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Cyclin D1-positive Mediastinal Large B-Cell Lymphoma With Copy Number Gains of *CCND1* Gene

A Study of 3 Cases With Nonmediastinal Disease

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Backgrounds

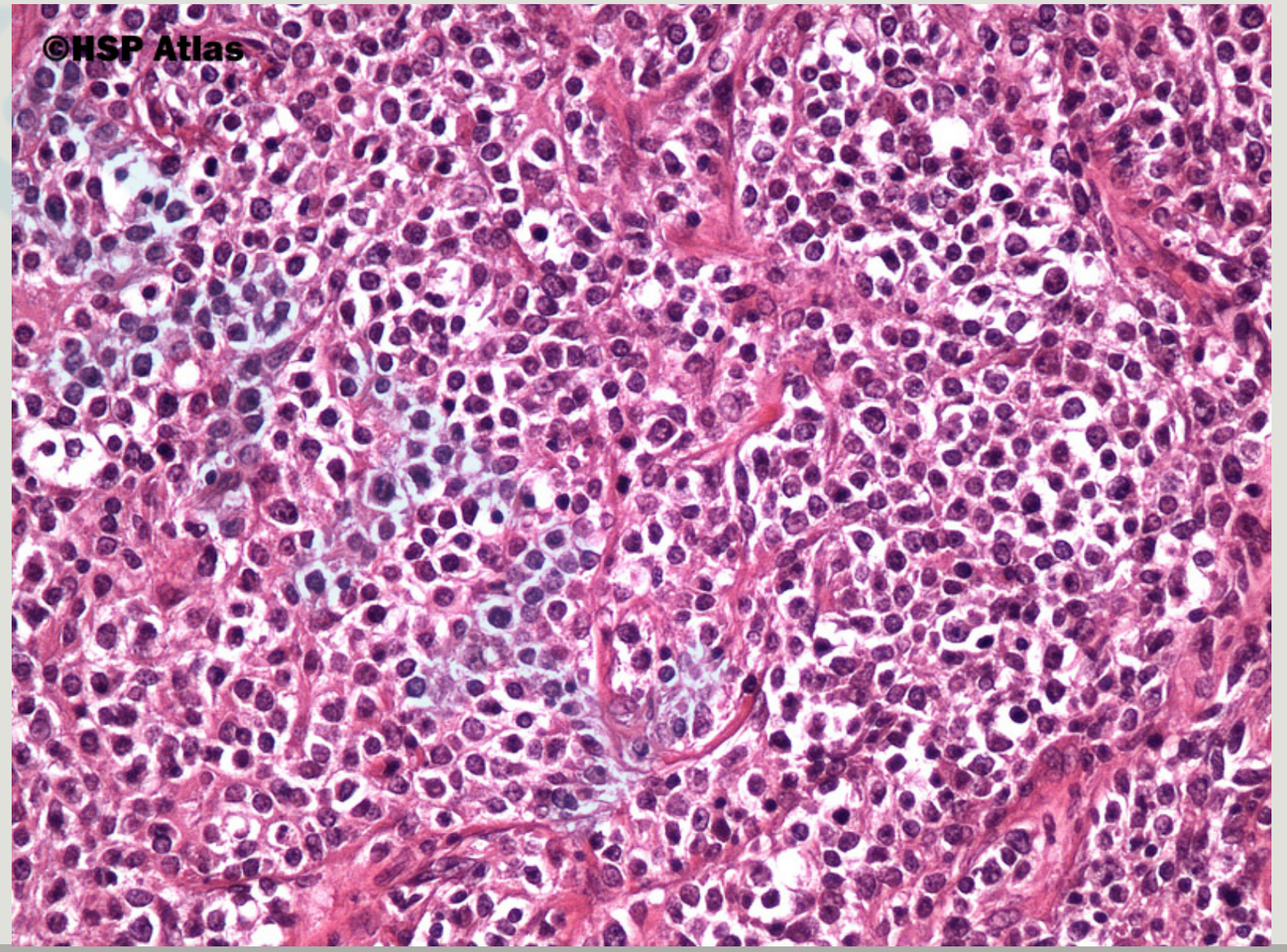
Primary mediastinal (thymic) large B-cell lymphoma (PMBL)

✓ a distinct entity of mature large B cells of putative thymic B-cell origin typically arising in the mediastinum

✓predominantly in young women （F:M=2:1） with a more favorable survival

Morphology of PMBL

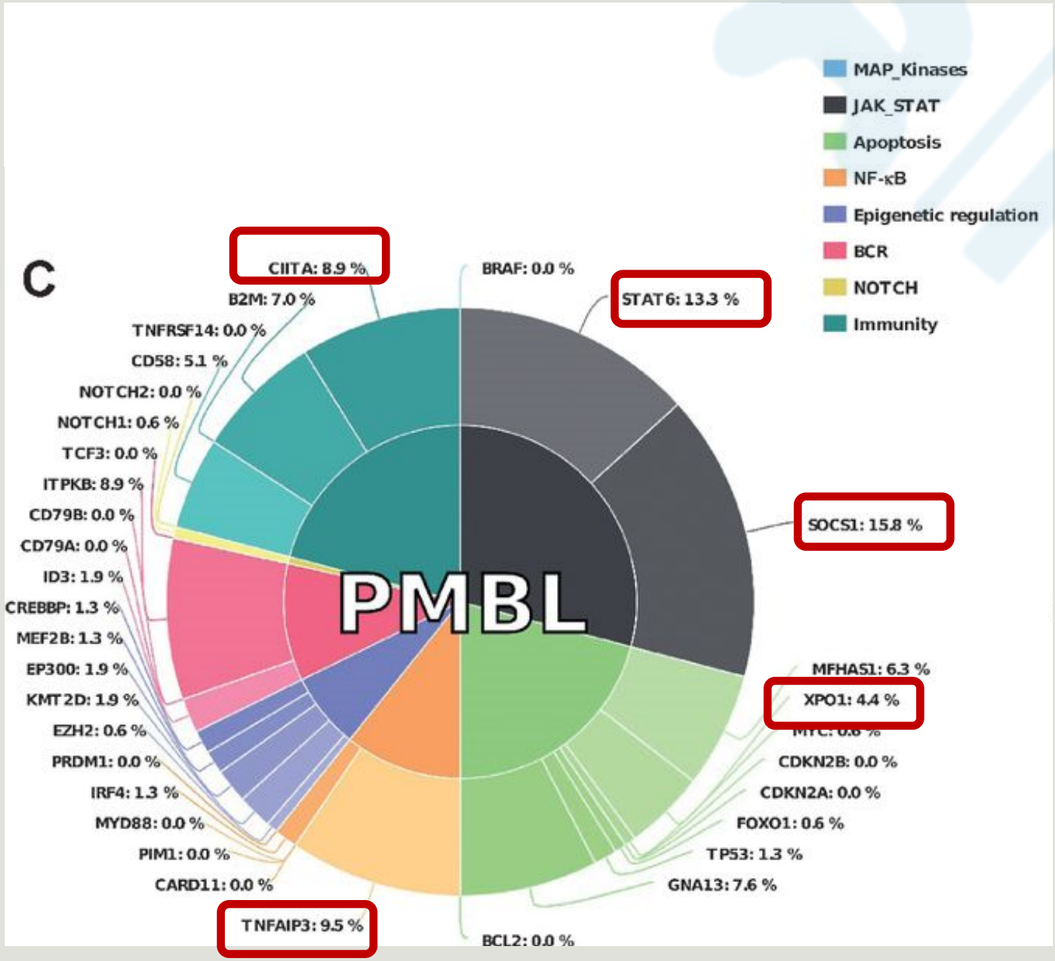
- ✓Morphologically, the tumor cells are usually medium-sized to large with round or oval nuclei and abundant pale or clear cytoplasm
- ✓Collagenous fibrosis compartmentalizing the tumor cells is frequently observed

