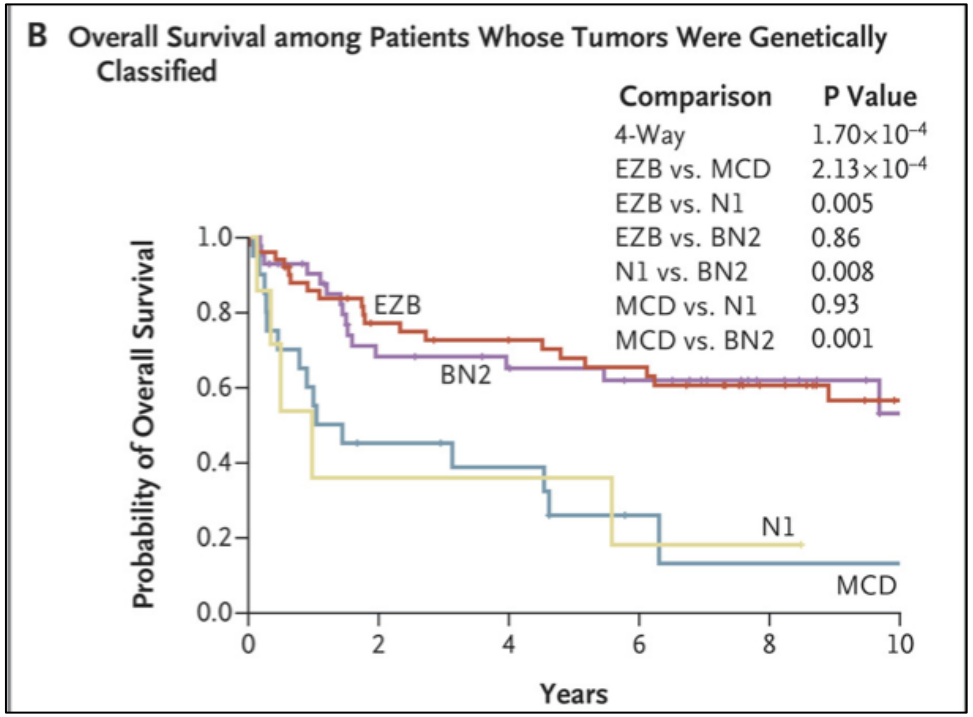
3. Pedrosa L, Fernández-Miranda I, Pérez-Callejo D, et al. Proposal and validation of a method to classify genetic subtypes of diffuse large B cell lymphoma. *Sci Rep*. 2021;11(1):1886. Published 2021 Jan 21.

4. Lacy SE, Barrans SL, Beer PA, et al. Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. *Blood*. 2020;135(20):1759-1771.

All these studies converge

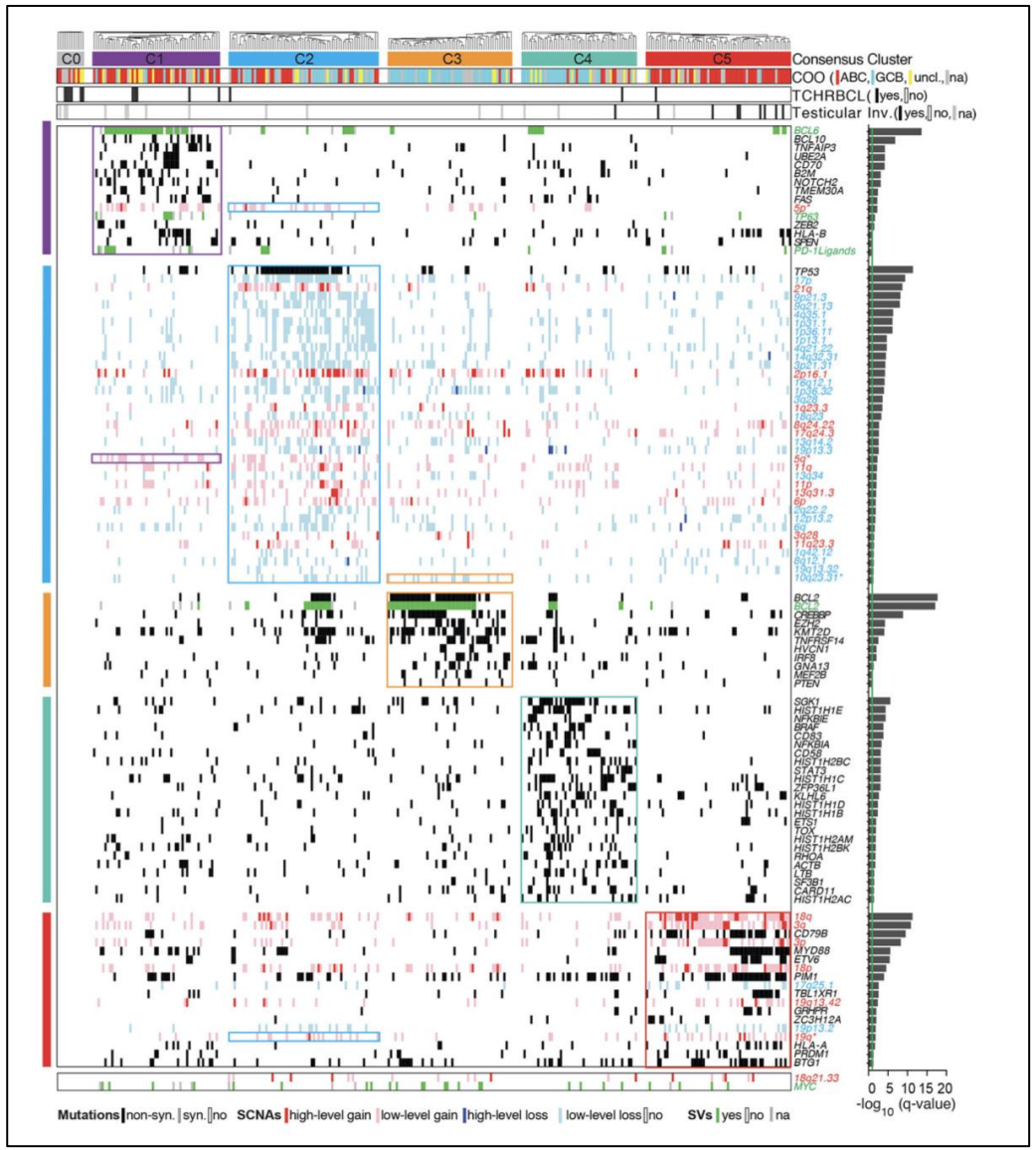
From Schmitz et al.,

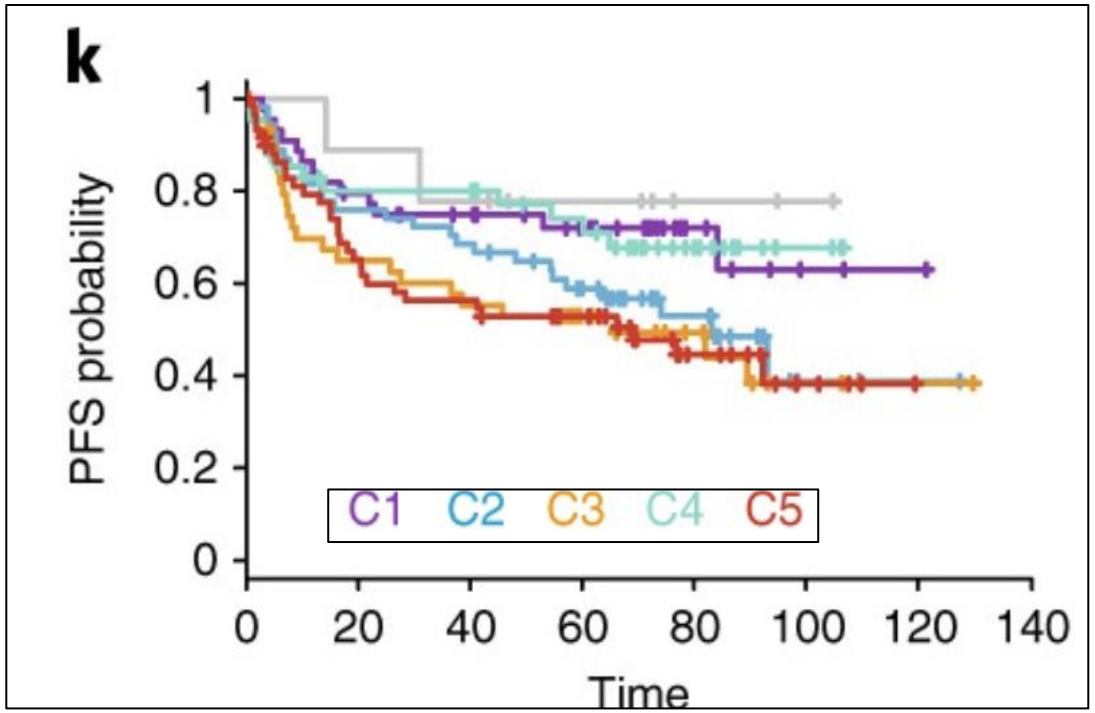




Schmitz R, Wright GW, Huang DW, et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. N Engl J Med. 2018;378(15):1396-1407. doi:10.1056/NEJMoa1801445

