Primary Mediastinal Large B-cell Lymphoma and B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL

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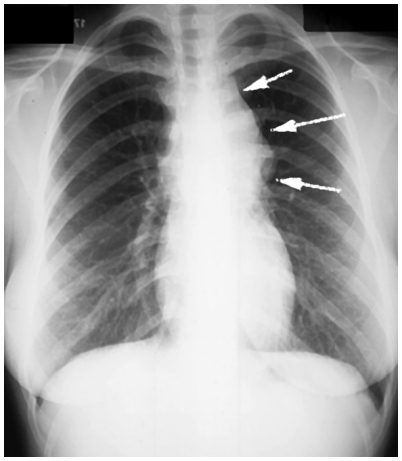
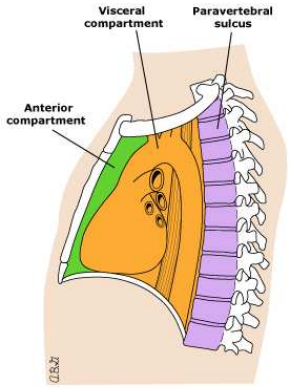
Case Summary



- **Twenty-six year old with a mediastinal mass**
- **Mediastinum- "The space between the lungs"**



UpToDate

Case Summary

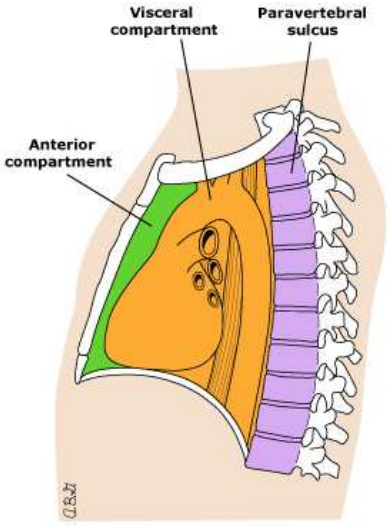
Differential diagnosis: Mediastinal mass

Anterior compartment:

Thymus-
- **Thymic hyperplasia**
- **Thymoma**
- **Thymic carcinoma**

Lymphoid-
- **Classical Hodgkin lymphoma**
- **Primary mediastinal (thymic) large B cell lymphoma**
- **T- lymphoblastic leukemia/ lymphoma**

Germ cell tumor-
- **Teratoma/ dermoid cyst**
- **Seminoma**
- **Yolk sac tumor/ Embryonial ca**



UpToDate

Case Summary

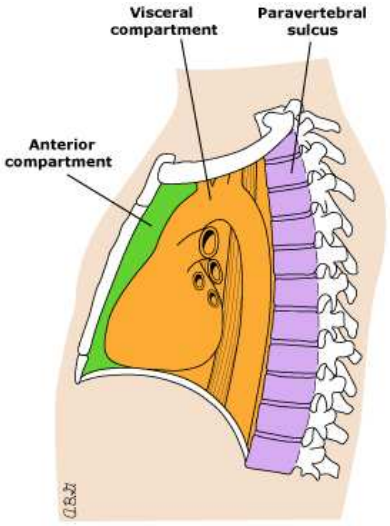
Differential diagnosis: Mediastinal mass

Middle compartment:

Lymphadenopathy-
- **Non-Hodgkin lymphoma**
- **Sarcoidosis**
- **Metastatic lung cancer**

Pericardial cyst

Bronchogenic cyst



UpToDate

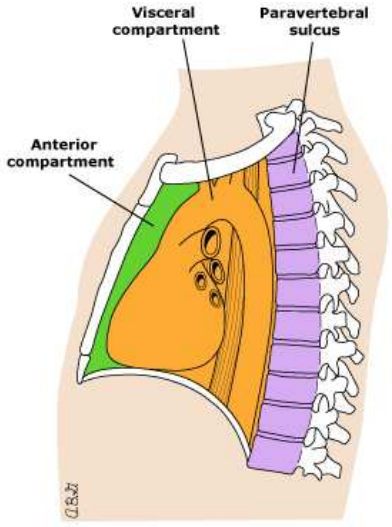
Case Summary

Differential diagnosis: Mediastinal mass

Posterior compartment:

Neurogenic tumors-

- **Neuroblastoma**
- **Ganglioneuroblastoma**
- **Ganglioneuroma**
- **Neurofibroma**
- **Pheochromocytoma**



UpToDate

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Differential diagnosis: Mediastinal mass

Posterior compartment:

Neurogenic tumors-

- **Neuroblastoma**
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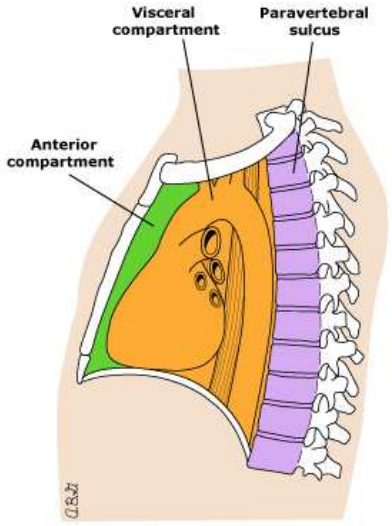
Diagnosis----

Need a biopsy!



UpToDate

Case Summary

Differential diagnosis: Mediastinal mass

Core needle biopsy vs Excisional biopsy

Utah hematopathology image bank

Case Summary

Differential diagnosis: Mediastinal mass

Core needle biopsy vs Excisional biopsy

Utah hematopathology image bank

Case Summary

Differential diagnosis: Mediastinal mass

Don't let an interventional radiologist serve you this...

When a surgeon will serve you this

Core needle biopsy vs Excisional biopsy

Case Summary

Differential diagnosis: Mediastinal mass

<u>Limitations-</u>

- May have not sampled lesion
- May have sampled only a portion of the lesion

- May have to ask for more

Difficult to resolve-

- Thymoma from thymic carcinoma
- Thymoma from T-lymphoblastic leukemia
- Classical Hodgkin lymphoma- no HRS cells
- Primary mediastinal (thymic) large B cell lymphoma from DLBCL, NOS
- Low-grade B-cell lymphoma from reactive

Case Summary

Differential diagnosis: Mediastinal mass

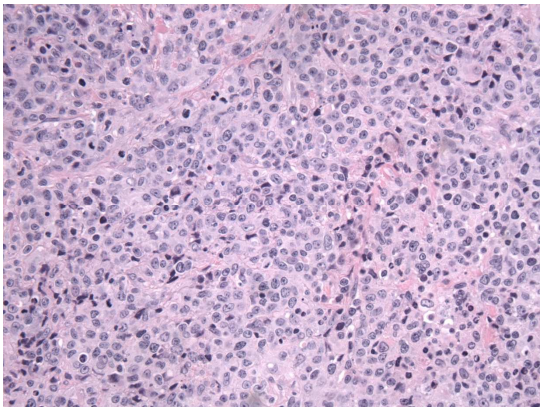
Need enough tissue to:

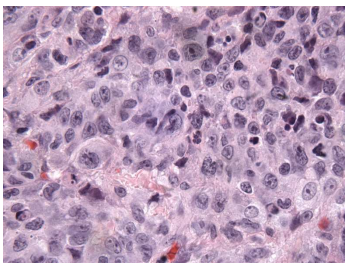
1. Find diagnostic Reed-Sternberg cells
2. Identify tumor architecture
3. Identify thymic remnants
4. Perform flow immunophenotyping
5. Perform molecular testing

