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Wang Lu

Enhancer of zeste homolog 2 is widely expressed in T-cell neoplasms, is associated with high proliferation rate and correlates with MYC and pSTAT3 expression in a subset of cases

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AJSP, 2019

DAVID M. DORFMAN
MD, PHD
Brigham And Women's Hospital

My research focuses on the diagnosis and analysis of hematopoietic neoplasms, particularly those of the immune system, to understand the relationship of these neoplasms to their normal cellular counterparts, and to identify new biological markers and approaches for their diagnosis, subtyping, and treatment. This work has included studies of a number of important biomarkers for the characterization of B cell and T cell neoplasms and other hematopoietic neoplasms, a number of which are widely utilized in clinical practice, and include: immune checkpoint receptor Programmed Death-1 (PD-1), including the first report that it is a marker of angioimmunoblastic T cell lymphoma, as well as a study of PD-L1 and PD-L2 ligands, which found that they are expressed in epithelial malignancies and play a role in attenuating the anti-tumor immune response, the foundational work for checkpoint inhibitor blockade as a treatment for a wide variety of solid tumors; chemokine receptors, which are involved in chemotaxis and cell migration of immune system cells, including neoplastic cells, in B cell and T cell neoplasms; OX-2 membrane glycoprotein (CD200), an immunomodulatory molecule, in angioimmunoblastic T cell lymphoma and a subset of B cell lymphomas, including hairy cell leukemia and mediastinal large B cell lymphoma, hairy cell leukemia and lymphoplasmacytic lymphoma; T cell transcription factors in T cell and B cell neoplasms; EZH2 methytransferase, which contributes to tumor development in hematopoietic malignancies, in T cell and B cell neoplasms as well as histiocytic and dendritic neoplasms and Hodgkin lymphomas; CD5 in B cell and T cell neoplasms and thymic epithelial neoplasms.

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EZH2, p-ERK1/2, p-STAT, and MYC Expression in the Differential Diagnosis of hematopoietic neoplasms

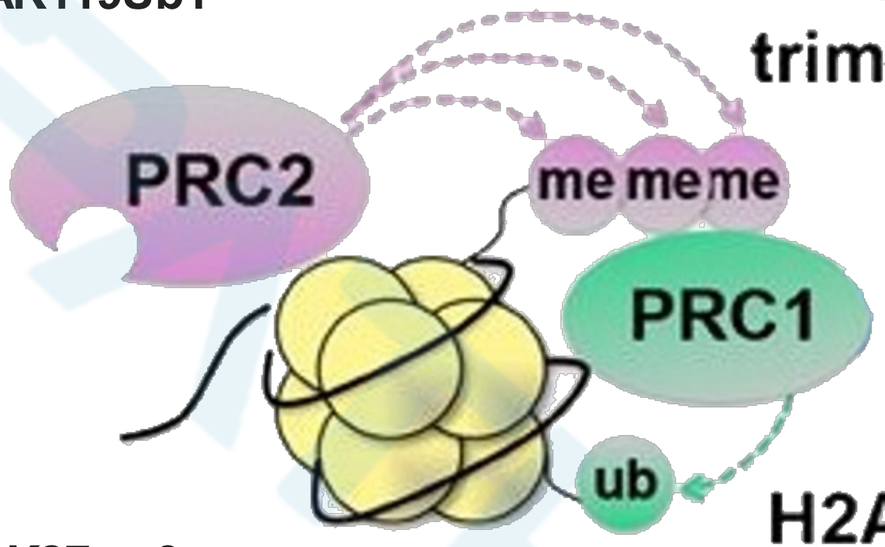
Background

PcG
Polycomb group proteins

polycomb repressive complex

PRC1 → **H2AK119Ub1**

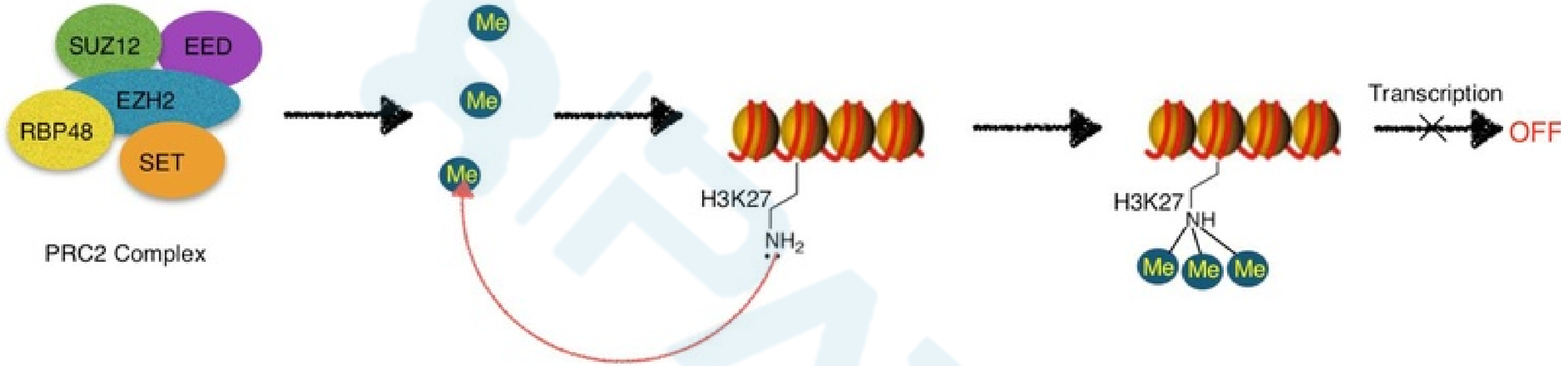
PRC2 → **H3K27me3**



H3K27 trimethylation

H2A monoubiquitination

Enhancer of zeste homolog 2，**EZH2**



EZH2 is expressed in stem cells and proliferating cells, which is down regulated in differentiated cells

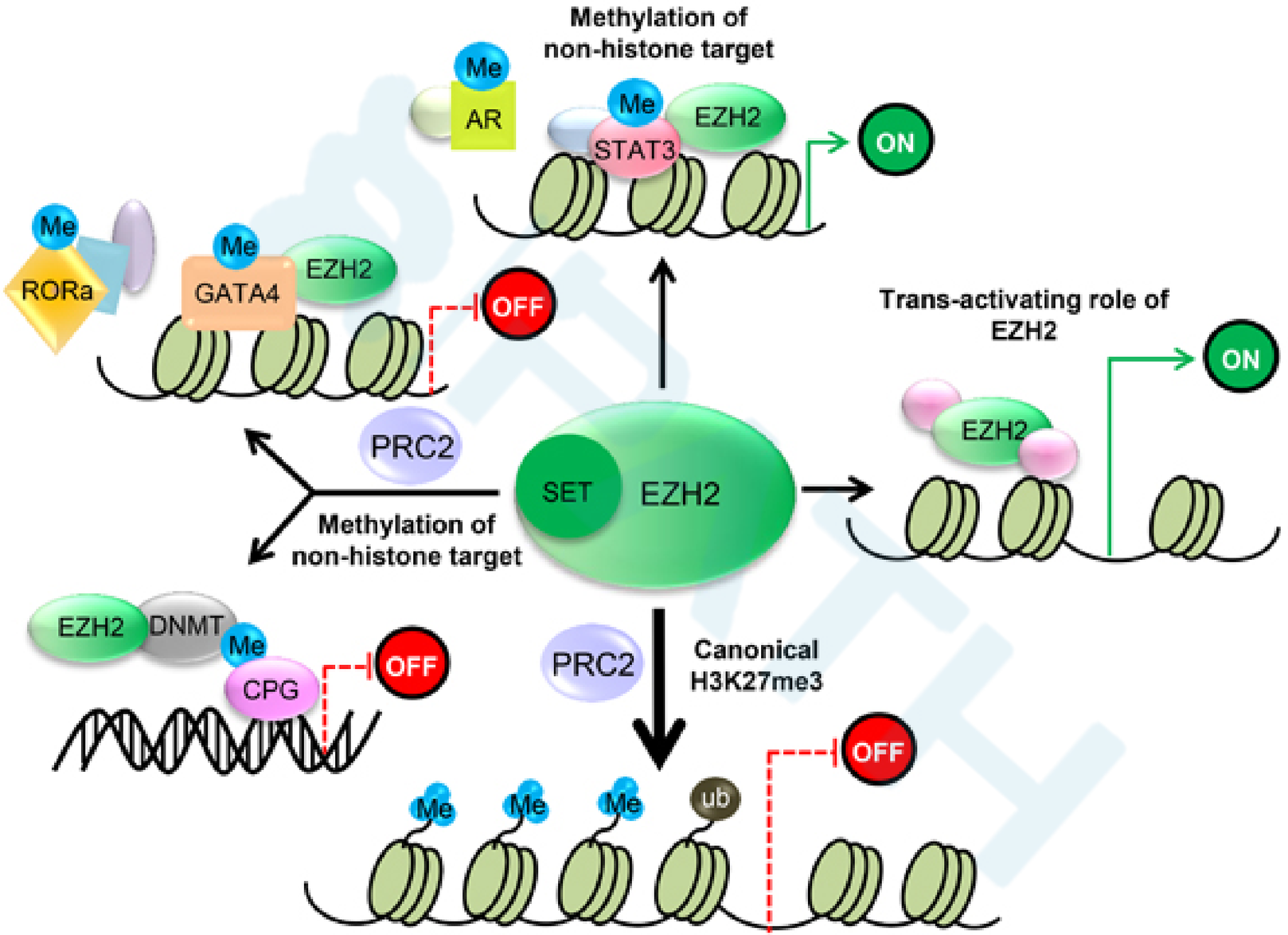
Mutation or over-expression of EZH2 has been linked to many forms of cancer

