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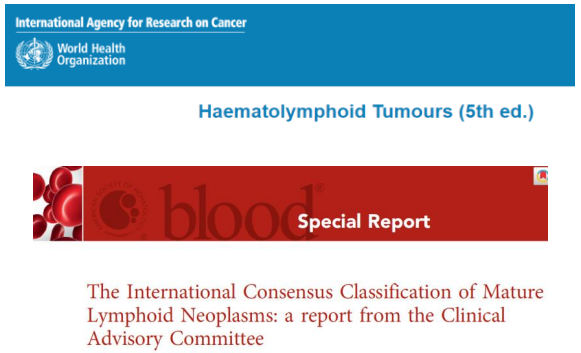
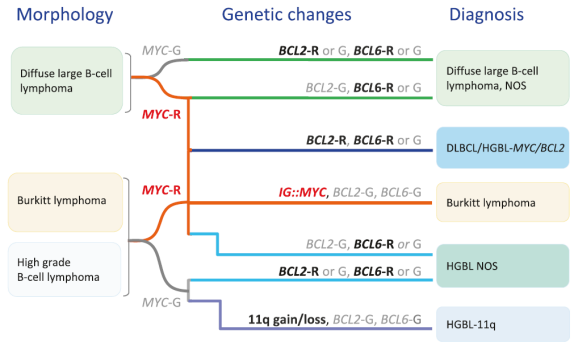
Update on Hematopathology Classification System(s!): Mature B-Cell Lymphomas

Elizabeth A. Morgan, MD
Pathologist, Brigham & Women's Hospital
Associate Professor of Pathology, Harvard Medical School

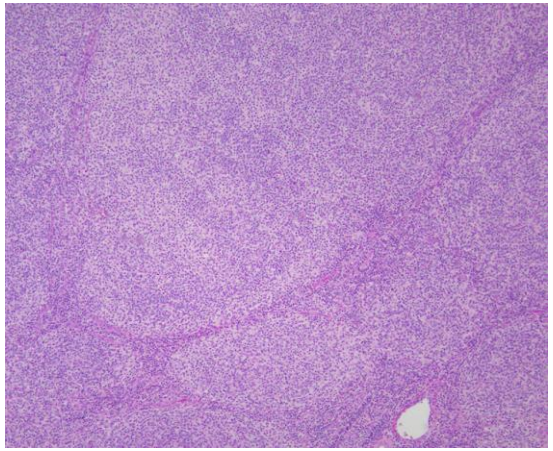
29th Annual Seminar in Pathology
Pittsburgh, PA
4/28/2023



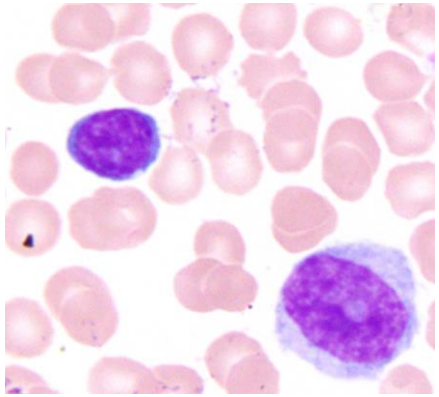
What's happening with the classification systems?



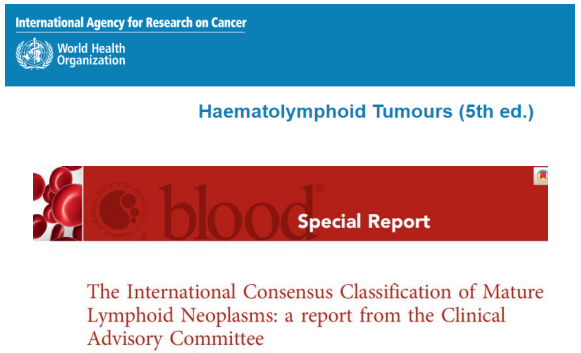
Large B-cell lymphomas with emphasis on double-hit lymphomas



Follicular lymphoma and related entitles



Other mature B-cell lymphomas



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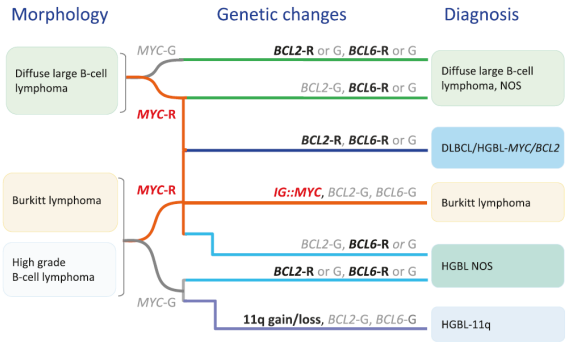

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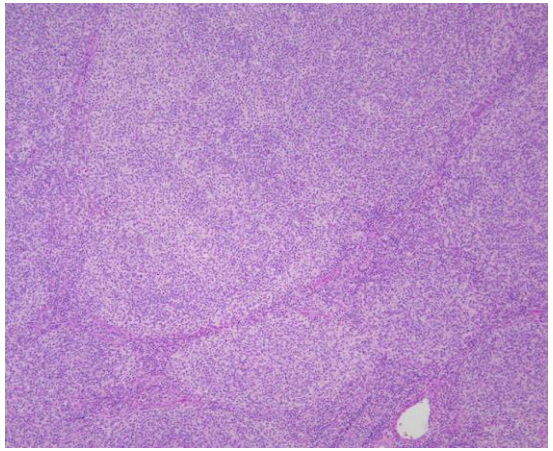
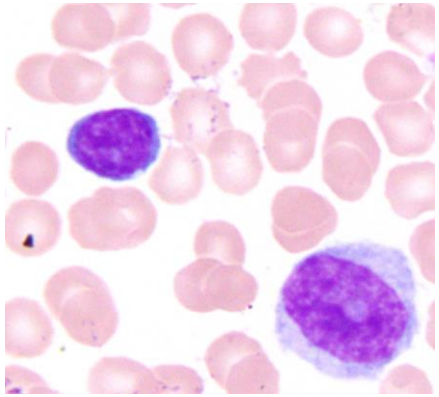


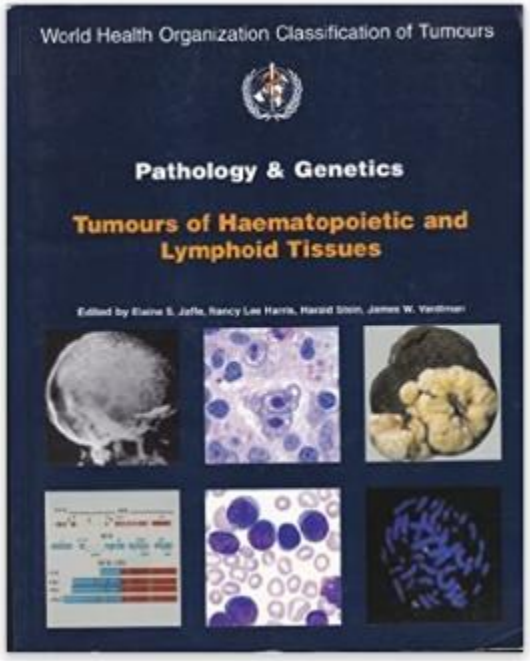
Follicular lymphoma and related entitles



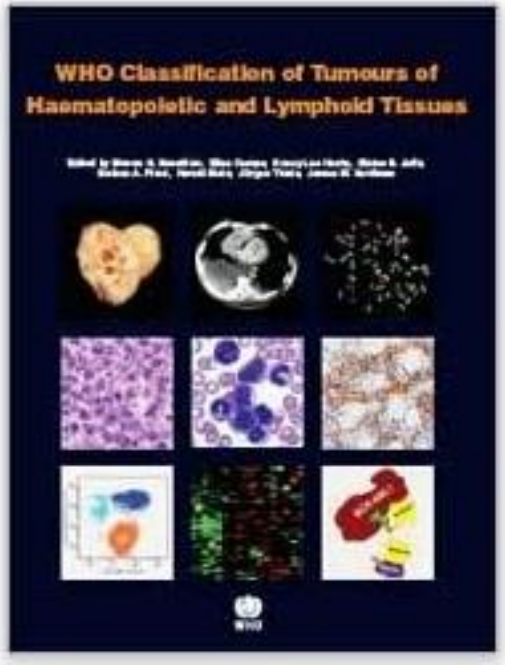
Other mature B-cell lymphomas

What's happening with the classification system(s)?

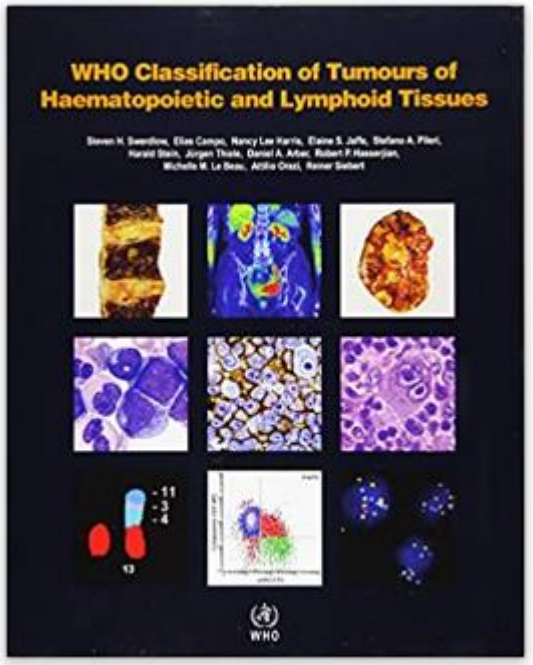
For 2 decades, the WHO "Blue Book" has been the global common language for hematopathologists



2001 = 3rd edition



2008 = 4th edition



2017 = 4th edition, revised

What's happening with the classification system(s)?

In preparation for the WHO 5th edition, there was a difference of opinion

Leukemia

EDITORIAL OPEN

The WHO Classification of Haematolymphoid Tumours

Ian A. Cree[1]✉

[1]International Agency for Research on Cancer (IARC), World Health Organization, 150 Cours Albert Thomas, Lyon 69372, France.

Received: 3 May 2022 Revised: 30 May 2022 Accepted: 31 May 2022
Published online: 22 June 2022

CORRESPONDENCE

LYMPHOMA

Response to "The WHO classification of haematolymphoid tumours" (Editorial)

Received: 21 July 2022 Revised: 15 August 2022 Accepted: 17 August 2022
Published online: 27 August 2022

Steven H. Swerdlow[1,16]✉, Elias Campo[2,16], Daniel A. Arber[3], Mario Cazzola[4], James R. Cook[5], Hartmut Döhner[6], Martin Dreyling[7], Robert P. Hasserjian[8], Elaine S. Jaffe[9], Attilio Orazi[10], Leticia Quintanilla-Martinez[11], David W. Scott[12], Ayalew Tefferi[13], Jane N. Winter[14] and Andrew D. Zelenetz[15]

[1]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [2]Hospital Clínic, IDIBAPS, Universitat de Barcelona, Barcelona, Spain. [3]University of Chicago, Chicago, IL, USA. [4]University of Pavia, Pavia, Italy. [5]Cleveland Clinic, Cleveland, OH, USA. [6]University Hospital Ulm, Ulm, Germany. [7]Ludwig Maximilians University Hospital, Department of Medicine III, Munich, Germany. [8]Massachusetts General Hospital, Boston, MA, USA. [9]National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. [10]Texas Tech University Health Sciences Center, El Paso, TX, USA. [11]University of Tuebingen, Tuebingen, Germany. [12]BC Cancer, Vancouver, BC, Canada. [13]Mayo Clinic, Rochester, MN, USA. [14]Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. [15]Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, NY, USA. [16]These authors contributed equally: Steven H. Swerdlow, Elias Campo.

CORRESPONDENCE OPEN

EPIDEMIOLOGY

The WHO classification of haematolymphoid tumours: response to Swerdlow et al.

Journal of Hematopathology (2021) 14:185–186
https://doi.org/10.1007/s12308-021-00465-5

EDITORIAL

What, how, and when for the WHO: will the clock be turned back for the next hematolymphoid tumor classification?

William R. Macon[1]

What is in a Name? Consequences of the Classification Schism in Hematopathology

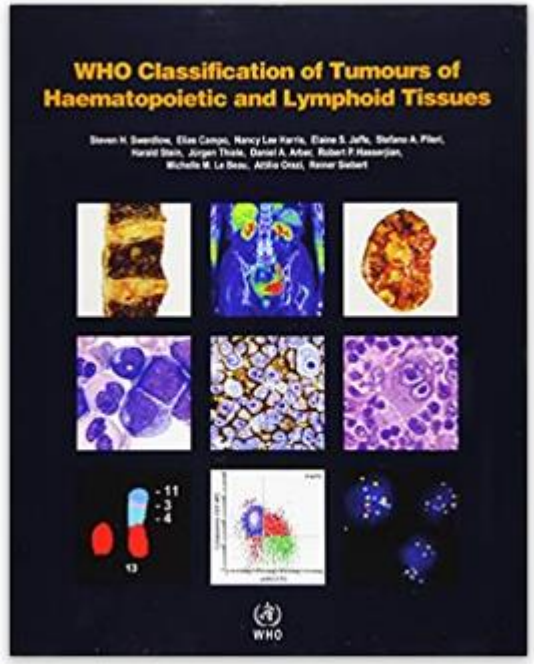
Jon C. Aster, MD, PhD[1]

Journal of Clinical Oncology®

Accepted on November 30, 2022 and published at ascopubs.org/journal/jco on December 8, 2022: DOI https://doi.org/10.1200/JCO.22.02680

What's happening with the classification system(s)?

We now are moving from our existing classification system…



WHO R4th

…to 2 new systems:

International Agency for Research on Cancer

World Health Organization

Haematolymphoid Tumours (5th ed.)

WHO 5th

blood Special Report

The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee

blood Special Report

International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data

International Consensus Classification (ICC)

WHO 5th: Where to find it

First available in two articles in *Leukemia*

- "The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms" (June 2022)
- "The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms" (June 2022)

Updates online (currently in Beta V2)

- https://tumourclassification.iarc.who.int/welcome/ (*requires subscription*)
- accepting feedback through online tool

Physical book should be out by the end of the calendar year

WHO 5th: Organization

Hierarchical system for classification

- category (*e.g. mature B-cell*)
- family/class (e.*g. large B-cell lymphomas*)
- entity/type (*e.g. diffuse large B-cell lymphoma, NOS*)
- subtype (*e.g. diffuse large B-cell lymphoma, NOS, germinal center B-cell-like*)

Place a disease in a broader diagnostic category initially while additional testing is underway

Naming convention

- lineage (*e.g. myeloid*) + dominant clinical attribute (*e.g. chronic, leukemic*) + dominant biologic attribute (*BCR::ABL1*)

Headings

Localization
Clinical features
Epidemiology
Etiology
Pathogenesis
Macroscopic appearance
Histopathology
Cytology
Diagnostic molecular pathology
Essential and desirable diagnostic criteria
Staging
Prognosis and prediction

WHO 5th: Organization

No provisional entities → emerging entities are listed as *other defined genetic alterations*

- used in AML, acute leukemia with ambiguous lineage, B-lymphoblastic leukemia/lymphoma

No use of the term “unclassifiable”

- represents a contradiction within a classification scheme

No use of the word “variant” except to refer to variant allele frequency

Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC) recommendations for the designation of gene fusions → double colon (::)

- *e.g. BCR::ABL1*

Essential and desirable diagnostic criteria

- essential: must-have features
  - aid in the applicability of the classification, particularly in limited resource settings
- desirable: nice-to-have features that support a diagnosis, but not mandatory

ICC: Where to find it

First available in multiple articles in *Blood*

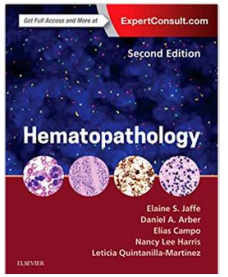
- "International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data" (September 2022)
- "Genomic profiling for clinical decision making in myeloid neoplasms and acute leukemia" (November 2022)
- "The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee" (September 2022)
- "Genomic profiling for clinical decision making in lymphoid neoplasms" (November 2022)

Subsequently 20 detailed articles published in *Virchows Archiv* (all of Volume 482, issue 1)

Additional papers have/will come out in *American Journal of Hematology* with clinician perspectives

ICC book expected to be published by end of 2024

Hematopathology 3rd ed. is structured by ICC and will also include WHO 5th (*Leukemia* papers)



ICC: Organization

Virchows Archiv (2023) 482:1–9
https://doi.org/10.1007/s00428-022-03487-1

EDITORIAL

Advances in the Classification of Myeloid and Lymphoid Neoplasms

Daniel A. Arber[1] · Elias Campo[2] · Elaine S. Jaffe[3]

Received: 19 December 2022 / Revised: 19 December 2022 / Accepted: 21 December 2022 / Published online: 31 December 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Provides a comprehensive "table of contents"

Provisional diagnoses are still included

Terms "unclassifiable" and variant are retained

New (::) designation for gene fusions is included

Similarities and differences

Many entities are largely similar to **WHO R4th**

Some entities have been updated from **WHO R4th** (especially genetic criteria) but same/similar in both **WHO 5th** and **ICC**

Some entities show significance differences between **WHO R4th** and one or both **WHO 5th** and **ICC**

- nomenclature changes
- diagnostic criteria
- new entities

Practical impact

"There is, unfortunately, no consensus for how best to cope with the current lack of consensus."

- Jon Aster (JCO editorial)

Most agree we need to provide both **WHO 5th** and **ICC** in our reports

- How?
  - cite both in top line equally? choose one and describe other in note?
  - significant differences – discuss first with clinician?
- What if it is a relapse of a disease diagnosed with **WHO R4th**?
- When?
  - **WHO 5th** is still in Beta V2

Minor nomenclature differences: **WHO 5th** will try to include **ICC** version as an "acceptable" related terminology

What about **WHO R4th**?

- still being used to enroll patients for clinical trials

ABPath primary and subspecialty exams

- 2023 exams: **WHO R4th**
- 2024 exams and beyond: **WHO 5th** and **ICC**

Looking forward

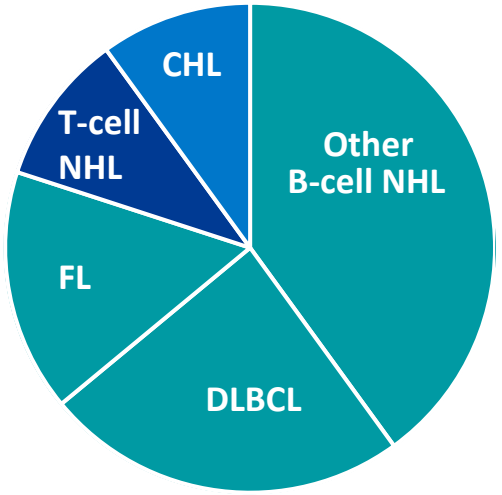
Overwhelming support from the community to go back to one classification system

WHO 6th is planned for 5 years from **WHO 5th**

Work is currently underway to achieve a unified classification process between ICC and WHO, and ensure that **WHO 6th** incorporates the best of both as well as recent hematopathology advances

Today's focus: Mature B-cell lymphomas

Distribution of lymphoma subtypes (~90K cases/yr)



- B-cell non-Hodgkin lymphoma
- T-cell non-Hodgkin lymphoma
- Classic Hodgkin lymphoma

Volume 482, issue 1, January 2023

Annual Review Issue: Advances in the classification of myeloid and lymphoid neoplasms as revealed in the International Consensus Classification

Issue editors

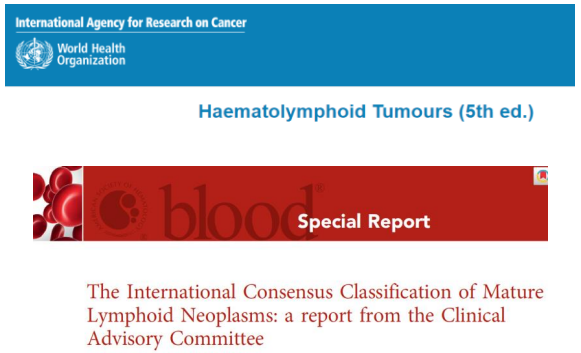
Daniel A Arber, Elias Campo & Elaine S. Jaffe

20 articles in this issue

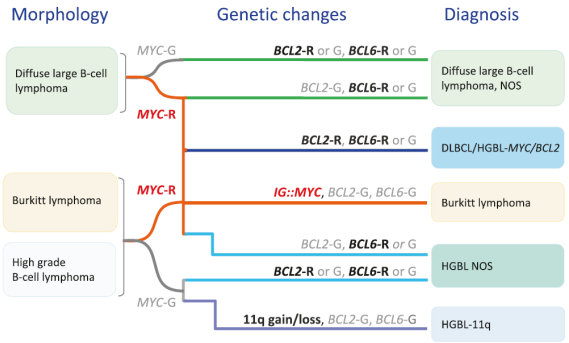
International Agency for Research on Cancer

World Health Organization

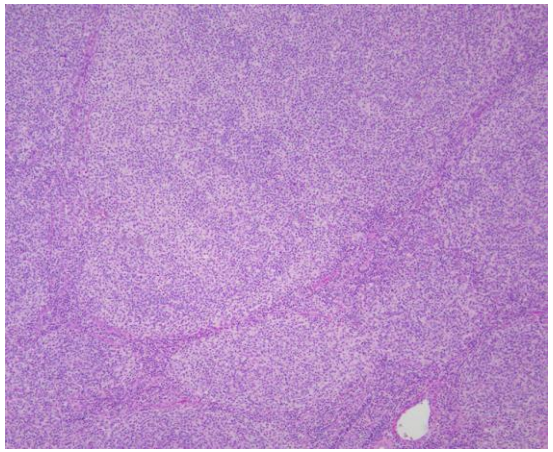
Haematolymphoid Tumours (5th ed.)



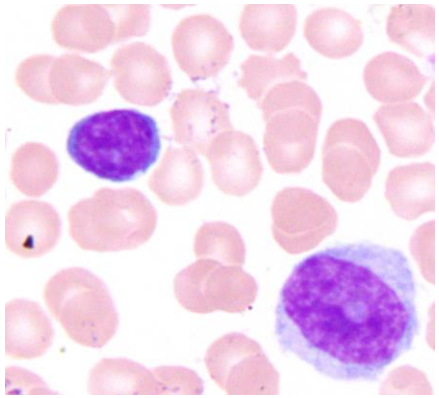
What's happening with the classification systems?



Large B-cell lymphomas with emphasis on double-hit lymphomas



Follicular lymphoma and related entitles



Other mature B-cell lymphomas

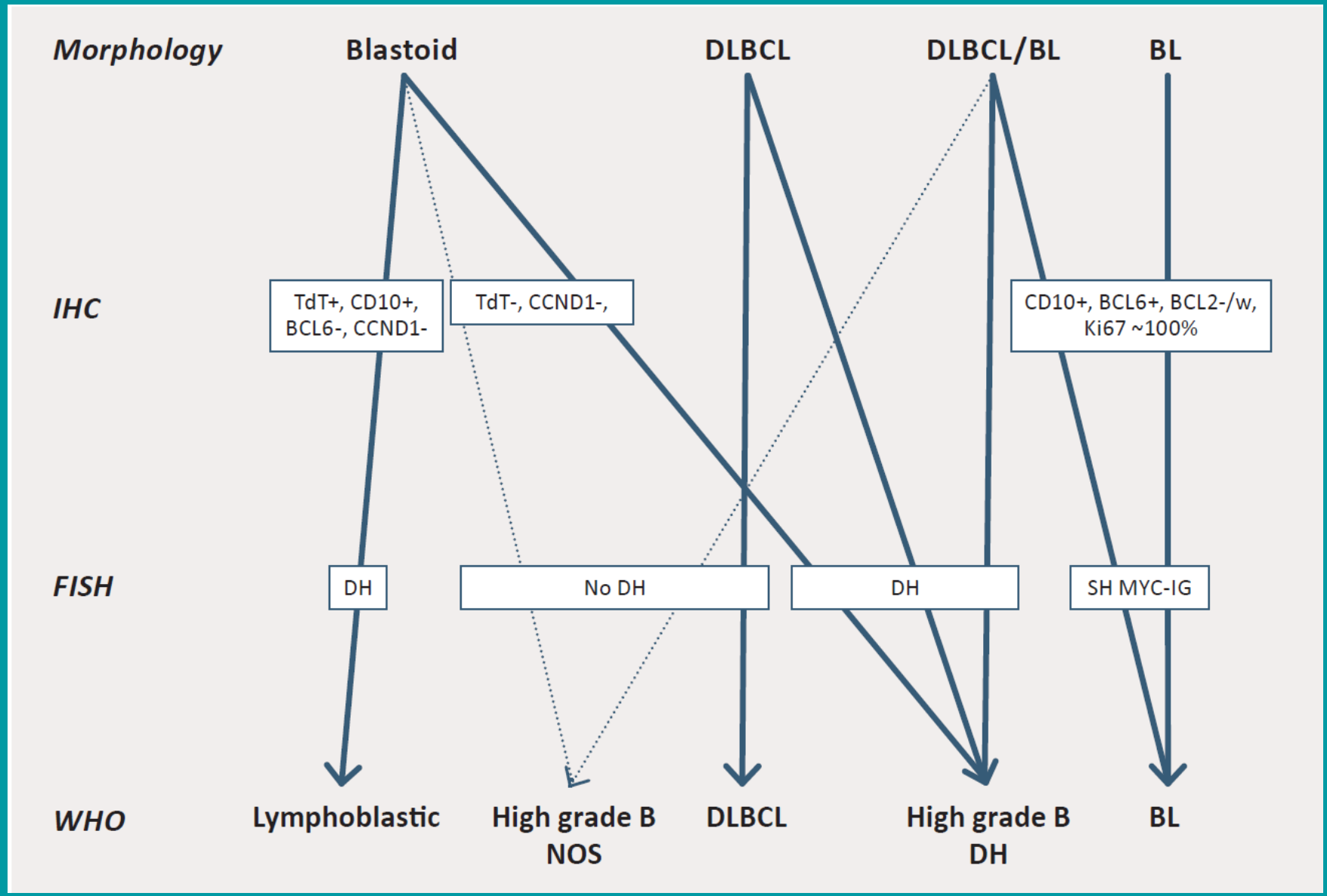
Large B-cell lymphomas: Comparison

WHO R4th	WHO 5th	ICC
Diffuse large B-cell lymphoma, NOS	(Same)	(Same)
T-cell/histiocyte-rich large B-cell lymphoma	(Same)	(Same)
Primary cutaneous diffuse large B-cell lymphoma, leg type	(Same)	(Same)
Intravascular large B-cell lymphoma	(Same)	(Same)
Primary mediastinal large B-cell lymphoma	(Same)	(Same)
High-grade B-cell lymphoma, NOS	(Same)	(Same)
Plasmablastic lymphoma	(Same)	(Same)
Diffuse large B-cell lymphoma associated with chronic inflammation	(Same)	(Same)
ALK-positive large B-cell lymphoma	(Same)	(Same)
Large B-cell lymphoma with *IRF4* rearrangement (provisional)	(Same) → No longer provisional	(Same) → Not provisional; grouped with FL-related entities
Lymphomatoid granulomatosis	(Same)	(Same)

Large B-cell lymphomas: Comparison

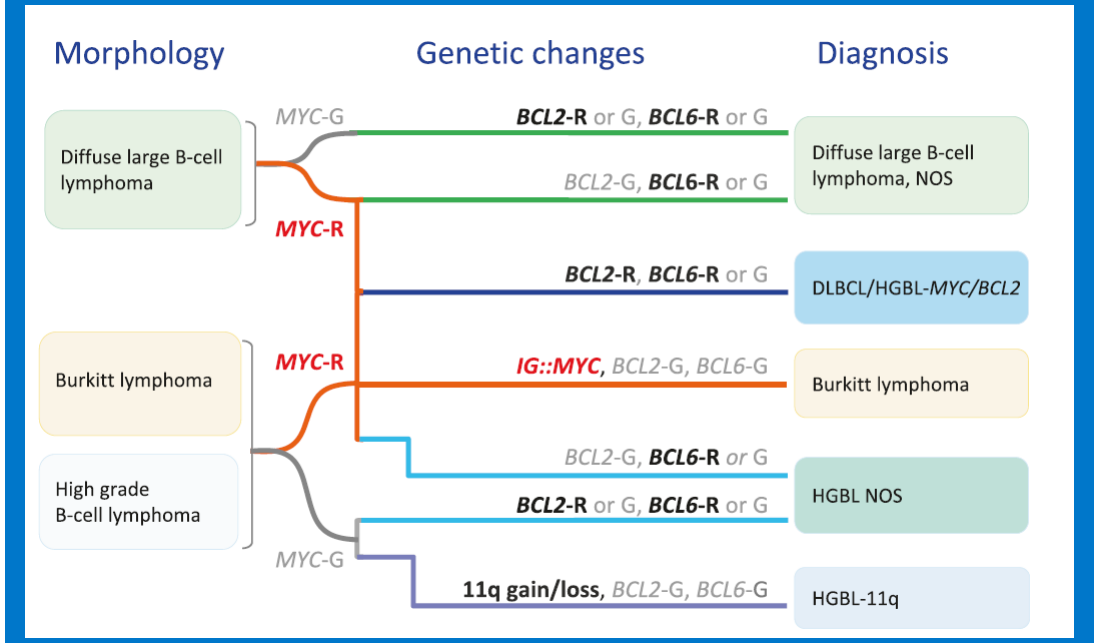
WHO R4th	WHO 5th	ICC
High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements	Diffuse large B-cell lymphoma/high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements	High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements
	Not included	High-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements (provisional)

