BDIAP 108th Symposium on Haematopathology Joint Meeting of the BDIAP and BLPG Bristol, 15th - 17th May 2014

# Lymphoma classification update
# Where are the grey zones now?

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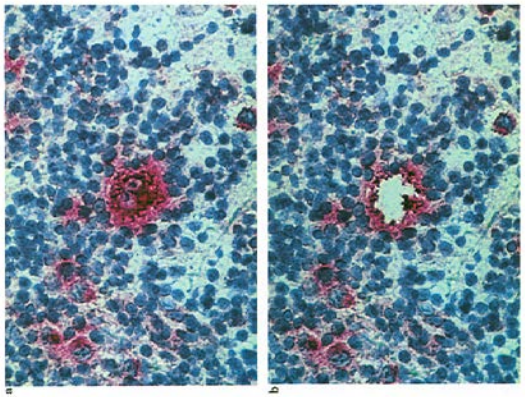
# Principles of the WHO classification

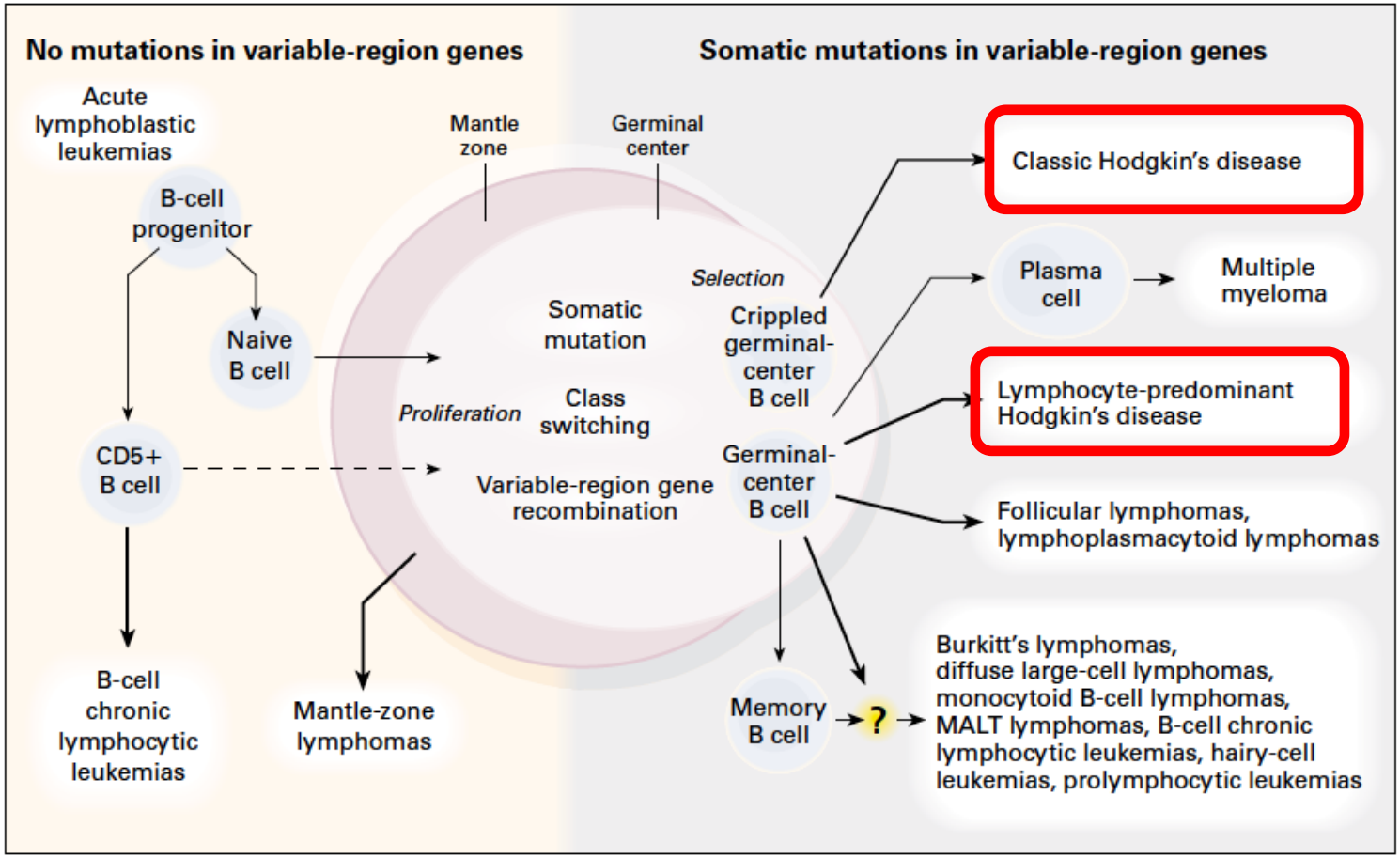
- Cell lineage as the starting point for disease definition: B, T/NK derivation
- Delineation of disease entities based on a constellation of clinical and pathological/biological findings
  - Morphology
  - Immunophenotype
  - Genetic features
  - Clinical presentation
- Importance of age and site of involvement

# « Gray zone » lymphomas

- With application of modern diagnostic tools, most lymphomas can be succesfully diagnosed and classified as one entity
- Gray zone lymphomas: lymphomas that exhibit transitional features (overlapping histology, immunophenotype and/or genetic features) that defy traditional diagnostic categories
- Synonymous: borderline lymphomas, unclassifiable
- First applied to lymphomas that exhibit features of both Hodgkin and non-Hodgkin (essentially large B-cell) lymphomas



- HRS cells have a B-cell genotype
- HL comprises a clonal proliferation of GC-derived B cells




*Kanzler et al. J Exp Med, 1996; Marafioti T et al. NEJM 1997; Kuppers et al. NEJM 1999*

- Identification of common GC B cell precursors in patients with both HL and NHL

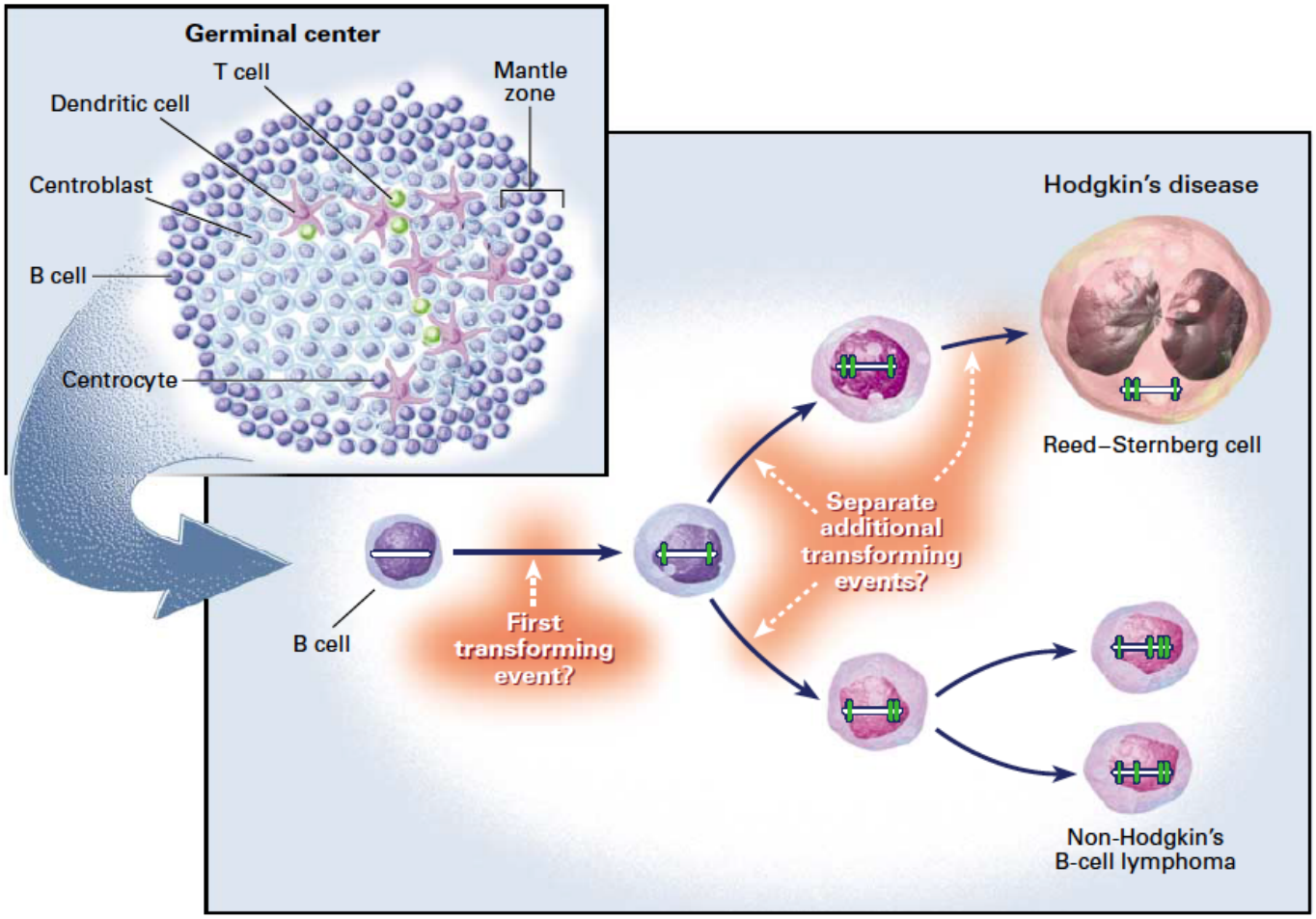


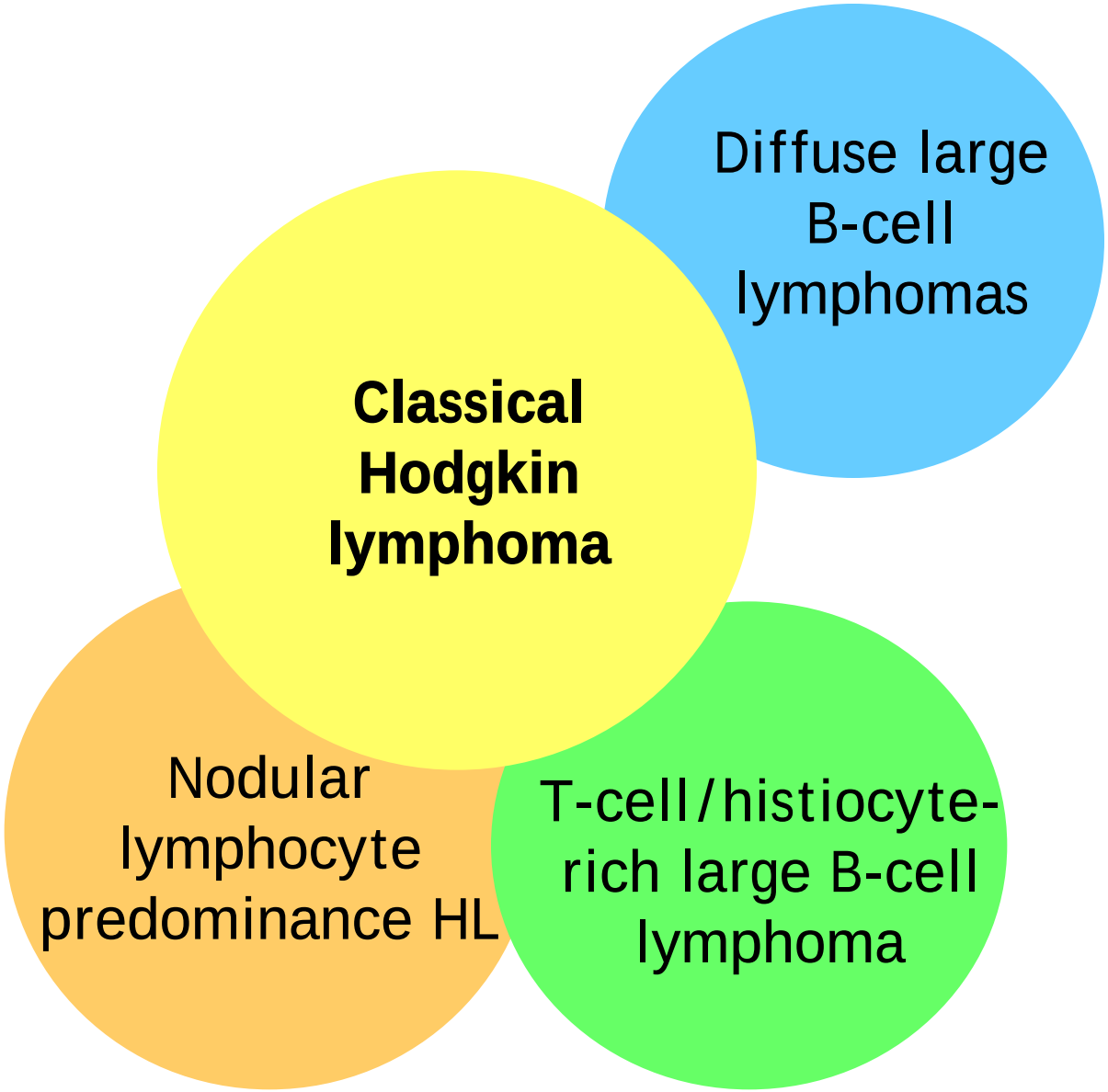

**Figure 3.** Scenario for the Generation of a Composite Lymphoma.
The horizontal lines within the circles indicate a *V* gene rearrangement; vertical lines within the circles indicate somatic mutations.

*Brauninger et al. NEJM 1999*

# « Gray zone » lymphomas with features of both Hodgkin and non-Hodgkin lymphomas

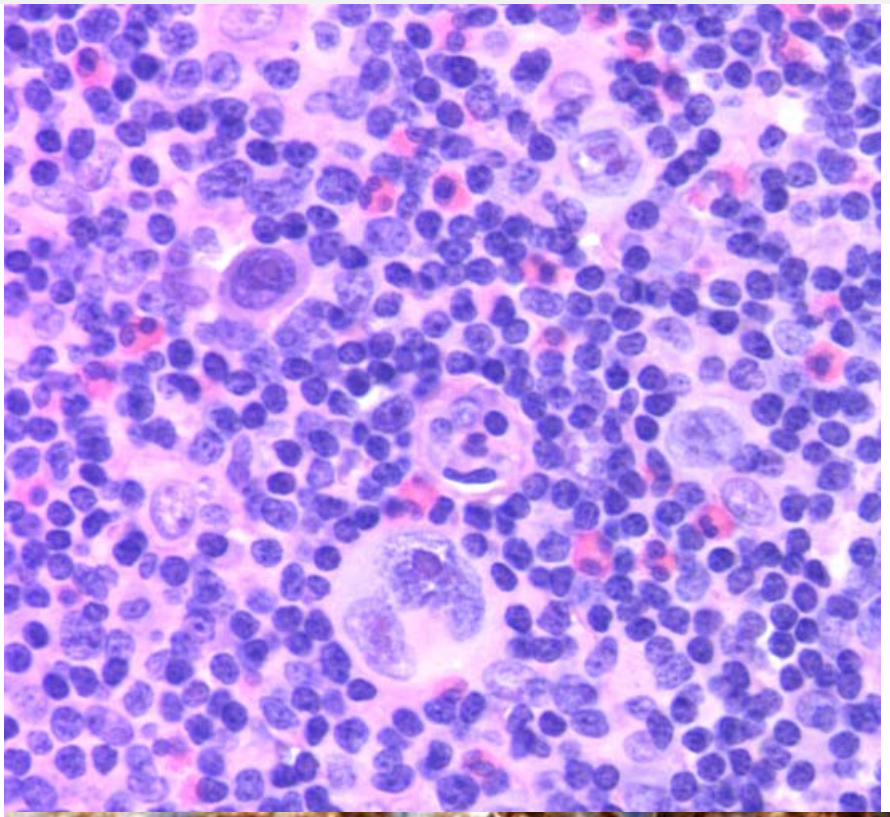
- Proceedings of the Workshop on Hodgkin's disease and related diseases (Ann Oncol 1998, 9:S31-38)
  - At the interface between classical HL and NLPHL
  - At the interface between cHL and NHL
  - At the interface between NLPHL and NHL
- Biological grey zone: cases representing true biological transition between related diseases versus morphological grey zones: reflecting the lack of criteria to reliably distinguish between lymphomas that are morphologically similar but biologically unrelated

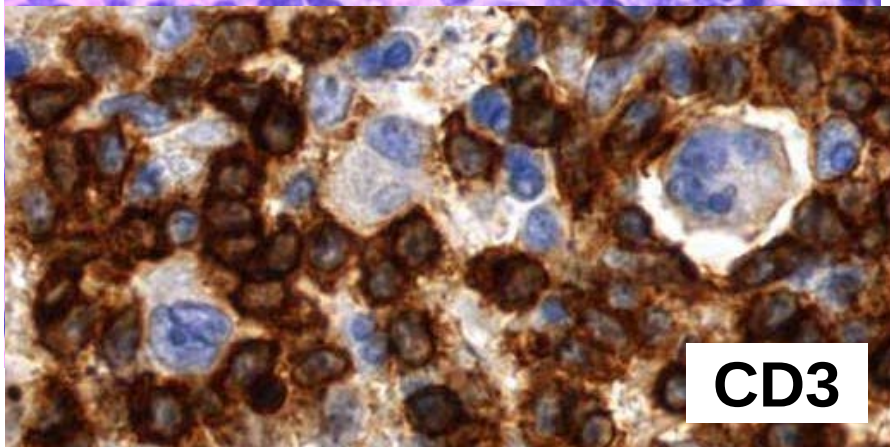
# The grey zones around classical HL



# Classical Hodgkin lymphoma

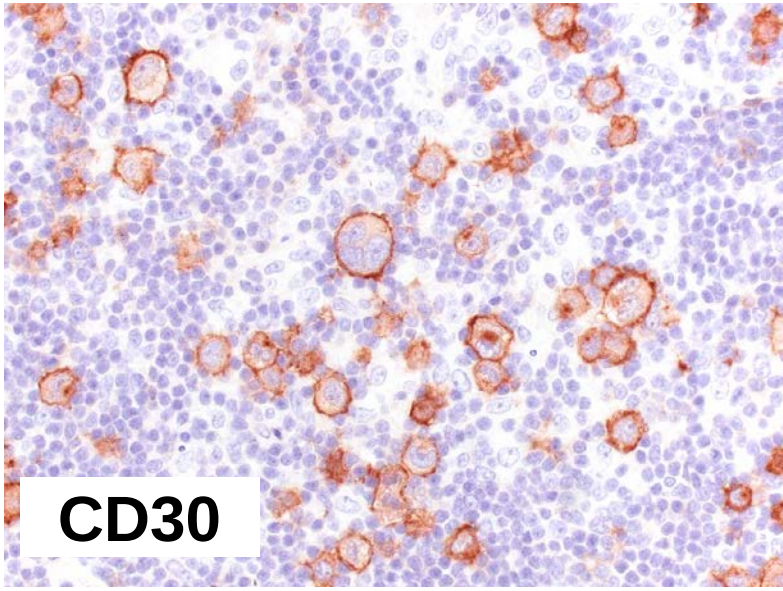
- Neoplasia comprising a small number of tumor cells (Hodgkin and Reed-Sternberg cells)
- In an abundant background of inlammatory cells, often ringed by T lymphocytes in a rosette-like manner
- Nodal disease

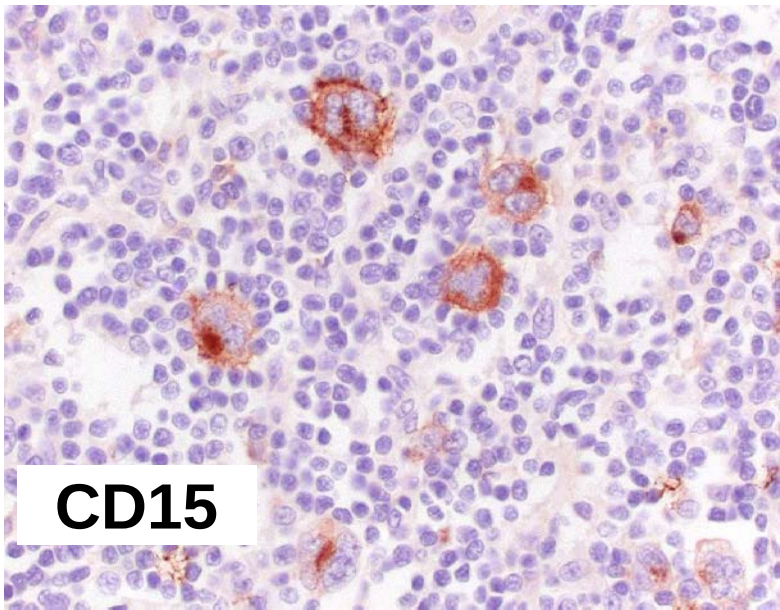


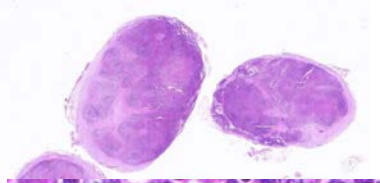


# Classical Hodgkin lymphoma: immunophenotype

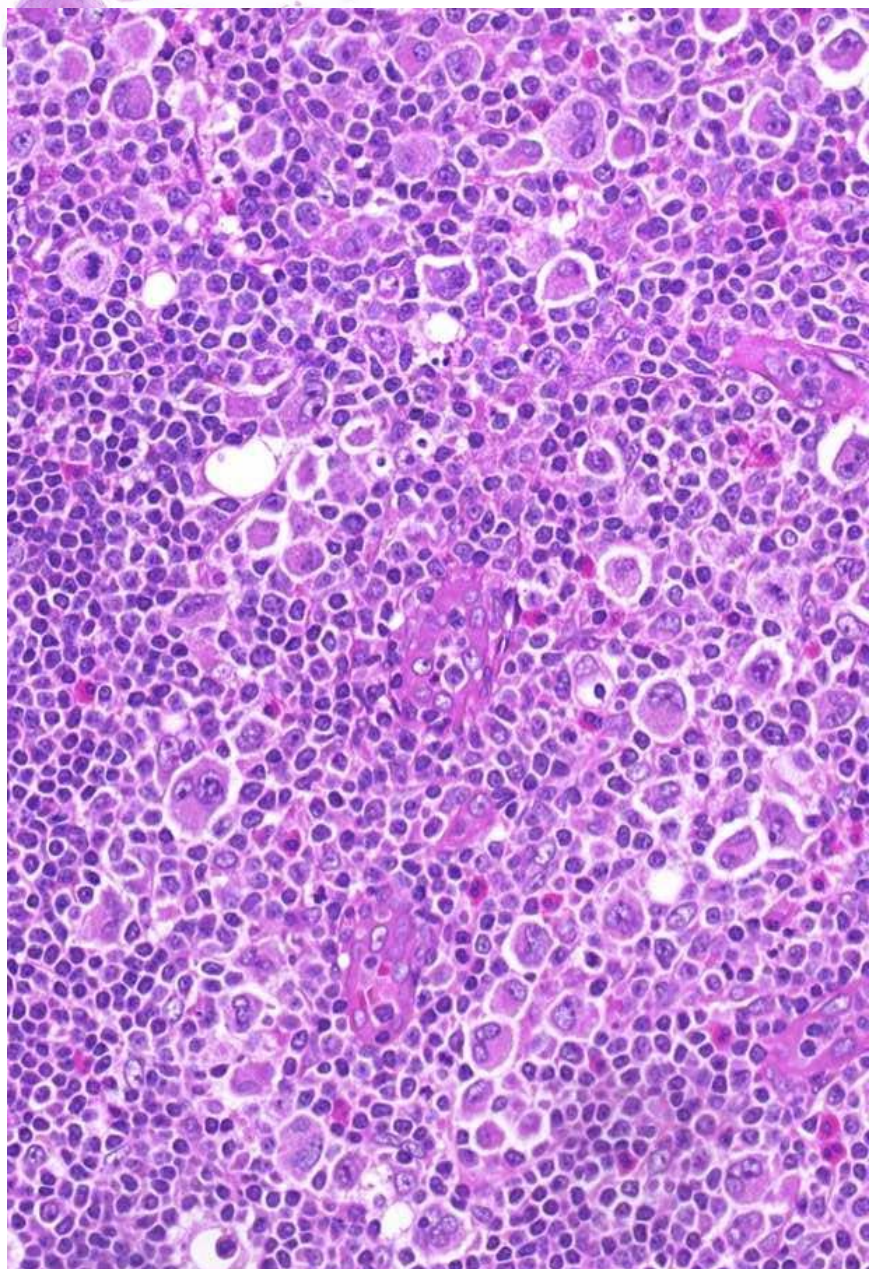
- CD30+: virtually all cases
- CD15+: 75-85% of cases
- Extinction of the B-cell differentiation programme
  - Pax5 weakly +
  - CD20 neg or weakly +
  - CD19- CD79a-
  - BOB.1 and/or OCT.2 –
  - CD45-
- MUM1+ CD138-
- EBV +/- (EBERs LMP1)

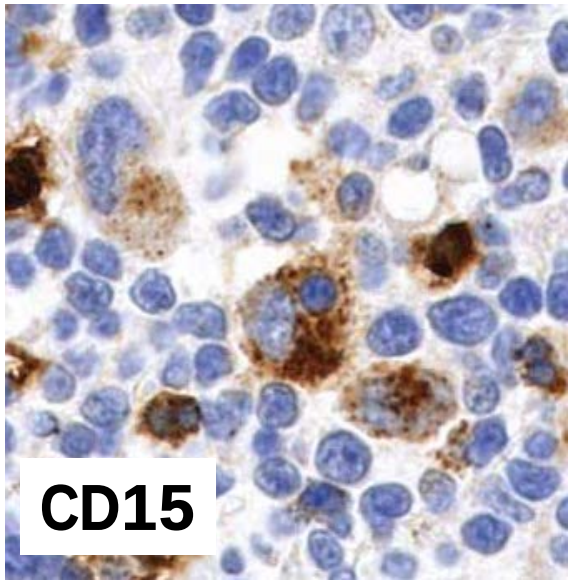


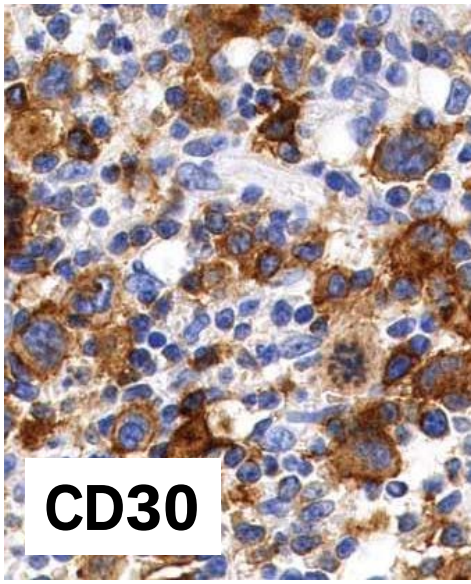



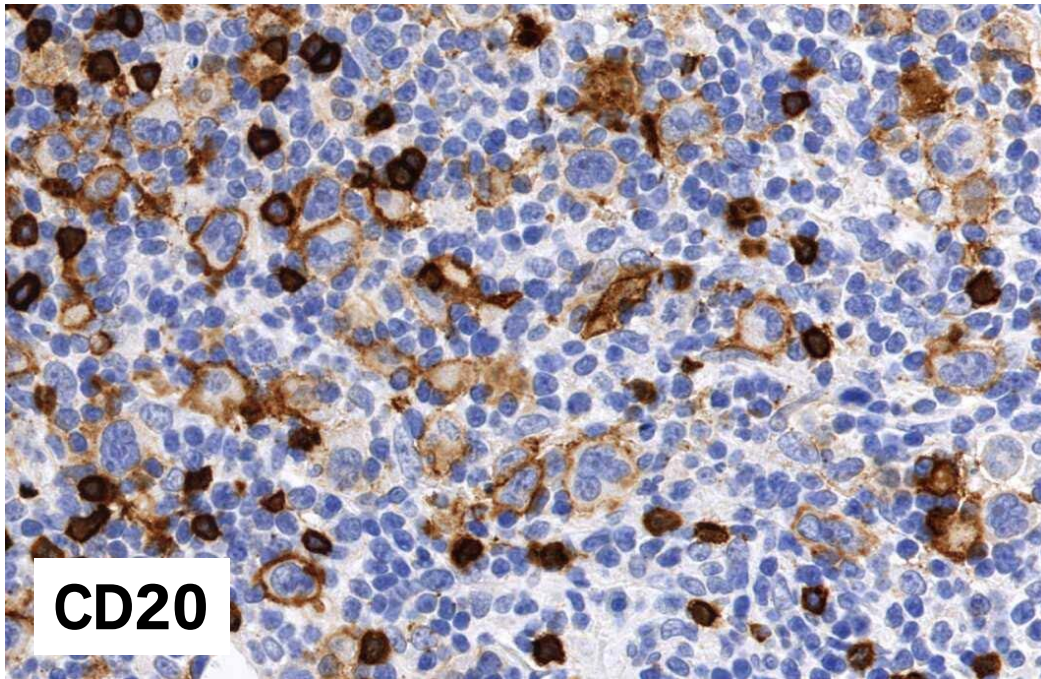


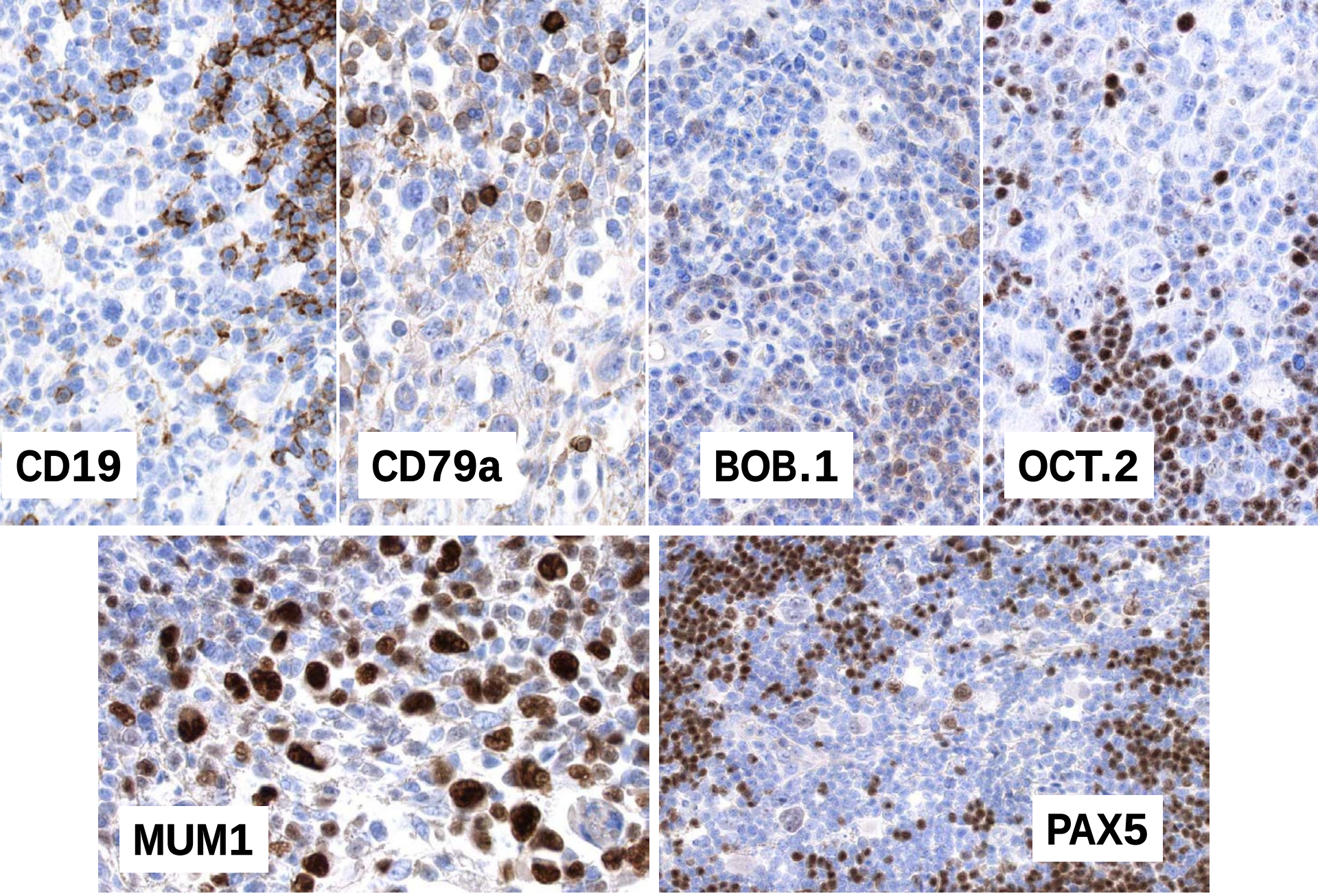
F 31 yrs supraclavicular lymph nodes





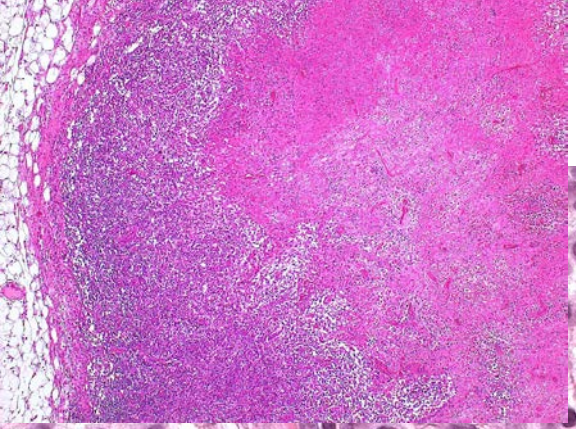



CD20-positive classical Hodgkin Lymphoma

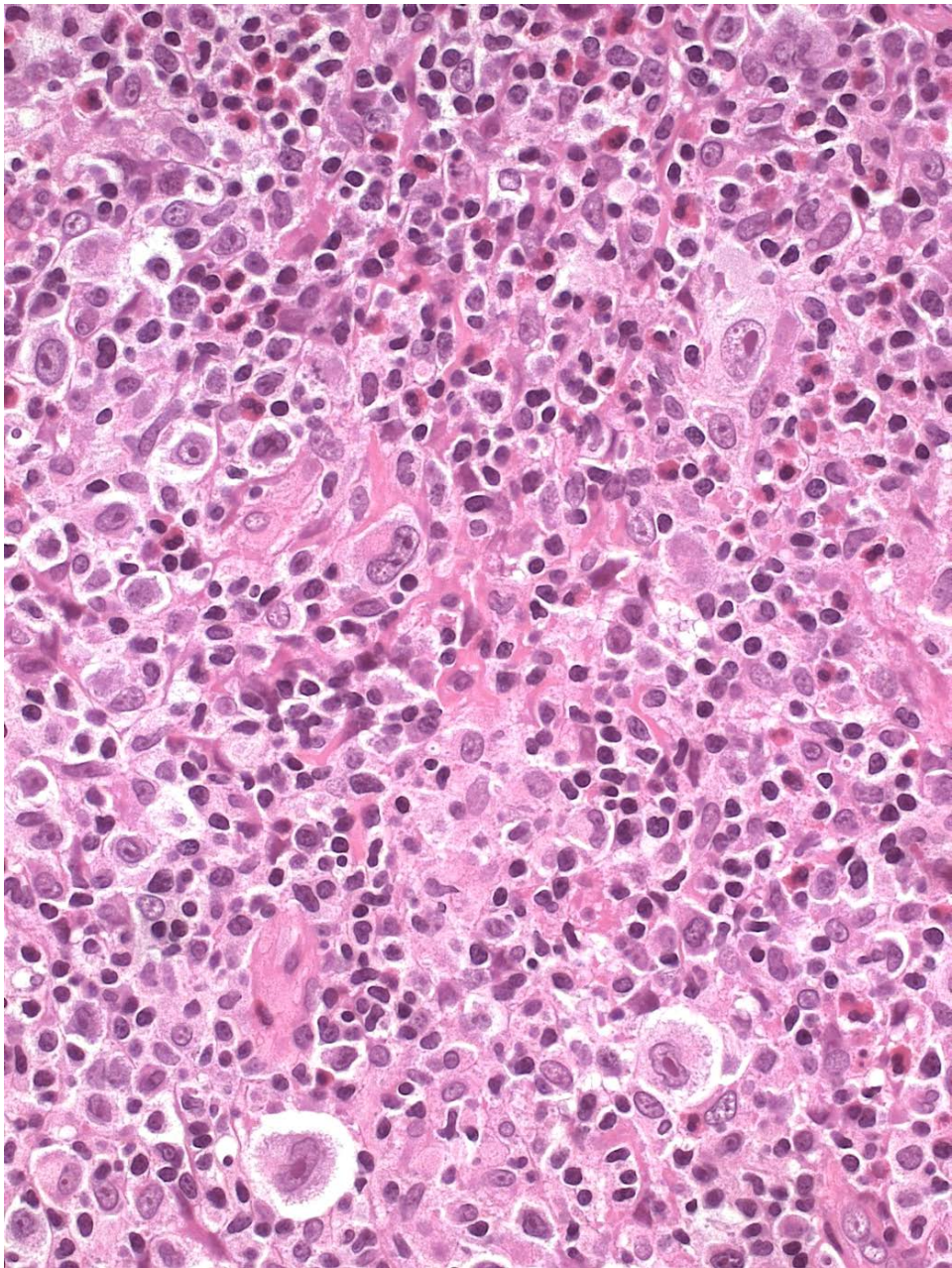
# EBV+ cHL - differential diagnosis

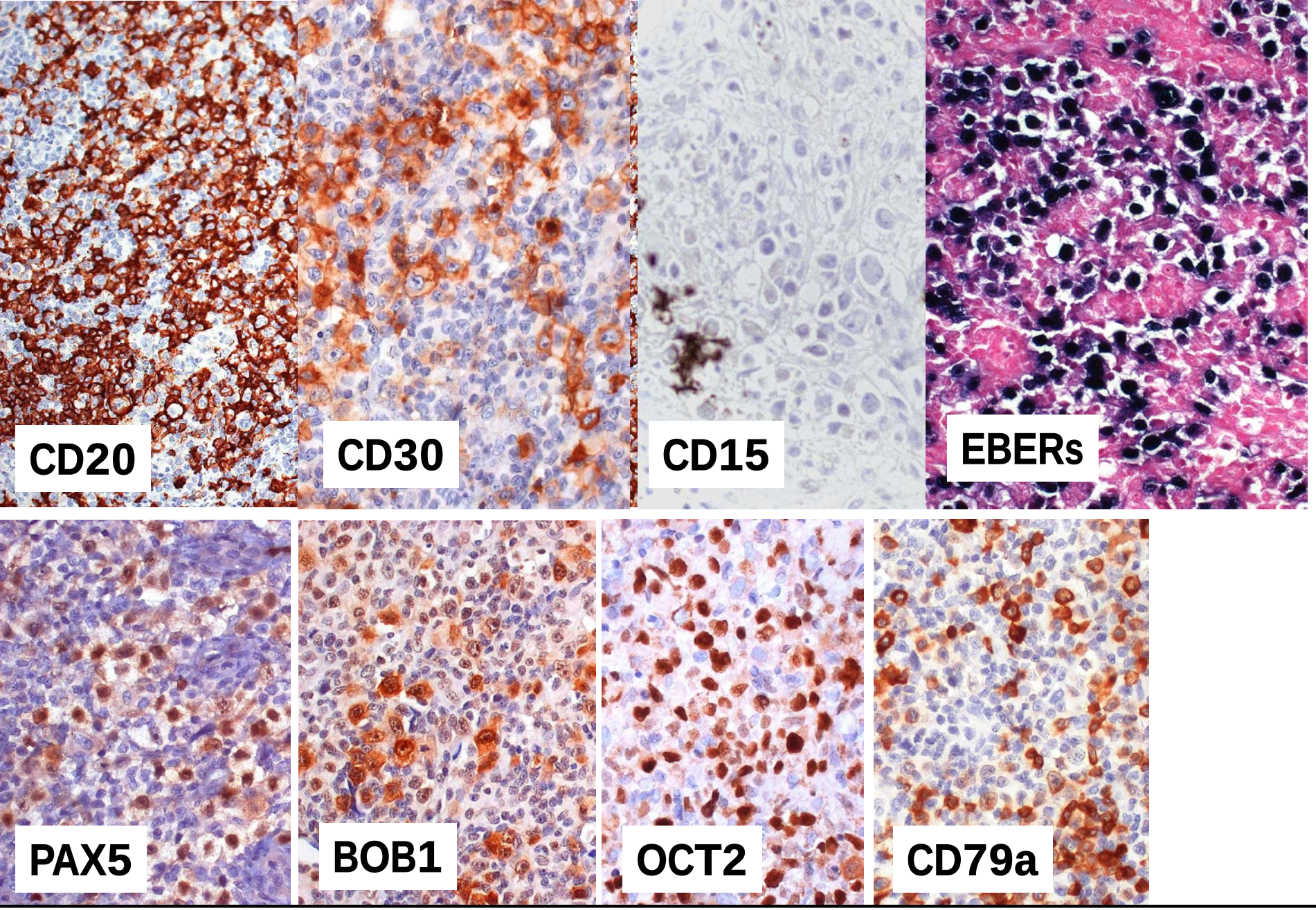
- **EBV-positive B-cell proliferations**
  - Infectious mononucleosis
  - Post-transplant/immunodeficiency-associated LPDs
  - Age-related EBV+ DLBCL of the elderly
  - EBV-positive mucocutaneous ulcer
- **EBV-positive RS-like cells in B-cell NHL**
  - Follicular lymphoma, CLL
  - Composite HL + NHL
  - Hodgkin variant of Richter syndrome
- **EBV-positive RS-like cells in T-cell NHL**

# M 84 yrs axillary lymph node



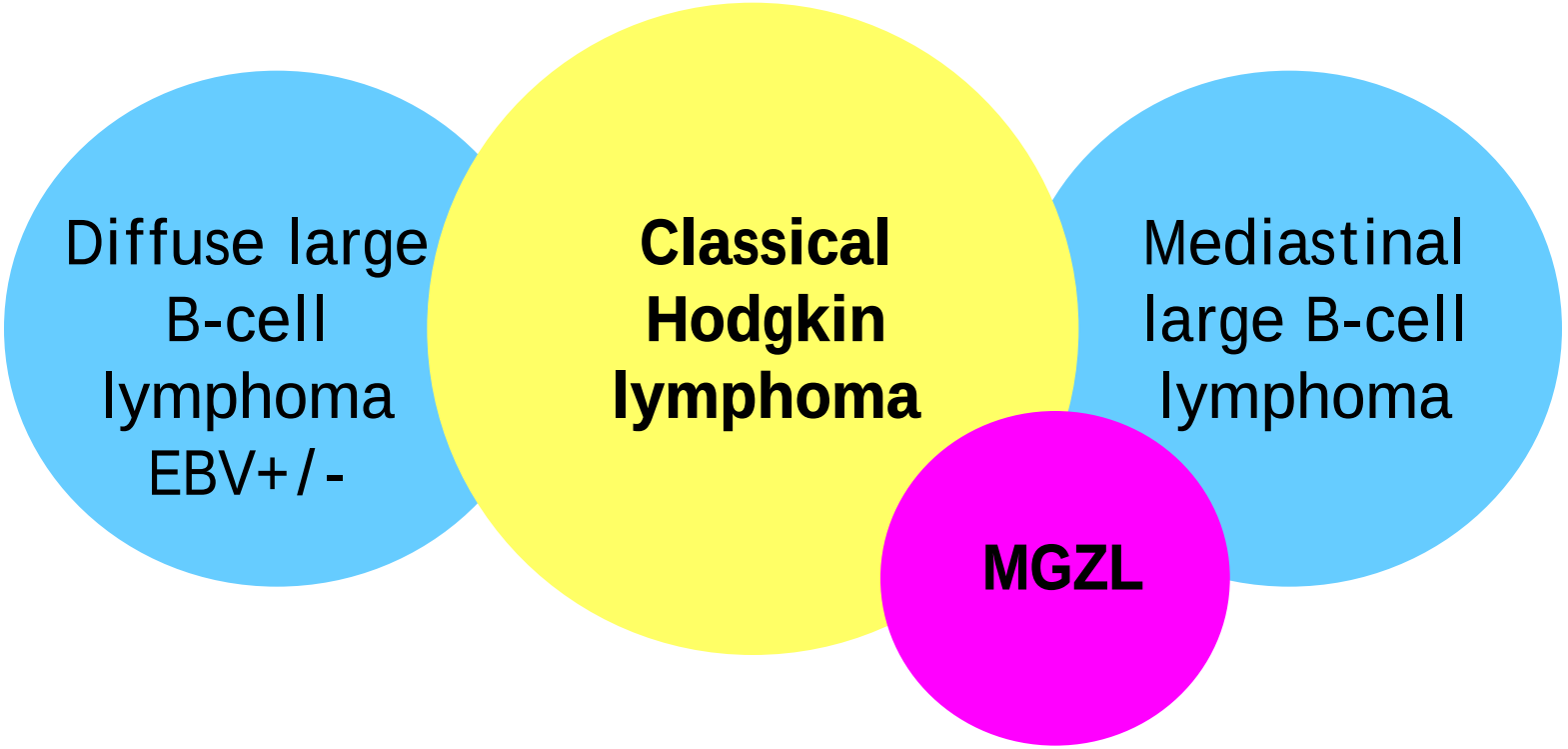







EBV-positive DLBCL of the elderly – polymorphic resembling cHL

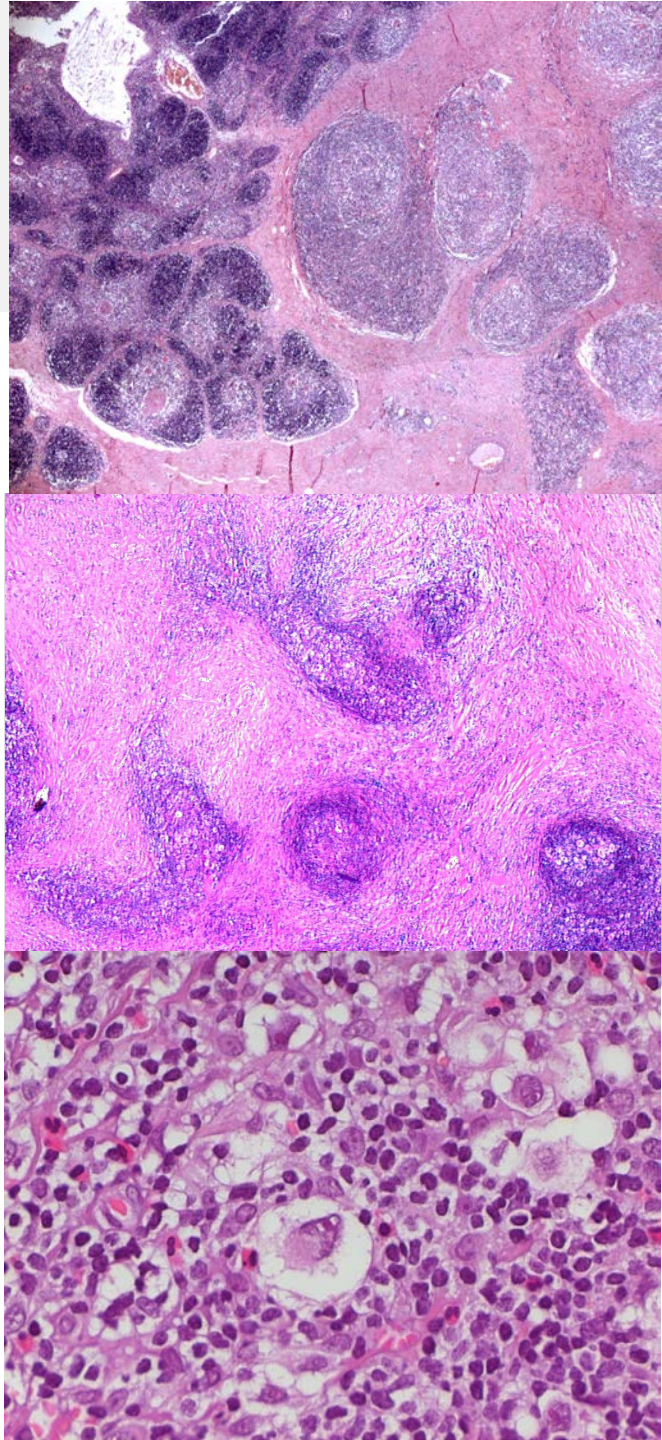
Lymphomas primarily involving the mediastinum in adults

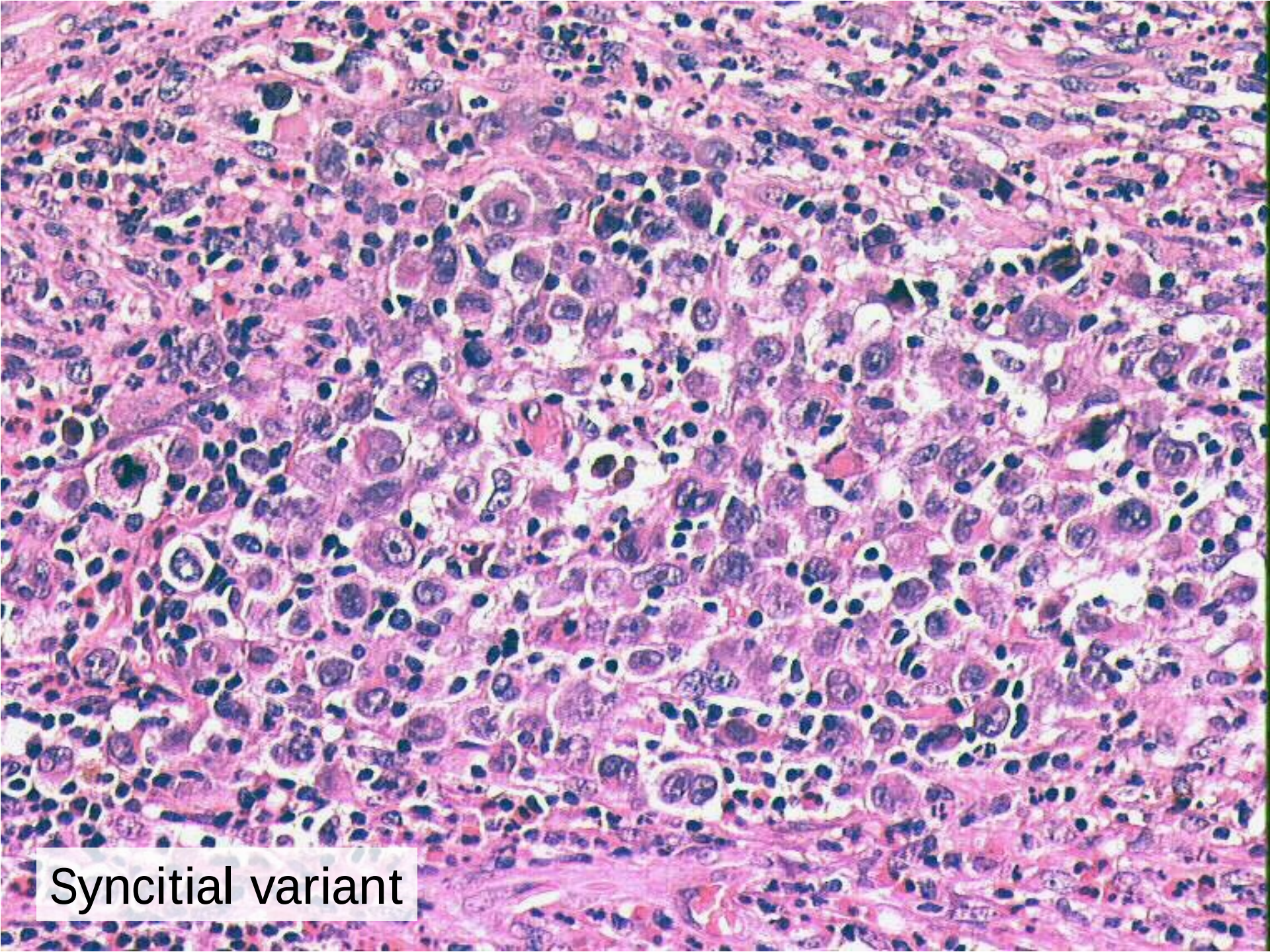



**B-cell lymphoma unclassifiable with features intermediate between DLBCL and cHL**

# Classical Hodgkin lymphomas, NS type

- Mediastinal involvement common, originating from the **thymus and/or from mediastinal lymph nodes**
- Adolescents and young adults, F>M
- Presenting symptoms due to a mediastinal mass, other cases discovered incidentally
- Collagenous bands surrounding at least one nodule
- HRS cells with lacunar morphology
- Necrosis can be prominent, syncitial variant
- EBV positivity




