Ancillary Testing in Lymphoma Diagnosis and the Challenges of Small Biopsies

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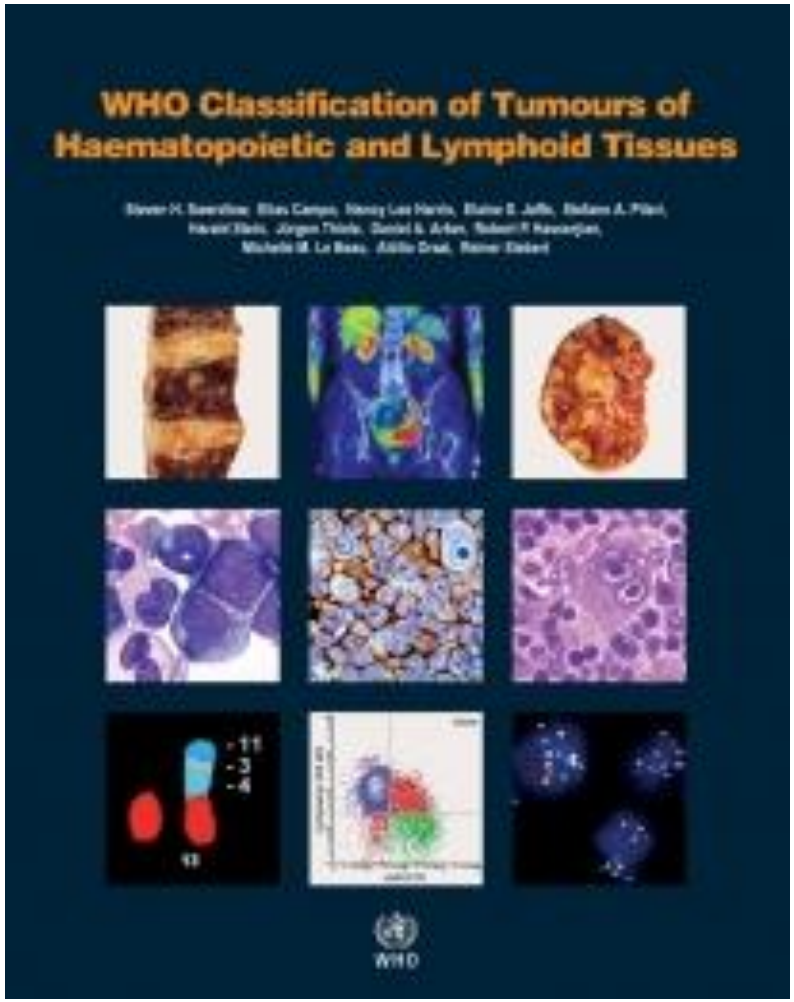
Objectives

- Review the utility and necessity of ancillary testing in the diagnosis of lymphoma.
- Discuss the use of FISH assays to provide prognostic information or precise classification in lymphoma diagnosis.
- Discuss the challenges and perils of small biopsies in lymphoma diagnosis.

2017 Revision of the WHO Classification of Leukemia and Lymphoma



Lymphoma: 86 types (+ subtypes)

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
γ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
*Large B-cell lymphoma with IRF4 rearrangement**
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV⁺ DLBCL, NOS*
*EBV⁺ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK⁺ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8⁺ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma
Mature T and NK neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK-cell leukemia
Systemic EBV⁺ T-cell lymphoma of childhood*
Hydroa vacciniforme–like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma

Table 1. (continued)

Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract**
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30⁺ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8⁺ aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8⁺ T-cell lymphoma**
*Primary cutaneous CD4⁺ small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large-cell lymphoma, ALK⁺
Anaplastic large-cell lymphoma, ALK⁻*
*Breast implant–associated anaplastic large-cell lymphoma**
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

Provisional entities are listed in italics.
*Changes from the 2008 classification.

small population, but in others associated with a lymphocytosis.[4] Whereas in 2008 it was unknown whether MBL was a precursor of CLL, we now know that MBL precedes virtually all cases of **CLL/small lymphocytic lymphoma** (SLL).[5] The updated WHO will retain the current criteria for MBL, but will emphasize that "low-count" MBL, defined as a PB CLL count of $<0.5 \times 10^9$/L, must be distinguished from "high-count" MBL because low count MBL has significant differences from CLL, an extremely limited, if any, chance of progression, and, until new evidence is provided, does not require routine follow-up outside of standard medical care.[6,7] In contrast, high-count MBL requires routine/yearly follow-up, and has very similar phenotypic and genetic/molecular features as Rai stage 0 CLL, although immunoglobulin heavy chain variable region (IGHV)-mutated cases are more frequent in MBL.[8] Also impacting our diagnostic criteria, the revision will eliminate the option to diagnose CLL with $<5 \times 10^9$/L PB CLL cells in the absence of extramedullary

How do we diagnose and classify lymphomas?

- Morphology forms the basis for the lymphoma classification system.
  - » Architecture: nodal effacement vs. preservation?
    - Diffuse, follicular, nodular
  - » Cell characteristics: large vs. small, blast vs. mature.
  - » Hodgkin vs. non-Hodgkin
- Role of ancillary testing continues to increase.
  - » Immunophenotype by flow cytometry or immunohistochemistry.
    - B-cell vs. T-cell, other characteristic antigen expression.
  - » Molecular/genetic characteristics: translocations, mutations.

Ancillary Tests Used in Lymphoma Diagnosis

- Immunophenotyping
  - » Flow cytometry and/or immunohistochemistry
- Cytogenetics
  - » Karyotype or FISH for translocations
- Molecular
  - » Clonality
  - » PCR for specific translocations or mutations
  - » Sequencing for mutations, translocations

Flow Cytometry

- Most sensitive and complete immunophenotyping.
  - » Determine expression of multiple antigens on one cell population.
- Detect clonal B-cells
  - » Polytypic: Mix of kappa and lambda expressing cells.
  - » Monotypic: Predominant expression of one or the other.
- Detection of abnormal T-cell populations.
- Detect low-level involvement.

CLL/SLL: Flow Cytometry Does This Well

- Characteristic immunophenotype: CD5, dim CD20, dim monoclonal light chains, CD23, CD200.
  - » Diagnostic in appropriate clinical setting.
  - » Imperfect phenotypes should raise consideration of other entities, especially mantle cell lymphoma.
- Only way to characterize monoclonal B-cell lymphocytosis (MBL).
  - » CLL type, atypical CLL type, non-CLL type.
  - » Low-count ($<0.5x10^9/L$) does not seem to progress.
  - » High-count ($\geq 0.5\text{-}5x10^9/L$) acts like Rai stage 0 CLL.

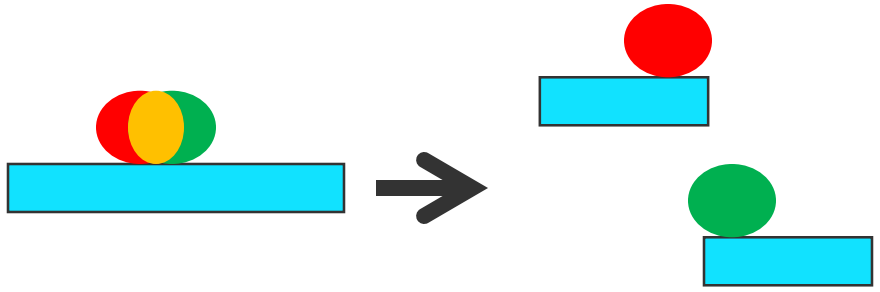
Limitations of Flow Cytometry

- Loss of morphologic features, which are key for the WHO classification system.
  - » Generally cannot give a specific WHO diagnosis.
  - » CD10 positive lymphoma, not “follicular lymphoma.”
- False negative: May lose some cell populations in processing, analysis, and/or sampling.
  - » Lymphoma cells, especially large cells, may be excluded.
  - » Sampling is critical.
- “False positive”: Small clonal B-cell populations and aberrant T-cell populations are not diagnostic of lymphoma.
- Discrepant flow results should make you think twice but should not necessarily change your mind.

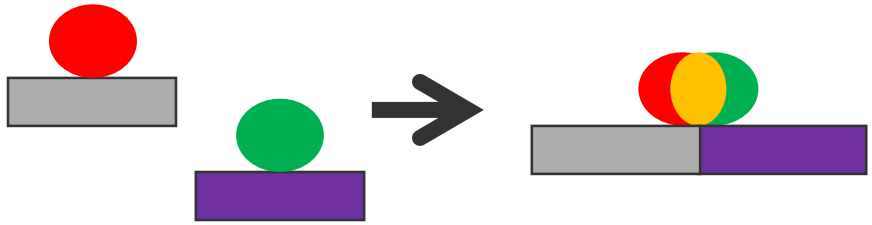
FISH: Fluorescence *in situ* Hybridization

- Detection of specific, defined abnormalities
- Relatively rapid turn-around (24-48 hrs)
- Can be performed on fixed, paraffin-embedded tissues

- Break-apart probes:
  - *Separation* of the signals is abnormal.

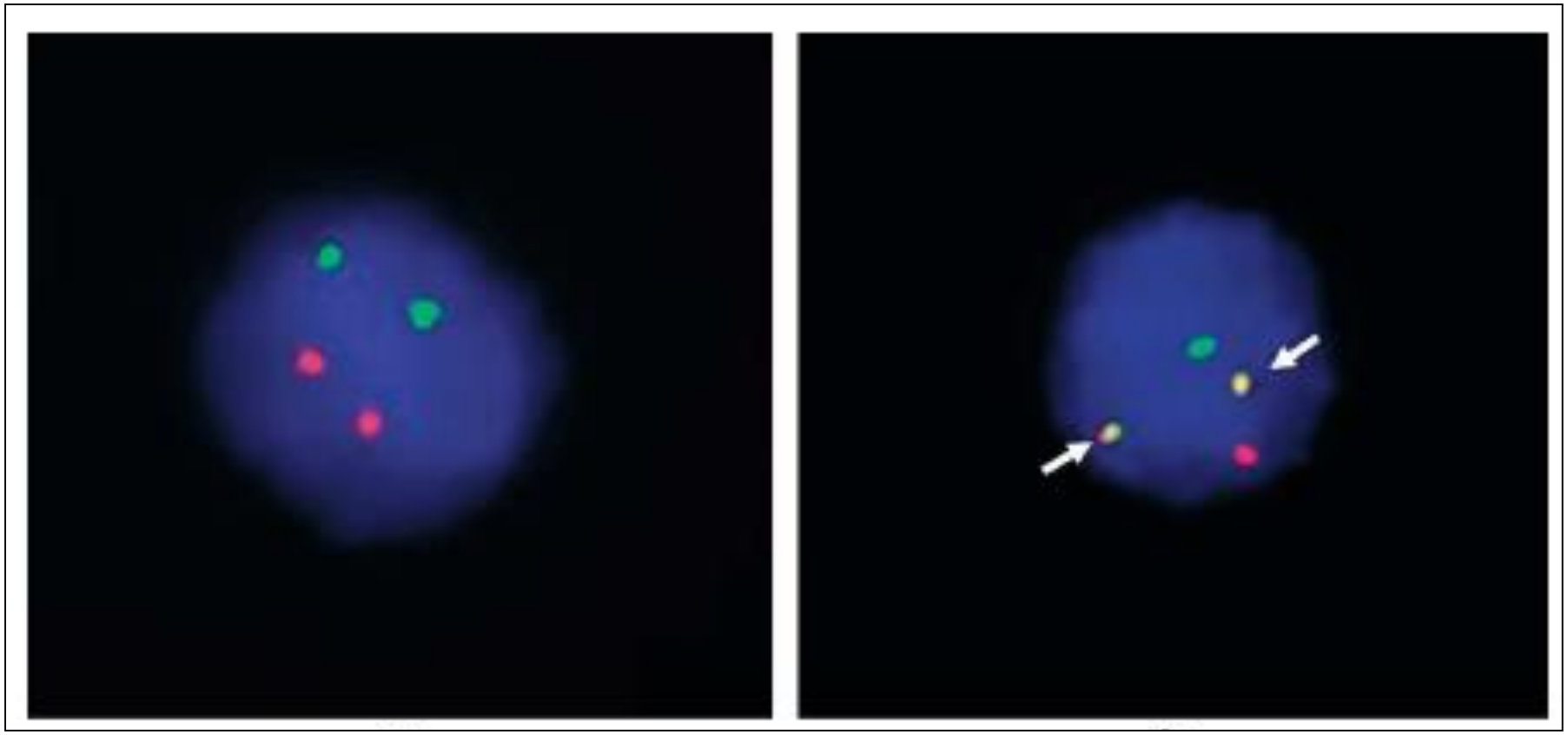


- Fusion probes:
  - *Fusion* of probe signals is abnormal.



FISH for t(14;18) *IGH/BCL2*

IGH/BCL2 fusion probe.

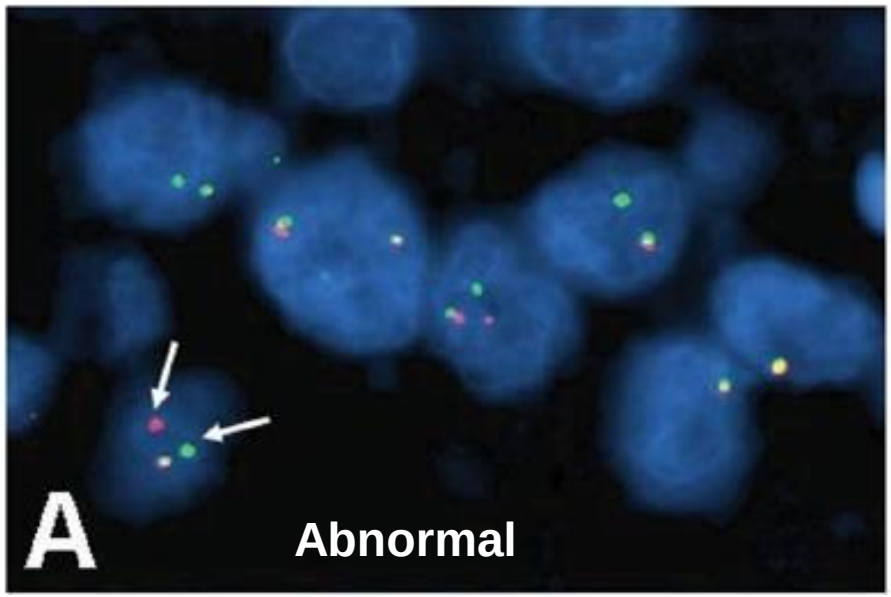


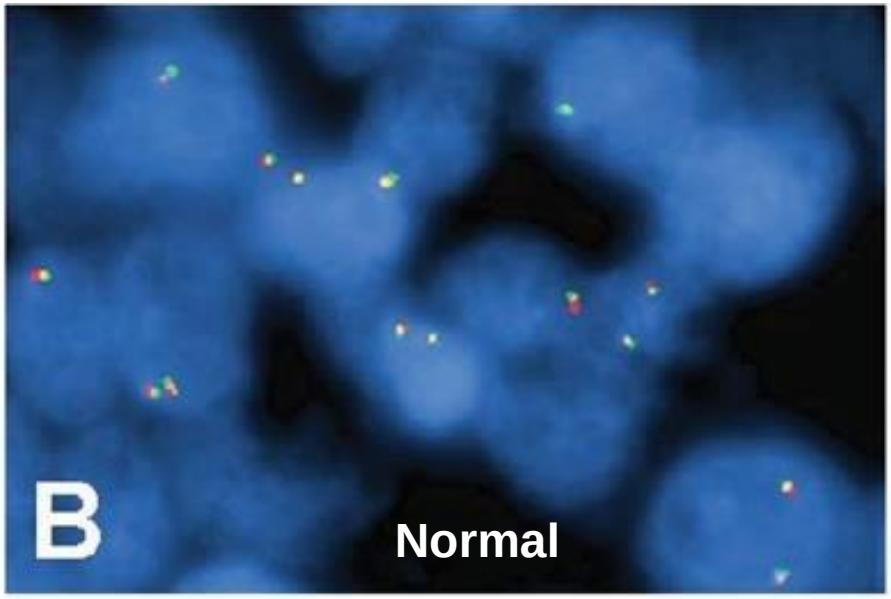
Normal

Abnormal

FISH for *MYC* Translocations

MYC break-apart probe





FISH Assays Used for Lymphomas

Probe	Type	Detects	Lymphoma Type
MYC-IGH	F	t(8;14) MYC-IGH	Burkitt, DLBCL
LSI-MYC	BAP	8q24 MYC	DLBCL, Burkitt
BCL6	BAP	3q27 BCL6	DLBCL, Follicular
IGH-BCL2	F	t(14;18) IGH-BCL2	Follicular, DLBCL
IGH-CCND1	F	t(11;14) Cyclin D1	Mantle Cell
ALK	BAP	2p23 ALK	ALK+ ALCL
6p25.3	BAP	DUSP22/IRF4	ALK- ALCL, B-cell
3q28	BAP	TP63	PTCL, ALK- ALCL