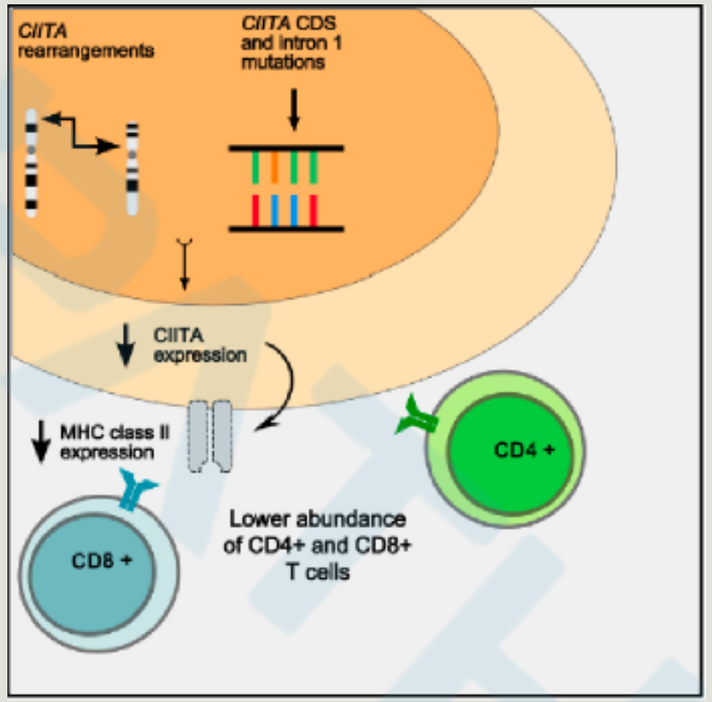


IHC of PMBL

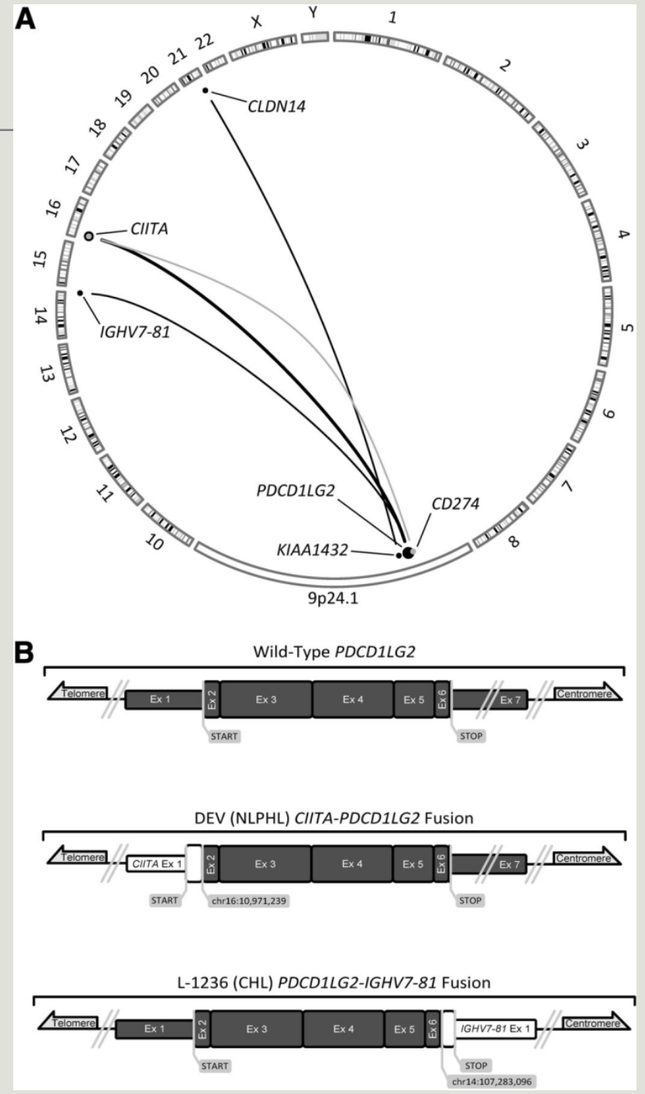
Antibodies	Expression
Positive expression	
pan-B-cell markers including CD20 and CD79a	Pos
CD30, IRF4/MUM1	Positive in majority of cases
BCL2, BCL6, and CD10	Variable expression
CD23, myelin and lymphocyte protein (MAL), PD-L1 and PD-L2	Pos, characteristic finding of PMBL, unlike other DLBCL
MYC, TNFAIP2, coexistent TRAF1 and nuclear REL	Pos
Negative expression	
IG, PAX5, OCT2, BOB1, PU1.2	Neg (despite a functional IG gene rearrangement)