Syncitial variant

# Primary mediastinal large B-cell lymphoma

- A subtype of DLBCL arising in the mediastinum, thought to derive from thymic B cells and with clinical, pathological and genetic features distinct from those of usual DLBCL
- Female predominance (2F:1M)
- Young adults (median 25-30 y)
- Bulky mediastinal mass, stage I/II
- Distant LN involvement rare
- Relapse in extranodal sites (lung, GI tract, ovaries, liver, kidney, CNS, breast)

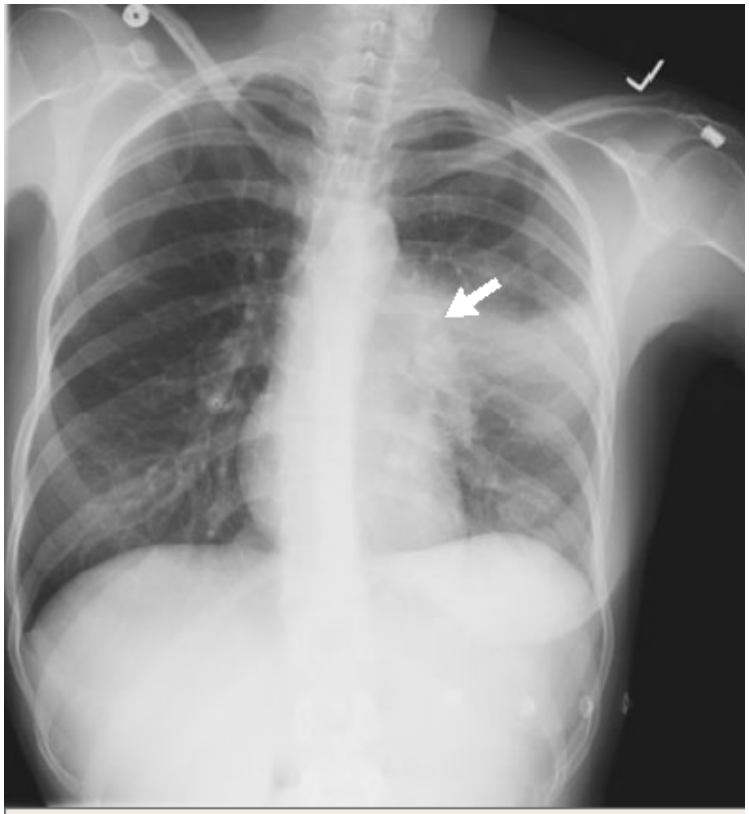
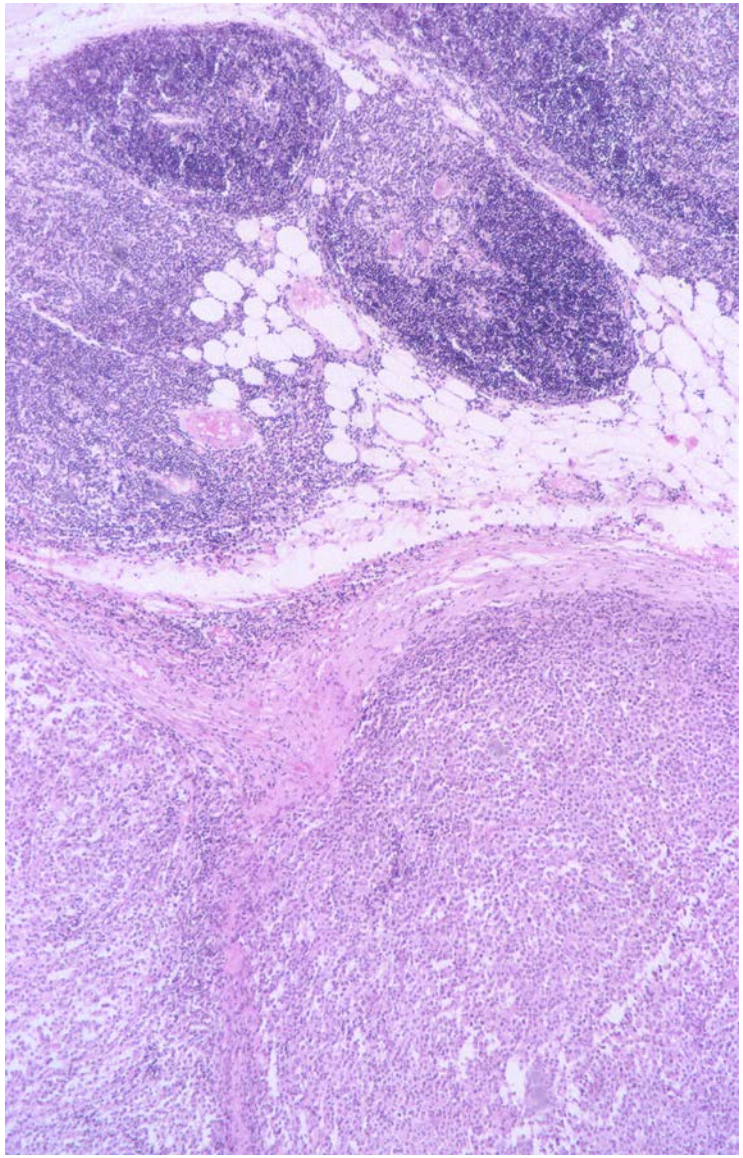


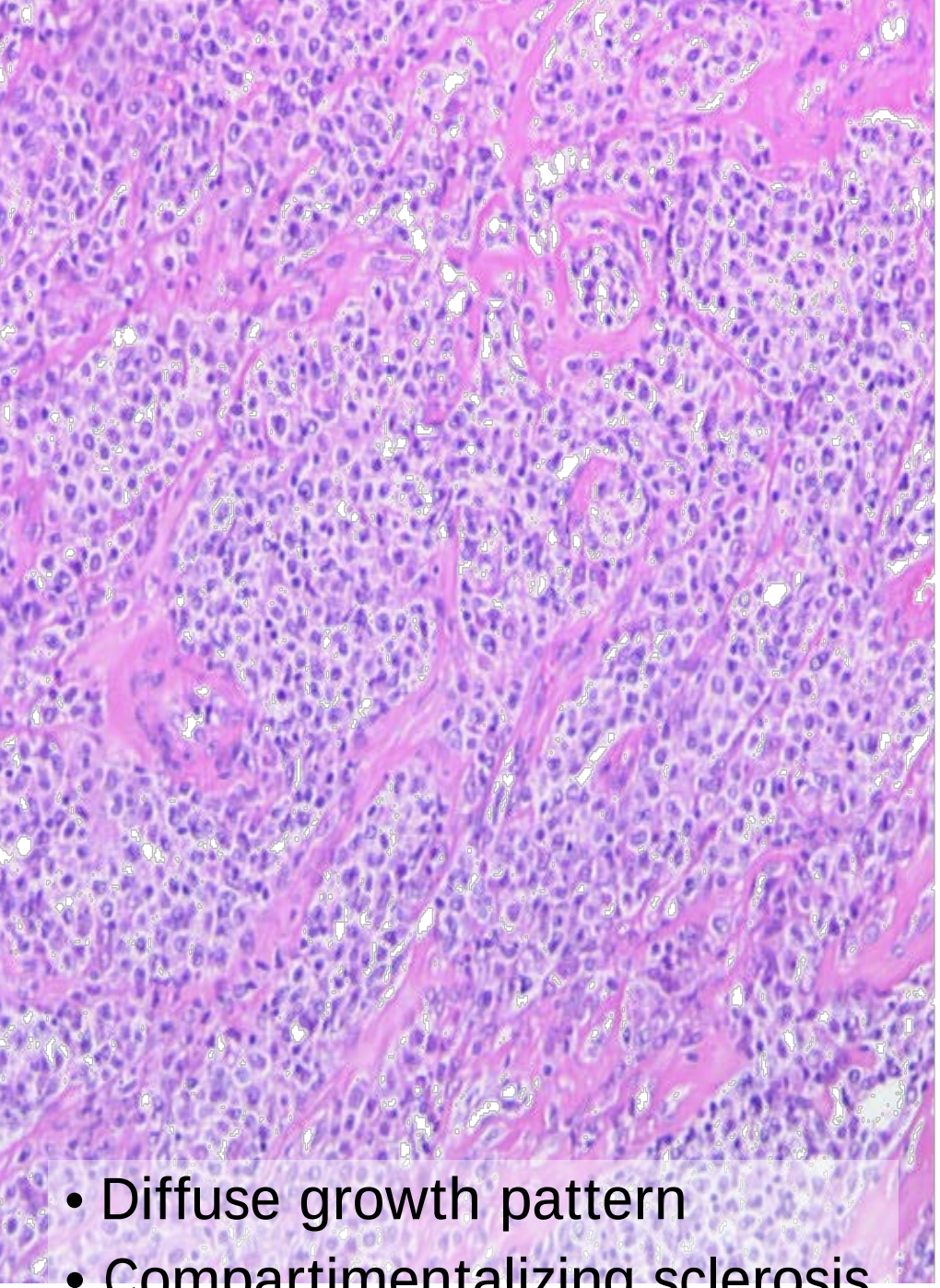
**Figure 1. Chest Radiograph.**
The initial chest radiograph shows soft-tissue fullness (arrow) along the left hilum and mediastinum that obscures the hilar anatomy. There is an air-space opacity in the middle left lung.

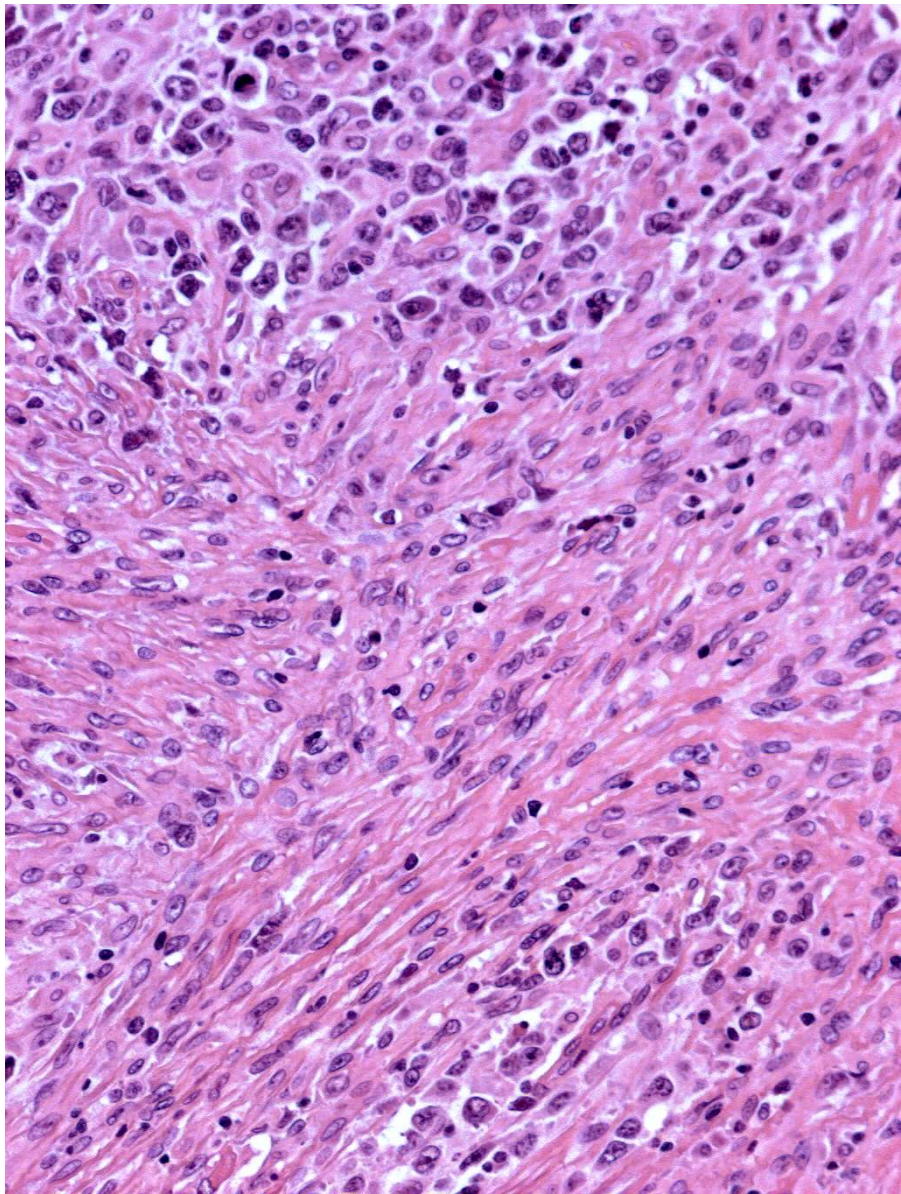
# Primary mediastinal large B-cell lymphoma

- **Morphology**
  - Large clear cells, pale cytoplasm
  - Sclerosis
  - Mixture of centroblasts, immunoblasts, ultilobated, anaplastic, HRS-like cells

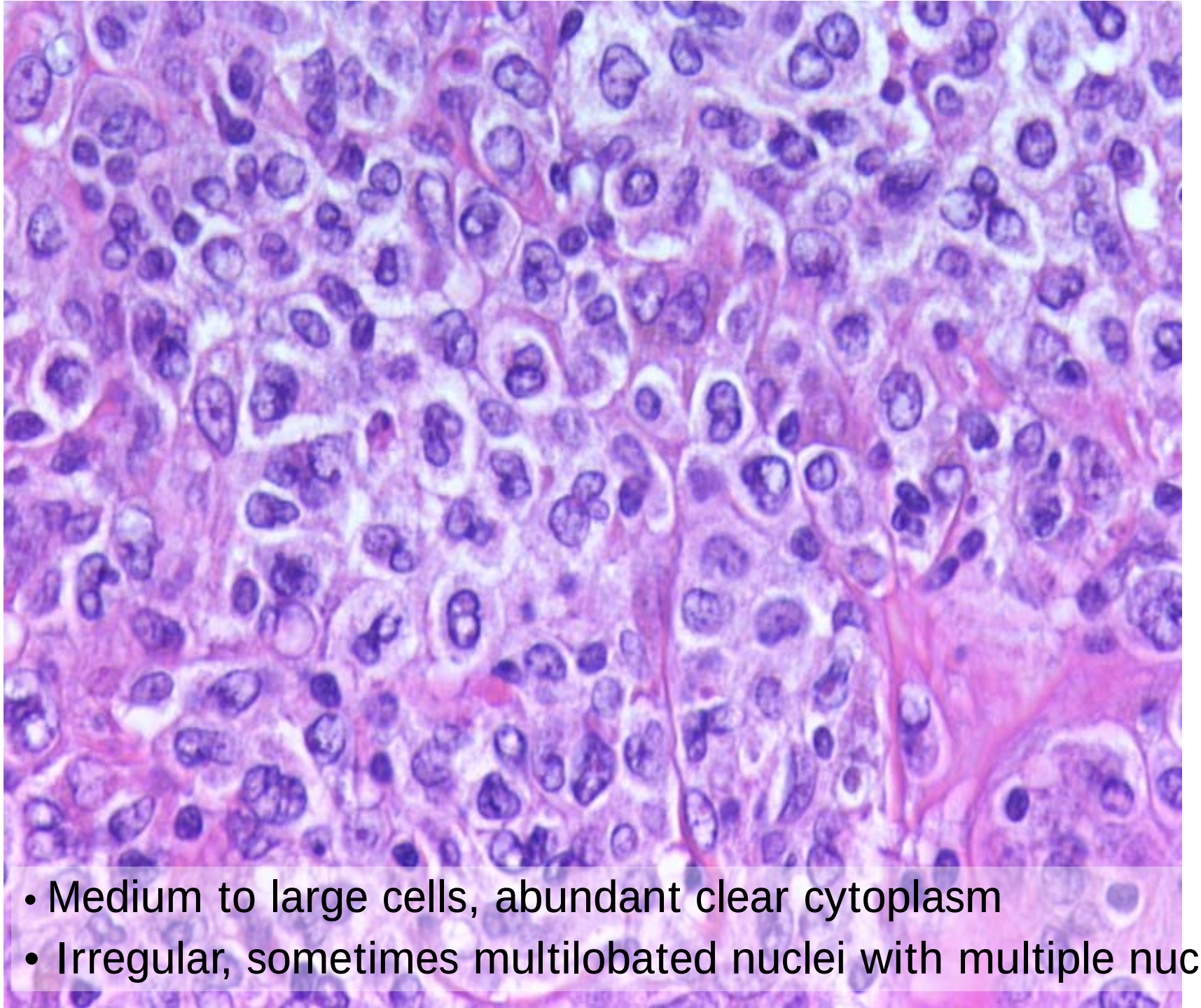
- **Immunophenotype**
  - B-cell phenotype: Pax5+ CD20+ CD79a+ OCT2+ BOB1+ but lack Ig expression
  - CD30 expression common, less intense than in cHL, CD15 usually negative
  - CD23 commonly coexpressed
  - Stage: CD10-/+ Bcl6+/- Mum1+ Bcl2+
  - Other: MAL+ TRAF+ nuclear cREL



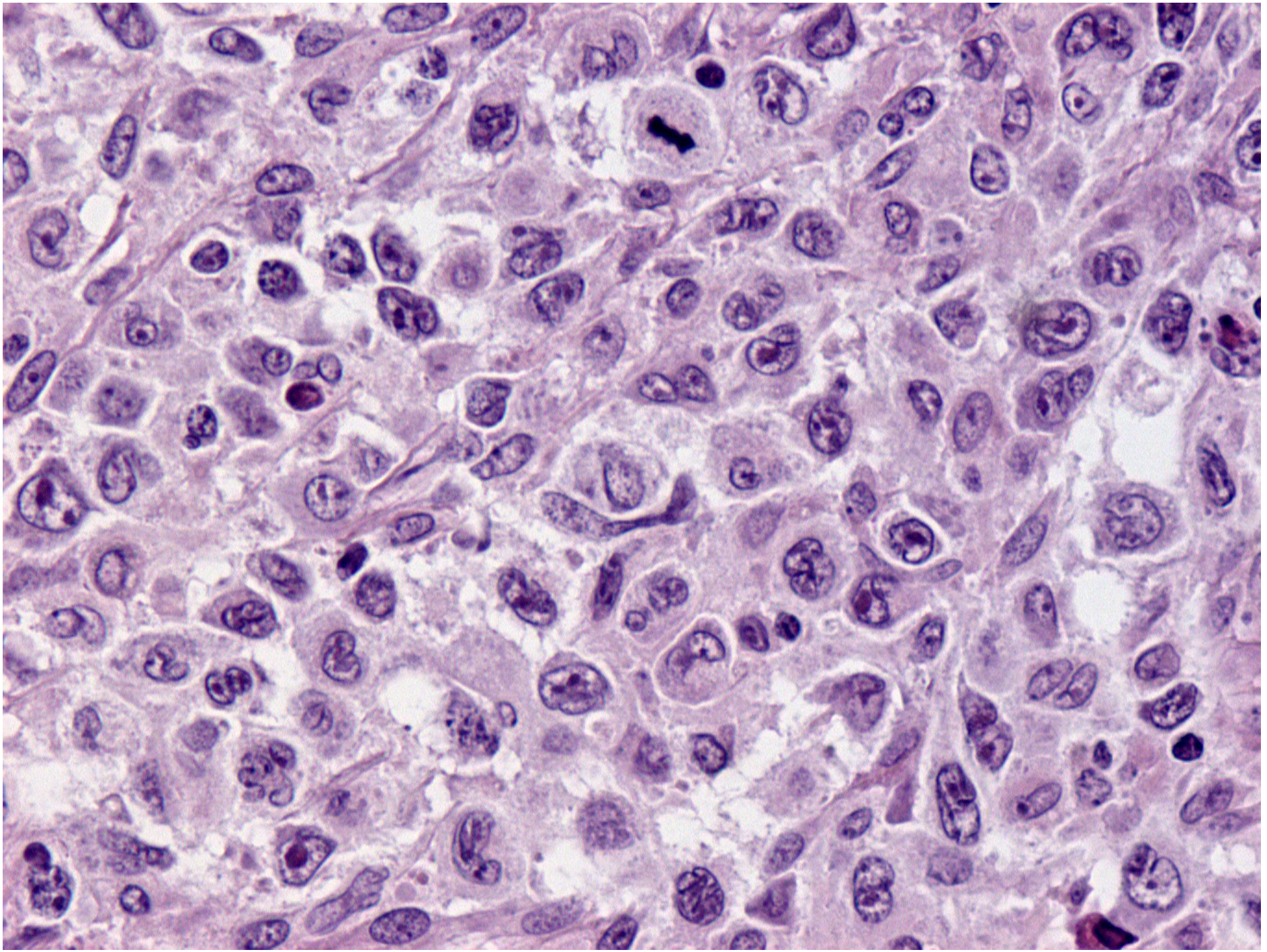


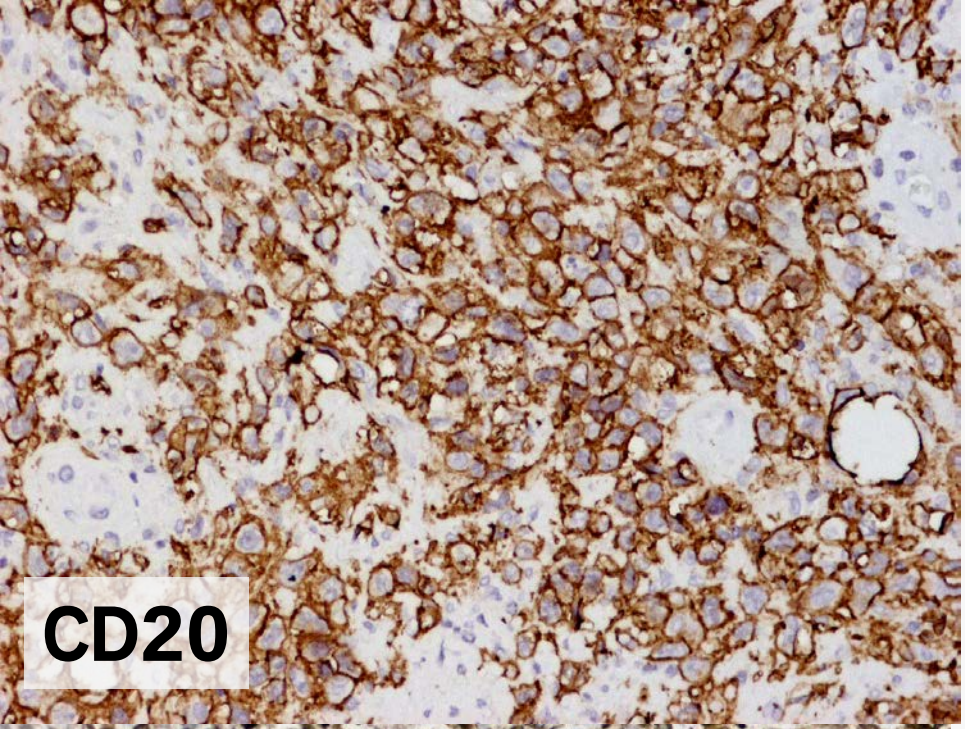


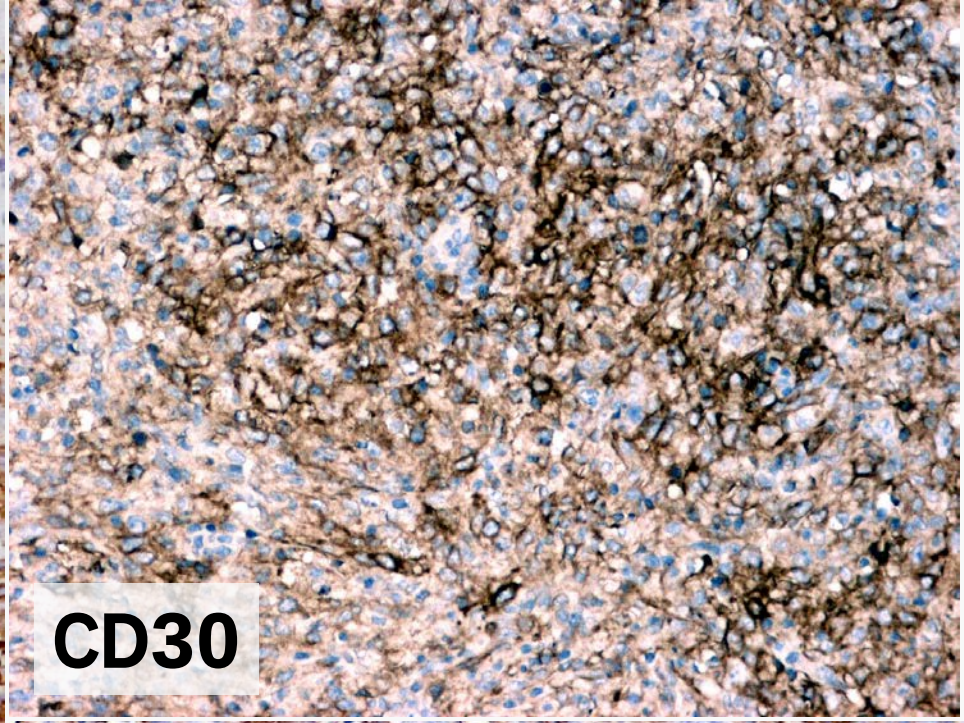
- Diffuse growth pattern
- Compartimentalizing sclerosis

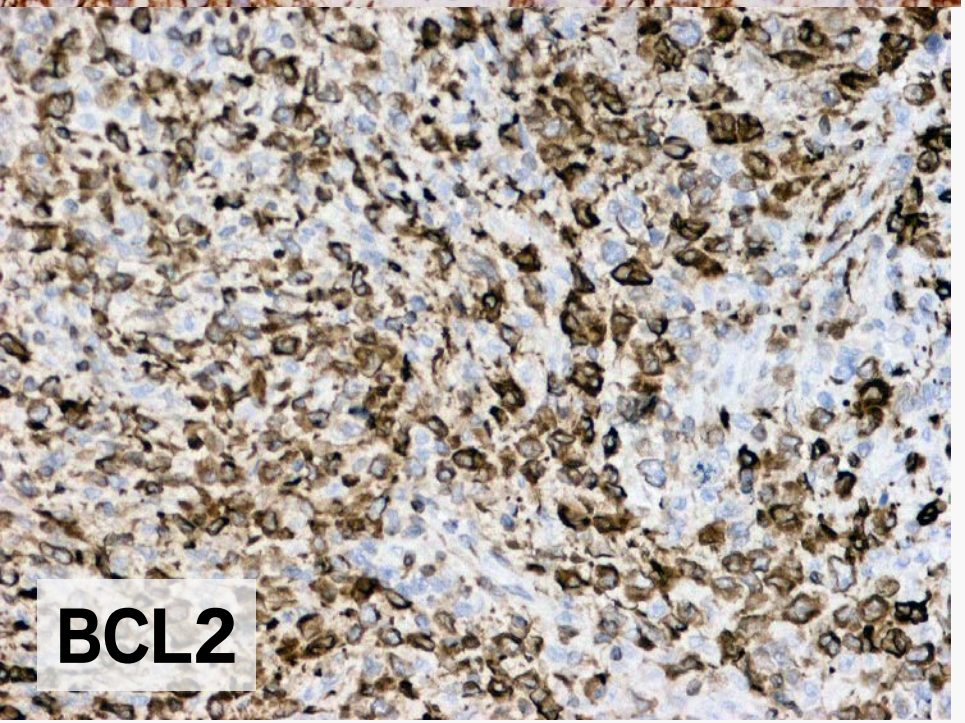


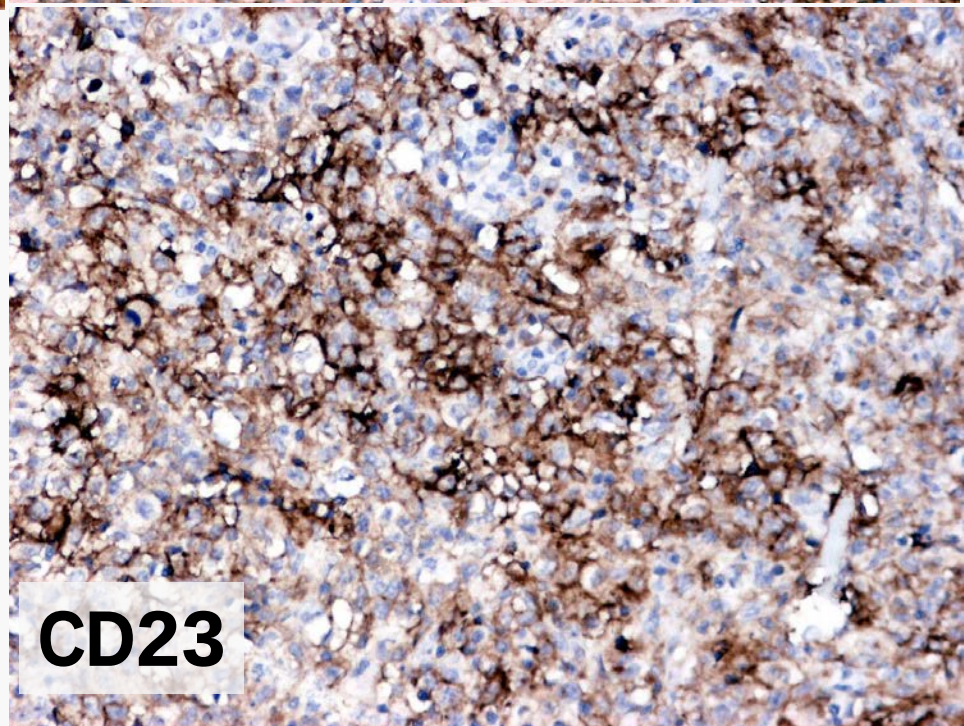
- Medium to large cells, abundant clear cytoplasm
- Irregular, sometimes multilobated nuclei with multiple nucleol




CD20

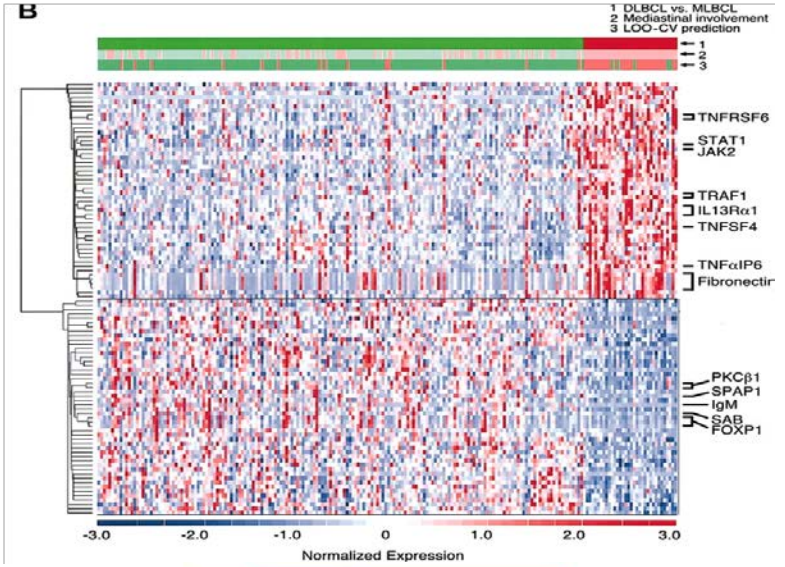

CD30

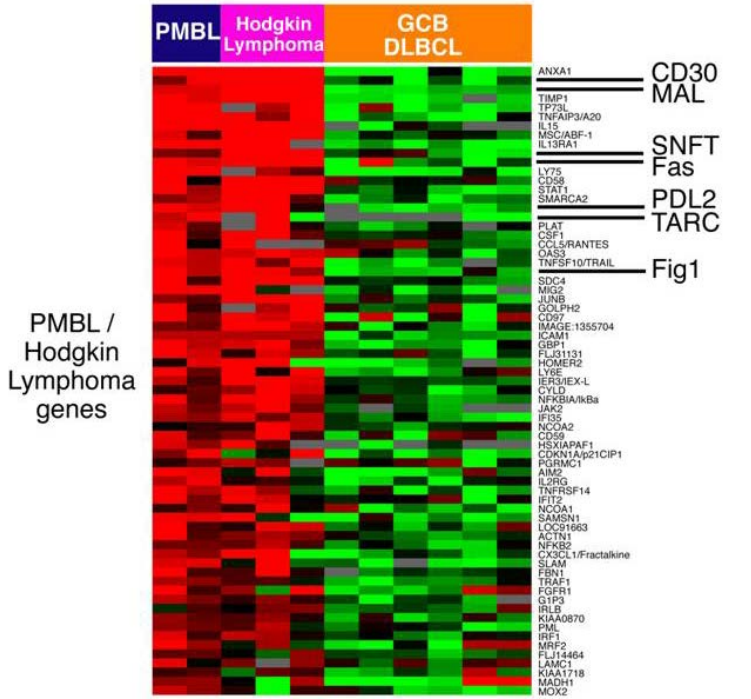

BCL2


CD23

# The genetics and molecular signature of PMLBCL differ from that of other DLBCL and resemble that of cHL

- Rearrangements of *BCL2 BCL6* rare
- Gains at 2p (*cREL*, *BCL11*), 9p (*JAK2*) also in cHL
- *CIITA (*MHC class 2 transactivator gene) rearr. in 38% PMBL and 15% of cHL
  - Fusion transcripts involving PDL1 and 2 (T-cell inhibition)
  - Decreased HLA-DR, increased PDL1/2 -> immune escape?
- Gene expression:
  - activation of NF kappa B pathway
  - cytokine pathway signature (importance of interactions with microenvironment)
  - downregulation of BCR pathway




*Savage K et al. Blood 2003; Rosenwald A et al. JEM 2003; Steidl C et al. Nature 2011*

# PMLBCL and mediastinal NS cHL overlap in clinical pathological molecular and genetic features

| | |
|---|---|
| **Clinical presentation** | Young adults |
| | Female predominance |
| | Mediastinal mass (thymus) |
| | supraclavicular nodes |
| **Morphology** | Fibrosis |
| | Cytologic overlap: RS cells and PMBL |
| **Immunophenotype** | Absence of sIg |
| | CD30 expression |
| **Genetic features** | Amplification of *REL* locus (2p) |
| | Amplification of *JAK2* locus (9p) |
| | *CIITA* translocations |
| | Molecular signature |

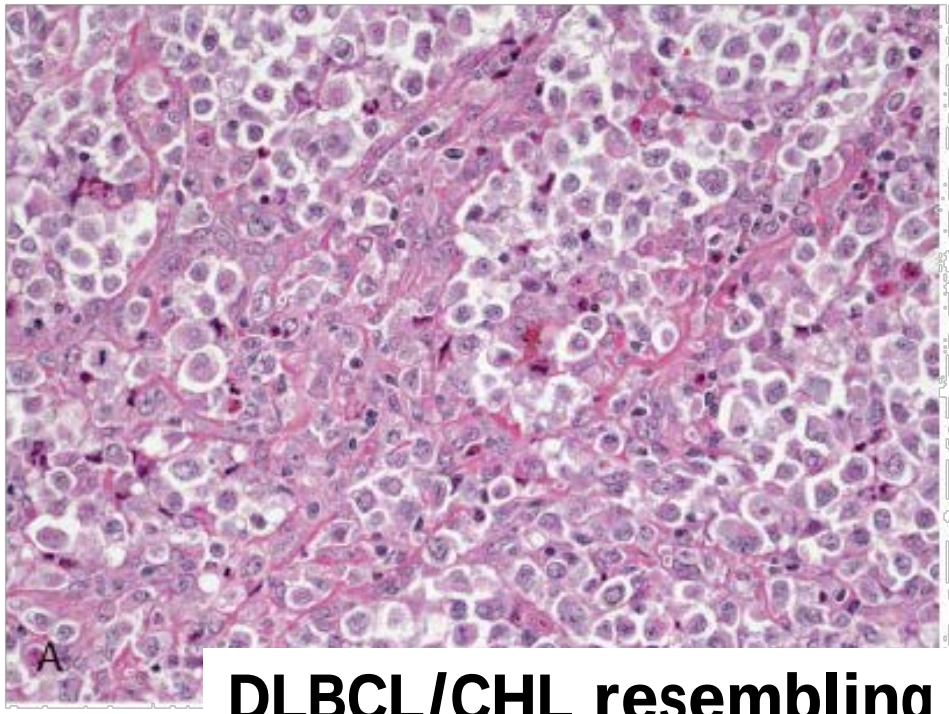
| | PMLBCL | NSHL |
|---|---|---|
| Pattern | diffuse | nodular |
| | clear cells | lacunar HRS |
| Inflammation | absent | present |
| CD45 | positive | negative |
| CD30 | often positive | positive |
| CD15 | negative | positive (85%) |
| B-cell antigens | | |
| CD20 | strong | weak |
| CD79a | positive | negative |
| Pax-5 | strong | weak |
| Mum-1 | positive | positive |
| Ig expression | | |
| sIg | negative | negative |
| BOB.1 | positive | usually negative |
| OCT.2 | positive | usually negative |
| MAL | usually positive | may be positive |
| EBV | absent | may be present |

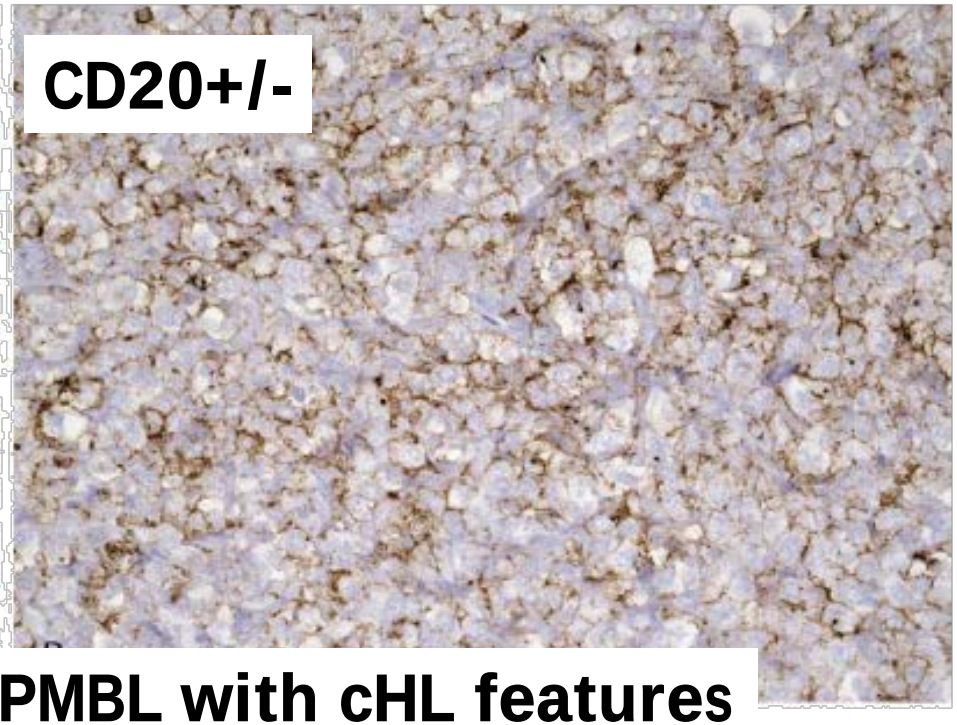
# B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL

- **New category (provisional) in 2008 WHO Classification**
- Definition
  - A B-lineage lymphoma with overlapping clinical, morphological and/or immunophenotypic features between CHL and DLBCL, especially PMBL
    - PMLBCL with cHL features
    - cHL with MLBCL features
  - Most commonly associated with mediastinal disease, but may occur in peripheral lymph nodes; extranodal sites uncommon
- Typically young men (20-40 y), but also older adults (non-mediastinal invovlement)

# B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL

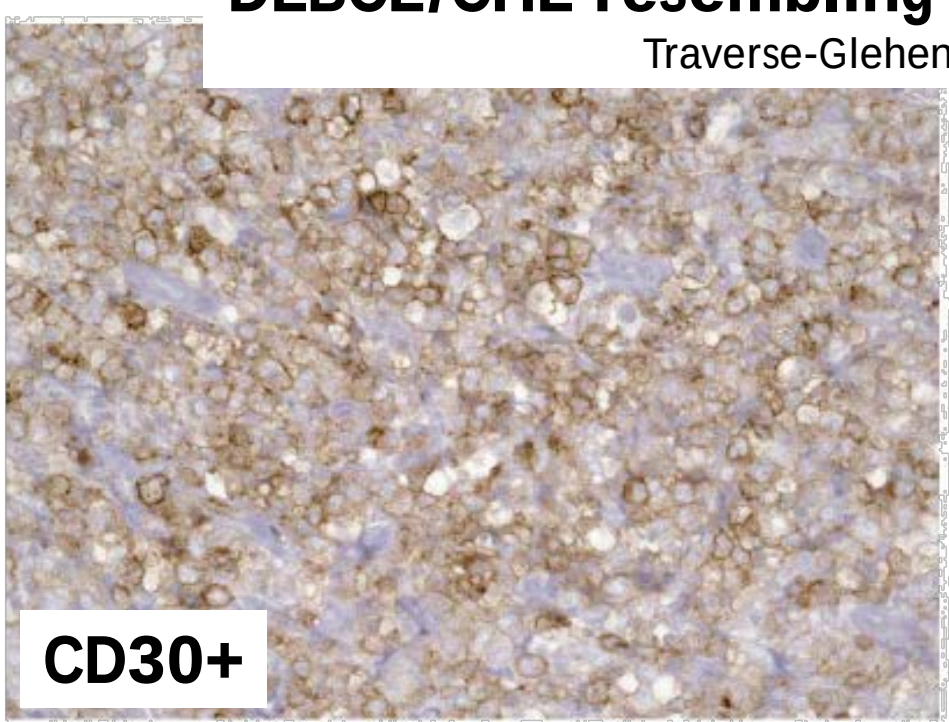
- Borderline morphology
- Architecture: sheets of tumor cells in sclerotic stroma, focal fibrous bands
- Cells larger and more pleomorphic than typical PMBL, lacunar-type cells may predominate
- Different areas show variations in appearance
    - some areas resemble CHL
    - others resemble DLBCL
- Inflammatory infiltrate usually sparse (eosinophils, lymphocytes, histocytes)
- Necrosis: common, absence of neutrophils (unlike CHL)