EZH2 inhibits genes responsible for suppressing tumor development, and blocking EZH2 activity may slow tumor growth

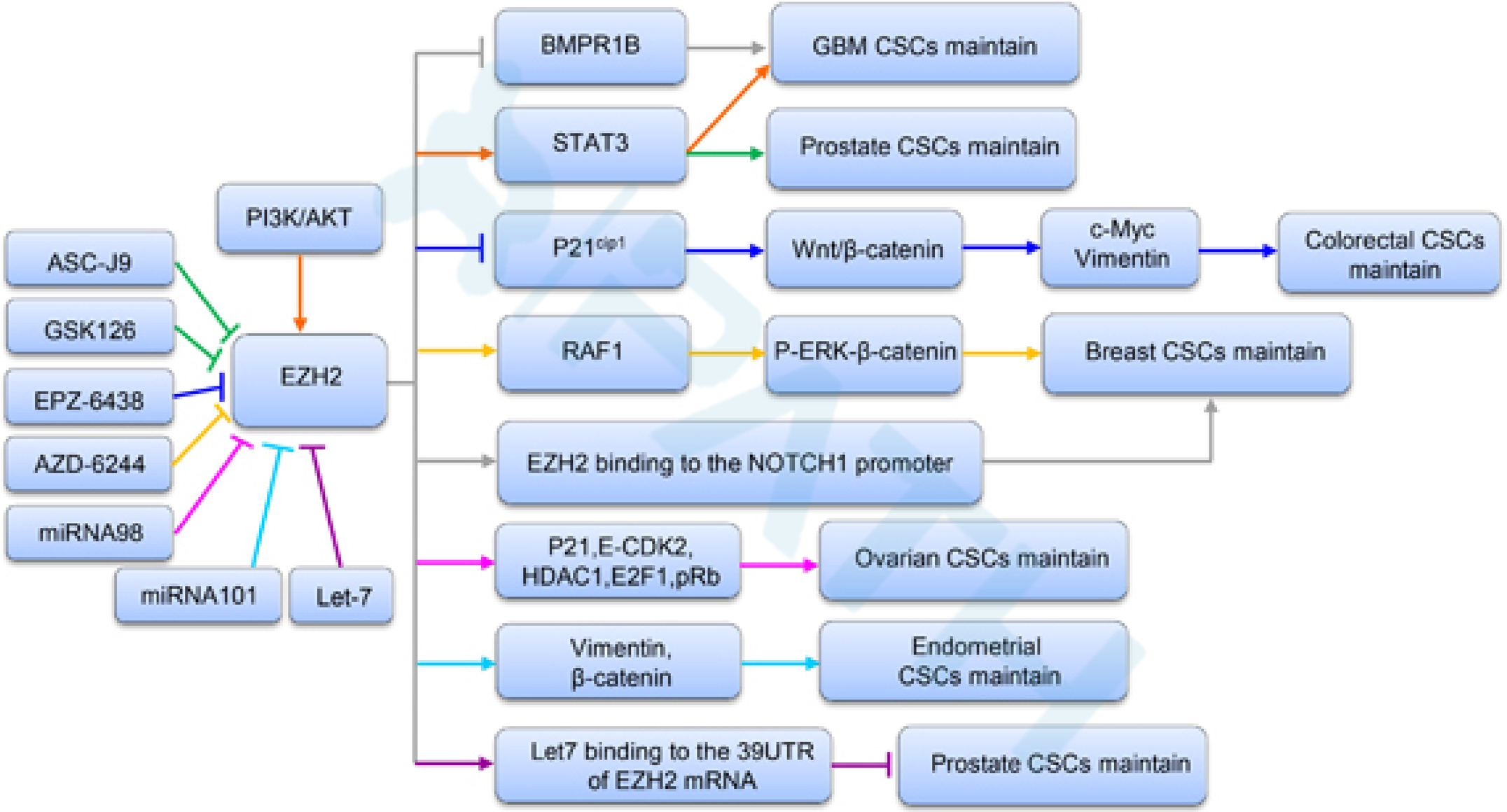
Expression of EZH2 in CSCs

Cancer type	Cell type	CSCs	EZH2	H3k27me3	Method
Breast cancer	HCC193, T47D, Primary tumor	CD44+CD24-	Upregulated	Upregulated	WB; qPCR
	Primary tumor	Spheroids	Upregulated	Upregulated	WB; qPCR
	SUM149, Primary tumor	ALDH+	Upregulated	Unknown	qPCR
	MCF7	CD44+CD24-	Downregulated	Downregulated	RT-PCR
Pancreatic cancer	HPAC, Panc-1, Primary tumor	EpCam+CD44+CD24+	Upregulated	Upregulated	WB; qPCR
	Primary tumor	Spheroids	Upregulated	Upregulated	WB; qPCR
Ovarian cancer	Primary tumor	CD44+CD117+	Upregulated	Unknown	qPCR
	Ascitic fluid	Side population	Upregulated	Unknown	qPCR
	IGROV1	Side population	Upregulated	Unknown	qPCR
Melanoma cancer	WM793, A375	Spheroids	Upregulated	Upregulated	WB
Skin cancer	SCC-13	Spheroids	Upregulated	Upregulated	WB
Colorectal cancer	SW480	CD133+CD44+	Upregulated	Unknown	qPCR
	SW480	Spheroids	Upregulated	Unknown	WB
Leukemia	CML mice cells	Lin-c-Kit+Sca1+	Upregulated	Unknown	qPCR
	CML patient cells	CD34+CD38-	Upregulated	Upregulated	qPCR
Hepatocarcinoma	HUH7	Side population	Downregulated	Downregulated	RT-PCR

Oncotarget. 2017



Oncotarget. 2017



Oncotarget. 2017

EZH2 **OVER-EXPRESSION**

- MEK/ERK/Elk-1(triple-negative and ERBB2+ breast cancer)
- AKT(Ser473) phosphorylation (breast carcinomas and NSCLC)
- MYC (prostate and hepatocellular carcinomas)
- NF-YA (ovarian cancer)
- STAT3 (colorectal carcinoma)

EZH2 **OVER-EXPRESSION**

is associated with tumor cell proliferation, metastasis, and poor prognosis

•Follicular lymphomas and DLBCL, GCB type: Y641 (recurrent somatic mutation) —— increased enzymatic activity

•B-cell lymphomas: A687V and A677G (gain of function mutations)

•can be blocked with small molecule inhibitors

EZH2 **LOSS OF TUMOR SUPPRESSOR FUNCTION**

- Myeloid malignancies: homozygous and heterozygous EZH2 deletions or inactivating mutations —— predictive of poor survival
- T-cell acute lymphoblastic leukemia: loss of function mutations and gene deletions —— inactivation of the EZH2 gene
- NK/T-cell lymphoma: JAK3 phosphorylation at Y244 —— promote oncogenesis by switching to a transcriptional activator
- Carcinomas of breast, colon, and other organs: EZH2 acts as a transcriptional activator

Previously work 1 (Leukemia Lymphoma, 2015)

Anaplastic large cell lymphoma, ALK positive 15

Anaplastic large cell lymphoma, ALK negative 15

Peripheral T-cell lymphoma, 18

Angioimmunoblastic T-cell lymphoma, 19

Extranodal NK/T-cell lymphoma, nasal type 19

Adult T-cell leukemia/lymphoma, 7

T-lymphoblastic leukemia/lymphoma, 10

- EZH2 and pSTAT3 was considered as positive if ⩾ 20%
- EZH2 ⩾ 60% (2 + or 3 +)
- Ki-67 high if ⩾ 40%
- MYC ⩾ 50%

Table I. EZH2 and Ki-67 immunoreactivity in T-cell lymphomas.

Neoplasm	EZH2*	Ki-67†
Anaplastic large cell lymphoma, ALK positive	14/15 (93%)	12/15 (80%)
Anaplastic large cell lymphoma, ALK negative	15/15 (100%)	12/13 (92%)
Peripheral T-cell lymphoma, NOS	16/18 (89%)	12/18 (67%)
Angioimmunoblastic T-cell lymphoma	16/19 (79%)	15/19 (79%)
Extranodal NK/T-cell lymphoma, nasal type	18/20 (90%)	15/19 (79%)
Adult T-cell leukemia/lymphoma	7/7 (100%)	6/7 (86%)
T-lymphoblastic leukemia/ lymphoma	10/10 (100%)	6/8 (75%)

