

Immunohistochemical classification of haematolymphoid tumours

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Malignant lymphoproliferative diseases

What are they?

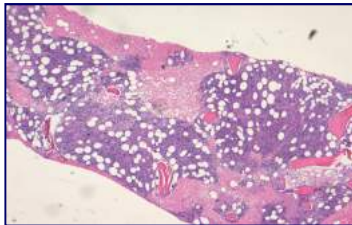
Haematolymphoid Neoplasias: Leukaemia vs Lymphoma

C L O N A L L Y M P H O I D M A L I G N A N C I E S

Haematolymphoid Neoplasias: Leukaemia vs Lymphoma

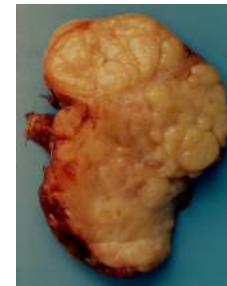
C L O N A L L Y M P H O I D M A L I G N A N C I E S

Bone marrow



Blood

- Lymph node
- Extranodal site



Leukaemia

Lymphoma

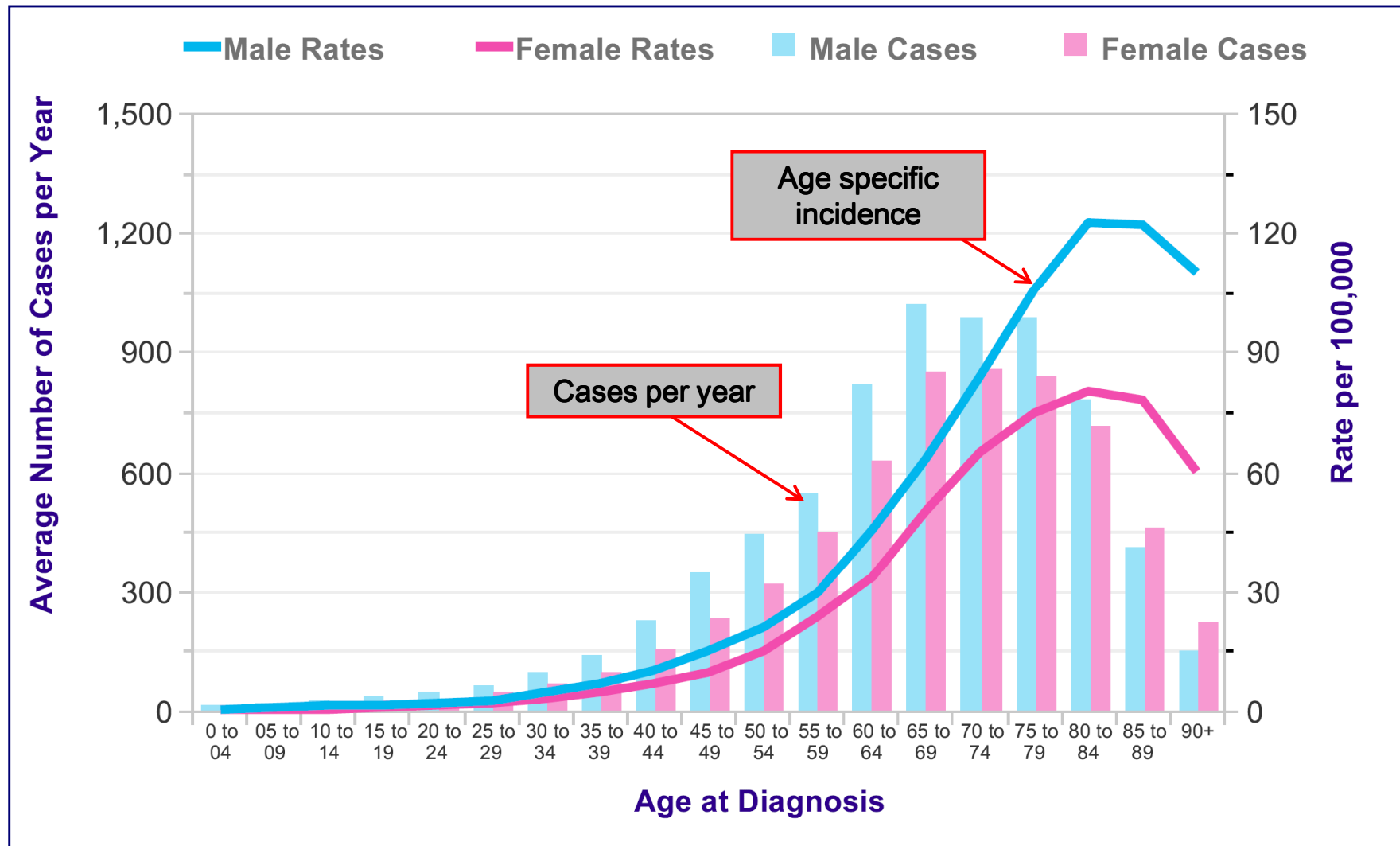
Malignant lymphoproliferative diseases

How common are they?

Malignant lymphoproliferative diseases

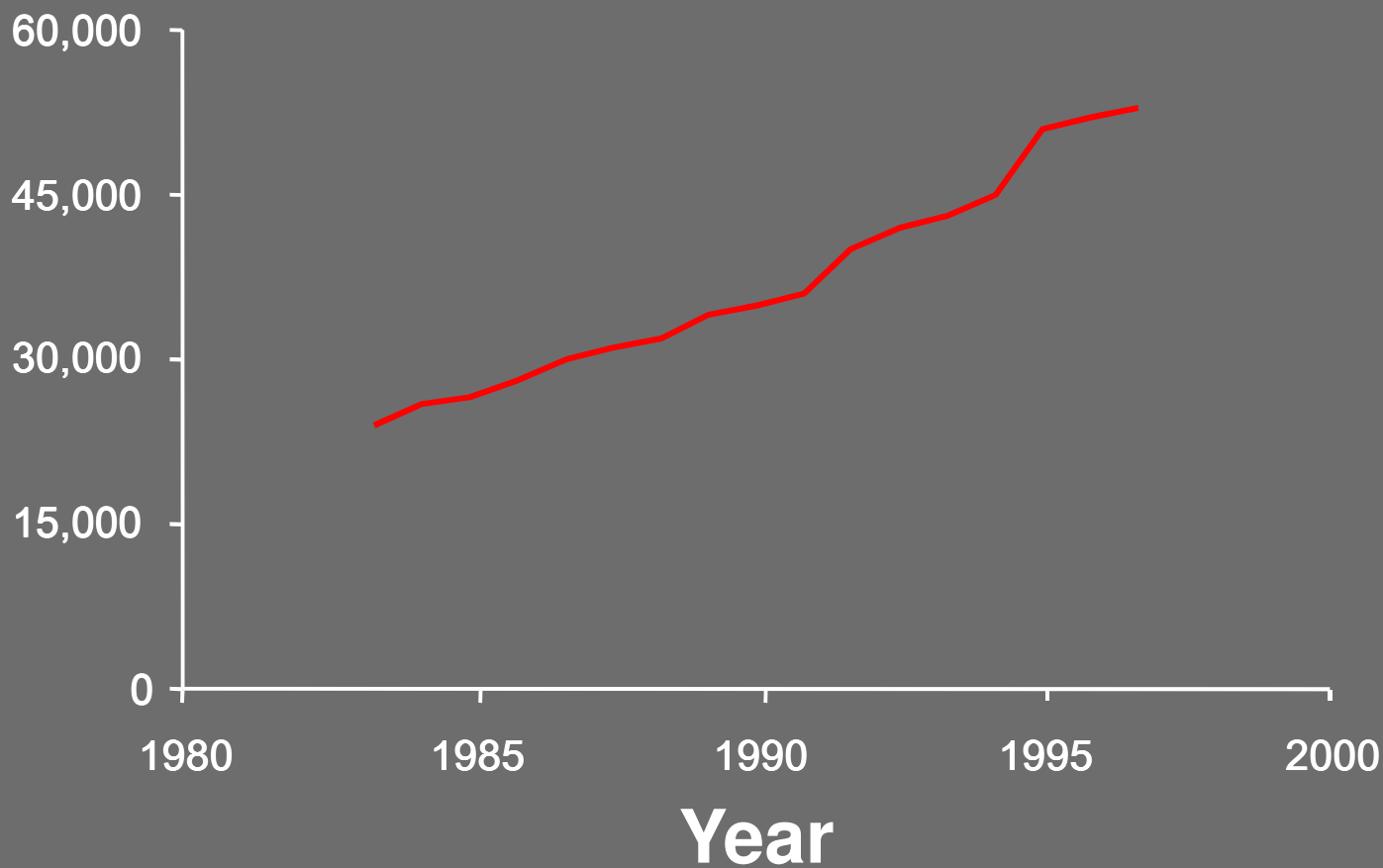
- **Malignant lymphoma**
- **Leukaemia**
 - Acute lymphoblastic leukaemia
 - Chronic lymphocytic leukaemia (CLL)
- **Ca. 1,600 per year in DK**
- **Ca. 700,000 per year in the world (?)**
- **8th commonest cancer globally**

Age & sex: Non-Hodgkins lymphoma (UK)



Estimated Incidence of NHL in the USA

Estimated Annual
Incidence



Malignant lymphoproliferative diseases

What causes them?

Largely unknown.....but involves:

- **Changes in genes**
 - e.g. mutations, translocations
 - inherited – radiation – chemicals – infections – sporadic
- **Changes in the immune system**
 - immune deficiencies
 - autoimmune diseases
 - chronic infections

Malignant lymphoproliferative diseases

What causes them?

HEALTH • CALIFORNIA

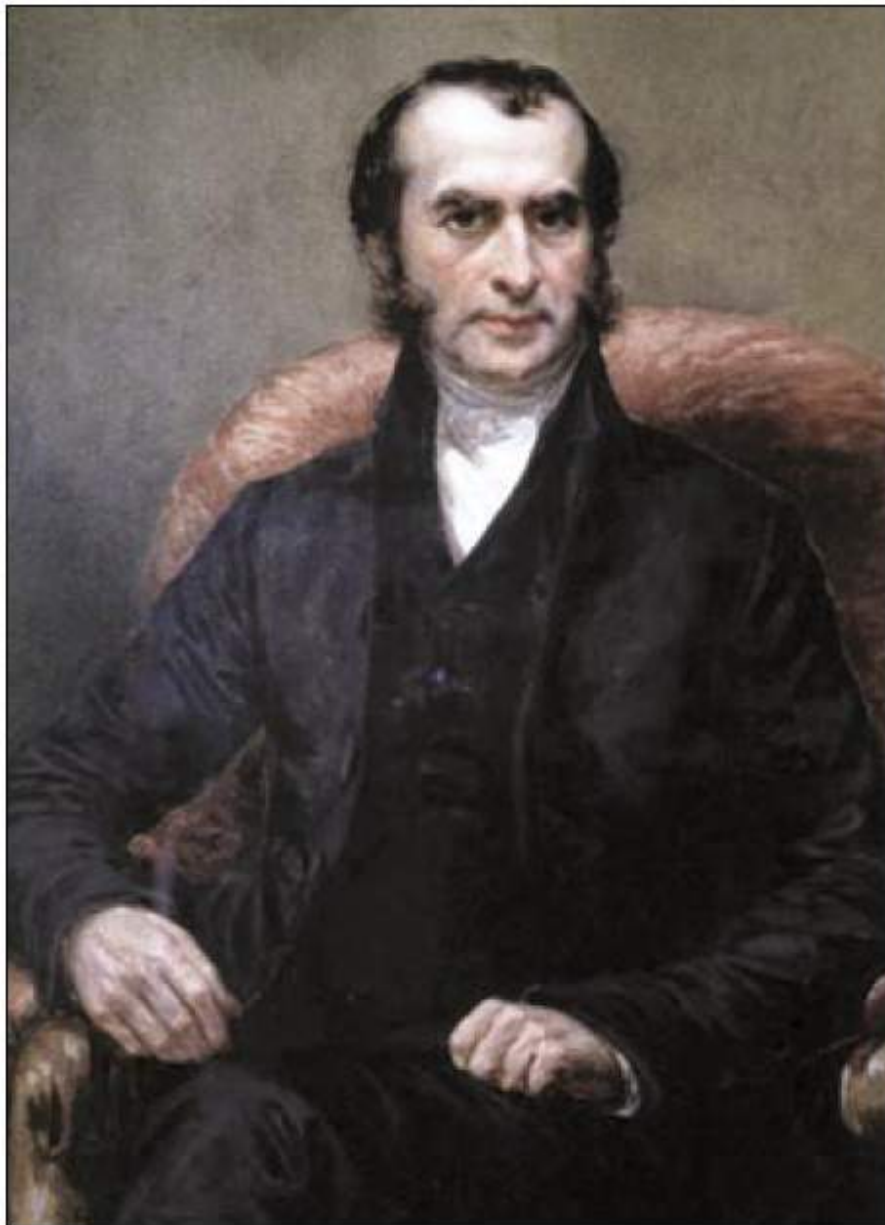
Jury Awards \$289 Million to Man Who Blames Monsanto's Roundup for Cancer



Malignant lymphoproliferative diseases

How are they classified?

Thomas Hodgkin 1798-1866



ON SOME
MORBID APPEARANCES
OF
THE ABSORBENT GLANDS
AND
SPLEEN.

BY DR. HODGKIN.

PRESENTED
BY DR. R. LEE.

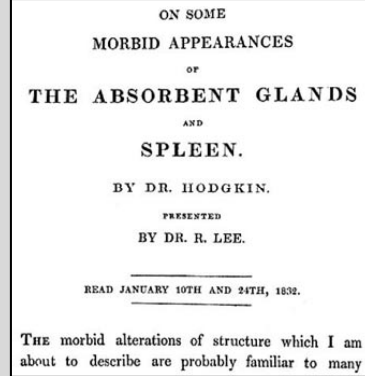
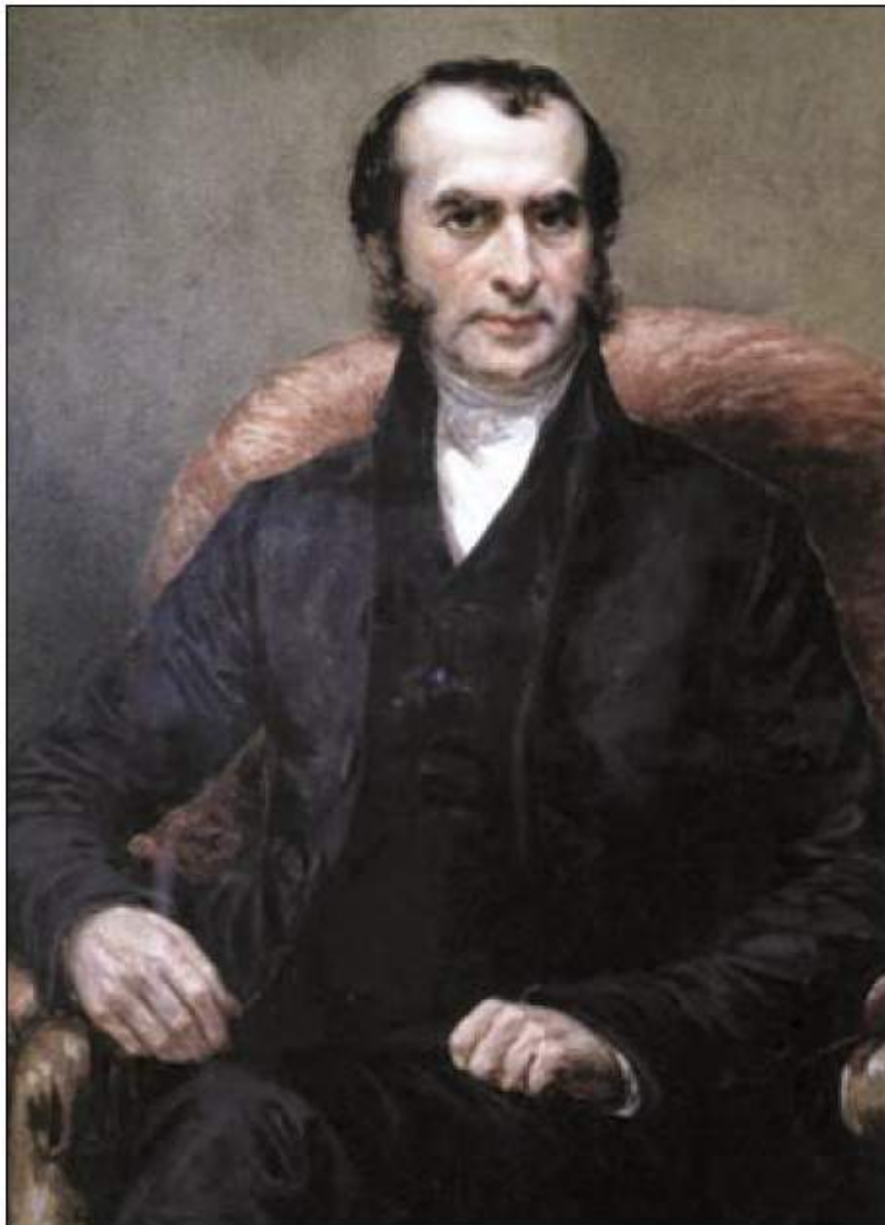
READ JANUARY 10TH AND 24TH, 1832.

