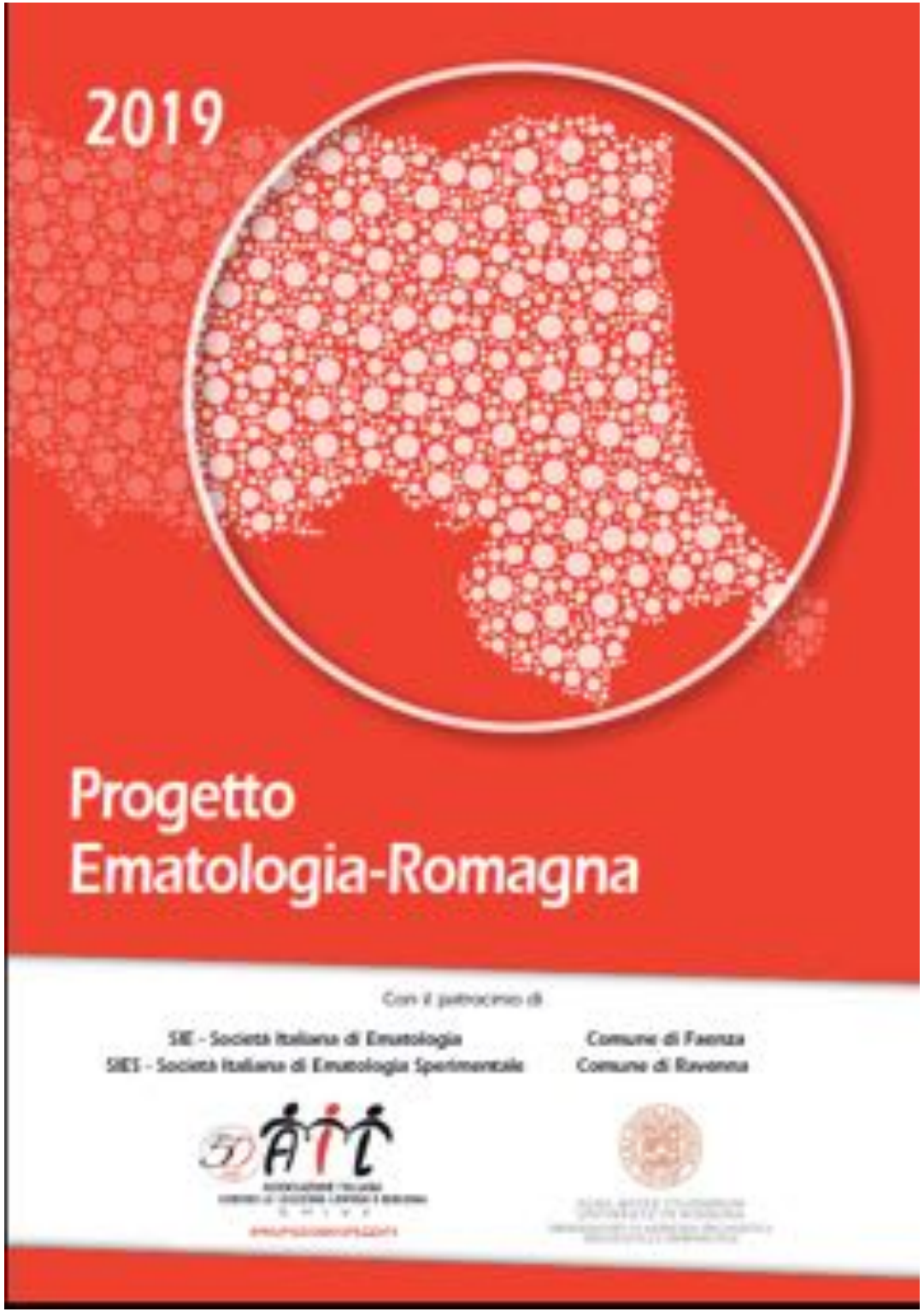
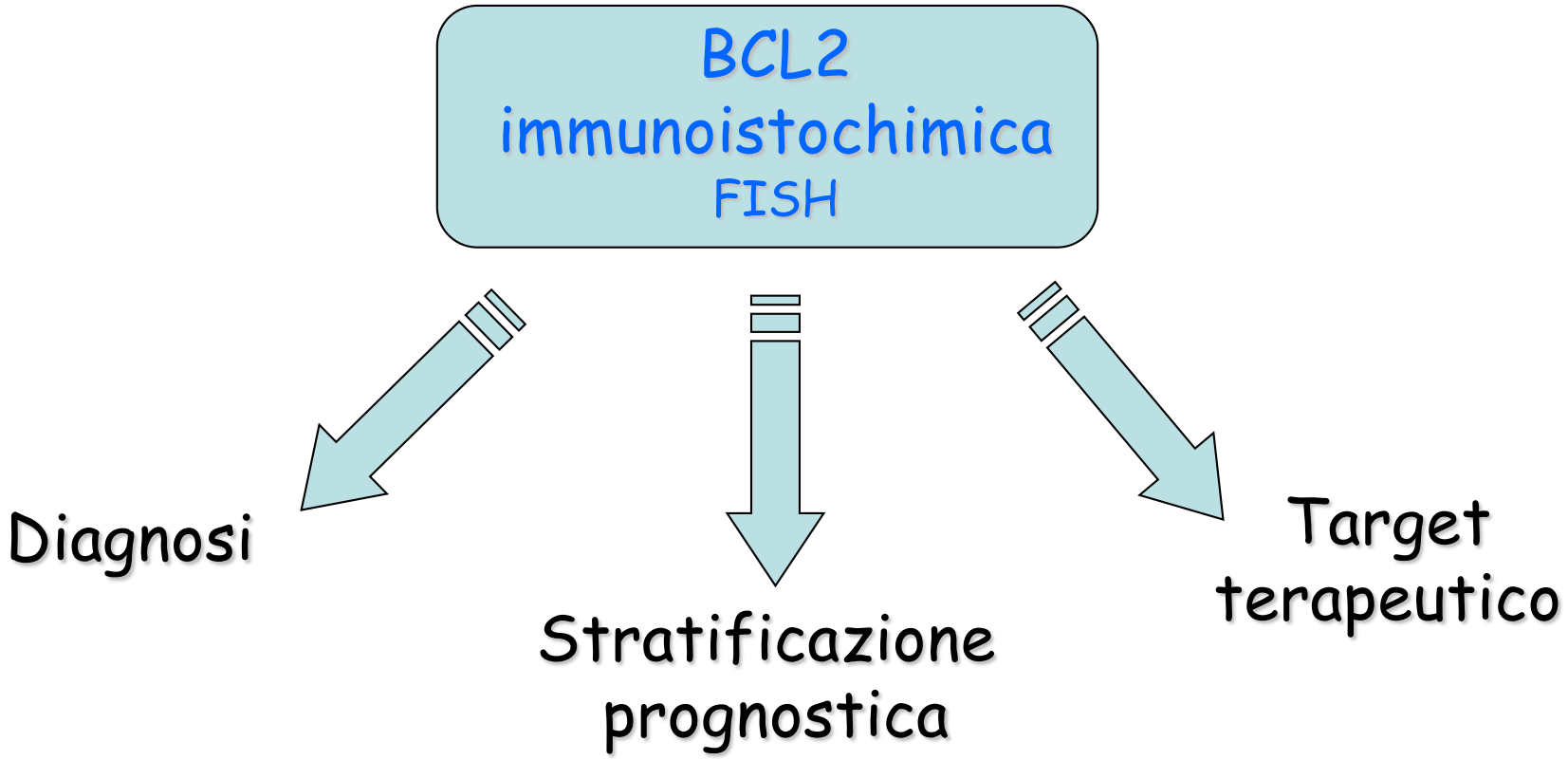


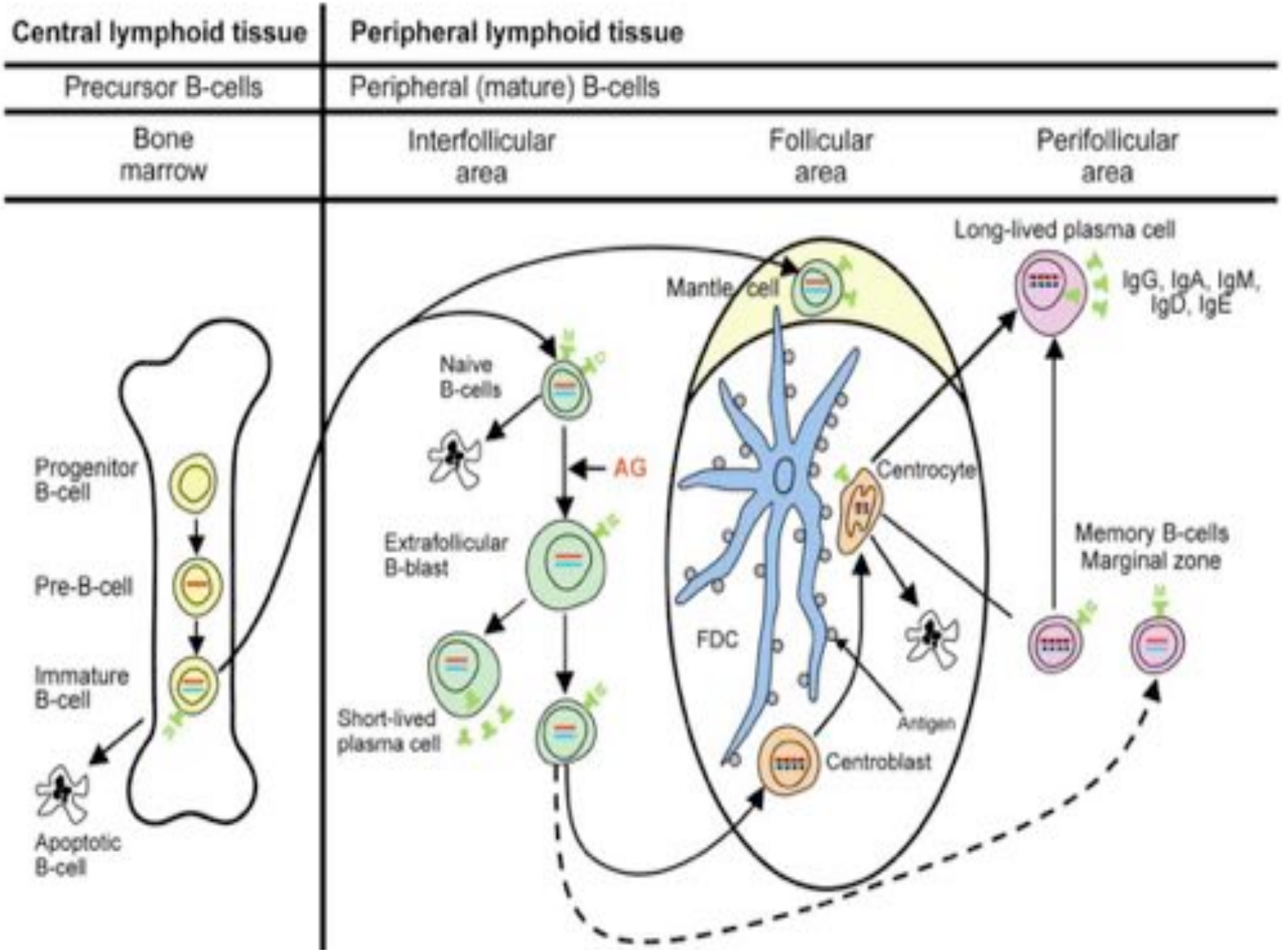
IL BCL2 È IL GENE DELLA "VITA";
LE SUE ALTERAZIONI
(MUTAZIONE, TRASLOCAZIONI E AMPLIFICAZIONI)
INDUCONO MALATTIE
LINFO-MIELOPROLIFERATIVE

Ruolo diagnostico dell'emolinfopatologo

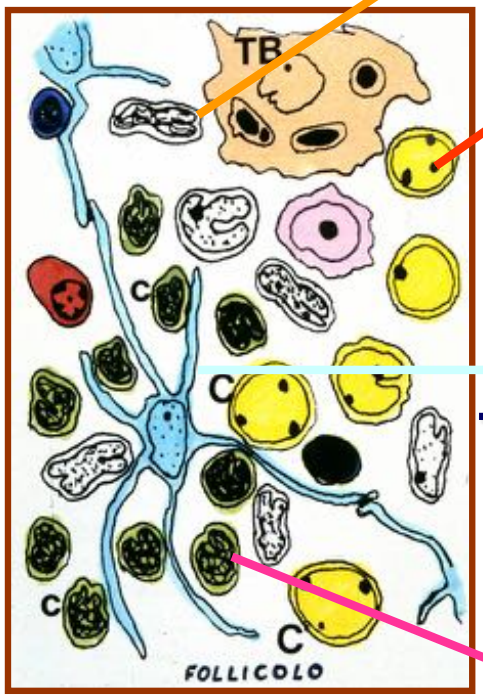
Agostinelli C,
UO Emolinfopatologia
Istituto Seragnoli
Policlinico S.Orsola
Univeristà di Bologna

BCL2 in ematopatologia




Central lymphoid tissue
Peripheral lymphoid tissue
Precursor B-cells
Peripheral (mature) B-cells
Bone marrow
Interfollicular area
Follicular area
Perifollicular area
Progenitor B-cell
Pre-B-cell
Immature B-cell
Apoptotic B-cell
Naive B-cells
AG
Extrafollicular B-blast
Short-lived plasma cell
Mantle cell
FDC
Centrocyte
Antigen
Centroblast
Long-lived plasma cell
IgG, IgA, IgM, IgD, IgE
Memory B-cells Marginal zone

