

OTTIMIZZAZIONE DIAGNOSTICA

Stefano A. Pileri

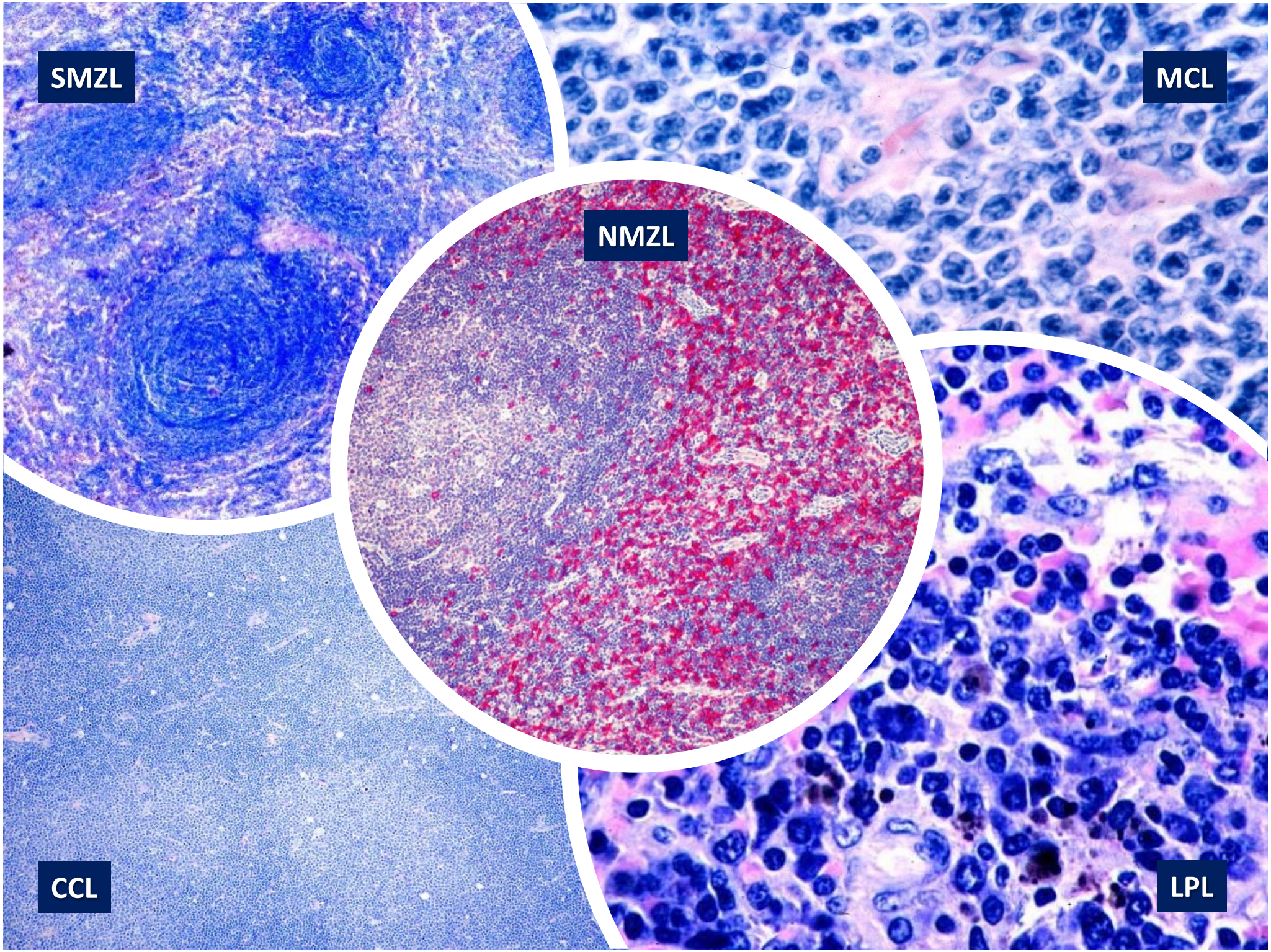
TORINO

5 Aprile 2022

Starhotels Majestic

Disclosures of Stefano A. Pileri

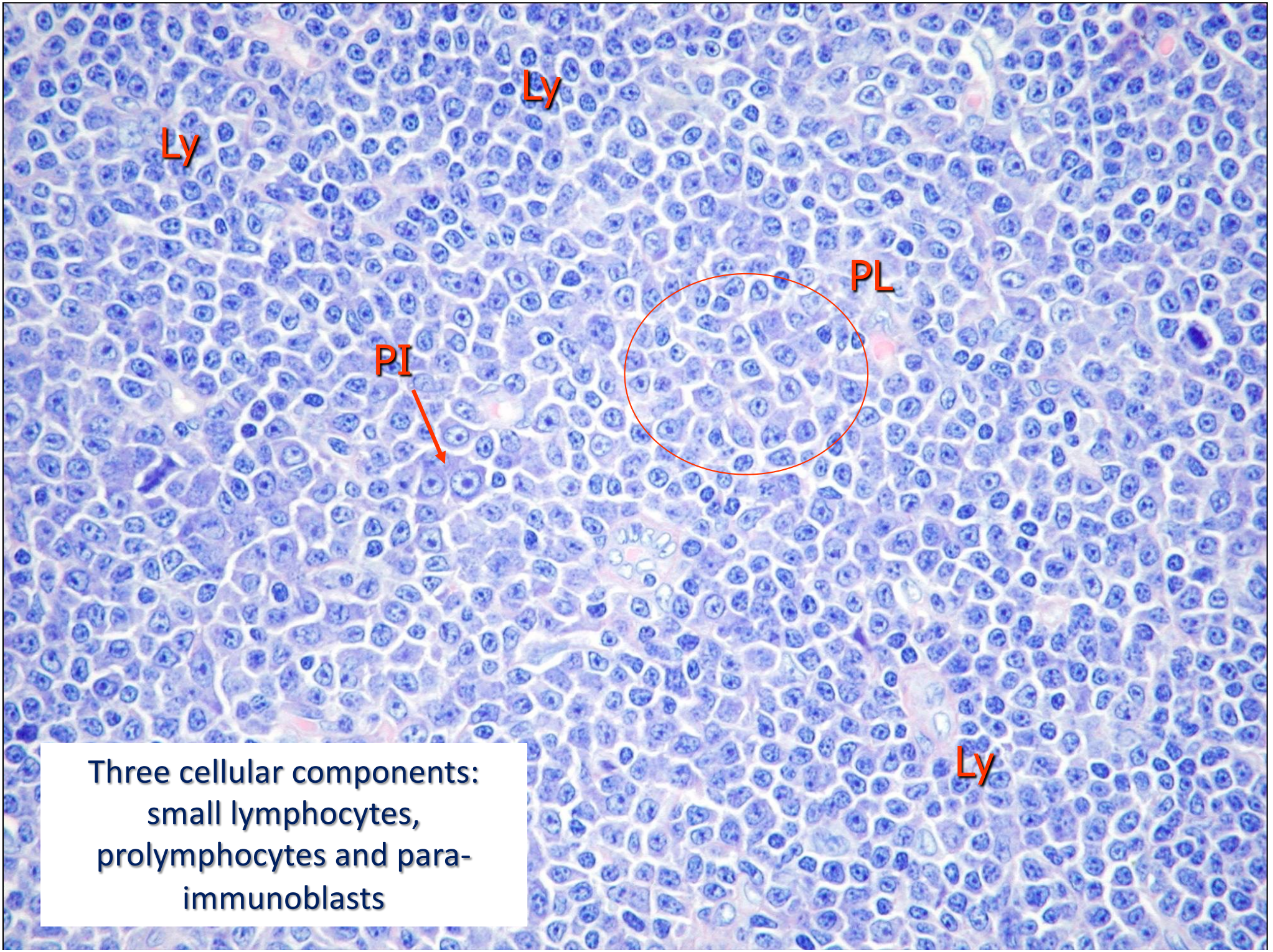
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
BeiGene						x	
Takeda						x	
Roche					x		
Diatech						x	


SMZL
MCL
NMZL
CCL
LPL

Chronic lymphocytic leukaemia/ small lymphocytic lymphoma

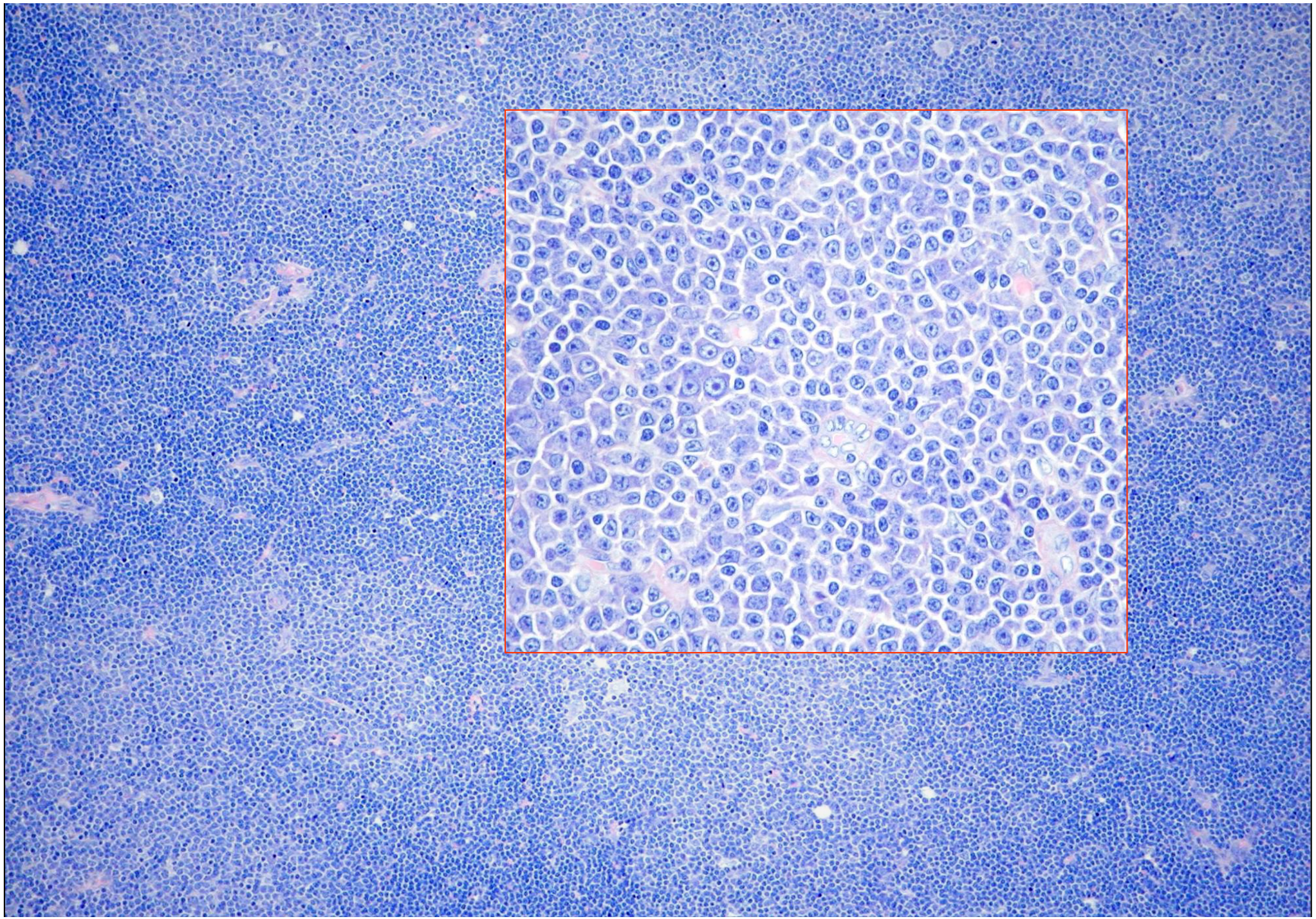
Definition

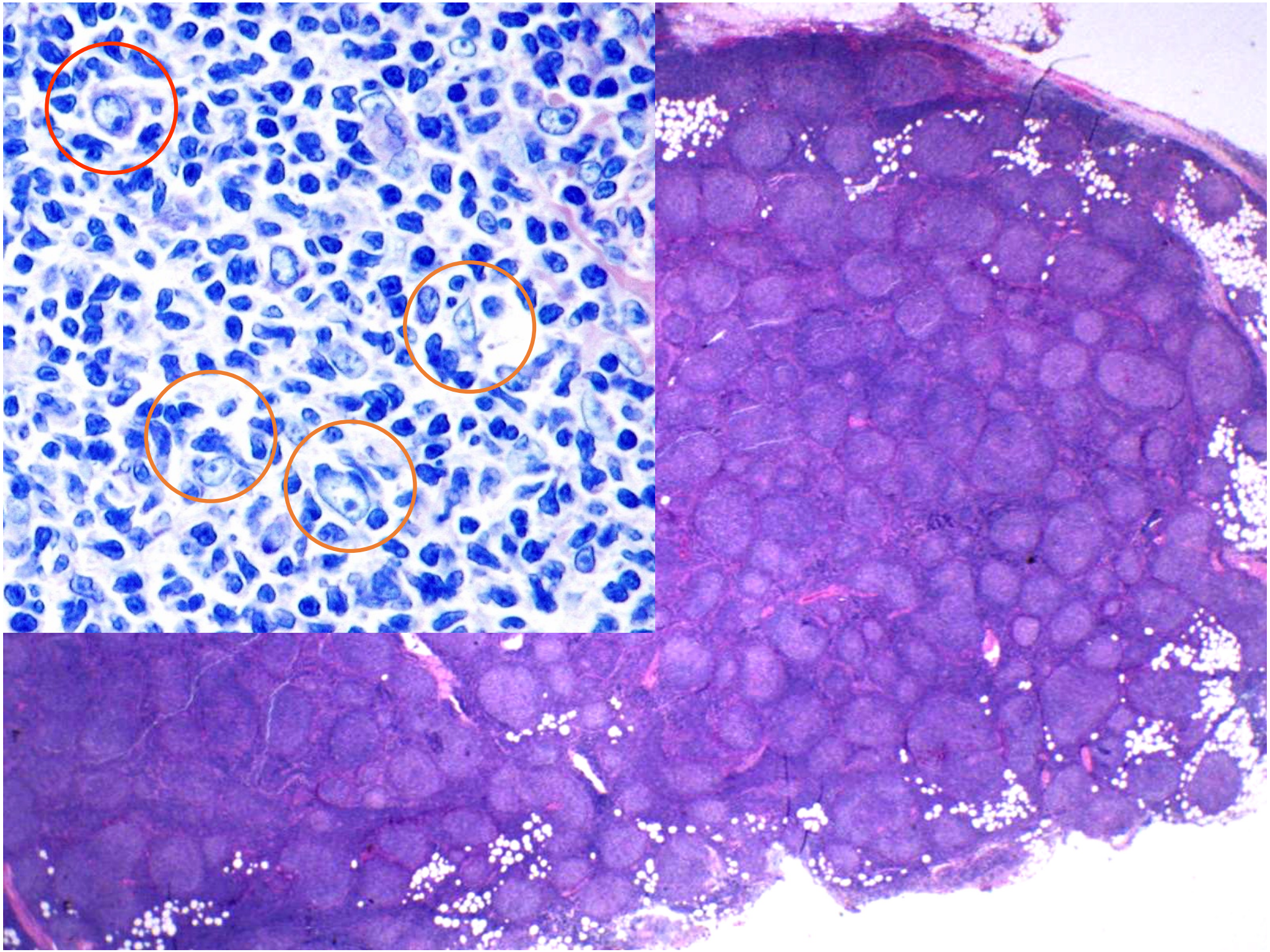
Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) is a neoplasm composed of monomorphic small mature B cells that coexpress CD5 and CD23. There must be a monoclonal B-cell count $\geq 5 \times 10^9$/L, with the characteristic morphology and phenotype of CLL in the peripheral blood. Individuals with a clonal CLL-like cell count $< 5 \times 10^9$/L and without lymphadenopathy, organomegaly, or other extramedullary disease are considered to have monoclonal B-cell lymphocytosis. Although CLL and SLL are the same disease, the term SLL is used for cases with a circulating CLL cell count $< 5 \times 10^9$/L and documented nodal, splenic, or other extramedullary involvement {1523}.



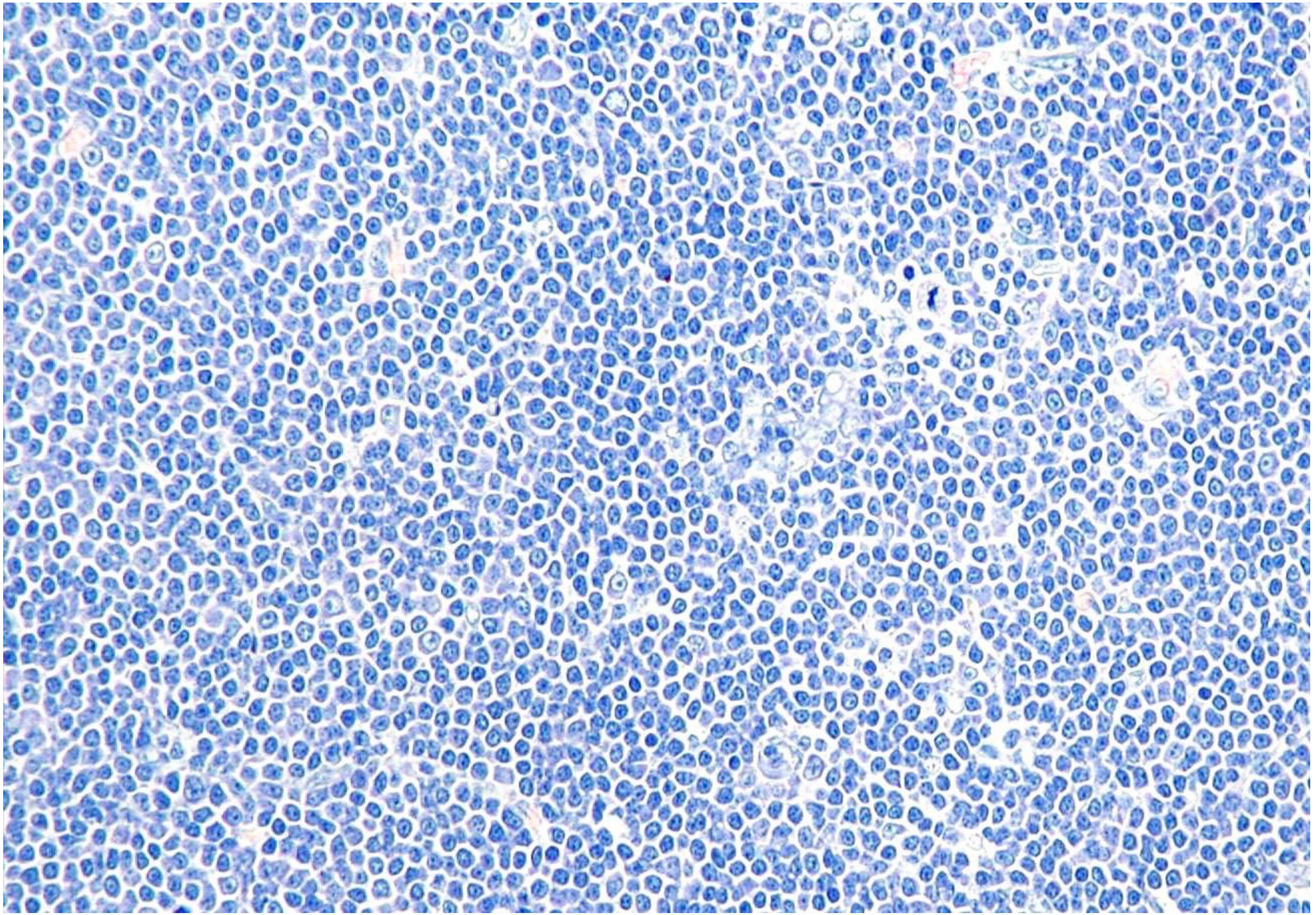
Three cellular components: small lymphocytes, prolymphocytes and para-immunoblasts

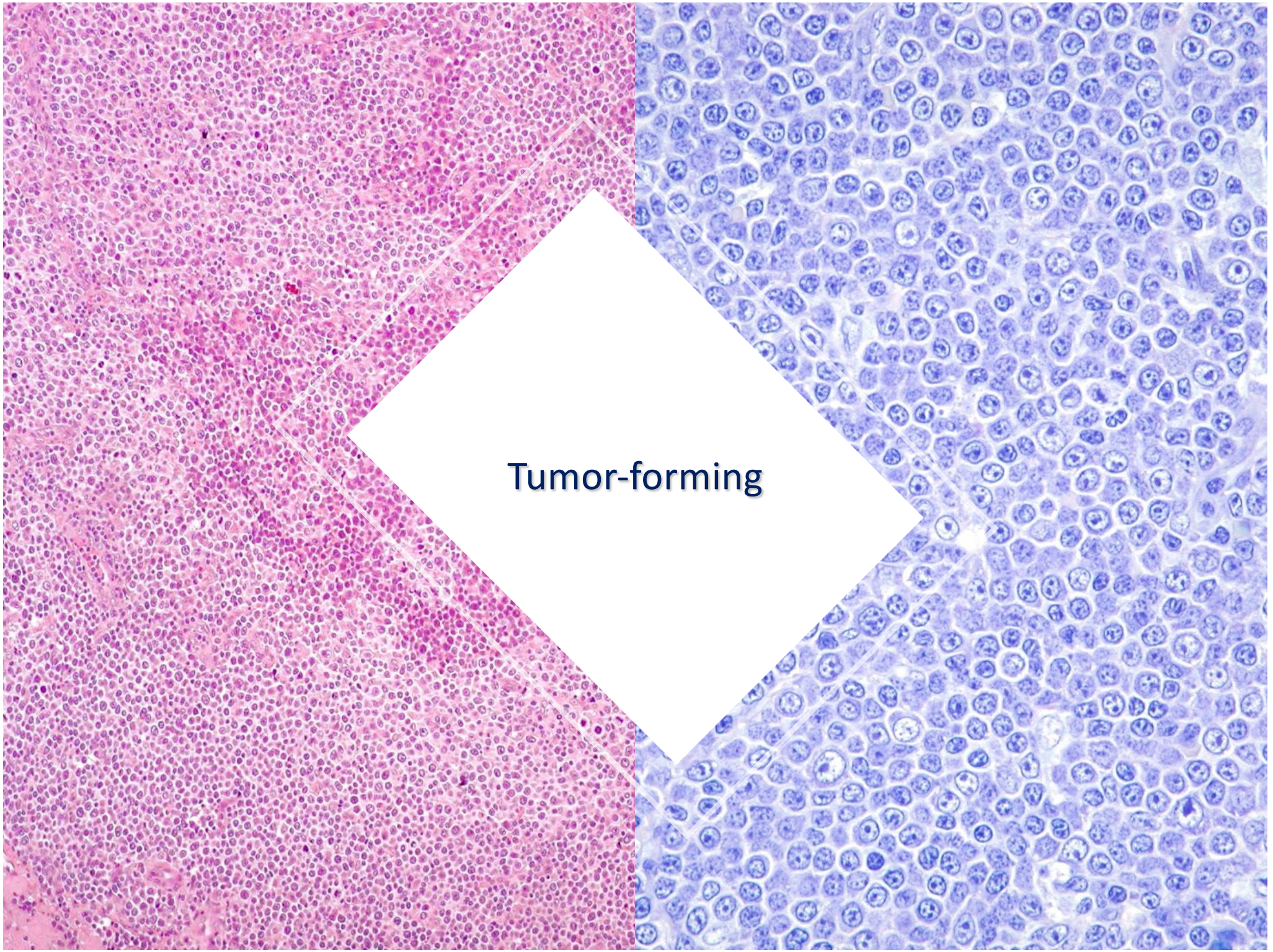
Pseudo-follicular pattern: 85% of cases (lymph node, bone-marrow, extranodal sites)