THE morbid alterations of structure which I am
about to describe are probably familiar to many



Thomas Hodgkin – dissection
(Prof. Robert Carswell)

Thomas Hodgkin 1798-1866

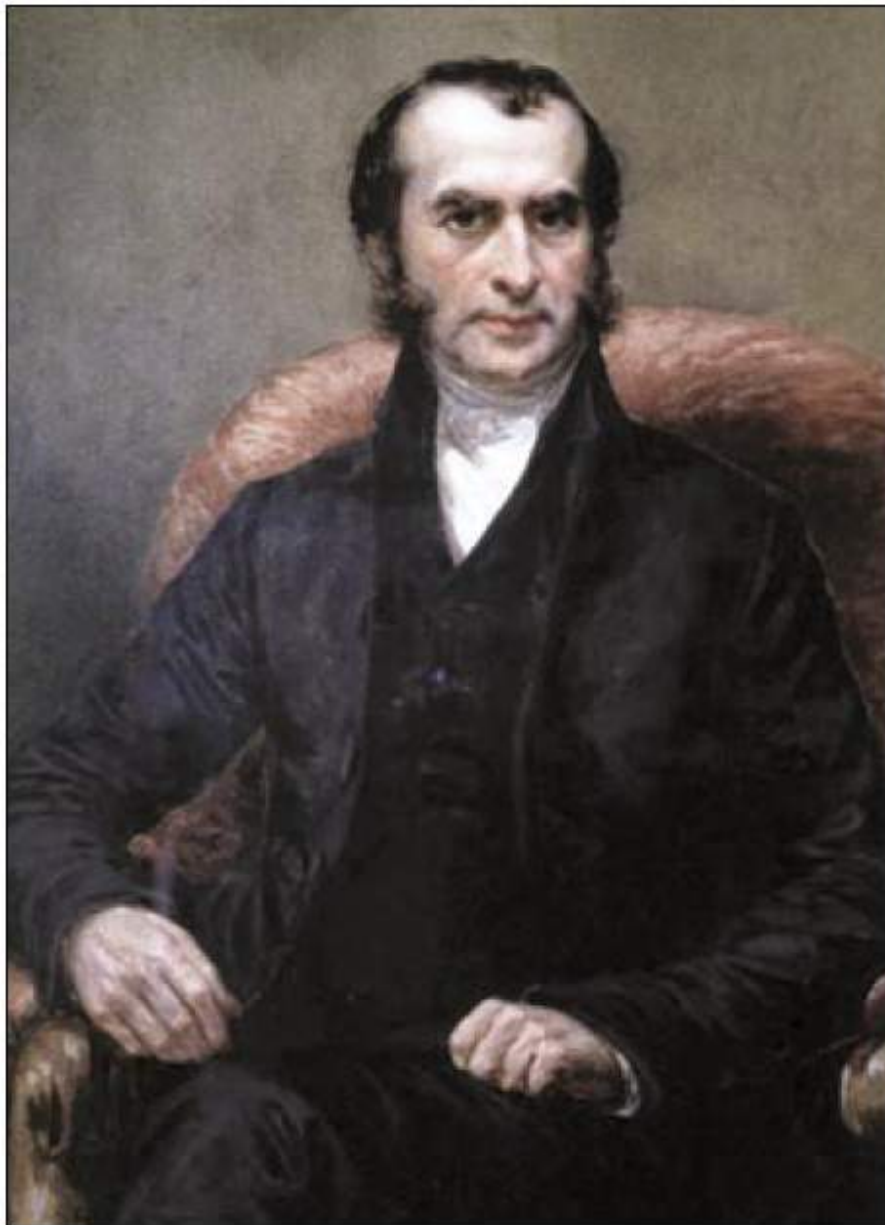


Hodgkin's original case:
abdominal nodes



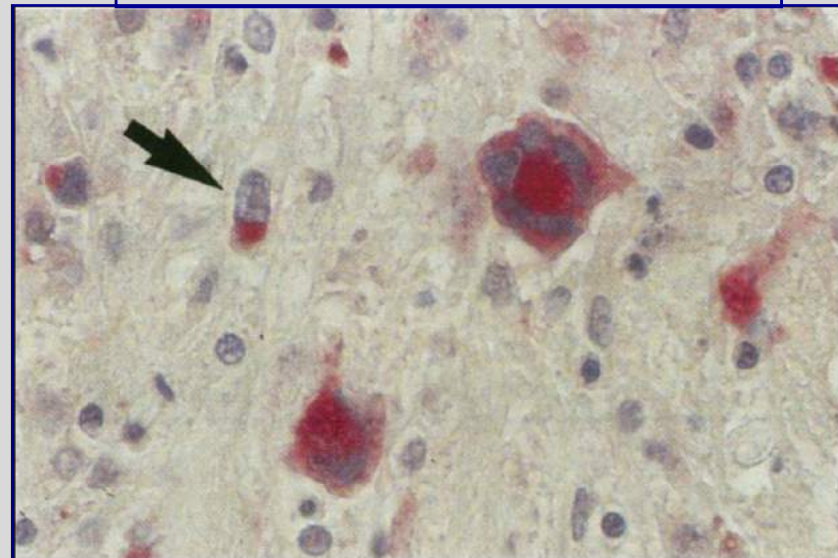
Gordon Museum,
King's College London

Thomas Hodgkin 1798-1866



ON SOME
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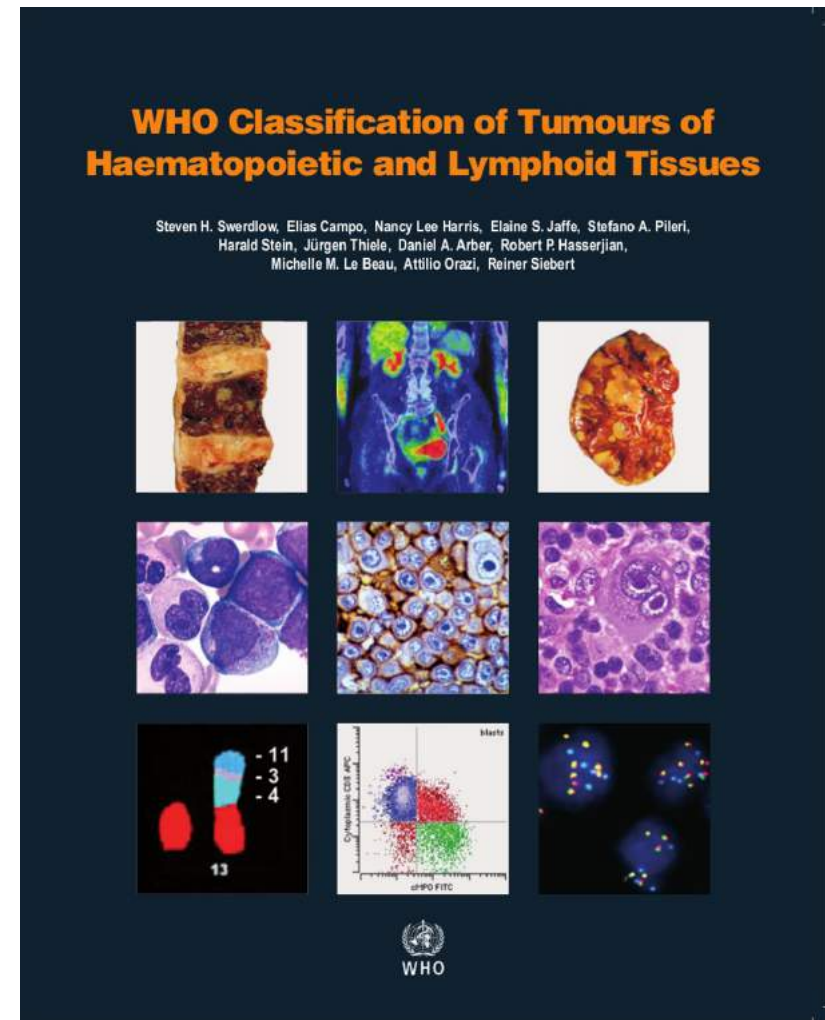
Hodgkin's original
case: CD15 (1991)



Paraffin-embedded tissue of one of the original cases by Thomas Hodgkin
immunostained with CD15 (David Mason)

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017

- 70s – 80s: Kiel classification
 - B vs T cells: IHC!!
- 90s: REAL classification
- WHO (latest2017)
 - "Real" disease entities
 - Clinical features
 - Morphology
 - Immunophenotype
 - Molecular genetics





Lymphoma



Hodgkins lymphoma

HL, LP
HL, NS
HL, MC
HL, LR
HL, LD

Non-Hodgkins lymphoma

B-cell

- precursor
- peripheral
 - ~32 subtypes

T/NK-cell

- precursor
- peripheral
 - ~20 subtypes

Updated WHO Classification – 2016 (2017)

Review Series

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

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. Jaffe¹¹

¹Division of Hematopathology, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Department of Pathology, Hospital Clinic, University of Barcelona, August Pi i Sunyer Biomedical Research Institute, Barcelona, Spain; ³Haematopathology Unit, European Institute of Oncology, Milan, and Department of Experimental, Diagnostic and Specialty Medicine, Bologna University Medical School, Bologna, Italy; ⁴Department of Pathology, Harvard Medical School and Massachusetts General Hospital, Boston, MA; ⁵Pathodiagnostik, Berlin, Germany; ⁶Institute of Human Genetics, Christian Albrechts University Kiel, Kiel, Germany; ⁷Division of Oncology, Department of Medicine, Stanford University, Stanford, CA; ⁸Department of Medical Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; ⁹Department of Hematology, Hospices Civils de Lyon, and Université Claude Bernard Lyon-1, Lyon, France; ¹⁰Department of Medicine, Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY; and ¹¹Hematopathology Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD

A revision of the nearly 8-year-old World Health Organization classification of the lymphoid neoplasms and the accompanying monograph is being published. It reflects a consensus among hematopathologists, geneticists, and clinicians regarding both updates to current entities as well as the addition of a limited number

of new provisional entities. The revision clarifies the diagnosis and management of lesions at the very early stages of lymphomagenesis, refines the diagnostic criteria for some entities, details the expanding genetic/molecular landscape of numerous lymphoid neoplasms and their clinical correlates, and refers to

investigations leading to more targeted therapeutic strategies. The major changes are reviewed with an emphasis on the most important advances in our understanding that impact our diagnostic approach, clinical expectations, and therapeutic strategies for the lymphoid neoplasms. (*Blood*. 2016;127(20):2375-2390)

Updated WHO Classification – 2016 (2017)

- It has not got any smaller!
- > 100 lymphoma entities

2376 SWERDLOW et al	BLOOD, 19 MAY 2016 • VOLUME 127, NUMBER 20
Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms	Table 1. (continued)
Mature B-cell neoplasms	Monomorphic epitheliotropic intestinal T-cell lymphoma*
Chronic lymphocytic leukemia/small lymphocytic lymphoma	Indolent T-cell lymphoproliferative disorder of the GI tract*
Monoclonal B-cell lymphocytosis*	Hepatosplenic T-cell lymphoma
B-cell prolymphocytic leukemia	Subcutaneous panniculitis-like T-cell lymphoma
Splenic marginal zone lymphoma	Mycosis fungoides
Hairy cell leukemia	Sézary syndrome
Splenic B-cell lymphoma/leukemia, unclassifiable	Primary cutaneous CD30 ⁺ T-cell lymphoproliferative disorders
Splenic diffuse red pulp small B-cell lymphoma	Lymphomatoid papulosis
Hairy cell leukemia-variant	Primary cutaneous anaplastic large cell lymphoma
Lymphoplasmacytic lymphoma	Primary cutaneous $\gamma\delta$ T-cell lymphoma
Waldenström macroglobulinemia	Primary cutaneous CD8 ⁺ aggressive epidermotropic cytotoxic T-cell lymphoma
Monoclonal gammopathy of undetermined significance (MGUS), IgM ⁺	Primary cutaneous $\alpha\alpha$ CD6 ⁺ T-cell lymphoma*
κ heavy-chain disease	Primary cutaneous CD4 ⁺ small/medium T-cell lymphoproliferative disorder*
λ heavy-chain disease	Peripheral T-cell lymphoma, NOS
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A ⁺	Angioimmunoblastic T-cell lymphoma
Plasma cell myeloma	Follicular T-cell lymphoma*
Solitary plasmacytoma of bone	Nodal peripheral T-cell lymphoma with TFH phenotype*
Extramedullary plasmacytoma	Anaplastic large-cell lymphoma, ALK ⁺
Monoclonal immunoglobulin deposition diseases*	Anaplastic large-cell lymphoma, ALK ⁻
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	Breast implant-associated anaplastic large-cell lymphoma*
Nodal marginal zone lymphoma	Hodgkin lymphoma
Pediatric nodal marginal zone lymphoma	Nodular lymphocyte predominant Hodgkin lymphoma
Follicular lymphoma	Classical Hodgkin lymphoma
In situ follicular neoplasia*	Nodular sclerosis classical Hodgkin lymphoma
Duodenal-type follicular lymphoma*	Lymphocyte-rich classical Hodgkin lymphoma
Pediatric-type follicular lymphoma*	Mixed cellularity classical Hodgkin lymphoma
Large B-cell lymphoma with IRF4 rearrangement*	Lymphocyte-depleted classical Hodgkin lymphoma
Primary cutaneous follicle center lymphoma	Posttransplant lymphoproliferative disorders (PTLD)
Mantle cell lymphoma	Plasmacytic hyperplasia PTLD
In situ mantle cell neoplasia*	Infectious mononucleosis PTLD
Diffuse large B-cell lymphoma (DLBCL), NOS	Ritid follicular hyperplasia PTLD*
Germinal center B-cell type*	Polymorphic PTLD
Activated B-cell type*	Monomorphic PTLD (B- and T-/NK-cell types)
T-cell/histiocyte-rich large B-cell lymphoma	Classical Hodgkin lymphoma PTLD
Primary DLBCL of the central nervous system (CNS)	Histiocytic and dendritic cell neoplasms
Primary cutaneous DLBCL, leg type	Histiocytic sarcoma
EBV ⁺ DLBCL, NOS*	Langerhans cell histiocytosis
EBV ⁺ mucocutaneous ulcer*	Langerhans cell sarcoma
DLBCL associated with chronic inflammation	Indeterminate dendritic cell tumor
Lymphomatoid granulomatosis	Interdigitating dendritic cell sarcoma
Primary mediastinal (thymic) large B-cell lymphoma	Follicular dendritic cell sarcoma
Intravascular large B-cell lymphoma	Fibroblastic reticular cell tumor
ALK ⁺ large B-cell lymphoma	Disseminated juvenile xanthogranuloma
Plasmablastic lymphoma	Erdheim-Chester disease*
Primary effusion lymphoma	
HHV8 ⁺ DLBCL, NOS*	
Burkitt lymphoma	
Burkitt-like lymphoma with t(1q aberration)*	
High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements*	
High-grade B-cell lymphoma, NOS*	
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	
Mature T and NK neoplasms	
T-cell prolymphocytic leukemia	
T-cell large granular lymphocytic leukemia	
Chronic lymphoproliferative disorder of NK cells	
Aggressive NK-cell leukemia	
Systemic EBV ⁺ T-cell lymphoma of childhood*	
Hidradenoma-like lymphoproliferative disorder*	
Adult T-cell leukemia/lymphoma	
Extranodal NK-/T-cell lymphoma, nasal type	
Enteropathy-associated T-cell lymphoma	

Provisional entities are listed in *italics*.
*Changes from the 2008 classification.

small population, but in others associated with a lymphocytosis.⁴ Whereas in 2008 it was unknown whether MBL was a precursor of CLL, we now know that MBL precedes virtually all cases of CLL/small lymphocytic lymphoma (SLL).⁵ The updated WHO will retain the current criteria for MBL, but will emphasize that “low-count” MBL, defined as a PB CLL count of $<0.5 \times 10^9/L$, must be distinguished from “high-count” MBL because low-count MBL has significant differences from CLL, an extremely limited, if any, chance of progression, and, until new evidence is provided, does not require routine follow-up outside of standard medical care.^{6,7} In contrast, high-count MBL requires routine/regular follow-up, and has very similar phenotypic and genetic/molecular features as Rai stage 0 CLL, although immunoglobulin heavy chain variable region (IGHV)-mutated cases are more frequent in MBL.⁸ Also impacting our diagnostic criteria, the revision will eliminate the option to diagnose CLL with $<5 \times 10^9/L$ PB CLL cells in the absence of extramedullary

Why does it all have to be so complicated!?