WHO R4th

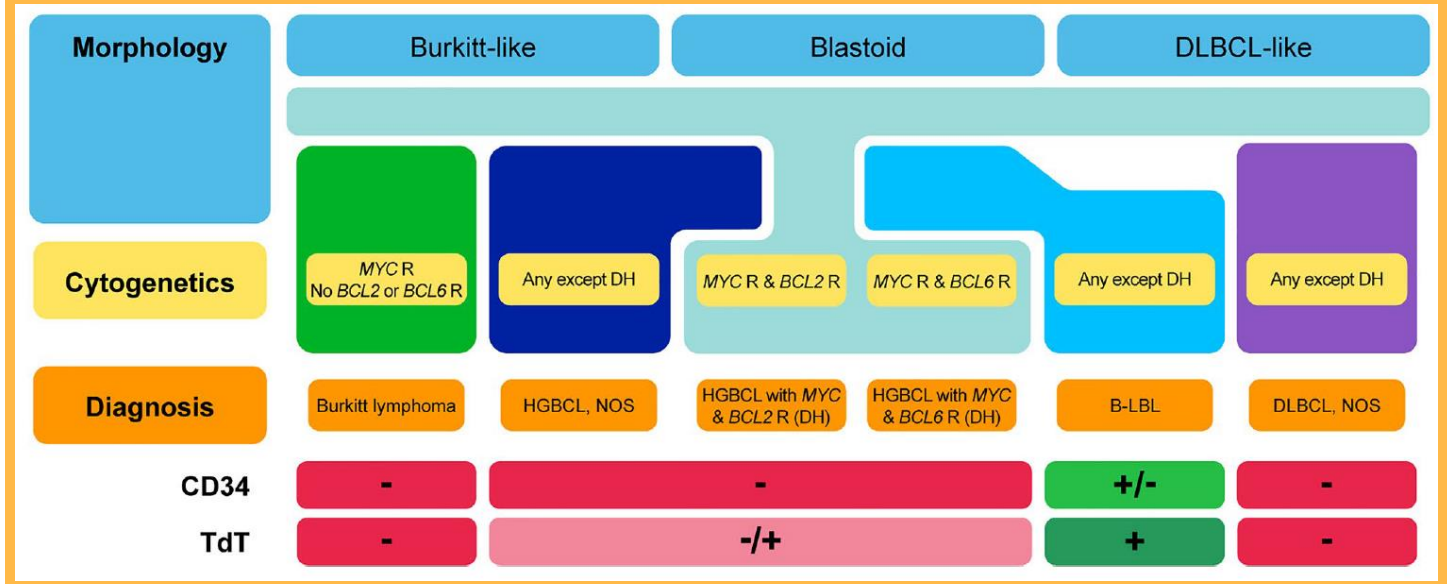


DH = Double hit

WHO 5th


Morphology
Genetic changes
Diagnosis
Diffuse large B-cell lymphoma
Burkitt lymphoma
High grade B-cell lymphoma
MYC-G
MYC-R
MYC-R
MYC-G
BCL2-R or G, BCL6-R or G
BCL2-G, BCL6-R or G
BCL2-R, BCL6-R or G
IG::MYC, BCL2-G, BCL6-G
BCL2-G, BCL6-R or G
BCL2-R or G, BCL6-R or G
11q gain/loss, BCL2-G, BCL6-G
Diffuse large B-cell lymphoma, NOS
DLBCL/HGBL-MYC/BCL2
Burkitt lymphoma
HGBL NOS
HGBL-11q

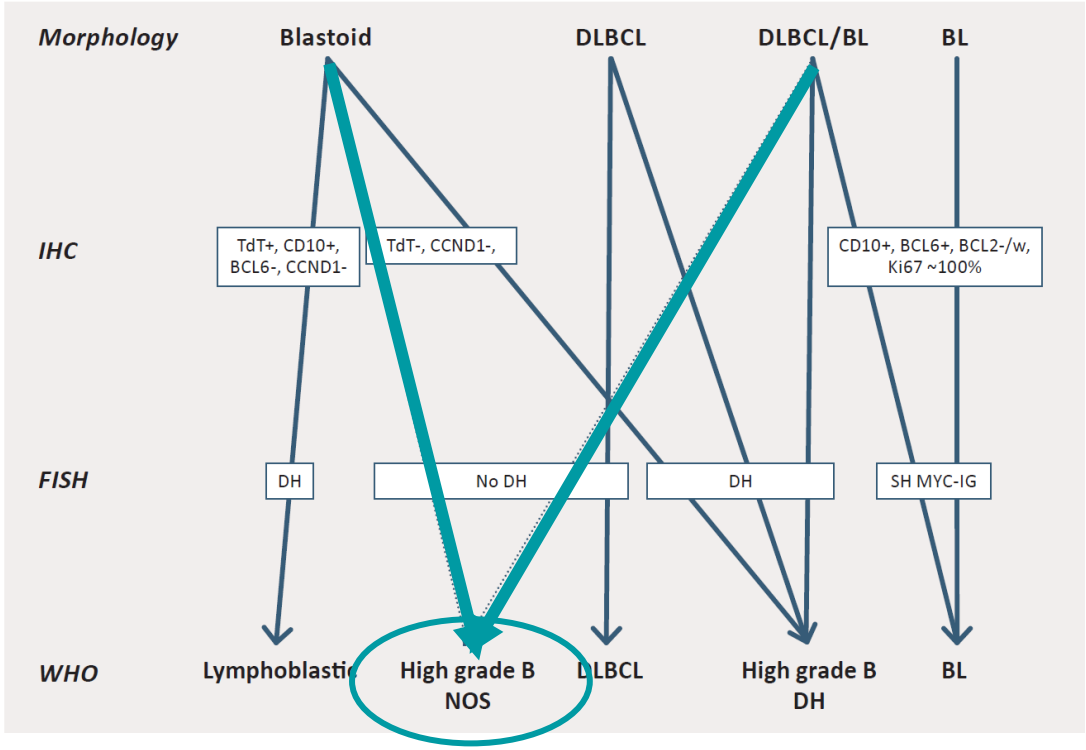
ICC


Morphology
Burkitt-like
Blastoid
DLBCL-like
Cytogenetics
MYC R No BCL2 or BCL6 R
Any except DH
MYC R & BCL2 R
MYC R & BCL6 R
Any except DH
Any except DH
Diagnosis
Burkitt lymphoma
HGBCL, NOS
HGBCL with MYC & BCL2 R (DH)
HGBCL with MYC & BCL6 R (DH)
B-LBL
DLBCL, NOS
CD34
-
-
+/-
-
TdT
-
-/+
+
-

Standard DLBCL work-up

Since **WHO R4th**, standard procedure for DLBCL work-up includes:

1. Characterize morphology:
   - "diffuse large B-cell lymphoma", or
   - "high grade" (intermediate between diffuse large B-cell lymphoma & Burkitt lymphoma, or blastoid)



- Needed a place to categorize non-double-hit cases that didn't fit perfectly with classic DLBCL morphology

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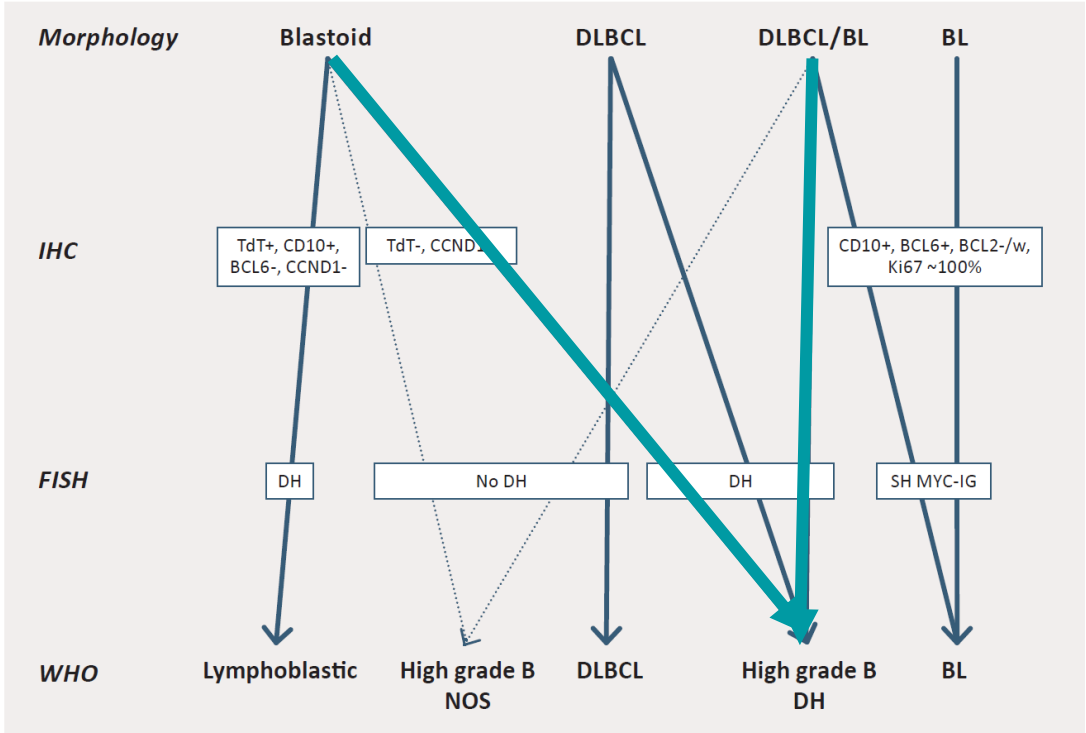

- Needed a place to categorize non-double-hit cases that didn't fit perfectly with classic DLBCL morphology
- Within DHL, some literature suggested a worse prognosis with high-grade morphology

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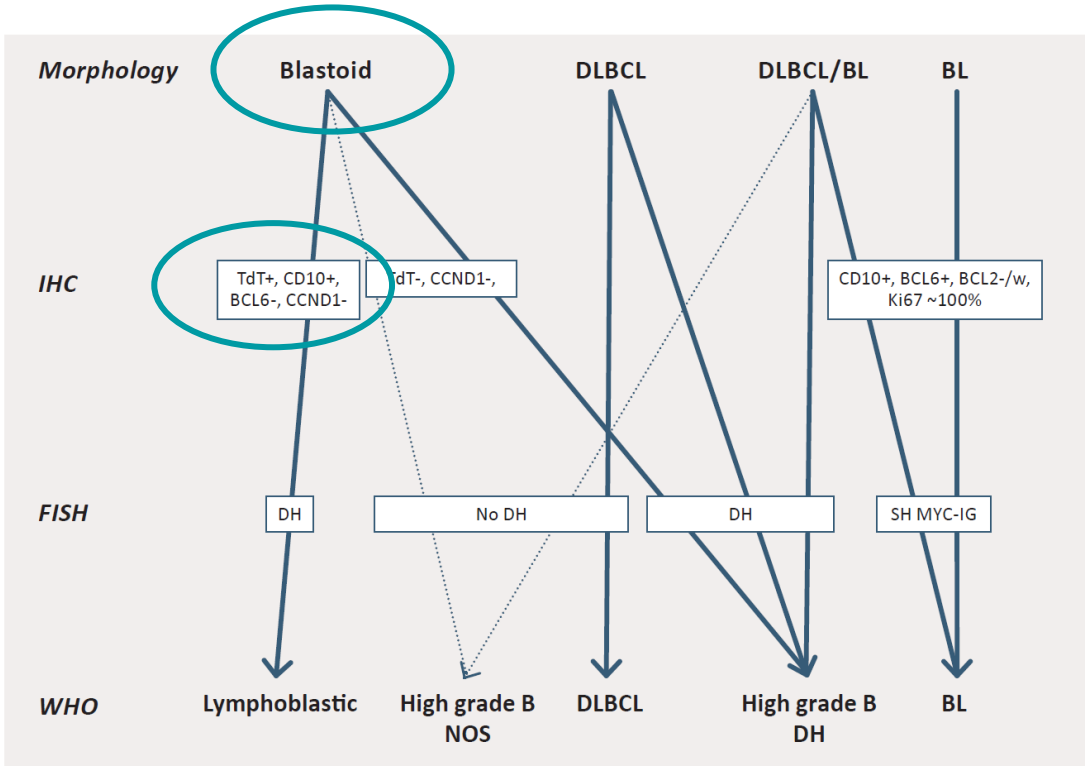
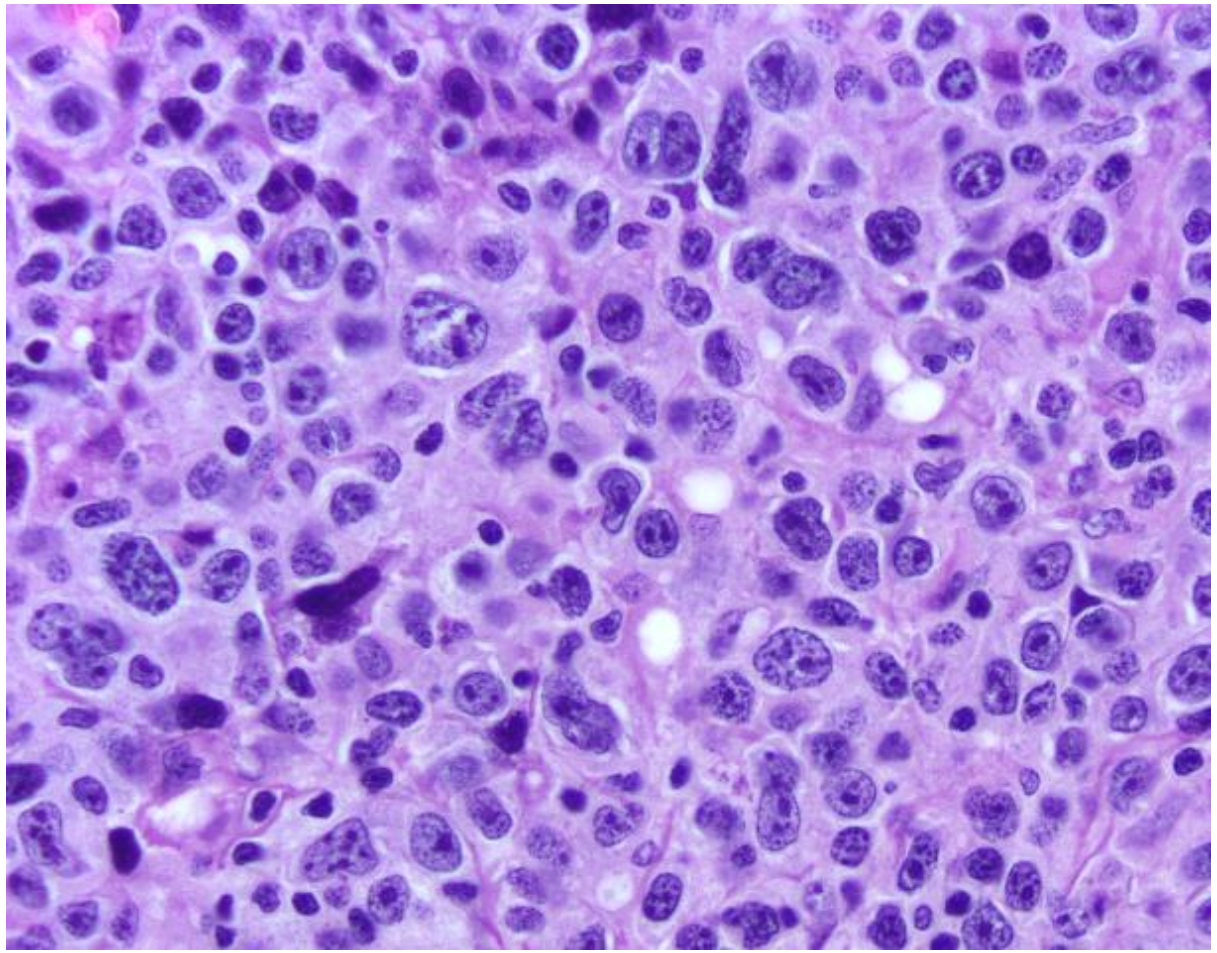
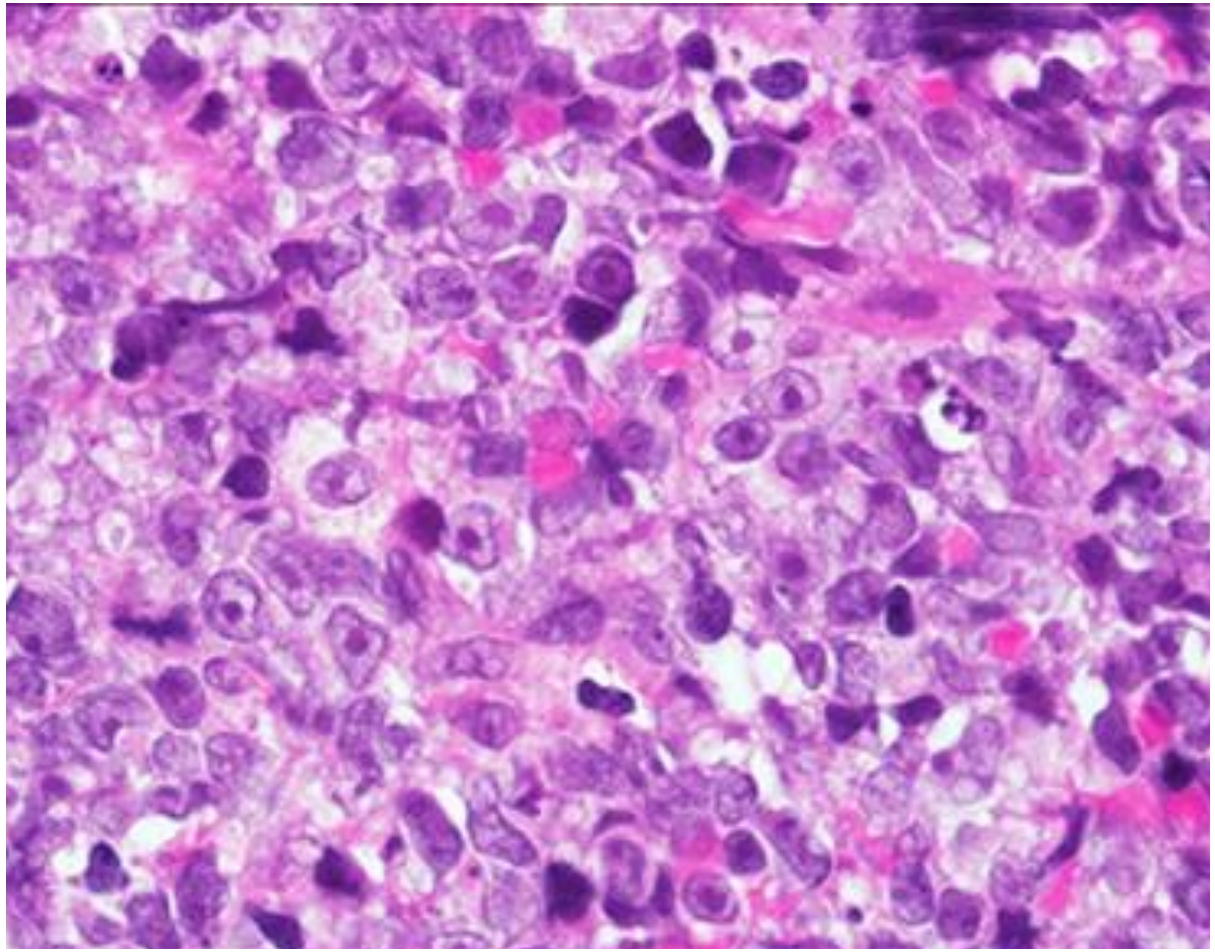


- Needed a place to categorize non-double-hit lymphoma (non-DHL) cases that didn't fit perfectly with classic DLBCL morphology
- Within DHL, some literature suggested a worse prognosis with high-grade morphology
- Some DHL are TdT+ with blastoid morphology → more to come on this!

Morphology



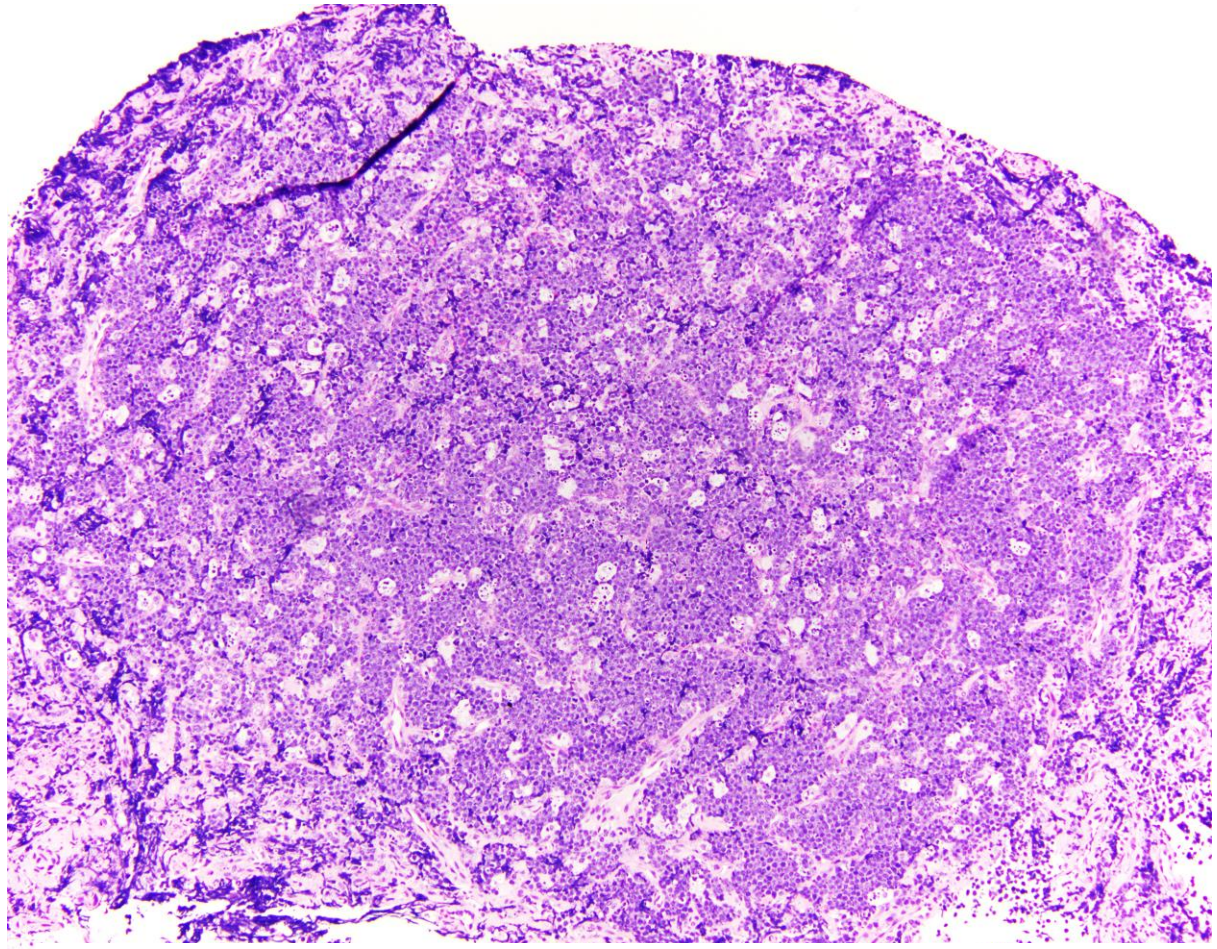


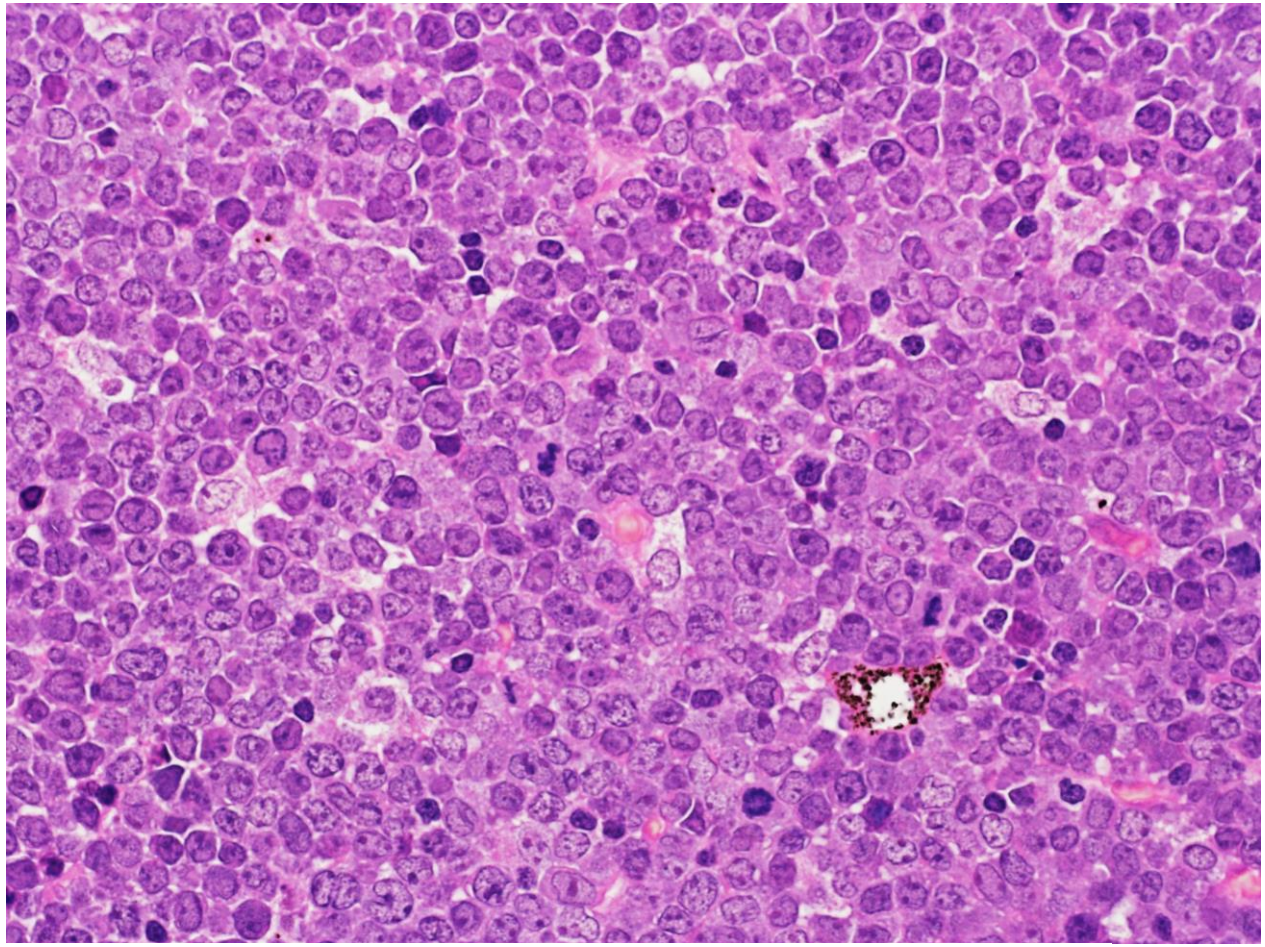
DLBCL morphology

sheets of large cells with centroblastic or immunoblastic-type cells, or anaplastic features

Swerdlow SH *et al*. *Blood* 2016 127(20): 2375-90.

Morphology



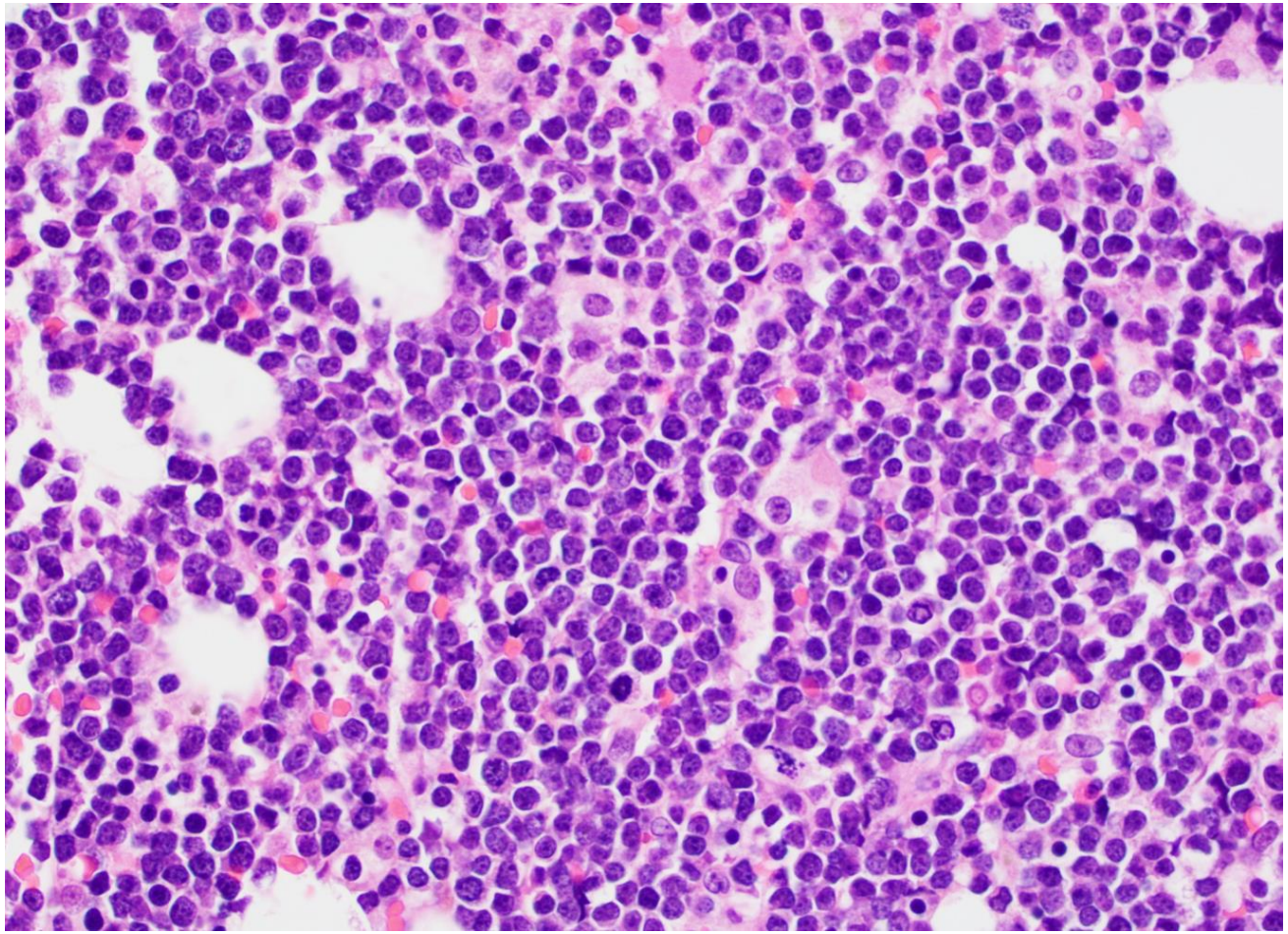


High-grade: DLBCL/BL morphology

Monotonous cells with features between DLBCL and BL; tingible-body macrophages, frequent mitoses

https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=32100

Morphology



High-grade: Blastoid morphology

Small nuclei, slightly irregular nuclear contours, small nucleoli, fine chromatin

https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=32117

Standard DLBCL work-up

Since **WHO R4th**, standard procedure for DLBCL work-up includes:

1. Characterize morphology:
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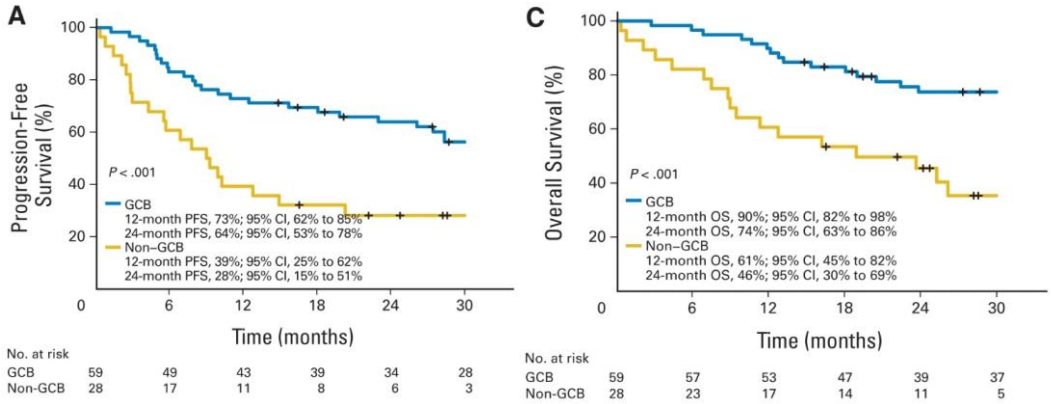
   - "high grade" (intermediate between diffuse large B-cell lymphoma & Burkitt lymphoma, or blastoid)
2. Establish cell-of-origin

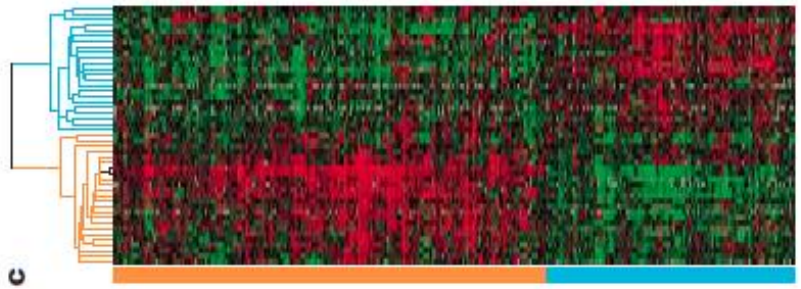


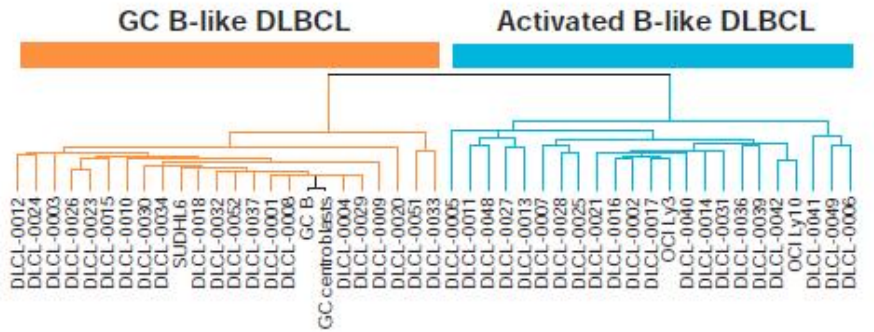
non-germinal center B-like (non-GCB) DLBCL have worse outcome compared to **germinal center B-like (GCB) DLBCL** treated with R-CHOP (standard therapy)

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

Ash A. Alizadeh[1,2], Michael B. Eisen[2,3,4], R. Eric Davis[5], Chi Ma[5], Izidore S. Lossos[6], Andreas Rosenwald[5], Jennifer C. Boldrick[1], Hajeer Sabet[5], Truc Tran[5], Xin Yu[5], John I. Powell[7], Liming Yang[7], Gerald E. Marti[8], Troy Moore[9], James Hudson Jr[9], Lisheng Lu[10], David B. Lewis[10], Robert Tibshirani[11], Gavin Sherlock[4], Wing C. Chan[12], Timothy C. Greiner[12], Dennis D. Weisenburger[12], James O. Armitage[13], Roger Warnke[14], Ronald Levy[6], Wyndham Wilson[15], Michael R. Grever[16], John C. Byrd[17], David Botstein[4], Patrick O. Brown[1,18] & Louis M. Staudt[5]

NATURE | VOL 403 | 3 FEBRUARY 2000 | www.nature.com





Grzegorz S et al. *J Clin Oncol* 2015 33:3, 251-257.