Genetic changes





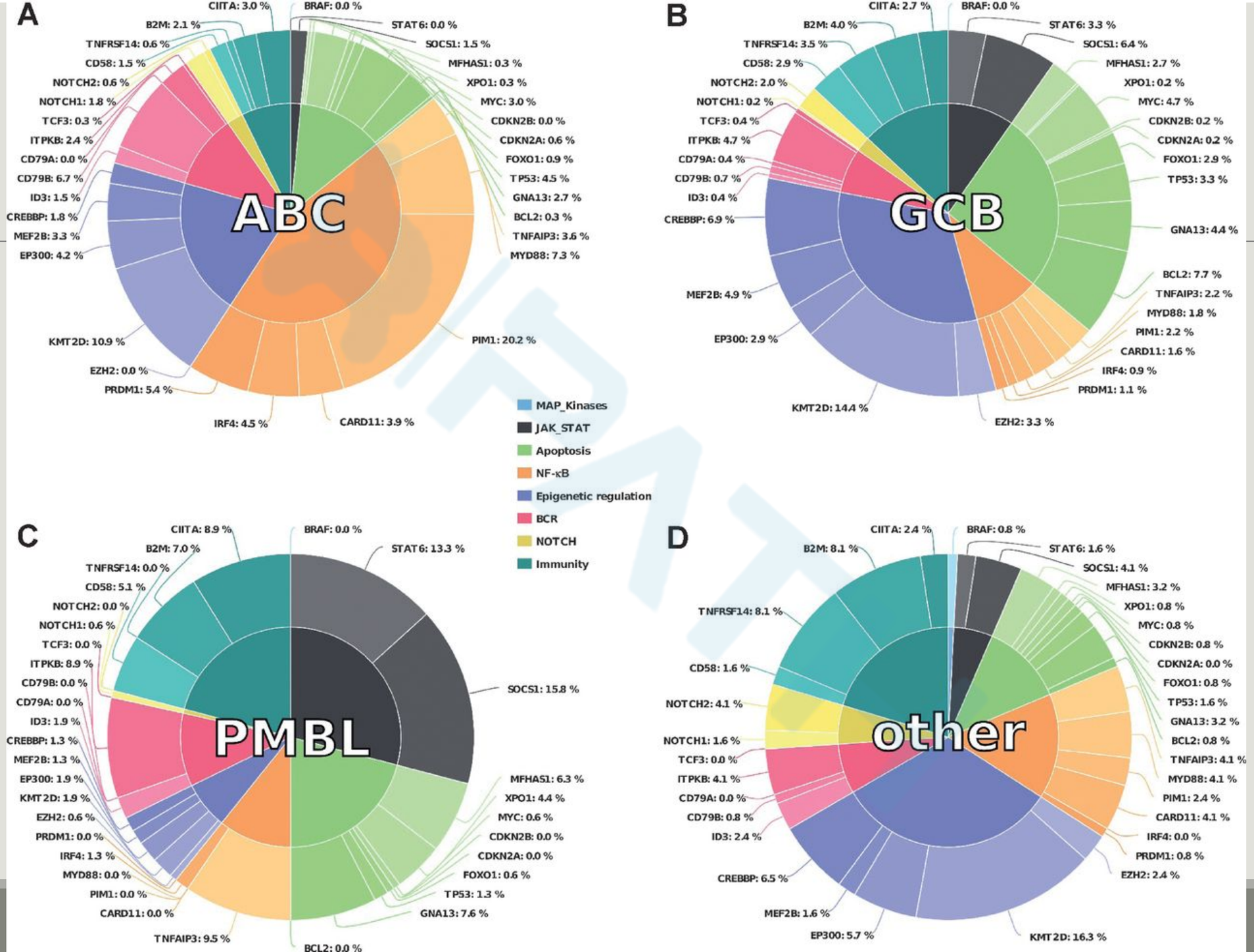
Rearrangements and mutations in the class II major histocompatibility complex (MHC) transactivator CIITA at 16p13 have been reported in half of the cases resulting in downregulation of MHC class II.



*Clin Cancer Res,2016,**22**(12): 2919-2928*

CIITA mutations

Translocation of PDL1 and PDL2 with CIITA


A
ABC
CIITA: 3.0 %
BRAF: 0.0 %
B2M: 2.1 %
STAT6: 0.0 %
TNFRSF14: 0.6 %
SOCS1: 1.5 %
CD58: 1.5 %
MFHAS1: 0.3 %
NOTCH2: 0.6 %
XPO1: 0.3 %
NOTCH1: 1.8 %
MYC: 3.0 %
TCF3: 0.3 %
CDKN2B: 0.0 %
ITPKB: 2.4 %
CDKN2A: 0.6 %
CD79A: 0.0 %
FOXO1: 0.9 %
CD79B: 6.7 %
TP53: 4.5 %
ID3: 1.5 %
GNA13: 2.7 %
CREBBP: 1.8 %
BCL2: 0.3 %
MEF2B: 3.3 %
TNFAIP3: 3.6 %
EP300: 4.2 %
MYD88: 7.3 %
KMT2D: 10.9 %
PIM1: 20.2 %
EZH2: 0.0 %
PRDM1: 5.4 %
IRF4: 4.5 %
CARD11: 3.9 %
B
GCB
CIITA: 2.7 %
BRAF: 0.0 %
B2M: 4.0 %
STAT6: 3.3 %
TNFRSF14: 3.5 %
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MFHAS1: 2.7 %
NOTCH2: 2.0 %
XPO1: 0.2 %
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MYC: 4.7 %
TCF3: 0.4 %
CDKN2B: 0.2 %
ITPKB: 4.7 %
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BCL2: 7.7 %
MEF2B: 4.9 %
TNFAIP3: 2.2 %
MYD88: 1.8 %
EP300: 2.9 %
PIM1: 2.2 %
CARD11: 1.6 %
KMT2D: 14.4 %
IRF4: 0.9 %
PRDM1: 1.1 %
EZH2: 3.3 %
C
PMBL
CIITA: 8.9 %
BRAF: 0.0 %
B2M: 7.0 %
STAT6: 13.3 %
TNFRSF14: 0.0 %
CD58: 5.1 %
NOTCH2: 0.0 %
NOTCH1: 0.6 %
TCF3: 0.0 %
ITPKB: 8.9 %
SOCS1: 15.8 %
CD79B: 0.0 %
CD79A: 0.0 %
ID3: 1.9 %
CREBBP: 1.3 %
MFHAS1: 6.3 %
MEF2B: 1.3 %
XPO1: 4.4 %
EP300: 1.9 %
MYC: 0.6 %
KMT2D: 1.9 %
CDKN2B: 0.0 %
EZH2: 0.6 %
CDKN2A: 0.0 %
PRDM1: 0.0 %
FOXO1: 0.6 %
IRF4: 1.3 %
TP53: 1.3 %
MYD88: 0.0 %
GNA13: 7.6 %
PIM1: 0.0 %
CARD11: 0.0 %
TNFAIP3: 9.5 %
BCL2: 0.0 %
D
other
CIITA: 2.4 %
BRAF: 0.8 %
B2M: 8.1 %
STAT6: 1.6 %
SOCS1: 4.1 %
MFHAS1: 3.2 %
TNFRSF14: 8.1 %
XPO1: 0.8 %
MYC: 0.8 %
CDKN2B: 0.8 %
CD58: 1.6 %
CDKN2A: 0.0 %
FOXO1: 0.8 %
NOTCH2: 4.1 %
TP53: 1.6 %
GNA13: 3.2 %
NOTCH1: 1.6 %
BCL2: 0.8 %
TCF3: 0.0 %
TNFAIP3: 4.1 %
ITPKB: 4.1 %
MYD88: 4.1 %
CD79A: 0.0 %
PIM1: 2.4 %
CD79B: 0.8 %
CARD11: 4.1 %
ID3: 2.4 %
IRF4: 0.0 %
PRDM1: 0.8 %
CREBBP: 6.5 %
EZH2: 2.4 %
MEF2B: 1.6 %
EP300: 5.7 %
KMT2D: 16.3 %
MAP_Kinases
JAK_STAT
Apoptosis
NF-κB
Epigenetic regulation
BCR
NOTCH
Immunity