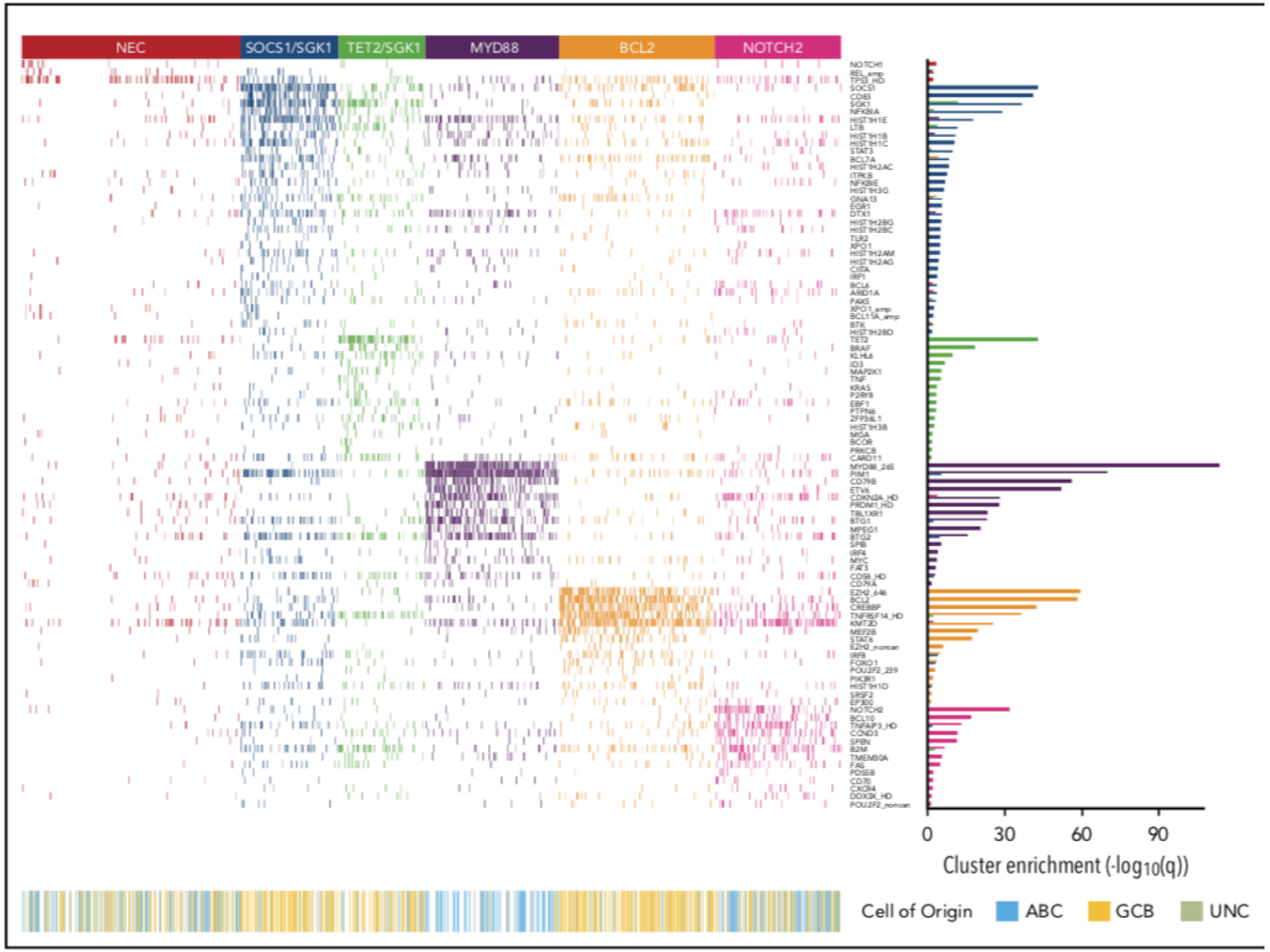
To Chapuy et al.,

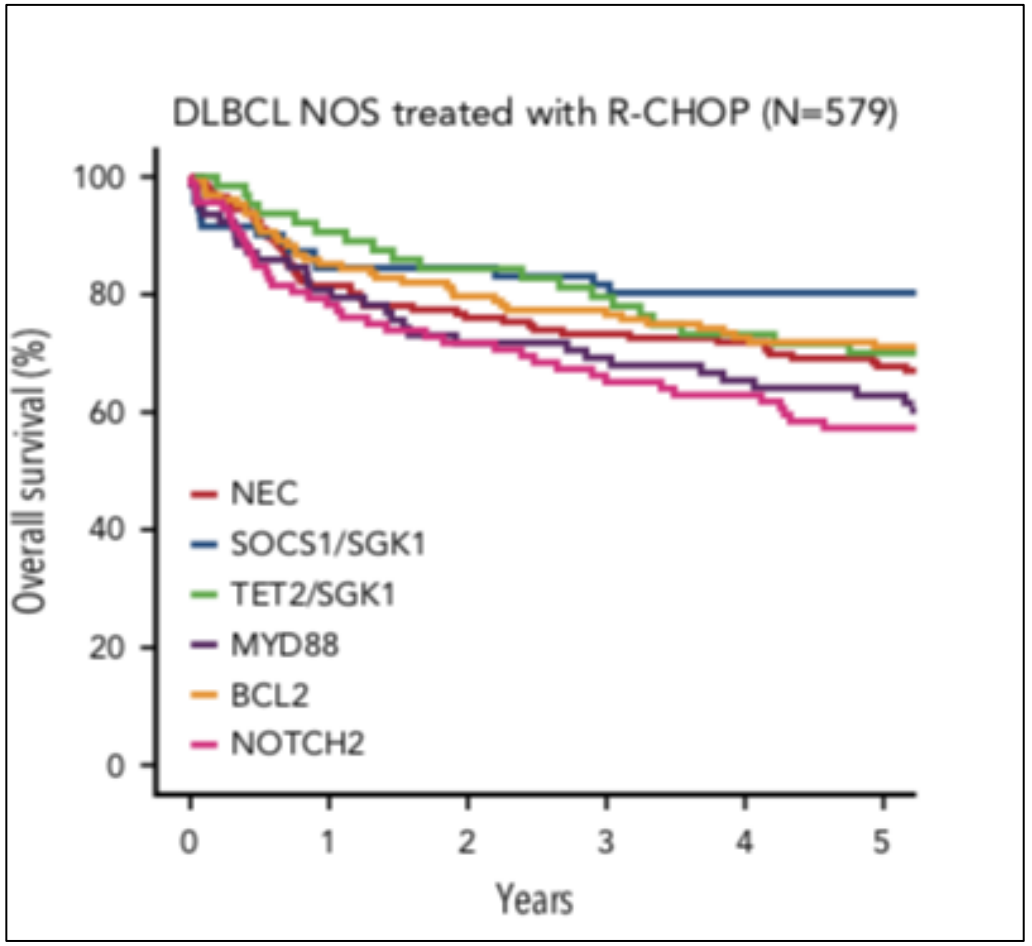




Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes.Nat Med. 2018 Aug;24(8):1290-1291]. Nat Med. 2018;24(5):679-690.