Because of "Personalized Medicine" –

- Many subtypes of lymphoma are rare**
- But.... they require specific treatments**

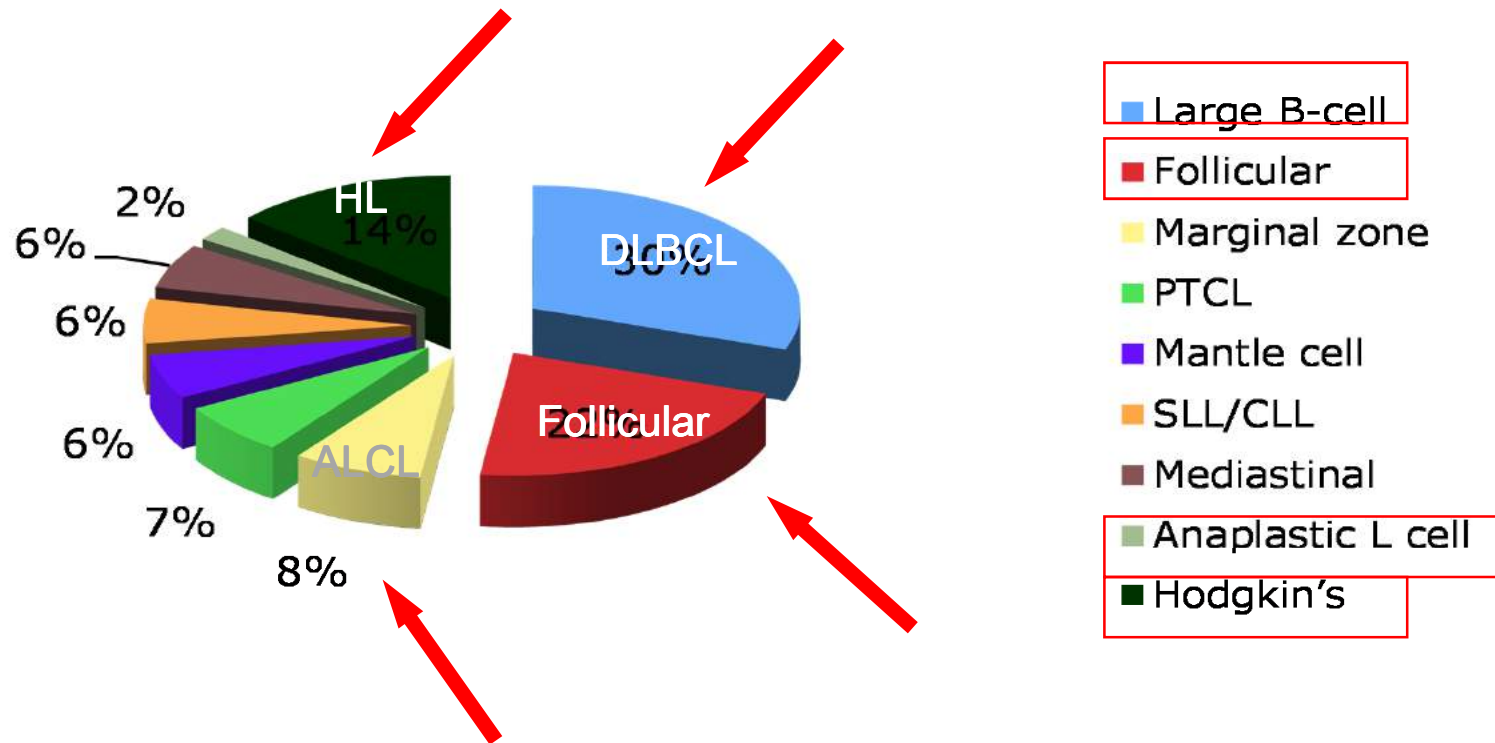
Updated WHO Classification – 2017)

Major immunophenotypic changes:

Diffuse large B-cell lymphoma

- COO – *cell of origin* analysis now required
 - to distinguish GCB vs *ABC*/non-GC types
 - either by gene expression profiling or immunohistochemistry
- IHC for MYC and BCL2 expression
 - to identify "*double -expressors*"

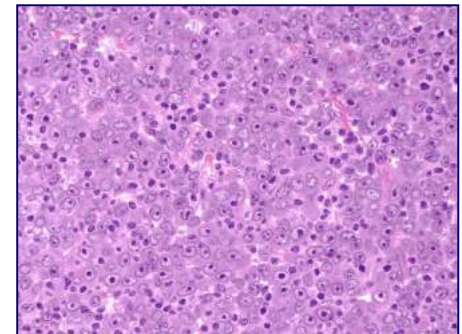
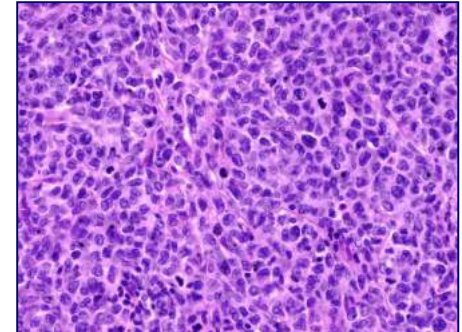
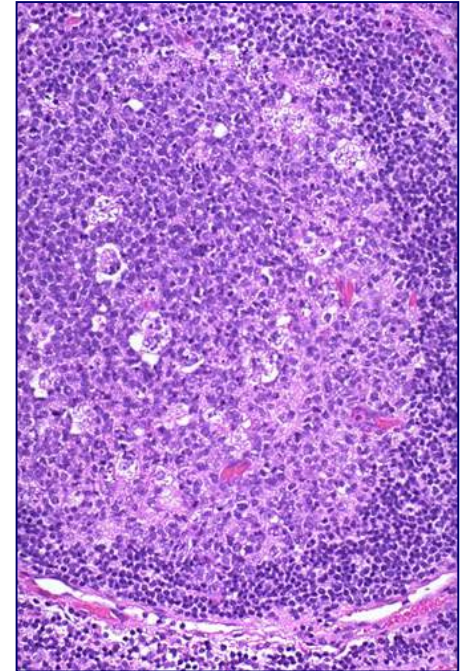
Lymphoma frequencies



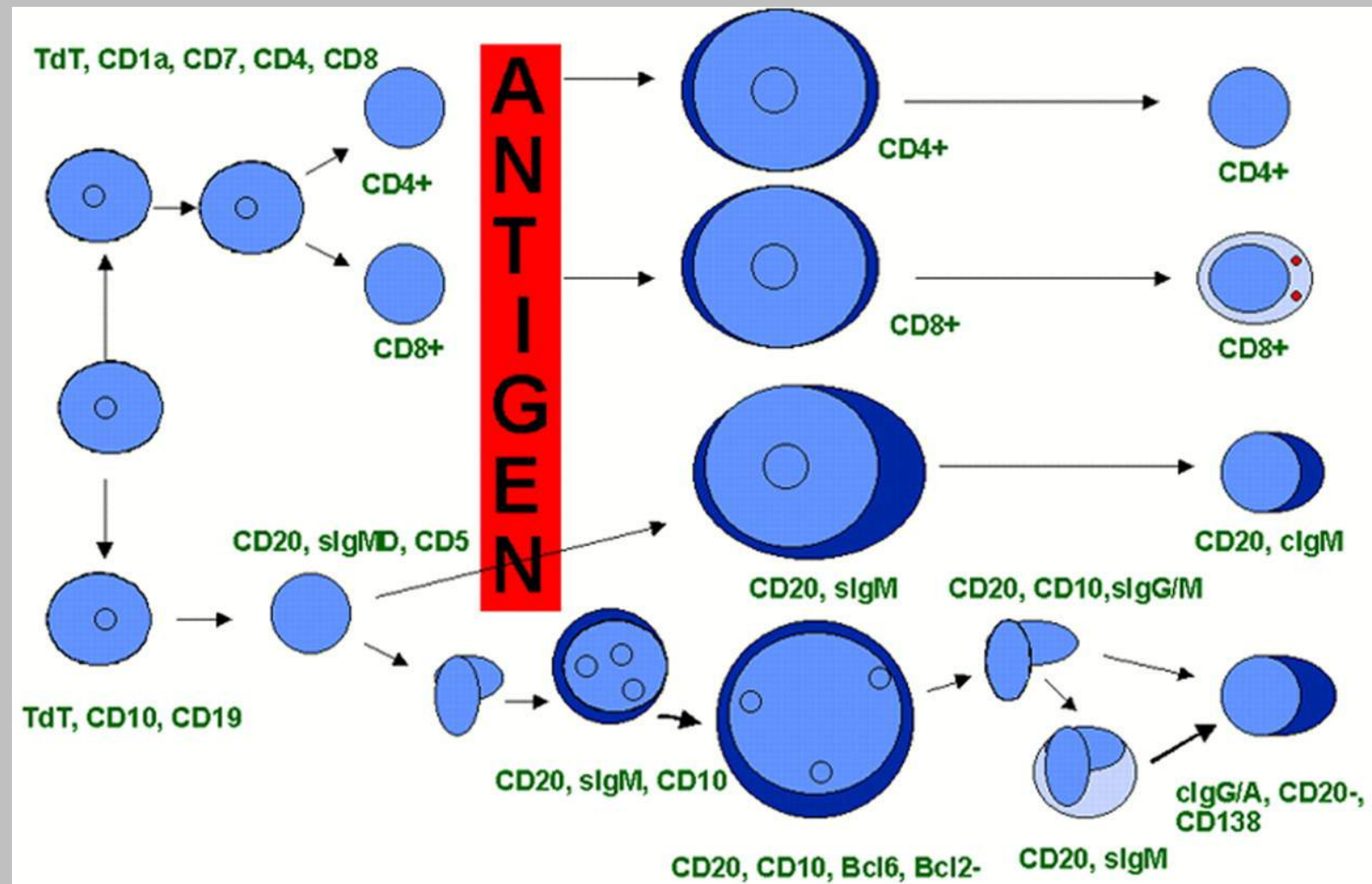
2002 SEER database. O'Connor

What is lymphoma?

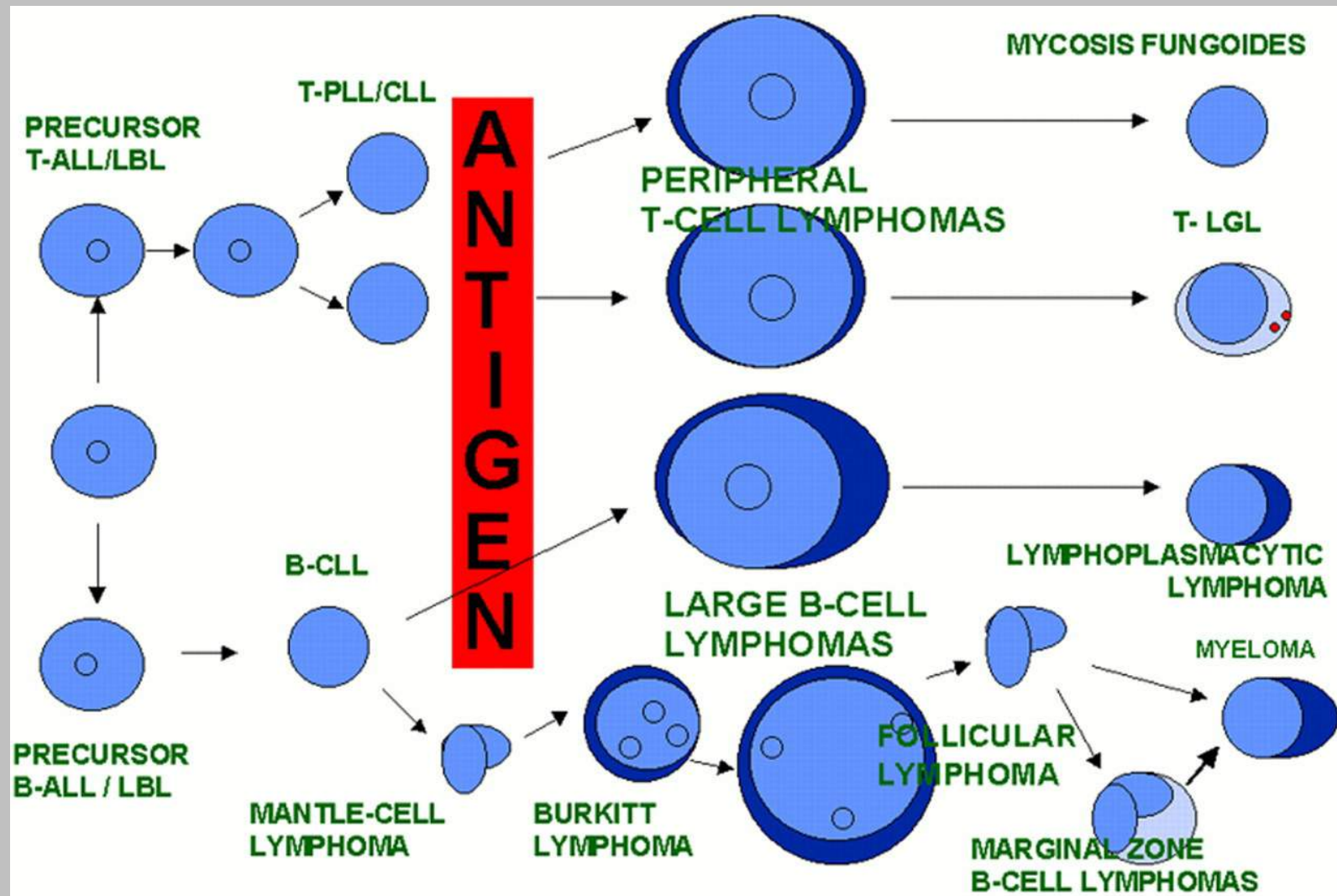
- **Clonal malignancy**
 - → mutational events cause cells to freeze at a single stage of normal lymphocyte differentiation
- **Morphology, immunophenotype & molecular features:**
 - mirror stages of normal lymphocyte development



T and B-cell differentiation: Stage-specific surface antigen expression

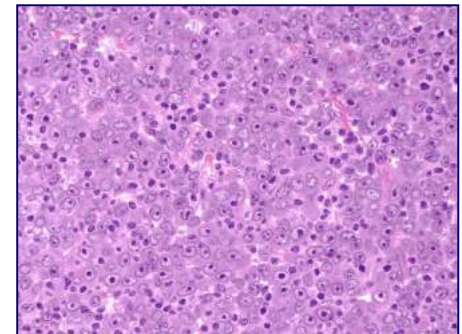
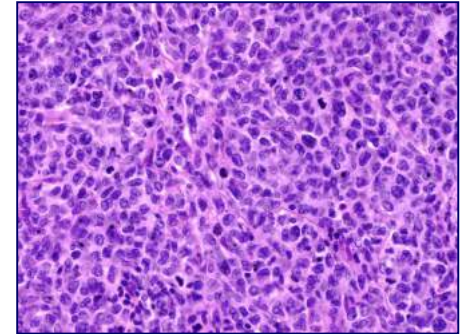
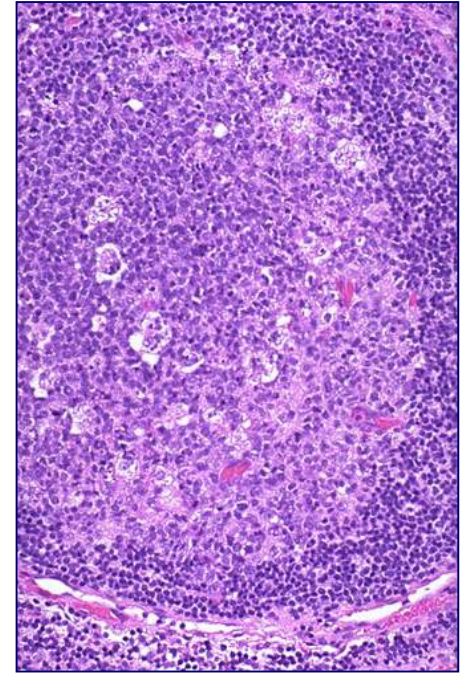


Lymphoid neoplasms: Correlation with normal T or B-cell differentiation



What is lymphoma?

- Clonal malignancy
 - → mutational events cause cells to freeze at a single stage of normal lymphocyte differentiation
- Morphology, immunophenotype & molecular features:
 - mirror stages of normal lymphocyte development
- **Resemble normal haematopoietic cells in their:**
 - morphology, **immunophenotype**, molecular genetics



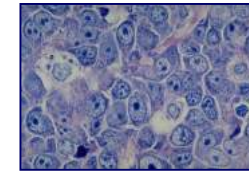
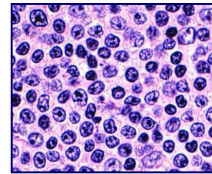
Lymphoma & Leukaemia diagnosis

- Clinical features
- **Morphology**
- Immunophenotype
- Molecular diagnosis

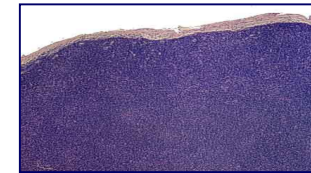
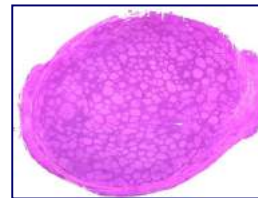
Lymphoma differential diagnosis

- **Assess morphology:**

- **cell size**



- **architecture**



But...that's not enough!