Backgrounds

PMBL has a distinct gene expression profile (GEP), which is different from DLBCL, NOS, but similar to classic Hodgkin lymphoma (CHL)

Primary nodal cases without mediastinal involvement with the typical morphology, phenotype, and GEP of PMBL have been recently described, indicating that rare cases outside the mediastinum do exist.

Aims of this study

In this study, we extend previous observations and report 3 cases :

- ✓ typical morphology and immunophenotype of PMBL
- ✓ diagnosed in lymph nodes (LN), including 2 cases without mediastinal mass that have the peculiarity of aberrant cyclin D1 expression

Distinguishing this unusual variant of PMBL from other cyclin D1-positive non-Hodgkin lymphomas entities such as mantle cell lymphoma (MCL) and DLBCL, NOS, may also have therapeutic implications.

MATERIALS AND METHODS

- ✓ **Lymphoma Samples and Clinical Data**
- ✓ **Immunohistochemistry and Fluorescence In Situ Hybridization**
- ✓ **Multiplex Ligation-dependent Probe Amplification, MLPA**

reverse transcriptase-MLPA (RT-MLPA) is a new mRNA-based technique that evaluates the expression of 21 genes to assess the probability of a DLBCL being of germinal center (GCB), activated B-cell (ABC) or PMBL derivation

This signature uses 3 genes to identify PMBL (FCER2 encoding CD23, MAL, and TNFRSF8 encoding CD30), and 3 genes to identify DLBCL (MME, which encodes for CD10, usually negative in PMBL), IGM (usually negative in PMBL) and IRF4

- ✓ **Next-generation Sequencing–based Mutational Analysis**

Results

Clinical Findings

TABLE 1. Clinical and Pathologic Findings of Nonmediastinal Primary Mediastinal Large B-cell Lymphoma

	Case No. 1	Case No. 2	Case No. 3
Age (y) and sex	72, female	80, male	48, male
Location of biopsy	Submandibular lymph node	Submandibular lymph node	Supraclavicular lymph node
Mediastinal mass	No	No	Yes
Compartmentalizing fibrosis	Yes	Yes	Yes
Pale or clear cytoplasm of tumor cells	Yes	Yes	Yes
BCL2	Positive	Positive	Positive
BCL6	Positive	Positive	Positive
CD3	Negative	Negative	Negative
CD5	Negative	Negative	Negative
CD10	Negative	Negative	Negative
CD20	Positive	Positive	Positive
CD23	Positive (partial)	Positive	Negative (rare +)
CD30	Positive	Negative	Positive
Cyclin D1	Positive (strong, 90%)	Positive (moderate to strong, 60%)	Positive (moderate, 60%)
Kappa light chain	Negative	Negative	ND
Lambda light chain	Negative	Positive	ND
MAL	Positive	Positive	Positive
MYC	Negative	Negative	Positive
MUM1	Negative	Negative	Positive
PD-L1	Negative	Negative	ND
SOX11	Negative	Negative	Negative
TP53	Negative	ND	ND
EBER	ND	ND	Negative
BCL2 FISH	Negative	Negative	Extra signals
BCL6 FISH	Negative	Rearranged	Extra signals
CIITA FISH	Negative	Negative	Negative
CCND1 FISH	Trisomy	Trisomy to tetrasomy	Tetrasomy
MYC FISH	ND	Negative	Extra signals
PD-L1/PD-L2 FISH	Negative	Negative	Negative
Probability of PMBL by RT-MLPA profile	87.6%	98.7%	99%
Stage (Ann Arbor)	IA	IA	IIB
Treatment	R-CHOP for 6 cycles	R-CHOP for 6 cycles	R-EPOCH for 6 cycles
Follow-up	CR for 25 mo	CR for 19 mo	CR for 25 mo

ND indicates not done.

