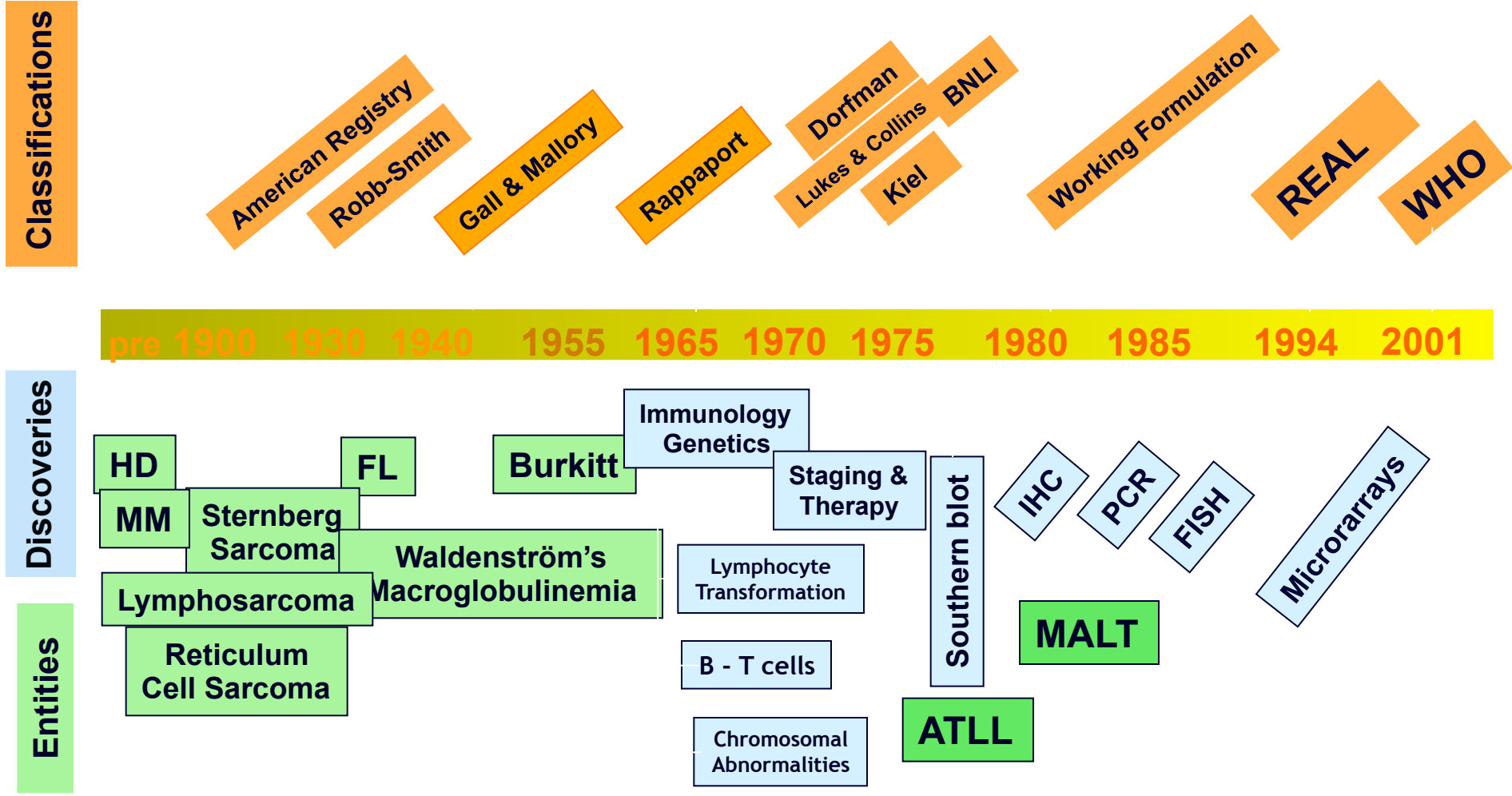


New Concepts and Clinical Implications
of the 2008 WHO Classification
Elaine S Jaffe

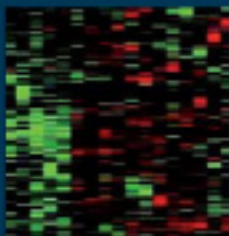
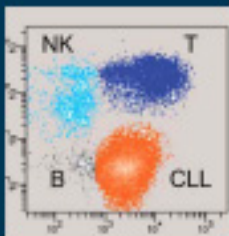
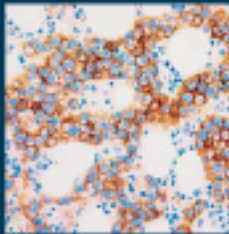
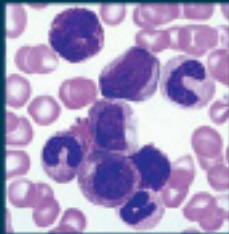
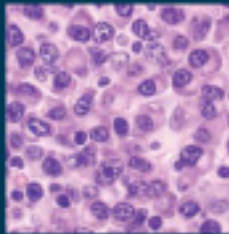


Time Line of Changes in Lymphoma Classification Impact of Clinical and Technical Advances



WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Edited by Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe,
Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman



WHO Monograph
4th Edition
IARC Press
September 2008

A **disease oriented**
approach to
classification in the
model of the REAL &
WHO 2001

Editors:

Steven H. Swerdlow, Elias Campo
Nancy Lee Harris, Elaine S. Jaffe
Stefano A Pileri, Harald Stein
Jurgen Thiele, James W. Vardiman
138 Authors from 22 countries

Clinical Advisory Committee
Airlie, Virginia March 2007



Implications of a Disease Oriented Classification

- Most insights into the pathogenesis of malignant lymphoma have followed on the heels of accurate pathological description
- Process is iterative
 - Knowledge of molecular pathways lead to development of new diagnostic tools
 - Tools further delineate disease & its borderlands

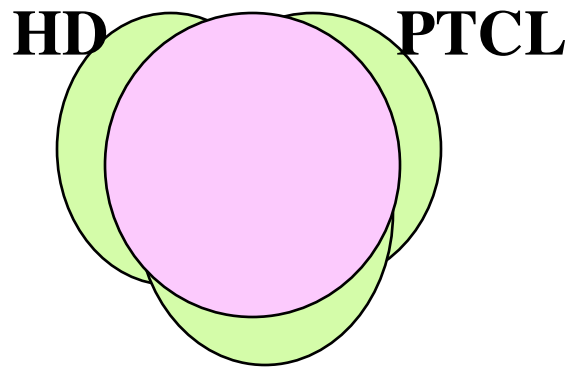
EVOLUTION OF ANAPLASTIC LARGE CELL LYMPHOMA

Initial
Description

Immunophenotypic
Studies

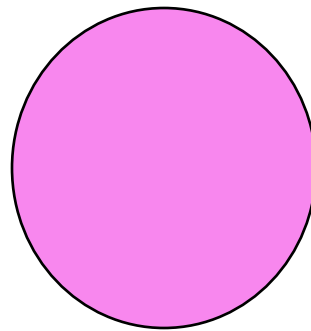
Molecular
Pathogenesis

ALK as a
Diagnostic Tool

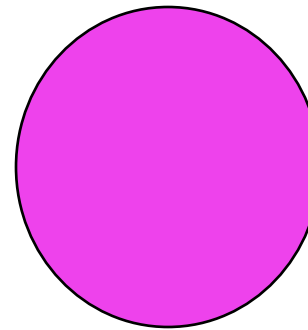


MH

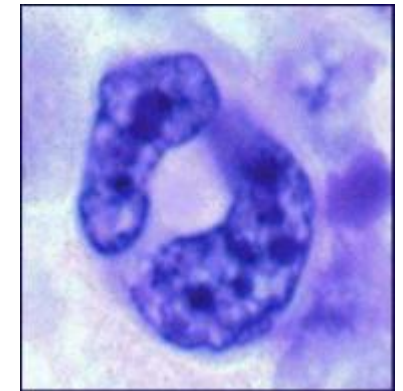
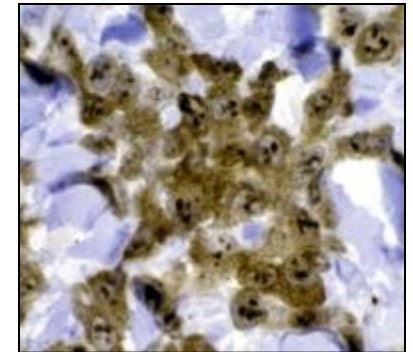
Ki-1+
Sinusoidal
lymphoma



CD30+
EMA+
LCA+
CD15-
CD3 -/+



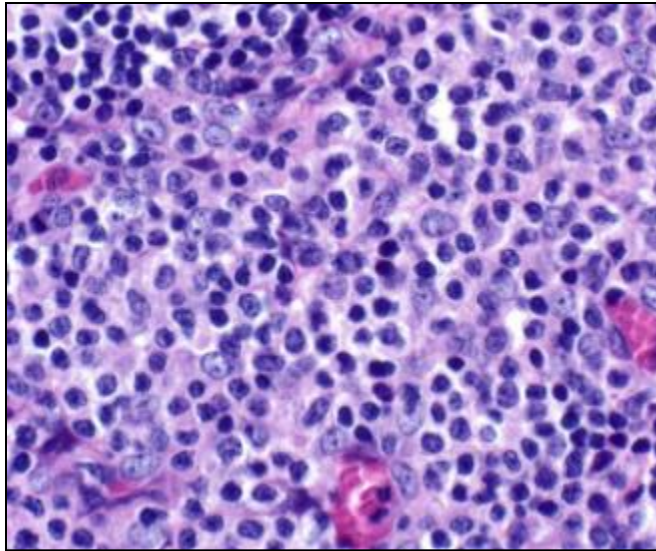
t(2;5)
NPM;ALK



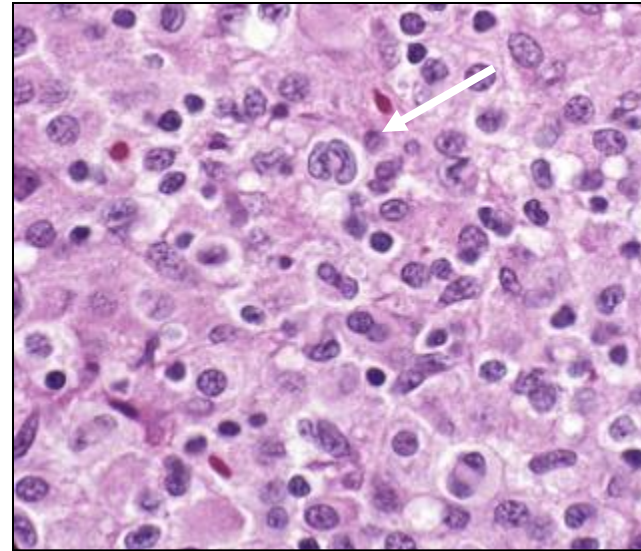
Hallmark cells

Ultimate spectrum of ALCL is broader & narrower than original concept
Diagnostic tools based on molecular phenotype have redefined ALCL

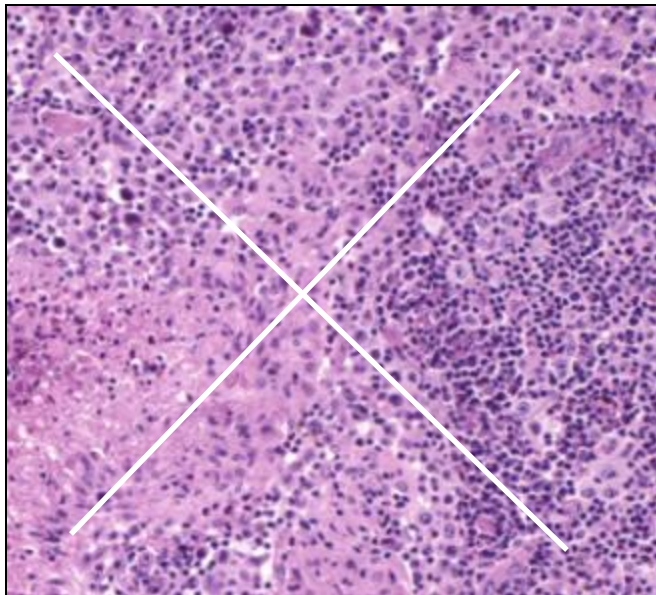
Small Cell
ALK pos



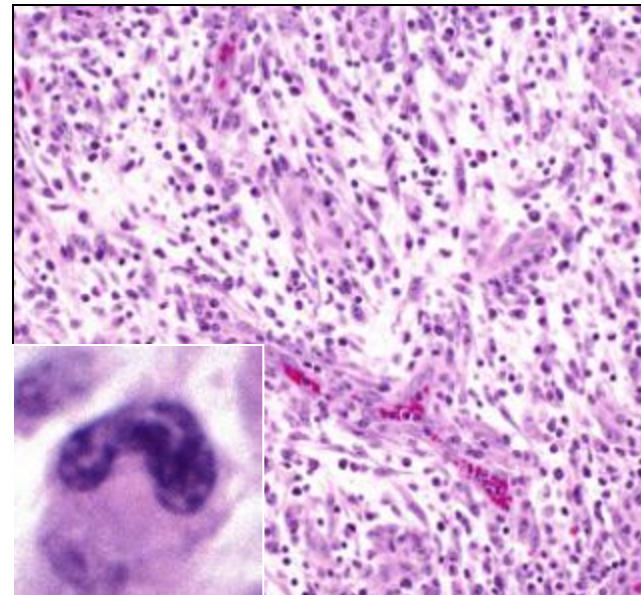
Lympho-
Histiocytic
ALK pos



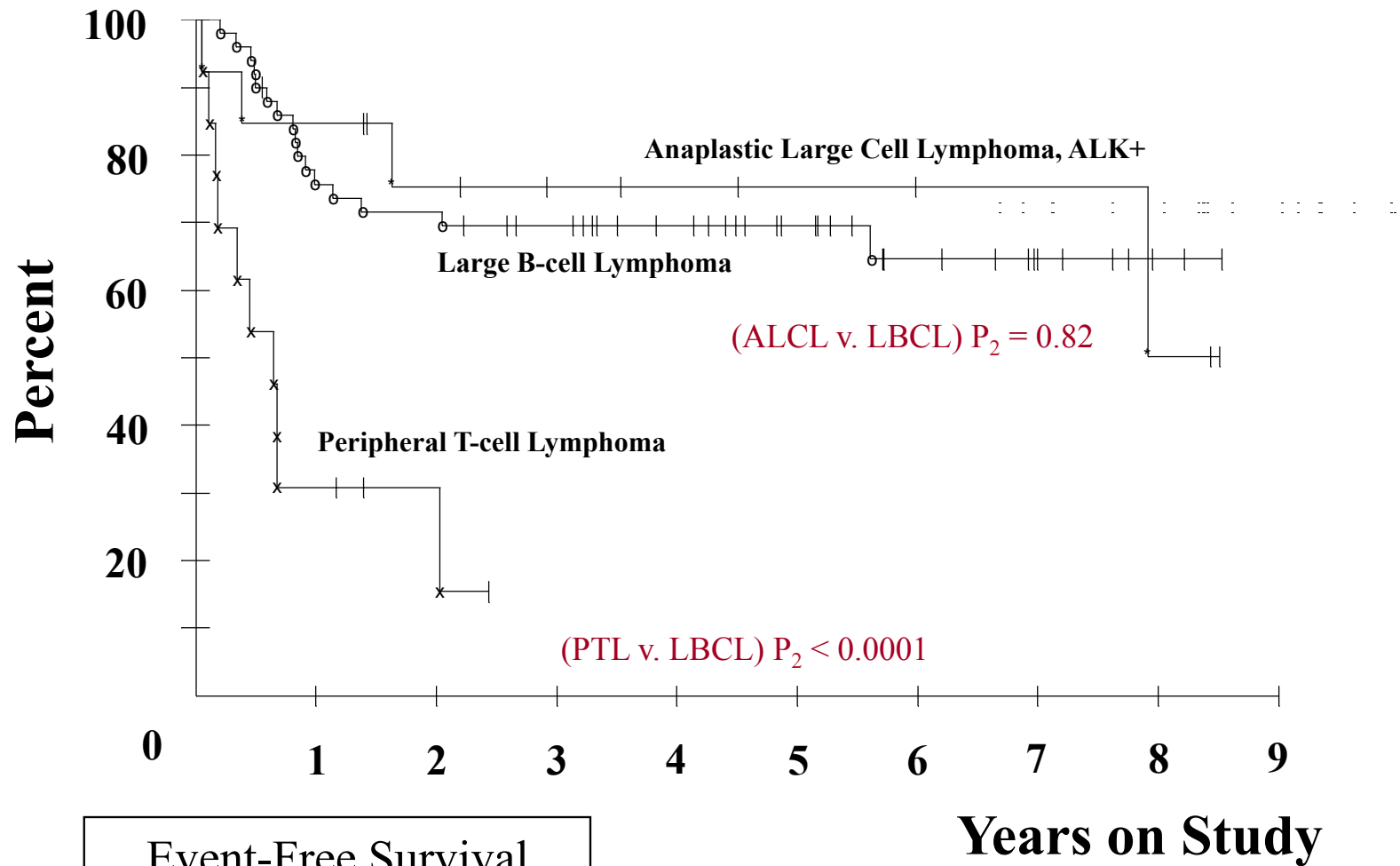
“Hodgkin’s like”
ALK neg



Hypocellular
Sarcomatoid
ALK pos



What we define as ALCL has major clinical significance



Event-Free Survival
NCI series

Years on Study

Pathogenetic Insights Based on Disease Discovery

Disease

Anaplastic large cell lymphoma

Adult T-cell leuk/lymphoma

Extranodal NK/T cell lymphoma

Primary effusion lymphoma

Mantle cell lymphoma

Gastric MALT lymphoma

Burkitt lymphoma

Follicular lymphoma

Pathogenesis

ALK kinase

HTLV1

Genetics, EBV

KSHV/ HHV-8

CCND1

H. pylori, *MALT1*

C-MYC

BCL2

New Aspects of WHO 2008

- Greater recognition of “early” lesions
 - Earliest steps in neoplastic transformation
- Age as a defining aspect of some neoplasms
 - Both pediatric and elderly
- Site-specific impact on disease definitions
 - Anatomic site or environmental influences
- Incorporation of borderline categories
 - At least a temporary measure

Early Lesions in Lymphoid Neoplasia

- WHO 2001- Lesions of uncertain malignant potential
 - Lymphomatoid papulosis
 - Lymphomatoid granulomatosis
- WHO 2008 – **greater recognition of early steps in lymphomagenesis**
 - In situ follicular lymphoma
 - Duodenal follicular lymphoma
 - In situ / indolent mantle cell lymphoma
 - Monoclonal B lymphocytosis (MBL)

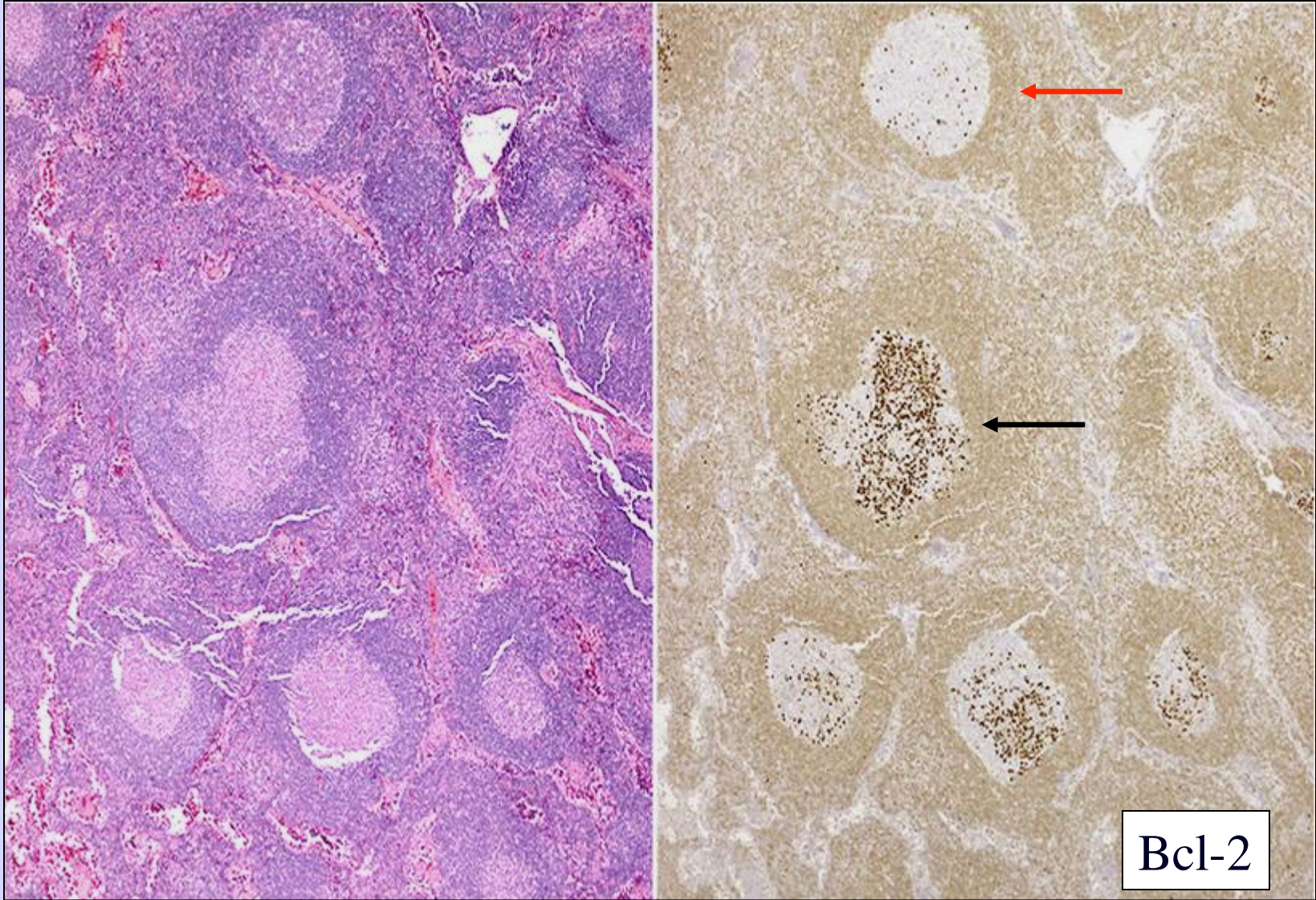
Monoclonal B Lymphocytosis (MBL)

- Clonal expansions of usually CD5+ B-cells in otherwise healthy persons (Marti 1992)
 - Identified first in familial CLL
- Progression to CLL occurs at a rate of 1.1% per year (Rawstron et al. NEJM 2008)
- 13q14 deletion found in ~ 50% of MBL & CLL
- Usually mutated but IG gene repertoire differs from that of typical CLL (Dagklis Blood 2009)

Revised Criteria for CLL (WHO 2008)

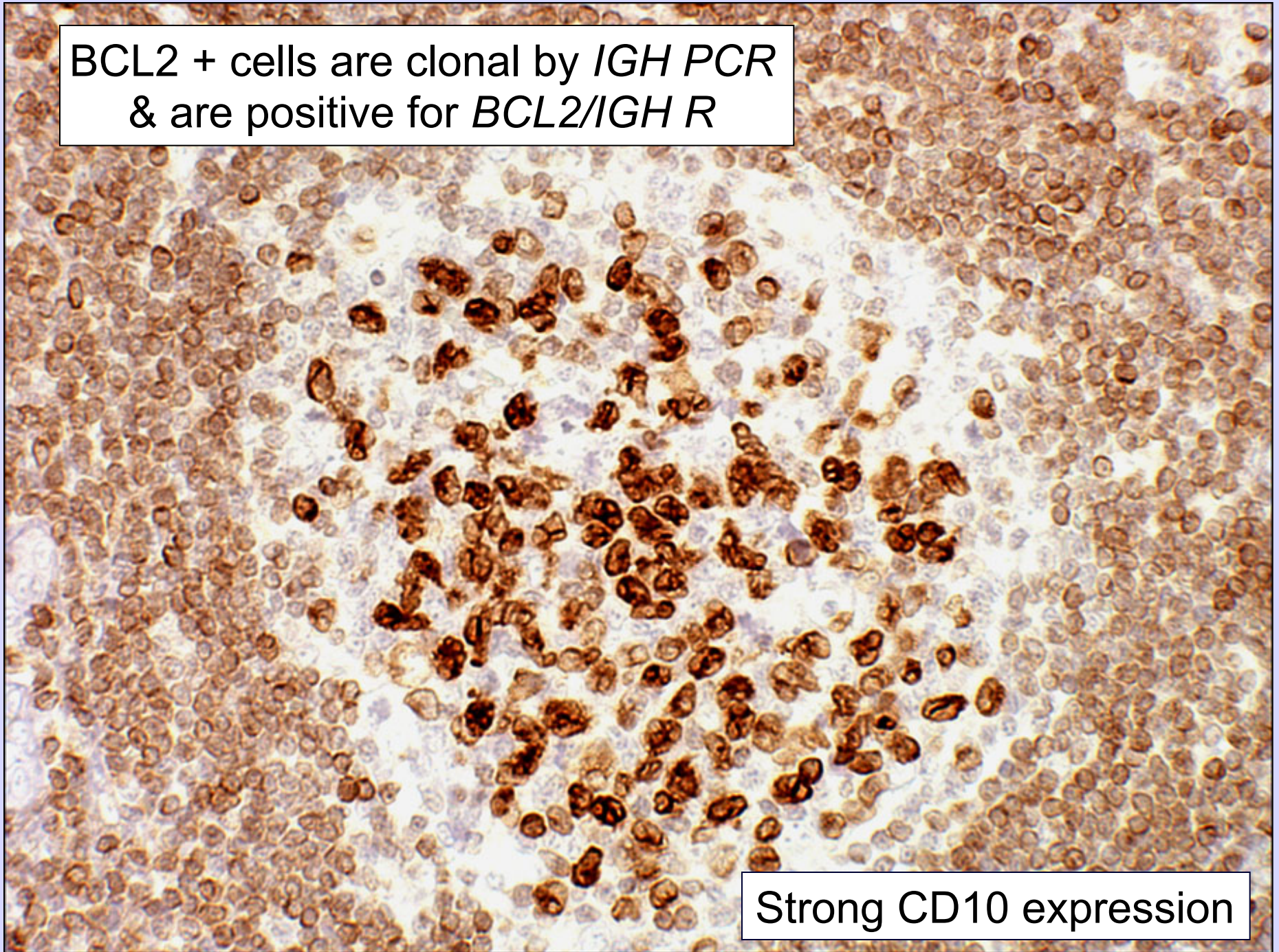
- Diagnosis of CLL requires
 - B-cell count greater than 5×10^9 or
 - Evidence of extramedullary disease
- Revised to permit distinction from MBL
- Based on B-cell count rather than absolute lymphocyte count
- Bone marrow involvement may be present
- Is there a tissue-based equivalent of MBL?