Do not hesitate to ask for more tissue if it is needed for a confident diagnosis

Case Summary

Differential Diagnosis: Lymphoma vs other





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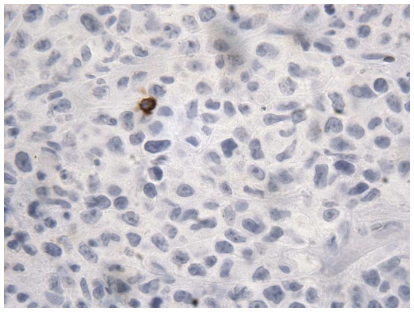
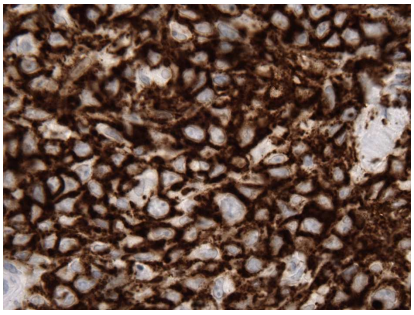


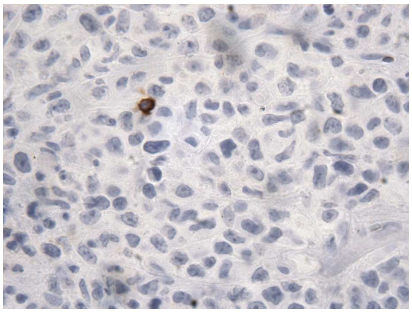
CD3



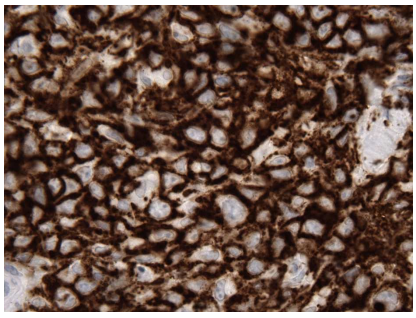
CD20

Case Summary

Diagnosis: Large B-cell lymphoma



CD3



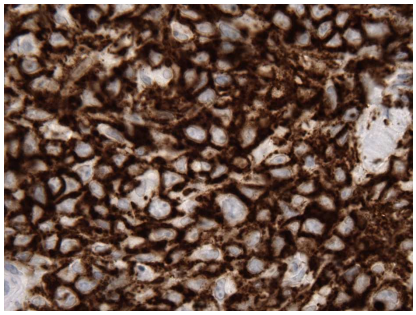
CD20

Case Summary

Diagnosis: Large B-cell lymphoma

1. But is it-

- Primary mediastinal (thymic) large B-cell lymphoma (PMLBCL)
- Diffuse large B-cell lymphoma, NOS (DLBCL) involving mediastinum (mediastinal LN)
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma (mediastinal grey zone lymphoma)



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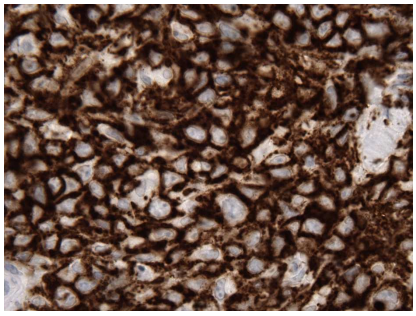
2. Does it matter?



CD20

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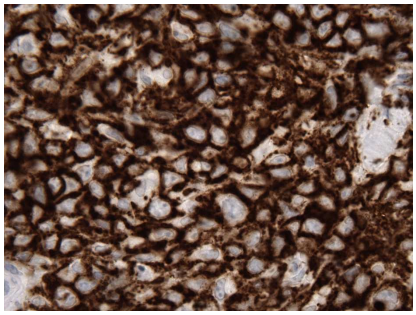


CD20

Anatomy

PMLBCL arises from a thymic B-cell

been described.[4-6] This tumour, which appears to arise in the thymus and is commonest in young women, was first thought to be derived from T cells. However, immunohistochemical studies have shown unequivocally that it is a B-cell tumour.[5,6] It was this finding that raised the possibility that the normal thymus contains a B-cell population and prompted the study described here.

Methods

Thymic tissue was obtained from 16 patients (aged 9 months to 66 years) during open heart surgery. In 12 cases only formalin-fixed paraffin-embedded tissue was available, but fresh tissue was obtained from 4 patients, and this was snap-frozen in liquid nitrogen and preserved at −70°C. In addition, paraffin-embedded thymic tissue removed at necropsy from 15 fetuses (at 15–40 weeks' gestation) and 3 infants aged 2, 3, and 4 months, respectively, was retrieved from the files of the Department of Histopathology, University College and Middlesex School of Medicine.

Both paraffin-embedded and frozen tissue were stained

FUNCTION?

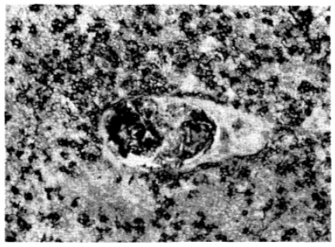


Fig 3—Cryostat section of thymus from a 5½-year-old child.

Anti-IgM staining shows B cells in medulla expressing IgM (immunoperoxidase ×140).

1489

...INING OF THYMUS

Use	Specificity	Reactivity of thymic B cells
PF	Immunoglobulin M	+ + + +
PF	Immunoglobulin D	+ + +
PF	Immunoglobulin A	+
PF	Immunoglobulin G	−
P	Most T cells	−
P	Most B cells	+ + +
F	B cells[9]	+ + +
PF	B cells[10]	+ + + +
F	B cells	+ + +
F	B cells (CD24 negative)	

Small numbers of B-cells, primarily within thymic medulla in the vicinity of Hassall's corpuscles.

Thymic B-cells are CD23+.

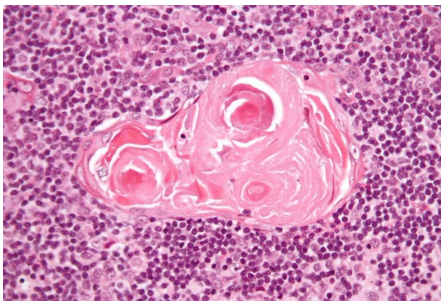
Ig loci are mutated, consistent with post-GC B-cells.

Isaacson and Addis, The Lancet, 1987

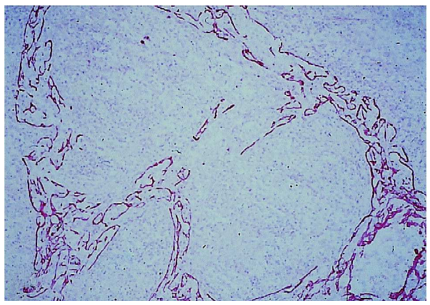
PMBLCL vs DLBCL, NOS

Histomorphology associated with PMLBCL :

Thymic remnants associated with the malignant cells



Hassall's corpuscles



Cytokeratin

PMBLCL vs DLBCL, NOS

Histomorphology associated with PMLBCL:

1. **Large lymphoid cells with abundant clear cytoplasm**
2. **Thin bands of fibrosis "compartmentalizing" aggregates of B cells**

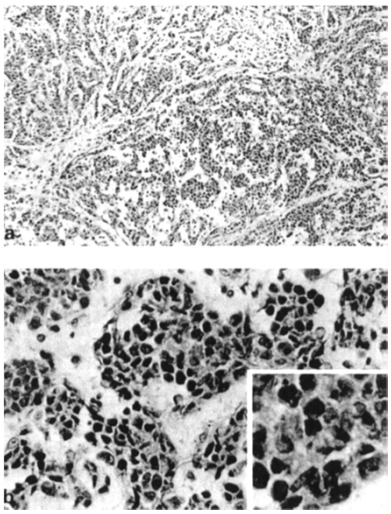


Figure 1. Case **3.** The sclerotic stroma separates tumour cells into groups or cords, mimicking the appearance of carcinoma.