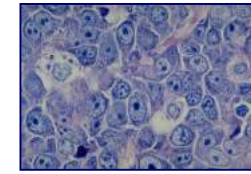
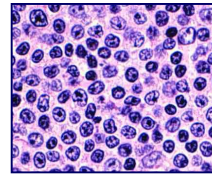
Lymphoma & Leukaemia diagnosis

- Clinical features
- Morphology
- **Immunophenotype**
- Molecular diagnosis

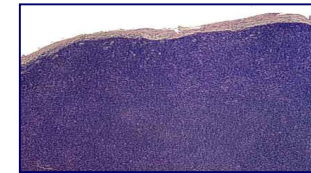
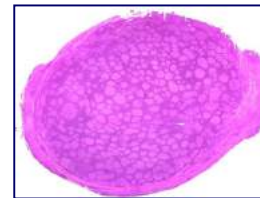
Lymphoma differential diagnosis

- Assess morphology:

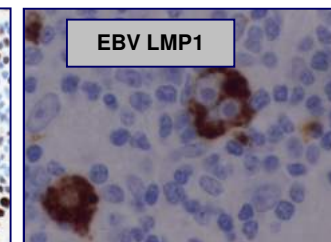
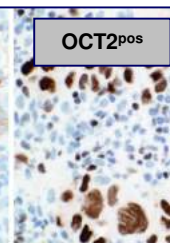
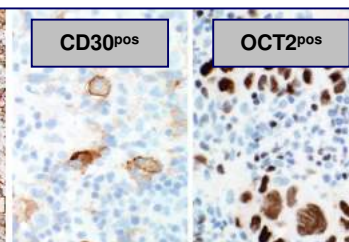
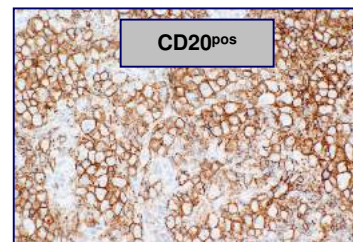
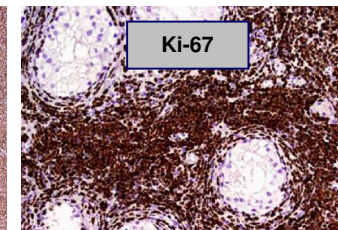
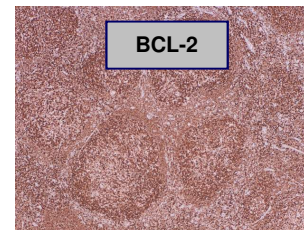
- cell size



- architecture



- Select appropriate immune panel(s)



Enlarged lymph node

Is it malignant?



- **Emphasis on lymphoma classification**
- **Reactive vs malignant**
 - often more challenging diagnosis
- **Use IHC to evaluate lymphoid tissue cytology and architecture**
- **Correlate immunophenotype with disease entity**

International recommendations for lymphoma diagnostics

Danish
lymphoma group

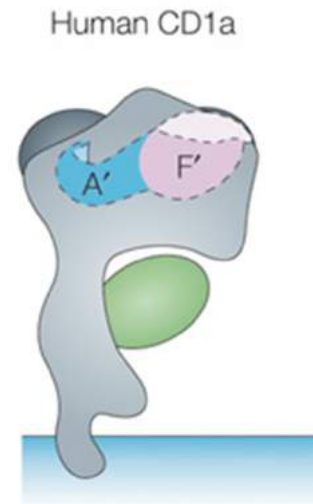
<http://www.lymphoma.dk/index.php?id=56,0,0,1,0,0>

See "Lymfomdiagnostik"

UK: RCPATH / BCSH

<https://www.rcpath.org/resourceLibrary/dataset-for-the-histopathological-reporting-of-lymphomas.html>

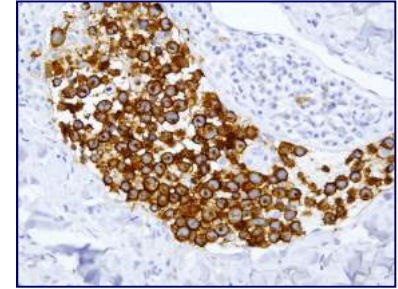
What are CD numbers?



- **CD: "clusters of differentiation"**
- **Classification system for antigens (and antibodies)**
- **Originally for surface antigens on leucocytes**
- **Now includes other cells and intracellular antigens (no CD no.)**
- **10 workshops since 1982**
- **Currently > 350 CD antigens**

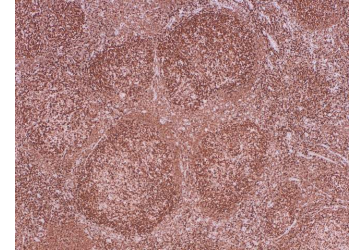
IHC Dogma

(also applies in diagnostic haematopathology)



- **IHC complements routine staining**
- **Helps characterize cells and architecture**
- **No single antibody is disease specific**
- **Antibodies should be used in panels**
- **Interpret findings in relation to the histology**

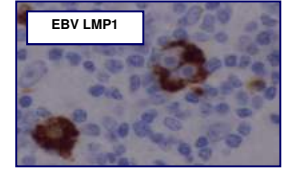
Diagnostic Applications of IHC 1



Reactive vs malignant

- Polyclonal vs monoclonal Ig
- Follicular hyperplasia vs follicular lymphoma
- Diff. diagnosis of small cell B-cell lymphomas
 - CLL/SLL vs MALT vs FL vs Mantle cell
- Aggressive B-cell lymphomas
 - DLBCL vs BL vs BL-like / grey-zone NHL
 - DLBCL – ‘cell of origin’ – GCB vs ABC

Diagnostic Applications of IHC 2



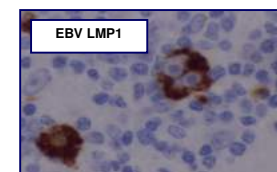
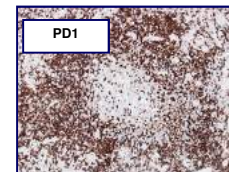
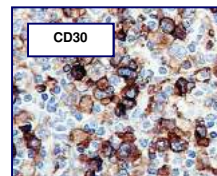
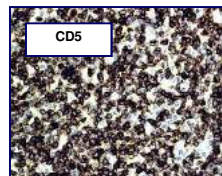
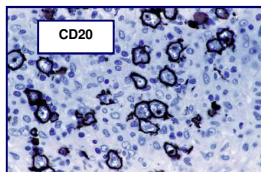
- **T-cell lymphoma vs B-cell lymphoma**
- **T-cell lymphoma vs T-zone hyperplasia**
- **Hodgkin lymphoma vs NHL**
- **Hodgkin lymphoma**
 - NLPHL vs classical HL
- **Lymphoblastic vs. Myeloblastic vs. Burkitt**
- **Undifferentiated malignant tumor**
- **Lymphoma prognosis**
 - e.g. Ki-67; ALK; c-myc
- **Targeted therapy**
 - e.g. CD20 / Rituximab; CD30 / Brentuximab; Alemtuzumab (anti-CD52)

Useful antigens in haematopathology

- **CD45**
- **B-cell 'specific'**
 - CD19
 - CD20
 - CD79 α
 - Pax-5
 - OCT-2 / BOB1
 - Ig
- **T-cell 'specific'**
 - CD3
 - CD5
 - CD2
 - CD7
 - CD1a
 - CD4
 - CD8
 - PD-1/CXCL-13 (TFH)

- **Other**
 - CD30
 - CD10
 - Bcl-2
 - Bcl-6
 - ALK
 - c-myc
 - CD21
 - CD23
 - CD15
 - TdT
 - Cyclin-D1
 - SOX-11
 - CD56
 - TIA-1, granzyme, perforin

- **Other**
 - EBV
 - LMP1
 - EBNA2
 - (EBER)
 - CD56
 - CD57
 - EMA
 - S100
 - CD68
 - CD163

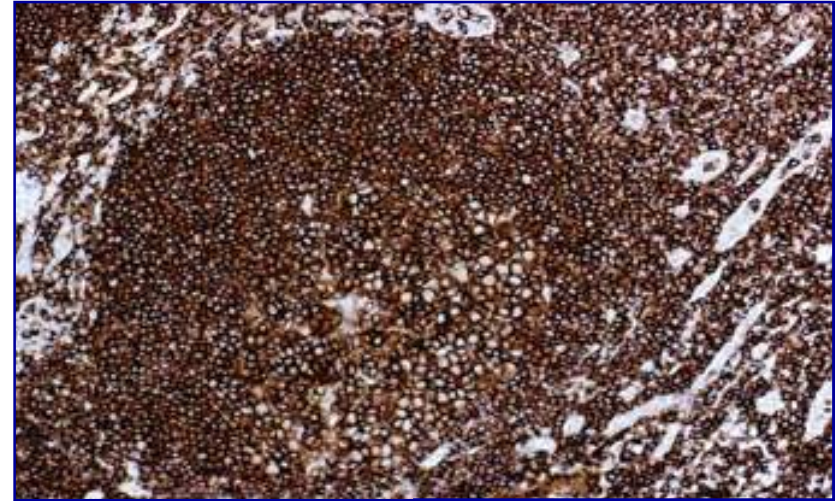


Basic IHC panel for lymphoma diagnosis

- CD45
- CD20
- CD79 α
- (PAX-5)
- kappa/lambda
- CD3
- CD5
- CD30
- CD43
- Bcl-2
- Bcl-6
- CD23 (CD21)
- Cyclin-D1
- Ki-67

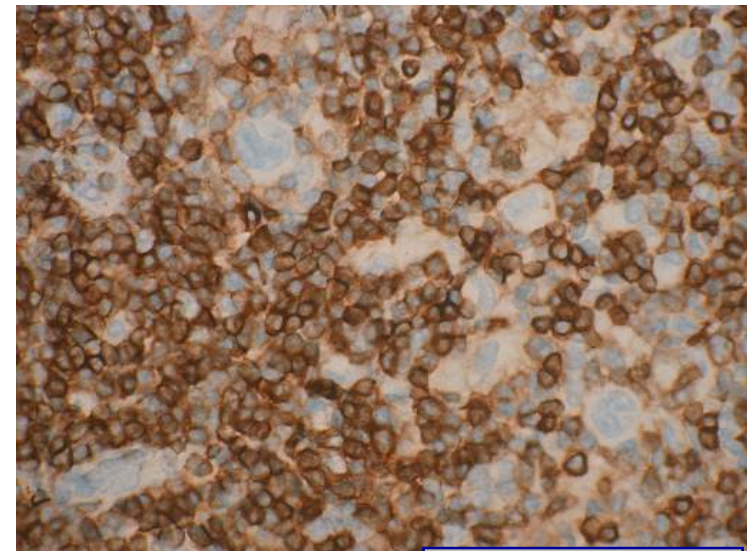
Basic stains: CD45

- Membrane glycoprotein family
- Positive in all (?) hæmopoietic cells
- Not expressed on non-BM-derived cells
- CD45 isoforms are more lineage specific



Reactive LN: CD45

- In lymphomas:
 - Most NHLs positive
 - Often/always negative in:
 - Precursor LB
 - Plasma cell neoplasia
 - Anaplastic large cell lymphoma
 - Hodgkins lymphoma:
 - LP: Popcorn cells positive
 - HRS cells in classical HL are negative



HL, NC: CD45

Basic stain: Immunoglobulin

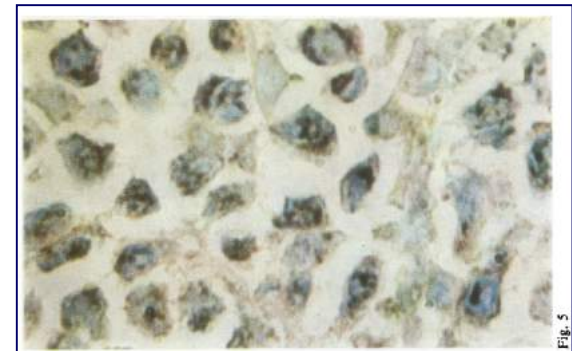
J. clin. Path., 1974, 27, 14-20

The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase-labelled antibody

C. R. TAYLOR AND J. BURNS

From the Department of Pathology, Gibson Laboratories, Radcliffe Infirmary, Oxford

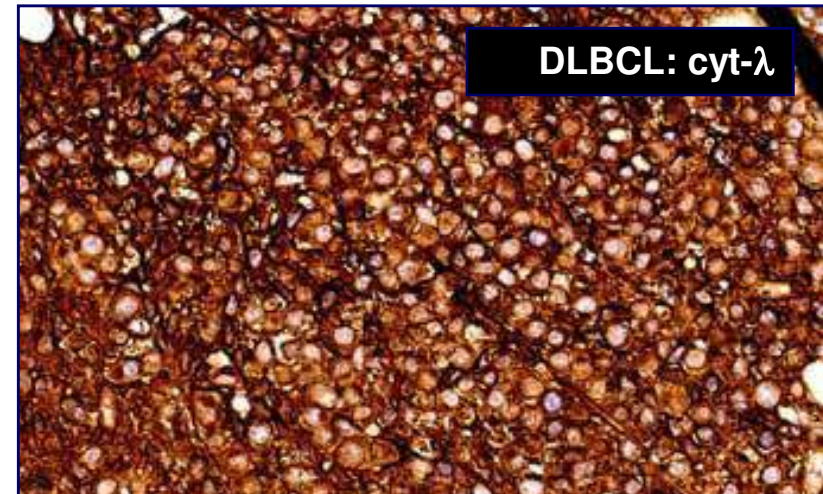
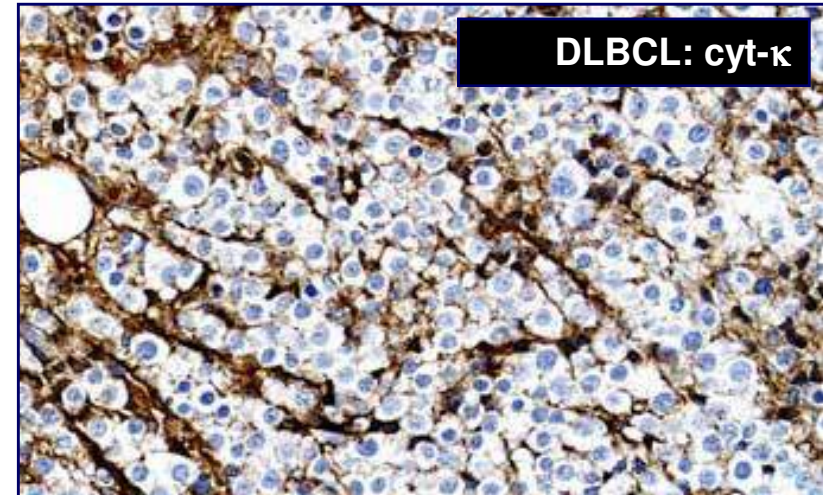
- IHC-Ig
 - first protocol for IHC in FFPE
 - still one of the hardest to perform & evaluate!