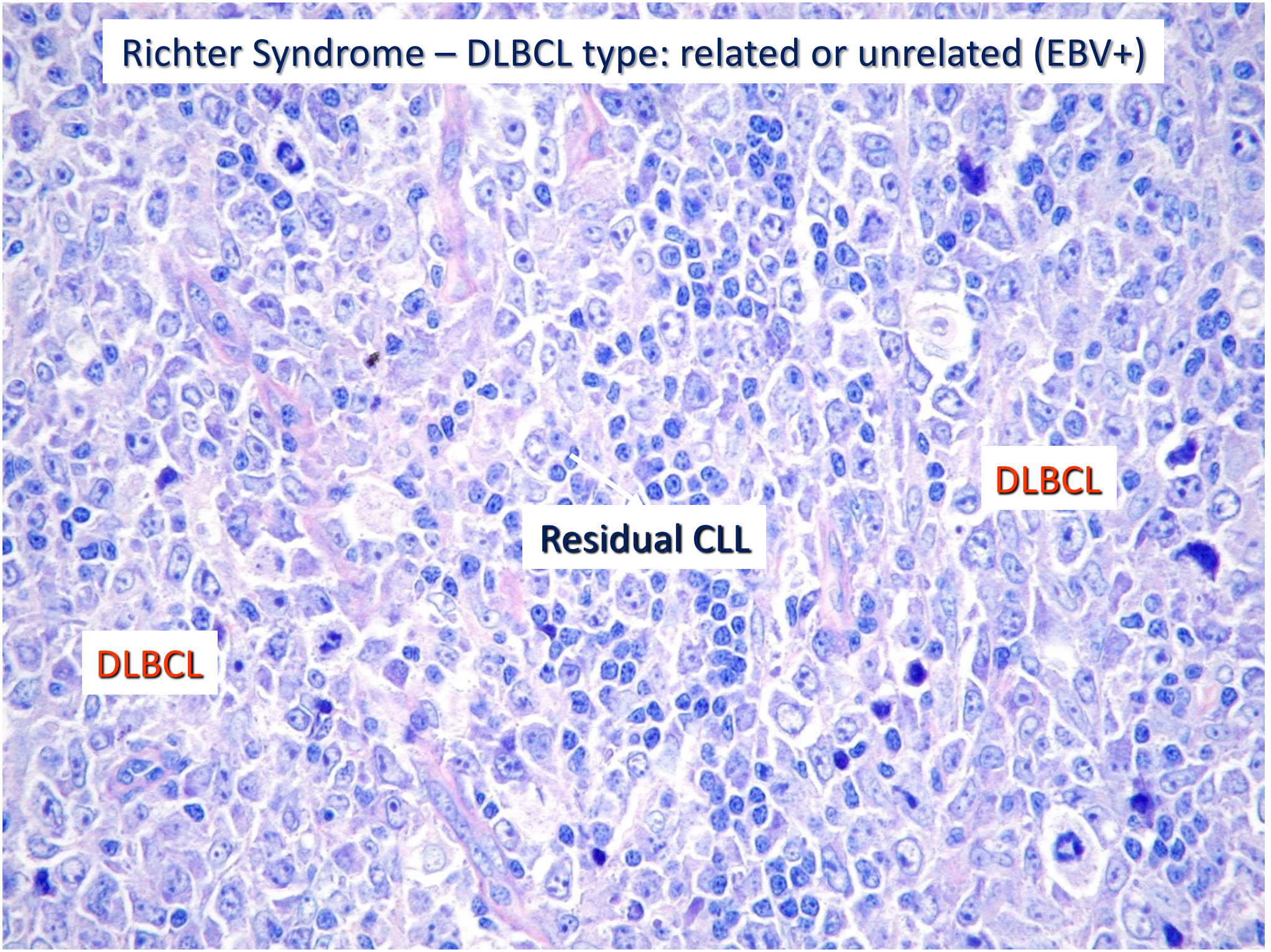


Diffuse growth pattern: 7% of cases; scanty prolymphocytes and paraimmunoblasts

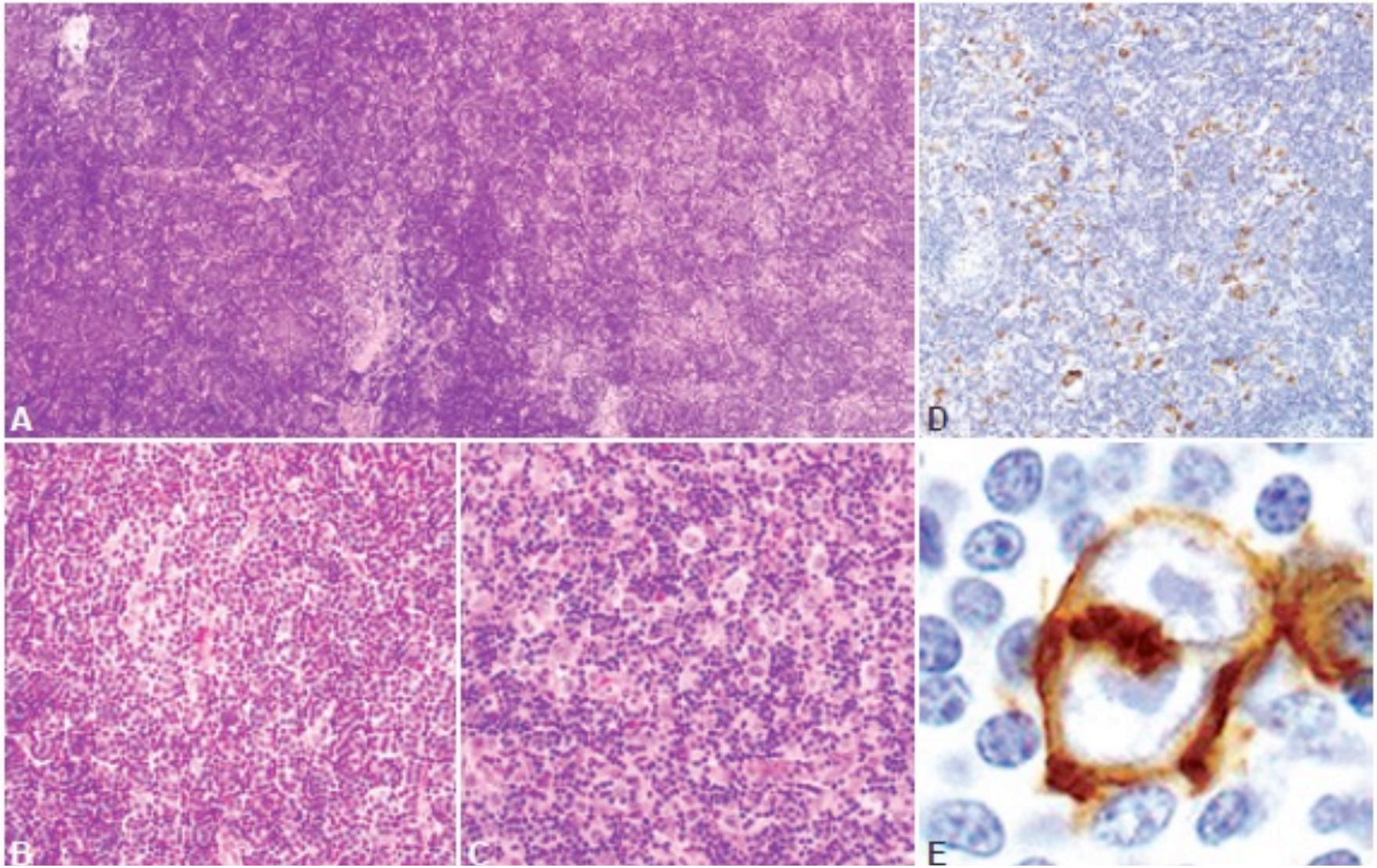


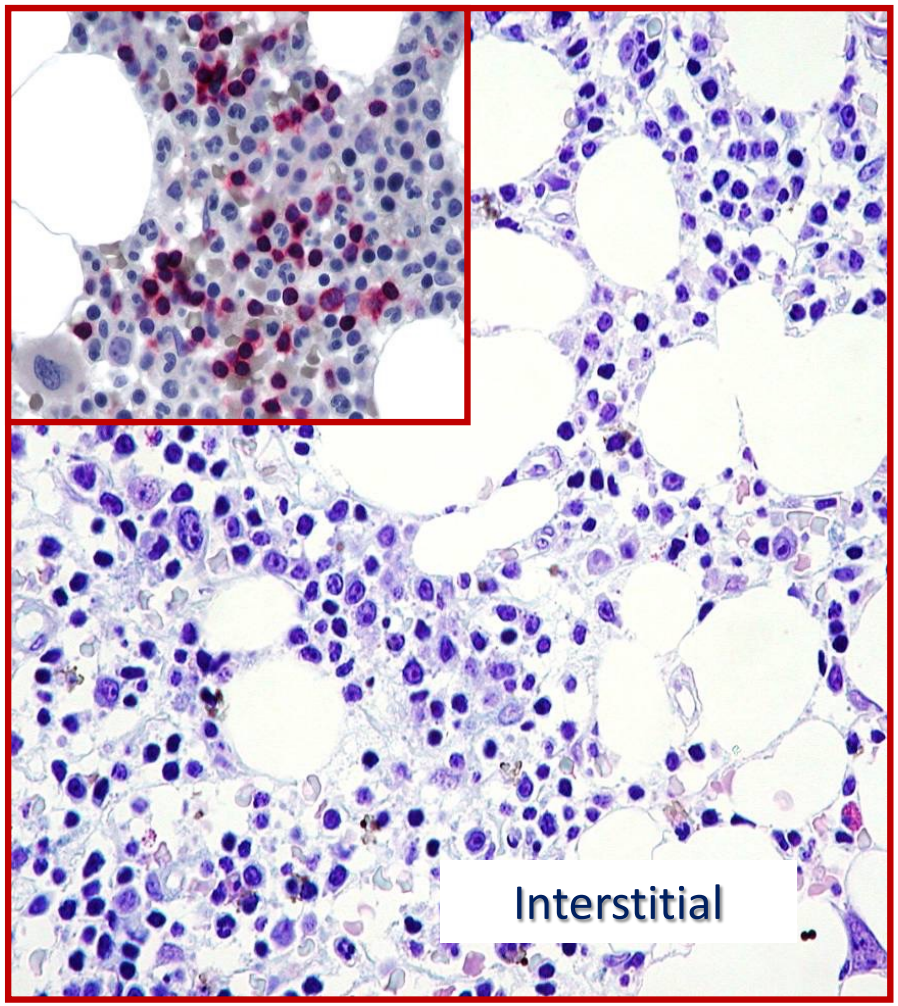

Tumor-forming

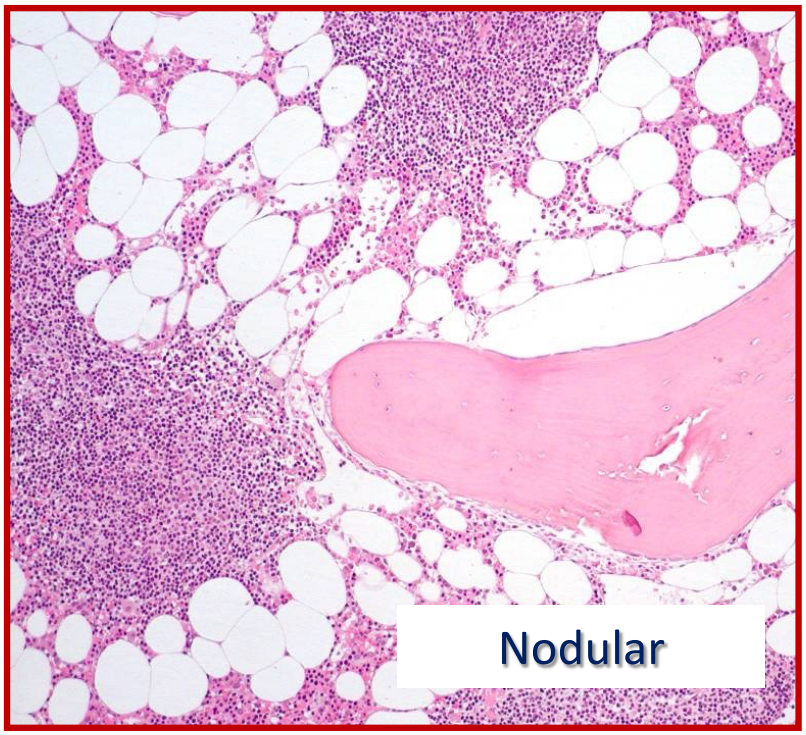
Richter Syndrome – DLBCL type: related or unrelated (EBV+)

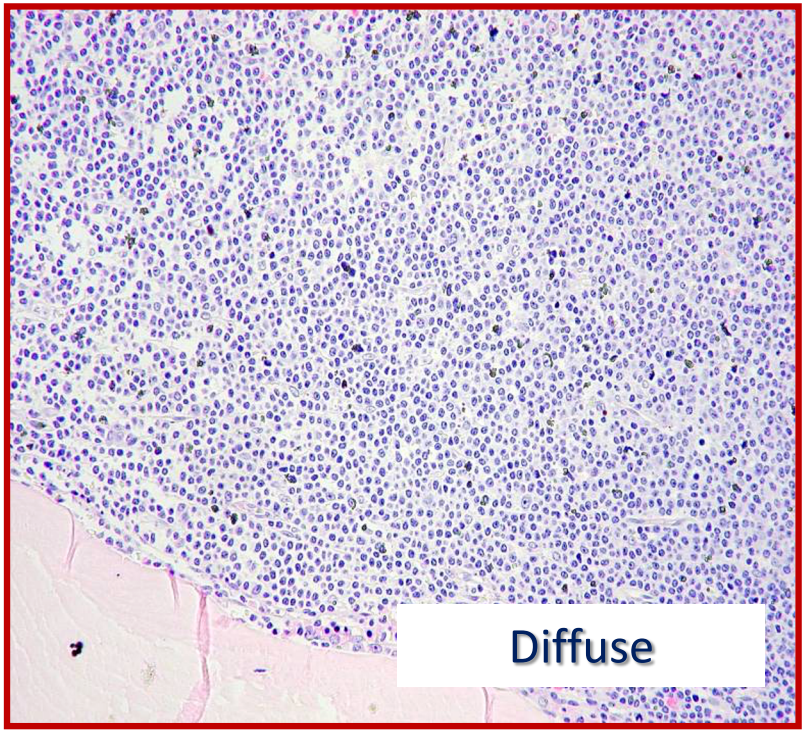


Richter Syndrome – Hodgkin type









Bone-marrow involvement

Pseudo-follicles (clear centres)
Useful for the diagnosis

Phenotype

CD20 + (weak but stronger in pseudo-follicles)

CD19, CD22, CD79a (homogeneously strong)

CD5 + (variable but stronger in pseudo-follicles)

CD23 + (variable but stronger in pseudo-follicles)

IgM/IgD+ (weak)

CD200 +

LEF1 +

ZAP70 (related to the IGVH mutational status: 85% concordance)

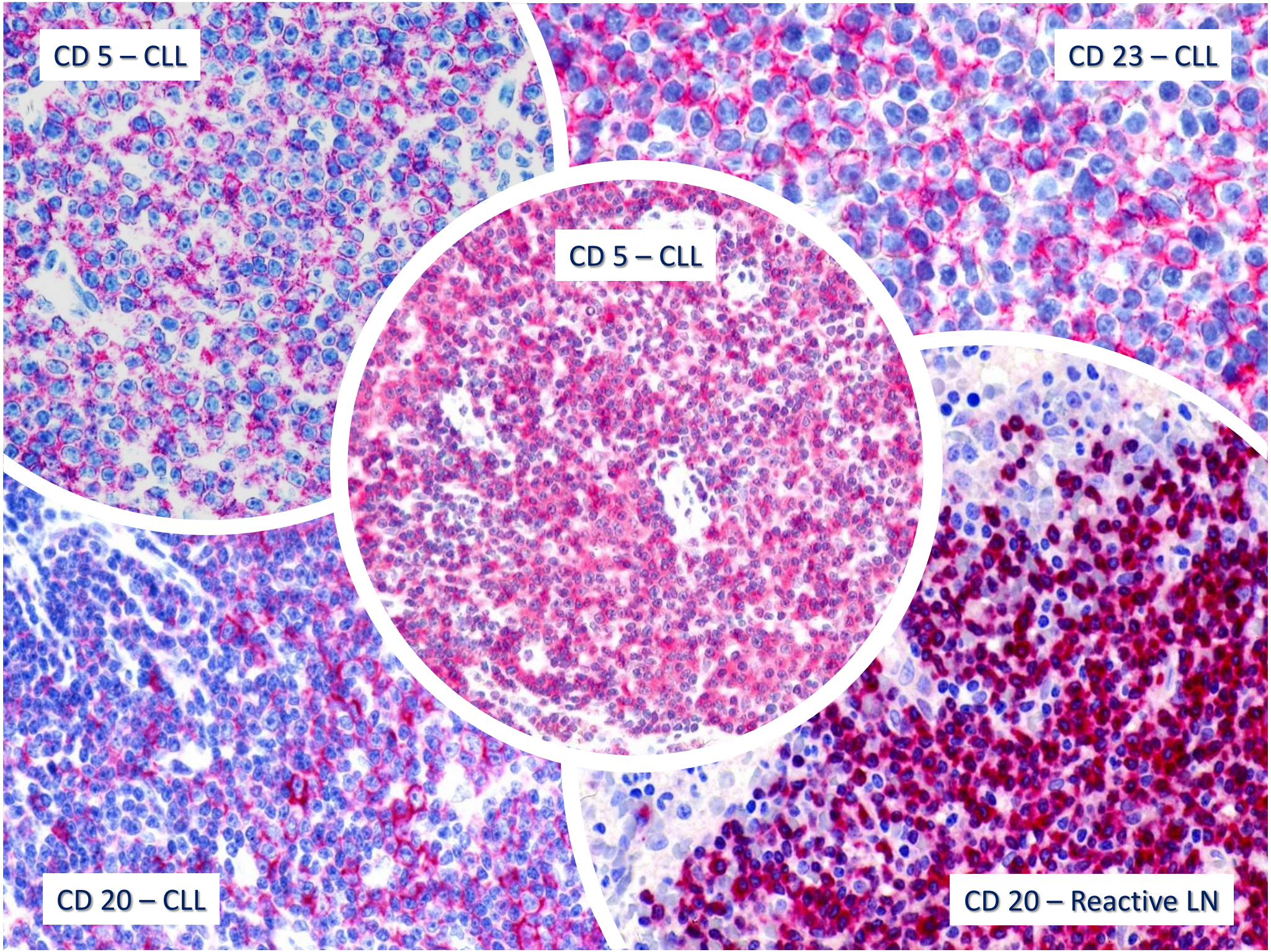
IRF4 (+ in pseudo-follicles)

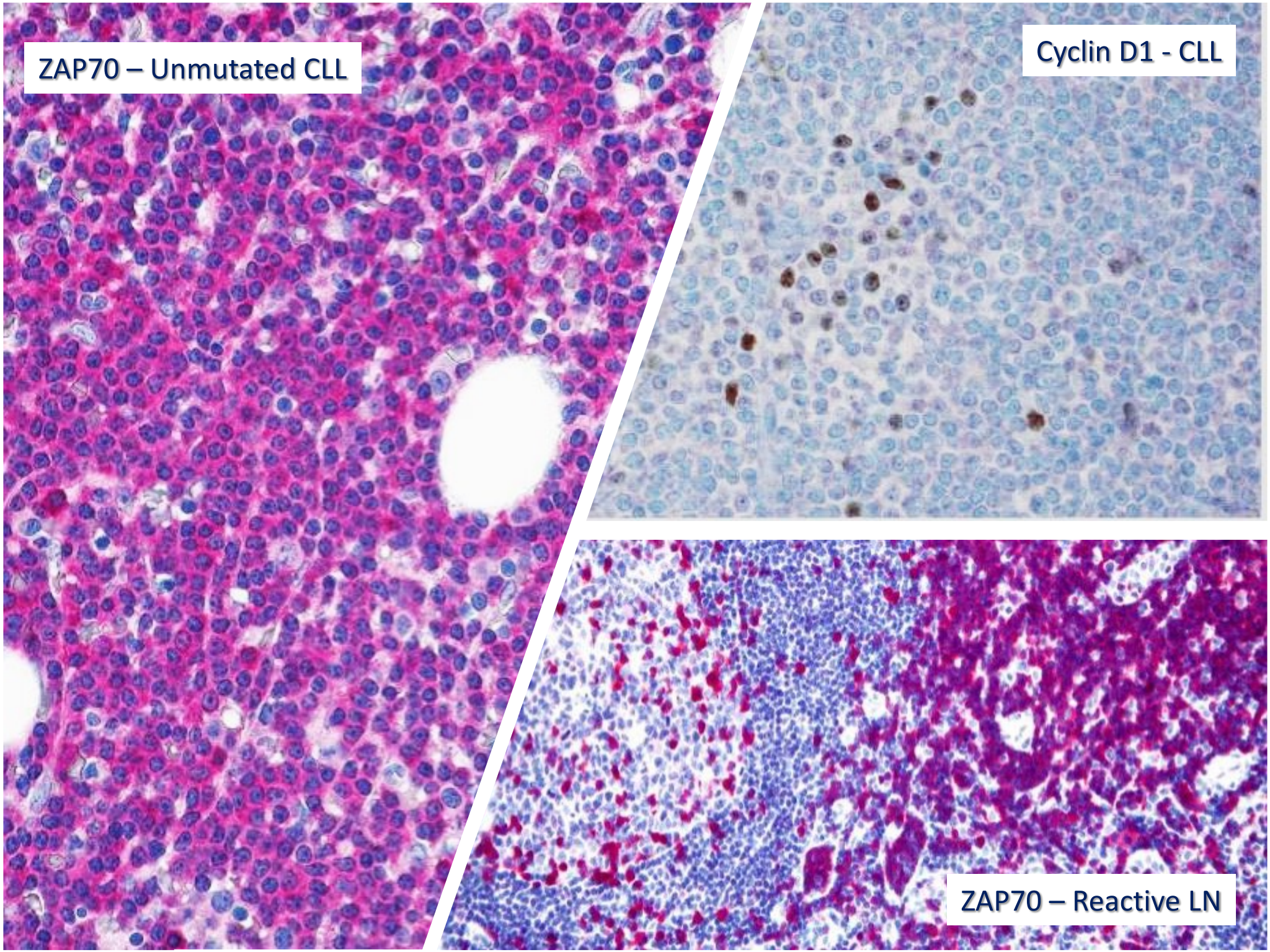
Cyclin D1 - (rare cases with weak, partial staining in the absence of t(11;14) and SOX11 positivity

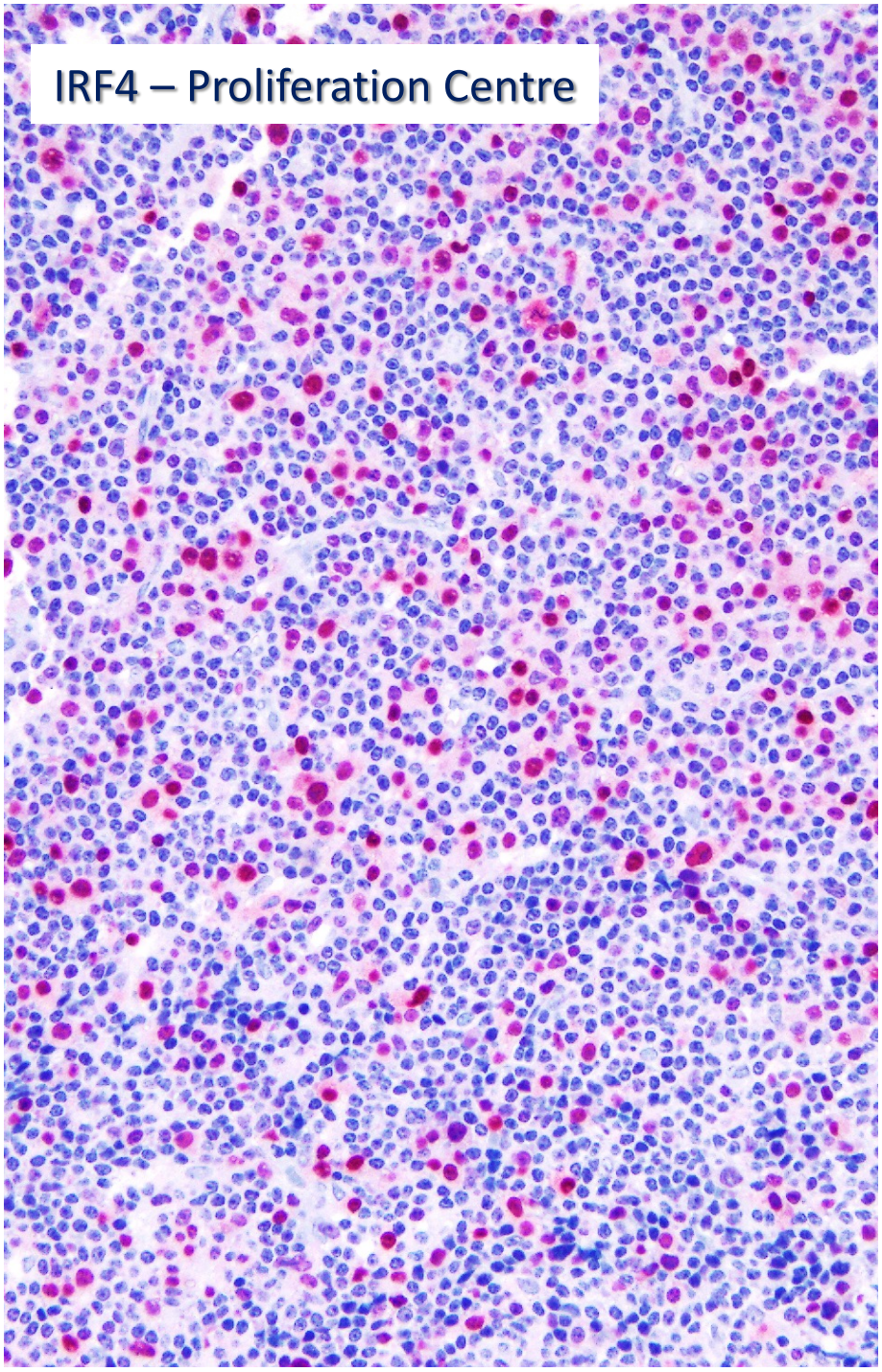
IRTA1, MNDA, T-bet -

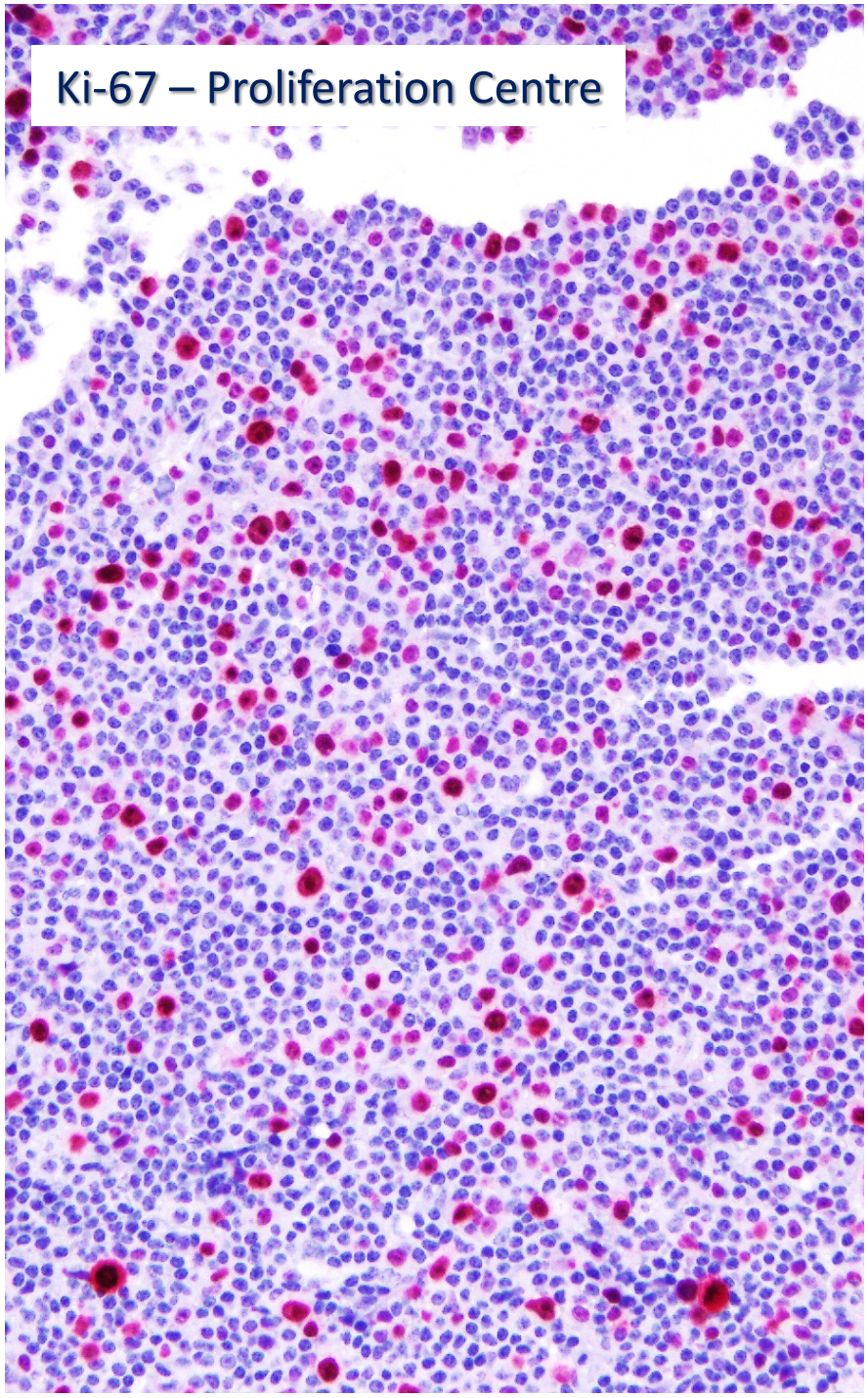
CD10, BCL6, LMO2 –

Ki-67 + (higher in pseudo-follicles)