F=Fusion, BAP= Breakapart probe

Genetically Defined Lymphomas

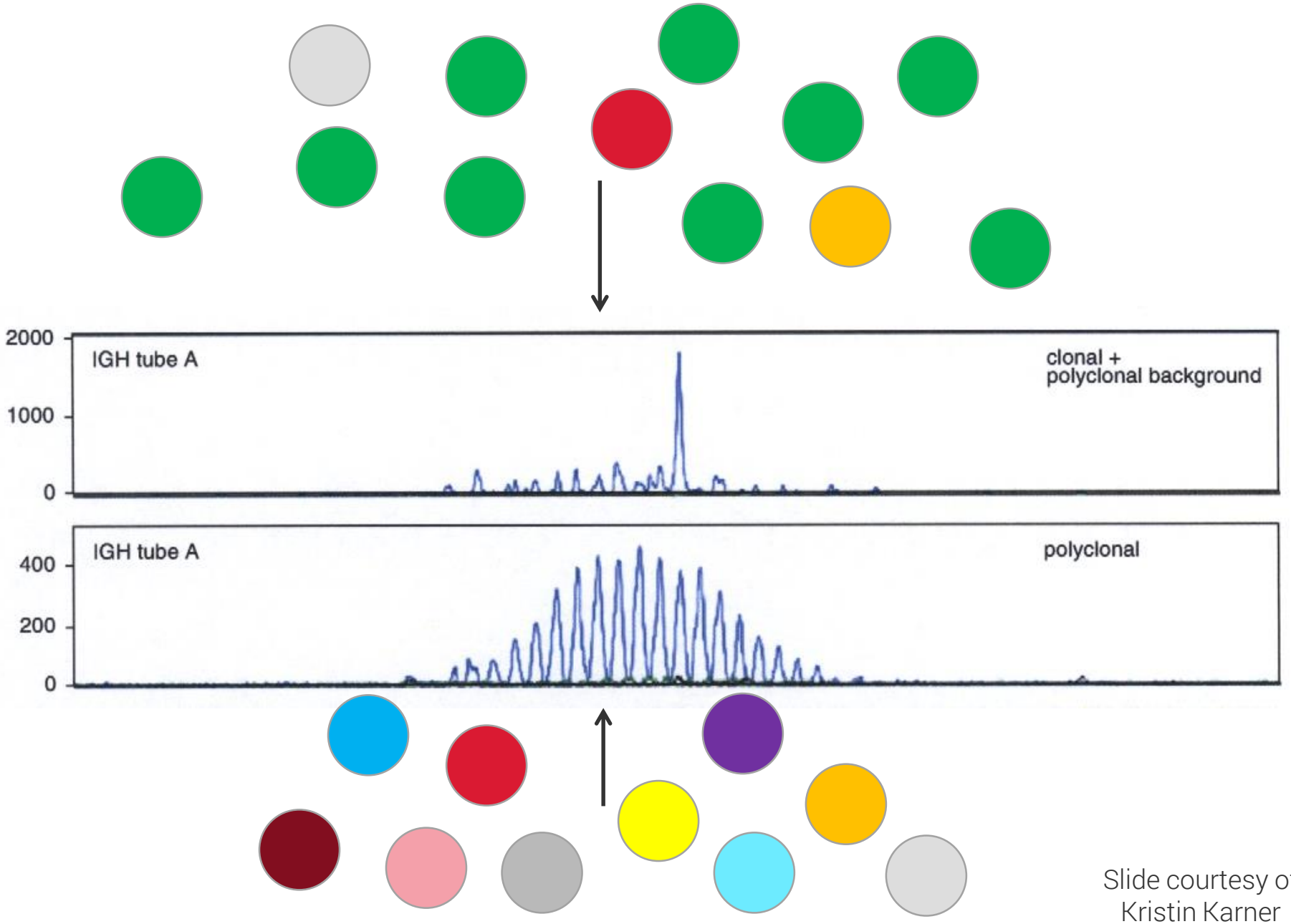
- ALK positive ALCL t(2;5) in ~85% of cases.
  - » Immunohistochemistry is a reliable surrogate and detects variant translocations as well.
- Large B-cell lymphoma with *IRF4* rearrangement
  - » Provisional entity, more later…
- Mantle cell lymphoma t(11;14) in >95% of cases.
  - » Immunohistochemistry is a reliable surrogate
    - Will detect rare variant translocations with light chain genes.
  - » Cyclin D1 negative cases exist.
- High grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangement

Lymphomas with highly characteristic genetic changes that do not define them:

- Burkitt lymphoma: *IG-MYC*, t(8;14), t(2;8), t(8;22)
- Follicular lymphoma: *IG-BCL2* t(14;18)
- Lymphoplasmacytic lymphoma: *MYD88* mutation.
- Hairy cell leukemia (*BRAF* V600E).

When should I send for clonality?

- Most diagnoses of lymphoma do NOT require molecular testing.
- Useful in difficult cases, usually where the differential includes an atypical reactive process.
- Comparing separate lesions (both spatially and chronologically).
- Will it change your diagnosis?
  - » Will you call it lymphoma if positive?
  - » Will you NOT call it lymphoma if negative?



Slide courtesy of
Kristin Karner

Pitfalls of Clonality Testing

- Failed amplification
  - » Low quantity, poor quality DNA
    - Small or poorly-fixed specimen
- False negatives
  - » Somatic hypermutation (85% sensitivity in follicular lymphoma)
  - » Sampling wrong area
- False positives
  - » Clonal selection in non-neoplastic processes
  - » Limited sampling area
- Determining lineage (T vs. B)
  - » Beware of lineage infidelity
    - Much more common in immature neoplasms
    - Bagg A. J Mol Diagn. 2006 Sep; 8(4): 426–429.

Genomic (NGS) Testing in Lymphoma: Role Still Limited

- Lymphoma diagnosis still based on morphology and immunophenotype.
  - » Point mutations associate with lymphoma subtypes but do not define them.
- Importance of translocations, which are technically challenging to assess for by NGS.
  - » RNA-based assays.
- Likely to increase as targeted therapy becomes a reality.

CLL Mutation Panel by NGS

- 27 gene panel:
  - » *ATM, BCL2, BIRC3, BRAF, BTG1, BTK, CARD11, CD79B, CXCR4, DDX3X, FBXW7, IKZF3, KRAS, MAP2K1, MED12, MGA, MYD88, NOTCH1, NRAS, PLCG2, POT1, RPS15, SAMHD1, SF3B1, TP53, XPO1, ZMYM3*
- Useful in conjunction with traditional CLL biomarkers (e.g., *IGHV* mutation status, 17p deletion)
- Assessment for BTK resistance mutations in CLL patients
  - » High frequency in patients who progress on BTK inhibitor therapy (e.g. ibrutinib, acalabrutinib)
    - Mutations in *BTK* (e.g., C481S) that confer resistance
    - Mutations in *PLCG2, CARD11, CD79B, CXCR4, MYD88* implicated in primary or acquired resistance

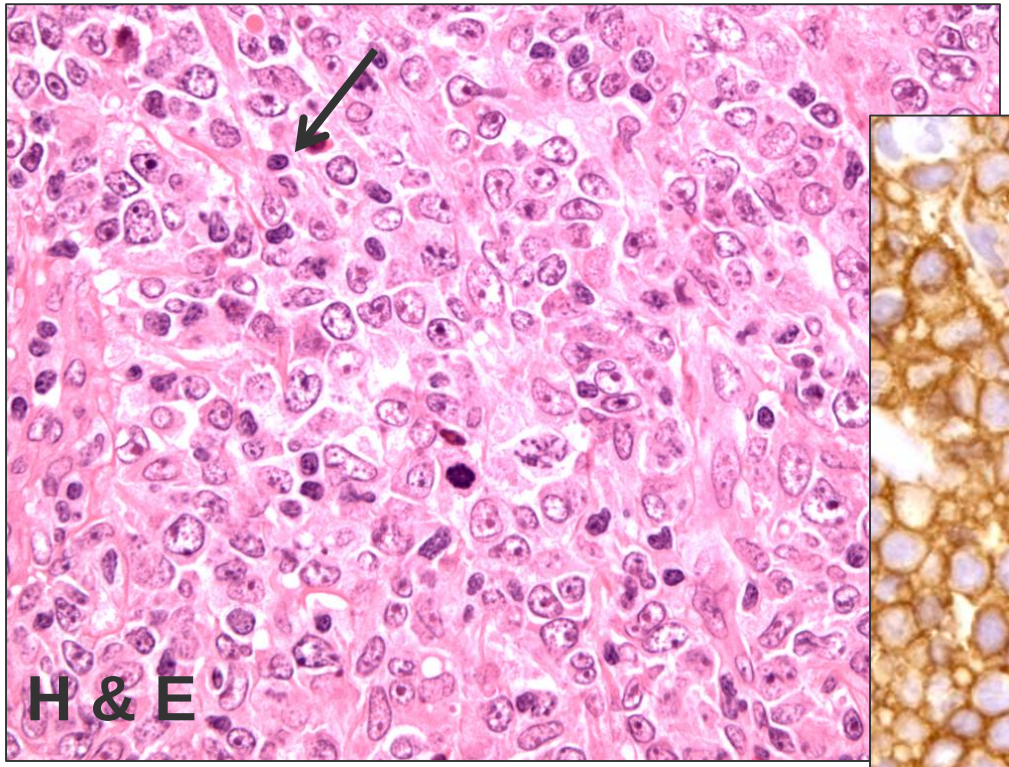
Slide courtesy of Jay Patel

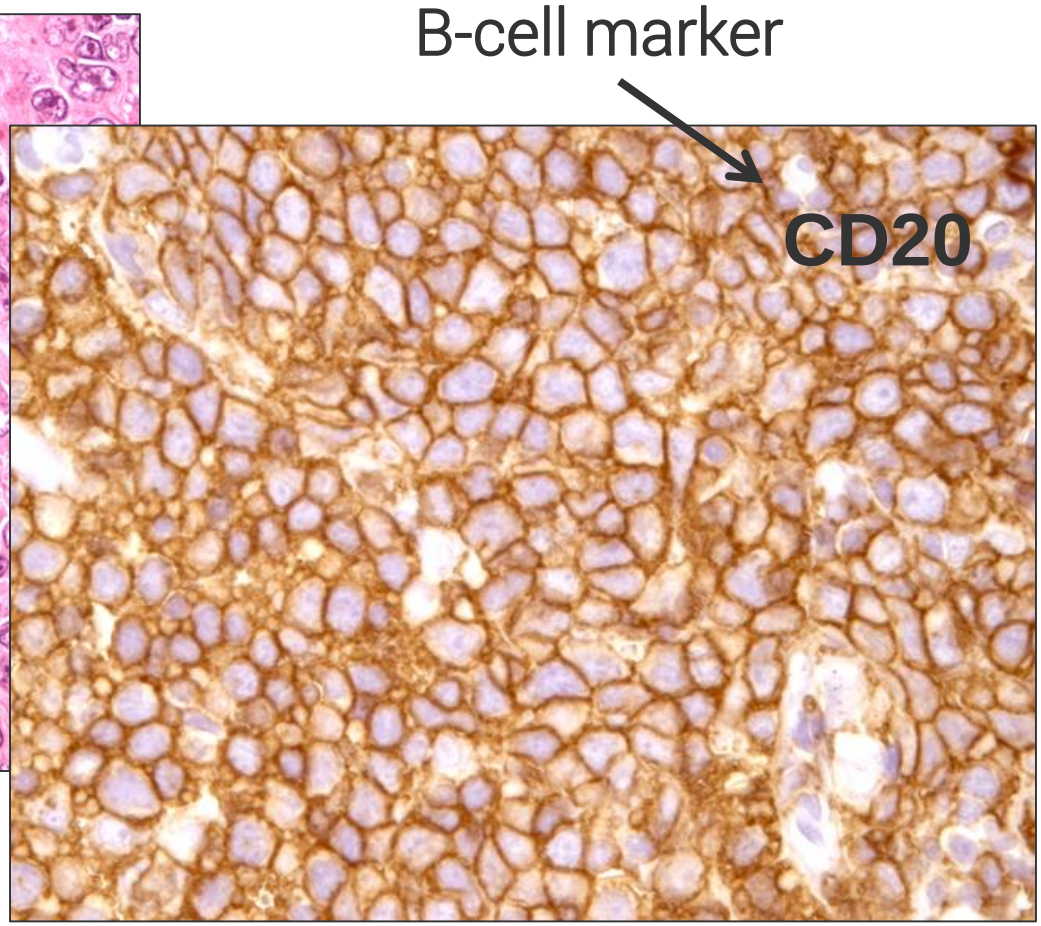
Is Ancillary Testing Required?

- Immunophenotyping considered essential for most diagnoses.
- It is not always necessary to do all testing to make a definitive diagnosis.
  - » Clinical guidelines like the NCCN advocate specific testing, often beyond what is required to diagnose.
- As more information becomes available (i.e., molecular profiling), more extensive testing may be required for some entities.

Diffuse Large B-cell Lymphoma

- Ancillary testing required for diagnosis: CD20.