Follicular Lymphoma In Situ (FLIS)
Cong et al Blood 2002



BCL2 + cells are clonal by *IGH* PCR
& are positive for *BCL2/IGH* R

Strong CD10 expression



In Situ FL vs. Partial Involvement

In situ FL

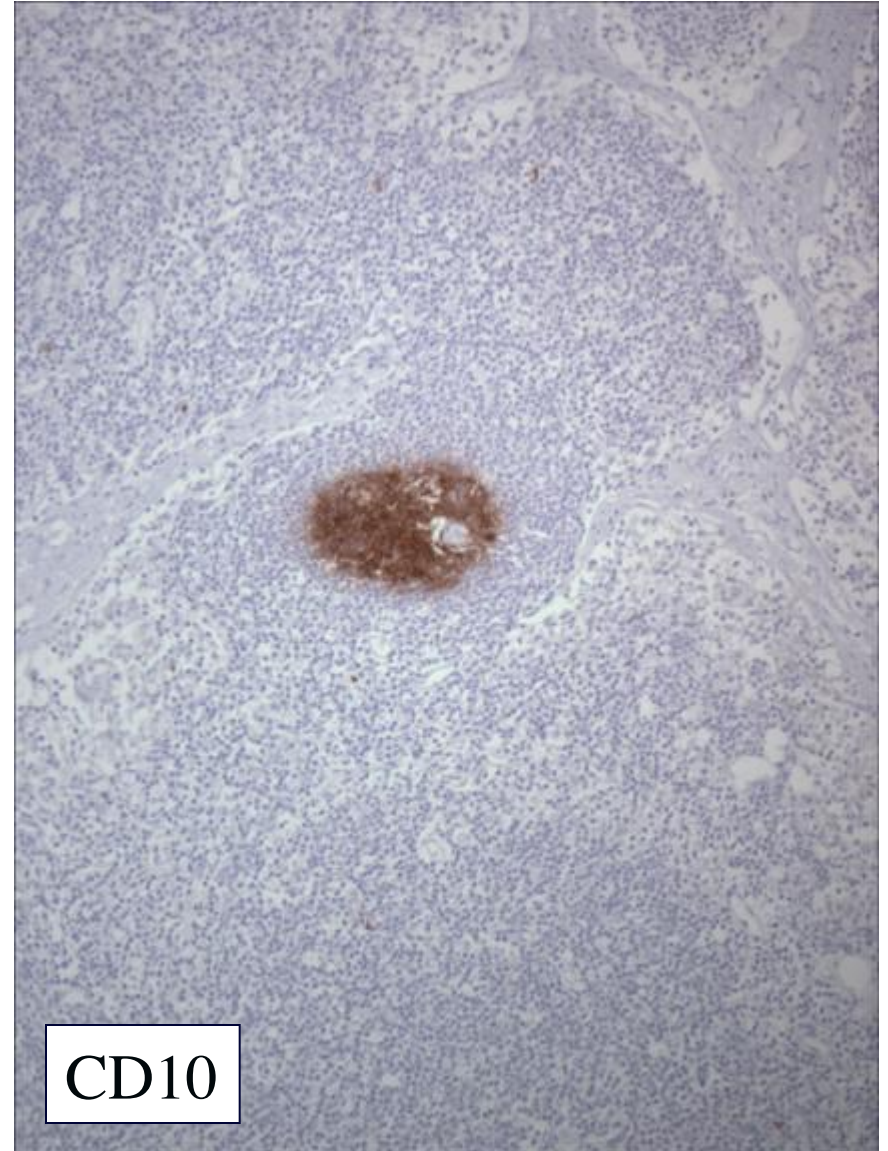
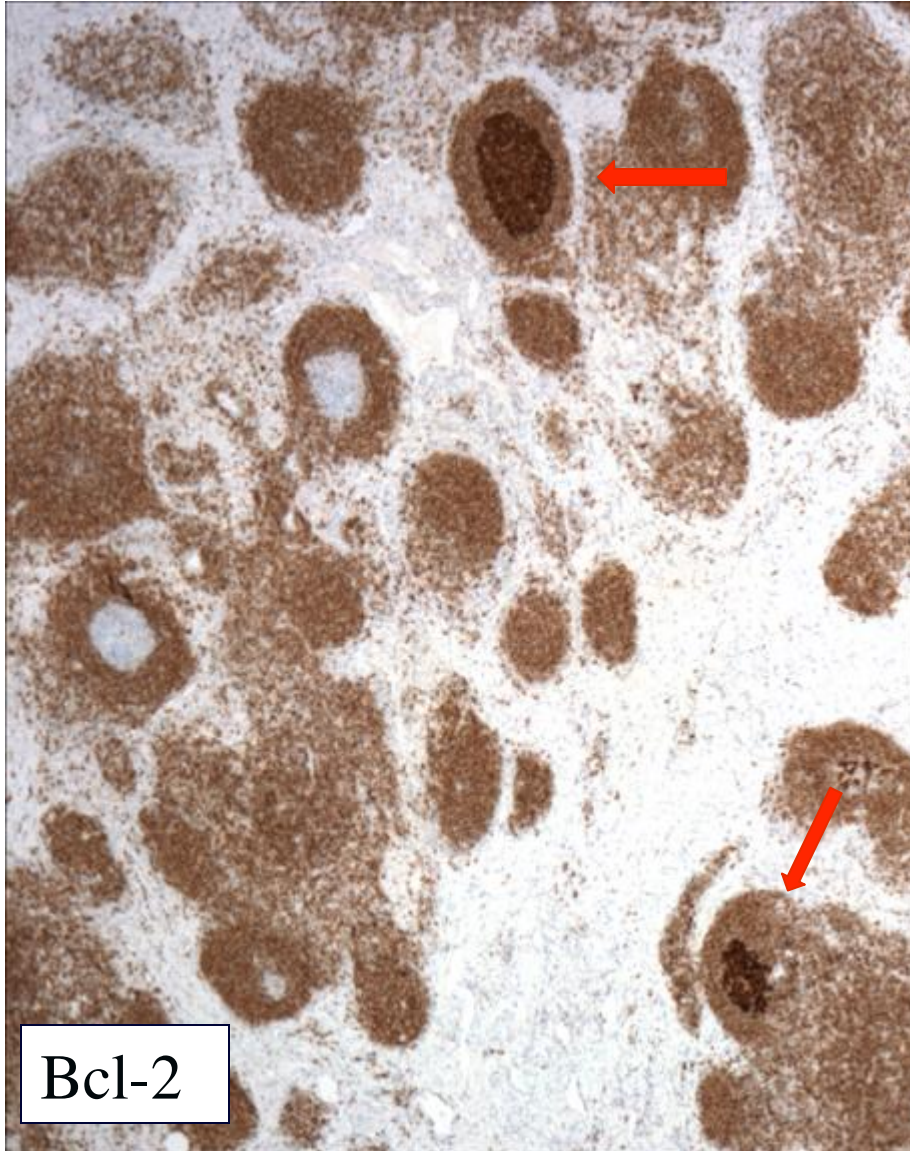
- Architecture intact
- Follicle size normal
- Involved follicles widely scattered
- Intact mantle cuff with sharp edge to GC
- Very strong BCL2 and CD10 expression
- Almost pure centrocytes

Partial FL

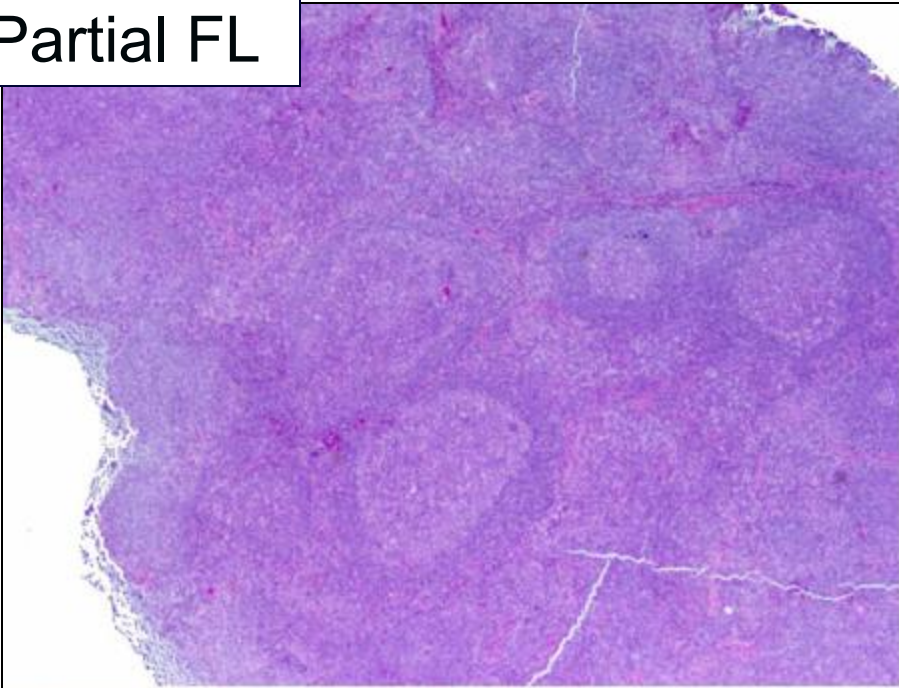
- Altered architecture
- Follicle size often expanded
- Involved follicles close together, adjacent
- Blurred edge to GC – attenuated mantle cuff
- BCL2 and CD10 not as strong and more variable
- Cytology more varied, CB/CC

BCL2+ follicles widely separated

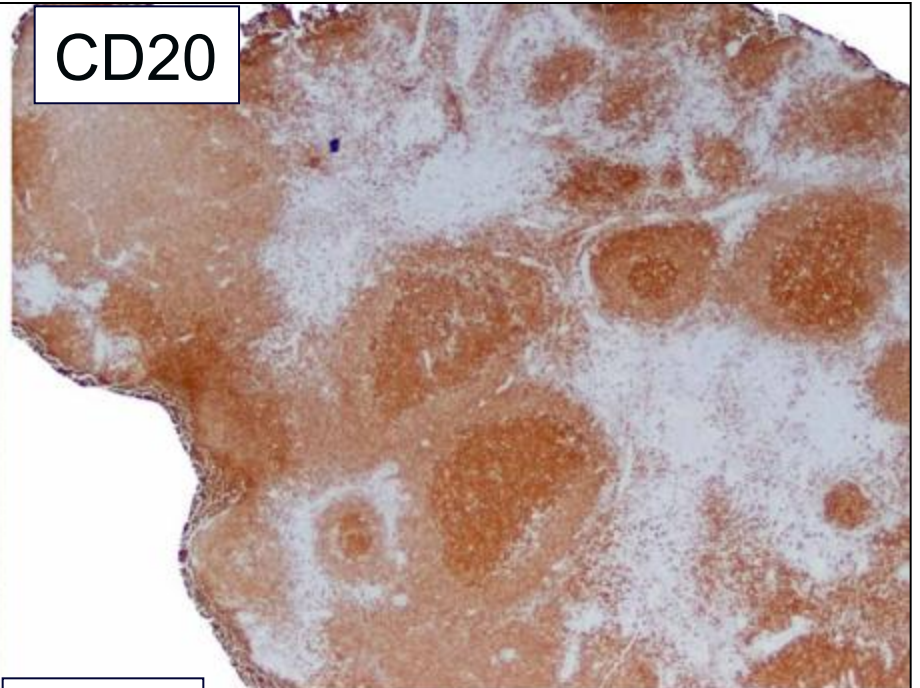
CD10 strongly positive



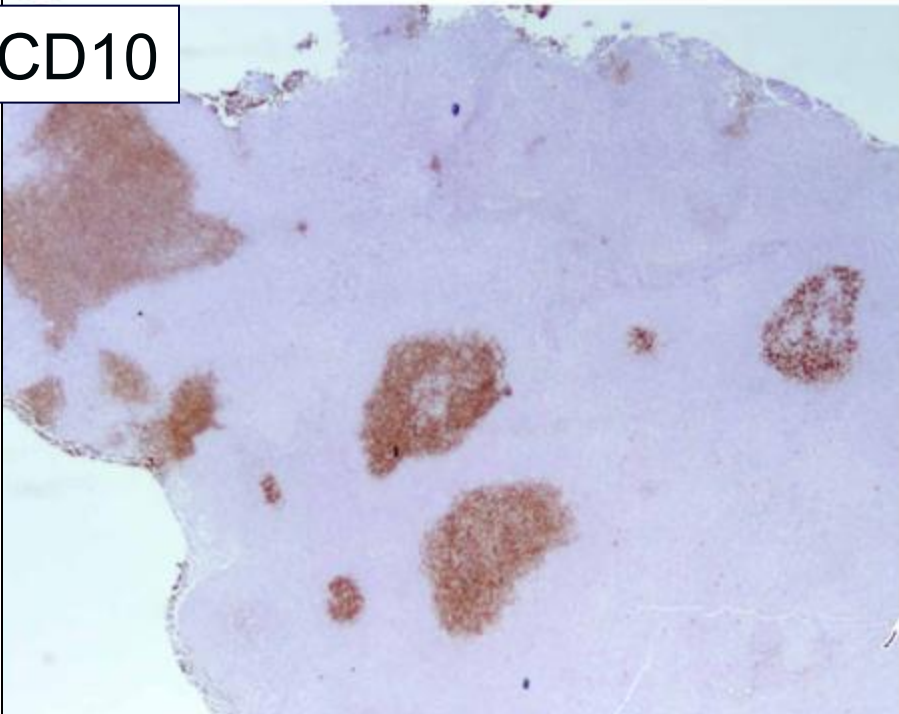
Partial FL



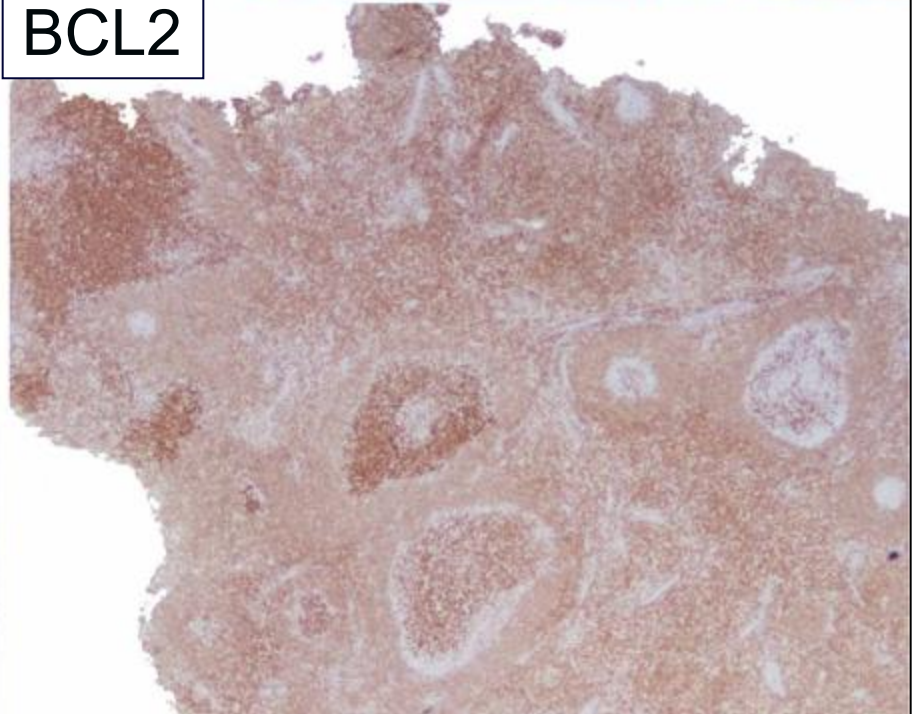
CD20



CD10



BCL2



Follicular lymphoma in situ (31 cases)
Clinical Outcome (2011 update)

- 4 (13%) had prior or concurrent BCL2+ FL
- 3 (10%) had concurrent BCL2 neg FL
- 1 (3%) developed FL at 4 yrs
- 4 (13%) had FLIS composite with another B-cell lymphoma
- 19 (61%) were NED with median follow up of 37 months (longest 118 months)

BCL2/IGH in Healthy Individuals

(Limpens et al. 1991; Roulland et al. 2006)

- *BCL2/IGH* is found in peripheral blood of up to 70% of normal adults over age 50
 - Numbers increase with age
 - Numbers increase with pesticide use in farmers
- *BCL2/IGH* + B-cells are not naïve B-cells
 - Memory B-cells, Class switched
 - Have encountered the germinal center reaction
 - Prone to intense trafficking among germinal centers

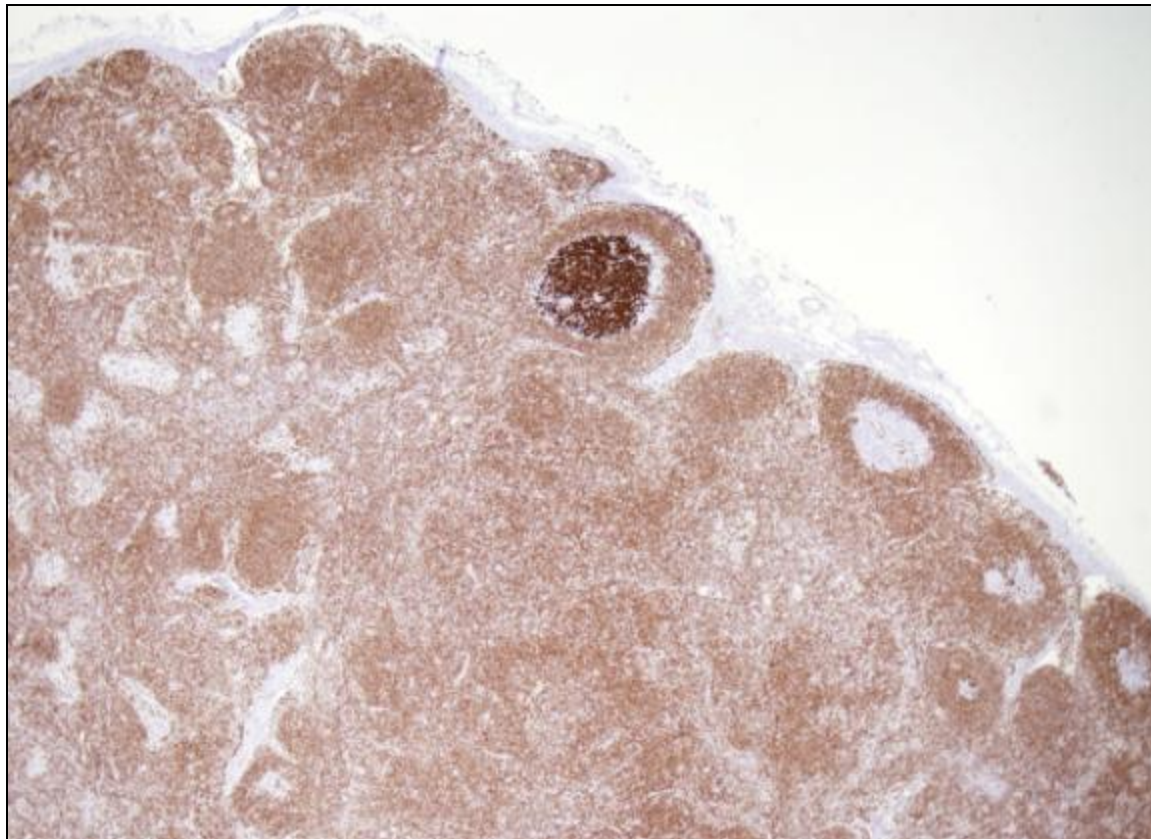


- FL-like B-cells home to the germinal center environment
- Lack of progression in most patients suggests *BCL2/IGH* is necessary but not sufficient for neoplastic transformation
 - *Second hit is required*
- FL-like PB B-cells & FL in situ are different manifestations of same molecular event

- **Terminology:** Proposal at EAHP/SH workshop 2010 to use
- the term: FL-like B-cells of uncertain significance
- Analogous to MGUS

Why do FLIS lymph nodes get biopsied?

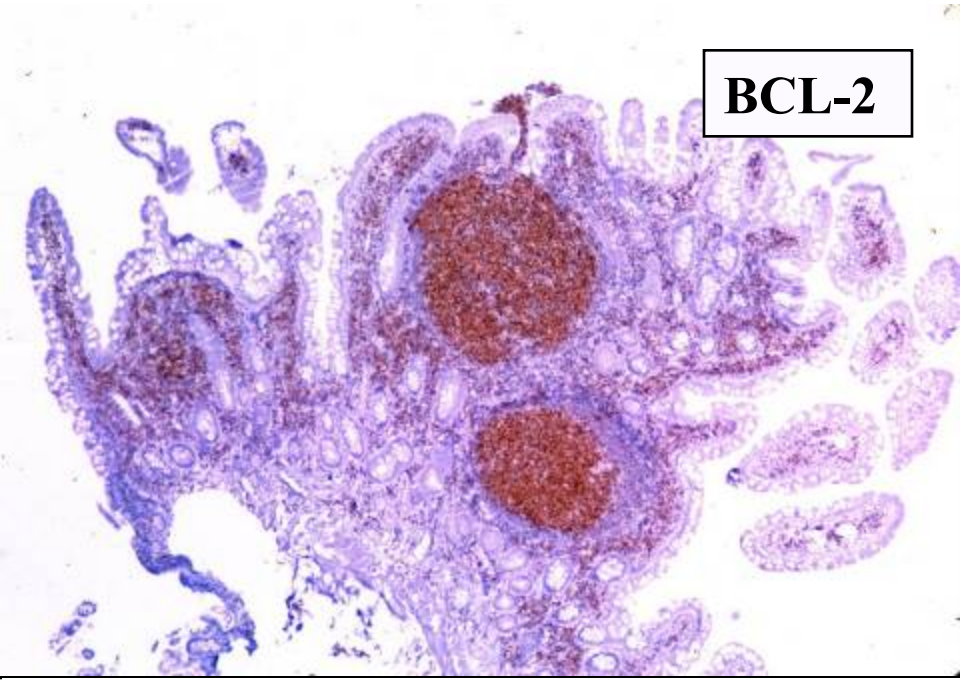
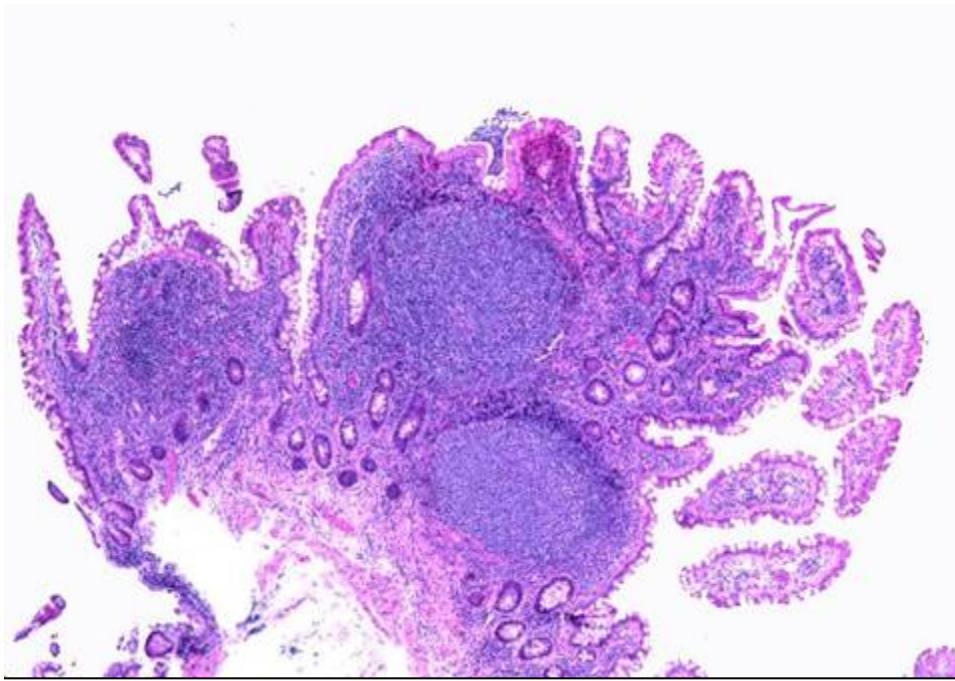
- Usually an incidental finding
- LN may show reactive hyperplasia or sometimes other lymphoma
- Unrelated immune stimulus may lead to increased trafficking of FL-like B-cells to germinal centers



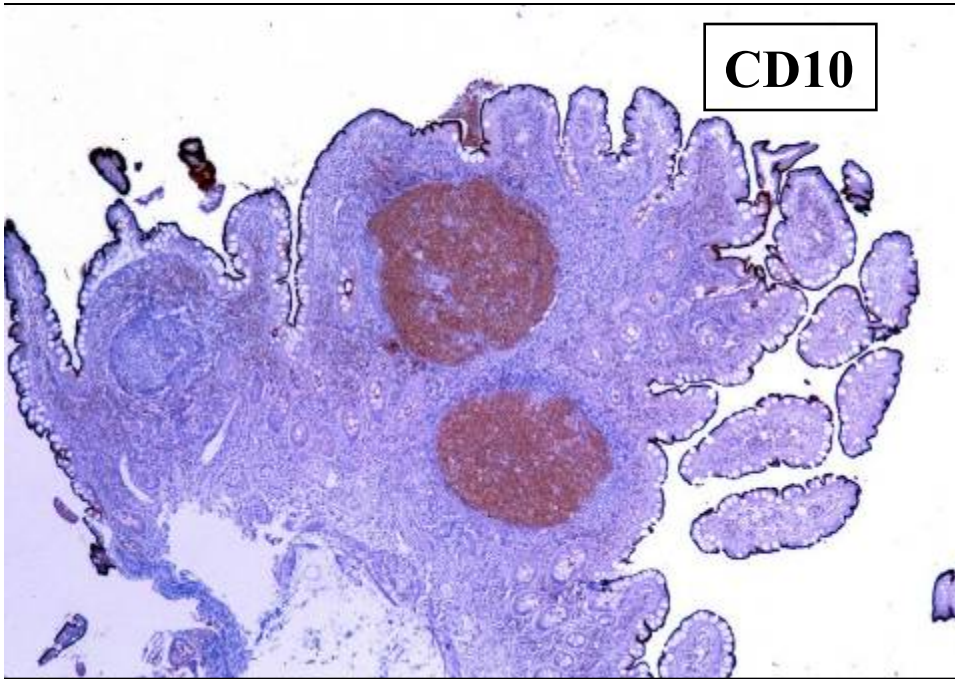
Primary FL of the Duodenum

(Schmatz JCO 2011)

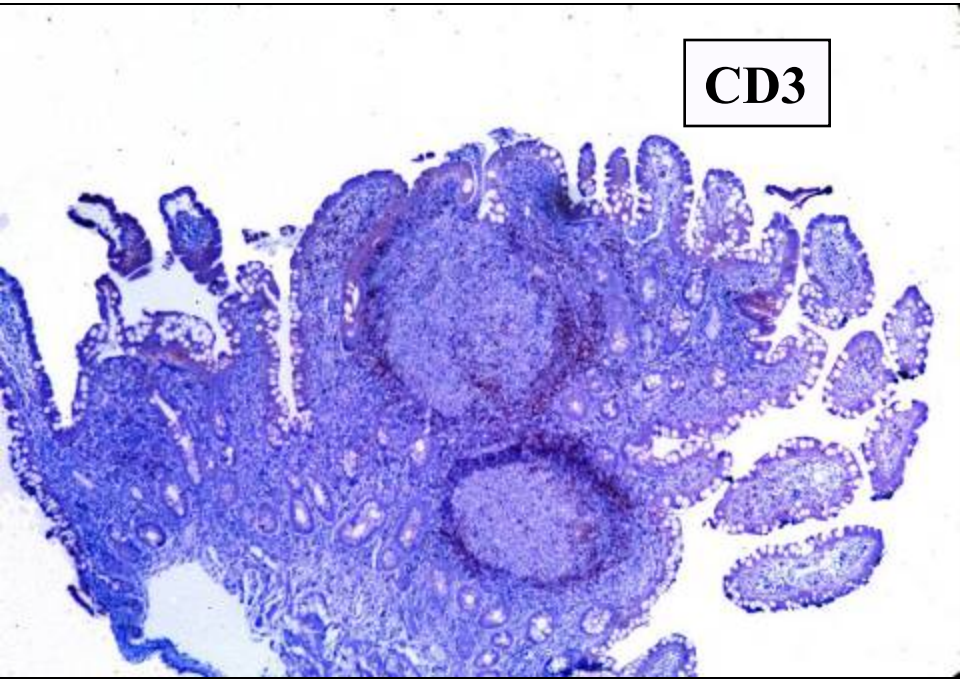
- Phenotypically and genetically similar to nodal FL (*BCL2/IGH*), but usually IgA+
- Commonly present in duodenum
 - other sites in distal small bowel
- Superficial polypoid lesions in mucosa
- Express homing receptor found on intestinal lymphocytes ($\alpha 4\beta 7$ integrin)
- Local recurrences without dissemination
 - *another type of in situ FL*



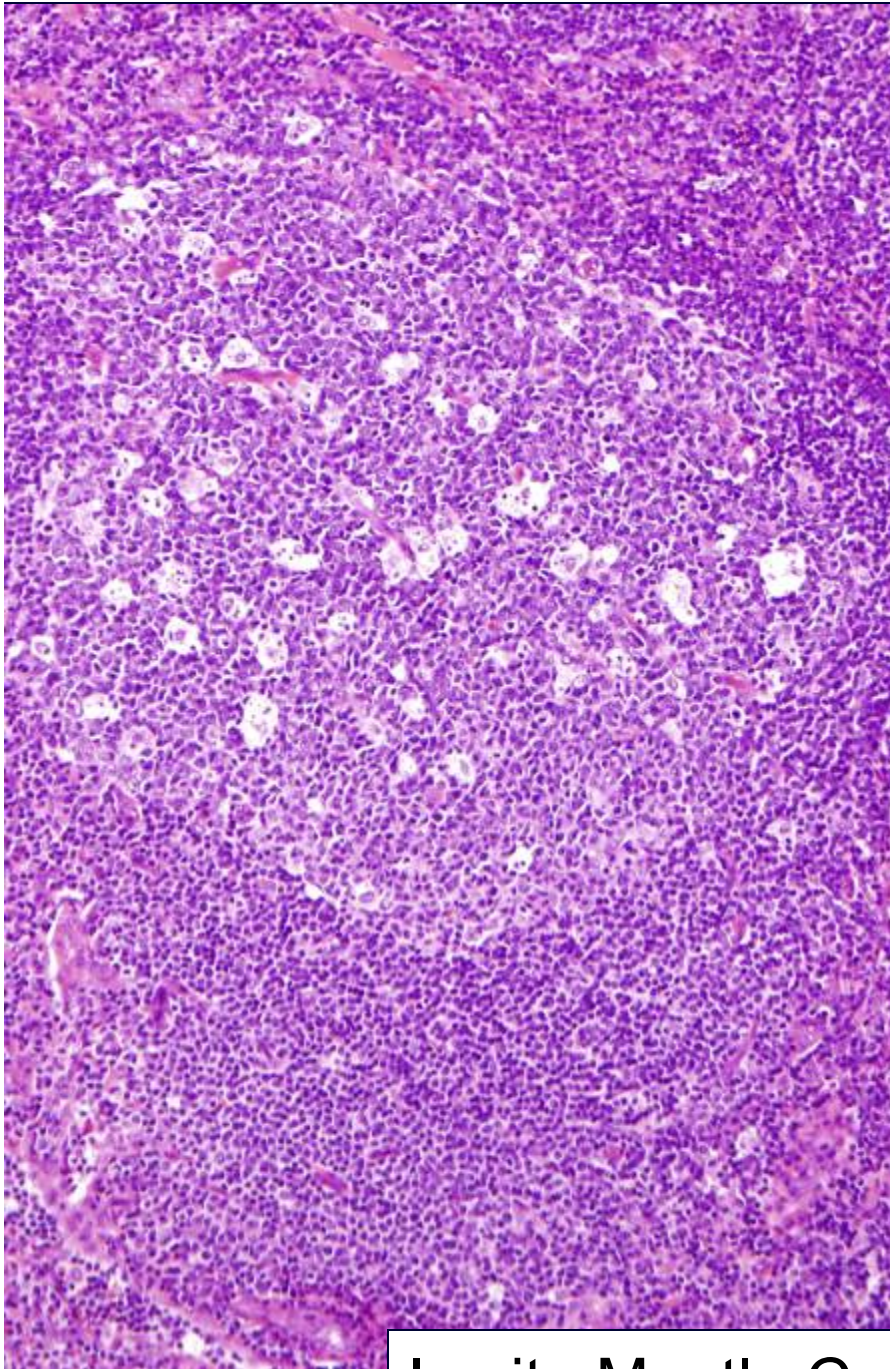
BCL-2



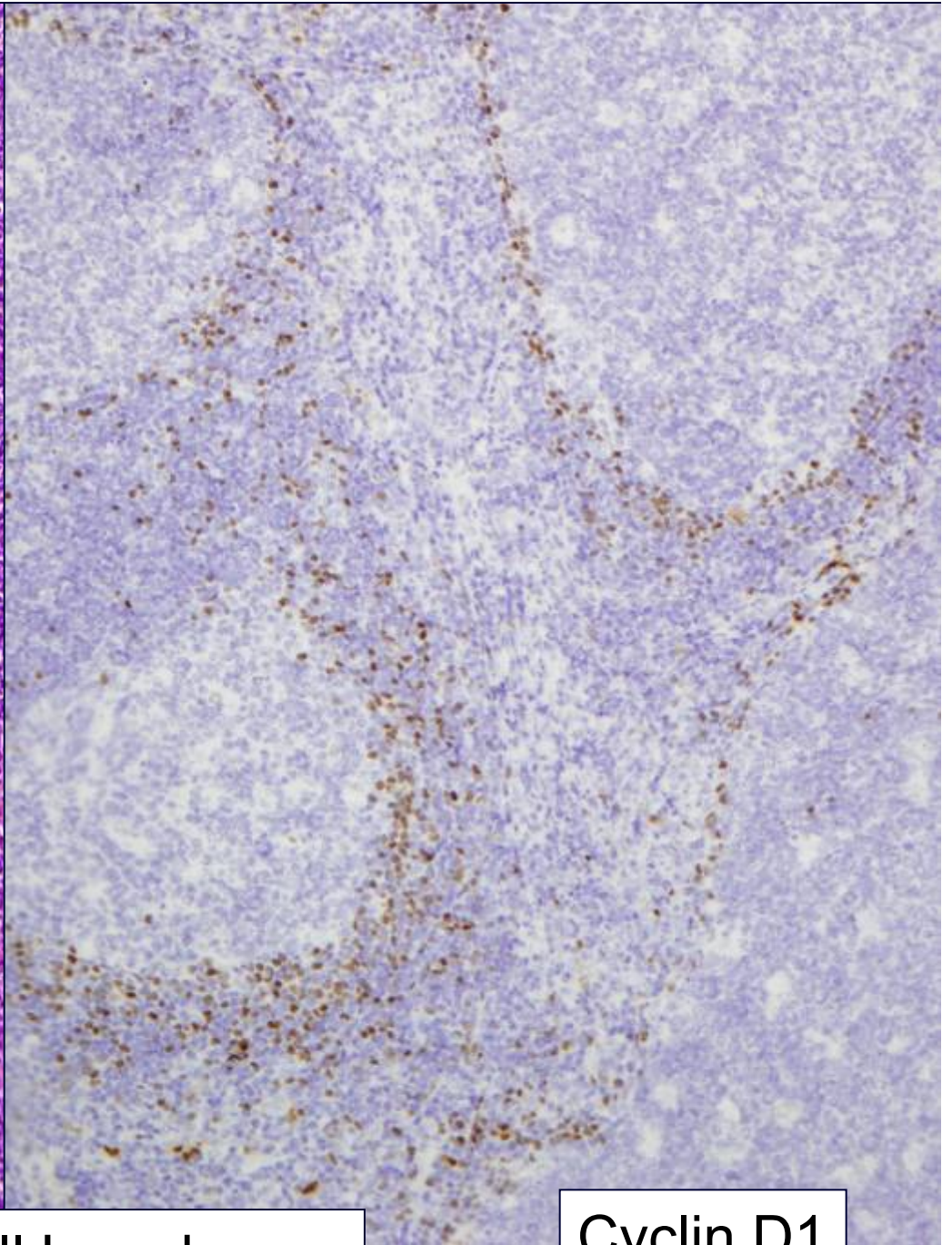
CD10



CD3



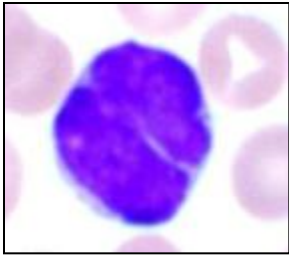
In situ Mantle Cell Lymphoma



Cyclin D1

“Malignant Lymphoma”

Why are there no benign lymphomas?