CD 5 – CLL
CD 23 – CLL
CD 5 – CLL
CD 20 – CLL
CD 20 – Reactive LN

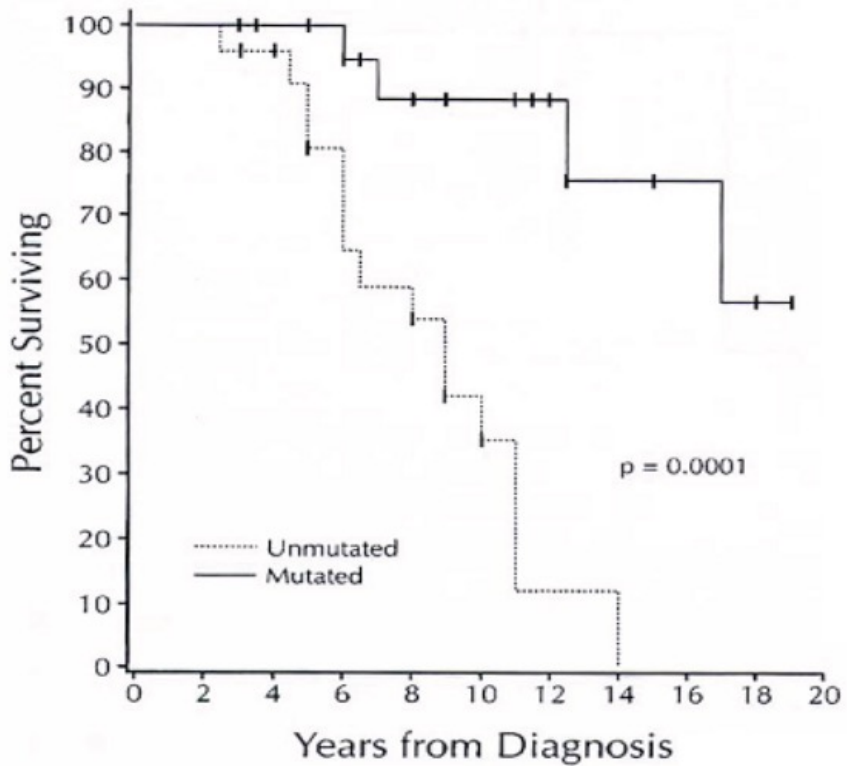

ZAP70 – Unmutated CLL
Cyclin D1 - CLL
ZAP70 – Reactive LN


IRF4 – Proliferation Centre

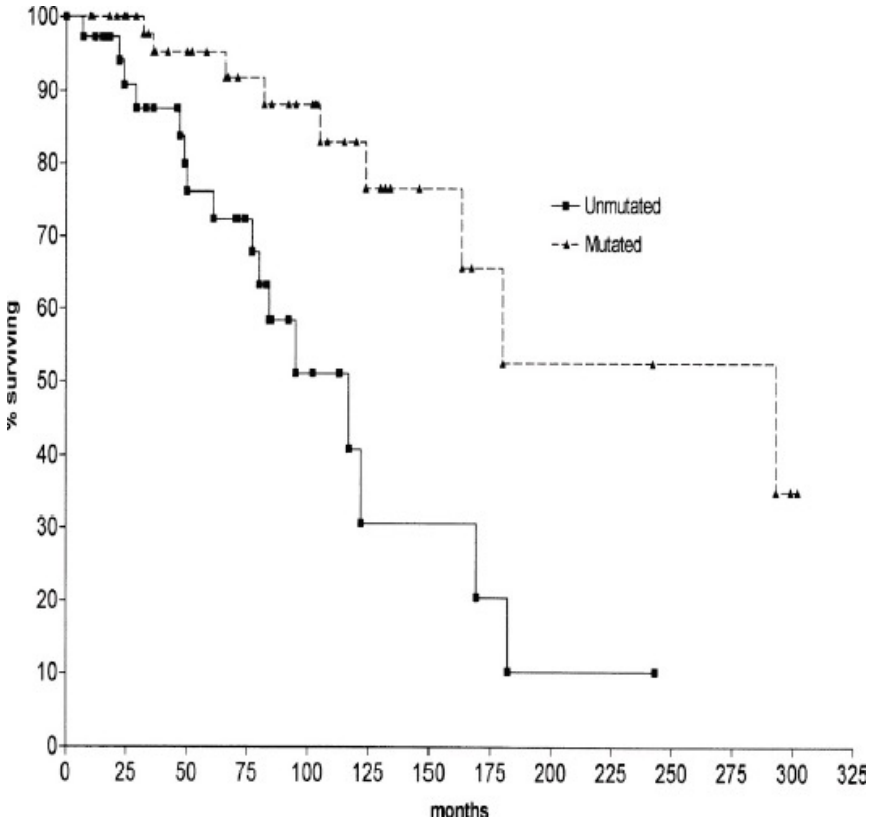

Ki-67 – Proliferation Centre

Molecular diagnostics

- BCR (IGHV) sequencing:
- Mutated (<98% identity with the germline configuration) (50 -70 %)
- Unmutated (>98% identity with the germline configuration) (30 -50%).
- Stereotyped subsets
- Cytogenetics/FISH
- Sequencing/NGS



Damle et al Blood 1999



Hamblin et al Blood 1999

Result summary: W299	Productive IGH rearranged sequence (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV3-72*01 F	score = 1299	identity = **93.54%** (275/294 nt)
J-GENE and allele	Homsap IGHJ3*01 F	score = 205	identity = 90.00% (45/50 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD2-2*01 F	D-REGION is in reading frame 2	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.11]	[8.10.17]	CAKVSGCSSIGCYYGLDAW
JUNCTION length (in nt) and decryption	57 nt = (10)-1{7}-6(22)-3{7}-8(11)	(3'V)3'{N1}5'(D)3'{N2}5'(5'J)	

Result summary: W86	Productive IGH rearranged sequence (no stop codon and in-frame junction)		
V-GENE and allele	Homsap IGHV5-10-1*03 F	score = 1440	identity = **100.00%** (288/288 nt)
J-GENE and allele	Homsap IGHJ4*02 F	score = 195	identity = 89.58% (43/48 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD6-19*01 F	D-REGION is in reading frame 3	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.11]	[8.8.14]	CAREQWLGPNSPFDYW
JUNCTION length (in nt) and decryption	48 nt = (9)0{3}-8(13)0{11}-5(12)	(3'V)3'{N1}5'(D)3'{N2}5'(5'J)	

KEY POINTS

- In a series of 29 856 CLL patients, the incidence of BcR stereotypy peaked at 41%.
- Higher-order relations exist between stereotyped subsets, particularly for those from U-CLL, for which satellite subsets were identified.

Chronic lymphocytic leukemia (CLL) is characterized by the existence of subsets of patients with (quasi)identical, stereotyped B-cell receptor (BcR) immunoglobulins. Patients in certain major stereotyped subsets often display remarkably consistent clinicobiological profiles, suggesting that the study of BcR immunoglobulin stereotypy in CLL has important implications for understanding disease pathophysiology and refining clinical decision-making. Nevertheless, several issues remain open, especially pertaining to the actual frequency of BcR immunoglobulin stereotypy and major subsets, as well as the existence of higher-order connections between individual subsets. To address these issues, we investigated clonotypic IGHV-IGHD-IGHJ gene rearrangements in a series of 29 856 patients with CLL, by far the largest series worldwide. We report that the stereotyped fraction of CLL peaks at 41% of the entire cohort and that all 19 previously identified major subsets retained their relative size and ranking, while 10 new ones emerged; overall, major stereotyped subsets had a cumulative frequency of 13.5%. Higher-level relationships were evident between subsets, particularly for major stereotyped subsets with unmutated IGHV genes (U-CLL), for which close relations with other subsets, termed "satellites," were identified. Satellite subsets accounted for 3% of the entire cohort. These results confirm our previous notion that major subsets can be robustly identified and are consistent in relative size, hence representing distinct disease variants amenable to compartmentalized research with the potential of overcoming the pronounced heterogeneity of CLL. Furthermore, the existence of satellite subsets reveals a novel aspect of repertoire restriction with implications for refined molecular classification of CLL. (*Blood*. 2021;137(10):1365-1376)

Cytogentics/FISH

Aberration(s)	Frequency	
	Mutated IGHV n = 132 (44% of cases)	**Unmutated IGHV** n = 168 (56% of cases)
Clonal aberrations	80%	84%
13q deletion*	65%	48%
Isolated 13q deletion*	50%	26%
Trisomy 12	15%	19%
11q deletion*	4%	27%
17p deletion*	3%	10%
17p or 11q deletion*	7%	35%

*Significant difference between cases with and without IGHV mutation.



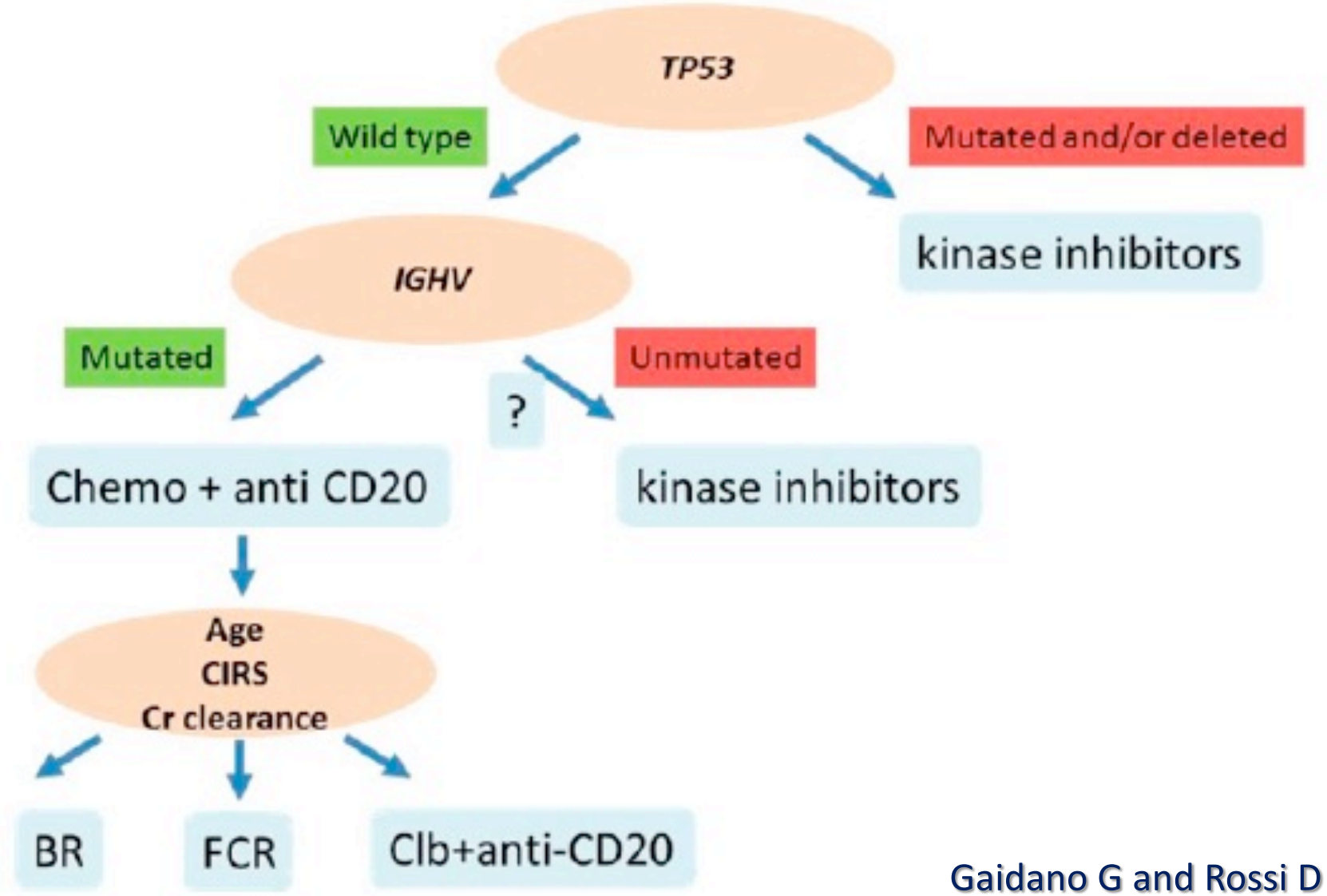


Gaidano G and Rossi D

Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia

Davide Rossi,[1] Silvia Rasi,[1] Valeria Spina,[1] Alessio Bruscaggin,[1] Sara Monti,[1] Carmela Ciardullo,[1] Clara Deambrogi,[1] Hossein Khiabanian,[2] Roberto Serra,[3] Francesco Bertoni,[4] Francesco Forconi,[5,6] Luca Laurenti,[7] Roberto Marasca,[8] Michele Dal-Bo,[9] Francesca Maria Rossi,[9] Pietro Bulian,[9] Josep Nomdedeu,[10] Giovanni Del Poeta,[11] Valter Gattei,[9] Laura Pasqualucci,[12-14] Raul Rabadan,[2] *Robin Foà,[15] *Riccardo Dalla-Favera,[12,13,16] and *Gianluca Gaidano[1]

(*Blood*. 2013;121(8):1403-1412)



Gaidano G and Rossi D

Lymphoplasmacytic lymphoma

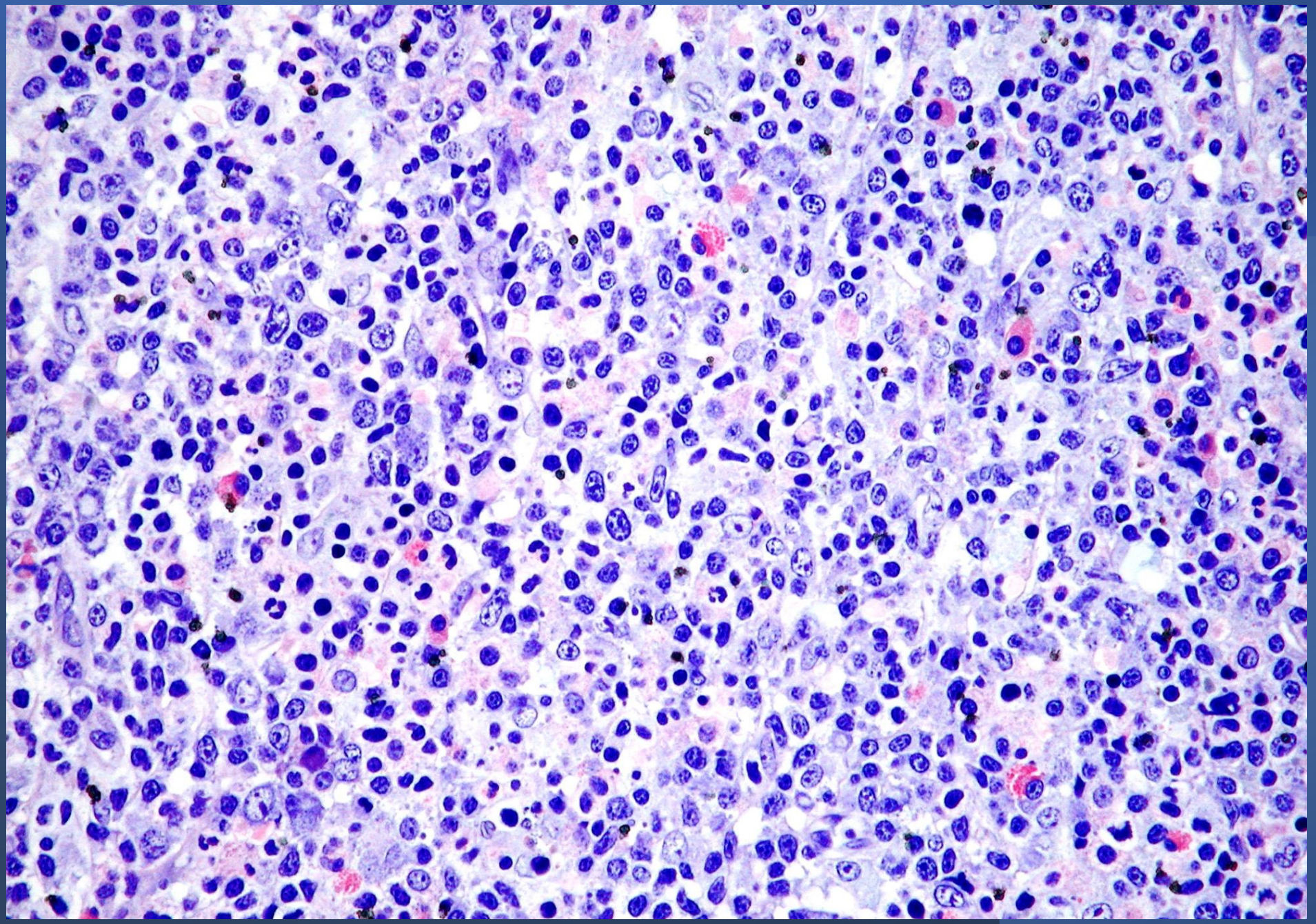
Small lymphocytes + plasmacytoid elements + plasma cells

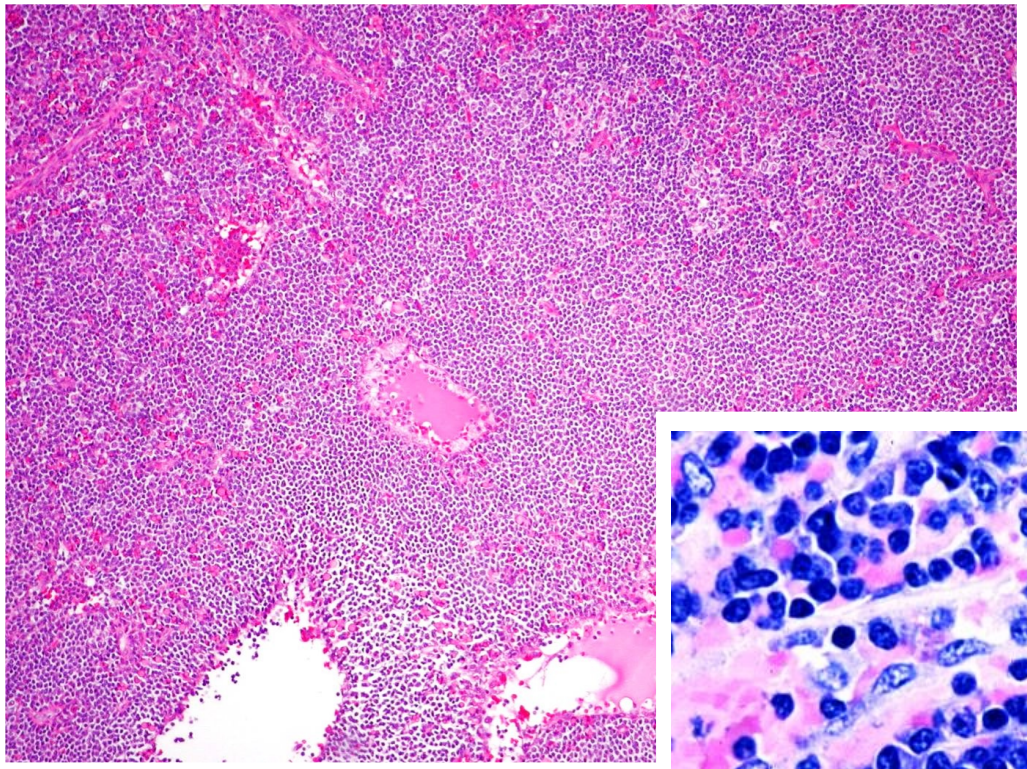
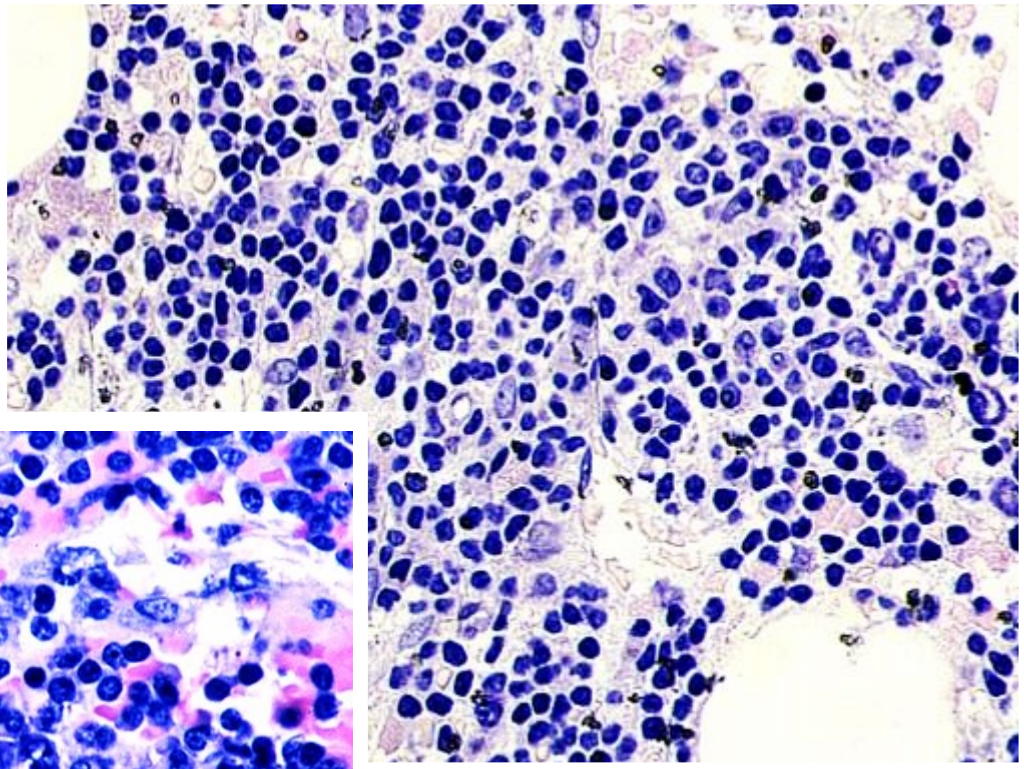
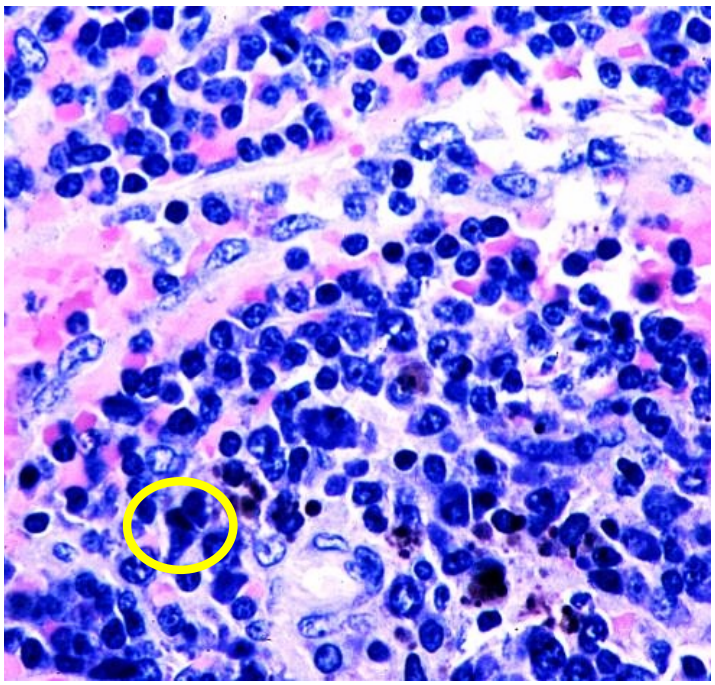
Exclusion diagnosis as other lymphomas can show plasmacellular differentiation

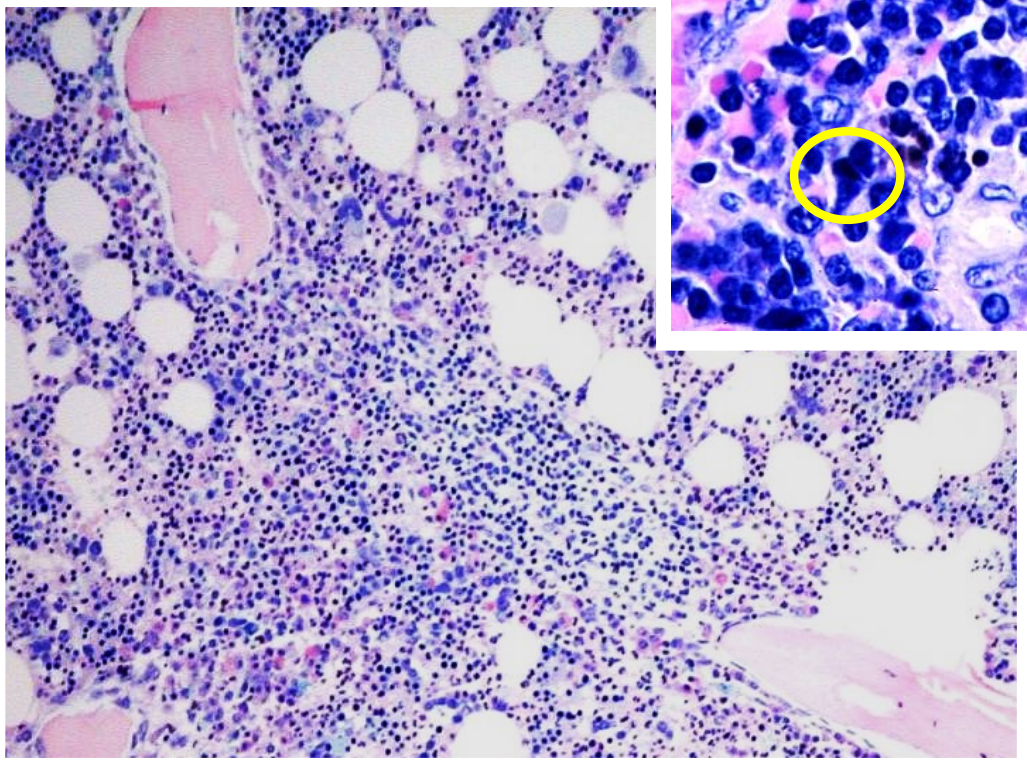
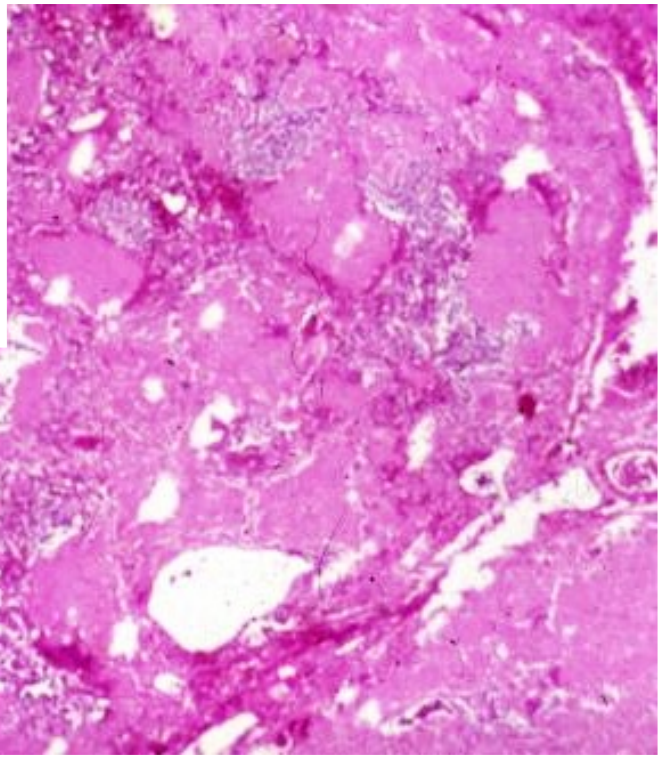
MYD88 L265P mutation characteristic but not exclusive

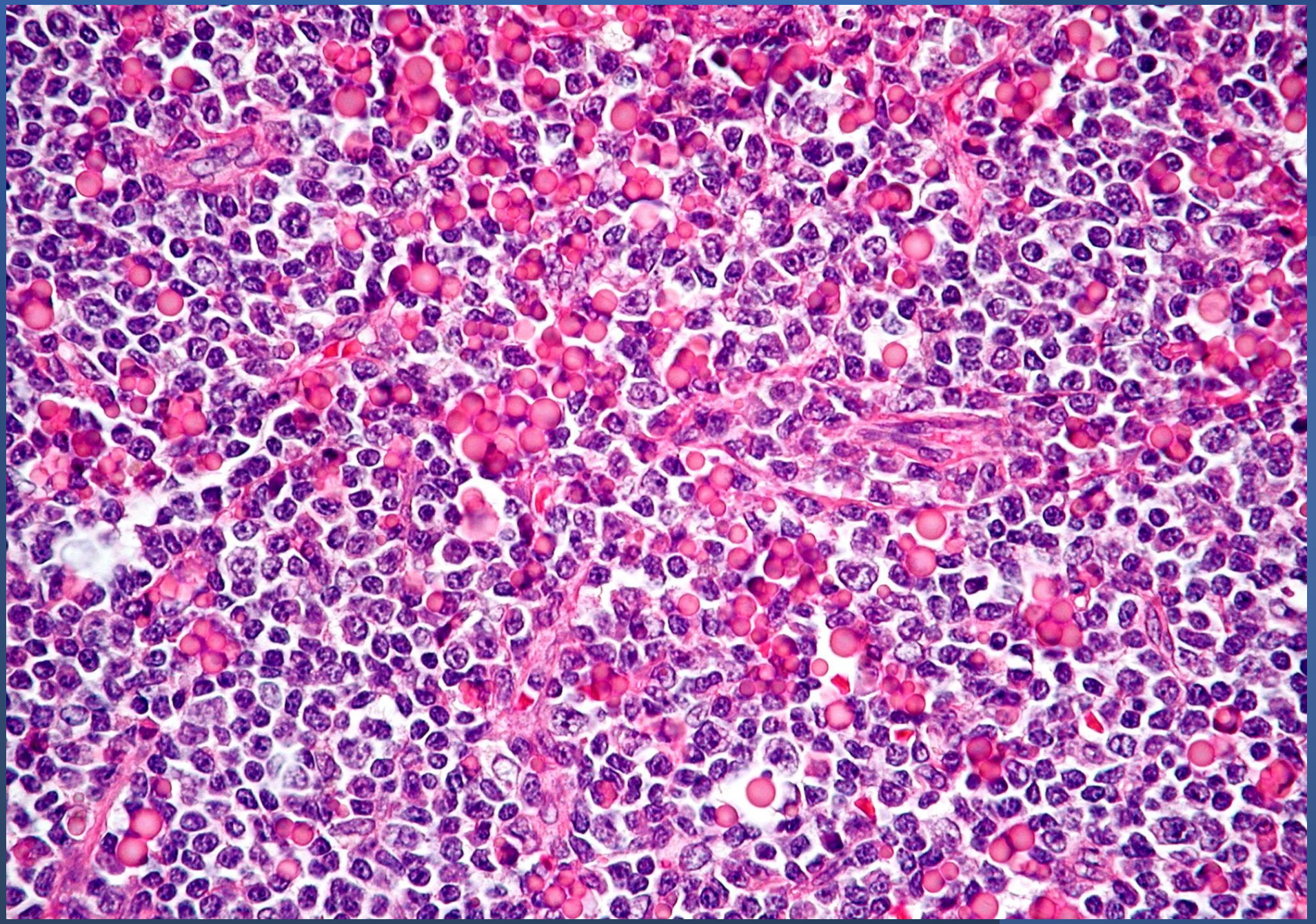
IgM paraprotein not necessarily required

WM found in a substantial subset, but not synonymous of LPL





Phenotype

CD19, CD20, CD22, CD79a, CD79b +

CD5 -

CD23 -/+

IgM+ (CYTOPLASMIC!)