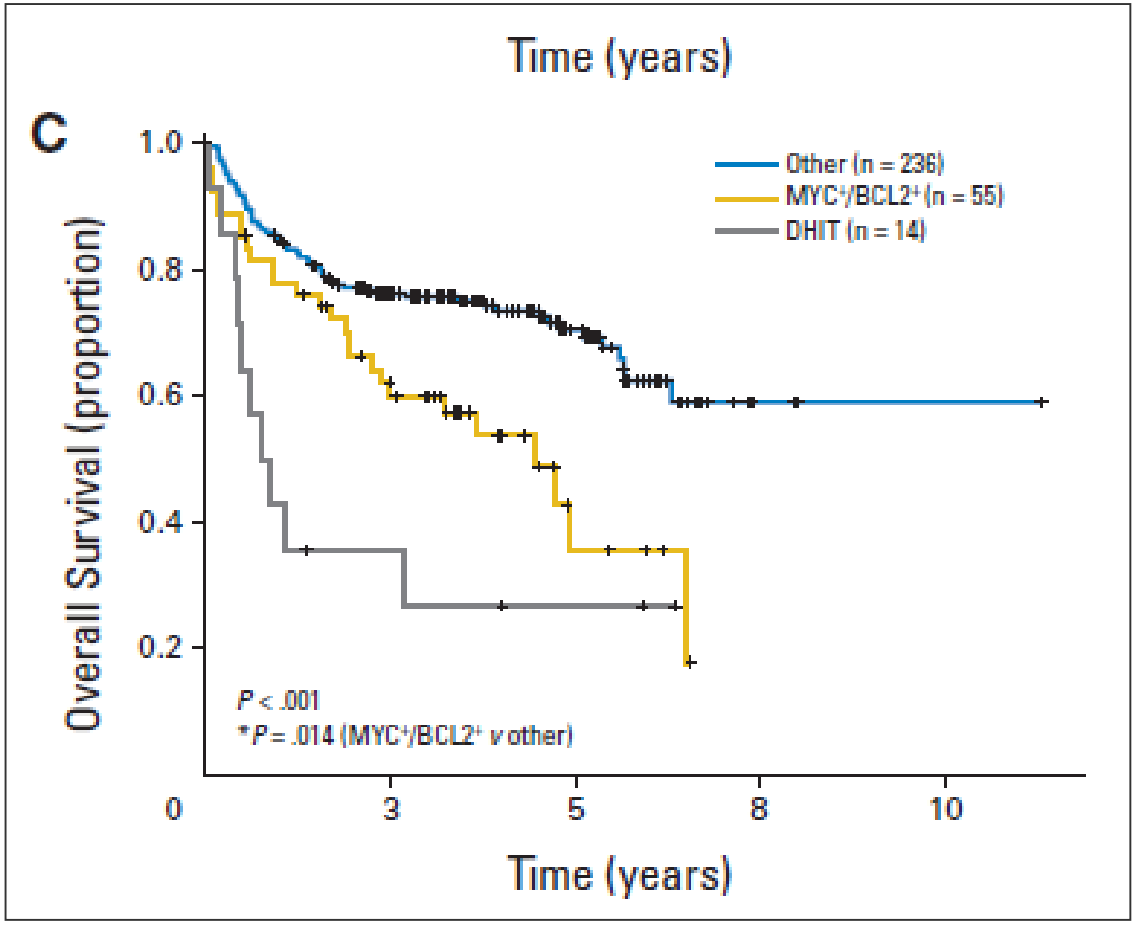
Diffuse Large B-cell Lymphoma Ancillary Testing

- Useful for sub-classification and/or prognostic information
  - » EBER
    - *EBV positive DLBCL, NOS*
  - » FISH for *MYC, BCL2, BCL6* translocations
    - *High grade B-cell lymphoma with MYC and BCL2+/-BCL6*
  - » CD10, BCL6, MUM1
    - *DLBCL NOS, germinal center vs. activated B-cell subtypes*
  - » MYC, BCL2 protein co-expression
    - Does not define an entity but has prognostic significance

MYC and *BCL2* Rearrangements and Protein Expression: Inform Prognosis and Guide Therapy

- Diffuse large B-cell lymphoma, NOS
- Double-expresser (DE) DLBCL, NOS
  - » Expresses MYC (>40%) and BCL2 (>50%) protein
  - » 30% of DLBCL
- High grade B-cell lymphoma double hit (HGBL-DH), 4-6% of DLBCL.
  - » *MYC/BCL2*, 80% (includes 20% triple hit).
  - » *MYC/BCL6*, 20%.

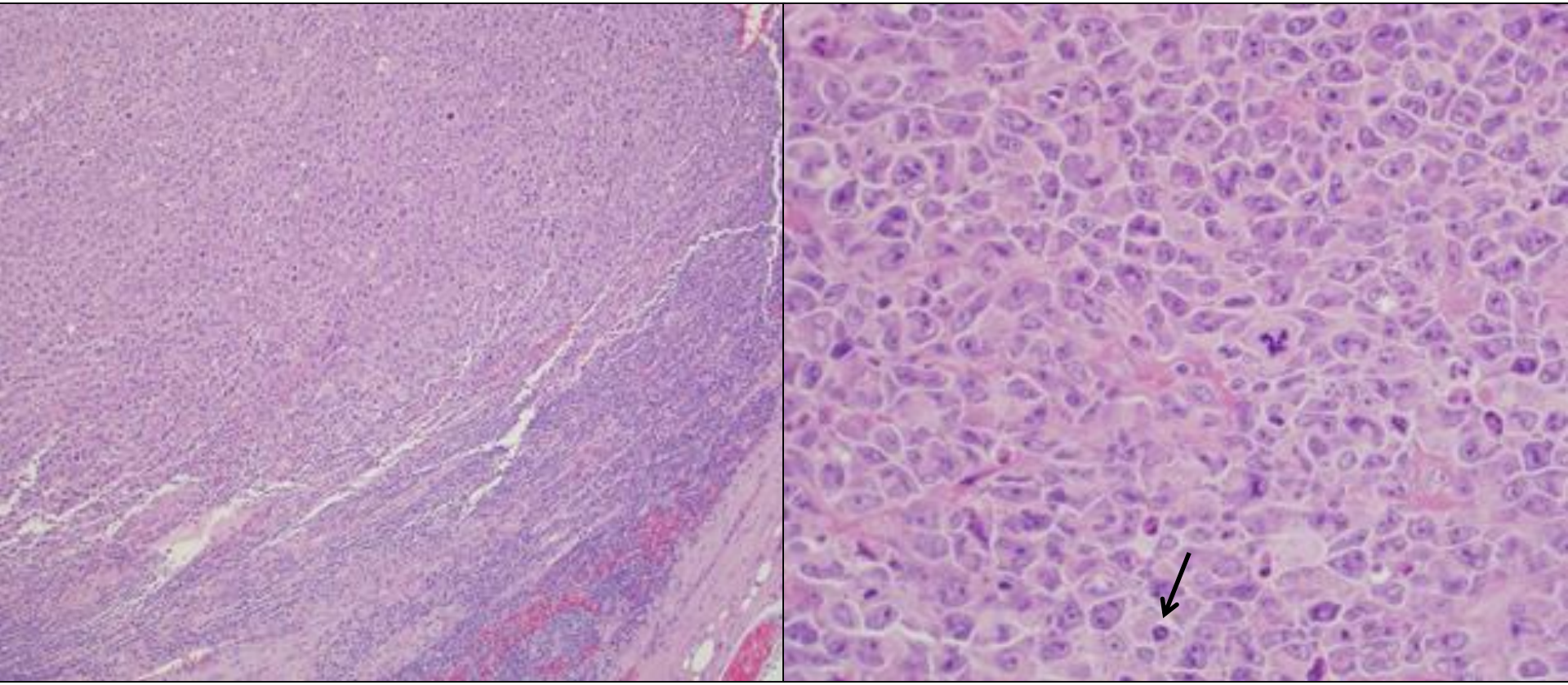
MYC and *BCL2* Double Hit or Double Expresser: Prognosis

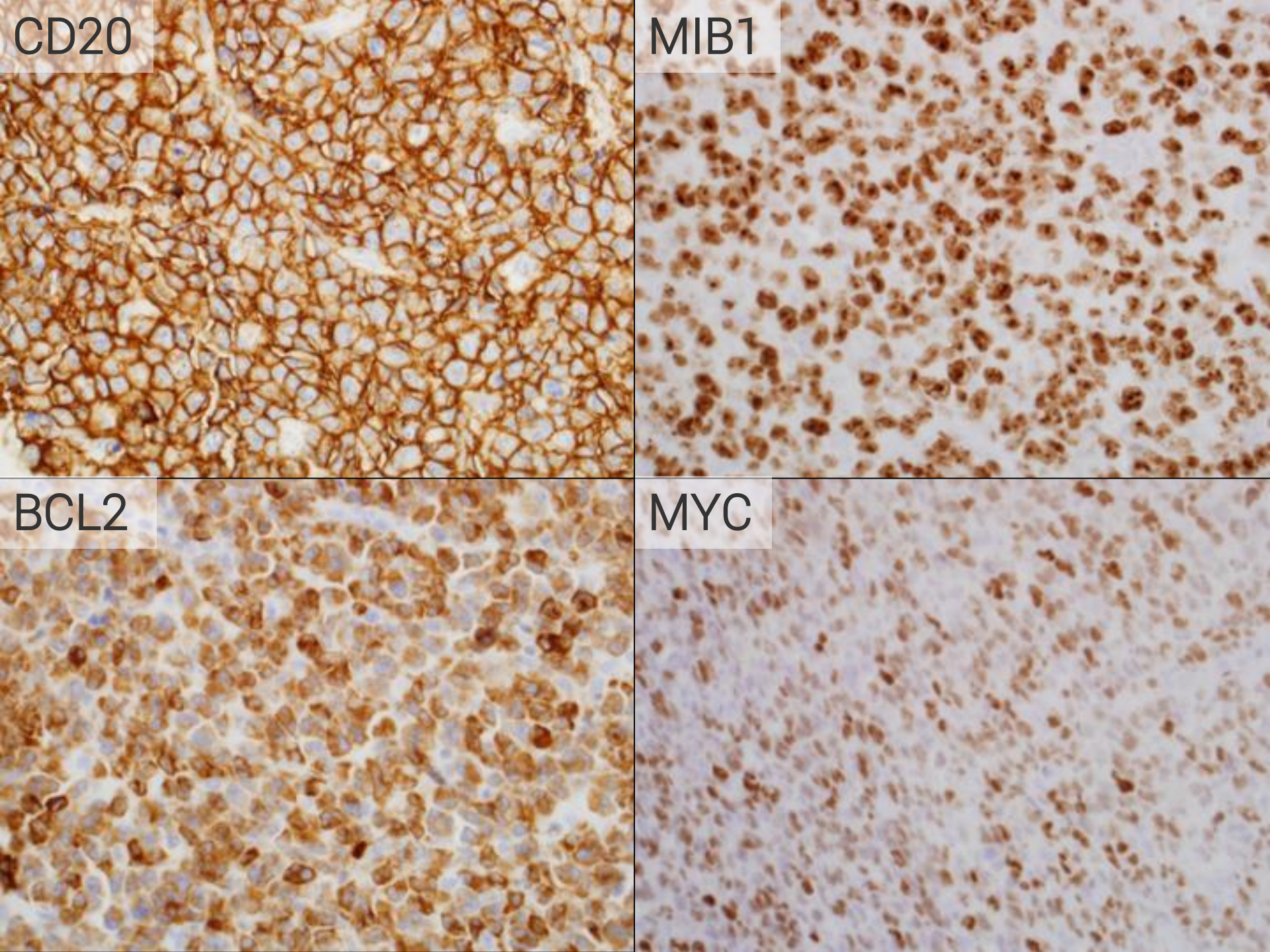


J Clin Oncol, 2012 30(28):3452-9.

79-year-old woman with recent breast cancer diagnosis and enlarged lymph nodes.

- Right neck mass.




CD20
MIB1
BCL2
MYC

Results of FISH and Final Classification

- *MYC* breakapart probe assay negative.
  - » *MYC* rearrangement excluded.
  - » *MYC/BCL2 or BCL6* double hit lymphoma excluded.
- Classification:
  - » Diffuse large B-cell lymphoma, activated B-cell subtype, with co-expression of MYC and BCL2.
- Prognosis: bad, but not as bad as *MYC/BCL2* double hit.
- Consider DA-EPOCH-R rather than R-CHOP.

DLBCL Ancillary Testing

- Diagnosis of DLBCL requires only morphology and immunophenotype.
- Ancillary testing can inform prognosis and identify recognized subtypes.
- Diagnosing or excluding the WHO 2017 category HGBL, with *MYC*+*BCL2* +/- *BCL6* rearrangement requires FISH.
  - » A genetically-defined lymphoma.
- Testing should be performed when results will affect patient care.

T-cell Lymphomas

- Diagnosis of lymphoma heavily dependent on morphology.
- Immunophenotyping can show aberrancies.
- Molecular or flow cytometry studies can show clonality.
- Neither aberrancies nor clonality is diagnostic of malignancy!

Immunophenotyping in T-cell Lymphomas

- Normal T-cells express CD2, CD3 (surface), CD5, CD7, and CD4 or CD8.
- Aberrancies:
  - » Deletion/absence
  - » Weaker or stronger than normal
- Relatively soft findings as reactive T-cells can show such aberrancies.
  - » Decreased CD7 is common.
- Multiple aberrancies increases suspicion.
- Loss of surface CD3 is highly suspicious.

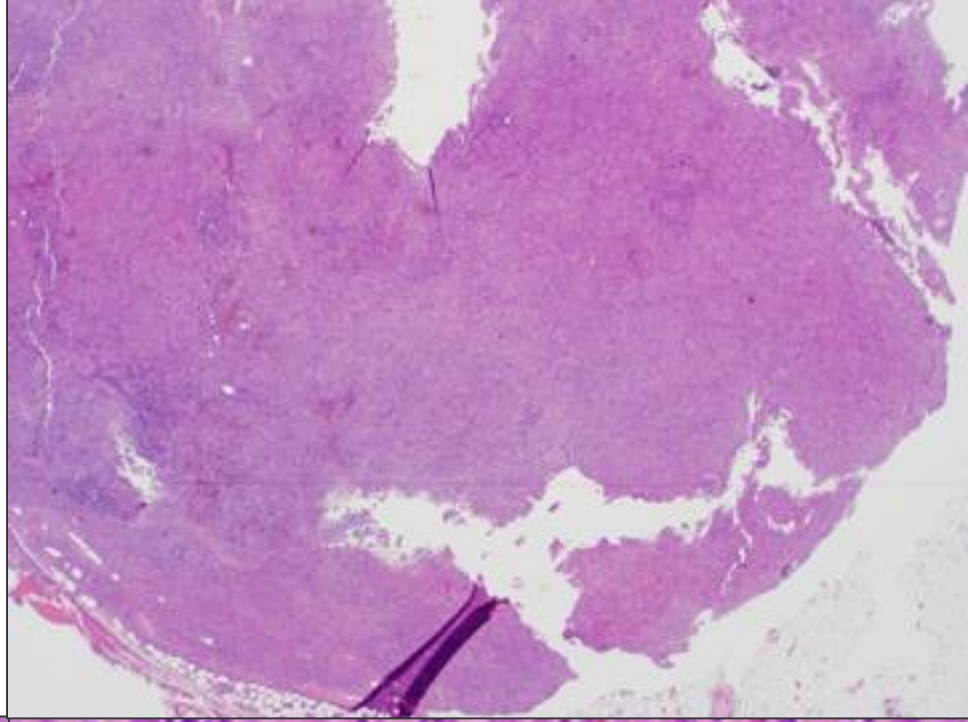
T-cell Clonality

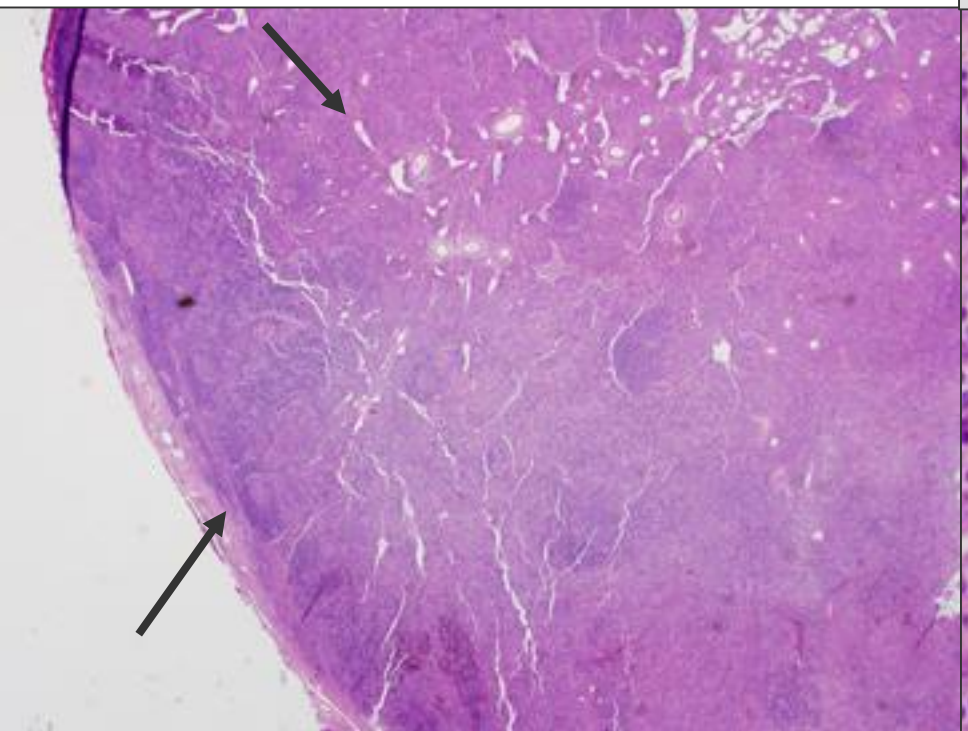
- Molecular/PCR
  - » Detects clonal TCR rearrangements.
  - » Fresh or fixed tissue.
- Flow cytometry: V-beta
  - » Identifies restricted use of TCR V-beta chains.
  - » Allows assessment of an aberrant population.
  - » Requires fresh tissue/blood/marrow.
- Both can be positive in reactive T-cell populations!