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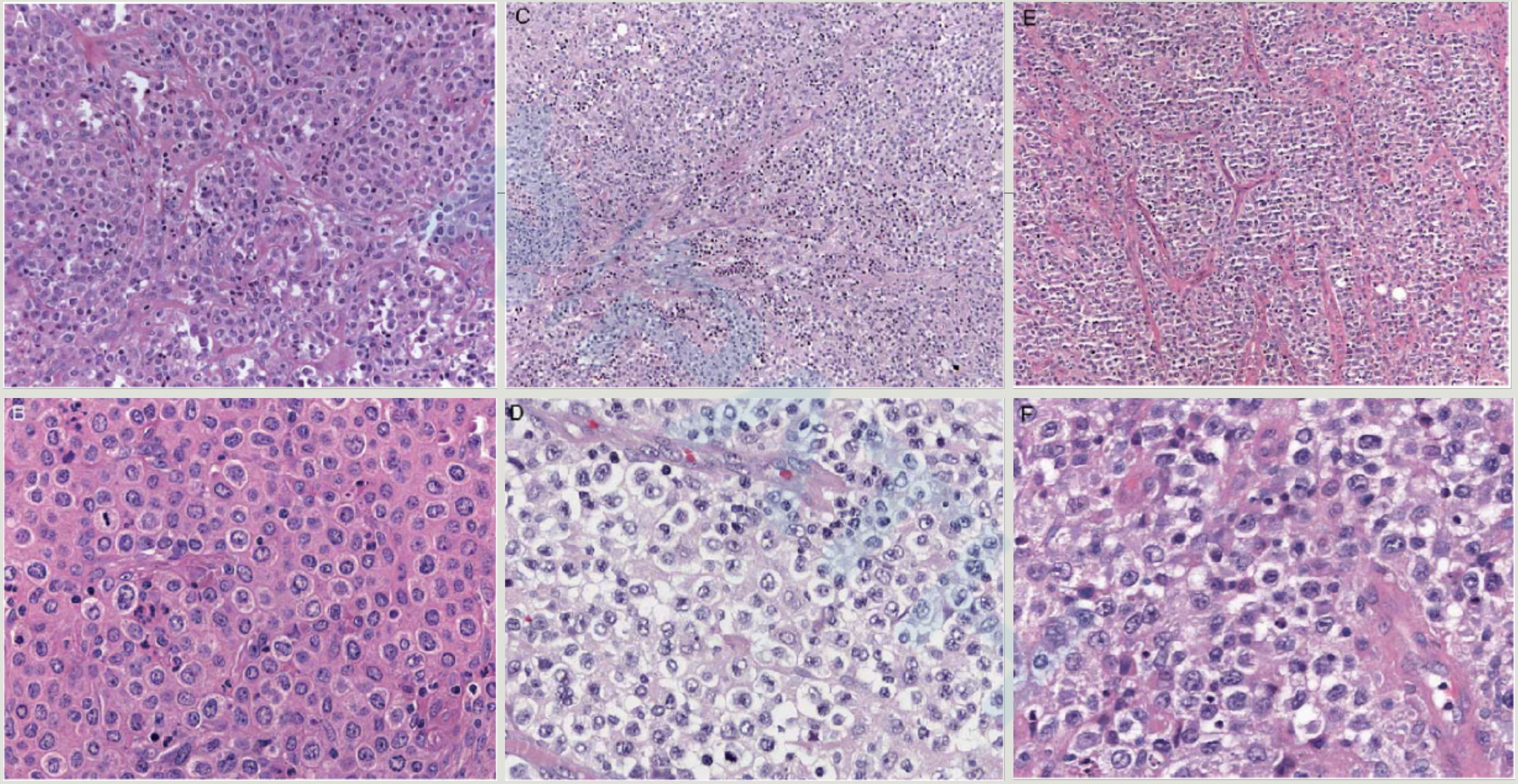
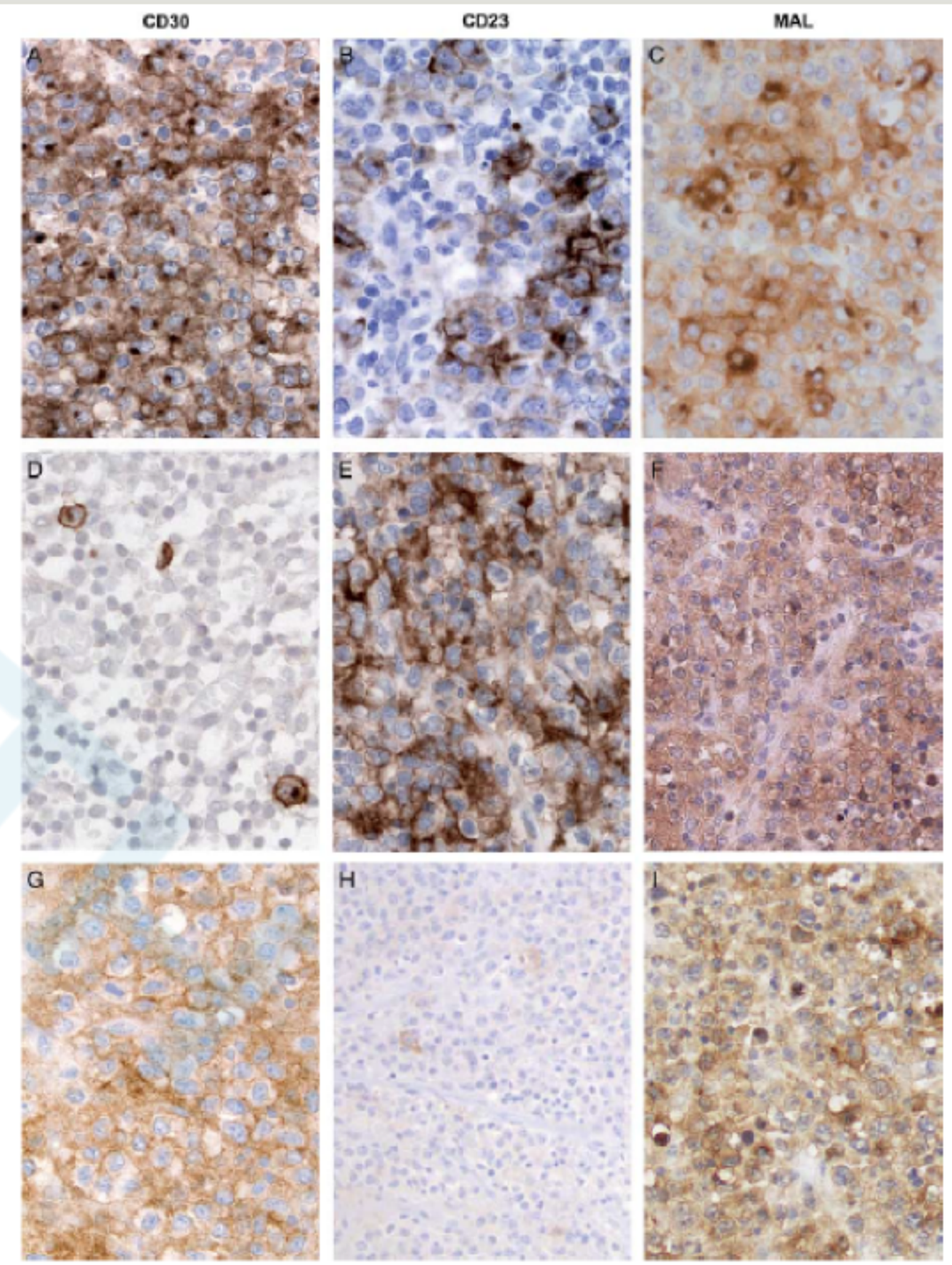


Case 1 Case 2 Case 3

Results-IHC

BCL2, BCL6,CD20, MAL, cyclin D1, CD23 (cases 1 &2), CD30 (cases 1 &3)