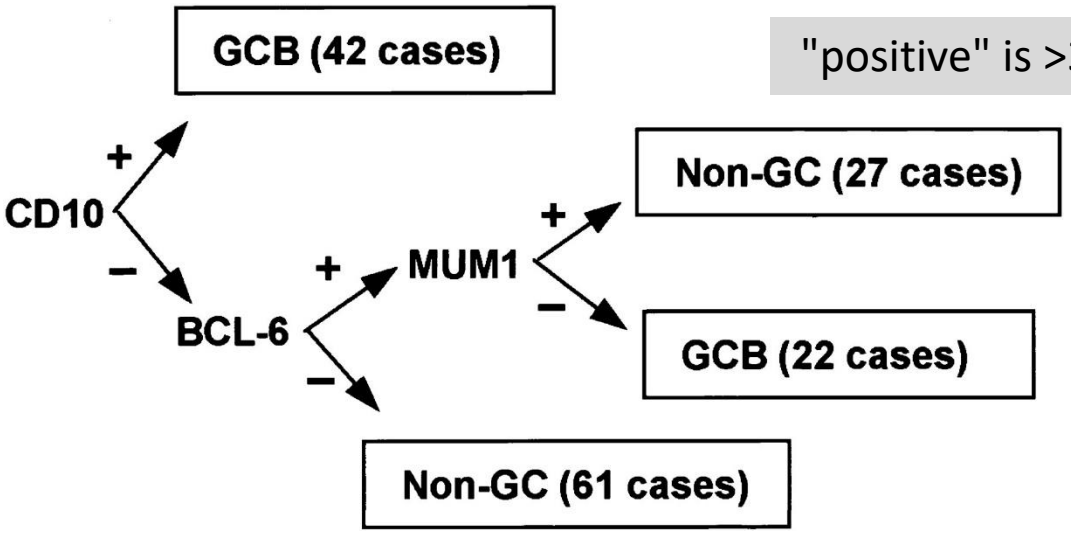
Cell-of-origin

Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray

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, and Wing C. Chan

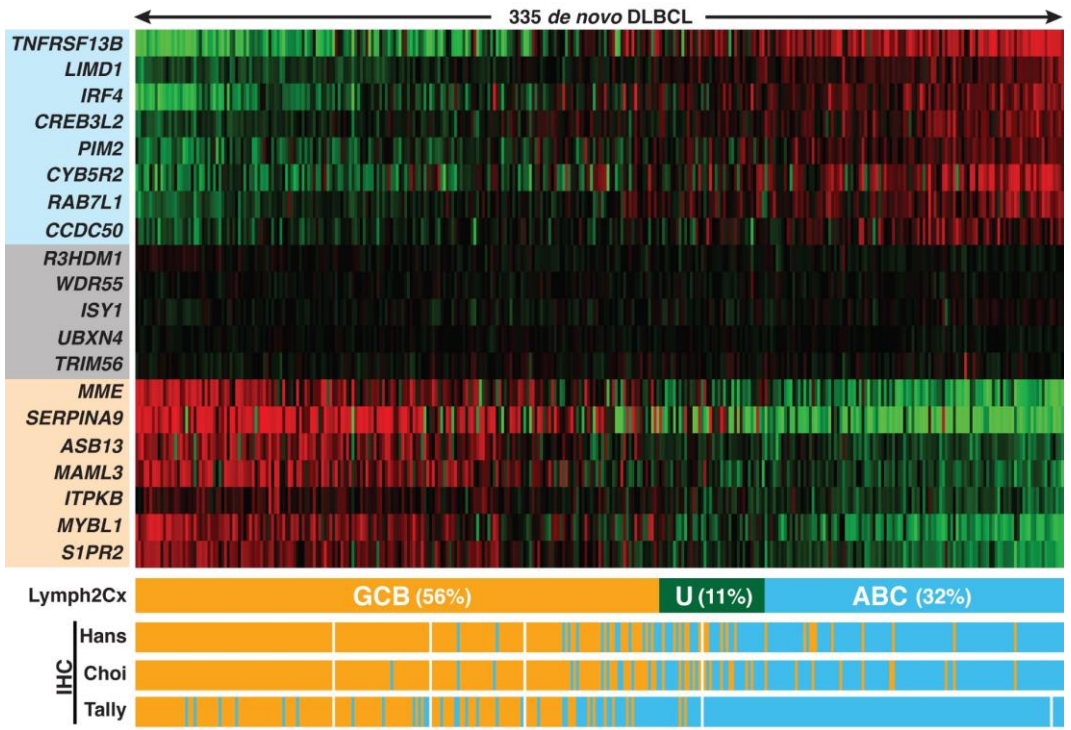
(Blood. 2004;103:275-282)

"Hans" algorithm



"positive" is >30%

Also "Choi" algorithm & "Tally" algorithm



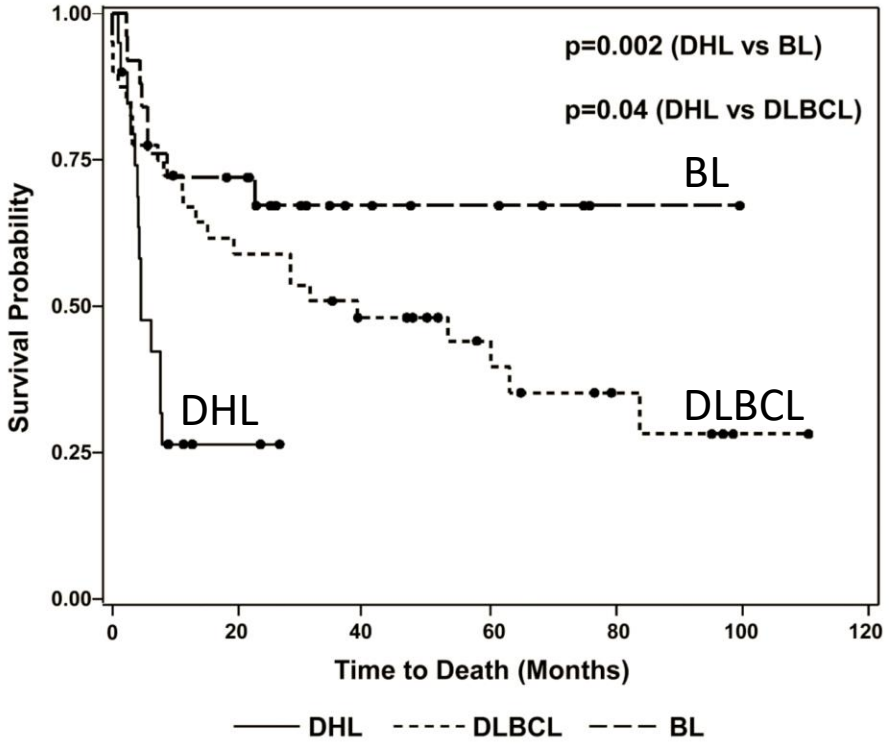
- cases classified as GCB or ABC by gene expression profiling (GEP) show relative concordance with IHC (some variability based on which algorithm)
- given lack of widespread availability of GEP, IHC algorithms are currently considered acceptable

Modified slide courtesy of Dr. Scott Lovitch

Standard DLBCL work-up

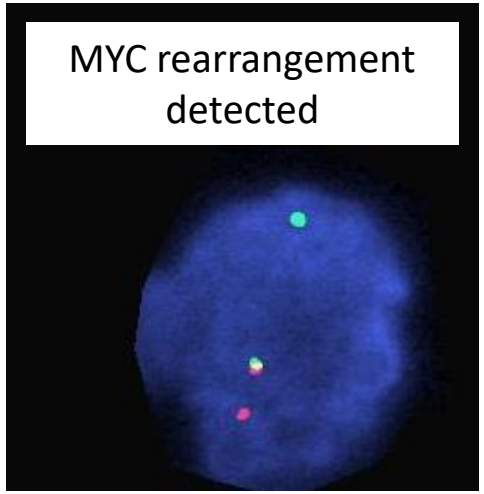
Since **WHO R4th**, standard procedure for DLBCL work-up includes:

1. Characterize morphology:
   - "diffuse large B-cell lymphoma", or
   - "high grade" (intermediate between diffuse large B-cell lymphoma & Burkitt lymphoma, or blastoid)
2. Establish cell-of-origin
3. Send for *MYC* FISH with concurrent (or reflex) *BCL2* and *BCL6* FISH to evaluate for "double-hit" or "triple-hit" lymphoma



Snuderl et al. *Am J Surg Pathol* 2010 Mar;34(3):327-40.

“Double-hit” genetics



MYC Dual Color, Break Apart Rearrangement Probe

+ *BCL2* rearrangement = **High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (aka double-hit lymphoma or DHL)**

+ *BCL6* rearrangement = **High-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements (aka double-hit lymphoma or DHL)**

+ *BCL2* and BCL6 rearrangements = **High-grade B-cell lymphoma with *MYC*, *BCL2* and *BCL6* rearrangements (aka triple-hit lymphoma or THL)**

→ DLBCL morphology without double/triple hit = **diffuse large B-cell lymphoma, not otherwise specified**

→ “High grade” morphology without double/triple hit = **high-grade B-cell lymphoma, not otherwise specified**

→ Follicular lymphoma morphology with double/triple hit = **follicular lymphoma**

High-grade B-cell lymphoma with *MYC* and *BCL2*

What have we learned? → **High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements is a distinct entity**

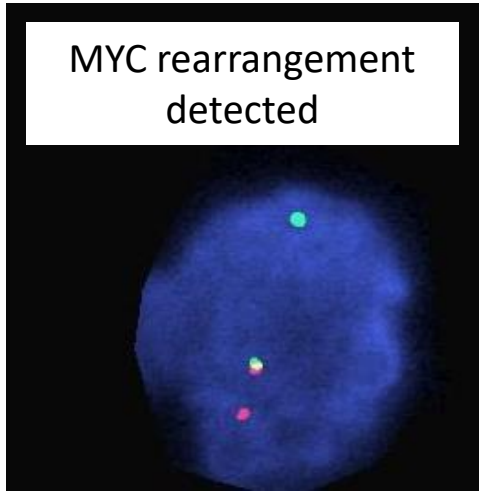
- accounts for 80-90% of "DHL" cases (2% of NHL)
- nearly 100% are "GCB" by Hans or GEP
  - usually positive for CD10, BCL6 and BCL2 IHC
  - usually positive for MYC IHC (positive is >40%)
- any morphology (large, intermediate, blastoid)
- uniform mutation profile (includes *BCL2*, *CREBBP*, *EZH2*, *TNFRSF14*, as well as *MYC*)
  - does overlap with some GCB DLBCL with poor prognosis
- dose-adjusted R-EPOCH (possibly other regimens for younger patients), CNS monitoring, consideration for novel therapies (e.g. CAR-T, targeted therapies)

Ennishi D et al. *JCO*. 2018

“Double-hit” genetics

+ *BCL2* rearrangement = **High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (ICC)**

→ **report that it is without *BCL6* rearrangement**



MYC Dual Color, Break Apart Rearrangement Probe

Diffuse large B-cell lymphoma/high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (WHO 5th)

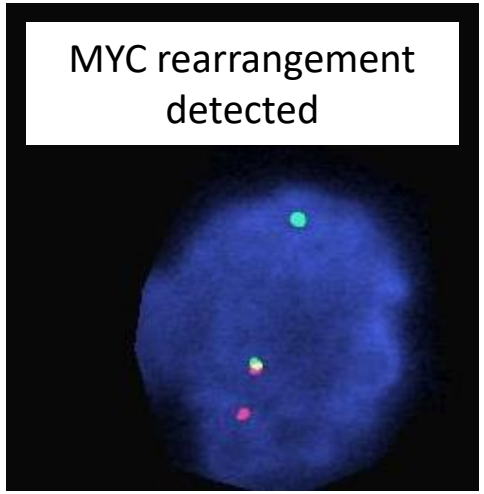
subtype: DLBCL/HGBL with rearrangements of *MYC* and *BCL2* without *BCL6* rearrangement

High-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements

What have we learned? → **High-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements is heterogenous and less well understood**

- accounts for 10-20% of “DHL” cases
- conflicting data on outcome
- variable cell-of-origin profile by IHC or GEP
  - ~50% GCB and ~50% ABC
- often large-cell morphology
- more diverse GEP and mutational profiles
- ~30% are pseudo-“double”-hit because *BCL6* is the *MYC* partner gene: *MYC::BCL6* translocation
  - significance of a pseudo-double-hit is uncertain; no requirement to perform FISH to discern true from pseudo

“Double-hit” genetics



MYC Dual Color, Break Apart Rearrangement Probe

DOUBLE HIT

+ *BCL2* rearrangement = High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (ICC)

is without *BCL6* rearrangement

Diffuse large B-cell lymphoma/high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (WHO 5th)

subtype: DLBCL/HGBL with rearrangements of *MYC* and *BCL2* without *BCL6* rearrangement

+ *BCL6* rearrangement = **High-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements (ICC) (provisional, to allow for data collection)**

Not included as a distinct entity (WHO 5th)

→ classify as DLBCL, NOS or high-grade B-cell lymphoma, NOS but report FISH findings

+ *BCL2* and *BCL6* rearr. = **High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (ICC)**

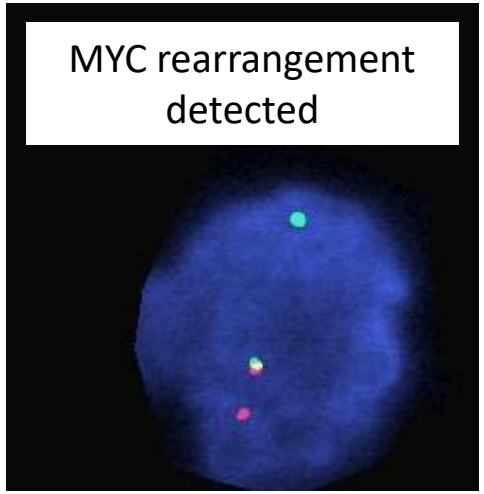
→ report that it is with *BCL6* rearrangement

Diffuse large B-cell lymphoma/high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (WHO 5th)

subtype: DLBCL/HGBL with rearrangements of *MYC* and *BCL2* with *BCL6* rearrangement

“Double-hit” genetics

What does this mean?



MYC Dual Color, Break Apart Rearrangement Probe

+ *BCL2* rearrangement = **High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (ICC)**

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Diffuse large B-cell lymphoma/high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (WHO 5th)

subtype: DLBCL/HGBL with rearrangements of *MYC* and *BCL2* without *BCL6* rearrangement

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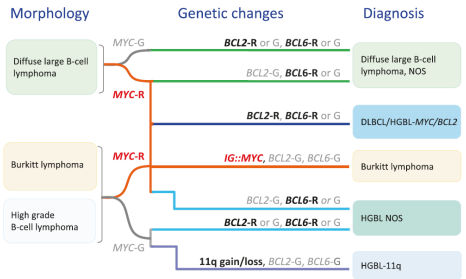
→ report that it is with *BCL6* rearrangement

Diffuse large B-cell lymphoma/high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (WHO 5th)

subtype: DLBCL/HGBL with rearrangements of *MYC* and *BCL2* with *BCL6* rearrangement



WHO 5th nomenclature provides flexibility to sign case out based on morphology

DLBCL morphology → **Diffuse large B-cell lymphoma, pending FISH**

High-grade morphology → **High-grade B-cell lymphoma, pending FISH**

Incorporate FISH (MYC, BCL2, BCL6)

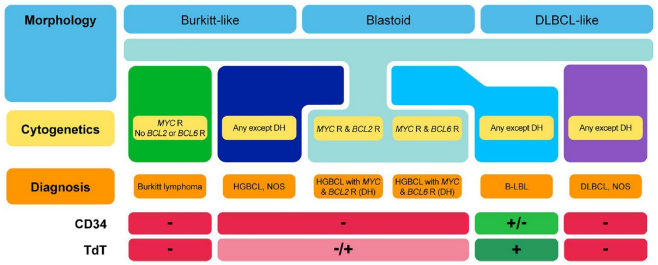
Any except concurrent *MYC*-R and *BCL2*-R → **Diffuse large B-cell lymphoma, NOS *(includes cases with concurrent MYC-R and BCL6-R)***

Concurrent *MYC*-R and *BCL2*-R, +/- *BCL6*-R → **Diffuse large B-Cell lymphoma with *MYC* and *BCL2* rearrangements [with or without] *BCL6* rearrangement**

Any except concurrent *MYC*-R and *BCL2*-R → **High-grade B-cell lymphoma, NOS* *(includes cases with concurrent MYC-R and BCL6-R)***

*consider BL if solo *MYC*-R and Burkitt-like morphology

Concurrent *MYC*-R and *BCL2*-R, +/- *BCL6*-R → **High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements [with or without] *BCL6* rearrangement**



ICC nomenclature does not allow DLBCL nomenclature to be associated with DHL

DLBCL morphology

High-grade morphology

[placeholder term e.g. aggressive B-cell lymphoma], pending FISH

Incorporate FISH (MYC, BCL2, BCL6)

Any except concurrent *MYC*-R and *BCL2*-R or concurrent *MYC*-R and *BCL6*-R

Diffuse large B-cell lymphoma, NOS

Concurrent *MYC*-R and *BCL2*-R, +/- *BCL6*

High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements *(note if with or without BCL6-R; specify morphology)*

Concurrent *MYC*-R and *BCL6*-R

High-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements (provisional)

Any except concurrent *MYC*-R and *BCL2*-R or concurrent *MYC*-R and *BCL6*-R

High-grade B-cell lymphoma, NOS*

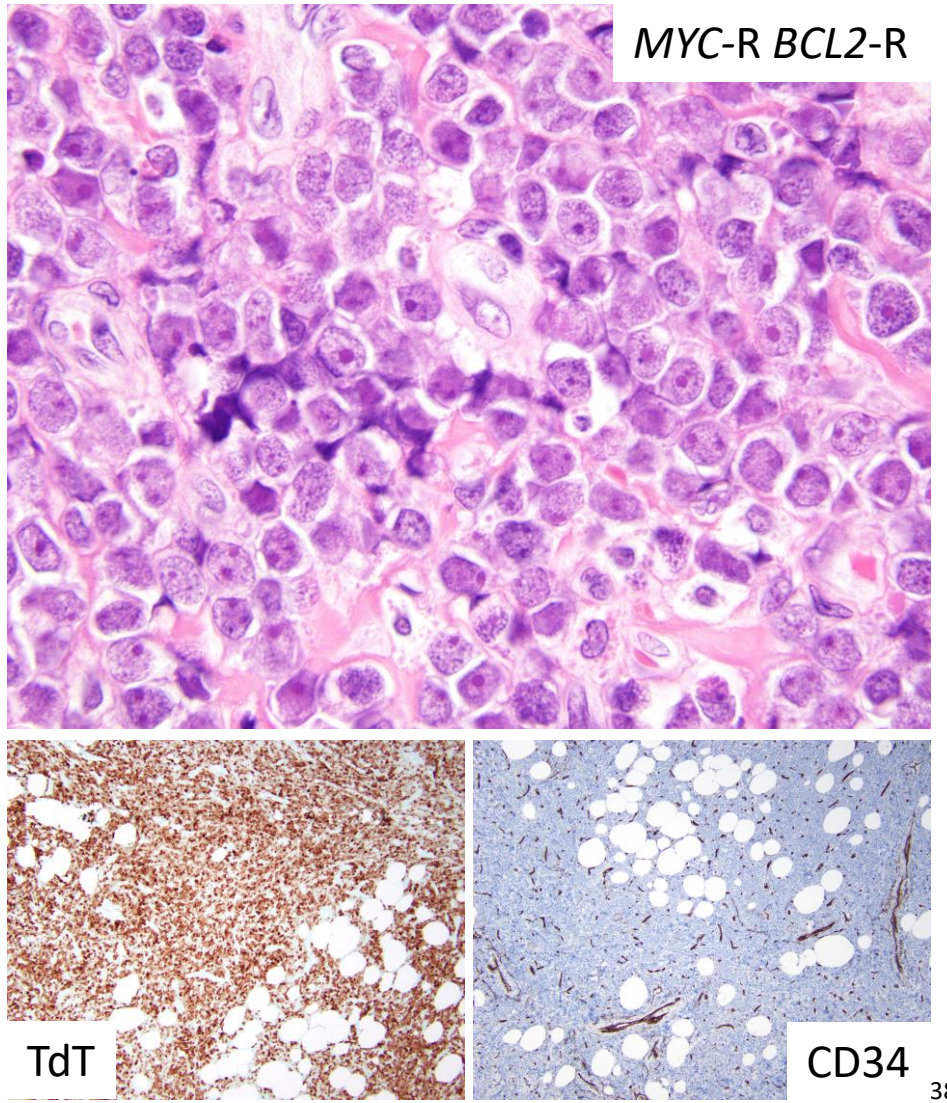
*consider BL if solo *MYC*-R and Burkitt-like morphology

TdT expression and "double-hit" genetics

The **WHO R4th** suggested blastoid morphology + TdT expression + double hit = B-lymphoblastic leukemia/lymphoma (B-ALL)

What have we learned?

- TdT expression has been reported in 2-15% of DHL
- **ICC** strongly advocates caution in TdT+ DHL cases
  - often transformed FL or relapse of prior aggressive TdT-neg disease
  - features not typical of B-ALL (no CD34; presence of light chain restriction, CD20 expression, somatic hypermutation)
- **WHO 5th** includes a subtype **DLBCL/HGBL with rearrangements of *MYC* and *BCL2* (with/without *BCL6* rearrangement) and TdT expression**
- true cases of B-ALL with DH genetics exist but are rare → apply strict clinical and pathological criteria



https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=32092

Q&A

If nearly all cases with double-hit genetics are GCB, do I need to perform FISH for ABC/non-GCB cases?

- yes, in order to capture cases classified as **high-grade B-cell lymphoma with *MYC* and *BCL6* rearrangements (ICC)** (~50% are ABC/non-GCB) and to capture cases misclassified by IHC algorithms (<5%)
- our clinicians want the prelim to include CD10 status; if they hear that the lymphoma is CD10-negative, they interpret this is a low chance of the case having DHL genetics

What is the best FISH strategy?

- *MYC* breakapart probe with reflex to *BCL2* and *BCL6* breakapart probes is acceptable
  - no requirement to identify *MYC* partner
    - conflicting data regarding prognosis depending on IG or non-IG partner genes
- *MYC* breakapart will miss cryptic rearrangements
  - *MYC::IGH* dual-color, dual-fusion increases sensitivity (but not to 100%)

When is a double-hit not a double-hit?

- cases with morphologic features of follicular lymphoma
- rare cases of B-ALL with double-hit genetics

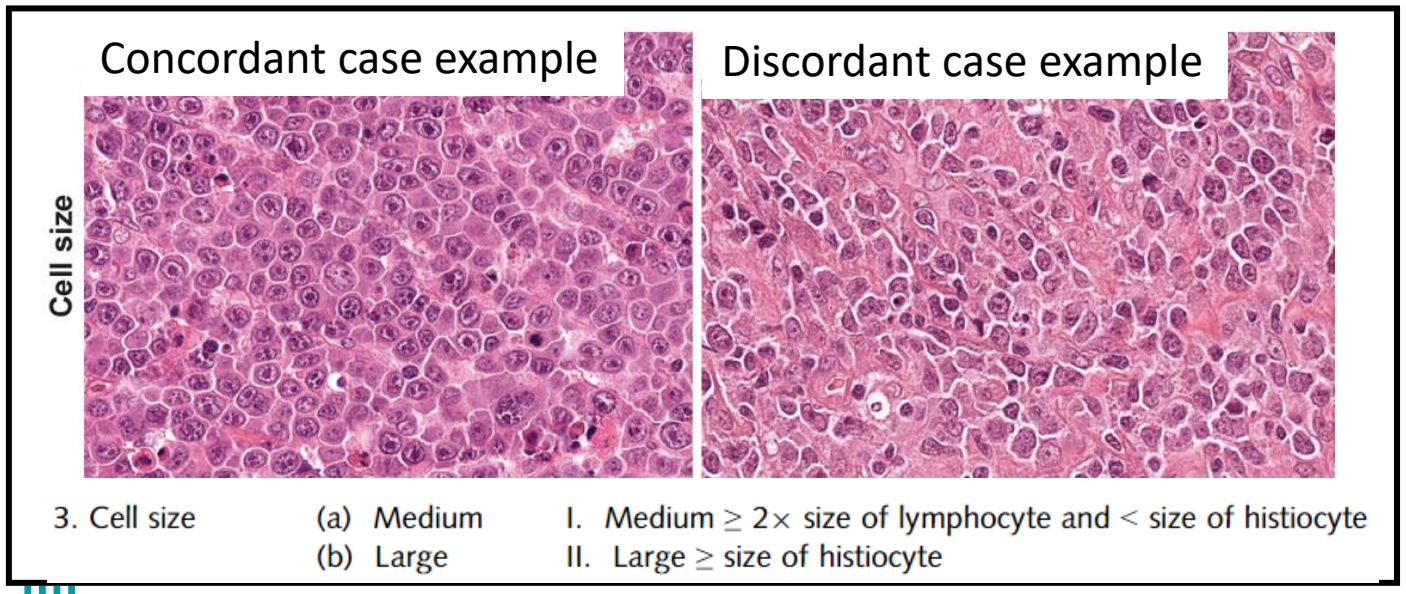
Q&A

What is high-grade B-cell lymphoma, NOS again?

- catch-all for cases with high-grade (intermediate; blastoid) morphology that are not double-hit, Burkitt lymphoma, mantle cell lymphoma, **large**/**high-grade** B-cell lymphoma with 11q aberration or B-ALL

How reproducible is high-grade morphology? **Not very**

- subjective; requires well-fixed, well-cut, well-stained sections
- recent study with central pathology review of 61 tumors submitted as high-grade B-cell lymphoma, NOS reclassified 48% to DLBCL and 5% to Burkitt lymphoma (Collinge BJ et al. Hematol Oncol (abstract). 2021.)



- 8 pathologists assessed *MYC*-R cases for 6 histopathological features associated with large B-cell lymphomas (architecture, cell size, cytology, nuclear pleomorphism, nucleoli, starry sky)
- despite standardization of scoring criteria, approximately 50% of large B-cell lymphoma cases had no majority score and spanned all histopathological features

Natkunam Y et al. *Histopathology*. 2023 Feb 27.

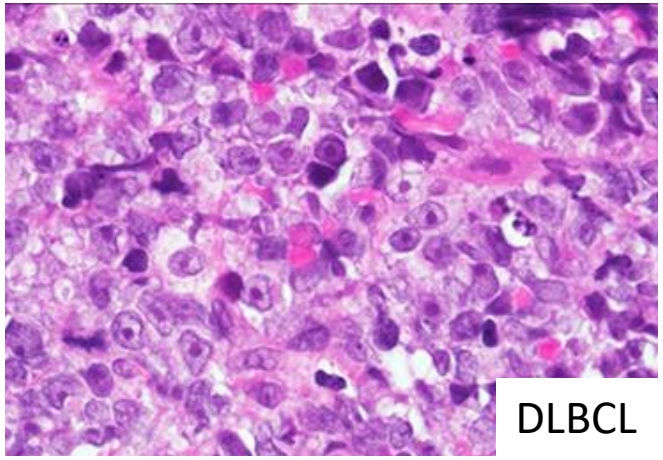
Q&A

How often should we use the diagnosis of HGBL,NOS?

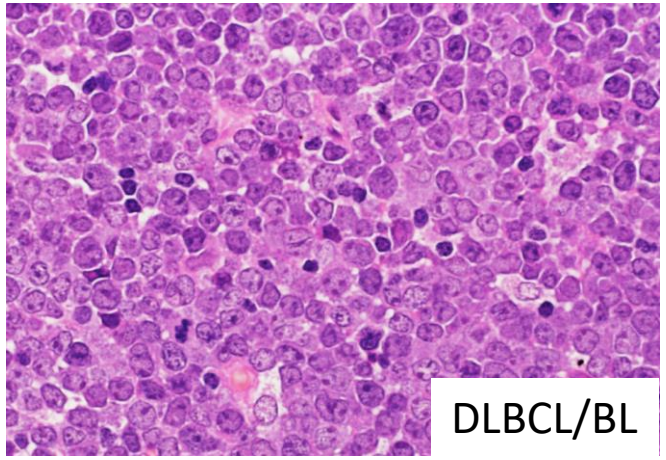
- sparingly

Should we continue to report high-grade morphology if a case is found to have DH genetics?