Follicolo reattivo



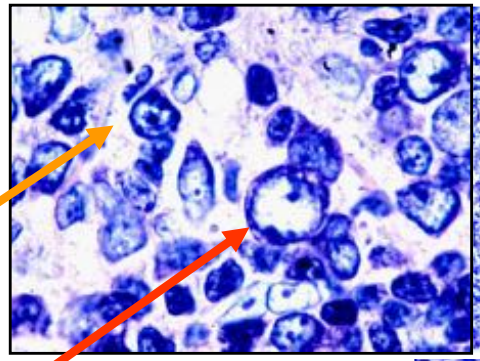
cc

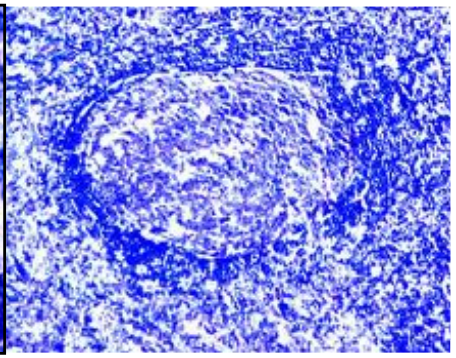
cb

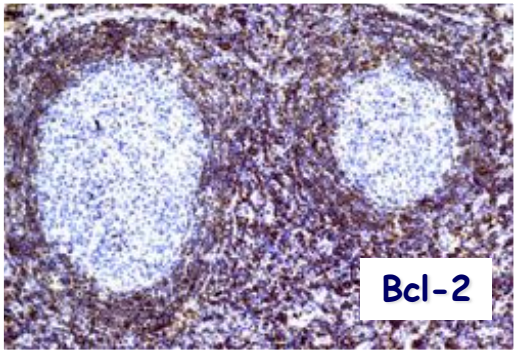
fdc

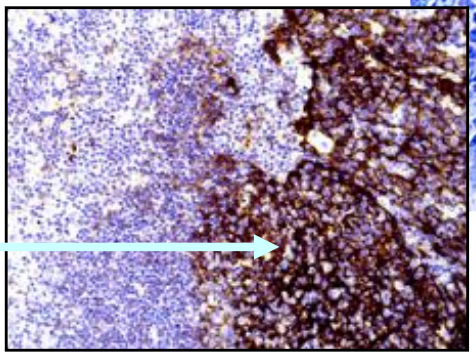
T

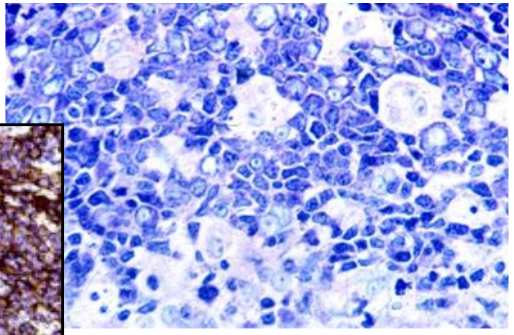
Bcl-2: anti-apoptotic protein

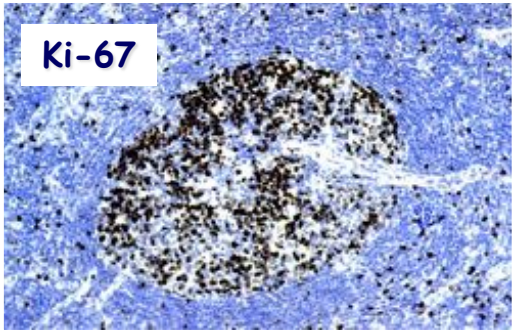




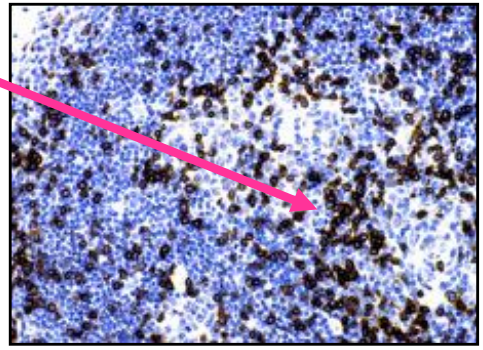


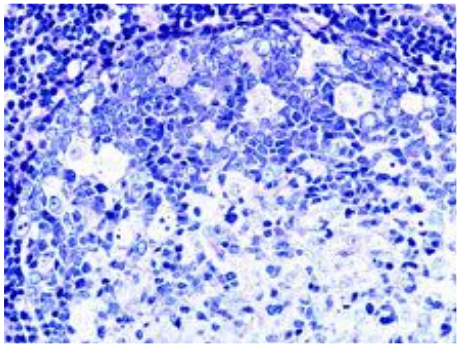


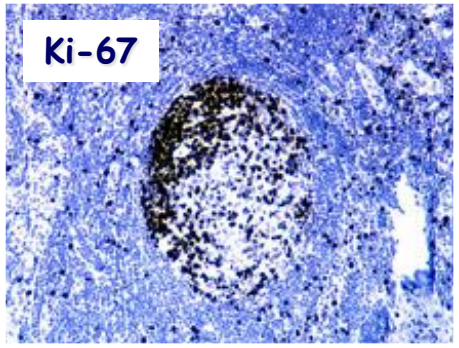


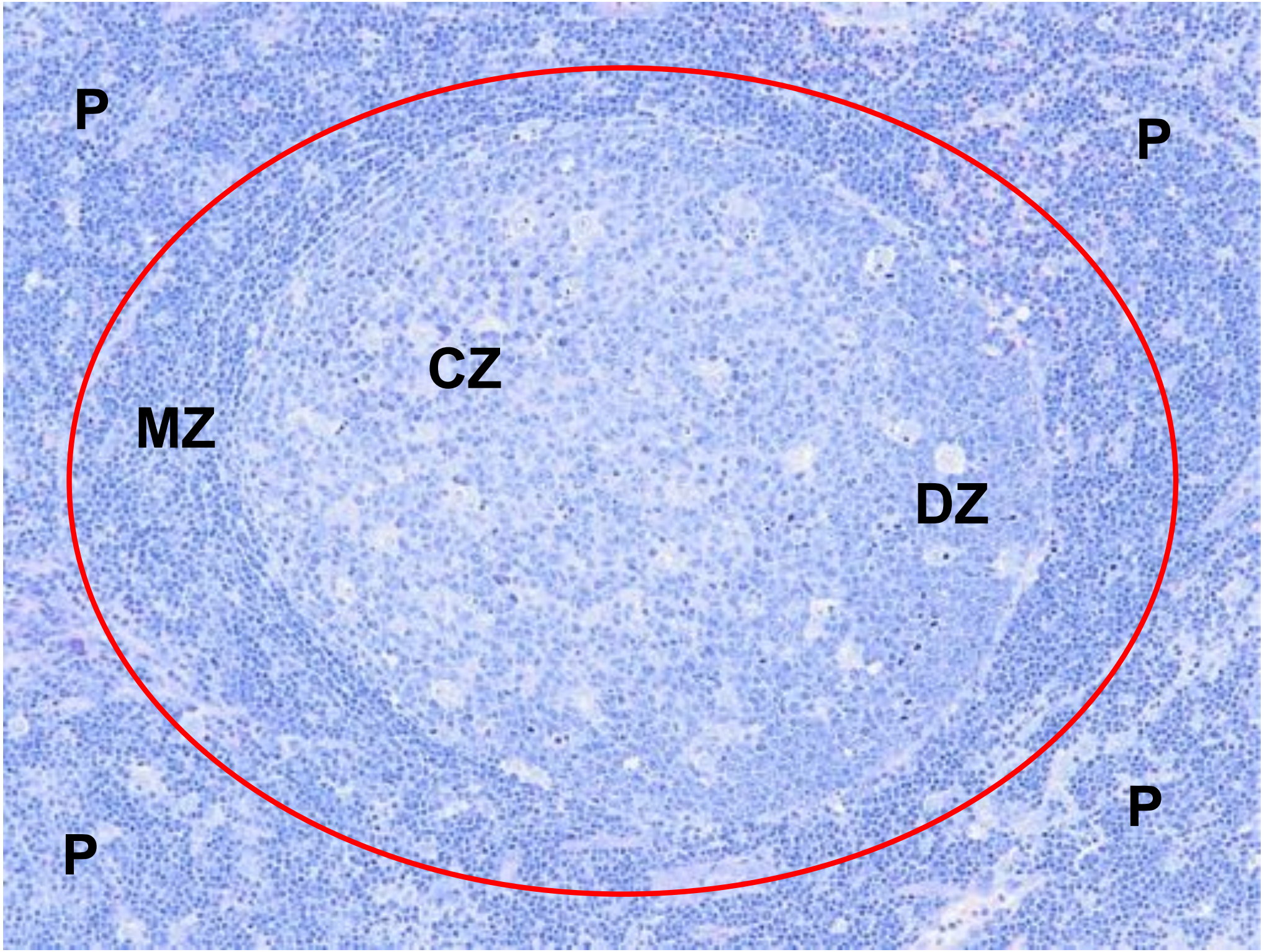


Polarizzazione del *GC*

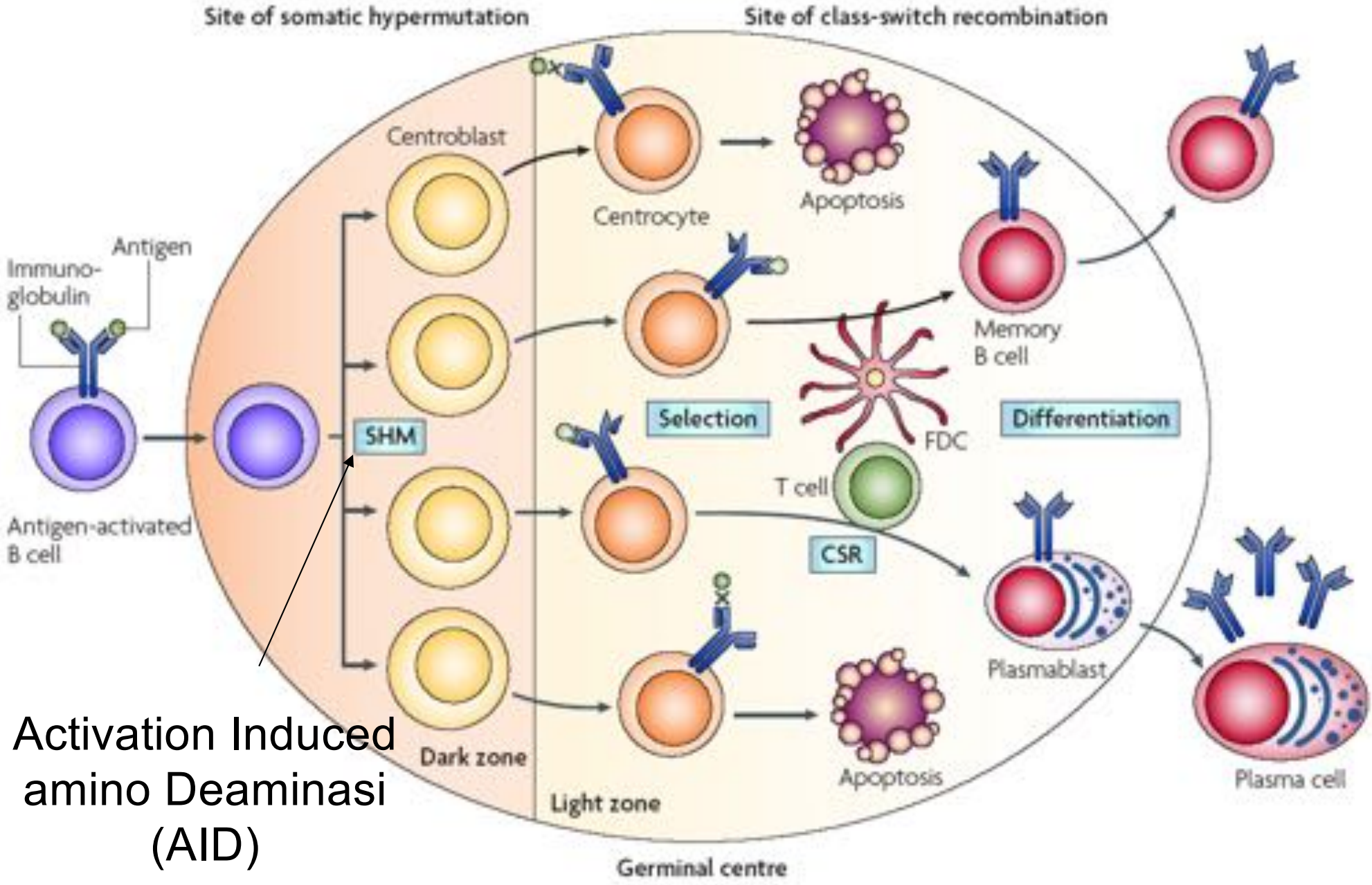







P
P
CZ
MZ
DZ
P
P

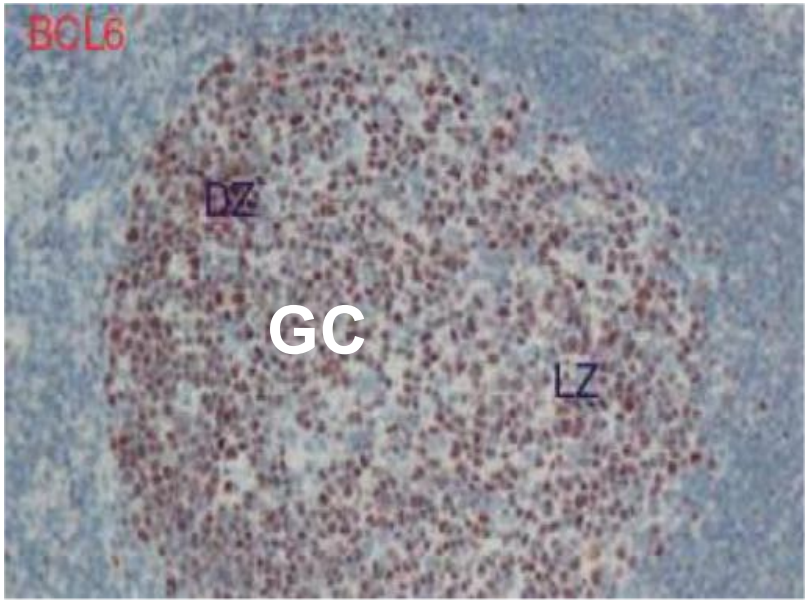
Fisiologia del centro germinativo

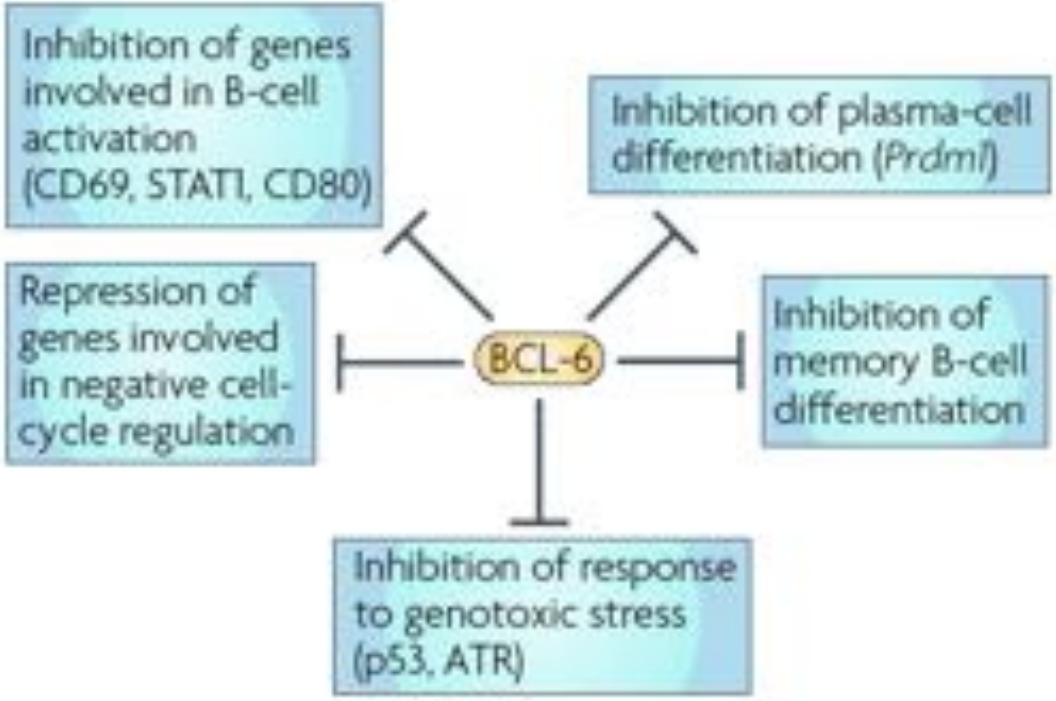


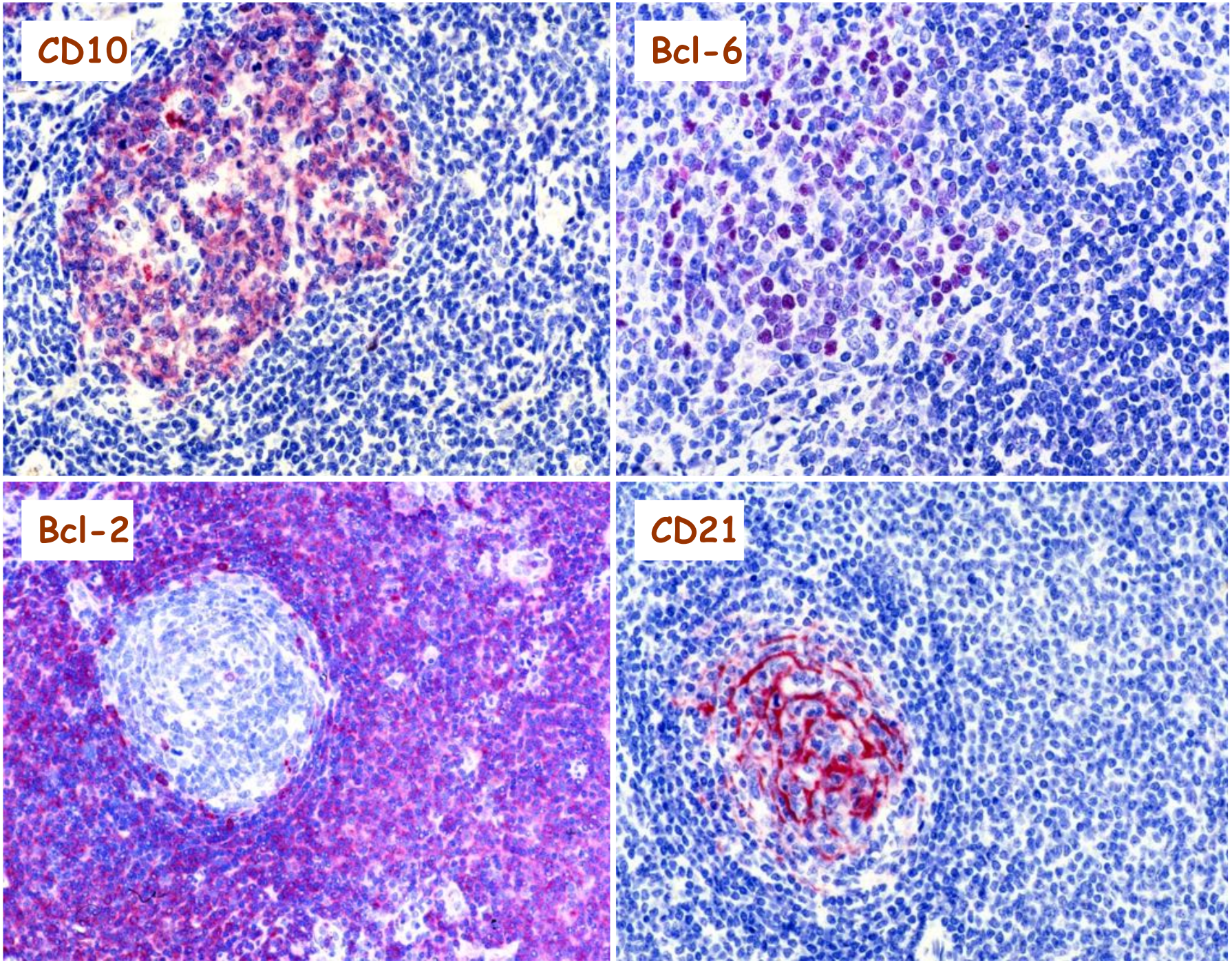
Activation Induced amino Deaminasi (AID)

Kluin Nat Rev Imm 2008

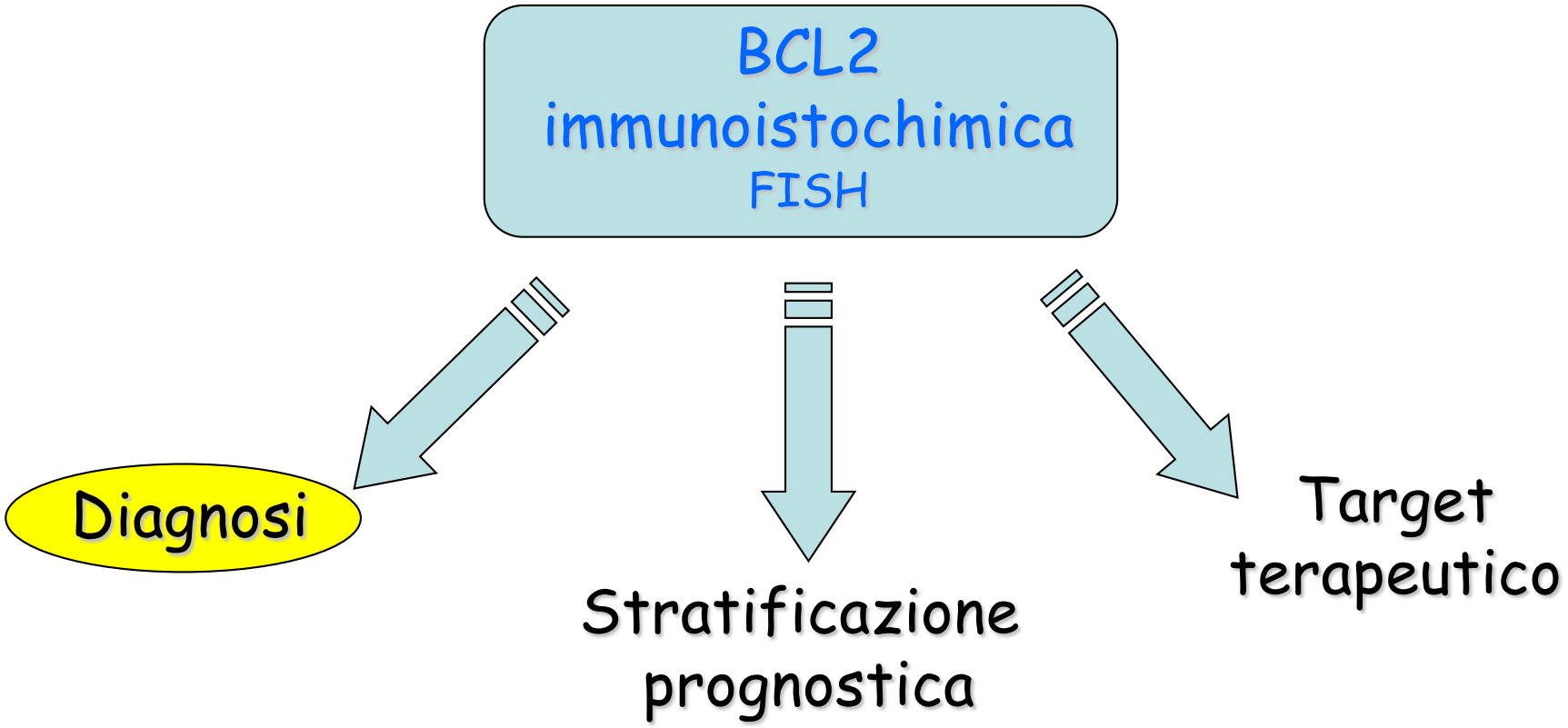
BCL6 è il gene regolatore principale dello sviluppo dei centroblasti e della maggior parte dei centrociti. Fosfoproteina che funziona da repressore della trascrizione reclutando complessi di co-repressori.