CD10, CD5, SOX11, PD-L1, TP53



Results-IHC & FISH

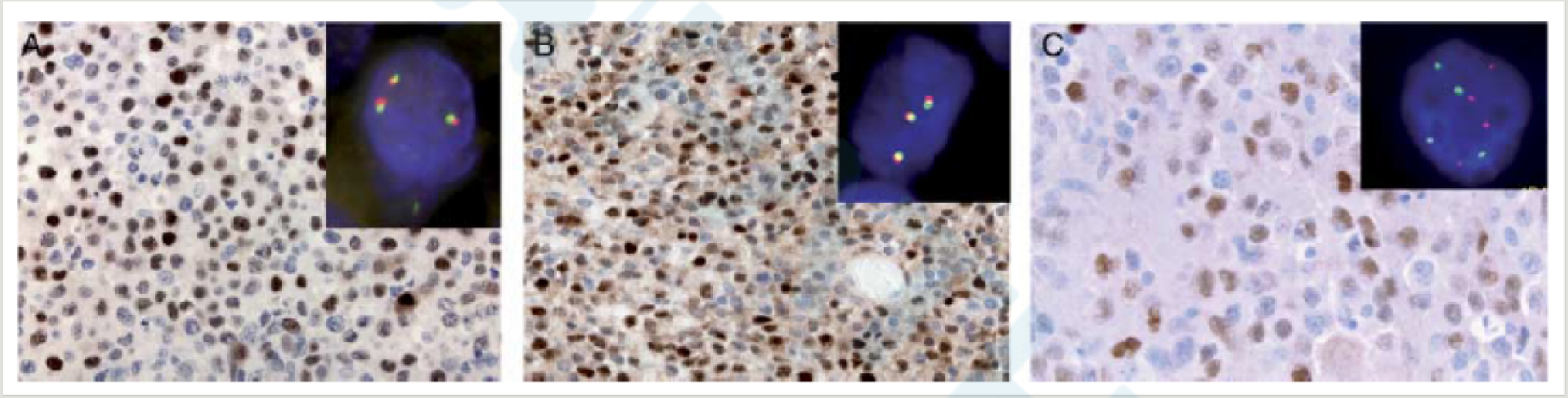
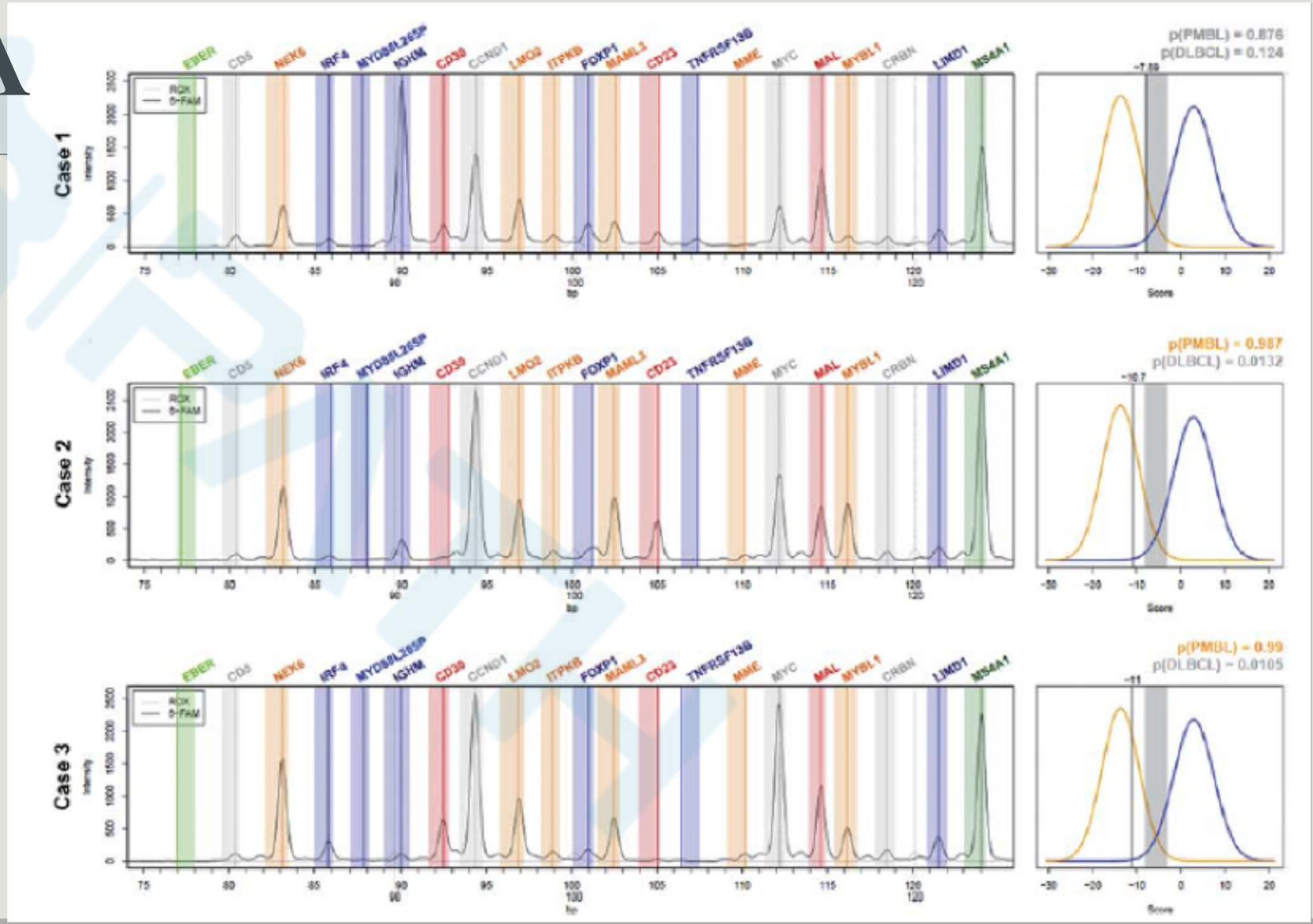


FIGURE 3. Cyclin D1 immunohistochemistry and CCND1 FISH analysis. All 3 cases expressed moderate to strong cyclin D1 protein. FISH analysis showed copy number gains of CCND1 gene without rearrangement (A, case 1 [inset, trisomy by CCND1 break-apart probe]; B, case 2 [inset, trisomy by CCND1 break-apart probe]; C, case 3 [inset, tetrasomy by IGH-CCND1 dual fusion probe, IGH, green signals; CCND1, red signals]).

Results- MLPA

FIGURE 4. Case 2 and 3 revealed a high probability of being PMBL (98.7% and 99%) rather than DLBCL, NOS (1.3% and 1%). Case 1 due to the high expression of IGM, and despite the expression of CD30, MAL, and CD23 reached only a marginal probability (87.6%) of being PMBL. The 3 cases revealed high CCND1 mRNA confirming the expression of the protein.