- Lymphocytes are cells that traffic or spread as part of their normal function
- Benign clonal expansions of lymphocytes do not remain localized, but disseminate based on normal lymphocyte homing
- Benign clonal expansions of plasma cells (sessile cells) do exist, e.g. extraosseous plasmacytoma

What is the minimal definition of malignant lymphoma in 2011?

- Clonality is not sufficient
 - There are many “benign” clonal proliferations
 - e.g. autoimmune disease, HCV, H. pylori gastritis
- Cytogenetic abnormalities are not sufficient
 - MBL has molecular alterations of CLL
 - MGUS has molecular alterations of myeloma
 - “FL in situ” has the minimal molecular alteration of FL (*IGH/BCL2*)

Age as a Significant Features in Lymphoid Malignancies

- Newly recognized mainly “pediatric” lymphomas
 - Pediatric follicular lymphoma
 - Pediatric nodal marginal zone lymphoma
 - EBV+ T-cell and NK-cell LPD’s of childhood (CAEBV)
 - Systemic EBV+ T-cell LPD of childhood
 - Hydroa vaccineforme-like lymphoma

Pediatric Follicular Lymphomas

Rare lymphoma subtype in children (1-2%)

- Tonsils, nasopharynx, GI tract, testis
- Less often Nodal, Usually Grade 3

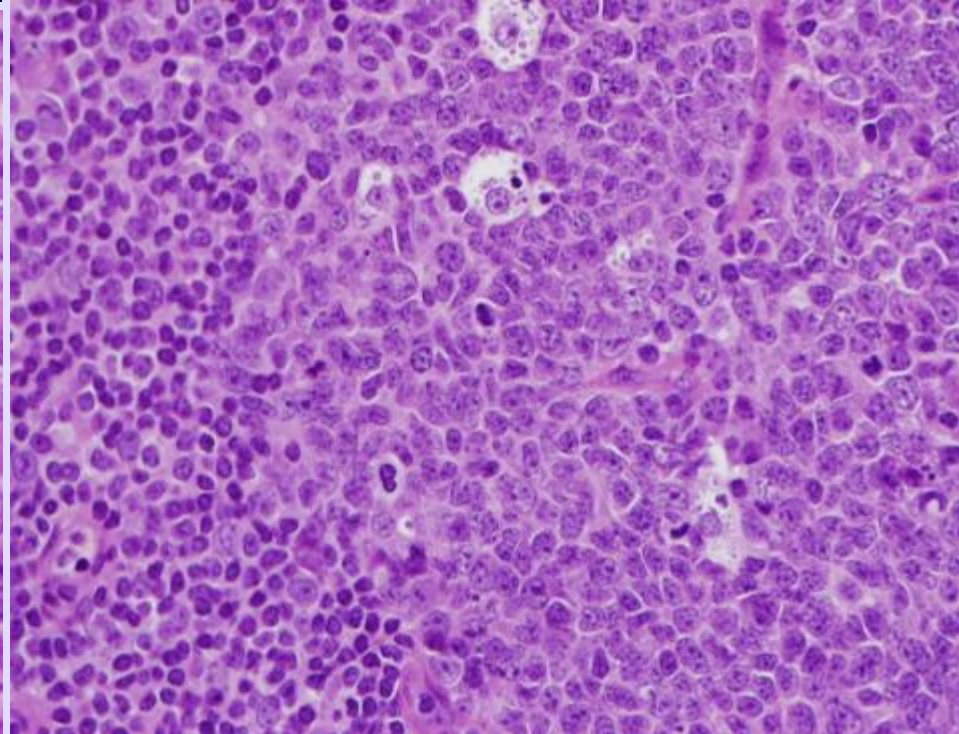
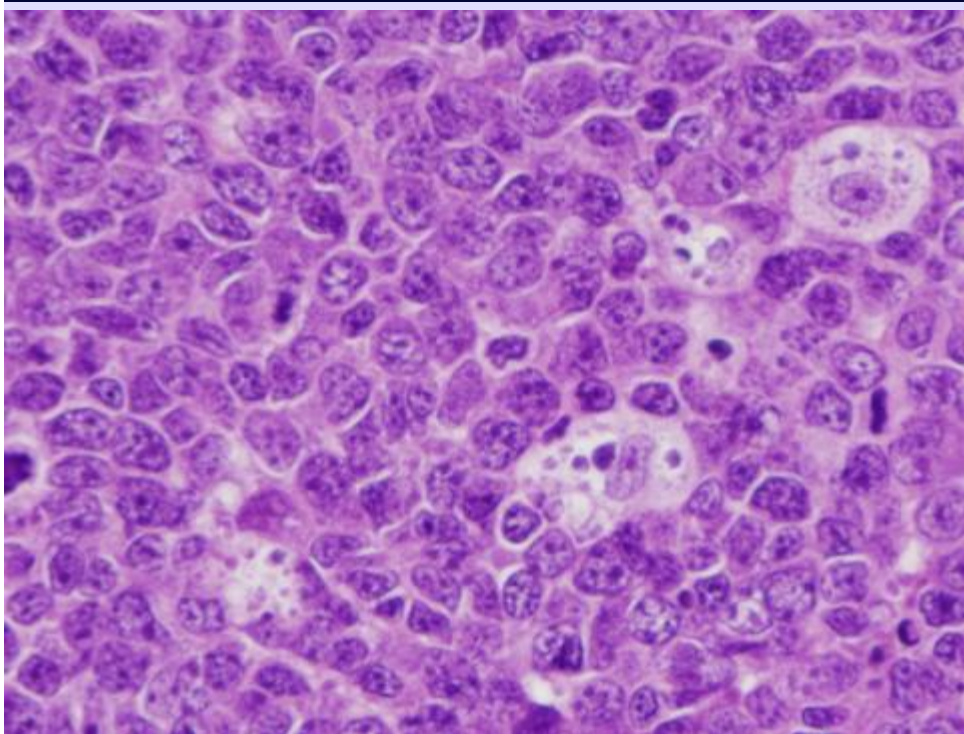
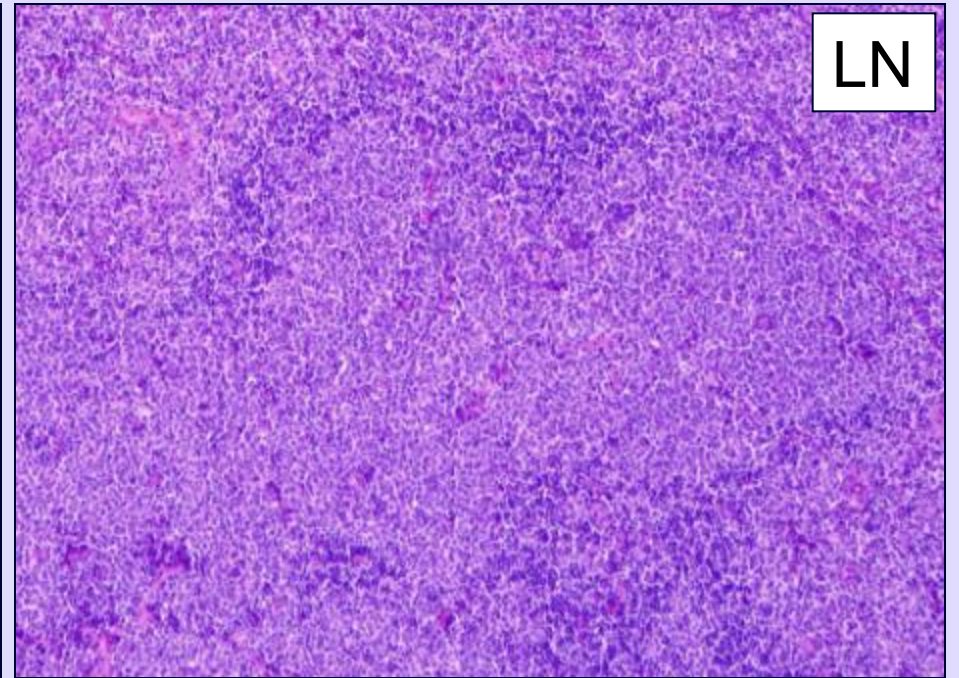
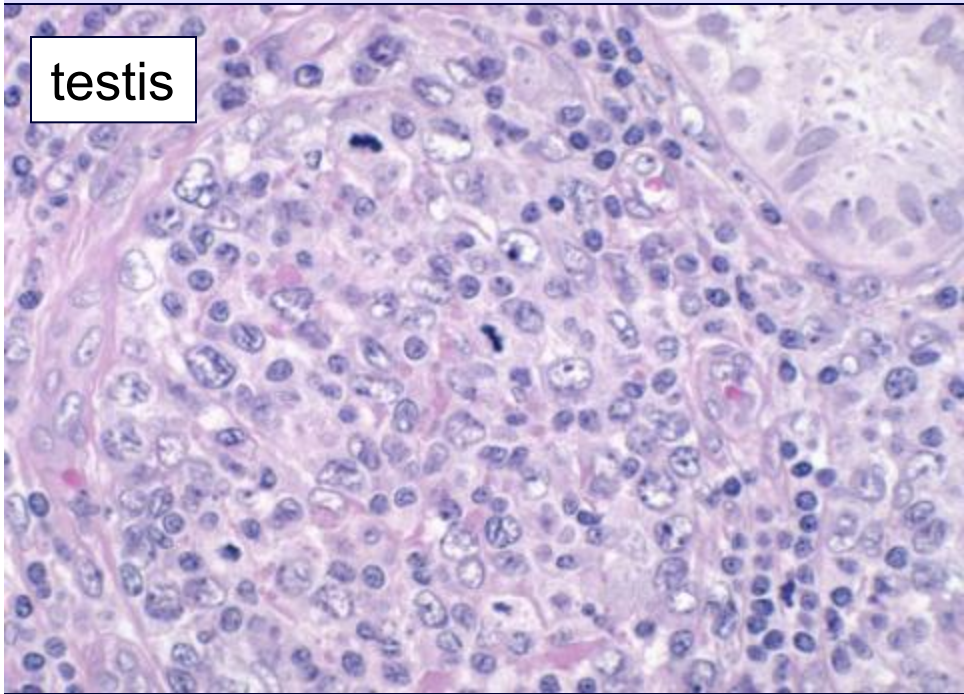
Male >> Female

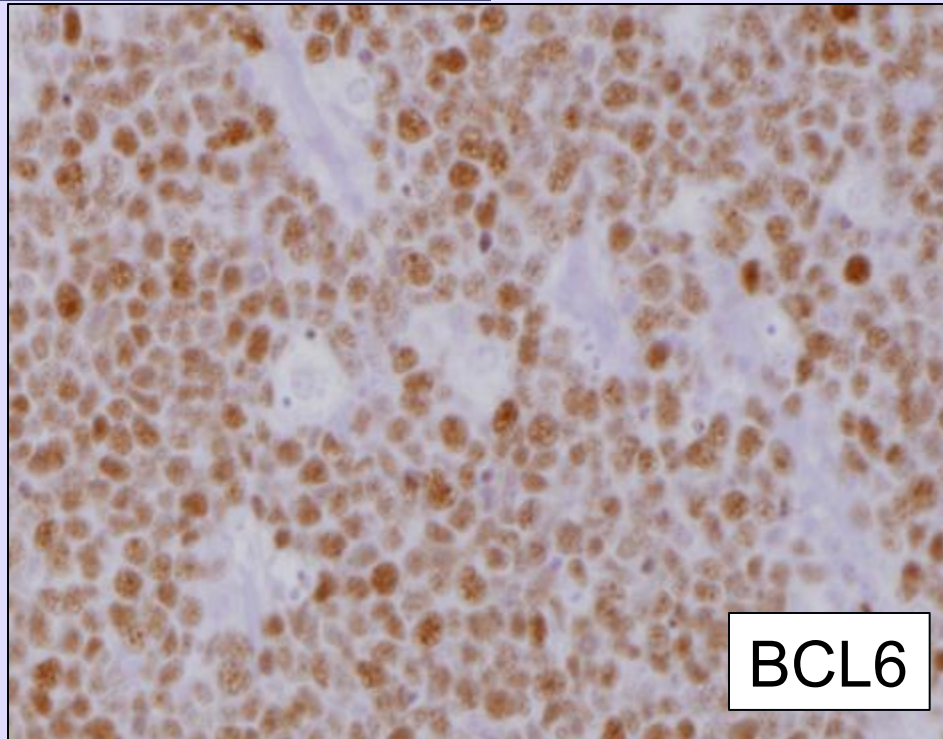
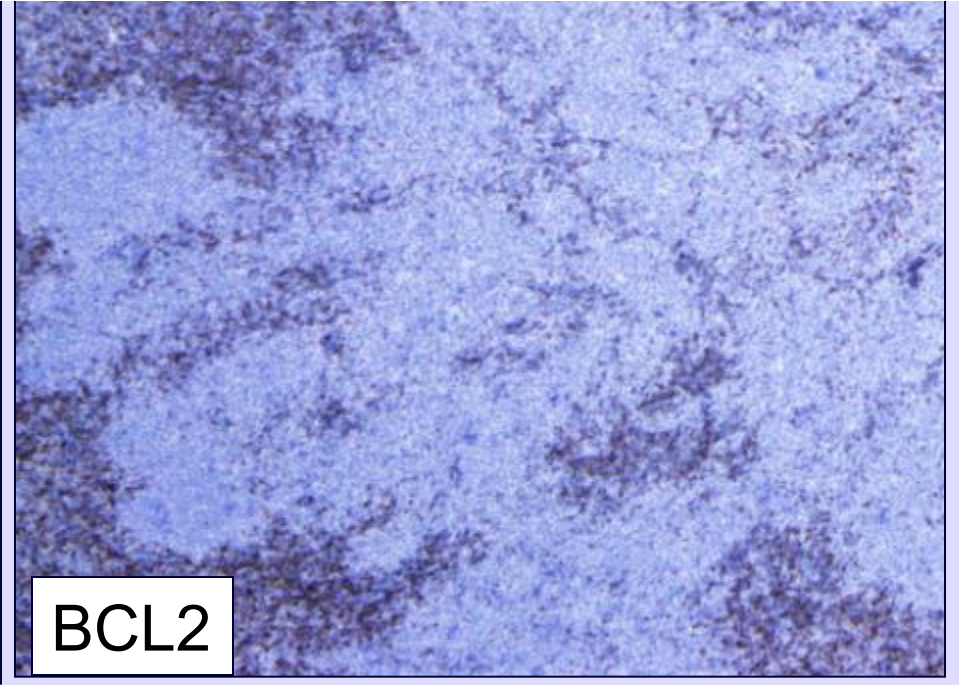
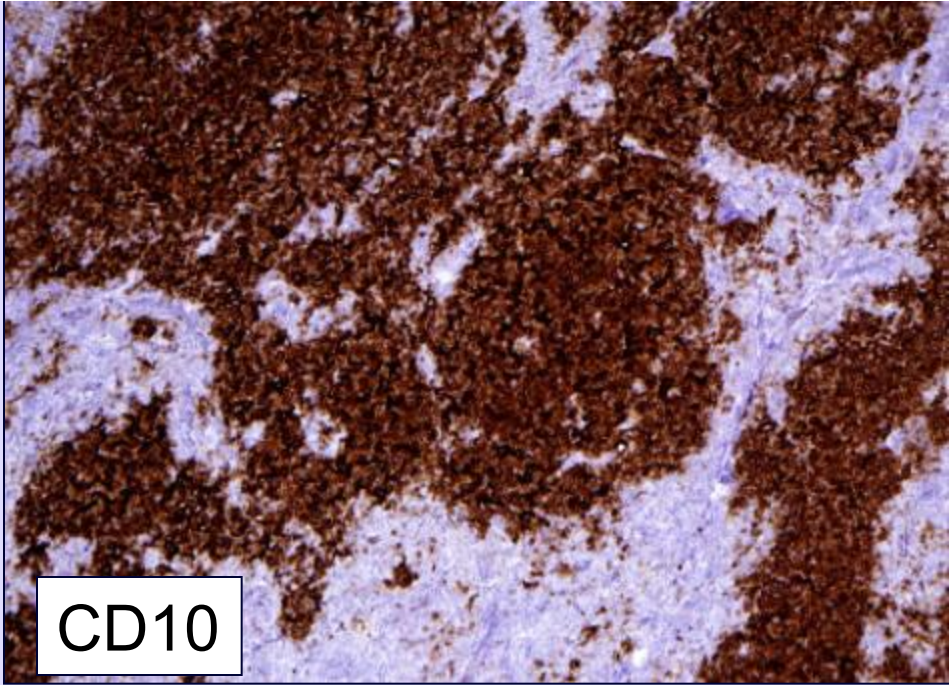
85% localized, Stage I or II

75% complete remissions with low relapse rate

Bcl-2 usually negative- both protein & BCL2/JH

- A different disease at the molecular and clinical levels

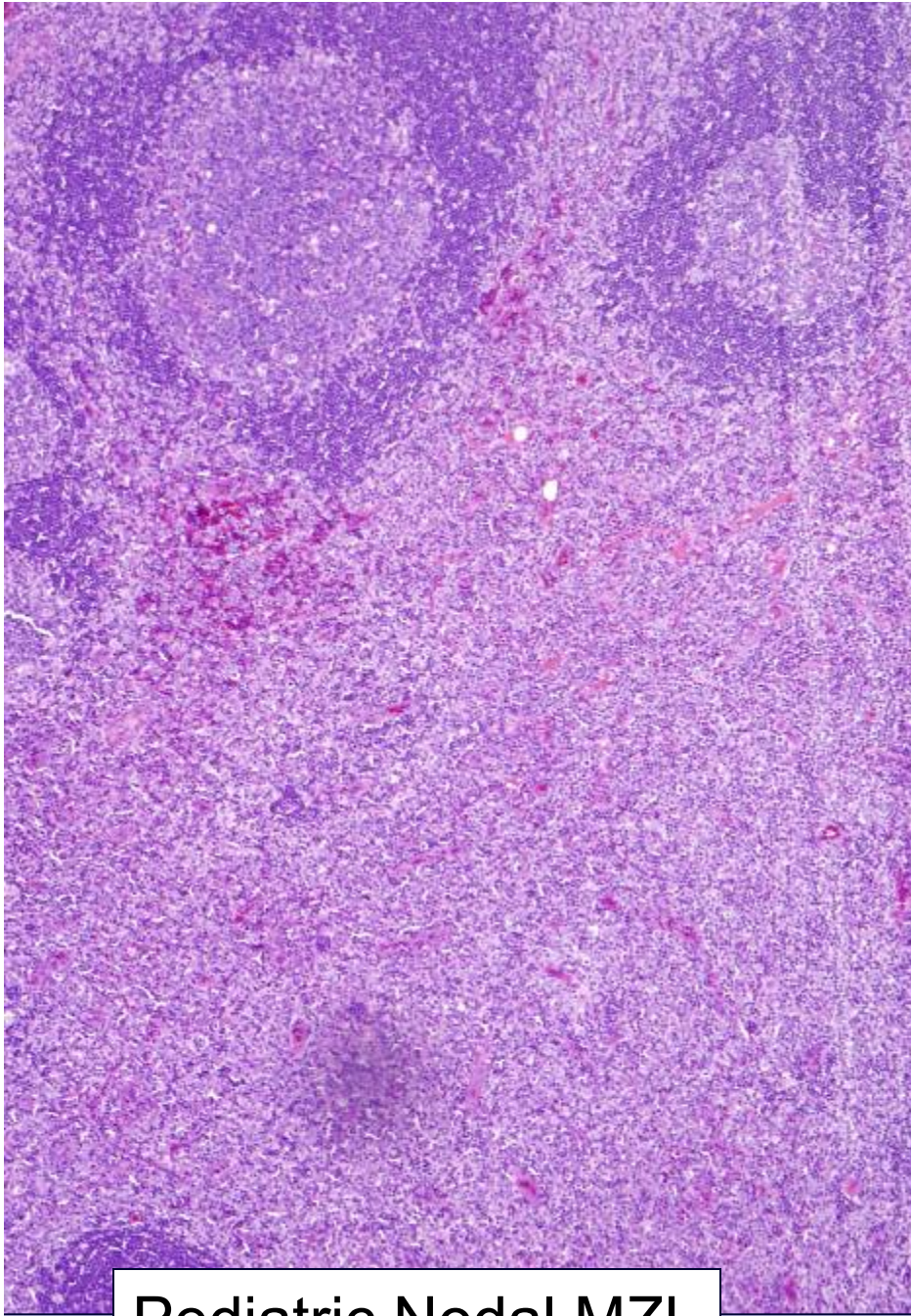




NMZL in pediatric age group

Distinctive Features (Taddesse-Heath, 2003)

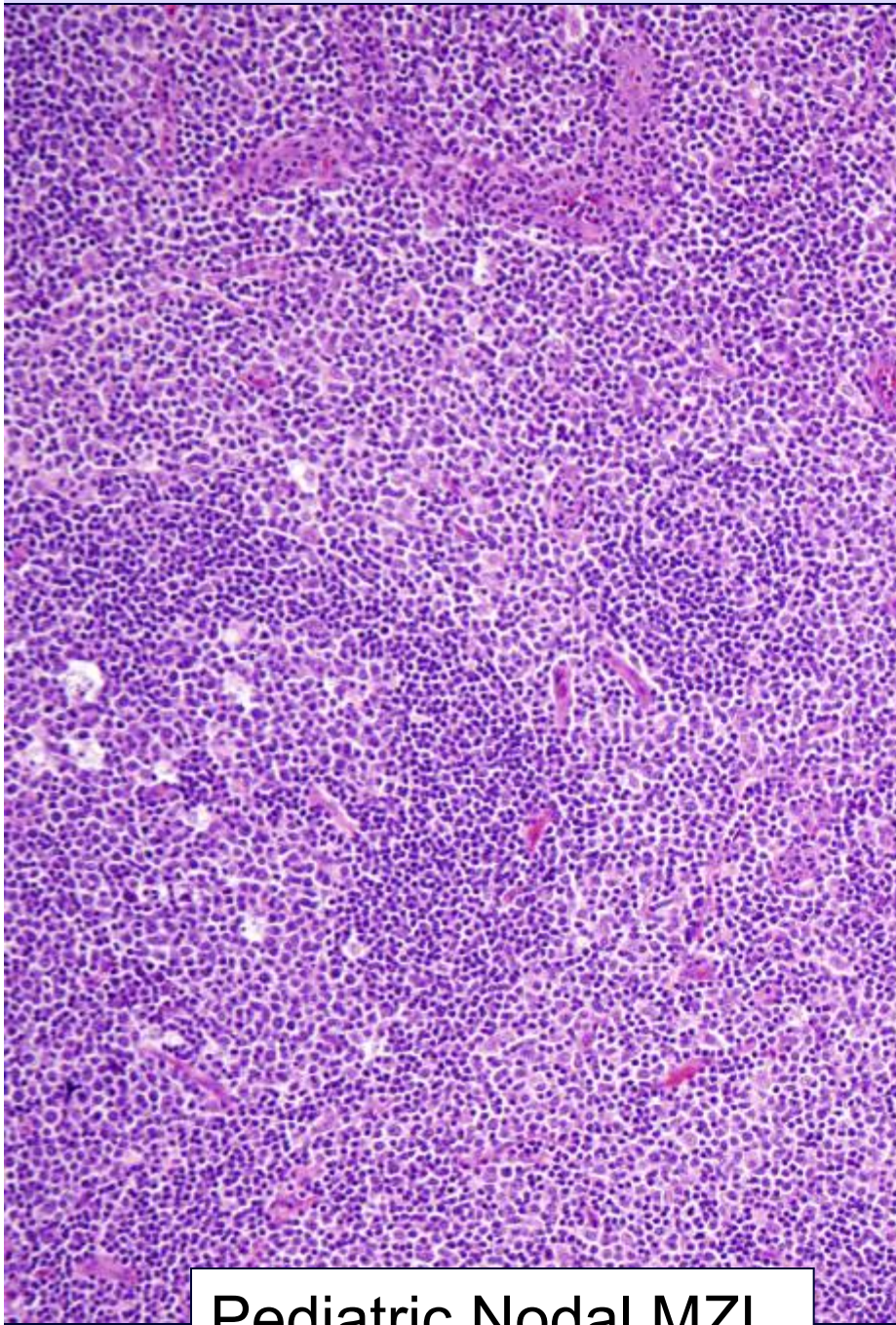
- Primary nodal more common than extranodal
- N-MZL in patients ≤ 18 yrs almost exclusively seen in males (20/21)
- Cervical LN most common site
- Morphologic association with PTGC in majority of cases
- Usually localized, Stage I, with uncommon recurrences
- Conservative management