Isaacson and Addis, Histopathology, 1986

PMBLCL vs DLBCL, NOS

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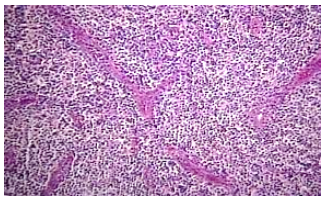
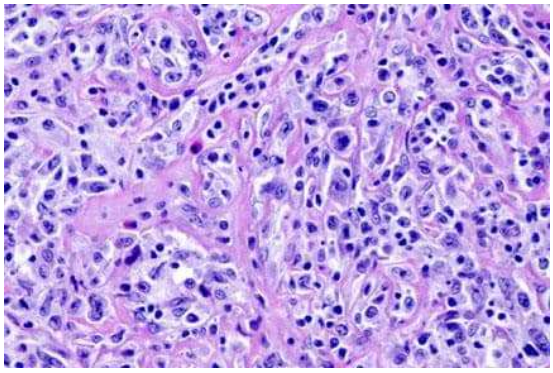
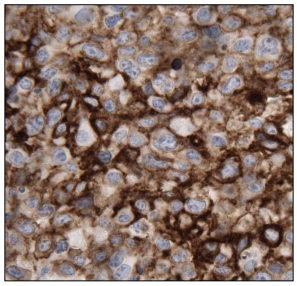




PMBLCL vs DLBCL, NOS

Immunophenotype associated with PMLBCL:

1. Thymic B-cell phenotype: CD19+ CD20+ IgM+ CD23+ Ki67+
2. MLBCL- **70% CD23+**
   DLBCL– 15% CD23+
   24 Cases PMLBCL and 100 cases DLBCL, NOS
3. MLBCL- **60% CD30+**
   18 cases PMLBCL



CD23

Helpful…
but more specific markers would be better!

Calaminici et al., Histopathology; 2004
Falini B et al., Br J Haematology; 1995

PMBLCL vs DLBCL, NOS- MAL

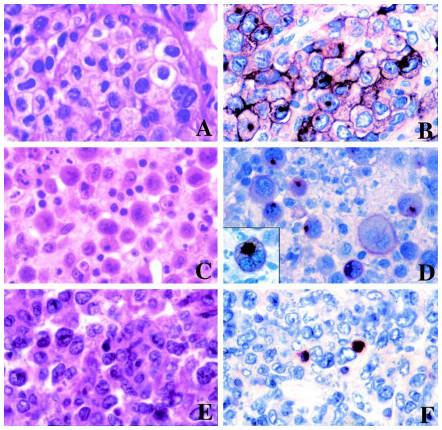
<u>Immunophenotype</u> associated with PMLBCL:

Experiment: Differential display of mRNAs between 3 MLBCLs and 3 DLBCLs

Differential expression of *MAL*
Differential expression of MAL protein was confirmed with non-commercial ab.

Validation: 33 MBLCL (70% positive)
33 DLBCL (3% positive)
41 CHL (7% positive)

Sensitivity= 70%
Specificity= 97%



MAL

Copie-Bergman et al., Blood; 1999.
Copie-Bergman et al., Mod Path, 2002.

PMBLCL vs DLBCL, NOS- MAL

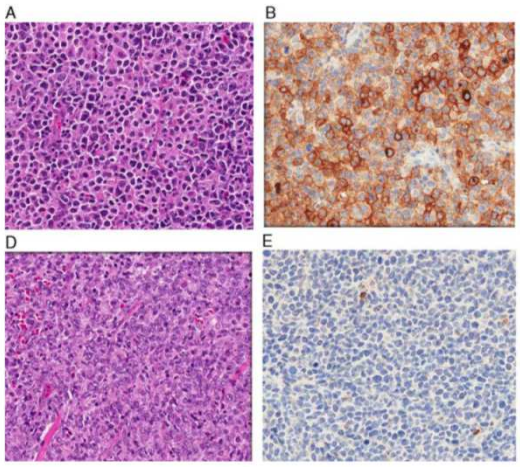
<u>Immunophenotype</u> associated with PMLBCL:

- Commercially available anti-MAL antibody
- Positive: tumor cells with ≥10% staining
- *43 Cases PMLBCL and 57 cases DLBCL, NOS*

Sensitivity= 72%
Specificity= 100%

PMLBCL

DLBCL



H&E MAL

Gentry et al., AJSP; 2017.

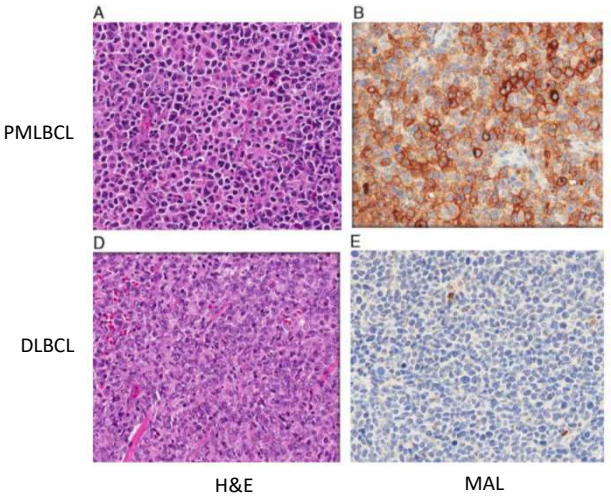
PMBLCL vs DLBCL, NOS- MAL

Immunophenotype associated with PMLBCL:

- Commercially available anti-MAL antibody
- Positive: tumor cells with >10% staining
- *43 Cases PMLBCL and 57 cases DLBCL, NOS*

Sensitivity= 72%
Specificity= 100%

Not bad, but almost 1/3 cases are MAL-negative



Gentry et al., AJSP; 2017.

PMBLCL vs DLBCL, NOS- TRAF/ cREL

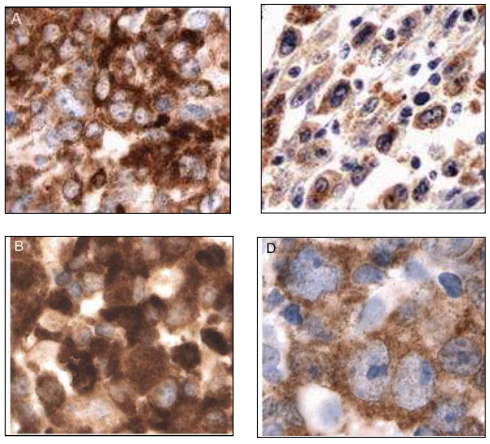
IHC markers derived from gene expression profiling signatures (GEP)

TRAF1 IHC

Sensitivity= 62%
Specificity= 88%

Nuclear cRel IHC

Sensitivity= 65%
Specificity= 82%



Rodig S et al., AJSP; 2007.