Blue for ABC-related genes, Orange for GCB-related genes, Red for PMBL-related genes, green for Epstein-Barr virus–positive DLBCL-specific EBER gene, dark green for MS4A1 (encoding CD20) internal control, and gray for other genes

Results - NGS

TABLE 2. Genetic Aberrations in Nonmediastinal Primary Mediastinal Large B-cell Lymphoma

Case No.	Gene	cDNA	Amino Acid	VAF (%)	Coverage	SIFT	Polyphen 2
1	SOCS1	c.621C > A	p.F207L	14	3077	Damaging	PD
	SOCS1	c.529C > G	p.L177V	31	1159	Damaging	PD
	SOCS1	c.428G > C	p.S143T	13	3976	Tolerated	Benign
	SOCS1	c.391C > T	p.Q131*	13	3999	Damaging	Damaging
	SOCS1	c.387C > G	p.H129Q	12	3992	Tolerated	Benign
	SOCS1	c.385C > T	p.H129Y	13	4000	Tolerated	pD
	SOCS1	c.374G > C	p.S125T	16	3996	Damaging	PD
	SOCS1	c.368C > T	p.P123L	13	4000	Damaging	PD
	SOCS1	c.364G > T	p.G122*	15	3990	Damaging	Damaging
	SOCS1	c.347G > A	p.S116N	14	3999	Damaging	PD
	SOCS1	c.283G > A	p.A95T	19	3764	Tolerated	Benign
	SOCS1	c.259C > T	p.H87Y	11	3762	Damaging	Benign
	SOCS1	c.197G > A	p.R66H	40	653	Tolerated	PD
	SOCS1	c.136C > T	p.P46S	33	1694	Tolerated	Benign
	SOCS1	c.49G > A	p.A17T	12	1033	Tolerated	Benign
	SOCS1	c.47C > T	p.A16V	27	1032	Tolerated	Benign
	SOCS1	c.37G > A	p.V13I	26	1035	Tolerated	Benign
	SOCS1	c.34G > T	p.A12S	26	1037	Tolerated	PD
	SOCS1	c.22G > A	p.A8T	26	1033	Tolerated	Benign
2	SOCS1	c.346A > T	p.S116C	26	158	Damaging	PD
	SOCS1	c.523C > T	p.Q175*	31	464	Damaging	Damaging
	ID3	c.189G > C	p.Q63H	38	327	Damaging	pD
3	SOCS1	c.199_209delATCACGCGCGC	p.I67fs	58	380	Damaging	Damaging
	TNFAIP3	c.1368_1369insGGGG	p.P457fs	15	1956	Damaging	Damaging
	TNFAIP3	c.111_112insT	p.H38fs	20	3919	Damaging	Damaging
	TNFAIP3	c.1251delA	p.K417fs	40	3951	Damaging	Damaging
	EZH2	c.1904_1905insAA	p.N635fs	10	440	Damaging	Damaging
	XPO1	c.1711G > A	p.E571K	32	1833	Damaging	pD
	PIM1	c.131C > A	p.A44D	34	200	Uncertain	pD
	GNA13	c.199C > T	p.Q67*	78	196	Damaging	Damaging
	EP300	c.4645dupA	p.K1549fs	23	322	Damaging	Damaging

*Stop codon mutation.
del indicates deletion; fs, frameshift; ins, insertion; pD, possibly damaging; PD, Probably damaging.

Results- NGS

- ✓ The mean read depth of the NGS analysis was 1694 (range, 158 to 4000).
- ✓ Aberrations in SOCS1 gene were found in all 3 cases (100%) :

  Eighteen of 22 (82%) were missense, 3 were nonsense mutations, and 1 was a frameshift deletion
- ✓ Three mutations in TNFAIP3 gene were identified in case 3; all of them are frameshift deletion/insertion
- ✓ Other mutated genes were ID3, XPO1, EZH2, PIM1, GNA13, and EP300

Discussion 1: aberrant expression of cyclin D1

Diseases with cyclin D1 expression	Genetic changes of Cyclin D1	DDx
MCL	IGH/CCND1：t(11;14)（q13; q32）	SOX11+, CD5+
hairy cell leukemia	/	CD20+,CD22+, Annexin A1+, CD5-, CD10-; VH基因体细胞突变
a subset of plasma cell myeloma	strong cyclin D1 expression is associated with t(11;14) and high CCND1 mRNA expression heterogenous cyclin D1 expression is mostly associated with polysomy 11	CD79a+, CD38+, CD138+
DLBCL	12% (7/60) revealed copy number gains of CCND1 gene	SOX11-, CD5-
NLPHL	Polysomy with increased copies of CCND1	/

TABLE 4. Cyclin D1 Expression and *CCND1* Gene Aberrations in DLBCL With Literature Review

References	Case No.	Cyclin D1	CD5	SOX11	*CCND1* FISH
Ehinger et al[32]	8 (3.5% of DLBCL)	Weak and focal to diffuse	Negative	ND	2 increased copies and 3 negative
Rodriguez-Justo et al[33]	1	60% of tumor cells	Negative	ND	3-4 copies
Teruya-Feldstein et al[57]	1	40% of tumor cells	Negative	ND	ND
Schneider et al[58]	1	30% of tumor cells	Positive	Negative	Negative
Metcalf et al[59]	4 (2% of DLBCL)	Strong and diffuse	ND	ND	Negative in 2 tested cases
Vela-Chavez et al[60]	17 (15% of DLBCL)	Weak and focal	Negative	ND	Negative
Lucioni et al[61]	1	Moderate in 60% of tumor cells	Negative	Negative	Negative
Hsiao et al[62]	4 (1.5% of DLBCL)	60%, 30%, 50%, and 10% of tumor cells	Negative	Negative	Negative
Izquierdo et al[34]	1	Strong and diffuse	Negative	ND	3 copies
Ok et al[35]	30 (2.1% of DLBCL)	30%-40% of tumor cells	Negative	ND	3 increased copies and 25 negative
Total	68 (1.5%-15% of DLBCL)	Weak and focal to strong and diffuse	Only 1 case (1.4%) positive	All negative	7/60 of cases (12%) with increased copies

ND indicates not done.

Discussion 1 : aberrant expression of cyclin D1

- ✓PMBL *V.S* CD5−, pleomorphic or blastoid MCL

IHC, GEP and Mutational analysis demonstrated SOCS1 mutations in the 3 cases, and TNFAIP3 and XPO1 mutations in case 3, supporting further the diagnosis of PMBL.