To Lacey et al.,

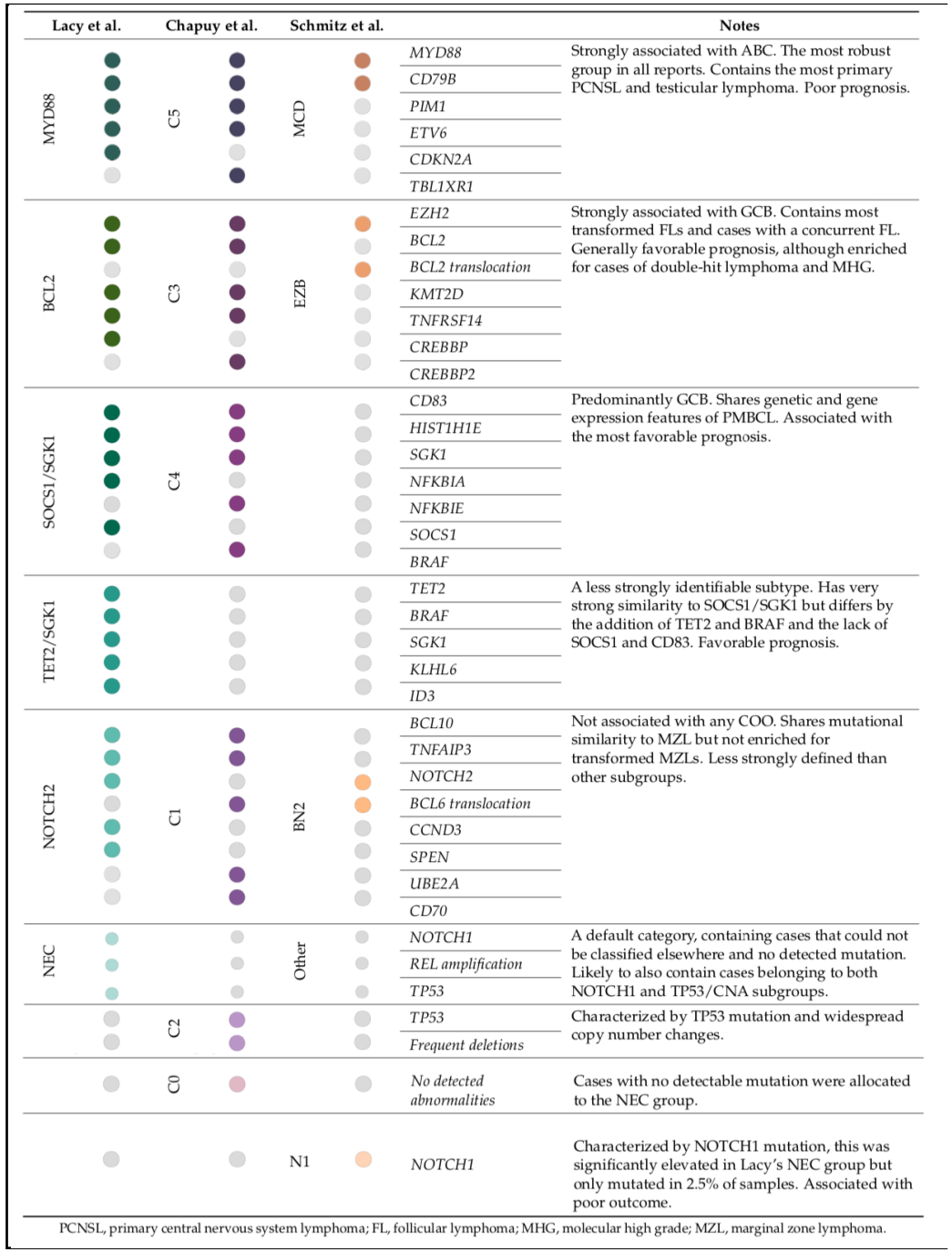




Lacy SE, Barrans SL, Beer PA, et al. Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. Blood. 2020;135(20):1759-1771.

There is good molecular concordance between studies

As summarized by: Pileri et al.,



Lacy et al.	Chapuy et al.	Schmitz et al.		Notes
MYD88	C5	MCD	*MYD88* *CD79B* *PIM1* *ETV6* *CDKN2A* *TBL1XR1*	Strongly associated with ABC. The most robust group in all reports. Contains the most primary PCNSL and testicular lymphoma. Poor prognosis.
BCL2	C3	EZB	*EZH2* *BCL2* *BCL2 translocation* *KMT2D* *TNFRSF14* *CREBBP* *CREBBP2*	Strongly associated with GCB. Contains most transformed FLs and cases with a concurrent FL. Generally favorable prognosis, although enriched for cases of double-hit lymphoma and MHG.
SOCS1/SGK1	C4		*CD83* *HIST1H1E* *SGK1* *NFKBIA* *NFKBIE* *SOCS1* *BRAF*	Predominantly GCB. Shares genetic and gene expression features of PMBCL. Associated with the most favorable prognosis.
TET2/SGK1			*TET2* *BRAF* *SGK1* *KLHL6* *ID3*	A less strongly identifiable subtype. Has very strong similarity to SOCS1/SGK1 but differs by the addition of TET2 and BRAF and the lack of SOCS1 and CD83. Favorable prognosis.
NOTCH2	C1	BN2	*BCL10* *TNFAIP3* *NOTCH2* *BCL6 translocation* *CCND3* *SPEN* *UBE2A* *CD70*	Not associated with any COO. Shares mutational similarity to MZL but not enriched for transformed MZLs. Less strongly defined than other subgroups.
NEC		Other	*NOTCH1* *REL amplification* *TP53*	A default category, containing cases that could not be classified elsewhere and no detected mutation. Likely to also contain cases belonging to both NOTCH1 and TP53/CNA subgroups.
	C2		*TP53* *Frequent deletions*	Characterized by TP53 mutation and widespread copy number changes.
	C0		*No detected abnormalities*	Cases with no detectable mutation were allocated to the NEC group.
		N1	*NOTCH1*	Characterized by NOTCH1 mutation, this was significantly elevated in Lacy's NEC group but only mutated in 2.5% of samples. Associated with poor outcome.

PCNSL, primary central nervous system lymphoma; FL, follicular lymphoma; MHG, molecular high grade; MZL, marginal zone lymphoma.

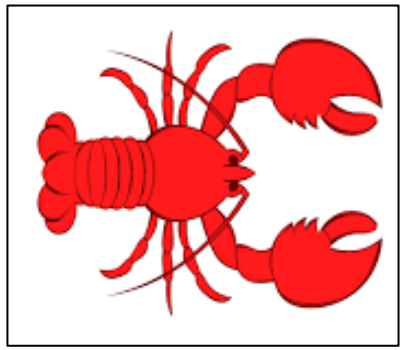
Pileri SA, Tripodo C, Melle F, Motta G, Tabanelli V, Fiori S, Vegliante MC, Mazzara S, Ciavarella S, Derenzini E. Predictive and Prognostic Molecular Factors in Diffuse Large B-Cell Lymphomas. Cells. 2021; 10(3):675.

Different name, same thing

- You say **soda**, she says **pop**, he says **coke**

It's all the same

- You say **crawfish**, she says **crayfish**, he says **crawdads**



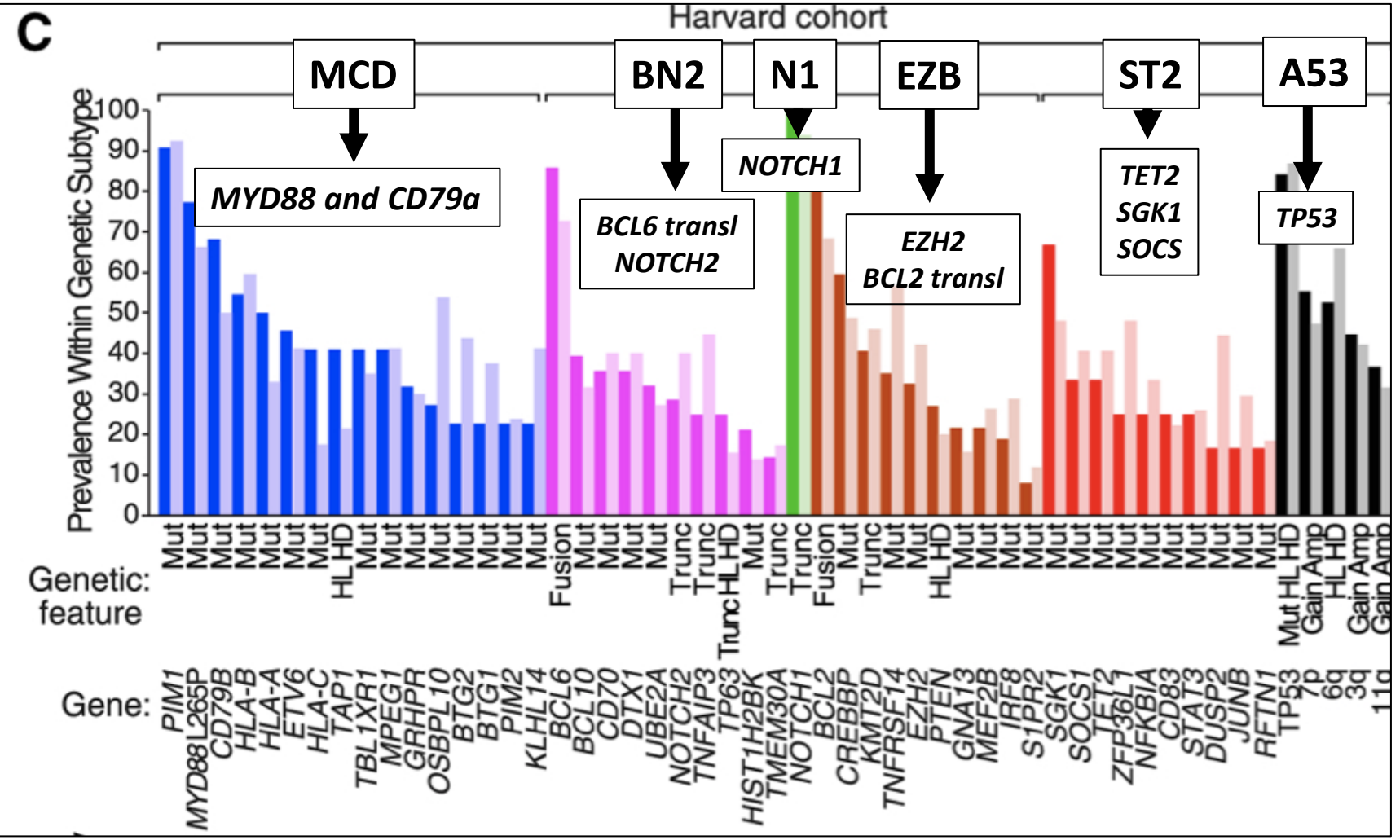
It's all the same

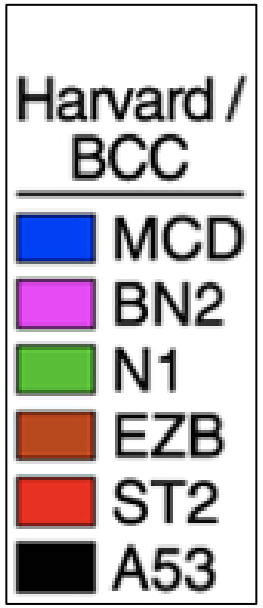
That said… let’s focus on one of the more commonly cited and used