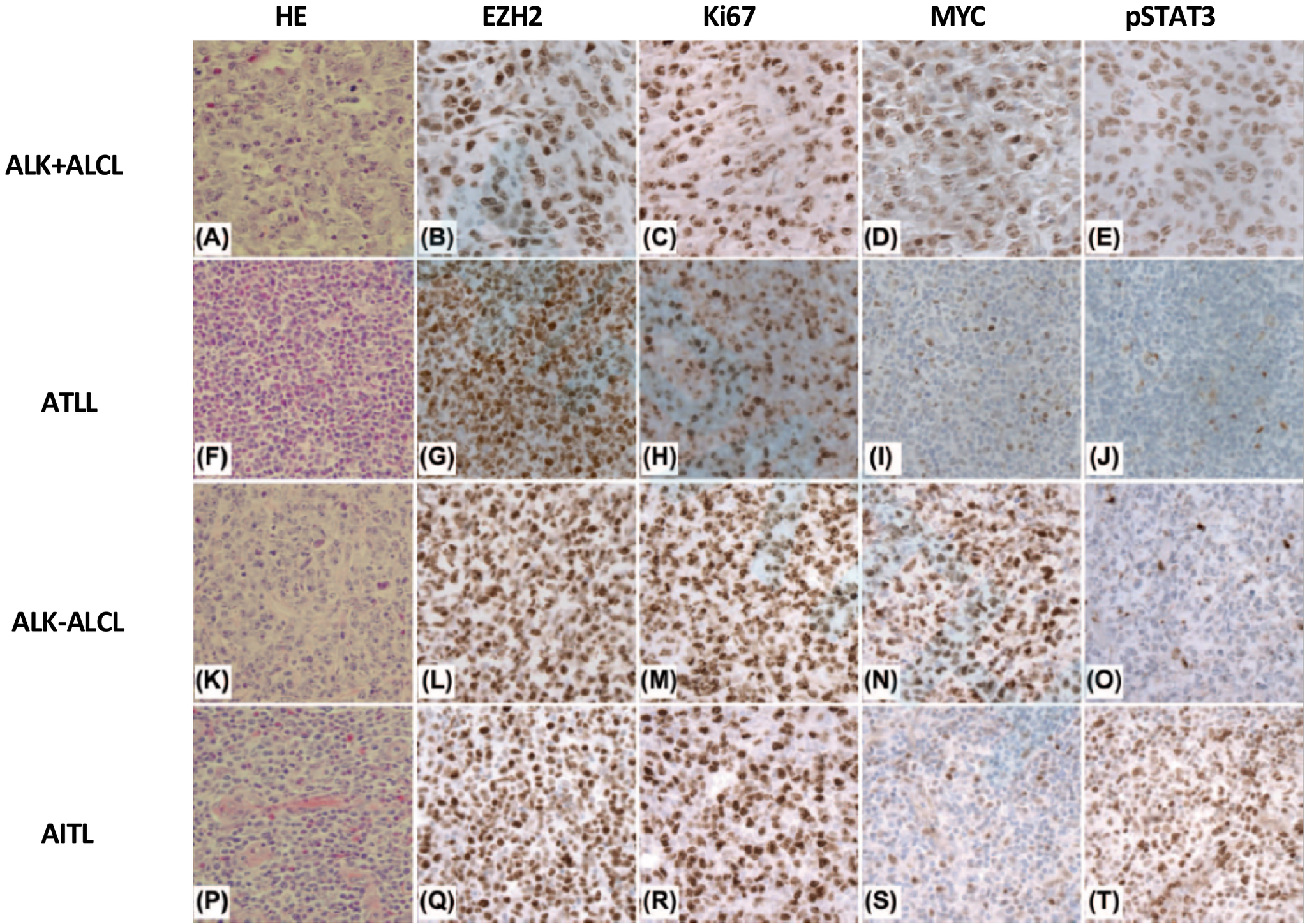

HE
EZH2
Ki67
MYC
pSTAT3
ALK+ALCL
ATLL
ALK-ALCL
AITL
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)
(P)
(Q)
(R)
(S)
(T)

Table II. EZH2, MYC and pSTAT3 immunoreactivity in T-cell lymphomas.

Neoplasm	EZH2*	MYC†	pSTAT3‡
Anaplastic large cell lymphoma, ALK-positive	14/15 (93%)	14/15 (93%)	12/15 (80%)
Anaplastic large cell lymphoma, ALK-negative	15/15 (100%)	12/14 (86%)	5/14 (36%)
Peripheral T-cell lymphoma, NOS	16/18 (89%)	10/18 (56%)	7/18 (39%)
Angioimmunoblastic T-cell lymphoma	15/19 (79%)	4/19 (21%)	10/19 (53%)
Extranodal NK/T cell lymphoma, nasal type	18/20 (90%)	11/20 (55%)	11/18 (61%)
Adult T-cell leukemia/lymphoma	7/7 (100%)	0/7 (0%)	0/7 (0%)
T-lymphoblastic leukemia/lymphoma	10/10 (100%)	0/10 (0%)	0/9 (0%)

- High Ki-67 proliferation index correlated with a high level of EZH2 expression
- MYC is highly expressed in most cases of ALCL, but not in ATLL and T-ALL， same as pSTAT3
- pSTAT3 expression is correlated with ALK expression, but not EZH2 expression
- EZH2 and MYC/pSTAT3 are coordinately expressed in only a subset of T-cell lymphomas, and that other mechanisms control the overexpression of EZH2 in many T-cell lymphomas

Previously work 2 (Modern Pathology, 2016)

- Histiocytic and dendritic cell neoplasm 65 cases

12 cases of blastic plasmacytoid dendritic cell neoplasm (BPDCN)

17 cases of histiocytic sarcoma (HS)

15 cases of follicular dendritic cell sarcoma (FDCS)

16 cases of Langerhans cell histiocytosis (LCH)

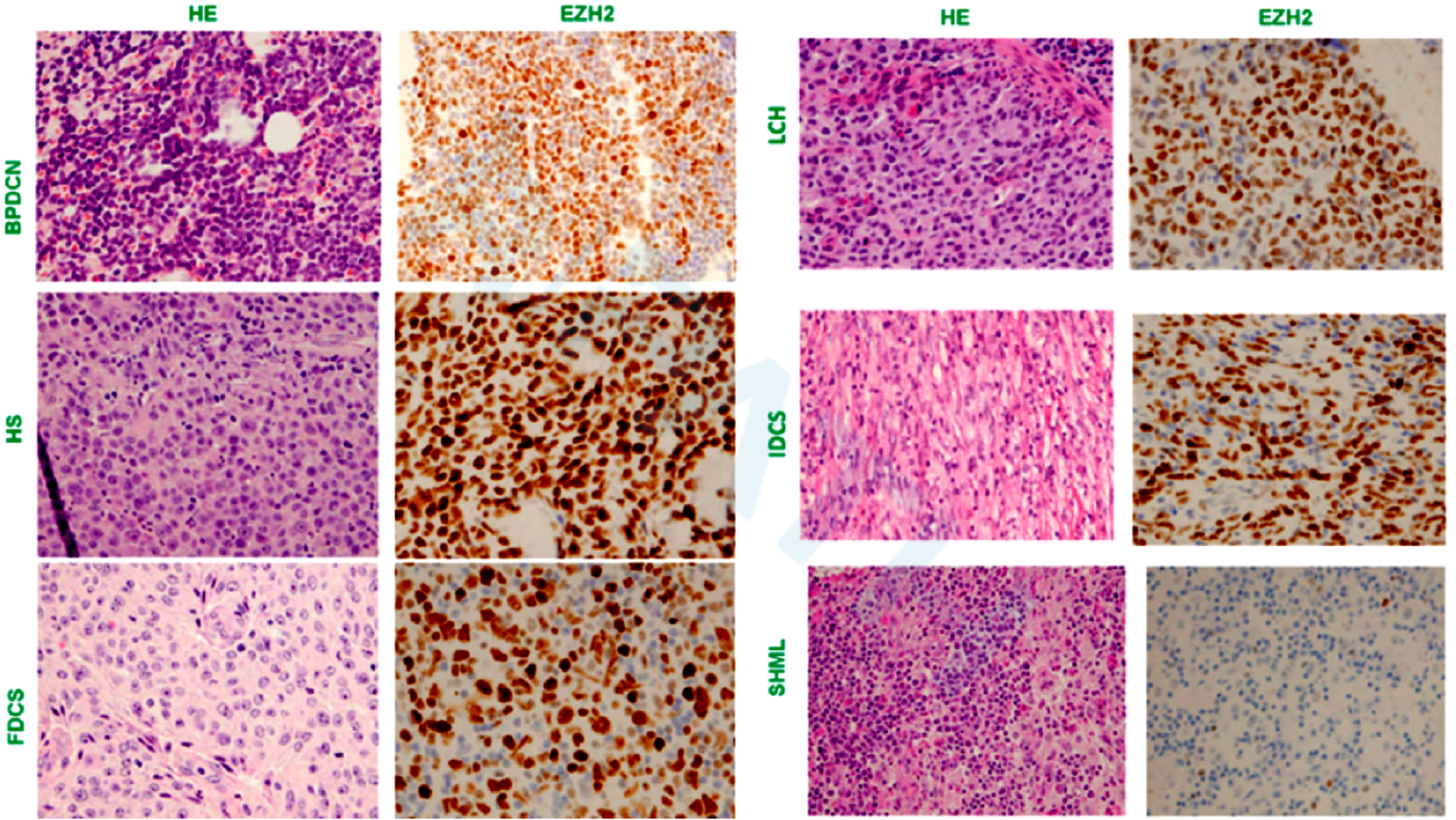
5 cases of interdigitating dendritic cell sarcoma (IDCS)