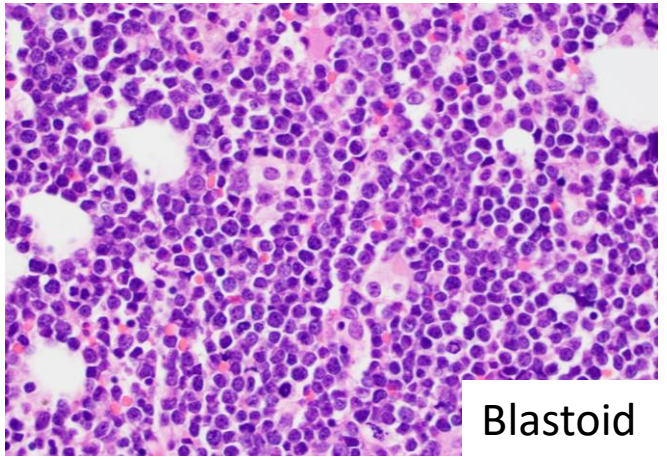
- **WHO 5th:** Yes, it is part of the naming strategy
- **ICC:** Yes, high-grade may have worse prognosis (as reported in **WHO R4th**)



DLBCL



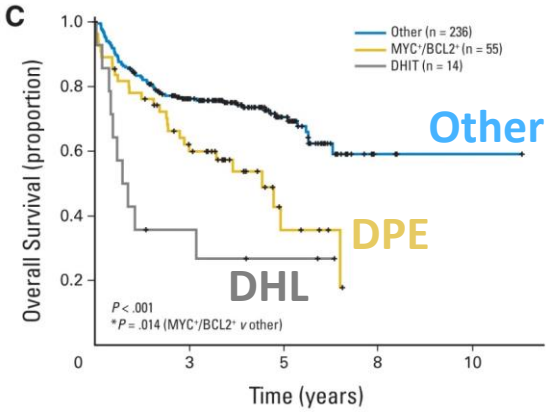
DLBCL/BL



Blastoid

High grade

Q&A



What about double-protein expressors (DPE) = BCL2 IHC >50% and MYC IHC >40%?

- DPE cannot predict double-hit genetics
- it was previously encouraged to report DPE status due to prognostic implications
  - newer studies show conflicting findings depending on genetics
- **ICC** "recommends deemphasizing DPE, since these cases most probably represent the final stage of different biological pathways"
- **WHO 5th** notes adverse prognosis of DPE "may not be independent of the prognostic impact of mutational subgroups"

What is the significance of MUM1 expression in DLBCL?

- DLBCL,NOS: 35-65%
  - ~30-50% of cases with CD10+ and MUM1+ (=GCB in Hans algorithm) show non-GCB profile by GEP
- double-hit: usually negative
- high-grade B-cell lymphoma with *MYC*-R and *BCL6*-R: 40-90%
- strong MUM1 and diffuse architecture, particularly in Waldeyer ring/cervical lymph node and with BCL6 co-expression, should prompt FISH screening for large B-cell lymphoma with *IRF4*-R

Should we continue to assess for cell-of-origin, and if so, is an IHC algorithm acceptable?

- yes to both for now → may change in future
- had been hope that non-GCB cases would respond to targeted therapies, but the results were disappointing

Johnson NA et al. *J Clin Oncol* 2012 30:28, 3452-3459.

Spoiler alert: it isn't getting less complicated…

nature medicine — RESOURCE
https://doi.org/10.1038/s41591-018-0016-8
Corrected: Publisher Correction; Author Correction

Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes Chapuy B et al. *Nat Med.* 2018;24(5):679-690.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

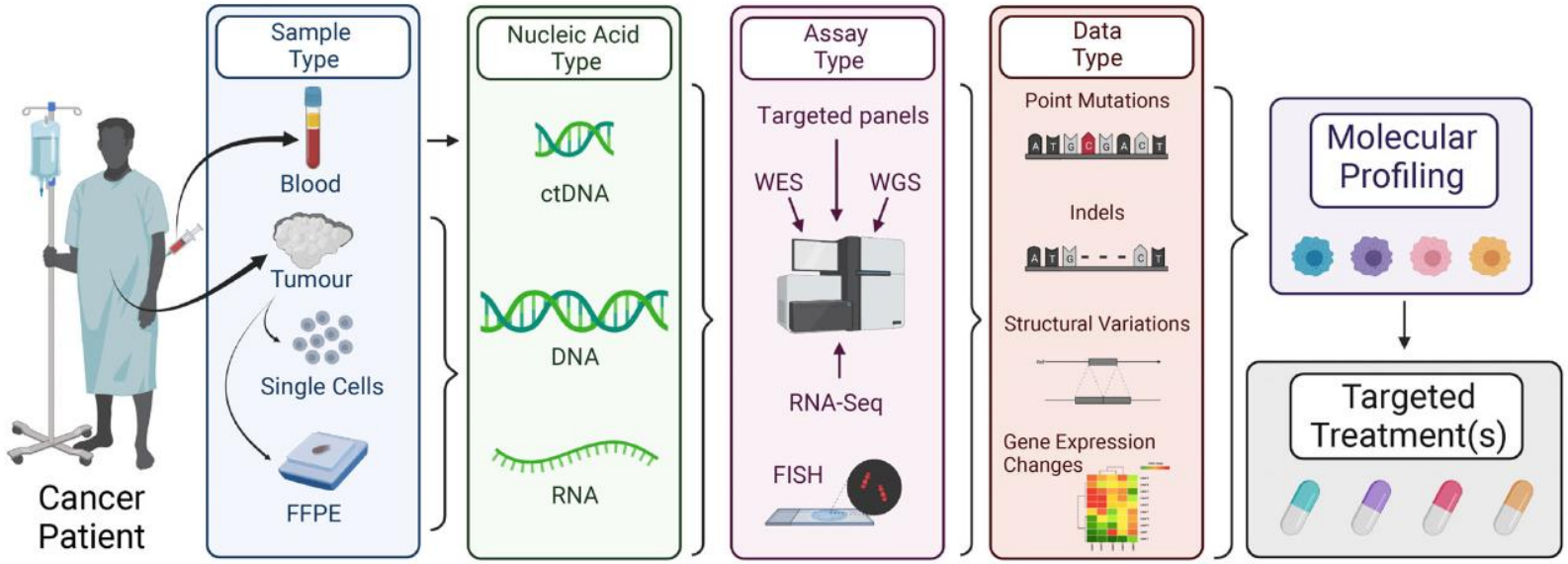
Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma
Schmitz R et al. *N Engl J Med.* 2018; 378(15):1396-1407.

blood — Regular Article

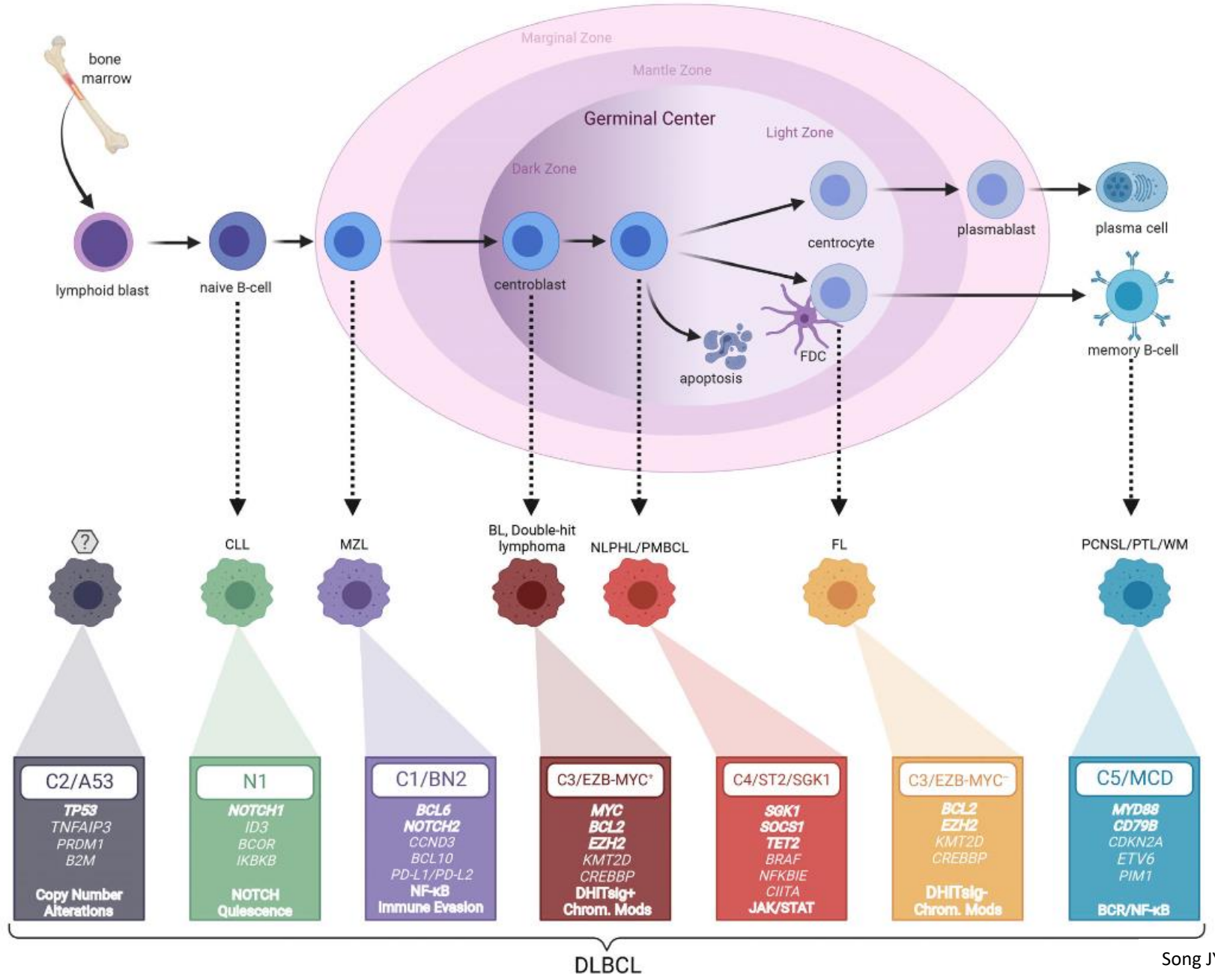
LYMPHOID NEOPLASIA

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

Classification into biological groups with clinical significance based on mutational profile, somatic copy number alterations, structural variants, gene expression changes



Morin RD et al. *Br J Haematol.* 2022;196:814–829.



Song JY et al. *Virchows Archiv.* 2023;482:179–192.
Morin RD et al. *Br J Haematol.* 2022;196:814–829.
Wright GW et al. *Cancer Cell.* 2020;37(4):551-568.e14.

"The **ICC** recommends retaining the COO classification at the present time with the expectation that transition to a more precise molecular genetic classification integrating the sequencing analysis of these tumors will be feasible in the near future."

"However, no unifying concept for proposed clusters and the significance of their genetic drivers has been established so far, precluding the definition of a unified genetic framework of DLBCL,NOS at the present time…Therefore, it was considered premature to introduce such molecular classifications in **WHO-HAEM5**."

Large B-cell lymphoma: Summary

Work-up of large B-cell lymphoma

- morphology (specify “large-cell”, or “high-grade” which includes intermediate and blastoid)
- cell-of-origin (IHC algorithm OK)
- FISH for MYC with reflex to/concurrent BCL2 and BCL6 (breakapart probes OK)
- consider FISH for *IRF4* if MUM1 is strong, BCL6 is also positive, and correct clinical context

Both classification systems recognize large B-cell lymphomas with *BCL2*-R and *MYC*-R to be a unique entity

- **WHO 5th** allows two names depending on morphology
  - Diffuse large B-cell lymphoma with *MYC* and *BCL2* rearrangements
  - High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements
- **ICC** also lists high-grade B-cell lymphoma with *MYC* and *BCL6* rearrangement as a provisional entity
- expression of TdT in a case with *BCL2*-R and *MYC*-R ≠ B-ALL unless strict clinical and pathologic criteria met

High grade B-cell lymphoma, NOS should be used sparingly

- “high-grade” morphology is not very reproducible
- rule-out double-hit, Burkitt lymphoma, mantle cell lymphoma, HGBCL with 11q aberration and B-ALL

The future is coming… and may negate a lot of these details (but we have a ways to go before clinical implementation!)

Sheets of large B cells: Recommended IHC work-up

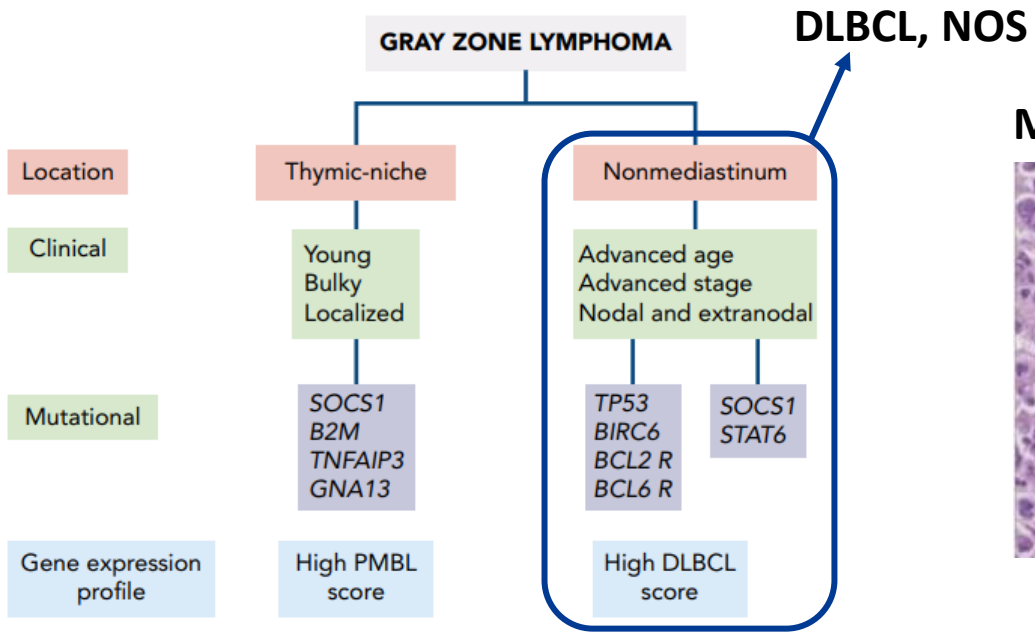
CD10	• Cell-of-origin classification • Clinicians like to know upfront a case is CD10-negative → unlikely FISH will reveal DH
BCL6	• Cell-of-origin classification • Cannot act as a screen for BCL6-R
MUM-1	• Cell-of-origin classification • If very strong, consider FISH for large B-cell lymphoma with *IRF4* rearrangement (particularly if BCL6+ & appropriate clinical setting)
BCL-2	• If negative, evaluate for Burkitt lymphoma • Evaluate for double-protein expression along with MYC (less emphasis in updated classifications) • Cannot act as a screen for *BCL2*-R
MYC	• If uniformly positive, evaluate for Burkitt lymphoma • Evaluate for double-protein expression along with BCL2 (less emphasis in updated classifications) • Cannot act as a screen for *MYC*-R
CD21, CD23, CD35	• Assess for lack of follicular dendritic cell (FDC) meshworks • If FDC meshworks present = evaluate for follicular lymphoma
EBER ISH	• Evaluate for EBV-positive DLBCL
TdT	• May be positive in DH cases (2-15%) • If +, carefully evaluate for B-ALL (CD34, CD20, surface light chain, clinical features)
CD5	• Can be positive in DLBCL (5-10%; often ABC) • Does not need to be reported in the top-line (not a true biologically distinct group)
Cyclin D1	• Always, to exclude mantle cell lymphoma (esp. blastoid or pleomorphic)
CD30	• Often positive with anaplastic morphology • May provide information regarding targeted therapeutics
Ki67	• DLBCL morphology + very high Ki67 and/or starry sky + no DH genetics = DLBCL, NOS (not high-grade B-cell lymphoma, NOS) • 100% → evaluate for Burkitt lymphoma

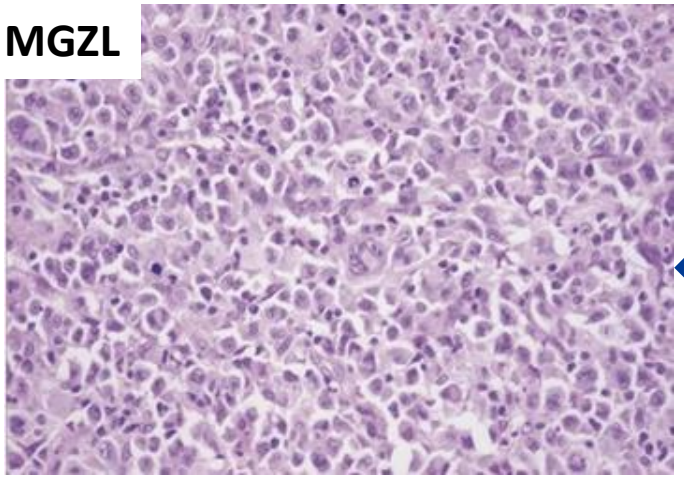
Large B-cell lymphomas: Comparison

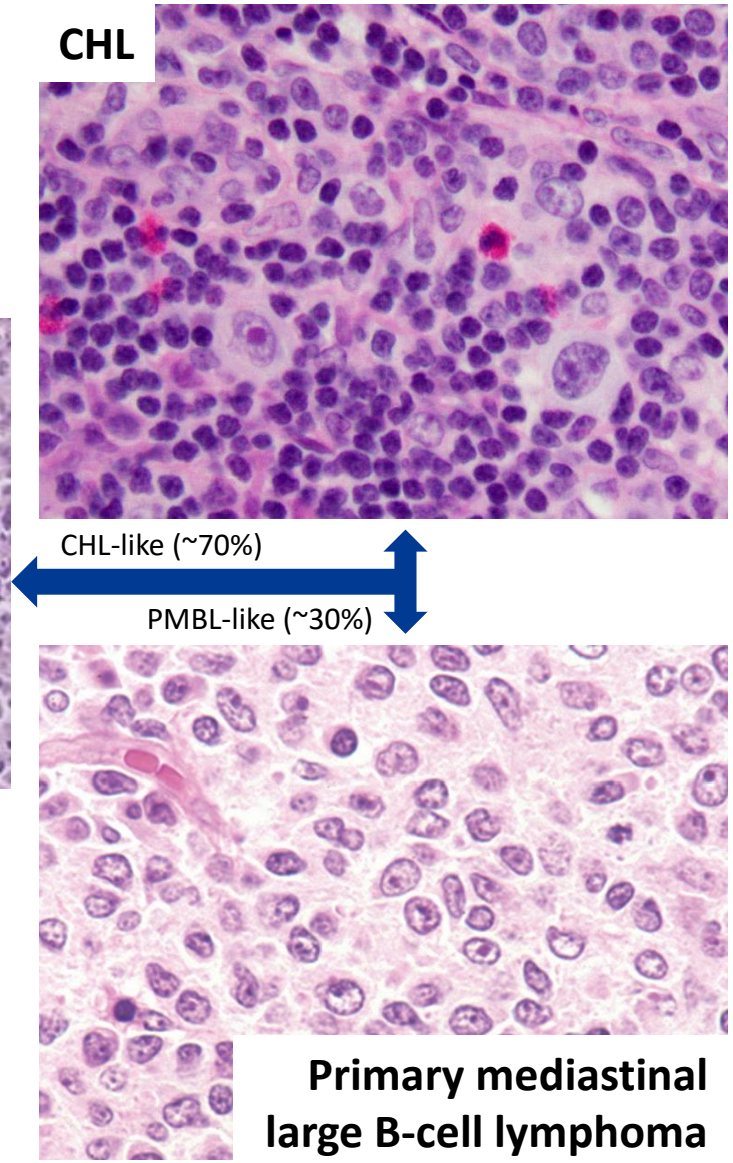
WHO R4th	WHO 5th	ICC
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma	Mediastinal grey zone lymphoma	Mediastinal gray-zone lymphoma
Burkitt-like lymphoma with 11q aberration	High-grade B-cell lymphoma with 11q aberrations	Large B-cell lymphoma with 11q aberration (provisional)
EBV-positive diffuse large B-cell lymphoma, NOS	EBV-positive diffuse large B-cell lymphoma	EBV-positive diffuse large B-cell lymphoma, NOS
Not previously included (subtype of DLBCL associated with chronic inflammation)	Fibrin-associated large B-cell lymphoma	Fibrin-associated diffuse large B-cell lymphoma (considered subtype of DLBCL associated with chronic inflammation)
Not previously included	Fluid overload-associated large B-cell lymphoma	HHV-8 and EBV-negative primary effusion-based lymphoma (provisional)
Partially encompasses Primary DLBCL of the CNS	Primary large B-cell lymphoma of immune-privileged sites: Primary LBCL of the CNS	Primary DLBCL of the central nervous system (includes vitreoretinal)
	Primary large B-cell lymphoma of immune-privileged sites: Primary LBCL of the vitreoretina	Not separate
	Primary large B-cell lymphoma of immune-privileged sites: Primary LBCL of the testis	Primary DLBCL of the testis

Mediastinal grey zone (gray-zone) lymphoma

- New name for **B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma**



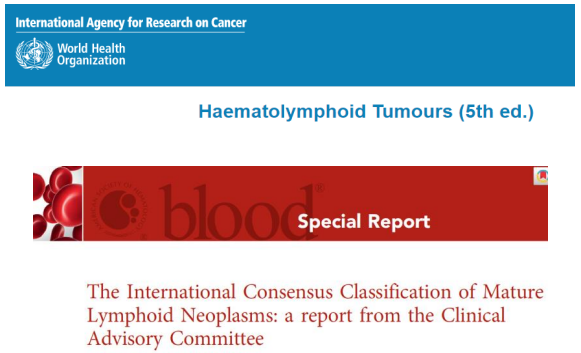




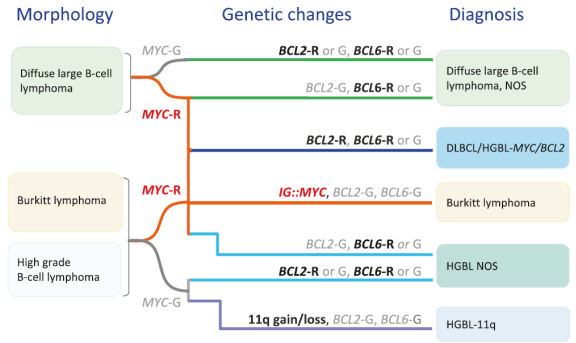
- CHL-like morphology + strong PAX5, CD20, one additional strong B-cell marker
- PMBL-like morphology + strong CD15 and partial or complete loss of B-cell markers

- EBER ISH should be negative → EBV+ DLBCL with Reed-Sternberg cells = EBV+ DLBCL

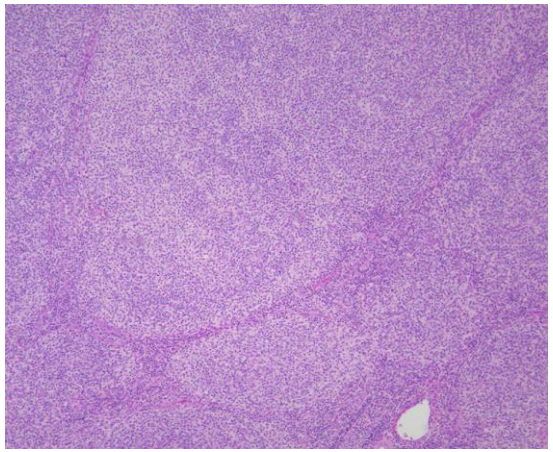
Sarkozy C et al. *Blood*. 2021 Apr 1;137(13):1765-1776. Gualco G et al. Mod Pathol 25, 661–674 (2012). Campo E, Jaffe ES. *Blood*. 2021 Apr 1;137(13):1703-1704.



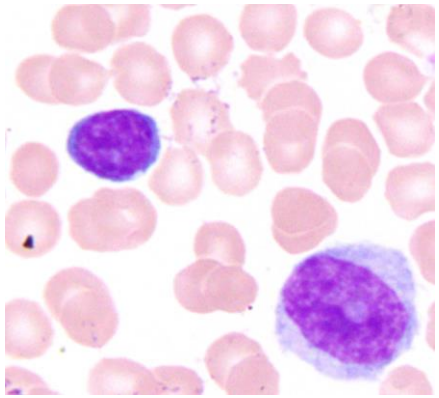
What's happening with the classification systems?



Large B-cell lymphomas with emphasis on double-hit lymphomas



Follicular lymphoma and related entitles



Other mature B-cell lymphomas

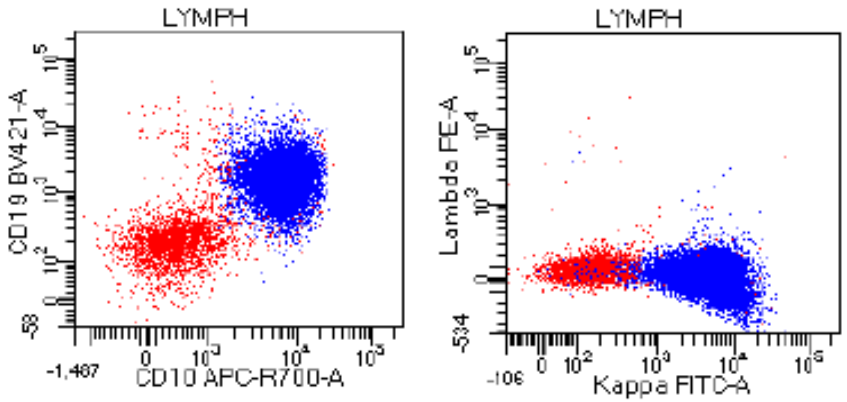
Follicular lymphoma: Definition

General features

- neoplasm of germinal center B cells (CD10+, BCL6+), often with at least a partial follicular pattern, typically driven by *IGH::BCL2* fusion
- lymph nodes, spleen, bone marrow, extranodal sites; typically widespread at diagnosis; not typically associated with B symptoms
- mean age: 6th decade
- 20% of all lymphomas

When to suspect follicular lymphoma

- work up of clonal CD10-positive B cells
- atypical-appearing follicles



Differential diagnosis of clonal CD10+ B cells

Follicular lymphoma

Diffuse large B-cell lymphoma, GCB type

"Double-hit" lymphoma

Burkitt lymphoma

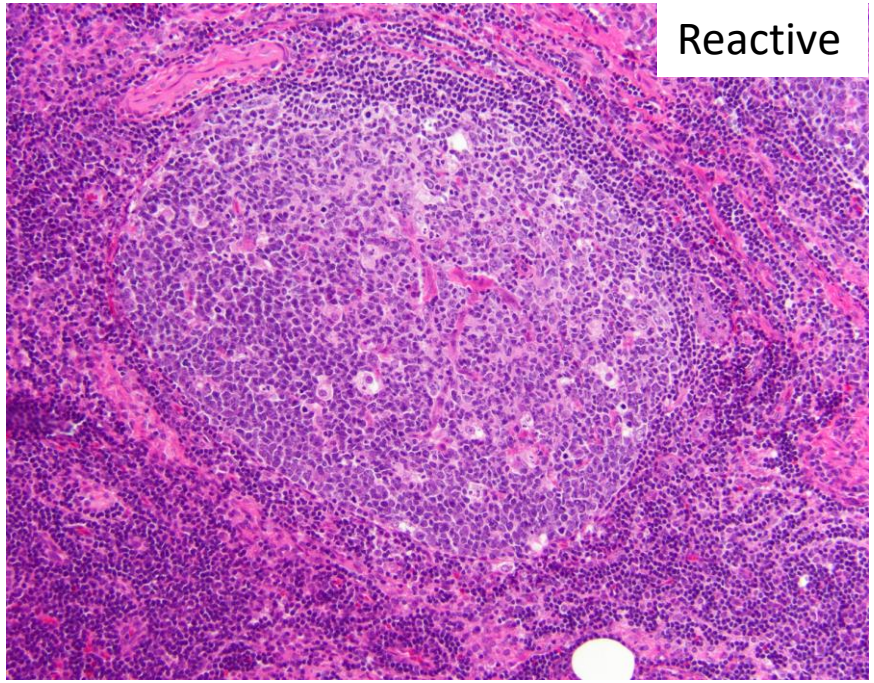
Aberrant CD10 in other small B-cell lymphomas (rare)

Follicular lymphoma: Diagnostic features

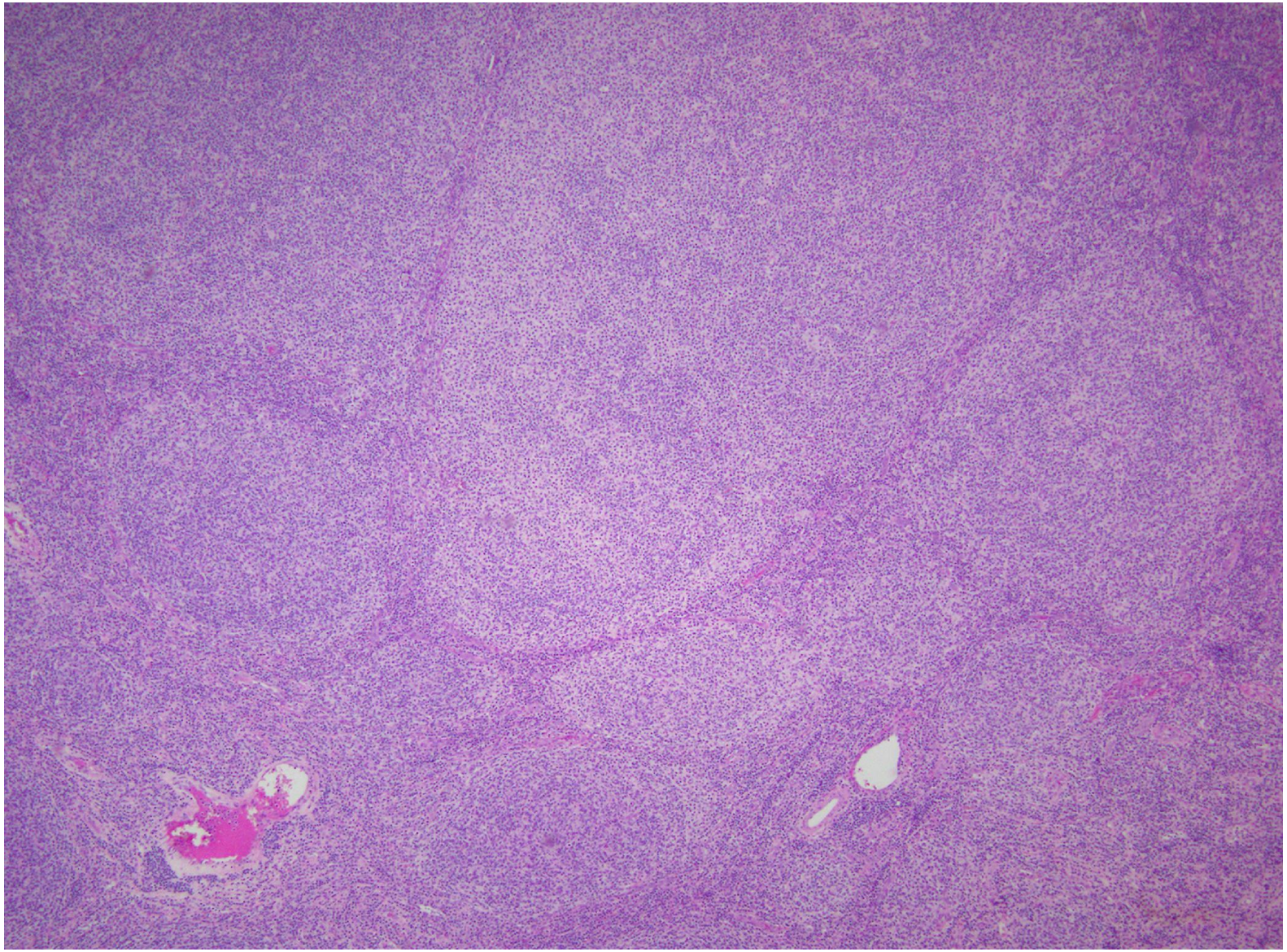
Atypical morphologic features:

- back-to-back follicles
- attenuated mantle zones
- loss of polarization

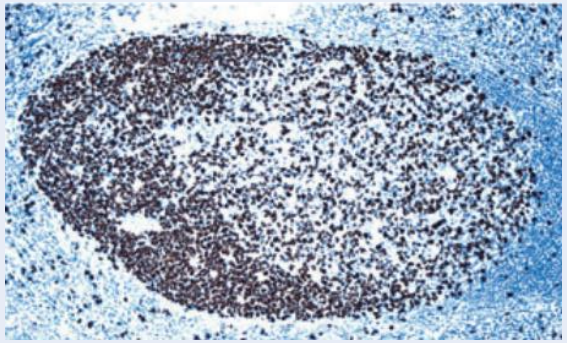
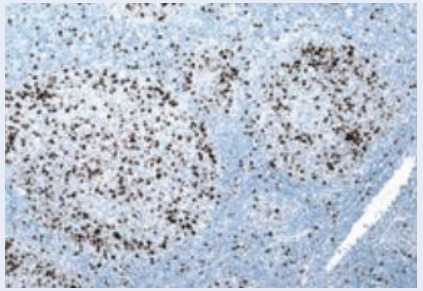
…should prompt IHC work-up



Reactive

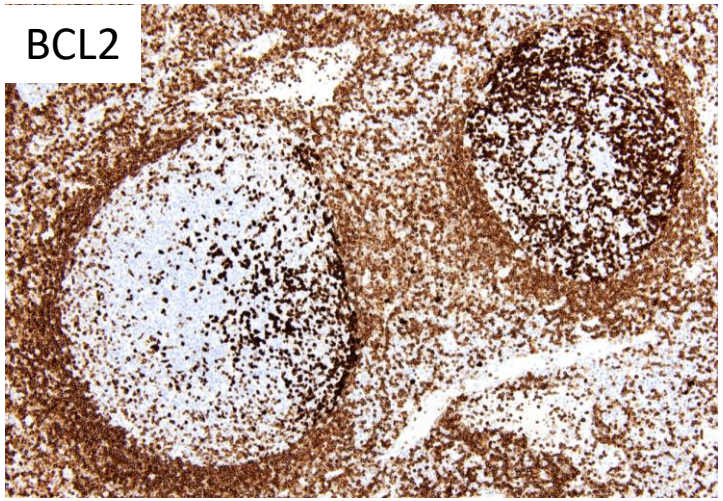


Follicular lymphoma: Diagnostic features

	Reactive	Follicular Lymphoma
CD10	positive in GC cells	positive in GC cells
BCL6	positive in GC cells	positive in GC cells
BCL2	**negative in GC cells*** *normal primary follicles are BCL2+ *normal T cells are BCL2+	**positive in GC cells**
Ki67	high  polarization	variable; typically lower than reactive GC  no polarization

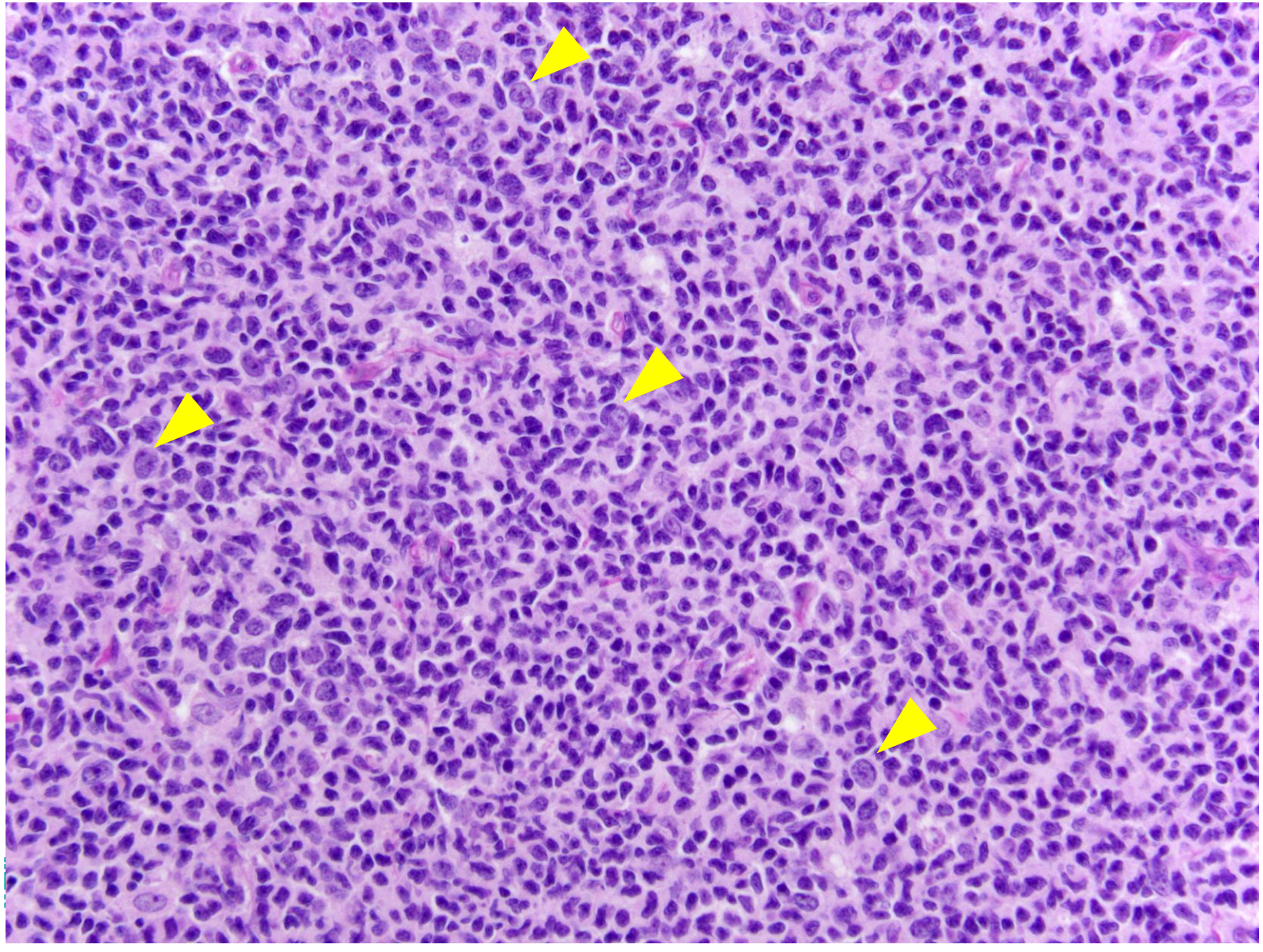
Occasionally strong BCL2 will be detected in a few follicles in otherwise normal reactive lymph nodes or lymphoid tissues at extranodal sites = **in situ follicular neoplasia** or **in situ follicular B-cell neoplasm**

BCL2



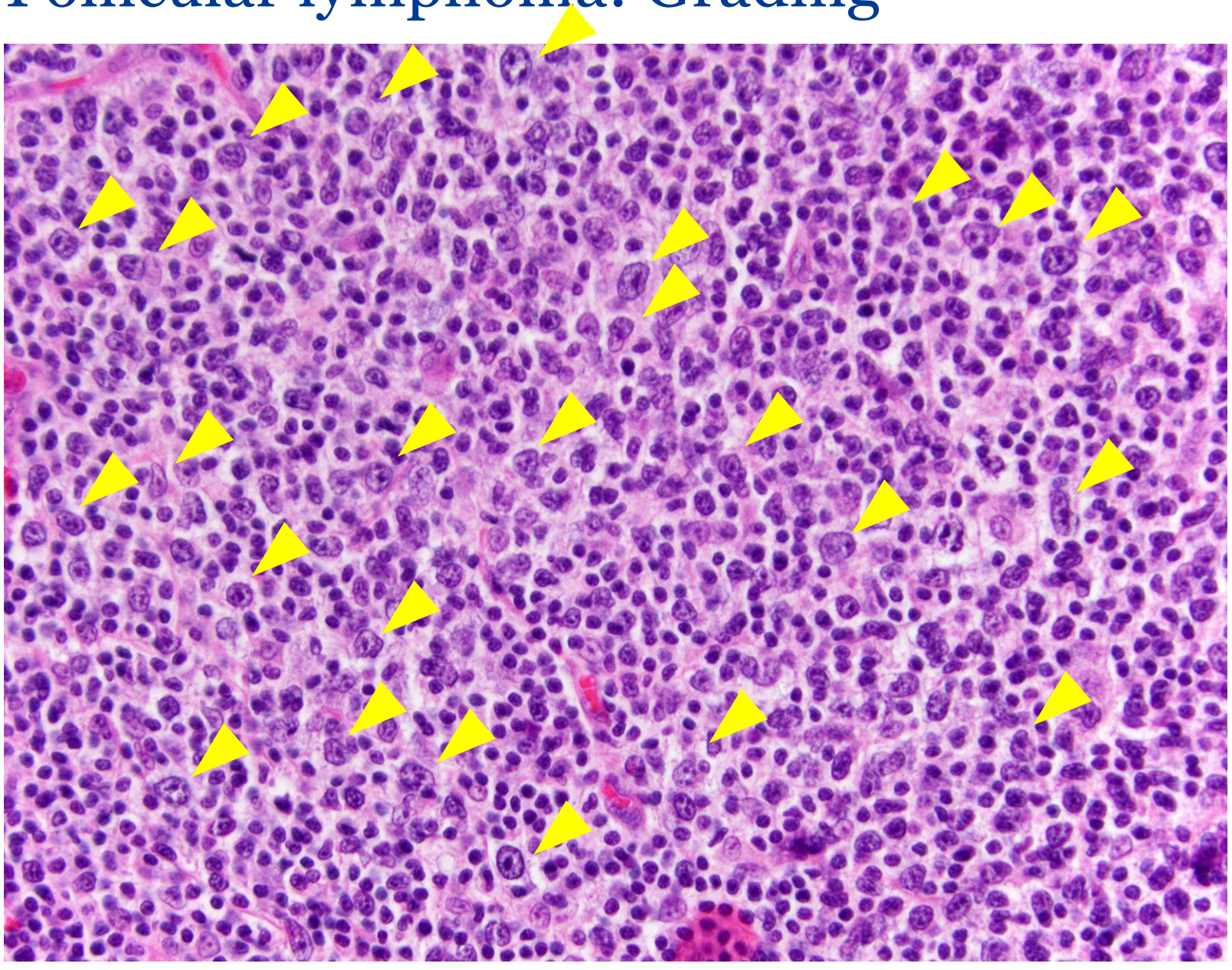
Bryant RJ et al. *Histopathology*. 2006 Apr;48(5):505-15.
https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=34243

Follicular lymphoma: Grading



WHO R4th	
Grading	
Grade 1-2 ✓	0-15 **centroblasts** per high-powered field (hpf)
Grade 3A	>15 **centroblasts** per hpf & admixed centrocytes
Grade 3B	>15 **centroblasts** per hpf, forming sheets

Follicular lymphoma: Grading



WHO R4th	
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Grade 1-2	0-15 **centroblasts** per high-powered field (hpf)
Grade 3A ✓	>15 **centroblasts** per hpf & admixed centrocytes
Grade 3B	>15 **centroblasts** per hpf, forming sheets

Follicular lymphoma: Grading

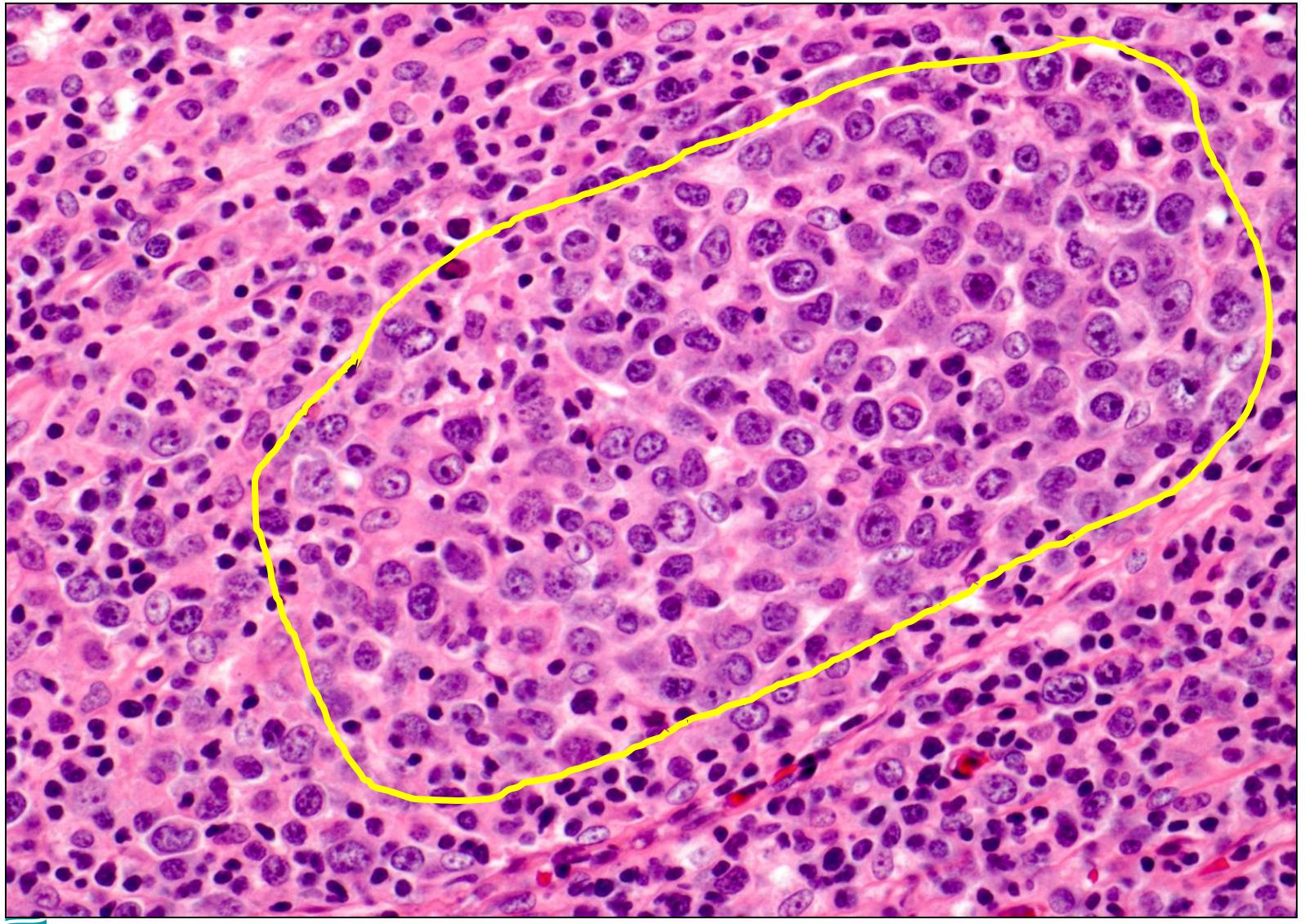
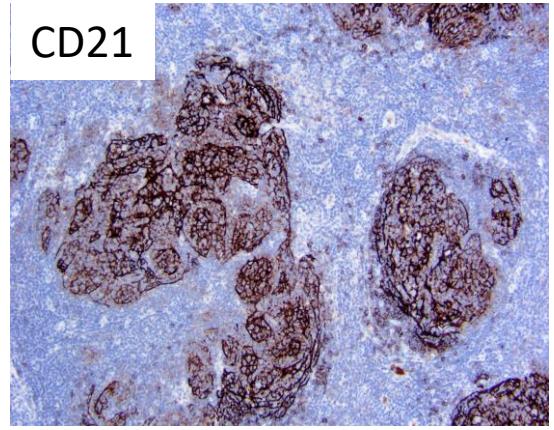


Image courtesy of Dr. A. Louissaint Jr (MGH)

WHO R4th	
Grading	
Grade 1-2	0-15 **centroblasts** per high-powered field (hpf)
Grade 3A	>15 **centroblasts** per hpf & admixed centrocytes
Grade 3B ✓	>15 **centroblasts** per hpf, forming sheets

Follicular lymphoma: Challenges



WHO R4th	
Grading	
Grade 1-2	0-15 **centroblasts** per high-powered field (hpf)
Grade 3A	>15 **centroblasts** per hpf & admixed centrocytes
Grade 3B	>15 **centroblasts** per hpf, forming sheets
Architecture	
Follicular	>75% follicular
Follicular & diffuse	25-75% follicular
Diffuse	<25% follicular

Is it this easy? No.

1. **Architecture also counts**
   - Grade 1-2 → any of the architecture patterns are allowed
   - Grade 3A or 3B → only follicular architecture is allowed
     - Grade 3 + diffuse architecture = DLBCL
2. **Intra- and interobserver variability is high!**
   - centroblasts can be difficult to distinguish from other larger cells (large centrocytes, follicular dendritic cell nuclei, macrophages)
3. **Grades 1, 2, and 3A**
   - studies suggest no statistically significant difference in clinical outcomes
4. **Pure grade 3B is very rare**
   - usually some diffuse areas
   - 3B diagnosis often treated like DLBCL

Follicular lymphoma: Updated classification

WHO R4th

Grade 1-2	0-15 **centroblasts** per high-powered field (hpf)
Grade 3A	>15 **centroblasts** per hpf & admixed centrocytes
Grade 3B	>15 **centroblasts** per hpf, forming sheets

↓

ICC

Grading and architectural pattern criteria are retained*

***Grade 3B acknowledged to have clinical and biologic behavior more like DLBCL; patients often managed like DLBCL**

WHO 5th

Classic follicular lymphoma (cFL) – grading is optional*
*classification of rare cases with 3A appearance + diffuse architecture is uncertain (FL vs DLBCL) → clinical correlation

Follicular large B-cell lymphoma (FLBCL)*
*requires a follicular architecture; extremely rare; can't diagnosis on core because insufficient tissue to rule-out diffuse component

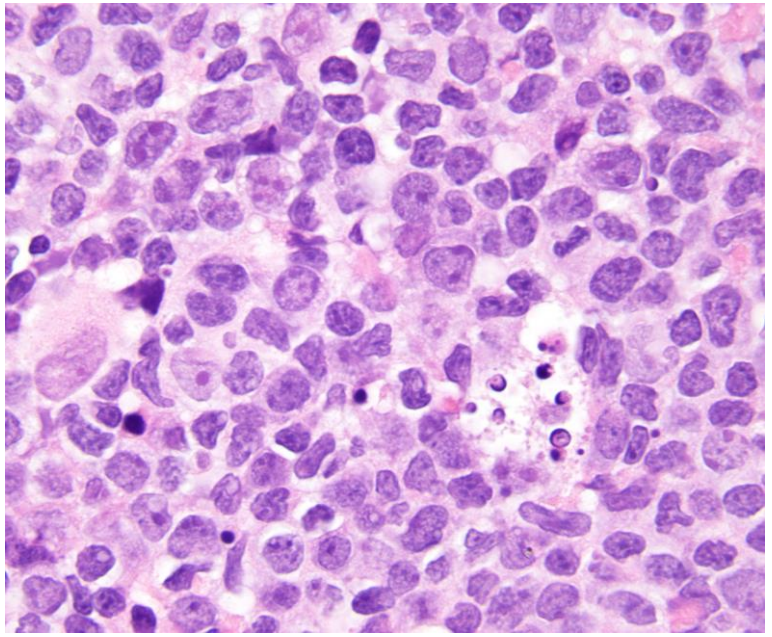
If debating between **3A**/**cFL** or **3B**/**FLBCL**

- expression of CD10 and presence of *IGH::BCL2* fusion by FISH favors **3A**/**cFL**
- lack of CD10 and lack of *IGH::BCL2* favors **3B**/**FLBCL** → often there is an associated diffuse (*i.e.* DLBCL) component → should perform DLBCL work-up including *MYC, BCL2, BCL6* FISH

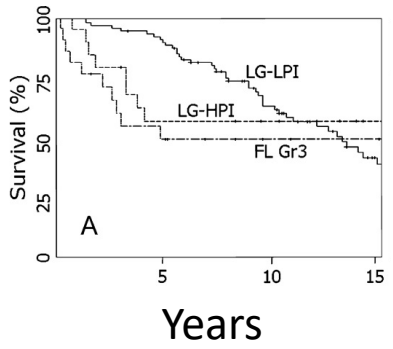
Follicular lymphoma: What if the cells look unusual?

Follicular lymphoma with unusual cytological features (uFL)

- new category only in **WHO 5th**
- either “blastoid” or “large centrocyte” morphology
- variability in immunophenotype (↑ Ki67, MUM1) compared to cFL
- differences in genetics (lower frequency *IGH::BCL2* fusion) compared to cFL
- prognostic impact is uncertain (may be inferior to cFL)
- use of this term will allow data collection



Follicular lymphoma: What if the Ki67 is really high?



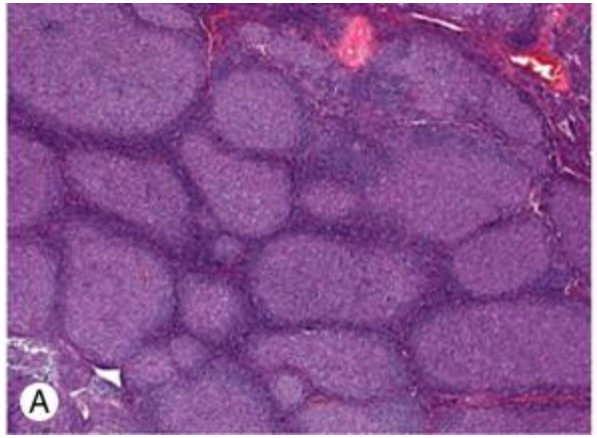
- studies nearly 20 years old suggest low-grade morphology + high proliferation index (>30%) shows more aggressive behavior
- but, uncertain clinical significance in individual cases
- still not used for grading

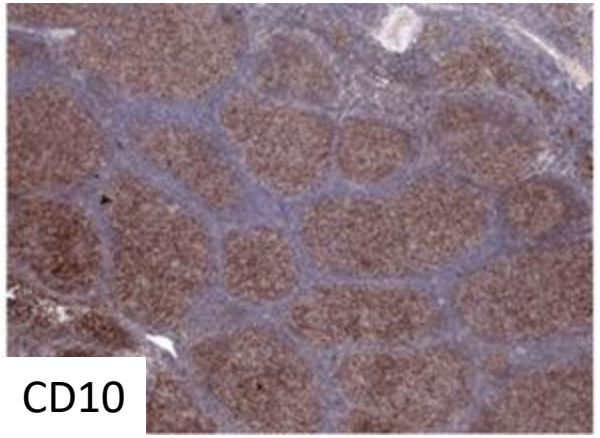
Wang S *The American Journal of Surgical Pathology* 29(11):p 1490-1496, November 2005.

Follicular lymphoma: What if BCL2 IHC is negative…?

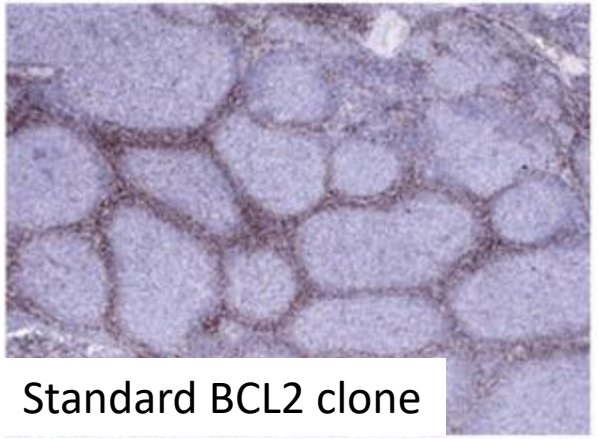
…and cytology/architecture is typical for **Grade 1, 2, 3A**/**cFL**?

- approximately 15% of cFL are negative for BCL2 IHC
- in some cases, the protein resulting from the *IGH::BCL2* fusion has lost the epitope recognized by the most common BCL2 antibody clone → try alternative BCL2 antibody clones and/or perform FISH for *IGH::BCL2* fusion

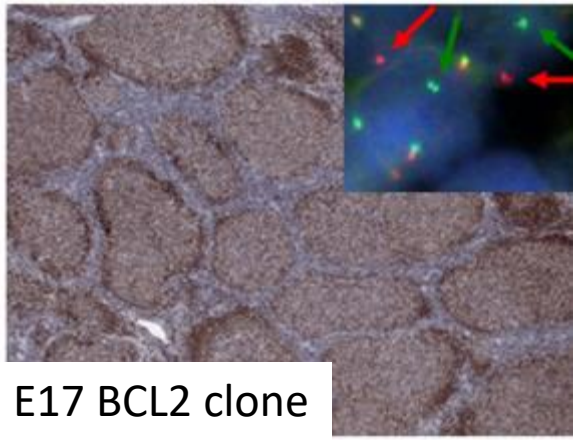




CD10



Standard BCL2 clone

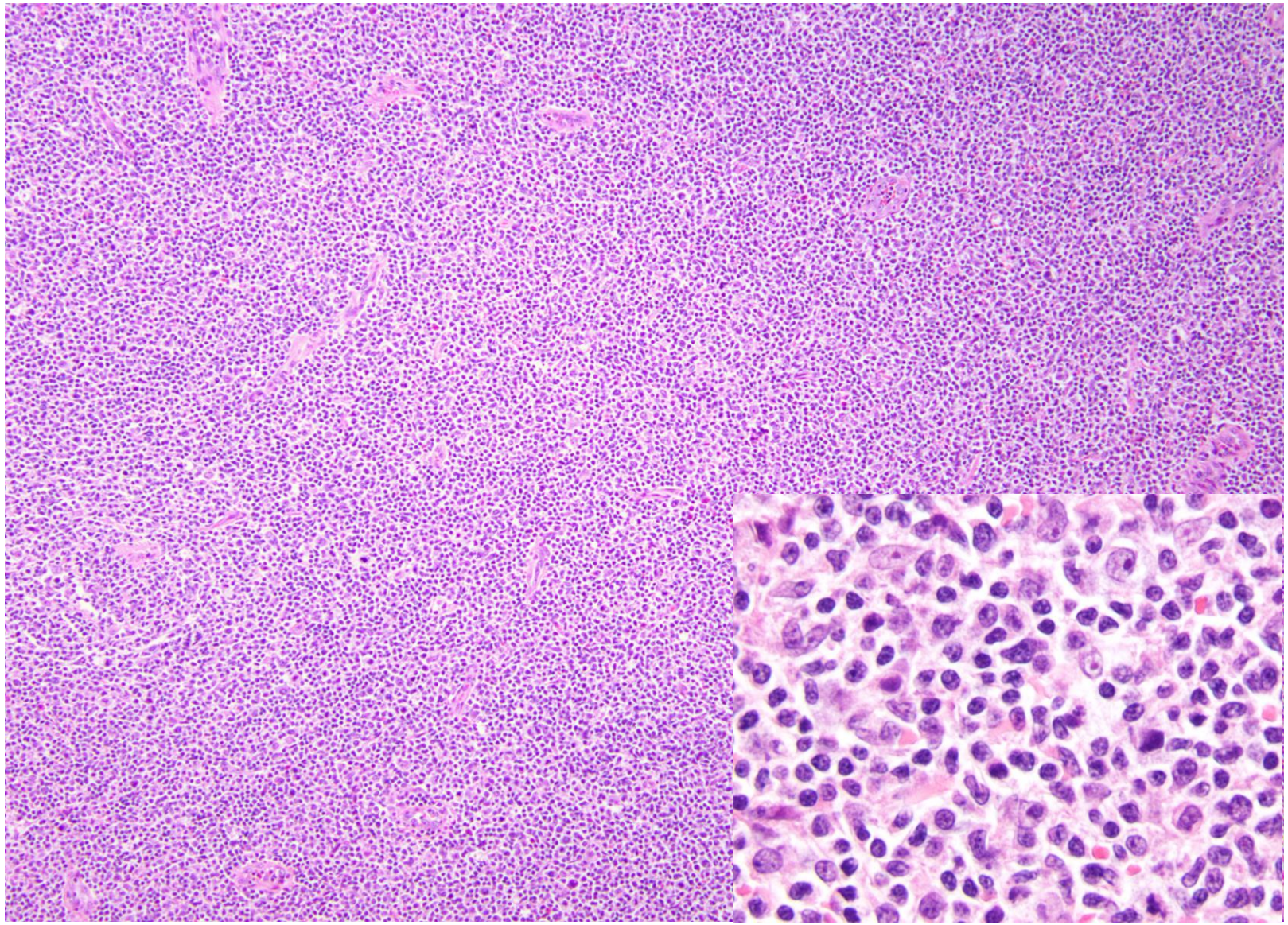


E17 BCL2 clone

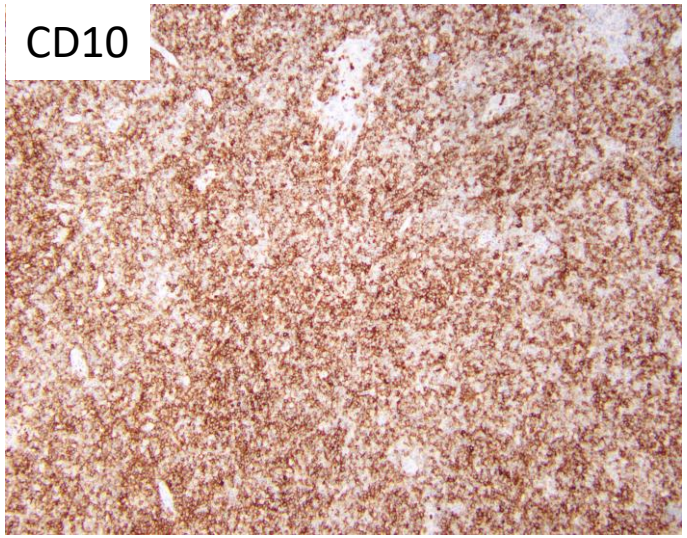
- or consider follicular lymphoma with *BCL6* rearrangement (confirm with FISH or karyotype) → may have more aggressive behavior

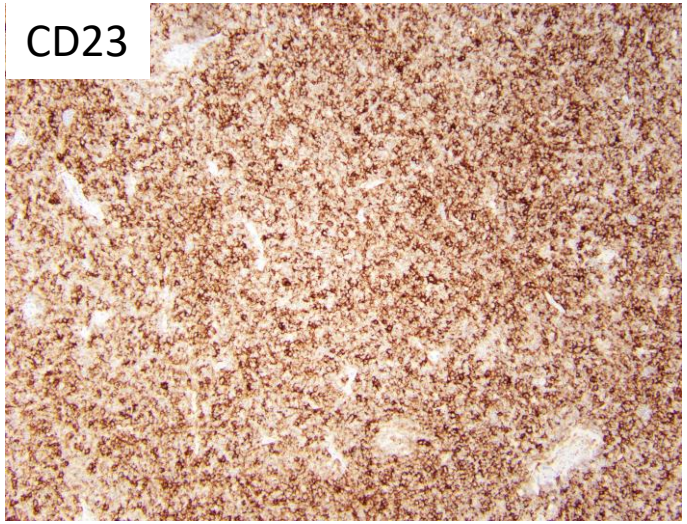
Adam P et al. *Hum Pathol*. 2013 Sep;44(9):1817-26.