- Wright et al.,
- Studied hundreds of DLBCL

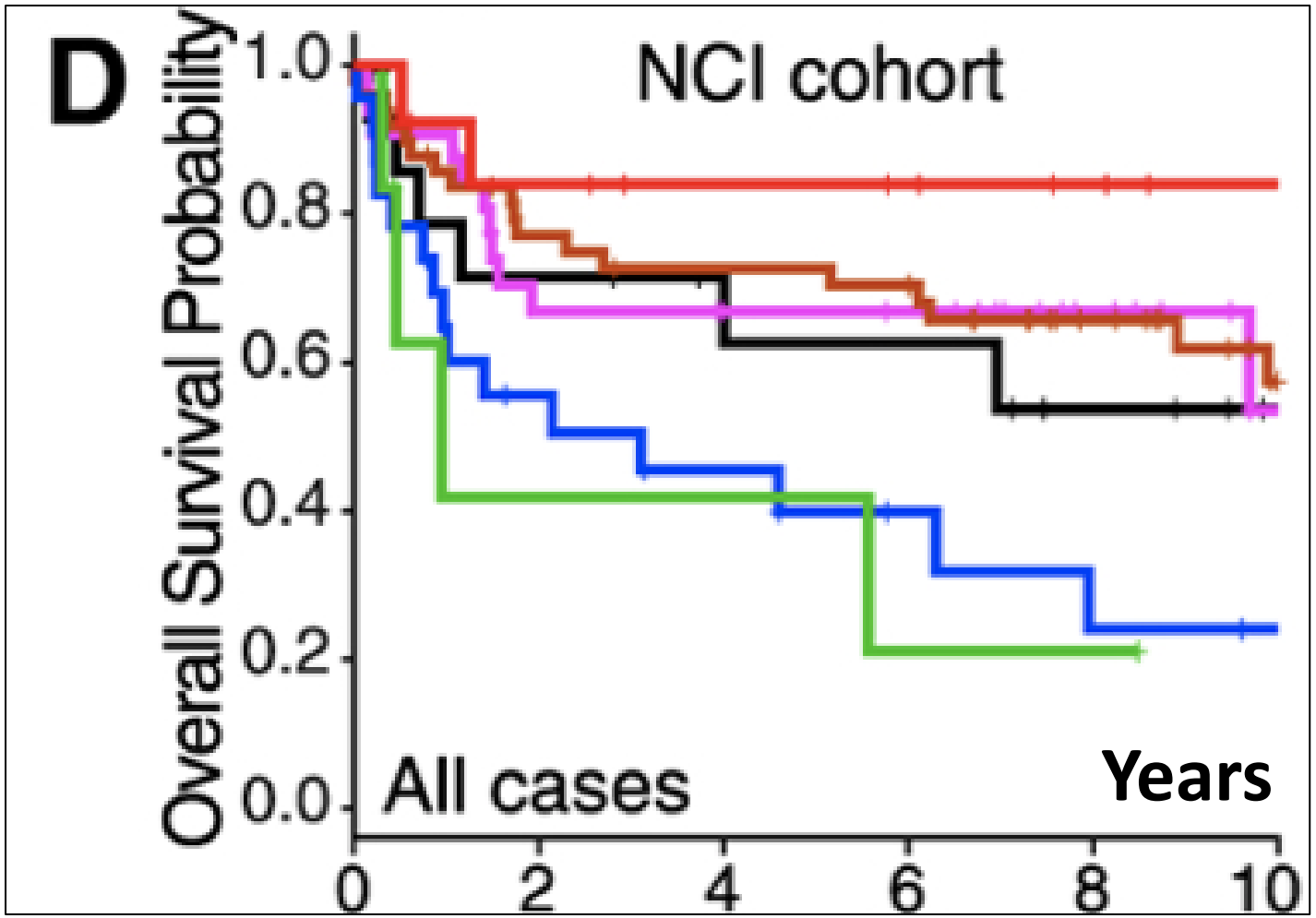
Wright, George W et al. “A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications.” Cancer cell vol. 37,4 (2020): 551-568.e14.

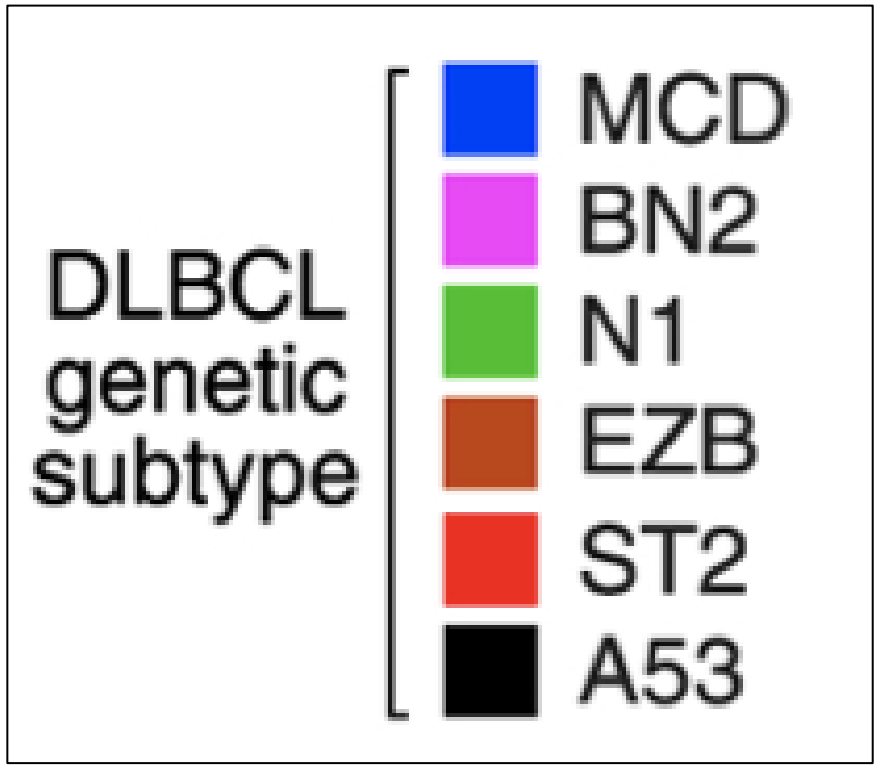
MCD, BN2, N1, EZB, ST2, A53 subtypes of DLBCL