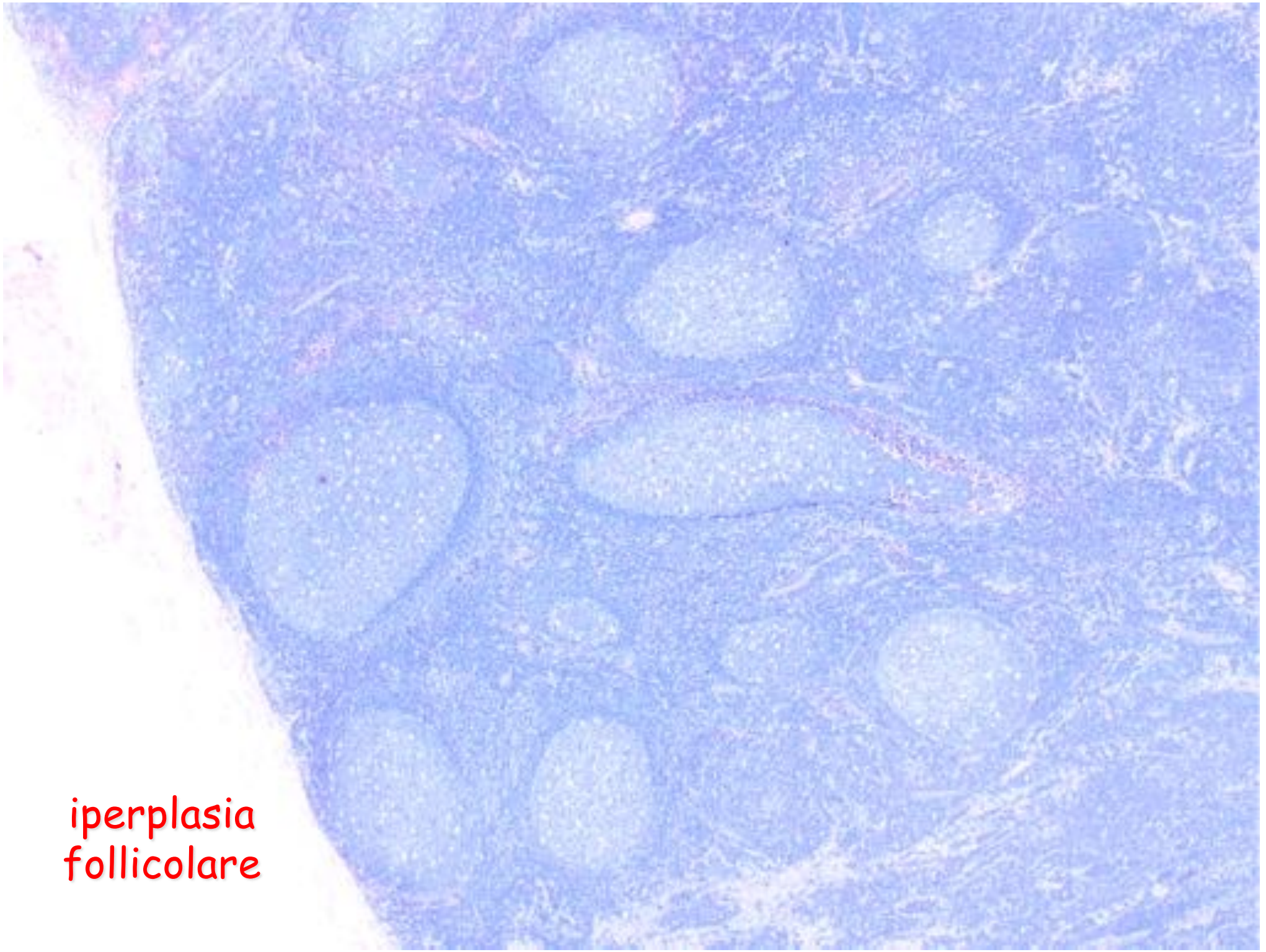





CD10
Bcl-6
Bcl-2
CD21

BCL2 in ematopatologia





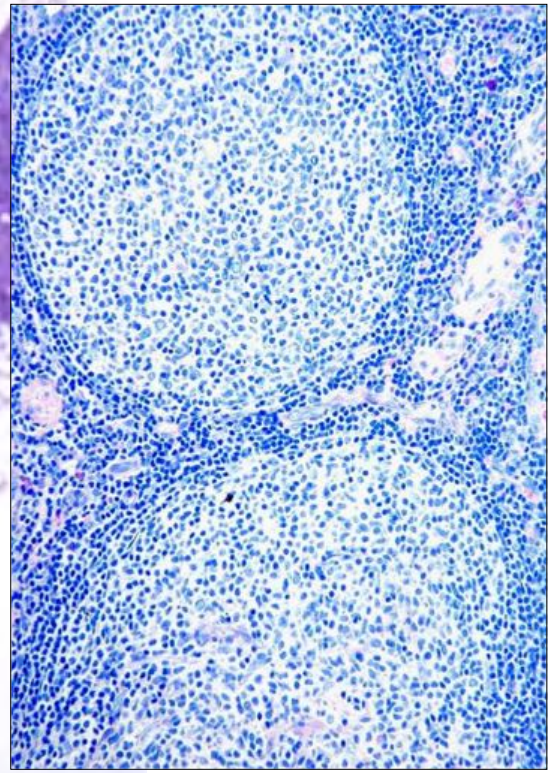
iperplasia
follicolare

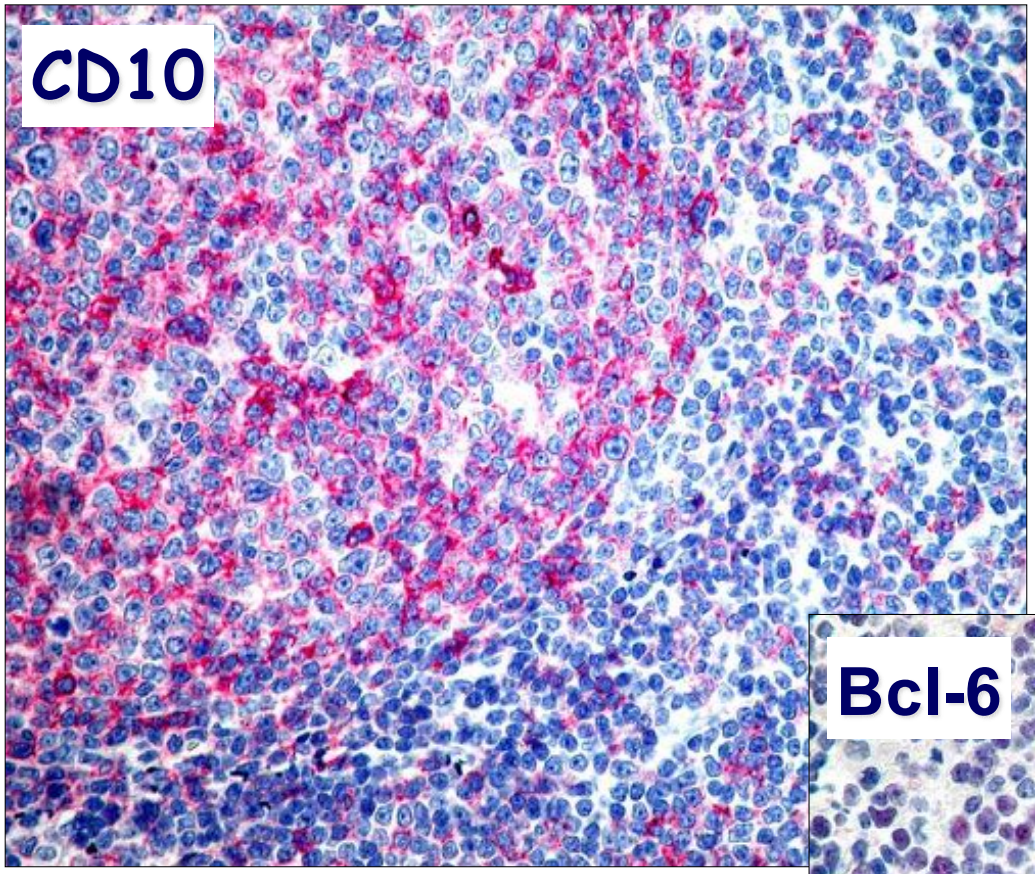
Follicoli addossati

mantelli attenuati o assenti mancanza

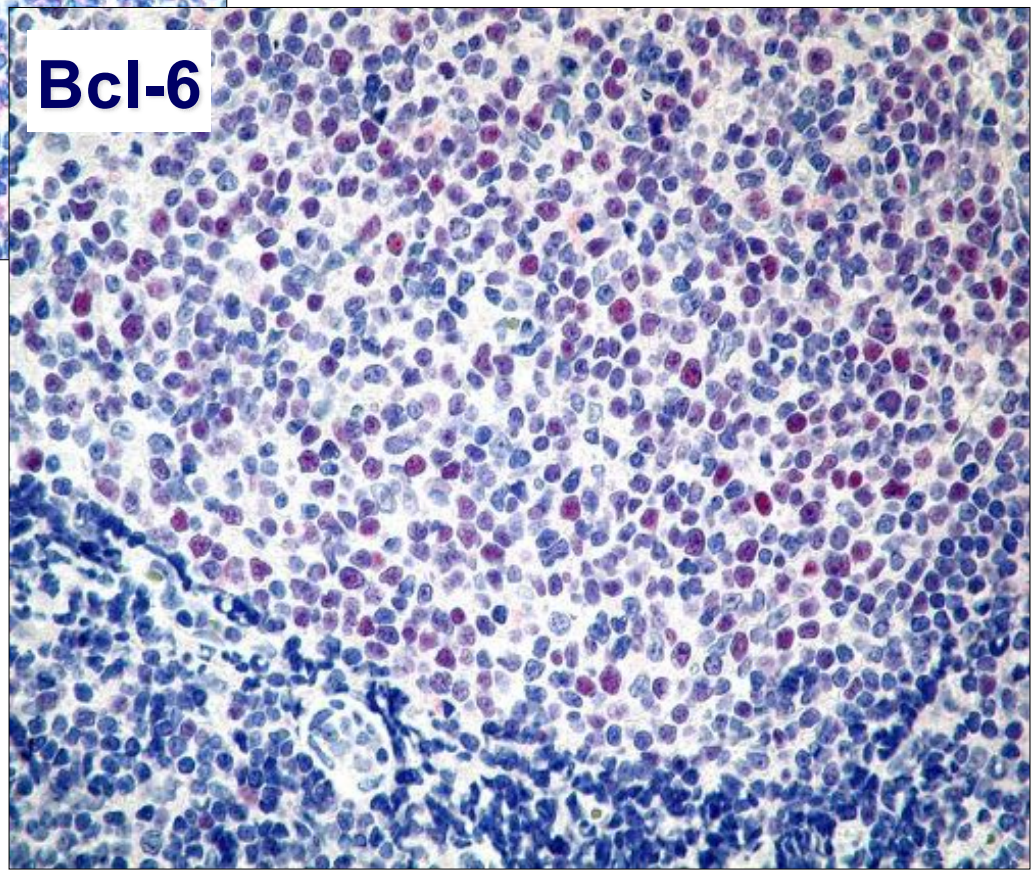
assenza polarizzazione di *GC*

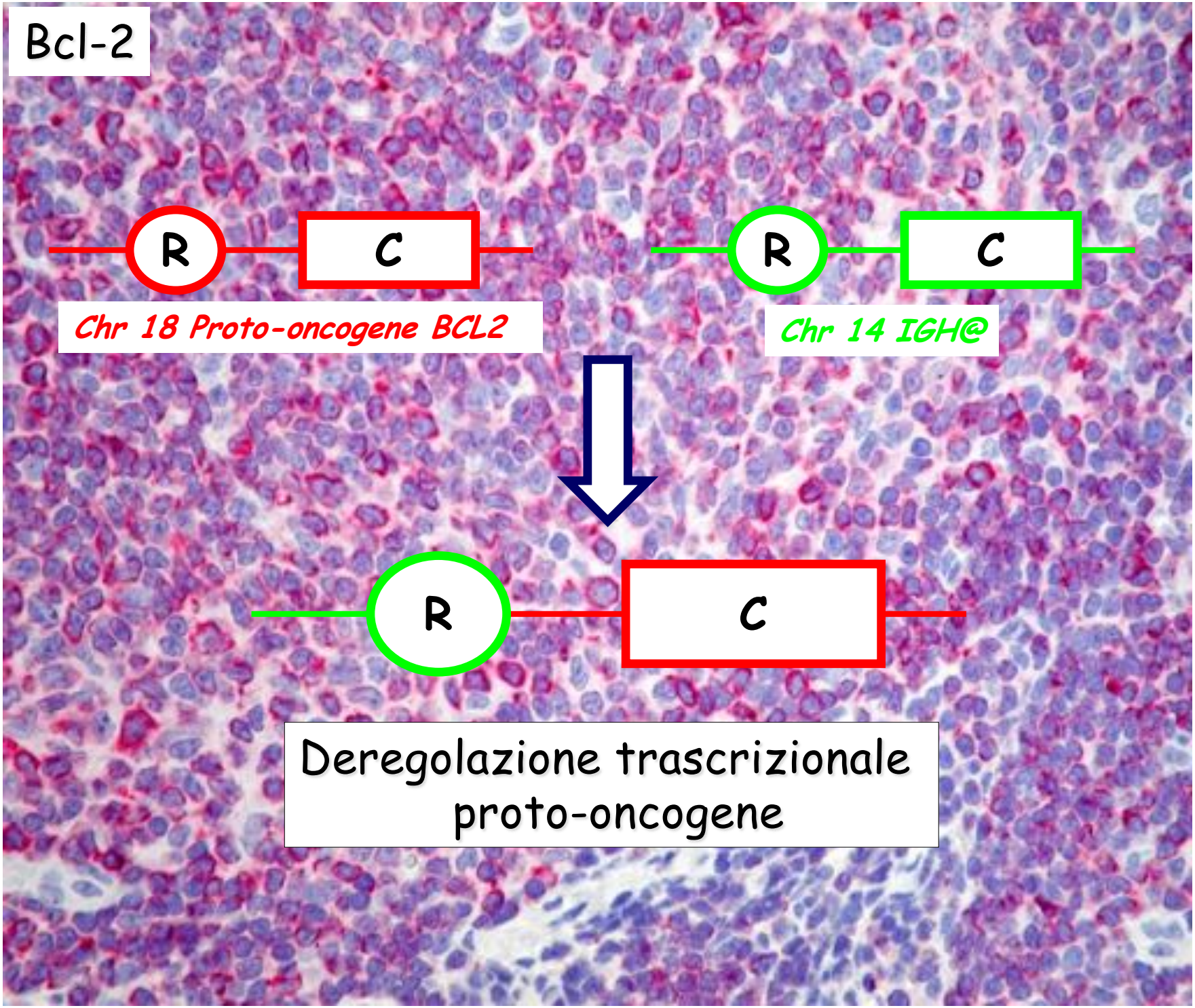
Linofoma follicolare

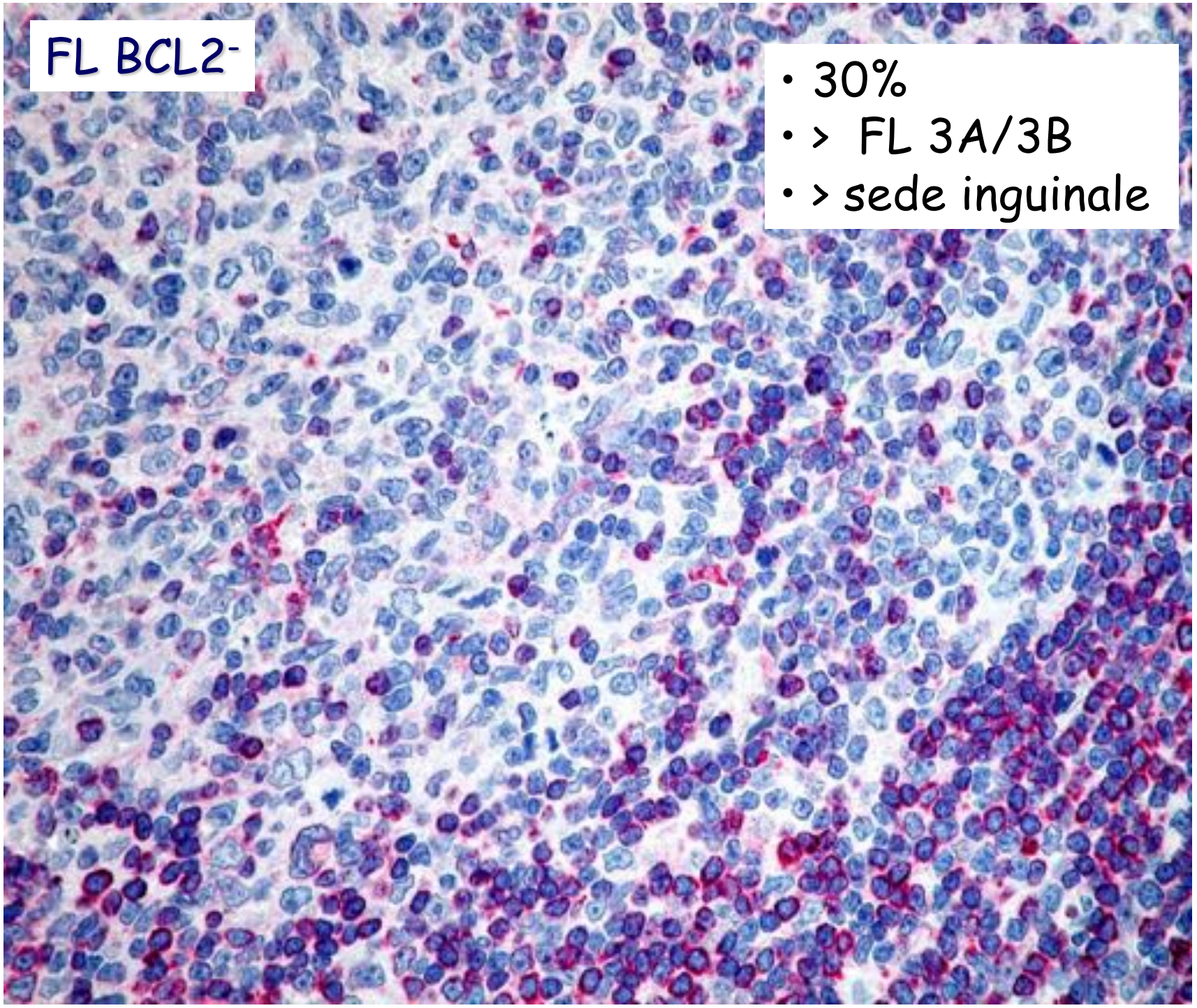




Germinal Center markers




Bcl-2
R
C
Chr 18 Proto-oncogene BCL2
R
C
Chr 14 IGH@
R
C
Deregolazione trascrizionale
proto-oncogene


FL BCL2-
• 30%
• > FL 3A/3B
• > sede inguinale

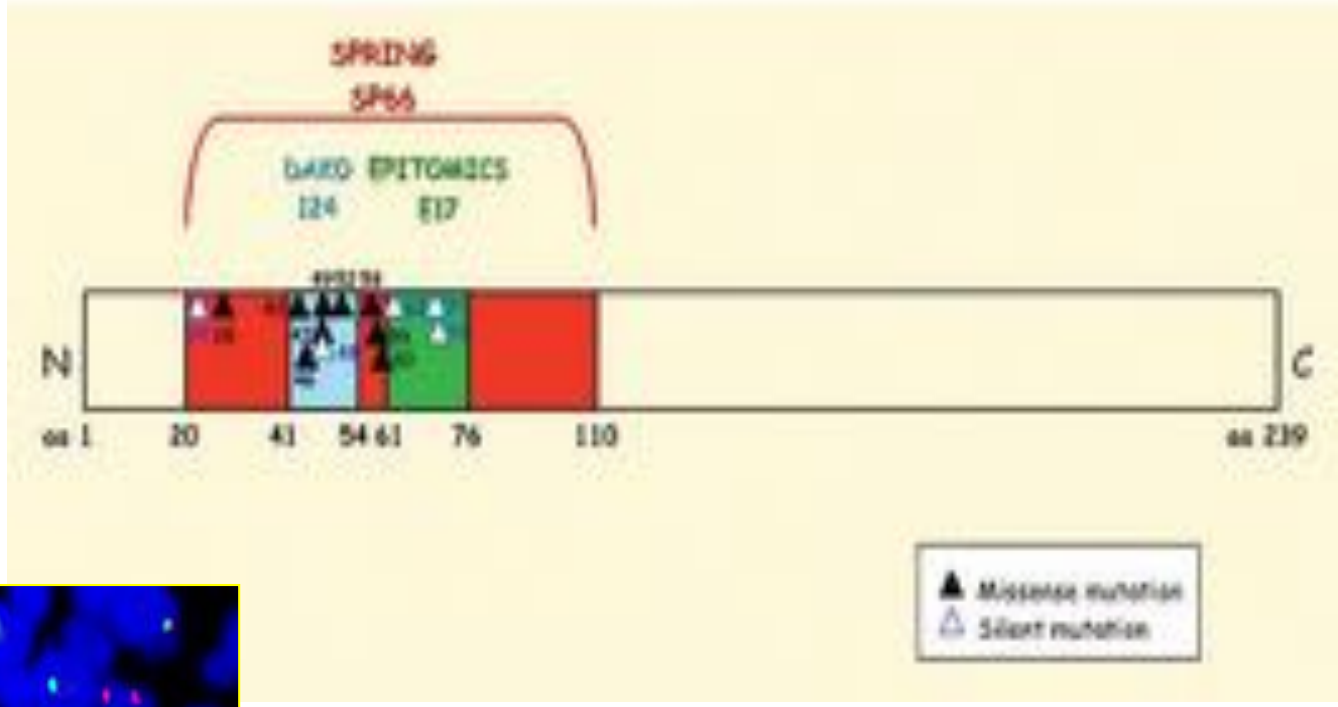
FL t(14;18)-

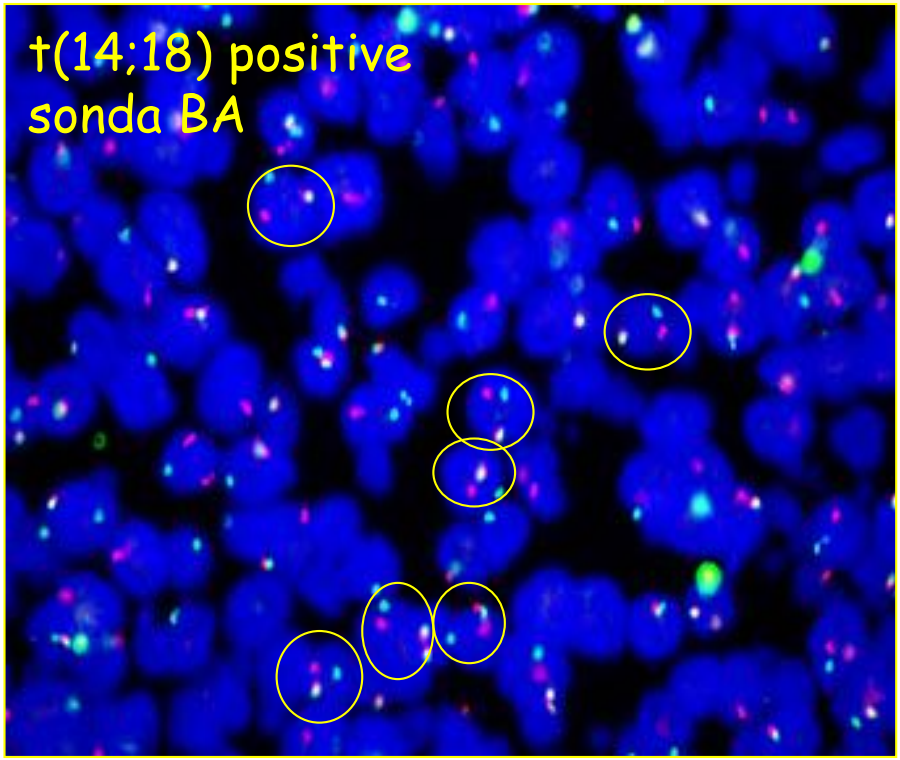
- perdita polarizzazione morfofunzionale CG
- Fenotipi aberranti ($CD10^-/BCL6^+/IRF4^+$)
- Aberrazioni BCL6
- Clonalità geni IG