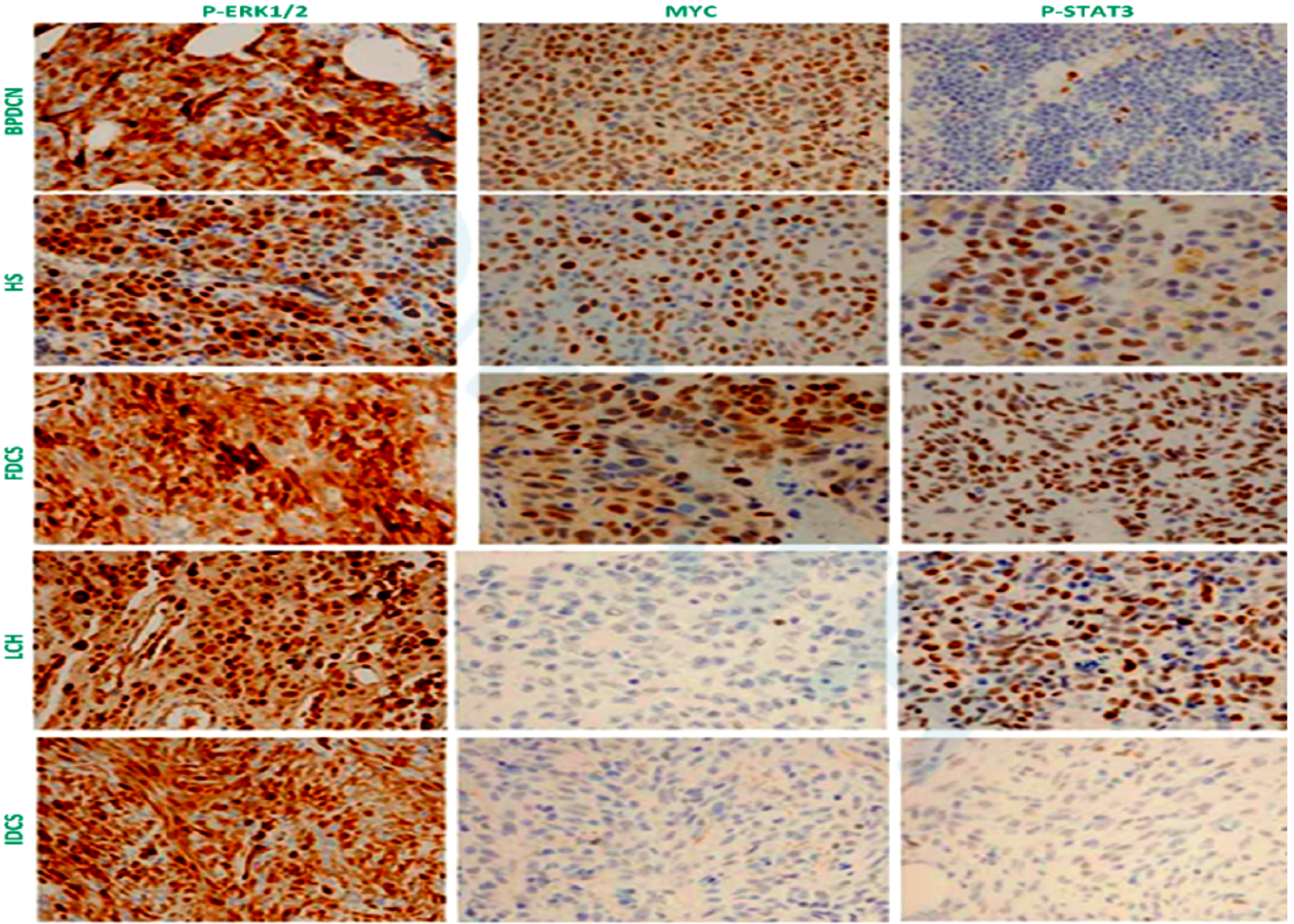
- Benign histiocytic diseases 9 cases

6 cases of sinus histiocytosis with massive lymphadenopathy (SHML)

3 cases of juvenile xanthogranuloma

- EZH2 overexpressed if ⩾60% (2+ or 3+)
- P-ERK1/2, MYC, and p-STAT3 ⩾5% as positive


HE
EZH2
HE
EZH2
BPDCN
LCH
HS
IDCS
FDCS
SHML


P-ERK1/2
MYC
P-STAT3
BPDCN
HS
FDCS
LCH
IDCS

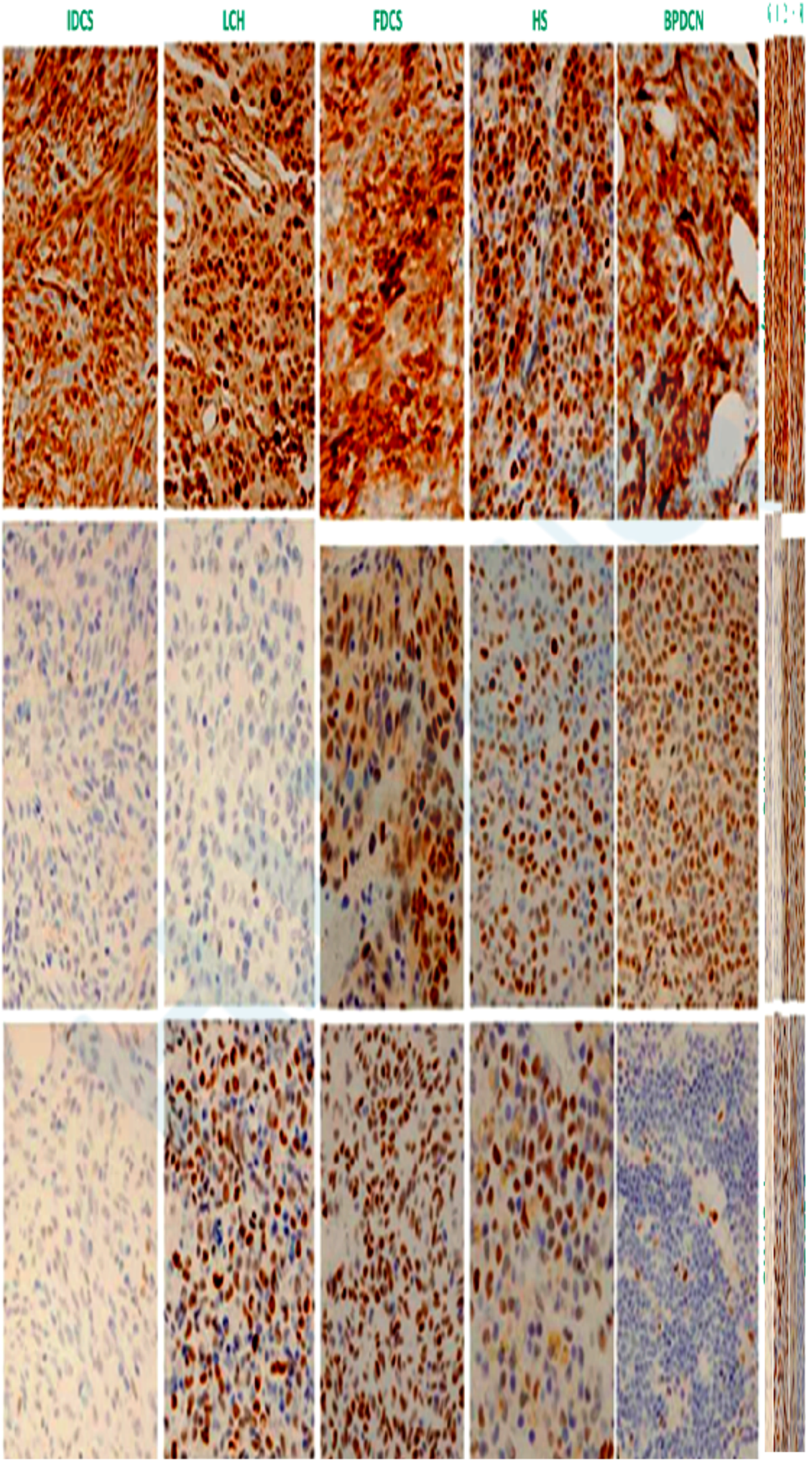
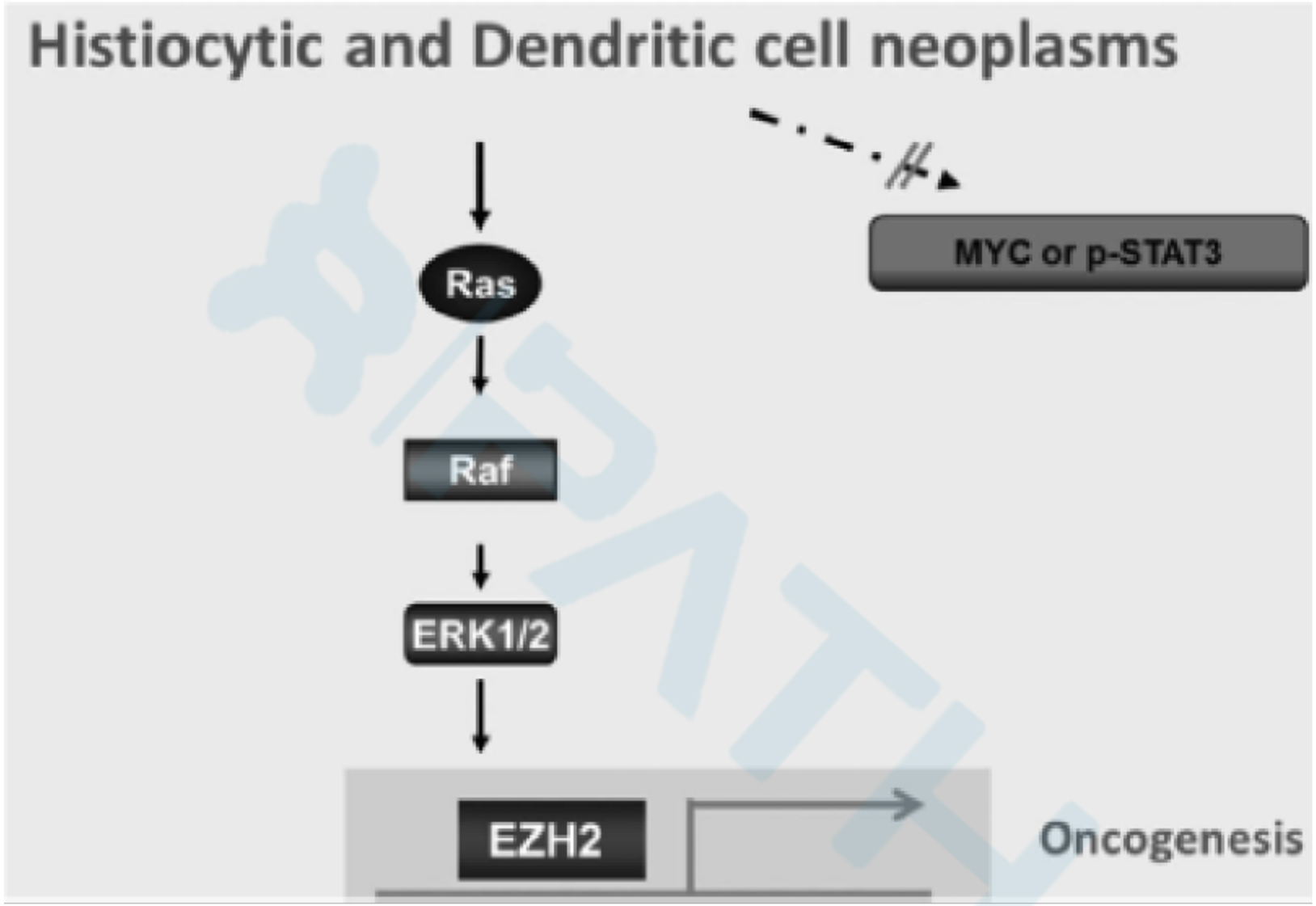