IgD-

CD38+

IRF4 +/-

CD45 +/-

CD138 -

CD200 -

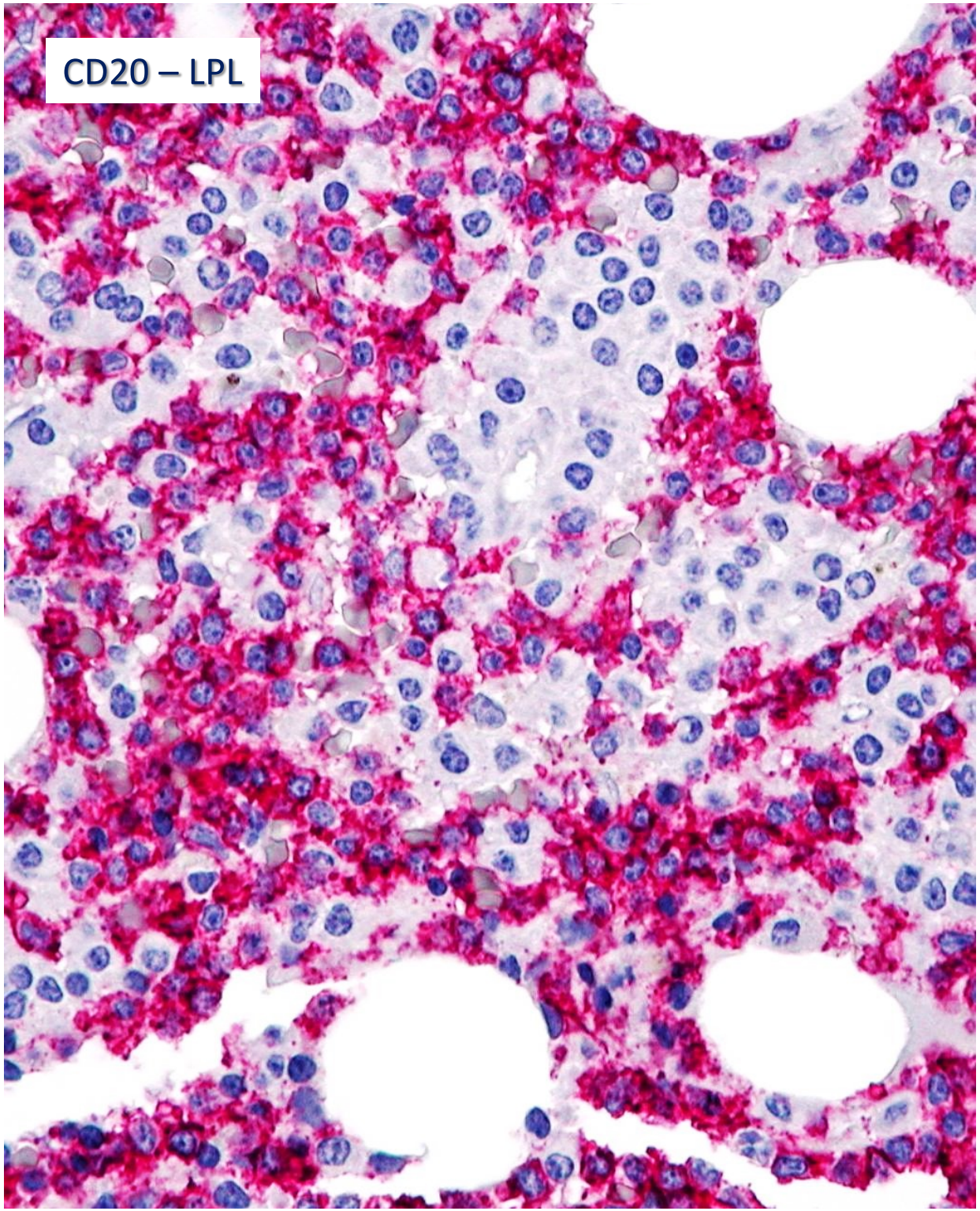
LEF1 -

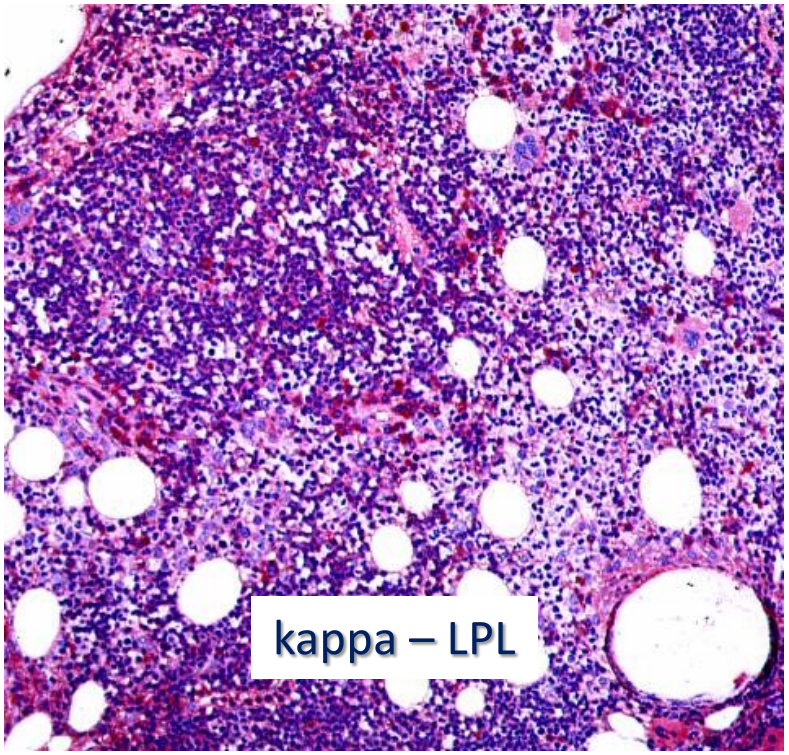
Cyclin D1 -

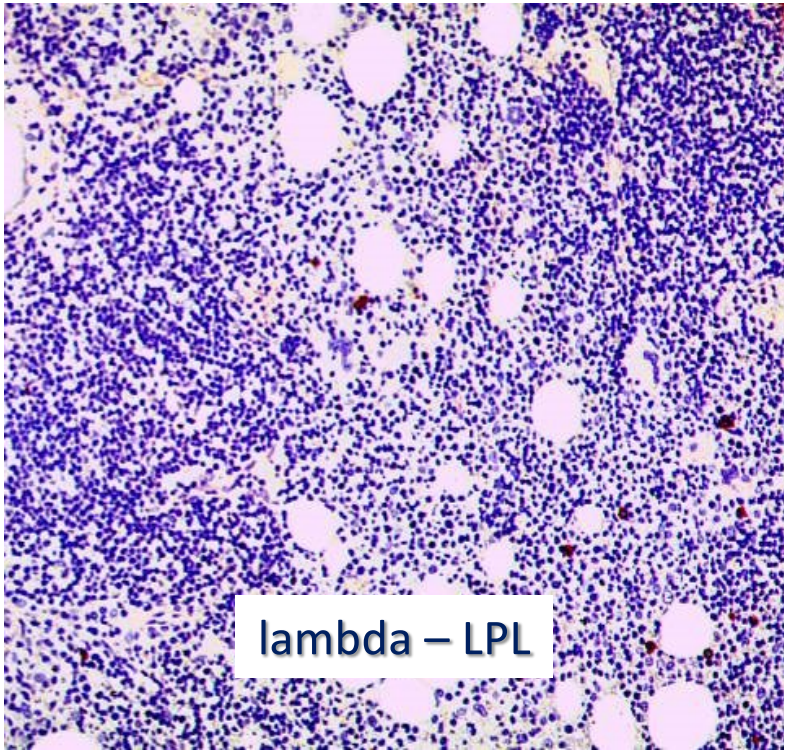
IRTA1, MNDA, T-bet -

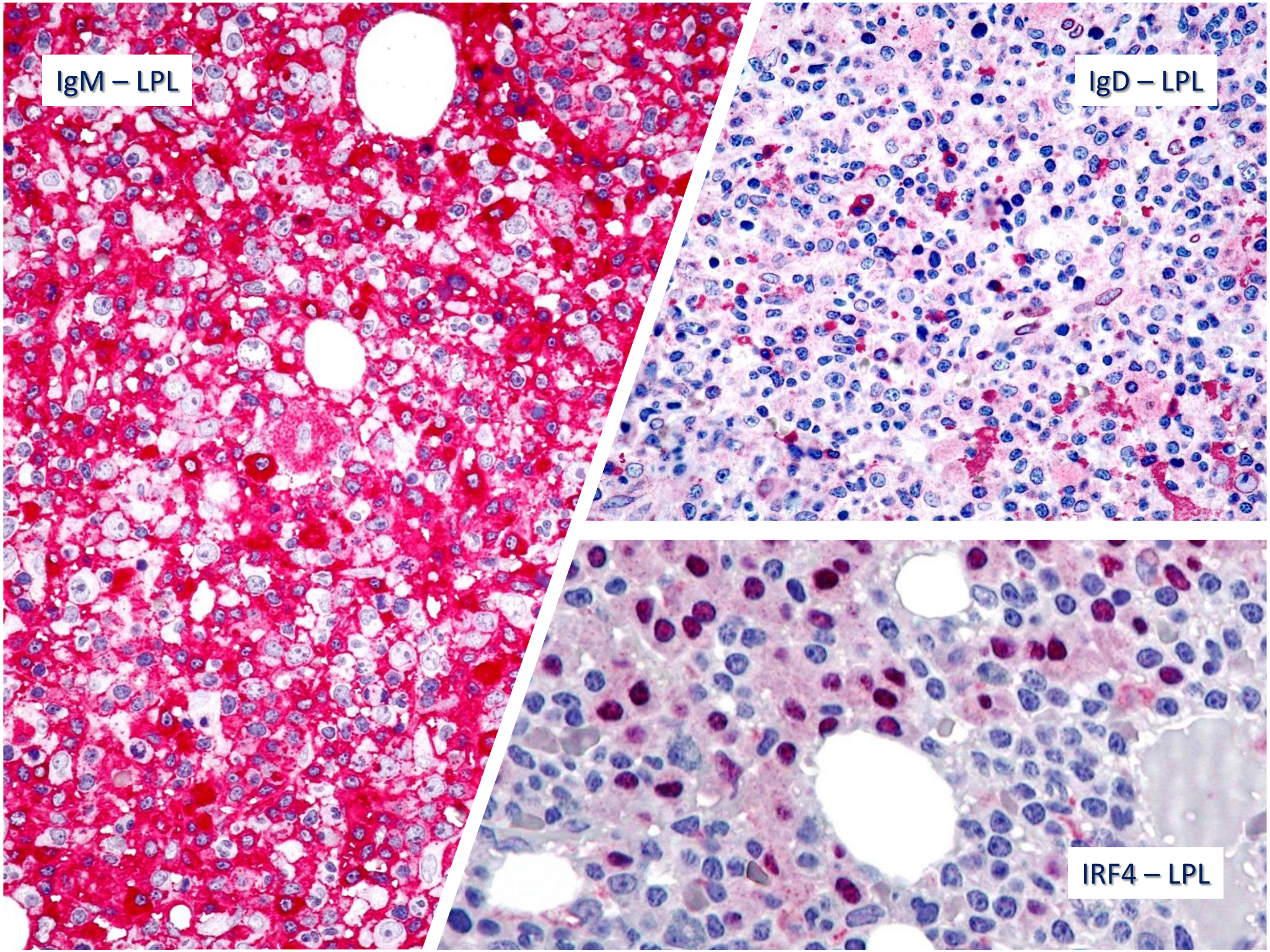
CD10, BCL6, LMO2 –

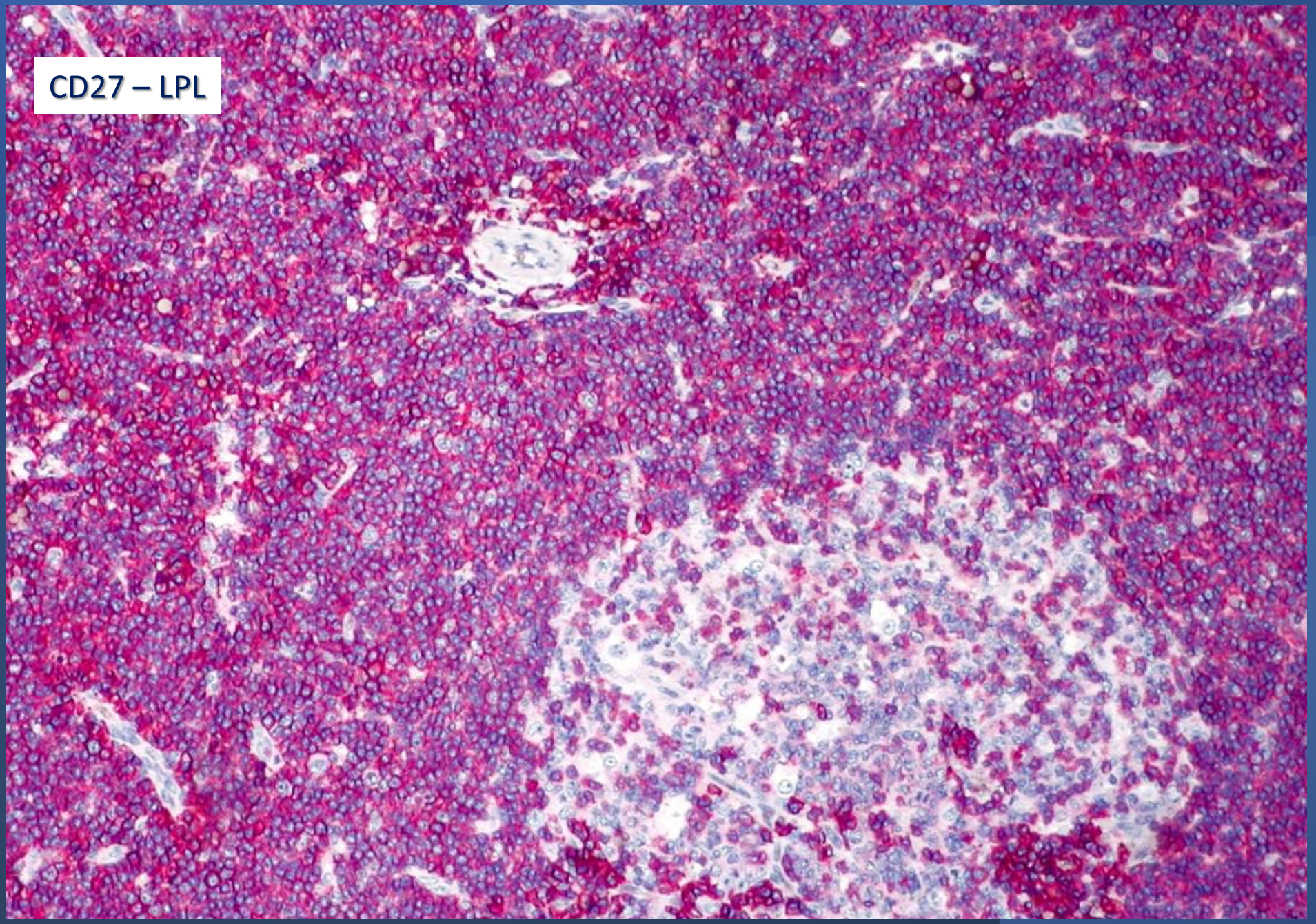
Ki-67: low

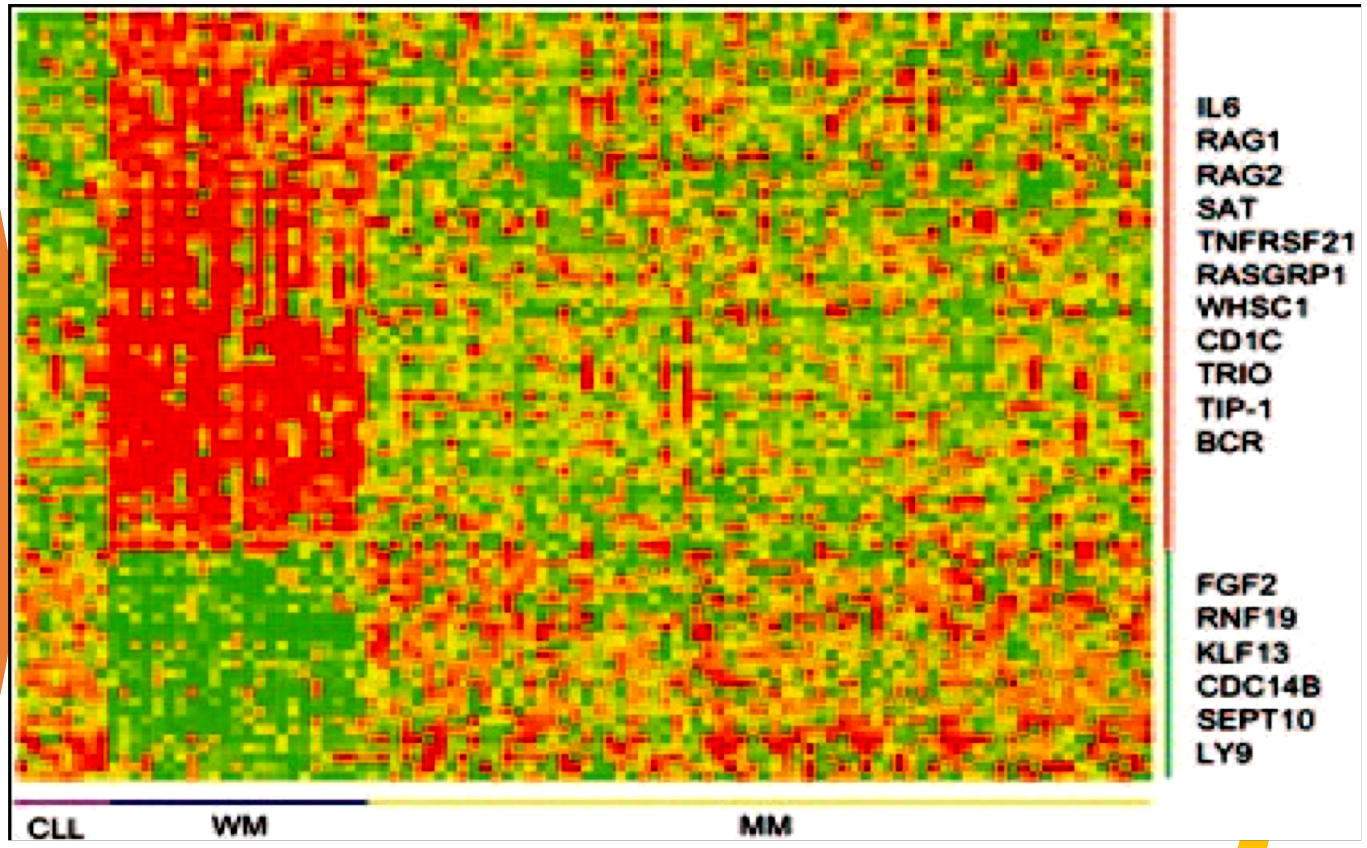

CD20 – LPL


kappa – LPL


lambda – LPL


IgM – LPL
IgD – LPL
IRF4 – LPL


CD27 – LPL

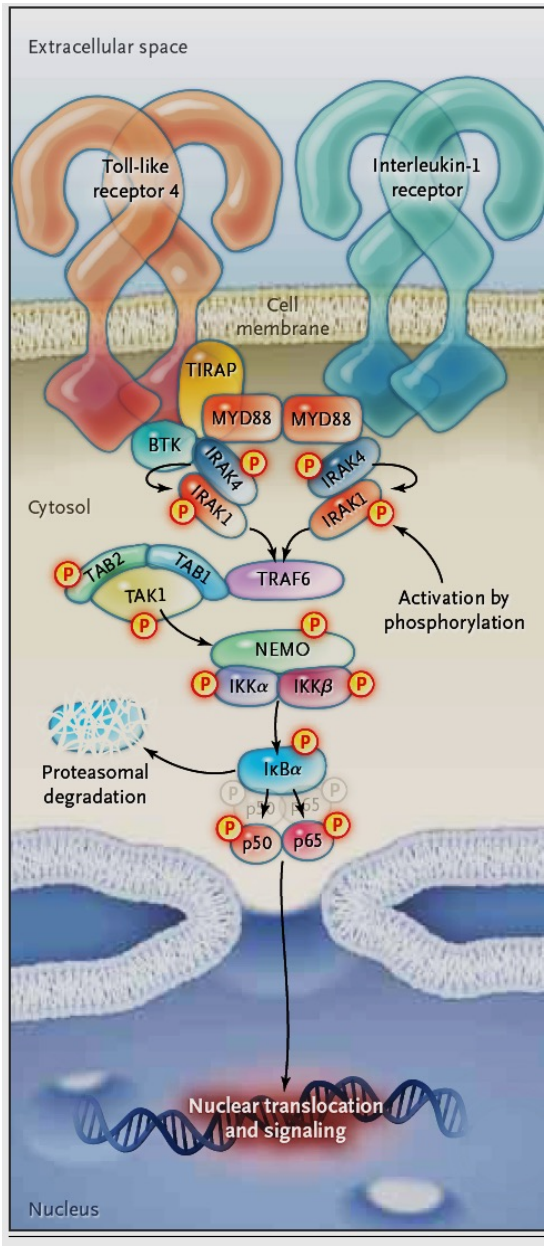

IL6
RAG1
RAG2
SAT
TNFRSF21
RASGRP1
WHSC1
CD1C
TRIO
TIP-1
BCR
FGF2
RNF19
KLF13
CDC14B
SEPT10
LY9
CLL
WM
MM

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia

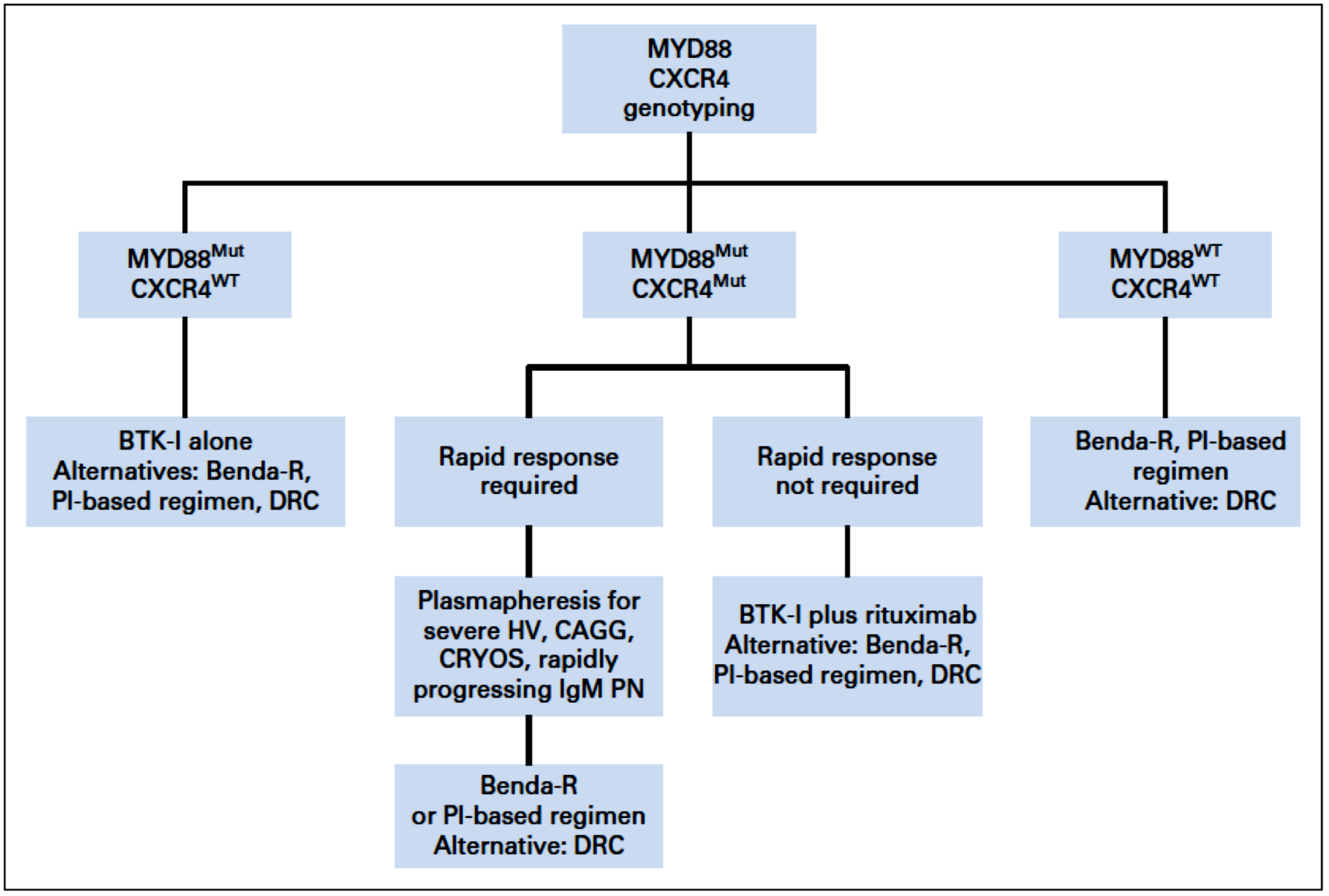
Steven P. Treon, M.D., Ph.D., Lian Xu, M.S., Guang Yang, Ph.D., Yangsheng Zhou, M.D., Ph.D., Xia Liu, M.D., Yang Cao, M.D., Patricia Sheehy, N.P., Robert J. Manning, B.S., Christopher J. Patterson, M.A., Christina Tripsas, M.A., Luca Arcaini, M.D., Geraldine S. Pinkus, M.D., Scott J. Rodig, M.D., Ph.D., Aliyah R. Sohani, M.D., Nancy Lee Harris, M.D., Jason M. Laramie, Ph.D., Donald A. Skifter, Ph.D., Stephen E. Lincoln, Ph.D., and Zachary R. Hunter, M.A.