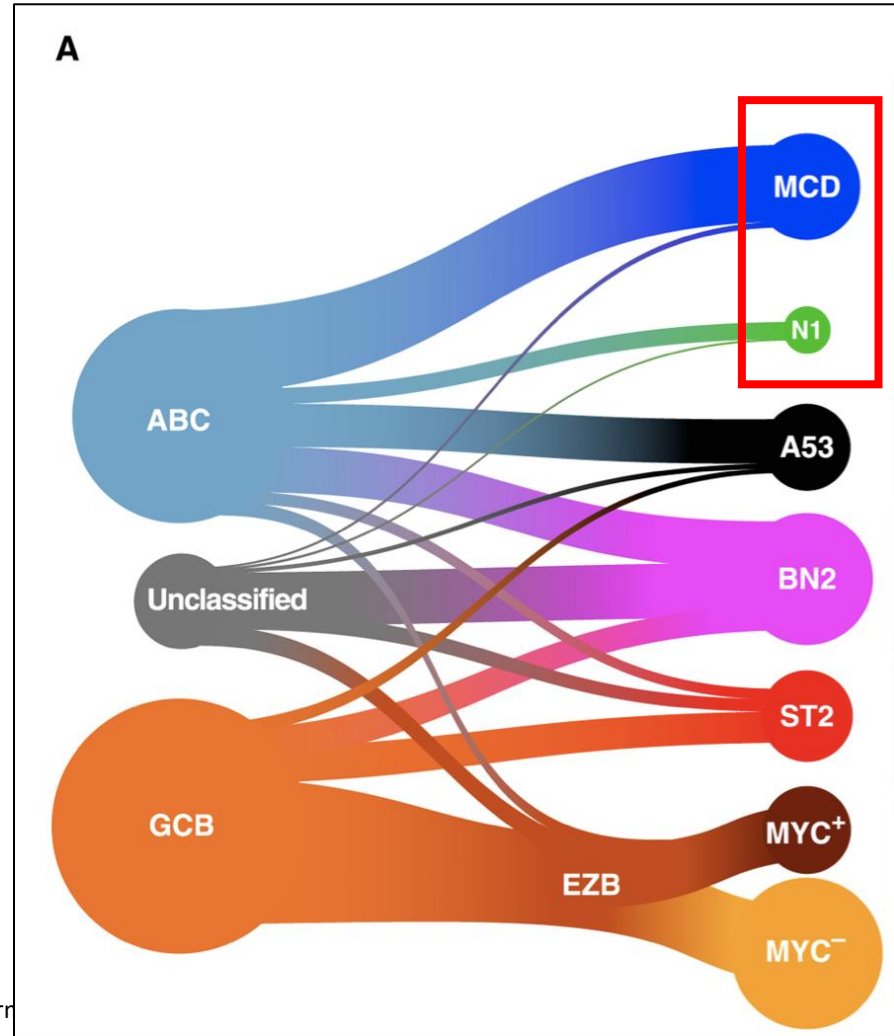


Molecular subtype and outcome





Molecular subtype and therapy



	Prevalence	OS	Genetic themes	Genetically related lymphomas	Gene expression signatures	Tx
MCD	8.7%	40% (All) 37% (ABC	My-T-BCR-dependent NF-κB Immune evasion-MHC class I Cell survival - *BCL2* expression Altered B cell differentiation G1-S cell cycle/p53 checkpoint BCR: IgM >> IgG; $IgV_H4\text{-}34^{++}$	Primary extranodal DLBCL Transformed WM	B cell activation NF-κB IRF4 Myc Proliferation	BCR-dep. NF-κB PI3 kinase mTORC1 BCL2-$BCLX_L$-MCL1 JAK1 IRAK4 IRF4
N1	1.7%	27% (All) 22% (ABC	NOTCH1 signaling Altered B cell differentiation BCR: IgM > IgG	NOTCH1-mutant CLL	NOTCH Quiescence Plasma cell T cell-myeloid-FDC	NOTCH1 Immune checkpoints
A53	5.8%	63% (All) 33% (ABC 100% (GCB	*TP53* inactivation/DNA damage Aneuploidy Immune evasion - *B2M* loss BCR: IgM >> IgG; $IgV_H4\text{-}34^{++}$	–	p53 Immune low	BCR-dep. NF-κB
BN2	13.3%	67% (All) 76% (ABC 100% (GCB 38% (UC)	NOTCH2 signaling Altered B cell differentiation BCR-dependent NF-κB Immune evasion - *CD70* loss Proliferation - Cyclin D3 BCR: IgM >> IgG; $IgV_H4\text{-}34^{++}$	MZL Transformed MZL	B cell activation NF-κB NOTCH Proliferation	BCR-dep. NF-κB PI3 kinase mTORC1 BCL2 NOTCH2
ST2	6.4%	84% (All) 81% (GCB	JAK/STAT3 signaling NF-κB activation *P2RY8 – GNA13* inactivation Altered B cell differentiation BCR: IgG >> IgM	NLPHD THRLBCL	GC B cell PI3K signaling JAK2 signaling Glycolysis Stromal	PI3 kinase JAK2
MYC+ / MYC−	5.9% (MYC^+) 17.6% (MYC^-)	48% (MYC^+) 82% (MYC^-)	Chromatin modification Anti-apoptosis PI3 kinase signaling *S1PR2 – GNA13* inactivation Altered T_{FH} interactions MYC (EZB-MYC^+) BCR: IgG > IgM	FL Transformed FL BL (EZB-MYC^+)	GC LZ (MYC^-) GC IZ (MYC^+) BCL6 (MYC^+) TCF3 (both) T_{FH} cells (MYC^-) Stromal (MYC^-) Immune low (MYC^+)	PI3 kinase mTORC1 EZH2 BCL2-MCL1

Returning to our case…

We can predict the following just based on mutations

- Diffuse large B-cell lymphoma, NOS, GCB, non-double expressor
- Molecularly classify it as: ST2 or TET2/SGK1
- Prediction:
  - Non-GCB
  - Good prognosis for patient
    - Indeed other markers: p53, ki67, c-myc and bcl2 all point to good prognosis
    - Patient is doing well 1 year after chemotherapy
- Therapy:
  - ?Potential targeted therapeutics- ?JAK/STAT?

There are other large B-cell lymphomas

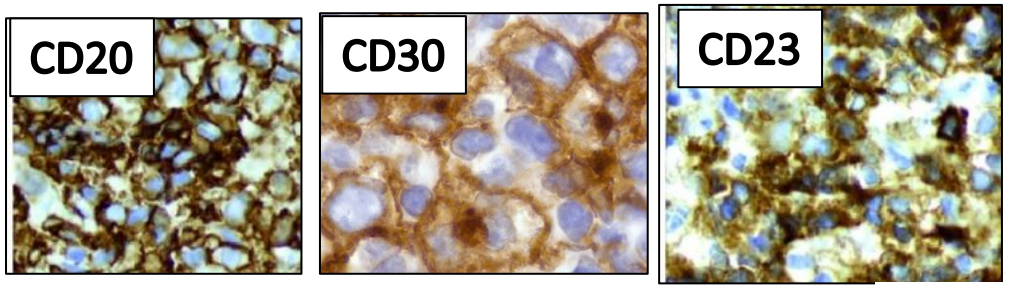
Primary mediastinal large B-cell lymphoma

Primary mediastinal large B-cell lymphoma

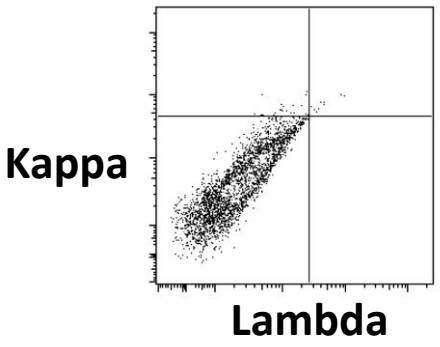
- **“PRIMARILY”** in Mediastinum

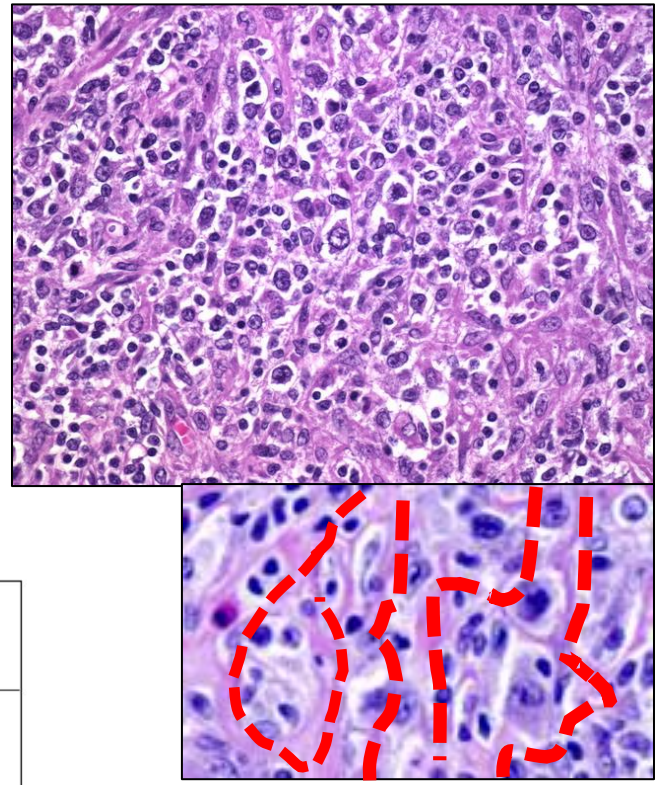
Primary mediastinal large B-cell lymphoma

- “PRIMARILY” in Mediastinum
- Fibrotic/Sclerotic background
- CD20+/ CD30+/ CD23+



- Often sIg negative
- Can be **sIg+** (Weinberg et al., 2016)