IDCS
LCH
FDCS
HS
BPDCN

Table 1 EZH2, p-ERK1/2, MYC, and p-STAT3 expression in histiocytic and dendritic cell neoplasms

	EZH2 (POS/ Total)	*p-ERK (POS/ Total)*	*MYC (POS/ Total)*	*p-STAT3 (POS/Total)*
BPDCN	5/12 (41%)	3/11(27%)	2/11(18%)	0/11(0%)
HS	10/17 (59%)	9/15 (60%)	3/11 (27%)	3/12 (25%)
FDCS	10/15 (67%)	12/15 (80%)	2/13 (15%)	4/13 (30%)
LCH	9/16 (56%)	10/16 (63%)	0/14 (0%)	1/15 (6%)
IDCS	3/5 (60%)	3/5 (60%)	0/5 (0%)	0/5 (0%)

Table 2 Co-expression of EZH2 with p-ERK1/2, MYC, and p-STAT3 proteins

	EZH2-positive cases (% positivity)	*p-ERK/EZH2*	*MYC/EZH2*	*p-STAT3/ EZH2*
BPDCN	5 (81 ± 10%)	2/5 (40%)	2/5 (40%)	0/5 (0%)
HS	10 (70 ± 13%))	7/10 (70%)	3/10 (30%)	2/10 (20%)
FDCS	10 (75 ± 12%)	9/10 (90%)	1/10 (10%)	3/10 (30%)
LCH	9 (67 ± 10%)	8/9 (89%)	0/9 (0%)	1/9 (11%)
IDCS	3 (71 ± 13%)	3/3 (100%)	0/3 (0%)	0/3 (0%)



EZH2 expression correlates with increased pERK1/2 expression, but not MYC or pSTAT3 expression, suggesting that the pERK signaling cascade may regulate EZH2 expression in these neoplasms

Previously work 3 (Modern Pathology, 2018)