NB: rischio pitfall diagnostico

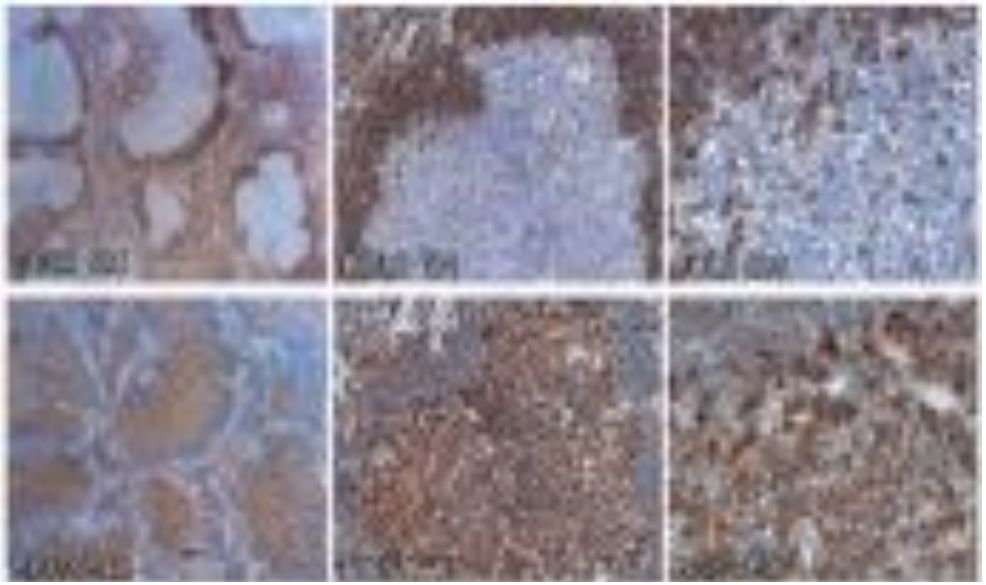
t(14;18) is present but BCL2 gene somatic mutations:

- produce stop codons
- modify epitope

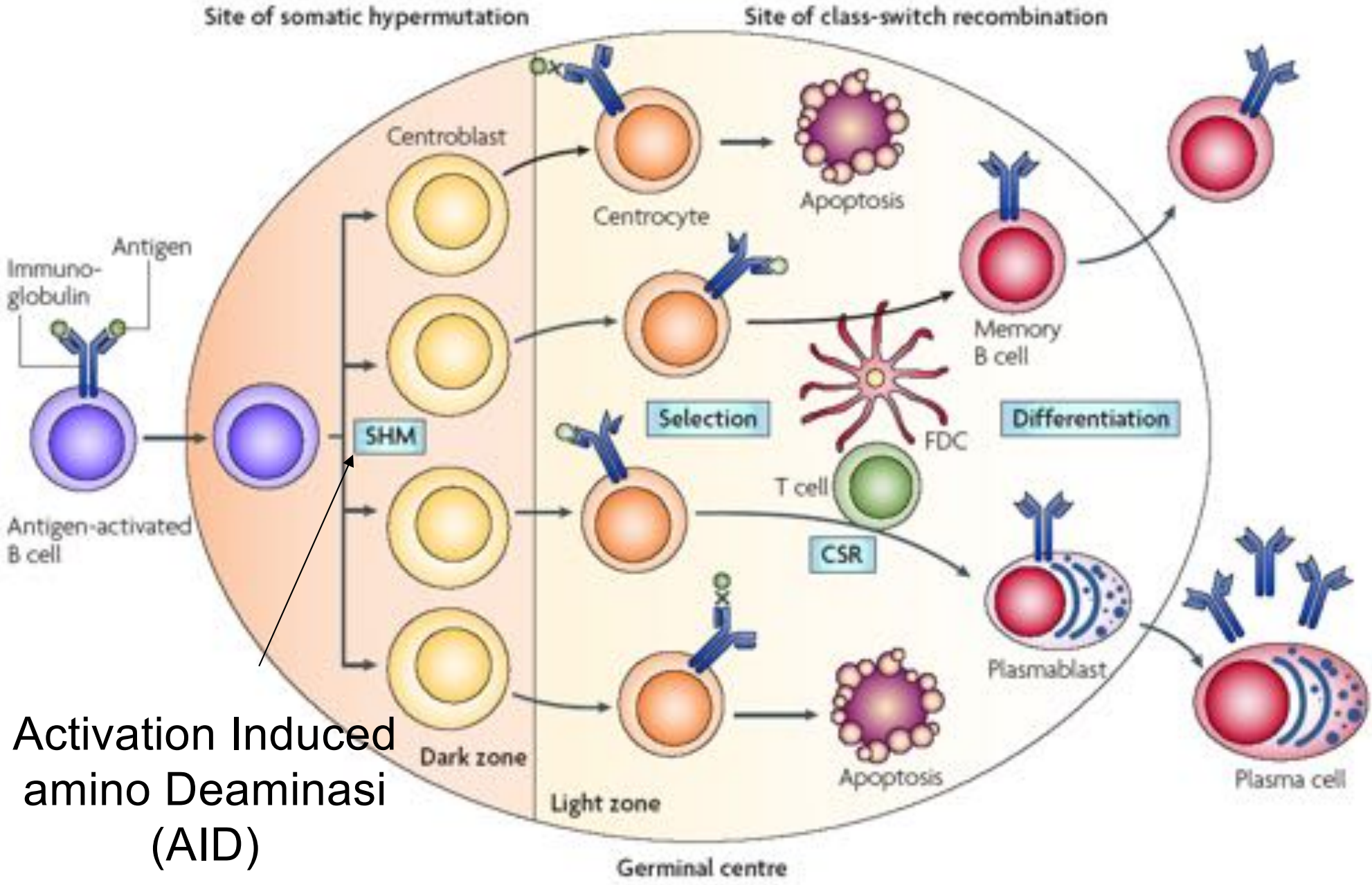




False negative BCL2



Fisiologia del centro germinativo



Activation Induced amino Deaminasi (AID)

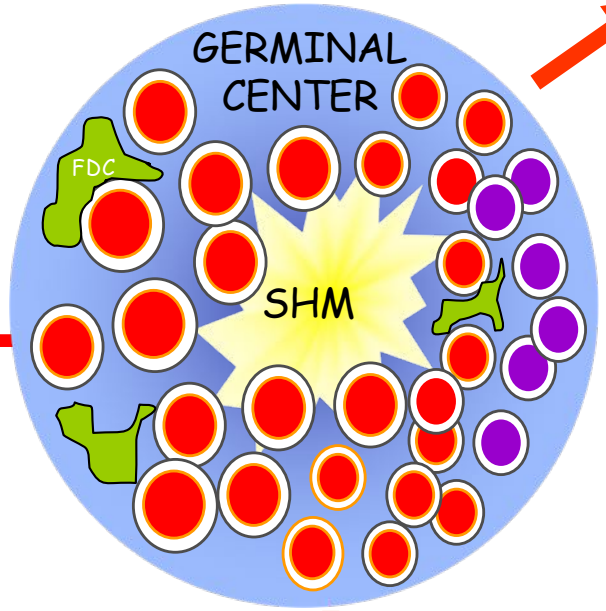
Kluin Nat Rev Imm 2008

SOMATIC HYPERMUTATION in GERMINAL CENTER

PHYSIOLOGICAL

IgV

BCL-6
FAS



PIM-1

- Serine kinase
- Prolongs survival of haematopoietic cells

PAX-5

- B-cell-specific transcription factor essential for B-lineage commitment and differentiation
- Implicated in translocations of NHL

RhoH/TTF

- Small GTP-binding protein belonging to the Ras superfamily
- Implicated in translocations of NHL

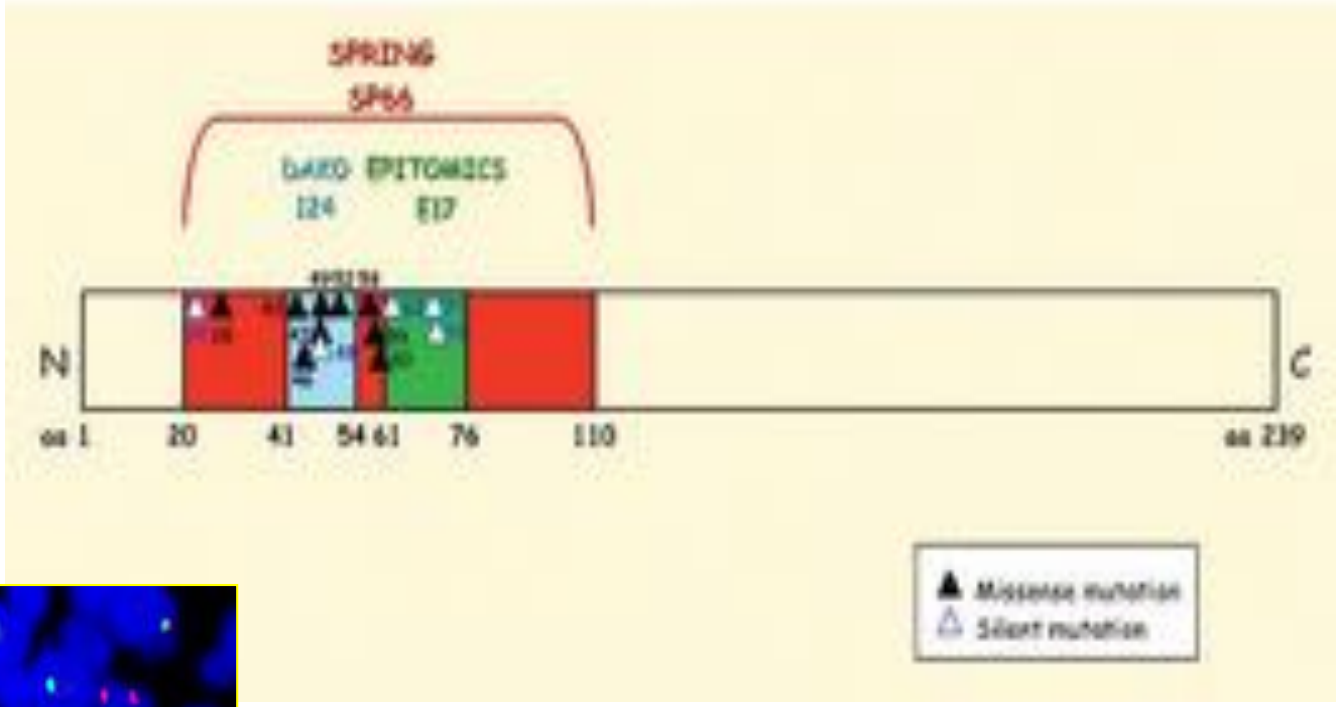
c-MYC

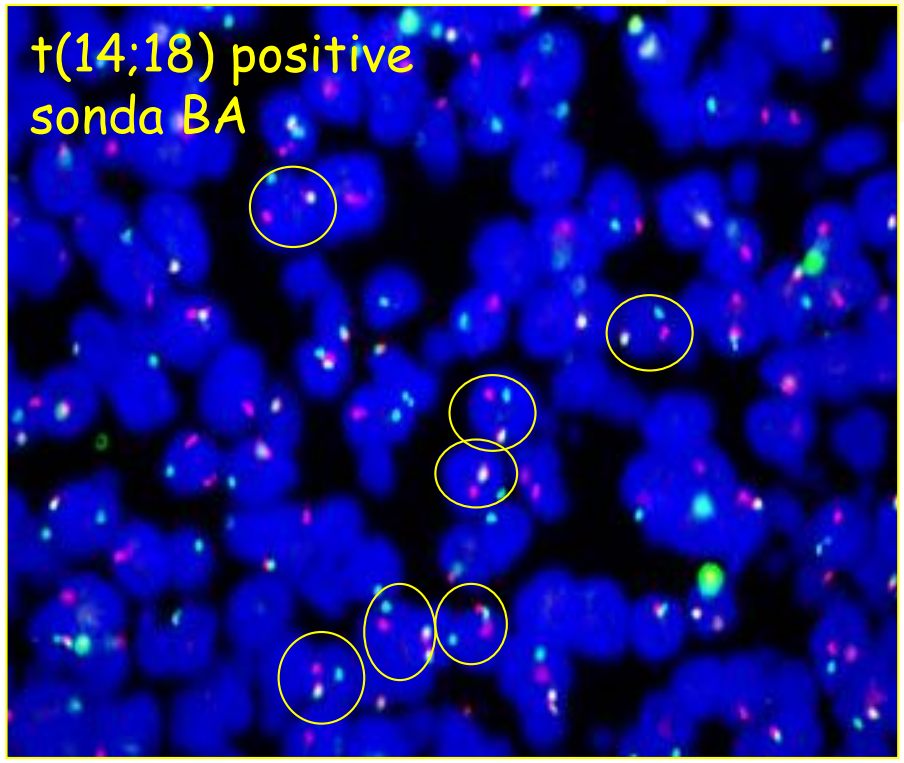
- Transcriptional activator
- Involved in the control of cell growth, proliferation, differentiation and apoptosis
- Implicated in translocations of NHL

ABERRANT
(SHM malfunctioning)

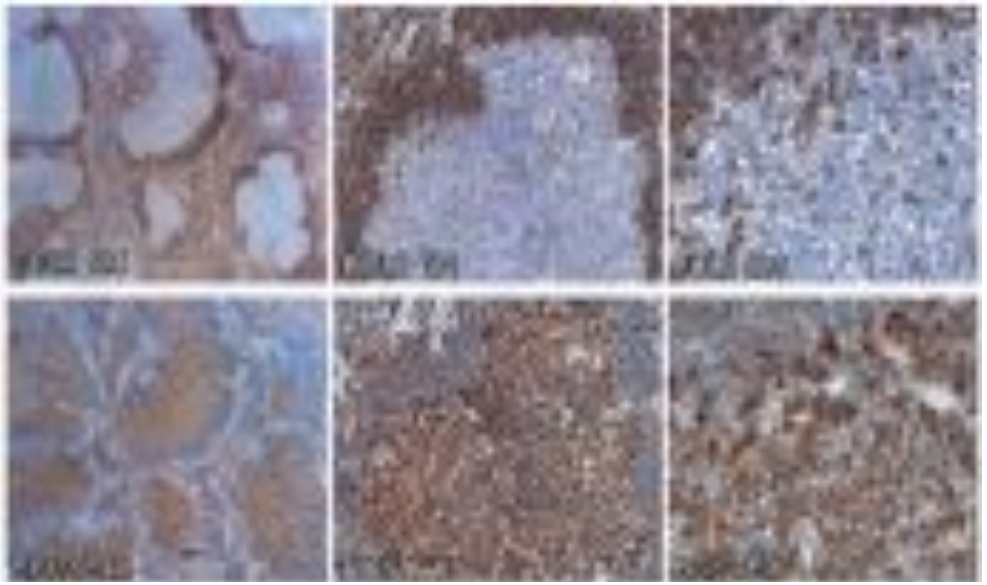
t(14;18) è presente ma BCL2 gene ha mutazioni somatiche :