Mediastinal grey zone lymphoma

- Also known as: MGZL

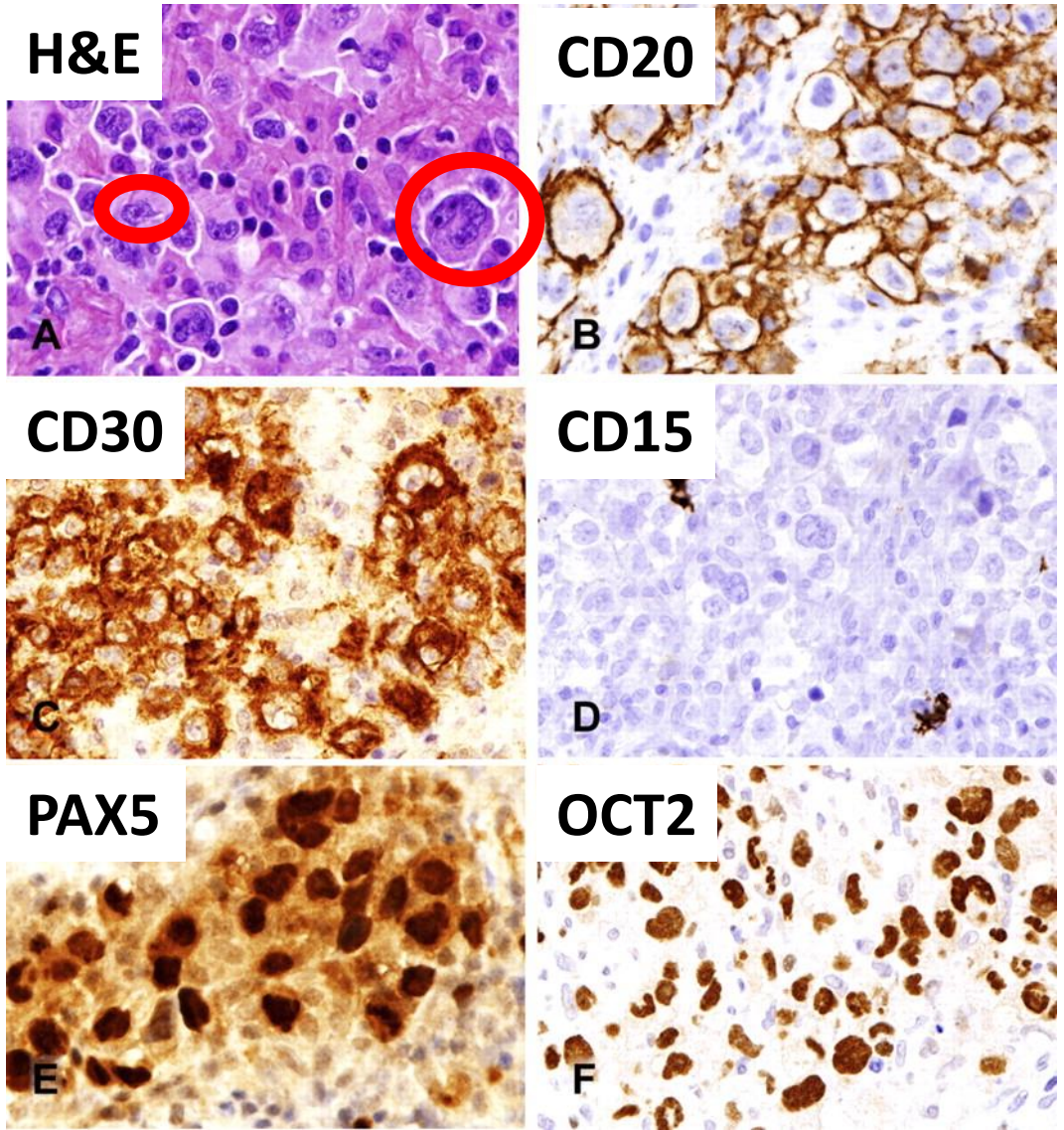
MGZL

- Essentially some features of DLBCL… some features of CHL…

MGZL

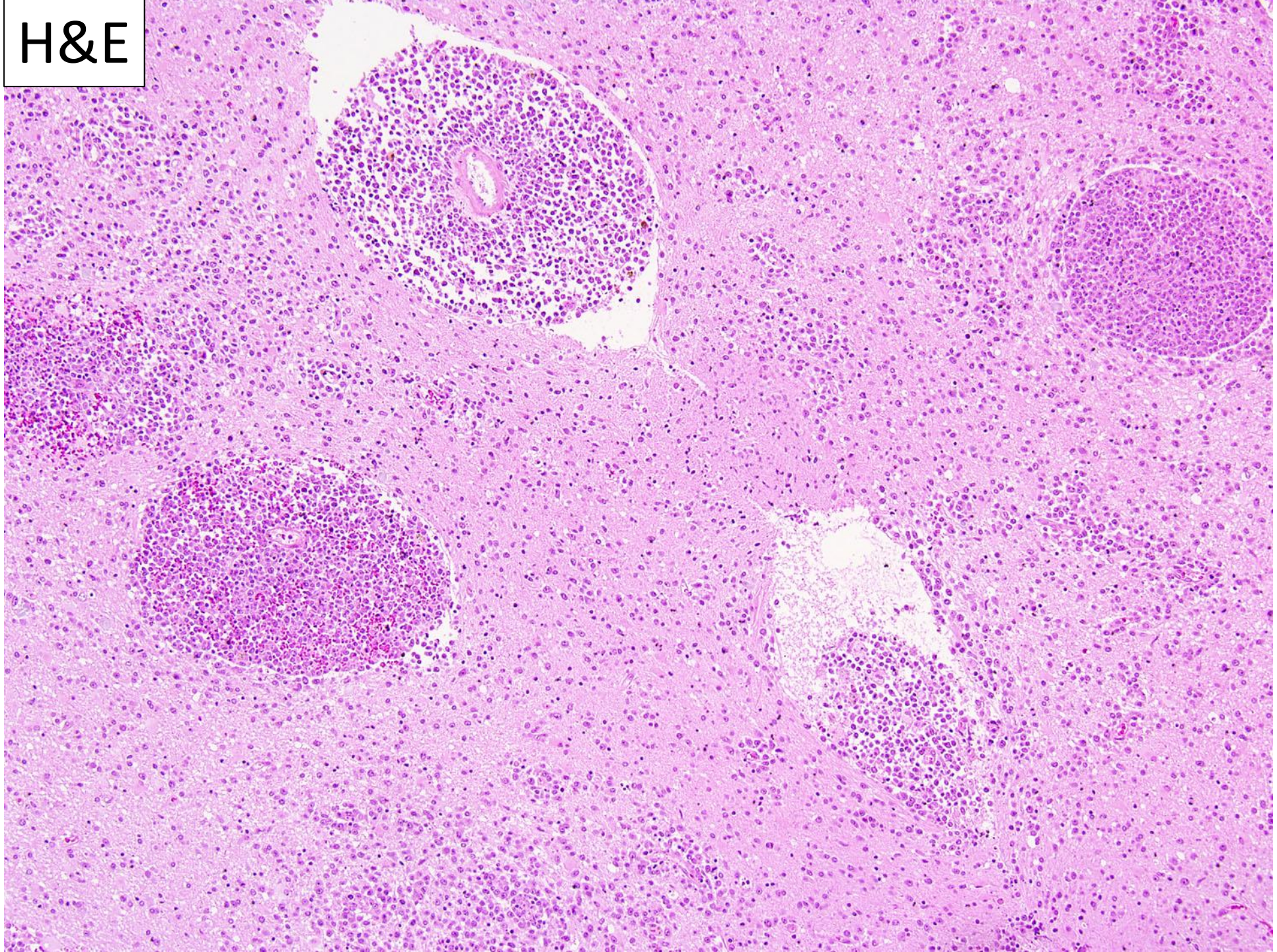
- More confluent sheets of large cells
- CD30+
- CD15-
- CD20+
- EBV-/+