- plasmacytoma
- monoclonal Ig-kappa

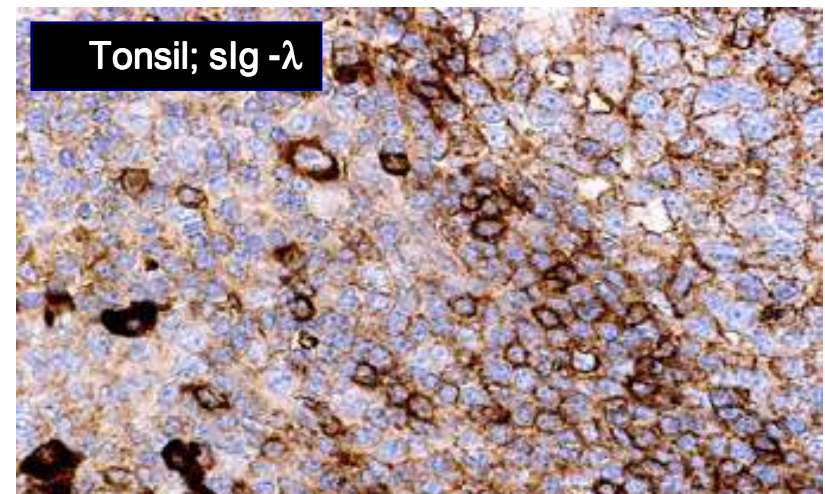
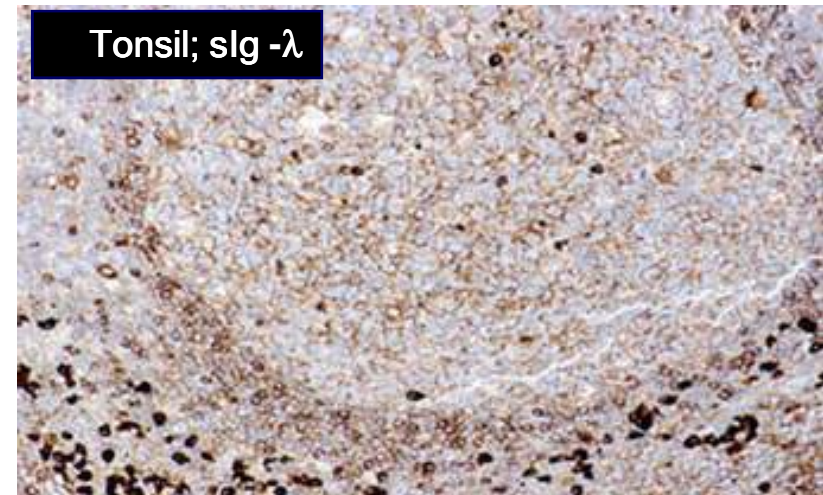
Basic stains: Immunoglobulin

- B-cell specific
- Normal $\kappa:\lambda$ ratio ca. 3-4:1
- Monotypic Ig restriction
 - Suggests clonality
 - $>10:1$ or $< 0.2:1$ = restriction
- Cytoplasmic Ig easily shown
- In lymphomas:
 - Cy Ig:
 - lymphoplasmacytic; myeloma; MZL; DLBCL, FL
 - Surface Ig



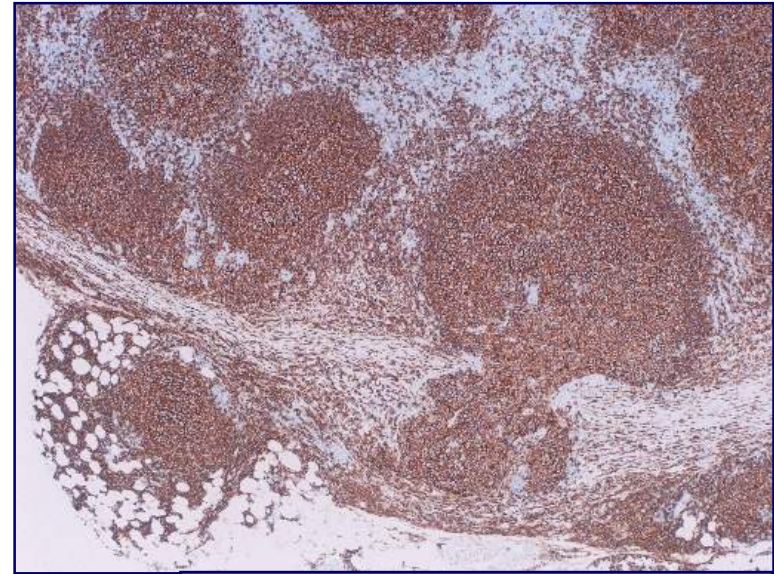
Basic stains: Immunoglobulin

- **Surface Ig**
 - B-NHL clonality
 - Requires sensitive, optimised technique
 - Interpretation difficult (serum Ig)



Basic stains: CD20

- **Many B-cell neoplasms**
- **Negative in:**
 - early precursor B-LB
 - plasma cell neoplasms
- **Negative in T-cell lymphomas**
 - rare cases positive
- **Hodgkins lymphoma**
 - HL-LP: 90% positive
 - Other types – variably positive
(10% - 30%; not all HRS cells)
- **Predictive marker for Rituximab therapy**



Follicular lymphoma: CD20

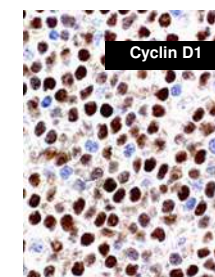
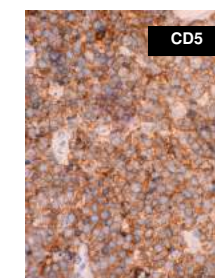
Usual staining pattern of B-cell neoplasms

	CD20	CD79	CD5	CD23	CD10	CD30	CD15	CyclinD1
Precursor B-cell neoplasms								
Precursor B-lymphoblastic leukaemia/lymphoma	–	+/-	–	–	+	–	–	–
Mature B-cell neoplasms								
B-cell chronic lymphocytic leukaemia/lymphoma	+	+	+	+	–	–	–	–
B-cell prolymphocytic leukaemia	+	+	–	+/-	–	–	–	-/+
Lymphoplasmacytic lymphoma	+	+	–	-/+	–	–	–	–
Mantle cell lymphoma	+	+	+	–	–	–	–	+
Follicular lymphoma,	+	+	–	-/+	+	–	–	–
Marginal zone B-cell lymphoma of mucosa associated lymphoid tissue type	+	+	–	–	–	–	–	–
Nodal marginal zone lymphoma +/- (monocytoid B-cells)	+	+	–	–	–	–	–	–
Splenic marginal zone lymphoma	+	+	–	–	–	–	–	–
Hairy cell leukaemia	+	+	–	–	–	–	–	–
Plasmacytoma	–	+	–	–	–	-/+	–	–
Plasma cell myeloma	–	+/-	–	–	–	-/+	–	–
Diffuse large B-cell lymphoma	+	+	-/+	-/+	-/+	-/+	–	–
Mediastinal (thymic)	+	+	–	+/-	-/+	-/+	-/+	–
Intravascular	+	+	-/+	–	-/+	-/+	–	–
Primary effusion lymphoma	–	+	–	–	–	+	–	–
Burkitt's lymphoma	+	+	–	–	+	–	–	

Key

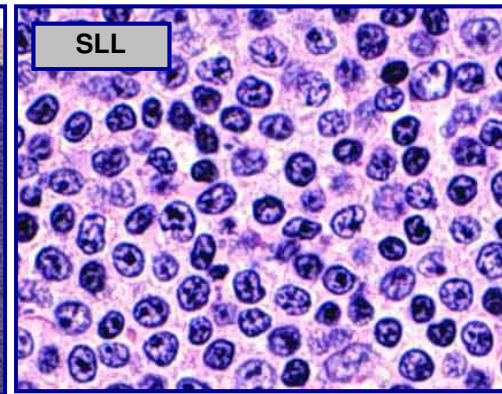
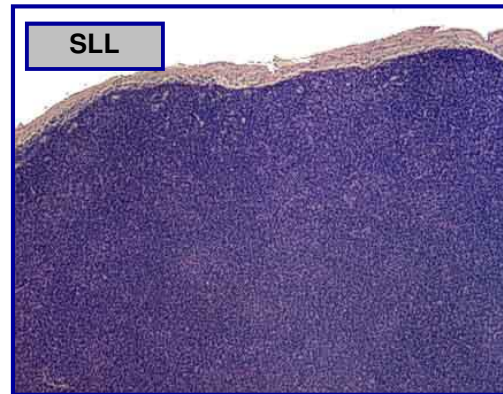
+/- The lymphoma cells are commonly but not always positive

-/+ The lymphoma cells are usually but not always negative



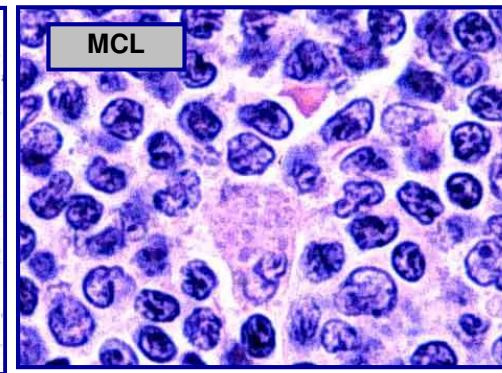
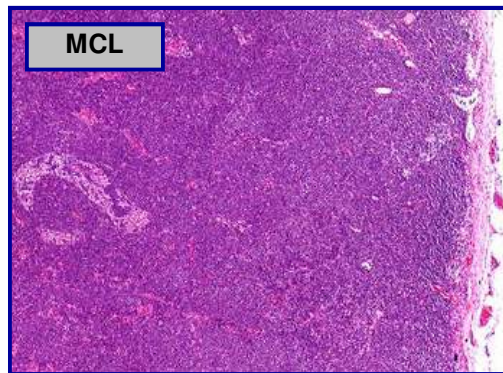
Small cell B-Cell lymphomas: Differential Diagnosis

Small lymphocytic NHL

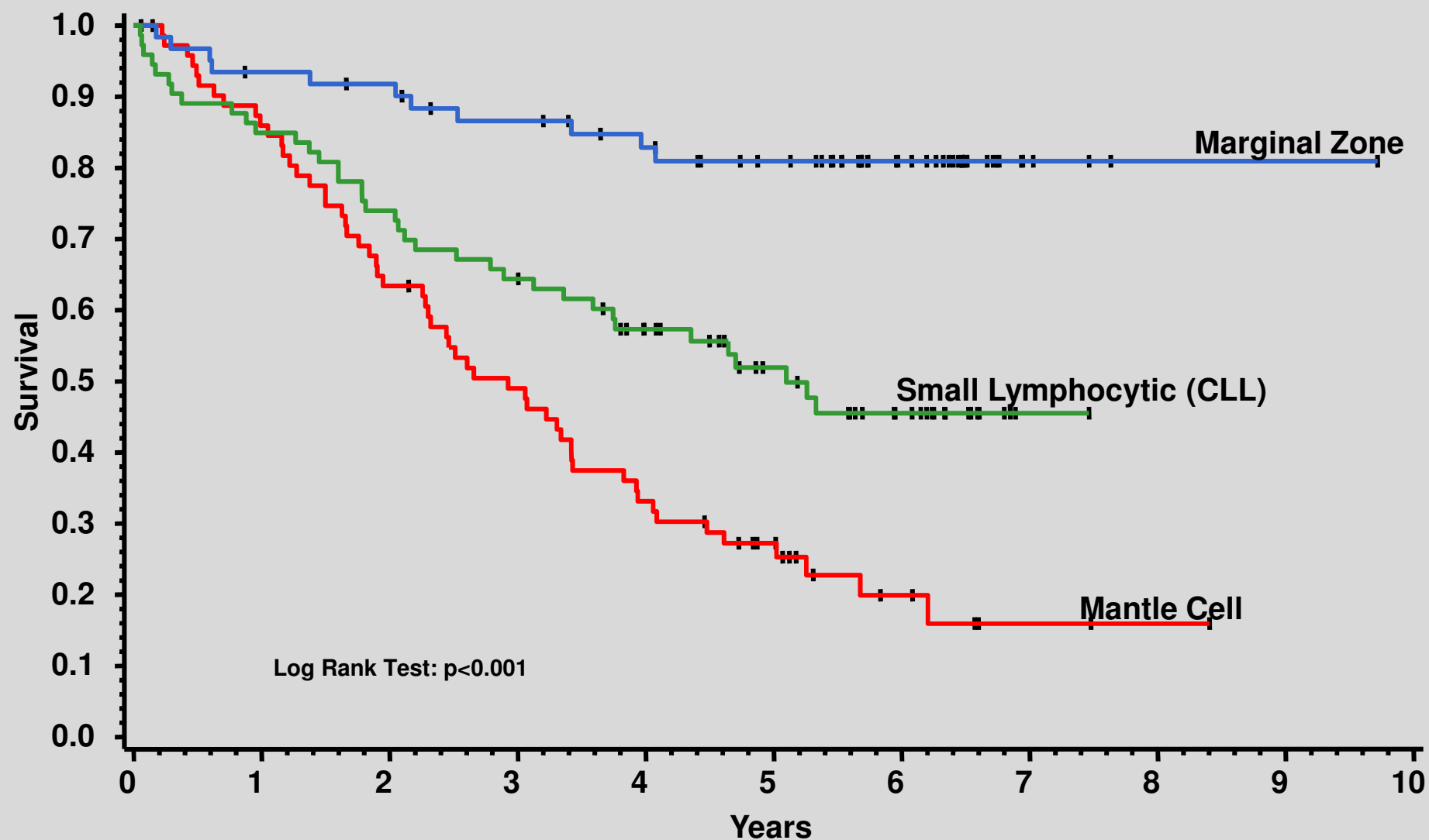


?

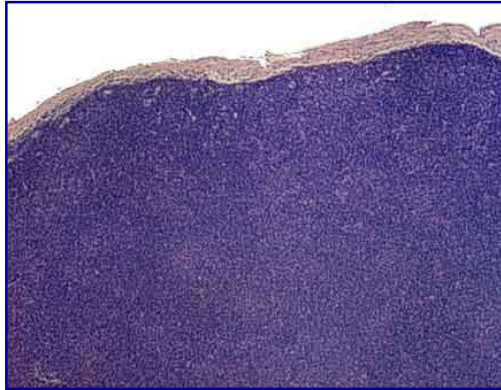
Mantle cell NHL



Small B-Cell Lymphomas: Overall Survival

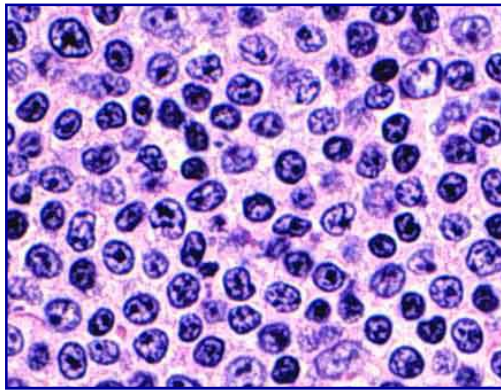
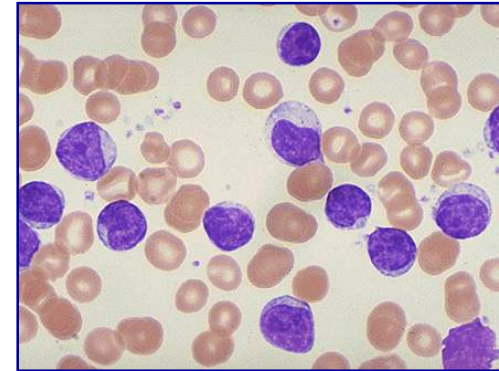


B-cell Small Lymphocytic Lymphoma (CLL)



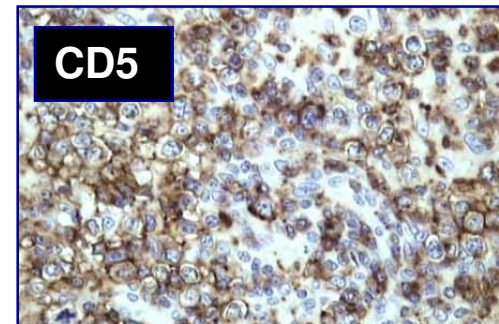
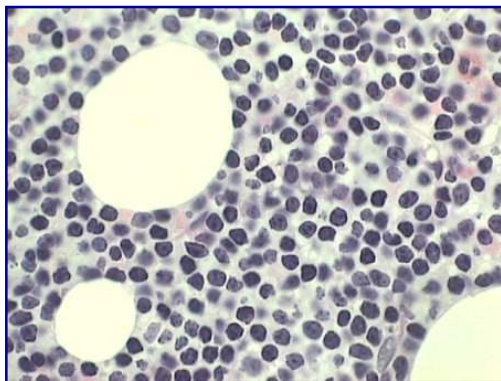
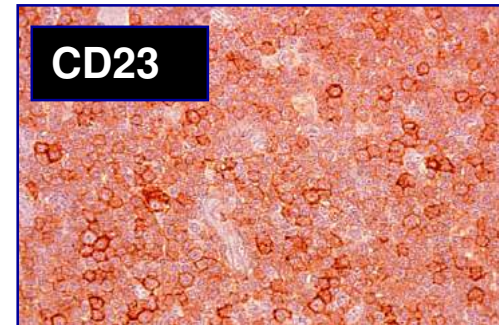
Morphology