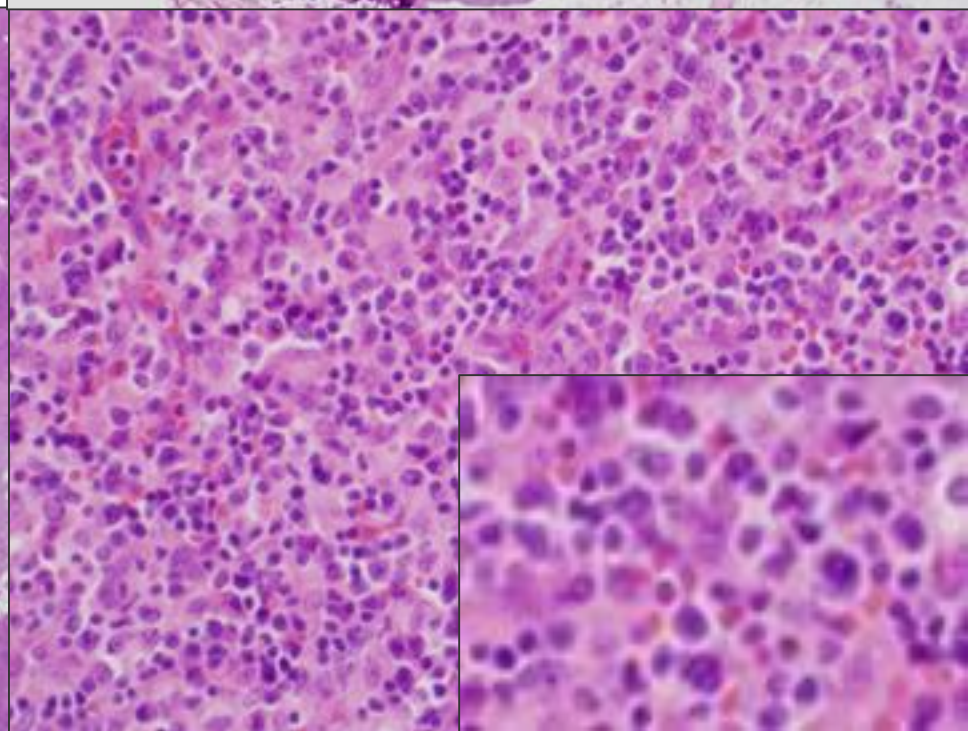
Be Aware of Non-Neoplastic Clonal T-cells

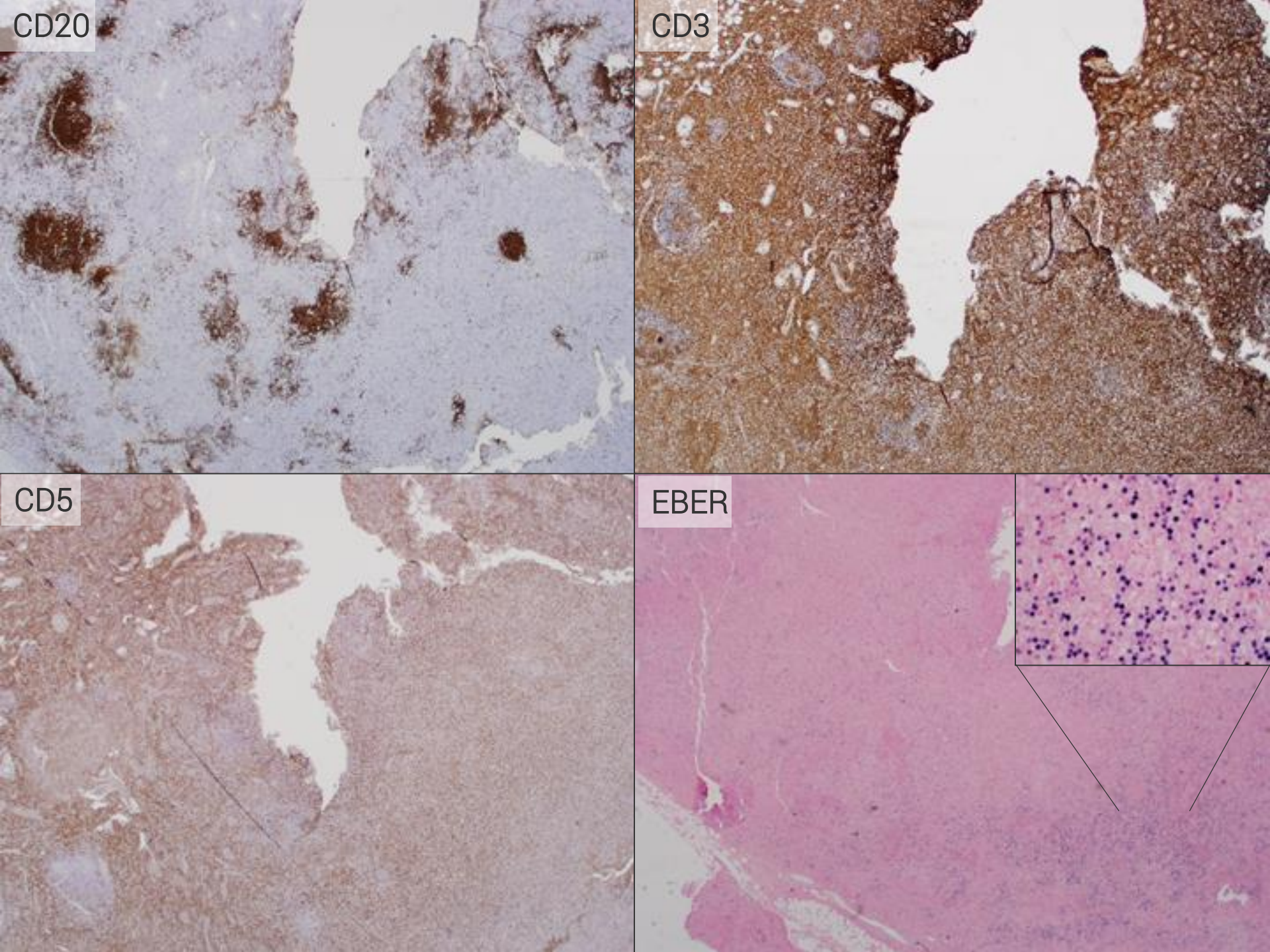
- There are MANY examples of clonal T-cell proliferations that are NOT neoplastic
  - Common in skin, peripheral blood
  - Post transplant
  - Various immune responses
    - Inflammatory (RA, Crohn's etc.)
    - Malignancy (CLL/SLL, etc.)
- T-cell repertoire decreases with age so clones more likely.

- 55-year-old man with a reported history of left groin lymphadenopathy,
- Fatigue, night sweats, fever, elevated LDH, mildly elevated AST and ALT.
- Mild lymphocytosis.








CD20
CD3
CD5
EBER

Difficult case!

- Diagnosis: EBV-positive T-cell lymphoproliferative disorder (not really a thing).
- What troubled me:
  - » Partial effacement, negative flow study, EBV positivity.
- Sent for clonality…came back positive.
  - » Remained stubborn.
- Sent to NIH, Dx: Peripheral T-cell lymphoma, NOS with associated EBV-positive B-cell proliferation.
  - » Remained stubborn.

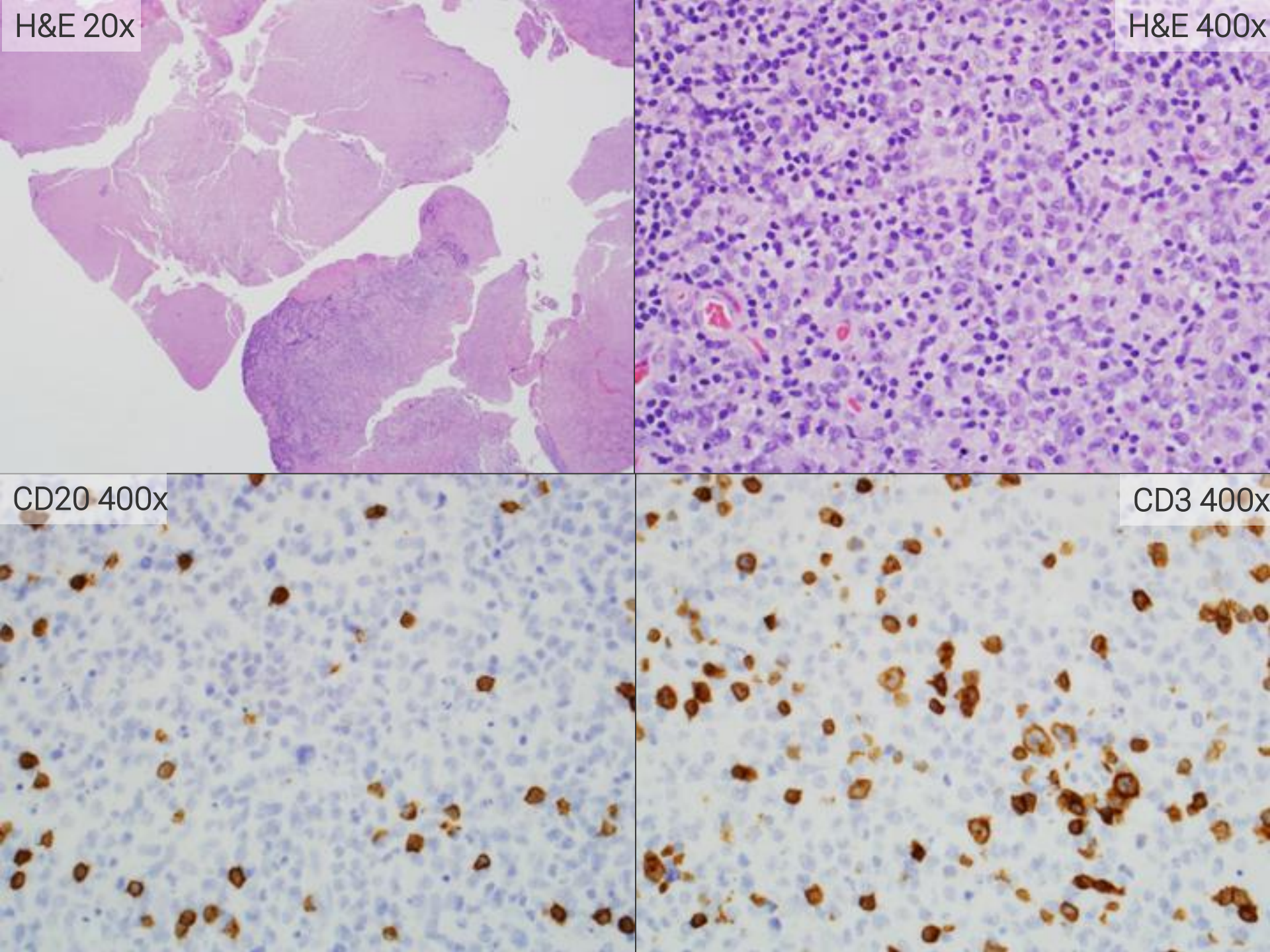
Clinical follow up 1 month later

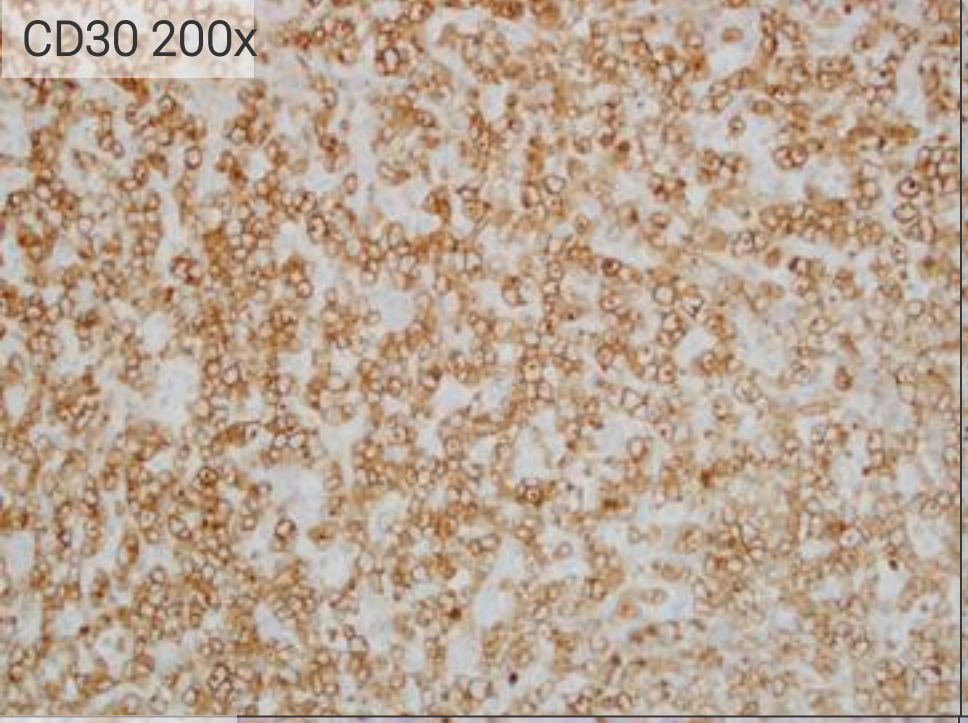
- Patient now totally asymptomatic.
- No abnormal lymphadenopathy.
- EBV titers at biopsy showed high IgM without IgG.
- Now convalescent titers with high IgG as well as IgM.
- Clinically fits best with a primary EBV infection, although unusual at this age.