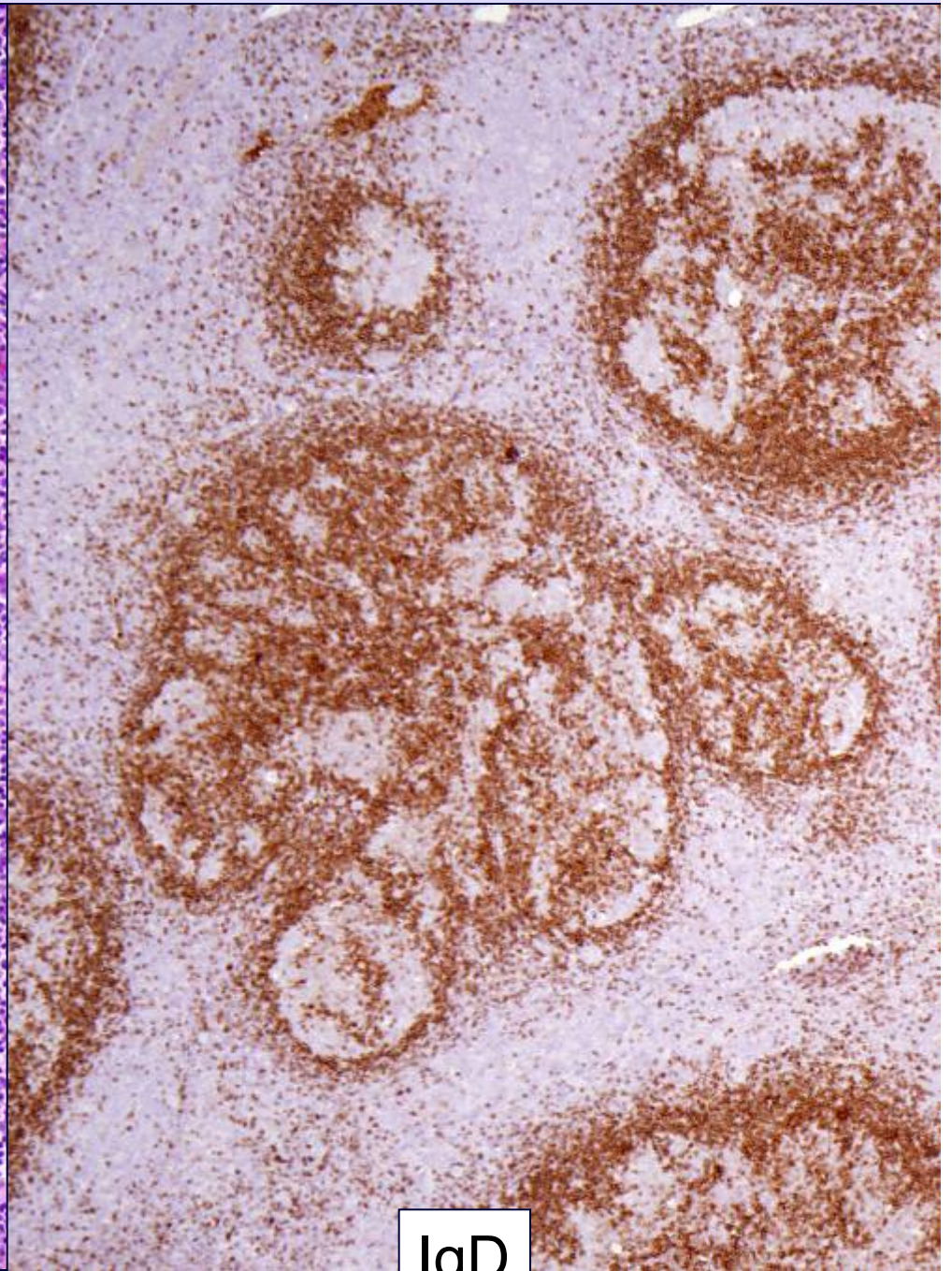
Pediatric Nodal MZL



CD20



Pediatric Nodal MZL



IgD

Systemic EBV+ T-cell LPD of Childhood

Asian or Hispanic children

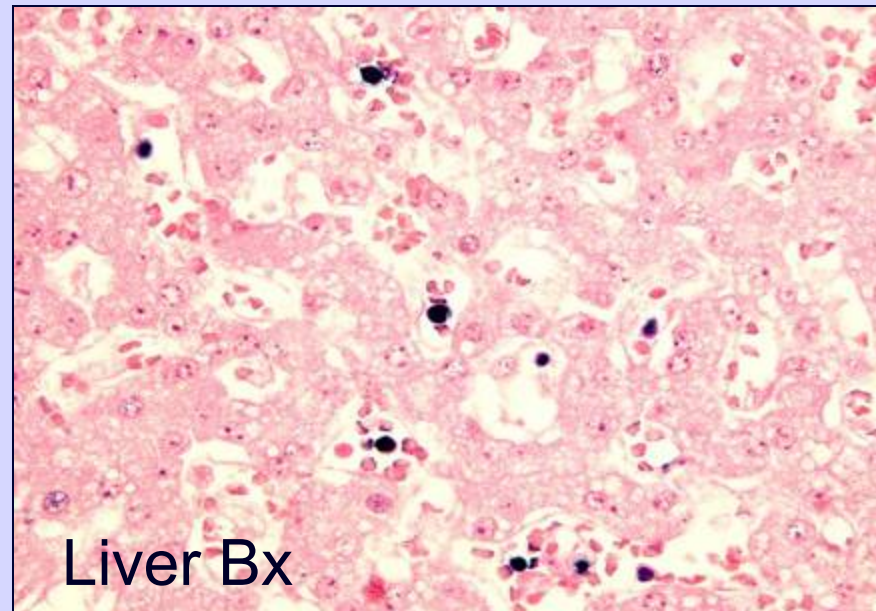
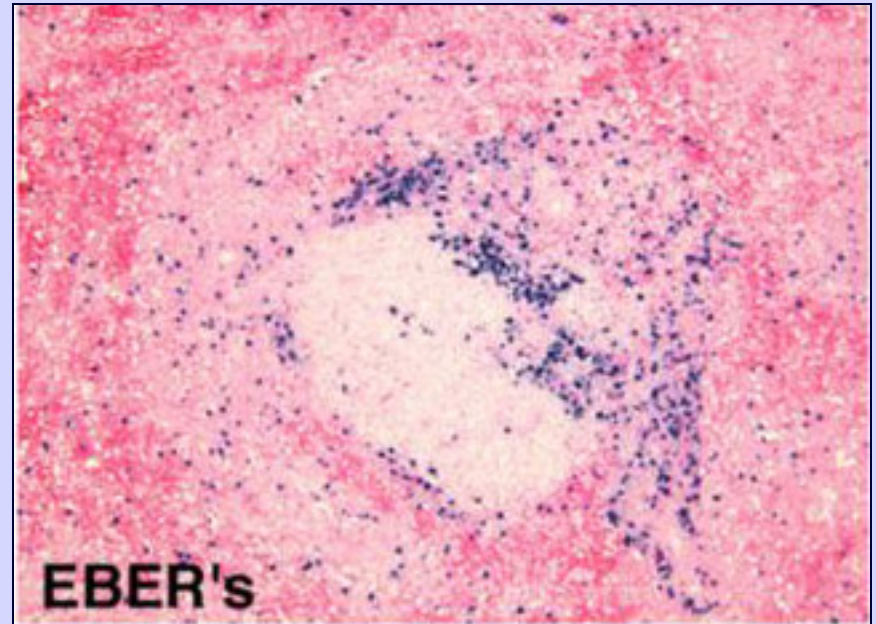
Acute systemic illness with hemophagocytic syndrome

Follows acute EBV infection
high viral loads

EBV+ T-cells are clonal

May follow chronic active EBV infection (CAEBV)

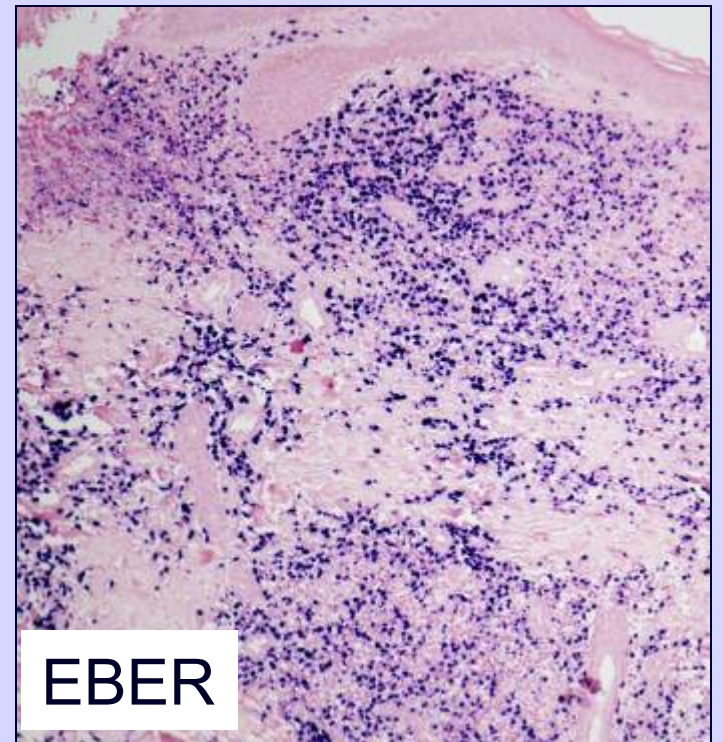
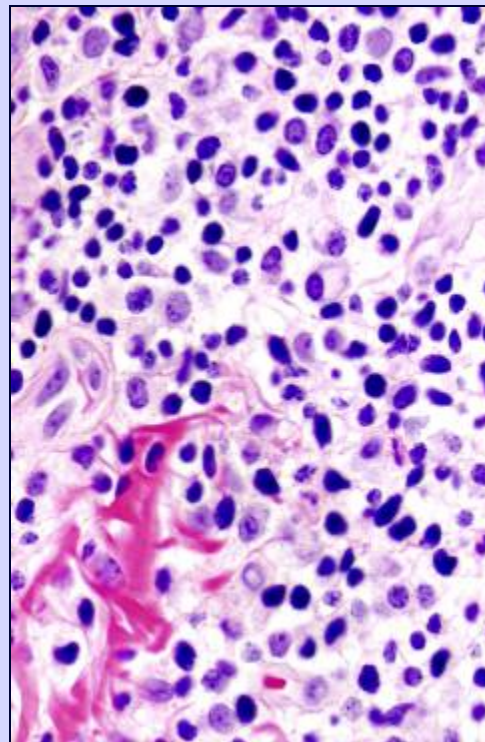
Overlaps with what has been termed *severe CAEBV*





- Hydroa-vacciniforme-like lymphoma
- Asian or Hispanic children
- Lesions in sun exposed areas
- Chronic course but may progress to acute phase with systemic disease

Cells of T-cells or
less often NK cell
origin



EBV-positive Diffuse Large B-cell Lymphoma of the Elderly (WHO 2008)

- Formerly senile EBV + LPD (Nakamura, et al.)

Median age, 71 ; M>F 1.5:1

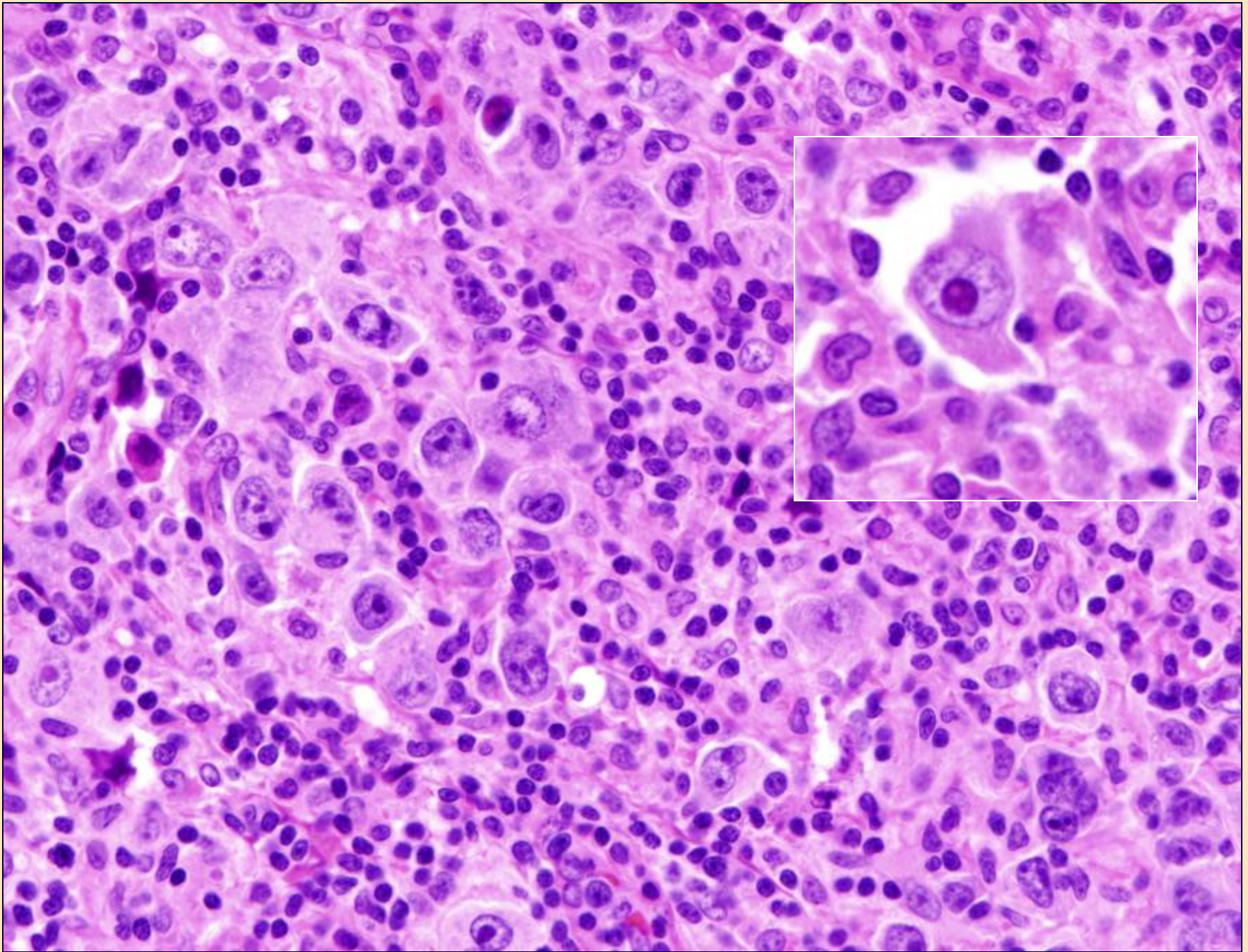
70% extranodal: skin, lung, stomach

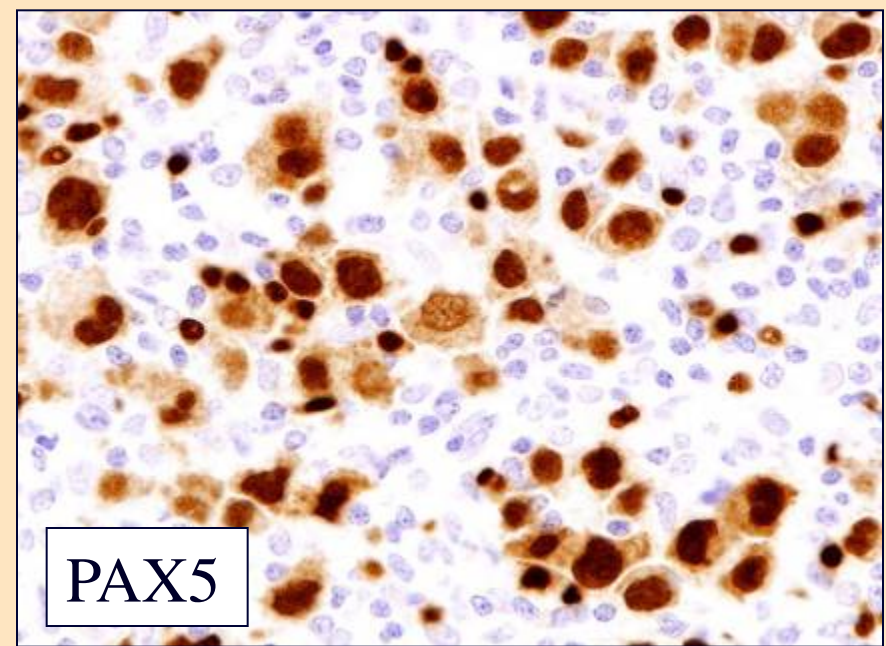
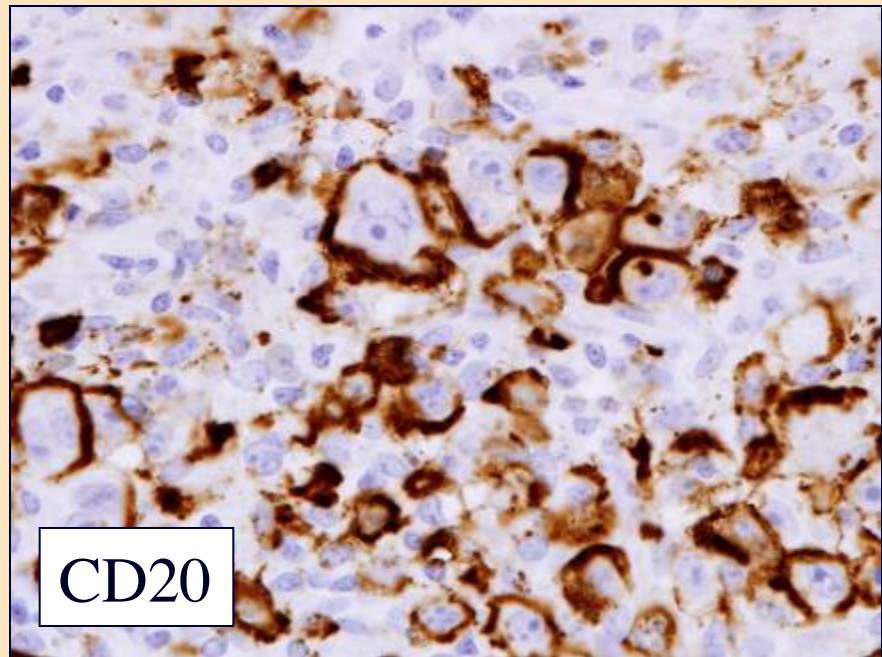
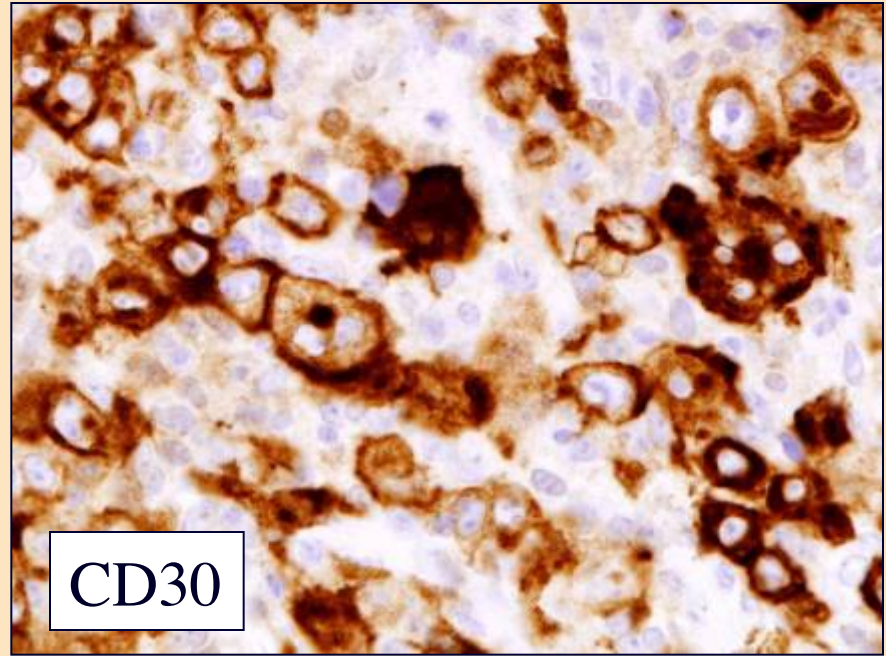
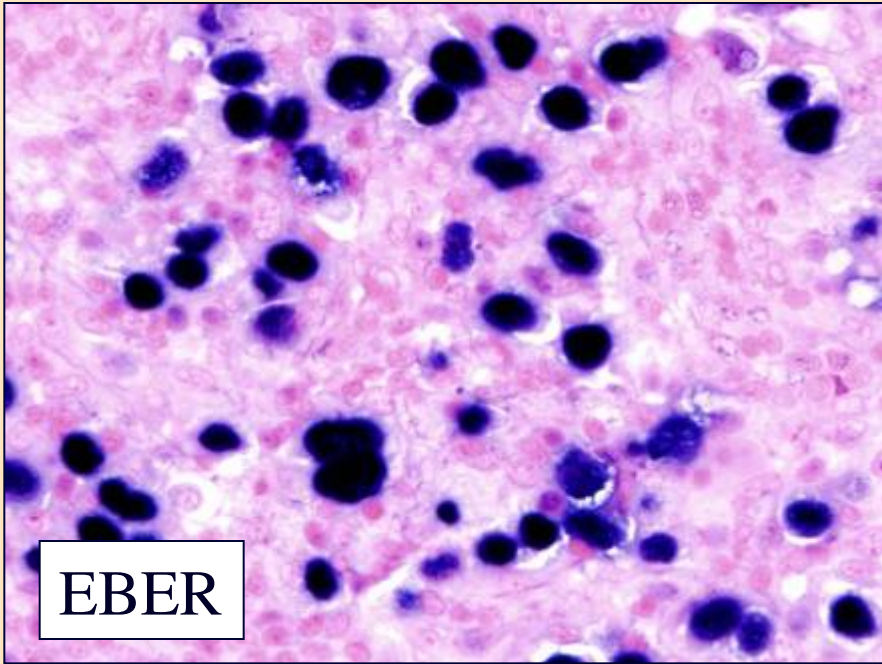
30% nodal disease alone

EBV + large B-cells in an inflammatory background

Frequent necrosis and H/RS-like cells

CD30+, MUM-1+, CD20+, negative for CD15



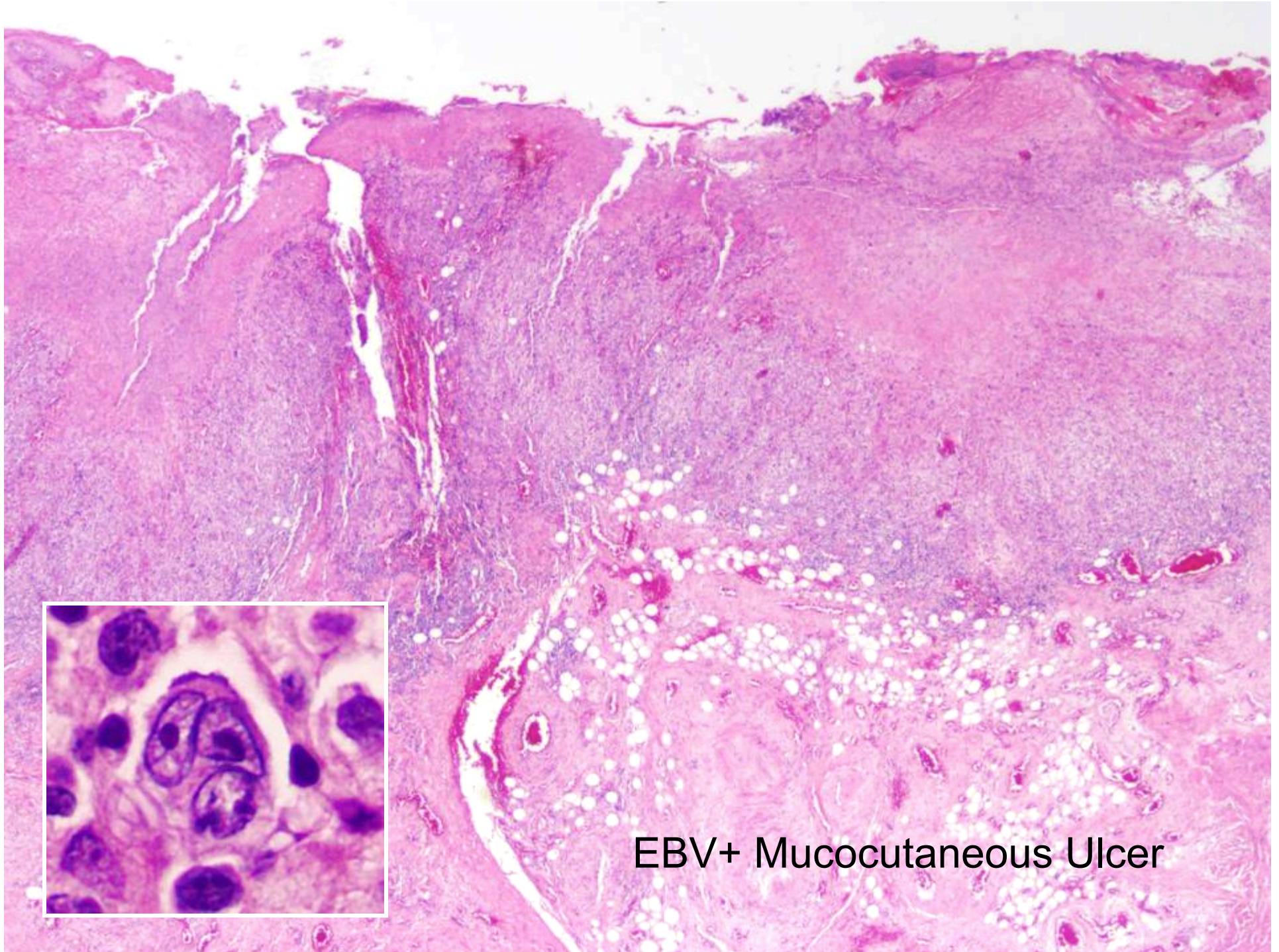


Mucocutaneous Ulcer – Clinical Spectrum

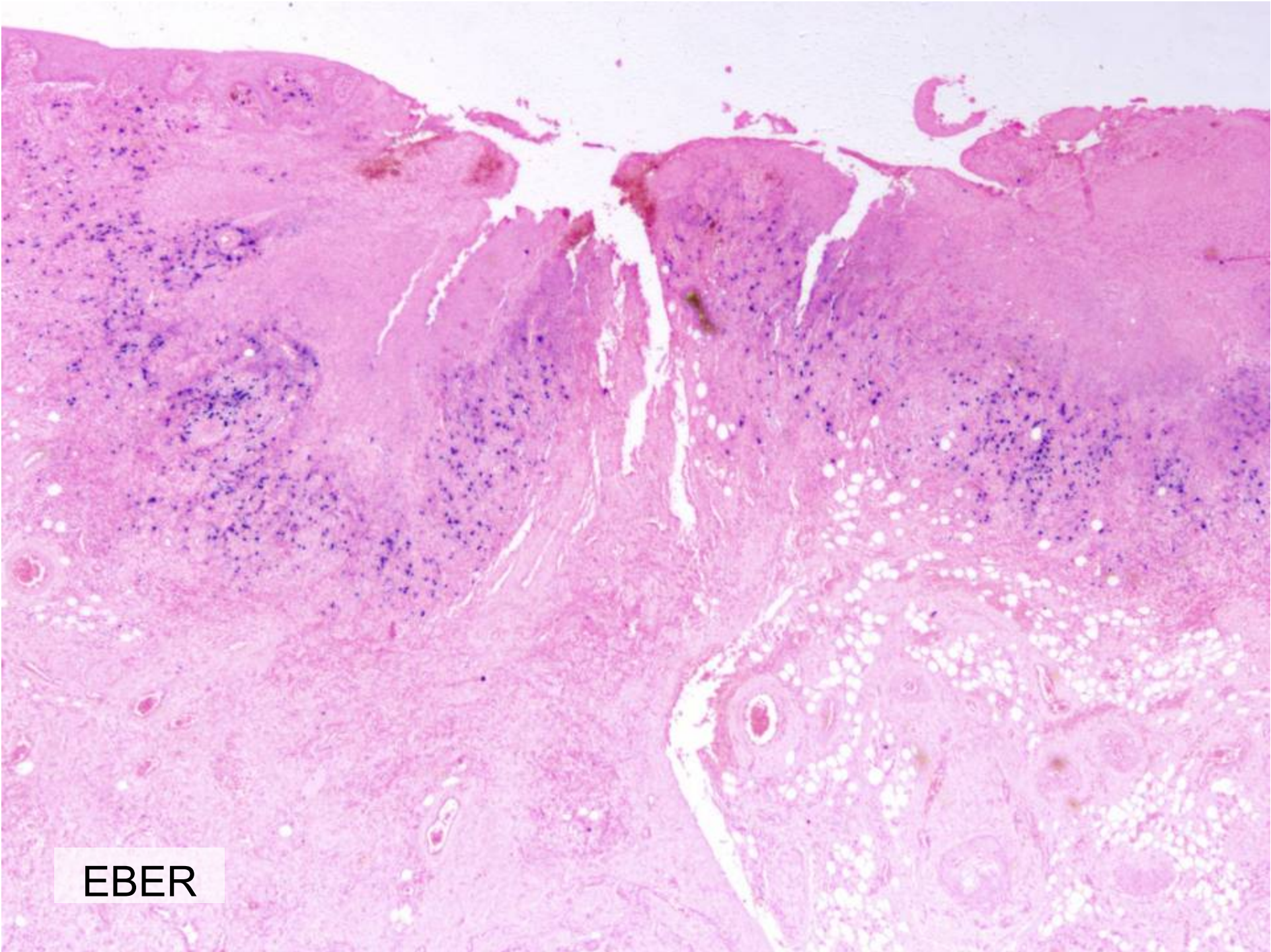
Dojcinov et al. AJSP 2010

24 patients 9 M 15 Fe; Median Age 77; R 42-101

- Age related only (16)
 - Median age 79; range 64-101
- MTX, AZA, Cyclosporin A (8)
 - Median age 72; range 42-80
 - Rheumatoid arthritis (5)
 - Ulcerative colitis (1); Myasthenia Gravis (1); SLE (1)
- Oropharyngeal mucosa (15)
- Skin (6)
- GI tract (3)