Lymphoplasmacytic Lymphoma and Nodal Marginal Zone Lymphoma

- *MYD88* L265P can distinguish LPL (70-100%) from NMZL (0-20%)
  - Frequency in NMZL may be overstated. Re-review reveals may of the cases are LPL
- Who to test for *MYD88* L265P
  - Classic WM diagnosis, testing likely will add little
  - Small B-cell lymphoma with plasmacytic differentiation, where LPL and other small B-cell lymphomas are in the differential diagnosis should be tested
  - *CXCR4* mutation may illuminate cases negative for MYD88
  - Further gene mutations: *ARID1A*, *CD79B*

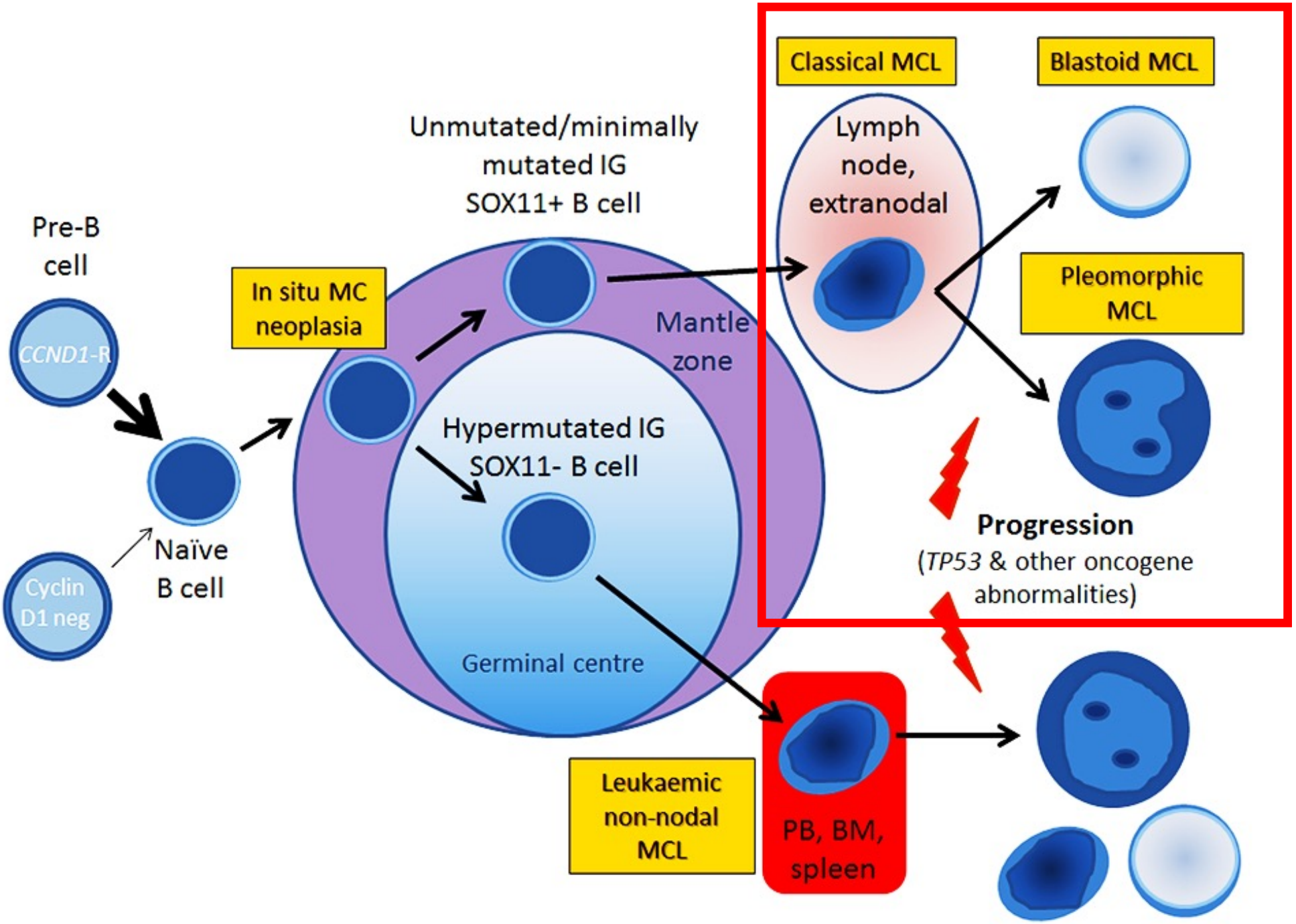


Treon et al. JCO, 2020

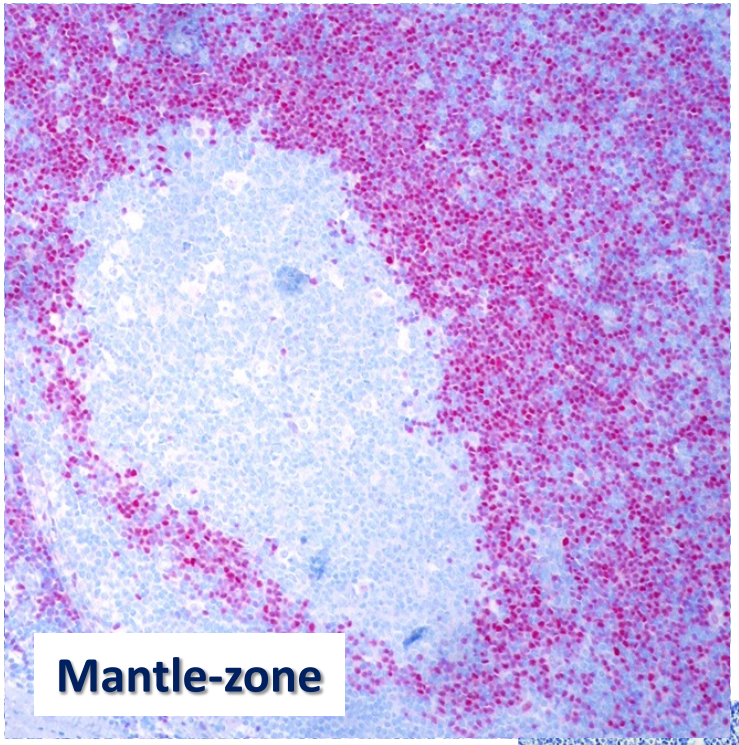
Mantle cell lymphoma

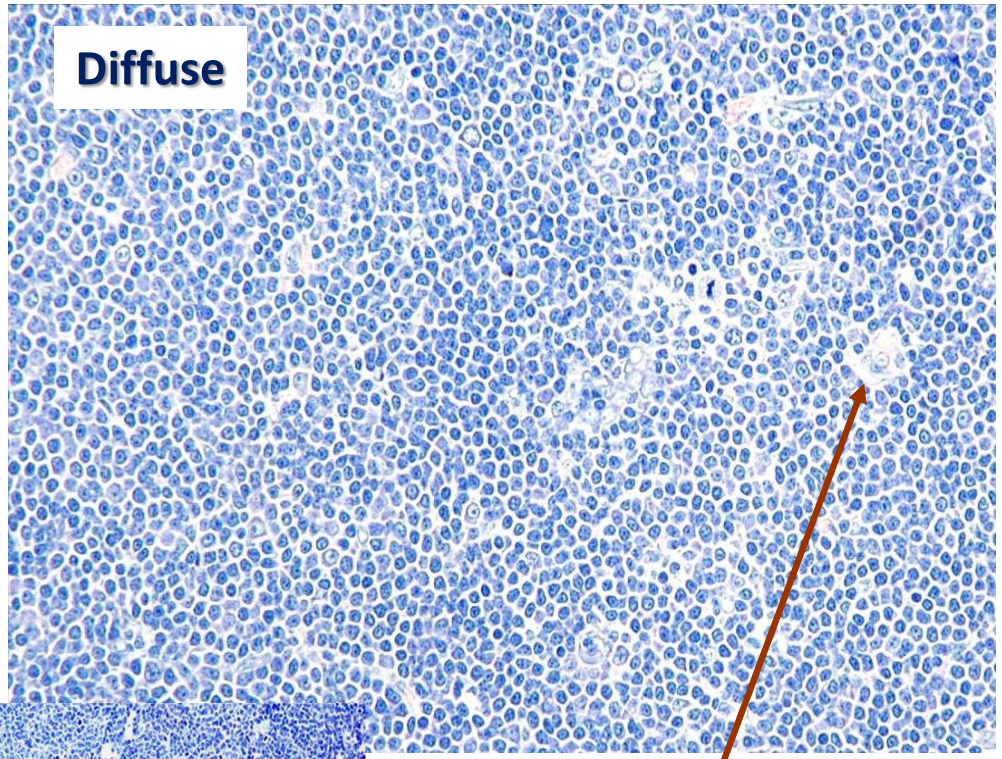
Definition

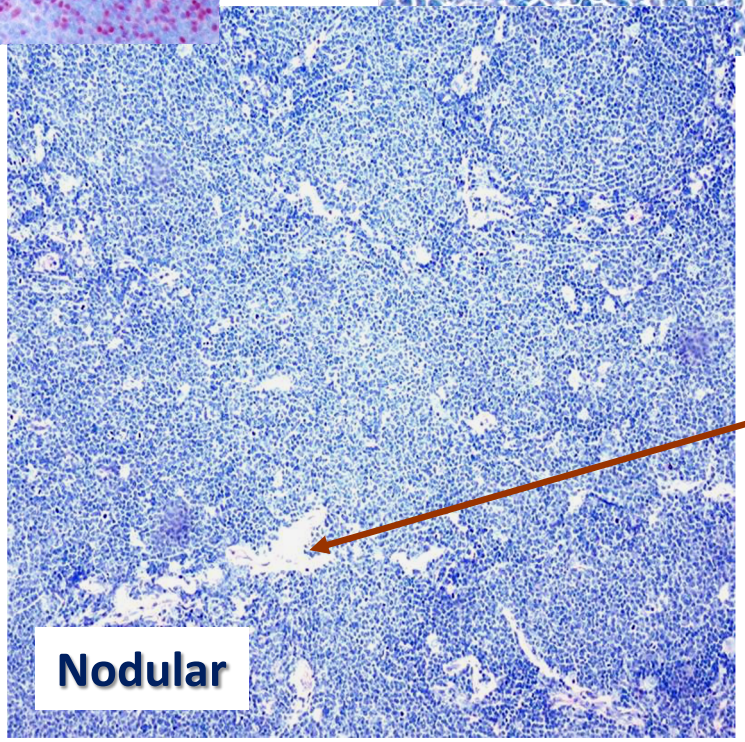
Mantle cell lymphoma is a mature B-cell neoplasm usually composed of monomorphic small to medium-sized lymphoid cells with irregular nuclear contours; in > 95% of cases, there is a *CCND1* translocation {245,543,2219,2269,3849,4018}. Neoplastic transformed cells (centroblasts), paraimmunoblasts, and proliferation centres are absent. Mantle cell lymphoma has traditionally been considered a very aggressive and incurable lymphoma, but more indolent variants, including leukaemic non-nodal mantle cell lymphoma and in situ mantle cell neoplasia, are now also well recognized.


Classical MCL
Blastoid MCL
Lymph node, extranodal
Unmutated/minimally mutated IG SOX11+ B cell
Pre-B cell
CCND1-R
In situ MC neoplasia
Mantle zone
Pleomorphic MCL
Hypermutated IG SOX11- B cell
Progression
(TP53 & other oncogene abnormalities)
Naïve B cell
Cyclin D1 neg
Germinal centre
Leukaemic non-nodal MCL
PB, BM, spleen

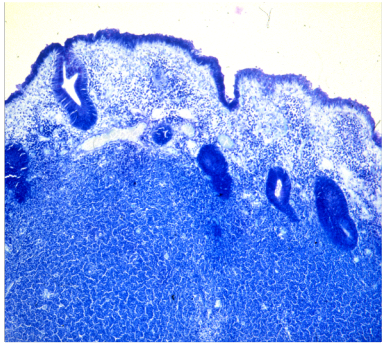
MCL Growth Patterns

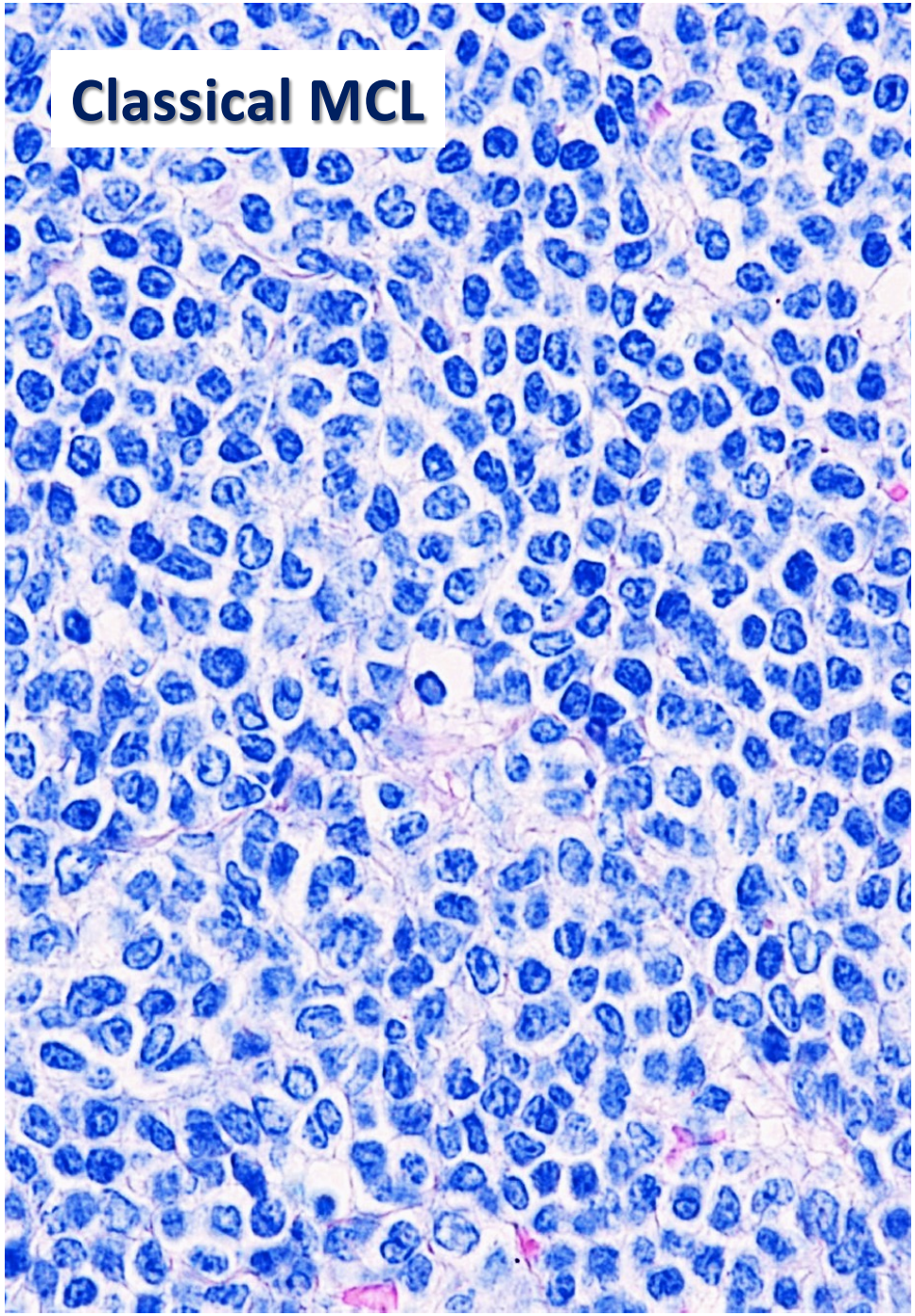


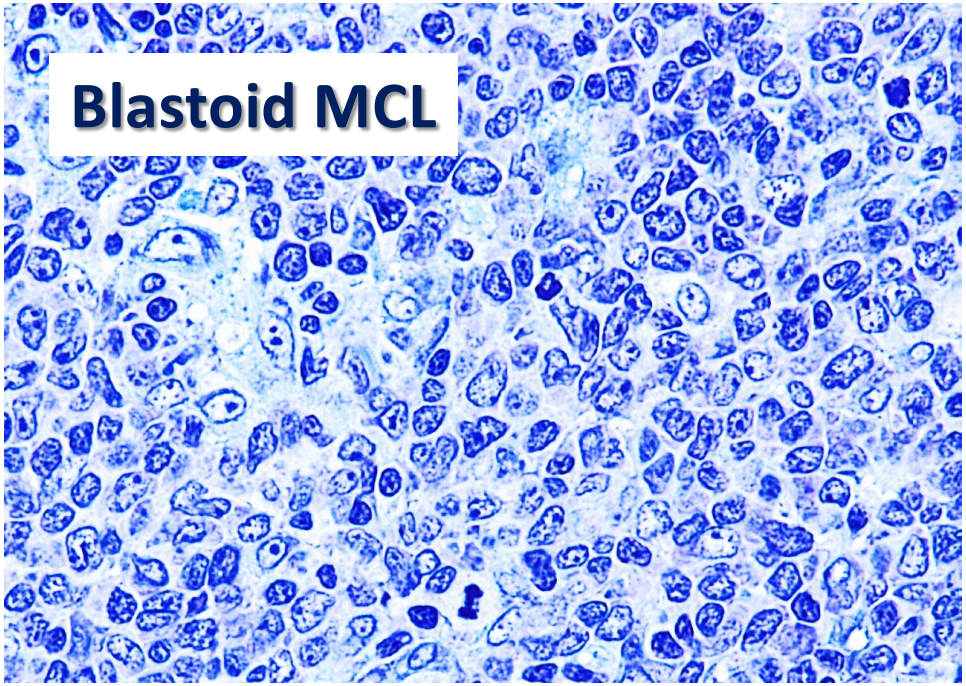


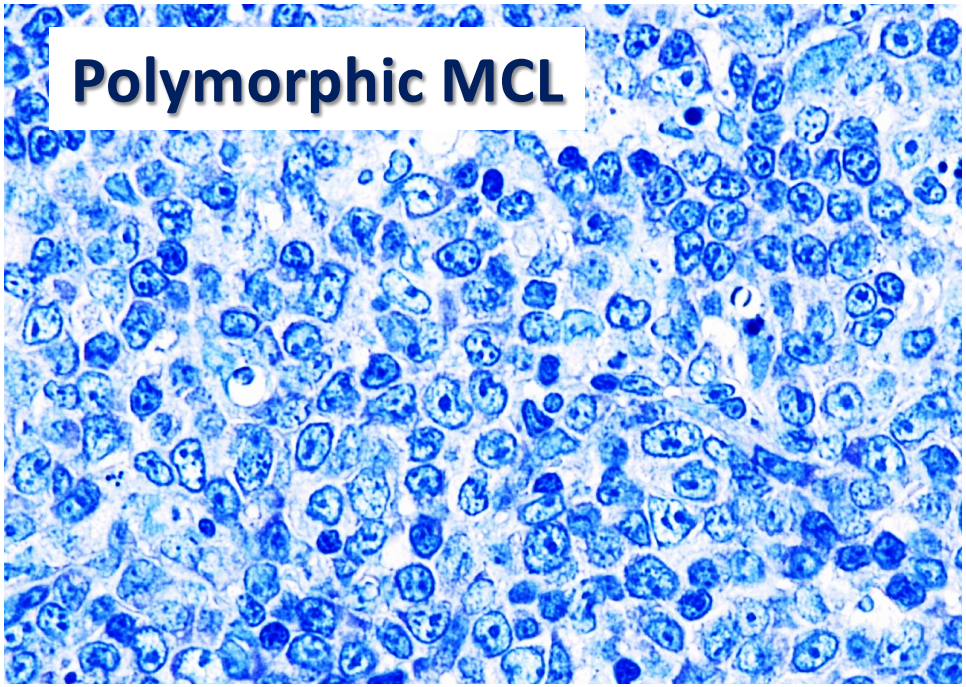


Epithelioid histiocytes




Classical MCL


Blastoid MCL


Polymorphic MCL

Phenotype

CD19, CD20, CD22, CD79a, CD79b +

CD5+

IgM+

IgD+

Cyclin D1+ (>95%)

SOX11+ (- in leukemic non nodal)

BCL2+

CD23 -

IRF4 -

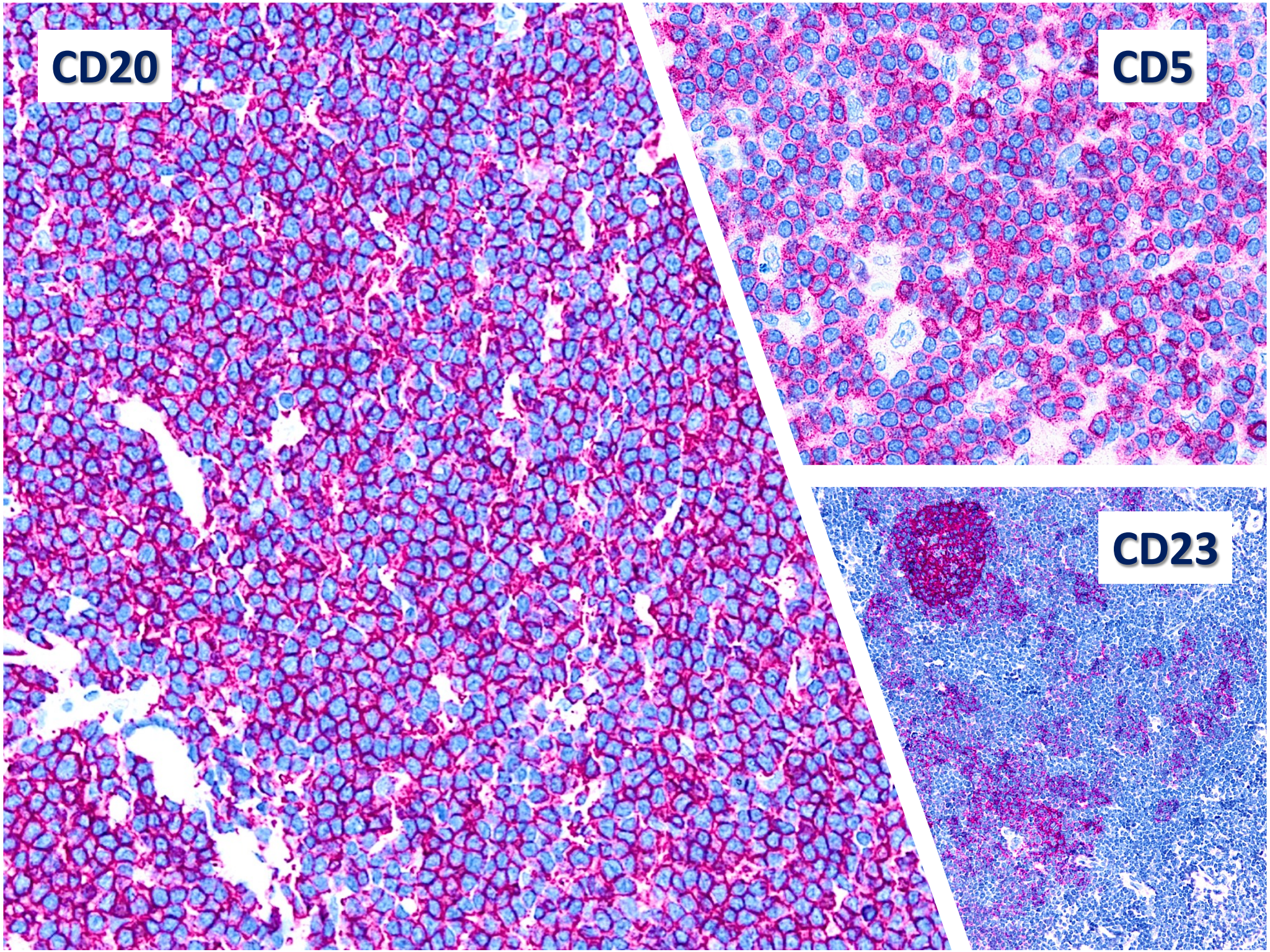
CD200 – (at times + in leukemic non nodal)

LEF1 – (at times + in blastoid/pleomorphic)

IRTA1, MNDA, T-bet -

CD10, BCL6, LMO2 –

Ki-67: variable


CD20
CD5
CD23

LYMPHOID NEOPLASIA

CCND2 and *CCND3* hijack immunoglobulin light-chain enhancers in cyclin D1^{-} mantle cell lymphoma

David Martín-Garcia,[1,2,*] Alba Navarro,[1,2,*] Rafael Valdés-Mas,[3] Guillem Clot,[1,2] Jesús Gutiérrez-Abril,[3] Miriam Prieto,[1,2] Inmaculada Ribera-Cortada,[4] Renata Woroniecka,[5] Grzegorz Rymkiewicz,[6] Susanne Bens,[7,8] Laurence de Leval,[9] Andreas Rosenwald,[10,11] Judith A. Ferry,[12] Eric D. Hsi,[13] Kai Fu,[14,15] Jan Delabie,[16,17] Dennis Weisenburger,[18] Daphne de Jong,[19] Fina Climent,[20] Sheila J. O'Connor,[21] Steven H. Swerdlow,[22] David Torrents,[23,24] Sergi Beltran,[25] Blanca Espinet,[26,27] Blanca González-Farré,[2,28] Luis Veloza,[28] Dolors Costa,[2,28] Estella Matutes,[28] Reiner Siebert,[7,8] German Ott,[29,30] Leticia Quintanilla-Martinez,[31] Elaine S. Jaffe,[32] Carlos López-Otín,[2,3] Itziar Salaverria,[1,2] Xose S. Puente,[2,3,†] Elias Campo,[1,2,28,33,†] and Sílvia Beà[1,2,†]

(*Blood.* 2019;133(9):940-951)