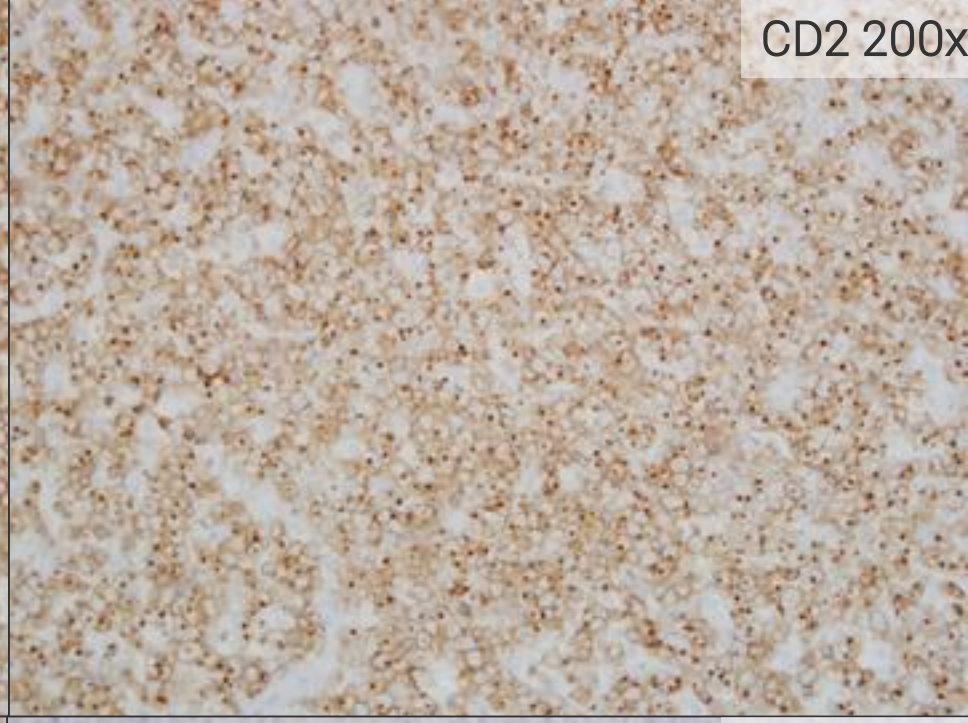
Considerations for T-cell Lymphoma Diagnosis

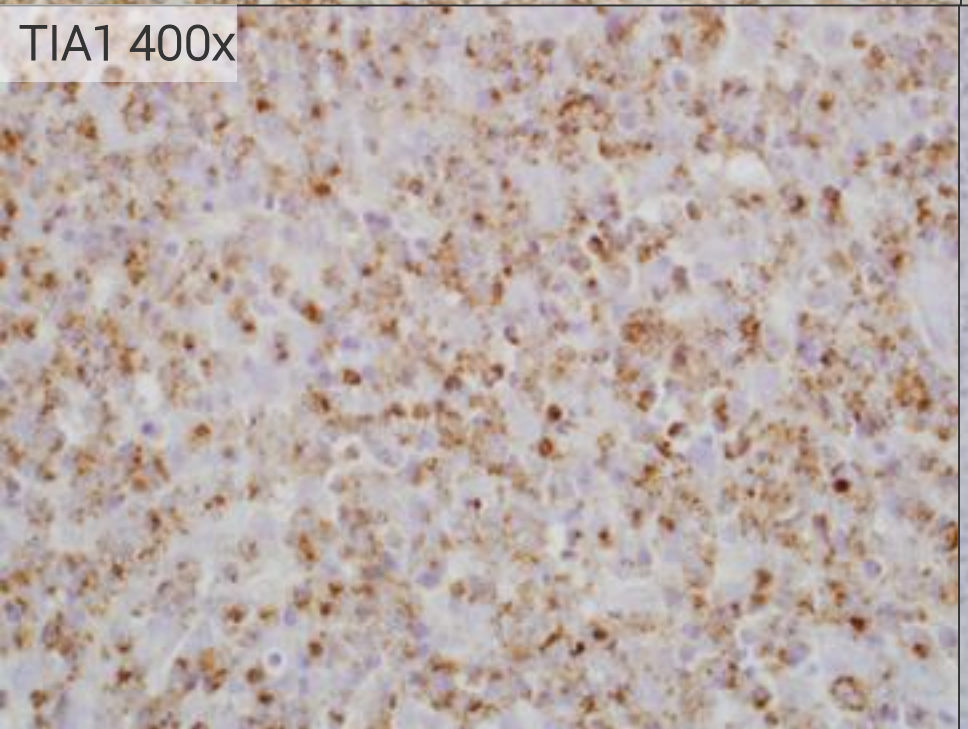
- Morphology: is there total effacement by monomorphic atypical cells, or are the atypical cells variable in size and mixed in?
- Ancillary Testing;
  - » Definitive phenotypic aberrancies by flow and immunohistochemistry?
  - » Strongly consider T-cell clonality.

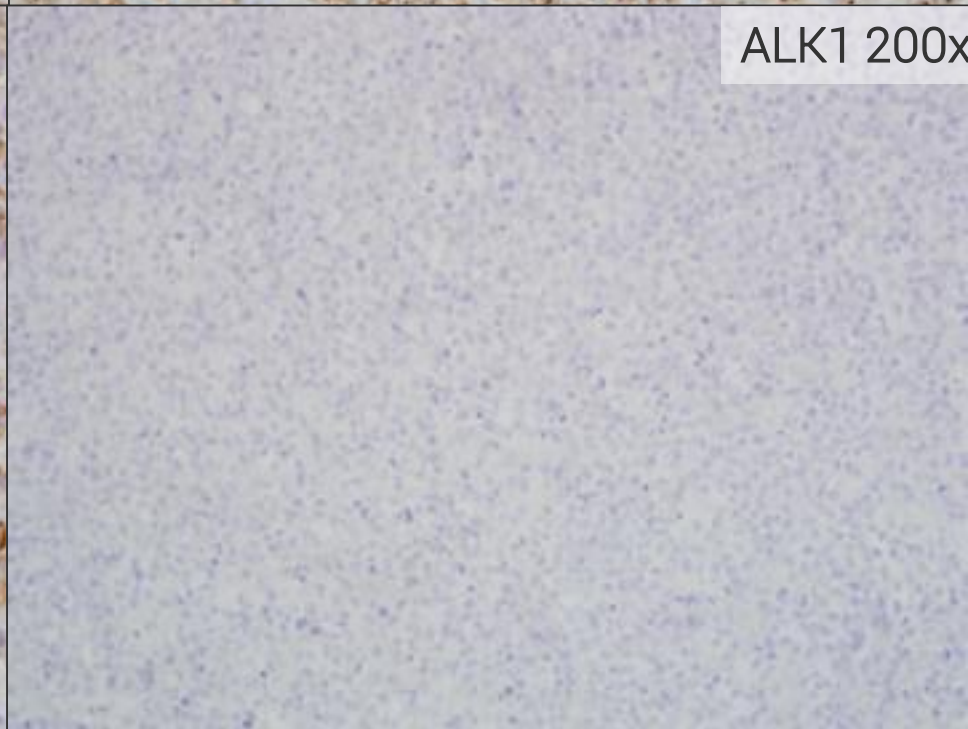
Case: 9-year-old boy with a mediastinal mass


H&E 20x
H&E 400x
CD20 400x
CD3 400x


CD30 200x


CD2 200x


TIA1 400x


ALK1 200x

Immunophenotype and Diagnosis

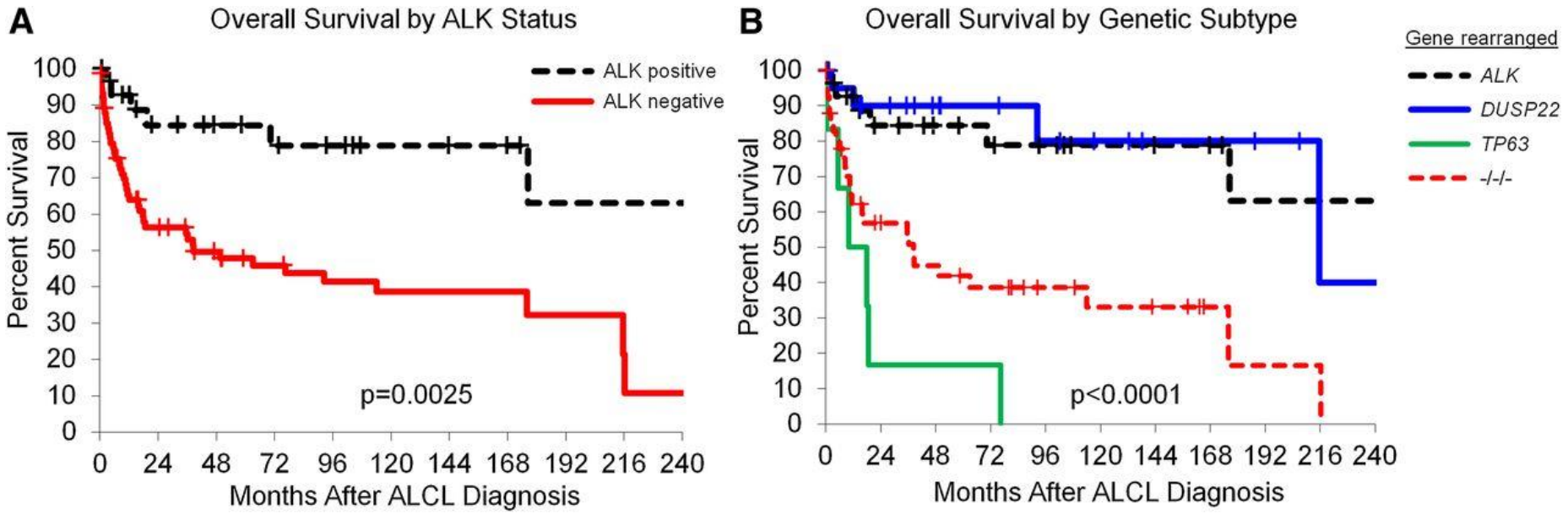
- Positive for CD2, CD30 (strong and diffuse), TIA1, CD45, CD4 (weak), CD7 (subset).
- Negative: CD20, PAX5, CD15, CD8, CD5.

- Diagnosis: Anaplastic Large Cell Lymphoma (ALCL), ALK1 negative.
- Other considerations:
  - » Hodgkin: morphology; CD15-, PAX5-, CD3+
  - » PTCL, NOS: CD30 too strong and diffuse.

Additional Studies

- FISH negative for rearrangement of *DUSP22/IRF4*.
- Immunohistochemical stain for p63 is negative in tumor cells.

IRF4/DUSP22 Rearranged ALK- ALCL Shows Outcomes Similar to ALK+ ALCL



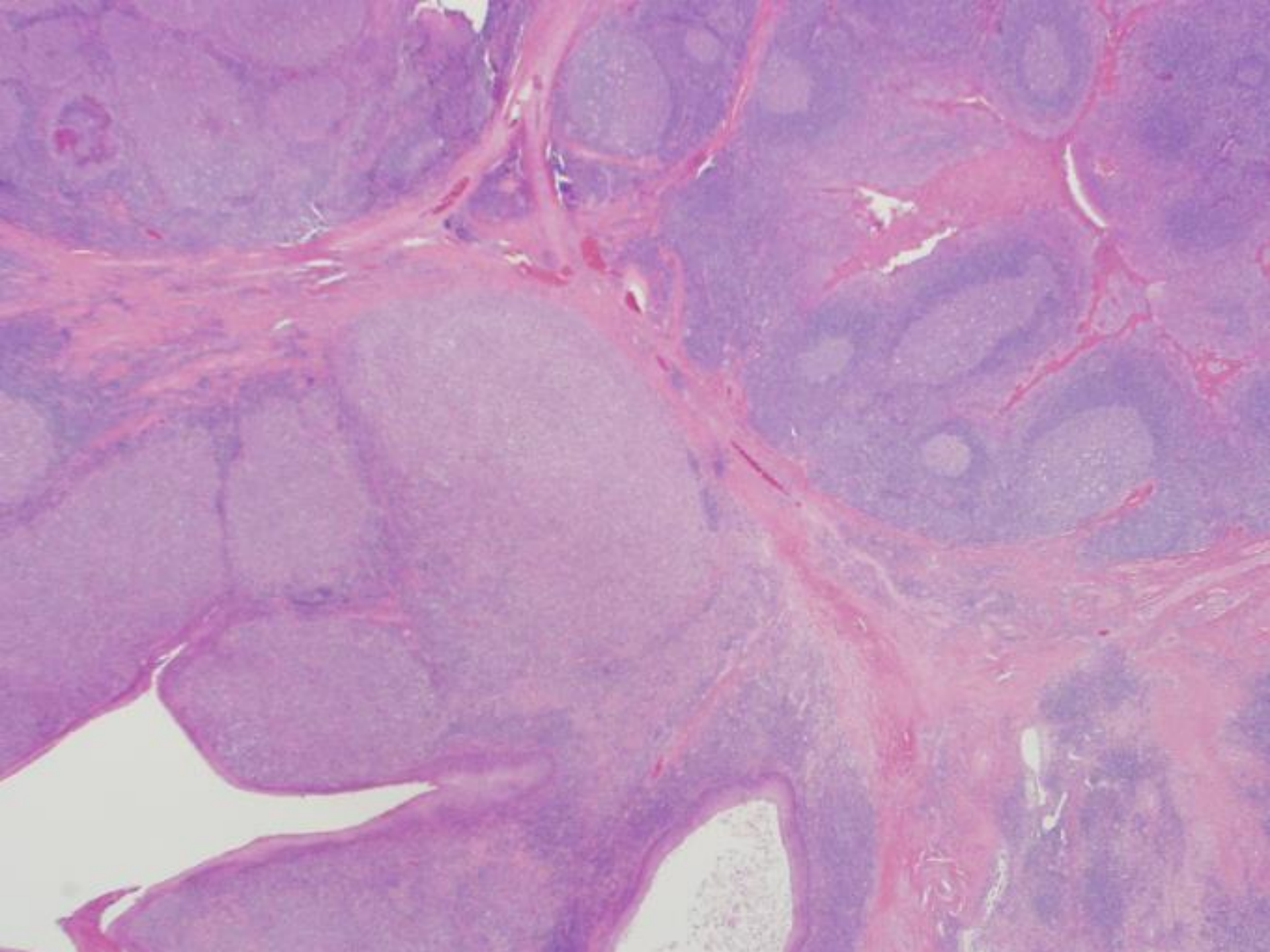
Edgardo R. Parrilla Castellar et al. Blood 2014;124:1473-1480