- small lymphocytes
- proliferation centres

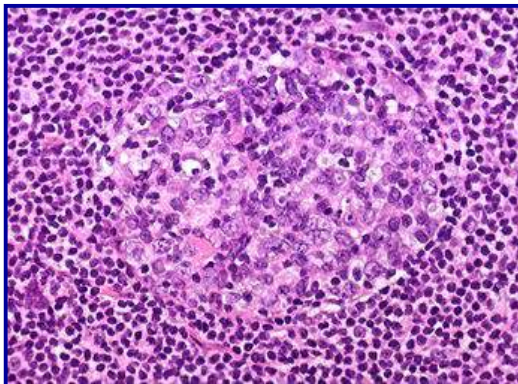
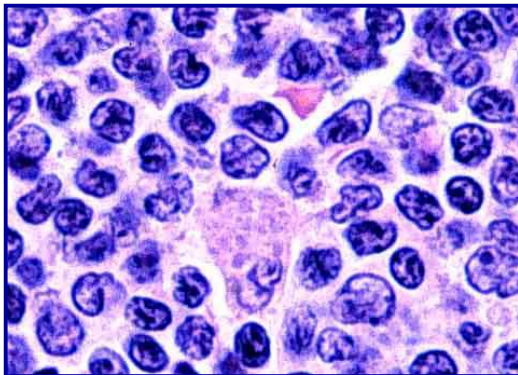
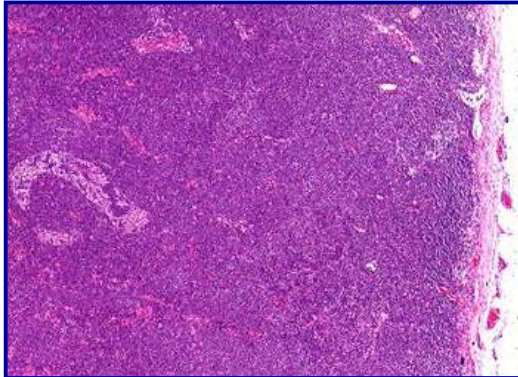


Immunology

- surface IgMD weak
 - CD19, 20, 79a
 - CD5
 - CD23
 - CD10, CysD1
- | | |
|--|---|
| | + |
| | + |
| | + |
| | - |



Mantle Cell Lymphoma

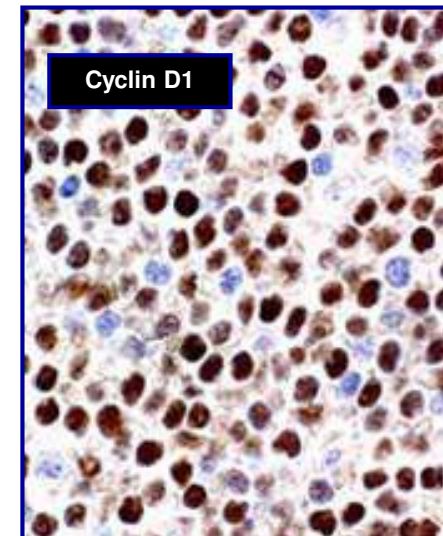
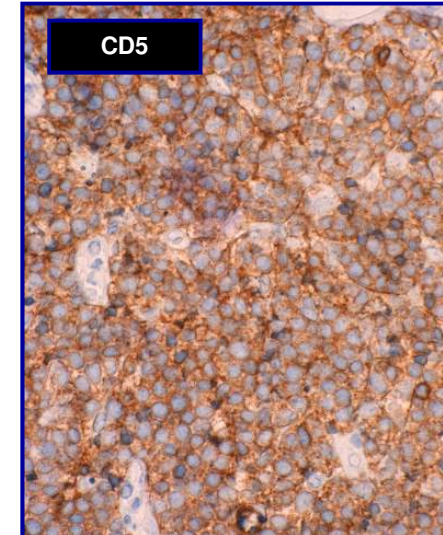


Morphology

- small-medium lymphocytes
- cleaved / irregular
- blastoid variant
- nodular / mantle / diffuse

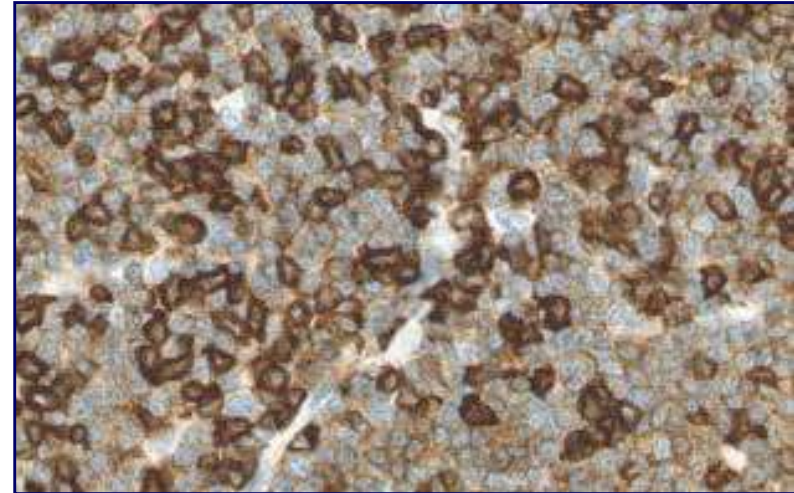
Immunology

- | | |
|---------------------|---|
| • surface Ig | + |
| • CD19, 20, 22, 79a | + |
| • CD5 | + |
| • CD23 | - |
| • Cyclin D1 | + |
| • CD10 | - |



Basic stains: CD5

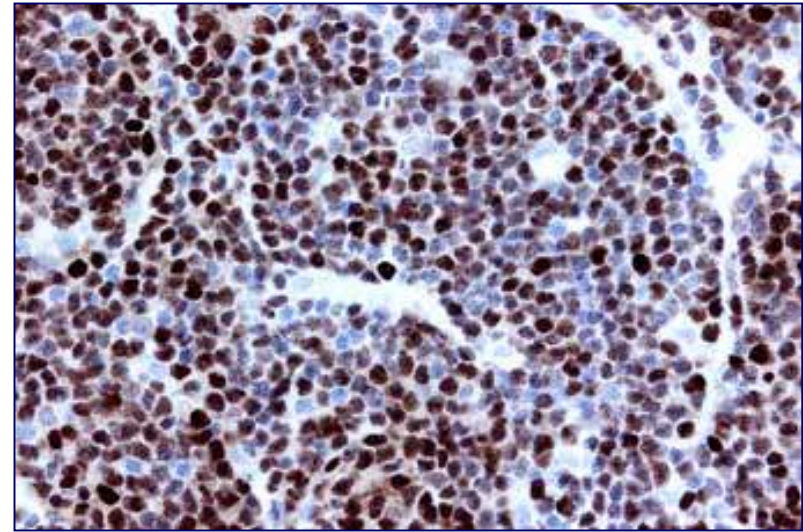
- **Modulates T & B cell signalling**
- **Pan-T cell marker**
 - 95% thymocytes
 - 100% post-thymic T-cells
 - ↑ expression with maturity
- **Minor population normal B-cells:**
 - ca. 10%+ peripheral B-cells
 - ↑ in autoimmunity
- **Lymphomas:**
 - 90% T-cell neoplasias
 - B-cell NHL
 - B-CLL / SLL (90%)
 - Mantle cell NHL (90%)
 - 10%+ DLBCL



- B-CLL
- B-cells 'dim'
- reactive T-cells 'strong'

Basic stains: Cyclin D1

- **cyclin family**
 - control cell cycle
- **normal proliferating cells, e.g. basal epidermal cells positive**
- **variable clone sensitivity**
- ***Bcl-1* gene product at 11q13**
- **upregulated in cells with t(11;14)**
- **>90% MCLs positive (nuclear)**
- **15% myelomas positive (nuclear)**

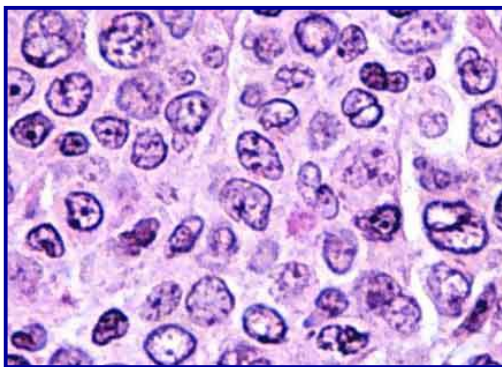
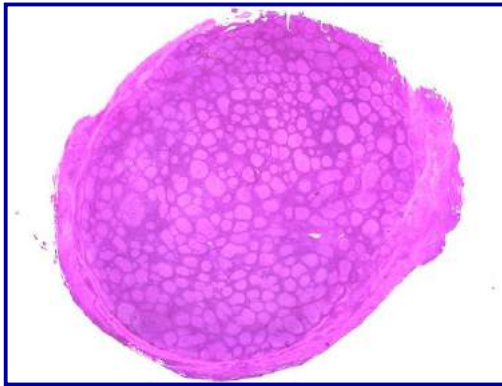


Mantle cell NHL: cyclin-D1

Immunophenotype: Small B-Cell Lymphomas

	CD20	CD79A	CD10	CD23	CD5	CD43	bcl-2	CyclinD1	TdT
CLL	+	+	-	+	+	+	+	-	-
FL	+	+	+	-	-	-	+	-	-
MCL	+	+	-	-	+	+	+	+	-
LPL	+	+	-	-	-	- / +	+	-	-
MZL	+	+	-	-	-	- / +	+	-	-
SMZ	+	+	-	-	-	- / +	+	-	-
MALT	+	+	-	-	-	- / +	+	-	-
HCL	+	+	-	-	-	-	+	-	-
BLB	- / +	+	+ / -	+ / -	-	-	+	-	+

Follicular Lymphoma

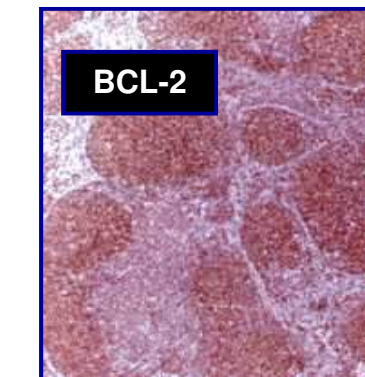
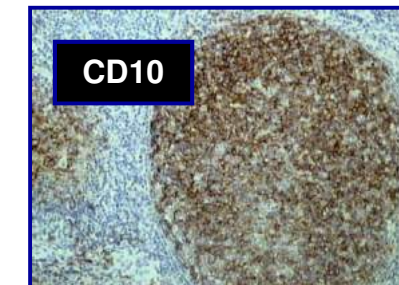
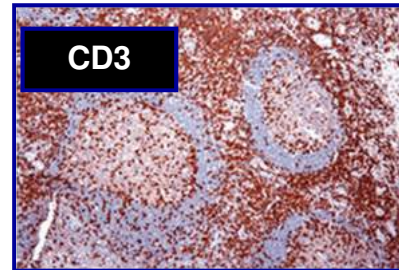
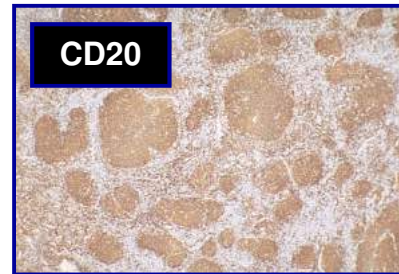


Morphology

- germinal centre cells
- CBs & CCs
- follicular

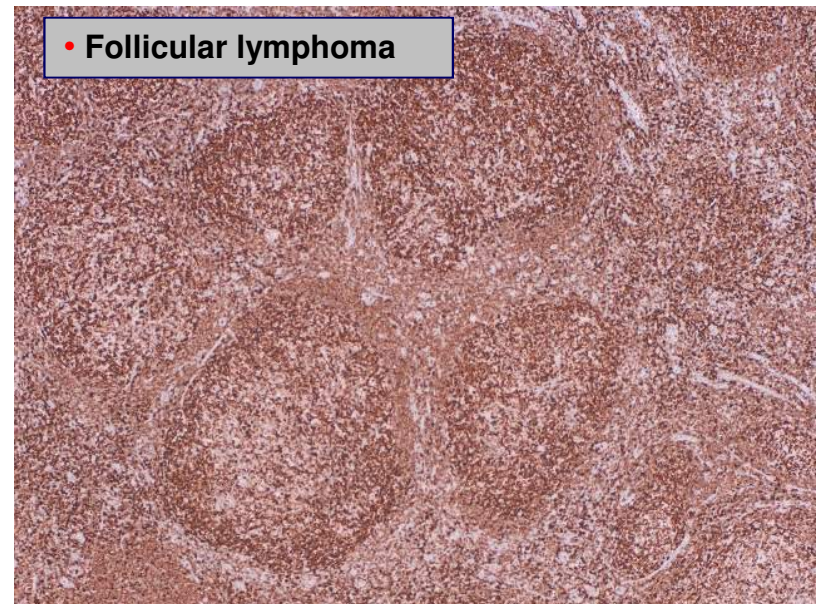
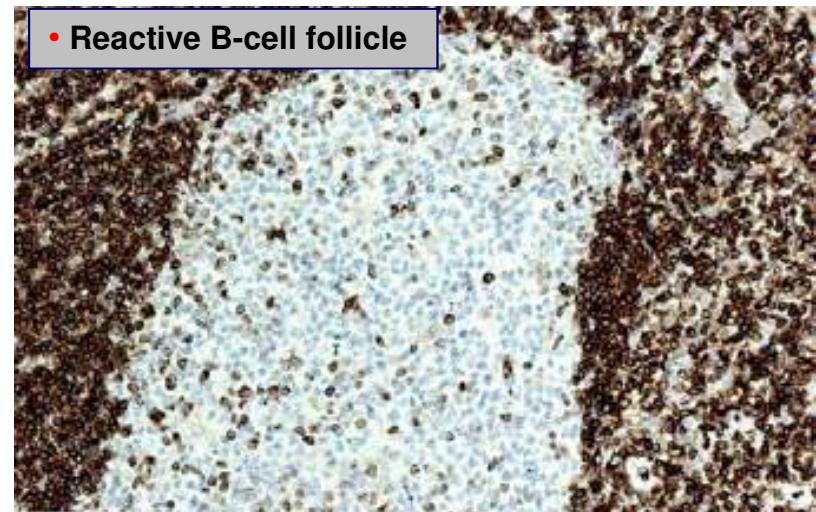
Immunology

- | | |
|---------------------|-----|
| • surface Ig | + |
| • CD19, 20, 22, 79a | + |
| • BCL-2 | + |
| • CD10 | +/- |
| • Bcl-6 | + |
| • CD5 | - |



Basic stain: bcl-2

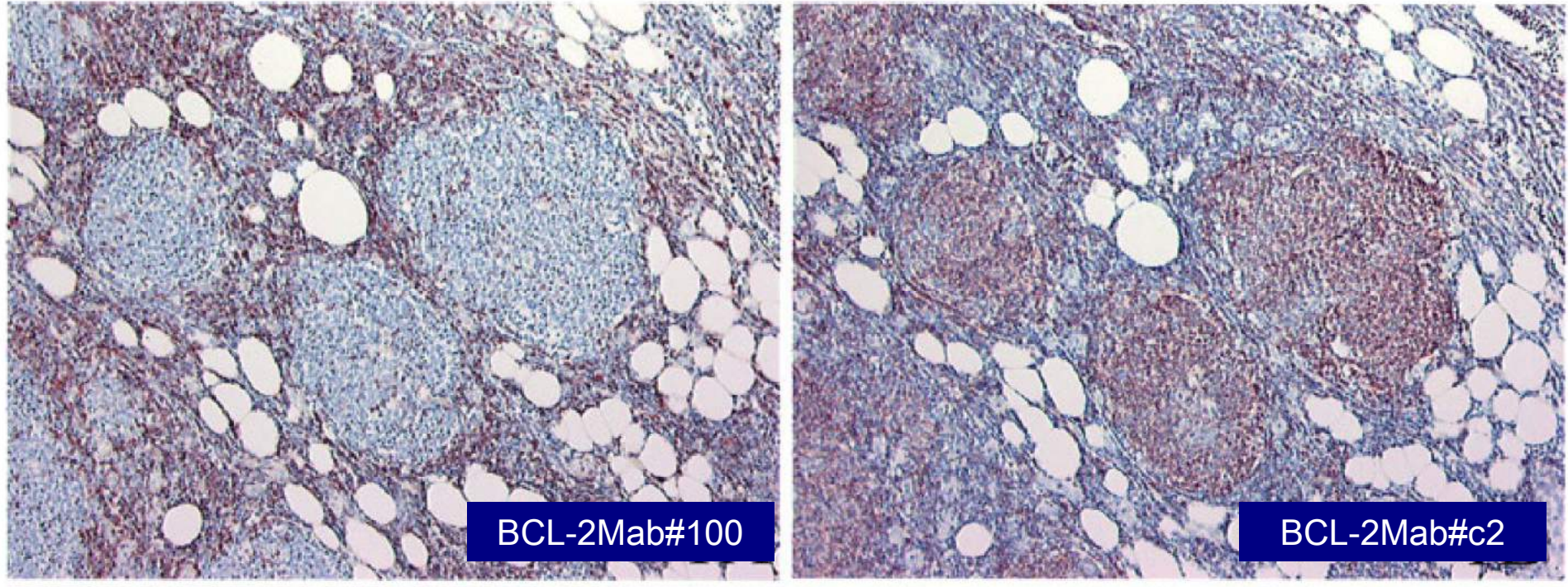
- Apoptosis inhibitor
- Nuclear and cytoplasmic stain
- Normal:
 - Mature B- and T-cells
 - Negative in cortical thymocytes and germinal centre cells
- In lymphoma:
 - Positive in most peripheral B-NHL and T-NHL
 - Negative in BL
 - Associated with, but not specific for t (14;18)
 - Positive in neoplastic germinal centres
 - Often negative in skin lymphoma
 - Ca 10% of follicular lymphomas re bcl-2 negative