Follicular lymphoma: What if BCL2 IHC is negative…?



…and CD23 is positive?





Follicular lymphoma: BCL2-negative/CD23-positive

FL with predominantly diffuse growth pattern (WHO 5th)

BCL2-rearrangement-negative, CD23+ follicle center lymphoma (ICC, *provisional*)

[criteria not identical between the two classification systems]

Clinical

- often limited to inguinal region (very large mass); typically low stage; favorable diagnosis

Morphology

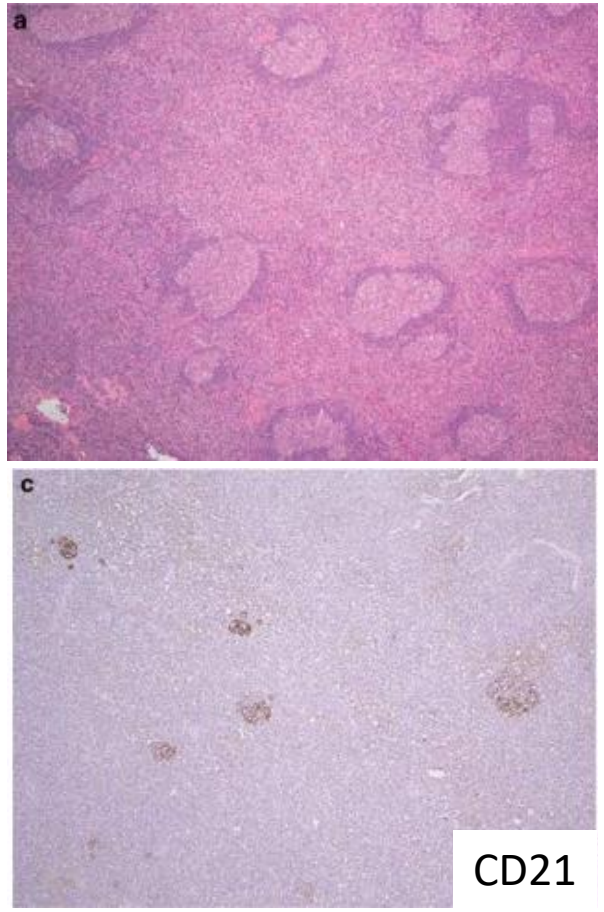
- predominantly diffuse growth pattern
  - may see small residual "microfollicles" (H&E, FDC markers)
  - pure follicular architecture accepted in **ICC** classification
- predominantly centrocytes

IHC

- at least one GC marker positive (CD10, BCL6, others)
- CD23+
- BCL2 IHC weak to absent

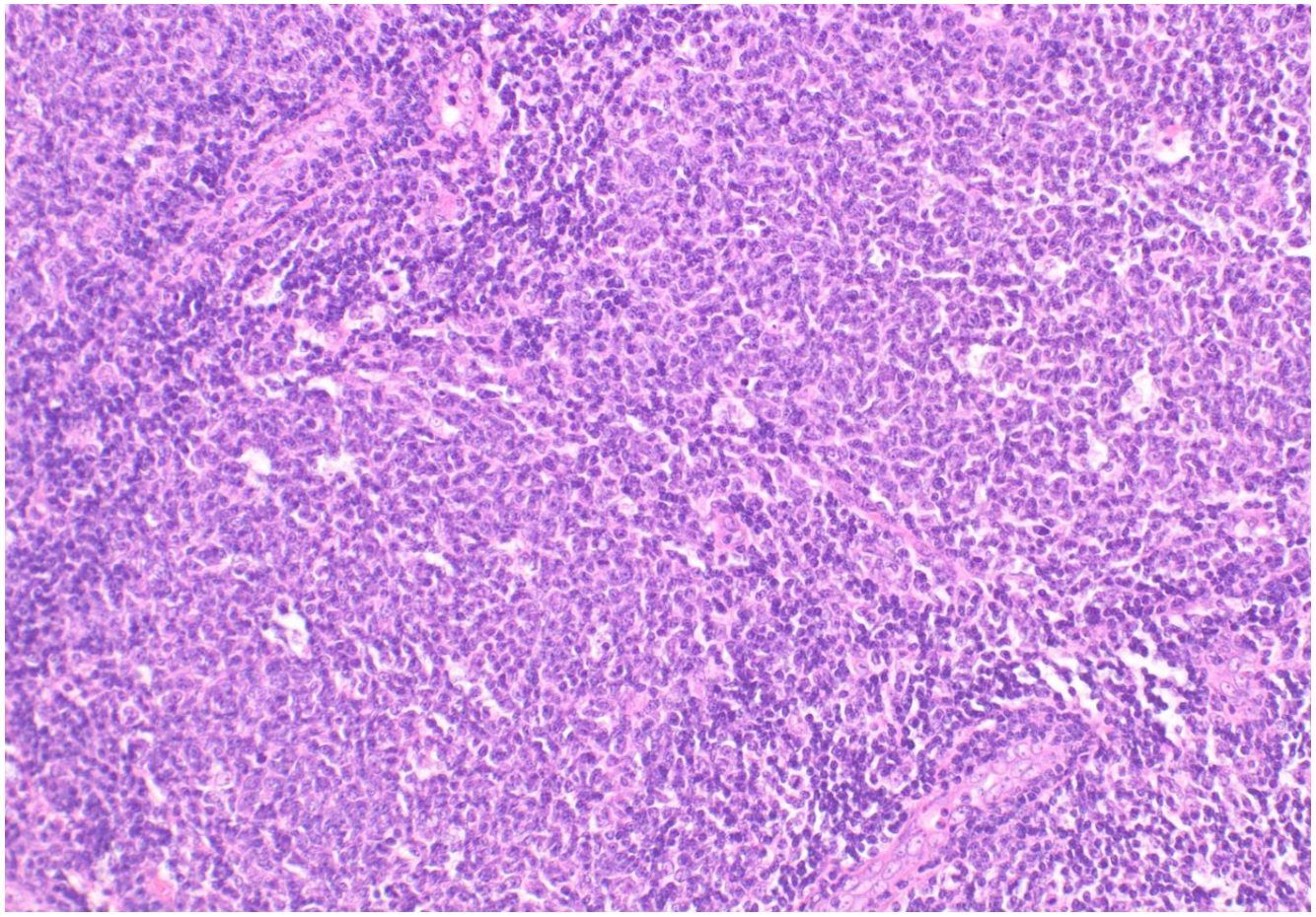
Genetics

- *CREBBP* and *STAT6* are highly recurrently co-mutated
- 1p36 loss in ~50%
- no *IGH::BCL2* fusion

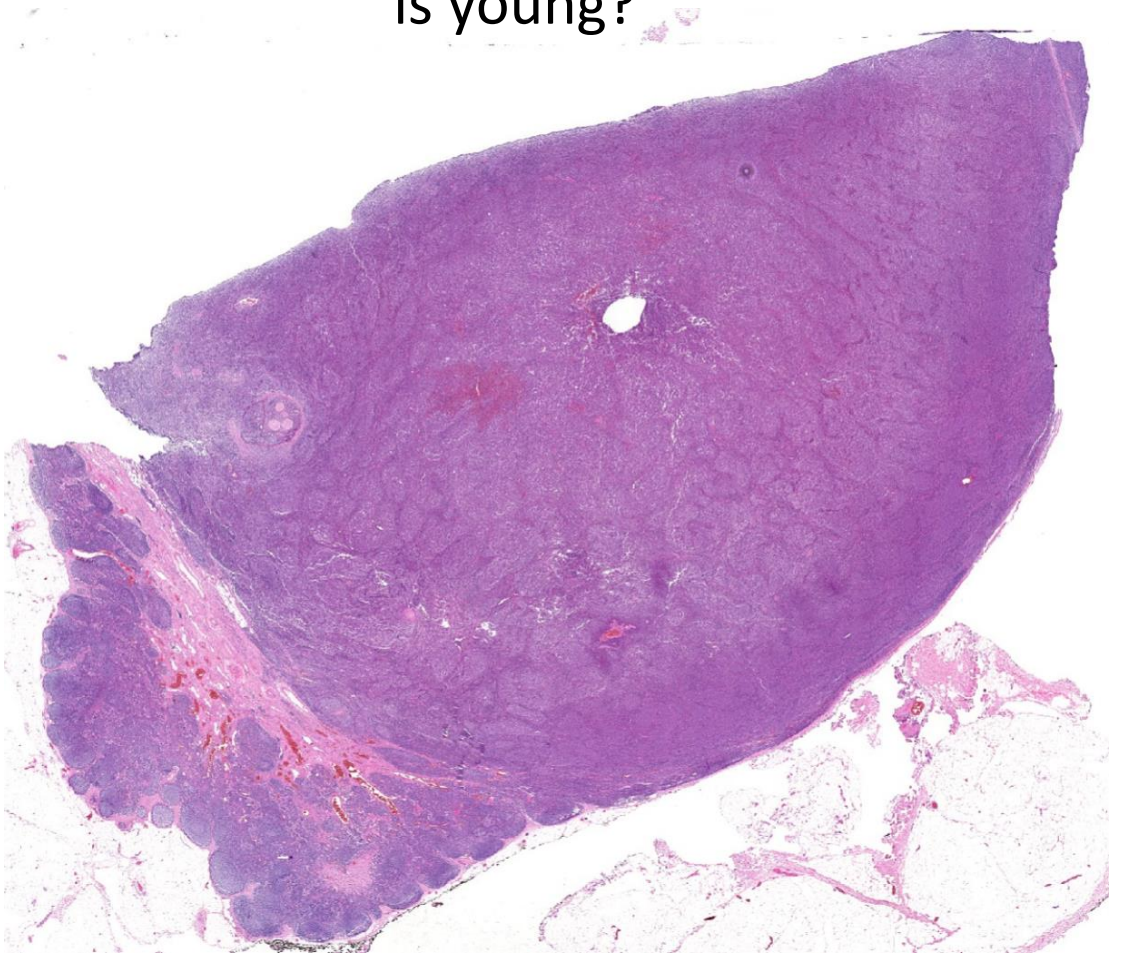


Siddiqi I et al. *Mod Pathol*. 2016 Jun;29(6):570-81.

Follicular lymphoma: What if BCL2 IHC is negative…?



…and the patient is young?



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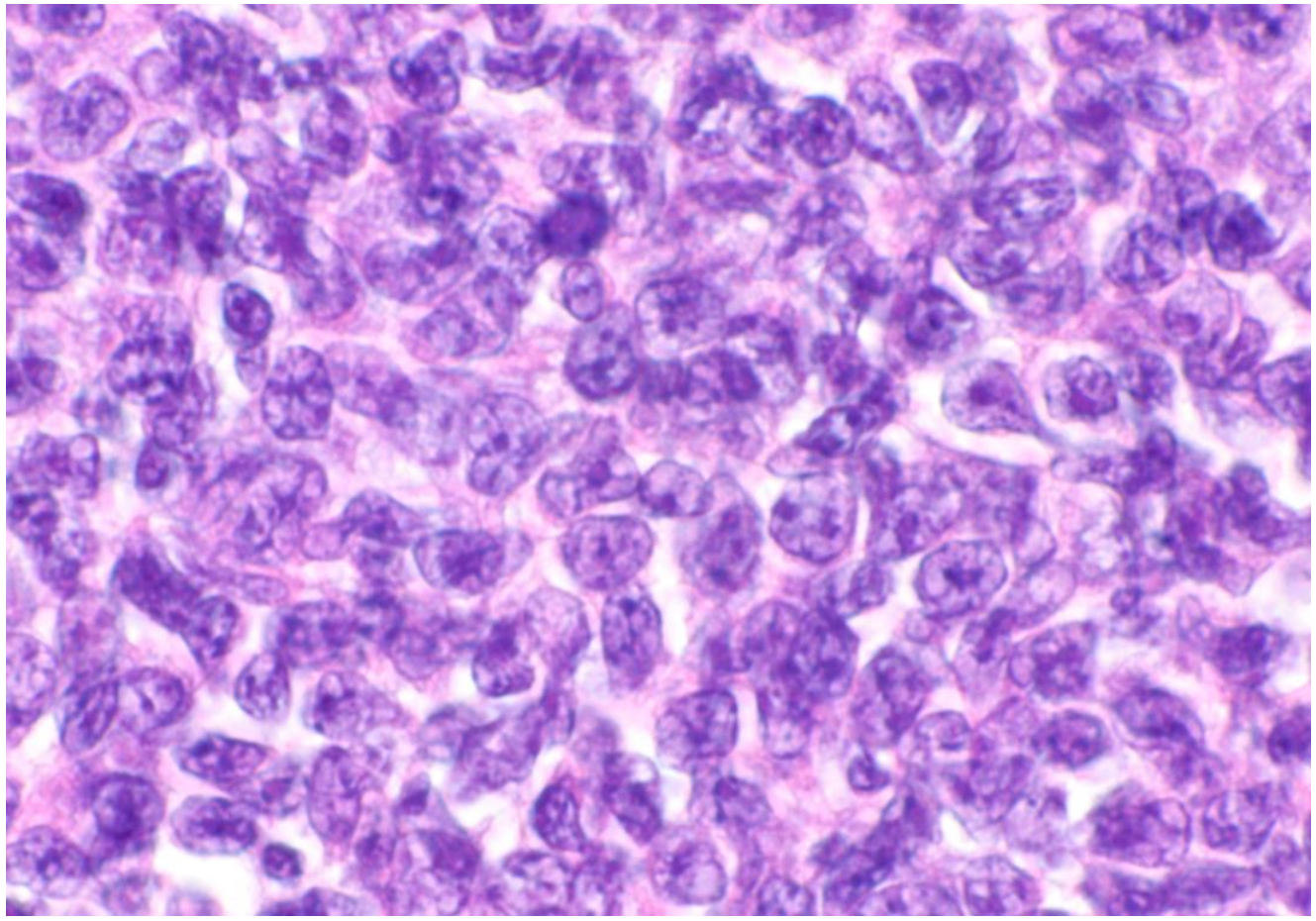
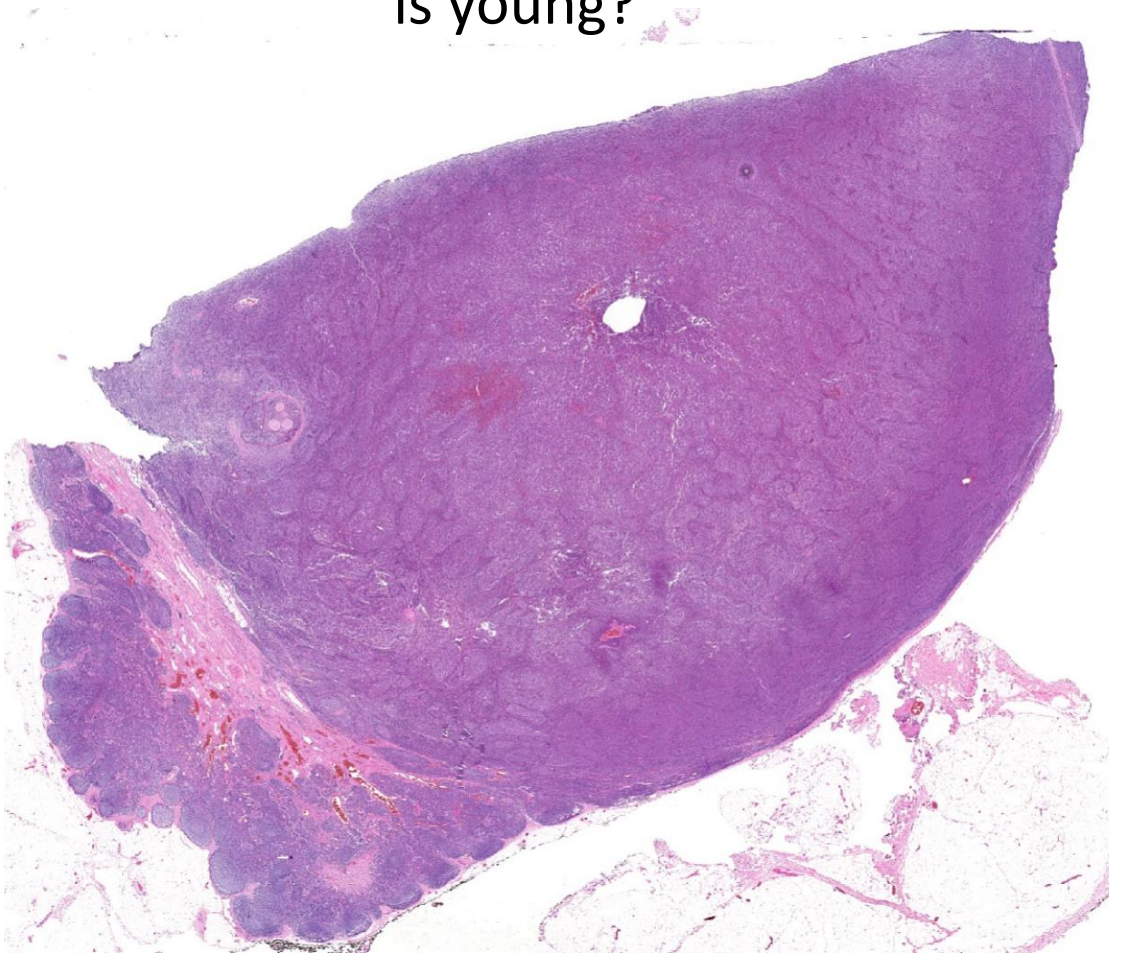
https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=29607
https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=29600

Follicular lymphoma: What if BCL2 IHC is negative…?



…and the patient is young?



https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=30901
https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=29607
https://tumourclassification.iarc.who.int/Viewer/DisplayImage2?f=29600

Follicular lymphoma: Pediatric-type (BCL2-negative)

Pediatric-type follicular lymphoma (WHO 5th)
Pediatric-type follicular lymphoma (ICC)

Clinical

- predominantly affects children, adolescents, young adults (M>>>F)
- single painless enlarged LN (typically H&N) → excellent prognosis; conservative management warranted

Morphology

- expanded, serpiginous to confluent follicles (no diffuse areas)
- may have rim of reactive follicles
- "blastoid" morphology (between centrocyte and centroblast)
- numerous tingible-body macrophages (starry sky pattern)

IHC

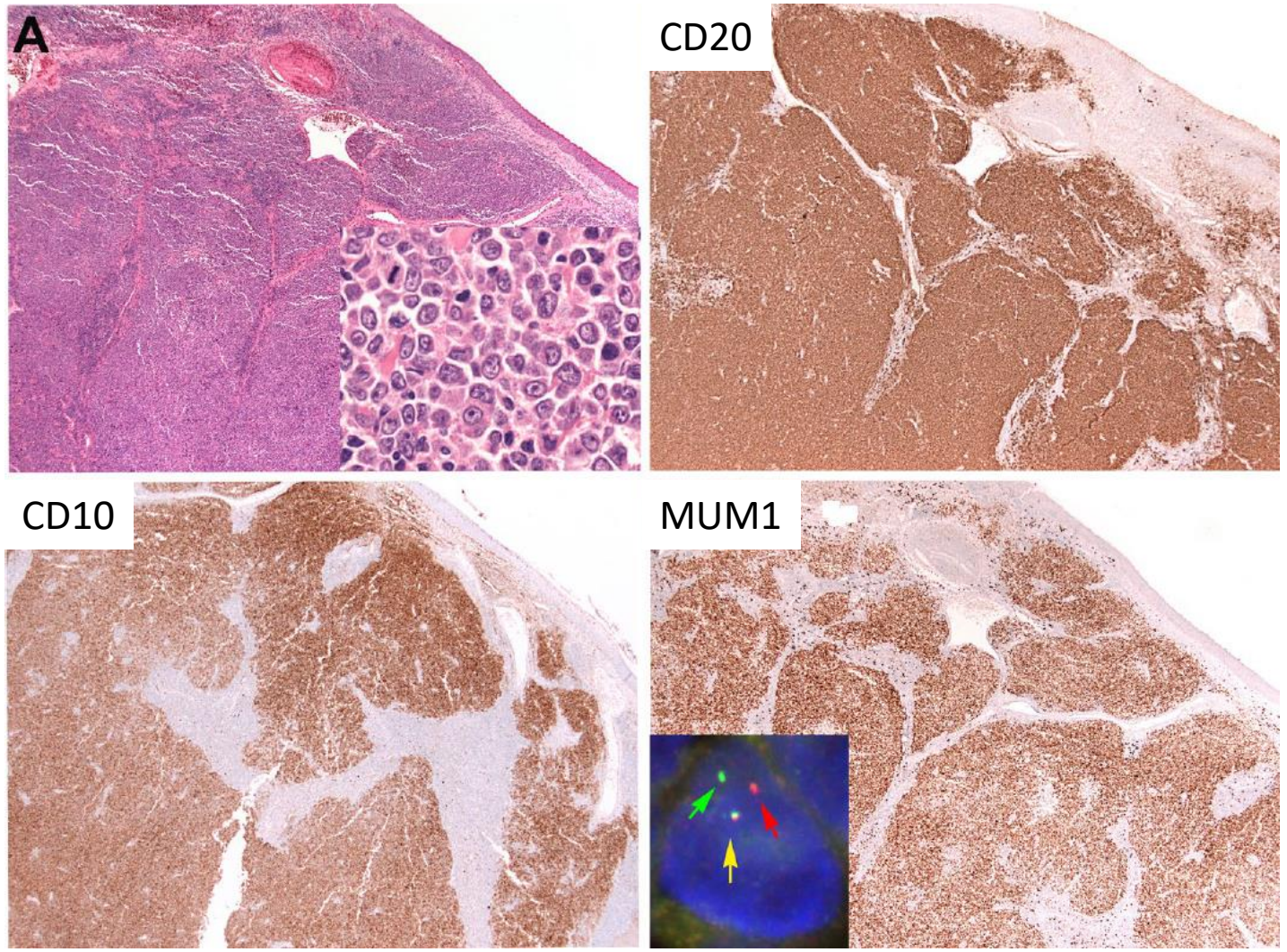
- CD10+, BCL6+
- BCL2 IHC weak to absent
- follicular dendritic cell meshworks (+ CD21, CD23 or CD35)
- Ki67 > 30%

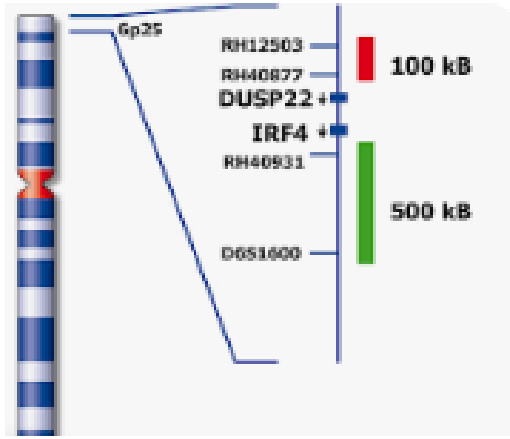
Genetics

- deletions and copy-neutral loss of heterozygosity at 1p36
- mutations of *TNFRSF14* and *MAP2K1*
- no *IGH::BCL2* fusion, no rearrangement of *BCL6* or *IRF4*

Advice from Dr. Louissaint:
Ages 0 to 18: conventional FL is extremely rare, likely PTFL
Ages 18-40: Rely on criteria
Age >40: Be cautious with diagnosis

Follicular lymphoma: What if MUM1 is strong?





Break apart probe at the 6p25 (*IRF4/DUSP22)* locus

Laurent C et al. *Virchows Archiv* (2023) 482:149–162.

Follicular lymphoma: *IRF4* rearrangement

Large B-cell lymphoma with *IRF4* rearrangement (WHO 5th) → placed under the "Large B-cell lymphoma" category

Large B-cell lymphoma with *IRF4* rearrangement (ICC) → placed under the "Follicular lymphoma" category

Clinical

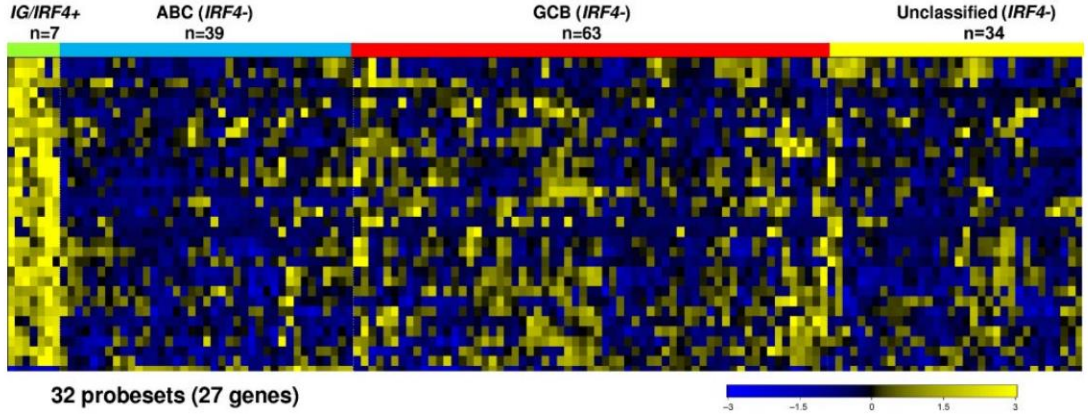
- rare overall; more common in children/young adults, M slightly > F
- Waldeyer's ring, isolated cervical LAD > intestine

Morphology

- follicular, diffuse or combined
- resembles **3B**/**FLBCL** or DLBCL; no starry sky

IHC

- conventional follicular lymphoma:
  - MUM1 IHC weak or negative
- Large B-cell lymphoma with *IRF4* rearrangement:
  - MUM1+ (strong); BCL6+; CD10 +/-; BCL2 +/-
  - Ki67 high



Genetics

- translocation of *IRF4* gene next to an *IG* locus is required for diagnosis
  - DLBCL (non-GCB) and rare **3B**/**FLBCL** can be MUM1+ (**IHC is not enough!**)
- may have *BCL6* rearrangement
- no *IGH::BCL2* fusion

Salaverria I et al. *Blood* (2011) 118 (1): 139–147.

Follicular lymphoma: Distinct extranodal entities

Testicular follicular lymphoma (ICC)

- **ICC** only (under **cFL** in **WHO 5th**)
- children/young adults
- no *IGH::BCL2*
- likely similar mutational profile to pediatric-type follicular lymphoma (more study needed)
- conservative management (like pediatric-type follicular lymphoma)

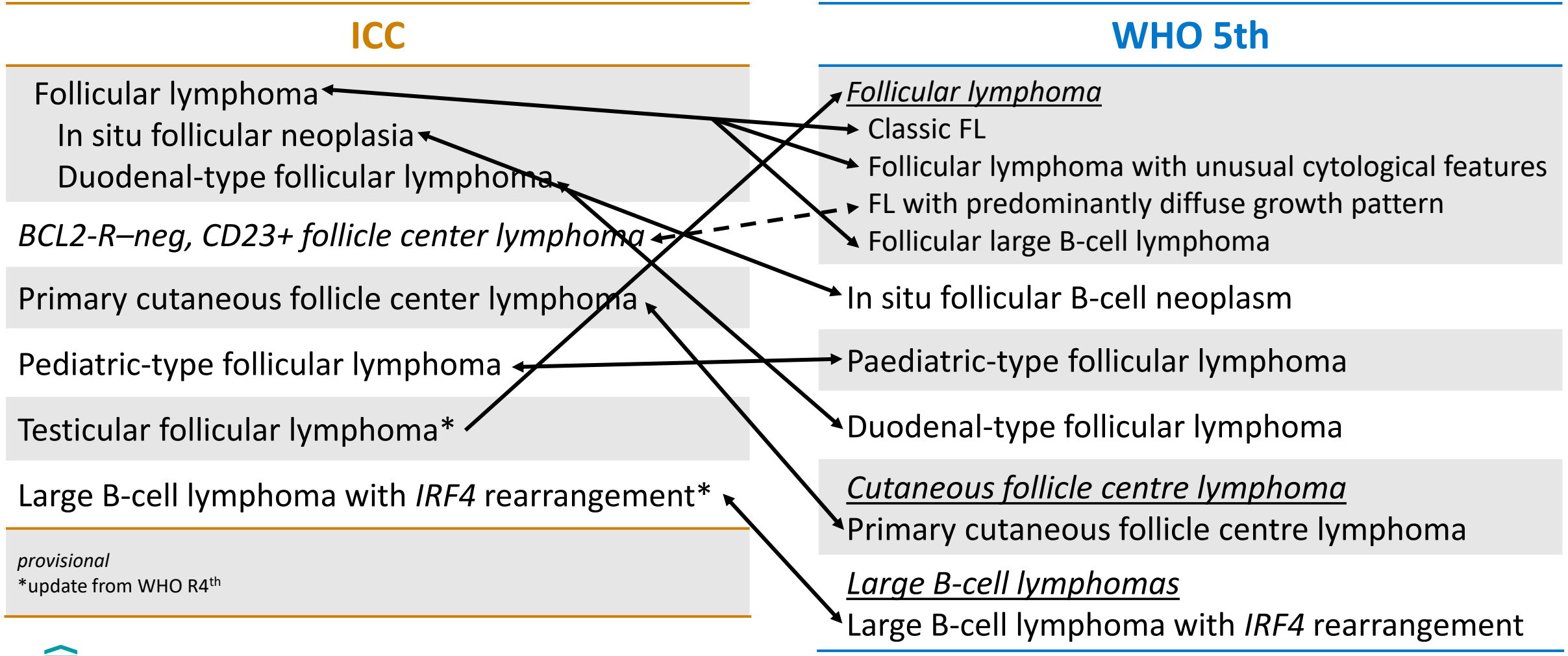
Duodenal-type follicular lymphoma (WHO 5th)
Duodenal type follicular lymphoma (ICC)

- no major changes from **WHO R4th**
- middle age; incidental
- polyps in 2nd portion of the duodenum
- low-grade cytology; follicular architecture
- BCL2+
- *IGH::BCL2* present
- localized disease → excellent prognosis

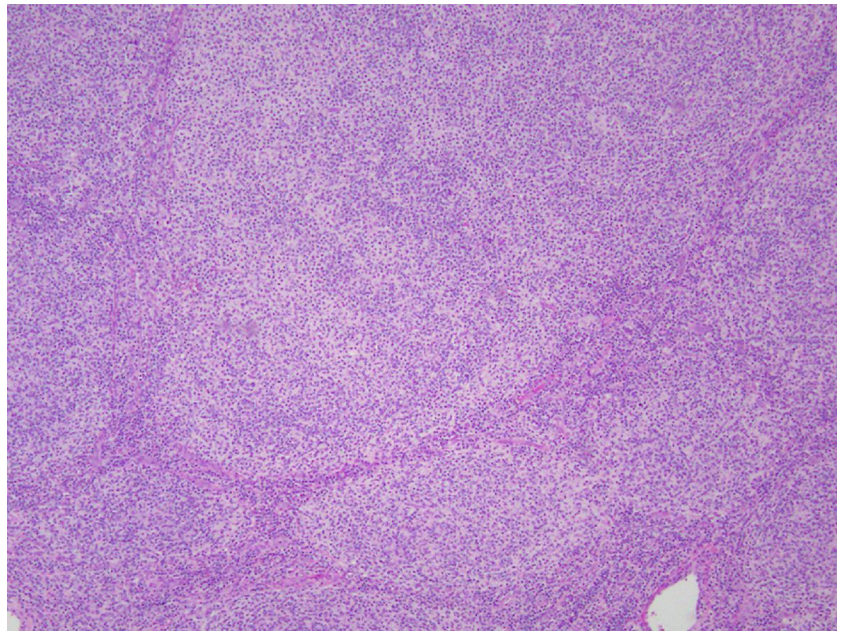
Primary cutaneous follicle centre lymphoma (WHO 5th)
Primary cutaneous follicle center lymphoma (ICC)

- no major changes from **WHO R4th**
- head/neck or trunk
- weak to negative BCL2 IHC
  - strong BLC2 IHC → think about 2° cutaneous involvement by systemic disease
- only ~10% harbor *IGH::BCL2*
- nearly 100% 5-year survival
- shows variable cytology/architecture:
  - large centrocytes + diffuse architecture → can be confused with DLBCL → clinical correlation essential

Follicular lymphoma: ICC and WHO 5th



Follicular lymphoma: Case approach



atypical follicles

grade 1, 2 or 3A morphology

CD10+

→ BCL2+ (Majority of cases) → **Classic follicular lymphoma, grading optional (WHO 5th)**
Follicular lymphoma, follicular pattern, grade ___ (ICC)

→ BCL2(-) (~15% of cases)

↓

Other BCL2 clones positive?