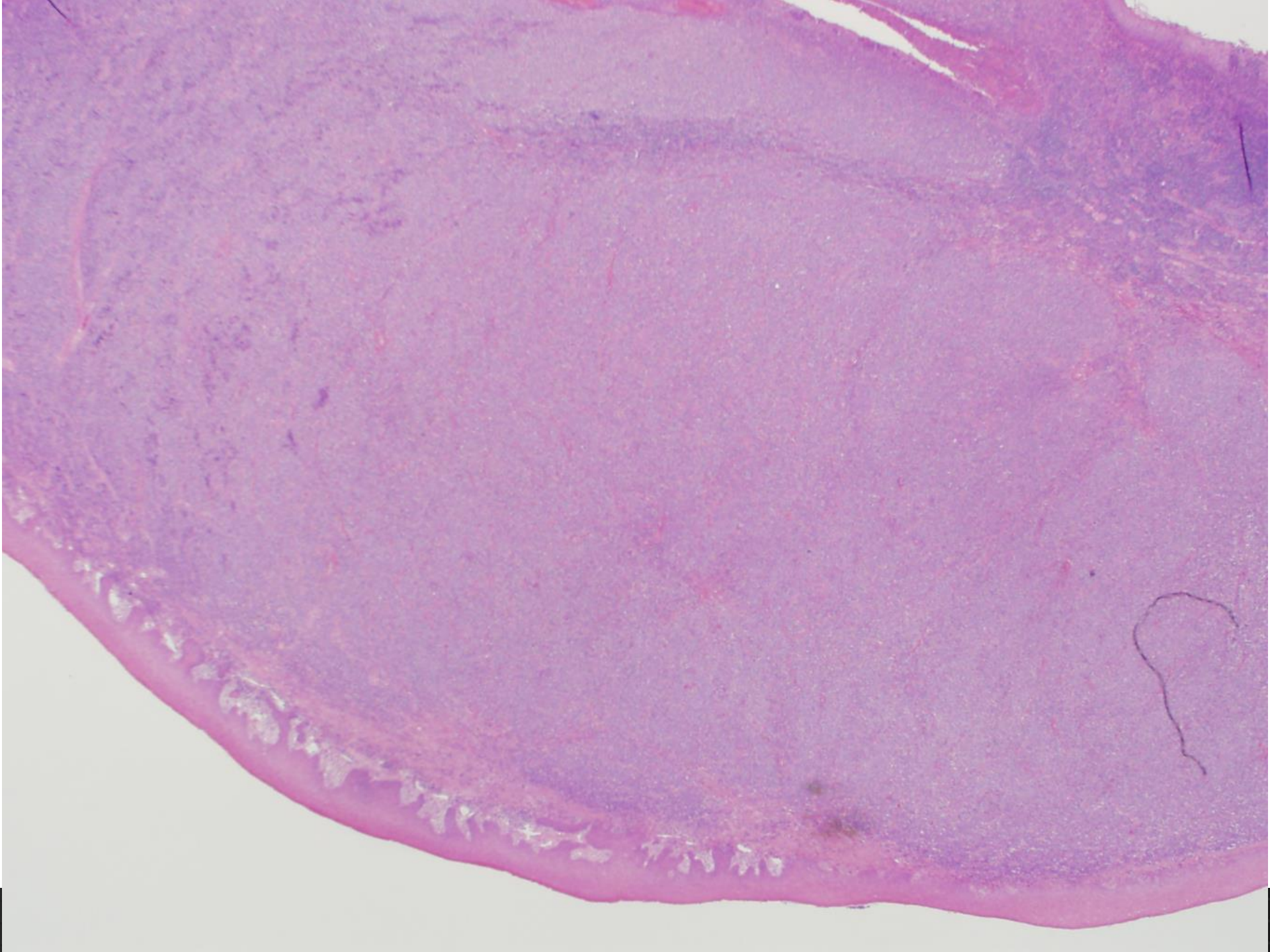
Summary: T-cell Lymphomas

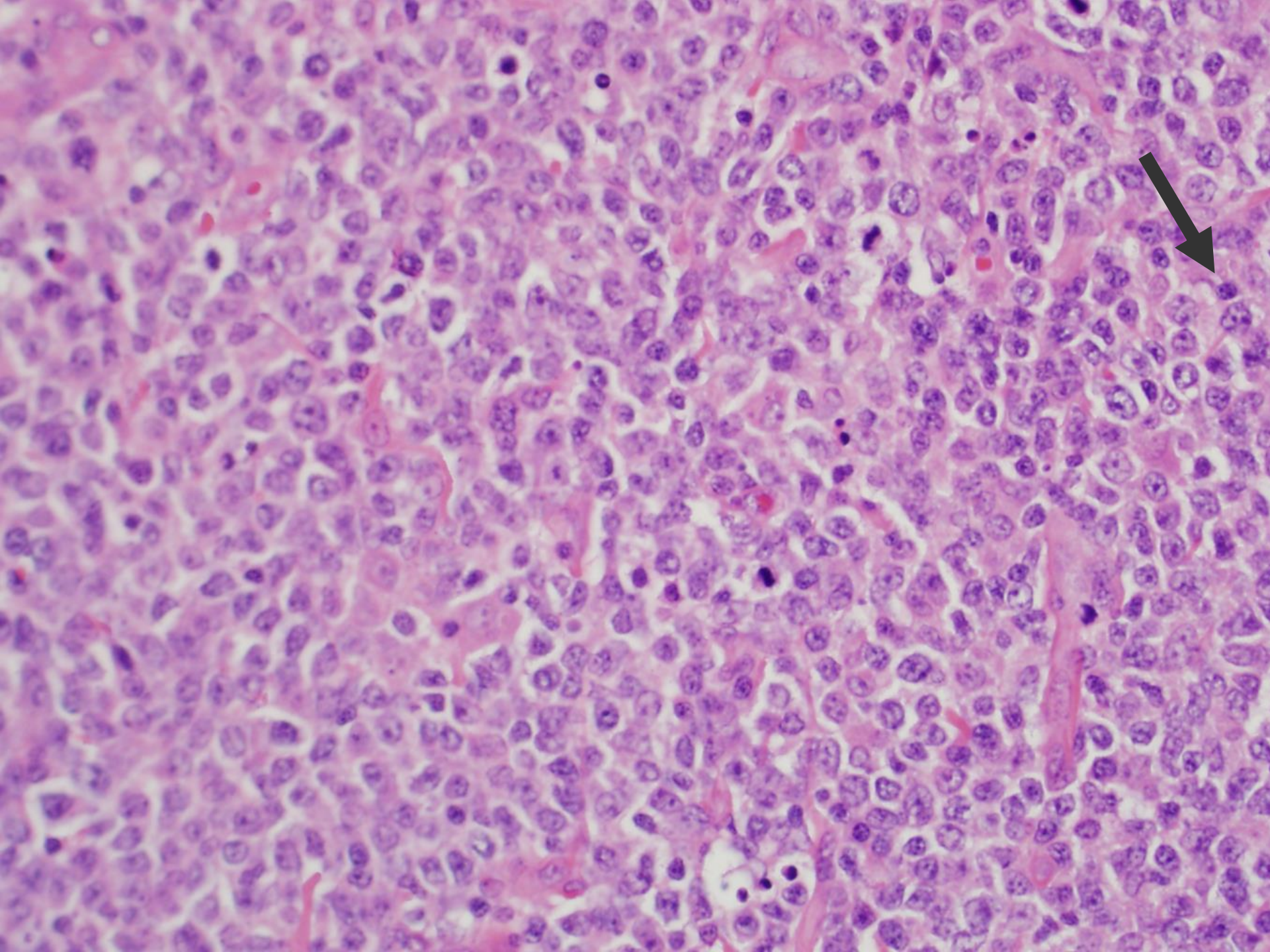
- Diagnosis should be based on morphology.
  - » Lymph node effaced by atypical cells.
- Immunophenotyping and clonality offer welcome support but beware of “false” positives.
- ALK negative ALCL should get additional prognostic testing.
  - » IRF4/DUSP22 FISH
    - Good prognosis if positive
  - » TP63 FISH or p63 IHC surrogate
    - Bad prognosis

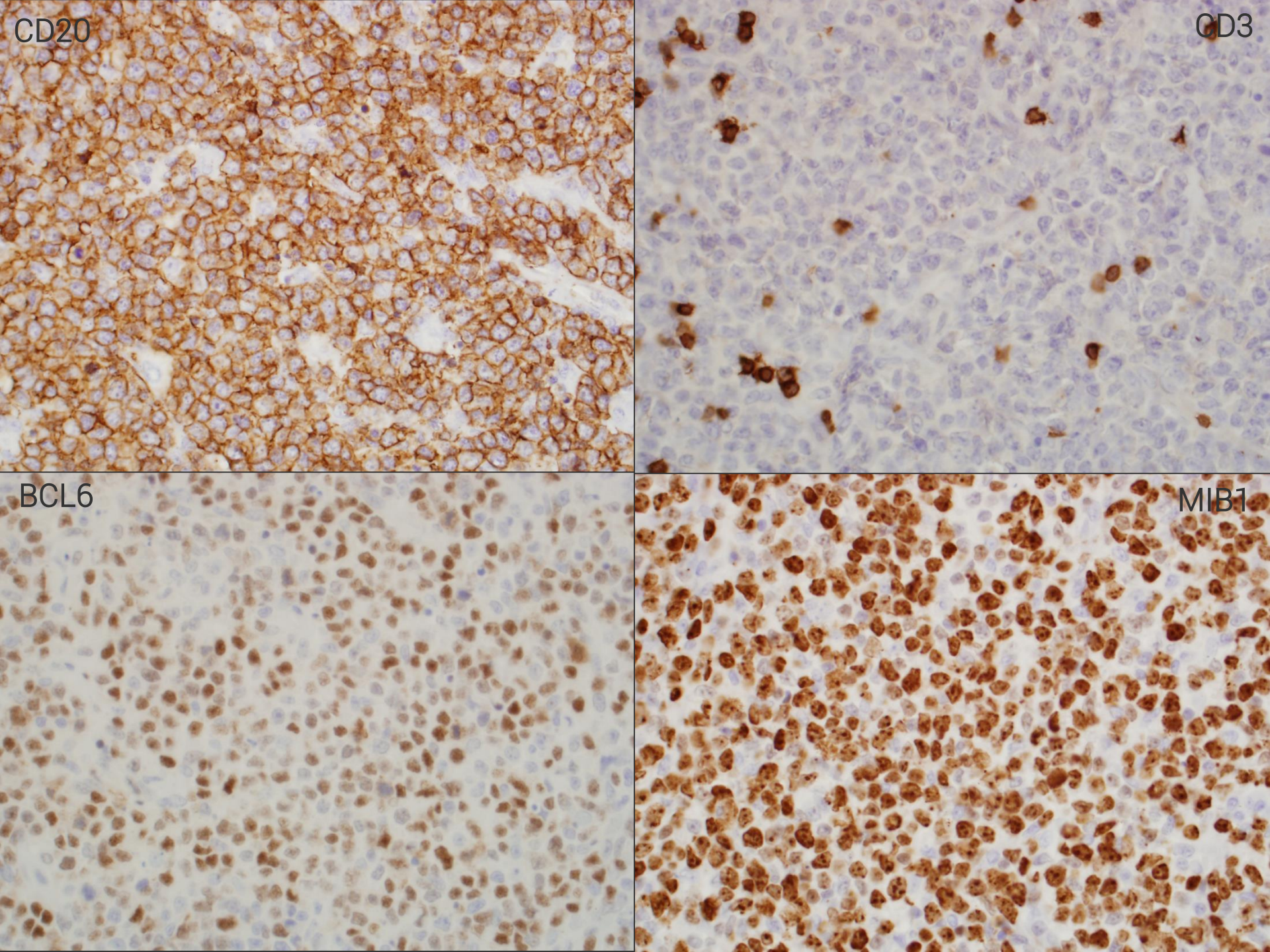
17-year-old girl with enlarged tonsils

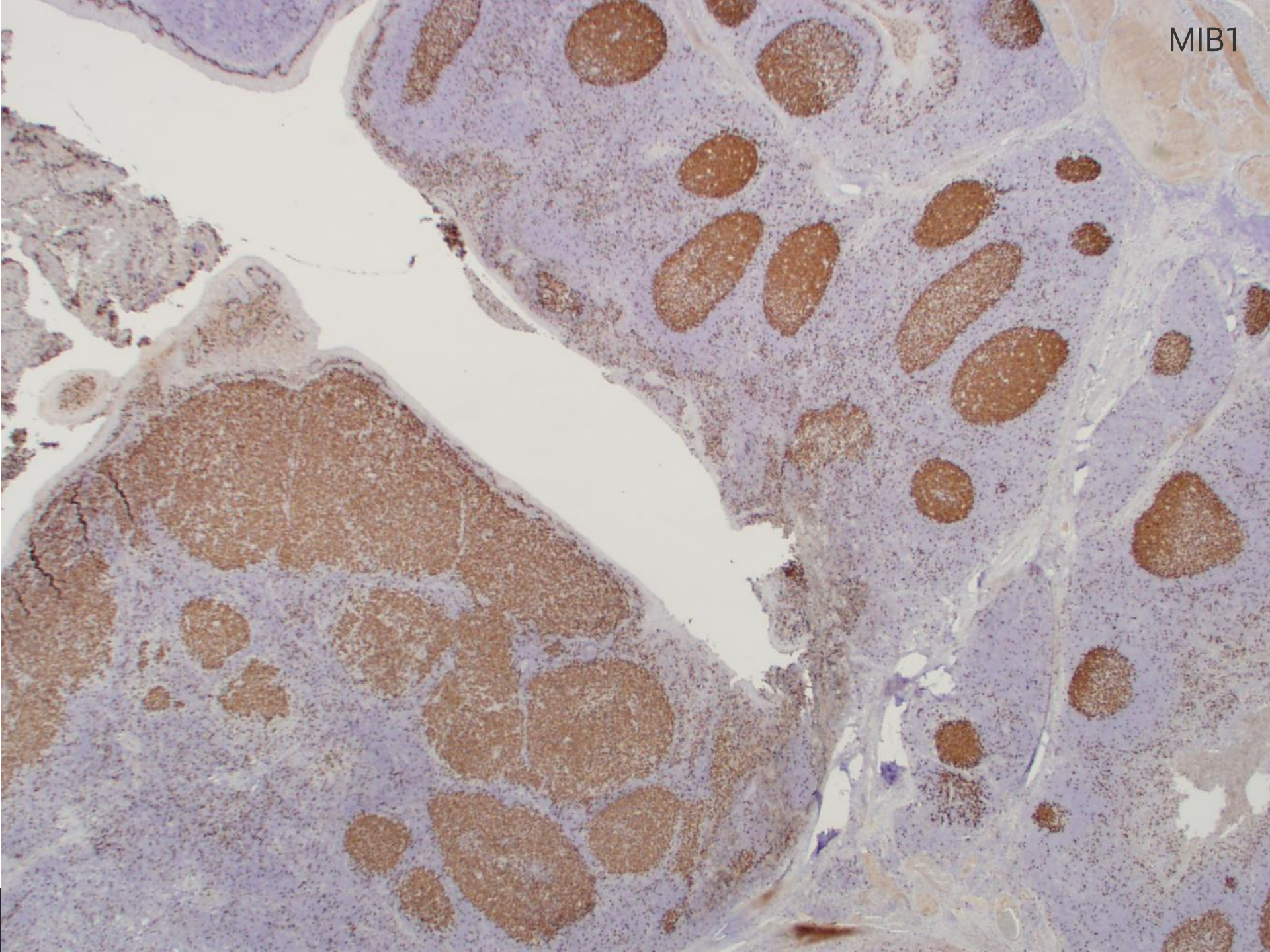
- Original pathology report:
  - » R tonsil: malignant lymphoma, favor high grade.
  - » L tonsil: follicular hyperplasia.
  - » “The overall features favor a high-grade lymphoma.”
- Implications: High-grade implies Burkitt lymphoma in this age group, could include double-hit lymphomas in older adults.
- Intensive chemotherapy regimen required.