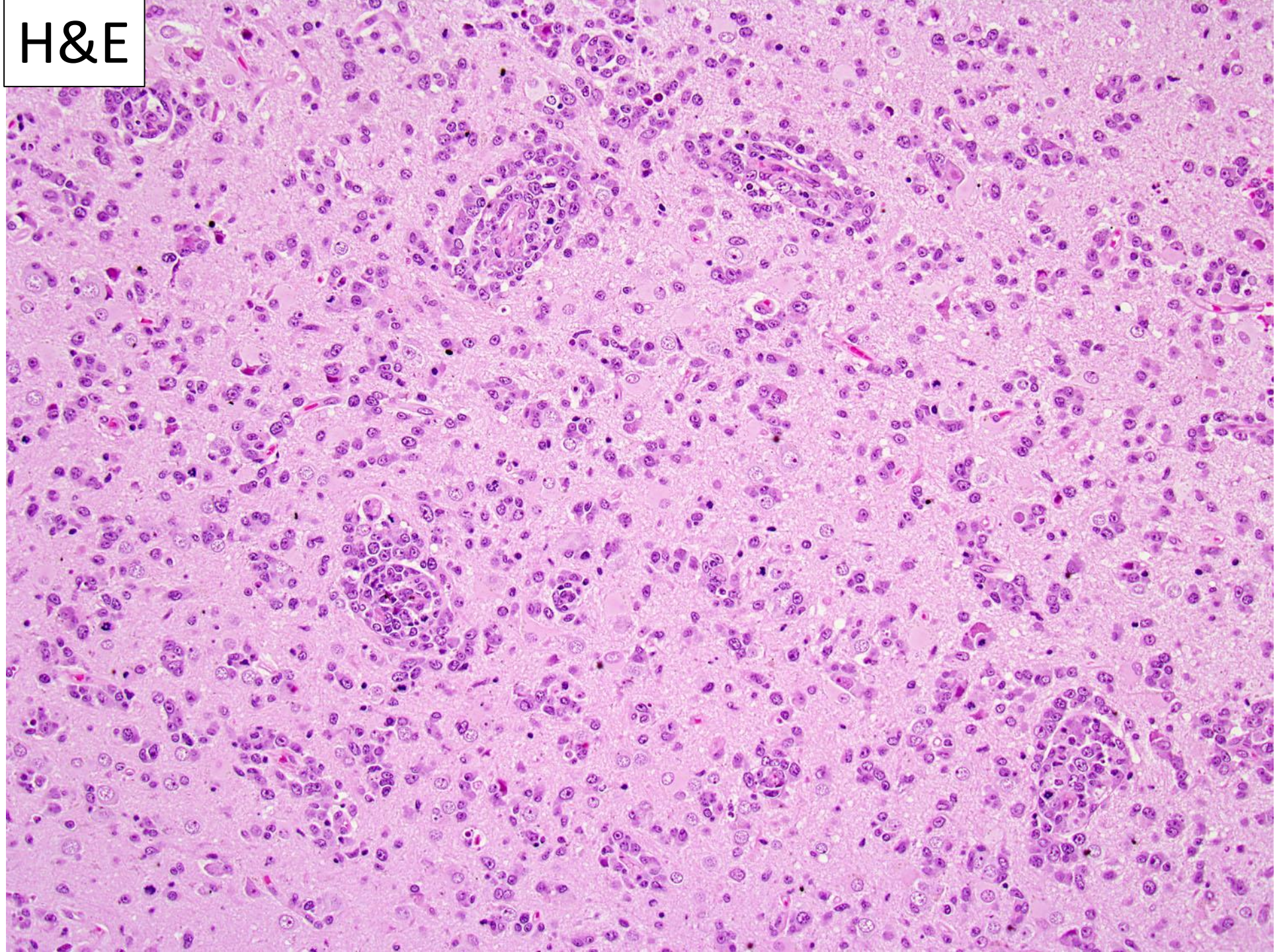
MGZL

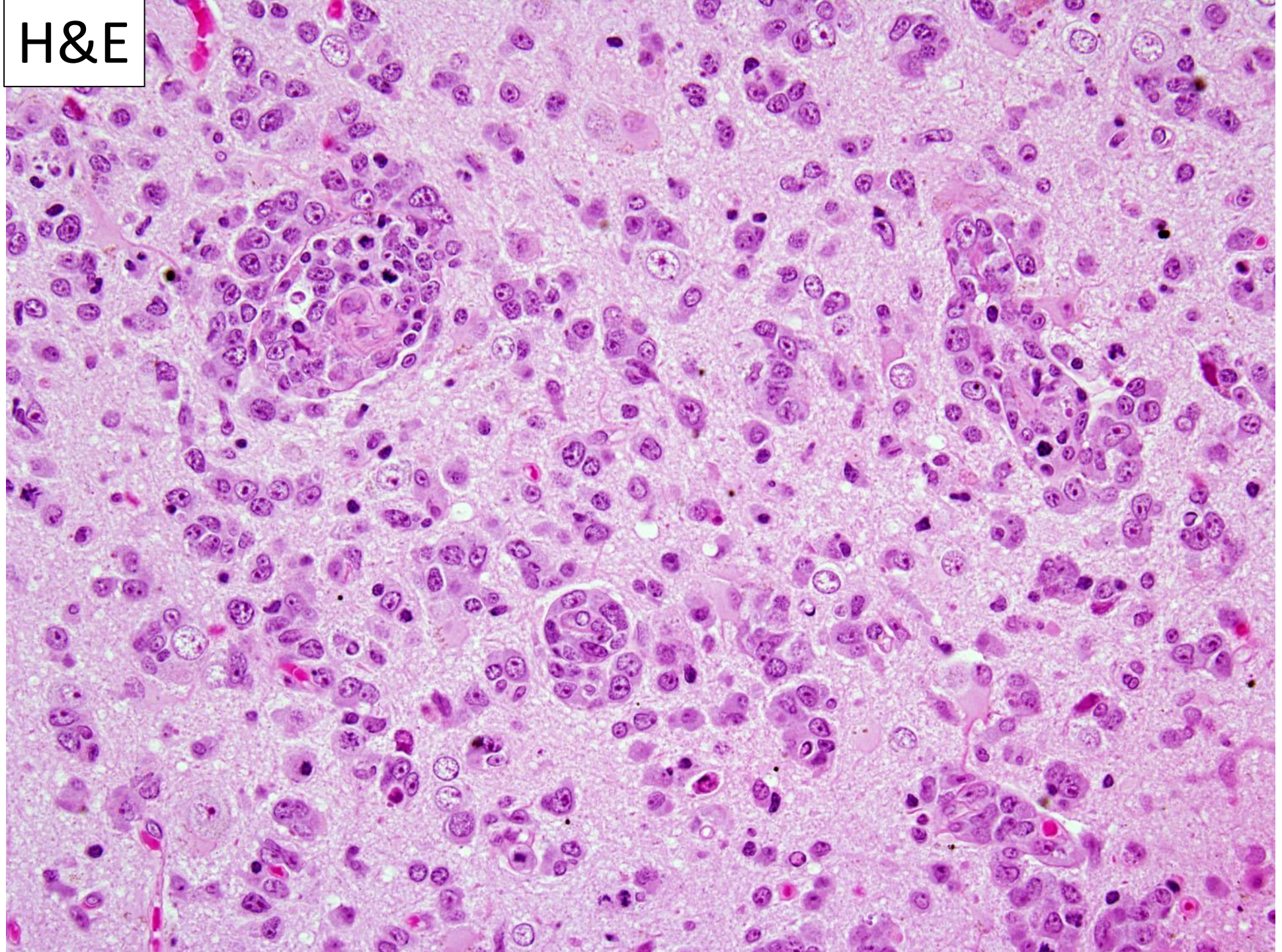


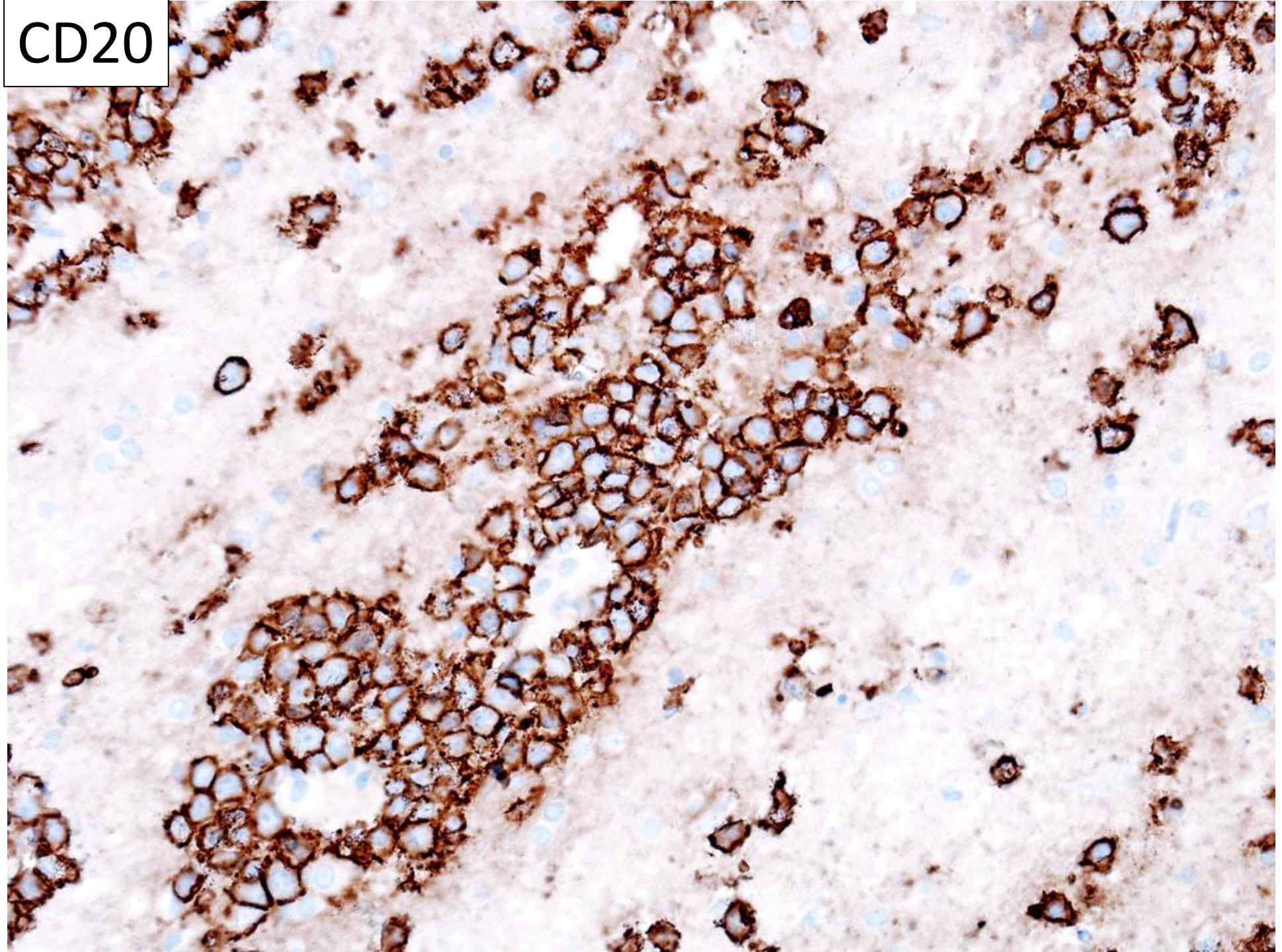
Case 7

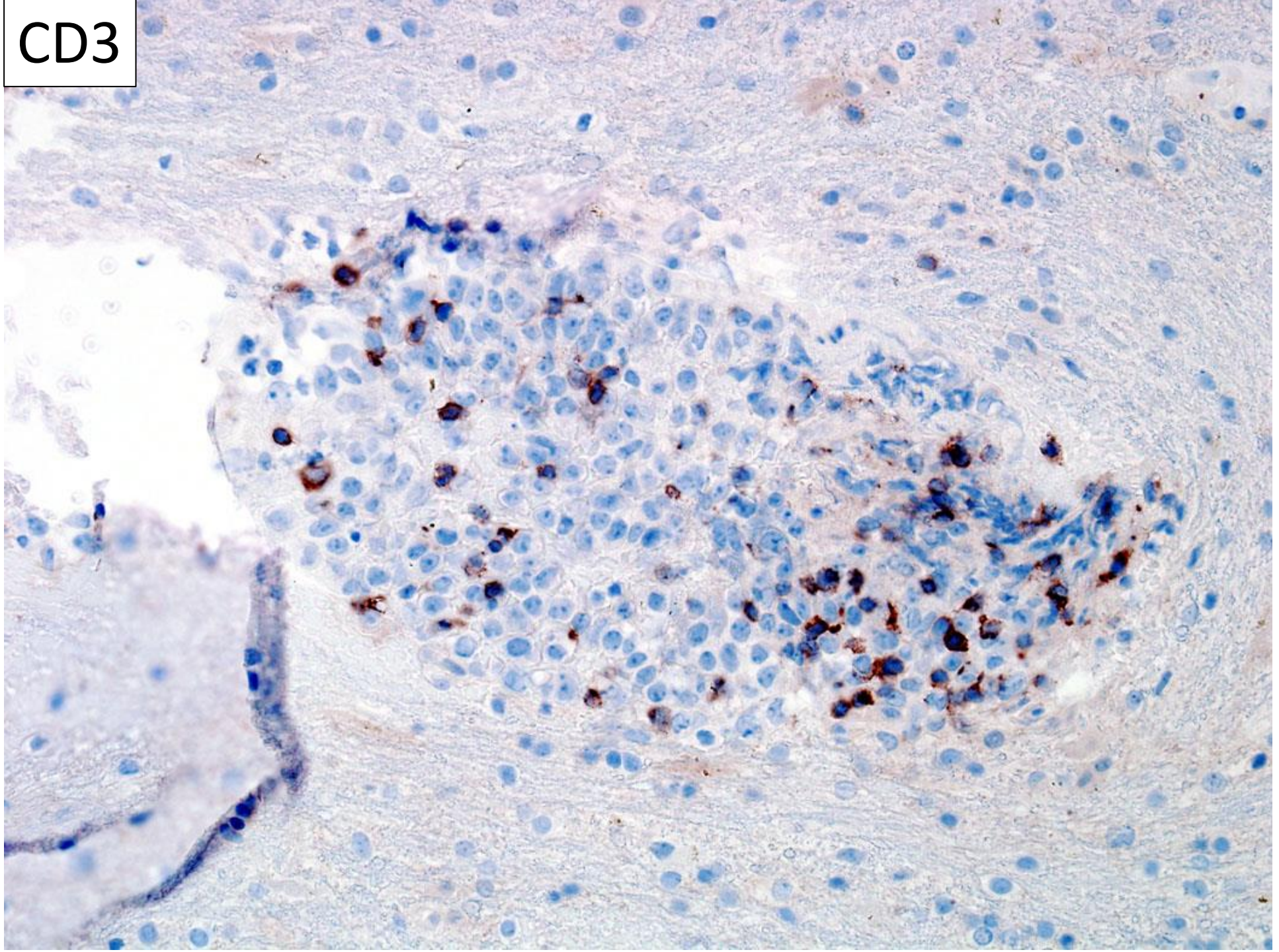
- 57 year old who complains of dizziness
- No lymphadenopathy, no organomegaly
- Imaging identifies a mass in the brain only











Other stains and information

- EBV negative
- No other lymphadenopathy or PB or marrow involvement

Diagnosis?

- Primary CNS lymphoma

Primary CNS lymphoma

- Primarily arises in the brain, spinal cord, leptomeninges, and vitreoretinal compartment of the eye
- No significant systemic involvement.
- 4–7% of all brain tumors
- Male predominance (ratio of 1.25); median age 66
- Two important risk factors of PCNSL are increasing age and human immunodeficiency
- Median age at diagnosis is 66 years
- Diffuse large B-cell lymphoma (DLBCL) accounts for 90–95% of all PCNSL

Primary CNS lymphoma

- Diffuse proliferation of medium-to-large-sized lymphoid cells with pleomorphic, round to oval, irregular, and vesicular nuclei with prominent nucleoli, morphologically consistent with centroblasts or immunoblasts
- Tumor cells usually exhibit perivascular arrangement (angiocentricity) by forming layers around blood vessels
- However, microvascular proliferation (frequently encountered in high-grade gliomas) is rare