Yes → ↑

No

↓

FISH for *BCL6*-R

BCL6-R detected → ↑

→ add note that this might portend a more aggressive course

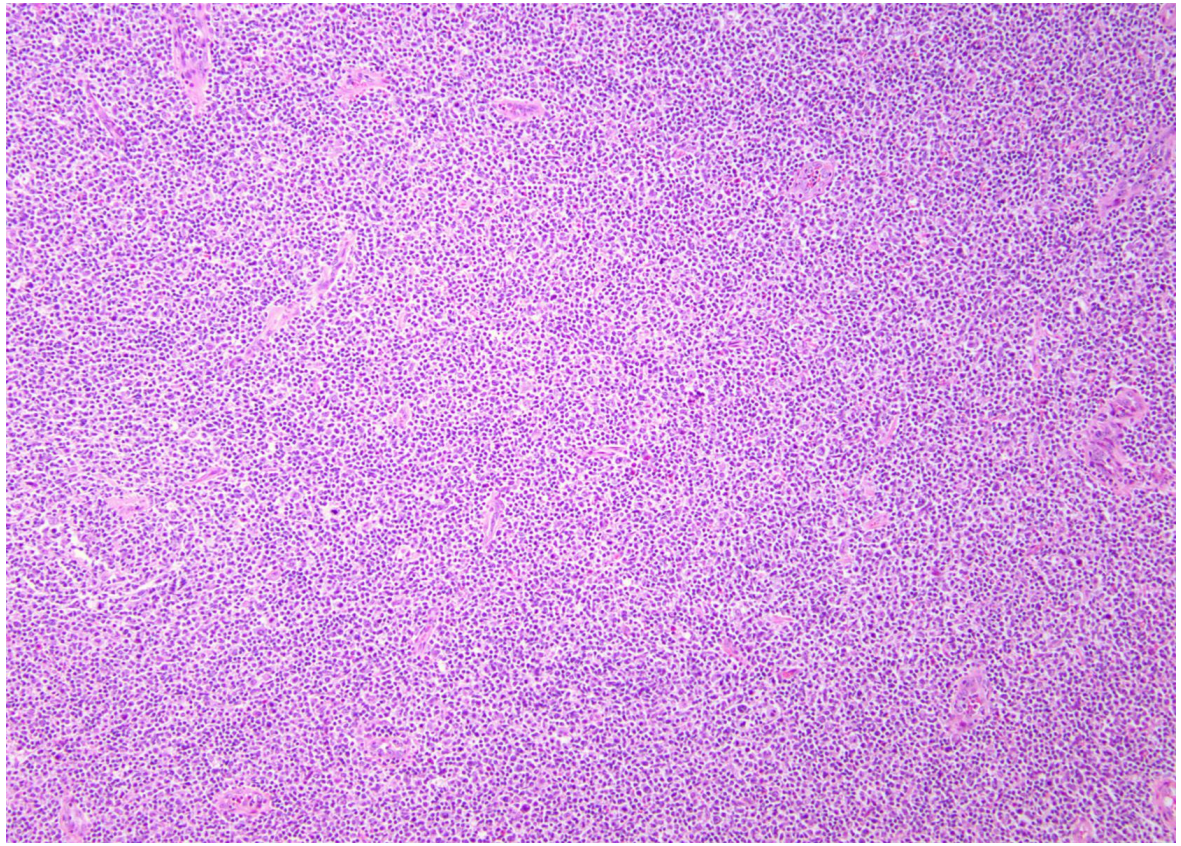
↓ Don't forget about…

no *IGH::BCL2*
low-grade cytology

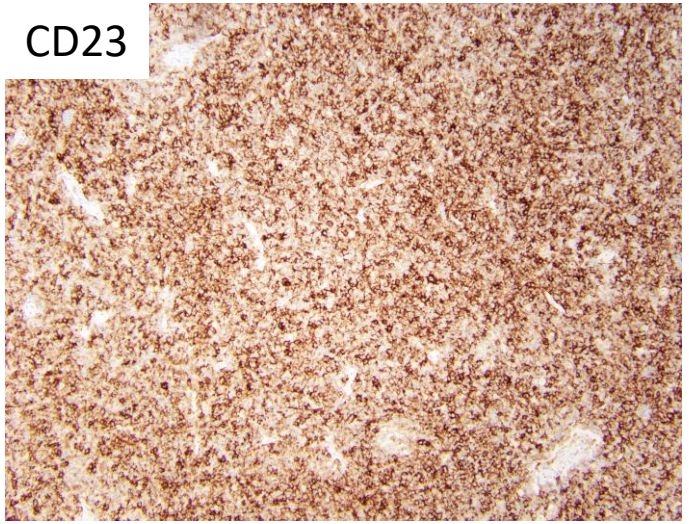
FL with predominantly diffuse growth pattern (WHO 5th)

BCL2-rearrangement-negative, CD23+ follicle center lymphoma (ICC, *provisional*)

[criteria not identical between the two classification systems]



CD23

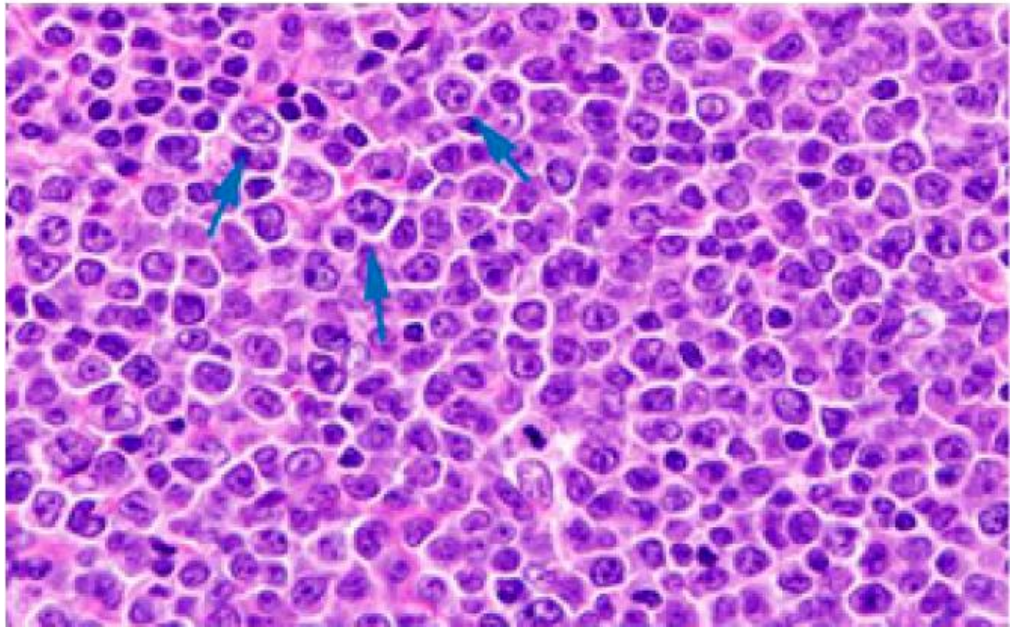


Supportive features:

- low stage (typically large inguinal LN)
- microfollicles
- *CREBBP* and *STAT6* mutations
- 1p36 loss

Follicular lymphoma: Case approach

Ambiguous morphology
(debating between 3A and 3B)



Campo E et al. *Blood* (2022) 140 (11): 1229–1253.

CD10+ → Yes → **Classic follicular lymphoma, grading optional (WHO 5th)**
Follicular lymphoma, grade 3A (ICC)

BCL2+ and/or
IGH::BCL2 fusion

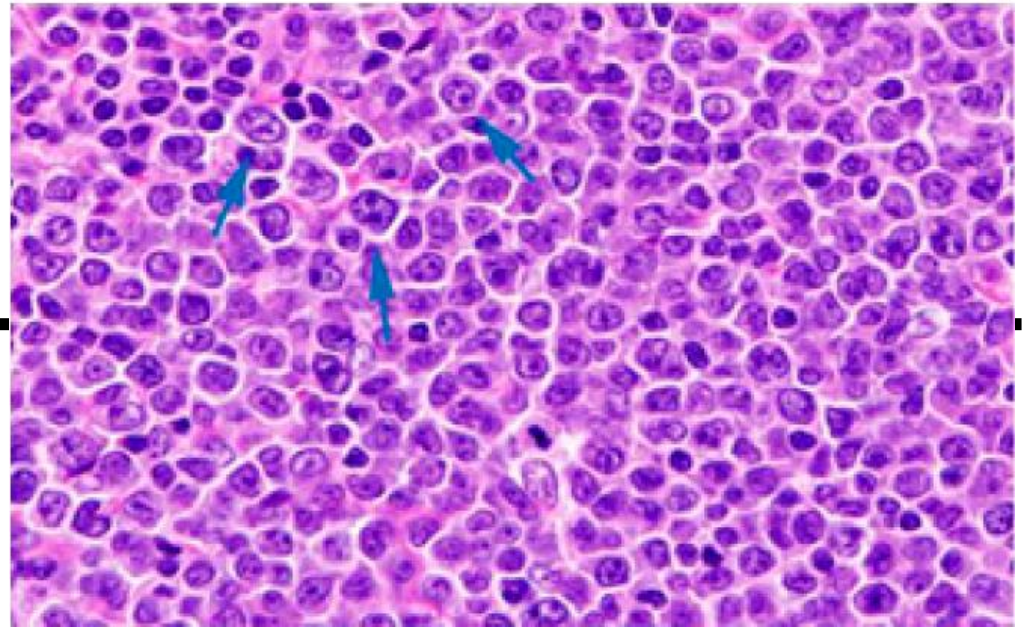
Additional supporting features:
- follicular pattern
- MUM1-negative
- bone marrow involvement

No CD10/*BCL2*-R → ---

Follicular lymphoma: Case approach

Don't forget about…

Ambiguous morphology
(debating between 3A and 3B)



Campo E et al. *Blood* (2022) 140 (11): 1229–1253.

Diffuse large B-cell lymphoma
(*MYC*, *BCL2*, *BCL6* work-up)

No CD10, *BCL2*-R → MUM-1 (+) → *IRF4* FISH (+) → **Large B-cell lymphoma with *IRF4* rearrangement**

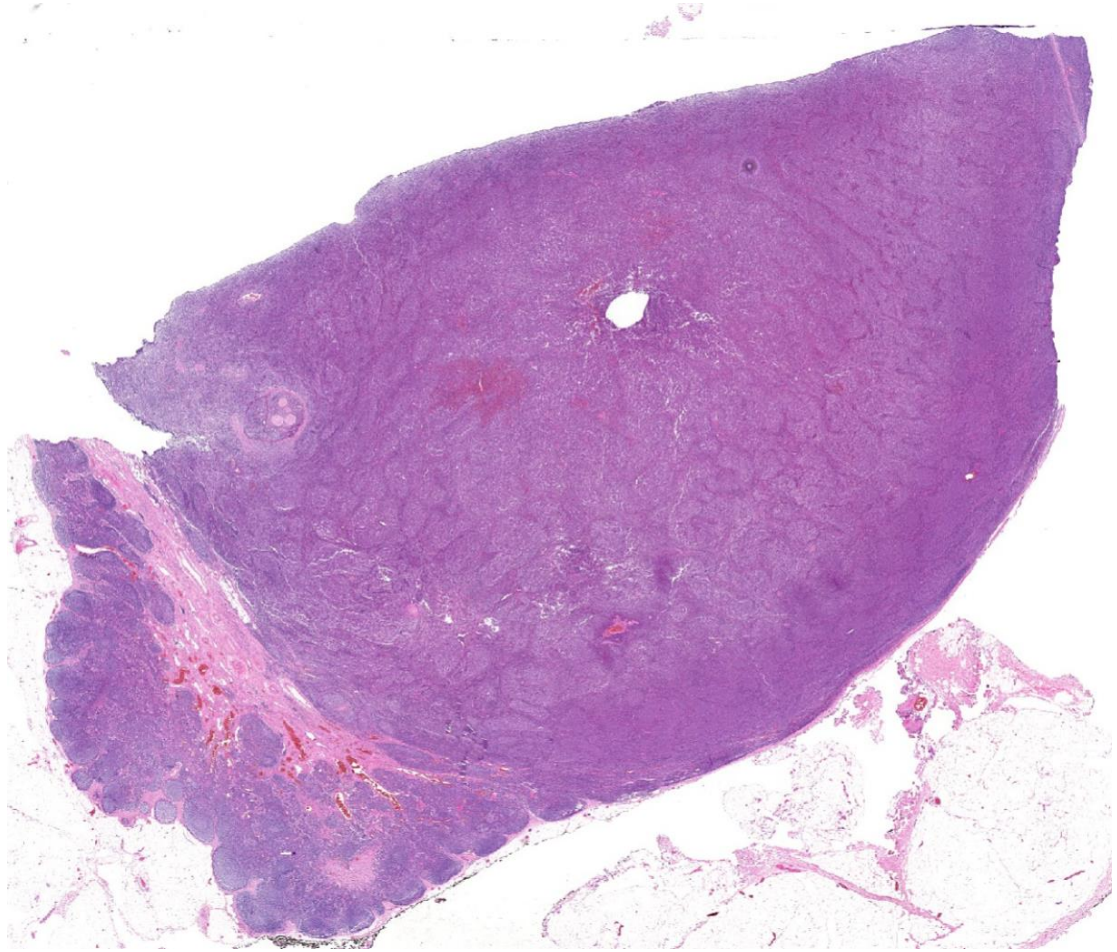
MUM-1 (-) → Predominantly diffuse pattern

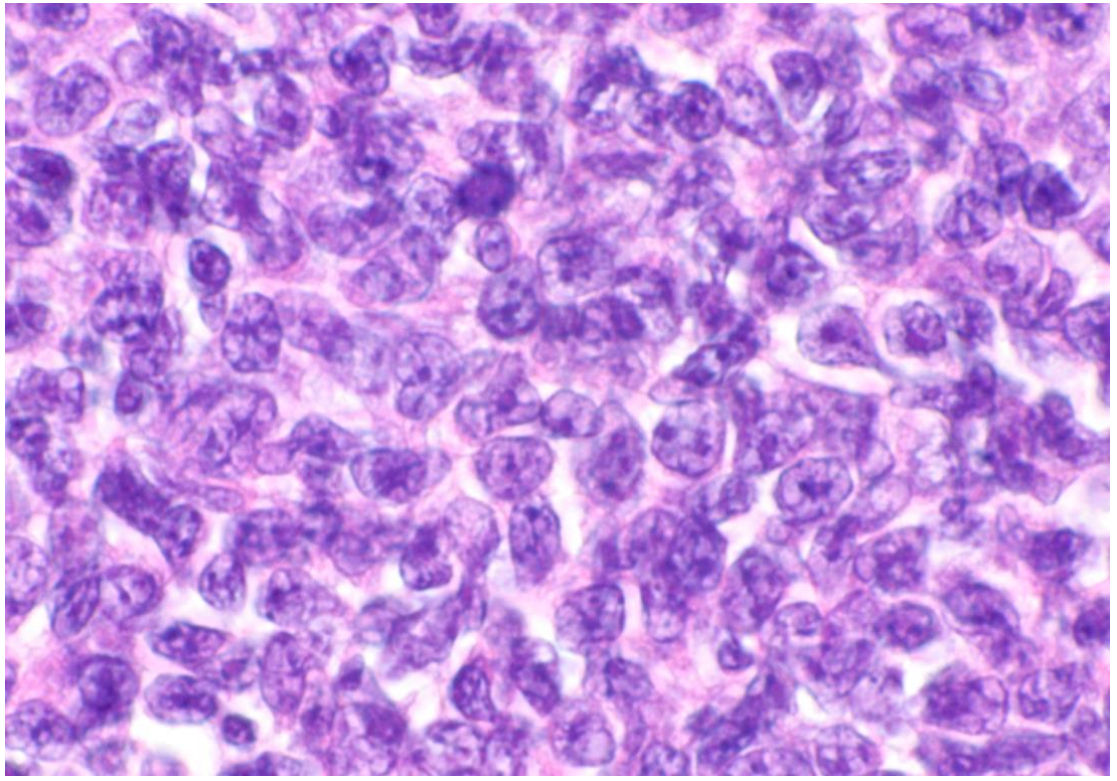
IRF4 FISH (-) → Predominantly follicular pattern

Favors:

Follicular large B-cell lymphoma (WHO 5th) **Rare!**

Follicular lymphoma, follicular pattern, grade 3B (ICC)





Pediatric-type follicular lymphoma

Supportive features

- limited stage; often head and neck region
- serpiginous and expansile follicles
- high Ki67
- no *BCL6* or *IRF4* rearrangement
- younger than 40 (usually)

no *IGH::BCL2*
blastoid cytology

Follicular lymphoma: Summary

Work-up of suspected follicular lymphoma

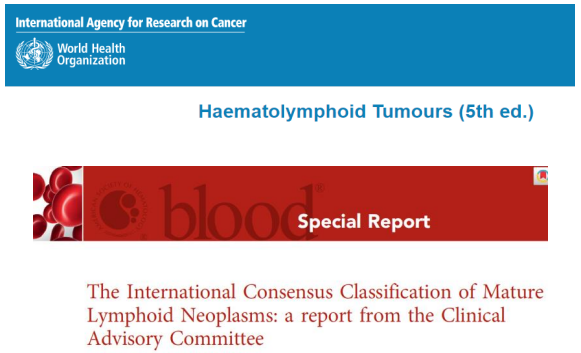
- assessment of cytologic features and architectural features; excisional biopsy is best
- IHC for CD10, BCL6, BCL2, MUM-1, FDC markers (CD21 and CD23; +/- CD35), Ki67
- fresh tissue for flow cytometry and karyotype (~0.5-1cm^3)
- FISH for *IGH::BCL2* fusion is not required in straightforward cases

Conventional follicular lymphoma has different names/approaches in the ICC and WHO 5th

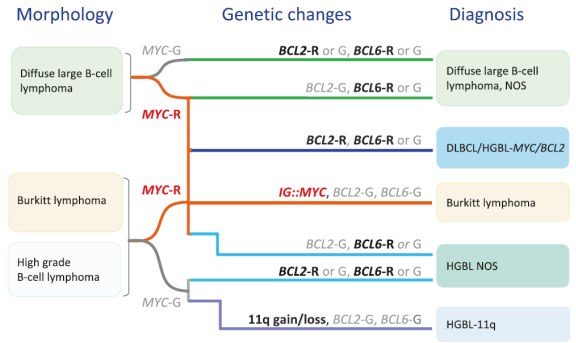
- **classic follicular lymphoma (WHO 5th)** → encompasses grades 1, 2 and 3A; stating grade is optional
- **follicular lymphoma (ICC)** → grading is retained
  - if debating between **3A**/**cFL** or **3B**/**FLBCL**, CD10 expression and *IGH::BCL2* fusion favors **3A**/**cFL**
- **follicular large B-cell lymphoma (WHO 5th)**
- **follicular lymphoma, follicular pattern, grade 3B (ICC)**
  - rare diagnosis; don't render on core biopsy; carefully evaluate for diffuse areas which would = DLBCL

If it seems like FL but is BCL2 IHC is negative, think about:

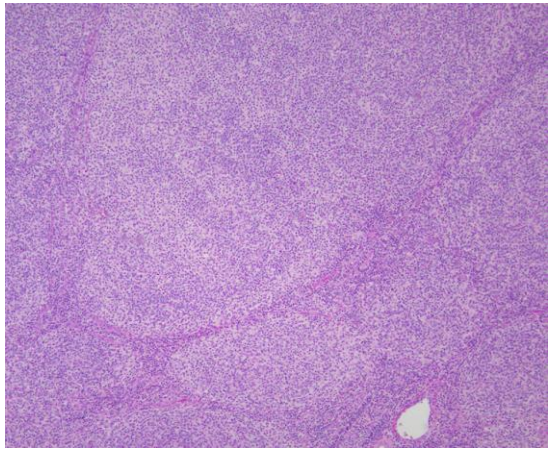
- alternative BCL2 IHC clones
- *BCL6* rearrangement
- CD23+ follicular lymphoma
- pediatric-type follicular lymphoma
- large B-cell lymphoma with *IRF4* rearrangement



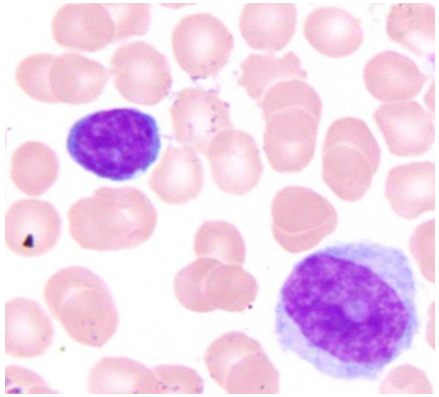
What's happening with the classification systems?



Large B-cell lymphomas with emphasis on double-hit lymphomas



Follicular lymphoma and related entitles

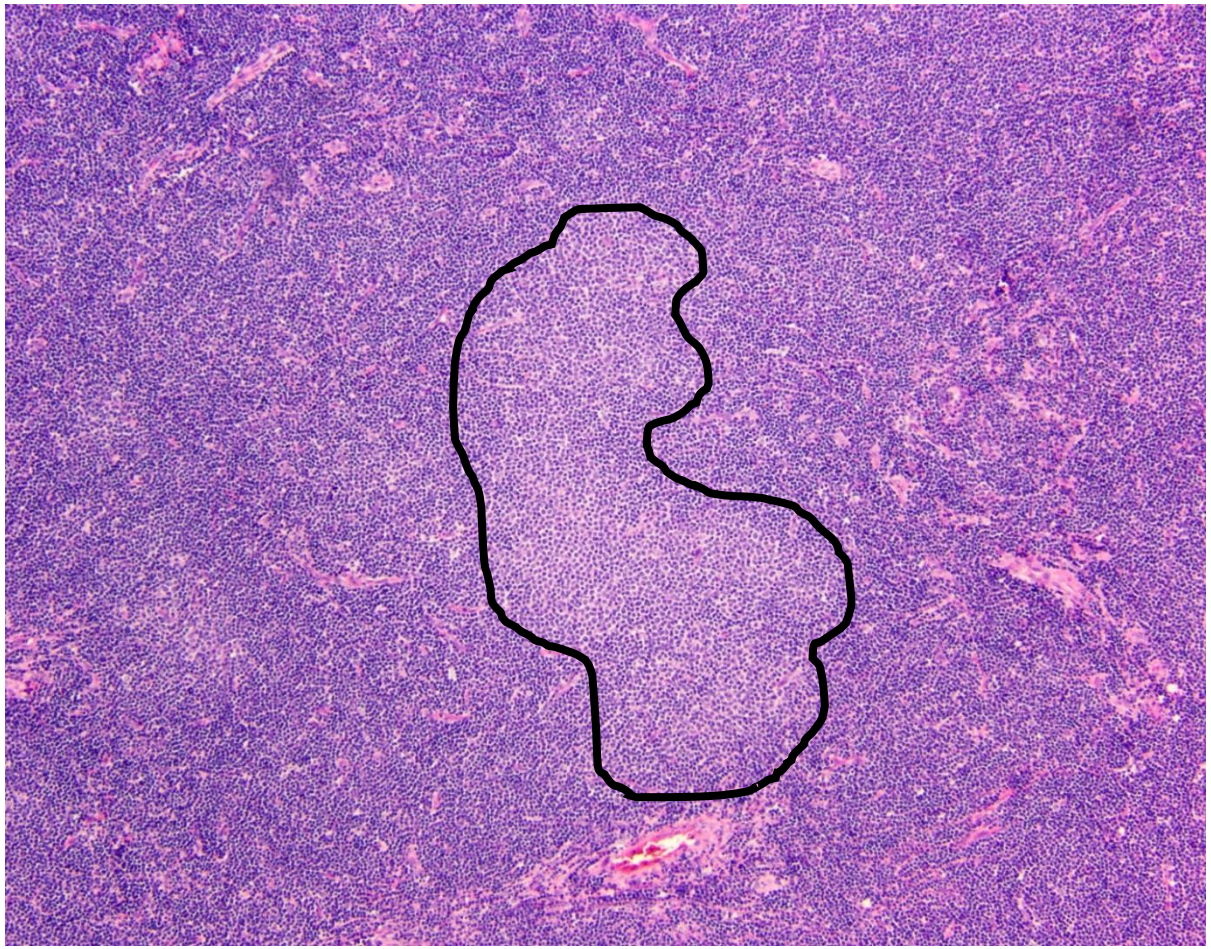


Other mature B-cell lymphomas

Mature B-cell neoplasms (not FL or DLBCL)

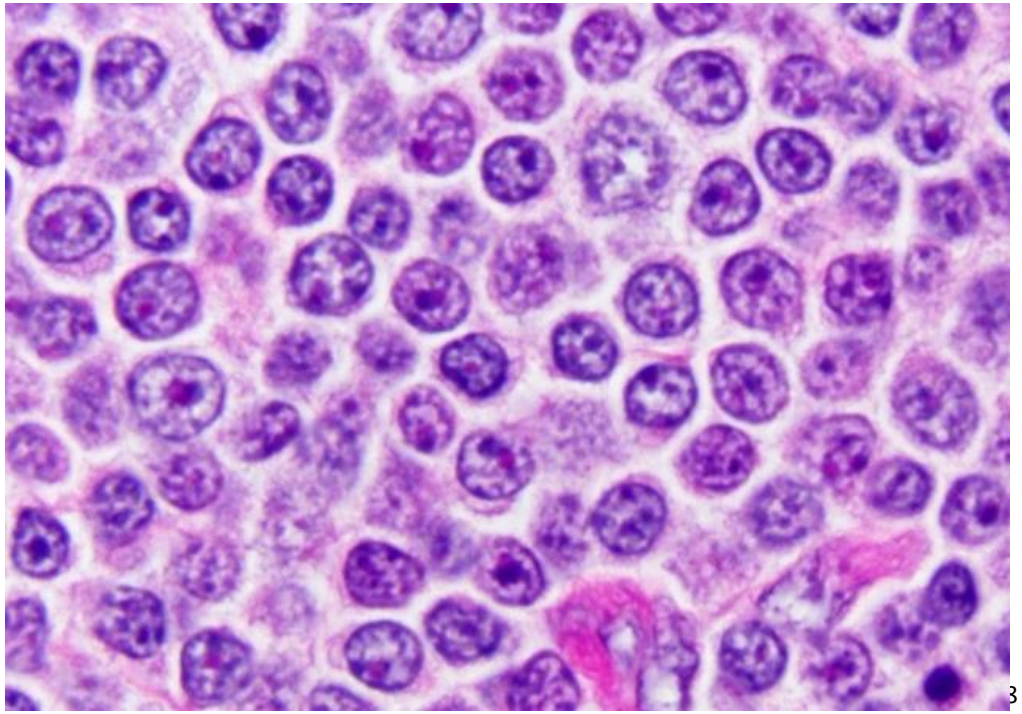
WHO R4th	WHO 5th	ICC
	Pre-neoplastic and neoplastic small lymphocytic proliferations	
Monoclonal B-cell lymphocytosis	(Same) → CLL/SLL-type (low count/high count); non-CLL/SLL type	(Same) → CLL-type; non-CLL type
Chronic lymphocytic leukemia/small lymphocytic lymphoma	(Same)	(Same)
B-cell prolymphocytic leukemia	(Entity deleted)	B-cell prolymphocytic leukemia
	Splenic B-cell lymphomas and leukemias	
Hairy cell leukemia	(Same)	(Same)
Splenic marginal zone lymphoma	(Same)	(Same)
Splenic diffuse red pulp small B-cell lymphoma	(Same)	(Same)
Hairy cell leukemia-variant	Splenic B-cell lymphoma/leukemia with prominent nucleoli (includes some cases formerly called B-cell prolymphocytic leukemia)	Hairy cell leukemia-variant