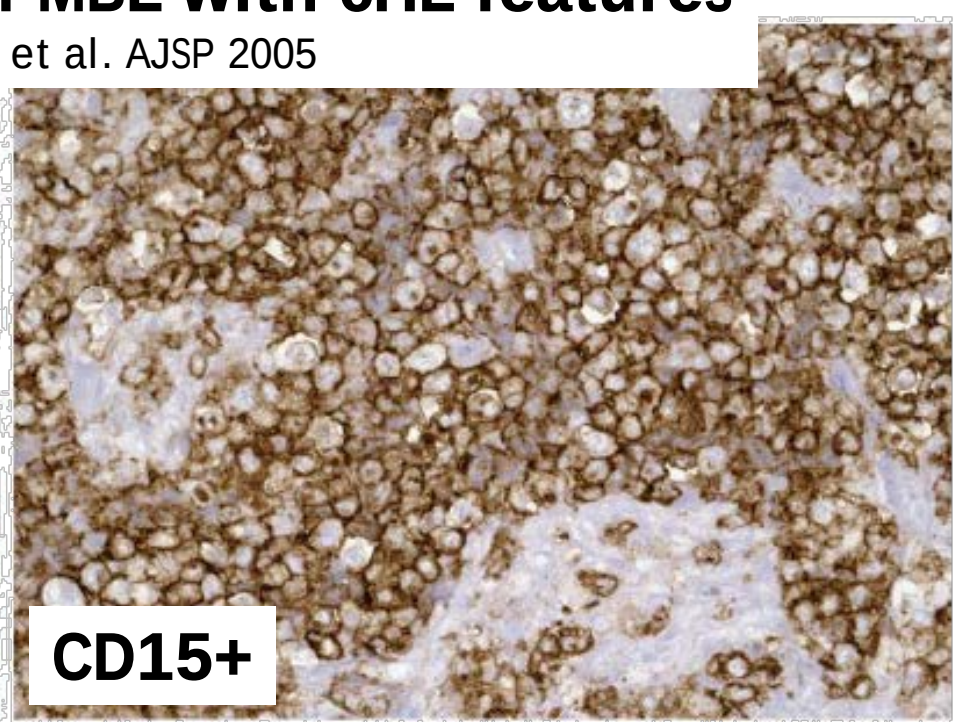


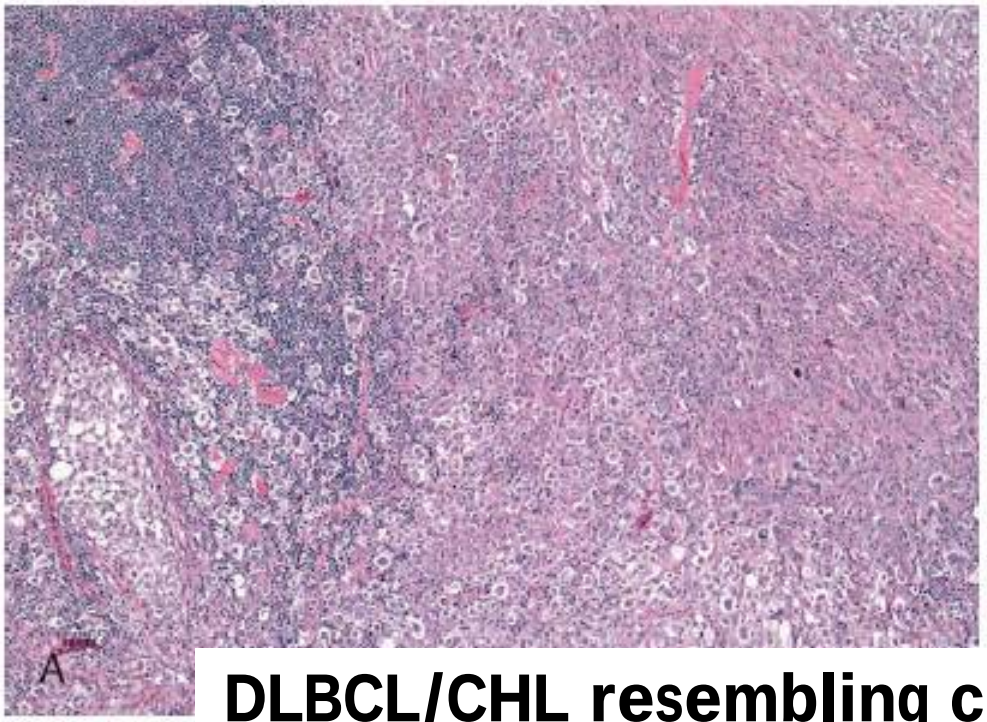


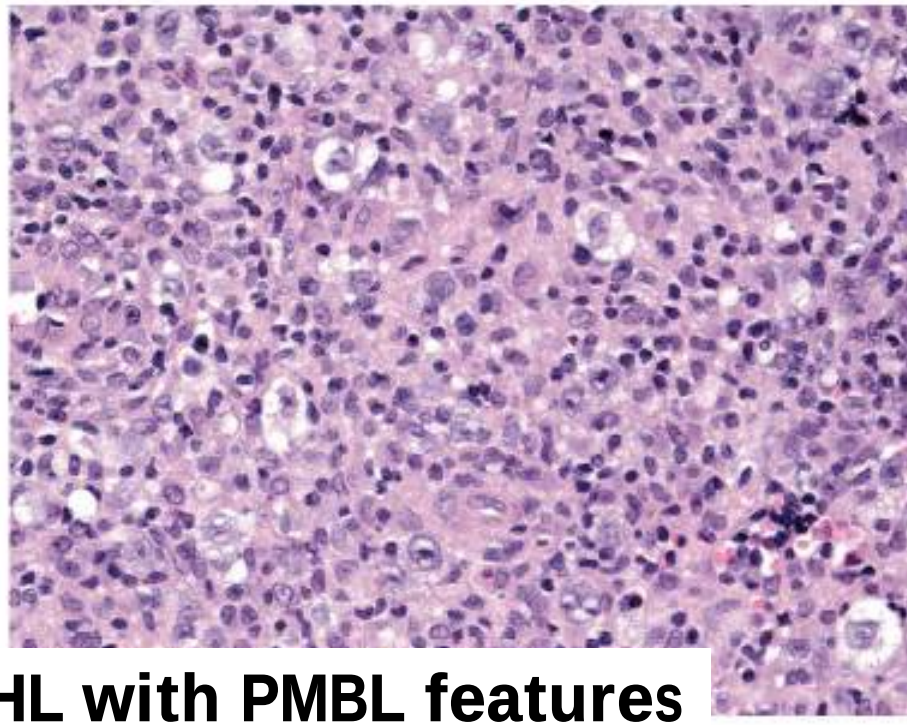

# DLBCL/CHL resembling PMBL with cHL features

Traverse-Glehen et al. AJSP 2005



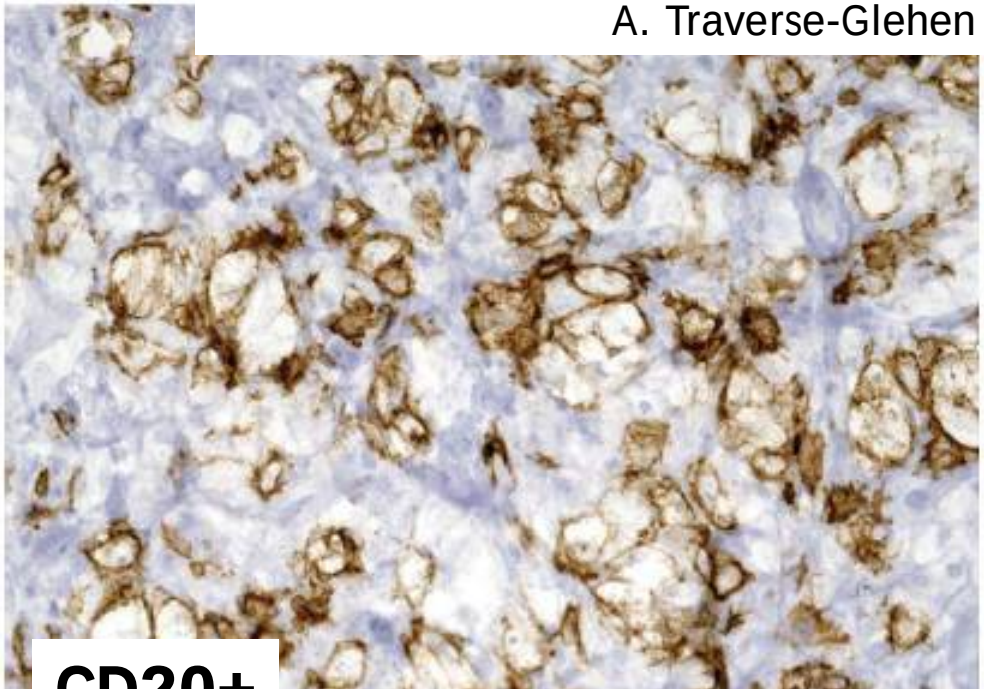





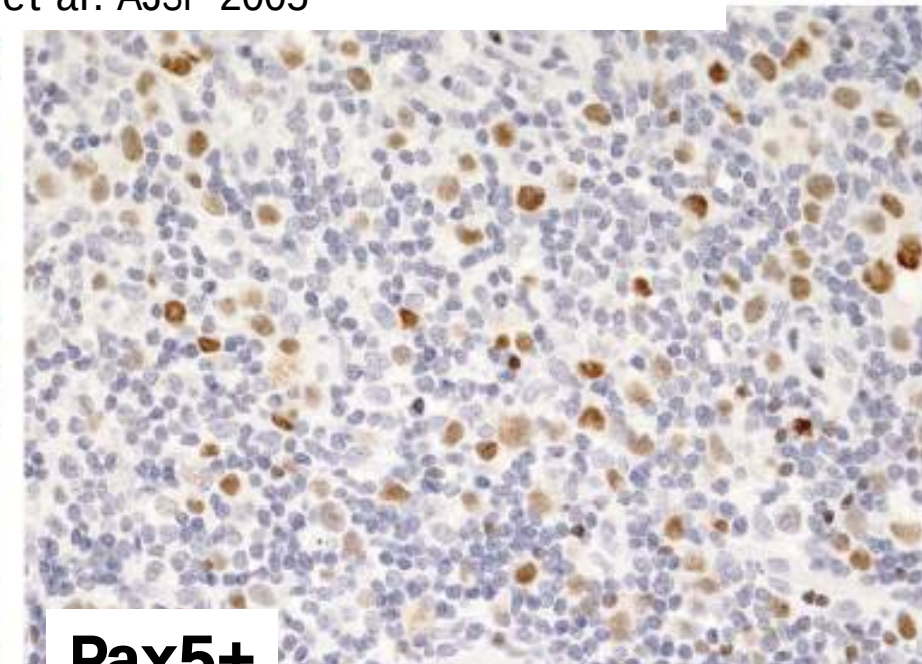



# DLBCL/CHL resembling cHL with PMBL features

A. Traverse-Glehen et al. AJSP 2005



CD20+



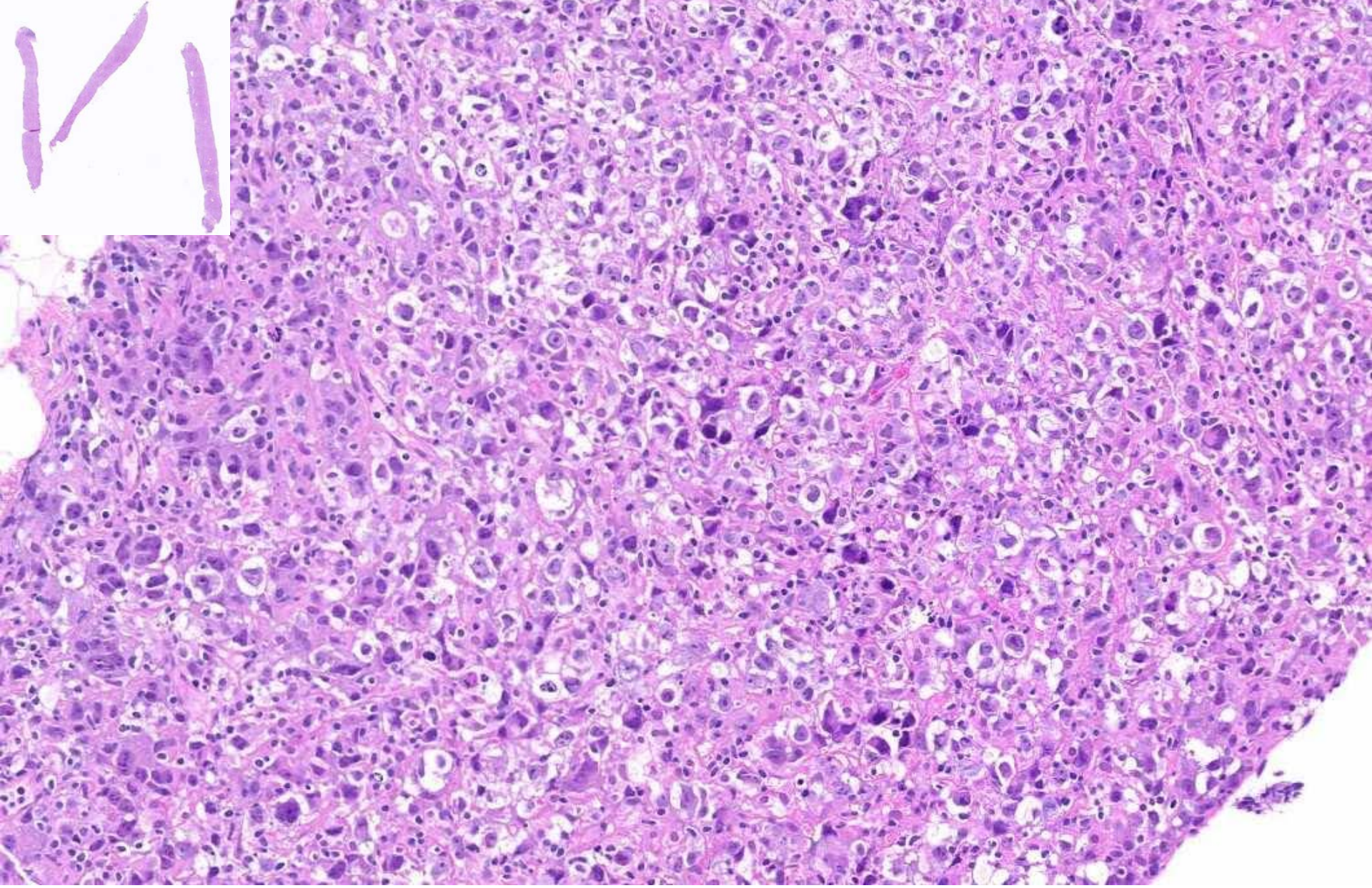
Pax5+

# B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL

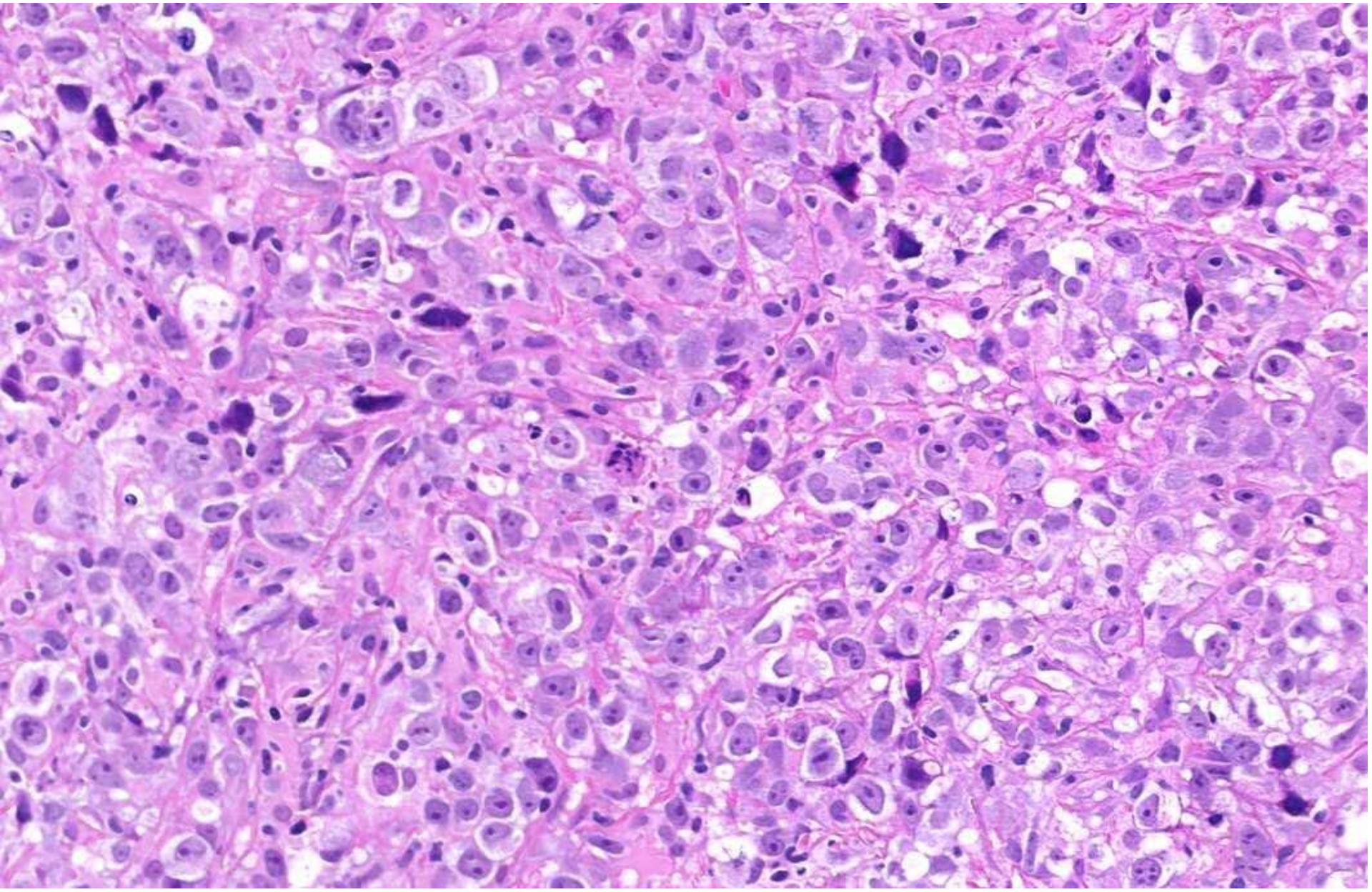
| | PMBL-like | cHL-like |
|---|---|---|
| CD20 | VARIABLE LOSS | POSITIVE |
| Pax5 | positive | positive |
| OCT2 | positive | positive |
| BOB1 | positive | positive |
| CD15 | POSITIVE | Positive or negative |
| CD30 | positive | positive |
| MAL | positive | positive |

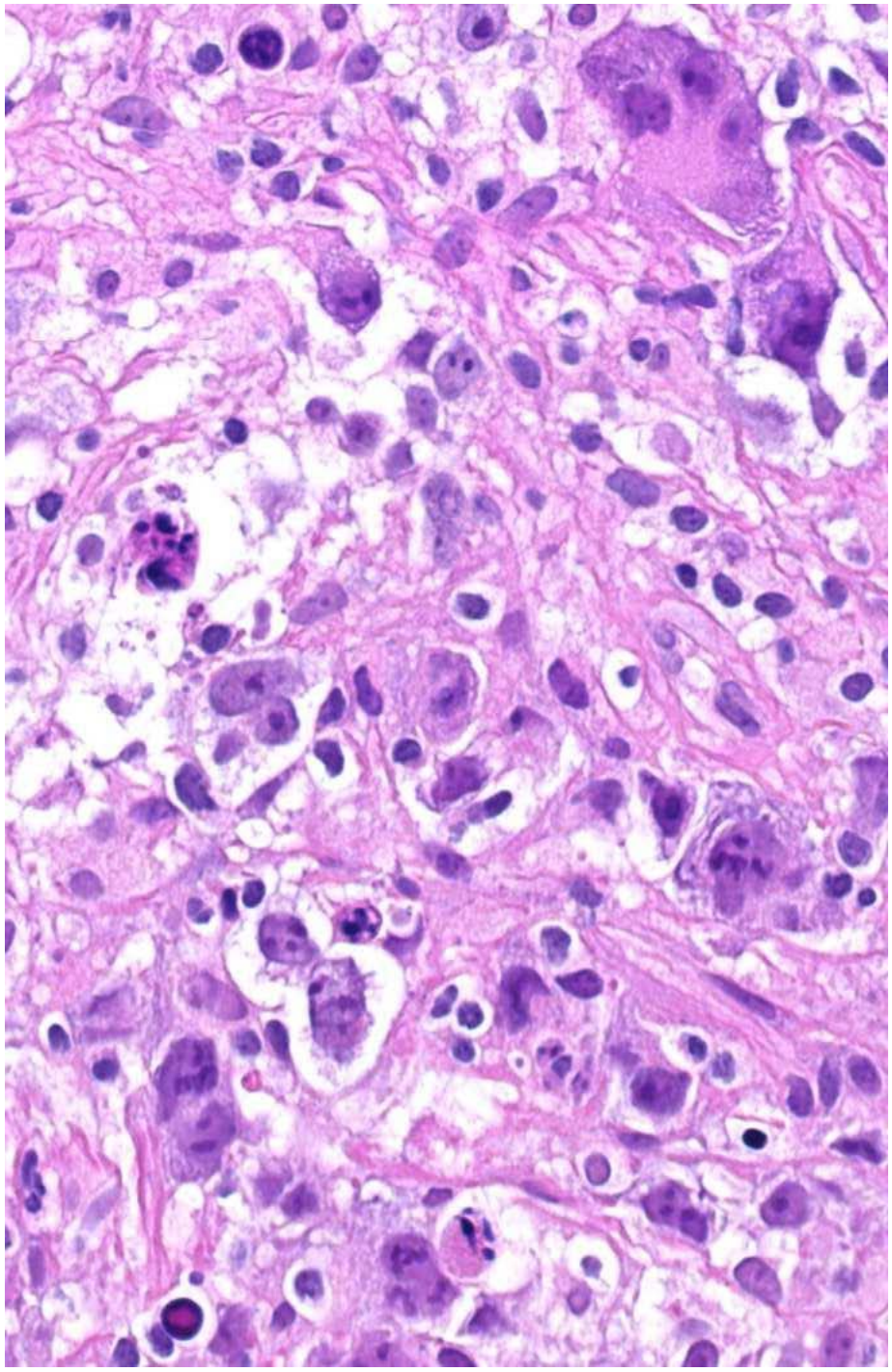
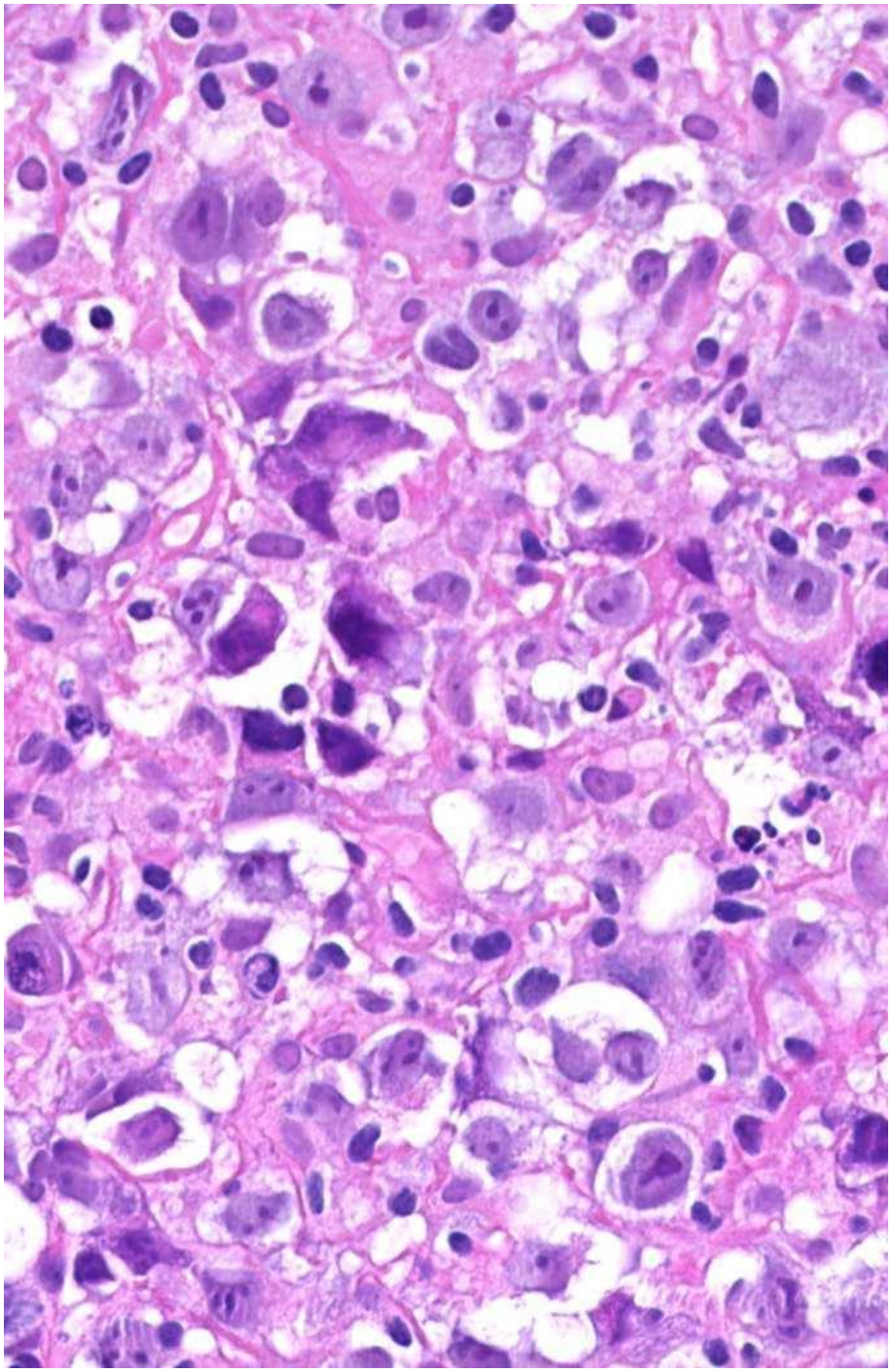
- Immunophenotype: discordant to morphology
- Genetic features: 2p 9q amplifications, *CIITA* translocations in a fraction of the cases; also in non-mediastinal cases

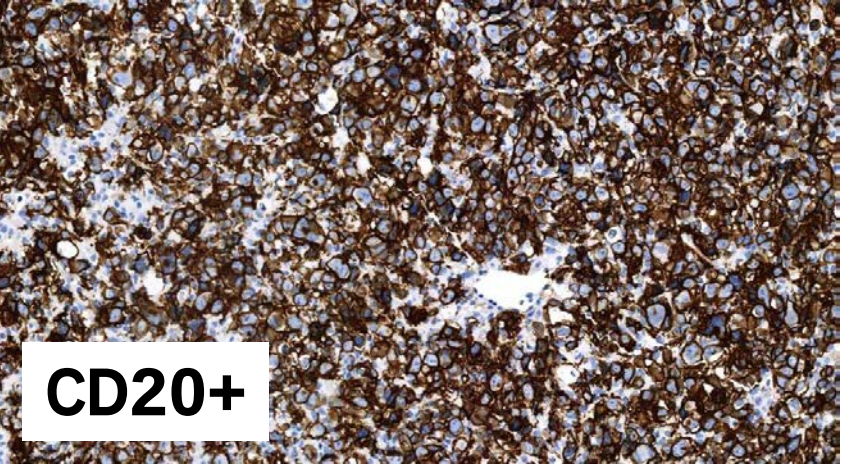
*Traverse-Glehen A et al. Am J Surg Pathol 1995; Eberle FC et al. Modern Pathol 2012*

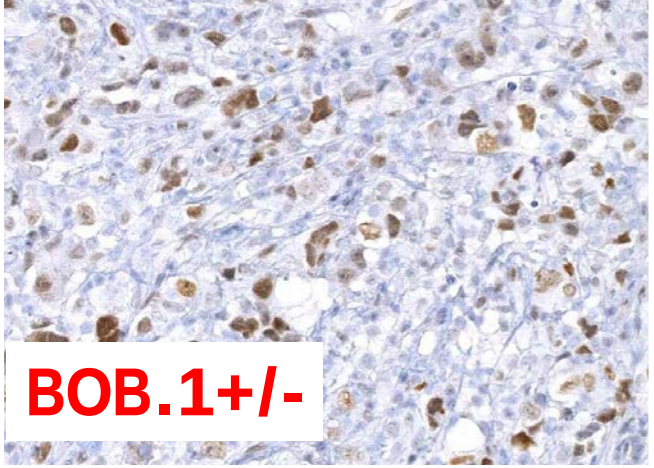


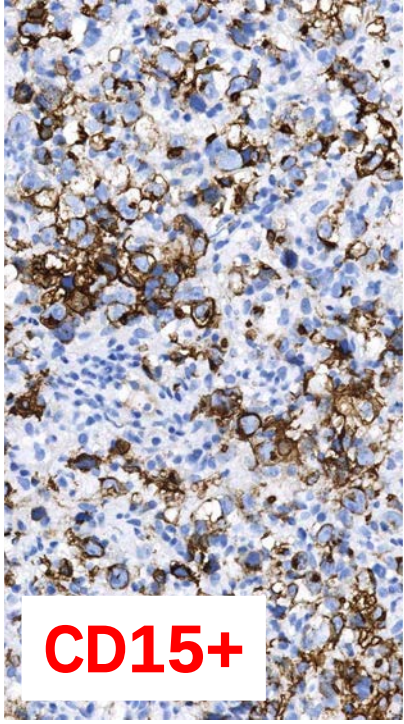
- M 82 yrs, inguinal lymph node biopsies
- Consultation case  HL or DLBCL?

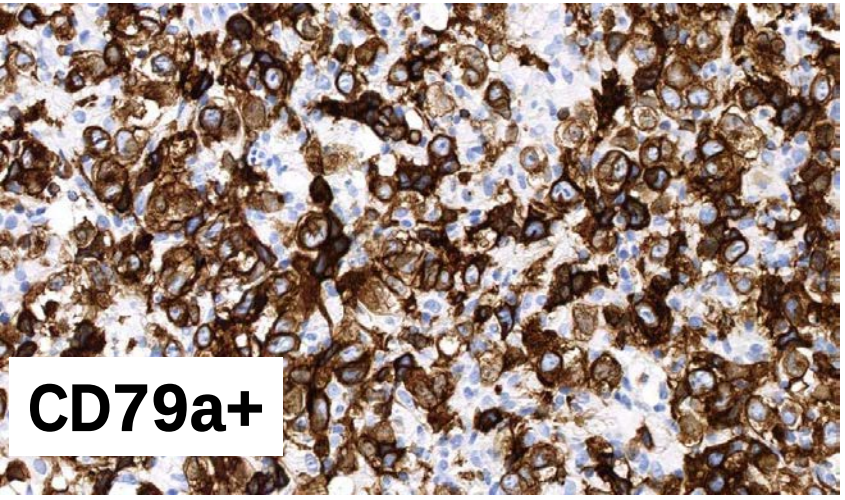


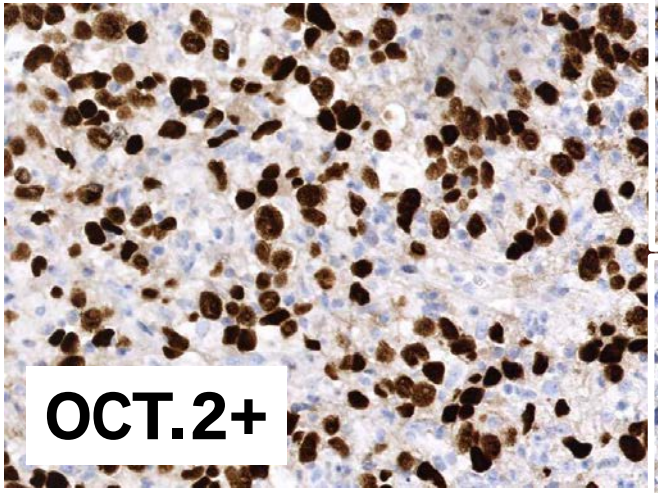



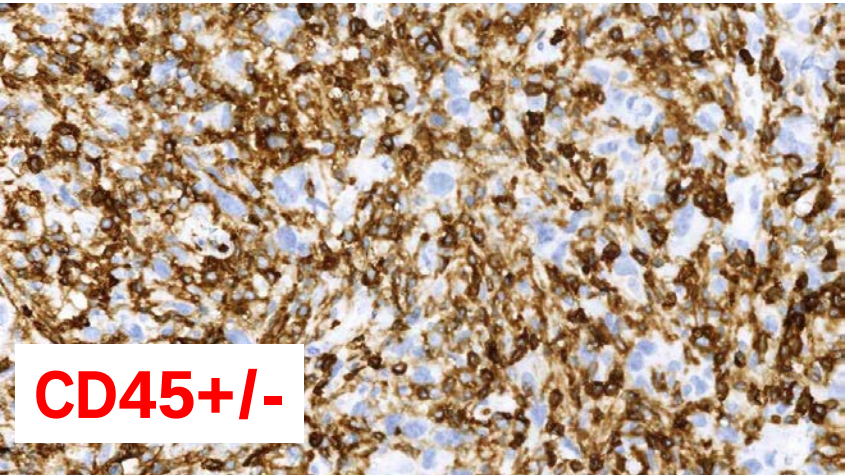

CD20+

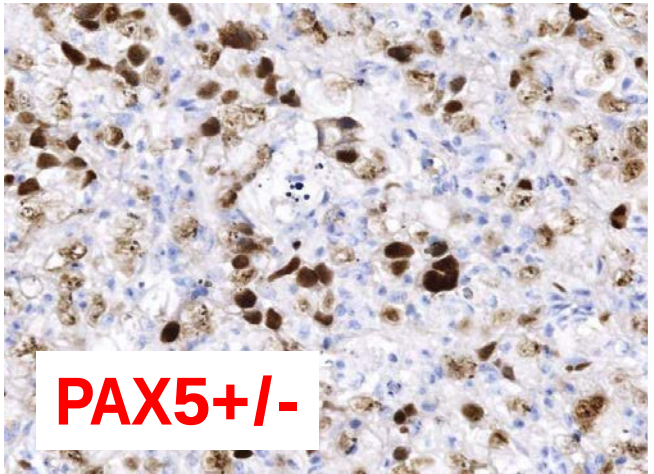

BOB.1+/-

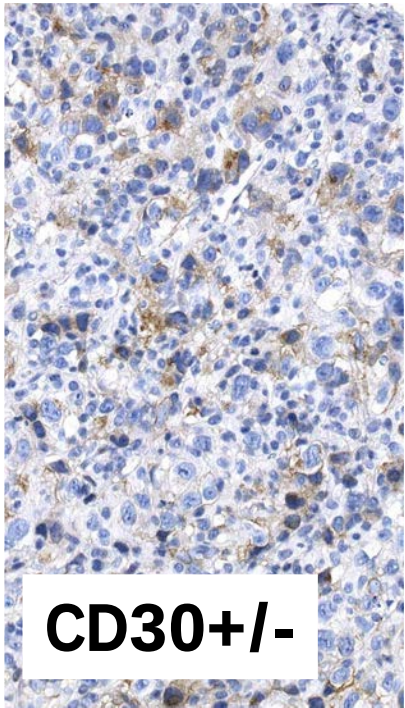

CD15+


CD79a+


OCT.2+


CD45+/-


PAX5+/-

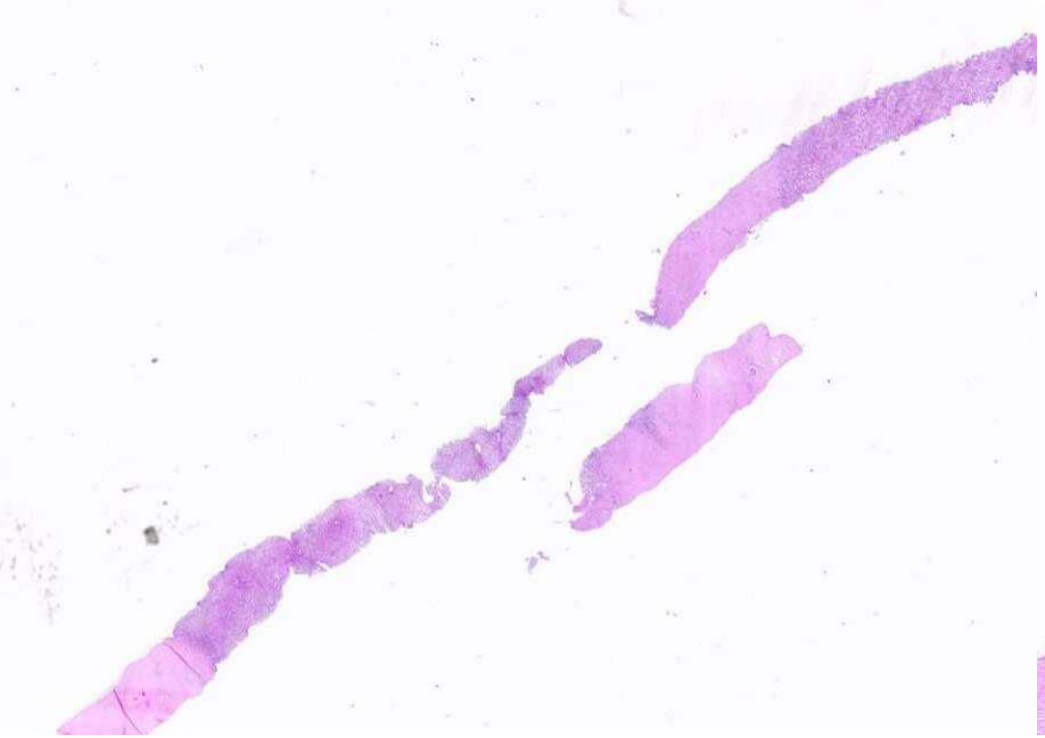

CD30+/-

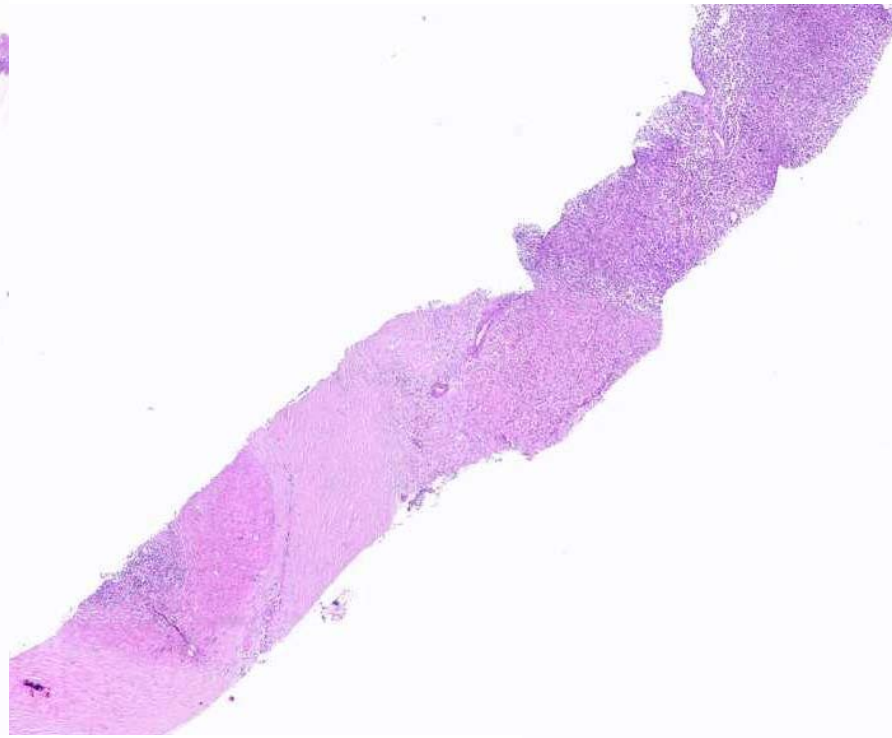
Diagnosis:

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

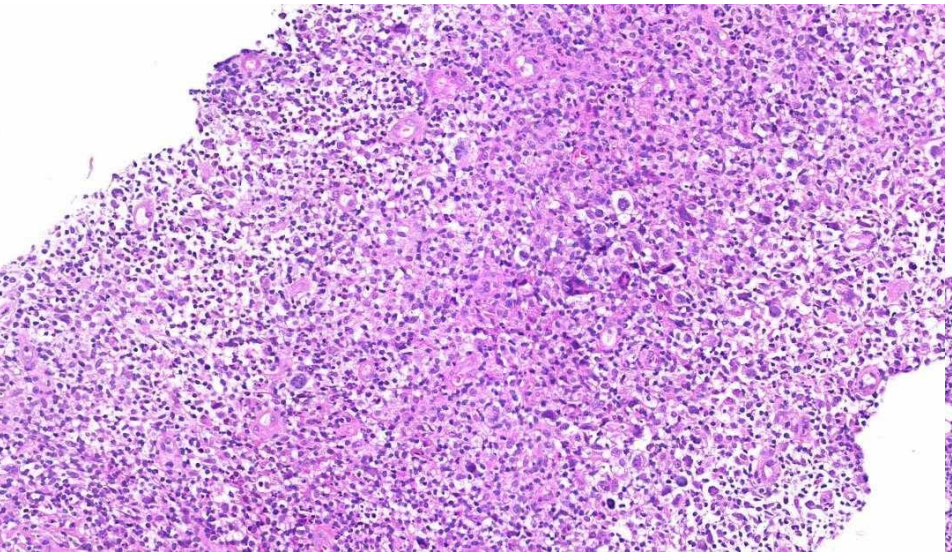
# Mediastinal “grey zone” lymphomas

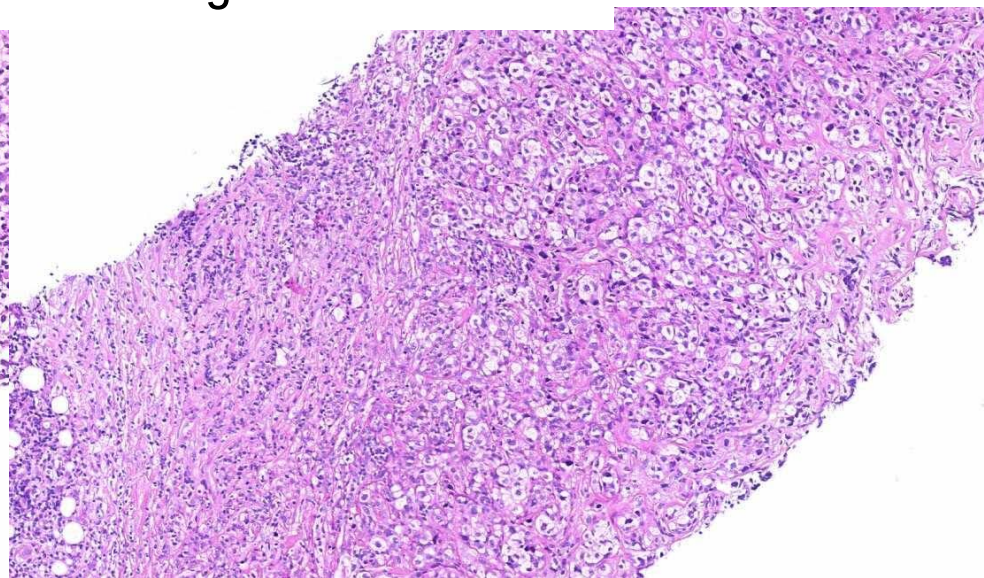
- **B-cell lymphoma, unclassifiable, with features intermediate** between DLBCL and cHL
- **Composite** mediastinal lymphomas
  - two distinct components and no transition between them (cHL-NS and MLBCL)
- **Sequential** mediastinal lymphomas:
  - MLBCL followed by cHL
  - cHL followed by MLBCL

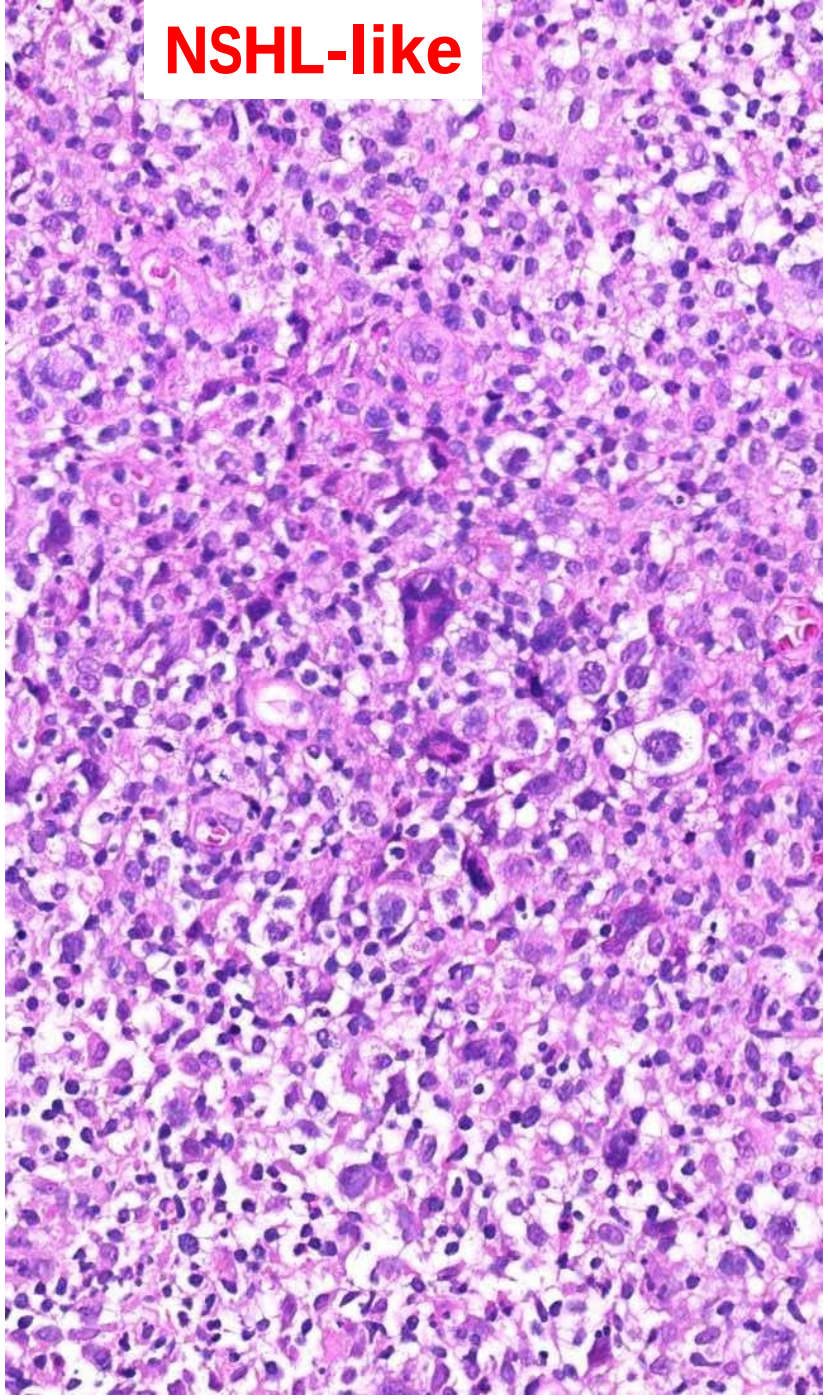


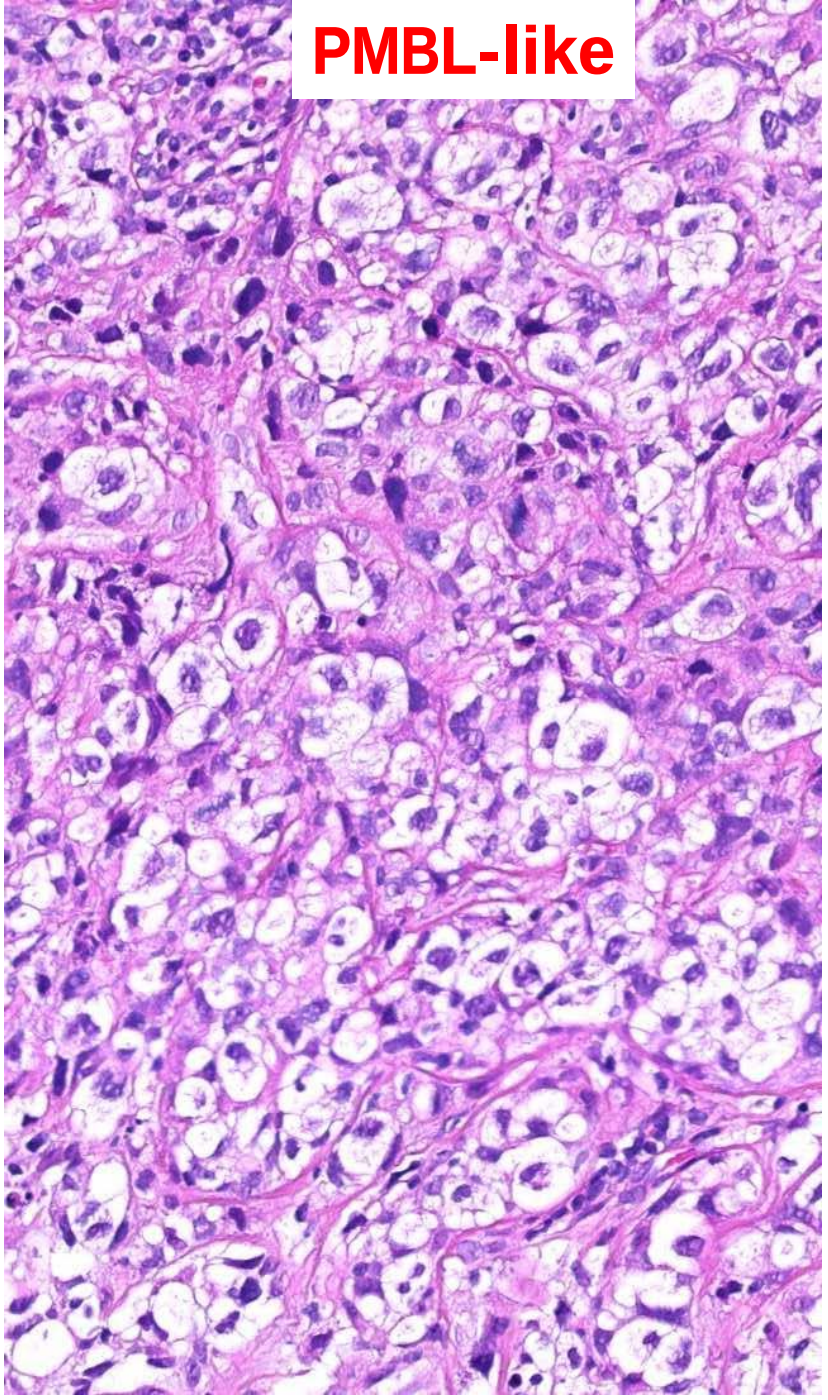


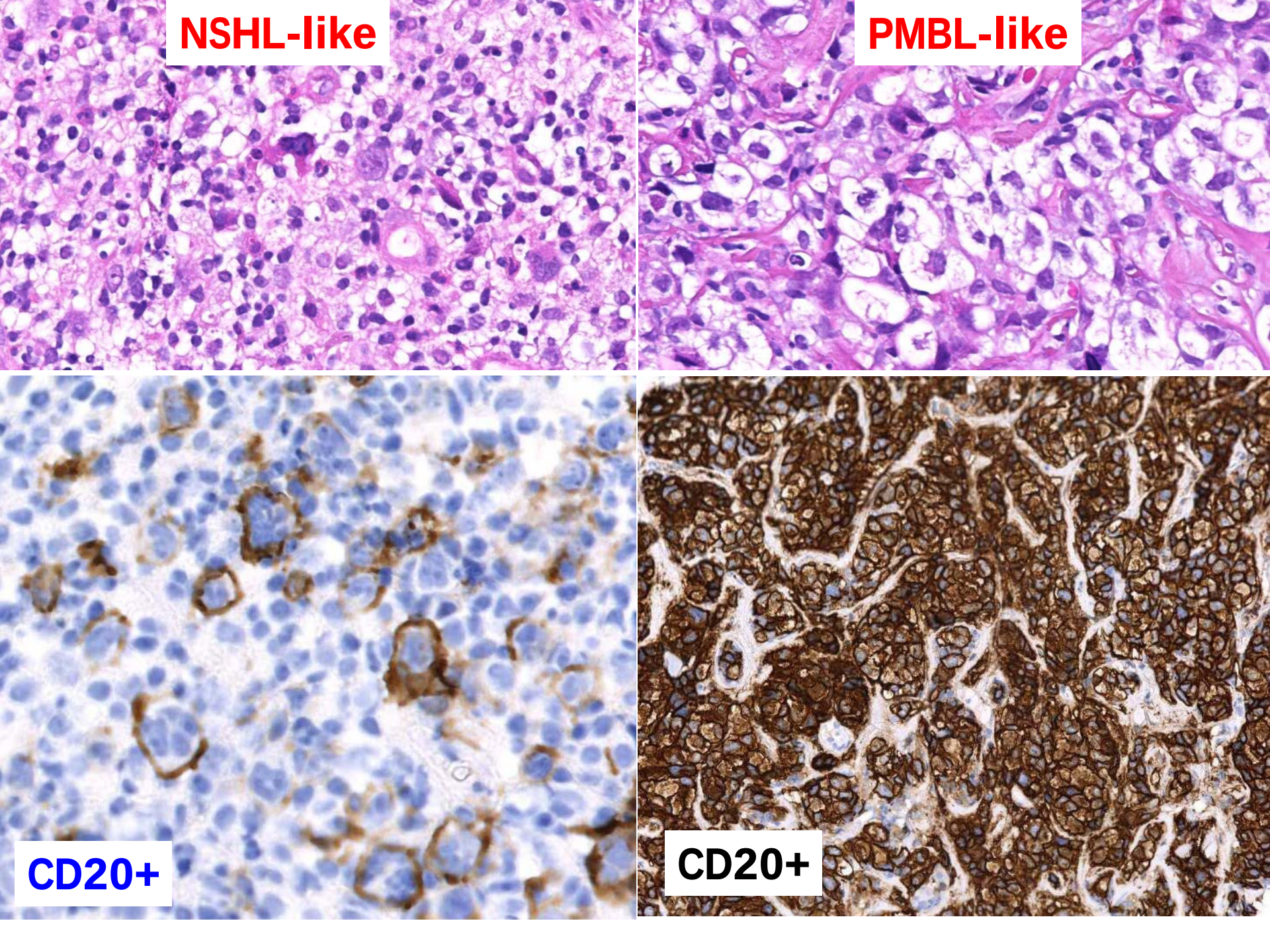
- M 30 yrs, voluminous anterior mediastinal mass, lymphoma vs. teratoma vs. thymoma? Bone marrow negative