- PCNS DLBCL is a mature B-cell neoplasm, and the tumor cells express B-cell markers, particularly CD19, CD20, CD22, CD79a, and PAX5

Primary CNS lymphoma: Prognostication

- A majority (67–96%) of cases are of an activated B-cell (ABC)/non-germinal center B-cell (non-GCB) subtype

- Co-expression of c-Myc and Bcl-2 (double-expressor) and/or co-rearrangement of *MYC* and *BCL2* and/or *BCL6* (double or triple hit lymphoma) may be seen

- Uncertain yet how any of the above impacts prognosis

Now in WHO-HAEM5 grouped in a new category

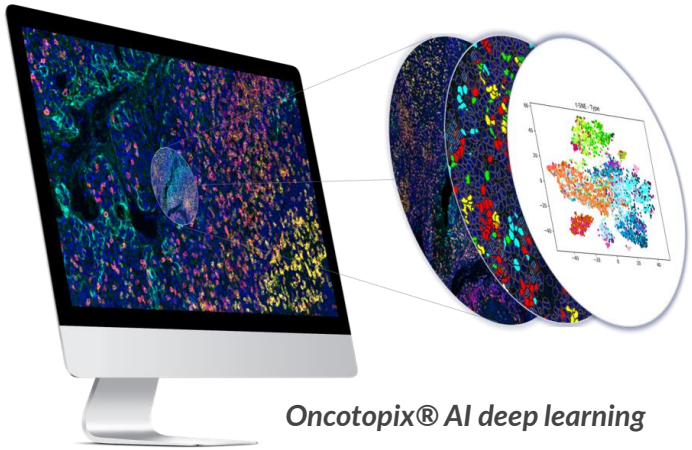
- Primary large B-cell lymphoma of immune privileged sites
  - Testis, CNS, vitreoretinal
  - Common genetic alterations for immune escape
  - Frequent *MYD88* and *CD79B* mutations; C5/MCD/MYD88 genetic signature
  - Frequent *CDKN2A*/9p21 inactivation

Future for B-cell lymphomas

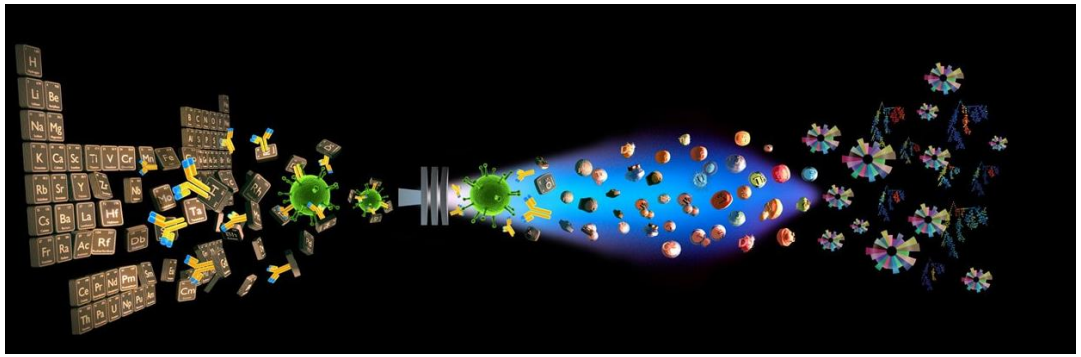
- Integrated diagnoses
  - Clinical, Anatomic, Laboratory data, Radiology
  - Histomorphology, Immunophenotype, Cytogenetics
- More molecular NGS testing
- Utilization and incorporation of novel technologies

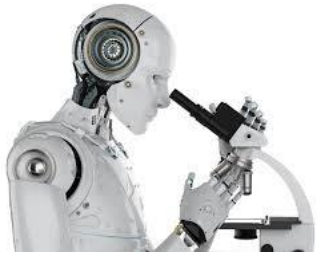
Future…

- Digital pathology/Digital Imaging
- High resolution immunophenotyping
- Advanced NGS molecular analysis
- Artificial intelligence, Machine learning



Oncotopix® AI deep learning





Thank you