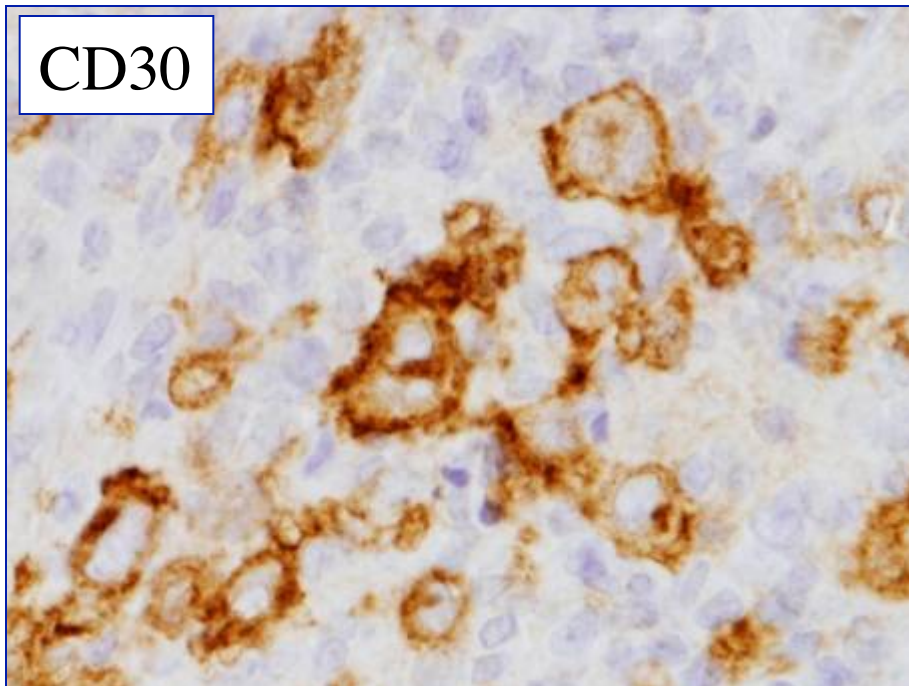


EBV+ Mucocutaneous Ulcer

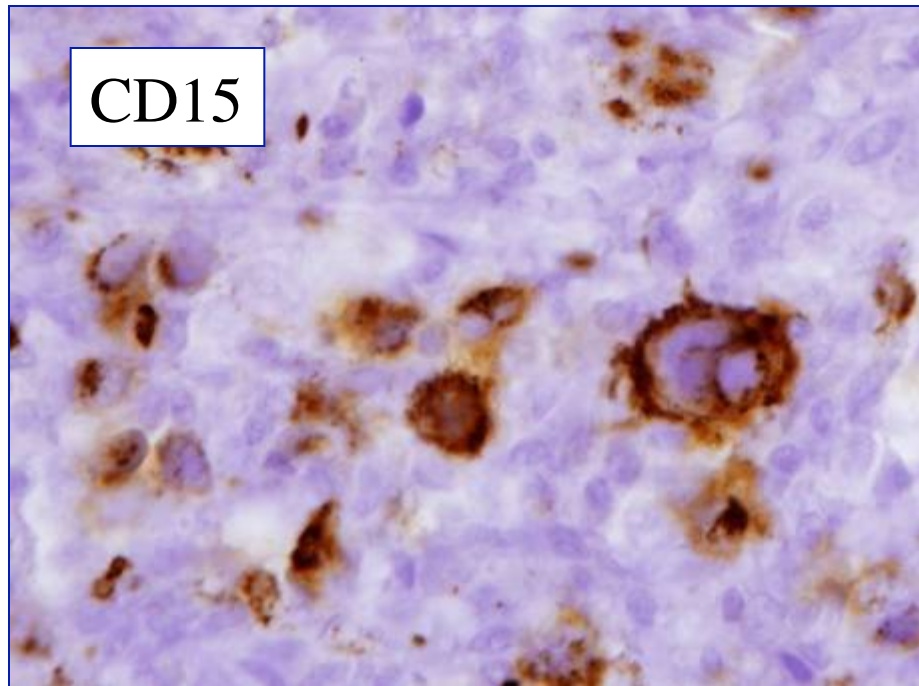


EBER

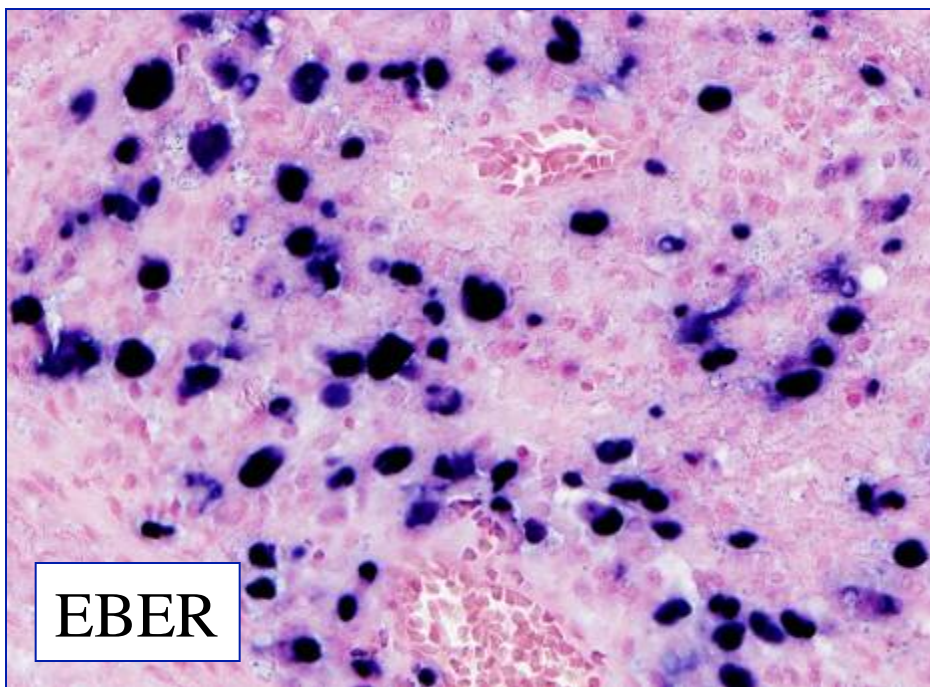
CD30



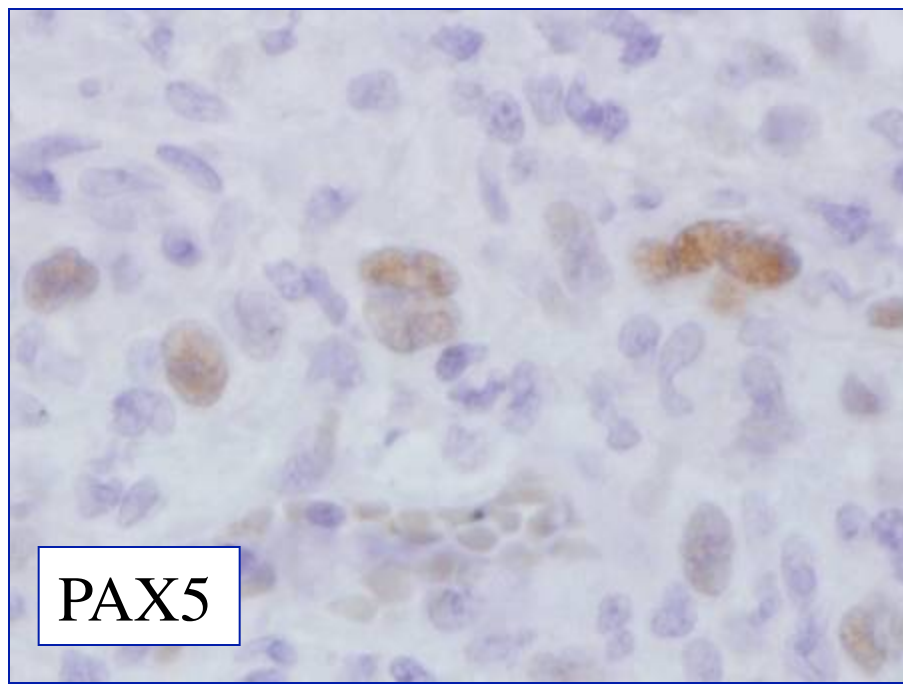
CD15



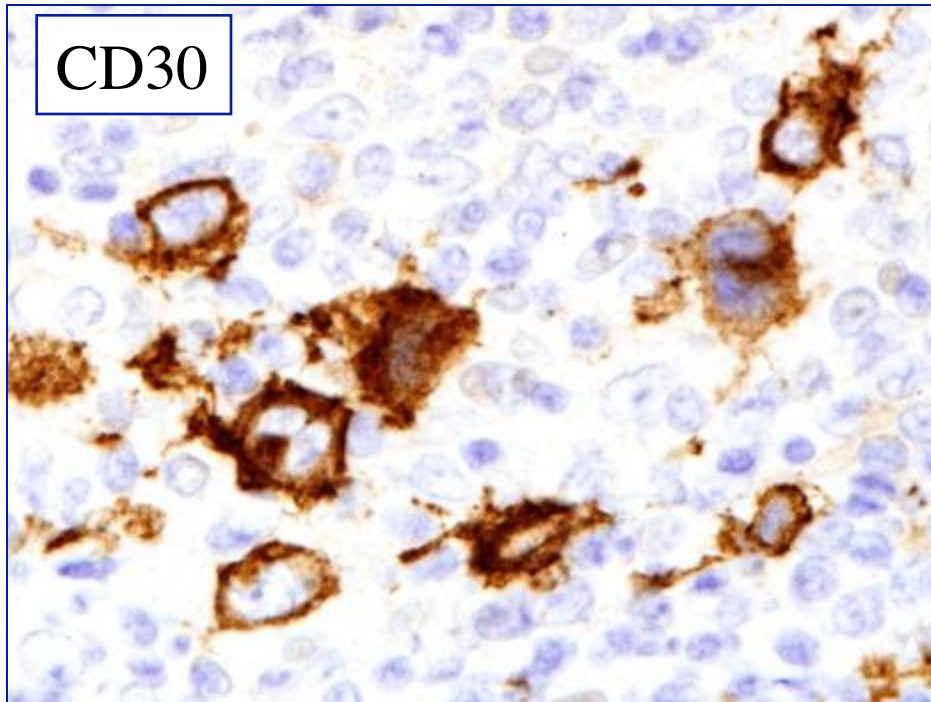
EBER



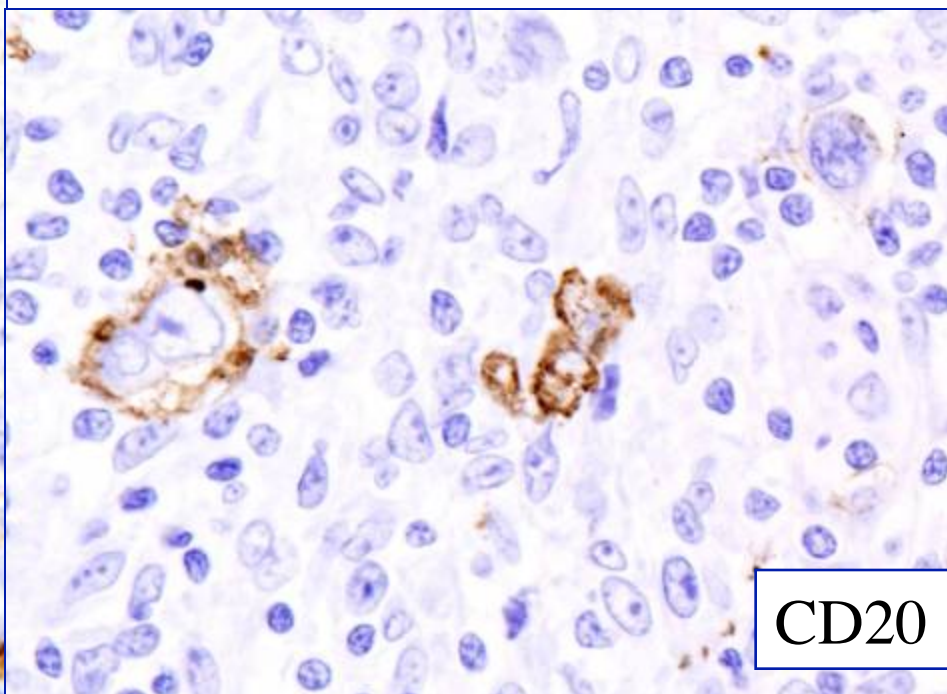
PAX5



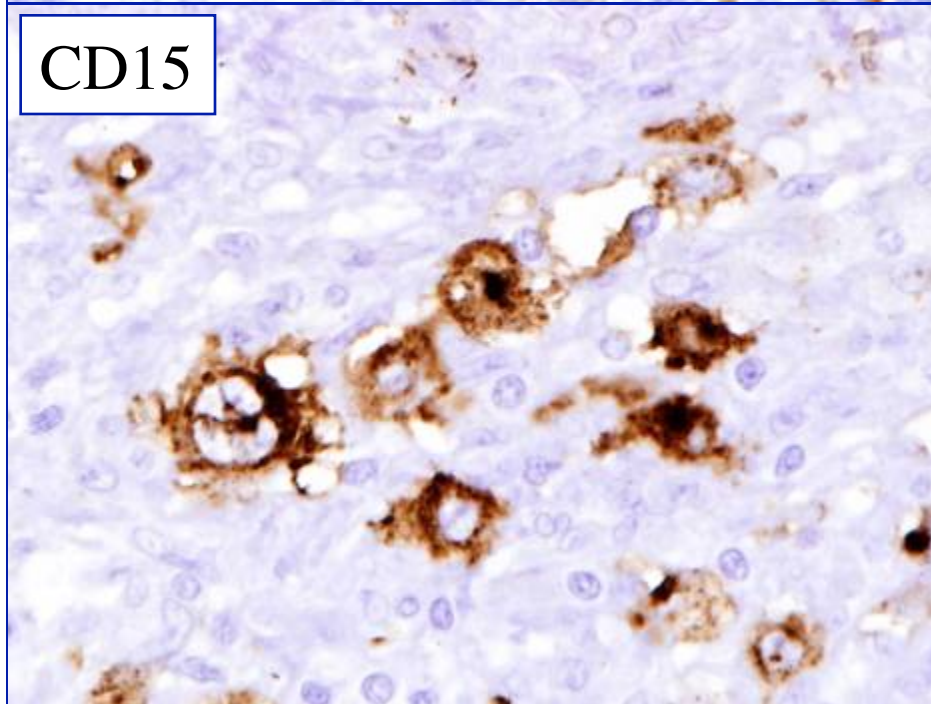
CD30



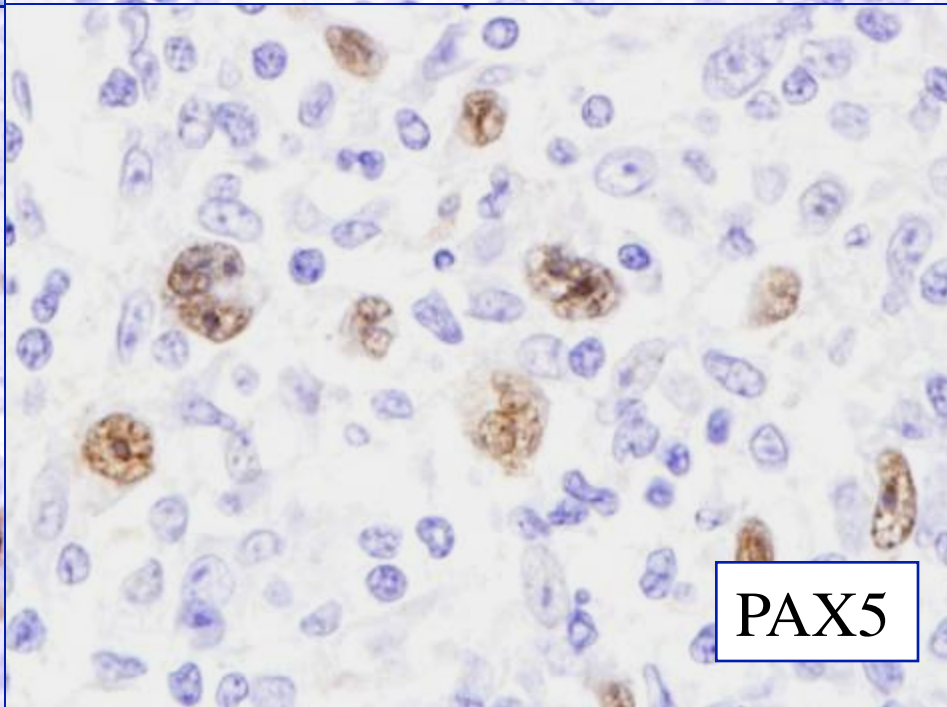
CD20



CD15



PAX5

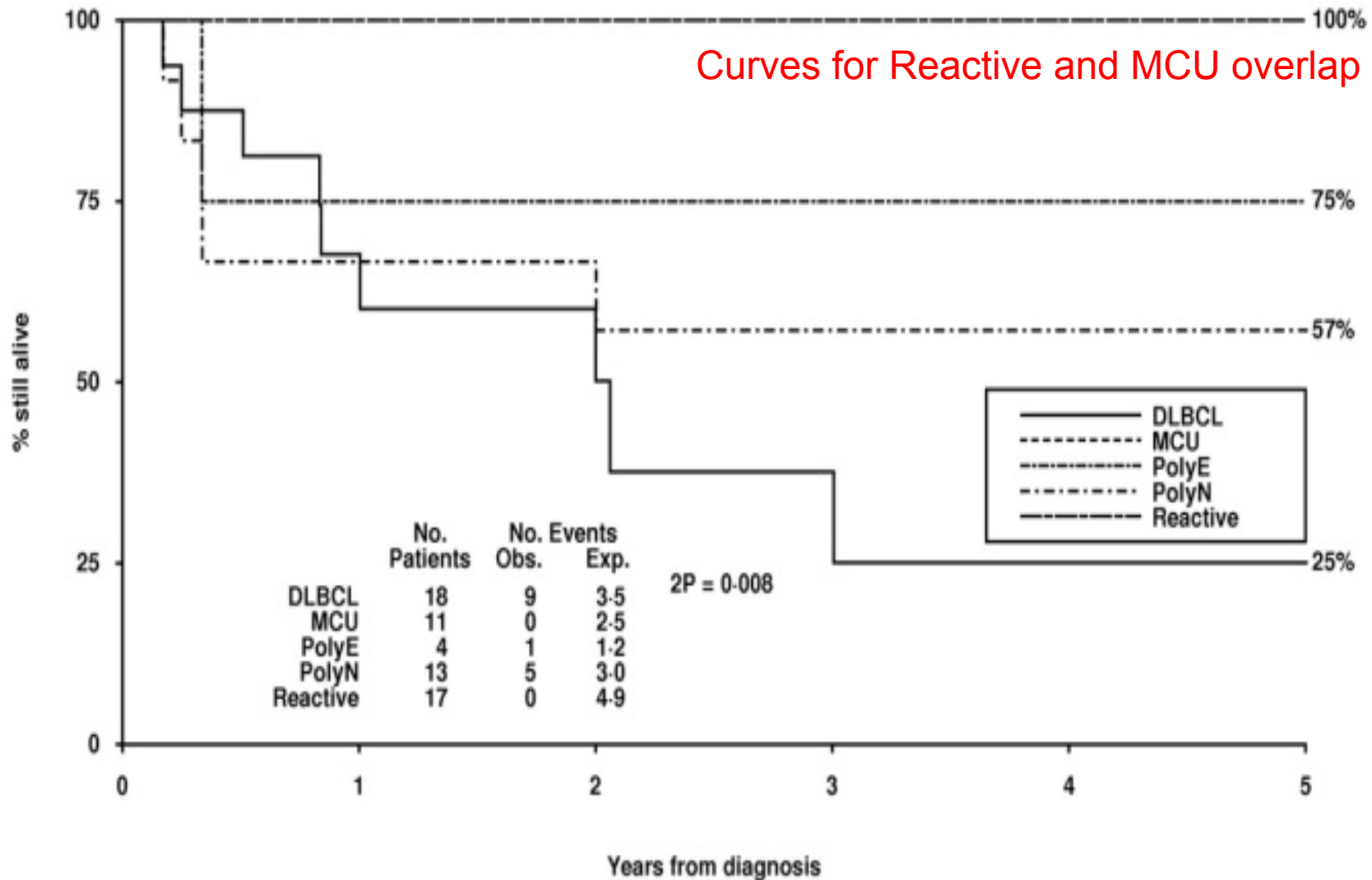


Spontaneous resolution of MCU in a patient with RA on MTX over the course of 8 weeks following withdrawal of drug



Age related EBV LPD – Dojcinov et al Blood 2011

Disease-Related Mortality by Pathology Subgroup



Site-specific or environmental impact on disease definitions

- Primary mediastinal large B-cell lymphoma
- Primary DLBCL of the CNS
- DLBCL associated with chronic inflammation
 - Pyothorax or other “confined” spaces
- Primary cutaneous follicle center lymphoma
 - May be composed of large centrocytes/centroblasts
- Primary cutaneous DLBCL, leg type

Primary Cutaneous FCL

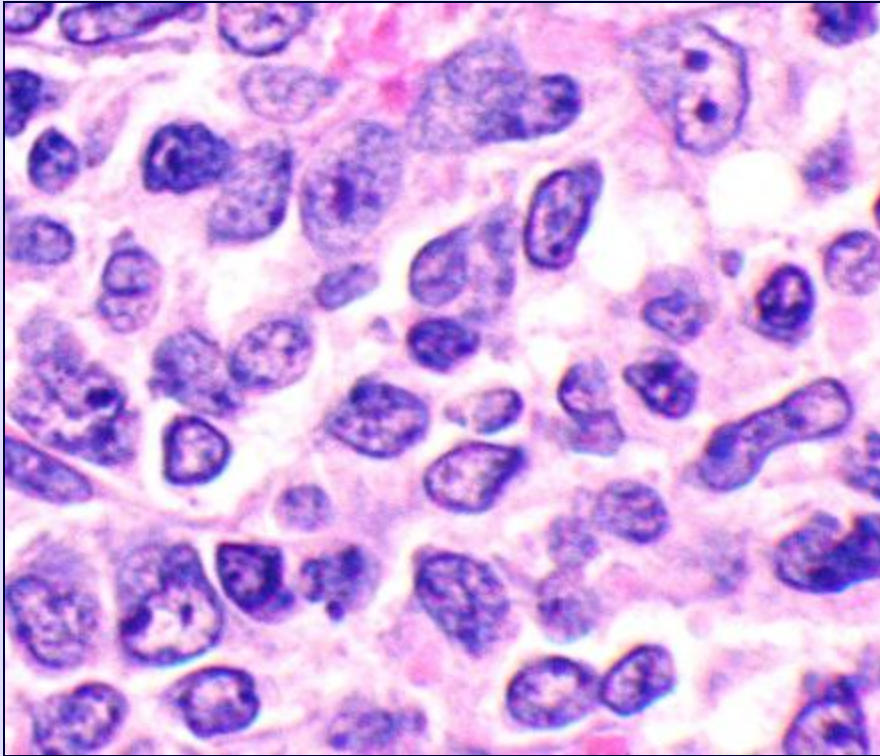


- *Most lesions on head or trunk*
- Middle aged adults
- Tumor nodules, sometimes with satellite lesions
- Local therapy an option in most cases

Primary Cut DLBCL Leg Type



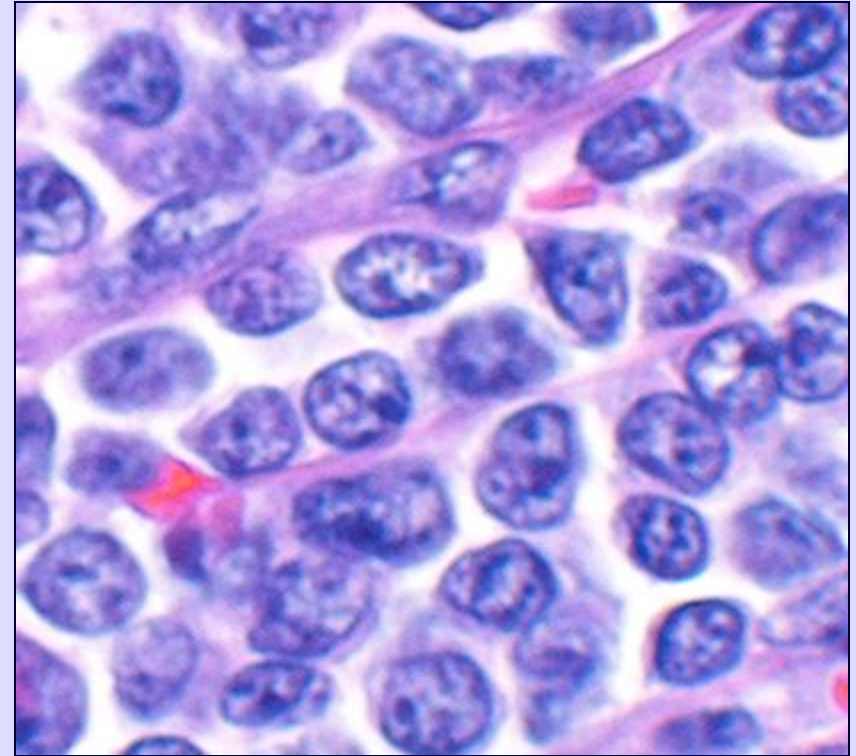
- *Lower extremity*
- Mainly elderly, F > M
- More aggressive clinical course
- Chemo recommended (for all but single lesions)



GCB

1° cutaneous FCL

BCL-2	-/+
BCL-6	+/-
CD10	+/-
MUM1/IRF4	-

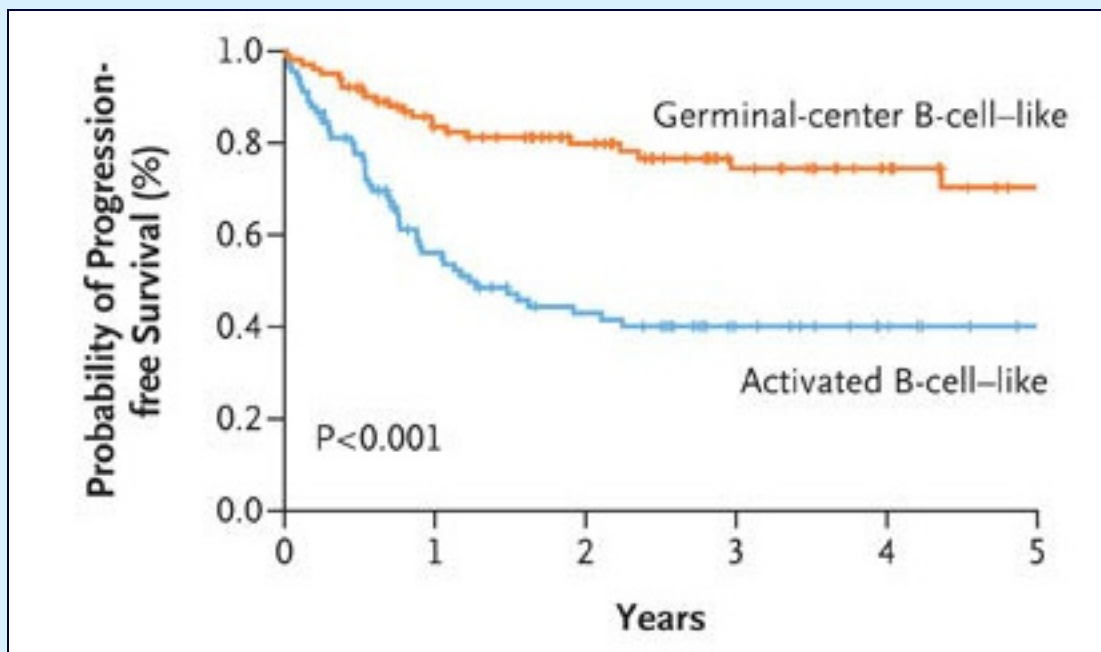
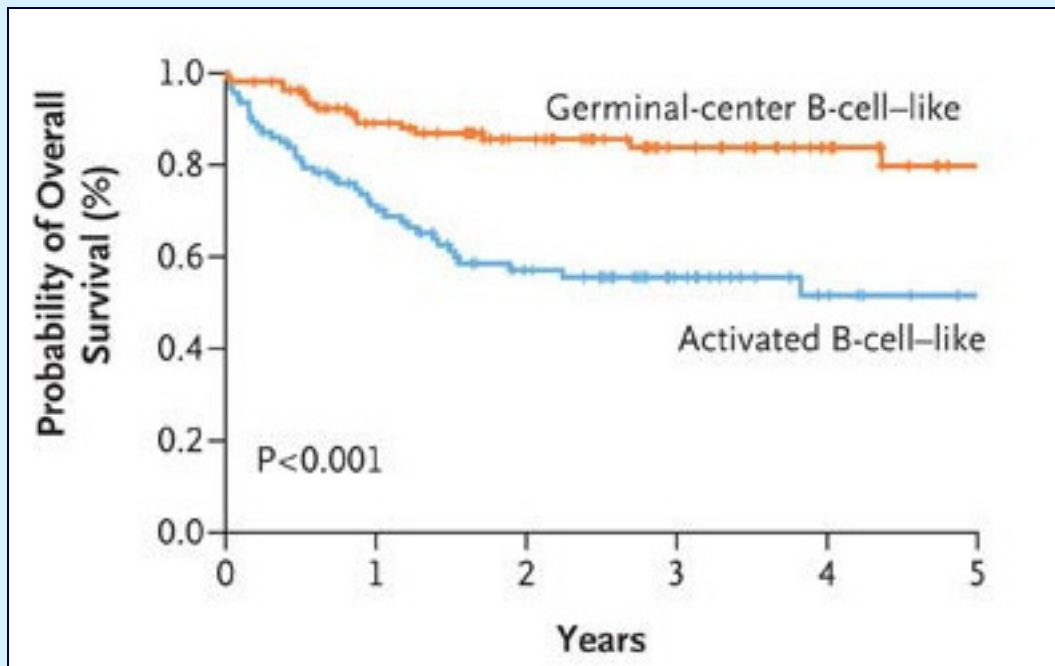