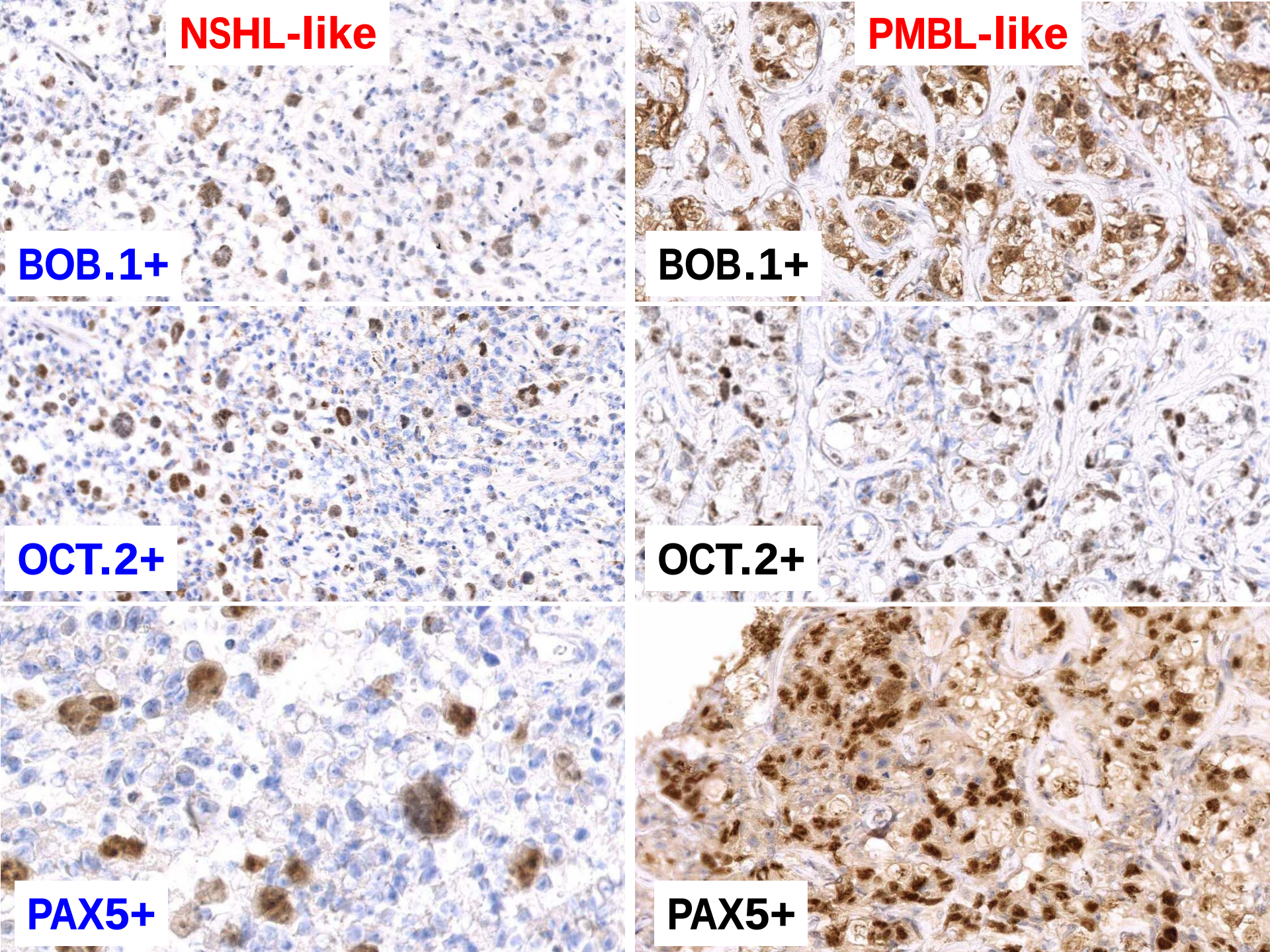


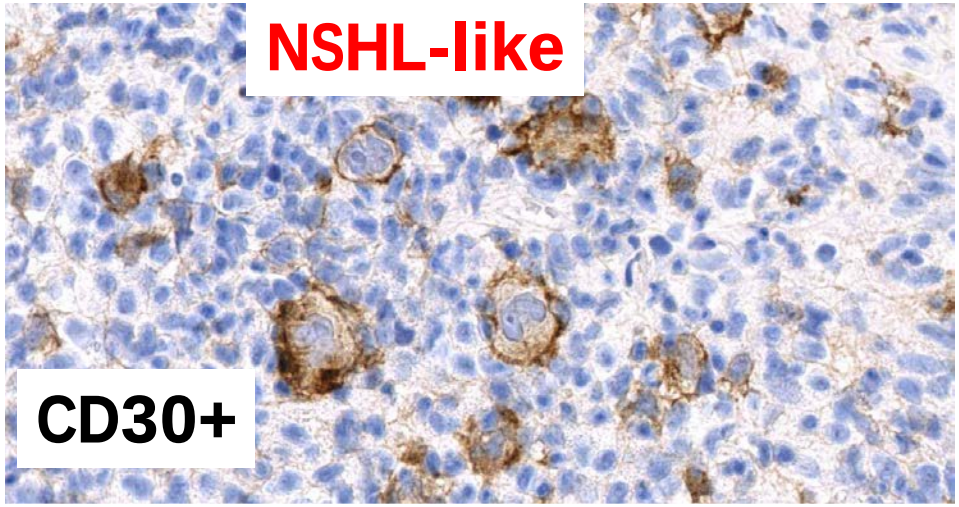


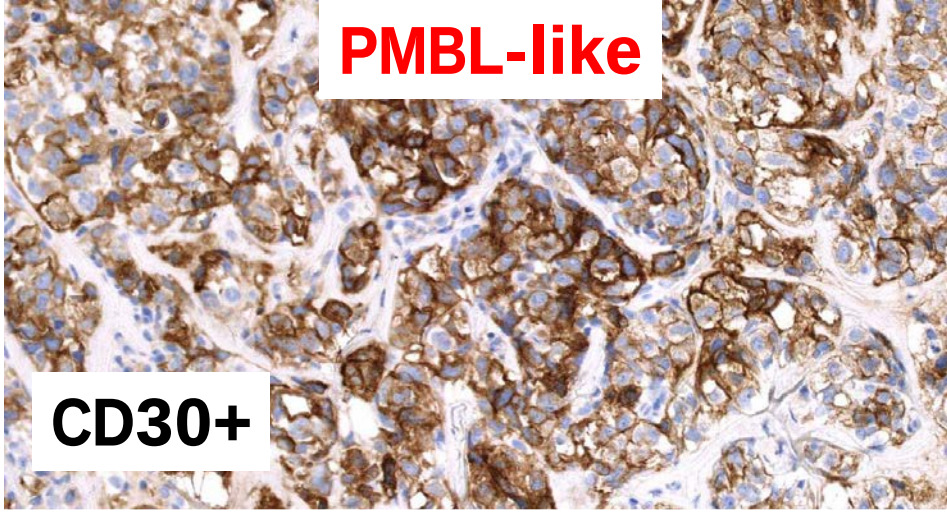

NSHL-like

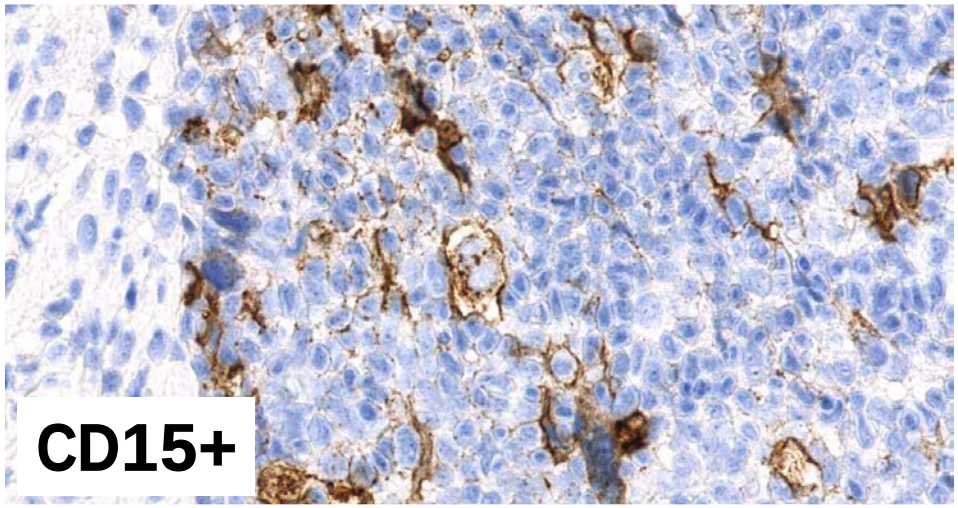

PMBL-like

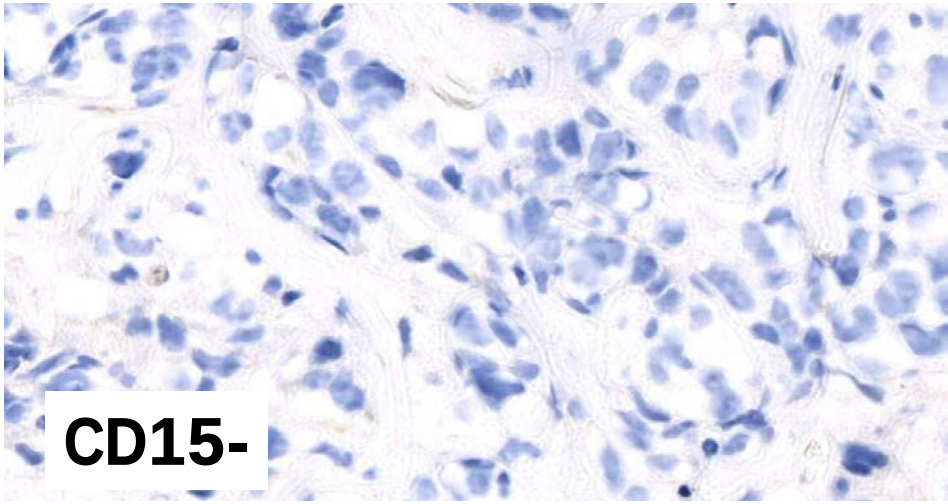

NSHL-like
PMBL-like
CD20+
CD20+

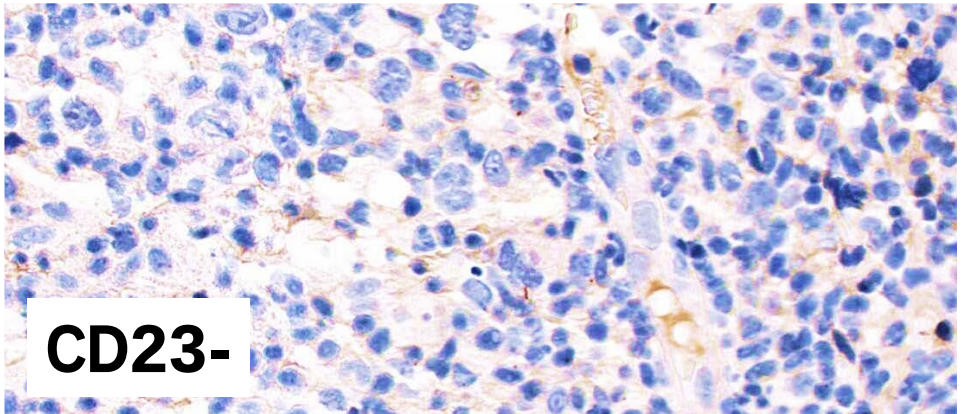

NSHL-like
BOB.1+
PMBL-like
BOB.1+
OCT.2+
OCT.2+
PAX5+
PAX5+

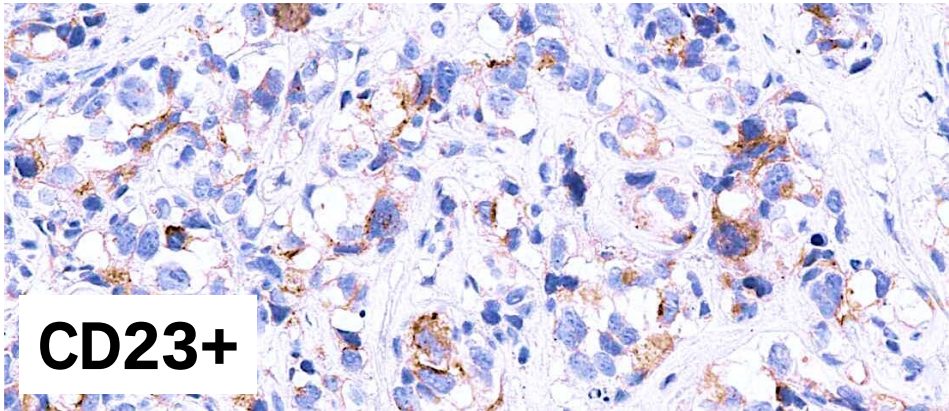

NSHL-like
CD30+


PMBL-like
CD30+


CD15+


CD15-


CD23-

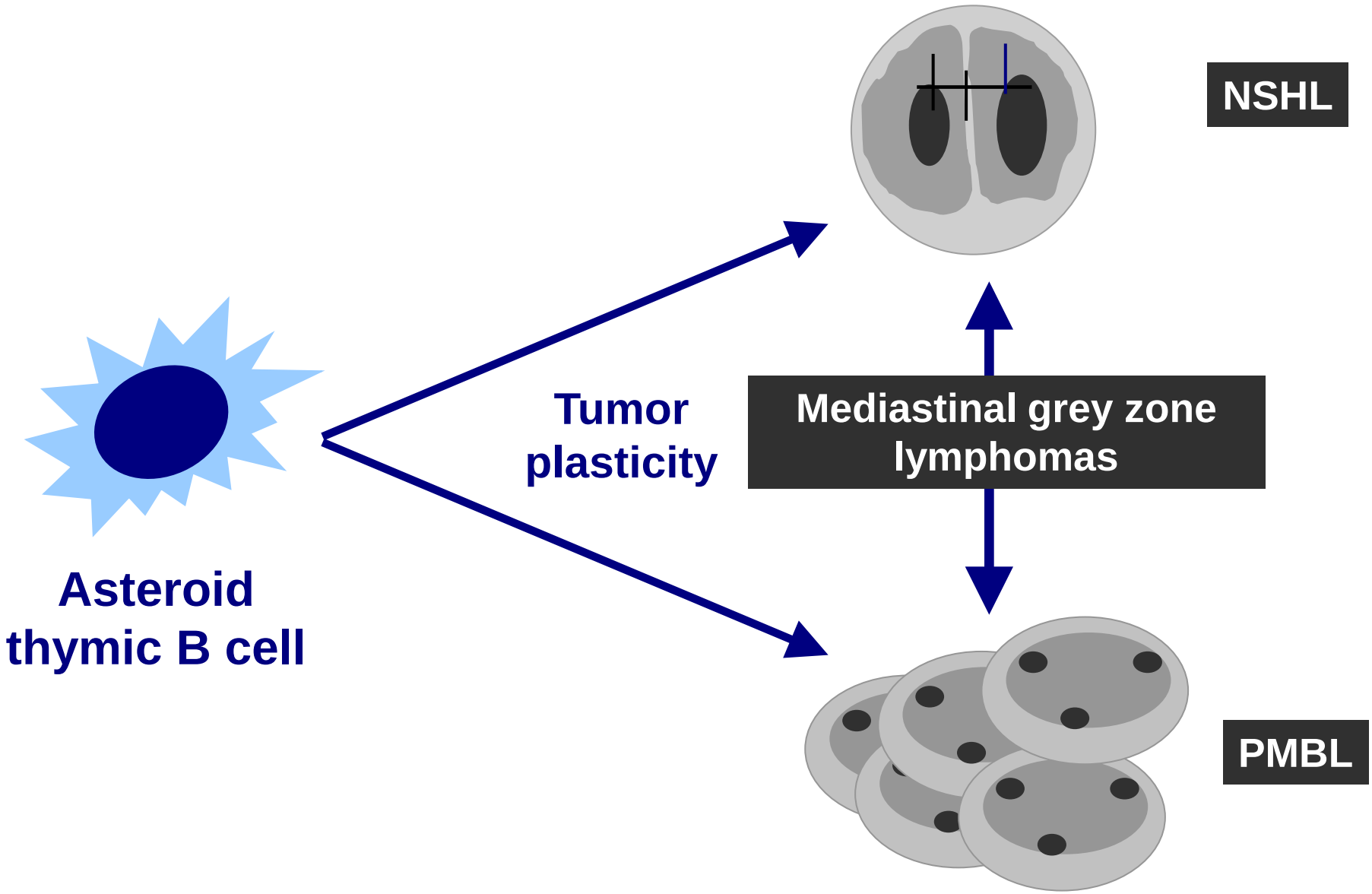

CD23+

Diagnosis:

Mediastinal grey zone lymphoma with composite histology:

cHL-like areas with discordant immunophenotype (intermediate)

PMBL-like areas


NSHL
Tumor plasticity
Mediastinal grey zone lymphomas
Asteroid thymic B cell
PMBL

# DNA methylation profiles in mediastinal lymphomas

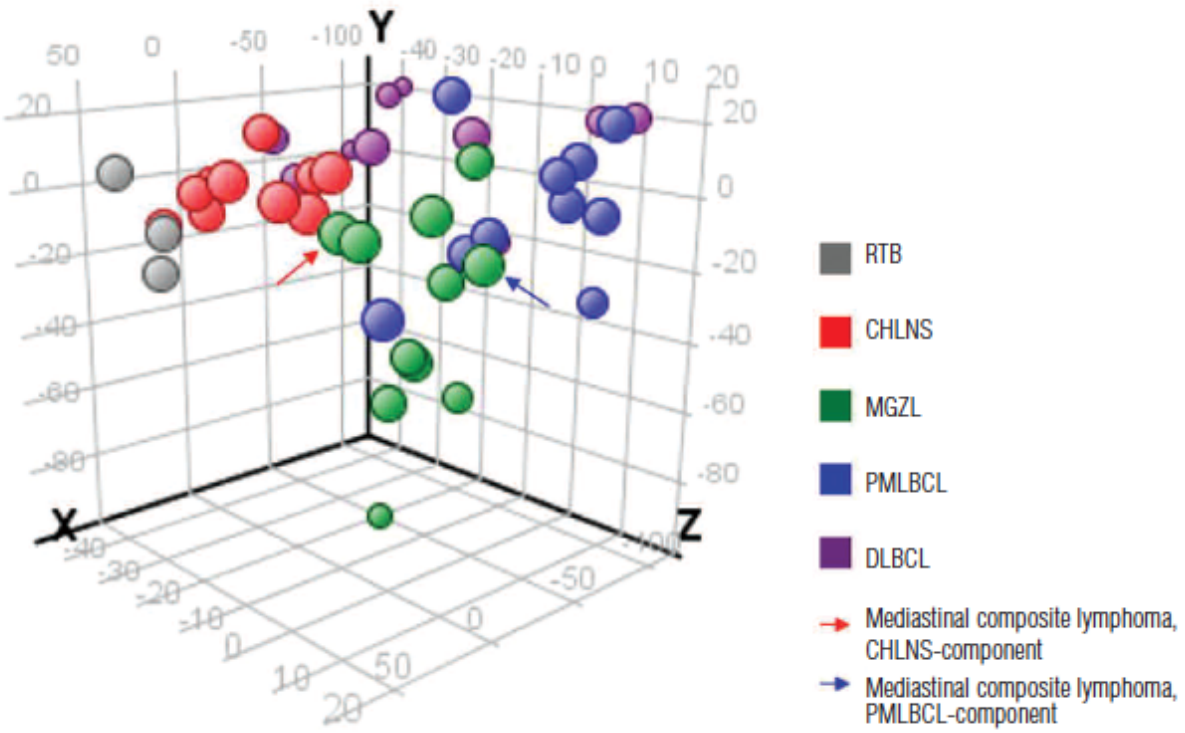



**Figure 1.** Distinct epigenetic profile of MGZL as assessed by principal component analysis. The methylation data for 1421 CpG targets from all studied tissue samples were subjected to principal component analysis and projected onto the first three principal components. MGZL appears to have a distinct epigenetic profile intermediate between CHLNS and PMLBCL, but clearly different from that of DLBCL. One of the lymphomas studied was a composite lymphoma, comprising two distinct components of CHLNS and PMLBCL in the same biopsy which were microdissected separately. Both elements of the composite lymphoma clustered with cases of MGZL, but the two components also demonstrated a particularly close association with cases of CHLNS or PMLBCL, respectively.

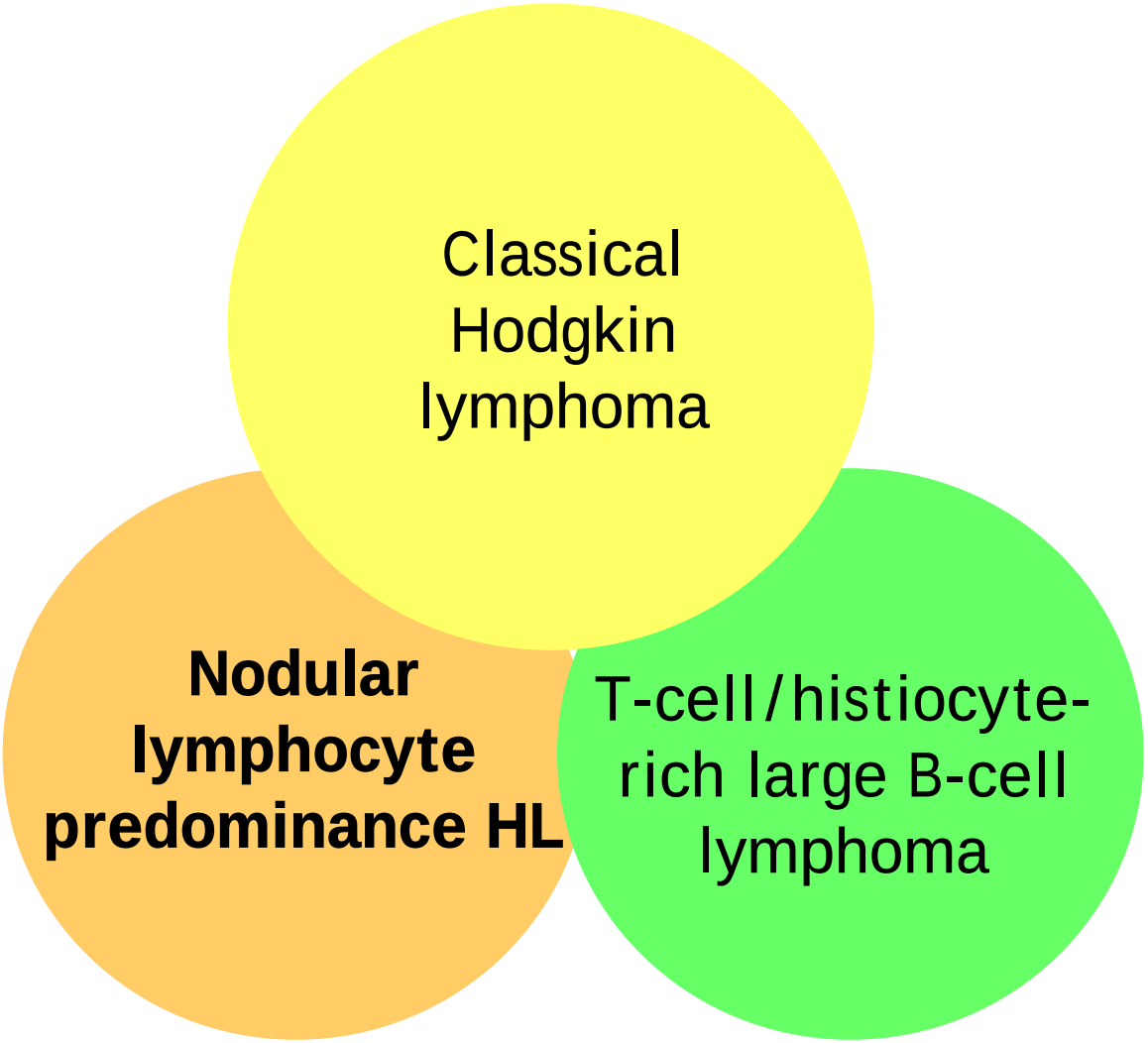
**The methylation profile of mediastinal grey zone lymphomas shares features with that of HL and PMBL and also shows distinctive features, validating a separate disease entity**

*Eberle FC et al. Haematologica 2011*

# Mediastinal “grey zone” lymphomas

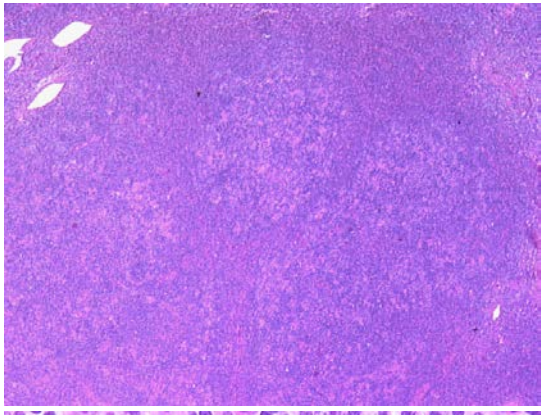
- **Diagnostic challenge**
  - Immuno panel: CD45, CD20, PAX5, CD15, CD30
  - CD79A, CD19 OCT.2, BOB.1, BCL6
- **Biological implications**: biological relationship between mediastinal cHL-NS and MLBCL
- **Medical implications**
  - Aggressive, treated either like DLBCL or cHL, worse outcome than cHL or PMBL
  - Clinical judgement required, treated with R-chemotherapy (NCI)
- A small number of cases have been reported in the literature. Is the actual frequency possibly underestimated due to small diagnostic samples

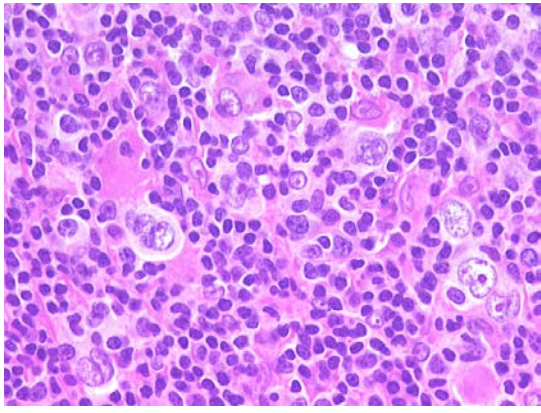
# The grey zones around Nodular NLPHL

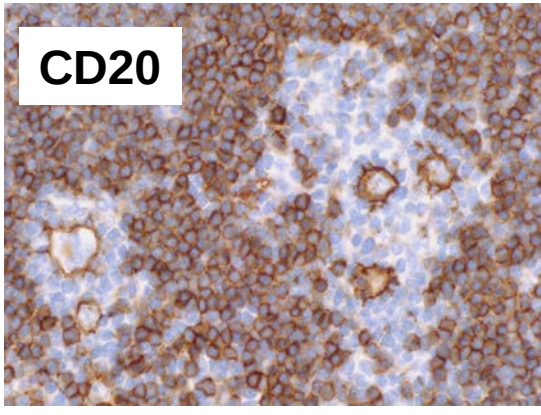


# Nodular Lymphocyte Predominance HL

- <10% HL; M>F, young adults
- Isolated peripheral lymphadenopathy
- Usually indolent, recurrence(s) in 20% of the cases, transformation to DLBCL
- Nodular (CD21+), LP (« popcorn ») cells
- **LP cells**
  - CD20, CD79a, CD45, BCL6, OCT-2, BOB-1+
  - EMA+/-, CD30-/+, CD15-, EBV-
- **Background reactive cells**
  - Small B cells (CD20+, BCL6-/+, IgD +/-)
  - Small T cells (CD3+, CD4+, CD57+, PD1+) and histiocytes



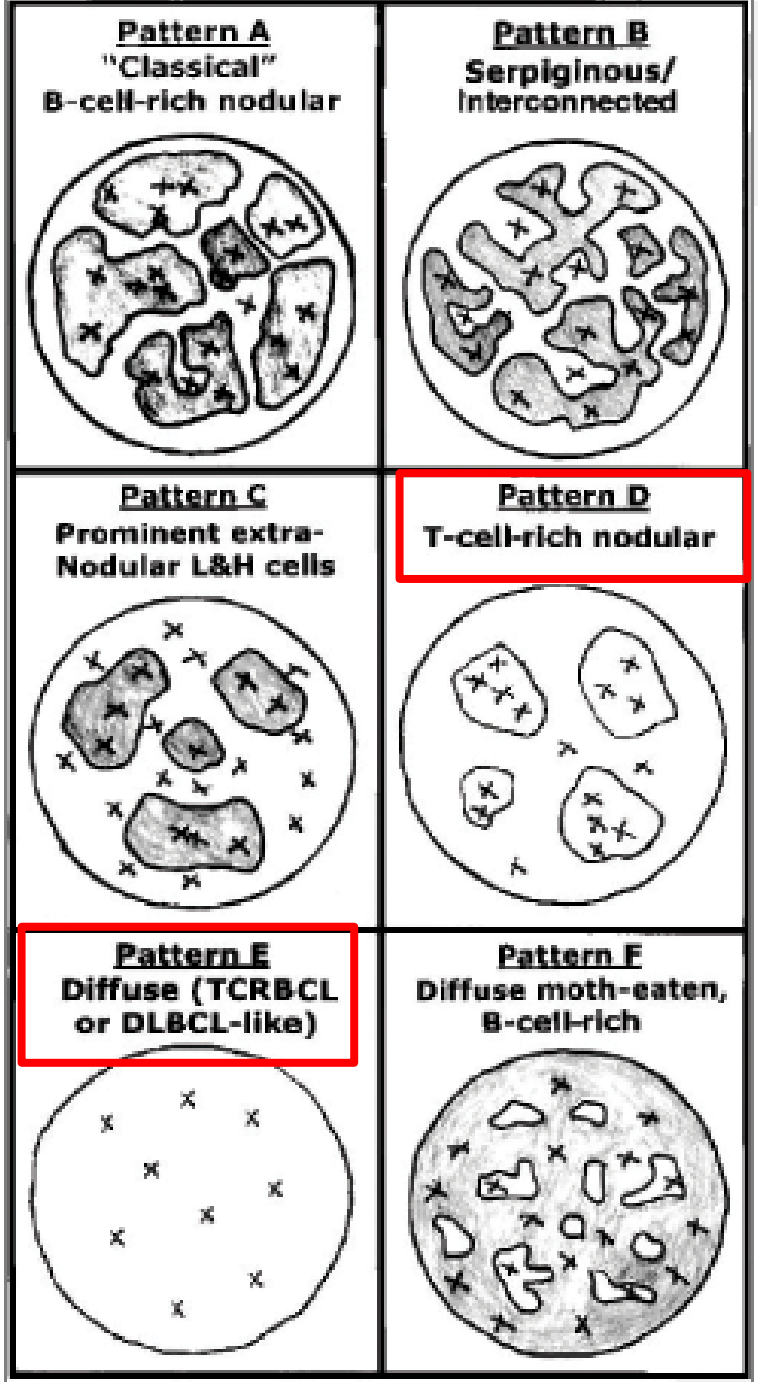




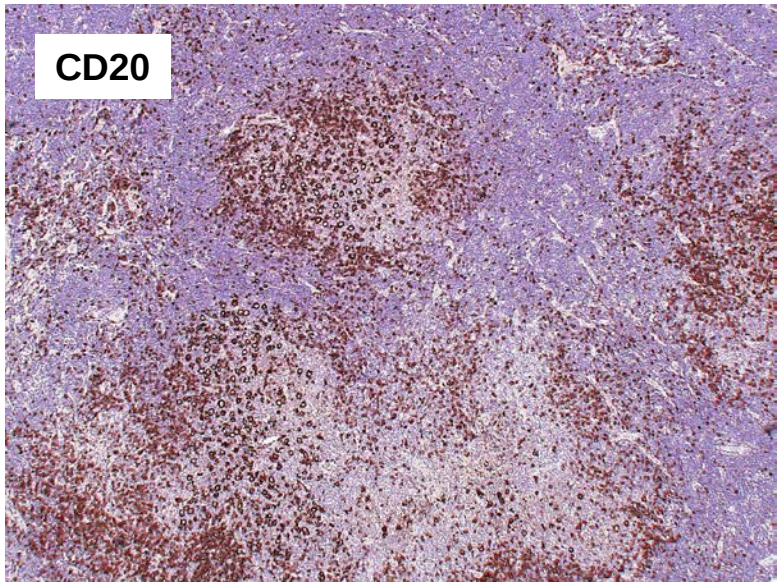
# Grey zones around NLPHL

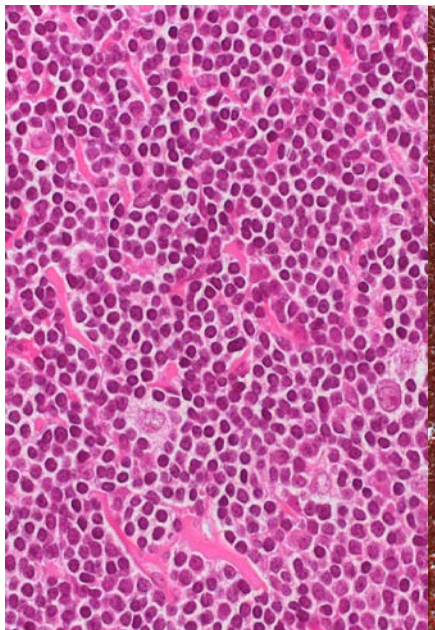
- **The interface between NLPHL and cHL**
  - Morphology immunophenotype and EBV association are distinct
  - LRcHL: morphologic overlap, more expression of B-cell markers
  - CD15 may be expressed and EBV may be present
  - Distinct diseases, rare cases clonally related
  - Not a true biologic grey zone
- **The interface between NLPHL and DLBCL**
  - Transformation to DLBCL in 5% of the cases, clonally related
  - **NLPHL with a diffuse pattern and THRLBCL**

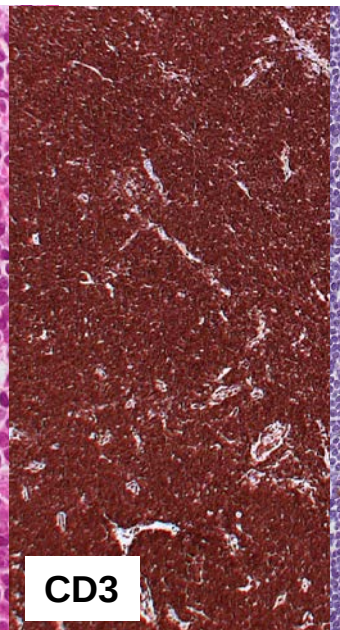
# NLPHL – patterns

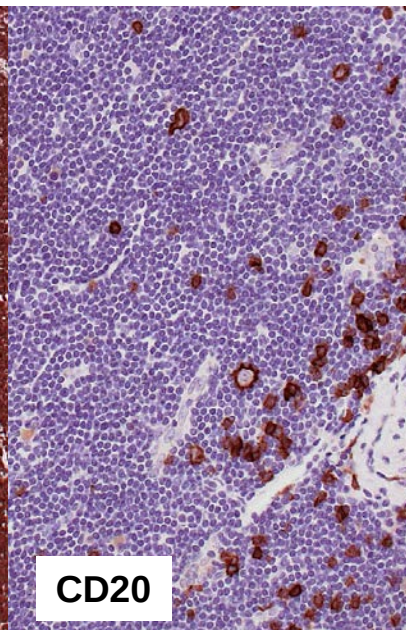



*Fan Y et al. Am J Surg Pathol 2003; 27: 1346*



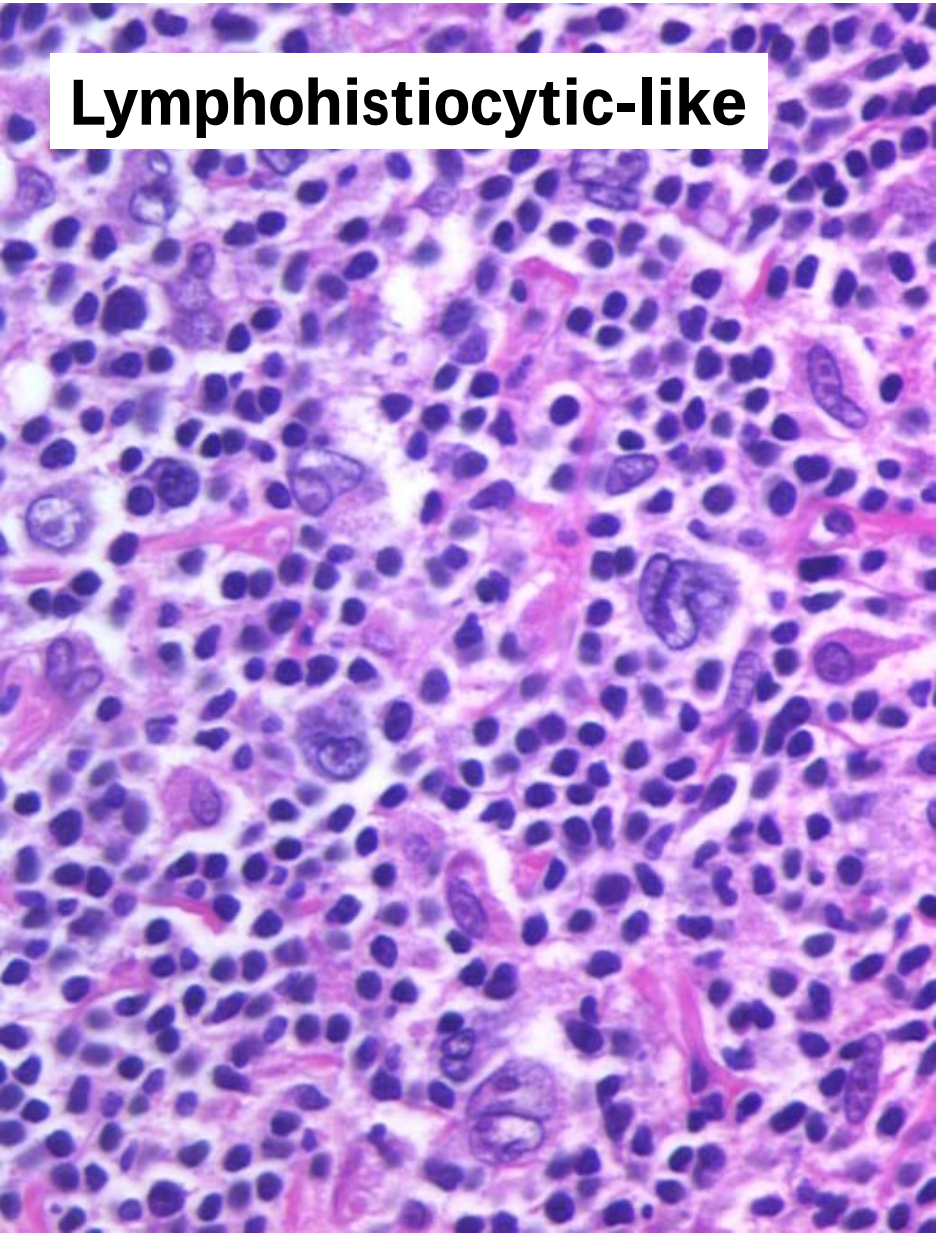



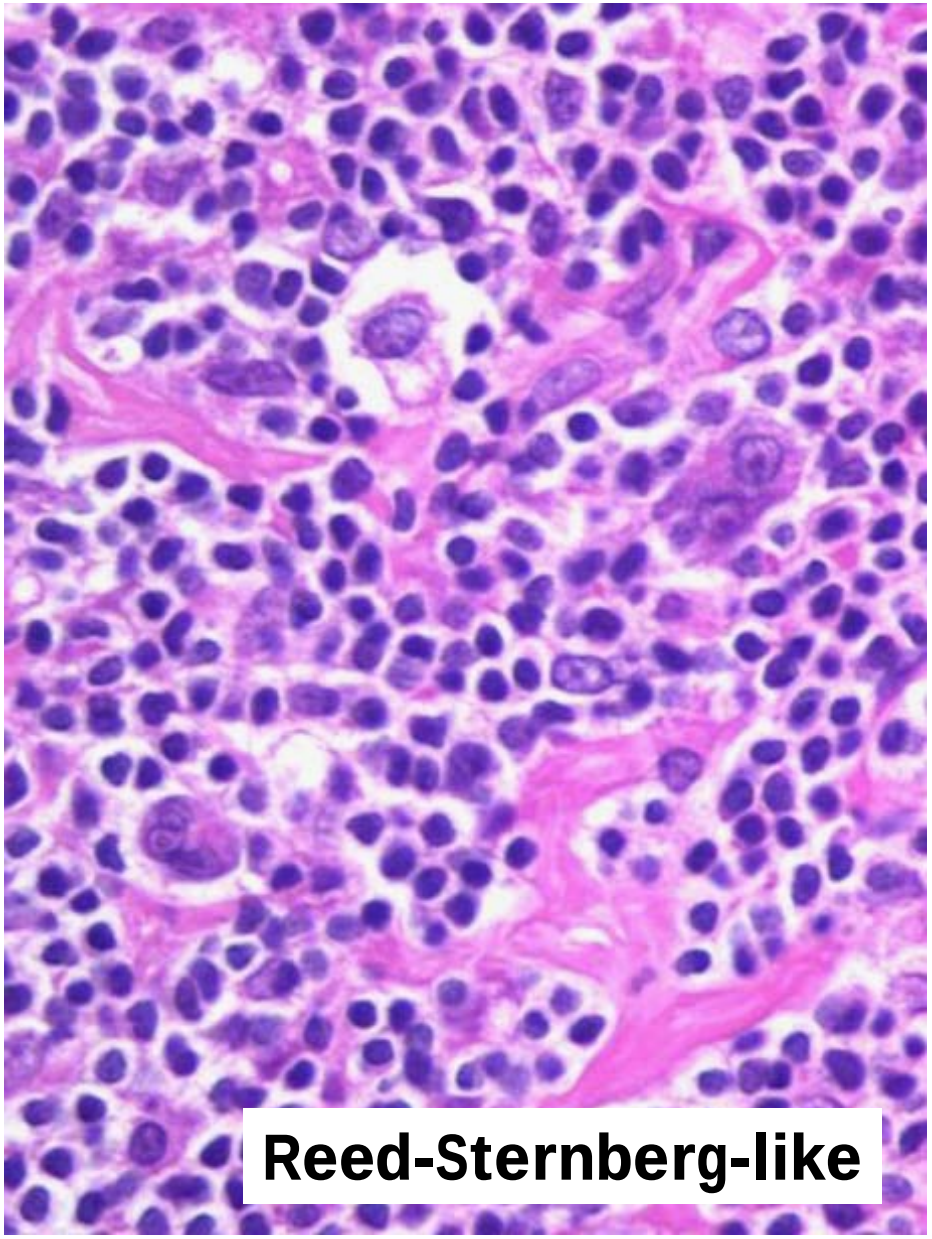


# Diffuse NLPHL versus THRLBCL

- A diffuse pattern may be seen in association with the nodular pattern at diagnosis or during progression/relapse
- Diffuse areas tend to acquire a T-cell rich background: THRLBCL-like areas and raise the differential diagnosis with THRLBCL
- **The distinction is clinically highly relevant given prognostic and therapeutic implications**
- **Is it a real biological grey zone?**
  - *BCL6* rearrangements common in NLPHL not in THRLBCL
  - Genomic complexity (aCGH) higher in NLPHL than THRLBCL

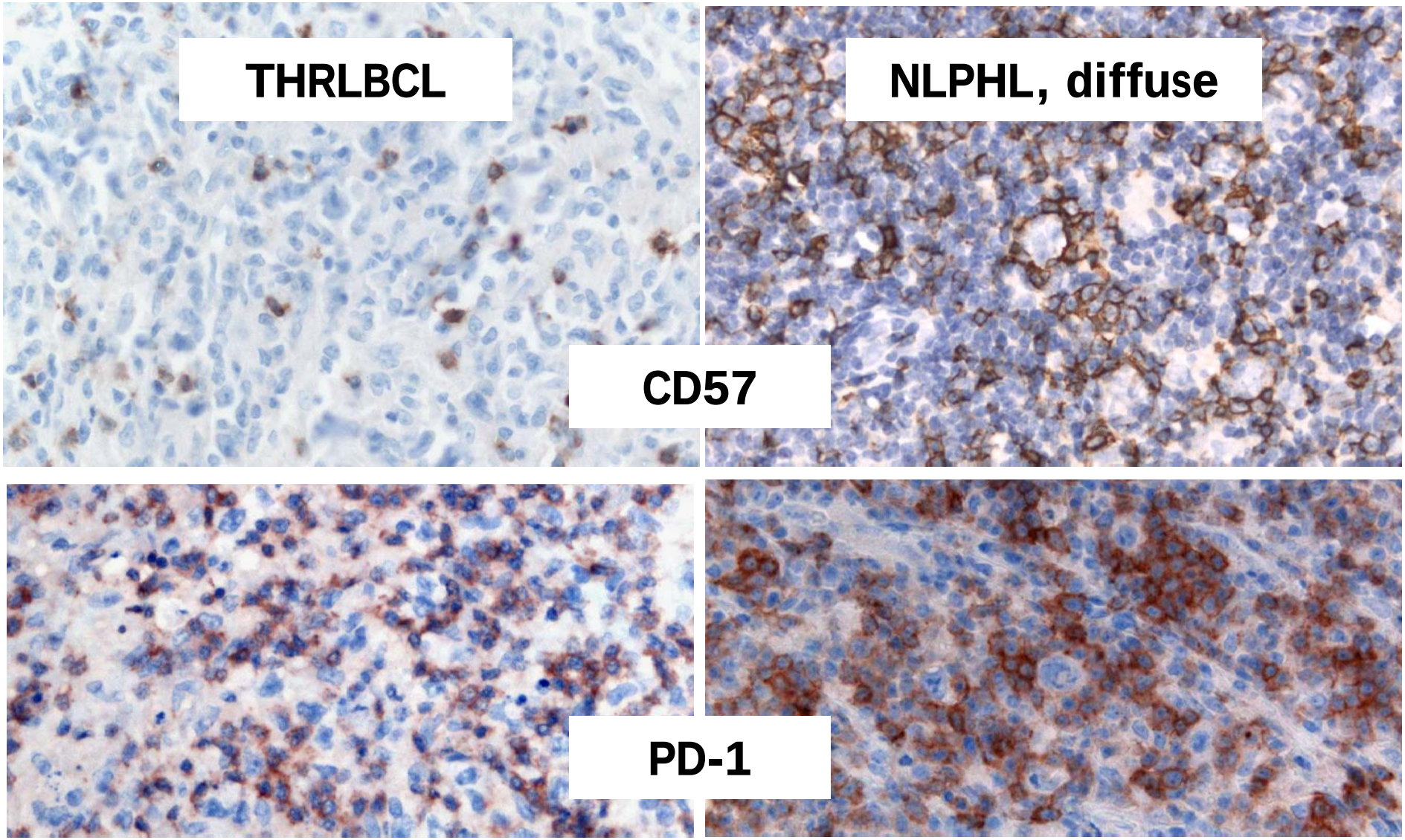


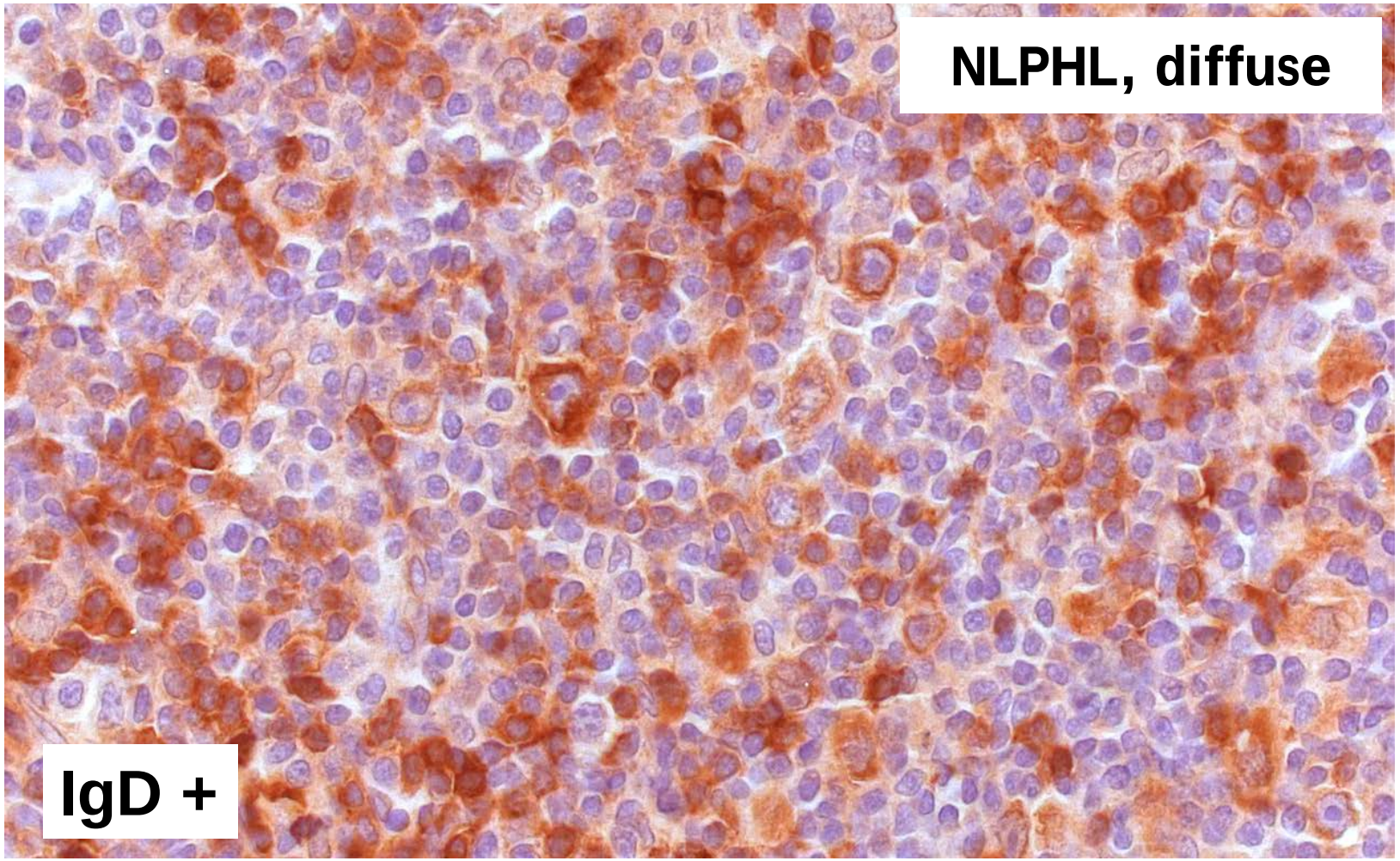




# THRLBCL

# Diffuse NLPHL versus THRLBCL
# Diagnostic clues

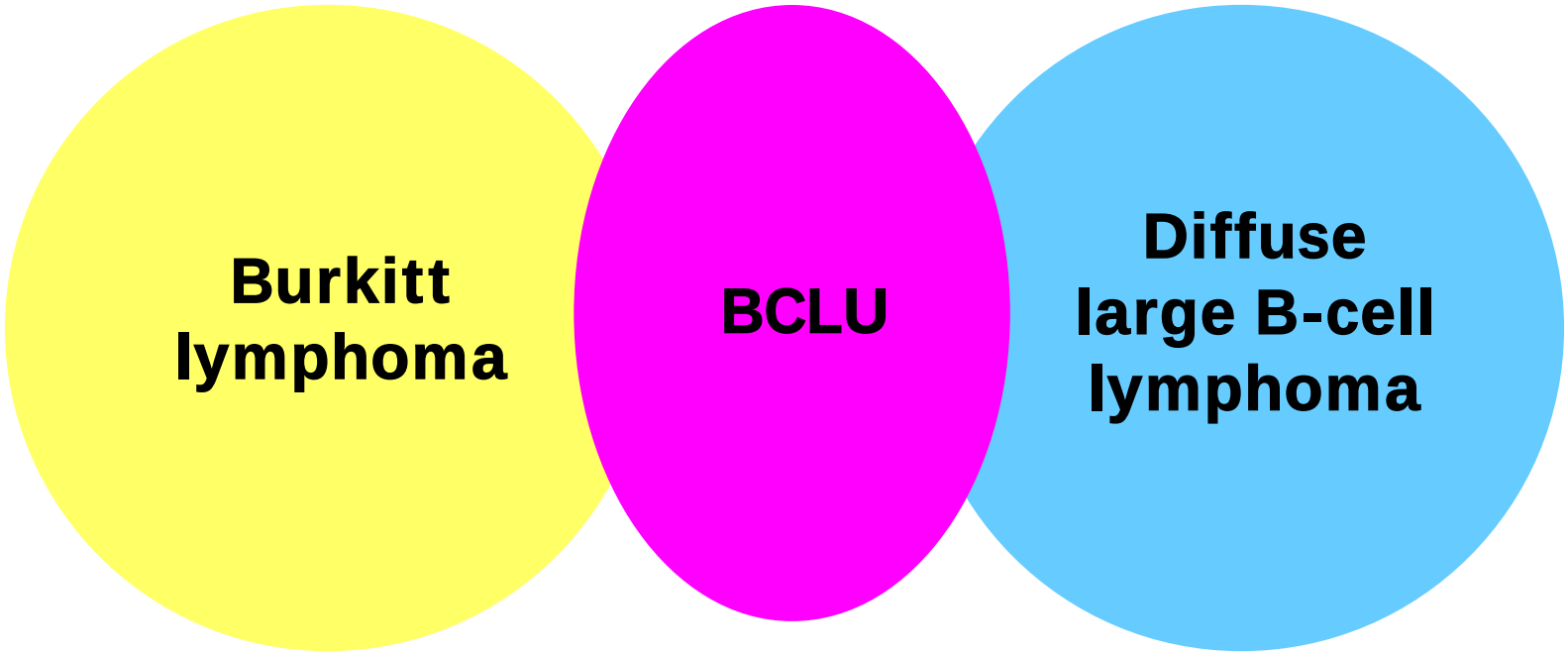
- **The diagnosis of THRLBCL should NOT be made in a patient with a history of NLPHL.** In that setting, a diagnosis of NLPHL, THRLBCL-like should be rendered.
- **Purely diffuse de novo NLPHL are exceptional**
  - The existence of purely diffuse NLPHL cases is questioned; the presence of a single nodule warrants the designation of NLPHL with THRLBCL-like areas
  - Search for focal nodular pattern, submit all tissue and ask for deeper sections, do IHC for demonstration of FDCs
- **THRLBCL in children and younger adults is rare**
  - Consider clinical history and presentation
  - Consider the possibility of predominantly diffuse NLPHL