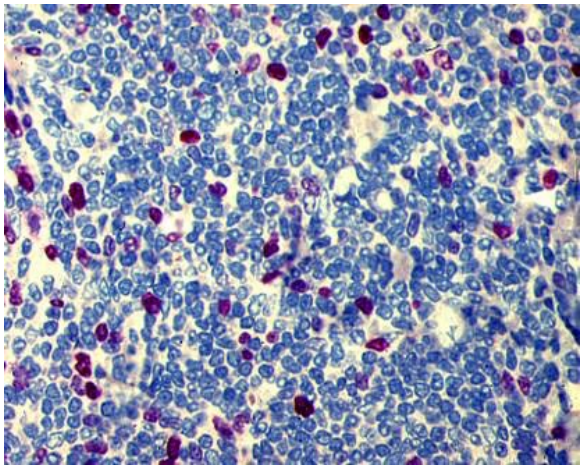
VOLUME 34 · NUMBER 12 · APRIL 20, 2016

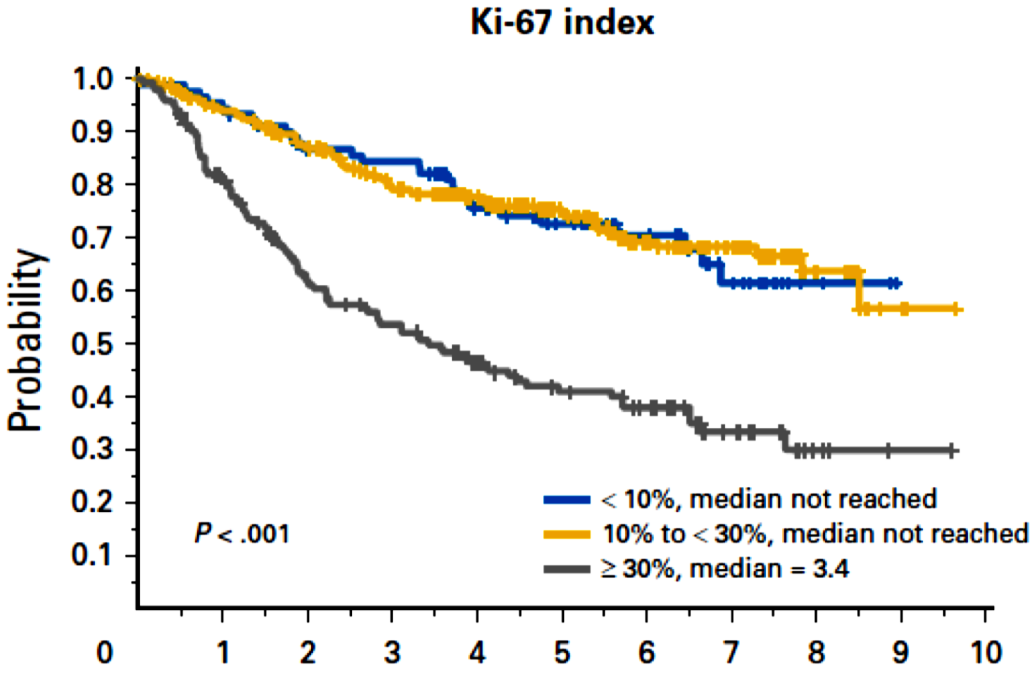
JOURNAL OF CLINICAL ONCOLOGY

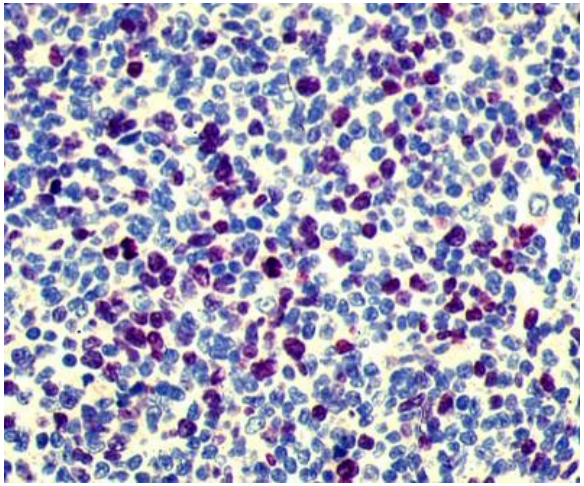
ORIGINAL REPORT

Prognostic Value of Ki-67 Index, Cytology, and Growth Pattern in Mantle-Cell Lymphoma: Results From Randomized Trials of the European Mantle Cell Lymphoma Network

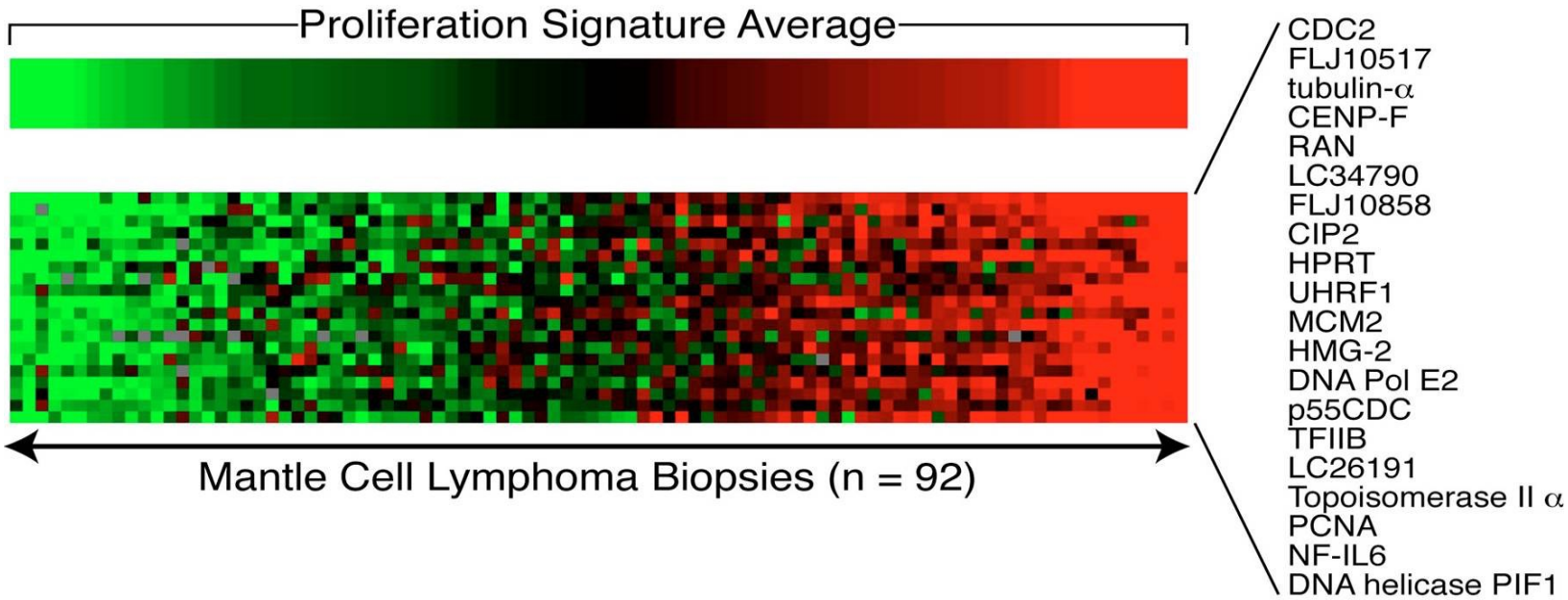
Eva Hoster, Andreas Rosenwald, Françoise Berger, Heinz-Wolfram Bernd, Sylvia Hartmann, Christoph Loddenkemper, Thomas F.E. Barth, Nicole Brousse, Stefano Pileri, Grzegorz Rymkiewicz, Roman Kodet, Stephan Stilgenbauer, Roswitha Forstpointner, Catherine Thieblemont, Michael Hallek, Bertrand Coiffier, Ursula Vehling-Kaiser, Réda Bouabdallah, Lothar Kanz, Michael Pfreundschuh, Christian Schmidt, Vincent Ribrag, Wolfgang Hiddemann, Michael Unterhalt, Johanna C. Kluin-Nelemans, Olivier Hermine, Martin H. Dreyling, and Wolfram Klapper

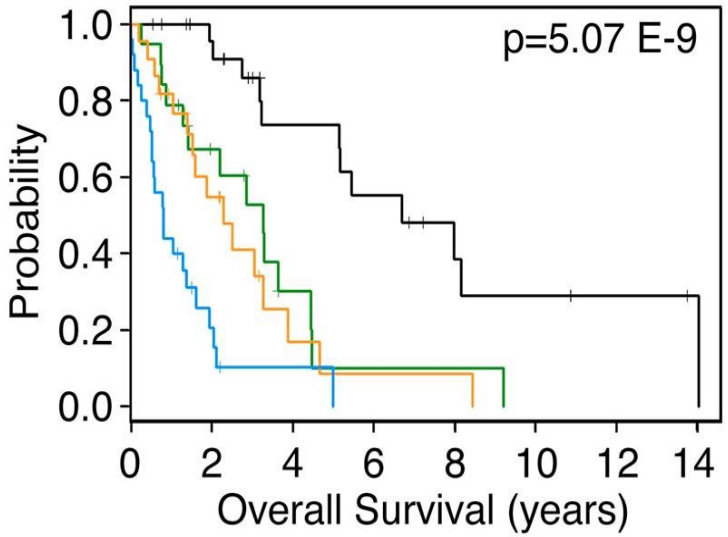




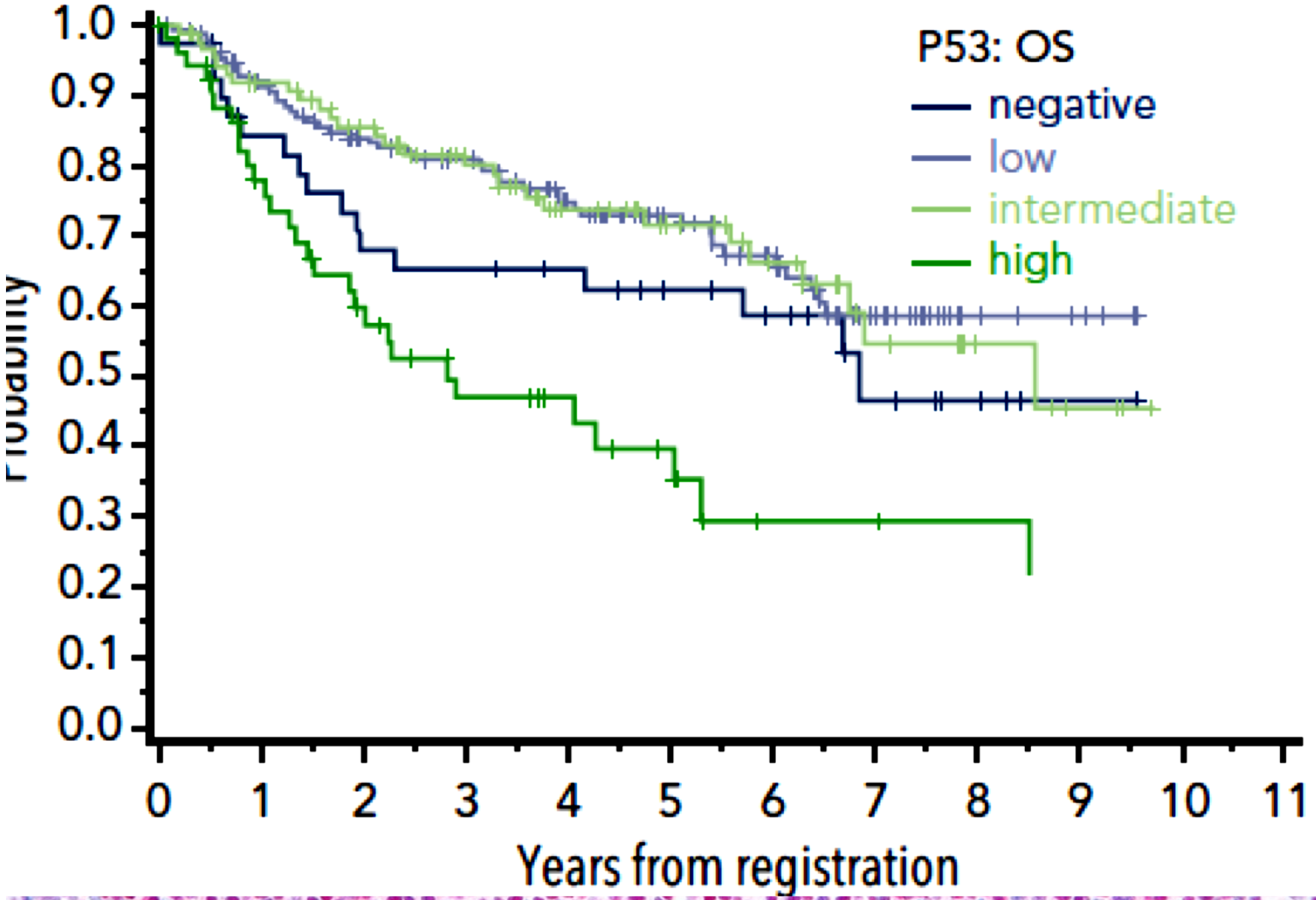


Variable Expression of Proliferation Signature Genes in Mantle Cell Lymphoma

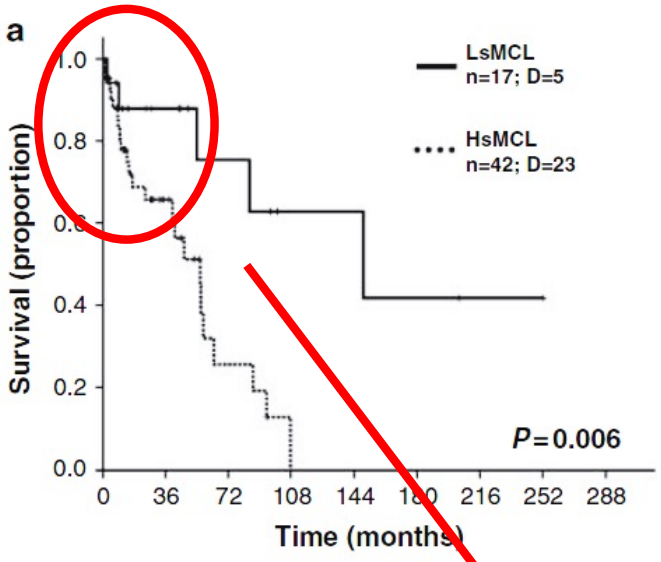




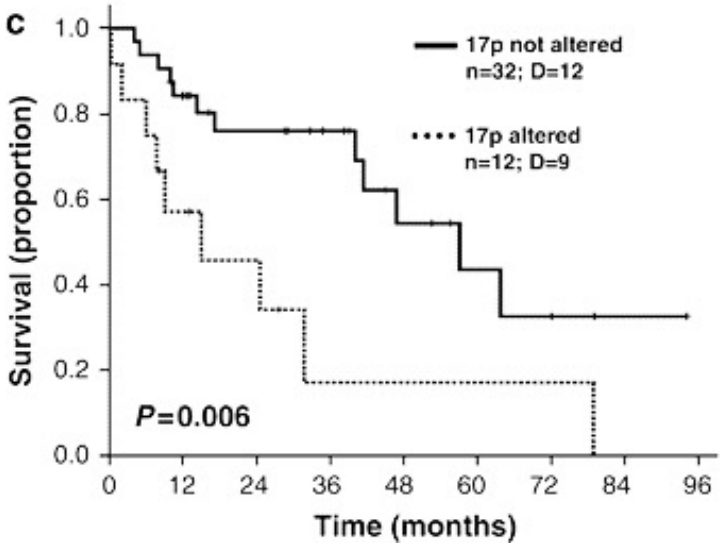
Rosenwald A et LLMPP, Cancer Cell 2003; 3(2):185-97.


P53: OS
negative
low
intermediate
high
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
11
Years from registration

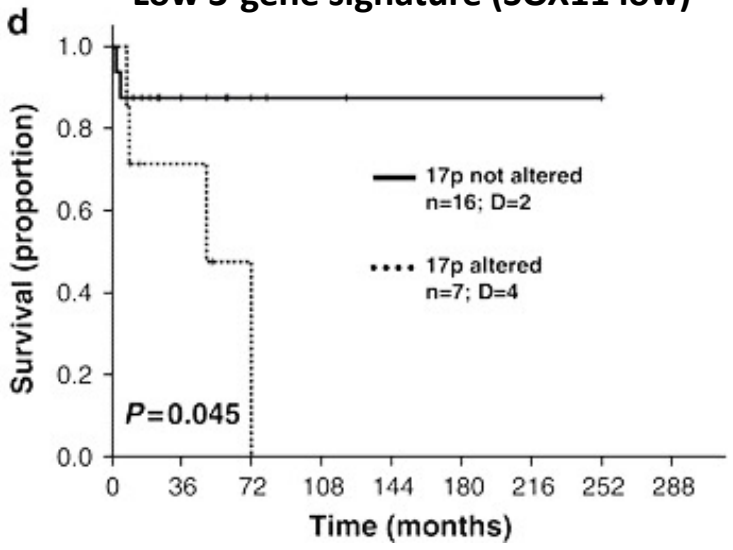
Prognostic impact of 17p del and *TP53* mutations in MCL



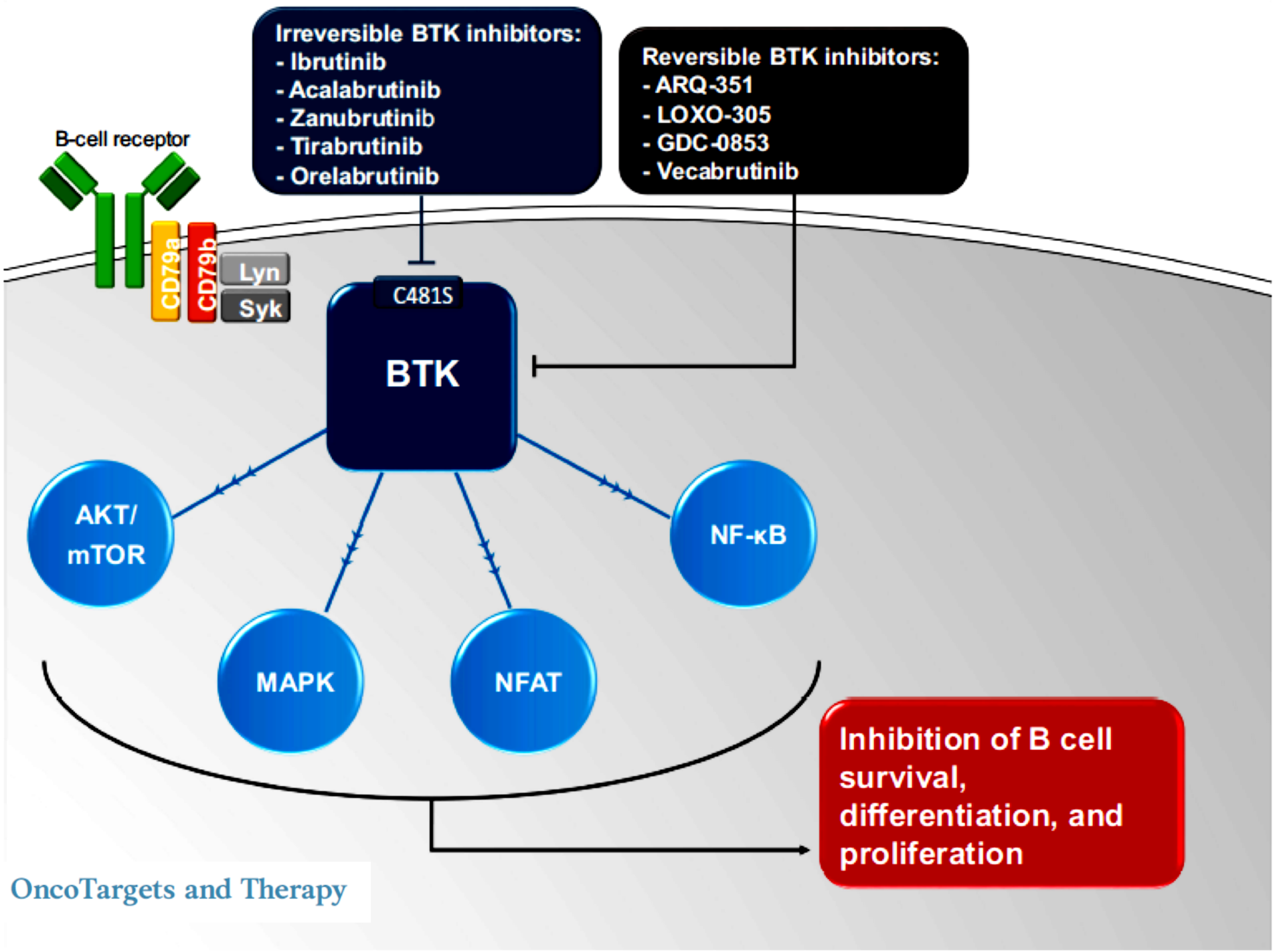
High 3-gene signature (SOX11high)

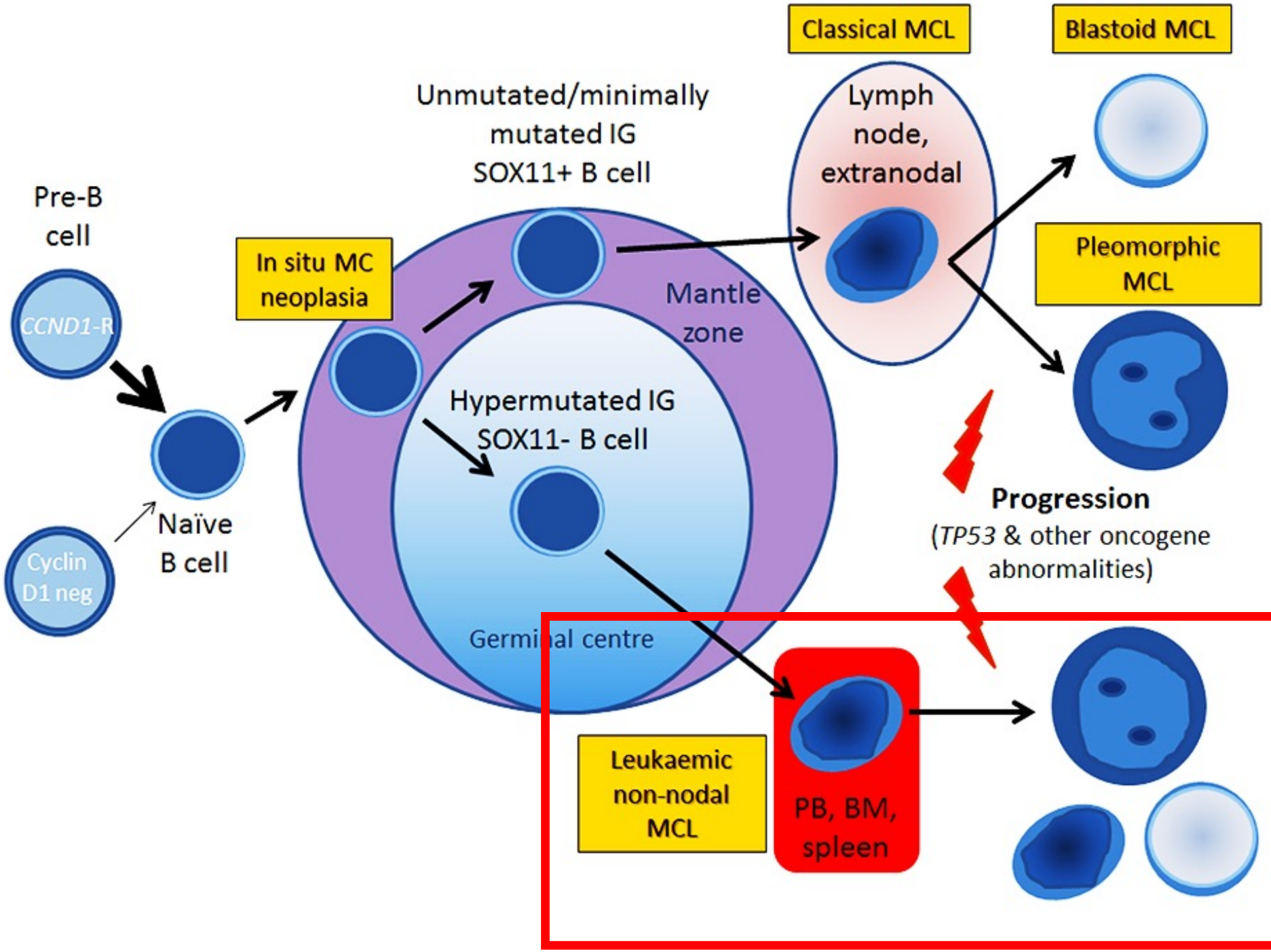


Low 3-gene signature (SOX11 low)



Royo C et al, Leukemia 2012


Irreversible BTK inhibitors:
- Ibrutinib
- Acalabrutinib
- Zanubrutinib
- Tirabrutinib
- Orelabrutinib
Reversible BTK inhibitors:
- ARQ-351
- LOXO-305
- GDC-0853
- Vecabrutinib
B-cell receptor
CD79a
CD79b
Lyn
Syk
C481S
BTK
AKT/
mTOR
MAPK
NFAT
NF-κB
Inhibition of B cell survival, differentiation, and proliferation


Classical MCL
Blastoid MCL
Unmutated/minimally mutated IG SOX11+ B cell
Lymph node, extranodal
Pre-B cell
In situ MC neoplasia
Mantle zone
Pleomorphic MCL
CCND1-R
Hypermutated IG SOX11- B cell
Progression
(TP53 & other oncogene abnormalities)
Naïve B cell
Cyclin D1 neg
Germinal centre
Leukaemic non-nodal MCL
PB, BM, spleen

Molecular and Cellular Pathobiology

Cancer Research

Genomic and Gene Expression Profiling Defines Indolent Forms of Mantle Cell Lymphoma

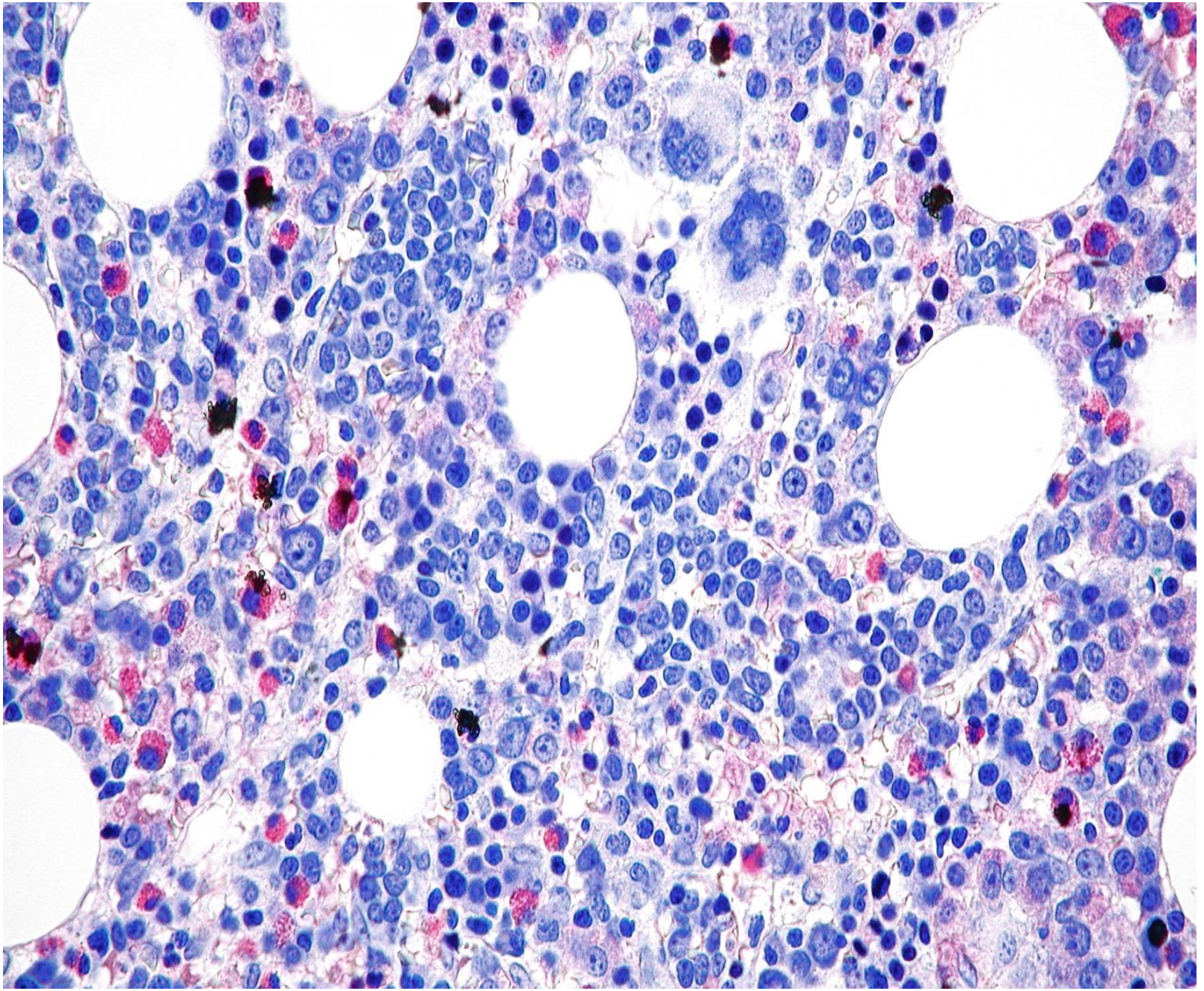
Verònica Fernàndez[1], Olga Salamero[2], Blanca Espinet[3], Francesc Solé[3], Cristina Royo[1], Alba Navarro[1], Francisca Camacho[4], Sílvia Beà[1], Elena Hartmann[5], Virginia Amador[1], Luis Hernández[1], Claudio Agostinelli[6], Rachel L. Sargent[7], Maria Rozman[1], Marta Aymerich[1], Dolors Colomer[1], Neus Villamor[1], Steven H. Swerdlow[7], Stefano A. Pileri[6], Francesc Bosch[2], Miguel A. Piris[4], Emili Montserrat[2], German Ott[8], Andreas Rosenwald[5], Armando López-Guillermo[2], Pedro Jares[1], Sergi Serrano[3], and Elías Campo[1]