CLL/SLL: Proliferation centers

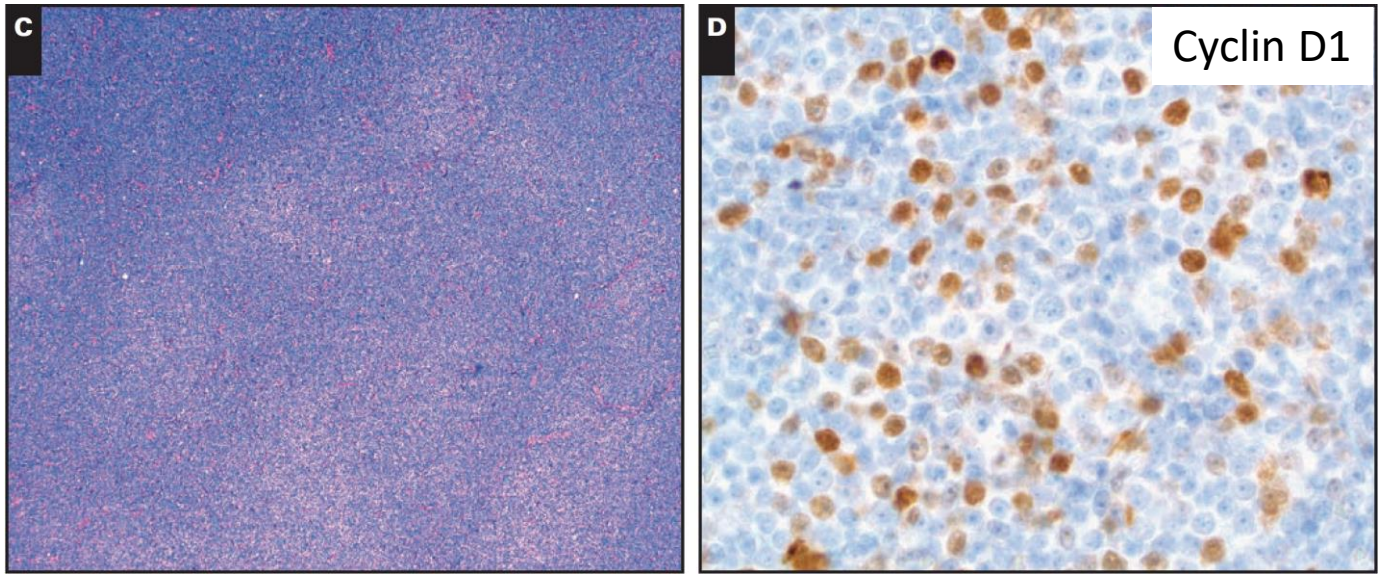


Proliferation centers:

- admixed small lymphocytes, prolymphocytes (1.5x size of a lymphocyte) and paraimmunoblasts
- may show mitotic activity

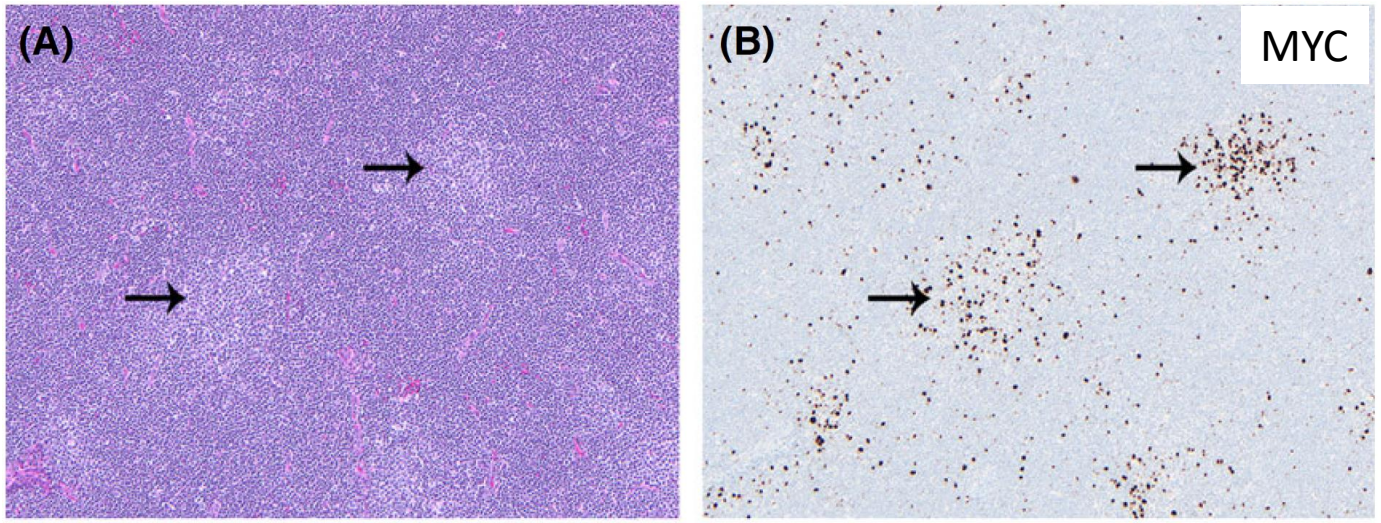


CLL/SLL: Proliferation centers



Cyclin D1+ proliferation centers in up to 30% of cases of CLL/SLL

- no t(11;14)
- no SOX11 expression by IHC
- does not = mantle cell lymphoma

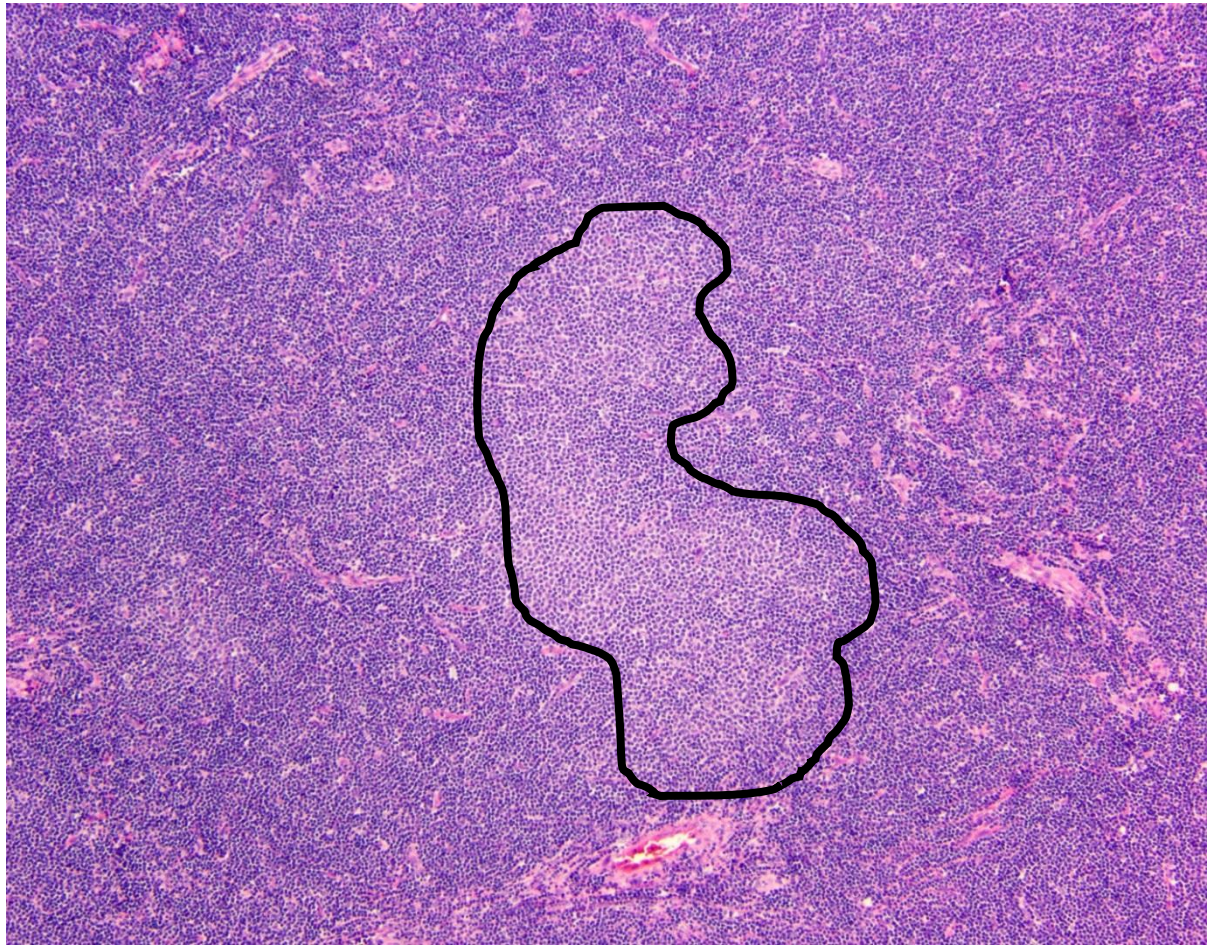


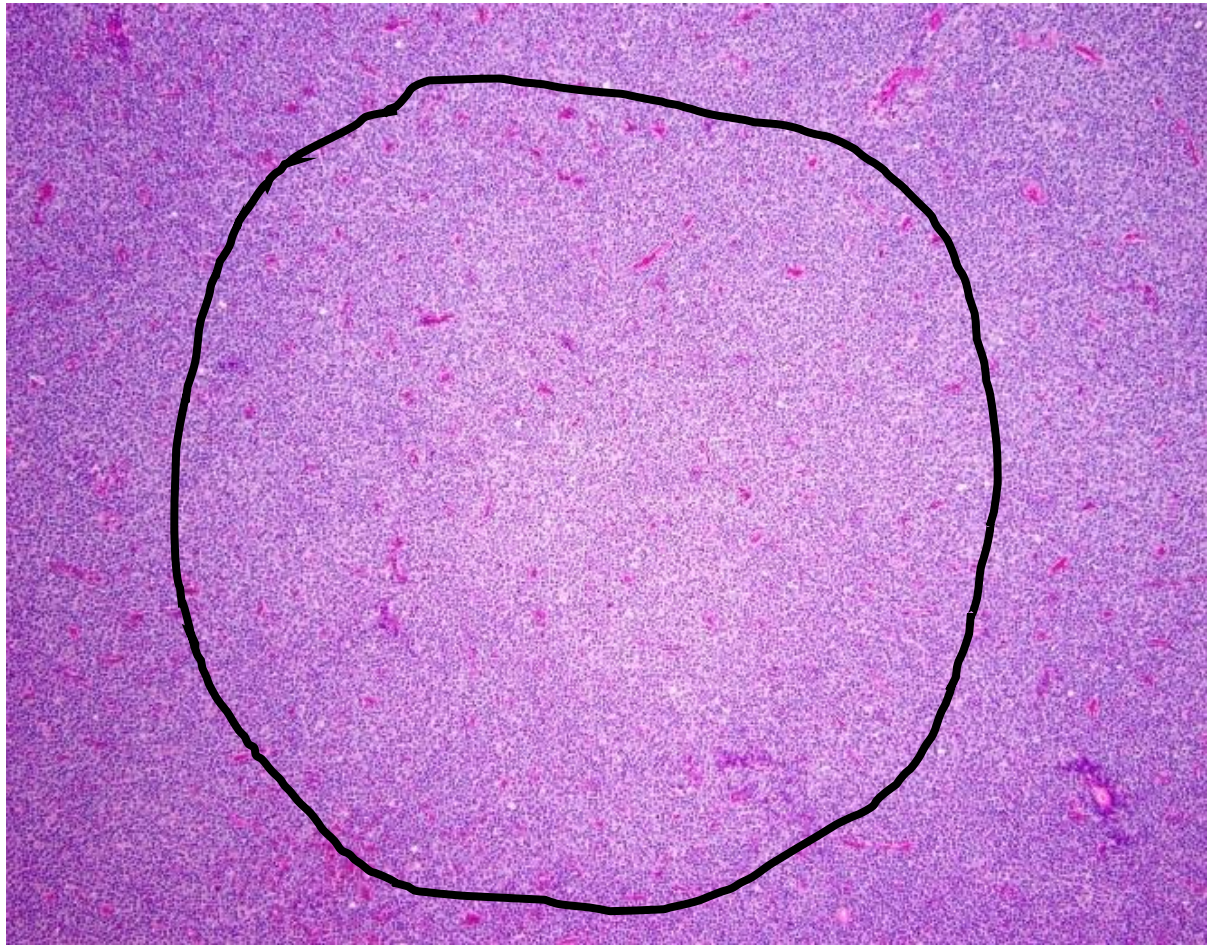
MYC IHC (at least subset) in the majority of cases of CLL/SLL

- no MYC rearrangement by FISH; few cases with MYC hyperdiploidy by FISH
- does not = transformation to large-cell lymphoma

Gibson SE et al. *Br J Haematol*. 2016;175, 161–175.
Gradowski JF et al. *Am J Clin Pathol.* 2012;138:132-139.

CLL/SLL: Proliferation centers





CLL/SLL: Proliferation centers

"histologically aggressive" CLL/SLL (WHO 5th)
"accelerated" CLL/SLL (ICC)

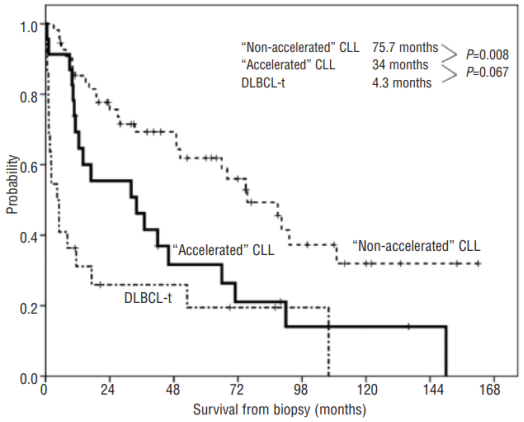
Descriptive terms

- proliferation centers broader than 20x field or becoming confluent and/or Ki67 >40% or mitoses >2.4/PC
- not official subtypes
- but warrants a comment

Clinical outcome between typical CLL/SLL and Richter transformation

- studies were before current therapy era

Association with deletion in 17p13 or trisomy 12



Challenging "gray zone" histologically

Distinct from DLBCL (Richter transformation, RT)

- often requires excisional biopsy)
  - DLBCL = confluent sheets of large B cells with a nuclear size equal to or exceeding that of normal macrophage nuclei or more than twice the size of a normal lymphocyte
- clinical trial study showed that only 33 of 40 (82.5%) cases submitted as RT were consistent with RT following expert central review
- morphologic mimics of RT
  - large, variably confluent and serpiginous proliferation centers
  - high proliferation index (sometimes thick section or associated normal bone marrow)

Ciccone M et al. *Leukemia.* 2012;26:499–508.
Soilleux EJ et al. *Histopathology*. 2016;69, 1066–76.
Gine E et al. *Haematologica*. 2010:95(9):1526-33.

Oscier D et al. *Br J Haematology.* 2016;174, 767–775.
Chabot-Richards D et al. Chp 14. *Hematopathology 2nd ed.* 2016.

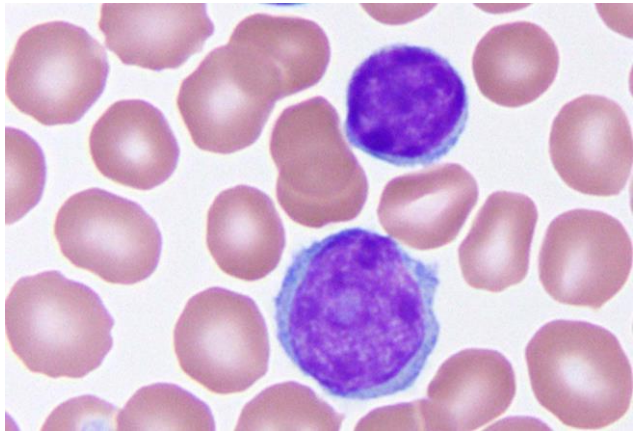
Prolymphocytes in peripheral blood

"Prolymphocytic progression" of CLL/SLL

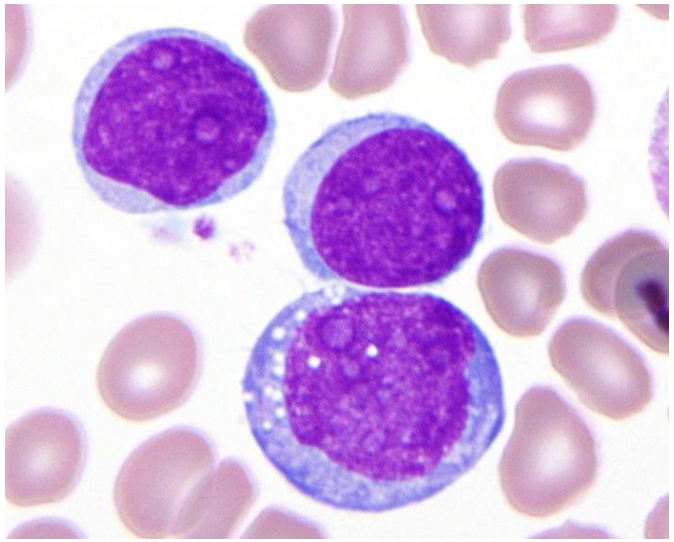
- Descriptor used in **WHO 5th**
  - >15% prolymphocytes among all lymphocytes
  - evaluate for underlying *TP53* alteration
  - exclude blastoid variant of mantle cell lymphoma
- No equivalent in **ICC**

B-cell prolymphocytic leukemia

- **WHO R4th**: B-cell prolymphocytic leukemia (>55% prolymphocytes in peripheral blood)
  - **ICC** retains this term for *de novo* cases
  - distinct phenotype and IGHV usage pattern
- **WHO 5th** eliminates category of B-cell prolymphocytic leukemia
  - cases distributed to:
    - mantle cell lymphoma
    - prolymphocytic progression of CLL/SLL
    - splenic B-cell lymphoma/leukemia with prominent nucleoli

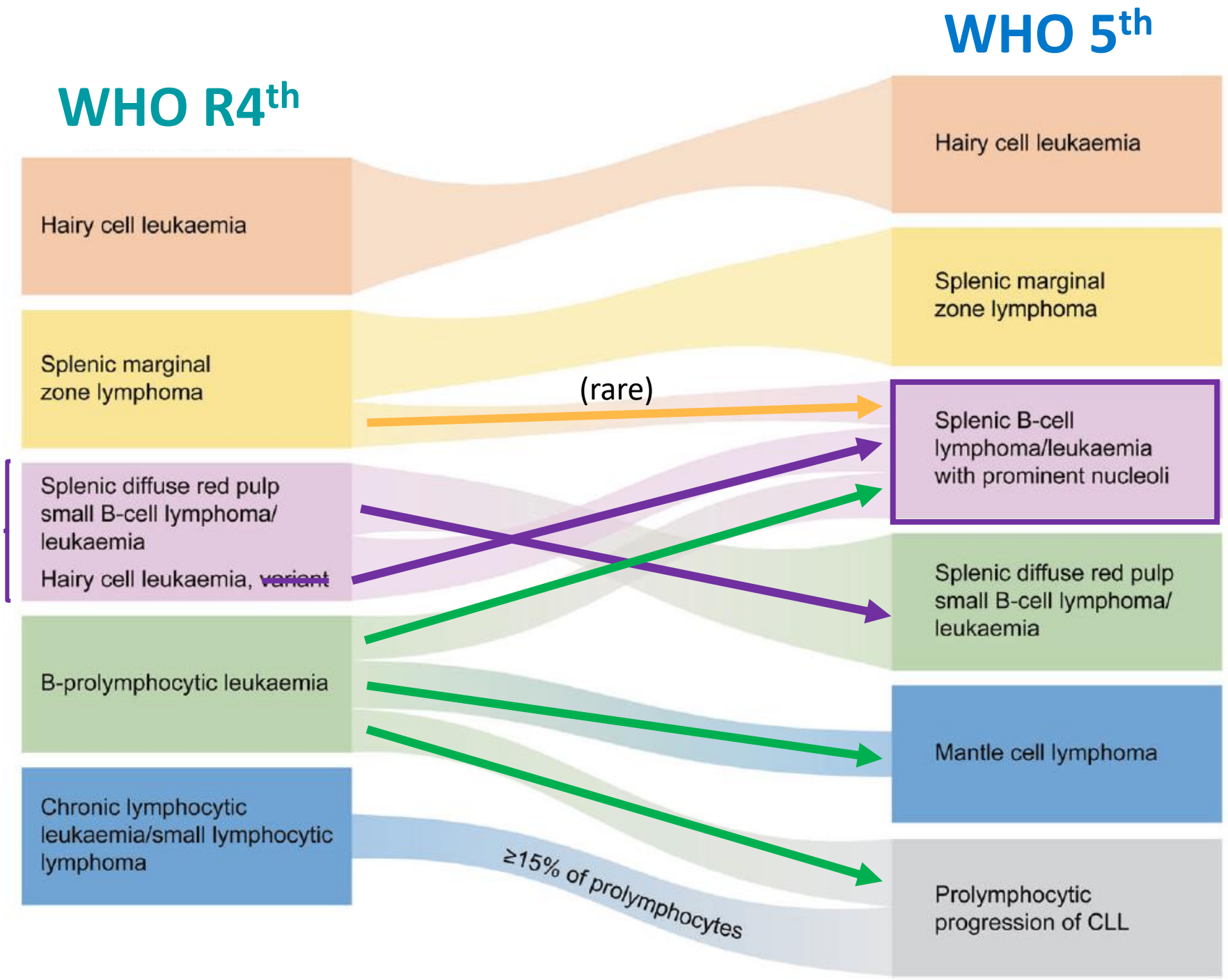


Prolymphocytes: intermediate-sized, prominent nucleolus

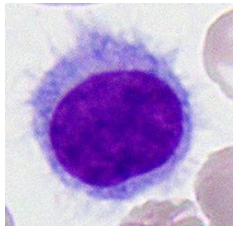
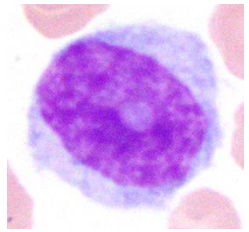
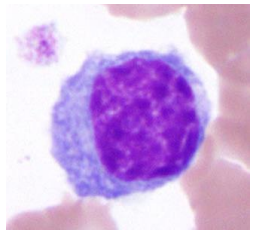
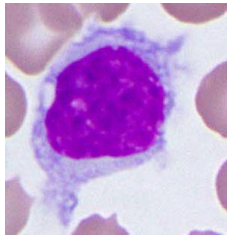


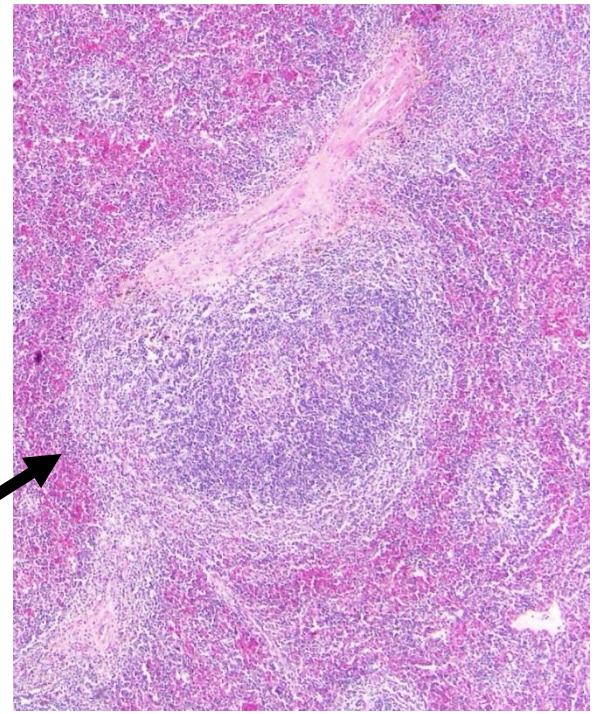
Need to exclude blastoid mantle cell lymphoma

~~Provisional~~ diagnoses under “Splenic B-cell lymphoma/leukemia, ~~unclassifiable~~”



Splenic B-cell lymphomas and leukemias

	Hairy cell leukemia	Splenic B-cell lymphoma/leukemia with prominent nucleoli (SBLPN)	Splenic diffuse red pulp small B-cell lymphoma	Splenic marginal zone lymphoma
				
Gender predominance	Male	Male (1.6)	Male	Equal
Age at diagnosis (years)	50	71	65	65
WBC, median (range)	Pancytopenia	35	11	19
Patients with Anaemia (<10 g/dl)	Frequent	30%	None	29%
Thrombocytopaenia	17%	45%	37%	18%
Monocytopenia	Yes	No	No	No
Spleen infiltration	Red Pulp	Red Pulp	Red Pulp	Marginal zone
Bone marrow infiltration	Interstitial spacing	Intrasinusoidal Interstitial	Interstitial Intrasinusoidal	Intrasinusoidal Interstitial
Median OS from diagnosis (years)	20	9	>15 years	>12



Matutes E et al. *Best Practice & Research Clinical Haematology*. 2015;28:253e263.

Splenic B-cell lymphomas and leukemias

Marker	HCL	HCL-v SBLPN	SDRPL	SMZL
DBA.44	+	+	+	+/-
CD11c	+	+	-/+	-/+
CD103	+	+	-/+	--/+
T-bet	+	+	ND	+/-
TRAP	+	-/+	-	+/-
CD123	+	-/+	+/-	-/dim+
CD25	+	-	-	-/+
Annexin A1	+	-	-	-
CD200	+	weak to -	ND	-/dim+
CD1d	+	-	ND	-/dim+
Cyclin D1	+	-	-	-

Splenic B-cell lymphomas and leukemias

Table 1. Main Recurrent Genetic Lesions in HCL and HCL-Like Neoplasms

Genetic Lesion	HCL	HCL-v/SBLPN	SDRPSBCL	SMZL
BRAF-V600E mutation	Present (> 97% of patients)	Absent	1 report	1 report
CDKN1B mutations	Present (16% of patients)	Absent	NA	NA
KLF2 mutations	Present (16% of patients) (missense)	Absent	NA	Present (approximately 20%-40% of patients)
KLF2 deletions	NA	NA	NA	Present (11% of patients)
MAP2K1 mutations	Absent*	Present (48% of patients)	8%	Rare
NOTCH2 mutations	4%	NA	10%	Present (approximately 10%-25% of patients)
7q deletions	Present (< 10% of patients)	Present (15% of patients)	Present (18% of patients)	Present (approximately 30% of patients)
TP53 deletions and/or mutations	Rare	Present (33% of patients)	Rare	Present (approximately 15%-20% of patients)
NF-κB pathway gene† alterations	NA	NA	NA	Present (approximately 35% of patients)
CCND3 mutations	Absent	13%	24%	13%
KMT2C/MLL3 mutations		Present		
U2AF1		Present		

Abbreviations: HCL, hairy cell leukemia; HCL-v hairy cell leukemia variant; NA, not assessed by targeted analyses specifically interrogating the concerned gene(s); NF-κB, nuclear factor-κB; SDRPSBCL, splenic diffuse red pulp small B-cell lymphoma; SMZL, splenic marginal zone lymphoma.

**MAP2K1* mutations have been observed in *BRAF* wild-type cases displaying a flow-cytometry immunophenotype compatible with HCL but almost always carrying an unmutated or lowly mutated *IGHV4-34* rearrangement.[43] This rearrangement seems to define a separate genetic group of *IGHV4-34*+ HCL-like neoplasms characterized by a poorer response to purine analogs and by a flow-cytometry immunophenotype which can be either that of HCL or that of HCL-variant[40,42] (see also text).

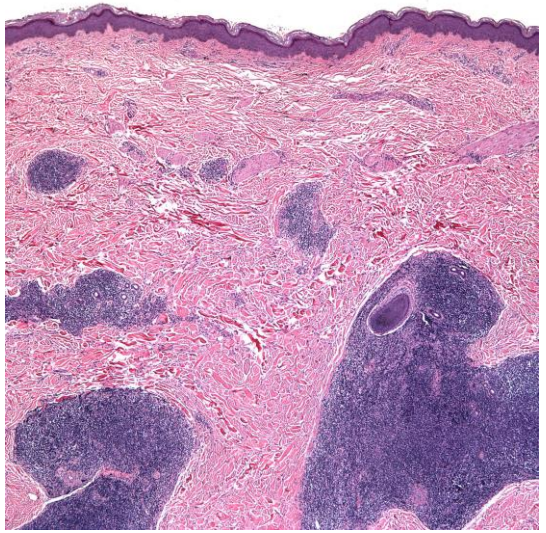
†Including *IKBKB*, *TNFAIP3*,*TRAF3*, *MAP3K14*, *TRAF2*, and *BIRC3*.

Adapted from Tiacci E et al. *JCO*. 2017;35(9):1002-1010.

Mature B-cell neoplasms (not FL or DLBCL)

WHO R4th	WHO 5th	ICC
	Lymphoplasmacytic lymphoma	
Lymphoplasmacytic lymphoma	(Same)	(Same)
	Marginal zone lymphoma	
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue	(Same)	(Same)
Not previously distinct (listed under "extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue)	Primary cutaneous marginal zone lymphoma	Primary cutaneous marginal zone lymphoproliferative disorder
Nodal marginal zone lymphoma	(Same)	(Same)
Pediatric nodal marginal zone lymphoma	(Same)	(Listed under "nodal marginal zone lymphoma")
	Mantle cell lymphoma	
In situ mantle cell neoplasia	In situ mantle cell neoplasm	In situ mantle cell neoplasia
Mantle cell lymphoma	(Same)	(Same)
Leukemic non-nodal mantle cell lymphoma	(Same)	(Same)

Primary cutaneous marginal zone



Primary cutaneous marginal zone lymphoma (WHO 5th)
Primary cutaneous marginal zone lymphoproliferative disorder (ICC)

- Taken out of "extranodal marginal zone lymphoma" in both systems
- Discontinuous lymphoid infiltrate separated from the epidermis by Grenz zone
- Translocations typical of MALT lymphoma (e.g. t(11;18) *BIRC3::MALT1*) not seen
- Mutations in *FAS*, *DAPK1*, *CDKN2A*

Heavy chain immunoglobulin class-switched (IgG+ > IgA+ or IgE+)

- ~90%
- T-cell rich background (may obscure B cells)
- reactive germinal centers common
- peripherally-located plasma cells; prominent plasmacytic differentiation
- dermally-located

❖ up to 40% are show IgG4 restriction → not associated with signs of IgG4-related disease

Non-class switched (IgM+)

- ~10%
- sheets of B cells
- fewer T cells
- scattered plasma cells
- frequently involves subcutis

❖ prominent monocytoid B cells and IgM → carefully exclude 2° cutaneous involvement by an extranodal marginal zone lymphoma

Non-FL/non-DLBCL mature B-cell lymphomas: Summary

Expanded proliferation centers in CLL/SLL

- distinction from DLBCL: sheets of lymphocytes at least 2x in size
- warrant descriptor terms: **"histologically aggressive" (WHO 5th)** or **"accelerated" (ICC)**

B-cell prolymphocytic leukemia

- no longer a diagnosis in **WHO 5th** (but recognizes prolymphocytic transformation of CLL/SLL if >15% of lymphocytes; may be associated with *MYC* and *TP53* mutations)
- retained in **ICC** for *de novo* cases
- distinguish from blastoid mantle cell lymphoma

Splenic B-cell lymphomas and leukemias

- hairy cell leukemia-variant has become splenic B-cell lymphoma/leukemia with prominent nucleoli in **WHO 5th**
  - this category also includes some cases formerly diagnosed as B-PLL
- clinical, morphologic, immunophenotypic (esp. CD103 expression) and cytogenetic/molecular features can help make the diagnosis without need for splenectomy

- **Primary cutaneous marginal zone lymphoma/lymphoproliferative disorder**
  - Two subtypes: class-switched (~90%) and non-class-switched (~10%)