THRLBCL
NLPHL, diffuse
CD57
PD-1



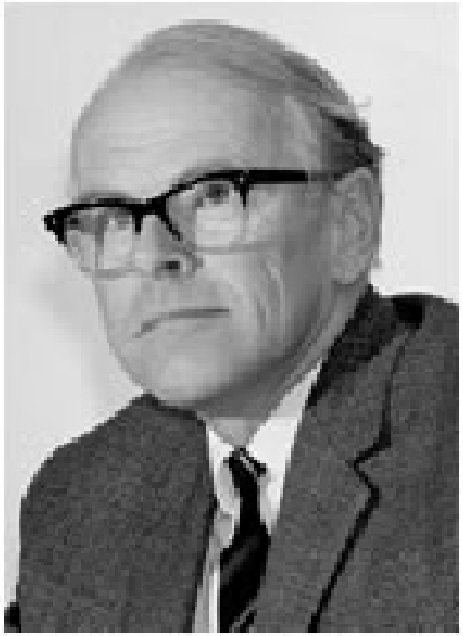

- **25-30% NLPHL have IgD+ LP cells**
  - Male predominance, younger age
  - ocalized disease in 75% of cases, cervical region
- Extrafollicular distribution & T-cell-rich background frequent

*Prakash S AJSP 2006*

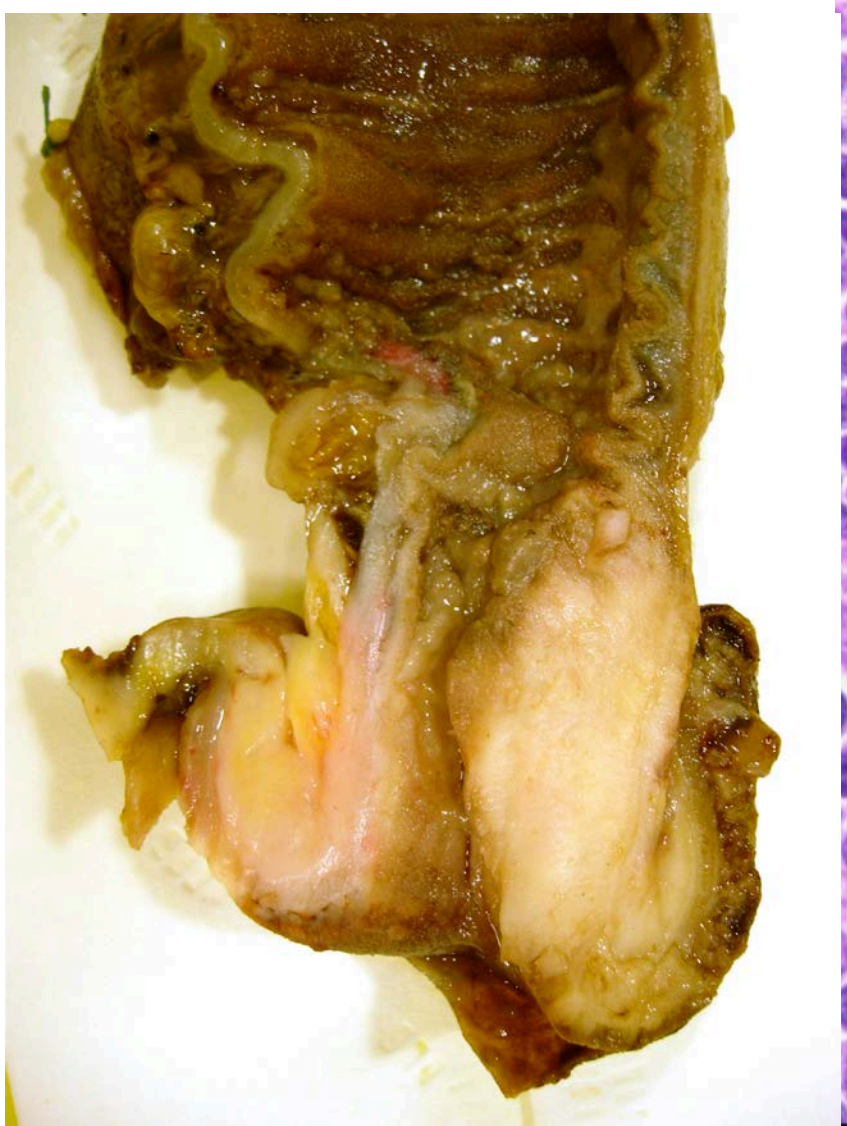


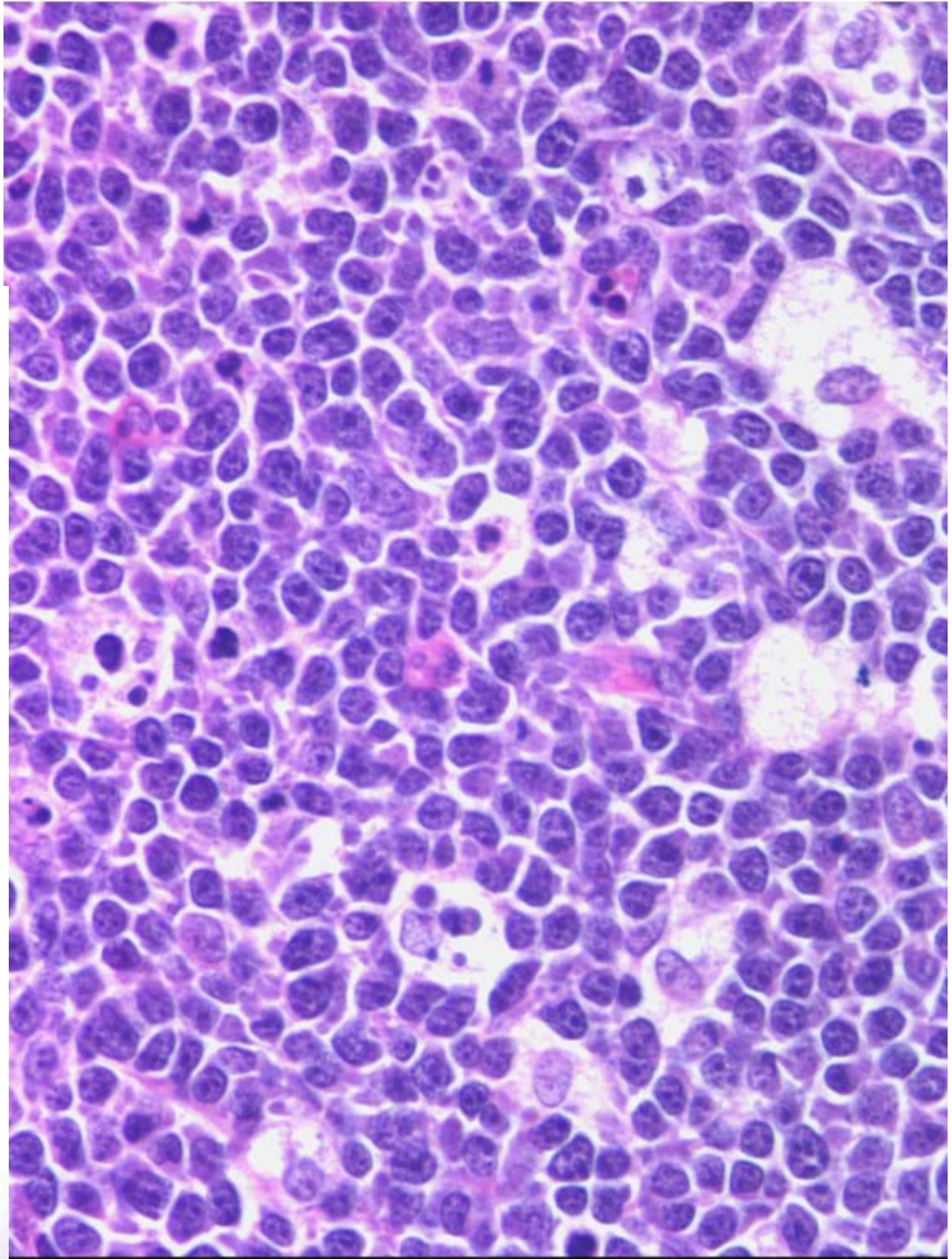

**B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL**

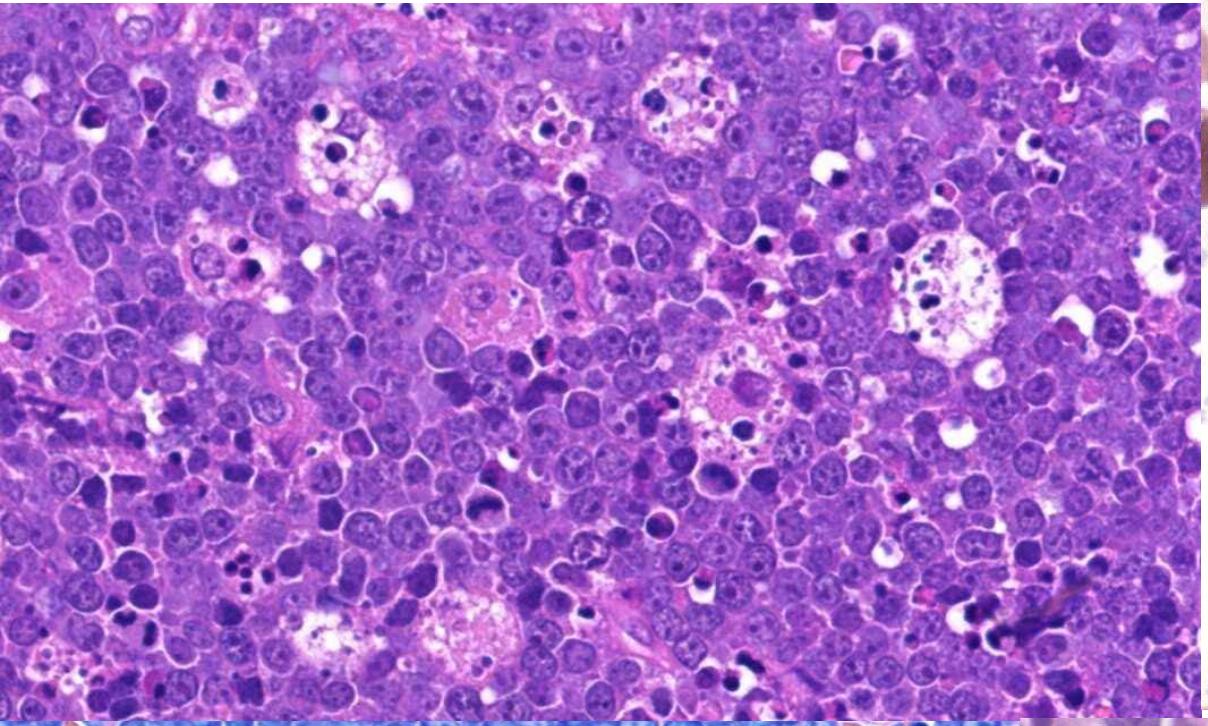
# Burkitt lymphoma

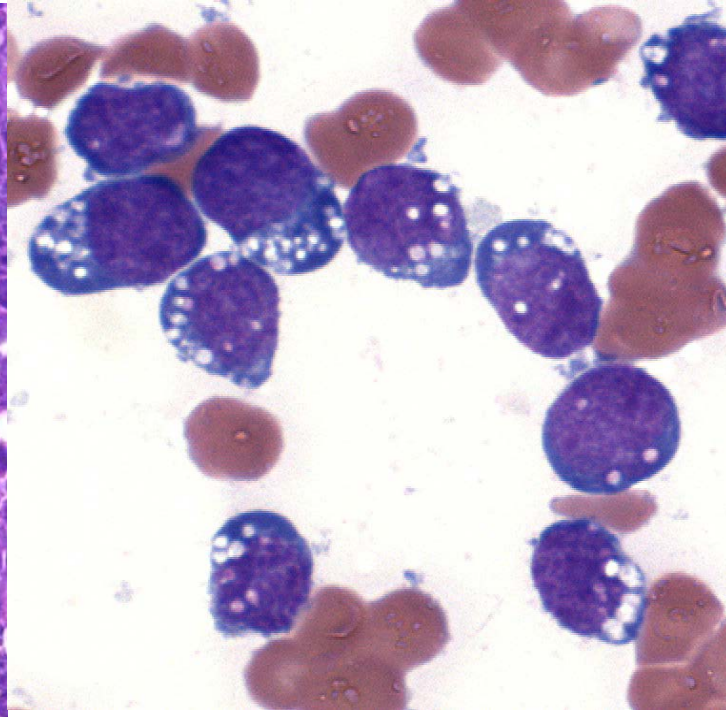


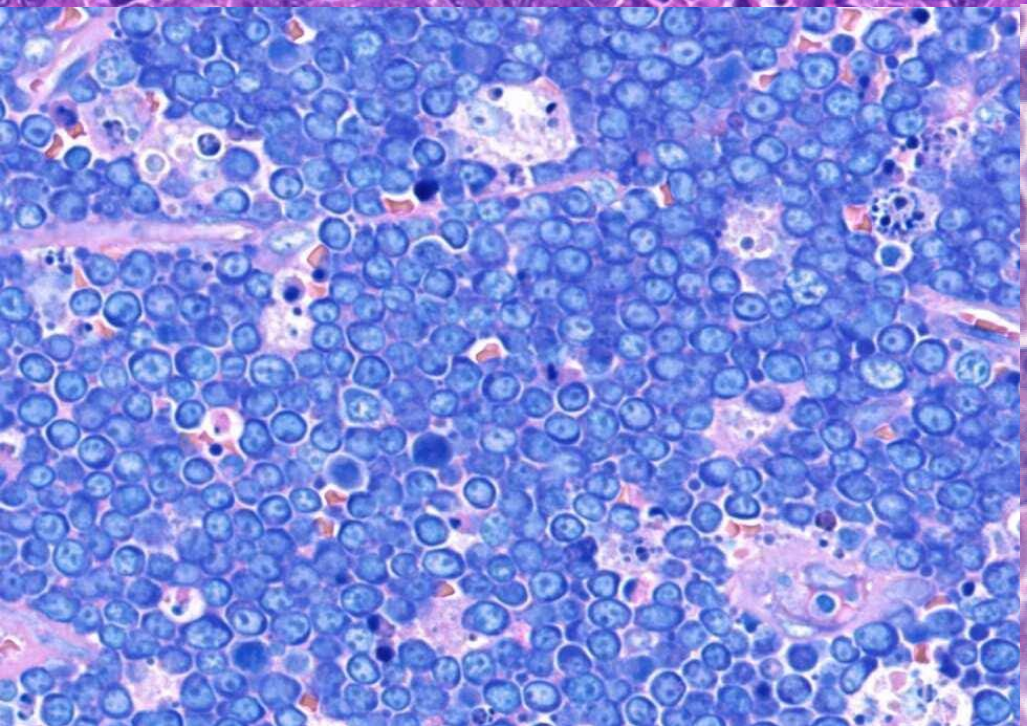
- Highly aggressive B-cell lymphoma often presenting in extranodal sites, composed of monomorphic medium-sized lymphoid cells with basophilic vacuolated cytoplasm
- Higly proliferative
- Endemic, sporadic and immunodeficiency-associated
- Common in the pediatric age group, also in adults
- Male predominance

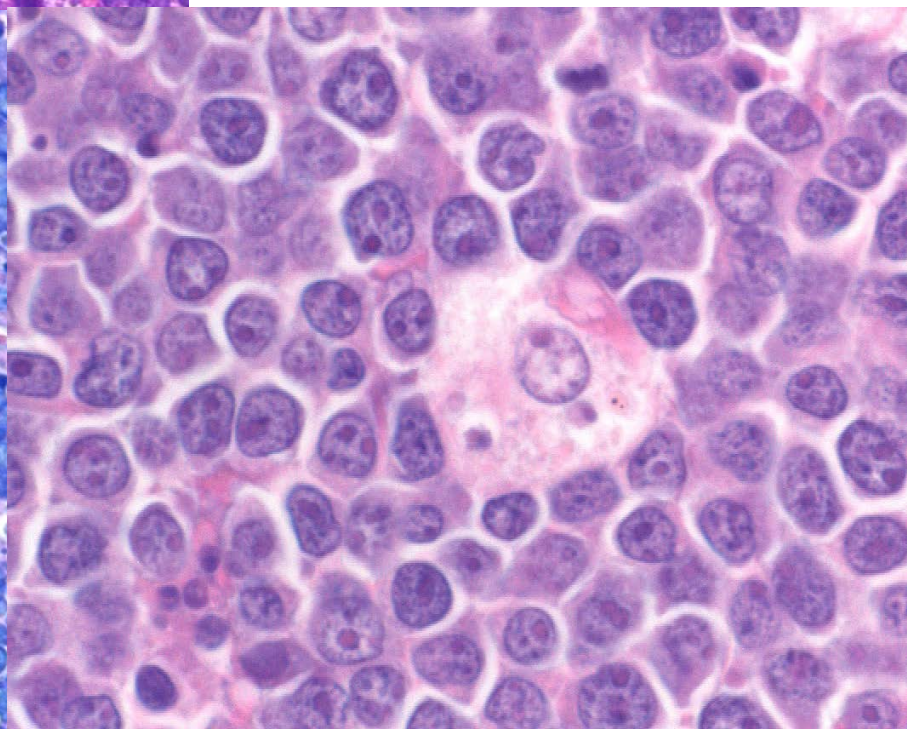






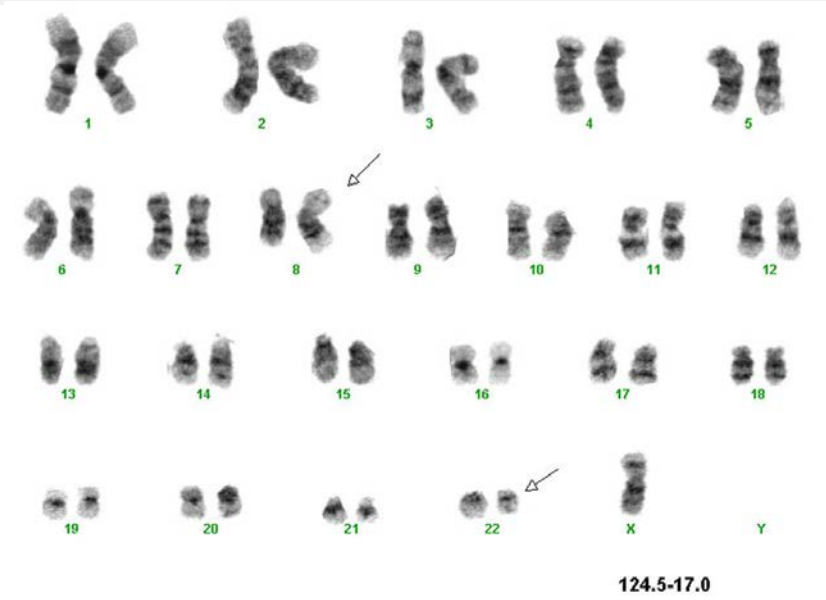


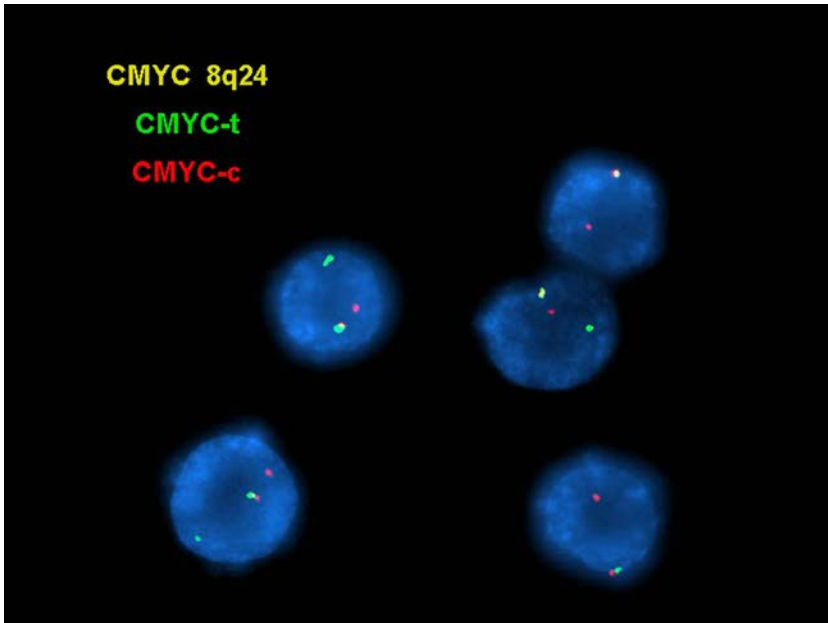




# Burkitt lymphoma

- CD20+
  BCL2- BCL6+ CD10+ MUM1-
- Ki67 100%
- IgMkappa or lambda, s+/-c
- EBV +/-
- MYC translocation is the genetic hallmark of BL
  - 80% t(8;14); 15% t(8;22); 5% t(2;8)
- Simple karyotype
- Damaging mutations *ID3* and/or *TCF3* resulting in activation of PI3K pathway in >70% of cases

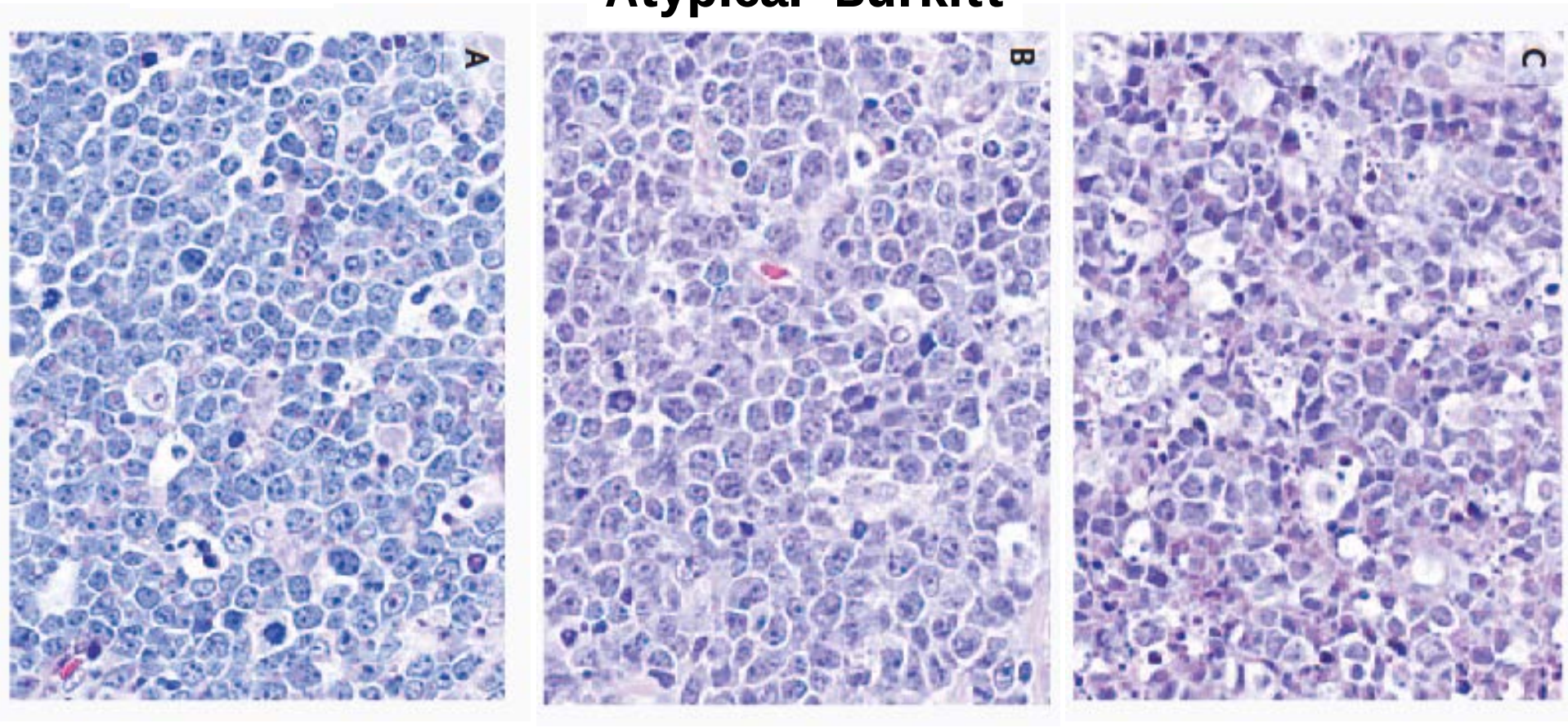


**Burkitt**

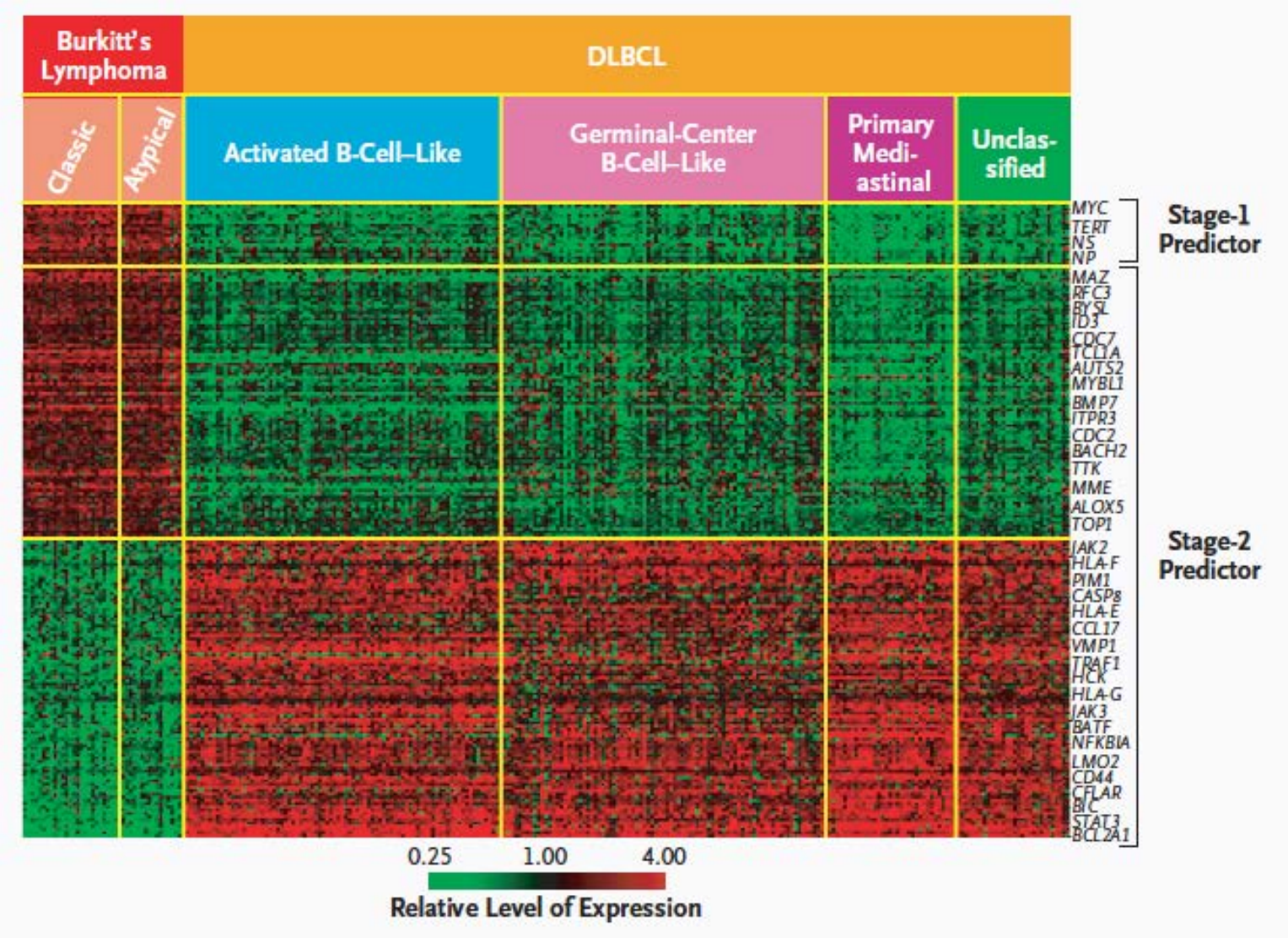
**Burkitt-like**
**Atypical Burkitt**

**DLBCL**




*Harris NL, Horning S NEJM 2006*

# Molecular classification of BL



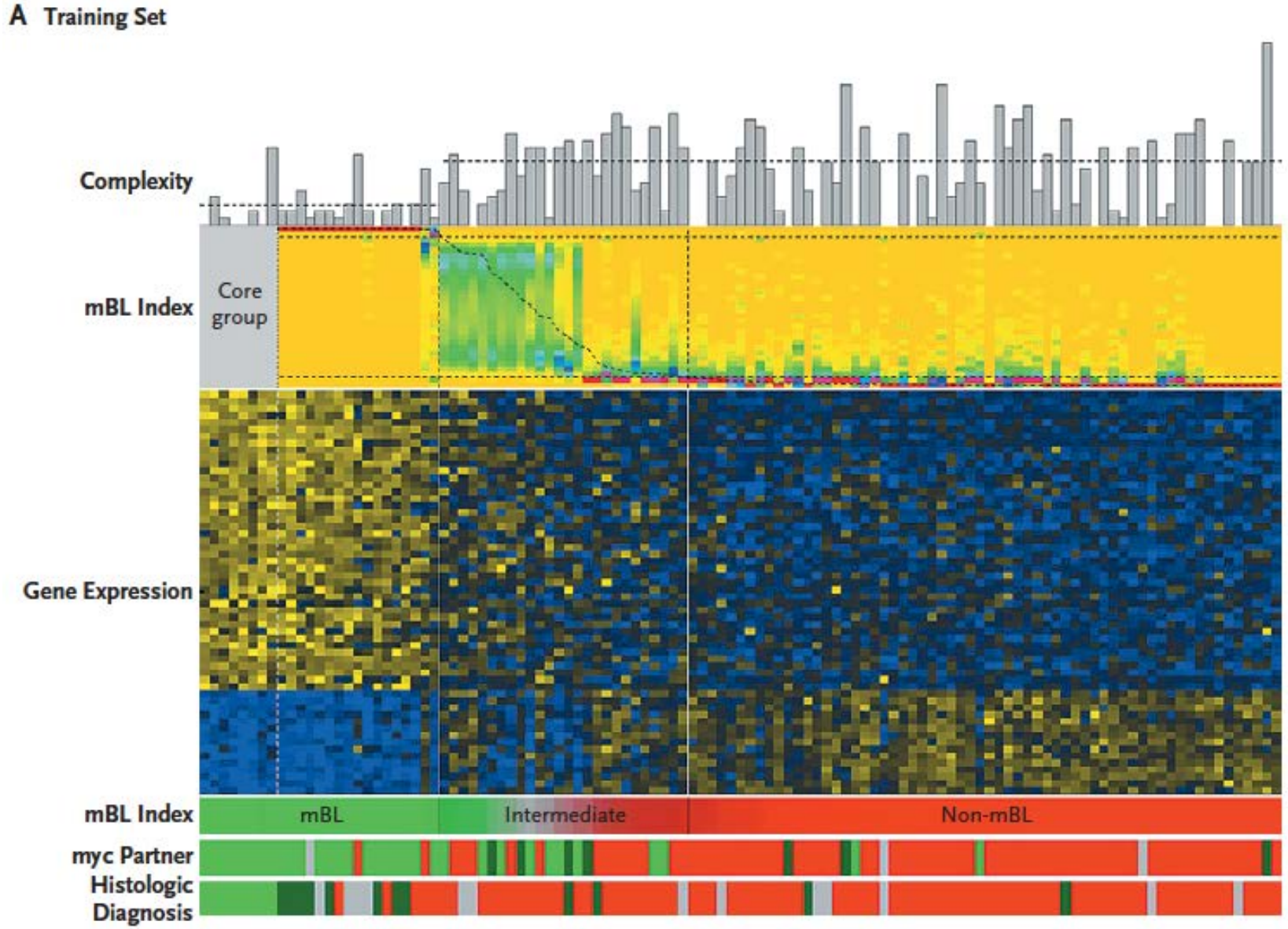

*Dave SS et al. NEJM 2006*

# Molecular classification of BL

| Total No. of Cases | Pathological Diagnosis† | Total No. of Cases | Molecular Diagnosis | Total No. of Cases |
|---|---|---|---|---|
| 71 | Classic Burkitt's lymphoma | 25 | Burkitt's lymphoma | 25 |
| | Atypical Burkitt's lymphoma | 20 | Burkitt's lymphoma | 19 |
| | | | DLBCL | 1 |
| | DLBCL | 20 | Burkitt's lymphoma | 7 |
| | | | DLBCL | 13 |
| | High-grade lymphoma, NOS | 6 | DLBCL | 5 |
| | | | Burkitt's lymphoma | 1 |
| 223 | DLBCL | 223 | Activated B-cell–like DLBCL | 78 |
| | | | Germinal-center B-cell–like DLBCL | 82 |
| | | | Primary mediastinal DLBCL | 33 |
| | | | Unclassified DLBCL | 30 |
| 9 | DLBCL | 9 | Activated B-cell–like DLBCL | 6 |
| | | | Germinal-center B-cell–like DLBCL | 2 |
| | | | Burkitt's lymphoma | 1 |

*Dave SS et al. NEJM 2006*

# Molecular classification of BL




*Hummel M et al. NEJM 2006*

# B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

P.M. Kluin
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**Definition**

B-cell lymphomas with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) are aggressive lymphomas that have morphological and genetic features of both DLBCL and BL, but for biological and clinical reasons should not be included in these categories. Some of these cases were previously classified as Burkitt-like lymphoma (BLL).

The majority of the cases in this category have morphological features that are intermediate between DLBCL and BL, with some cells that are smaller than typical DLBCL, resembling BL, and some cells that are larger than typical BL, resembling DLBCL, as well as a high proliferation fraction, starry-sky pattern, and an immunophenotype consistent with BL. Some cases may be morphologically more typical of BL but have an atypical immunophenotype or genetic features that preclude a diagnosis of BL. The diagnosis of this type of unclassifiable B-cell lymphoma category should not be made in cases of morphologically typical DLBCL that have a *MYC* rearrangement, or in otherwise typical BL in which a *MYC* rearrangement cannot be demonstrated. Some transformed follicular lymphomas may fall into this category. This is a heterogeneous category that is not considered a distinct disease entity, but is useful in allowing the classification of cases not meeting criteria for classical BL or DLBCL.

**ICD-O code** 9680/3

- Intermediate features , cases that should not be diagnosed as BL or DLBCL
- Biological basis
- Clinical implications
- Heterogeneous group of aggressive lymphomas, not a distinct entity

**Table 10.18** Morphologic, immunophenotypic, and genetic features that may be useful in distinguishing BL from DLBCL

| Characteristic | BL | Intermediate BL/DLBCL | DLBCL |
|---|---|---|---|
| **Morphology** | | | |
| Only small/medium-size cells | Yes | Common | No |
| Only large cells | No | No | Common |
| Mixture | No | Sometimes | Rare |
| **Proliferation (Ki67/MIB1)** | | | |
| >90% and homogeneous | Yes | Common | Rare |
| <90% or heterogeneous | No | Sometimes | Common |
| **Bcl-2 expression** | | | |
| Negative / weak | Yes | Sometimes | Sometimes |
| Strong | No | Sometimes | Sometimes |
| **Genetic features** | | | |
| *MYC* rearrangement | Yes* | Common | Rare |
| *IG-MYC*** | Yes | Sometimes | Rare |
| Non *IG-MYC*** | No | Sometimes | Rare |
| *BCL2* but no *MYC* rearrangement | No | Rare | Sometimes |
| *BCL6* but no *MYC* rearrangement | No | Rare | Sometimes |
| Double hit# | No | Sometimes | Rare |
| *MYC*-Simple karyotype*** | Yes | Rare | Rare |
| *MYC*-Complex karyotype*** | Rare | Common | Common |

# Burkitt lymphoma, diffuse large B-cell lymphoma or intermediate category?

- Burkitt Lymphoma morphology but atypical features
  - Atypical immunophenotype
  - Lack of *MYC* translocation
- Pediatric cases
- Intermediate morphology
- Diffuse large B-cell lymphoma morphology with *MYC* translocation
- « Double hit » lymphomas

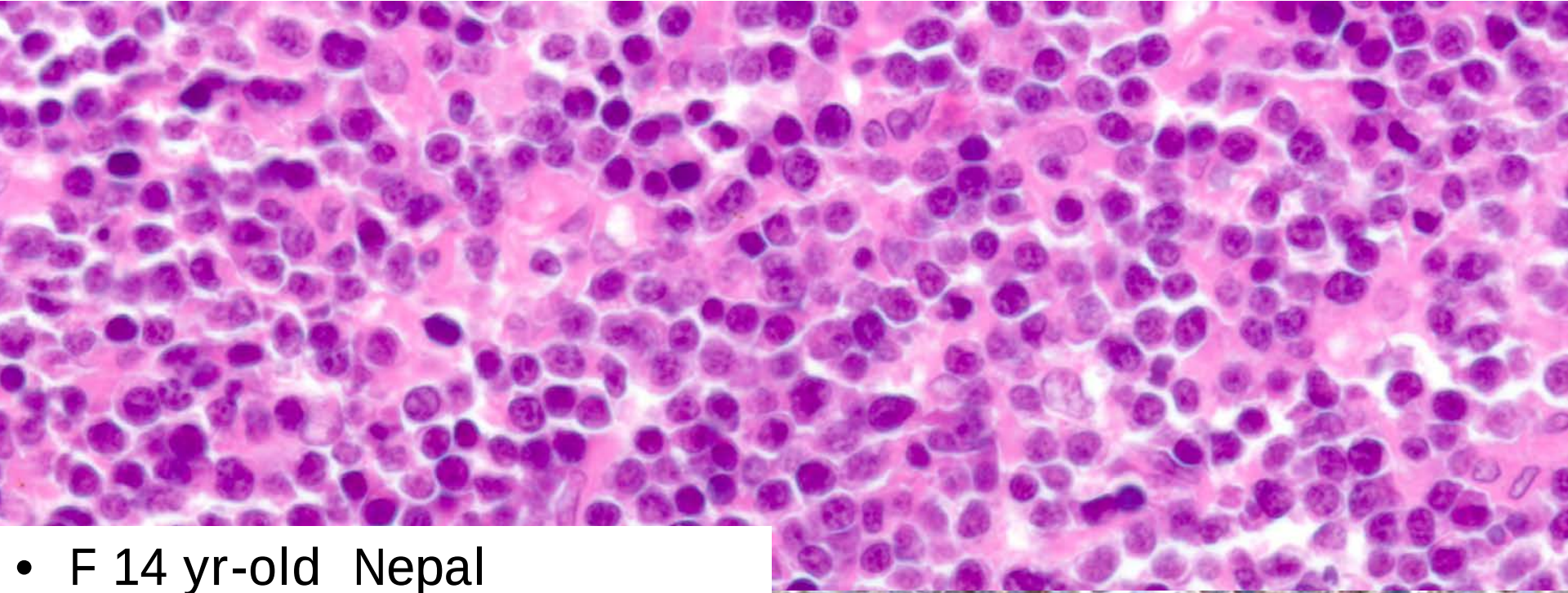
**Table 9**
**Morphologic, immunophenotypic, and genetic features used to distinguish Burkitt lymphoma from DLBCL/BL and DLBCL**

| Expected BL Finding | Relative Contraindication for a Diagnosis of BL | Absolute Contraindication for a Diagnosis of BL[a] |
|---|---|---|
| Uniform, medium-sized cells | Mild or moderate cellular pleomorphism | Blastic morphology[b]<br>Large cell size or marked cellular pleomorphism[c] |
| CD10 strongly positive | CD10 negative | NA |
| BCL6 positive | BCL6 negative | NA |
| BCL2 negative | NA | BCL2 strongly positive[a] |
| Ki67 | NA | <95% proliferation index[a] |
| *MYC* rearrangement with *LG* locus (usually *LGH*, but sometimes with kappa or *lambda* loci) | Absent or shown to be with a non-*LG* locus | NA |
| Simple karyotype | Complex karyotype (3 or more abnormalities in addition to 8q24) | NA |
| No *BCL6* rearrangement | NA | BCL6 rearrangement present[a] |
| No *BCL2* rearrangement | NA | BCL2 rearrangement present[a] |

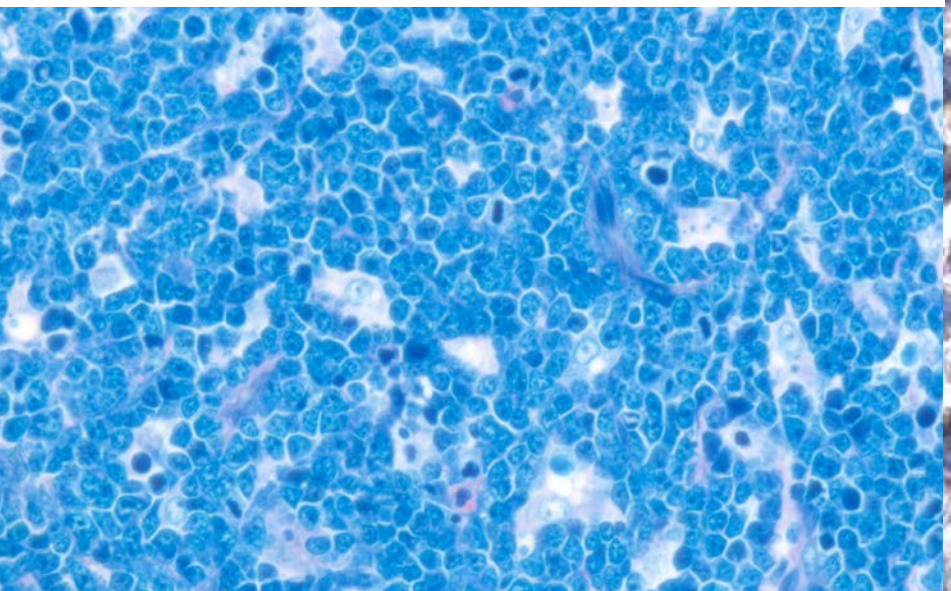
*de Leval L, Hasserjian RP Hematol Oncol North Am 2009*

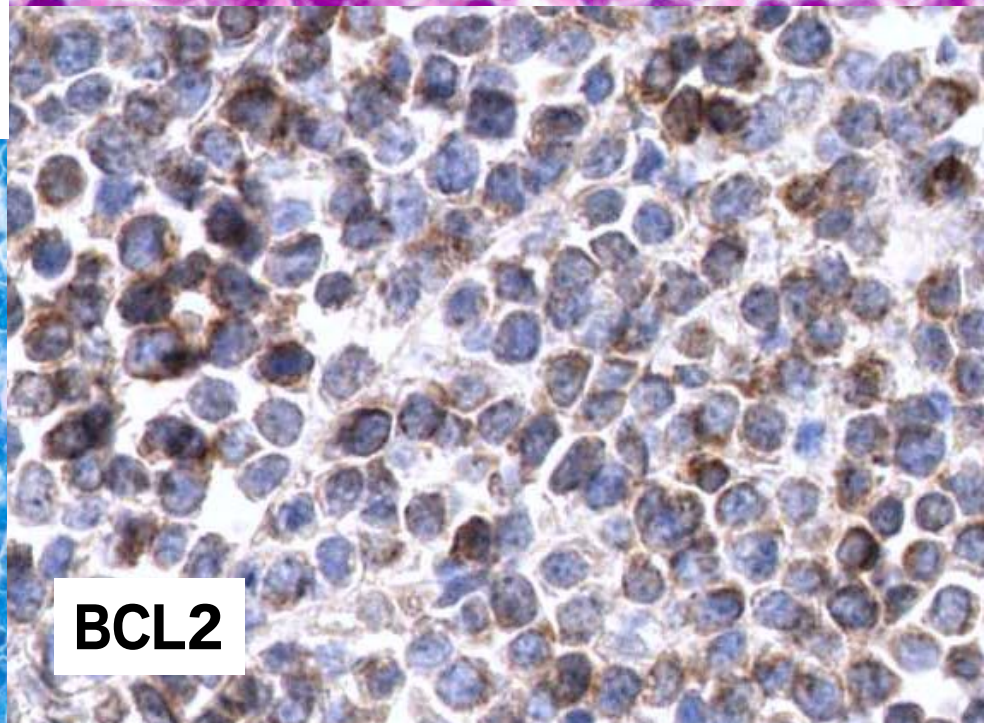
# Burkitt lymphoma with atypical immunophenotype

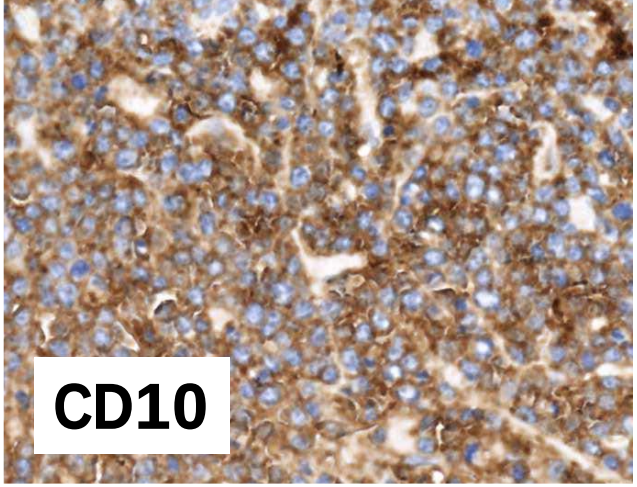
- Rare cases CD10 weak or neg
- Rare cases BCL-6 neg
- Less than 95% proliferation fraction
- Up to 20% BCL2+
  - Upregulation of BCL2 upon EBV infection
  - No strong expression
  - Exclude *BCL2* translocation
- OK for BL if otherwise perfect

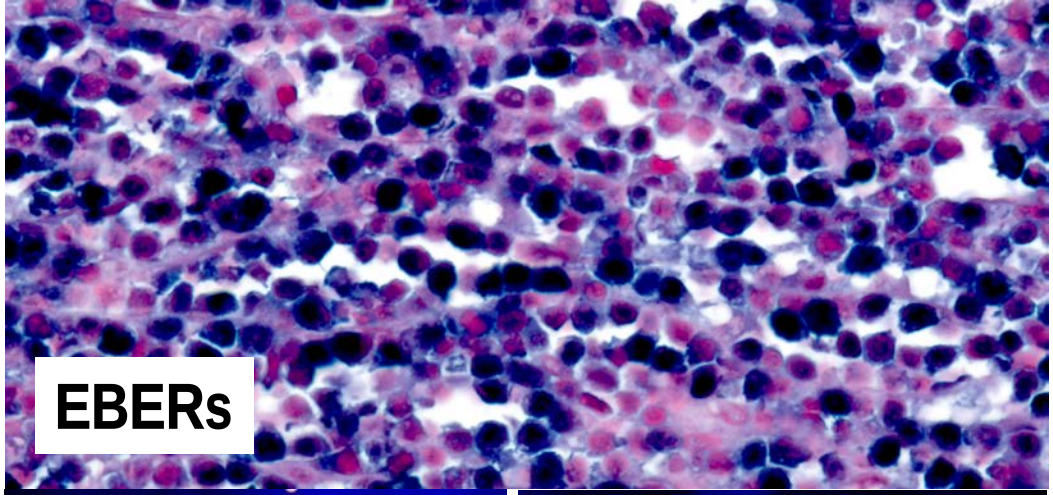


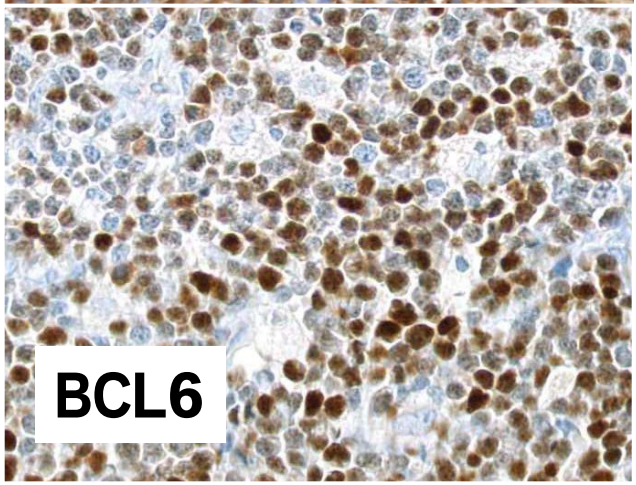
- F 14 yr-old  Nepal
- Bilateral ovarian masses