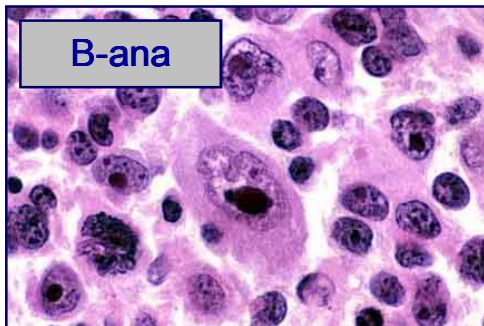
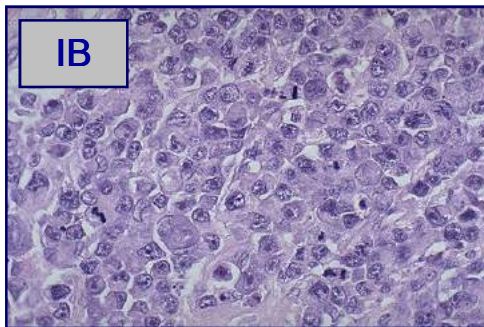
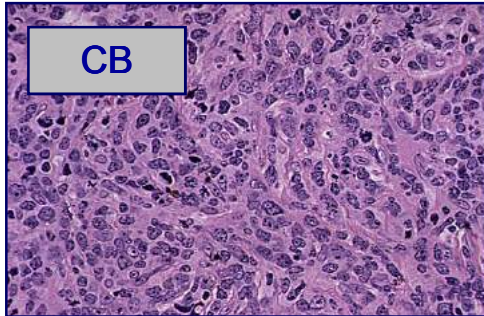
Original Paper

Lack of Bcl-2 expression in follicular lymphoma may be caused by mutations in the *BCL2* gene or by absence of the t(14;18) translocation

Margit Schraders,^{1*} Daphne de Jong,² Philip Kluin,³ Patricia Groenen¹ and Han van Krieken¹



Diffuse Large B-cell Lymphoma

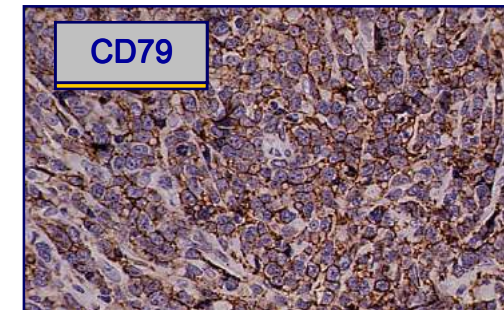
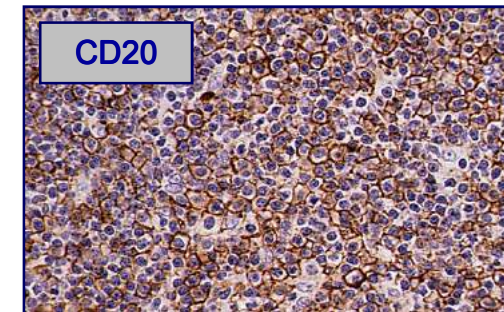
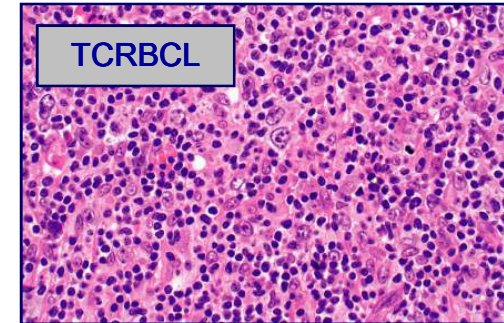


Morphology

- large cells
- nucleoli
- diffuse

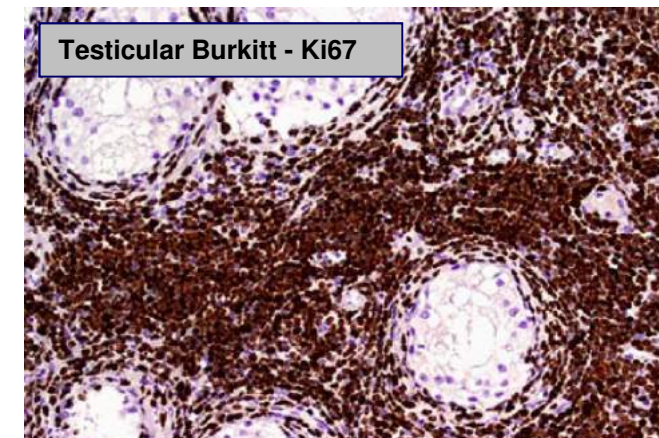
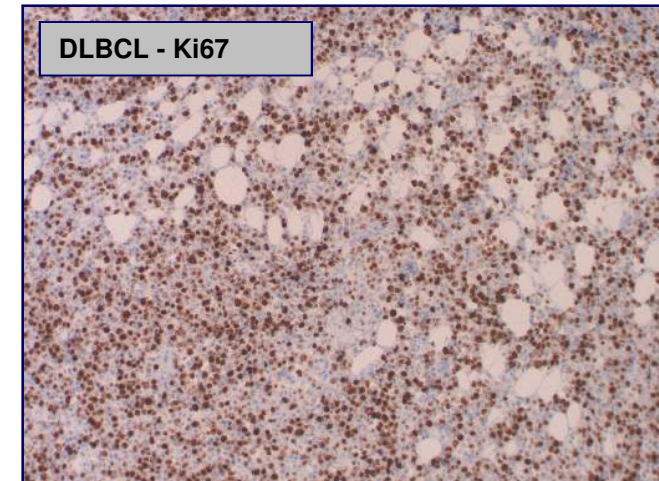
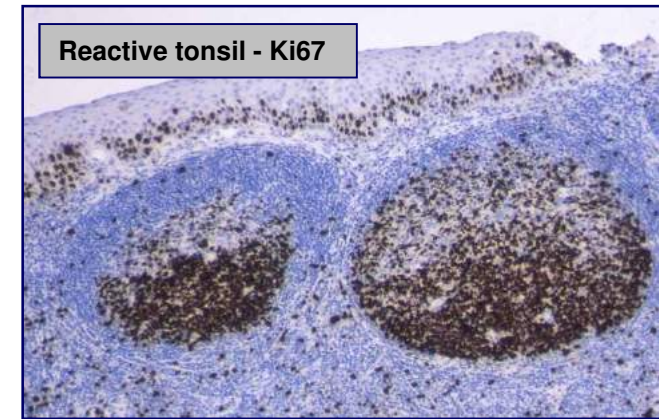
Immunology

- | | |
|---------------------|-----|
| • surface Ig | +/- |
| • cytoplasmic Ig | -/+ |
| • CD19, 20, 22, 79a | + |
| • CD30 | -/+ |
| • CD38, CD138 | pc |
| • CD5 | 10% |
| • CD10 | 40% |
| • bcl6 | 79% |
| • mum1 | 50% |



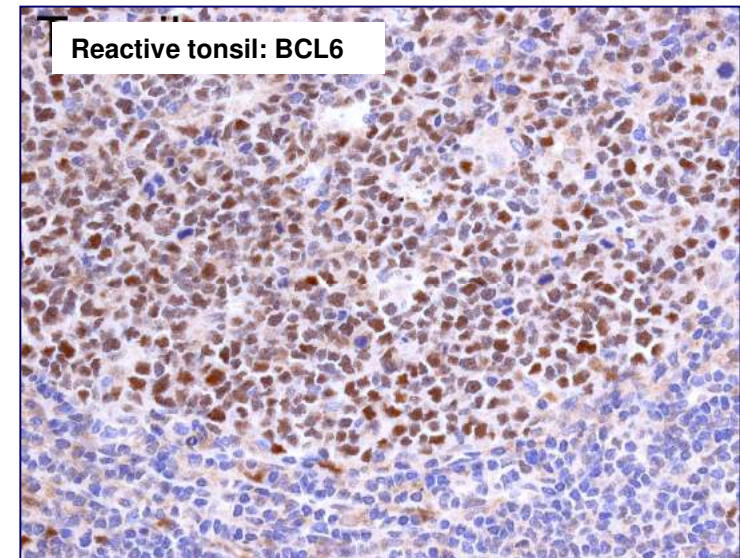
Basic stain: Ki- 67

- Nuclear protein
- Expressed in all cell cycle stages except G0
- In lymphomas:
 - 'Roughly'
 - indolent / aggressive / highly aggressive NHL
 - Prognosis?
 - Characteristic pattern in HRS cells in HL



Basic stain: Bcl-6

- Nuclear protooncogene product
- Normal:
 - germinal centre cells
- In lymphomas:
 - follicular lymphoma
 - most BL
 - variable DLBCL
 - 'cell of origin' staining in DLBCL
 - HL-LP (not classical)
 - SLL, MCL, MZL, HCL: negative



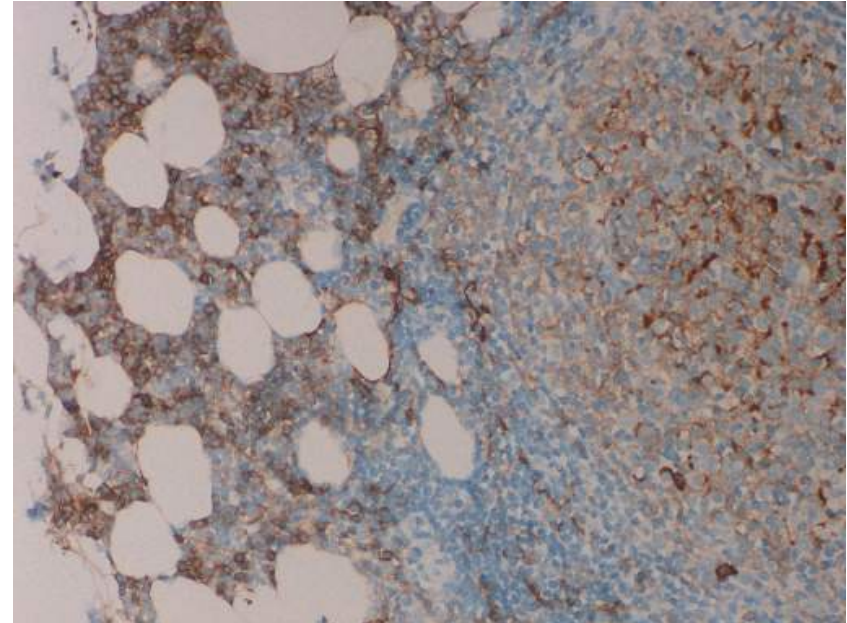
IHC for DLBCL

Add to basic panel:

- **CD10**
- **CD138**
- **MUM1**

Secondary stain: CD10

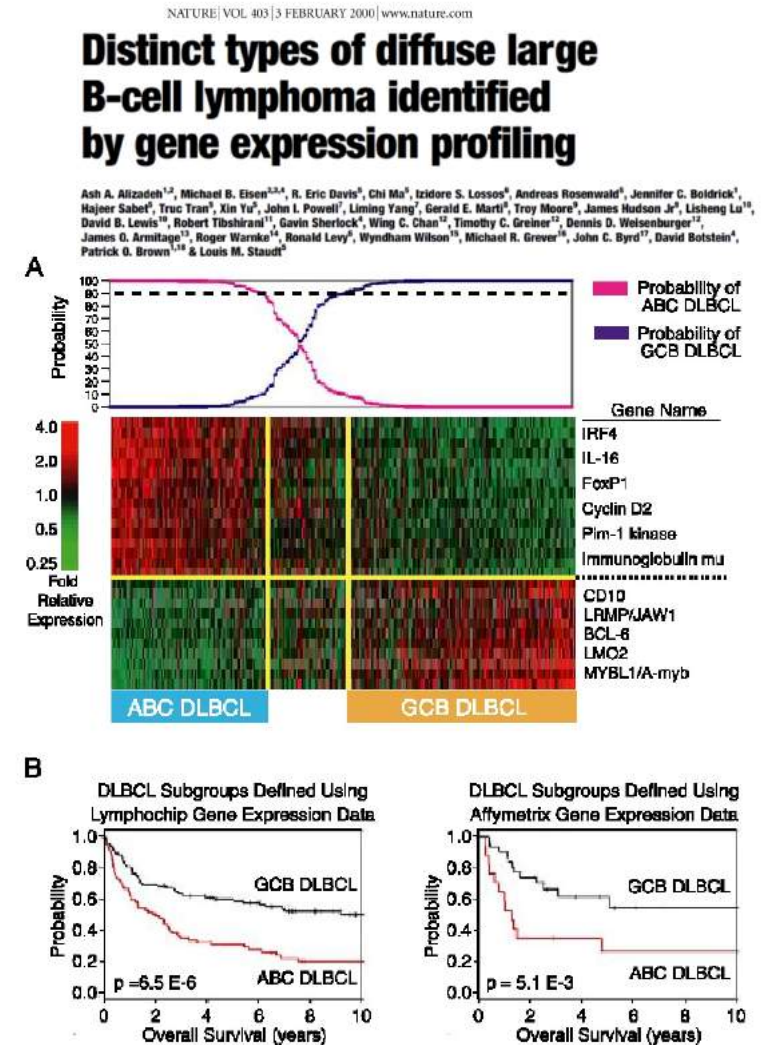
- >90% precursor B-LB (membrane & paranuclear stain)
- ca. 25% precursor T-LB
- Burkitt lymphoma
- Follicular lymphoma
 - Interfollicular CD10+ cells suggests lymphoma
- Some DLBCL
 - 'Cell of origin' algorithm in DLBCL
 - GCB vs ABC



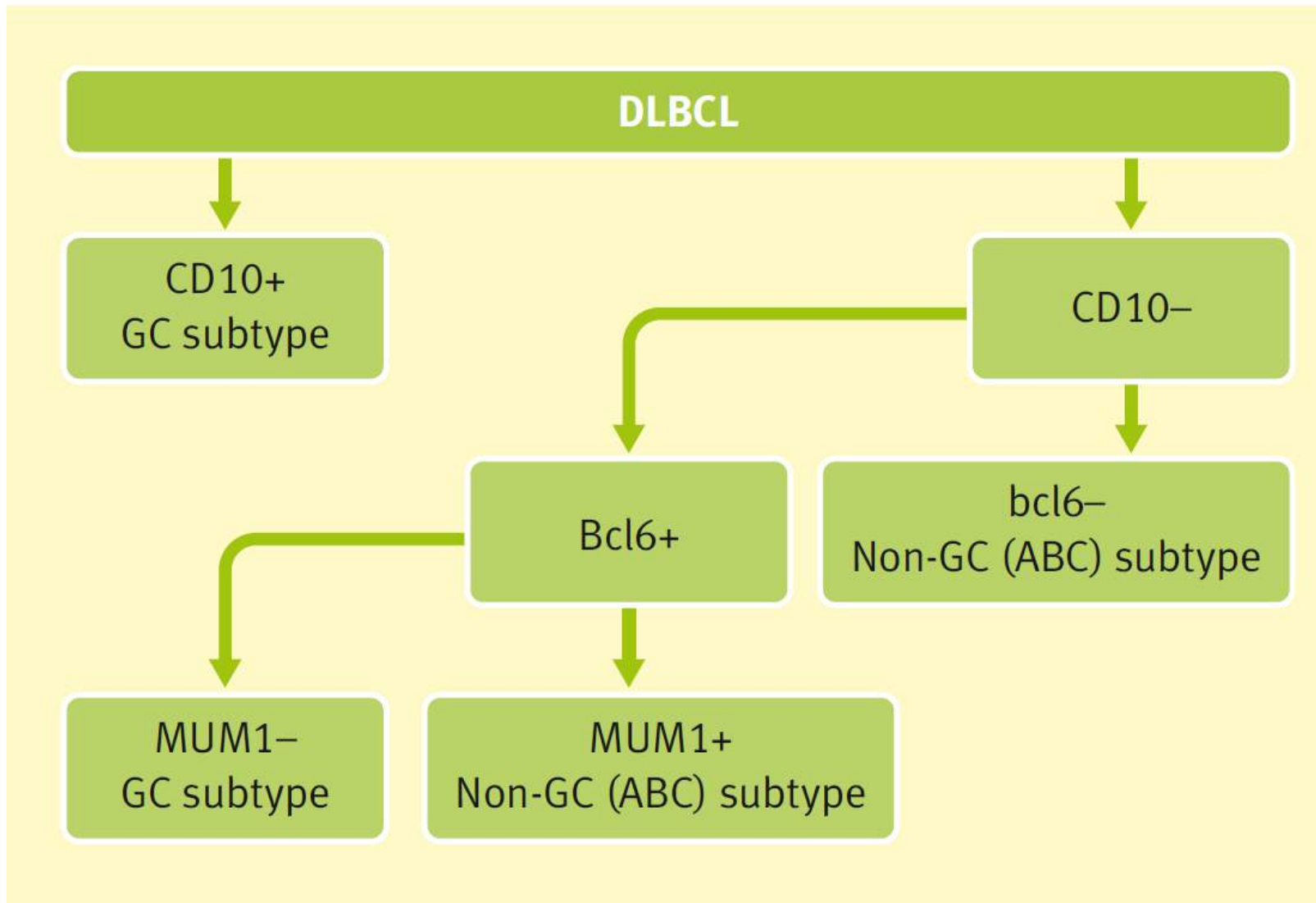
- Follicular lymphoma – CD10
- Interfollicular tumour cells