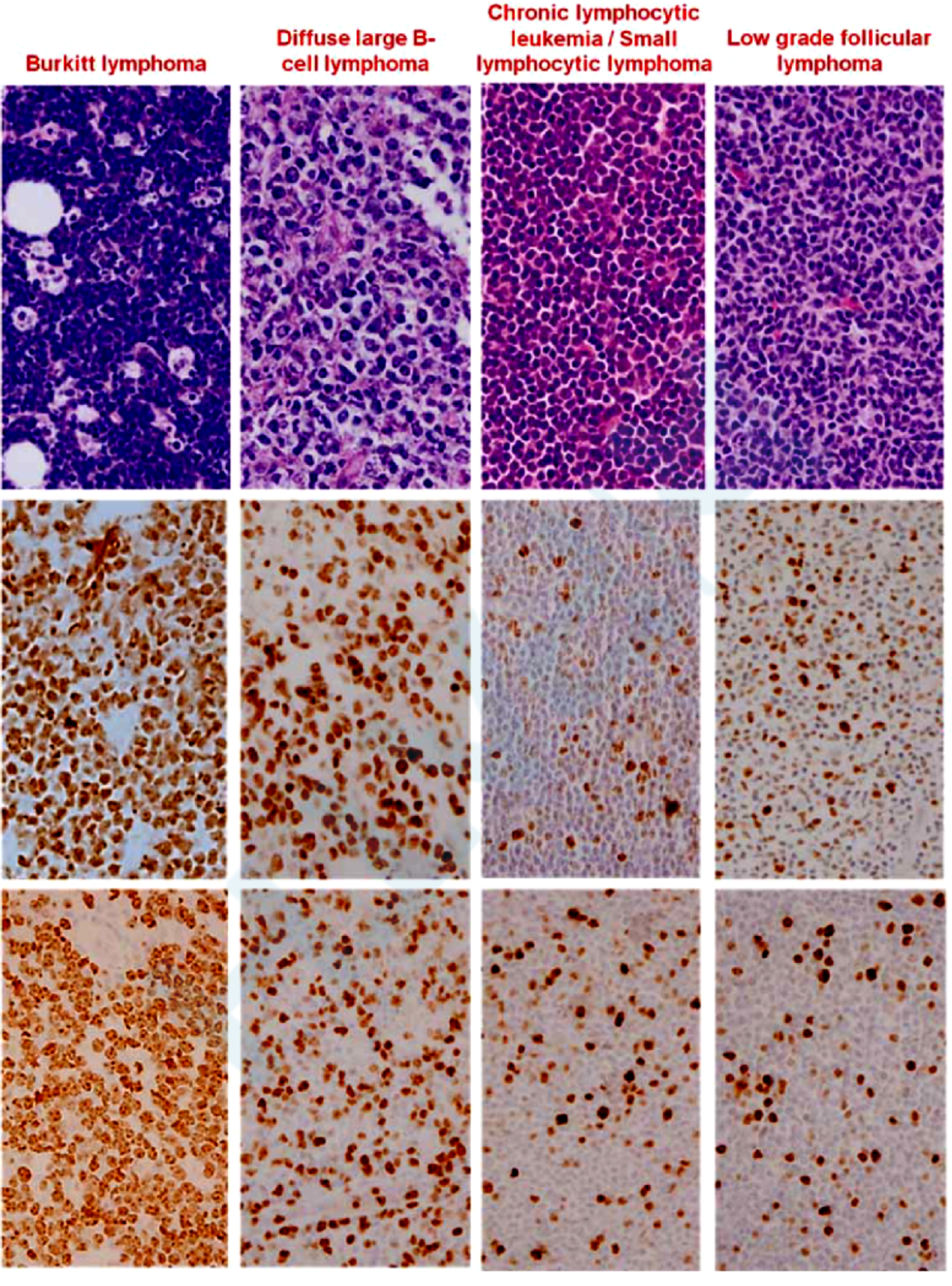
- High-grade B-cell lymphomas

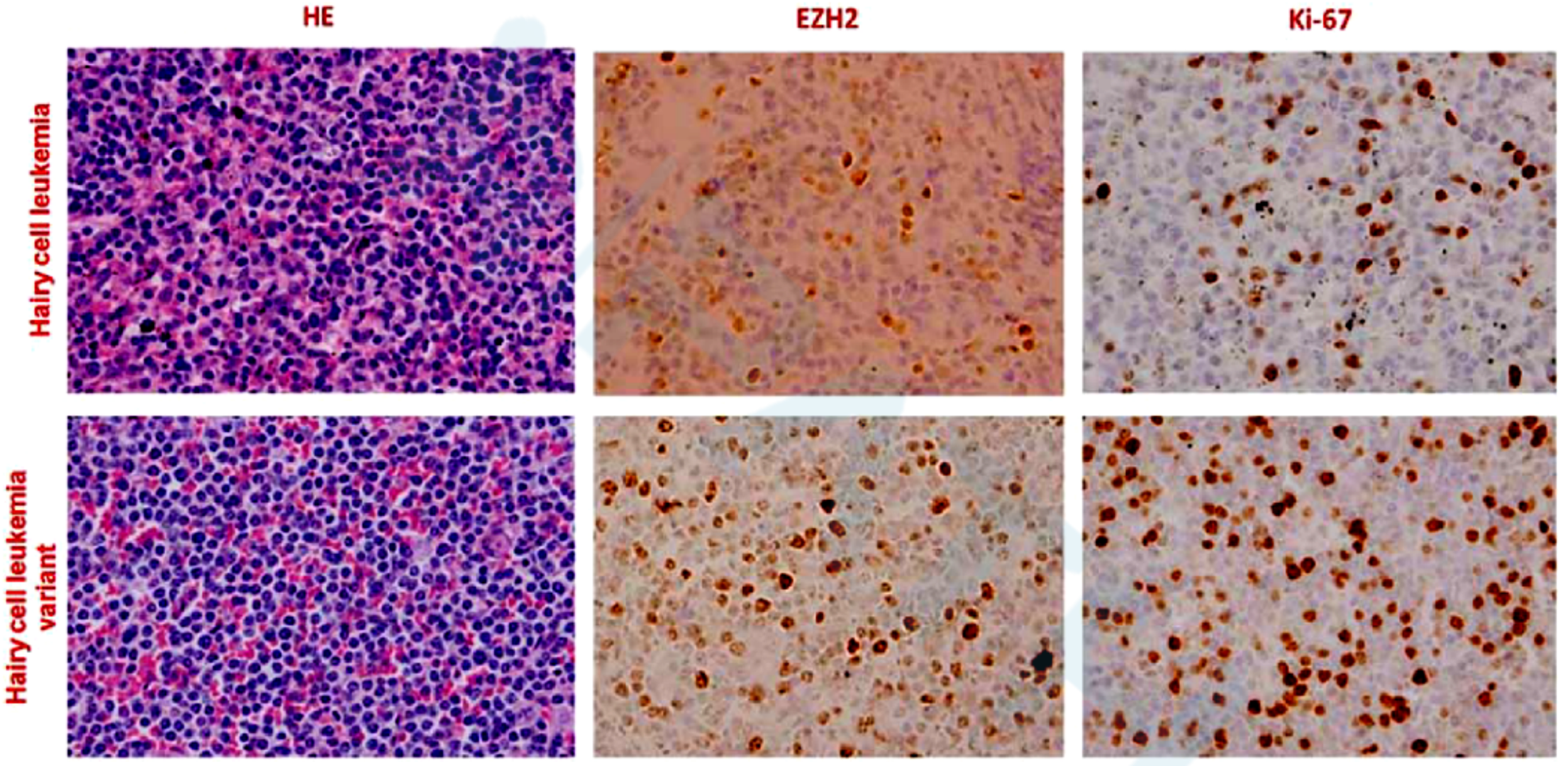
33 cases of DLBCL

19 cases of BL

22 cases of DHL 13 MYC+BCL2

9 MYC+BCL6


Burkitt lymphoma
Diffuse large B-cell lymphoma
Chronic lymphocytic leukemia / Small lymphocytic lymphoma
Low grade follicular lymphoma
HE
EZH2
Ki-67


HE
EZH2
Ki-67
Hairy cell leukemia
Hairy cell leukemia variant

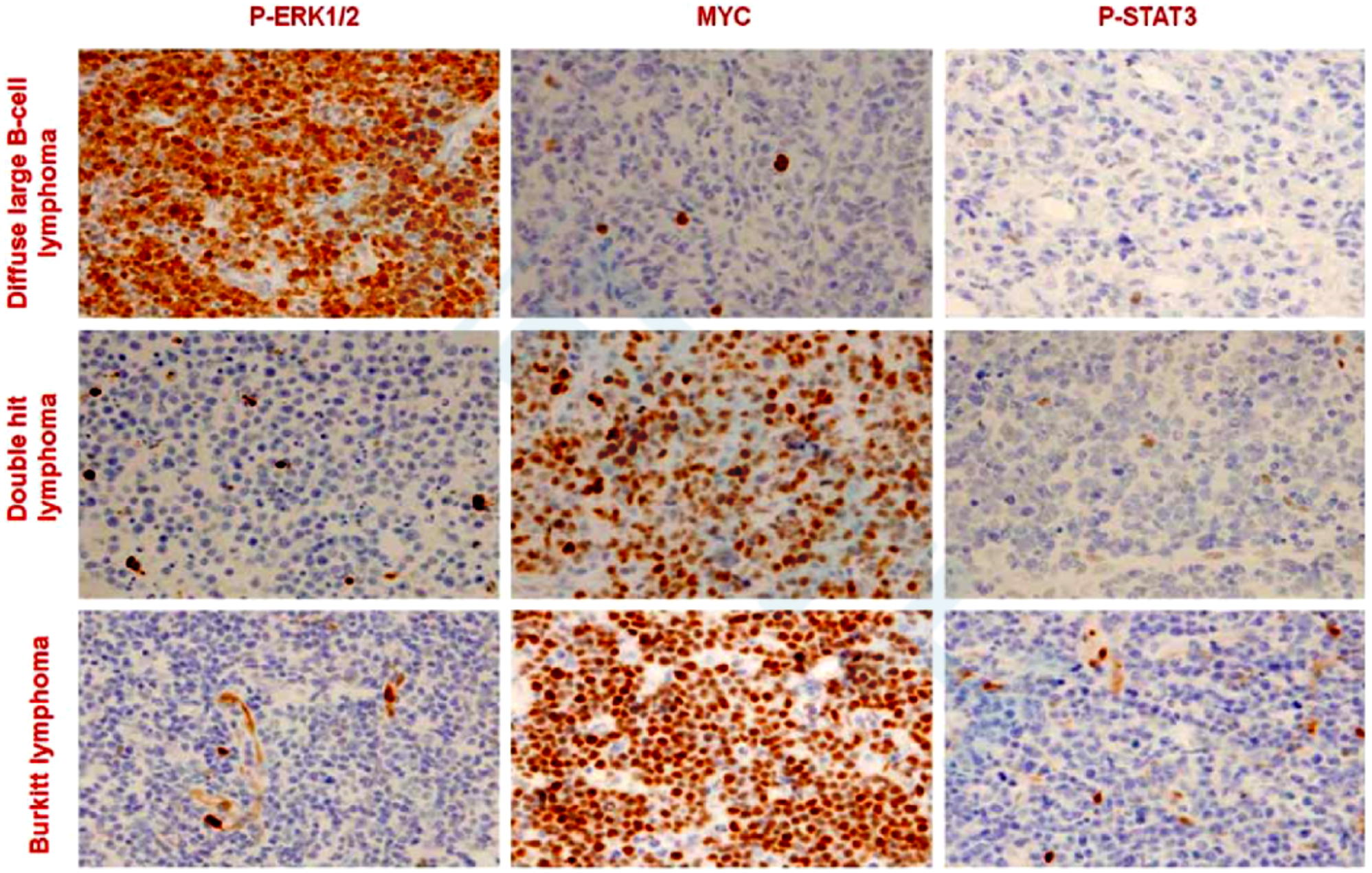
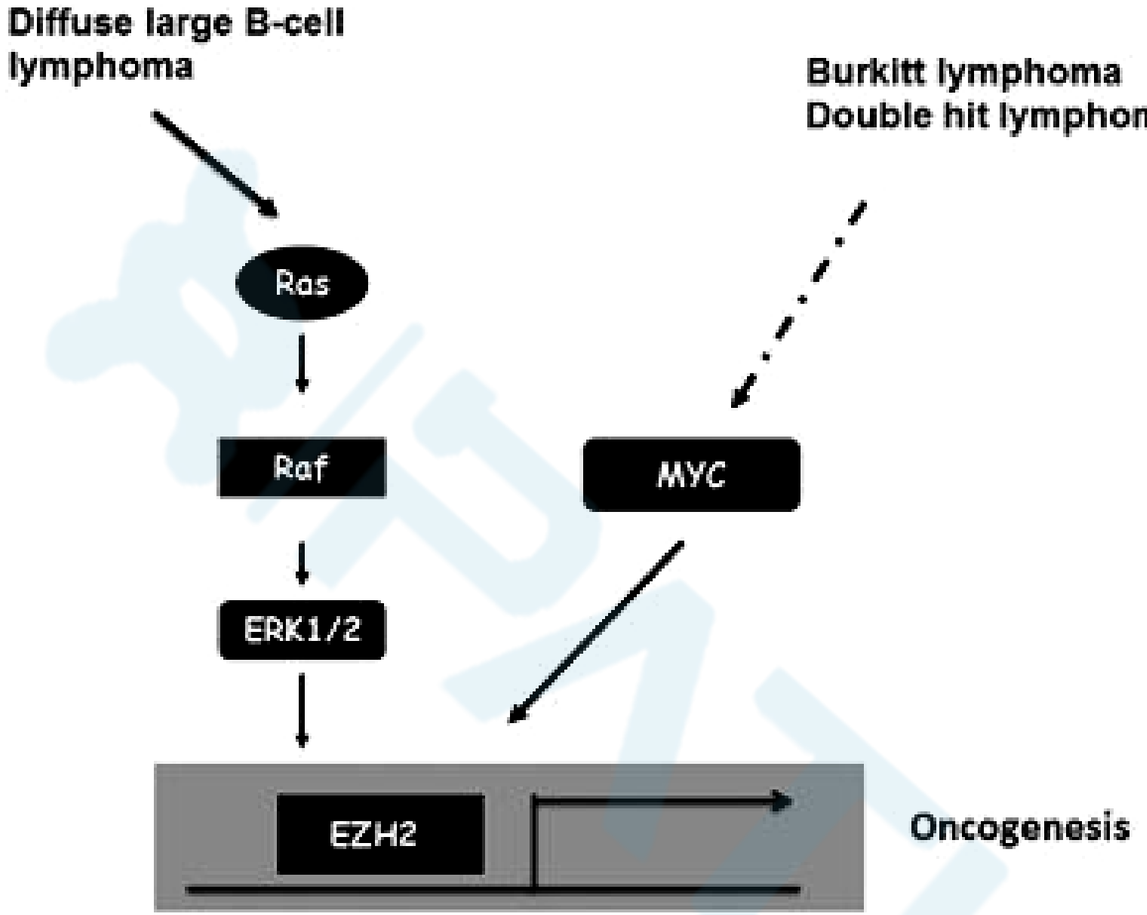

P-ERK1/2
MYC
P-STAT3
Diffuse large B-cell lymphoma
Double hit lymphoma
Burkitt lymphoma