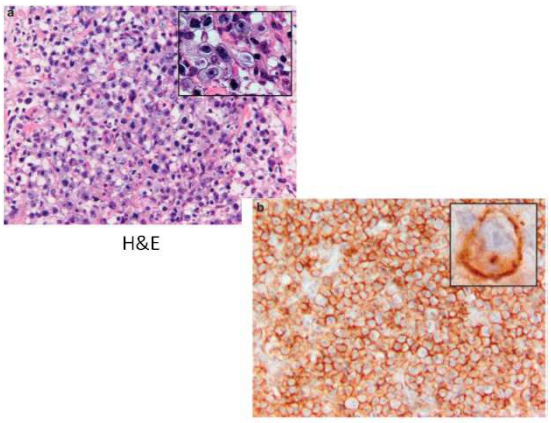
PMBLCL vs DLBCL, NOS- CD200

<u>Immunophenotype</u> associated with PMLBCL:

- Commercially available anti-CD200 antibody
- Positive: tumor cells with >20% staining
- *43 Cases PMLBCL and 42 cases DLBCL, NOS*

Sensitivity= 94%
Specificity= 93%

PMLBCL



H&E

CD200

Dorfman DM et al., Mod Path; 2012.

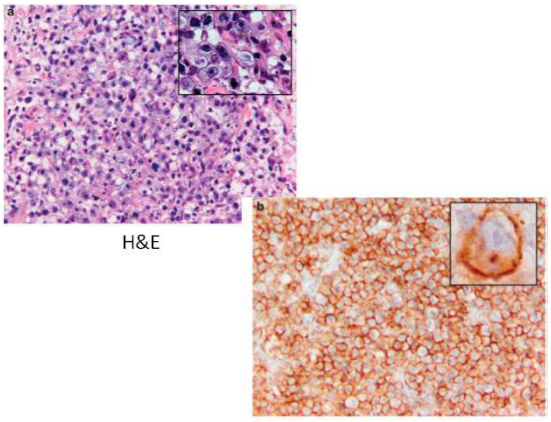
PMBLCL vs DLBCL, NOS- CD200

<u>Immunophenotype</u> associated with PMLBCL:

- Commercially available anti-CD200 antibody
- Positive: tumor cells with >20% staining
- *43 Cases PMLBCL and 42 cases DLBCL, NOS*

Sensitivity= 94%
Specificity= 93%

PMLBCL



H&E

CD200

Dorfman DM et al., Mod Path; 2012.

Pretty good!!

PMBLCL vs DLBCL, NOS

Immunophenotype associated with PMLBCL:

- Commercially available antibodies

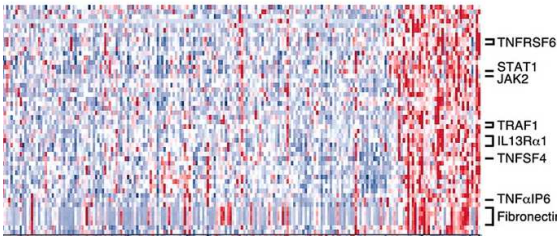
IHC Marker	Sensitivity	Specificity
CD200	.94	.93
MAL	.86	.97
CD23	.69	.93
CD30	.60	-
TRAF	.86	.77
Nuclear cRel	.77	.83

Dorfman DM et al., Mod Path; 2012.

PMBLCL vs DLBCL, NOS, Alternative Approaches

Transcriptional programs associated with PMLBCL:

- Affymetrix arrays (frozen), nanostring (FFPE)



BCR signaling (IgM)

JAK-STAT signaling (JAK2)

TNFR and NF-kB signaling (Traf1; cRel)

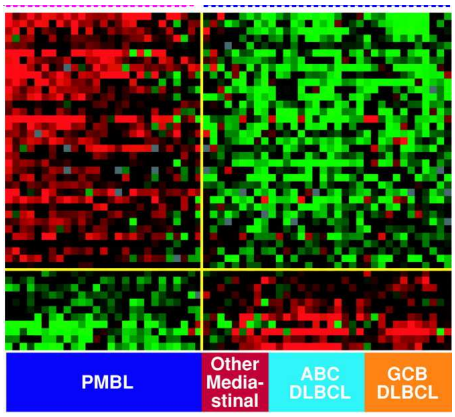
PMLBCL shows GEP signature more closely resembling classic Hodgkin lymphoma (CHL) than DLBCL, NOS

Savage KJ et al., Blood; 2003
Feuerhake F et al., Blood; 2005

PMBLCL vs DLBCL, NOS, Alternative Approaches

<u>Transcriptional programs</u> associated with PMLBCL:

- Affymetrix arrays (frozen), Nanostring (FFPE)

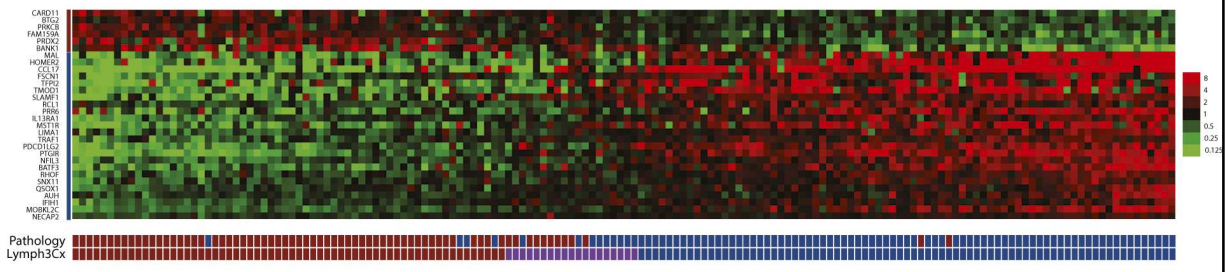


- ⬇ BCR signaling (IgM)
- ⬆ JAK-STAT signaling (JAK2)
- ⬆ TNFR and NF-kB signaling (Traf1; cRel)
- ⬆ PD-1 Ligands (PD-L1; PD-L2)

Rosenwald A et al., JEM; 2003

PMBLCL vs DLBCL, NOS- Lymph3Cx

<u>Translation of transcriptional signatures to a diagnostic test Lymph3Cx</u>:



Differentially expressed targets include:
MAL, TRAF, PD-L2

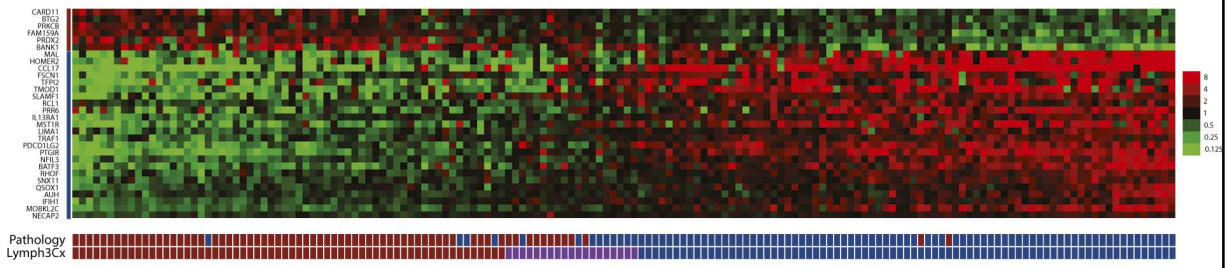
Targeted transcriptional profiling using FFPE tissues can accurately distinguish PMLBCL from DLBCL, NOS

Misclassification rate- 3.8%

Mottok A et al., Blood; 2018.