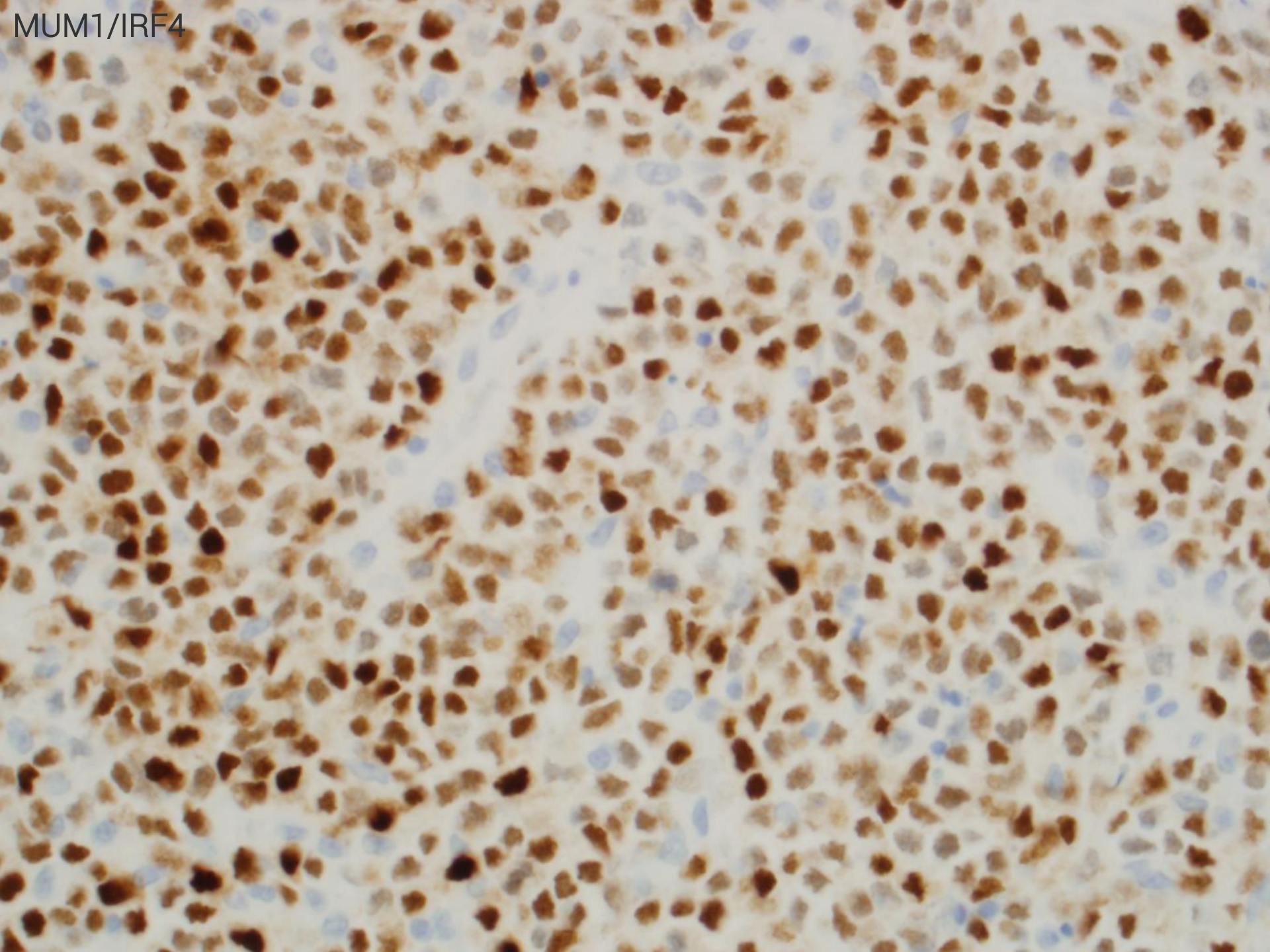







CD20
CD3
BCL6
MIB1


MIB1


MUM1/IRF4

Differential Diagnosis

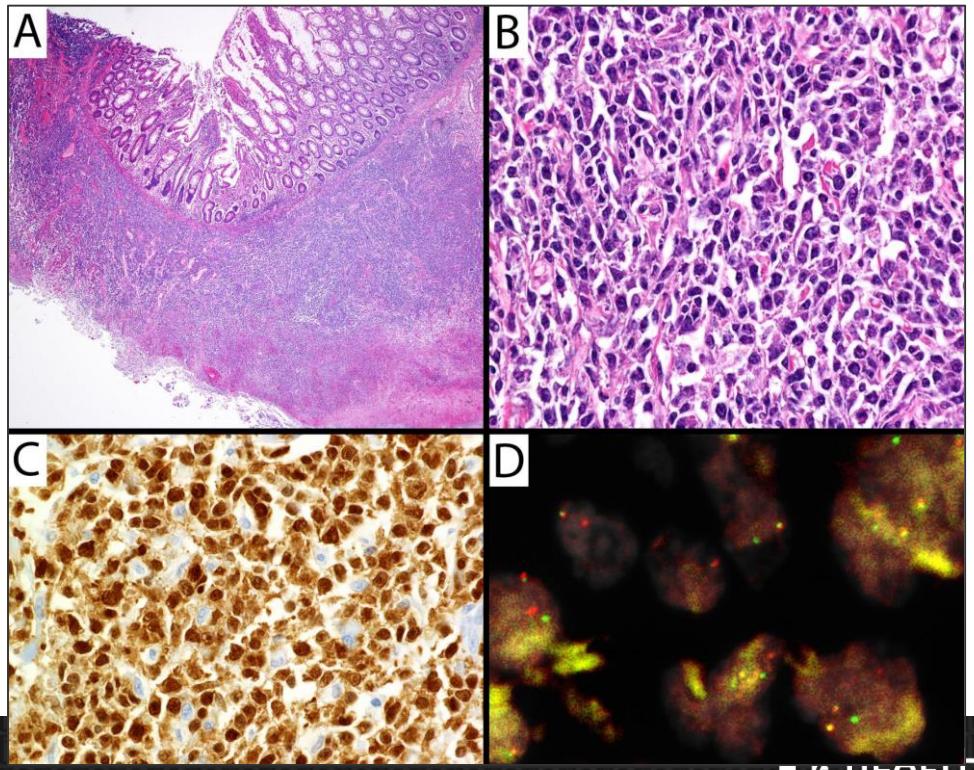
- Burkitt lymphoma: Excluded by morphology
  - » Lacks tingible body macrophages, too pleomorphic.
- Follicular lymphoma, grade 3.
- Diffuse large B-cell lymphoma.
- Large B-cell lymphoma with *IRF4* rearrangement.
  - » New entity in WHO 2017.
- FISH results:
  - » FISH for *MYC*, *BCL6,* and *BCL2* negative.
  - » FISH for *IRF4/DUSP22* positive.

Large B-cell lymphoma with *IRF4* rearrangement.

- Localized in head and neck.
- Median age 12 (range 4-79).
- Morphologically fit into DLBCL, follicular lymphoma grade 3, or pediatric type follicular lymphoma.
- Positive for BCL6 and IRF4/MUM1.
- Good outcome after chemotherapy.
  - » Less intensive therapy than Burkitt lymphoma.
- In the appropriate clinical context, FISH for *IRF4/DUSP22* should be performed.

Overall uncommon (<1%), but more common in younger patients

- We studied 32 patients from Children's Oncology Group protocols.
- FISH for *IRF4/DUSP22* positive in 2/32 cases (6%).
  - » One in tonsils.
  - » One in ileum.



Chisholm KM, *et al.* Pediatr Blood Cancer 2019.

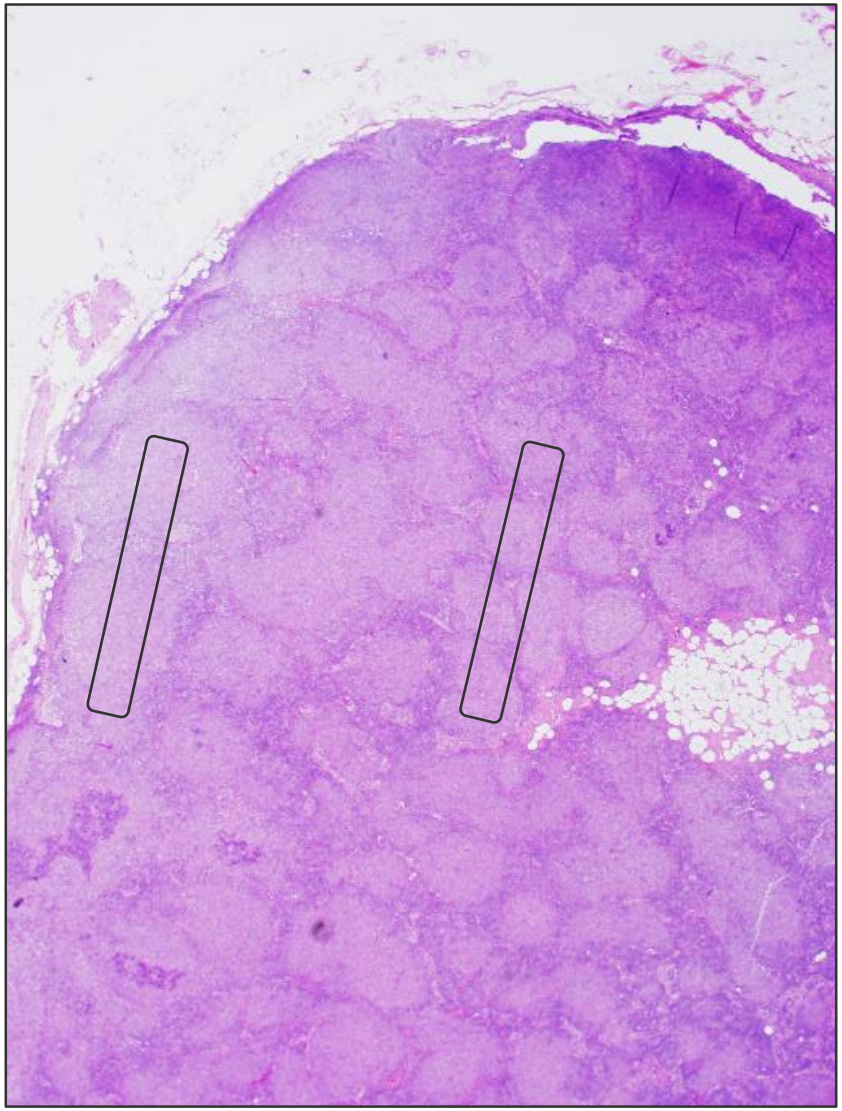
Summary

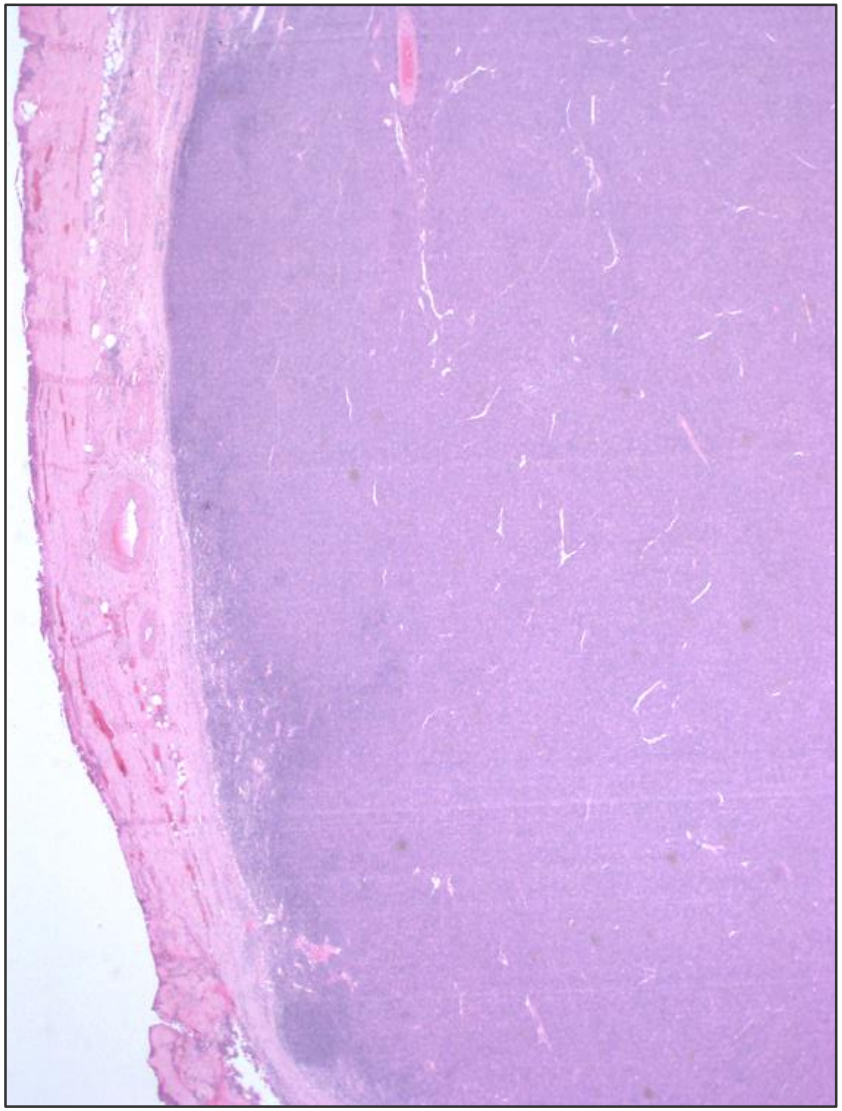
- Ancillary testing contributes greatly to lymph node diagnosis.
  - » Flow/IHC: in almost every case.
  - » FISH: specific applications.
  - » Clonality: important in some cases but be aware of false positives and negatives.
  - » Genomics: largely still in the future.
- Important to keep the limitations in mind.
  - » Stick to your morphologic impression!

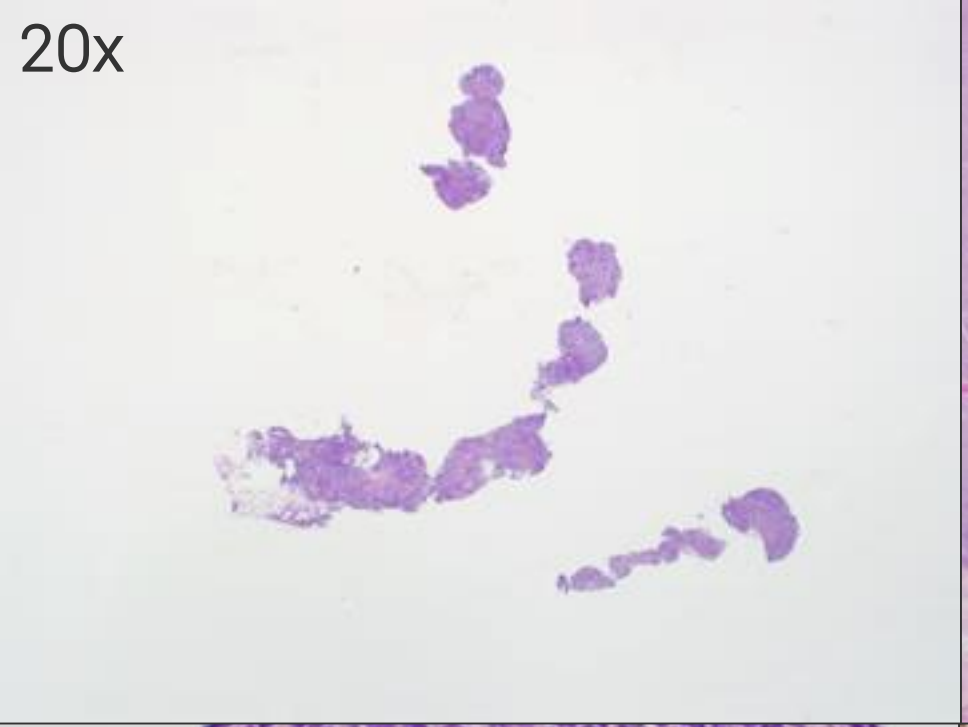
Small Needle Core Biopsies

- Small biopsies and cytology lose many morphologic clues.
- Architecture critical to our classification system.
  - » Aspirations only: difficult to say much.
  - » In lymphoma diagnosis, FNA means “For No Answer.”
- Needle cores at least offer a glimpse of architecture, but the tiny snapshot can be misleading.