- produce stop codons
- modifica epitopi

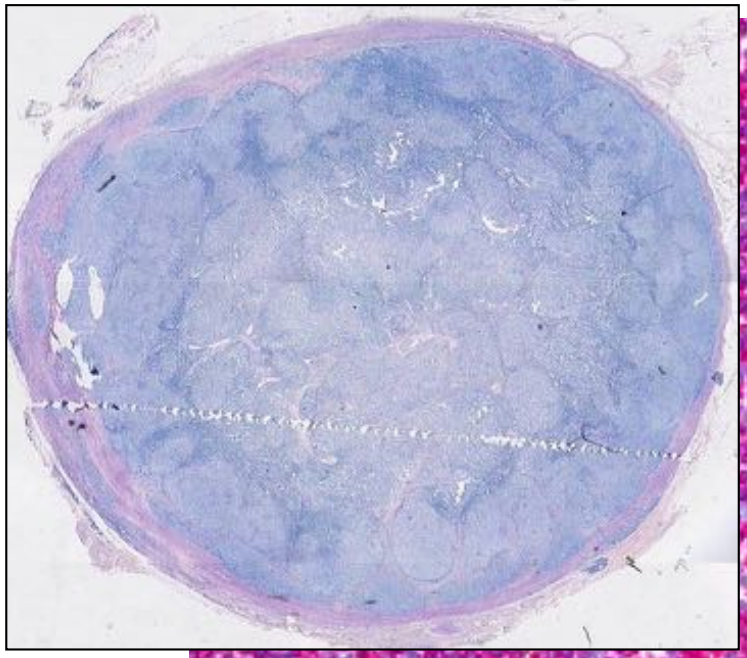




BCL2 falso negative

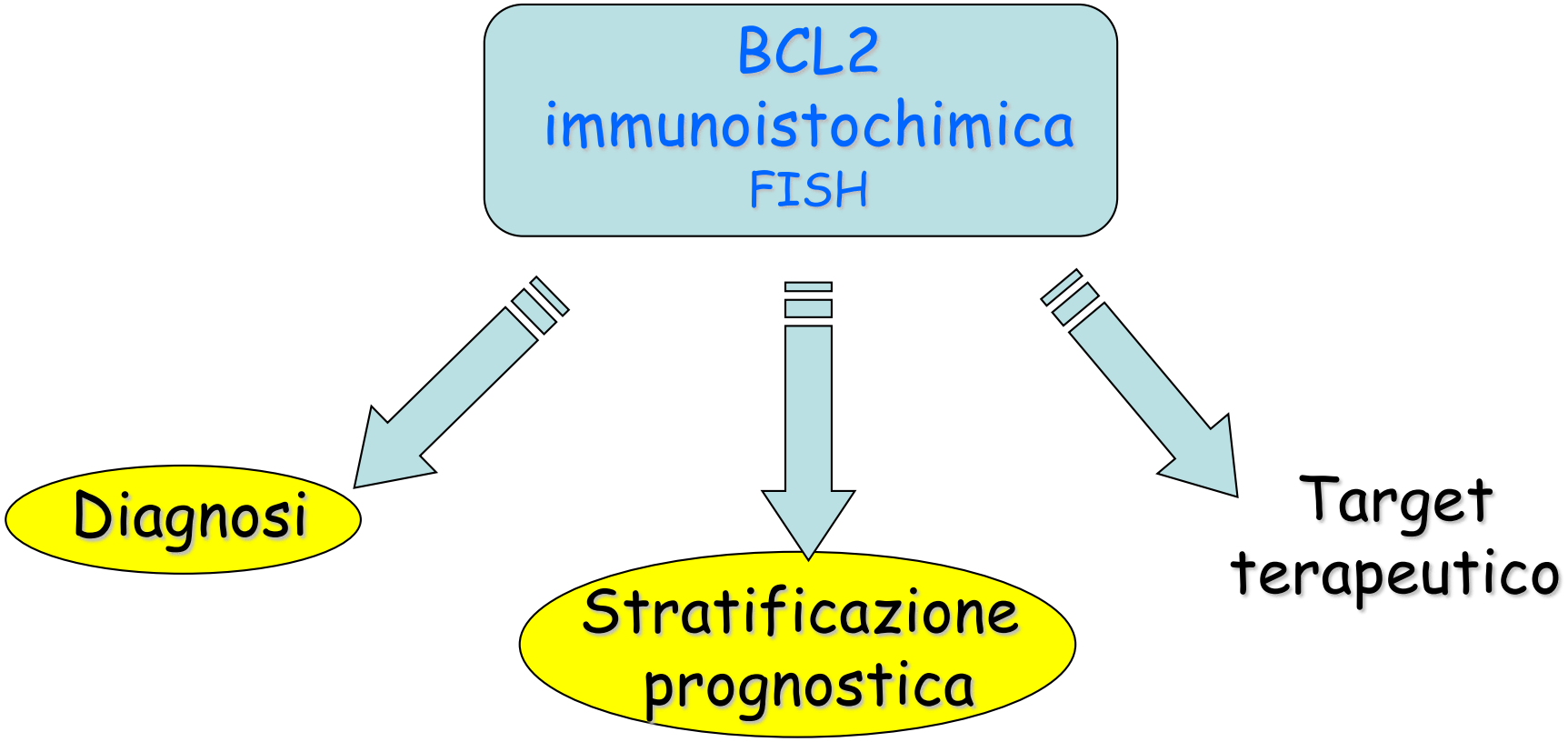


Linfoma marginale Nodale Vs Linfoma Follicolare

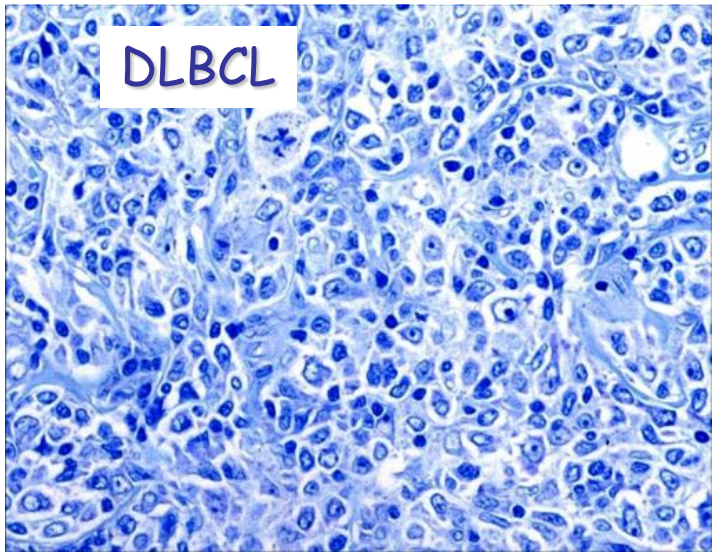


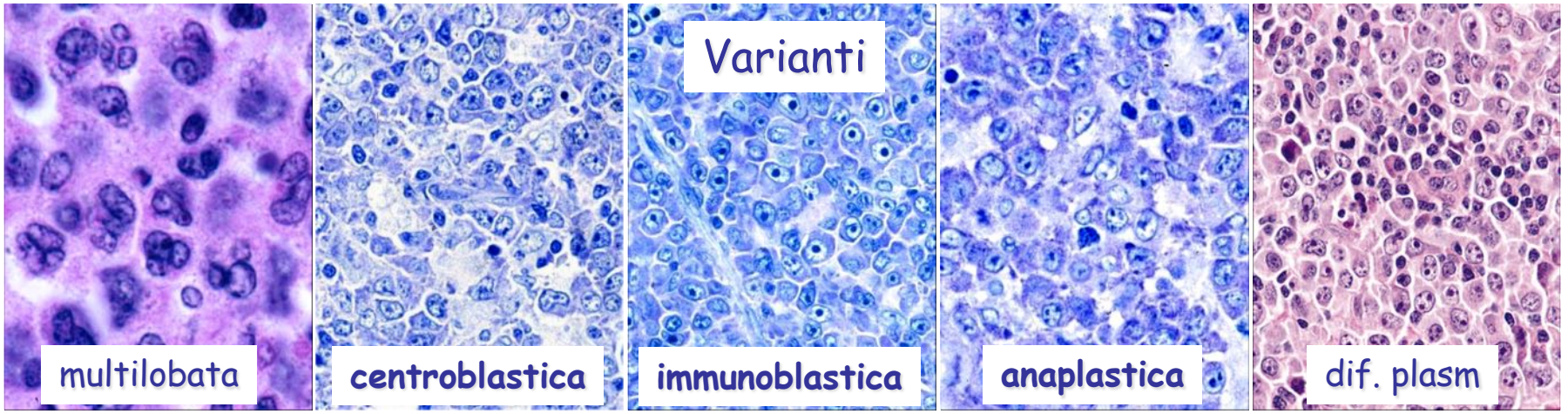
BCL2^{+} (assenza della t14;18)

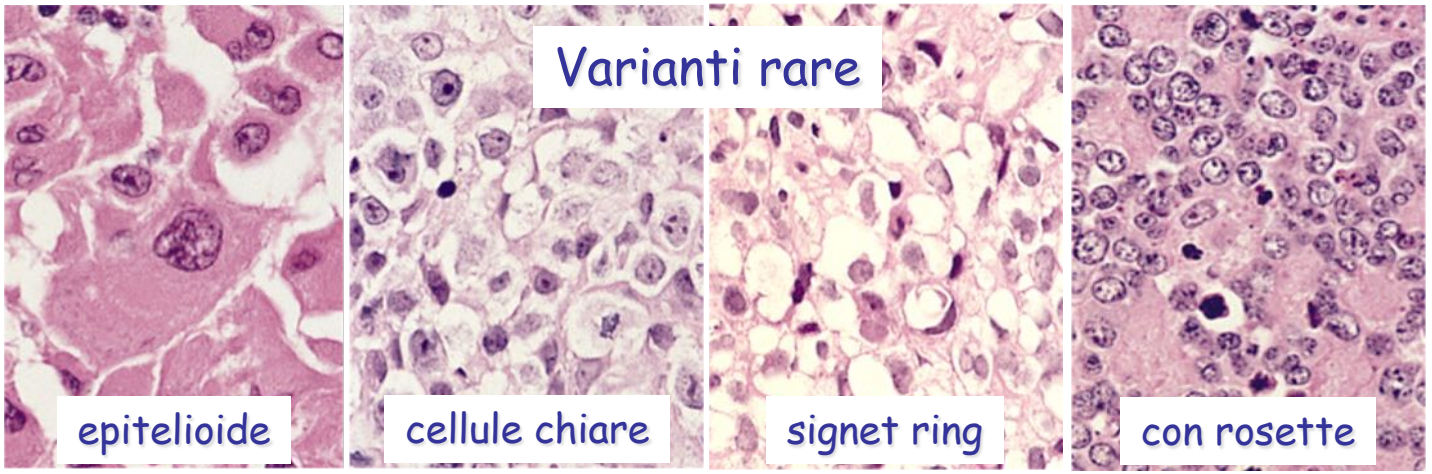
BCL2 in ematopatologia



BCL2: classificazione e stratificazione prognostica linfomi B aggressivi







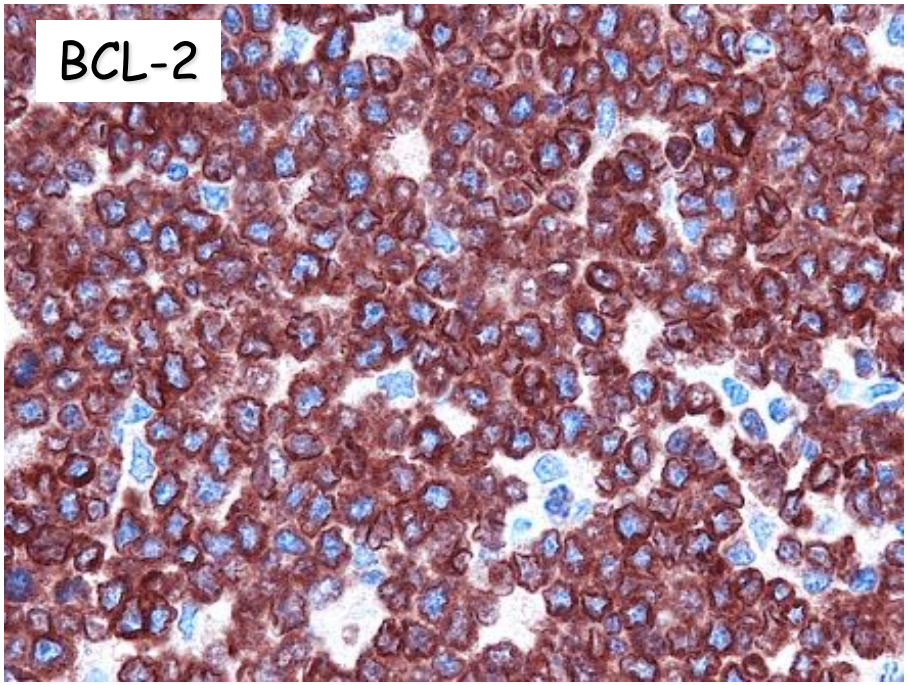
The 2016 revision of the World Health Organization classification of lymphoid neoplasms

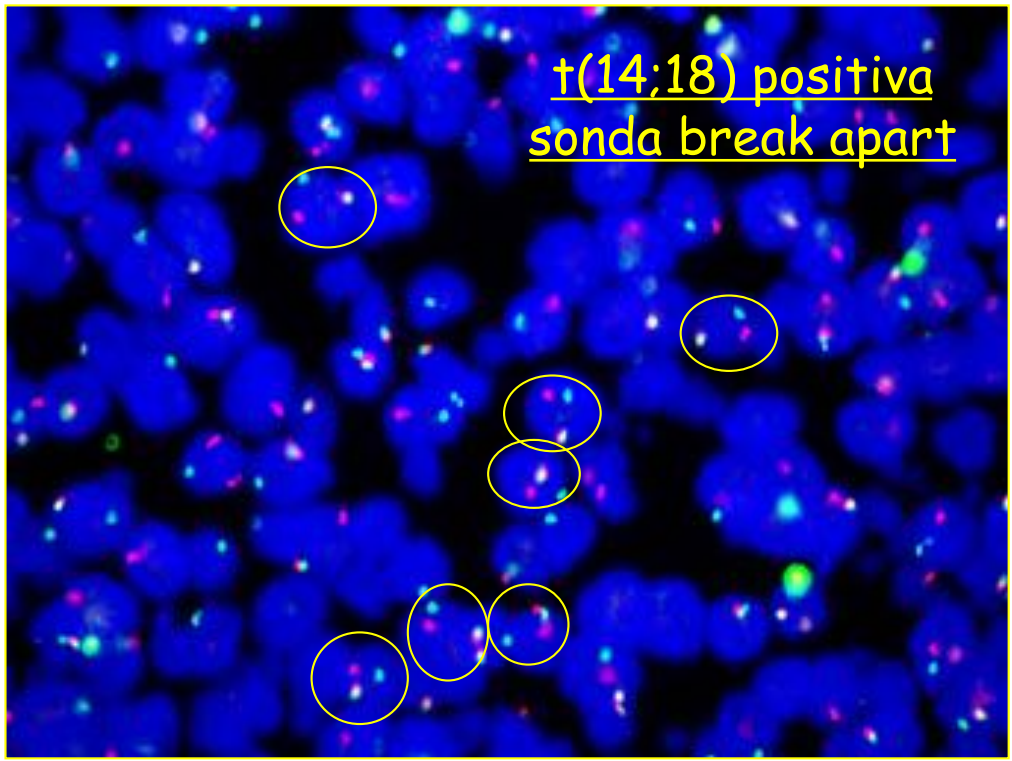
Steven H. Swerdlow,[1] Elias Campo,[2] Stefano A. Pileri,[3] Nancy Lee Harris,[4] Harald Stein,[5] Reiner Siebert,[6] Ranjana Advani,[7] Michele Ghielmini,[8] Gilles A. Salles,[9] Andrew D. Zelenetz,[10] and Elaine S. Jaffe[11]

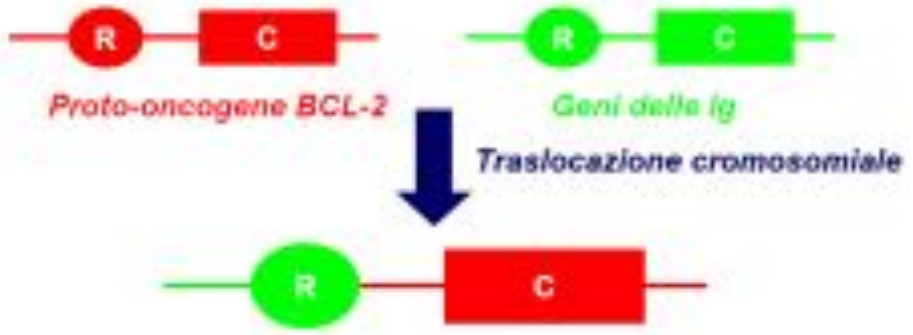
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
*EBV$^+$ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK$^+$ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8$^+$ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*

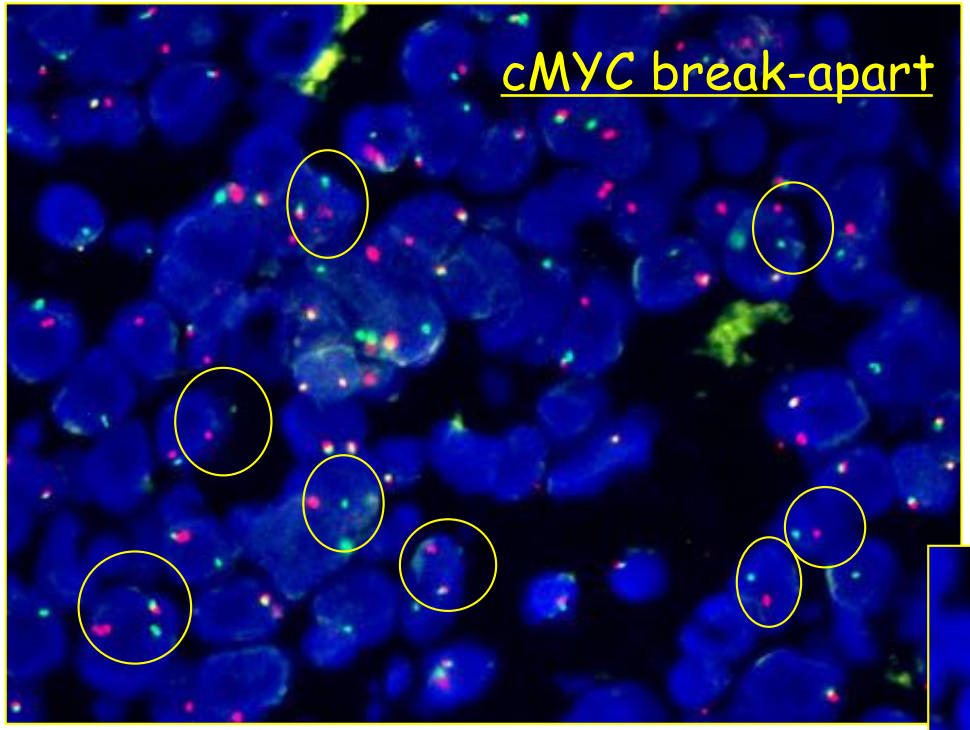
Riarrangiamenti del gene BCL2 sul cromosoma 18:

- 20%-30% DLBCL
- possibili copy gain e amplificazioni

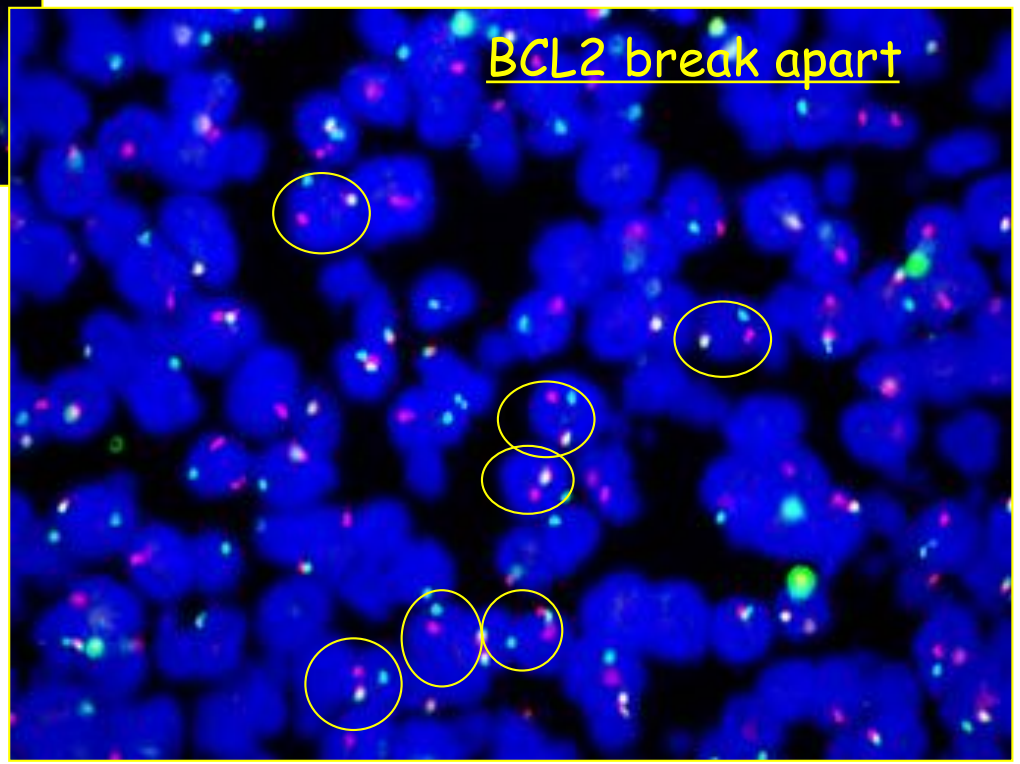








analysis of *MYC*, *BCL2* and *BCL6* gene rearrangements by FISH is indicated



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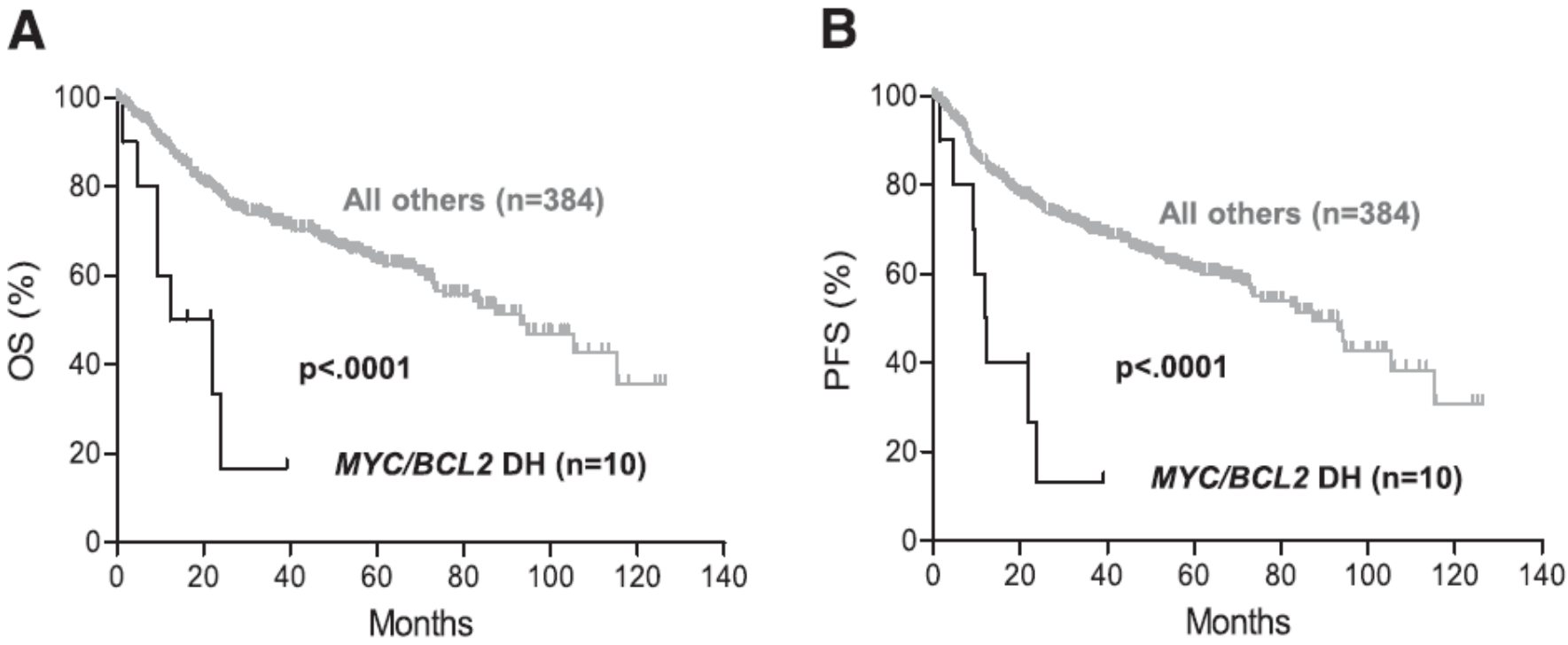
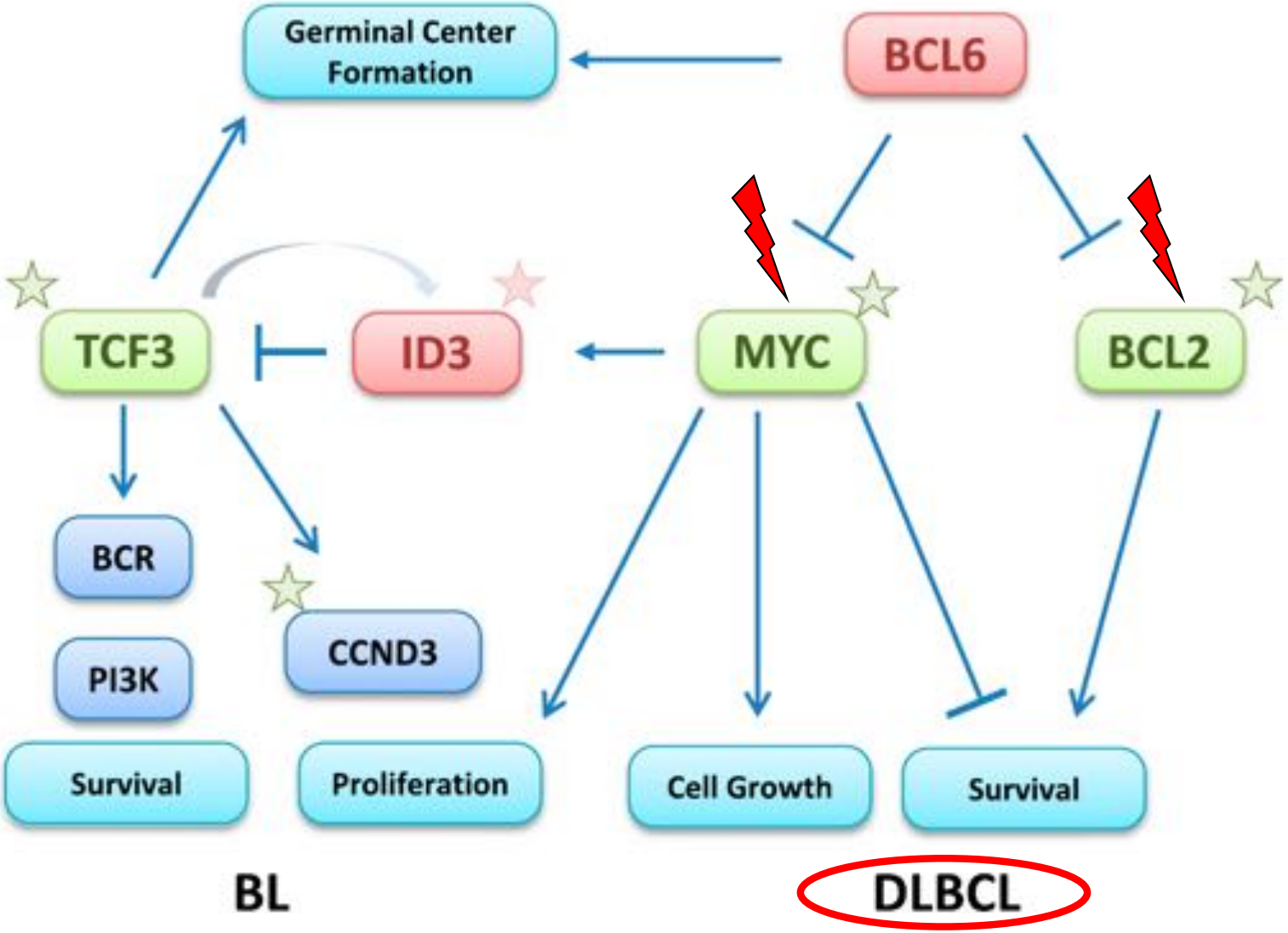
High-grade B-cell lymphoma, NOS*

LGCBD double-hit riarrangiamento MYC/BCL2



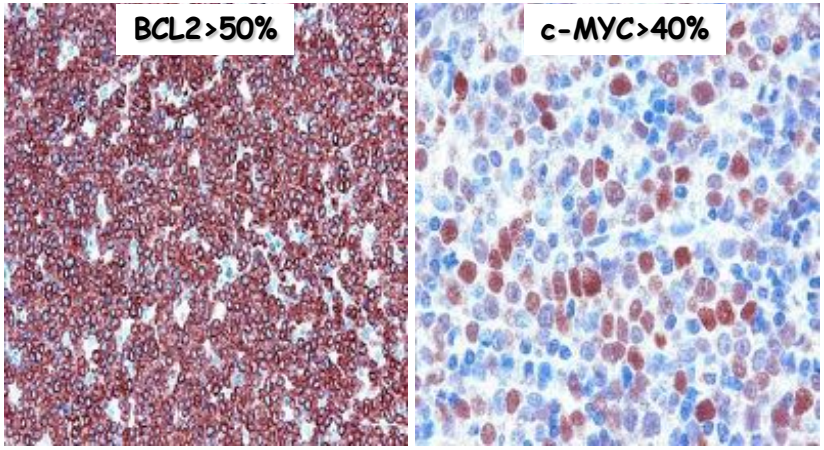
- Circa il 10% dei LGCBD presentano doppio riarrangiamento per MYC/BCL2
- > Frequenti in GCB
- Stadio avantato
- Età avanzata
- IPI alto

LGCBD double-hit riarrangiamento MYC/BCL2



Possiamo usare DE per identificare i DH/TH ?

Con cut-off BCL2>50% e MYC>40%: *NO*



- DE non si osserva solo in DH/TH
- anche DLBCL non MYC/BCL2r possono essere DE
- 30% dei DLBCL sono DE
- in genere DE belong to non-GCB
- **DE impatta comunque con la prognosi**

Concurrent Expression of MYC and BCL2 in Diffuse Large B-Cell Lymphoma Treated With Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone

Nathalie A. Johnson, Graham W. Slack, Kerry J. Savage, Joseph M. Connors, Susana Ben-Neriah, Sanja Rogic, David W. Scott, King L. Tan, Christian Steidl, Laurie H. Sehn, Wing C. Chan, Javeed Iqbal, Paul N. Meyer, Georg Lenz, George Wright, Lisa M. Rimsza, Carlo Valentino, Patrick Brunhoeber, Thomas M. Grogan, Rita M. Braziel, James R. Cook, Raymond R. Tubbs, Dennis D. Weisenburger, Elias Campo, Andreas Rosenwald, German Ott, Jan Delabie, Christina Holcroft, Elaine S. Jaffe, Louis M. Staudt, and Randy D. Gascoyne

Double expressor Lymhpoma MYC+/BCL2+

MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program

Shimin Hu,[1] Zijun Y. Xu-Monette,[1] Alexander Tzankov,[2] Tina Green,[3] Lin Wu,[4] Aarthi Balasubramanyam,[4] Wei-min Liu,[4] Carlo Visco,[5] Yong Li,[6] Roberto N. Miranda,[1] Santiago Montes-Moreno,[7] Karen Dybkaer,[8] April Chiu,[9] Attilio Orazi,[10] Youli Zu,[11] Govind Bhagat,[12] Kristy L. Richards,[13] Eric D. Hsi,[14] William W. L. Choi,[15] Xiaoying Zhao,[16] J. Han van Krieken,[17] Qin Huang,[18] Jooryung Huh,[19] Weiyun Ai,[20] Maurilio Ponzoni,[21] Andrés J. M. Ferreri,[21] Fan Zhou,[22] Graham W. Slack,[23] Randy D. Gascoyne,[23] Meifeng Tu,[24] Daina Variakojis,[25] Weina Chen,[26] Ronald S. Go,[27] Miguel A. Piris,[7] Michael B. Møller,[3] L. Jeffrey Medeiros,[1] and Ken H. Young[1]

Key Points

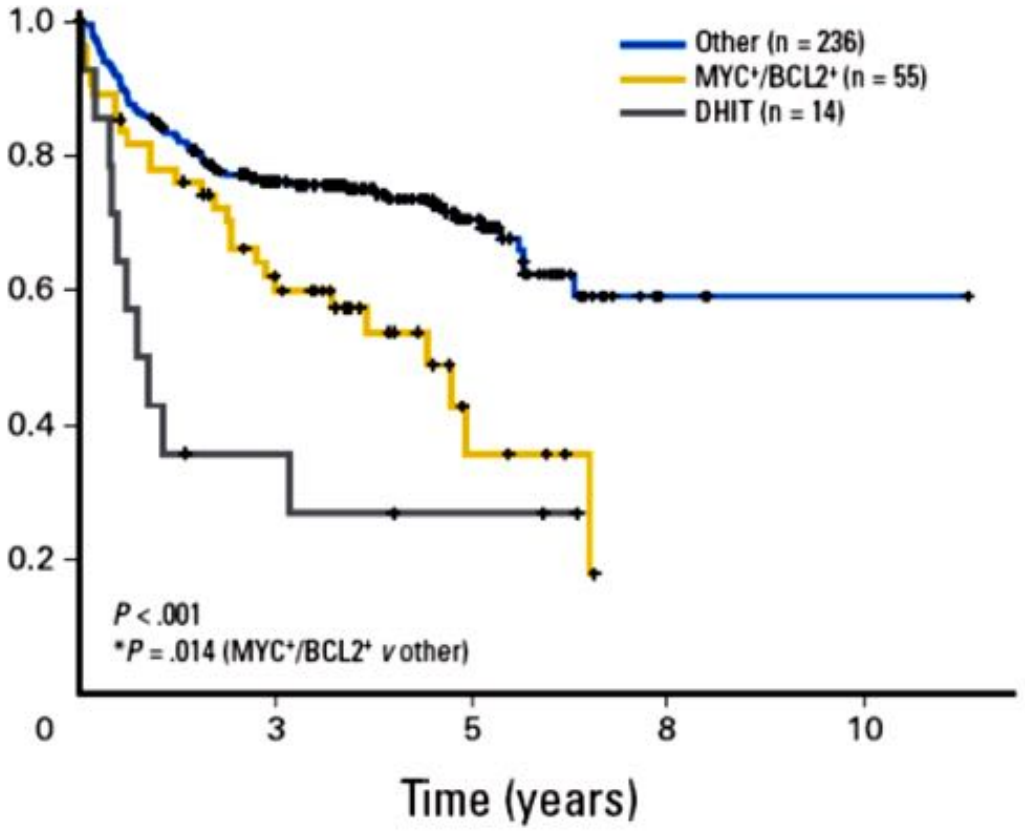
- DLBCL patients with MYC/BCL2 coexpression demonstrate inferior prognosis and high-risk gene expression signatures.

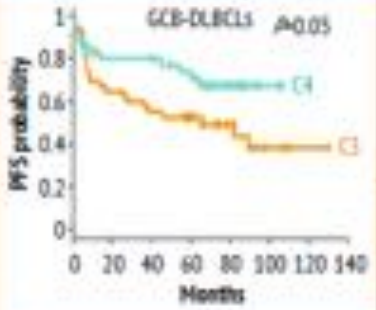
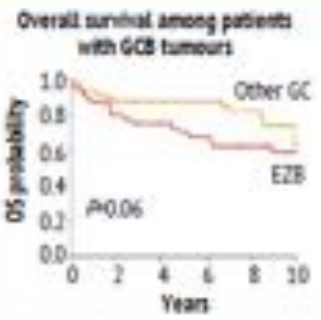
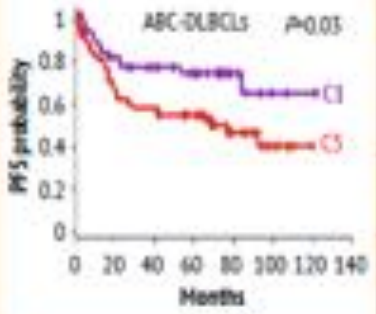
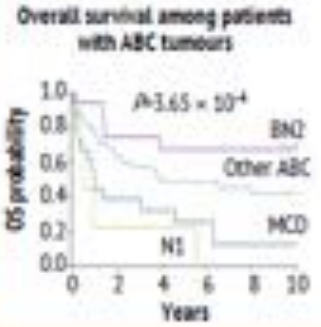
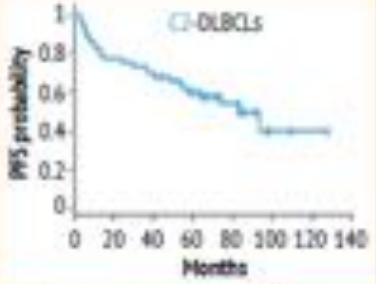
MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma

(*Blood.* 2013;121(12):2253-2263)

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

DHL vs DE vs non DH/DE



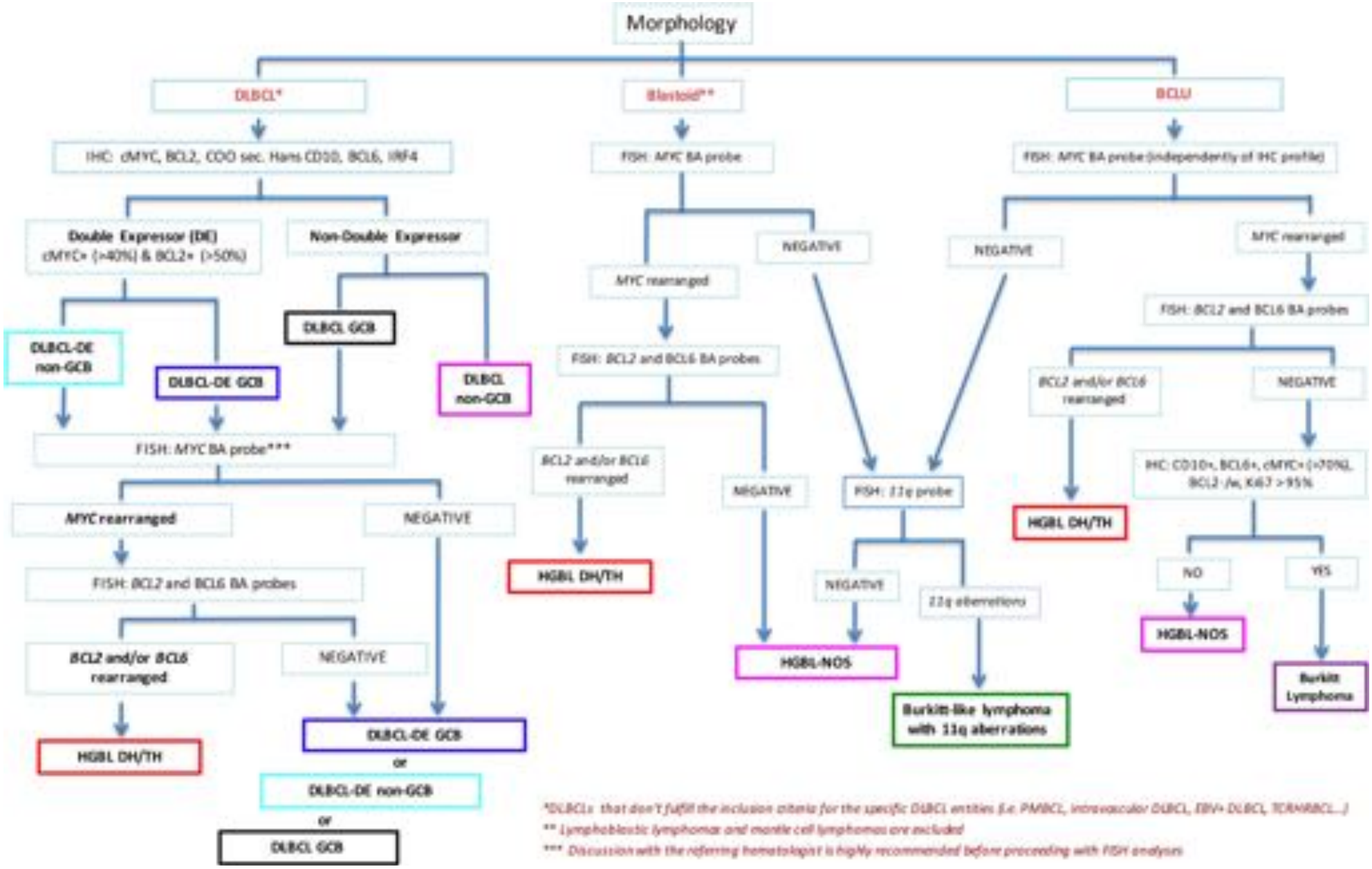
COO	Risk	Chapuy & Shipp[1]		Schmitz & Staudt[2]		Possible Agent
Germinal Center	Lower Risk	Cluster 4 Histone mutations JAK/STAT and PI3K signalling NF-κB mutations		Other GC		PI3K or JAK inhibitor
	Higher Risk	Cluster 3 Bcl-2 translocations EZH2 mutations PI3K signalling		EZB Bcl-2 translocations EZH2 mutations		Bcl-2 and/or EZH2 inhibitor PI3K inhibitor
ABC	Lower Risk	Cluster 1 Immune evasion NOTCH2/NF-κB mutation Bcl-6 translocations MYD88$^{non-L265P}$ mutation		BN2 Immune evasion NOTCH2/NF-κB mutation Bcl-6 alterations		Proteasome inhibitor Checkpoint inhibitor
	Higher Risk	Cluster 5 CD79B, MYD88^{L265P} mutation 18q gains Bcl-2/MALT-1 expression		N1 NOTCH1 mutations MCD CD79B, MYD88^{L265P}		Btk inhibitor
Other	Higher Risk	Cluster 2 Inactivation of p53, CDKN2A loss				Tissue-agnostic treatment