PMBLCL vs DLBCL, NOS- Lymph3Cx

Translation of transcriptional signatures to a diagnostic test Lymph3Cx:



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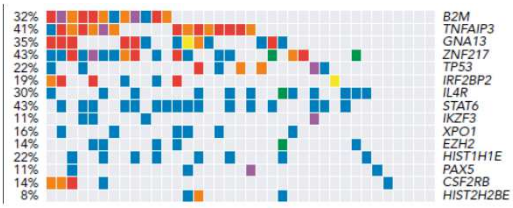
Misclassification rate- 3.8%

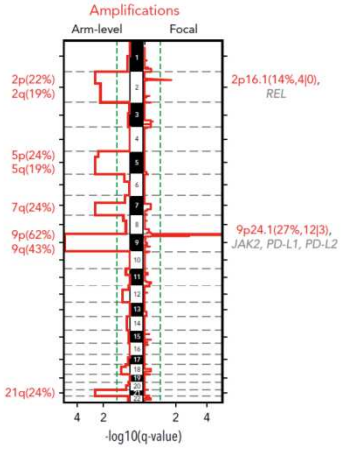
Commercial test coming soon?

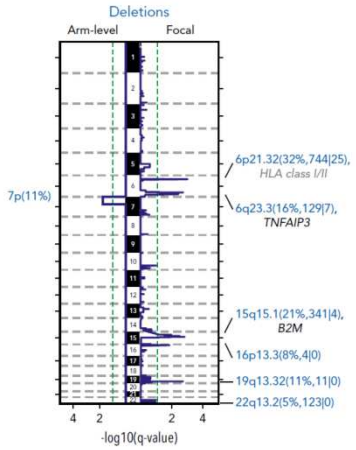
Mottok A et al., Blood; 2018.

PMBLCL vs DLBCL, NOS

Genetic signatures of PMLBCL:







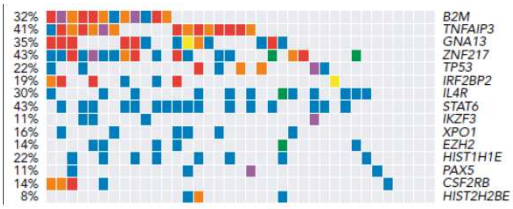
Notable for:

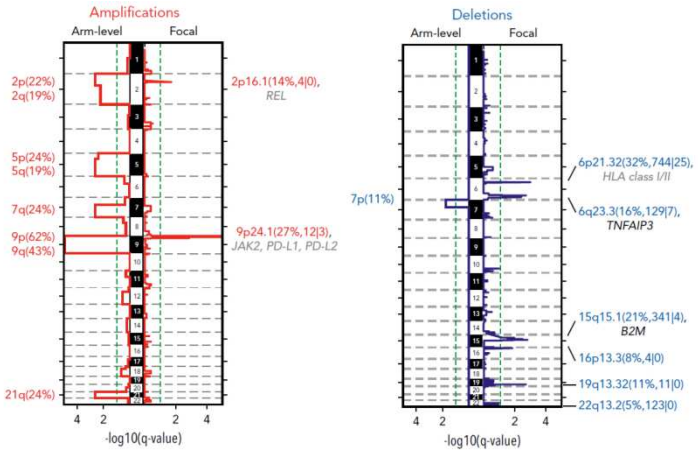
- B2M mutations
- MHC I/ II loss
- PD-L1/PD-L2 gains/amplification

Chapuy B et al., Blood; 2019.

PMBLCL vs DLBCL, NOS

Genetic signatures of PMLBCL:





Notable for:

- B2M mutations*
- MHC I/ II loss*
- PD-L1/PD-L2 gains/amplification*

***These are also seen in CHL!!!**

Chapuy B et al., Blood; 2019.

PMBLCL vs DLBCL, NOS- PD-L1

Translation of genetic signatures to IHC:

PD-L1 IHC

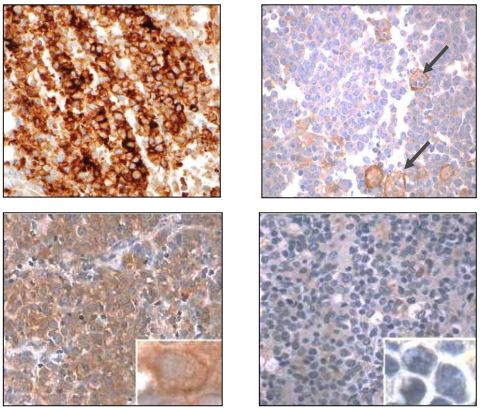
Sensitivity= 71%
Specificity= 89%

Chen BJ et al., CCR; 2013

PD-L2 IHC

Sensitivity= 72%
Specificity= 98%

Shi M et al., AJSP; 2014



PMBLCL vs DLBCL, NOS- PD-L1

Translation of genetic signatures to IHC:

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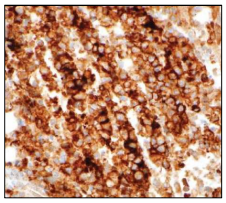

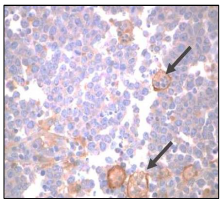

PD-L2 IHC*

Sensitivity= 72%
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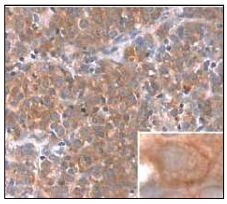
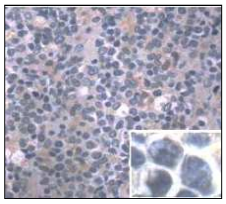
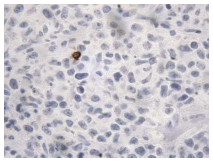




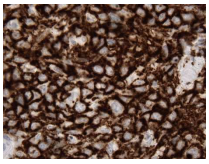
*** PD-L1 IHC is positive on Hodgkin Reed-Sternberg cells in >90% of CHLs!**

Case Summary

Diagnosis: Large B-cell lymphoma



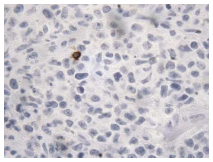
CD3-



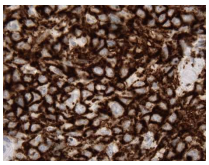
CD20+

Case Summary

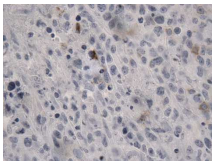
Diagnosis: Primary mediastinal (thymic) large B-cell lymphoma



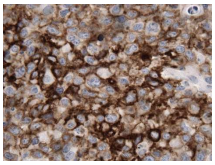
CD3-



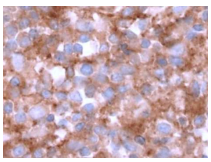
CD20+



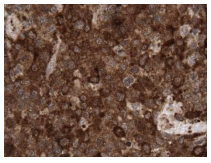
CD23-



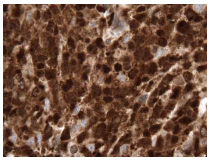
CD30+



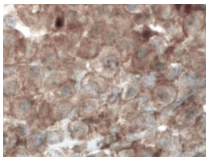
PD-L2+



TRAF1+



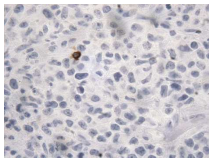
Nuclear cRel+



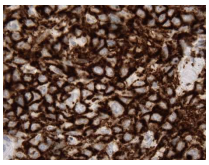
PD-L1 (weak)+

Case Summary

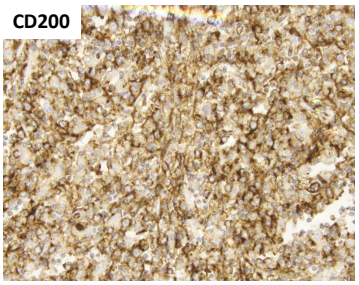
Diagnosis: Primary mediastinal (thymic) large B-cell lymphoma