ABC

1° cut DLBCL leg-type

BCL-2	++
BCL-6	+/-
CD10	-
MUM1/IRF4	+

Lenz et al.
NEJM 2008



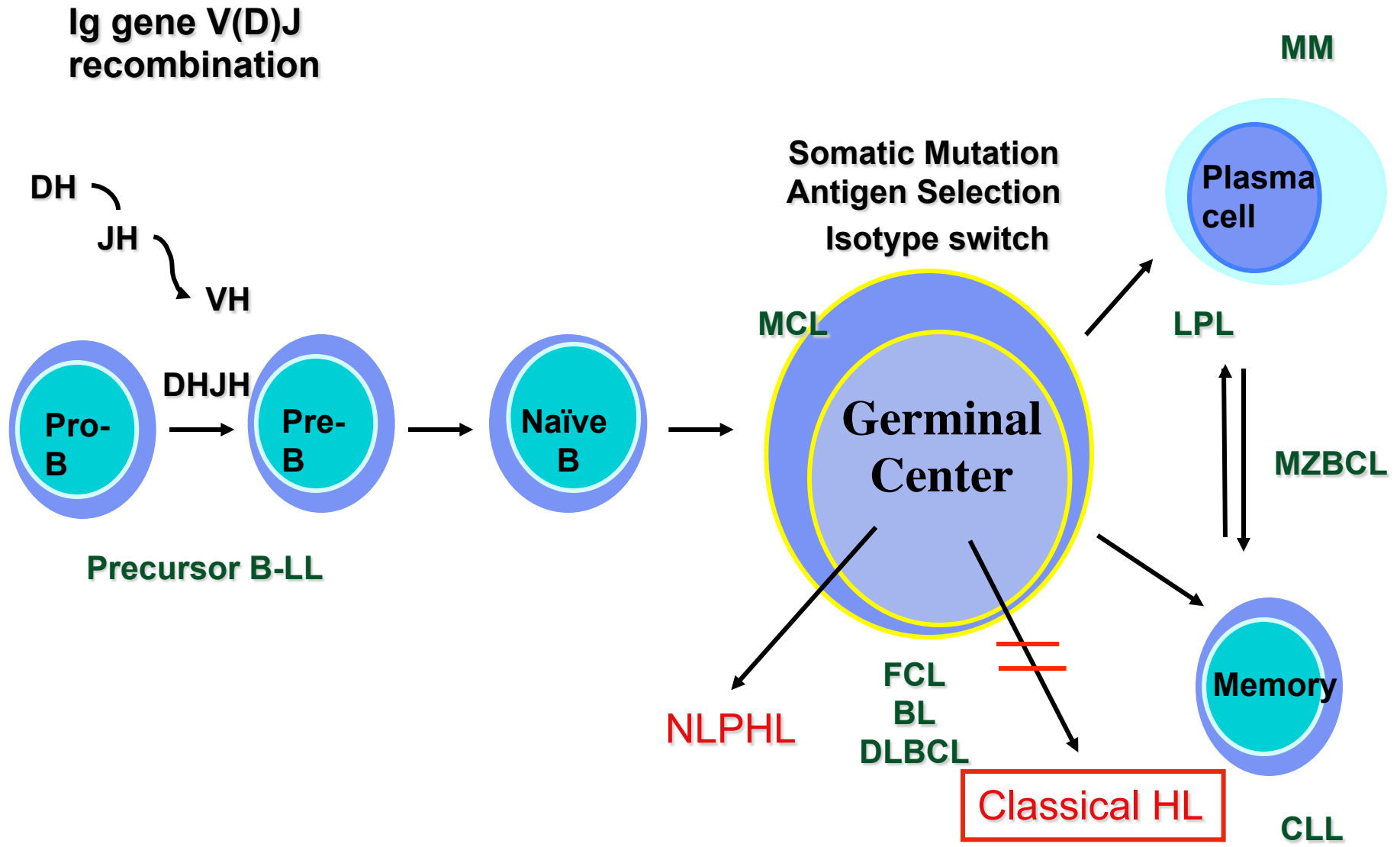
Site-specific or environmental impact on disease definitions

- Anatomic site may play a role in the initial identification of the entity
- However, there are biological underpinnings that drive the distinctive clinical and biological features
 - Primary mediastinal large B-cell lymphoma is distinct at the molecular level from other DLBCL
 - DLBCL, leg type shares many features with the ABC type of DLBCL

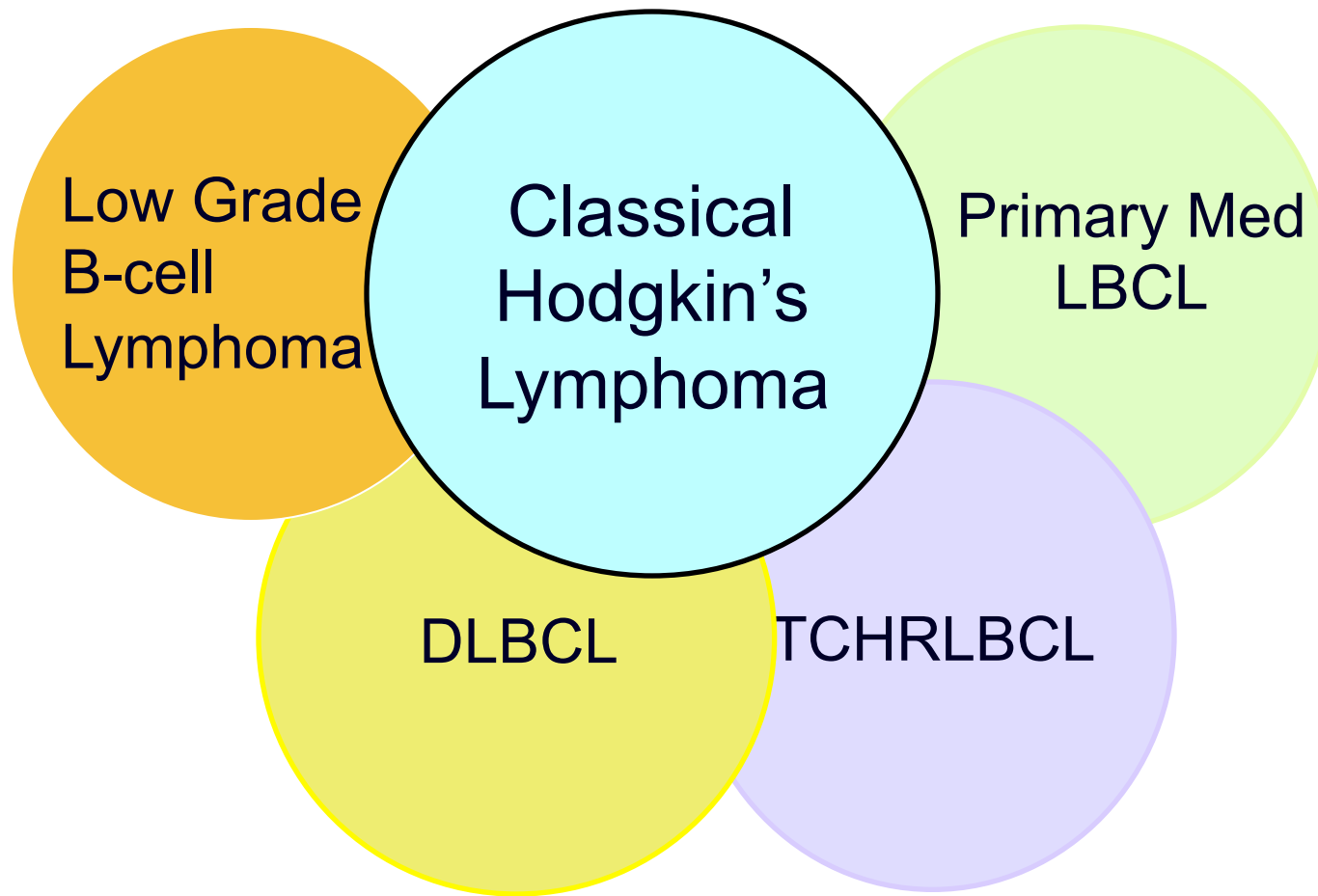
Borderline Categories

- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

B-cell Lymphomas Related to Stage of Differentiation



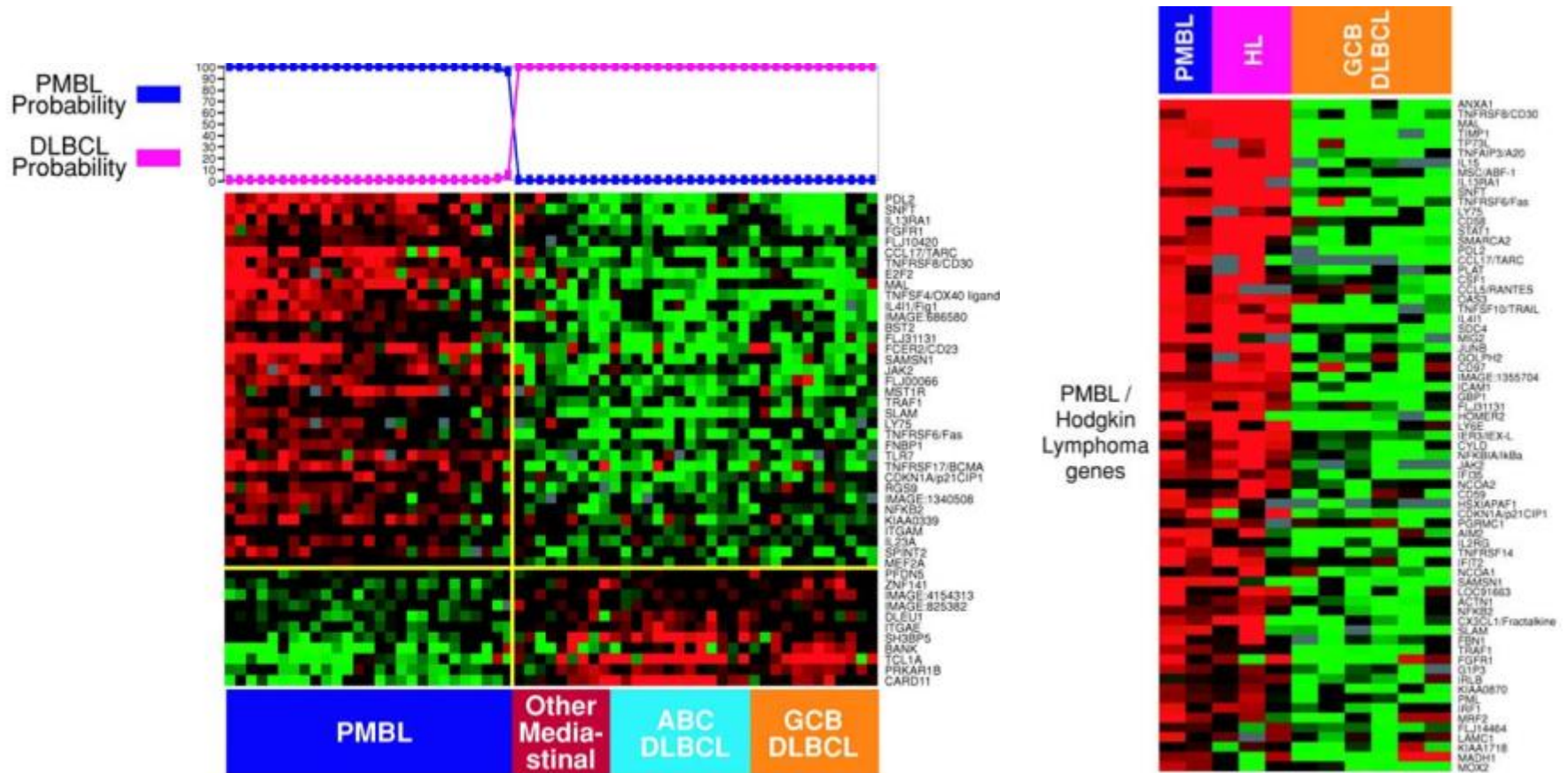
Classical Hodgkin's Lymphoma Biological Interfaces or Grey Zones



Common features of Primary Mediastinal (Thymic) Large B-cell Lymphoma & Classical Hodgkin's Lymphoma - Nodular Sclerosis

- Females > Males, 2:1 ratio
- Adolescents, young adults
- Mediastinal mass +/- Supraclavicular LN
 - May be composite or sequential in same pt.
- Common cytogenetic alterations
 - Gains at 9p24 (JAK2) & 2p16 (REL)
- Similar gene expression profile with Activation of NFκB pathway
- *Thought to arise from a “thymic B-cell”*

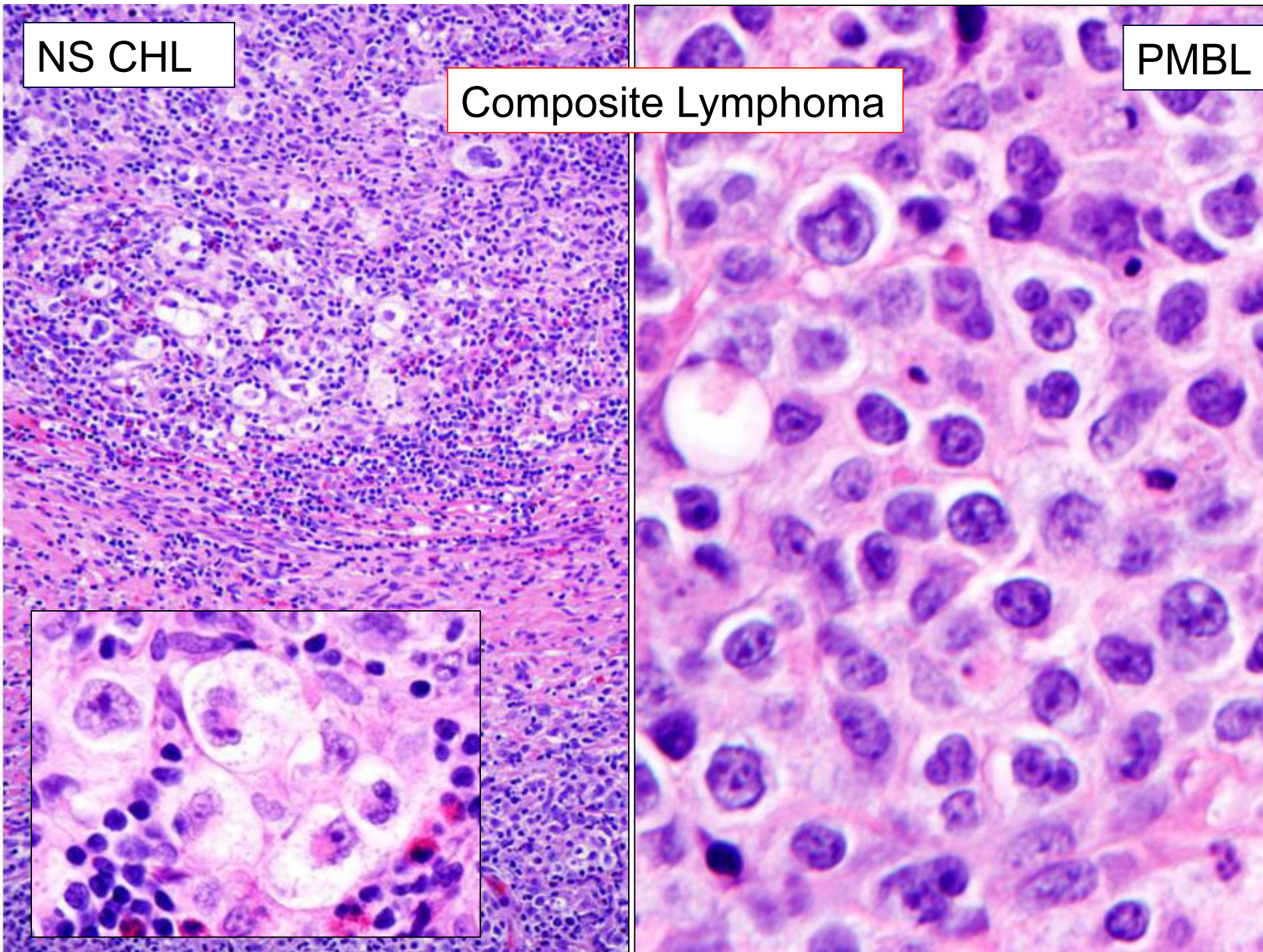
Primary Mediastinal Large B-cell Lymphoma has a gene expression signature that distinguishes it from other DLBCL, and many elements of that signature are shared by Classical Hodgkin's lymphoma cells (Rosenwald, JEM, 2003)

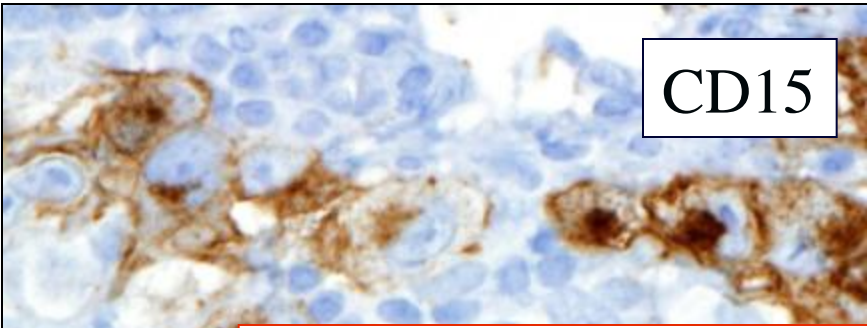


NS CHL

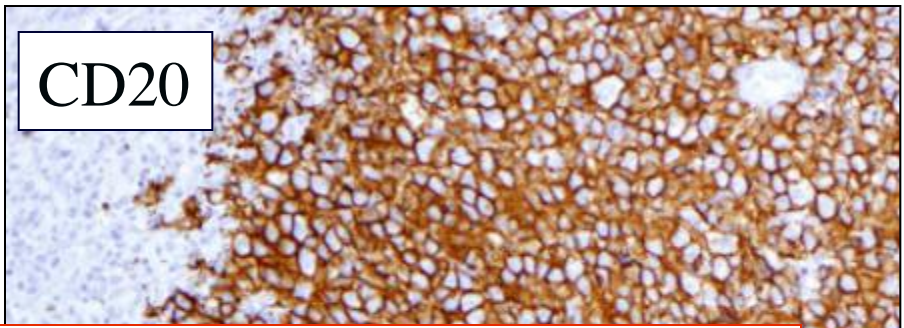
Composite Lymphoma

PMBL



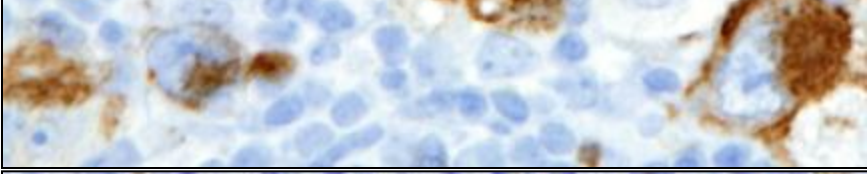


CD15

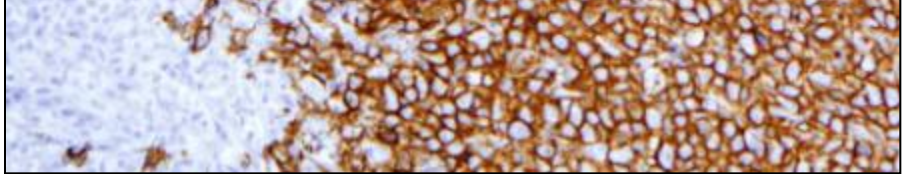


CD20

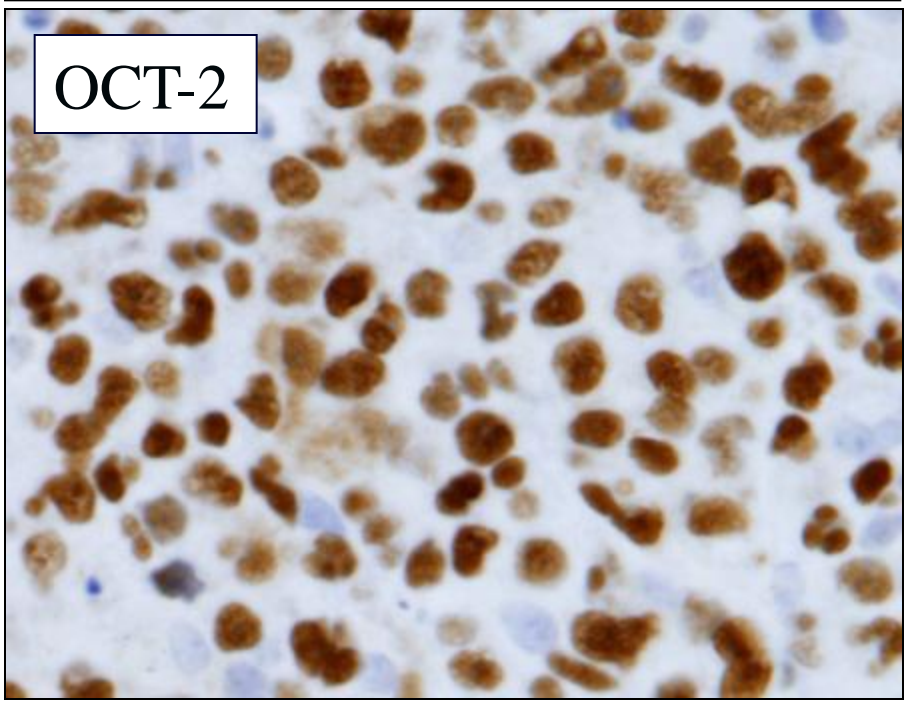
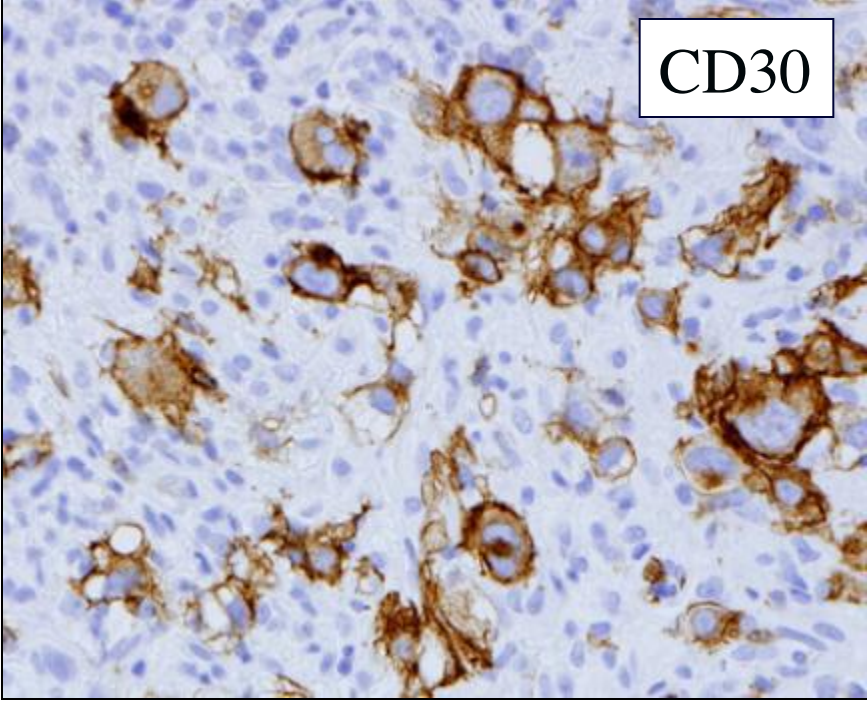
Single Cell Microdissection of both component shows that they are clonally related



CD30



OCT-2

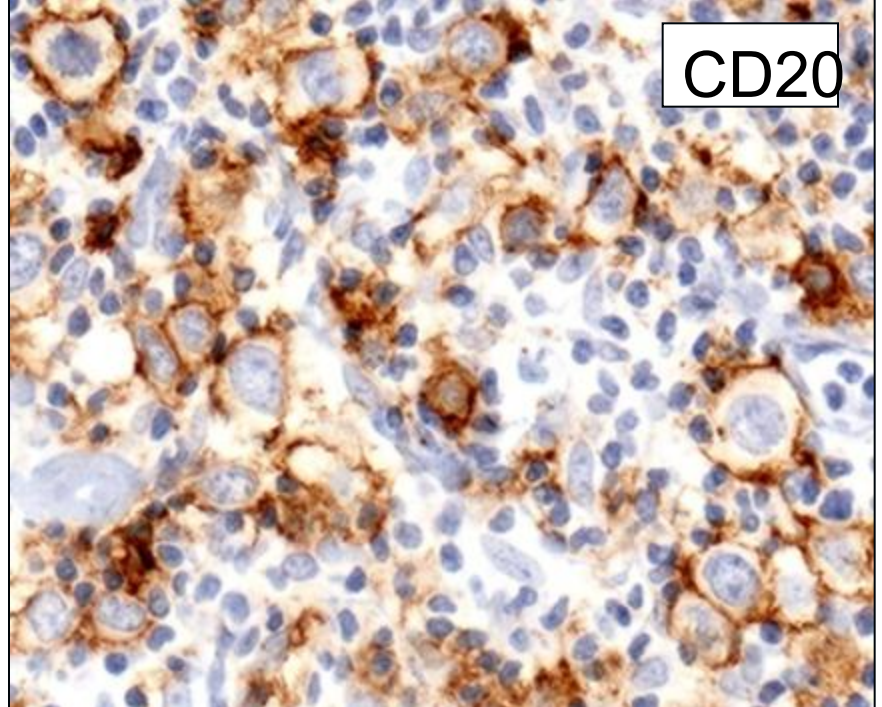
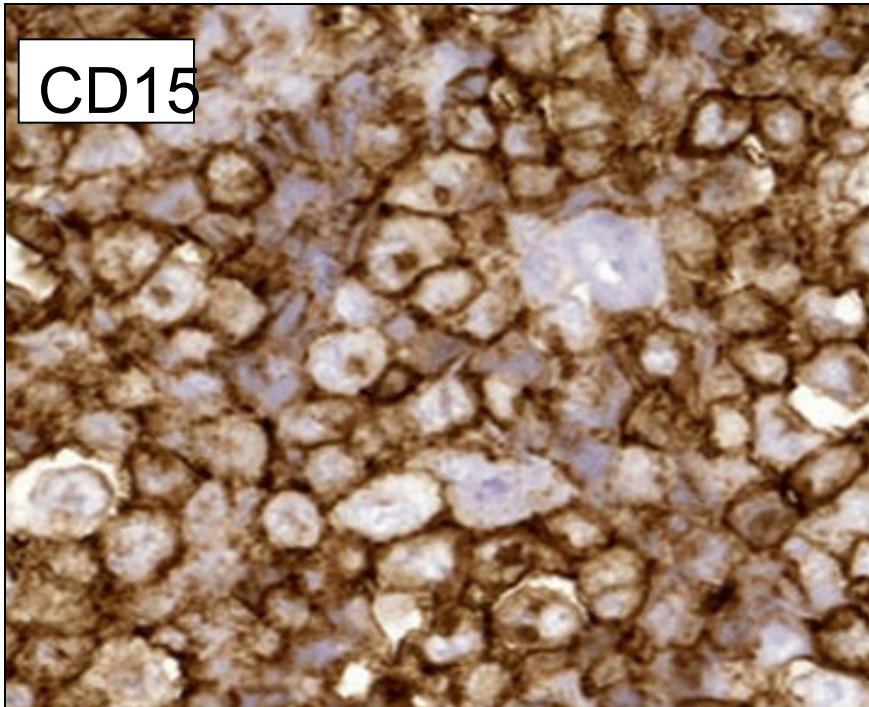
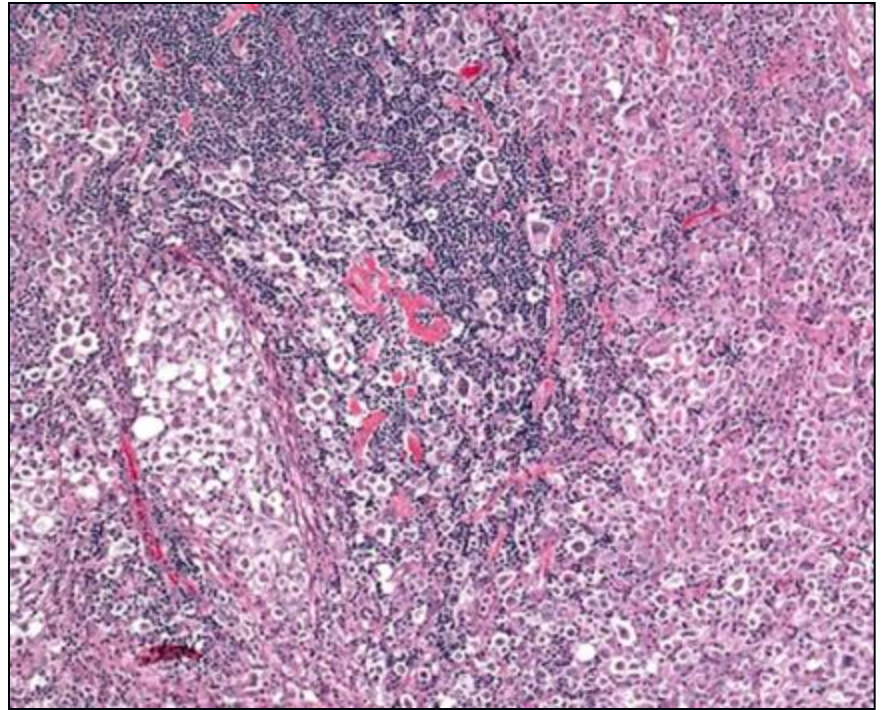
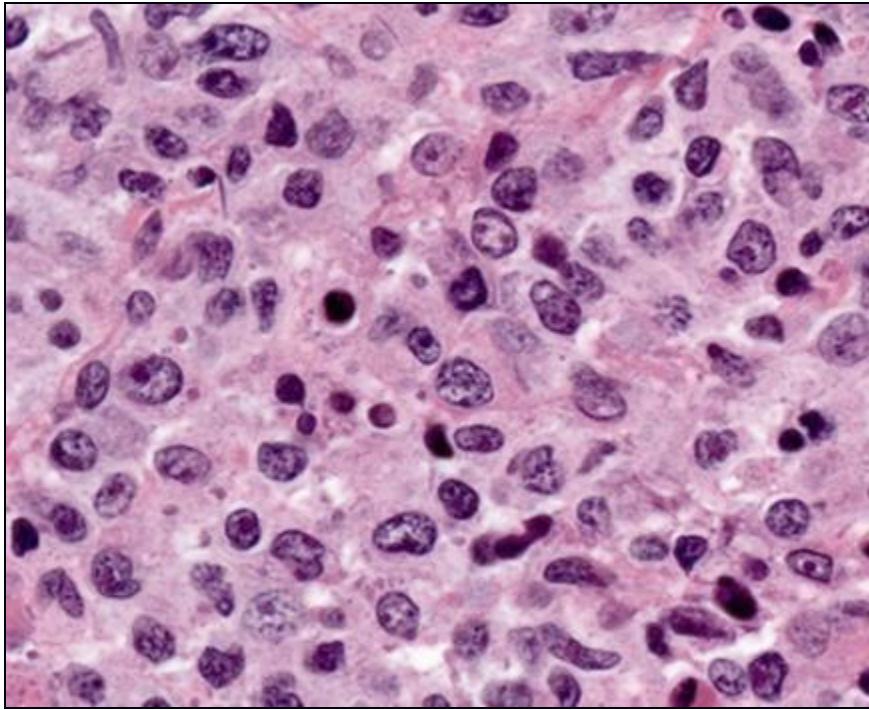