Follicular vs. Diffuse



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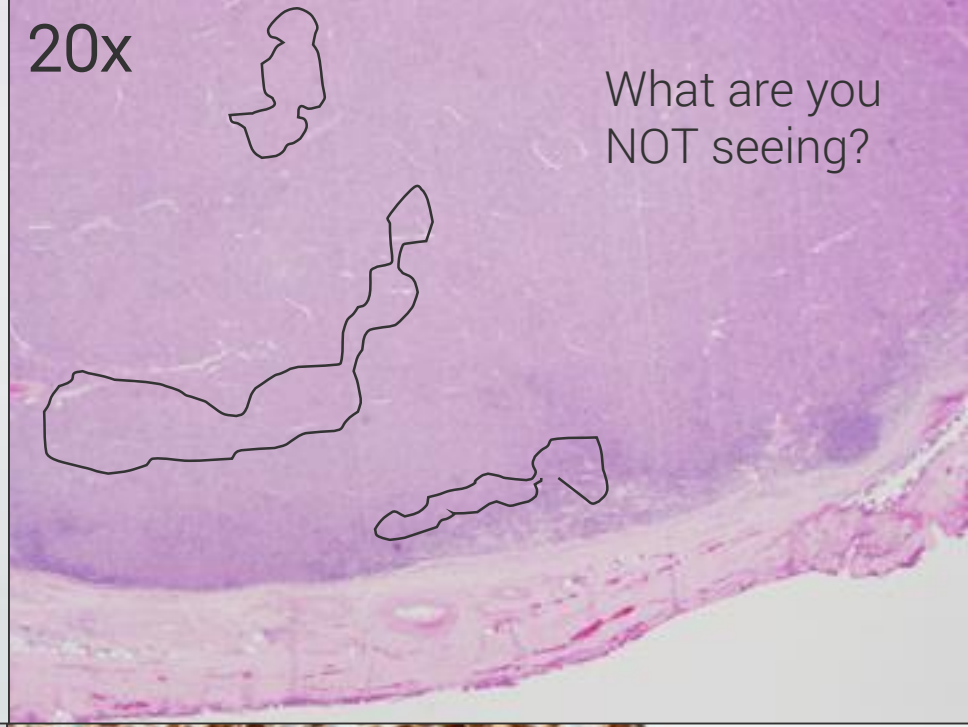
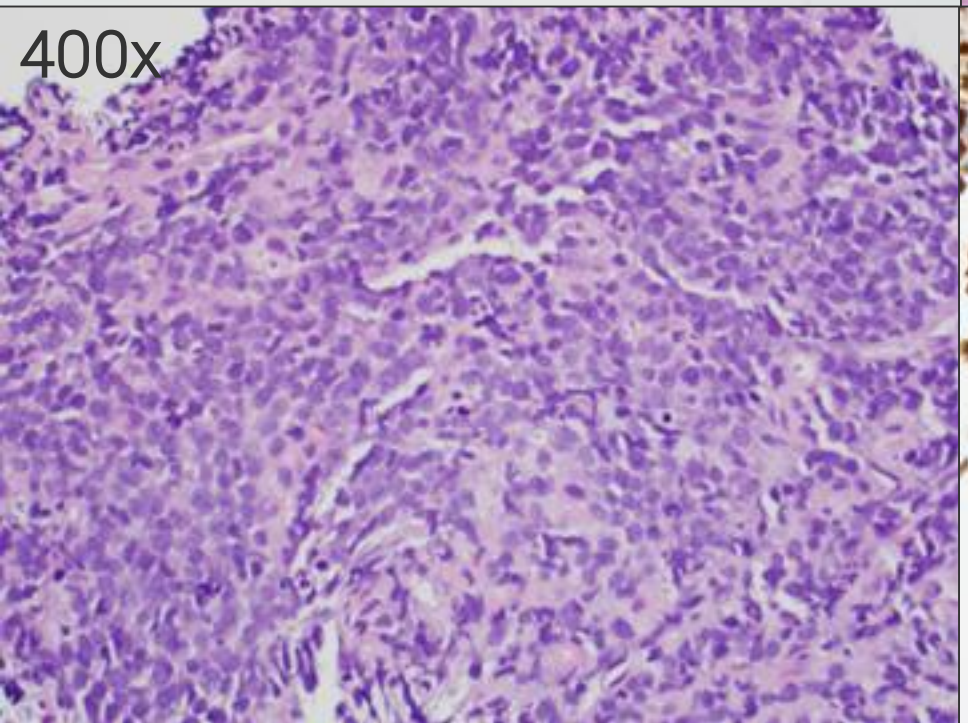

20x
What are you
NOT seeing?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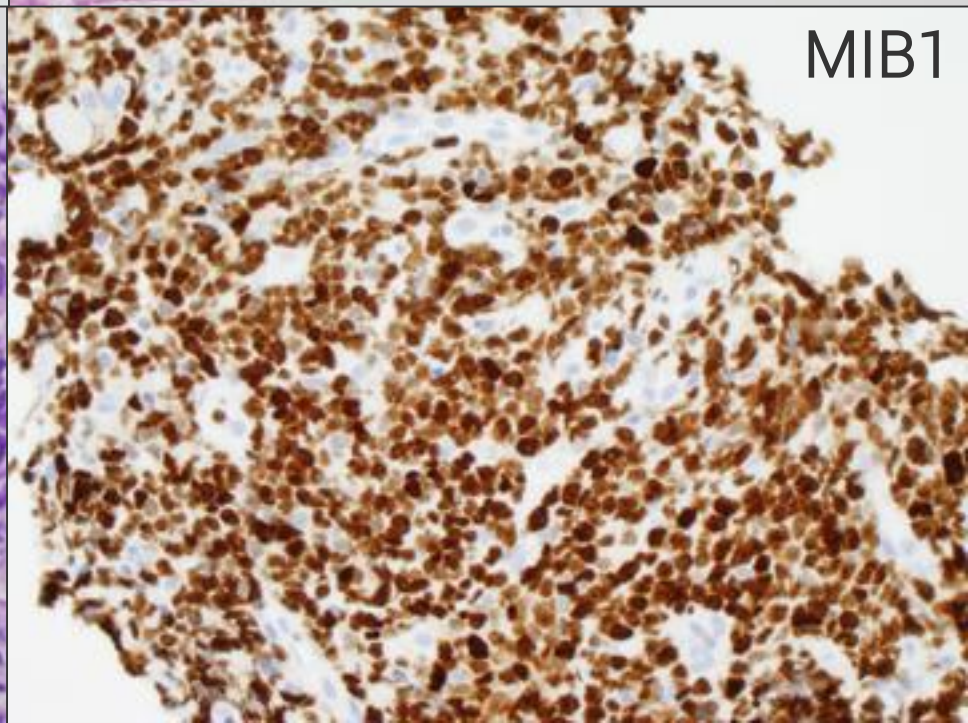
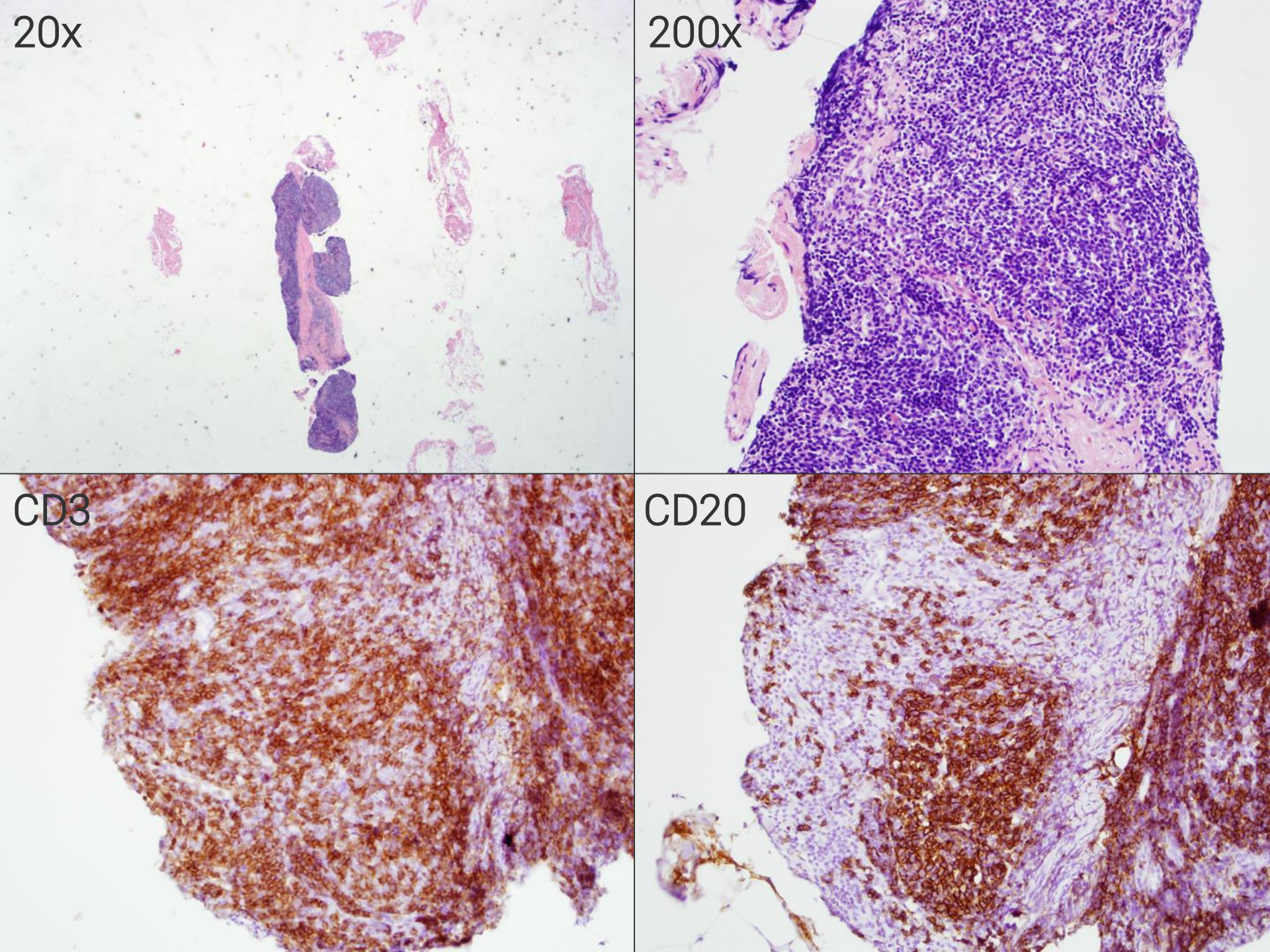
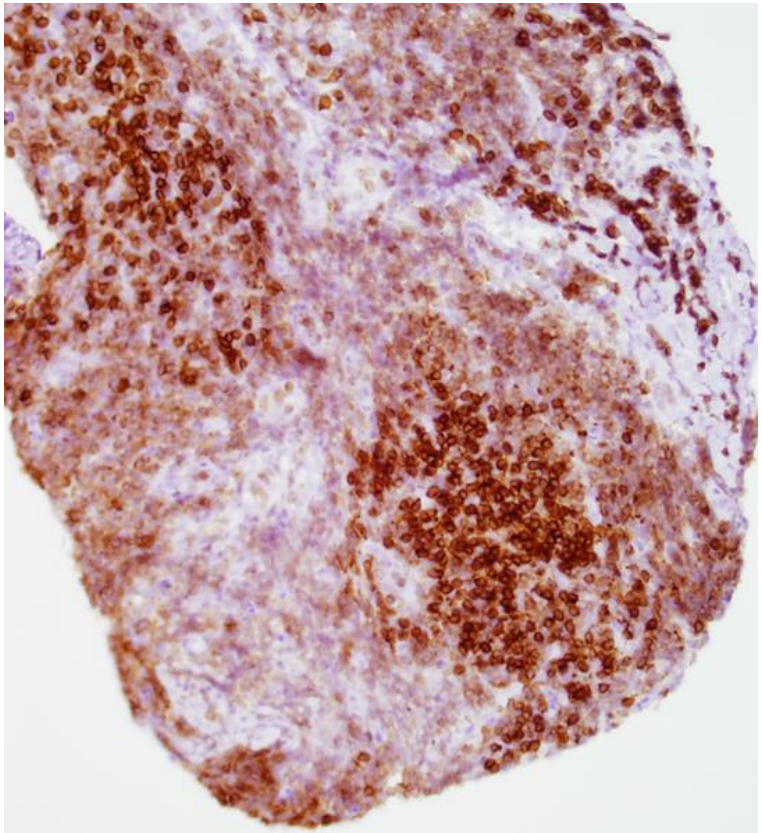

MIB1

How specific should I be?

- CD10 positive B-cell lymphoma (flow cytometry was positive).
- This is probably a DLBCL.
- Consider other possibilities:
  - » Grade 3B follicular lymphoma
  - » Follicle of florid follicular hyperplasia with a small B-cell clone by flow cytometry?


20x
200x
CD3
CD20

How specific should I be?

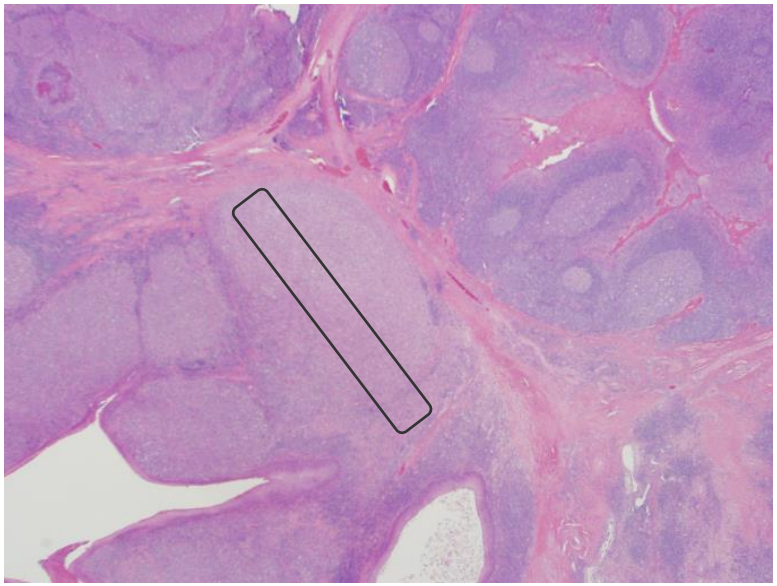


BCL2

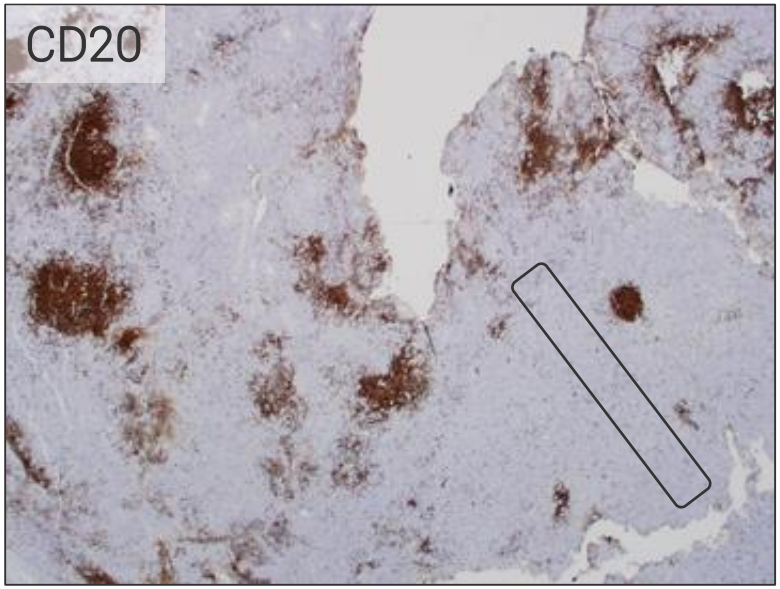
- Follicular lymphoma?
- Consider other possibilities:
  - » Missing areas of transformation not sampled.
  - » BCL2+ B-cells are normal in mantle zones/primary follicles.
    - Confirm BCL6 co-expression.
  - » Follicular neoplasia *in situ* (formerly FL *in situ*).
- Avoid formal grading.
  - » “No high grade seen.”

Problematic Diagnoses in Cores

Large B-cell Lymphoma with IRF4 rearrangement.



EBV lymphadenitis



Problematic Diagnoses in Cores

- Nodular lymphocyte predominant Hodgkin lymphoma vs. T-cell histiocyte rich large B-cell lymphoma.
  - » NLPHL can have extensive diffuse areas that look just like TCHR.
- Hodgkin lymphoma with few HRS cells.
  - » Ok in right clinical setting with multiple cells, typical immunophenotype.
  - » Beware if primary mediastinal or EBV driven LPD is in the differential.
- Lymphoma vs. *in situ* neoplasia (FL, MCL).
- T-cell lymphoma—architecture assessment from low power is critical.

Summary

- Needle core biopsies present a significant challenge in lymphoma diagnosis.
- Cannot assess architecture.
  - » Critical in classification system.
  - » Limits ability to assign precise diagnosis.
- Limited sampling may not represent entire node.
  - » Ask yourself: What am I not seeing?
- Use caution and consider a less specific diagnosis, e.g. “B-cell lymphoma.”
- More and more common due to less invasive nature.

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