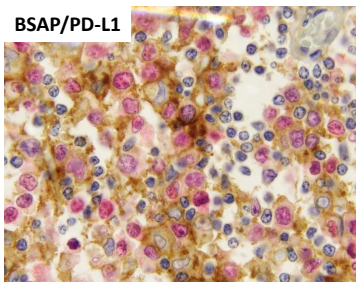
CD3-



CD20+



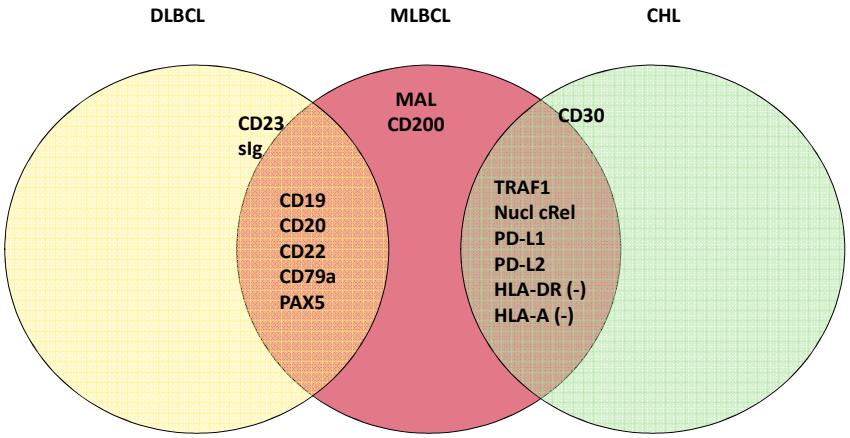
CD200+



PD-L1+

DLBCL, NOS vs PMLBCL vs CHL

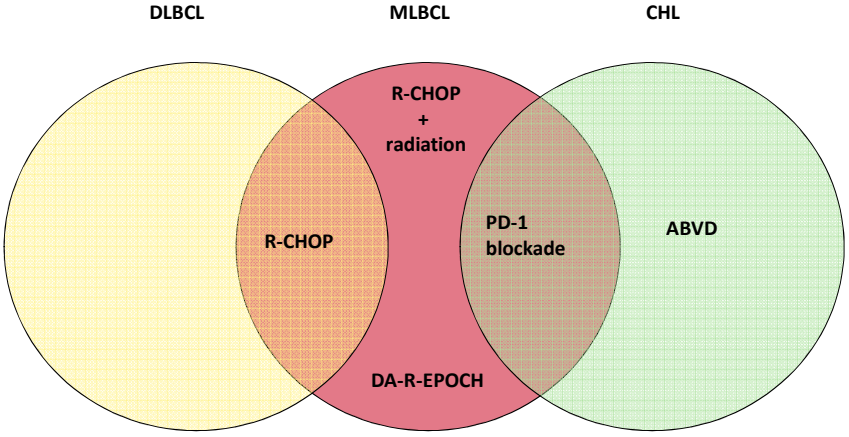
What does all this mean diagnostically if we use IHC ??



Diagnosis of MLBCL, like CHL, requires a synthesis of clinical, histologic, phenotypic and data

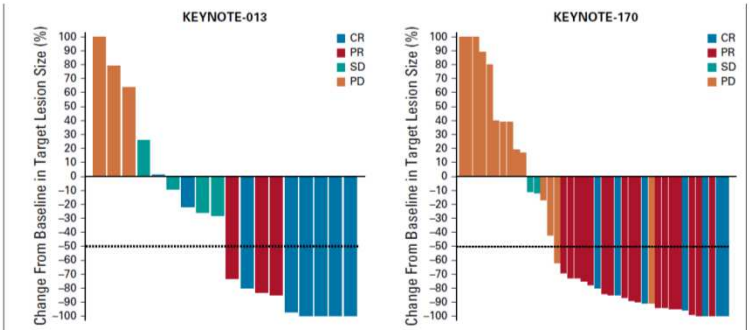
DLBCL, NOS vs PMLBCL vs CHL

What does all this mean therapeutically ??



More accurate diagnosis will facilitate the design of better clinical trials!