Mediastinal GZL represent the “missing link” between CHL-NS and PMBL

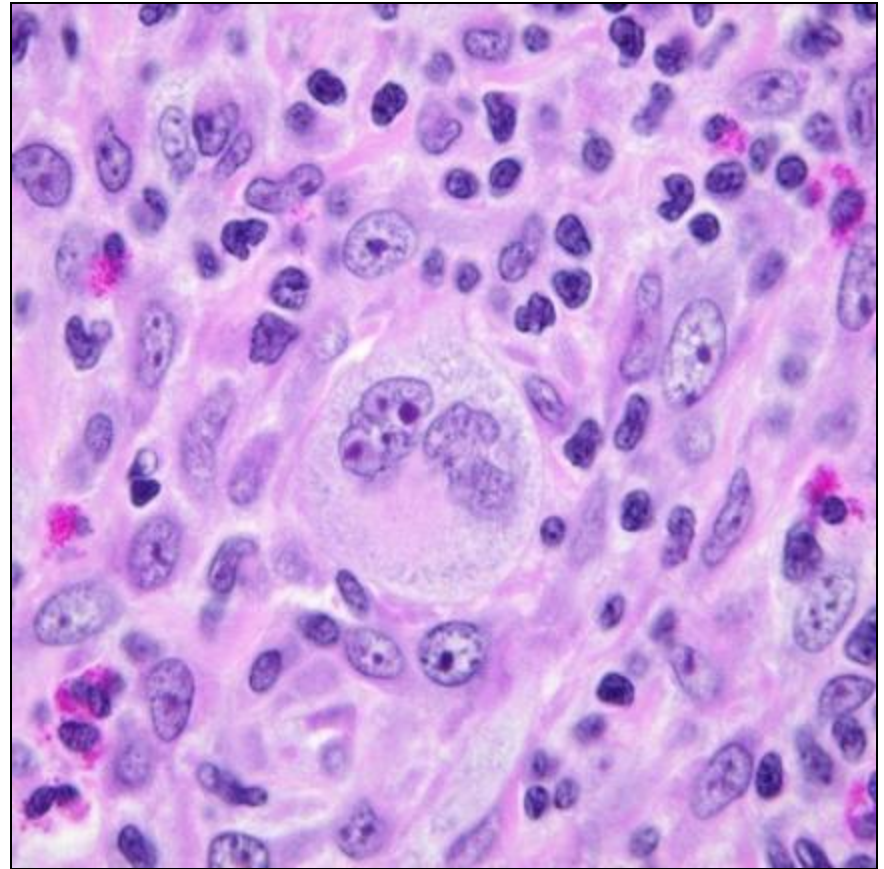
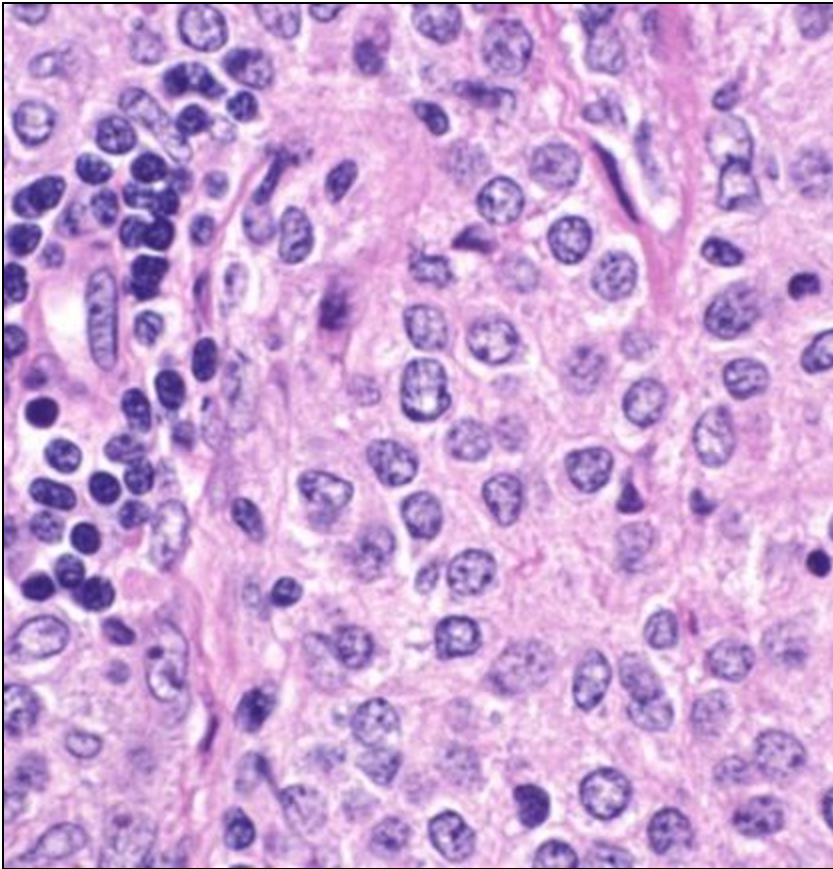
Traverse-Glehen et al AJSP 2005



- Gray zone lymphomas exhibit a morphological and immunophenotypic continuum
- MGZL cannot be readily classified as either PMBL or CHL, with frequent asynchronous histology & immunophenotype
- Clinical features similar to NS-CHL & PMBL, except for male predominance
- Composite and sequential lymphomas are a related phenomenon



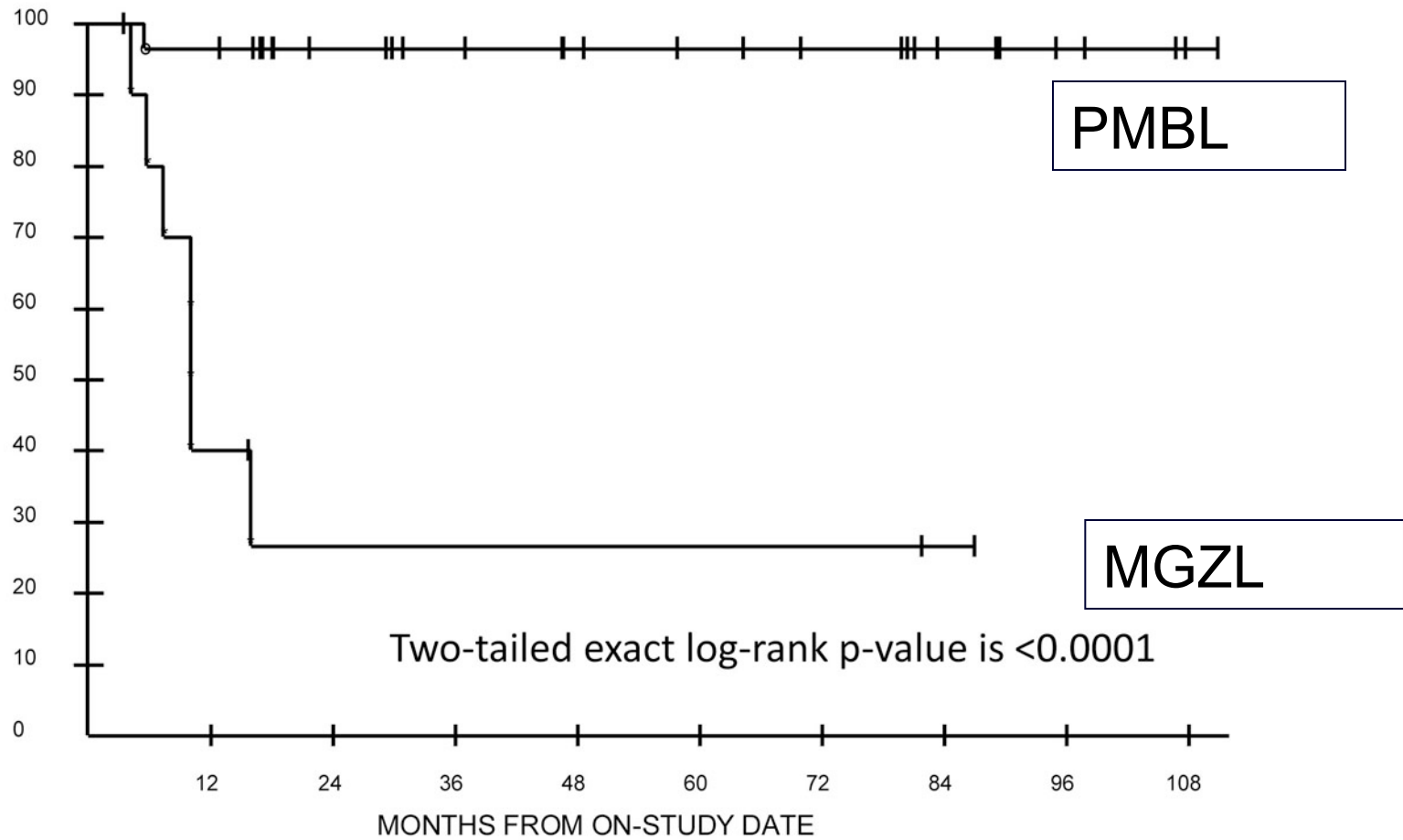
What is the optimal treatment for PMBL,
CHL-NS & Gray zone Lymphoma?



Clinical Features of PMBL and Med GZL treated with DA-EPOCH-R (Dunleavy et al. NCI)

Characteristics	PMBL	MGZL
Patients	35	11
Gender (F/M)	23:12	4:7
Median Age (range)	32 (19-52)	34 (14-52)
Median Mass cm (range)	10.9 (5-16.8)	10.8 (6.2-19.7)
ECOG PS > 1	1 (3%)	1 (9%)
Stage III or IV	11 (31%)	1 (9%)
LDH > Normal	25 (71%)	7 (64%)
Extranodal Sites	21 (60%)	4 (36%)
Pleural Effusion	17 (48%)	2 (18%)
IPI Score > 2	4 (11%)	1 (9%)

EPOCH-R PMBL VS. GRAY ZONE EVENT FREE SURVIVAL

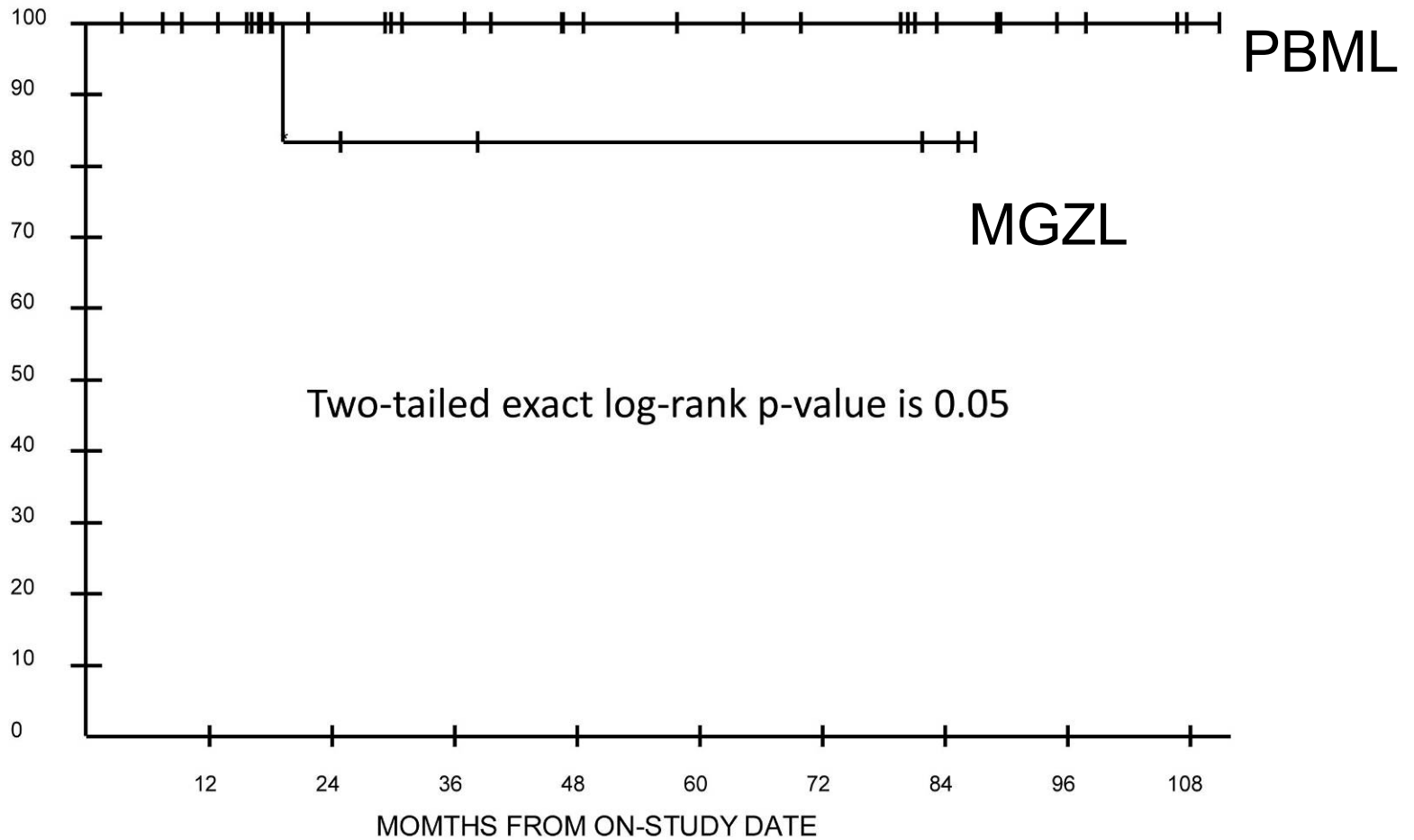


Legend: * EPOCH-R/EF/GRAY o EPOCH-R/EF/PMBL

7/10 failed 1/30 failed

Dunleavy, et al ASH 2009

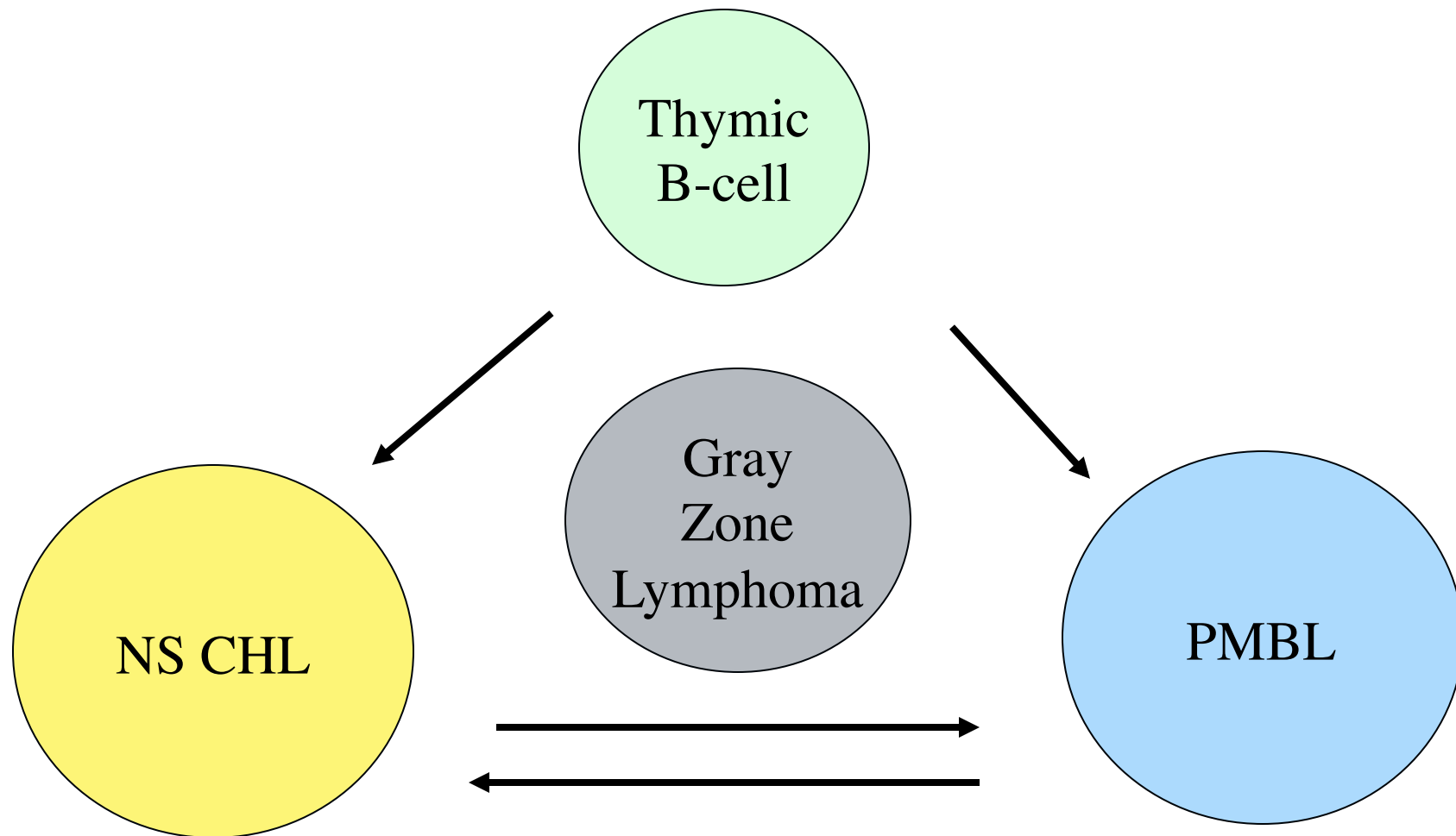
EPOCH-R PMBL VS. GRAY ZONE SURVIVAL



Legend: * EPOCH-R/OS/GRAY o EPOCH-R/OS/PMBL

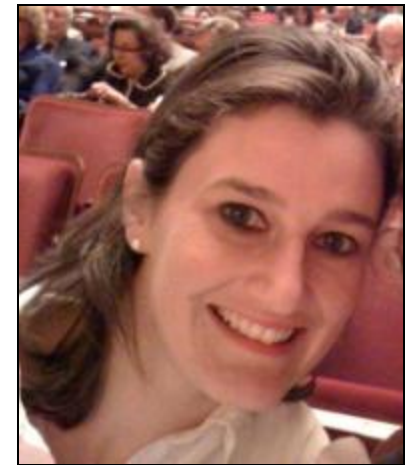
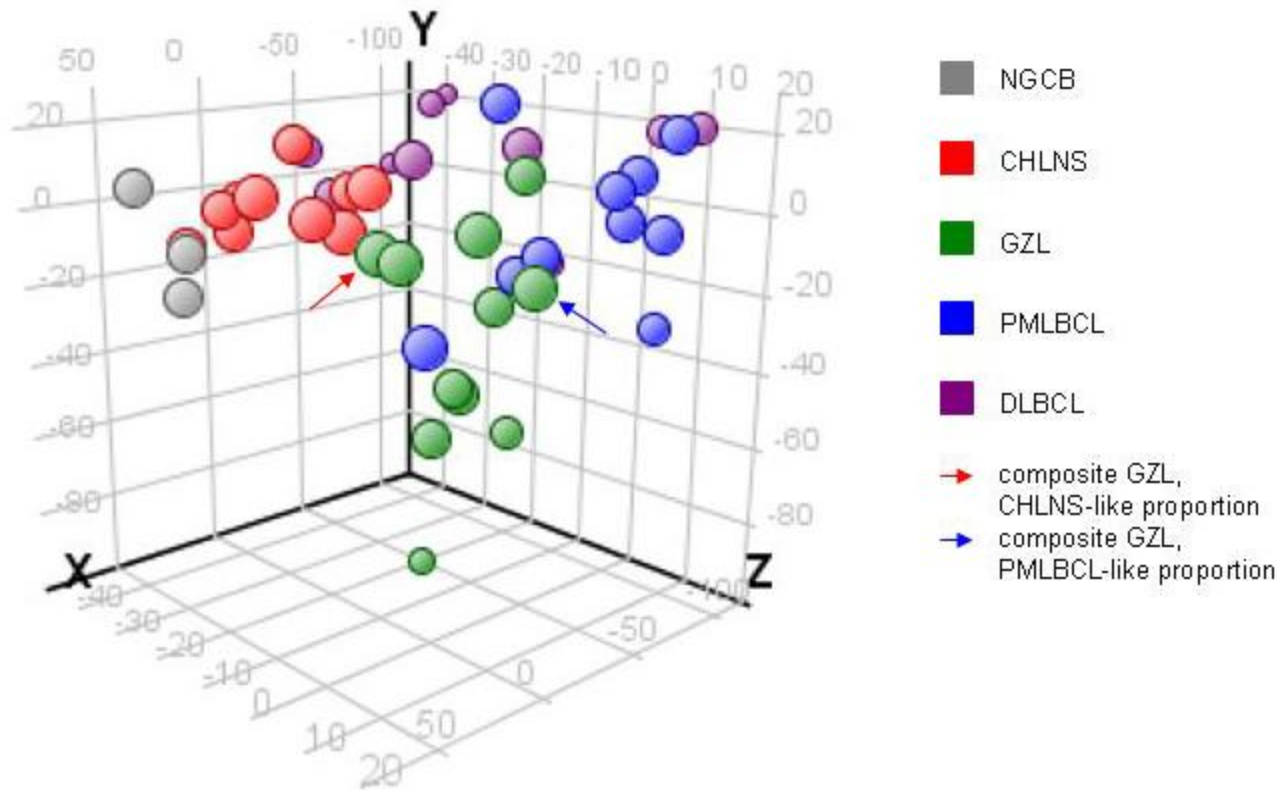
1/10 failed 0/30 failed

Molecular Events that drive the transformation of Thymic B-cells are unknown



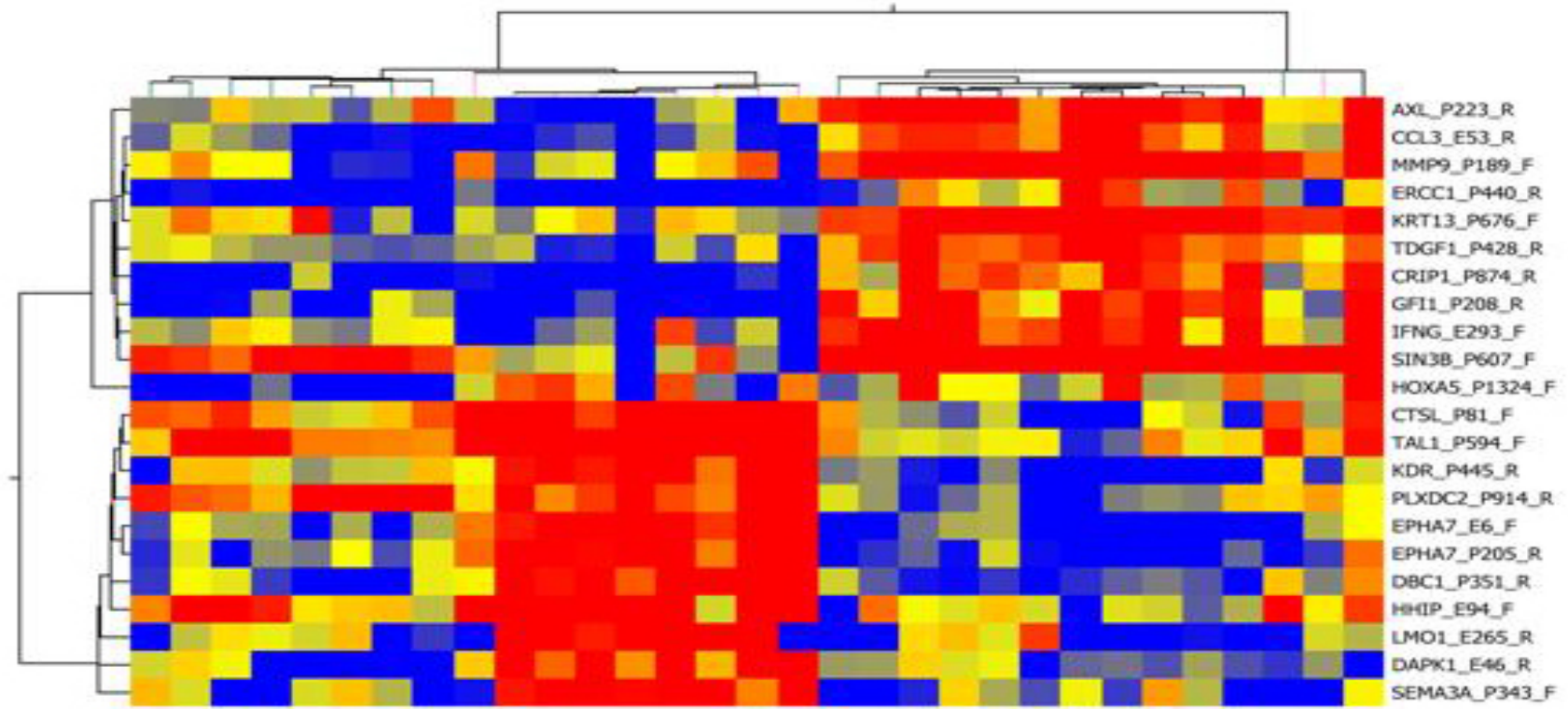
Epigenetic Profiling of MGZL, CHLNS & PMBL

Principal Component Analysis (PCA) of Microdissected Tumor



Epigenetic profile of MGZL lies between CHL and PMBL
Does not allow assignment to either “parent entity”

Unsupervised hierarchical cluster analysis of 22 DMTs



Methylation level (β values)



MGZL

PMLBCL

CHLNS

Composite Lymphoma PMBL

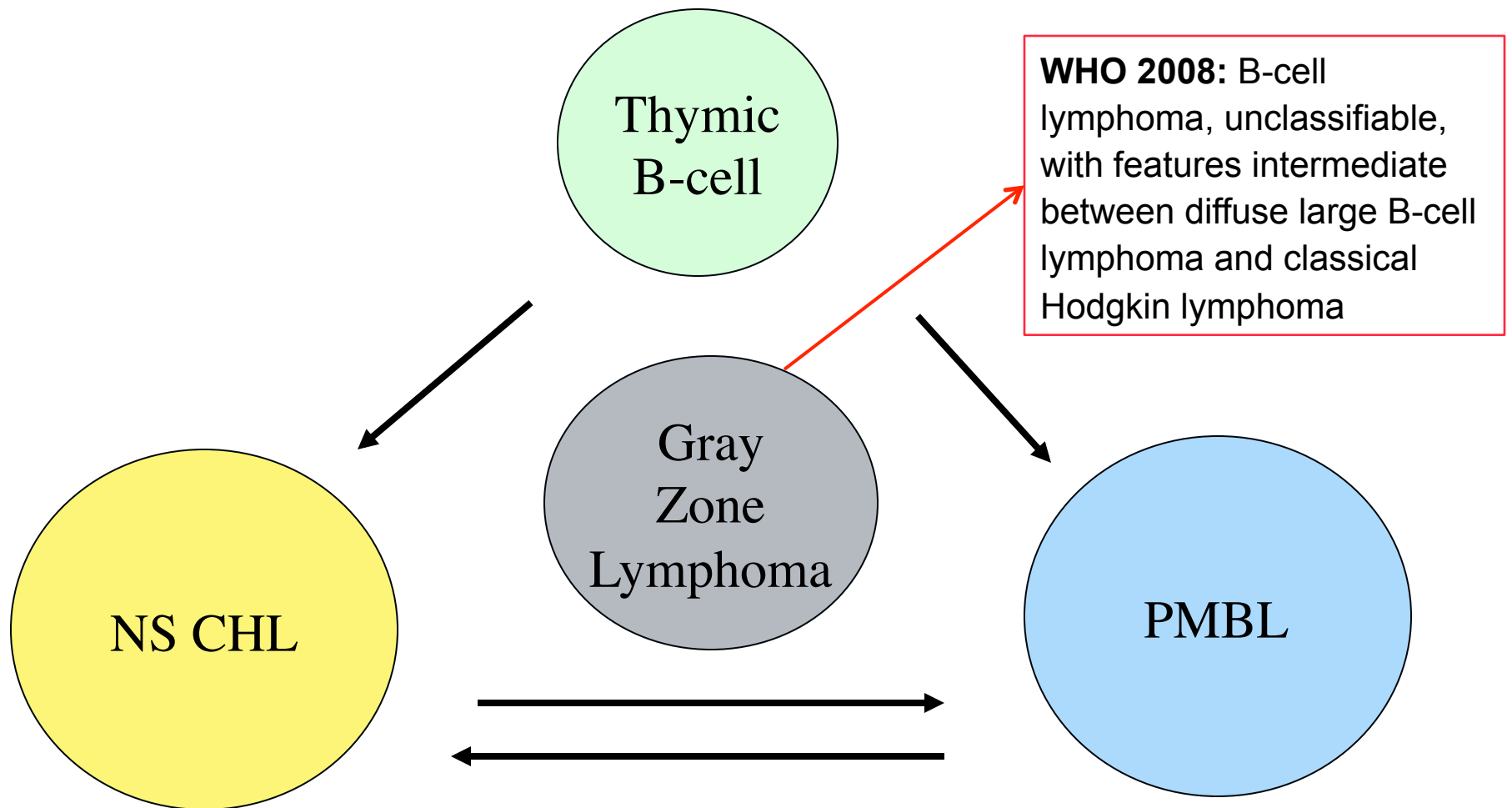
Composite lymphoma NS CHL

Methylation Profiling of MGZL

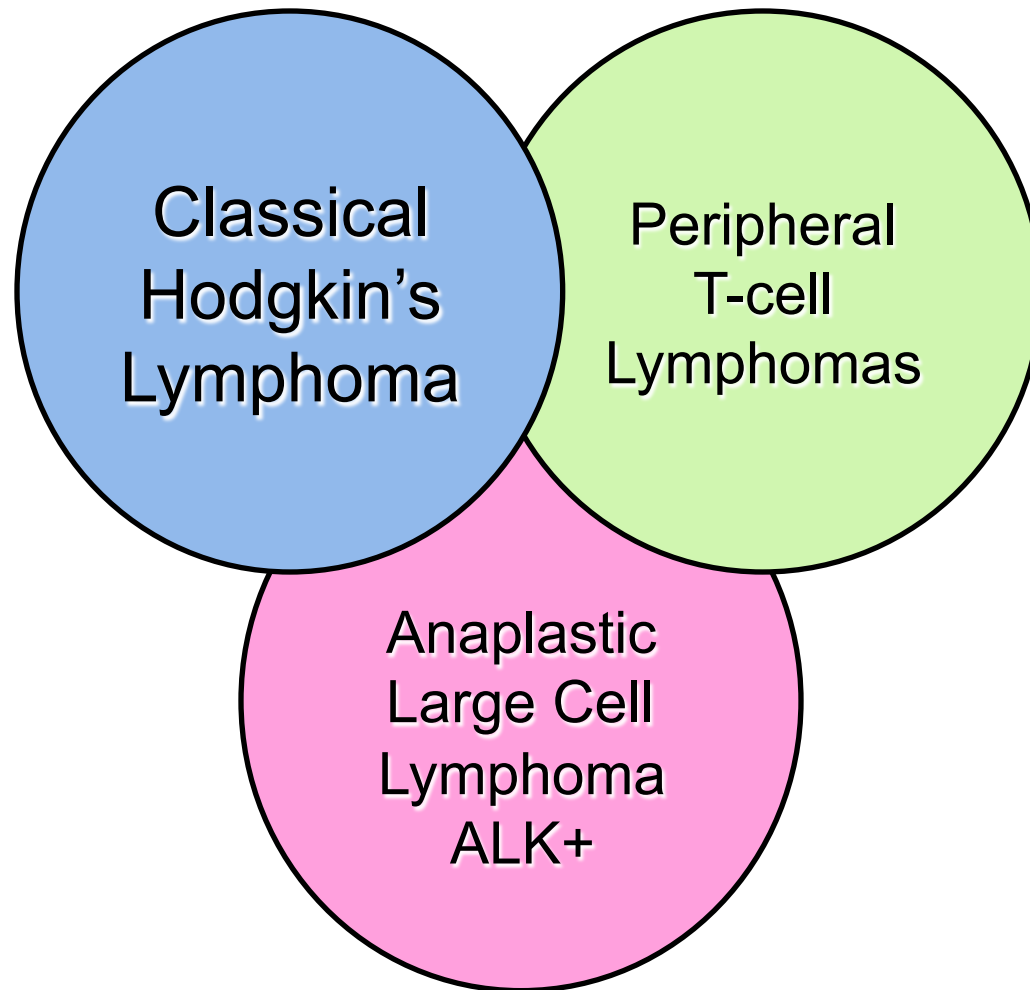
Eberle et al. Haematologica 2011

- Principal component analysis showed that MGZL has a distinct epigenetic profile that is intermediate between CHLNS and PMBL, but distinctly different from other DLBCL.
- Class prediction models using selected CpG sites could distinguish between CHLNS, PMBL & MGZL
- MGZL cannot be re-assigned to one of the parent entities, and differs from both
- Gray zone features are present at the epigenetic level, in addition to morphology and immunophenotype

Molecular Events that drive the transformation of Thymic B-cells are still unknown ...