ABC, activated B-cell; C, cluster; COO, cell-of-origin; DLBCL, diffuse large B-cell lymphoma; GC, germinal center; GCB, germinal center B-cell; OS, overall survival; PFS, progression-free survival

1. Chapuy B, et al. Nat Med. 2018;24:679-690; 2. Schmitz R, et al. N Engl J Med. 2018;378:1396-1407

Di Napoli A/GIE Virchows Archiv 2019

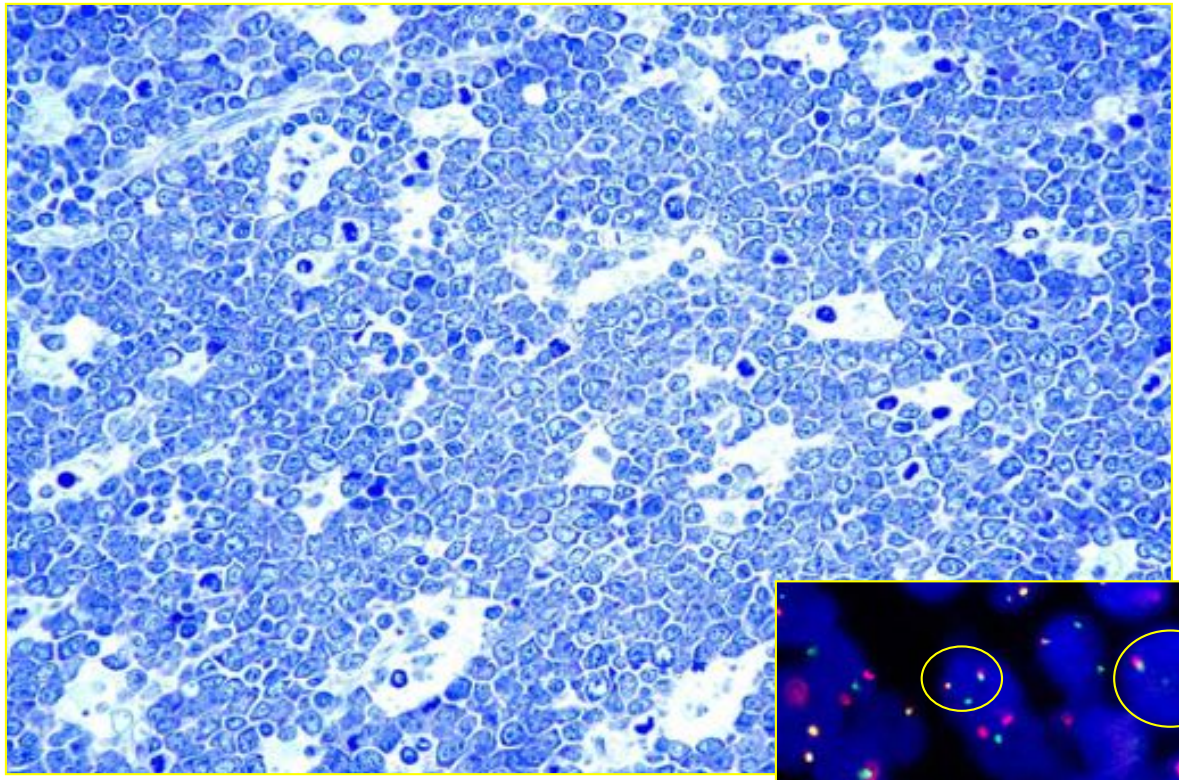
DLCLvs HGBCL vs DH-TH vs BL

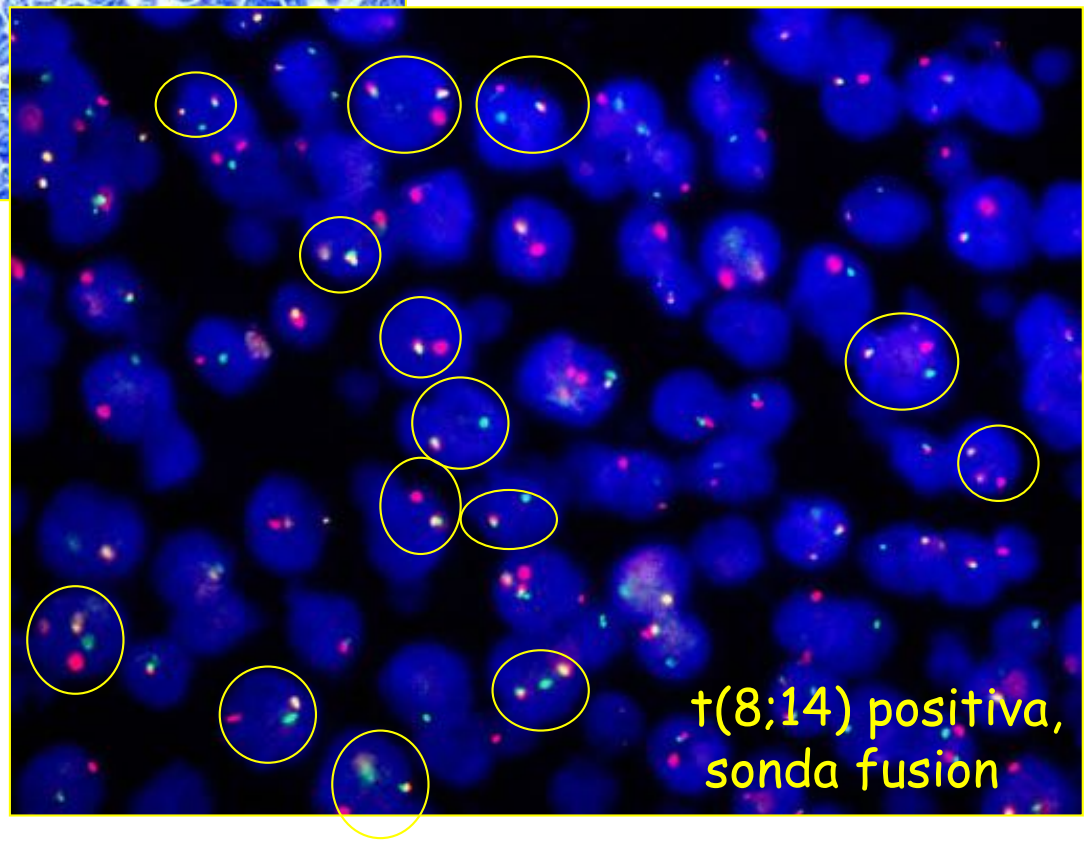


*DLBCLs that don't fulfill the inclusion criteria for the specific DLBCL entities (i.e. PMBCL, intravascular DLBCL, EBV+ DLBCL, TCRAIRBCL...)

** Lymphoblastic lymphomas and mantle cell lymphomas are excluded

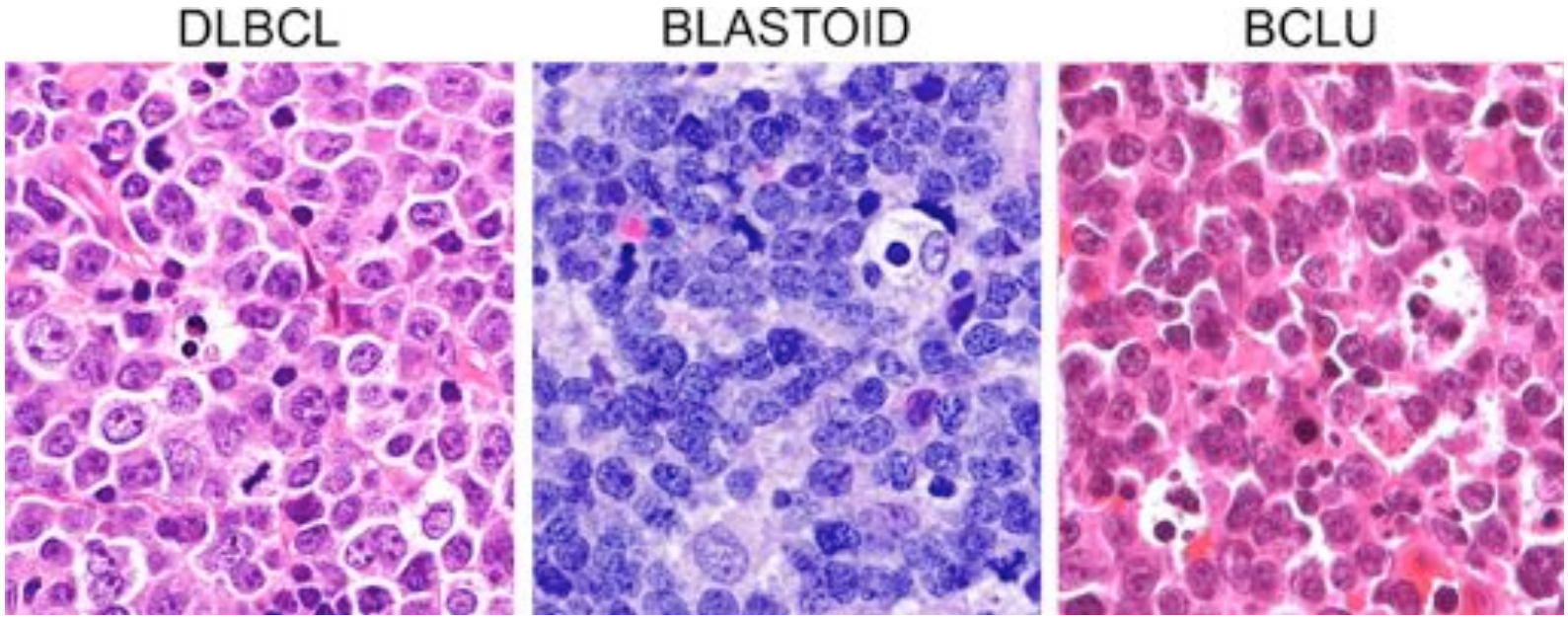
*** Discussion with the referring hematologist is highly recommended before proceeding with FISH analysis