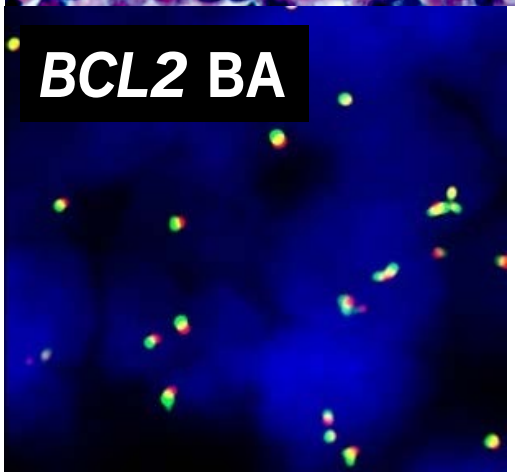


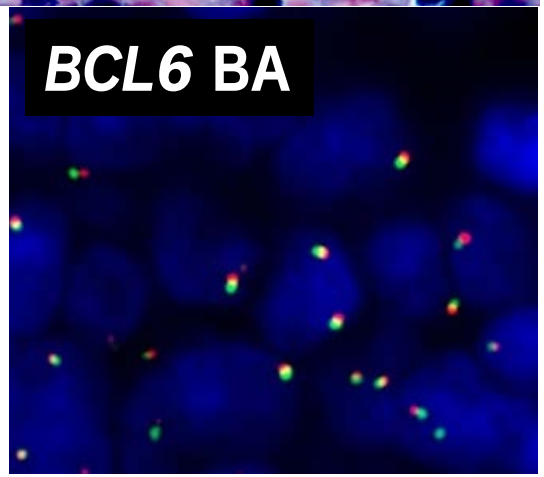


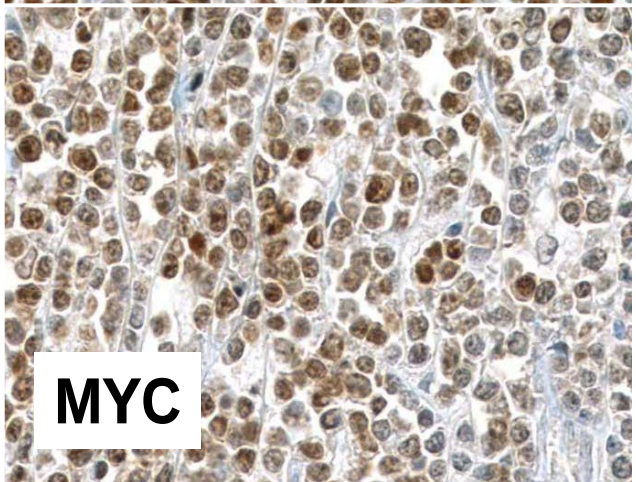


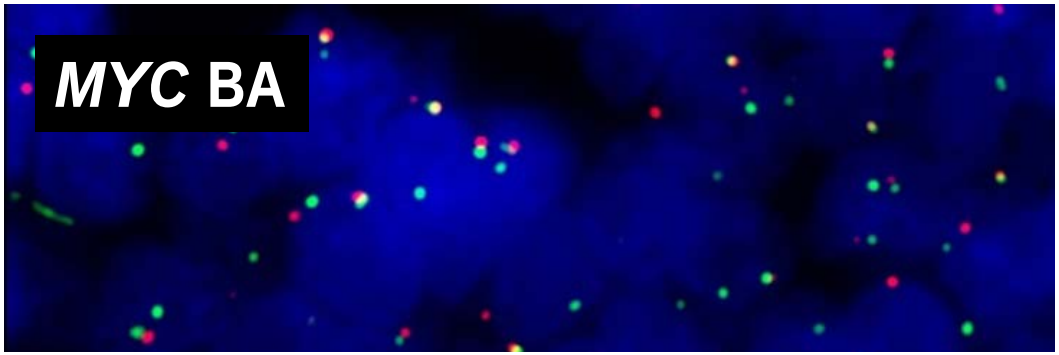



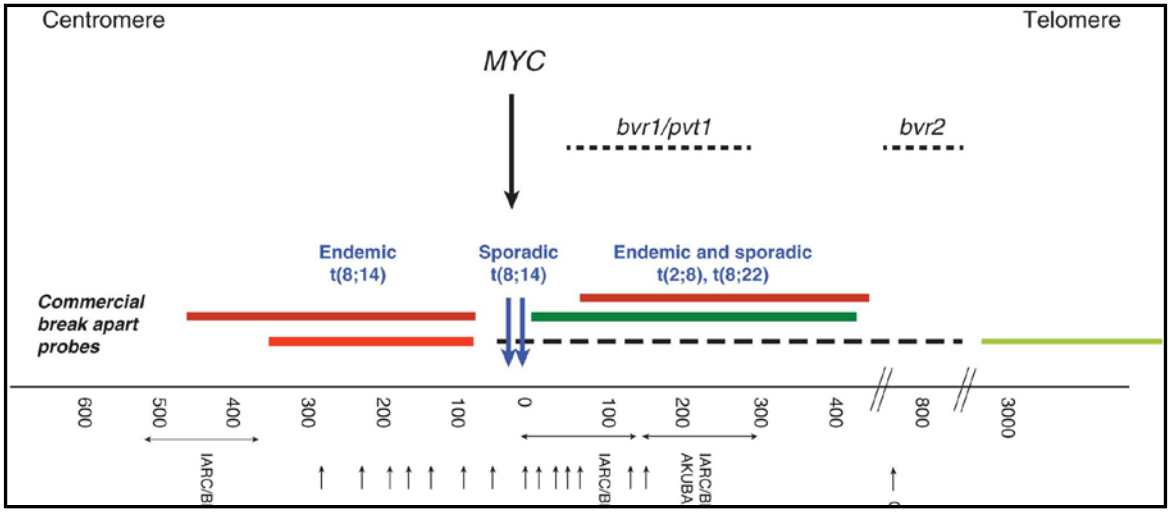



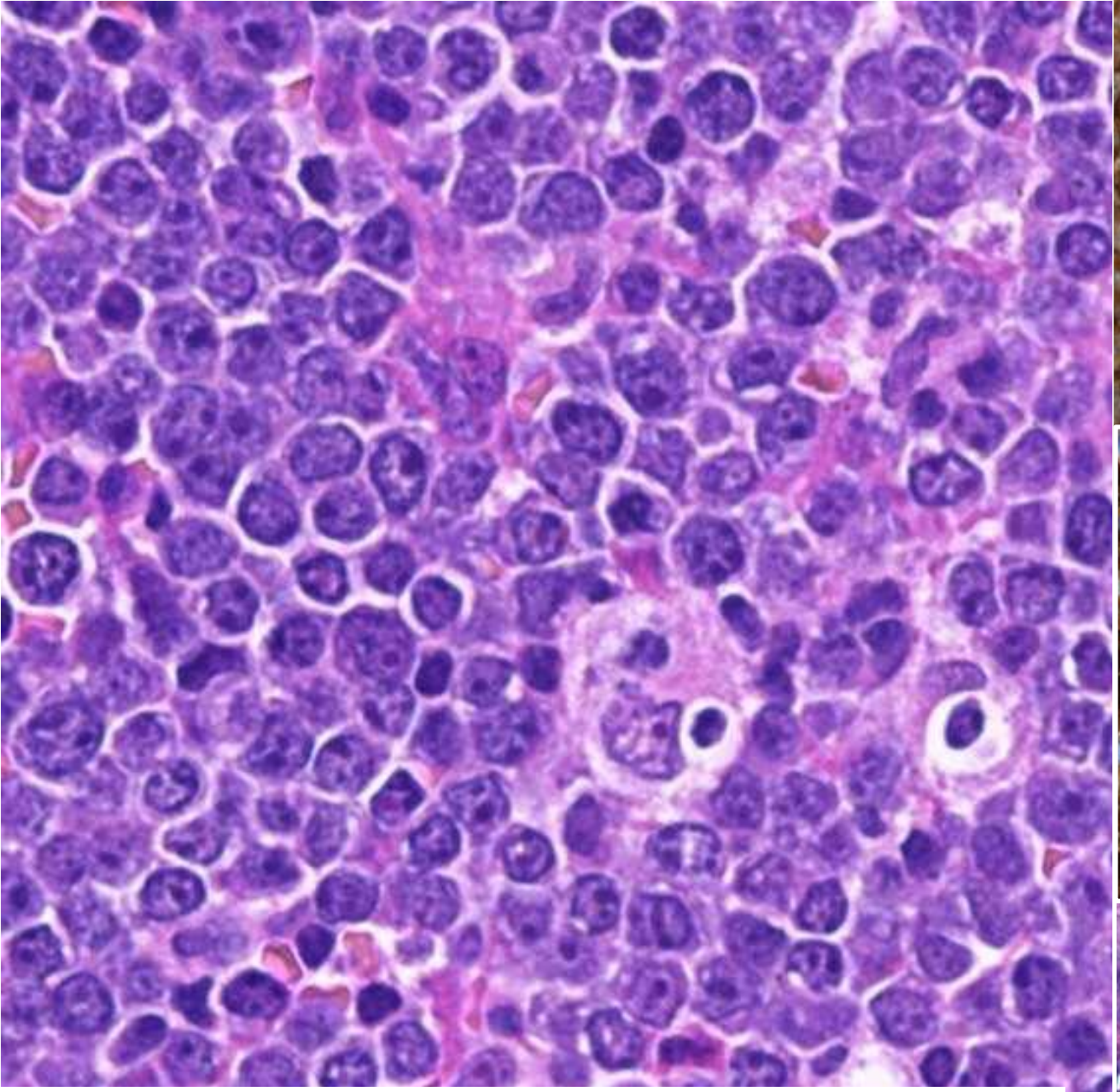

- PTLD, EBV+ Burkitt lymphoma, without demonstrable MYC rearrangement

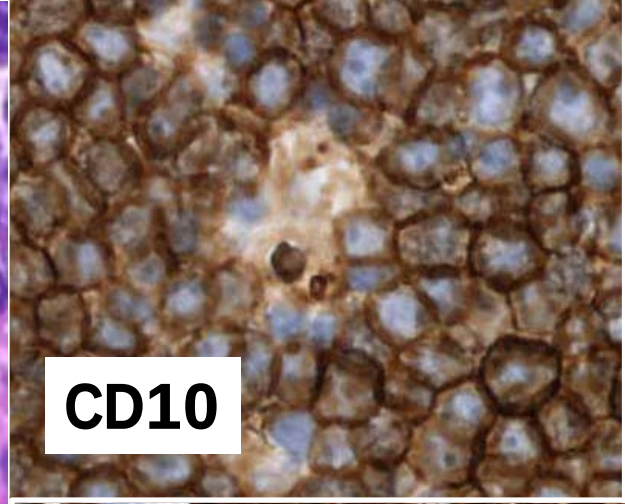
# BL with no demonstrable *MYC* translocation

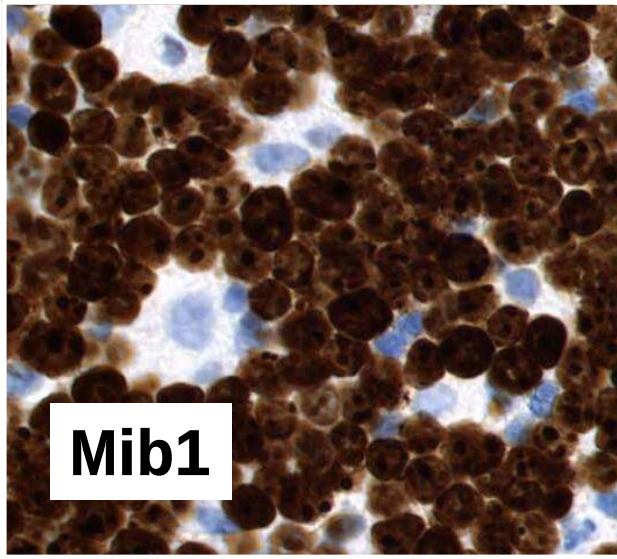
- Approximately 10% of BL *MYC* negative in all age groups
- Cases with *MYC* translocation missed by FISH probes
  - Cryptic insertions of *IG* into *MYC* locus
  - Distal 5' and 3' breaks
- miRNA hsa-mir-34B deregulation
- MYC-negative high-grade B-cell lymphomas resembling BL with 11q aberrations (proximal gains + telomeric loss)

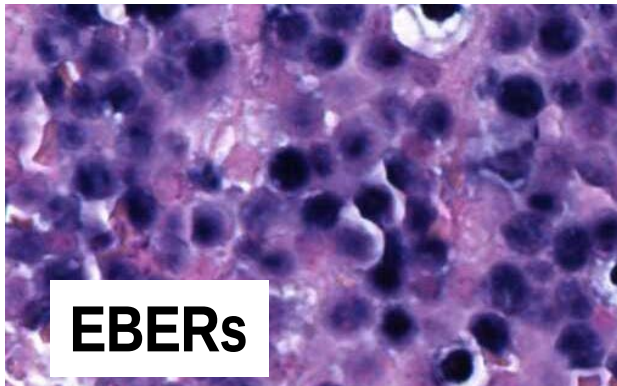



*Leucci E J Pathol 2008; Salaverria I Blood 2014; Knowles D Neoplastic hematopathology 2013*

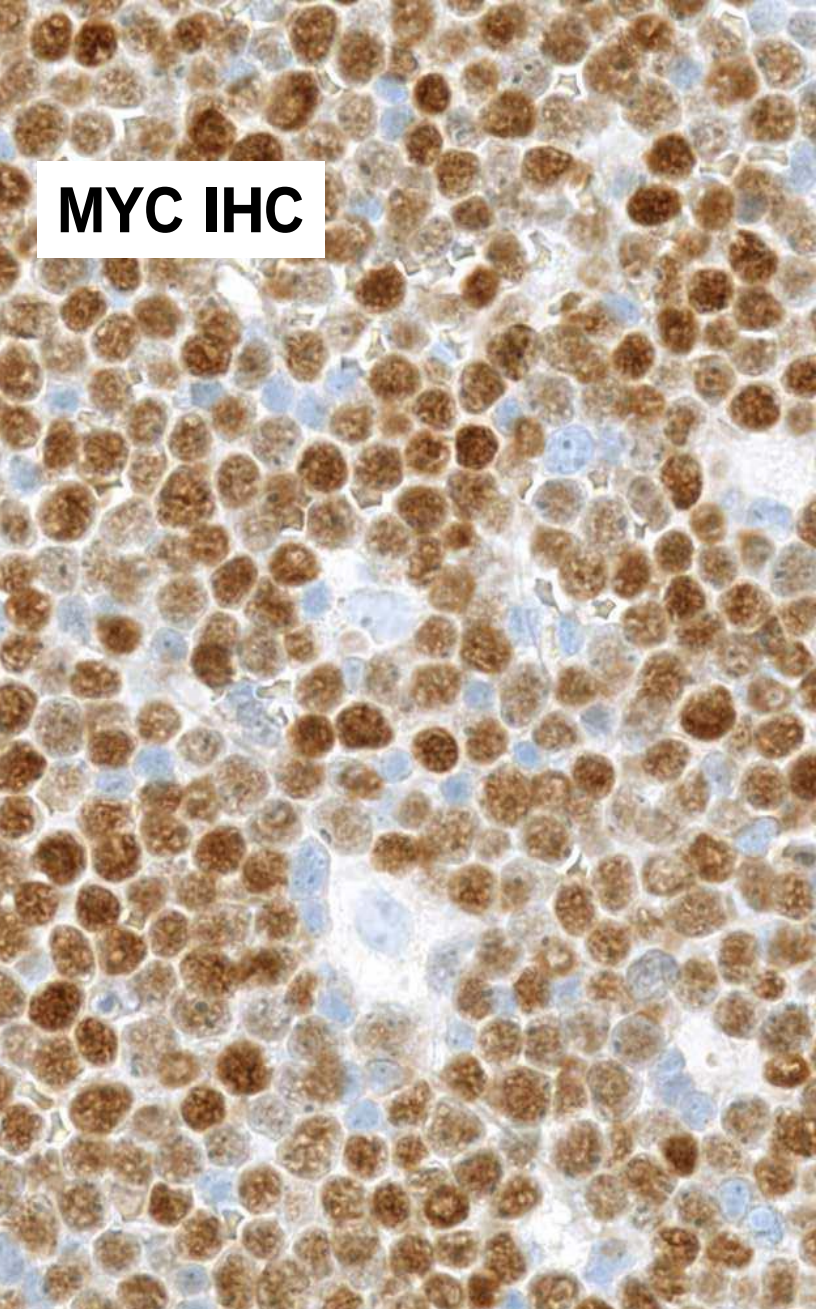


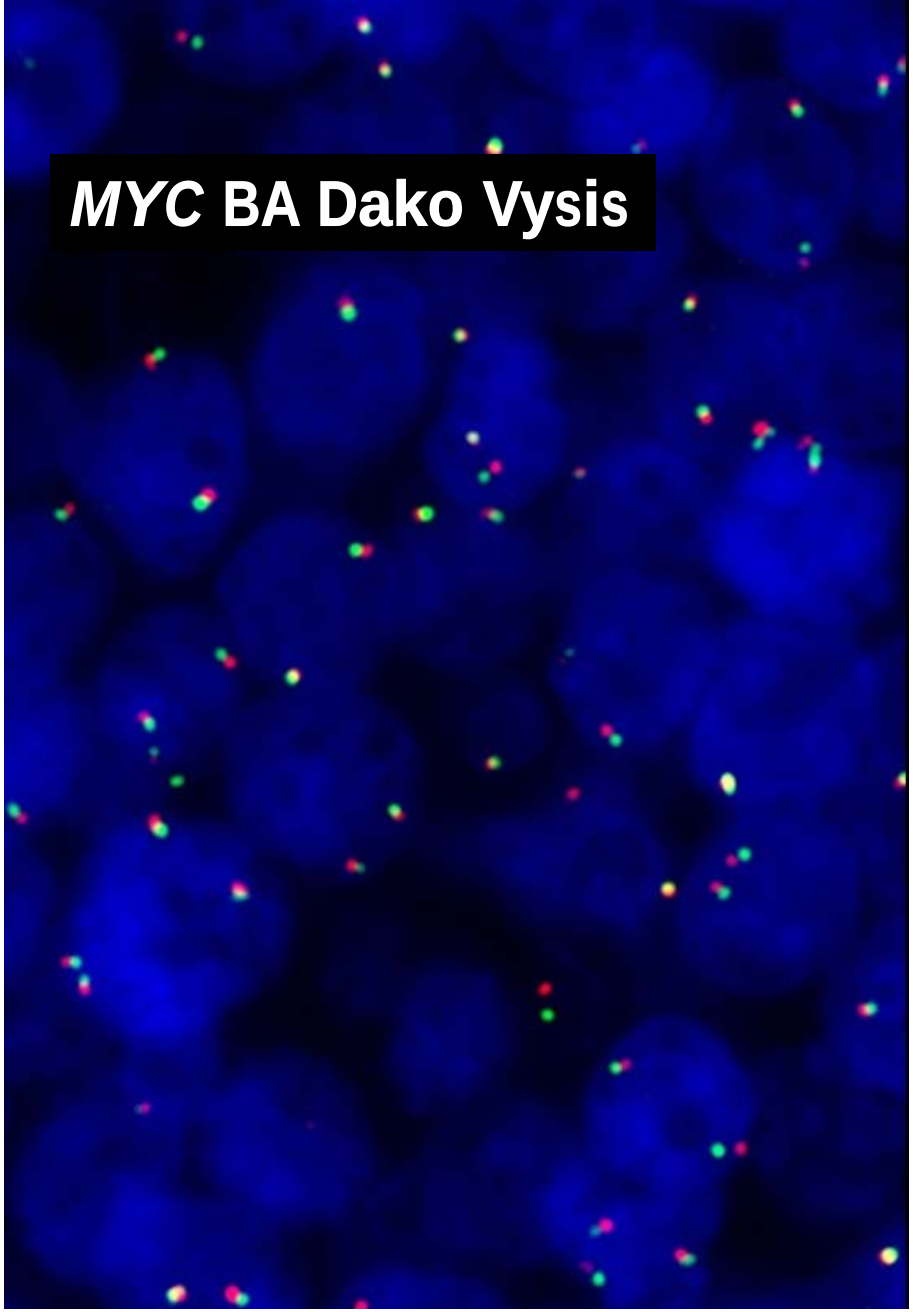



- M 20 yr-old  10 yrs post cardiac transplant
- Cervical lymph node enlargement

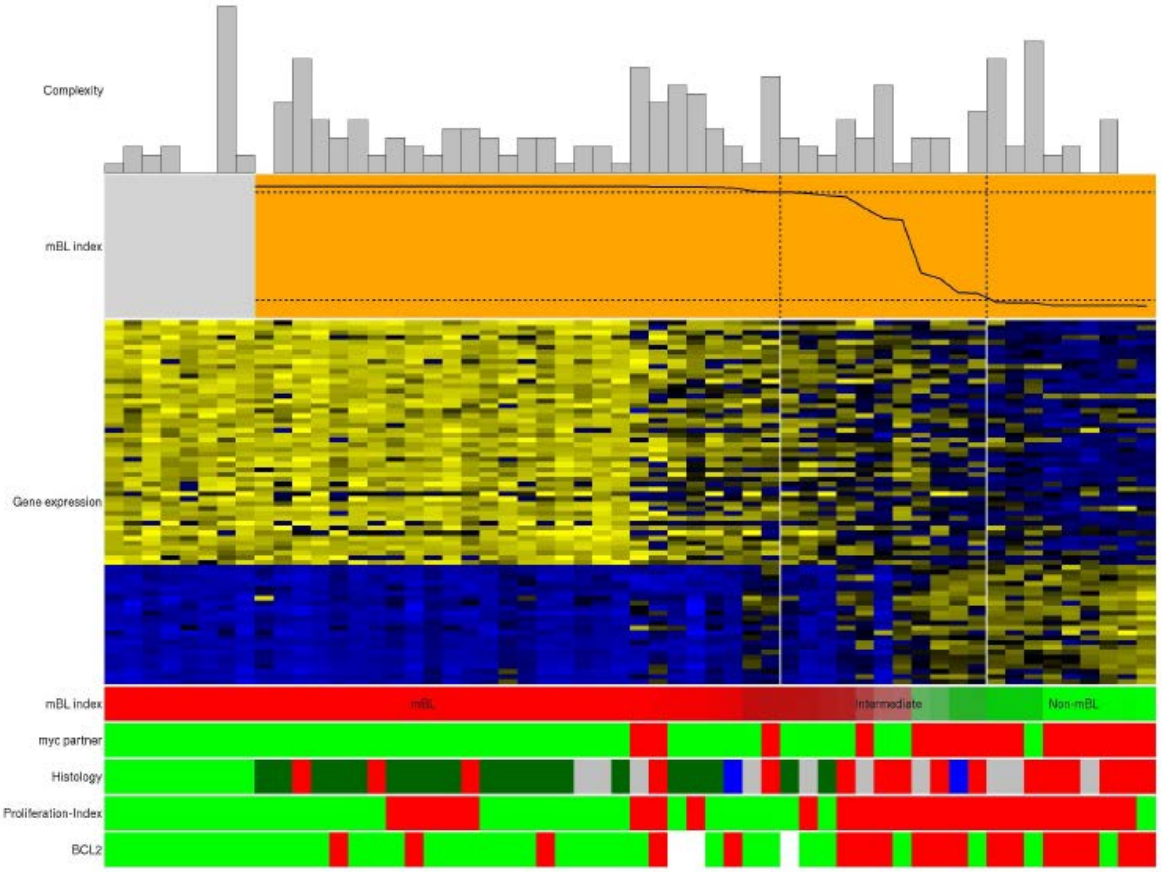


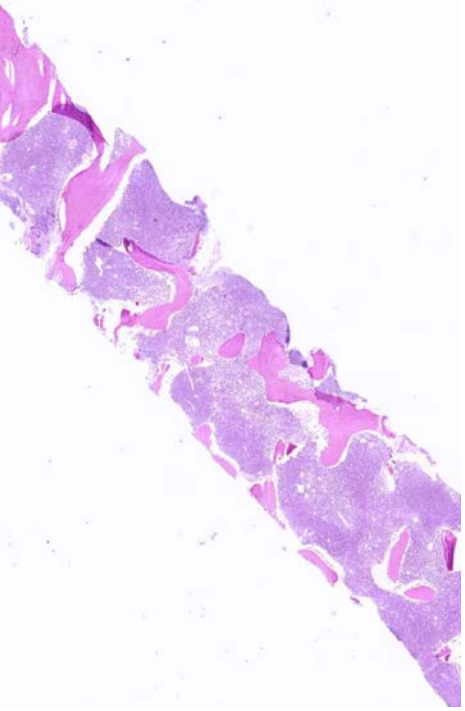

- PTLD, EBV+ Burkitt lymphoma, without demonstrable MYC rearrangement

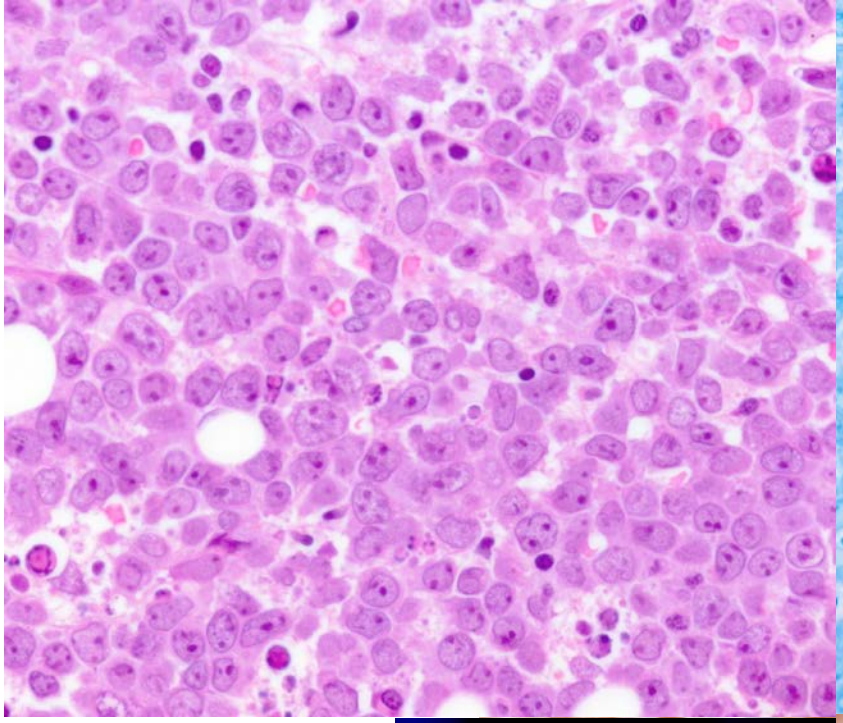
# High-grade B-cell lymphomas in children

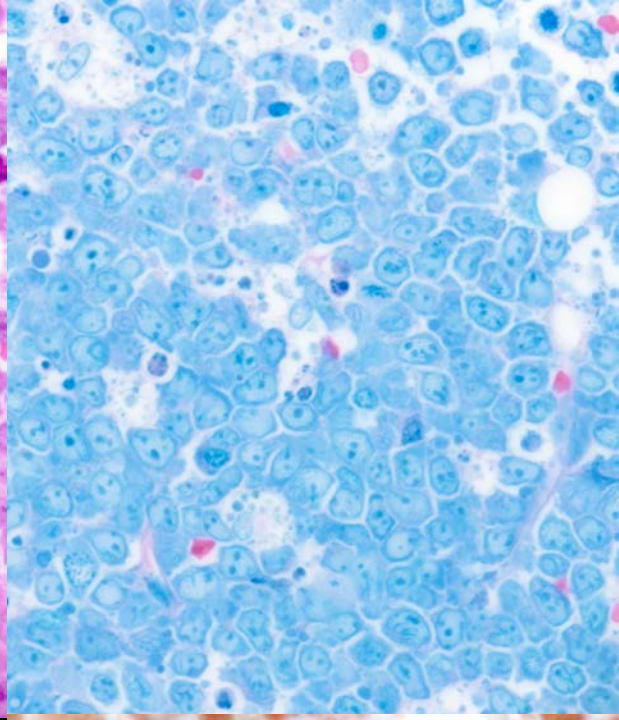
- Same treatment for high-grade B-cell lymphomas with overall good outcome
- Majority are mBL
- BL and aBL have a mBL signature
- DLBCL with *MYC* break have a mBL signature



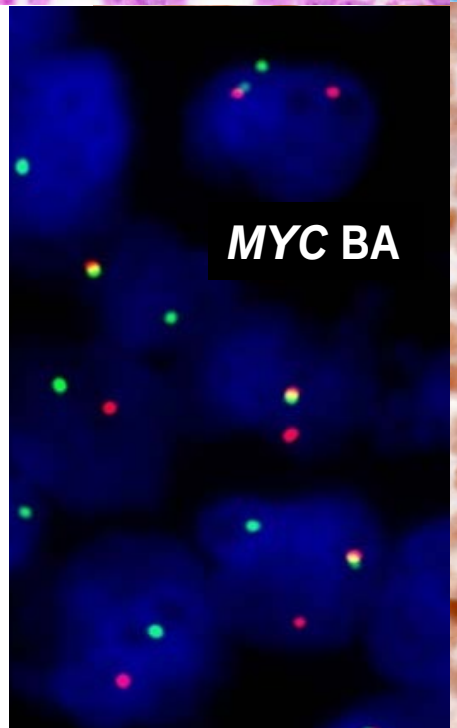

*Klapper W et al. Blood 2008*

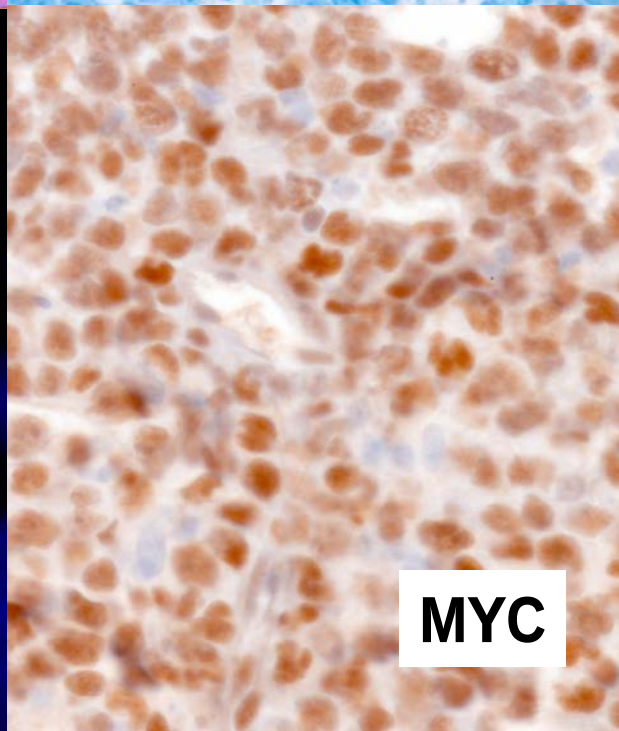






- M 19 yrs  hepatosplenomegaly, LDH 22’000, circulating blasts, ALL vs BL?
- CD20+ CD10+ BCL6+ BCL2- MUM1-
- TdT- Ki67 100% EBER-
- FISH: *BCL2* nl, *BCL6* nl
- **DLBCL with *MYC* break**
- **Treated with BL protocole**



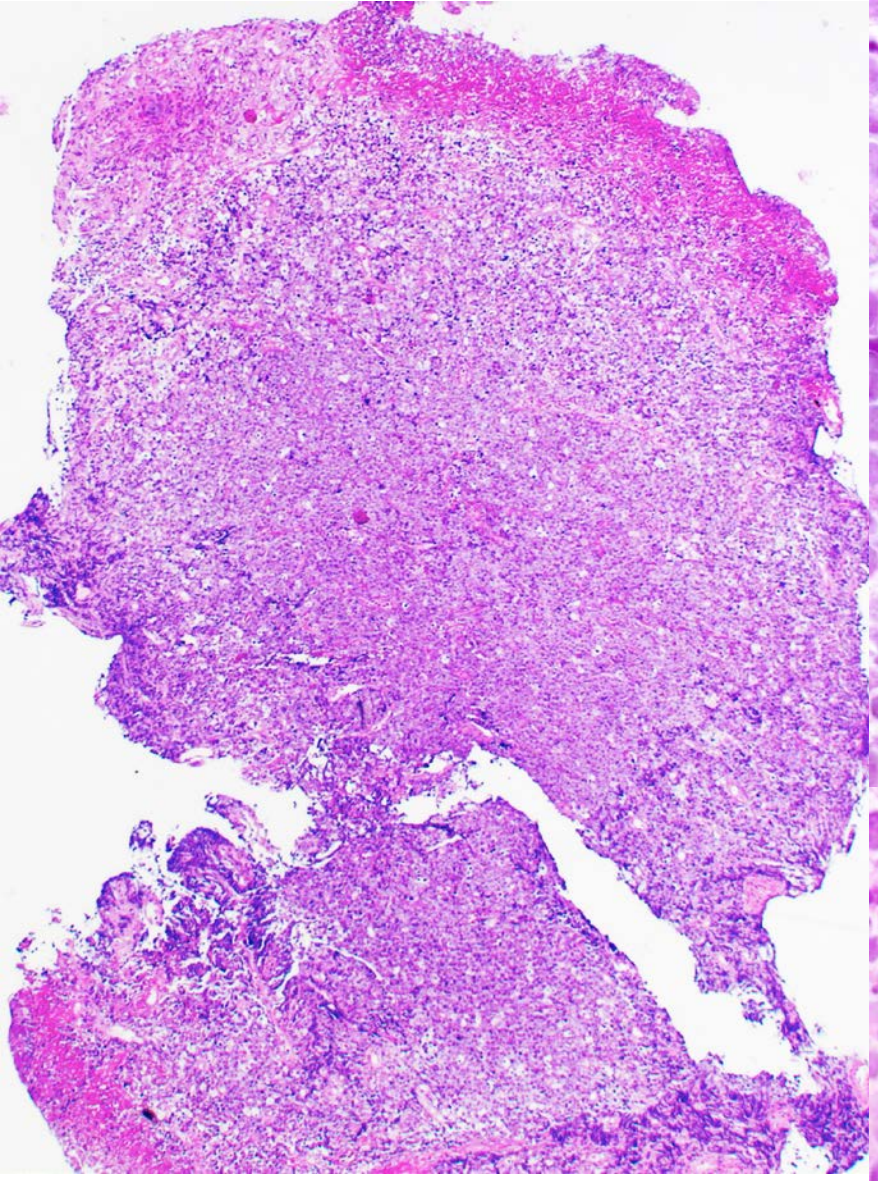



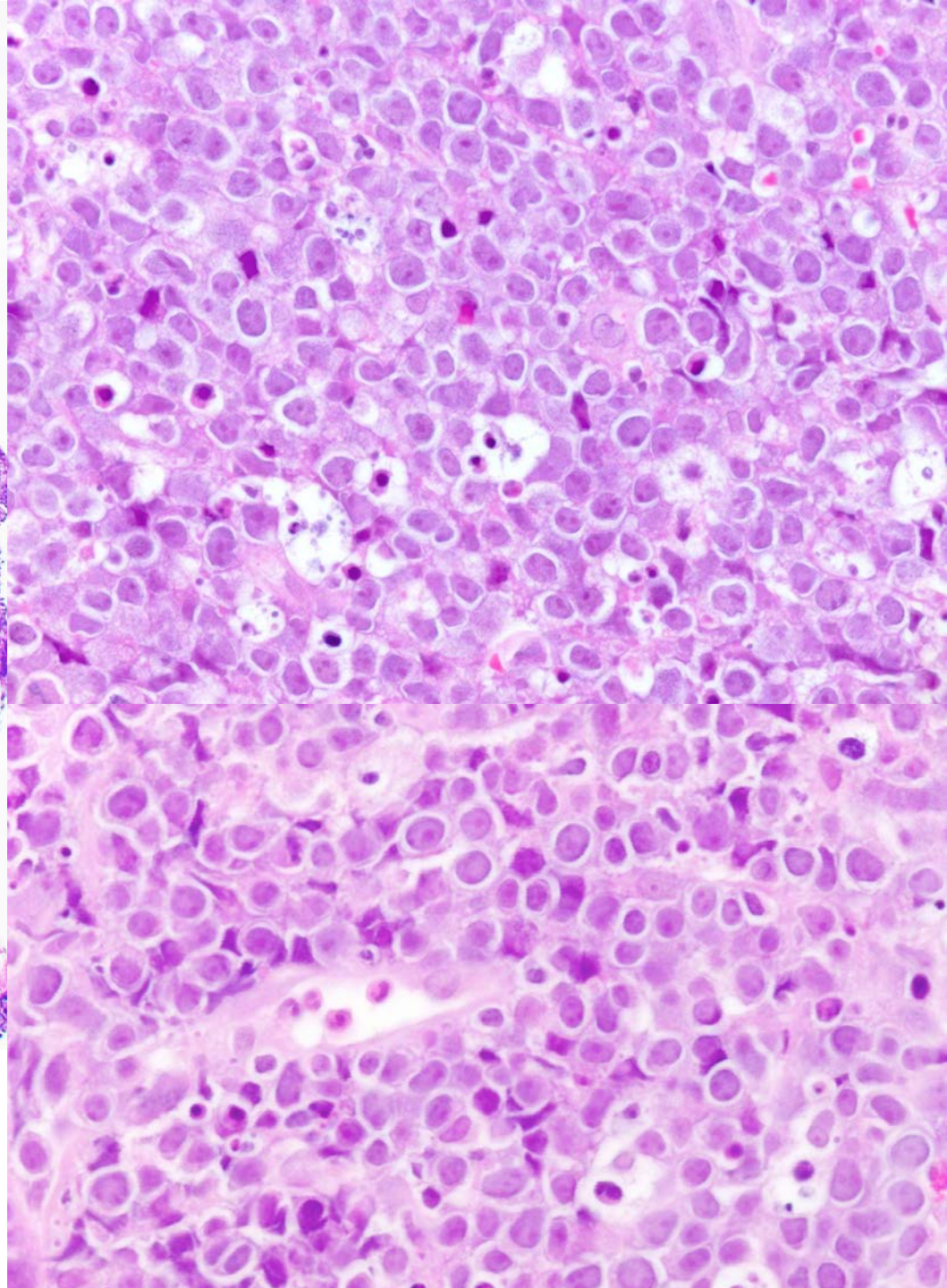
# B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL

- **Rare**
- **Clinical features**
  - Adults, median age 50-65
  - Widespread, often extranodal presentation
  - Bone marrow and CNS often involved
  - High IPI index and poor survival
- **Morphology intermediate**
  - Medium to large cells, coarse to fine chromatin
  - Starry sky frequent
  - Some pleomorphism
  - Some cases closer to BI, others closer to DLBCL

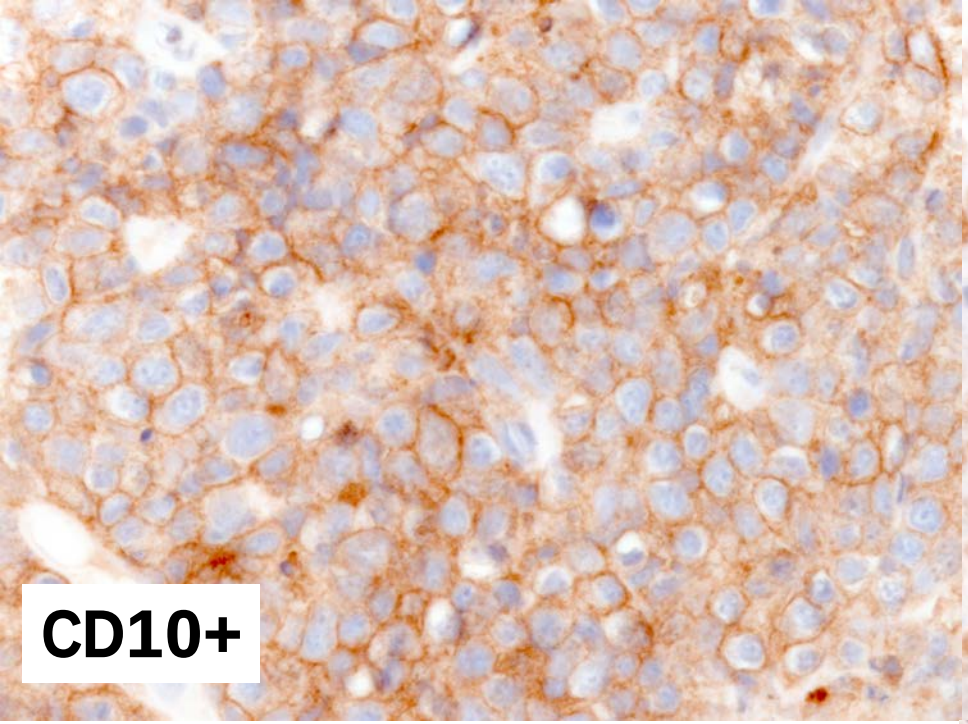
# B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL

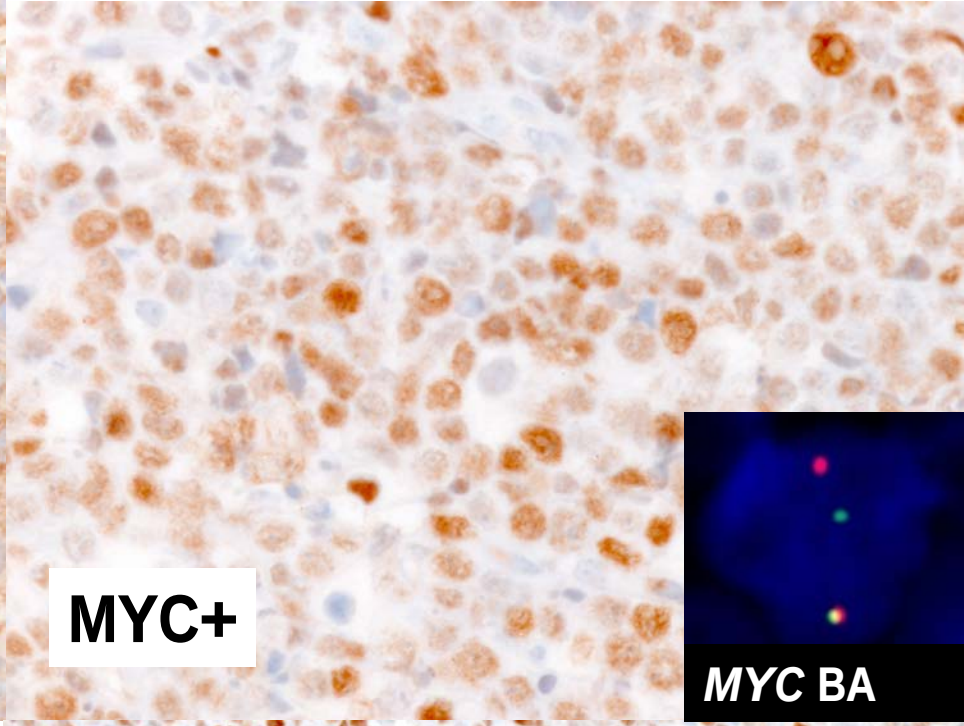
- **Immunophenotype**
  - CD20+
  - CD10 often positive, GC-like (Hans algorithm)
  - Ki67 proliferation fraction variable, often <95%
  - TdT-negative (that implies lymphoblastic lymphoma)
- **Genetic features**
  - 35 to 50% have a *MYC* translocation
  - *IG* and non-*IG* parteners
  - Frequent complex karyotype
  - *BCL2* and/or *BCL6* translocation in addition to *MYC* (double hit/ triple hit) (approximately 15% of the cases)

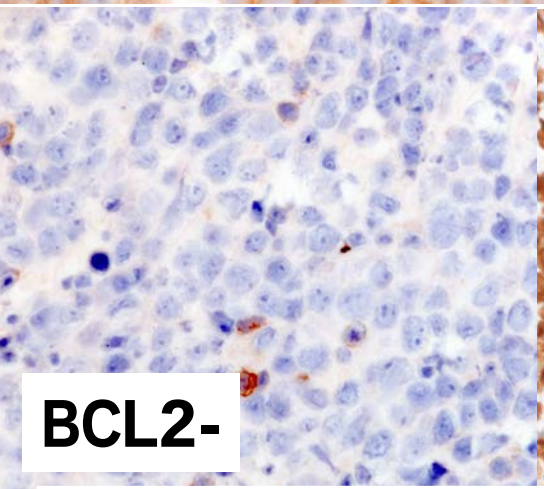


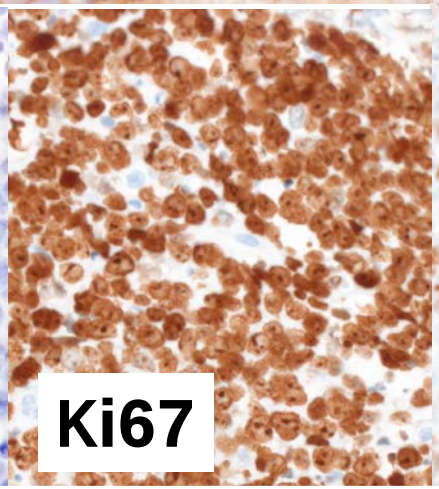


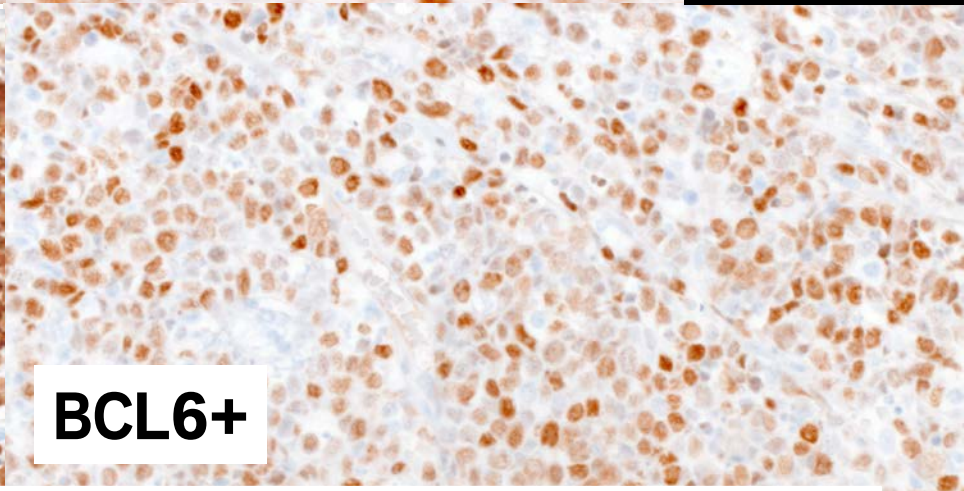
- M 57 yrs, right colon mass
- Endoscopic biopsies



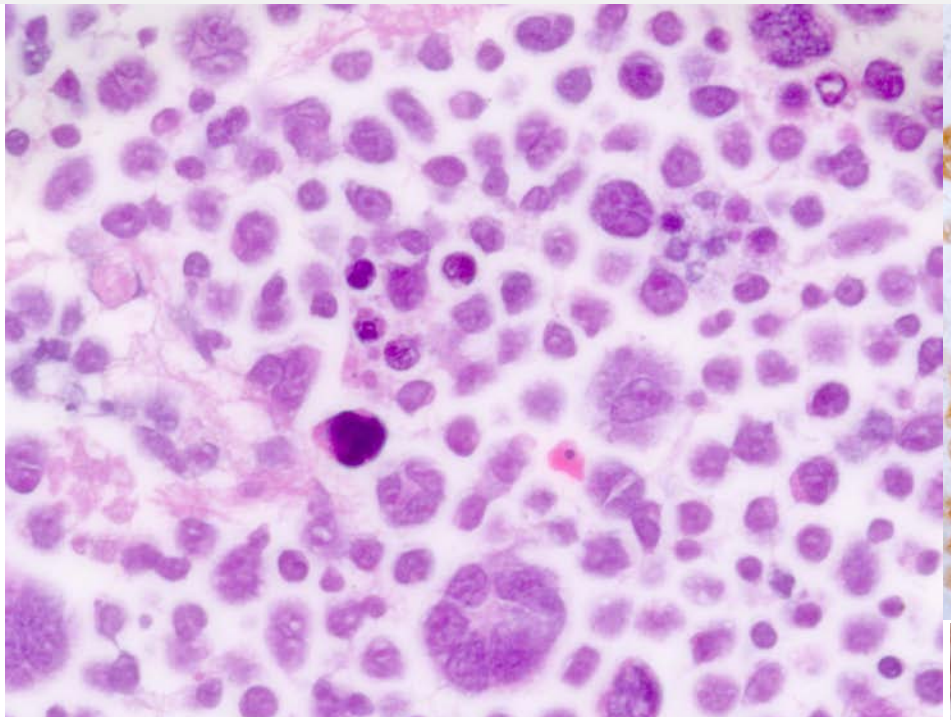



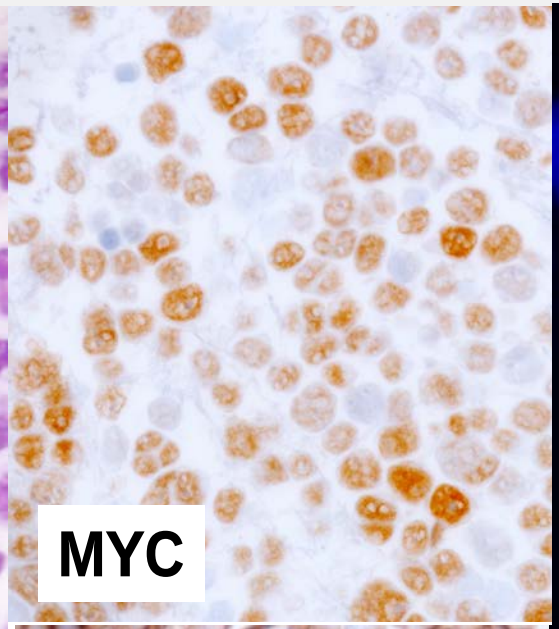



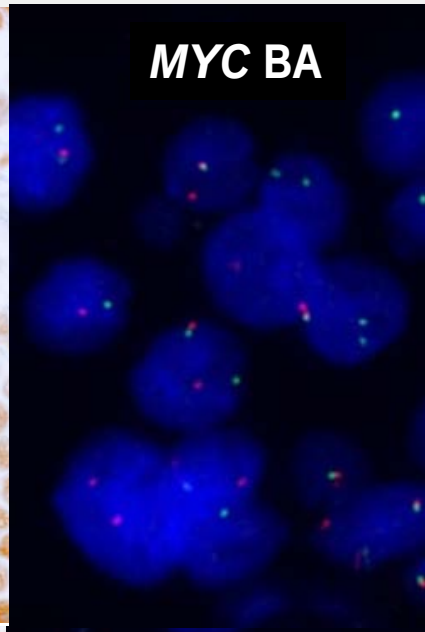



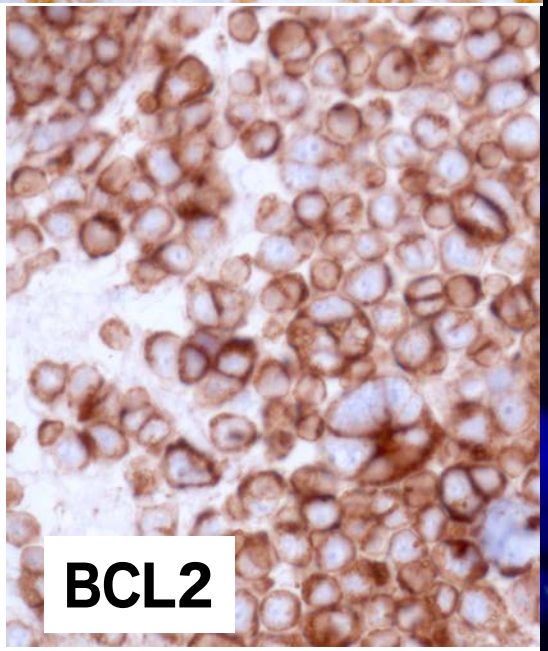

- Other findings: EBER negative, FISH BCL2 BCL6 normal
- Diagnosis: **B-cell lymphoma unclassifiable with features intermediate between DLBCL and HL, with *MYC* rearrangement**