Hodgkin's Lymphoma & T-cell Lymphomas
Morphological "Grey Zones"



Hodgkin's Lymphoma & T-cell Lymphomas
- *not Biological* "Grey Zones"

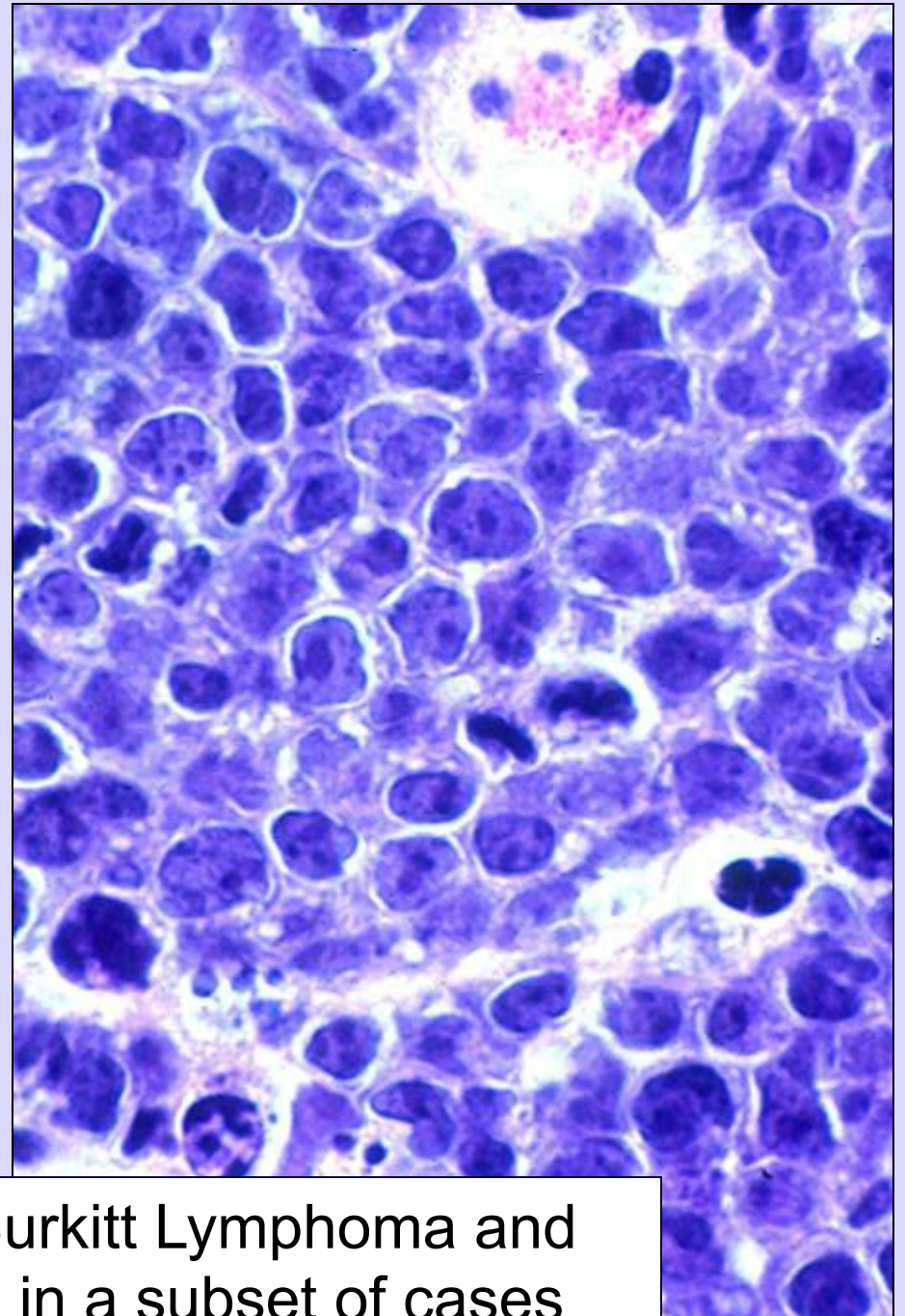
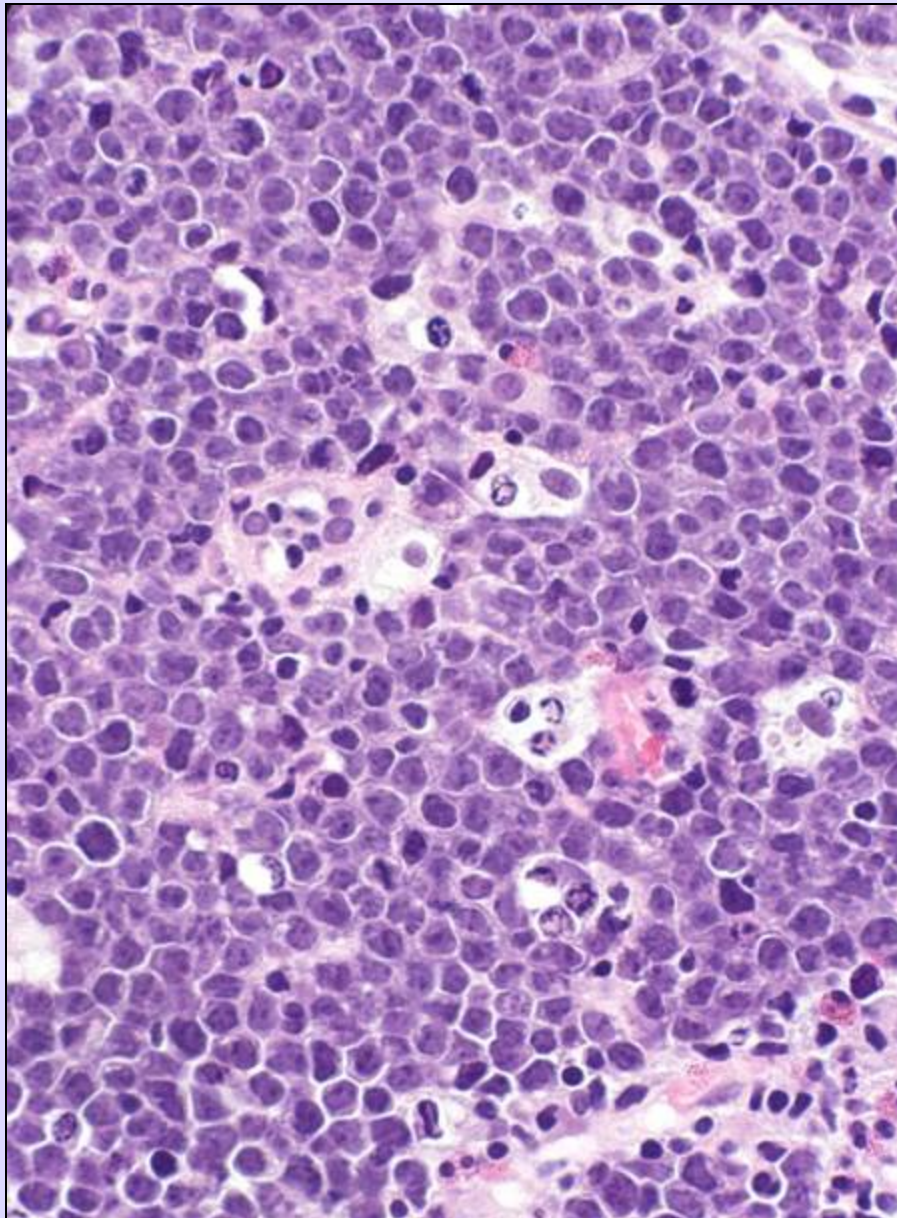
Classical
Hodgkin's
Lymphoma

Peripheral
T-cell
Lymphomas

Anaplastic
Large Cell
Lymphoma
ALK+

Borderline Categories

- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma



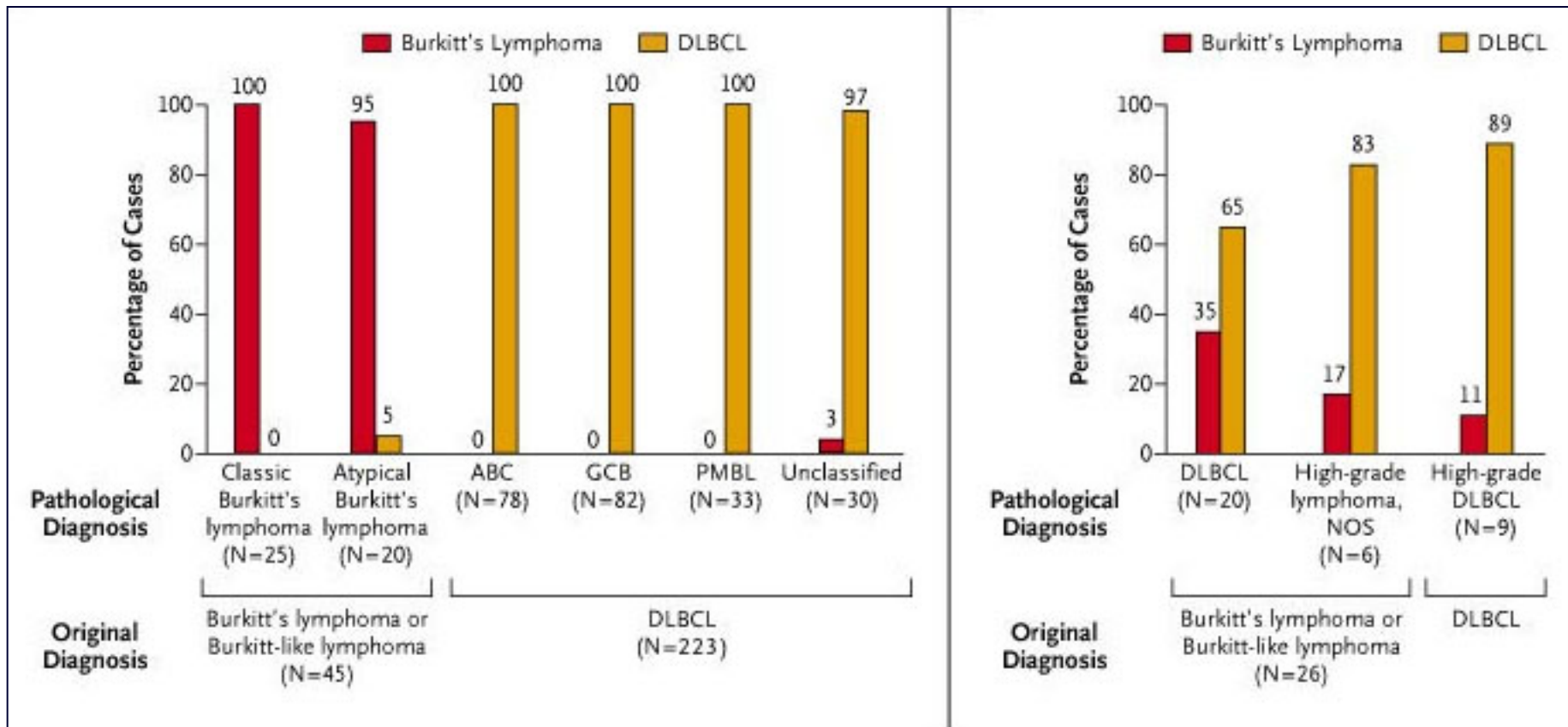
Differential Diagnosis of Burkitt Lymphoma and DLBCL is still challenging in a subset of cases

Gene Expression Profiling in the Diagnosis of Burkitt Lymphoma

Dave et al. NEJM 2006

In the majority of cases good correlation between GEP & Dx

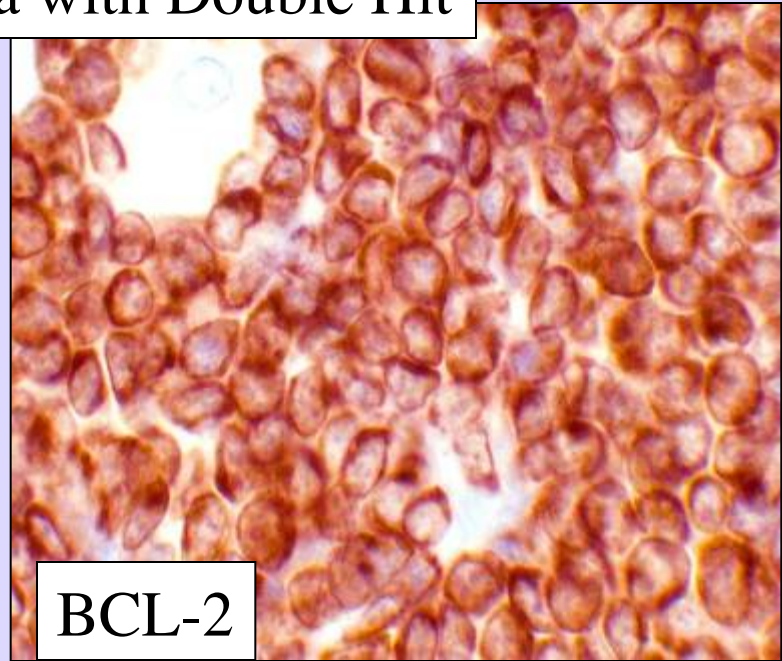
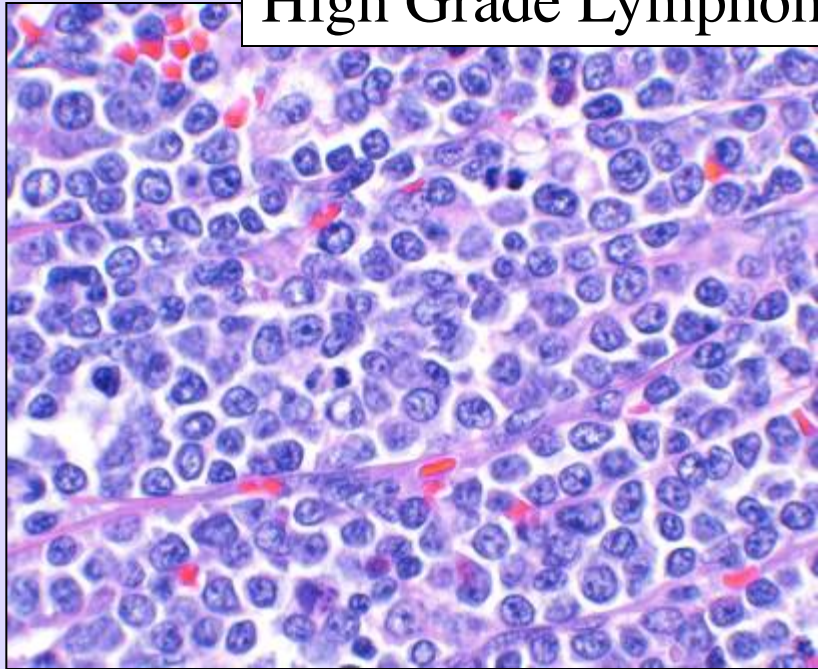
Subset of cases show discordance between GEP & Path Dx



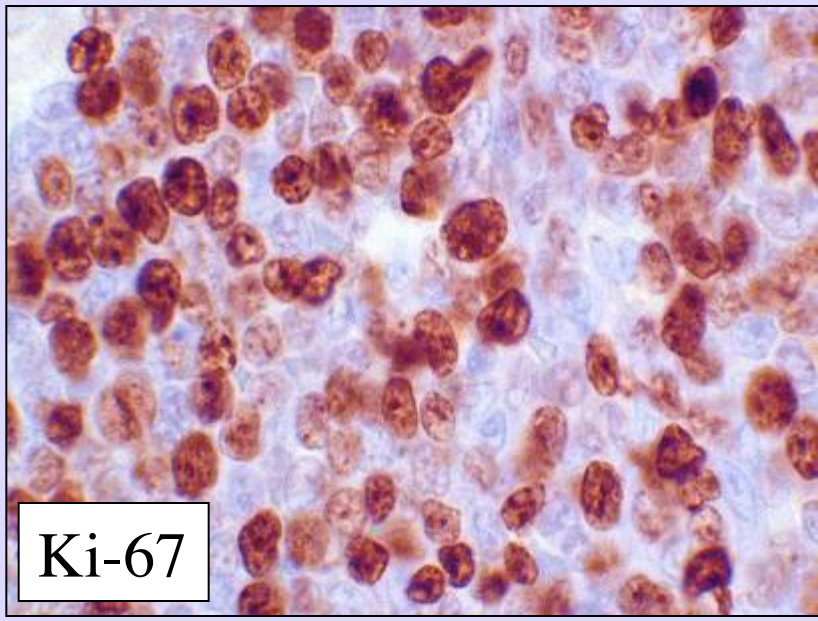
Cases at the Borderline of BL and DLBCL

- Concurrent BCL2 and MYC translocations
 - Tomita et al. Haematologica 2009
 - Johnson et al. Blood 2009
- Most have complex karyotypes
 - BCL6 translocation in a subset
- Histology not that of typical Burkitt lymphoma
 - DLBCL or high grade NOS
- Poor prognosis with conventional therapy
- High risk of CNS involvement

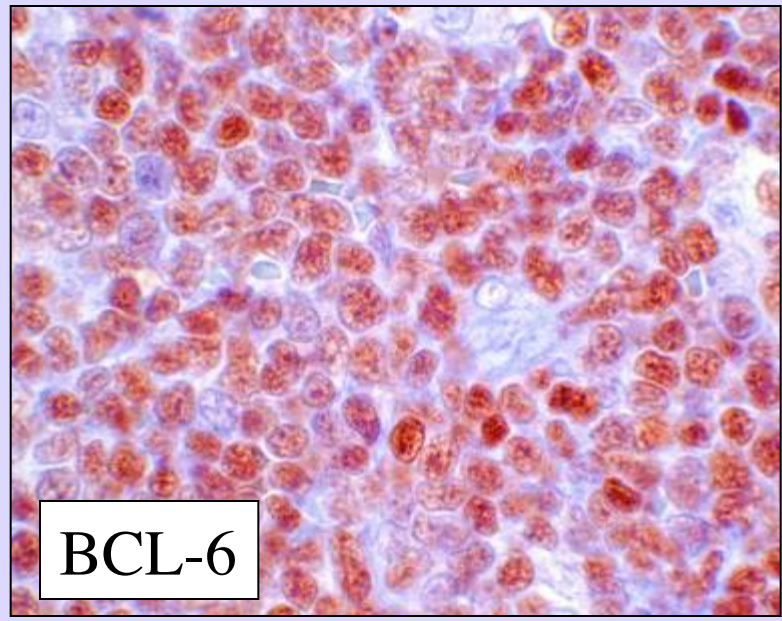
High Grade Lymphoma with Double Hit



BCL-2



Ki-67

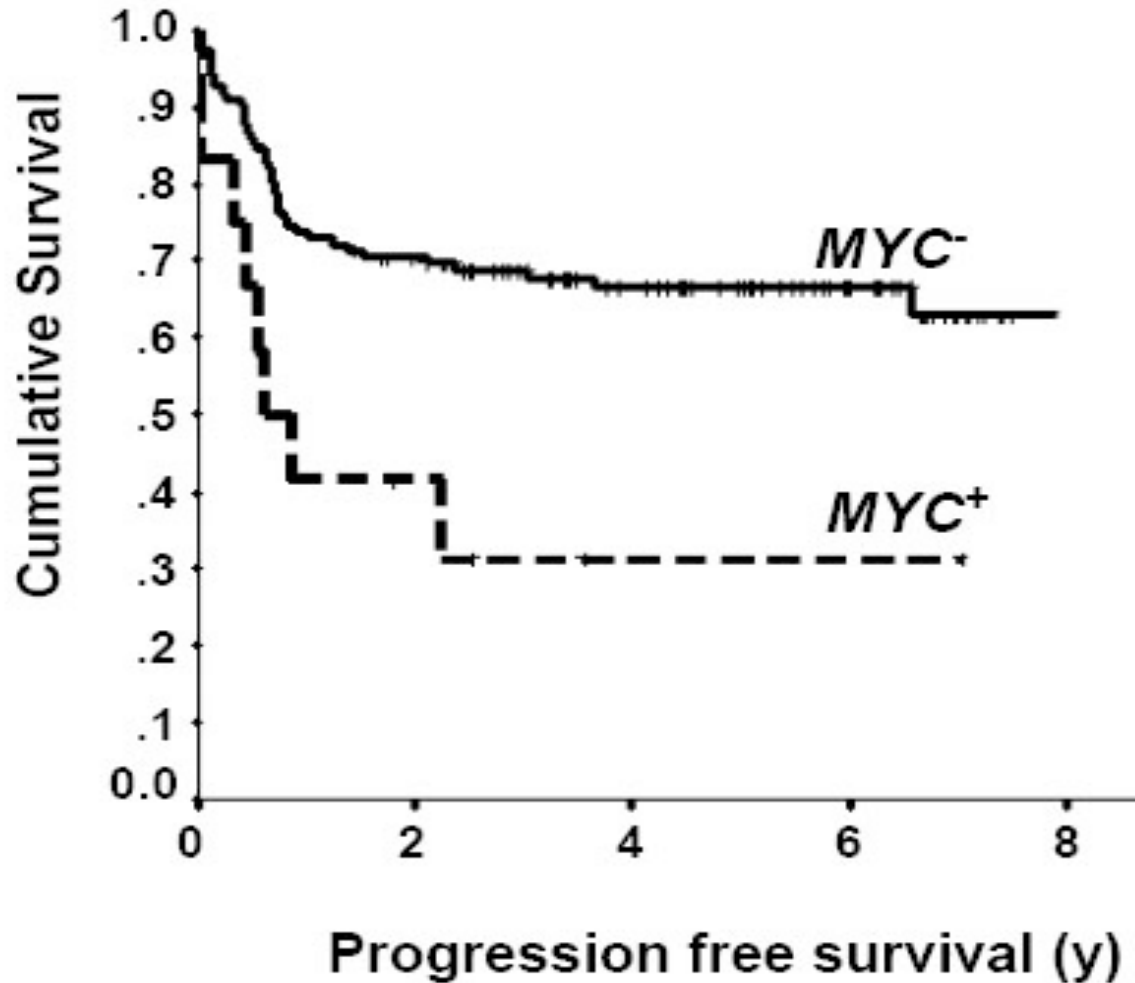


BCL-6

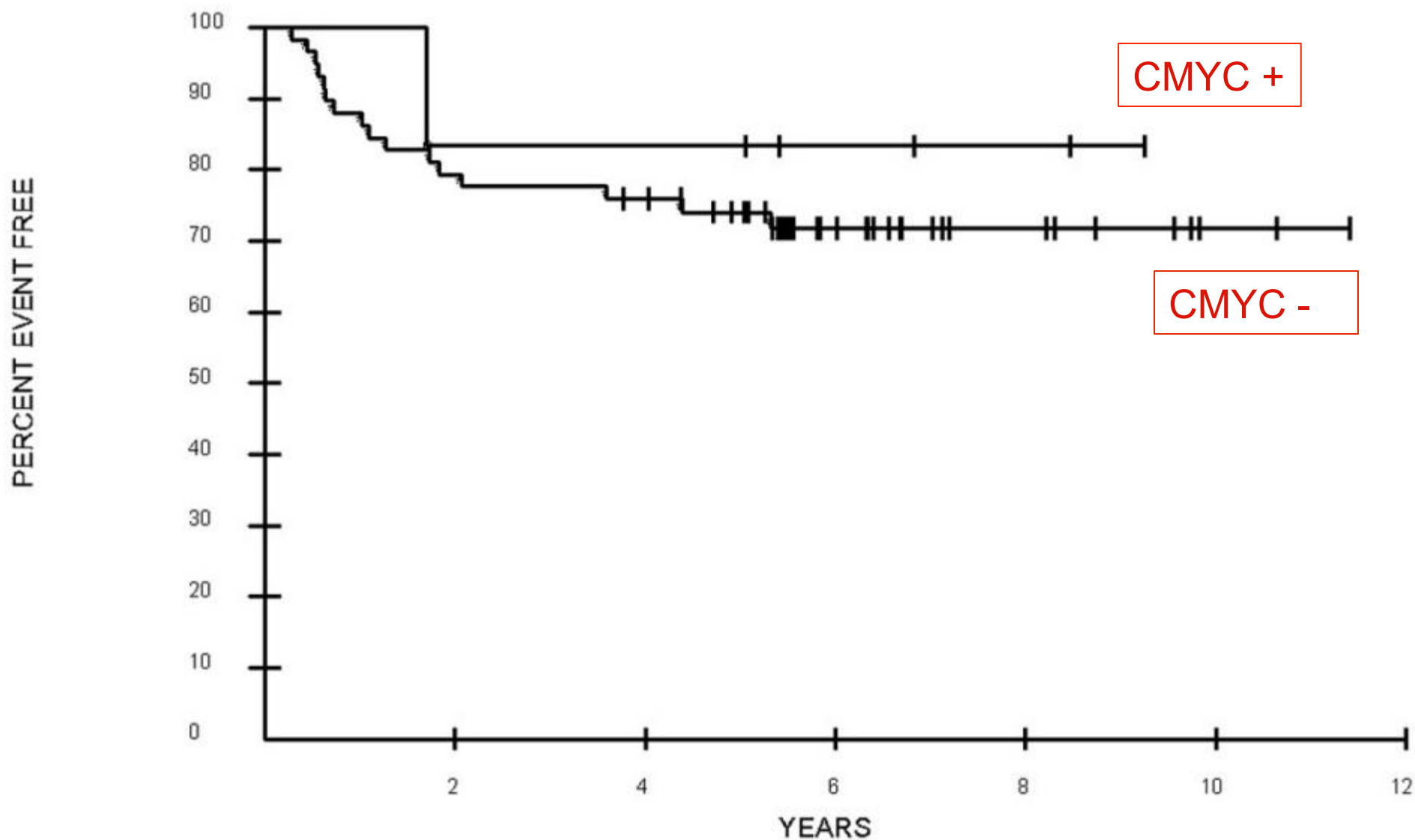
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

- Not a single disease, but a category to use when one cannot make a definite diagnosis of either Burkitt Lymphoma or DLBCL
- Generally high grade (Ki-67 > 90%)
- Many cases carry a MYC translocation
 - Often double hit – both MYC and BCL2
 - MYC with a non-Ig partner – t(8;9)
 - MYC Complex, rather than MYC simple
- A diagnosis to be made sparingly – not just DLBCL with a high growth fraction
- Does not include all DLBCL with MYC translocation

Influence of MYC gene R in DLBCL treated with R-CHOP
(3 cases had double hit)
Savage et al. Blood 2009



DLBCL Rx with DA-EPOCH R – impact of C-MYC on Event Free Survival



Legend: * CMYC - o CMYC +
16/58 failed 1/6 failed

WH Wilson, NCI

New Aspects of WHO 2008

- Greater recognition of “early” lesions
 - Earliest steps in neoplastic transformation
- Age as a defining aspect of some neoplasms
 - Both pediatric and elderly
- Site-specific impact on disease definitions
 - Anatomic site or environmental influences
- Incorporation of borderline categories
 - At least a temporary measure

We are coming in for a landing

Acknowledgements

Stefania Pittaluga

Mark Raffeld

Wyndham H. Wilson

Kieron Dunleavy

Peijie Cong

Leki Taddesse-Heath

Alexandra Traverse-

Glehen

Armin Jegalian

Joo Song

Franziska Eberle

Svetlana Pack