Molecular and Cellular Pathobiology

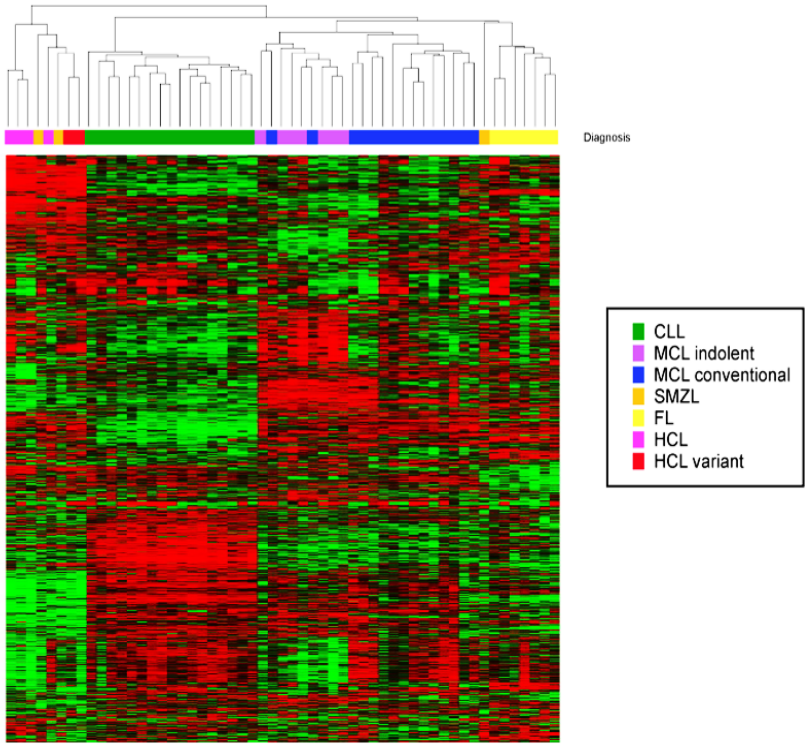
Cancer Research

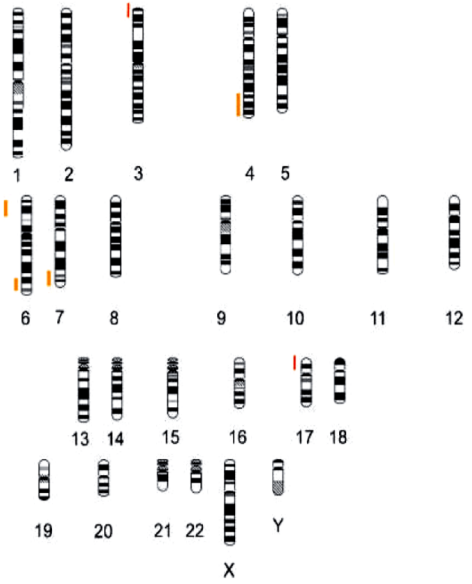
Molecular Subsets of Mantle Cell Lymphoma Defined by the *IGHV* Mutational Status and SOX11 Expression Have Distinct Biologic and Clinical Features

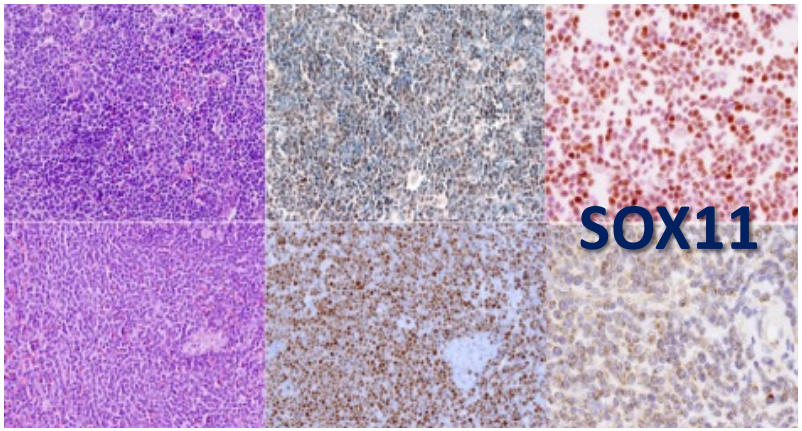
Alba Navarro[1], Guillem Clot[1], Cristina Royo[1], Pedro Jares[1], Anastasia Hadzidimitriou[4], Andreas Agathangelidis[4,5], Vasilis Bikos[4], Nikos Darzentas[4], Theodora Papadaki[7], Itziar Salaverria[1,8], Magda Pinyol[1], Xavier Puig[2], Jara Palomero[1], Maria Carmela Vegliante[1], Virgina Amador[1], Alejandra Martinez-Trillos[1], Lenka Stefancikova[12], Adrian Wiestner[13], Wyndham Wilson[13], Christiane Pott[9], Maria Jose Calasanz[3], Nicola Trim[14], Wendy Erber[15], Birgitta Sander[16], German Ott[10], Andreas Rosenwald[11], Dolors Colomer[1], Eva Giné[1], Reiner Siebert[8], Armando Lopez-Guillermo[1], Kostas Stamatopoulos[4,6], Sílvia Beà[1], and Elías Campo[1]

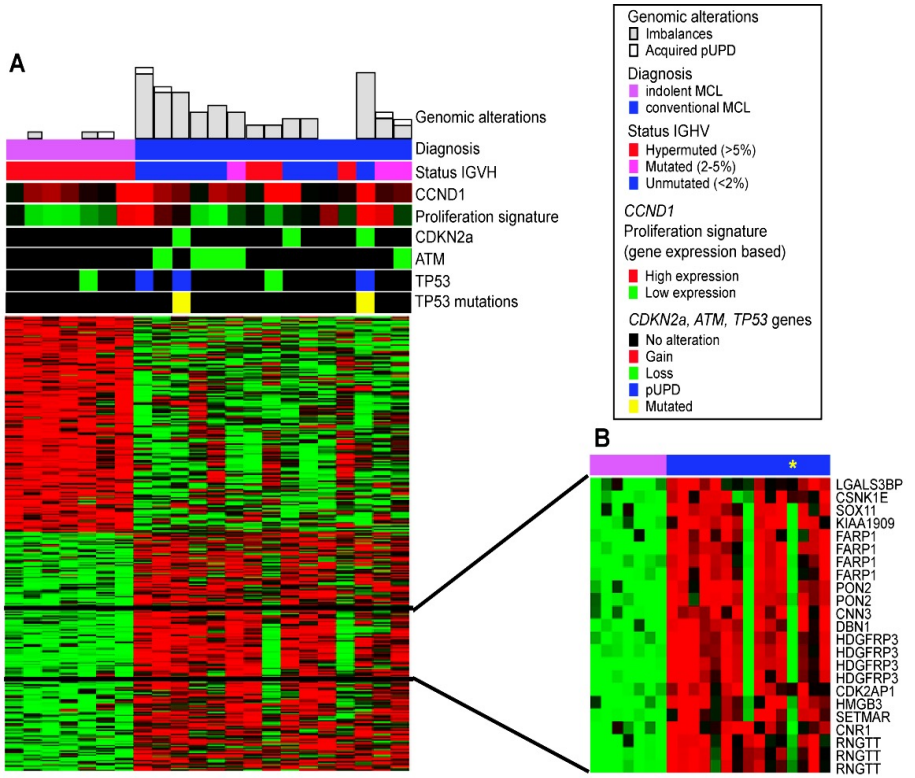


	cMCL (n=15)	iMCL (n=12)	P value
B symptoms (%)	33	0	0.03
Non-ambulatory performance status ECOG≥2 (%)	70	0	0.01
Nodal presentation (lymph nodes >1 cm) (%)*	**93**	**17**	**<0.001**
High serum LDH* (%)	46	0	0.03
Intermediate or high-risk MIPI	46	0	0.016
Morphology	13	67	0.007
Small cell (%)	74	33	
Classical	13	-	
Blastoid			
***IGHV* gene hypermutations (>5%)**	**20**	**70**	**< 0.04**
Genomic Profile			
1.imbalance	**13**	**100**	**<0.001**
≥ 2 imbalances	**87**	**0**	
Chemotherapy at any time (%)	100	17	
Dead patients (%)	**47**	**0**	**<0.001**
5-year overall survival (%)	49	100	0.03

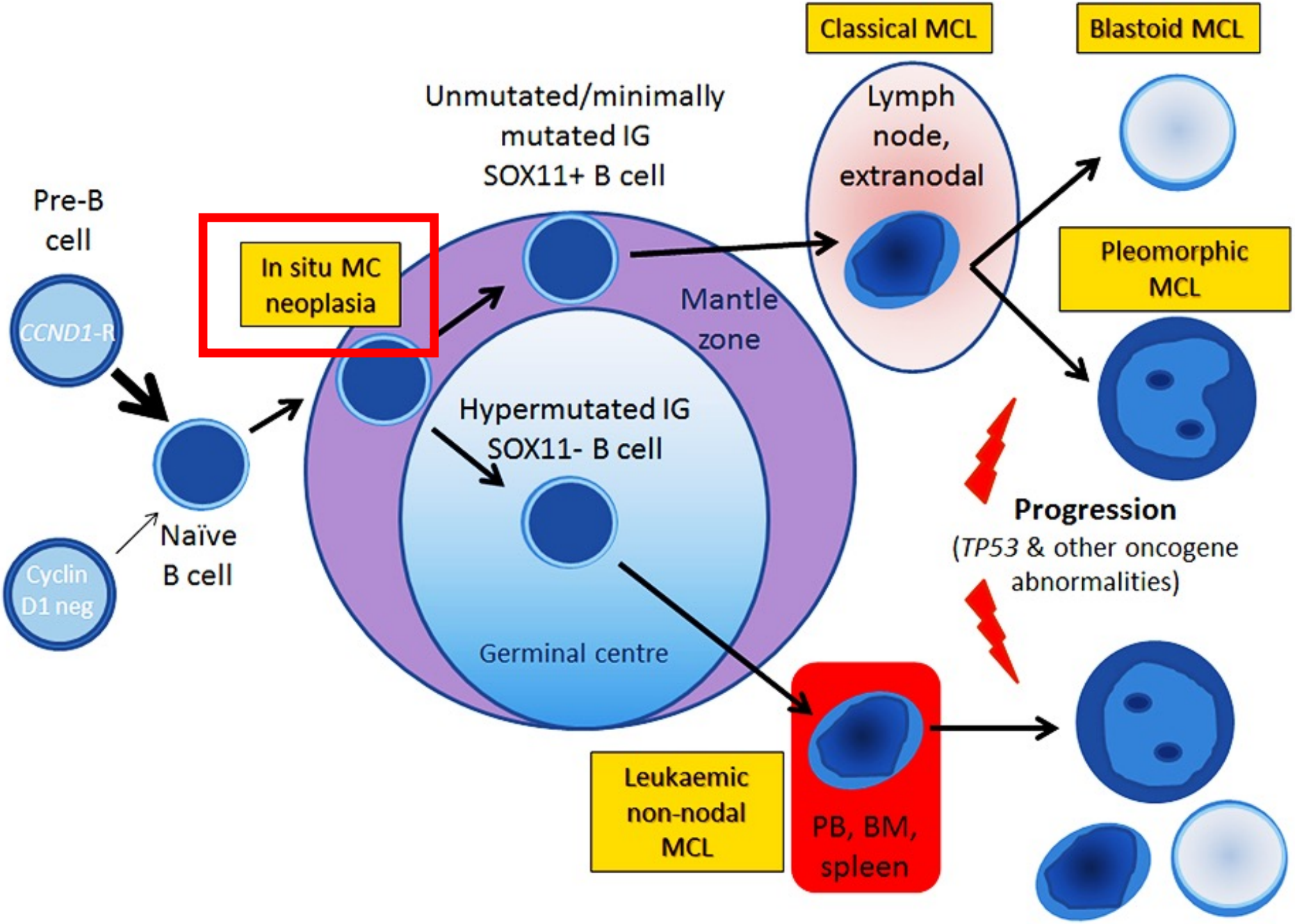


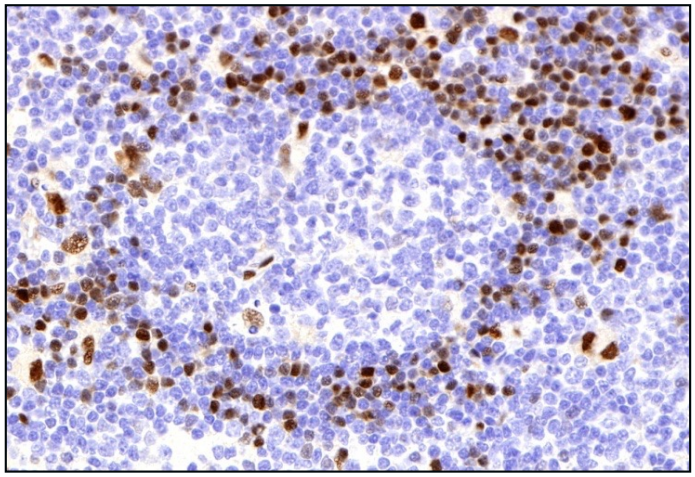






LNMCL shows a specific gene signature and SOX11 negativity


Classical MCL
Blastoid MCL
Unmutated/minimally mutated IG SOX11+ B cell
Lymph node, extranodal
Pre-B cell
In situ MC neoplasia
Mantle zone
Pleomorphic MCL
CCND1-R
Hypermutated IG SOX11- B cell
Progression
(TP53 & other oncogene abnormalities)
Naïve B cell
Cyclin D1 neg
Germinal centre
Leukaemic non-nodal MCL
PB, BM, spleen



LN with Cyclin D1+
In Situ Pattern

SOX11 negative

May be CD5 negative
Rare event: <1% of LNs
Low risk of Progression (<10%)

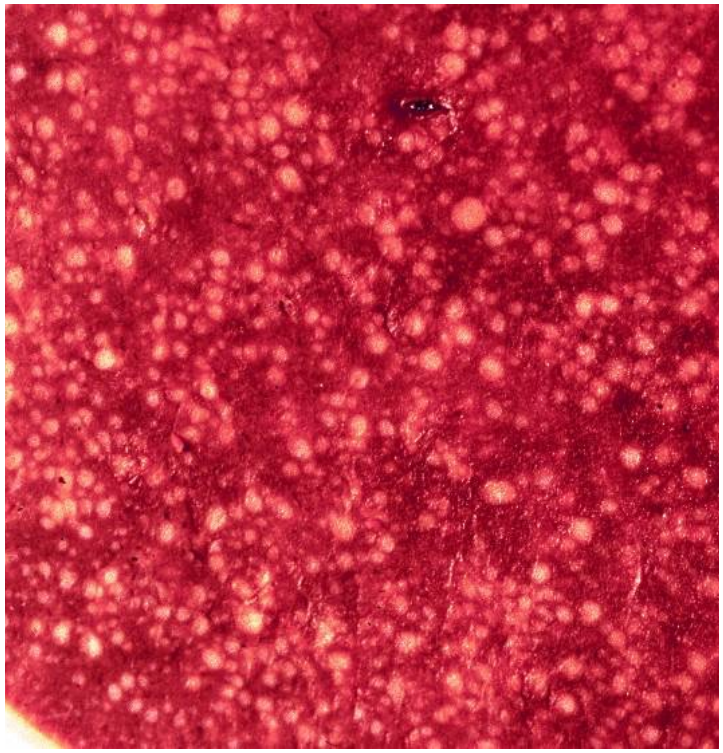
SOX11 positive

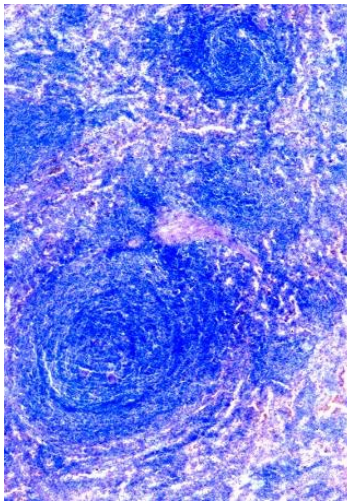
More often CD5 positive
Higher risk of progression
Similar pattern can be seen
at relapse or at distant sites

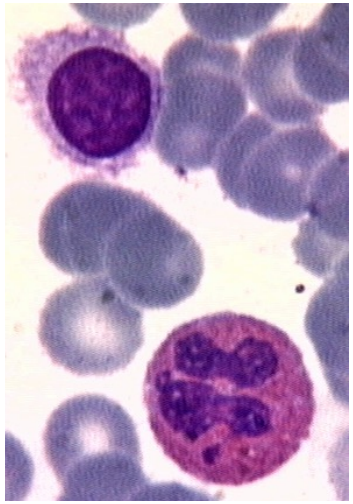
Splenic marginal zone lymphoma

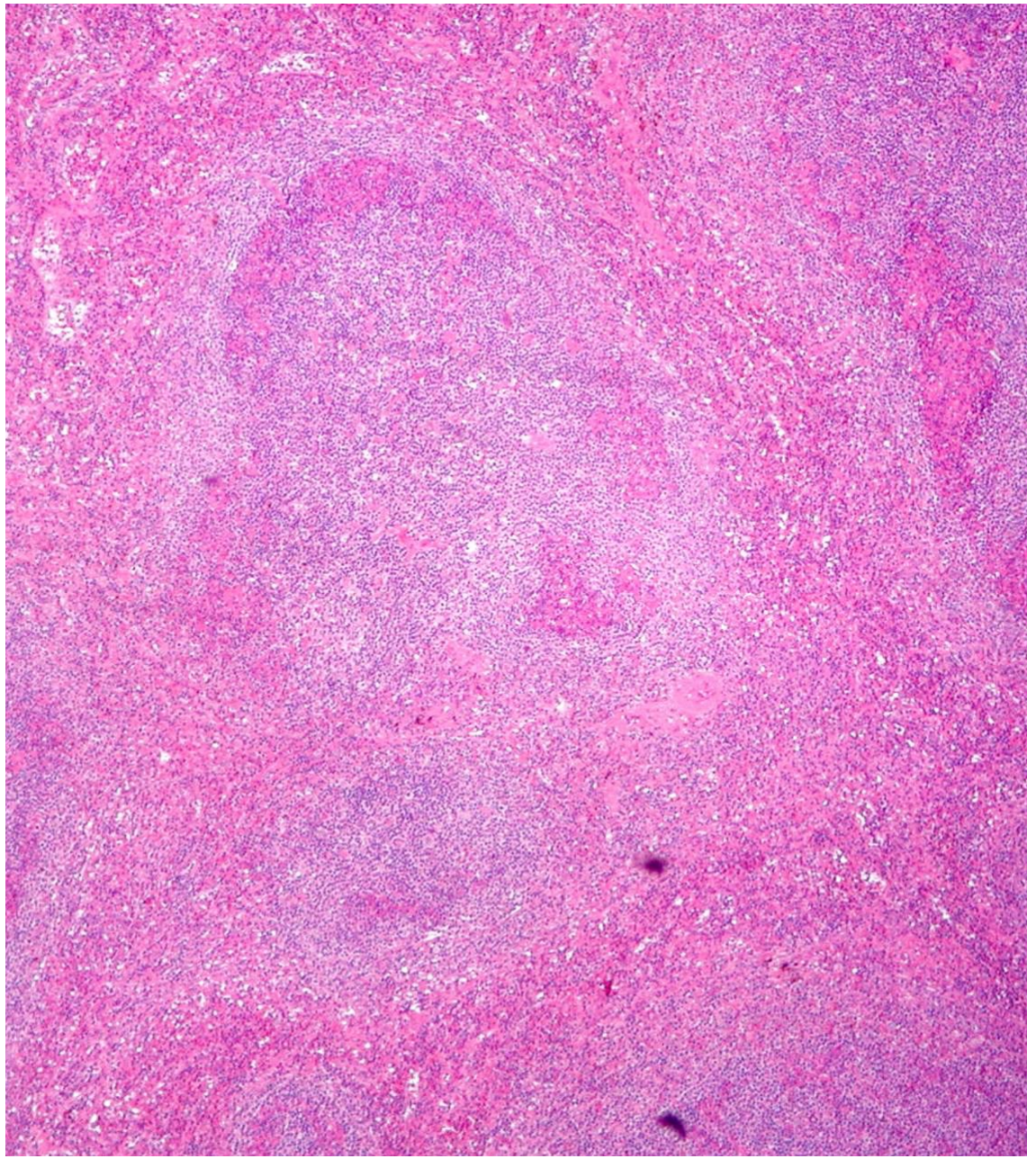
Definition

Splenic marginal zone lymphoma (SMZL) is a B-cell neoplasm composed of small lymphocytes that surround and replace the splenic white pulp germinal centres, efface the follicle mantle, and merge with a peripheral (marginal) zone of larger cells, including scattered transformed blasts; both small and larger cells infiltrate the red pulp. Splenic hilar lymph nodes and bone marrow are often involved; lymphoma cells are frequently found in the peripheral blood as villous lymphocytes.









Phenotype

CD19, CD20, CD22, CD79a, CD79b +

IgM+

IgD+

MNDA+

DBA44+/-

IRF4 -/+

BCL2+ (weak)

Annexin A-

CD5- (exceptions)