Discussion 2: the possibility of PMBL at nonmediastinal sites

TABLE 3. Nonmediastinal Primary Mediastinal Large B-Cell Lymphoma With Literature Review

References	Age (y)/Sex	Location	Molecular Findings	Stage	Treatment	Outcome (mo)
Chen et al[8,38,39]	39/M	Right lung	ND	IE	Pneumonectomy without chemotherapy	Bilateral adrenal spreading after 3 mo
Saarinen et al[37]	53/F	Between urinary bladder and uterus	MLL gene mutation	IV	NA	NA
Yuan et al[20]	53/M	Cervical LN	GEP signal of PMBL	I	R-CHOP	NED (100)
Yuan et al[20]	85/F	Periaortic and inguinal LN	GEP signal of PMBL	II	R-CHOP	DOD (66)
Yuan et al[20]	39/F	Kidney, adrenal gland, pancreas, small intestine	GEP signal of PMBL	IV	R-EPOCH	DOD (34)
Yuan et al[20]	19/M	Parotid gland	GEP signal of PMBL	I	R-CHOP	NED (93)
Case 1, current study	72/F	Submandibular LN	RT-MLPA and mutational analysis of PMBL	IA	R-CHOP	NED (25)
Case 2, current study	80/M	Submandibular LN	RT-MLPA and mutational analysis of PMBL	IA	R-CHOP	NED (19)

DOD indicates die of disease; F, female; M, male; NA, not available; ND, not done; NED, no evidence of disease.

Discussion 2: the possibility of PMBL at nonmediastinal sites

- ✓ ectopic thymus location
- ✓ common sites of PMBL involvement
- ✓ a recent study has hypothesized the origin of PMBL from a B cell that also gives origin to CHL with the propensity to migrate into the thymus but also to other organs

- ➢ According to the WHO classification, in order to establish a diagnosis of PMBL at nonmediastinal sites without evident mediastinal disease, **GEP analysis is mandatory to confirm the diagnosis**
- ➢ RT-MLPA classified 85% of the samples into the expected subtype

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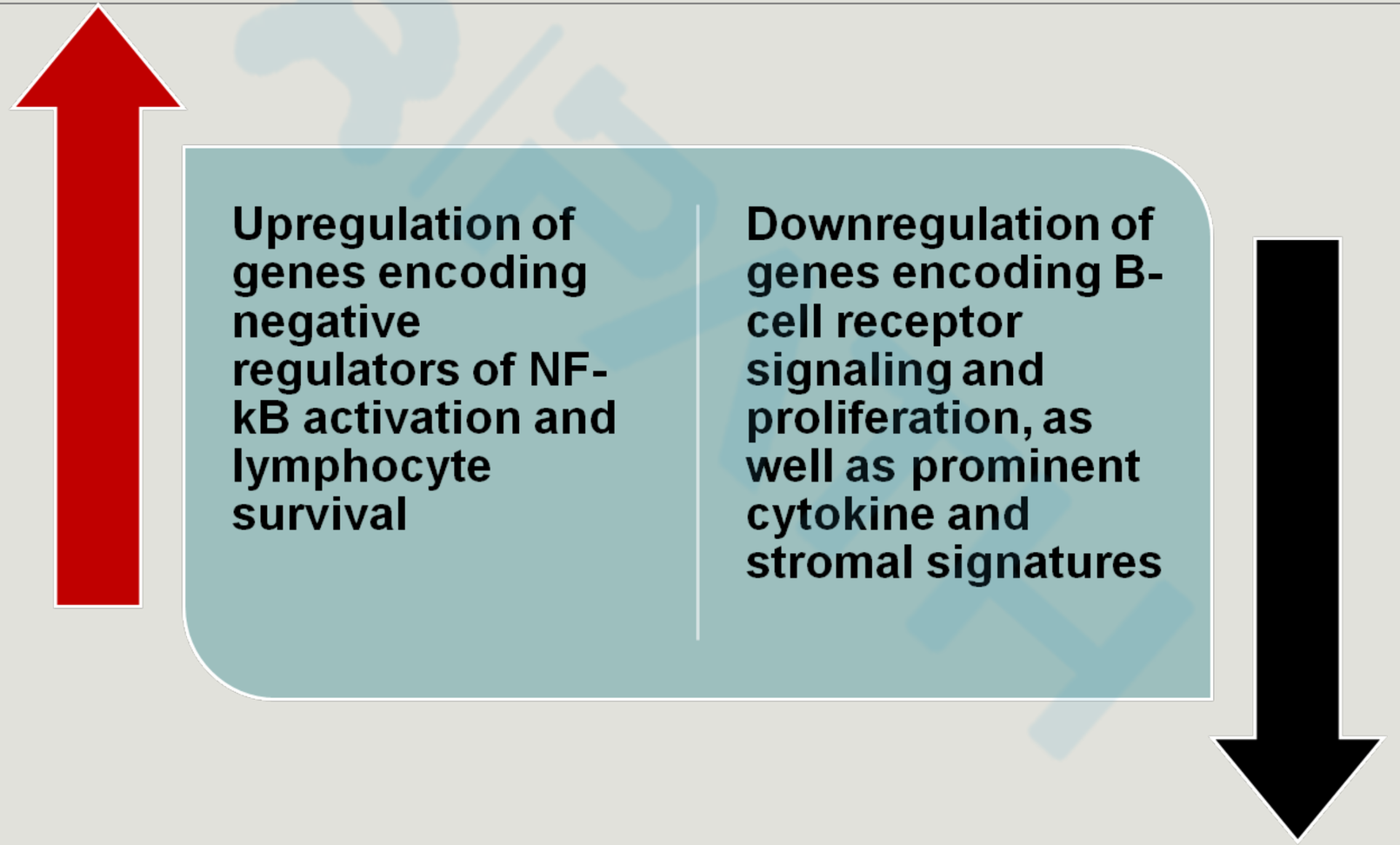
Discussion 3: CD30 $^{+}$DLBCL had a favorable prognosis



Discussion 4: PMBL & CHL

- ✓Similar in their mutational profile and show frequent mutations in TNFAIP3, SOCS1, STAT6, XPO1, and PTPN1 genes
- ✓SOCS1 mutations are reported in up to 56% of PMBL, 42% of CHL, and 50% of NLPHL, incontrast to 16% in GCB DLBCL, NOS
- ✓**a recent study has hypothesized the origin of PMBL from a B cell that also gives origin to CHL with the propensity to migrate into the thymus but also to other organs**

Conclusion

- ✓We report 3 cases of nodal mature large B-cell lymphomas with cyclin D1 expression and copy number gains of CCND1 gene but without translocation.
- ✓The morphology, phenotype, mutational landscape, and GEP supported the diagnosis of PMBL, although in 2 of the cases no mediastinal mass was present.
- ✓Our study also underlies the importance of investigating CD30, CD23, and MAL expression in DLBCL to identify possible cases of PMBL without evidence of mediastinal involvement to gain insight into this rather unusual nodal presentation.

THANK YOU!