Table 1 EZH2 and Ki-67 expression in small cell and aggressive B-cell lymphomas

Lymphoma type	*Cases, positive/total (%)*	*EZH2 positive (%)*	*Ki-67 proliferation rate (%)*
Small cell			
Multiple myeloma	10/12 (83%)	10	5–10
Lymphoplasmaytic lymphoma/Waldenstrom macroglobulinemia	9/12 (75%)	5–10	5–10
Chronic lymphocytic leukemia/small lymphocytic lymphoma	15/18 (83%)	10–15	10–15
Mantle cell lymphoma[a]	8/9 (89%)	30	20–25
Marginal zone lymphoma	20/21 (95%)	15–20	10
Follicular lymphoma (grades 1–2)	8/10 (80%)	20	15–20
Hairy cell leukemia[b]	13/14 (93%)	5–10	5–10
Hairy cell leukemia variant[b]	9/10 (90%)	30–40	30–35
Aggressive			
Diffuse large B-cell lymphoma	29/33 (87%)	90	70–75
Burkitt lymphoma	19/19 (100%)	100	95–100
Chronic lymphocytic leukemia—Richter transformation	5/5 (100%)	80–90	85
Follicular lymphoma (grade 3)[c]	6/6 (100)	80	85–90
Double hit lymphoma	22/22 (100)	90–100	75–80
Primary mediastinal large B-cell lymphoma	19/19 (100%)	60–80	90
B lymphoblastic leukemia/lymphoma	11/11 (100%)	95–100	75

Table 2 Co-expression of EZH2 and p-ERK1/2, MYC, or p-STAT3 in aggressive B-cell lymphomas

Lymphomas	*EZH2+p-ERK1/2 mean positivity (positive/total)*	*EZH2+MYC mean positivity (cases)*	*EZH2+p-STAT3 mean positivity (cases)*
Diffuse large B-cell lymphoma	57 ± 17% (24/30)[a]	20 ± 17% (30)[a]	< 10% (31)
Double hit lymphoma	10 ± 9% (17)[a]	76 ± 14% (29)[a]	< 10% (18)
Burkitt lymphoma	13 ± 10% (15)[a]	91 ± 7% (19)[a]	< 10% (17)



There is evidence for regulation of EZH2 by different signaling cascades in different types of ABCLs

p-ERK-related signaling in DLBCL

MYC-related signaling in Burkitt lymphoma and double hit lymphoma

Recently Work (AJSP, 2019)

- By comparing EZH2 and associated signaling molecules (p-ERK1/2, MYC, and p-STAT3) expression patterns in

Hodgkin lymphomas (NLPHL and cHL)

T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL)

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma (BCLu-DLBCL/cHL)

- To further understanding the relationship of these B-cell neoplasms to other aggressive B-cell lymphomas, DLBCL, and the Hodgkin lymphomas

M&M

- Case Selection (84 cases)