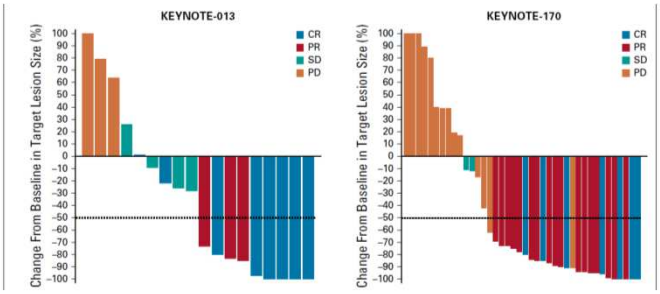
PMLBCL- Pembrolizumab

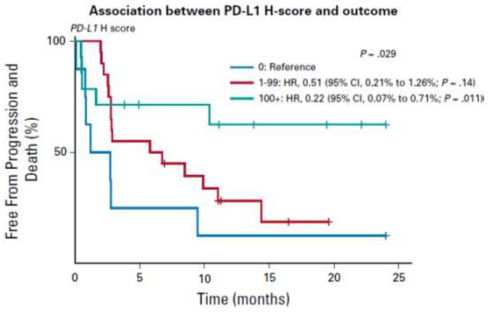


Anti-PD-1 is FDA-approved for patients with relapsed/refractory PMLBCL

Armand and Rodig et al., JCO; 2019

PMLBCL- Pembrolizumab

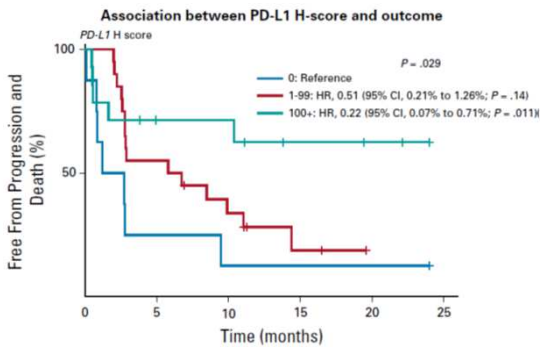




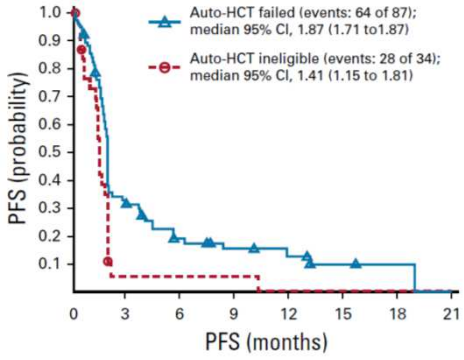
Armand and Rodig et al., JCO; 2019

PMLBCL vs DLBCL, NOS- Pembrolizumab

PMLBCL



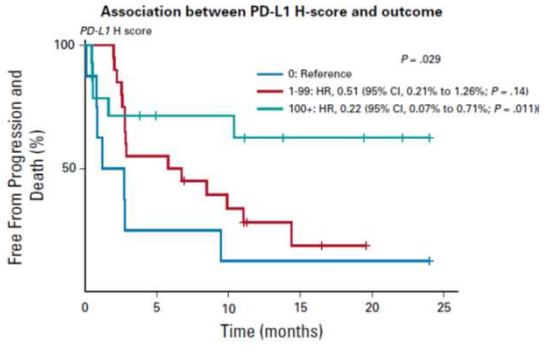
DLBCL, NOS



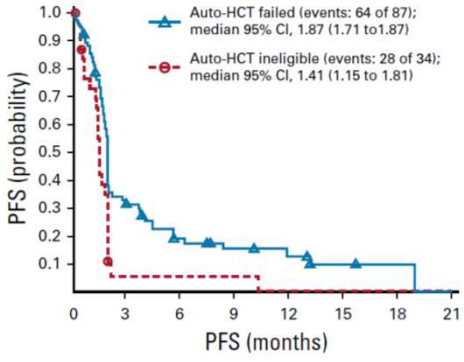
Armand and Rodig et al., JCO; 2019
Ansell et al., JCO; 2019

PMLBCL vs DLBCL, NOS- Pembrolizumab

PMLBCL



DLBCL, NOS



PD-L1 IHC is not necessary, but a correct diagnosis of PMLBCL is critical

Armand and Rodig et al., JCO; 2019
Ansell et al., JCO; 2019

B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL

What about Gray zone tumors?

Tumors with over-lapping features between PMLBCL and CHL

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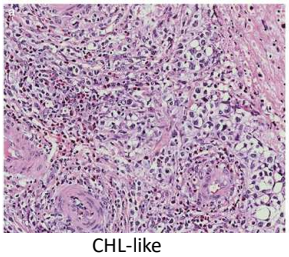
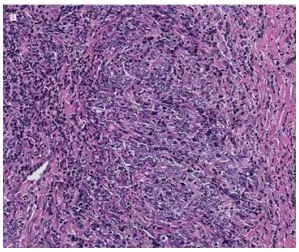
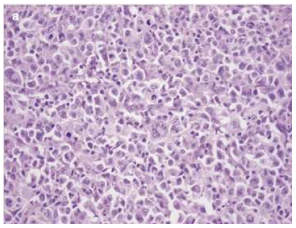


CHL-like



PMLBCL-like



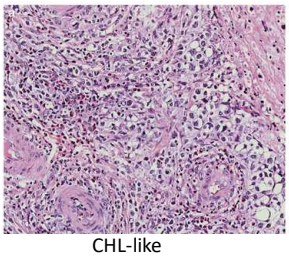
Mixed CHL/PMLBCL-like

1. Abnormal histomorphology

2. Abnormal immunophenotype

Gualco et al., Mod Path; 2012

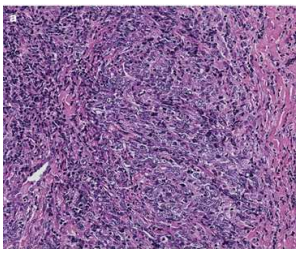
B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL



CHL-like

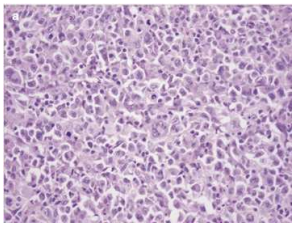
What about Gray zone tumors?

Tumors with over-lapping features between PMLBCL and CHL



PMLBCL-like

1. Abnormal histomorphology



Mixed CHL/PMLBCL-like

2. Abnormal immunophenotype

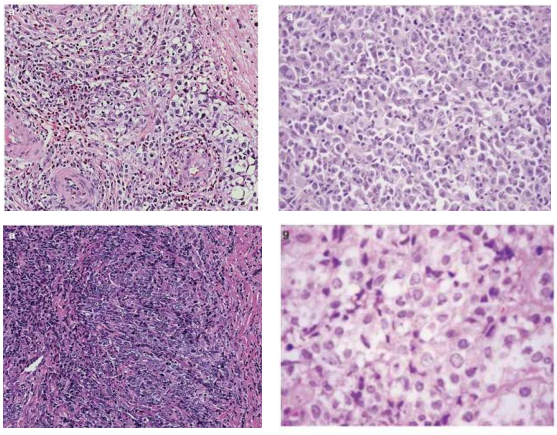
Can occur in the mediastinum or not!

Gualco et al., Mod Path; 2012

B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL

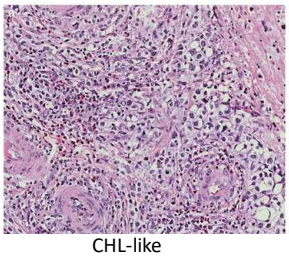
<u>Four</u> proposed patterns:

1. **Morphology of CHL but IHC has strong B-cell antigens expression**
2. **Morphology of PMLBCL but IHC as loss B-cell antigens, expression CHL markers**
3. **Morphology is mixed with CHL and PMLBCL areas present with inconsistent IHC results**
4. **True composite lymphoma of morphologically and phenotypically typical PMLBCL and CHL present**



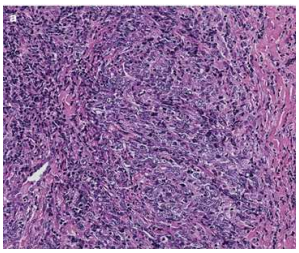
Gualco et al., Mod Path; 2012

B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL



CHL-like

Tumors with over-lapping features between PMLBCL and CHL



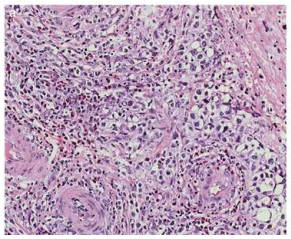
PMLBCL-like

Patterns (number of cases)	*Morphology*	*CD45*	*CD20*	*CD79a*	*CD30*	*CD15*	*EBV*
1 (1 case)	CHL-like	+	+ strong, diffuse	+	+	+	–
2 (2 cases)	PMBL-like	–	–	–/+	+/–	+	+
3 (6 cases)	Mixed CHL-like and PMBL-like	+/–	+/–	+	+	+/–	+/–
4 (1 case)	Sequential composite lymphoma: CHL followed by PMBL	CHL– PMBL+	CHL– PMBL+	CHL– PMBL+	CHL+ PMBL+	CHL+ PMBL–	CHL– PMBL–

CHL: classical Hodgkin lymphoma; PMBL: primary mediastinal B-cell lymphoma.