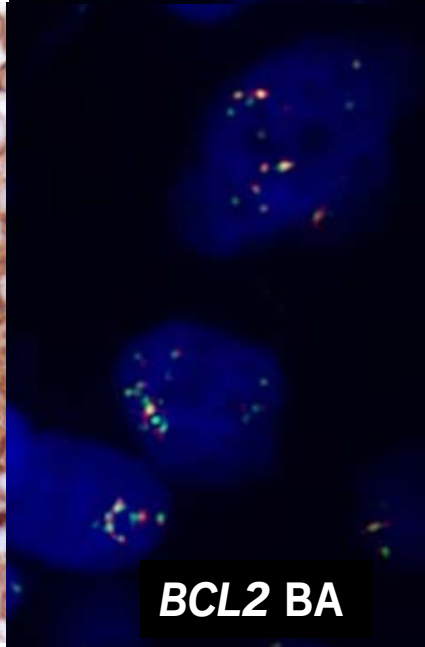
# DLBCL with *MYC* rearrangement



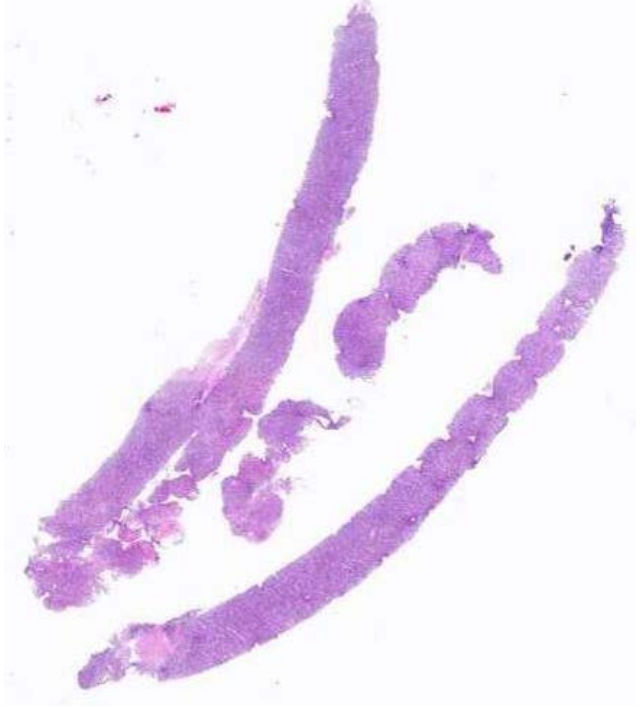


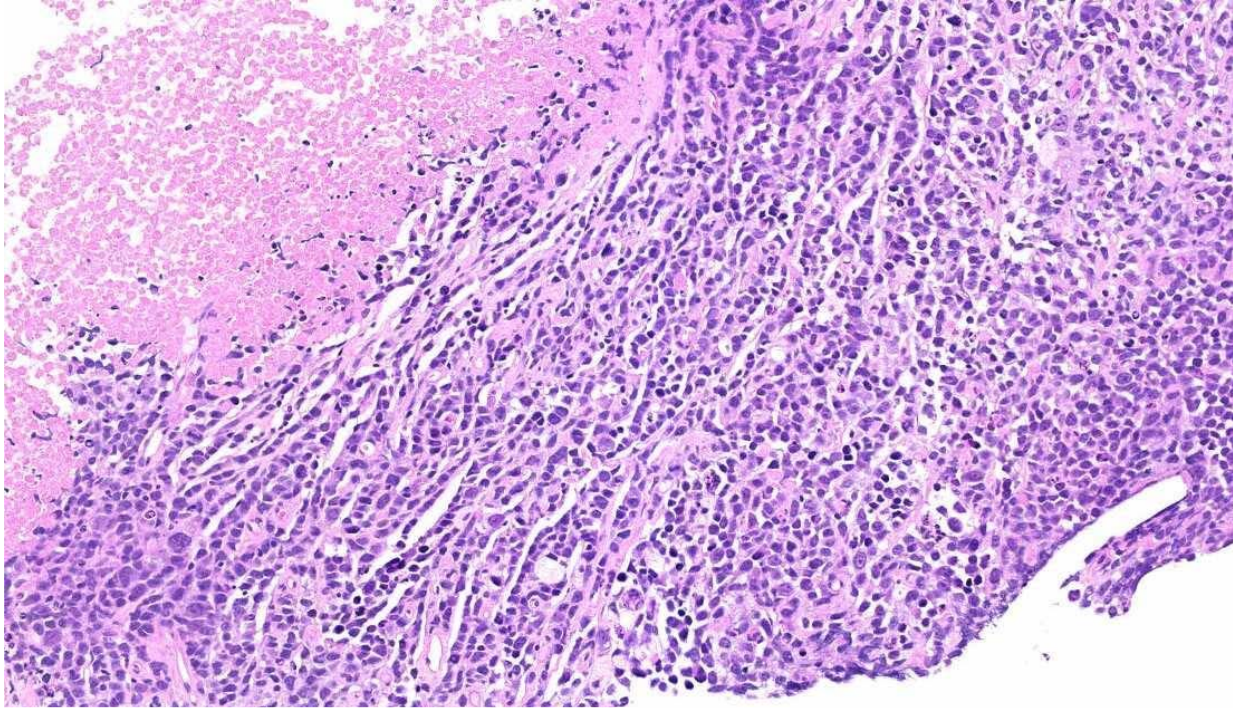



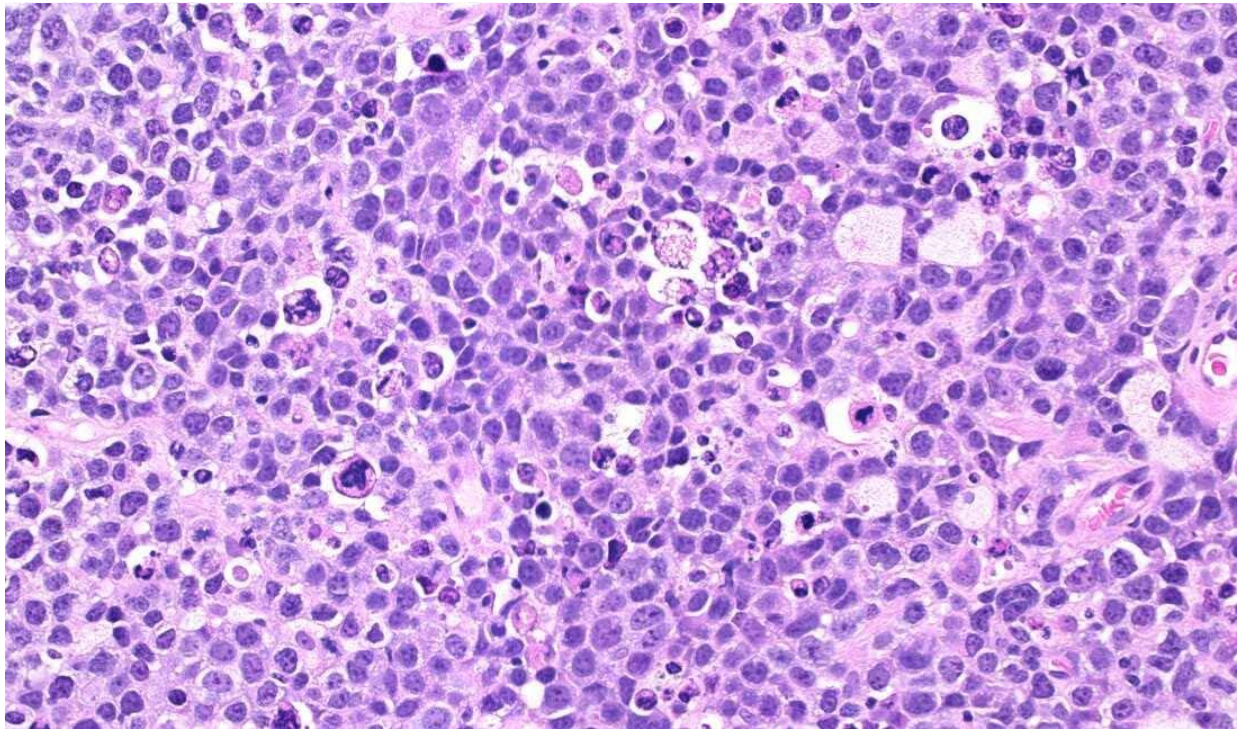



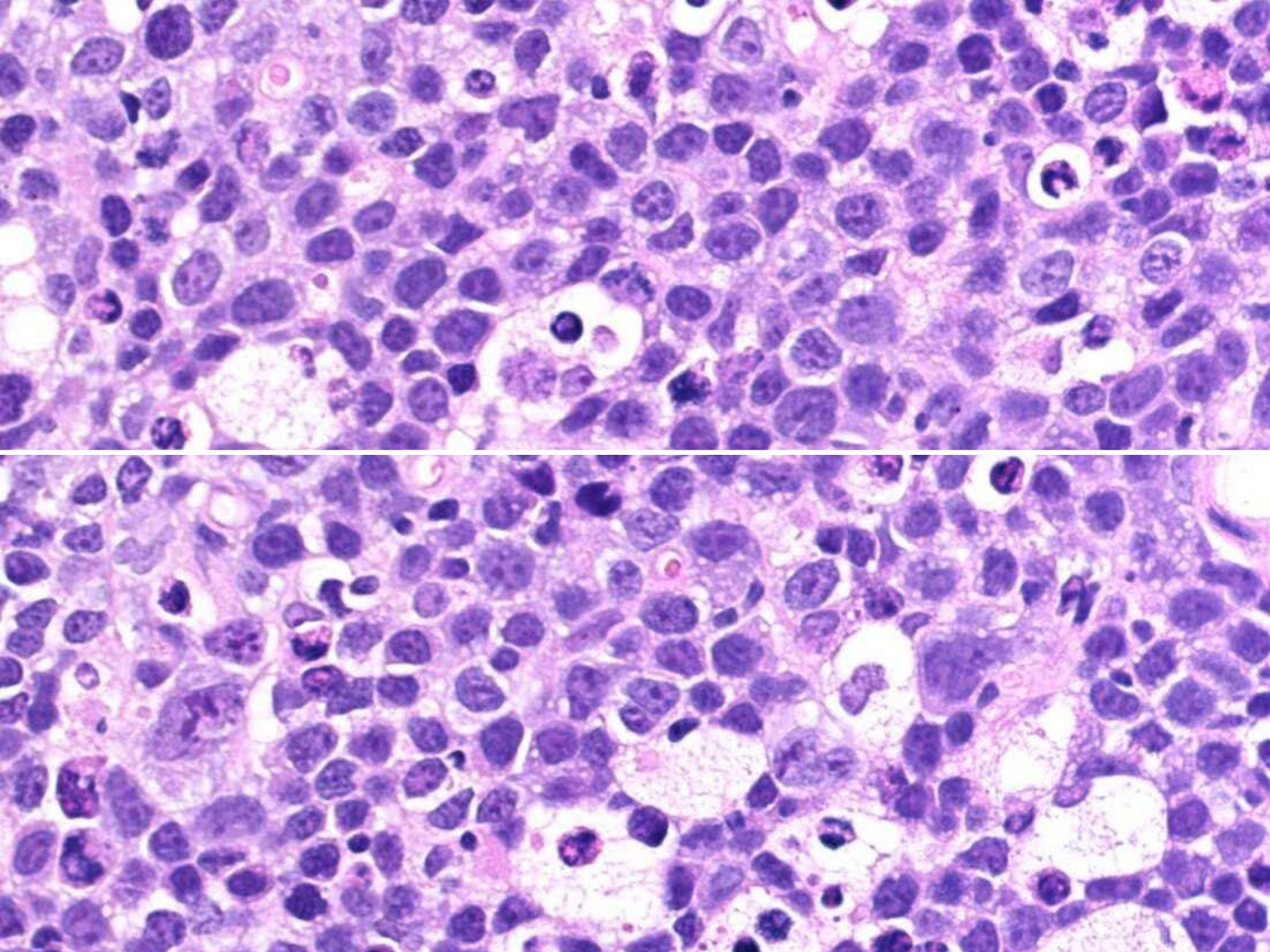

- M 81 yrs  15 cm mass inferior to left kidney
- CD20+ CD10+ BCL6- MUM1+
- MYC+ BCL2+ Ki67 85%
- EBER-
- *MYC* break, *BCL2* ampl, *BCL6* nl
- DLBCL with  *MYC* break (IHC DH)

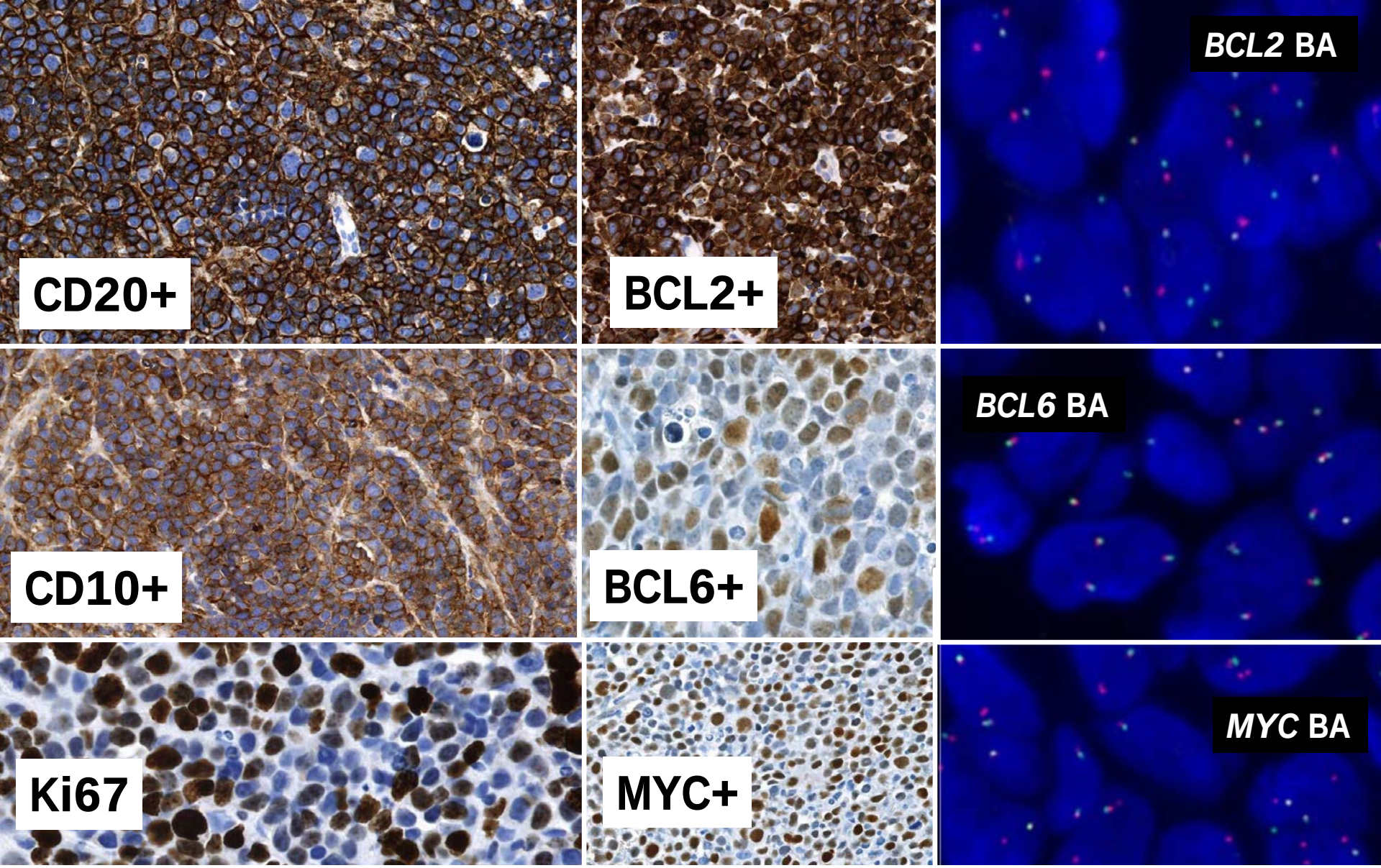




- F 57 yrs, large retroperitoneal mass, increased LDH





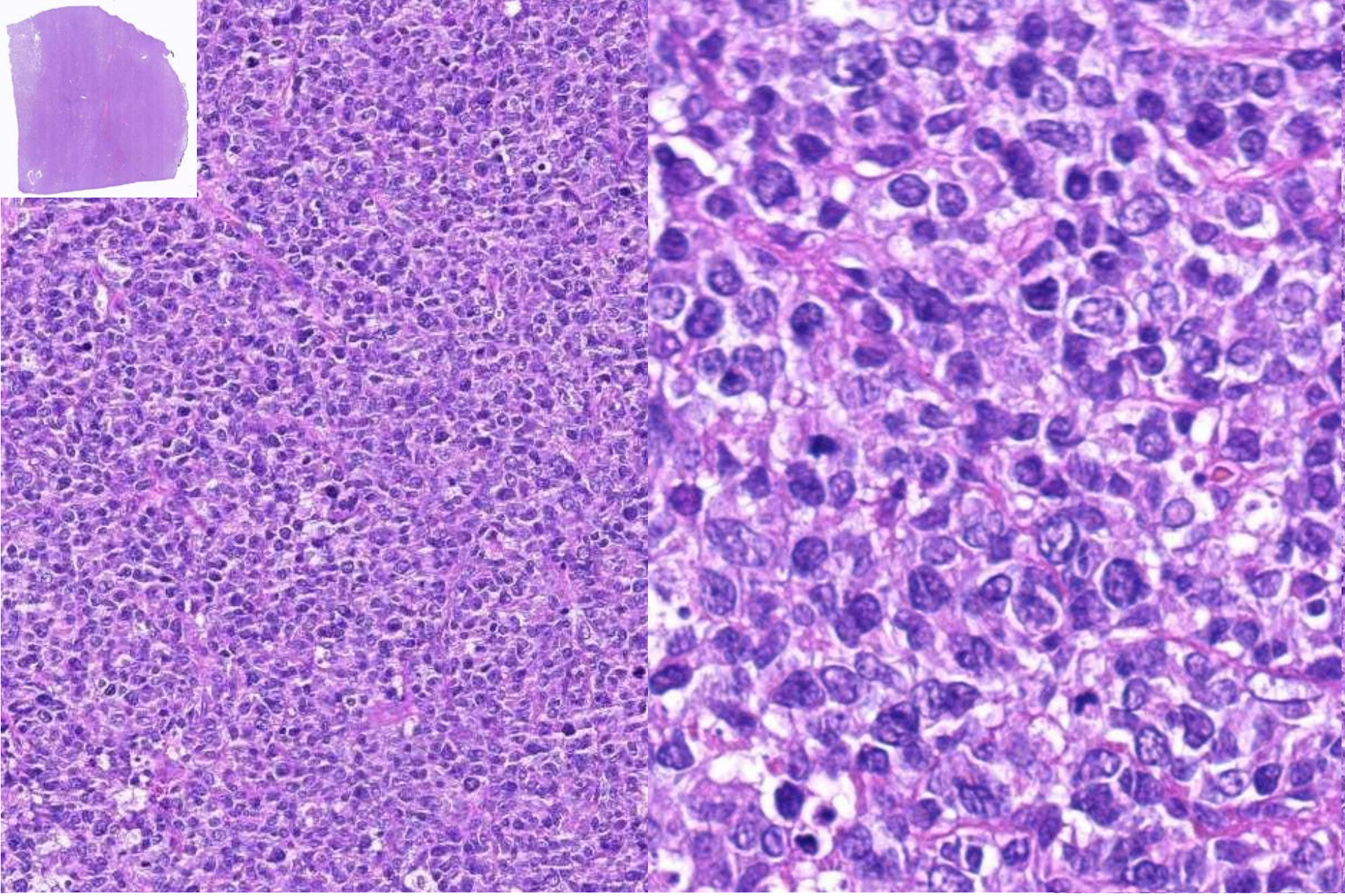



- Other findings: EBER negative
- Diagnosis: **B-cell lymphoma unclassifiable with features intermediate between DLBCL and HL, with *MYC*/BCL2 double hit**

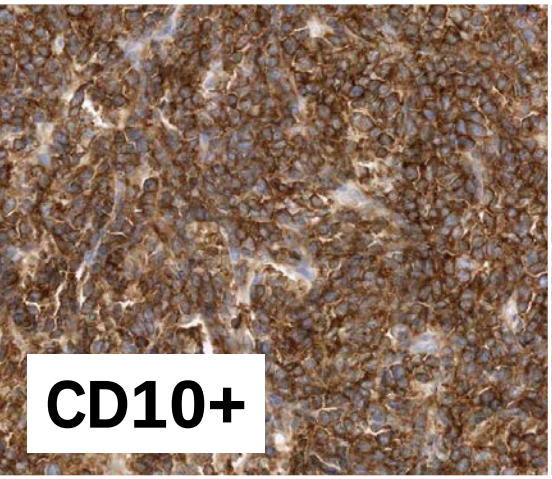
# « Double hit » lymphomas

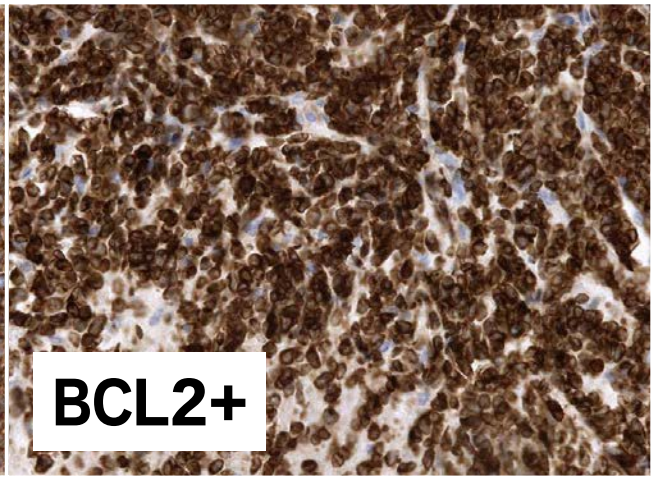
- **Concurrent rearrangement of MYC and another oncogene**
  - *MYC/BCL2*
  - *MYC/BCL6*
  - *MYC/BCL2/BCL6* (triple hit)
  - Others (*MYC/CCND1*, ..)
- Elderly patients
- **Morphologic spectrum**
  - B-cell lymphoma, unclassifiable, with features intemediate between BL and DLBCL
  - Diffuse large B-cell lymphoma
  - Progressed/transformed follicular lymphomas
  - Lymphoblastic morphology

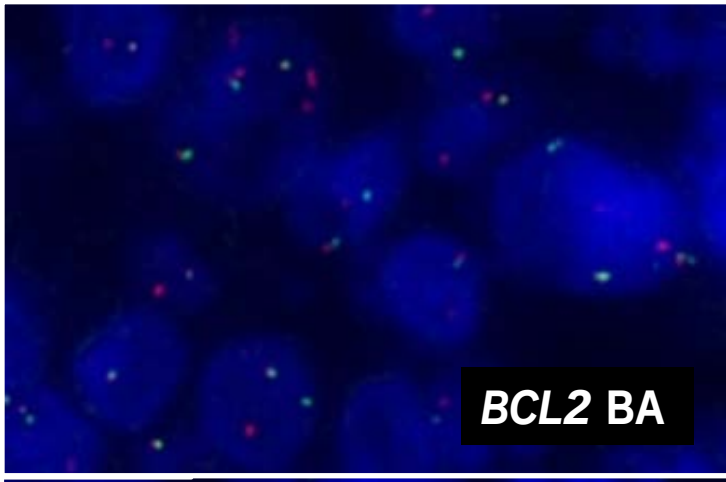
*Aukema et al. Blood 2011*

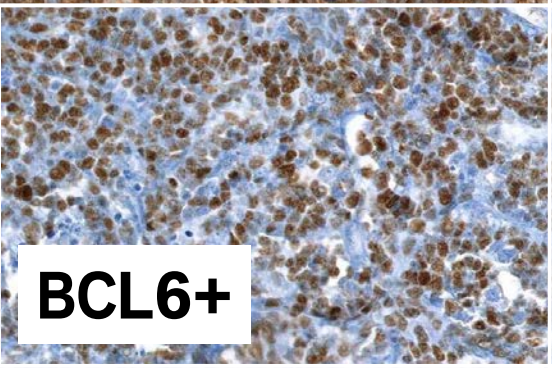


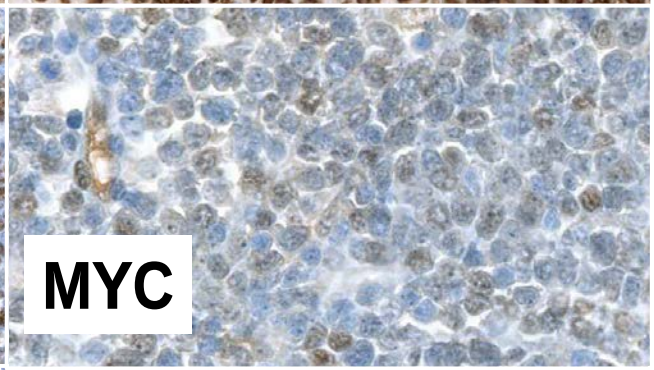
- F 68 yrs, clinically peritoneal carcinomatosis, bilateral ovarian masses, multiple mesenteric nodules

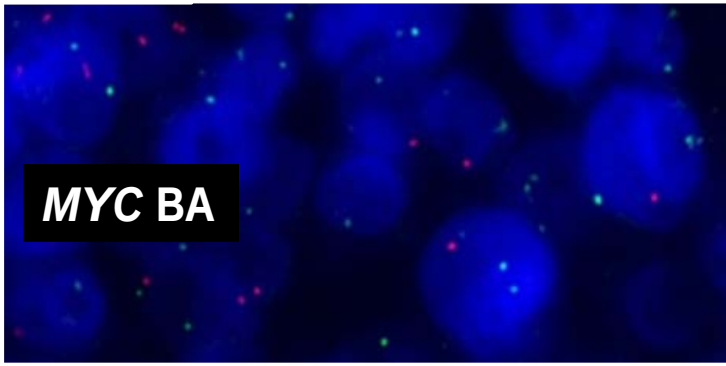


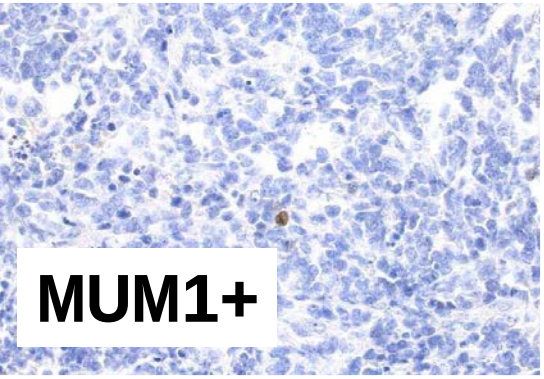



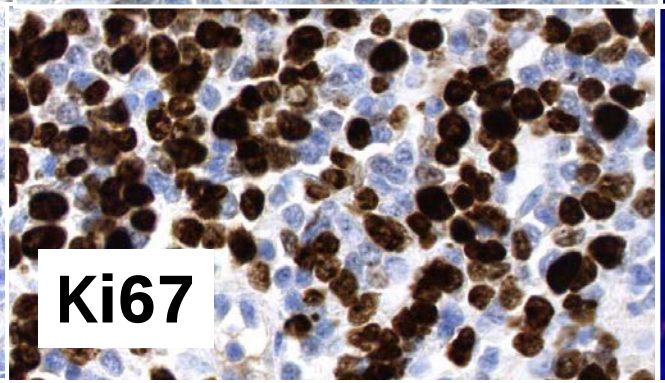



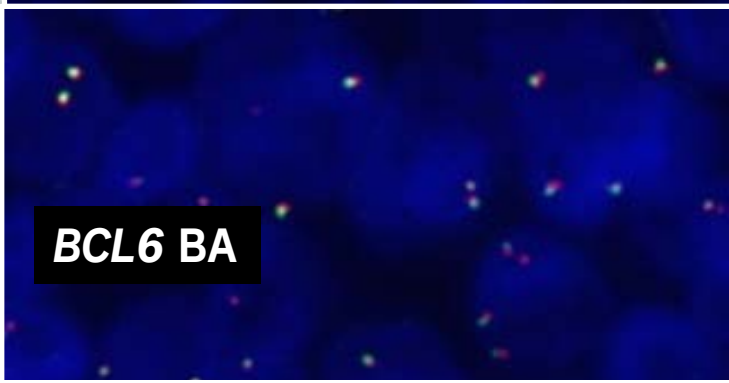



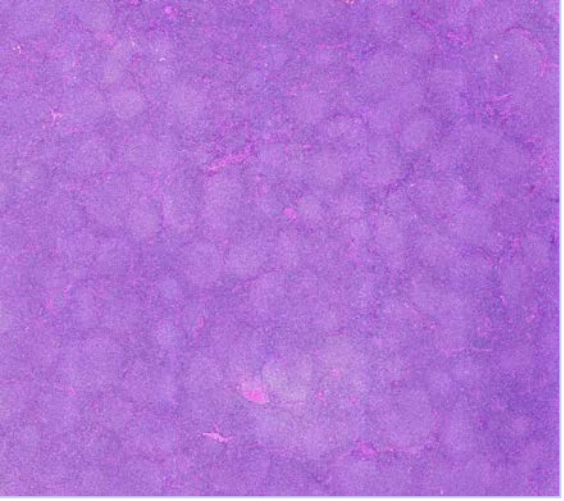
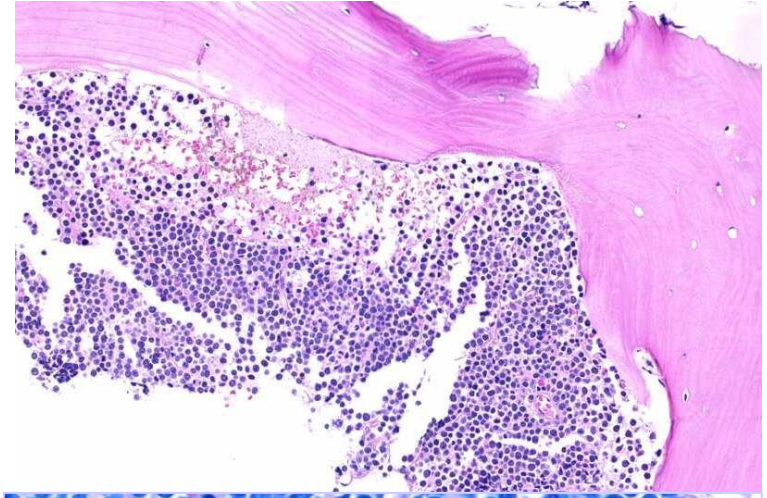
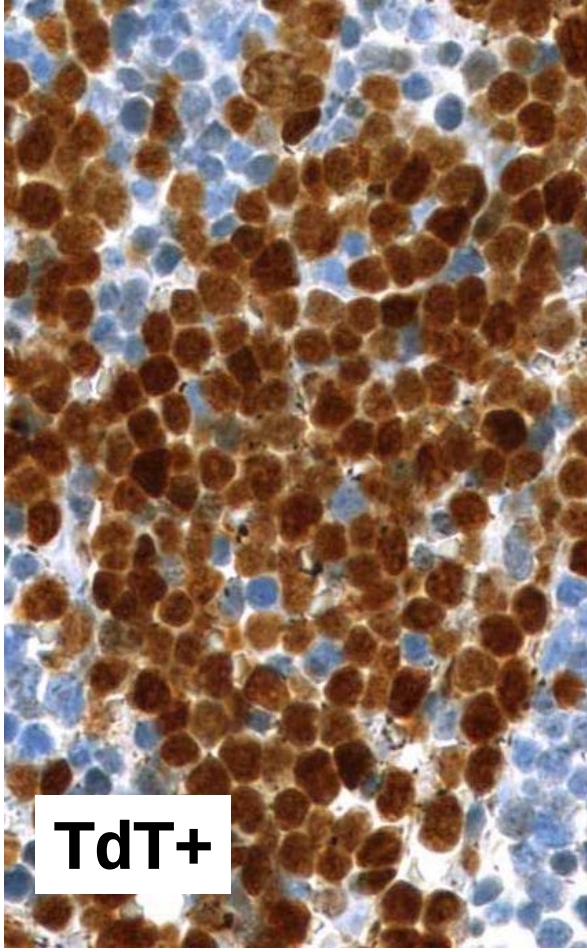



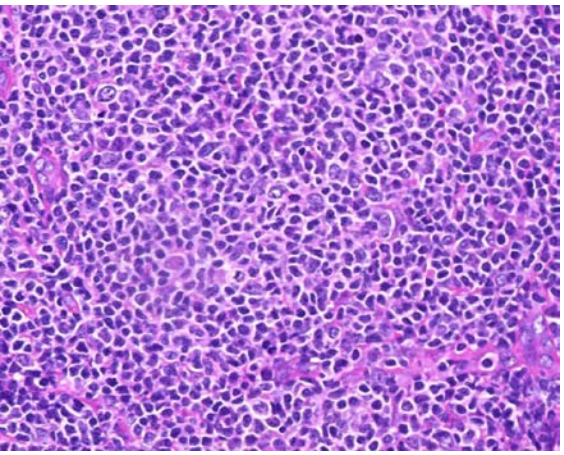
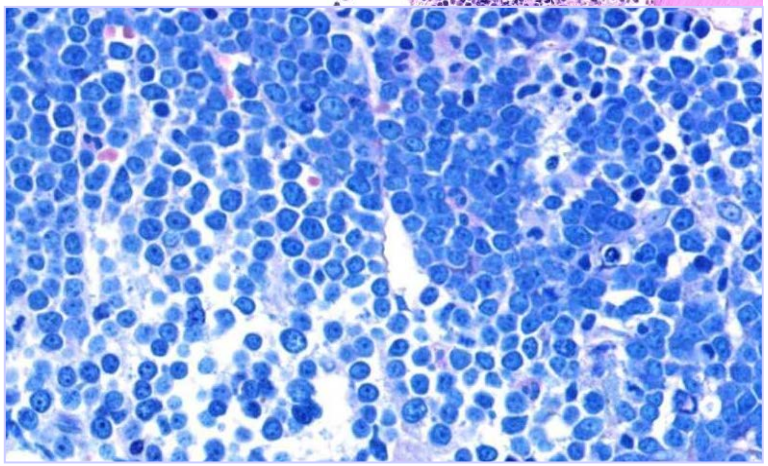




- Other findings: EBER negative
- Diagnosis: **DLBCL, GC-like, with *MYC*/BCL2 double hit**

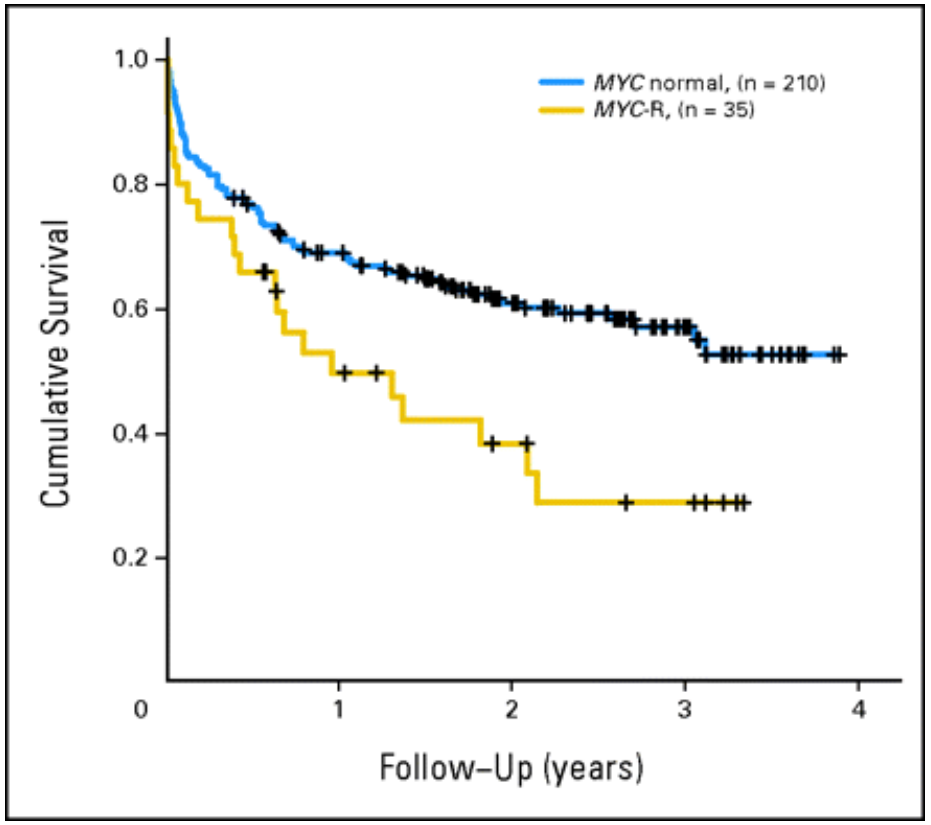




- 64 year-old man with pancytopenia and multiple adenopathies
- LN: grade I-II FL BCL2+ *BCL2* break
- BM: lymphoblastic (TdT+) B-cell neoplasm; FISH *MYC/BCL2* DH
- **Transformation of FL into lymphoblastic B-cell neoplasm with *MYC/BCL2* DH**

# *MYC* rearrangement

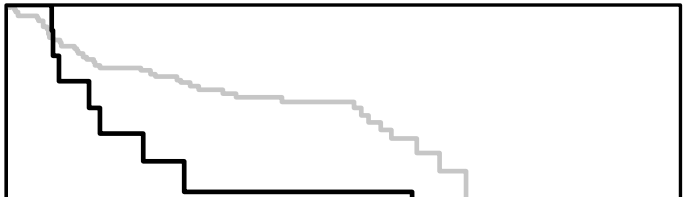
- **7-14% of de novo DLBCLs**
  - association with GCB immunophenotype
  - no correlation with proliferation
- Tend to be higher stage at presentation
- Predictive of aggressive disease, poor response to therapy
- **Independent predictor of reduced OS**
- Negative impact in relapsed/refractory patients (CORAL)

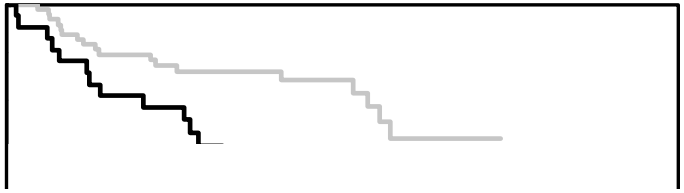



*Savage K. et al. Blood 2009; Barrans S et al., J Clin Oncol 2010; 28:3360-3365; Cuccuini et al. Blood 2012*

# MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma

Heike Horn,[1] Marita Ziepert,[2] Claudia Becher,[3] Thomas F. E. Barth,[4] Heinz-Wolfram Bernd,[5] Alfred C. Feller,[5] Wolfram Klapper,[6] Michael Hummel,[7] Harald Stein,[7] Martin-Leo Hansmann,[8] Christopher Schmelter,[9] Peter Möller,[4] Sergio Cogliatti,[10] Michael Pfreundschuh,[11] Norbert Schmitz,[12] Lorenz Trümper,[13] Reiner Siebert,[3] Markus Loeffler,[2] Andreas Rosenwald,[9] and German Ott,[1] for the German High-Grade Non-Hodgkin Lymphoma Study Group

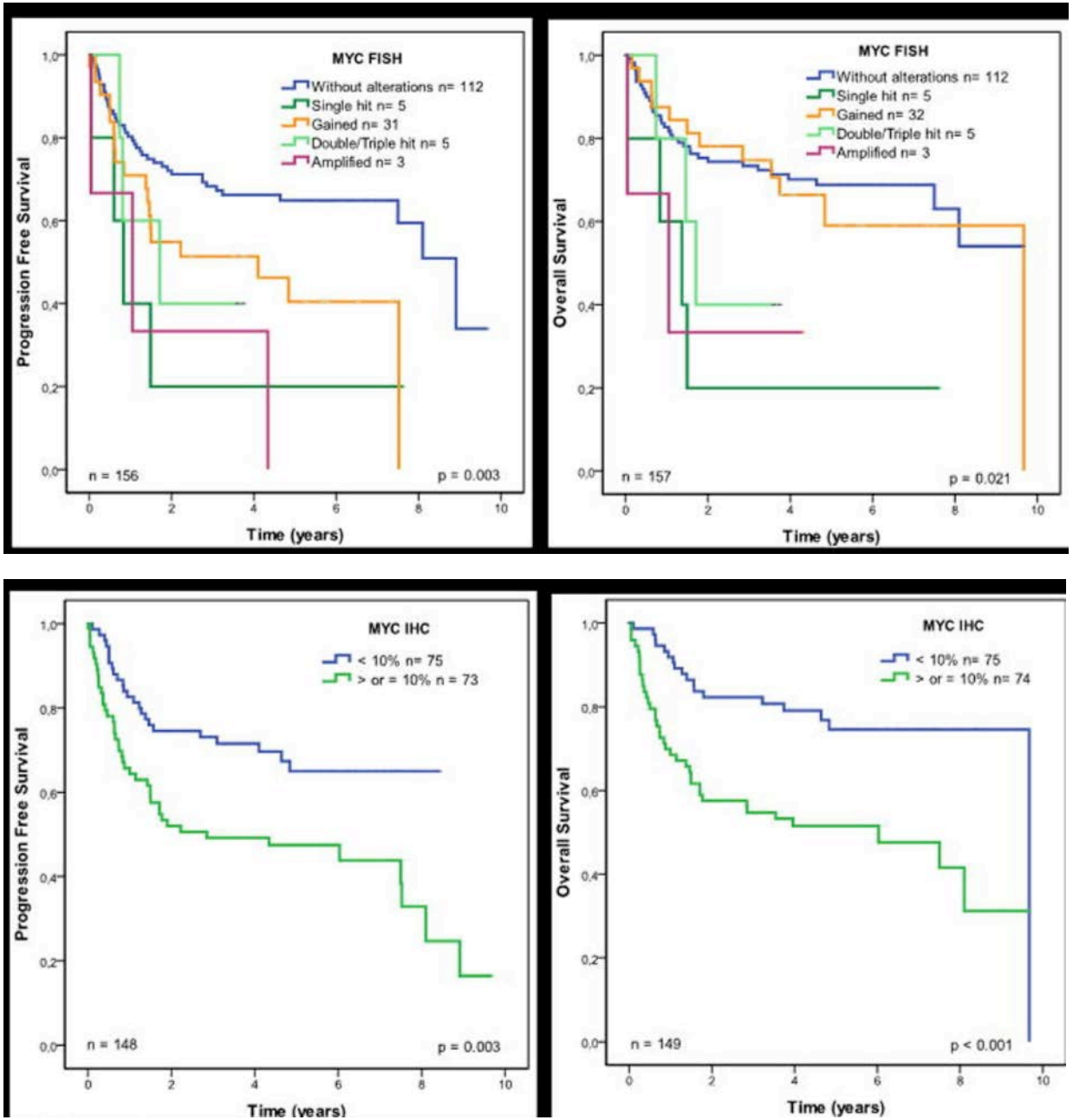




IHC
BCL2high
BCL6low
MYChigh
FISH
*MYC*

- ***MYC* rearrangement** (SH DH TH), MYC amplifications (>4 copies) but not MYC gains (3-4 copies) are associated with poor OS

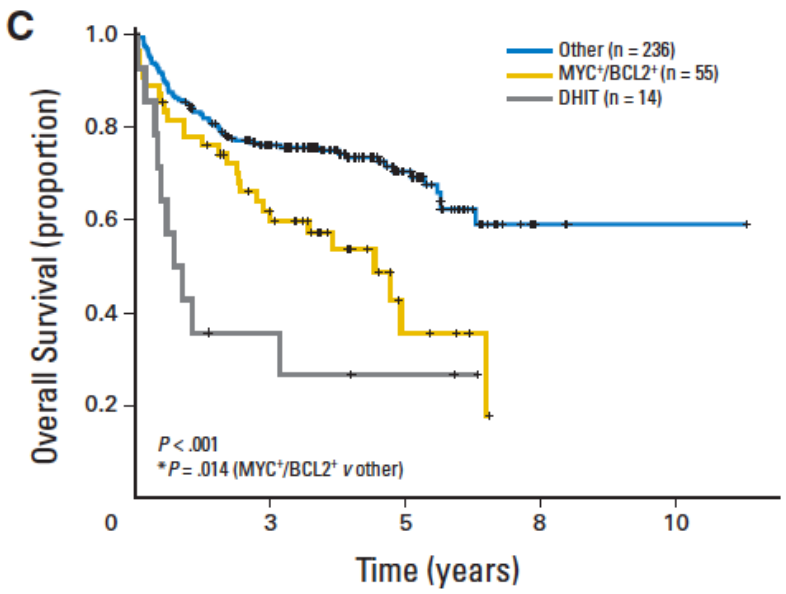
- **IHC (>10% MYC positive cells)** captures cases with MYC alterations and a subset of patients with no gene alteration and similar poor prognosis

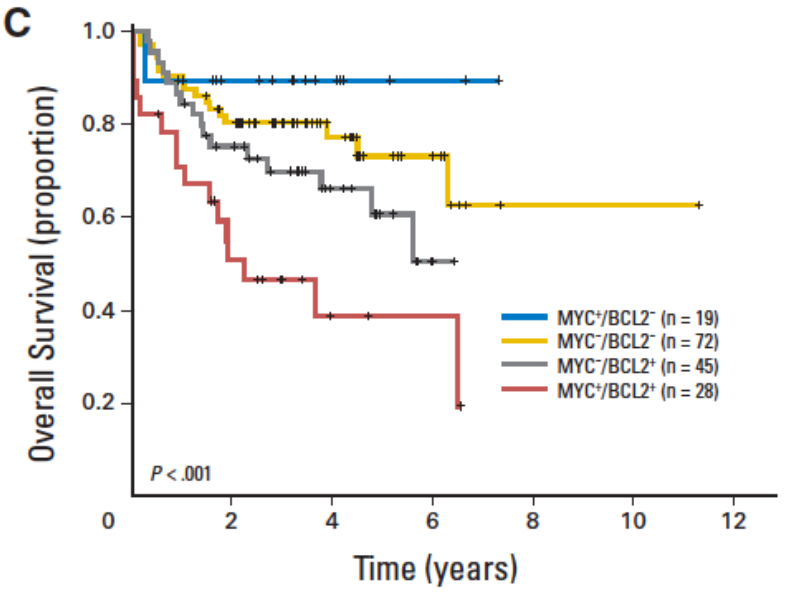



*Valera A. et al. Haematologica 20132012*

# DLBCL with concurrent *MYC* and *BCL2* deregulation

- 25-50% DLBCL with *MYC* rearrangement have concurrent *BCL2* rearrangement ("double hit") and these patients do very poorly
- Immunohistochemistry for MYC (cutoff 40%) and BCL2 (cutoff 70%) as a robust rapid and inexpensive approach to risk-stratify patients at diagnosis
- 20-30% of patients are MYC+ BCL2+ by IHC




*Johnson N et al., JCO 2012; Green TM et al., JCO 2012; Valera A et al. Haematologica 2013*

# *MYC* SH versus DH

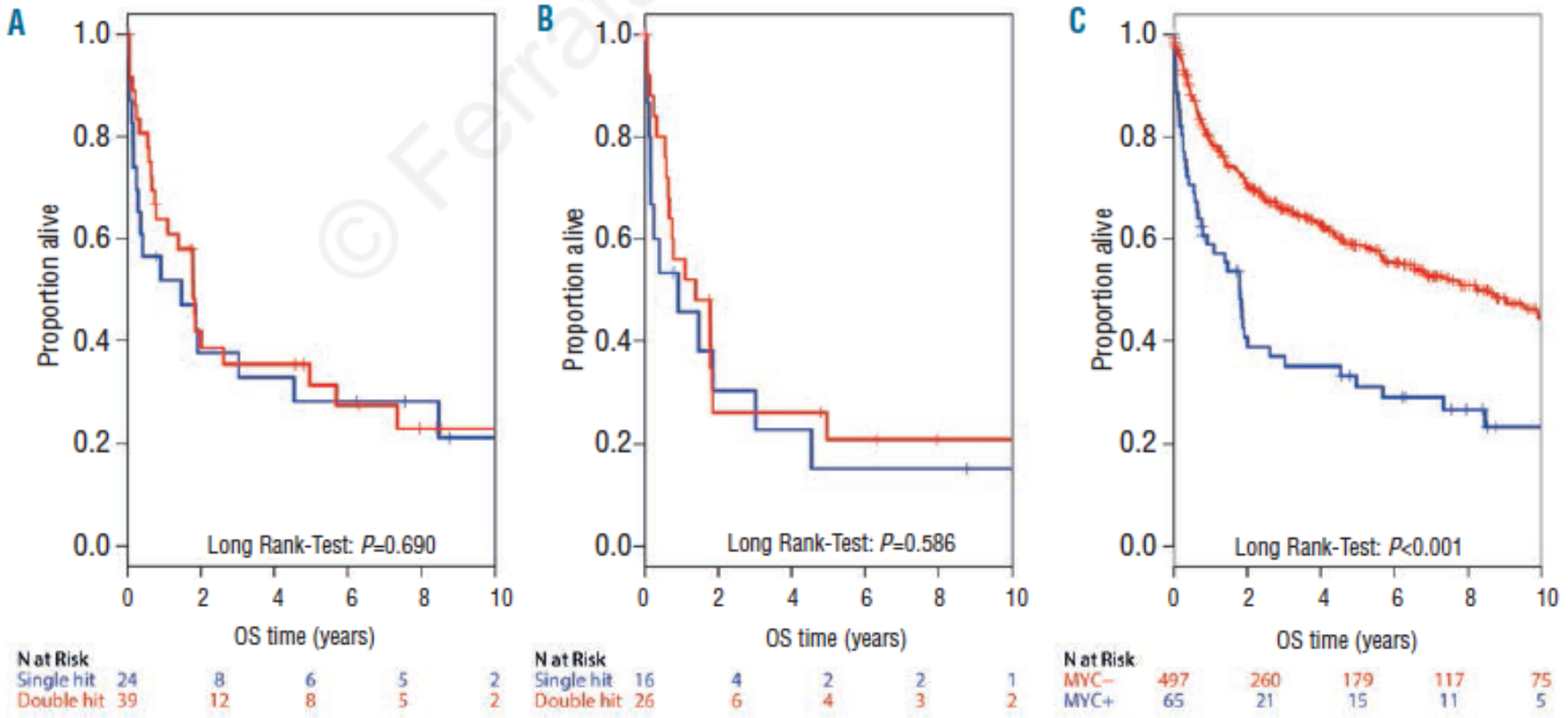



**Figure 2.** (A) Comparison of survival between DHL and SHL shows no significant differences between the two groups ($P$=0.690). The blue line represents SHL, the red line represents DHL. (B) Comparison of survival between DHL and SHL restricted to lymphomas with a morphological diagnosis of DLBCL (without any follicular lymphoma component). No difference was seen in survival ($P$=0.586). The blue line represents SHL, the red line represents DHL. (C) Overall survival of patients with *MYC*$^+$ and *MYC*$^-$ lymphomas with non-mBL or Intermediate gene-expression profile in the MMML cohort. Patients with *MYC*$^+$ lymphomas show markedly inferior survival compared to those with *MYC*$^-$ lymphomas ($P$<0.001). The blue line represents *MYC*$^+$ lymphomas, the red line represents *MYC*$^-$ lymphomas.