CD23-

Cyclin D1 –

SOX11-

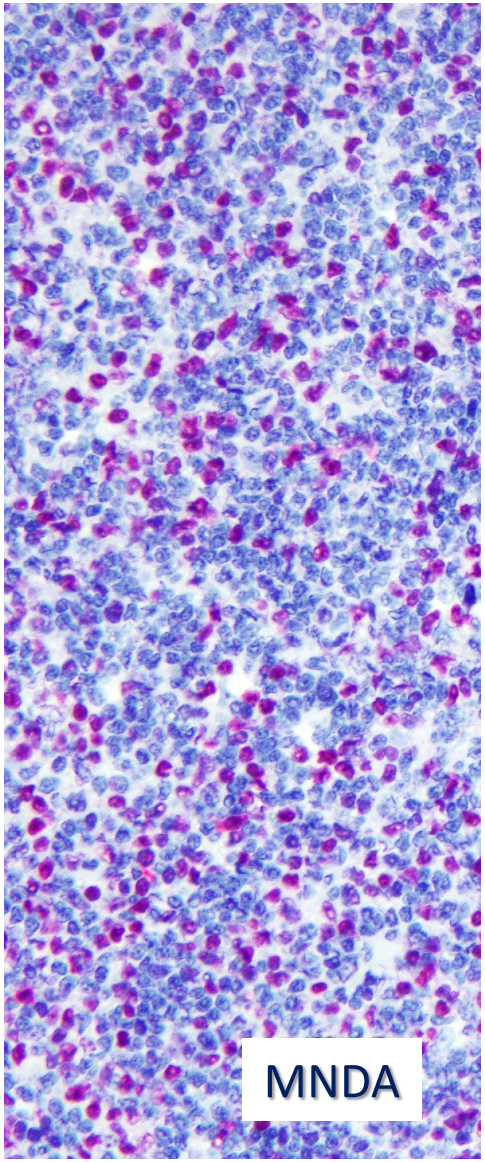
CD200 –

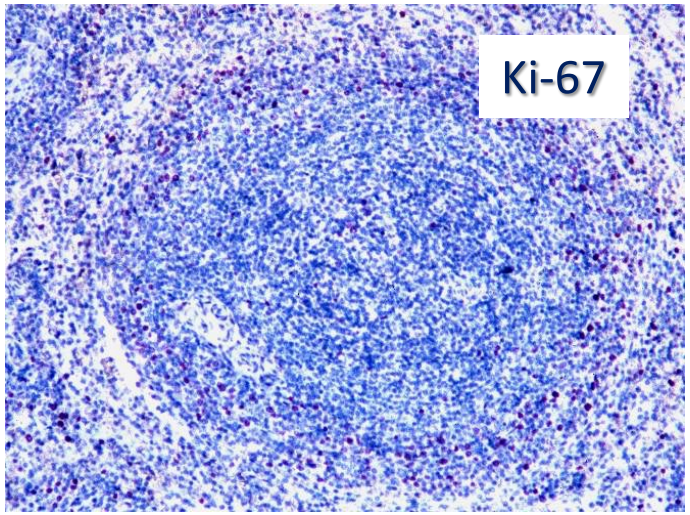
LEF1 –

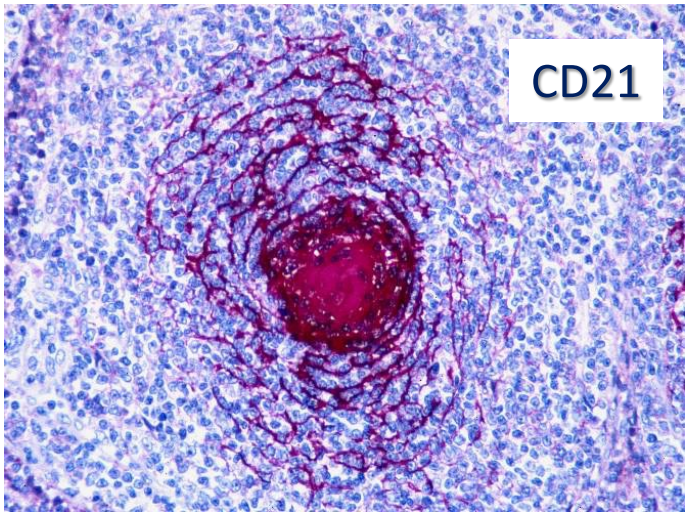
IRTA1, T-bet -

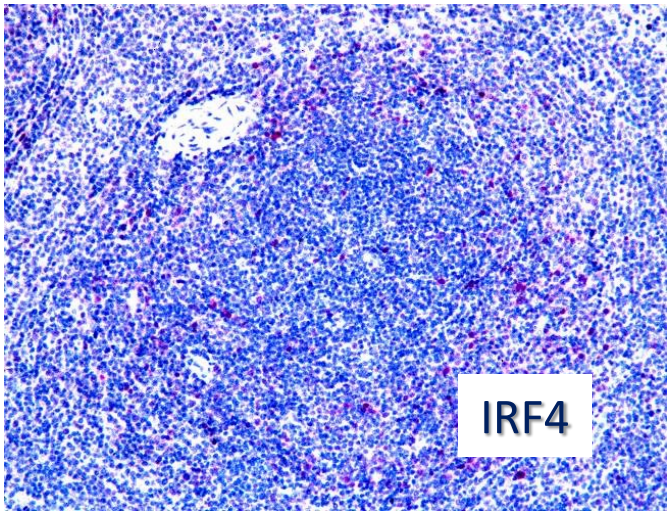
CD10, BCL6, LMO2 –

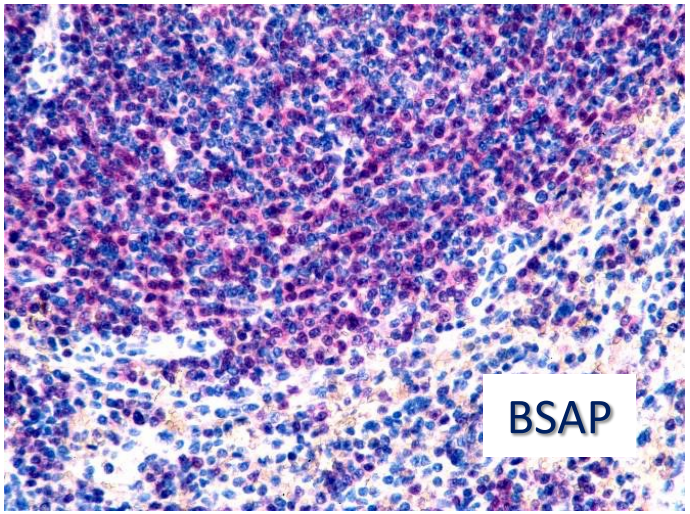
Ki-67: variable


MNDA


Ki-67


CD21


IRF4

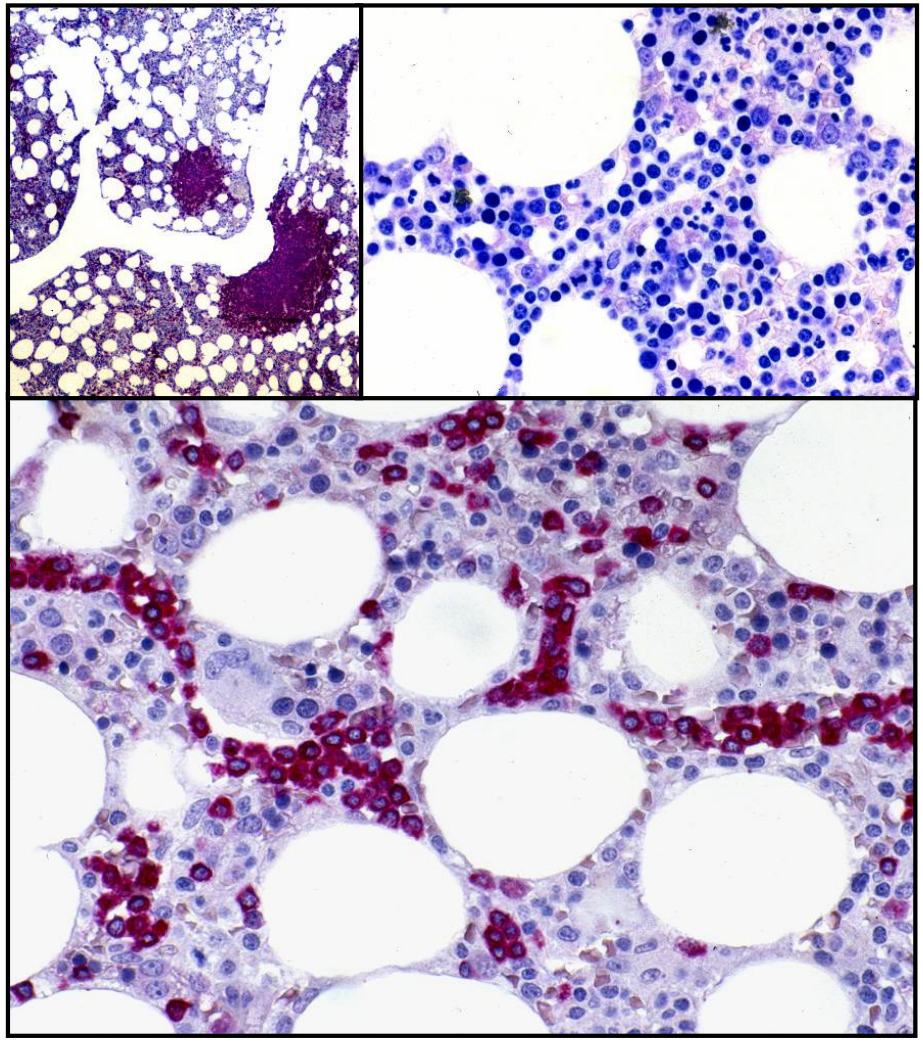

BSAP

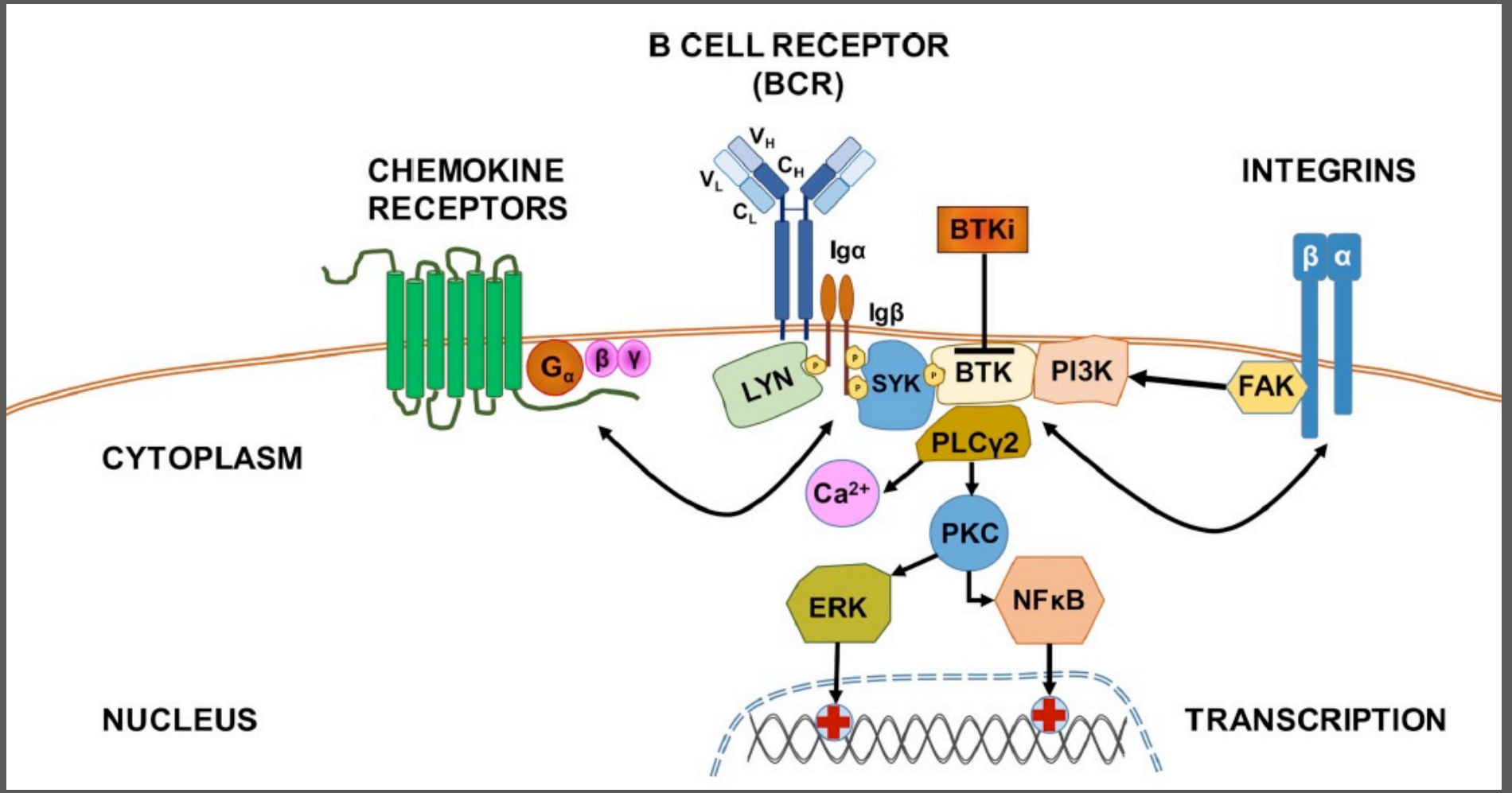
BM involvement

nodular,

Interstitial.

Intra-sinusoidal

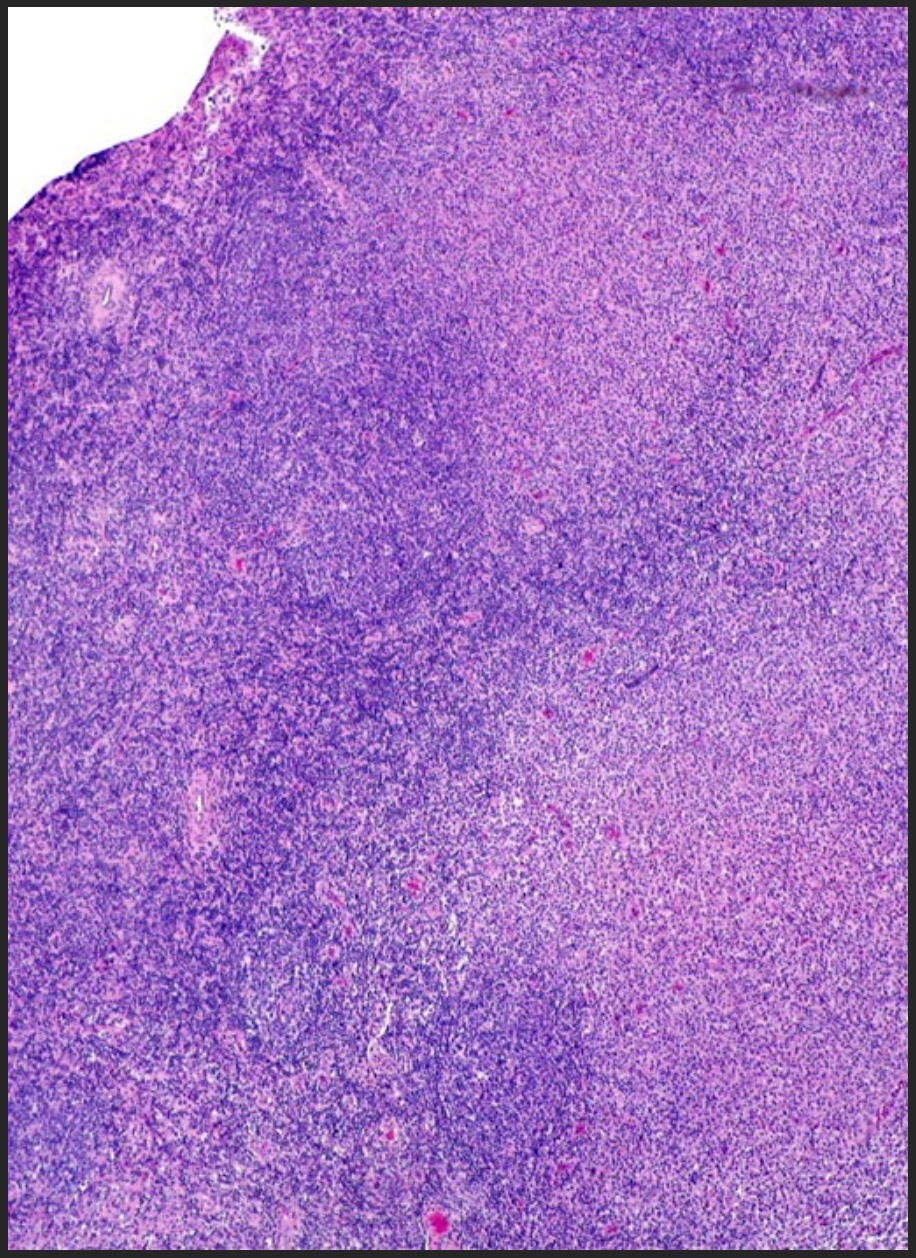


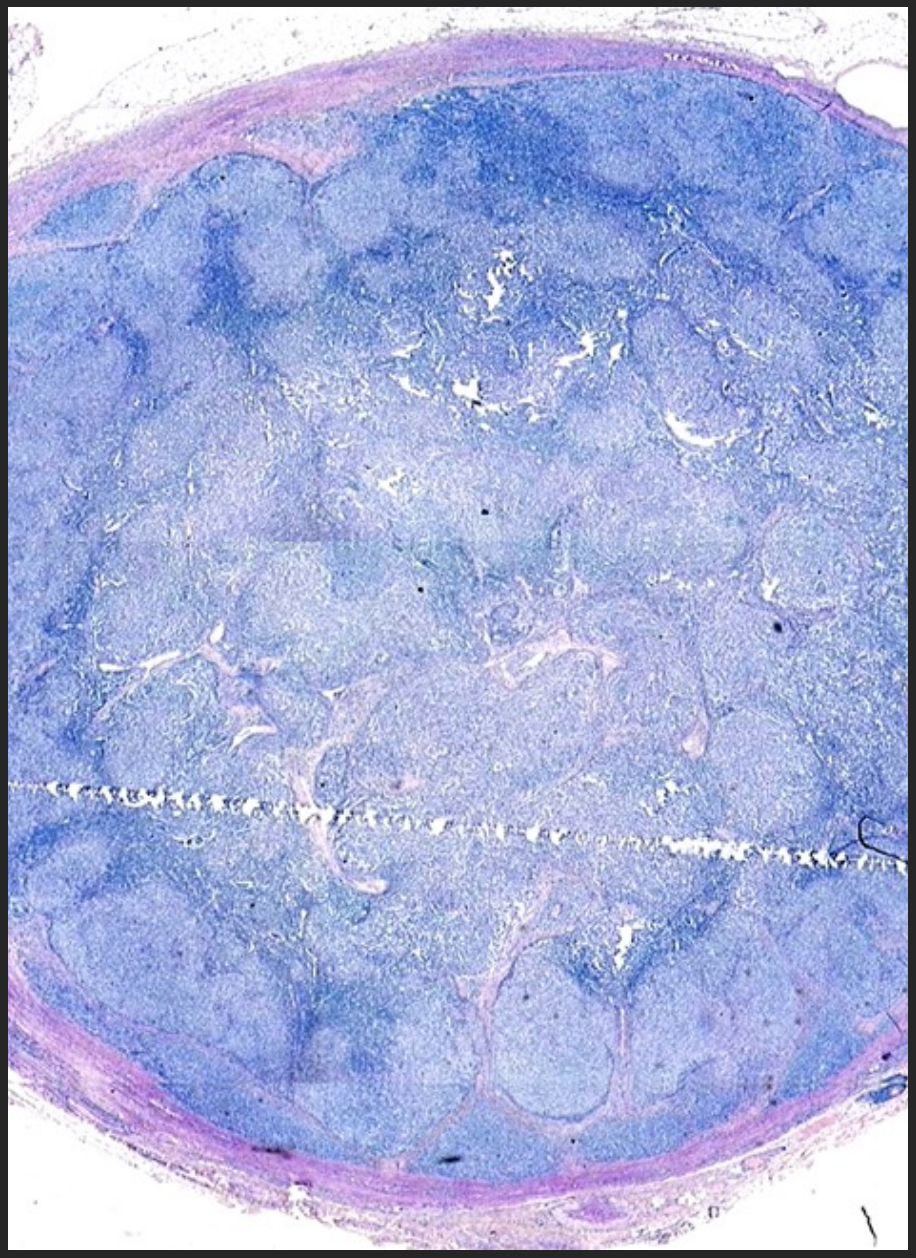

B CELL RECEPTOR
(BCR)
CHEMOKINE
RECEPTORS
INTEGRINS
V_H
C_H
V_L
C_L
Igα
Igβ
BTKi
β α
G_α
β γ
LYN
SYK
BTK
PI3K
FAK
PLCγ2
Ca^{2+}
PKC
ERK
NFκB
CYTOPLASM
NUCLEUS
TRANSCRIPTION

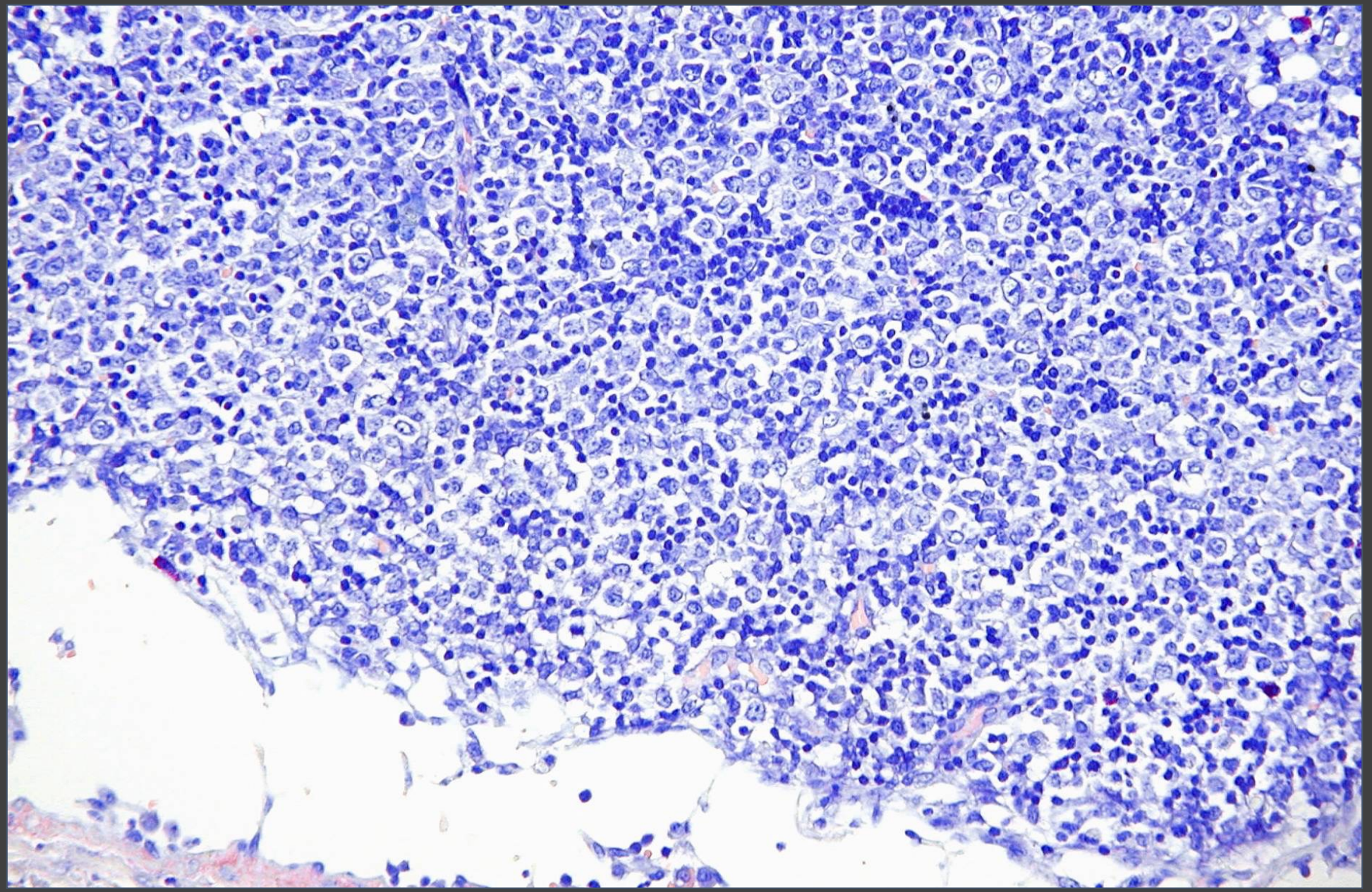
Nodal marginal zone lymphoma

Definition

Nodal marginal zone lymphoma (NMZL) is a primary nodal B-cell neoplasm that morphologically resembles lymph nodes involved by marginal zone lymphoma (MZL) of the extranodal or splenic types, but without evidence of extranodal or splenic disease.







Phenotype

CD19, CD20, CD22, CD79a, CD79b +

IgM/G+

IgD-/+

IRTA1+, T-bet+ (monocytoid); MNDA+ (splenic-type)

IRF4-/+ (plasma cell differentiation)

BCL2+ (weak)

CD5- (rarely +)

CD23-

Cyclin D1 –

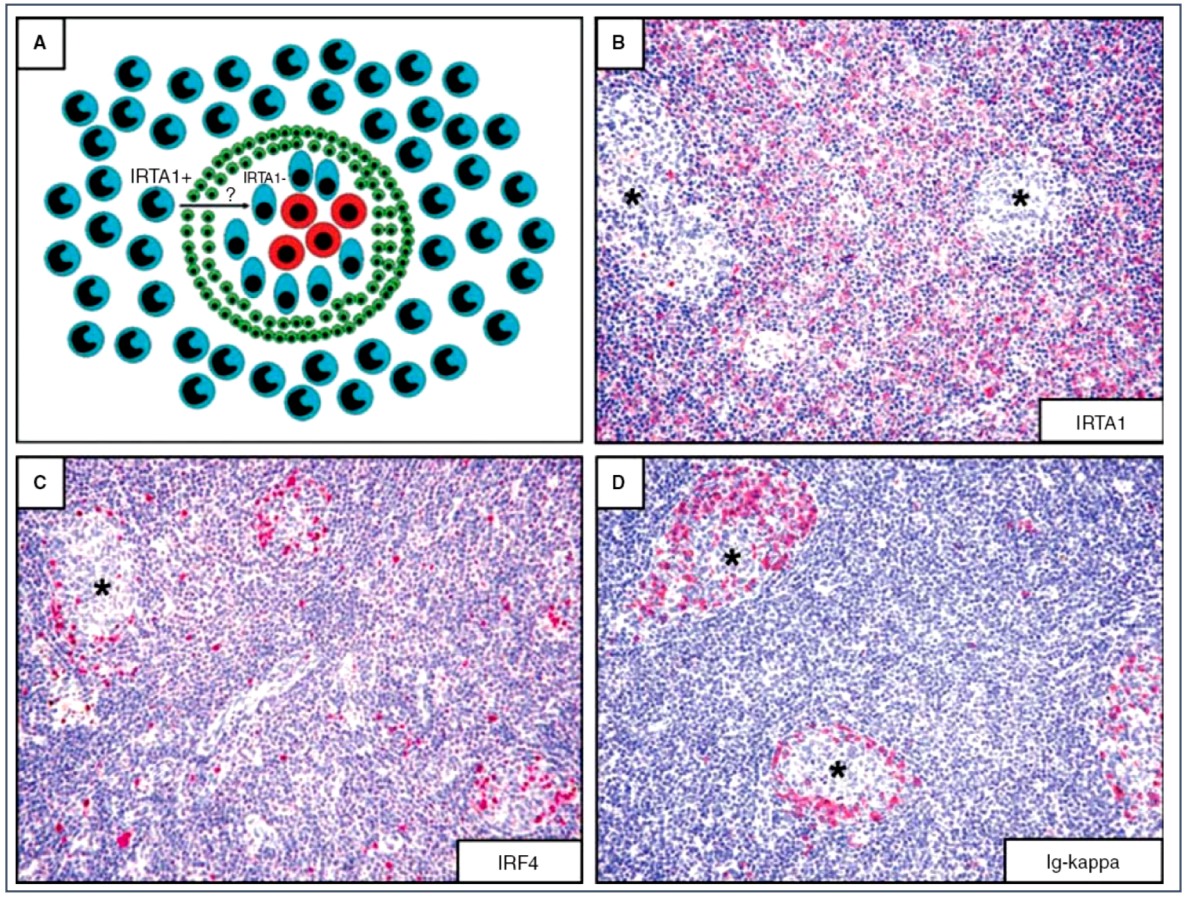
SOX11-

CD200 –

LEF1 –

CD10, BCL6 (colonization), LMO2 –

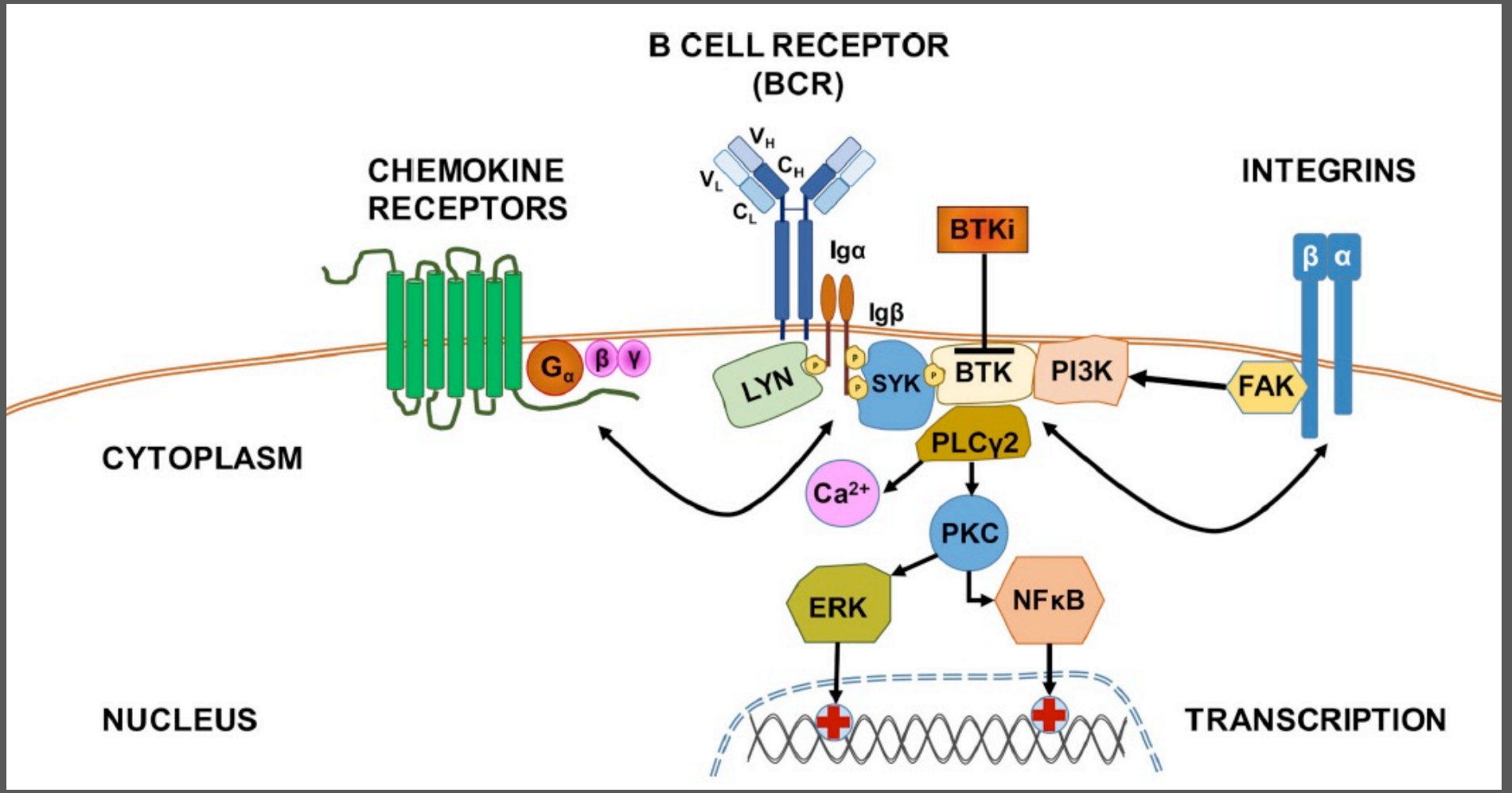
Ki-67: variable



Histopathology 2012, **61**, 930–941. DOI: 10.1111/j.1365-2559.2012.04289.x

IRTA1 is selectively expressed in nodal and extranodal marginal zone lymphomas

Brunangelo Falini, Claudio Agostinelli,[1] Barbara Bigerna, Alessandra Pucciarini, Roberta Pacini, Alessia Tabarrini, Flavio Falcinelli, Milena Piccioli,[1] Marco Paulli,[2] Marcello Gambacorta,[3] Maurilio Ponzoni,[4] Enrico Tiacci, Stefano Ascani,[5] Maria Paola Martelli, Riccardo Dalla Favera,[6] Harald Stein[7] & Stefano A Pileri[1]


B CELL RECEPTOR
(BCR)
CHEMOKINE
RECEPTORS
INTEGRINS
V_H
C_H
V_L
C_L
Igα
Igβ
BTKi
β α
G_α
β γ
LYN
SYK
BTK
PI3K
FAK
PLCγ2
Ca^{2+}
PKC
ERK
NFκB
CYTOPLASM
NUCLEUS
TRANSCRIPTION



- V. Tabanelli
- S. Fiori
- D. Lorenzini
- A. Calleri
- F. Melle
- G. Motta
- S. Mazzara
- M.R. Sapienza
- P. Antoniotti
- S. Spagnolo
- M. Giuffrida
- V. Rossi
- E Derenzini