26 cases of NLPHL

31 cases of cHL

19 cases of THRLBCL

9 cases of BCLu-DLBCL/cHL

- Previously studied high-grade B-cell lymphomas

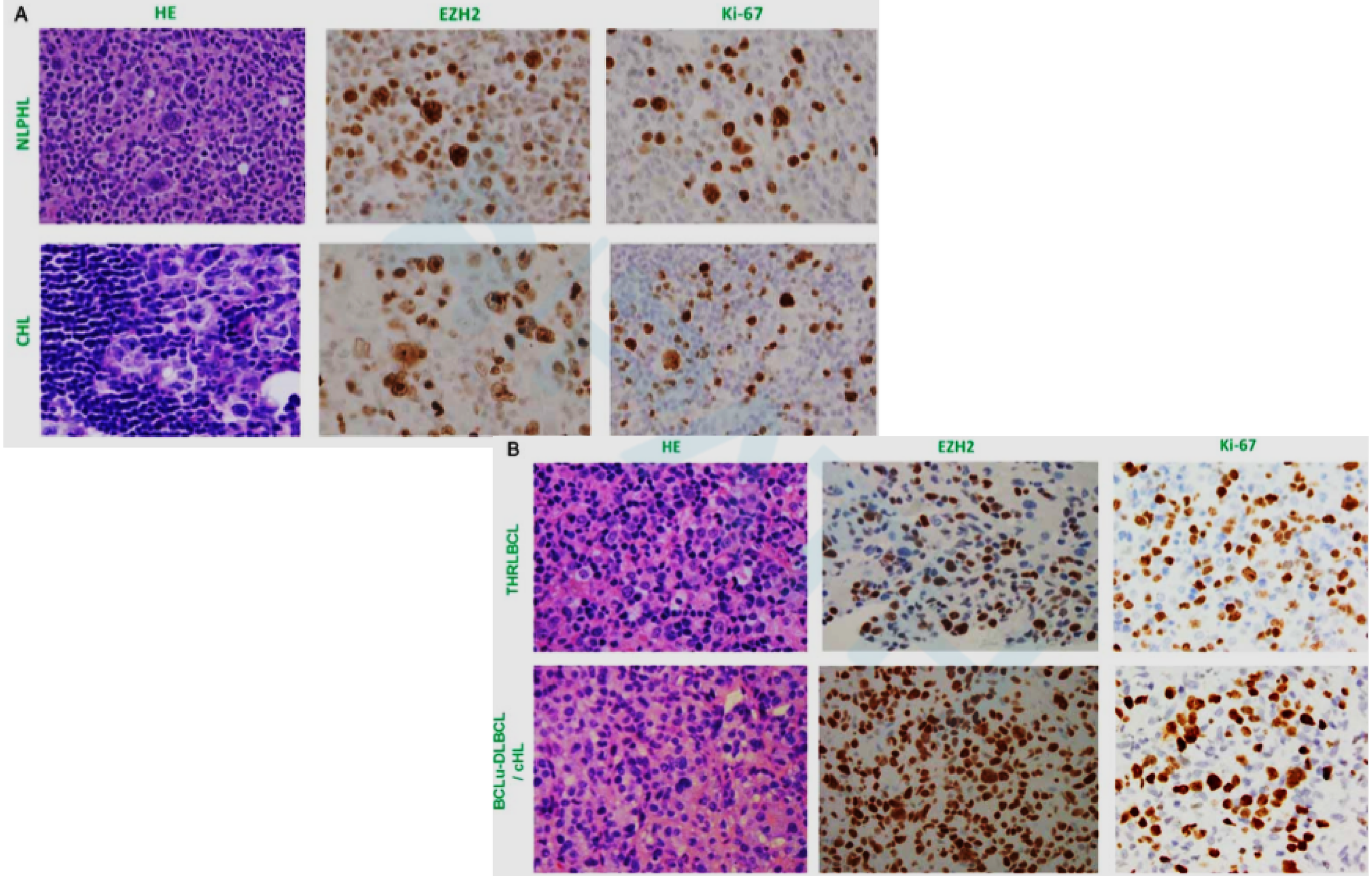
33 cases of DLBCL

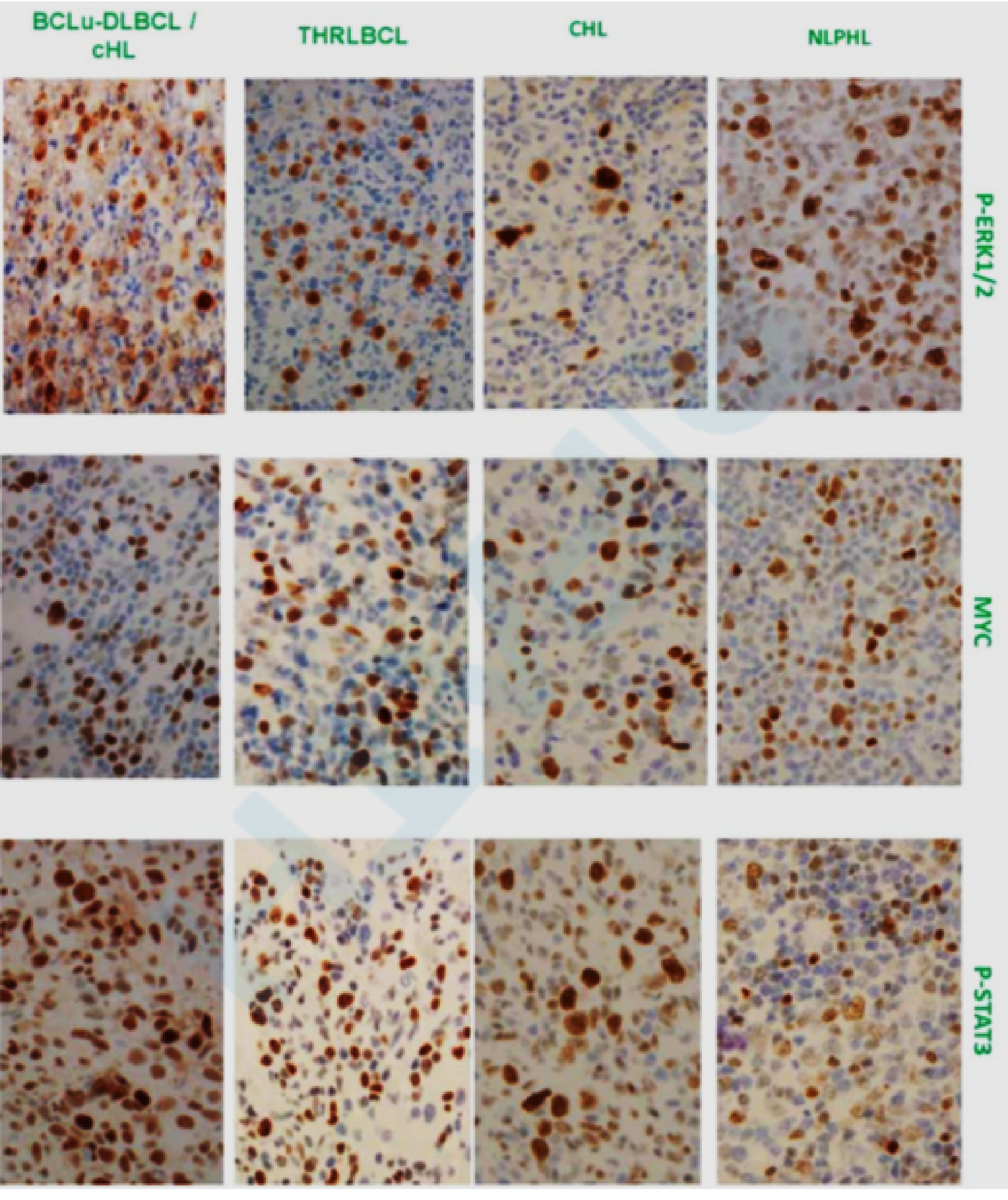
19 cases of BL

22 cases of DHL 13 MYC+BCL2

9 MYC+BCL6

- EZH2 ⩾60% 2+ or 3+
- MYC ⩾40%
- p-ERK1/2 and p-STAT3 ⩾30%


A
HE
EZH2
Ki-67
NLPHL
CHL
B
HE
EZH2
Ki-67
THRLBCL
BCLu-DLBCL / cHL


BCLu-DLBCL / cHL
THRLBCL
CHL
NLPHL
P-ERK1/2
MYC
P-STAT3

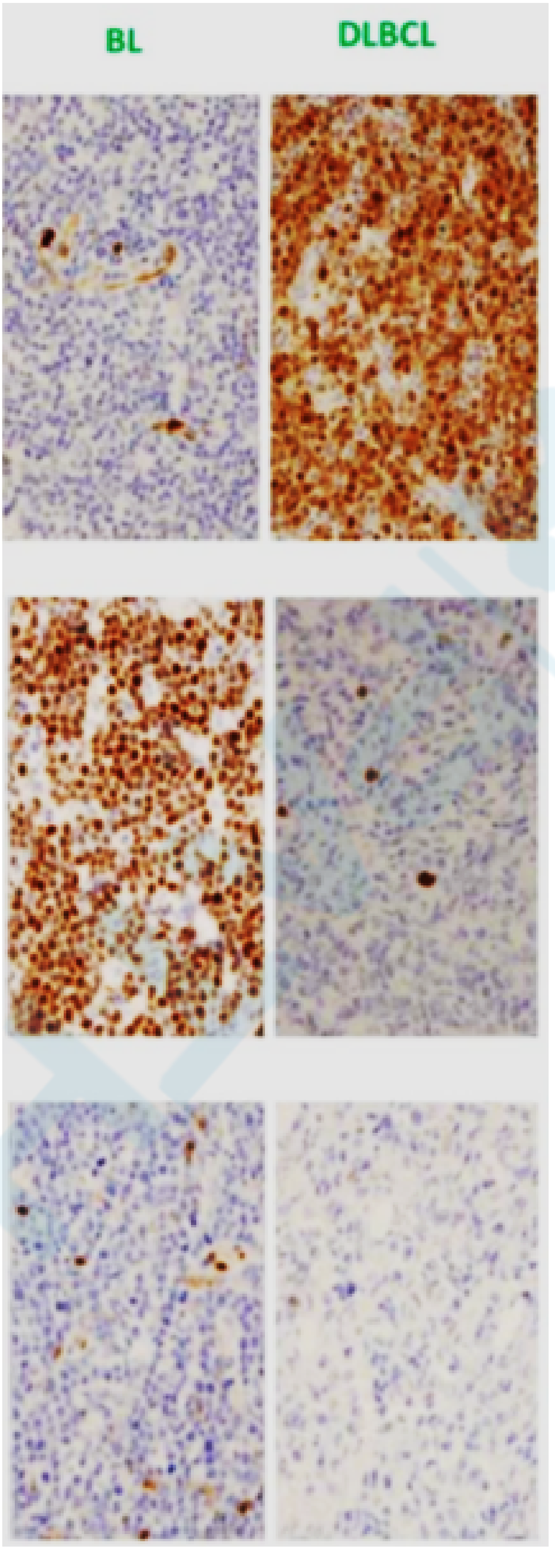
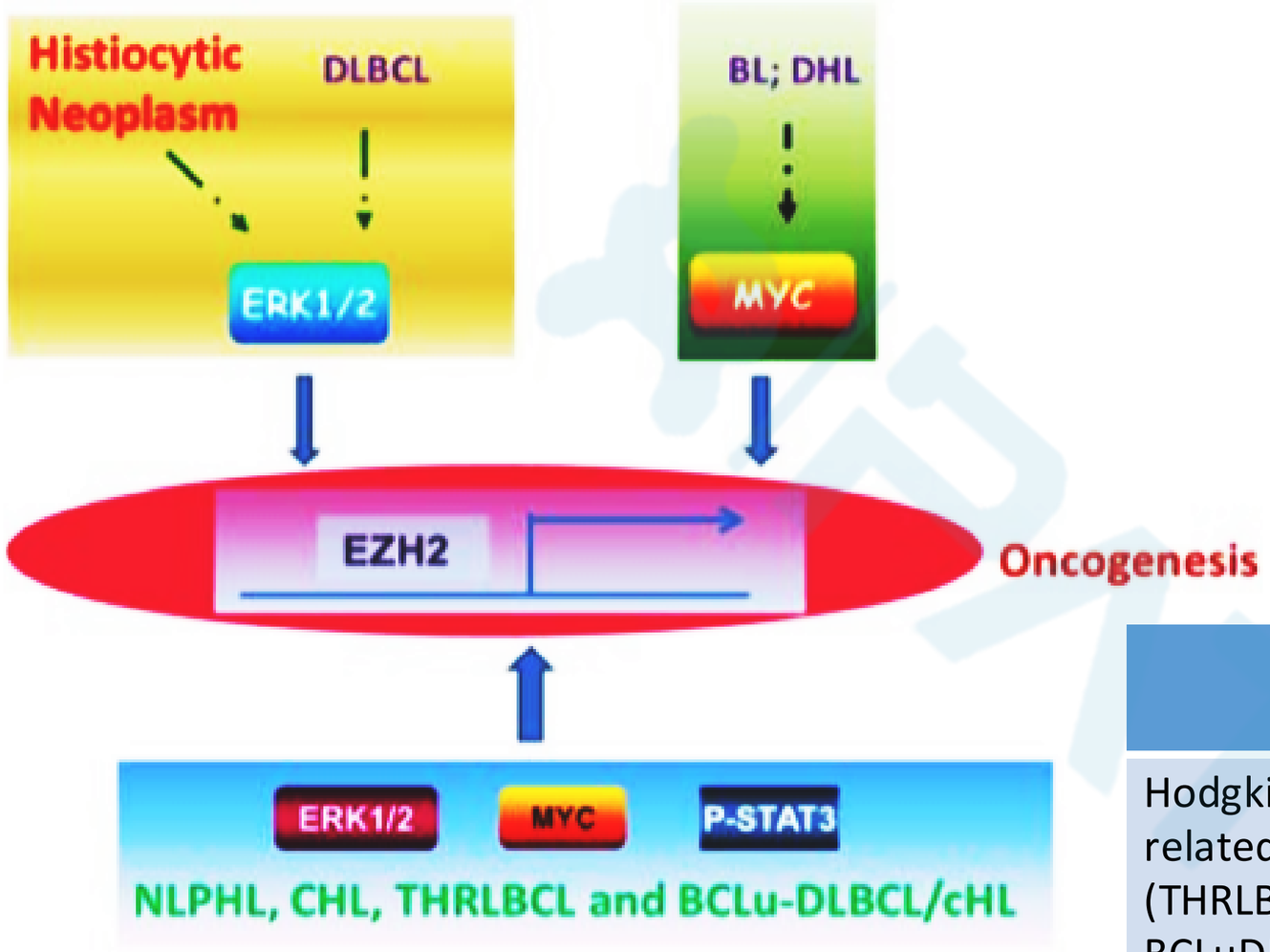

BL
DLBCL

TABLE 1. EZH2, p-ERK, MYC, and p-STAT3 Expression in NLPHL, cHL, THRLBCL, and BCLu-DLBCL/cHL, in Comparison With DLBCL, BL, and DHL

	n/N (%)				
	EZH2 (POS/Total)	**p-ERK (POS/Total)**	**MYC (POS/Total)**	**p-STAT3 (POS/Total)**	**p-ERK+MYC+p-STAT3 (POS/Total)**
NLPHL	26/26 (100)	26/26 (100)	25/25 (100)	19/19 (100)	19/19 (100)
cHL	31/31 (100)	30/30 (100)	27/27 (100)	24/25 (96)	23/24 (96)
THRLBCL	19/19 (100)	19/19 (100)	17/19 (89)	18/19 (100)	16/19 (84)
BCLu-DLBCL/cHL	8/9 (89)	6/9 (67)	9/9 (100)	7/8 (100)	5/8 (63)
TOTAL	84/85 (99)	81/84 (96)	78/80 (98)†	68/71 (96)†	63/70 (90)†
DLBCL*	29/33 (87)	28/30 (93)	8/30 (27)†	4/30 (13)†	2/25 (8)
BL*+DHL*	41/41 (100)	7/34 (21)	48/48 (100)	2/36 (6)	0/26 (0)
Total	70/74 (95)	—	—	—	2/51 (4)†



A possible connection between EZH2 and the potential intracellular signaling molecules p-ERK, MYC, and p-STAT3 on the basis of the IHC data

Type	p-ERK1/2	MYC	pSTAT-3
Hodgkin lymphomas and related lymphomas (THRLBCL and BCLuDLBCL/cHL)	+	+	+
DLBCL	+	-	-
BL and DHL	-	+	-
histiocytic and dendritic cell neoplasms	+	-	-


Thanks