*Aukema et al. Haematologica 2014*

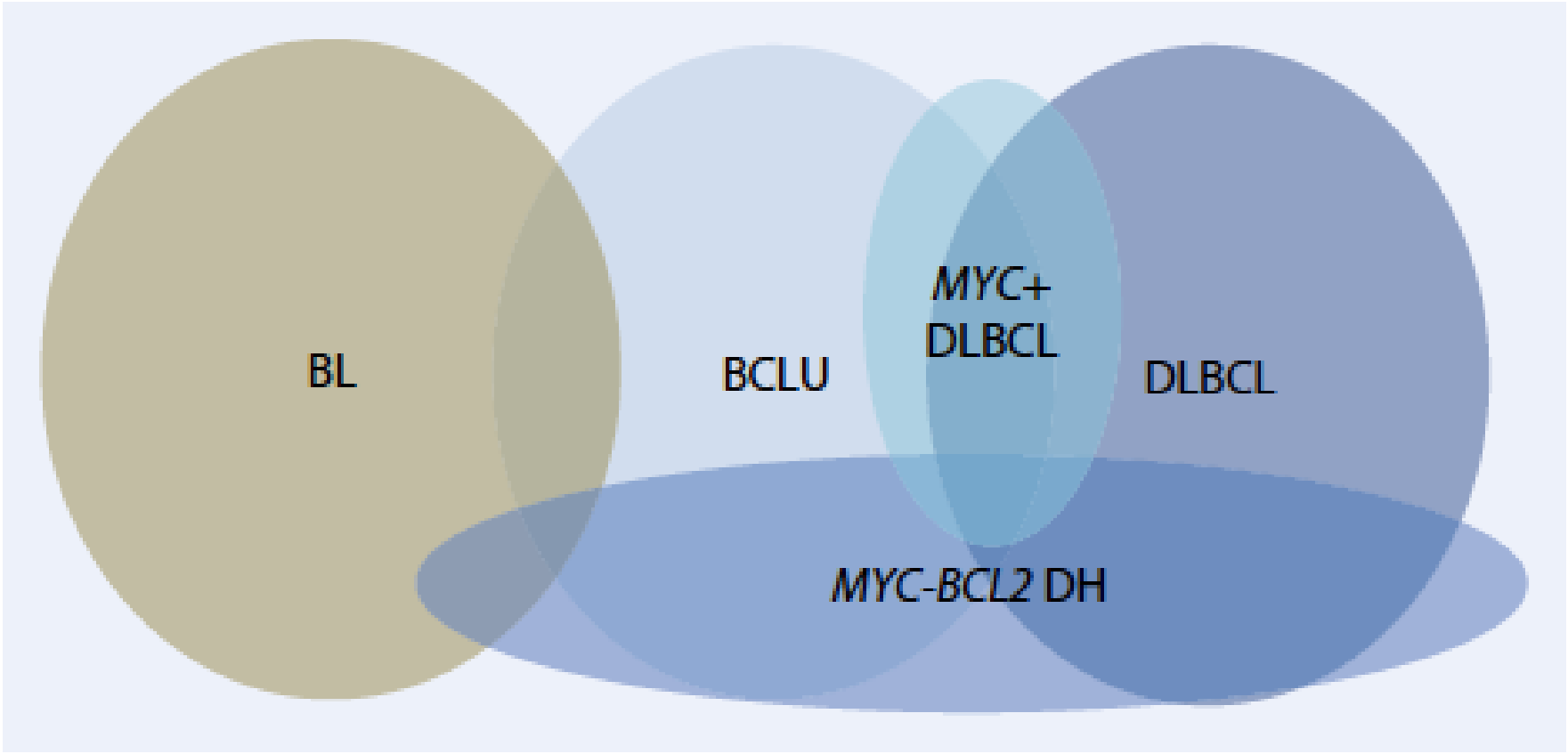



**Abb. 6** ▲ Graphische Darstellung der Überlappung von Burkitt-Lymphom (*BL*), B-Zell-Lymphom, unklassifiziert (*BCLU*) und diffusem großzelligem B-Zell-Lymphom (*DLBCL*). Mit unterschiedlicher Häufigkeit können in allen Subtypen Double-hit-Translokationen (*MYC-BCL2 DH*) auftreten

*Ott, Rosenwald, Pathologe 2013*

# Grey zones in aggressive B-cell neoplasms

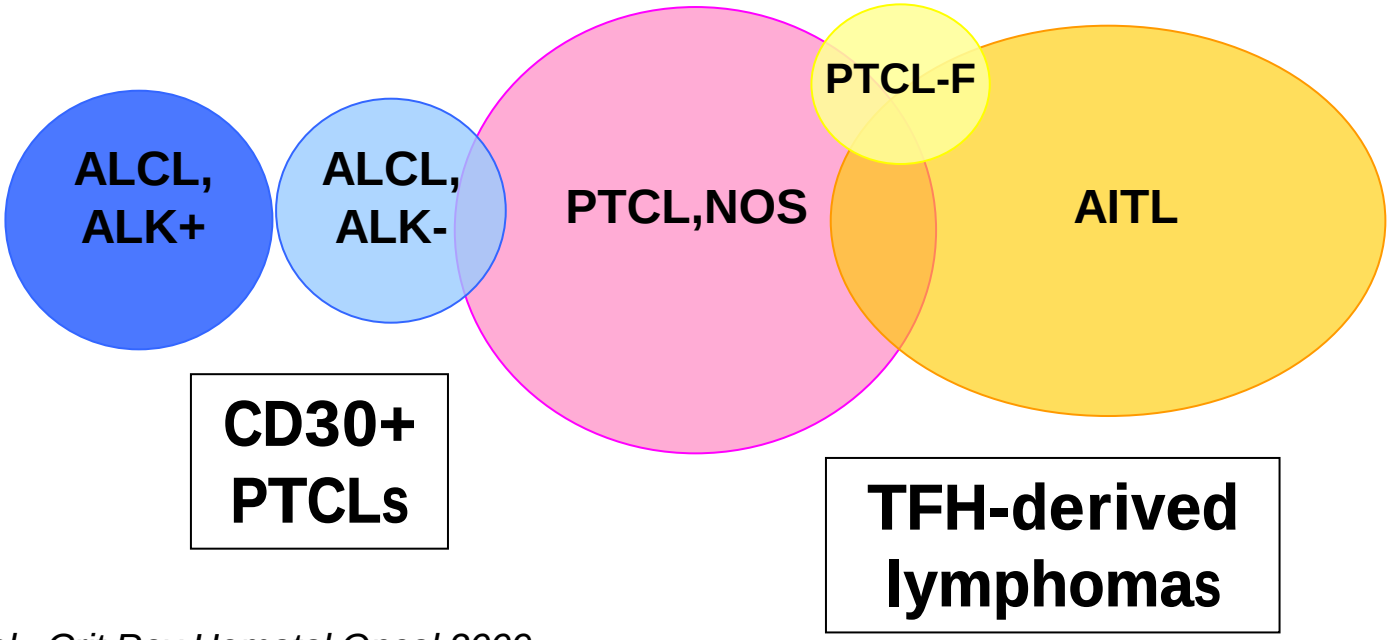
- More than the « intermediate » category!
- **Clinical correlations:**
  - Identify subgroups of patients who may benefit the most appropriate therapy
  - What is driving the biological aggressive behaviour of these tumors, which features are most relevant to subgroup definition
  - Importance of genetic alterations and genetic complexity
- **Should there be subgroups/entities defined on genetic/molecular, and if so…**
  - *MYC* rearrangement, *MYC* translocation partner
  - *MYC* alone or DH
  - Immunohistochemistry versus FISH
- **Which patients should be tested and how?**

# Other grey zones…

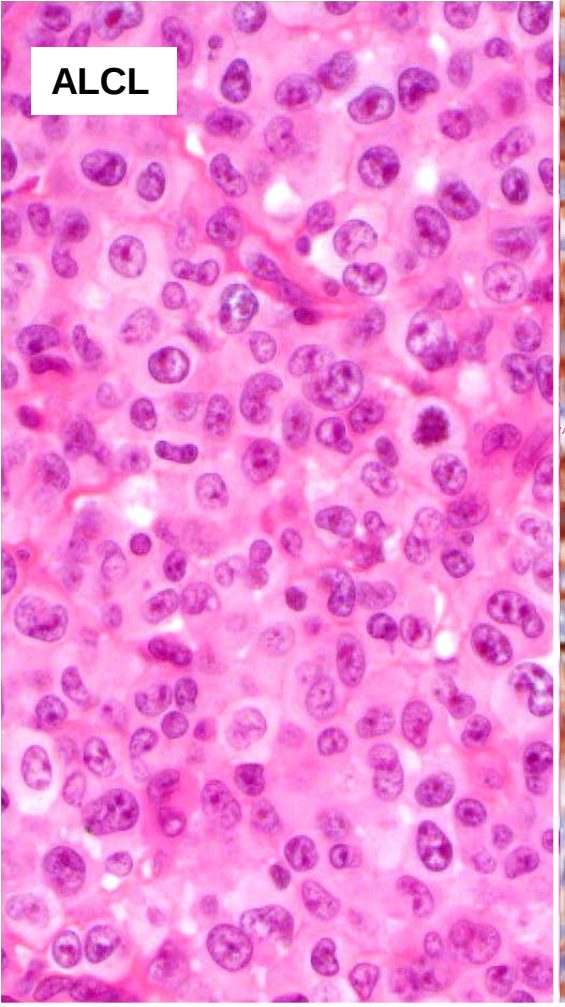
- EBV-positive lymphomas
  - Hodgkin versus non-Hodgkin
  - Different DLBCL entities
- Atypical lymphoproliferations, benign versus malignant lymphoproliferations
- Early lymphoid neoplasias
- Follicular versus marginal zone lymphomas
- Biological continuum

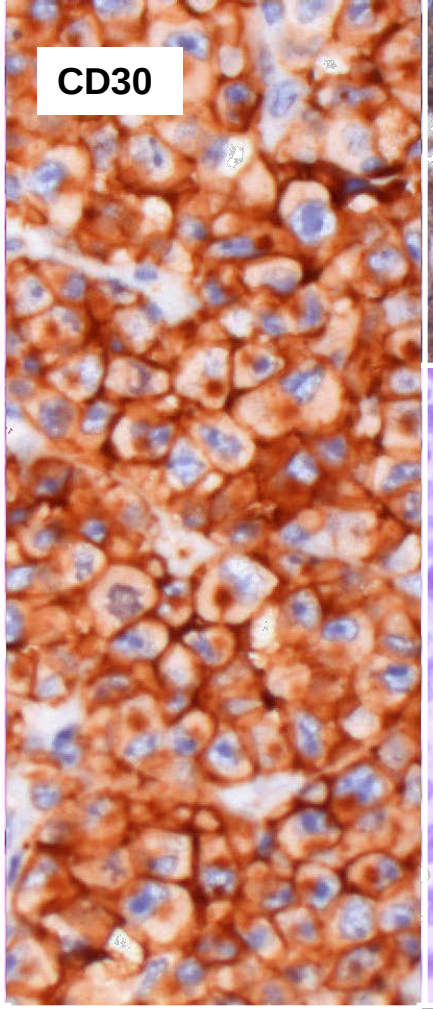
# Grey zones in T-cell lymphomas

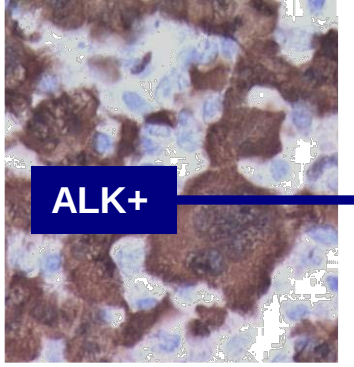
- Rare diseases, heterogeneous
- Cell of origin and mechanisms of transformation poorly understood; entities grouped according to clinical presentation
- **Several provisional entities in the current WHO classification**
- **Peripheral T-cell lymphoma, not otherwise specified**

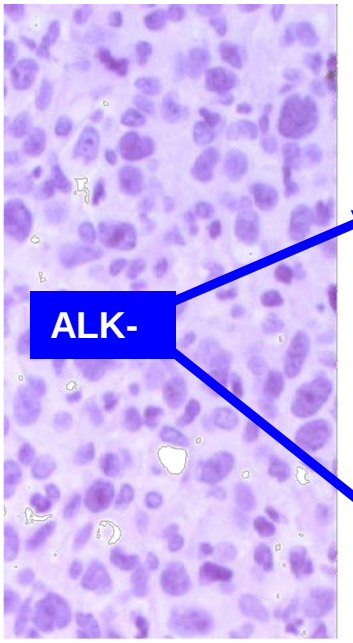


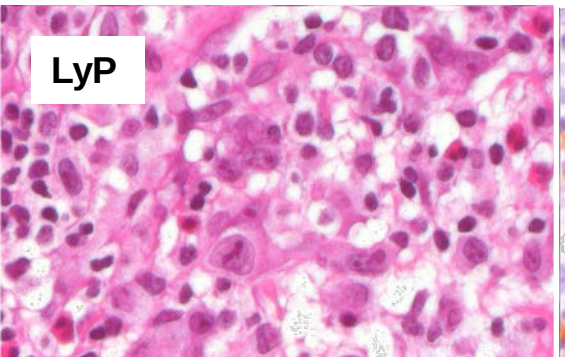

*de Leval L et al., Crit Rev Hematol Oncol 2009*

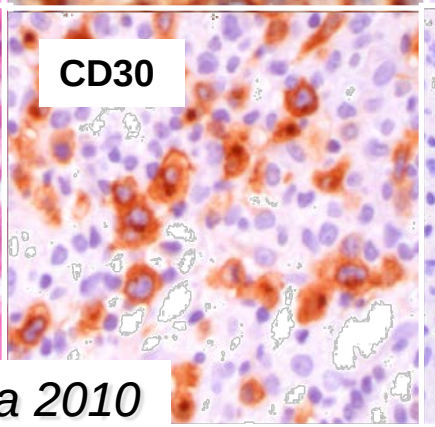


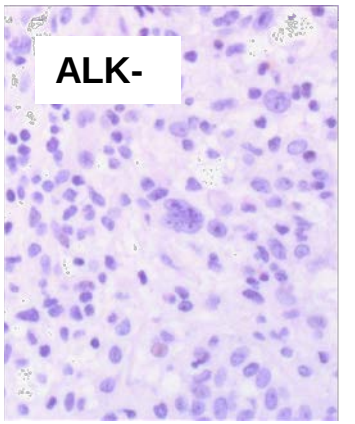



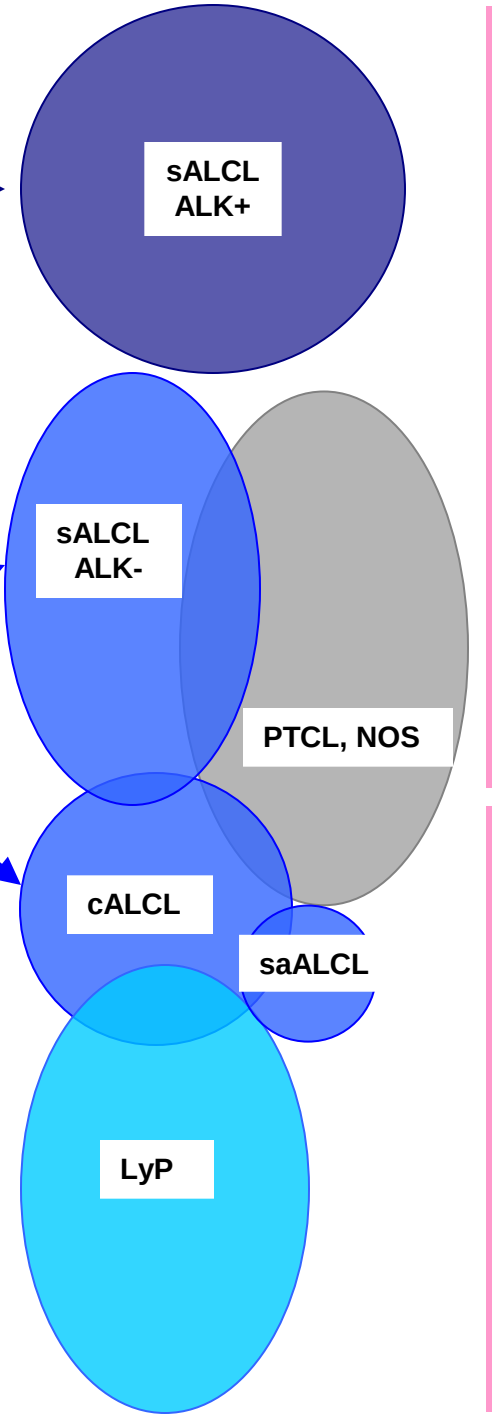



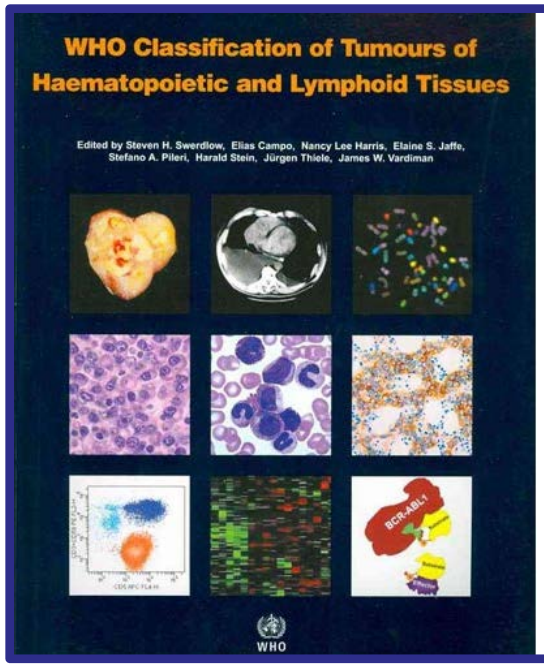




SYSTEMIC

CUTANEOUS

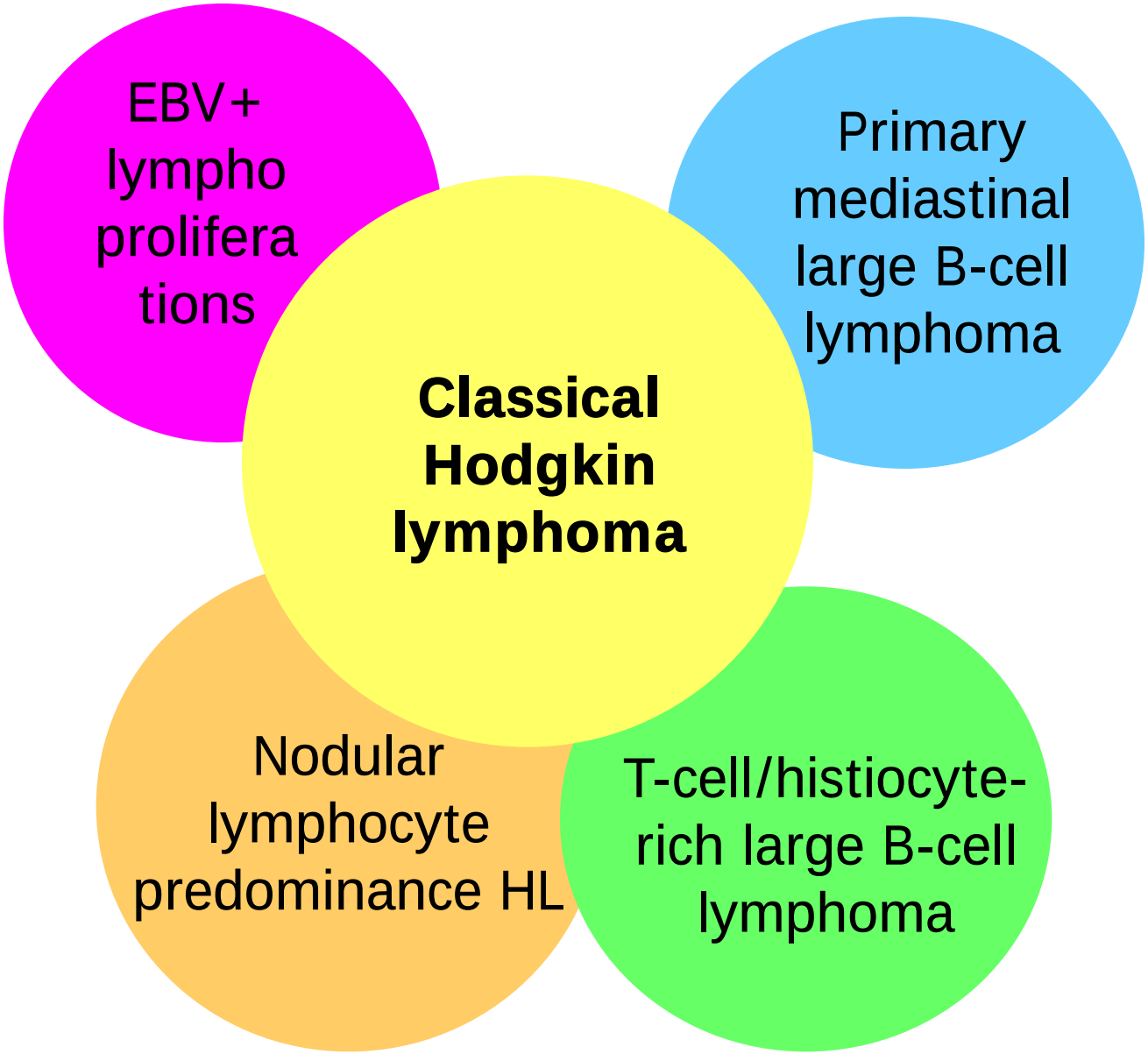
*de Leval L Haematologica 2010*

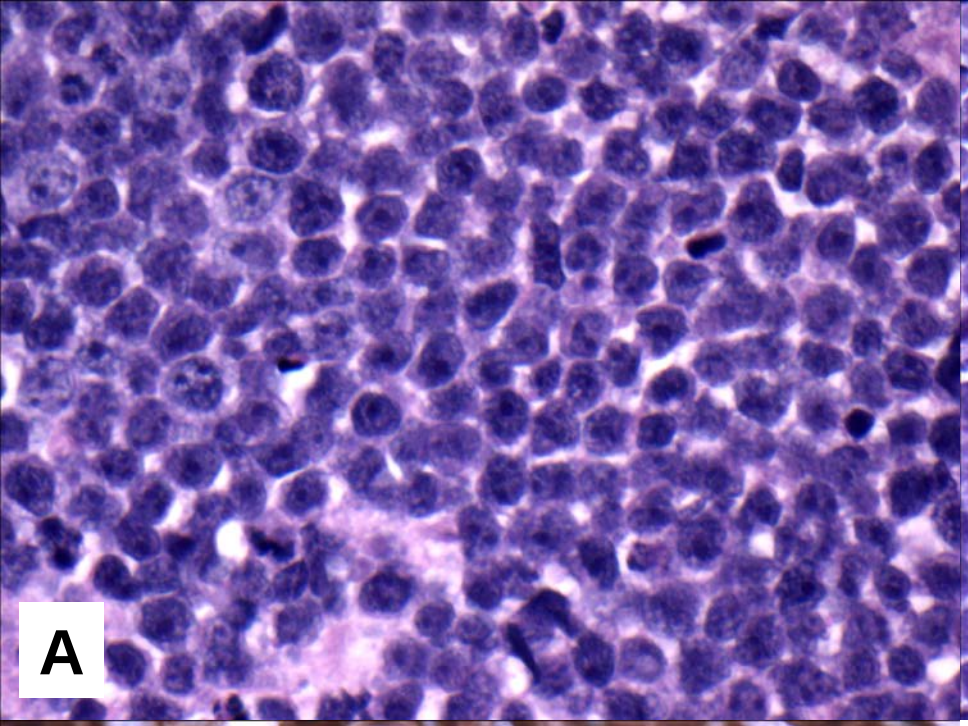
- Grey zone lymphomas: an evolving concept
- Better knowledge of the biology leading to better definition of disease entities
- Recognition of novel clinico-pathological entities
- Intermediate and provisional categories: some may represent real disease entities, others need to be sorted out




- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma

**5th Edition**


EBV+ lympho prolifera tions
Primary mediastinal large B-cell lymphoma
Classical Hodgkin lymphoma
Nodular lymphocyte predominance HL
T-cell/histiocyte-rich large B-cell lymphoma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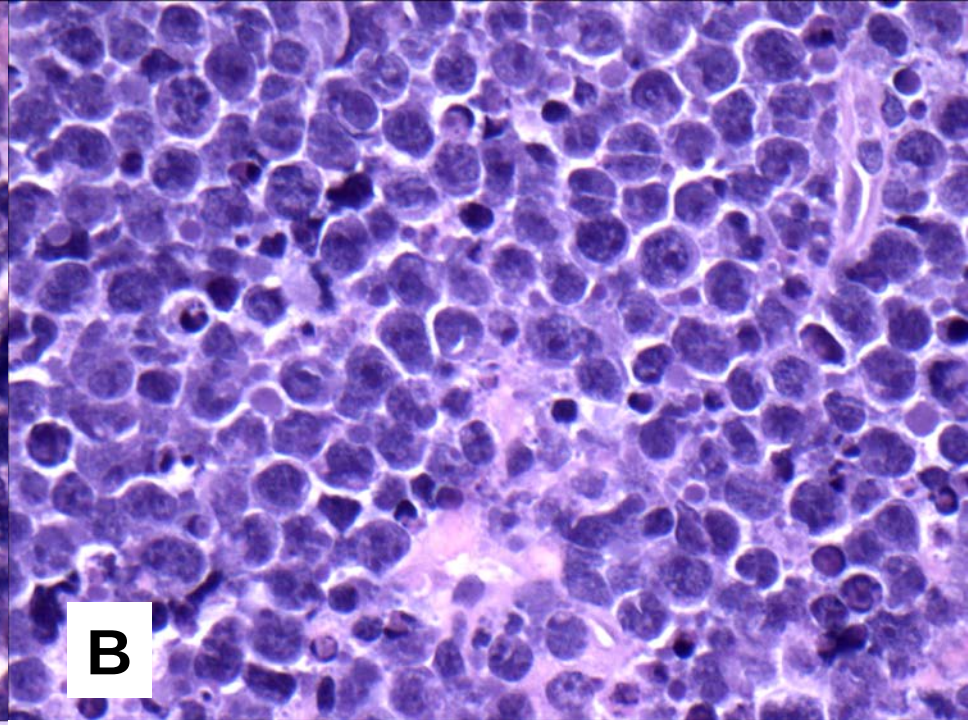
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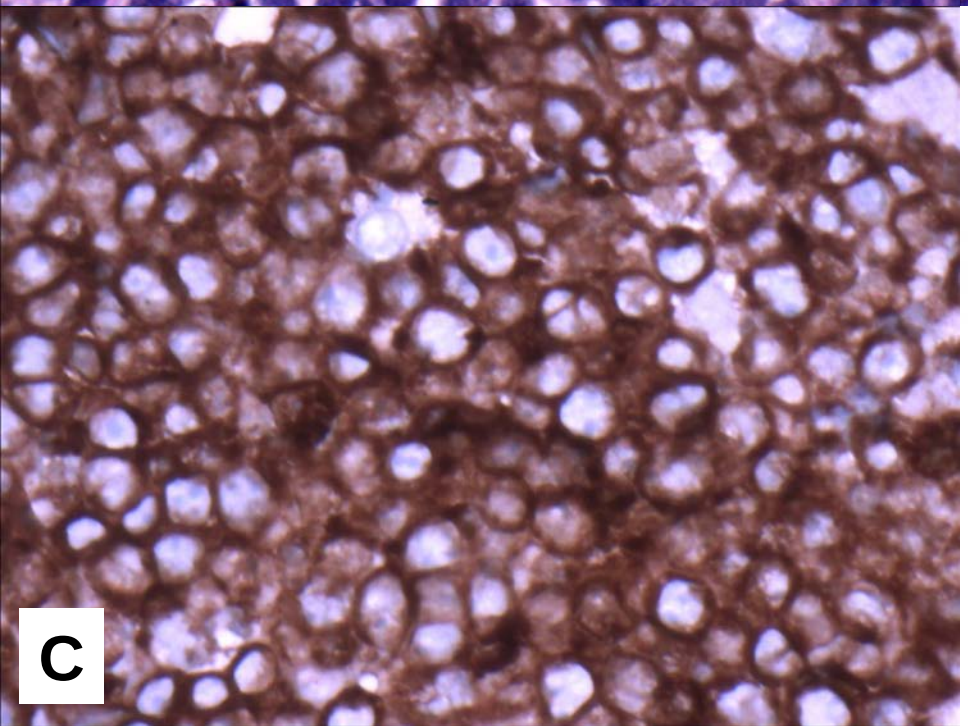
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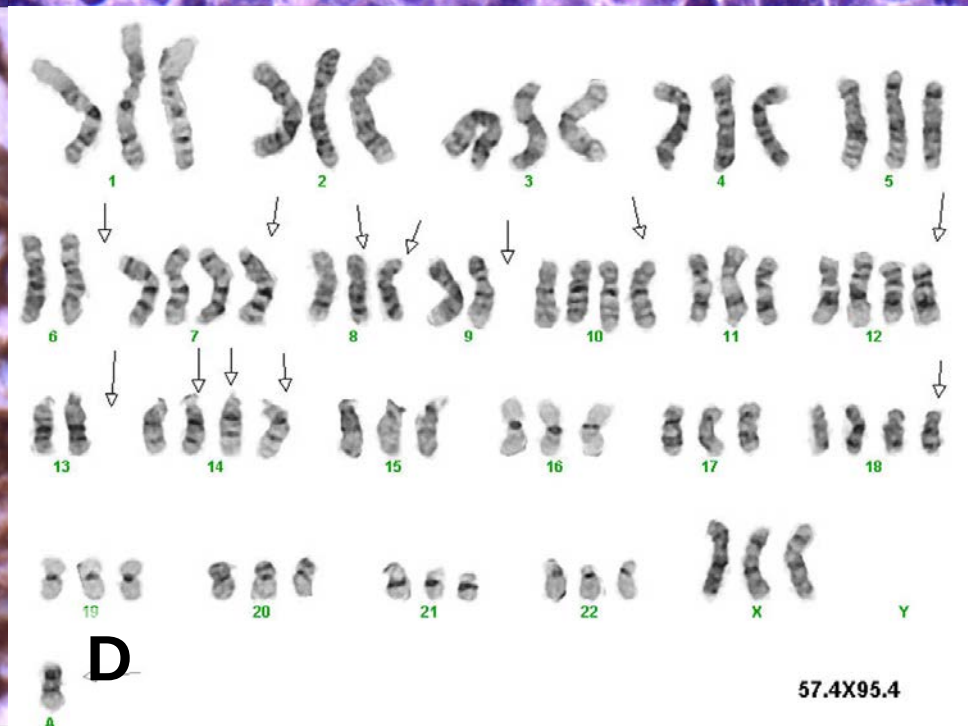
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# 6. Lymphomes de Hodgkin

# DLBCLs (WHO 2008)

- **DLBCL, NOS ****
- **DLBCL subtypes**
  - T-cell/histiocyte-rich LBCL
  - Primary DLBCL of the CNS
  - Primary cutaneous DLBCL, leg type
  - EBV+ DLBCL of the elderly
- **DLBCL entities**
  - Primary mediastinal LBCL
  - Intravascular LBCL
  - DLBCL arising in chronic inflammation
  - Lymphomatoid granulomatosis
  - ALK+ DLBCL
  - Plasmablastic lymphoma (PBL)**
  - LBCL arising in HHV8-associated MCD
  - Primary effusion lymphoma
- **B-cell lymphoma, unclassifiable, intermediate between DLBCL and Burkitt, or DLBCL and cHL**

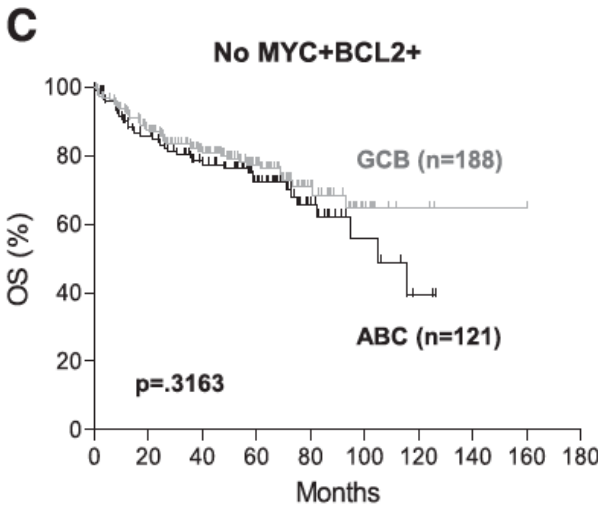
**DLBCLs in specific anatomic sites**

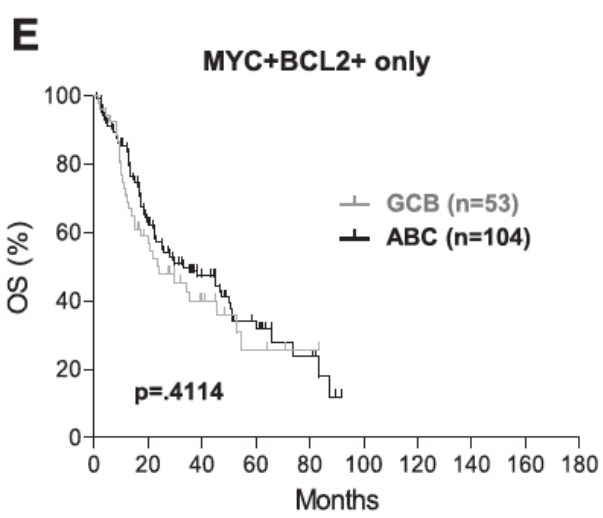
**EBV+ DLBCLs**

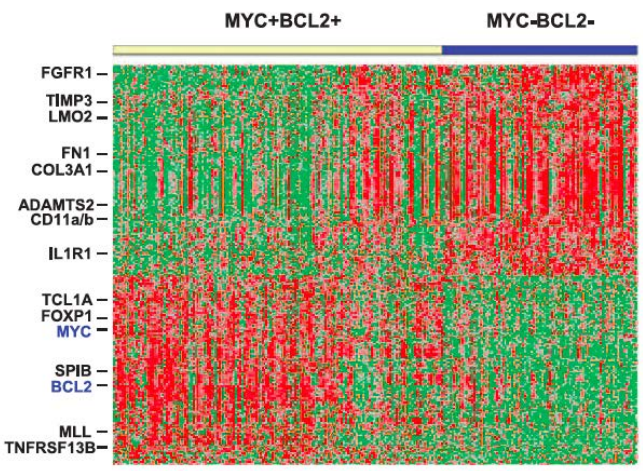
**DLBCLs with plasmablastic features**

# Is MYC/BCL2 coexpression better than COO to predict prognosis?

- 34% DLBCL coexpress MYC and BCL2, more frequently ABC (>50%) than in GCB (<25%)
- Patients with or without MYC/BCL2 coexpression have similar prognoses irrespective of the GCB/ABC




- GEP: DLBCL with MYC/BCL2 coexpression: downregulation of ECM genes and increased proliferation signature

*Hu S et al., Blood 2013*

blood 1994 84: 1361-1392

**A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group [see comments]**

NL Harris, ES Jaffe, H Stein, PM Banks, JK Chan, ML Cleary, G Delsol, C De Wolf- Peeters, B Falini and KC Gatter



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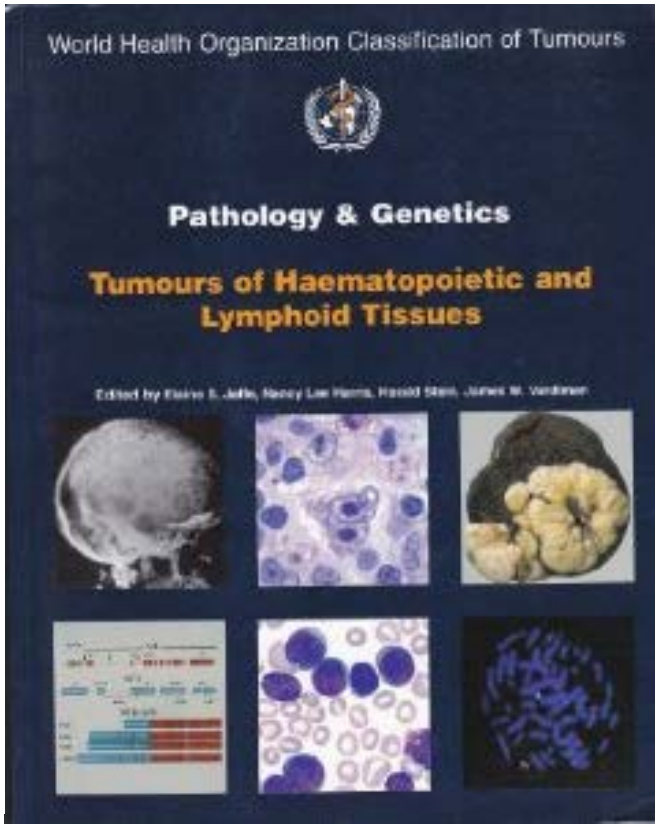
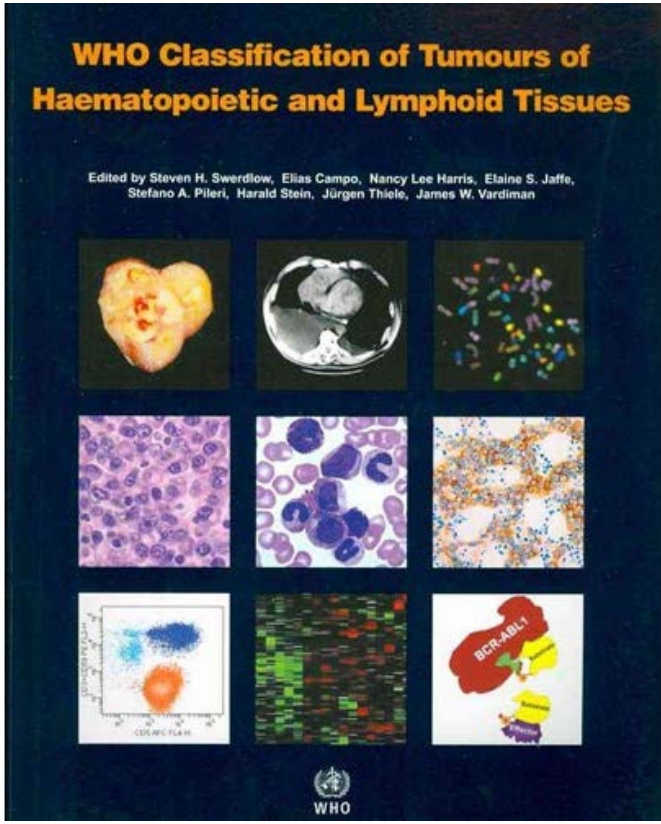


# PMBL – pathological features

- Morphology
  - Often “clear” cytoplasm, sclerosis
  - Mixture of centroblasts, immunoblasts, multilobated, RS-like
- Immunophenotype
  - B-cell antigens: Ig-, CD20+ Pax5+ Oct.2+ Bob.1+
  - Stage: CD10-/+ Bcl6+/- CD23+/- Mum1+ Bcl2+ CD30+ CD15-
  - Other: MAL+ CD54+ TRAF+ nuclear cREL
- Genetics
  - Ig genes: rearranged, somatic hypermutation
  - Genetic abnormalities:
    - Rearrangements of *BCL2 BCL6* rare
    - Gains at 2p (*cREL*, *BCL11*), 9p (*JAK2*)
    - Translocations involving MHC class 2 transactivator gene (*CIITA)* (38%)*
      - 15% of CHL
      - Fusion transcripts involving PDL1 and 2 (T-cell inhibition)
      - Decreased HLA-DR, increased PDL1/2 -> immune escape?
  - Gene expression: activation of NF kappa B pathway
    - Unlike DLBCL of other sites
    - Like classical Hodgkin’s lymphoma (CHL)

*Steidl et al, Nature: epub 3/2/11

**Table 1. Recurrent gene alterations involved in the pathogenesis of primary mediastinal B-cell lymphoma**

| Gene | Pathway/function | Frequency of alteration in PMBCL, % |
|---|---|---|
| **Copy number gain** | | |
| *REL* | NF-κB pathway | 75 (45) |
| *PDL1/PDL2* | Induction of T-cell exhaustion/apoptosis | 63 (10) |
| *JAK2* | IL/JAK-STAT pathway/histone modification | 63 (10) |
| *JMJD2C* | Histone modification | 63 (61) |
| **Chromosomal translocation/rearrangement** | | |
| *CIITA* | Transcriptional regulation of HLA class II/antigen presentation | 38 (11) |
| **Coding sequence mutation** | | |
| *SOCS1* | IL/JAK-STAT pathway | 45 (64) |
| *STAT6* | IL/JAK-STAT pathway | 36 (68) |
| *TNFAIP3* | NF-κB pathway | 36 (4) |
| *MYC* | Transcriptional regulation/chromatin remodeling | 25 (42) |
| *TP53* | p53 pathway | 13 (42) |
| **Promoter hypermethylation** | | |
| *p16/INK* | Cell-cycle progression, p53 pathway | 9 (42) |

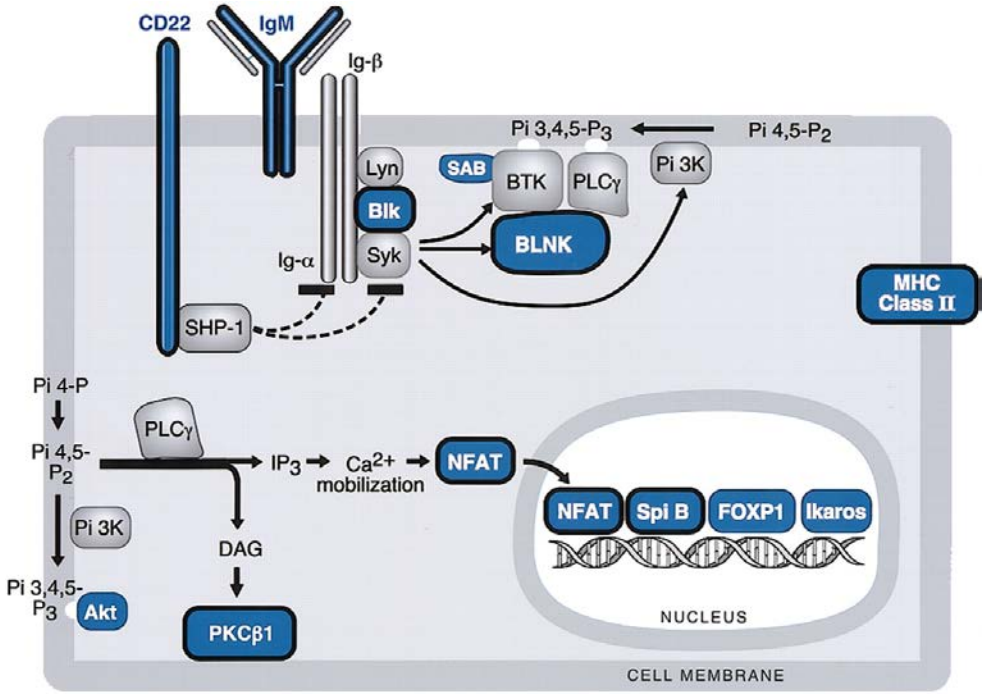
# Similar molecular pathways are involved in MLBCL and cHL-NS

- **Activation of the cytokine-JAK-STAT pathway**
  - Constitutive JAK2 activation
  - STAT1 overexpression
  - Constitutive STAT6 activation
- **TNF family members**
  - CD30, CD40, OX40L, HVEM, CD95
  - TRAF1 overexpression by IHC
- **Constitutive NF-kappa B activation**
  - Essential for survival
  - cREL nuclear localization
- **Aberrant activation of tyrosine kinases and activation of the PI3K/ATK pathway**
  - Renne C Leukemia 2007: 21:780

# The molecular signature of MLBCL shares features with cHL

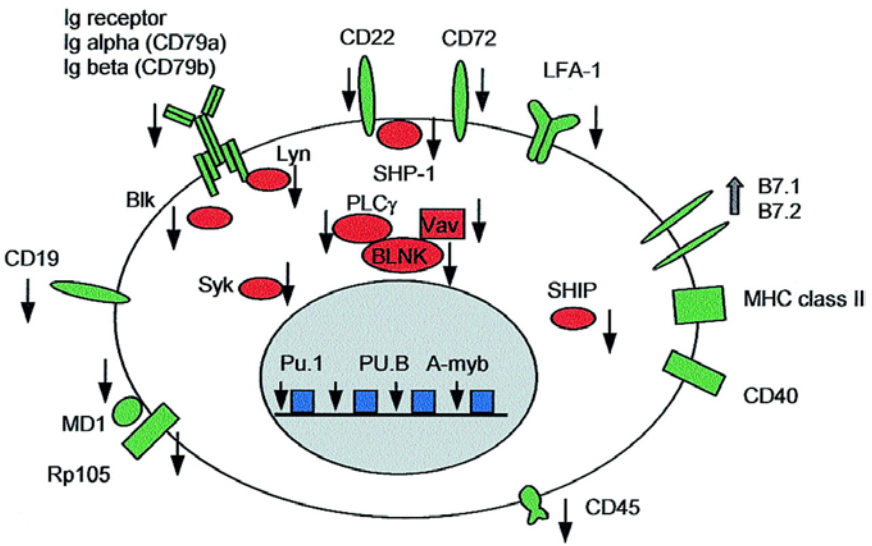
Savage K et al.
Blood 2003: 102, 3871

## MLBCL




**Downregulation of genes of the BCR signalling pathway**

## HRS cell of cHL

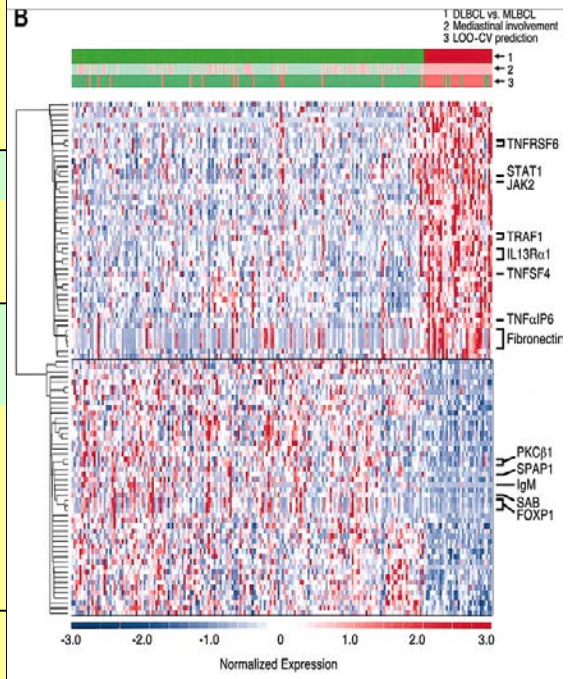



Schwering, I. Blood 2003: 101,1505

**Loss of the B-lineage-specific gene expression program**

# The genetics and molecular signature of MLBCL differ from that of other DLBCL and resmble that of HL

| | DLBCL | PMBL |
|---|---|---|
| CLINICAL FEATURES | Adults, M>F<br>Stade I-II: 50% | Young women<br>Stade I-II >50%<br>Bulky |
| MORPHOLOGY | Sheets of large cells | |
| | Variable | Clear cells<br>Sclerosis |
| IMMUNO-PHENOTYPE | CD45+ CD20+ CD79a+<br>B-cell transcription factors: Pax5 Bob2 Oct1 | |
| | sIg+ | sIg-<br>CD30 +/-<br>CD23+<br>MAL+ |
| GENETICS | R BCL6: 30%<br>R BCL2: 20% | gains at 2p: cREL, BCL11<br>gains at 9p: JAK |
| MOLECULAR SIGNATURE | DISTINCT | BCR signalling<br>NF-kappa B<br>Extracellular matrix<br>Cytokines/JAK/STAT |




Savage K et al. Blood 2003

# SOME CASES REMAIN DIFFICULT TO CLASSIFY

**Composite** and **metachronous** lymphomas in the same patient (clonal identity proven in some cases)

**Mediastinal gray zone lymphomas** with features transitional between NSHL and PMLBCL

For clinical management, should be treated as aggressive B-cell lymphomas

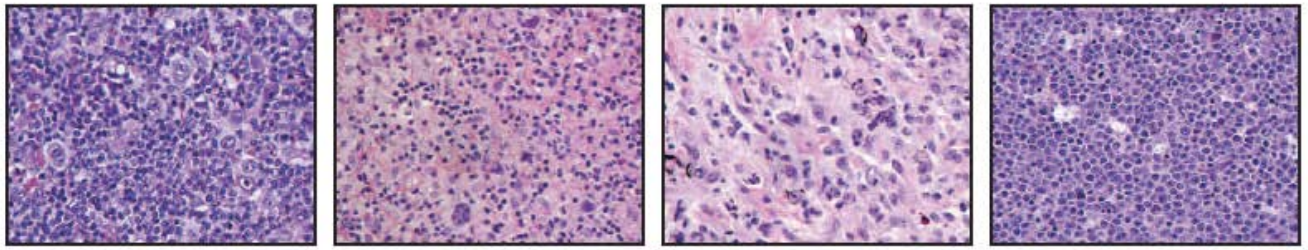
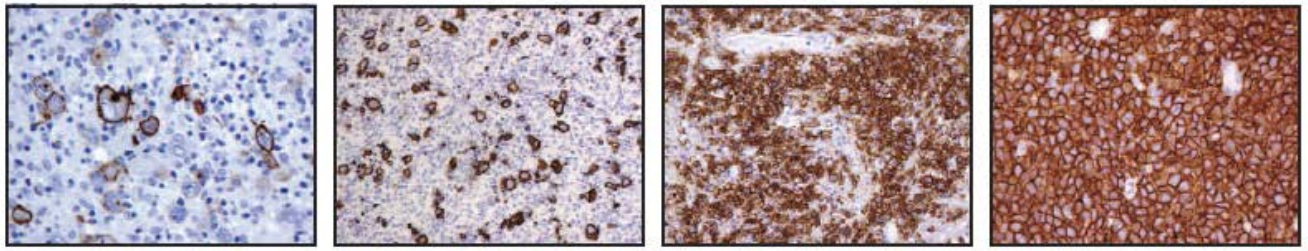
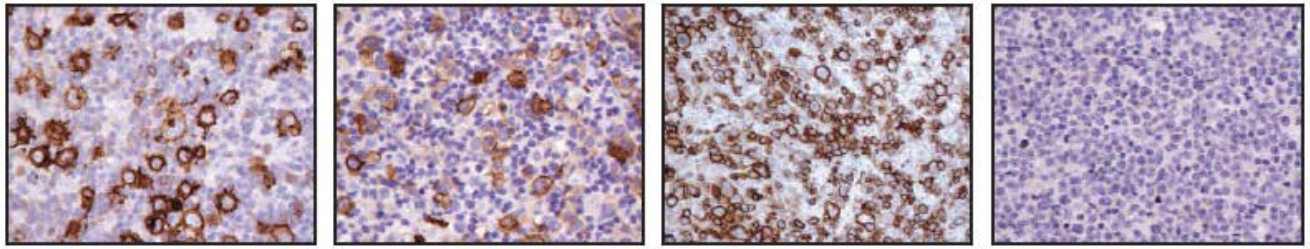
## Mediastinal Gray Zone Lymphoma

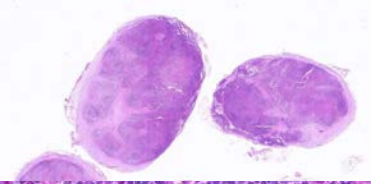
*The Missing Link Between Classic Hodgkin's Lymphoma and Mediastinal Large B-Cell Lymphoma*

*Alexandra Traverse-Glehen, MD,* Stefania Pittaluga, MD, PhD,* Philippe Gaulard, MD,† Lynn Sorbara, PhD,* Miguel A. Alonso, PhD,‡ Mark Raffeld, MD,* and Elaine S. Jaffe, MD*

**TABLE 3.** Summary of Immunophenotypic Findings of Mediastinal Gray Zone Lymphoma: Asynchrony Between Morphology and Immunophenotype

| Antigen | MLBCL-Like | cHL-Like |
|---|---|---|
| CD20 | Variable | +++ |
| CD30 | ++ | + |
| CD15 | + | + |
| Pax5 | + | + |
| BOB.1 | + | + |
| Oct2 | + | + |
| MAL | + | + |

| | Nodular sclerosis Hodgkin lymphoma | Mediastinal gray zone lymphoma | Primary mediastinal B cell lymphoma | Diffuse large B cell lymphoma |
|---|---|---|---|---|
| **Pathological features** | | | | |
| **Hematoxylin and Eosin** |  | | | |
| **CD20** |  | | | |
| **CD30** |  | | | |
| **Clinical features** | | | | |
| **Approximate median age** | 30 years | 30 years | 35 years | 65 years |
| **Gender predominance** | female | male > female | female | male ≥ female |
| **Typical manifestation** | supraclavicular LN / mediastinal | mediastinal | mediastinal / supraclavicular LN | nodal |
| **Bone marrow involvement** | uncommon | rare | rare | 16% |



F 31 yrs supraclavicular lymph nodes