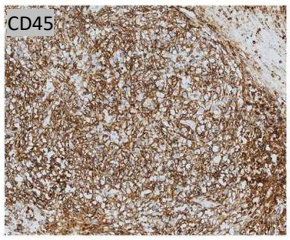
Gualco et al., Mod Path; 2012

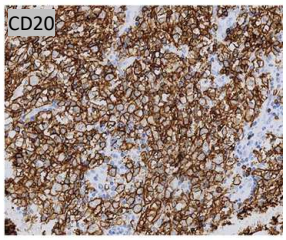
B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL

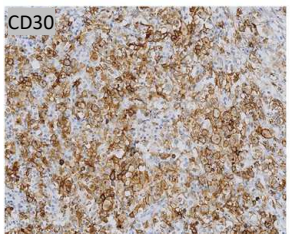


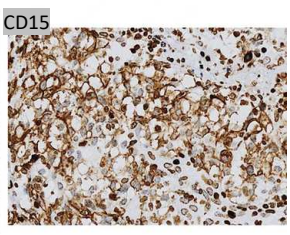
CHL-like

1. HRS-like cells in a mixed inflammatory background.
2. CD45+, CD20+(strong), CD30+, CD15+



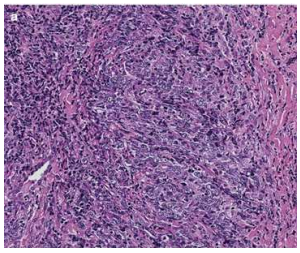






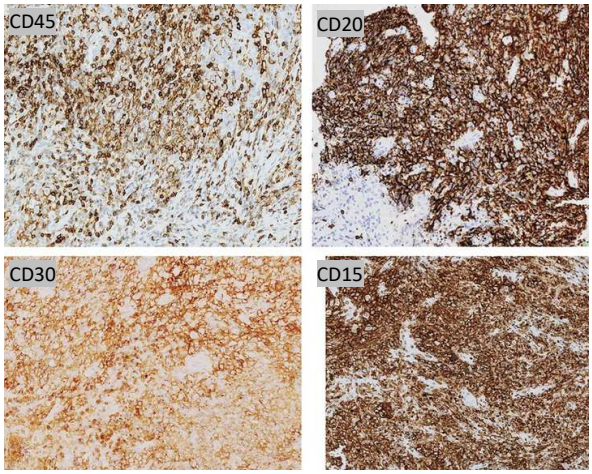
Gualco et al., Mod Path; 2012

B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL



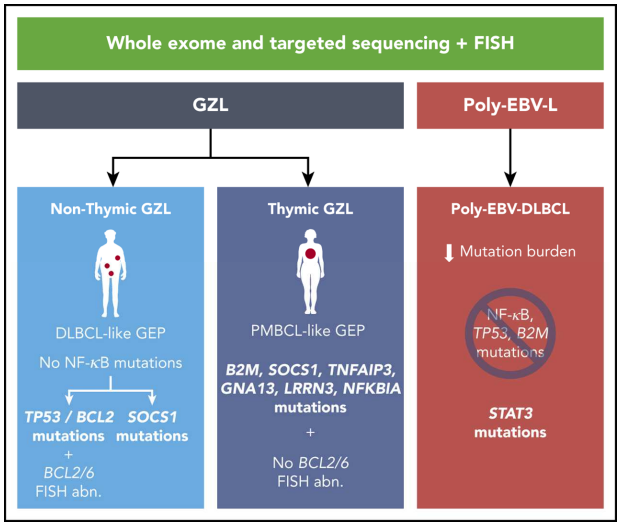
PMLBCL-like

1. Sheets of atypical lymphoid cells.
2. CD45variable, CD30+, CD15+, CD20+



Gualco et al., Mod Path; 2012

B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL

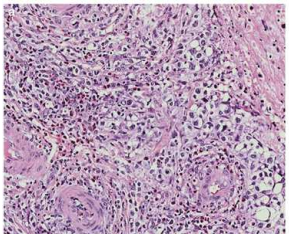


1. **Mediastinal GZL- mutations resemble CHL and PMLBCL**
2. **Non-mediastinal GZL- mutations are different, maybe distinct tumor and cell-of-origin**

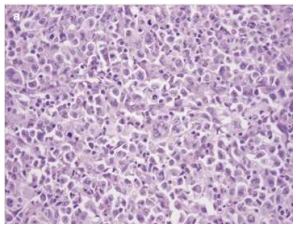
Sarkozy et al., Blood 2021.

B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and CHL

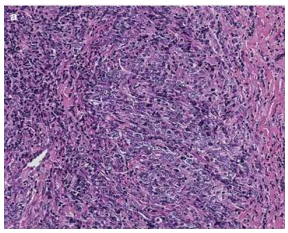
CHL-like



Mixed CHL/PMLBCL-like



PMLBCL-like



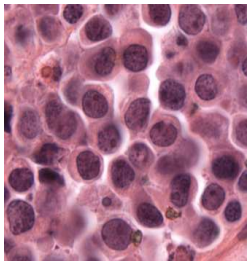
How to treat?

Unclear, but most clinicians favor DLBCL-directed therapies

1. **R-CHOP or DA-R-EPOCH**
2. **+/- Radiotherapy**
3. **R/R dz chemo + auto-HSCT**

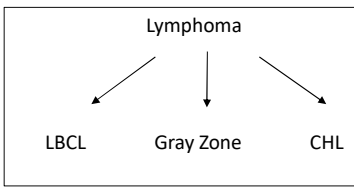
Krtharis A et al., Br J Haematol., 2016.

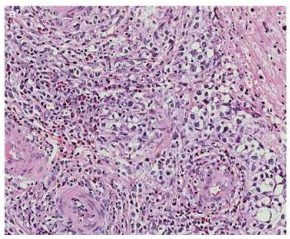
Diagnostic Algorithm

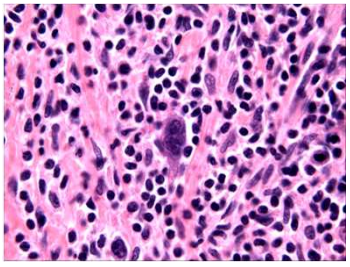


LBCL

1. $CD3^-$, $CD20^+$, $PAX5^+$, LCA^+
2. $OCT2^+$, $BOB1^+$, $CD79a^+$
3. $CD30^{+/-}$, $CD15^-$, EBV^-



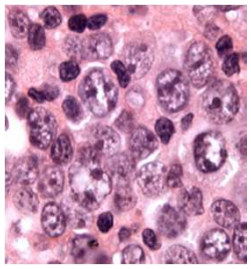


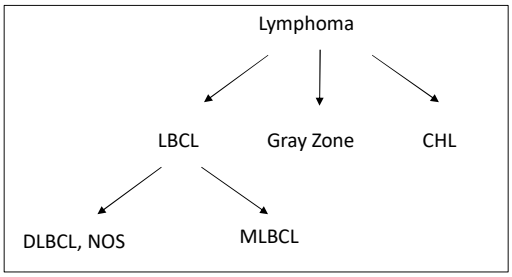


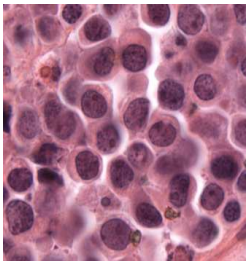
CHL

1. $CD3^-$, $CD20^{-/+}$, $PAX5^{+weak}$, LCA^-
2. $OCT2^-$, $BOB1^-$, $CD79a^-$
3. $CD30^+$, $CD15^+$, $EBV^{+/-}$

Diagnostic Algorithm







DLBCL, NOS

1. CD3^{-}, CD20^{+}, PAX5^{+}, LCA^{+}
2. CD30$^{-/+}$, CD15^{-}, EBV$^{+/-}$
3. CD200^{-}, PD-L1^{-}
4. TRAF1^{-}, nucl-cREL^{-}

Diagnoses of MLBCL, CHL, GZL, and DLBCL require a <u>synthesis</u> of clinical, histologic, phenotypic and (soon) genetic data

MLBCL

1. CD3^{-}, CD20^{+}, PAX5^{+}, LCA^{+}
2. CD30$^{+/-}$, CD15^{-}, EBV^{-}
3. CD200^{+}, MAL^{+}, PD-L1^{+}
4. TRAF1^{+}, nucl-cREL^{+}

Thank you!