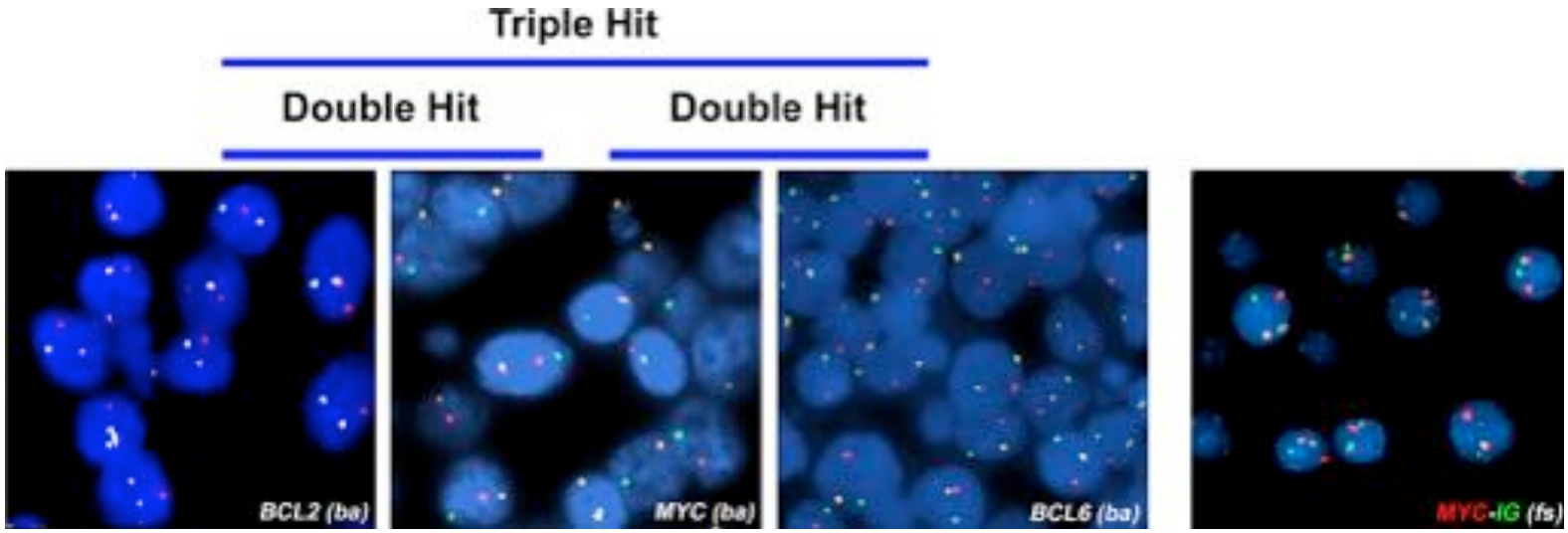


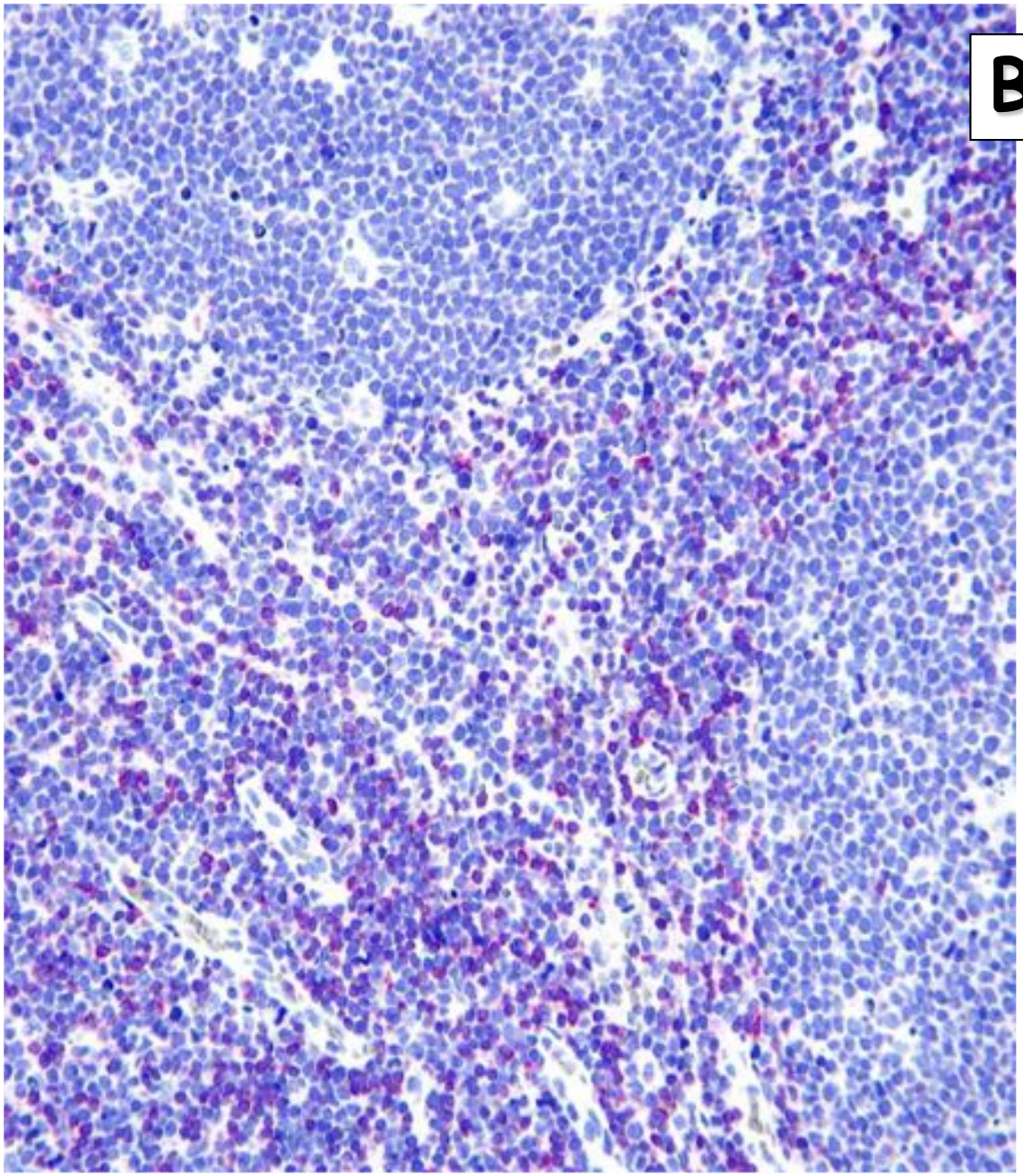


Linfoma di Burkitt
ricerca t(8;14) , t(2;8), t(8;22)
a scopo diagnostico

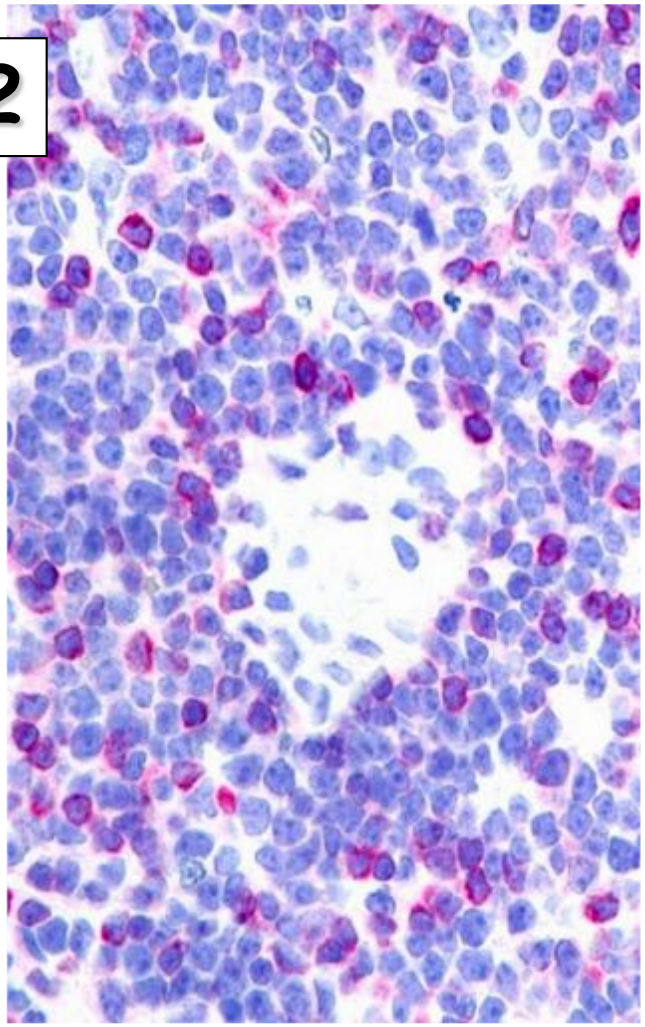
Classificazione B aggressivi





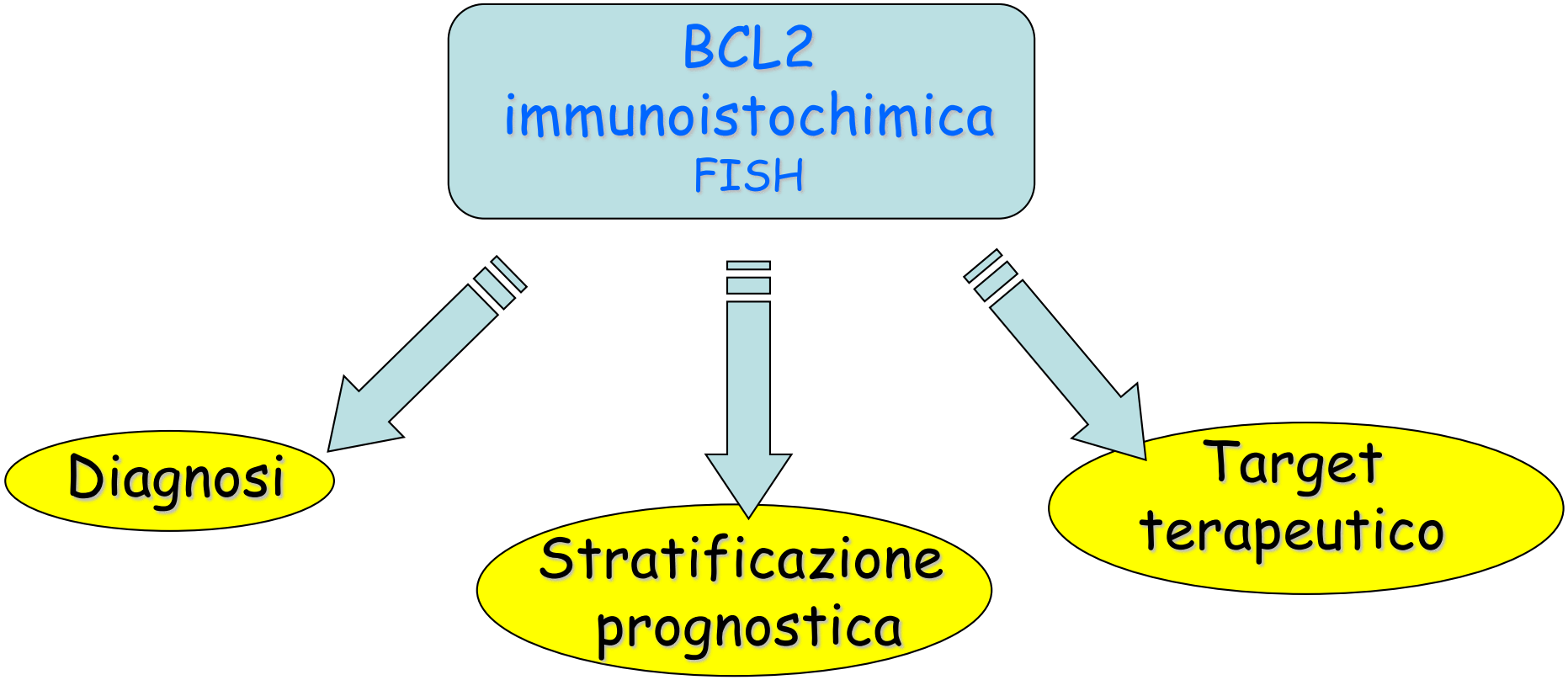


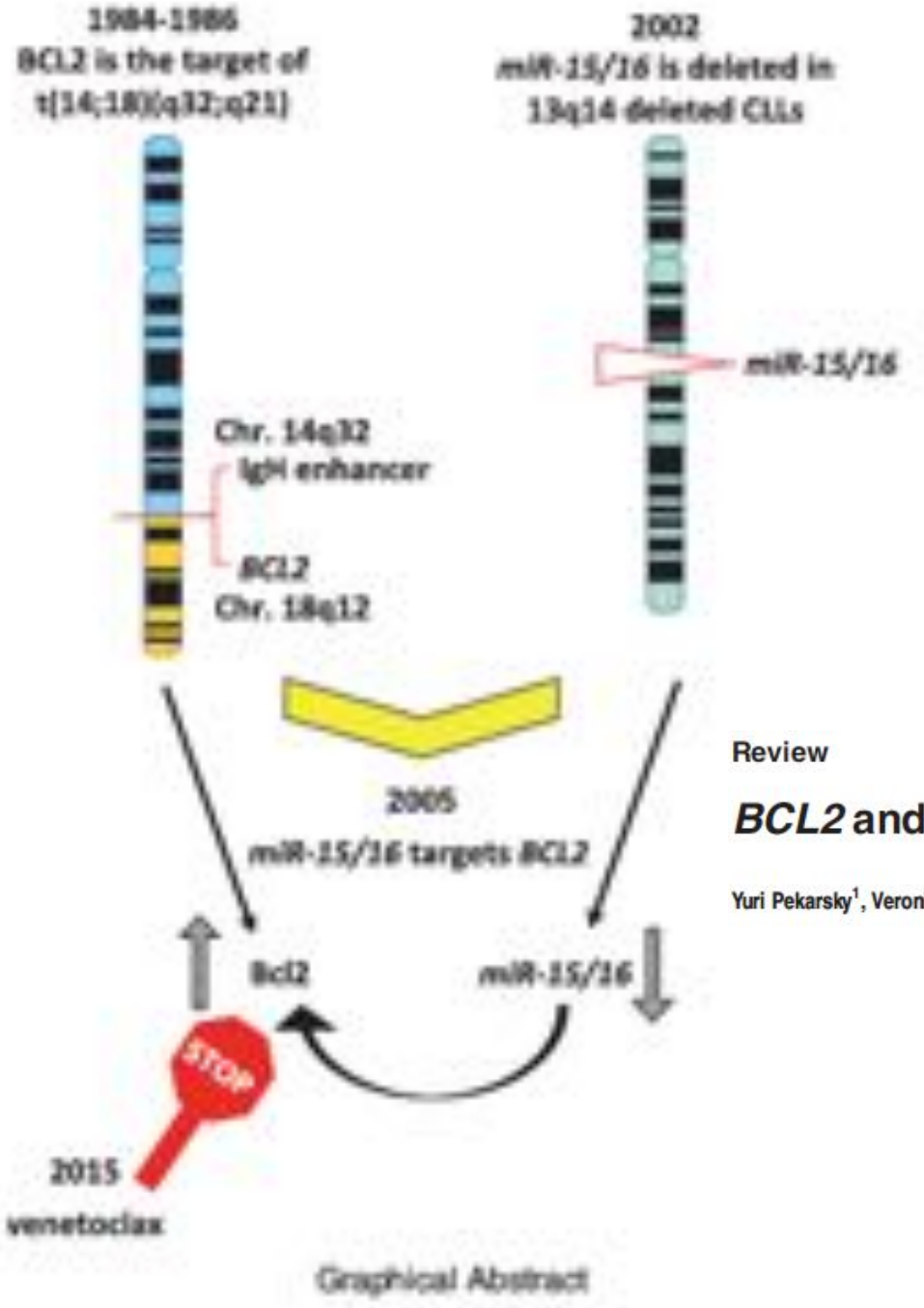
BCL2



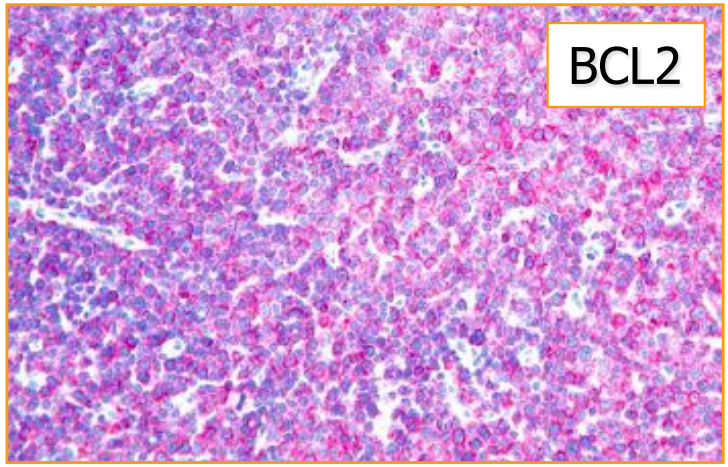
BL: BCL2^{-}/CD10^{+}/BCL6^{+}

BCL2 in ematopatologia





Leucemia Linfoatica cronica BCL2^{+}

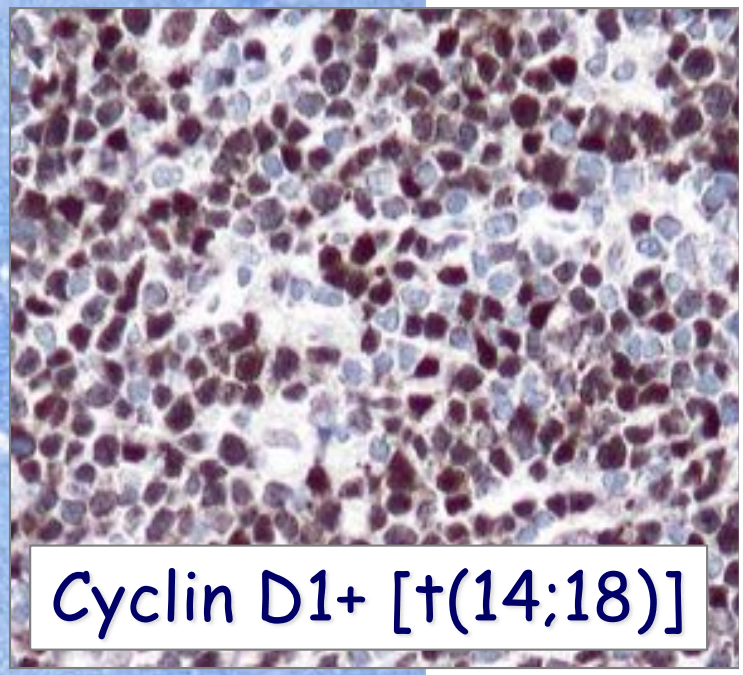


Review

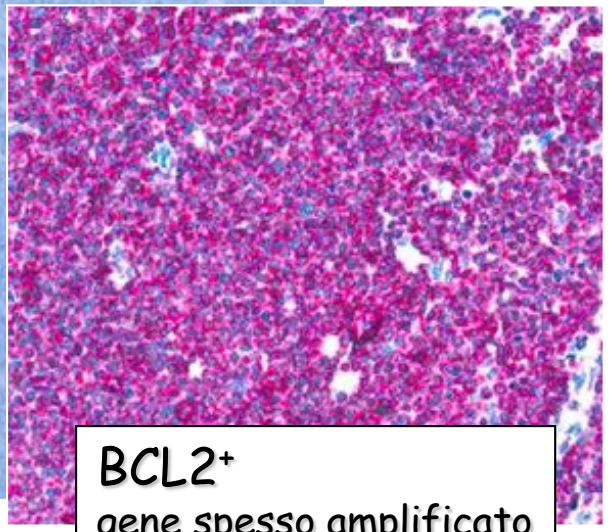
BCL2 and *miR-15/16*: from gene discovery to treatment

Yuri Pekarsky[1], Veronica Balatti[1] and Carlo M Croce*[,1]

Mantle cell lymphoma

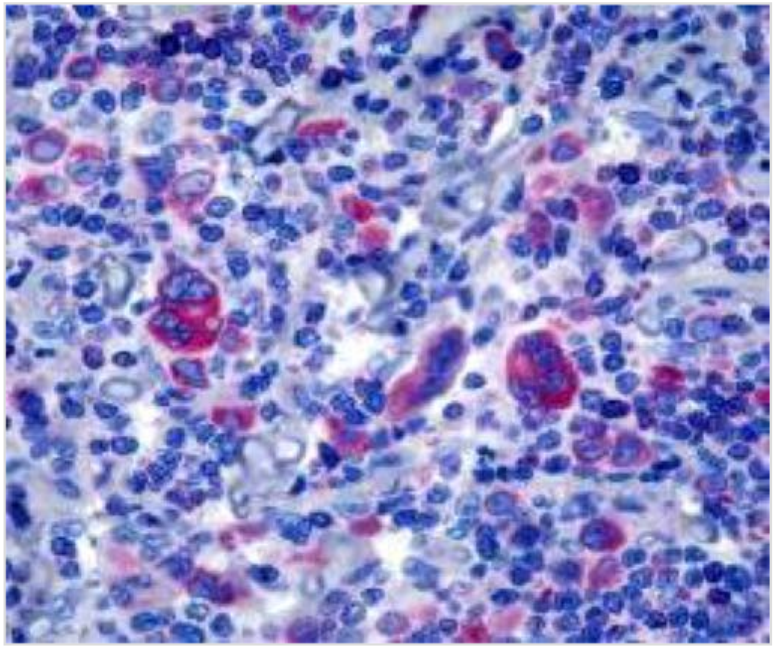


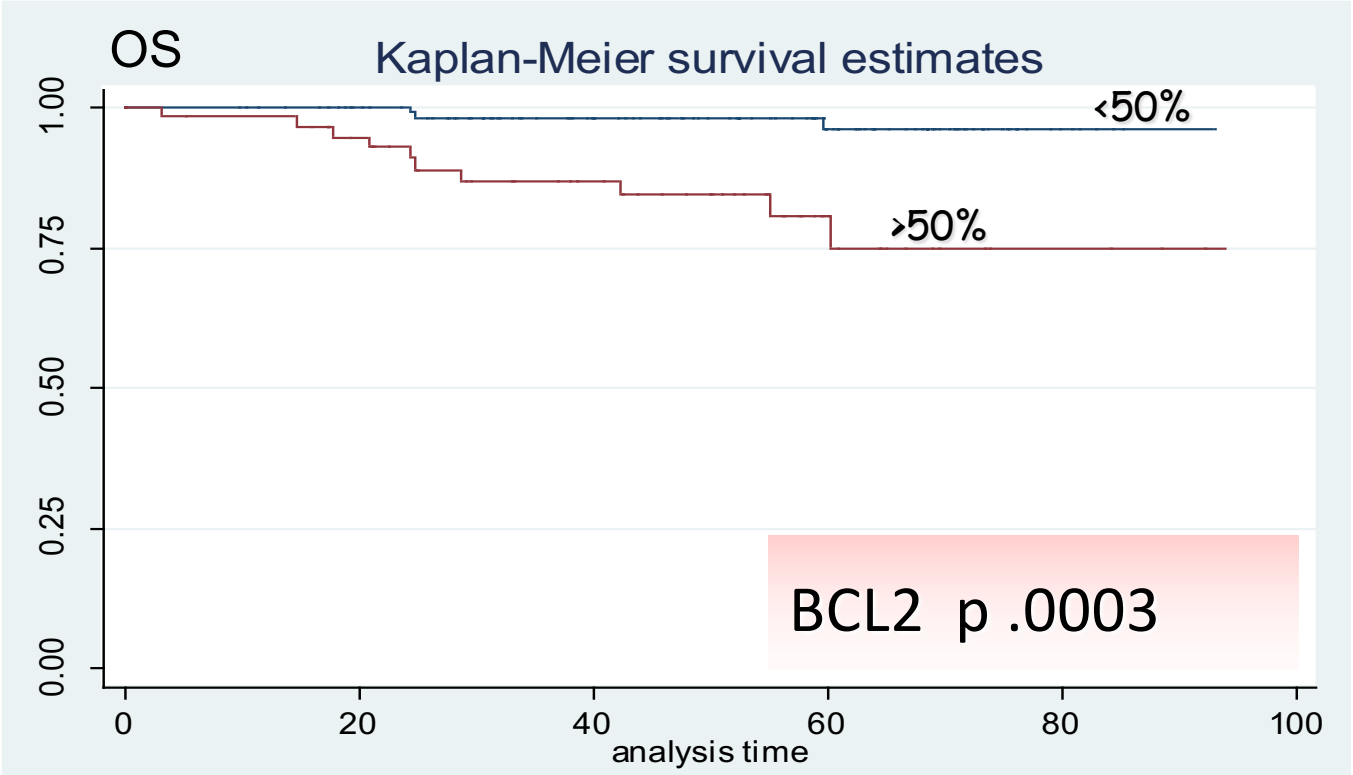
Cyclin D1+ [t(14;18)]



BCL2+
gene spesso amplificato

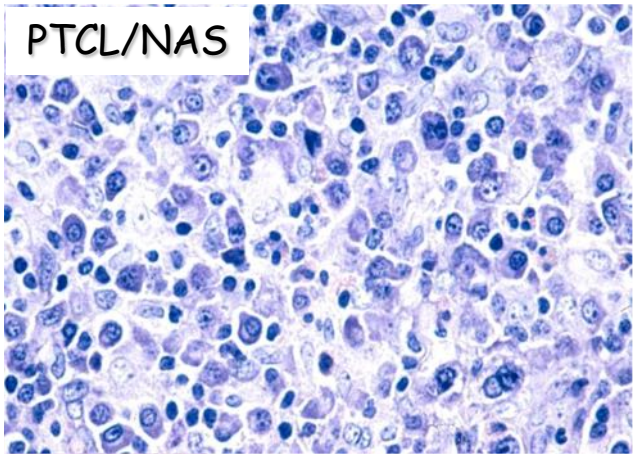
Linfoma di Hodgkin Classico

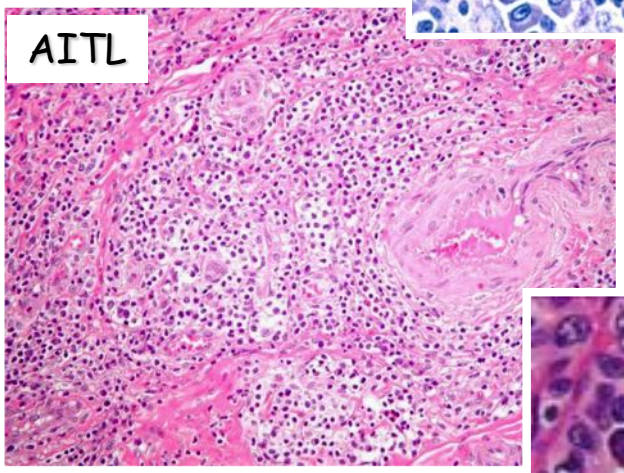


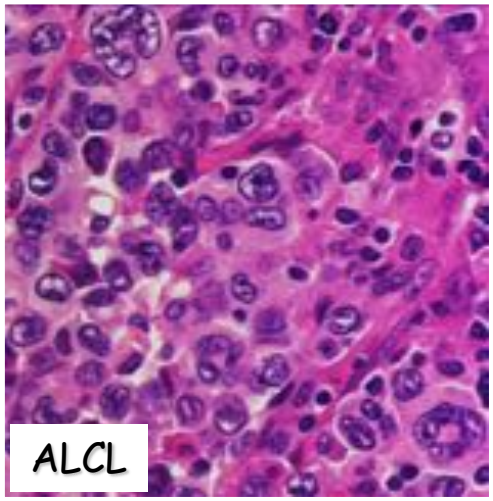


Agostinelli et al Lancet Hematol 2016

CD38, BCL-2, PD-1, and PD-1L expression in nodal peripheral T-cell lymphoma: Possible biomarkers for novel targeted therapies?







	ALCL-ALK⁻	ALCL-ALK⁺	PTCL-NOS	AITL
Total cases	**48**	**25**	**73**	**27**
CD38				
N. of evaluable cases	36	10	42	25
CD38 positive cases	6 (17%)	0	24 (57%)	20 (80%)
Positivity score:				
4	3		9	2
3	1		3	5
2	0		2	4
1	2		10	9
0	30		18	5
BCL-2				
N. of evaluable cases	37	13	44	26
BCL-2 positive cases	21 (58%)	4 (31%)	35 (79.5%)	23 (88%)
Positivity score:				
4	13	2	22	10
3	3	0	5	3
2	3	1	5	4
1	2	1	3	6
0	16	9	9	3
PD-1				
N. of evaluable cases	36	18	46	27
PD-1 positive cases	0	2 (11%)	28 (61%)	19 (70%)
Positivity score:				
4		2	9	5
3		0	9	9
2		0	5	1
1		0	5	4
0		16	18	8
PD-1L				
N. of evaluable cases	35	11	44	27
PD1-L positive cases	16 (46%)	8 (72%)	0	0
Positivity score:				
4	9	4		
3	2	1		
2	2	2		
1	3	1		
0	19	3		

Positivity score: 4 = >75% stained cells; 3 = 75–50%; 2 = 25–49%; 1 = 5–24%; 0 = <5%.

E Sabattini, Bacci F, C Agostinelli, C Bertuzzi, C Sagramoso, M Rossi, S Righi, A Gazzola, M Piccioli, C Mannu, F Sandri, G De Biase, S Giusti, G Da Pozzo