Large B-cell Lymphomas Molecular Variants

- Gene profiling identified 2 types of DLBCL (*Cell Of Origin – COO*)
 - Germinal Centre B-cell
 - Activated B-cell
- Molecular profiling not applicable in routine setting
- IHC
 - surrogate molecular profiling
 - Hans 'cell of origin' classifier



DLBCL - the HANS Classifier: Germinal centre (GC) & Activated B cell (ABC) types



DLBCL - 'cell of origin': Competing IHC classifiers

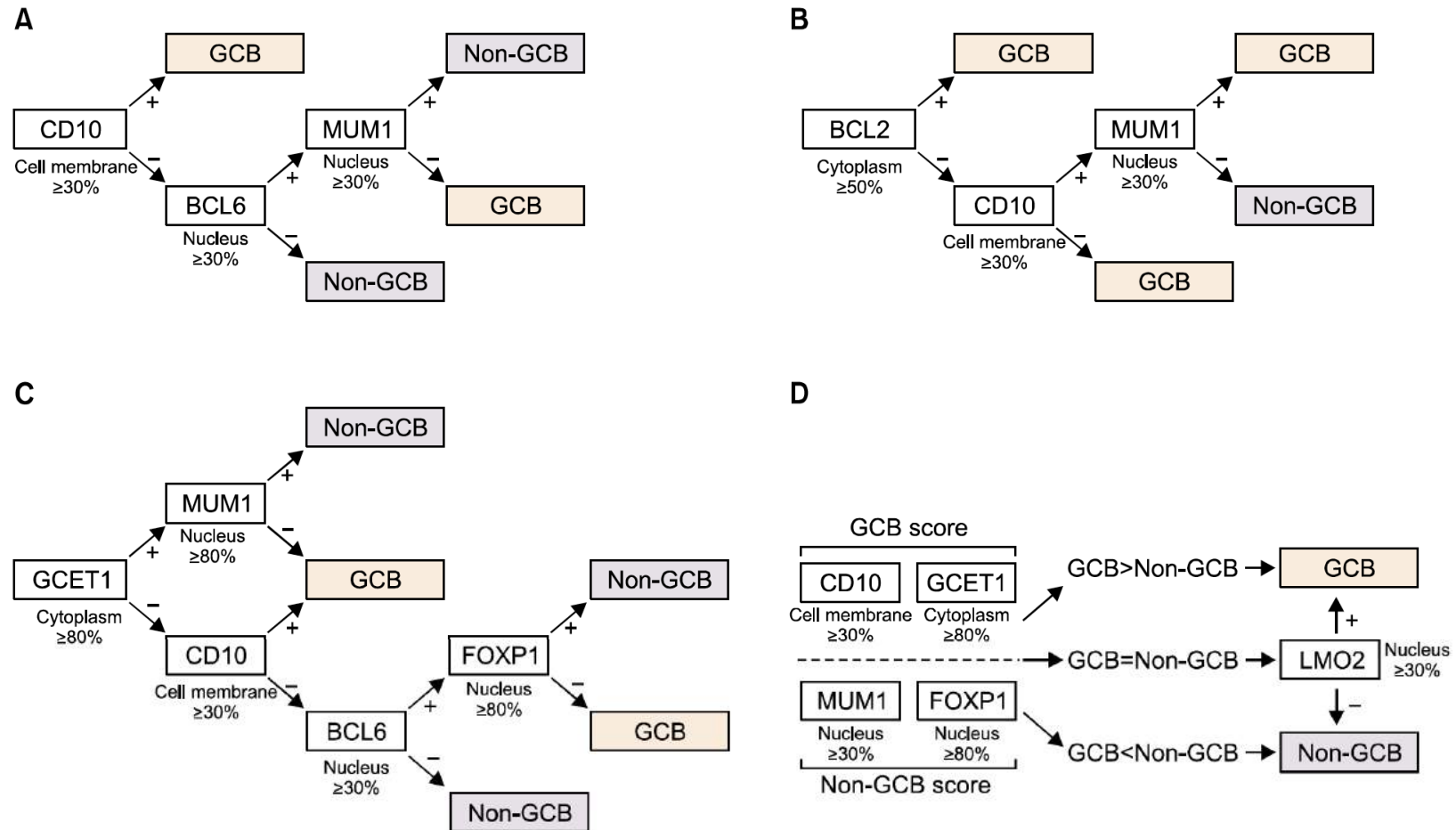
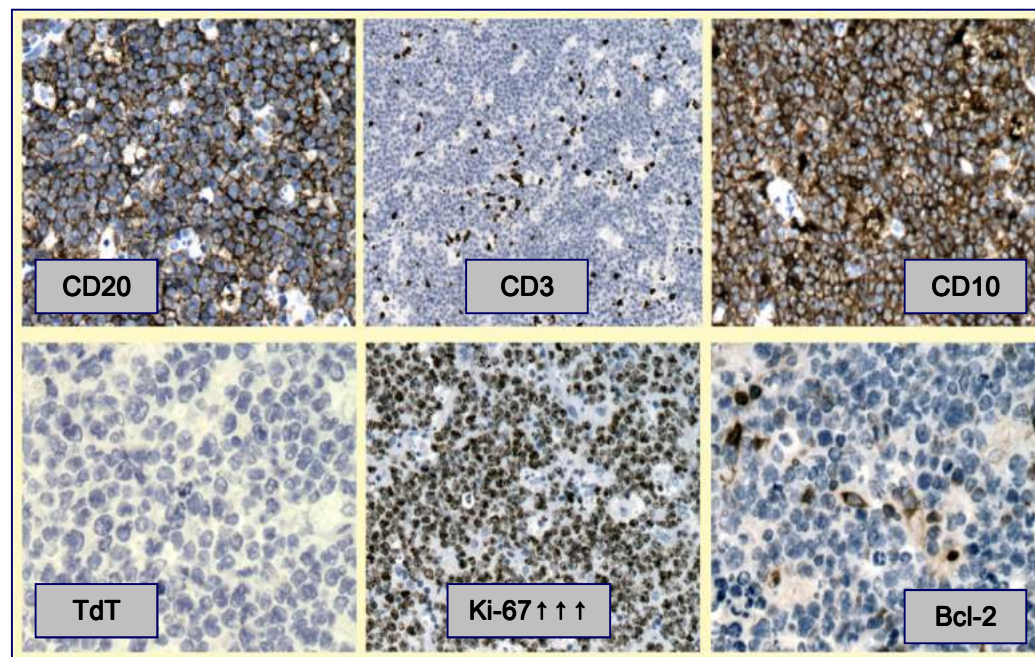
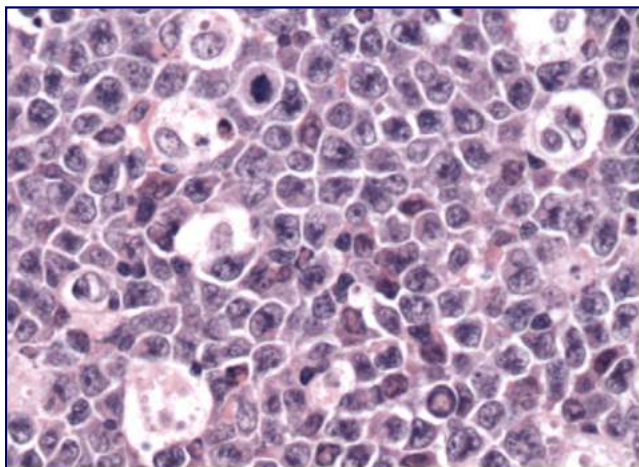


Fig. 2. Summary of the (A) Hans, (B) Muris, (C) Choi, and (D) Tally algorithms, and criteria for a positive signal for individual immunohistochemical markers (below or to the right of the white-filled box). Note that the positive criterion for MUM1/IRF4 in the Choi algorithm (more than 80%) is different from that of the other algorithms (more than 30%).

Immunophenotyping in Aggressive B-NHL

	CD20	CD79a	CD5	CD10	CD23	Ki67	TdT	bcl-2	CyclinD1
Diffuse large B	+	+	-/+	-/+	-	<90%	-	+/-	-
Burkitt	+	+	-	+	-	>95%	-	-	-
Blastic mantle cell	+	+	+	-	-	<90%	-	+/-	+
B lymphoblastic	+	+	-	+	-	<90%	+	+/-	-
Blastic myeloma	-	+	-	-	-	<90%	-	+/-	-/+

OFTEN TRICKY!!



- DLBCL-like morphology
- BL-like immunophenotype (BCL2^{neg})
- ↑↑ proportion of double-hit B-NHL (e.g. c-myc / bcl-2 rearranged)

IHC for c-myc and bcl-2 identifies double-hit & double-expressor B-NHL

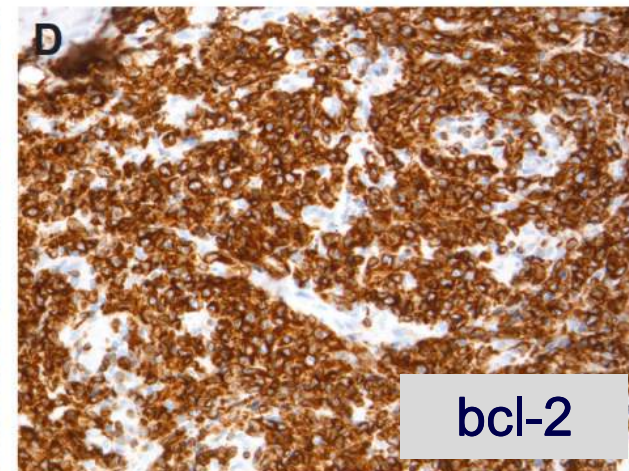
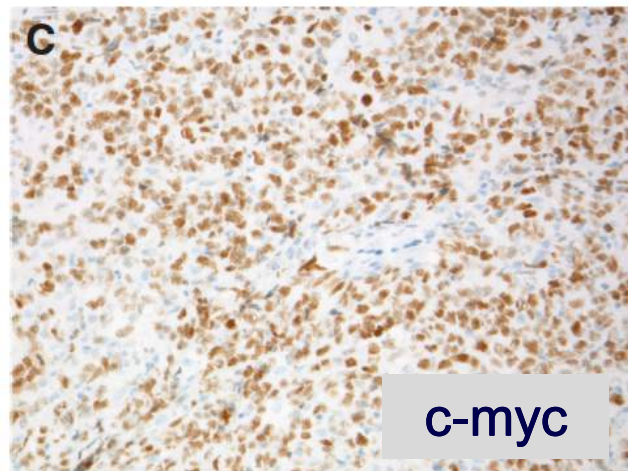
VOLUME 30 • NUMBER 28 • OCTOBER 1 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Immunohistochemical Double-Hit Score Is a Strong Predictor of Outcome in Patients With Diffuse Large B-Cell Lymphoma Treated With Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone

Tina Marie Green, Ken H. Young, Carlo Visco, Zijun Y. Xu-Monette, Attilio Orazi, Ronald S. Go, Ole Nielsen, Ole V. Gadeberg, Torben Mourits-Andersen, Mikael Frederiksen, Lars Møller Pedersen, and Michael Boe Møller



Updated WHO Classification – 2017

Major immunophenotypic changes:

Diffuse large B-cell lymphoma

- COO – *cell of origin* analysis now required
 - to distinguish GCB vs *ABC*/non-GC types
 - either by gene expression profiling or immunohistochemistry
- IHC for MYC and BCL2 expression
 - to identify "*double -expressors*"

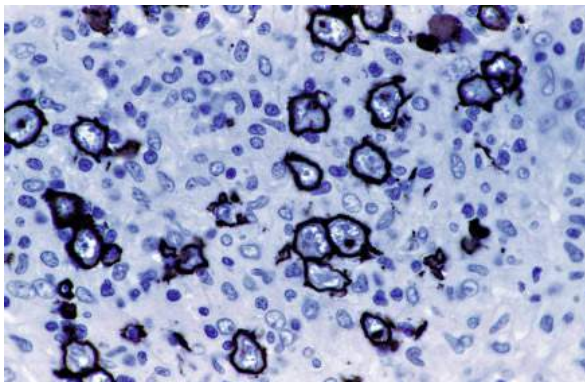
Hodgkins lymphoma: differential diagnosis

	CD20	CD79a	T-cell antigen	CD4 CD8	CD30	CD15	EMA
Nodular lymphocyte predominant HL	+	+	–	–	–/+	–	+
Classical HL	–/+	–/+	–	–	+	+	+
T-cell rich large B-cell lymphoma	+	+	–	–	–	–	–
Anaplastic large cell lymphoma	–	–	+/-	CD8>CD4> CD4&8 -ve	+	–	+

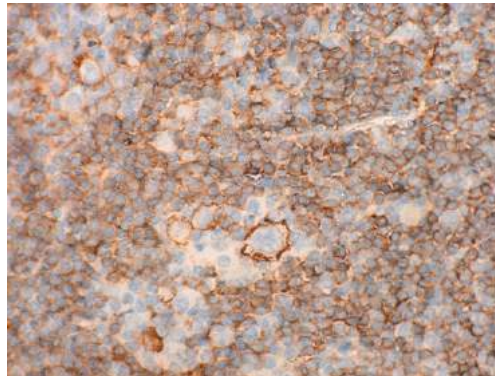
Key

- +/- The lymphoma cells are commonly but not always positive
 -/+ The lymphoma cells are usually but not always negative

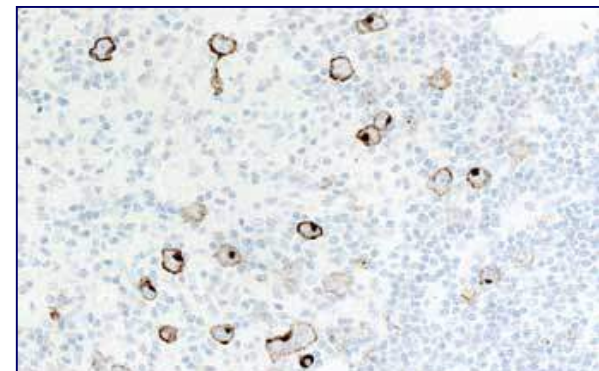
T-cell rich, B-cell lymphoma: CD20



Hodgkins lymphoma, LP: CD20

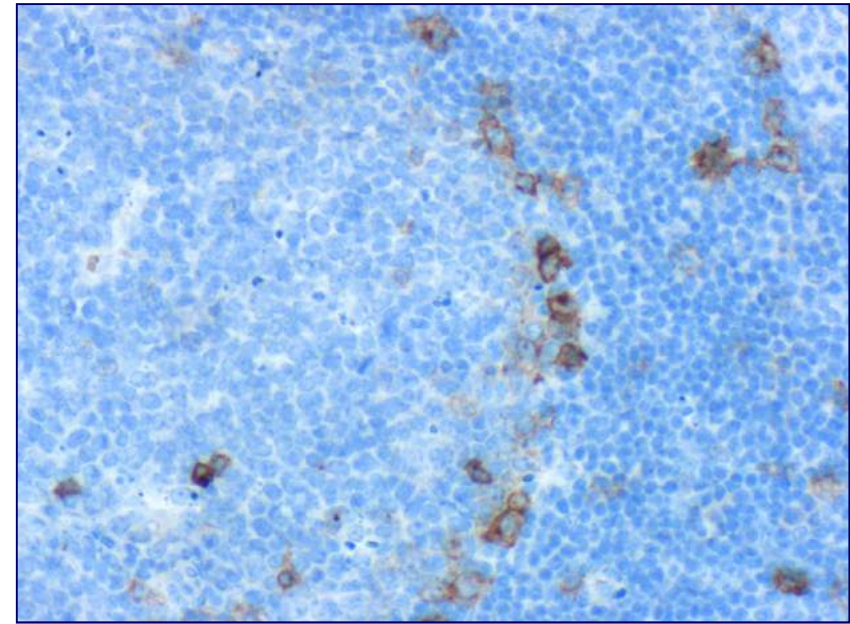


Classical Hodgkin lymphoma, MC: CD30



Basic stain: CD30

- **TNF-R family**
- **'Ki-1 antigen'**
- **Activation antigen**
- **Normal expression:**
 - activated parafollicular immunoblasts
 - virally infected cells (EBV)
 - some clones stain plasma cells (Ber-H2)
- **Pattern:**
 - Membrane with dot-like Golgi

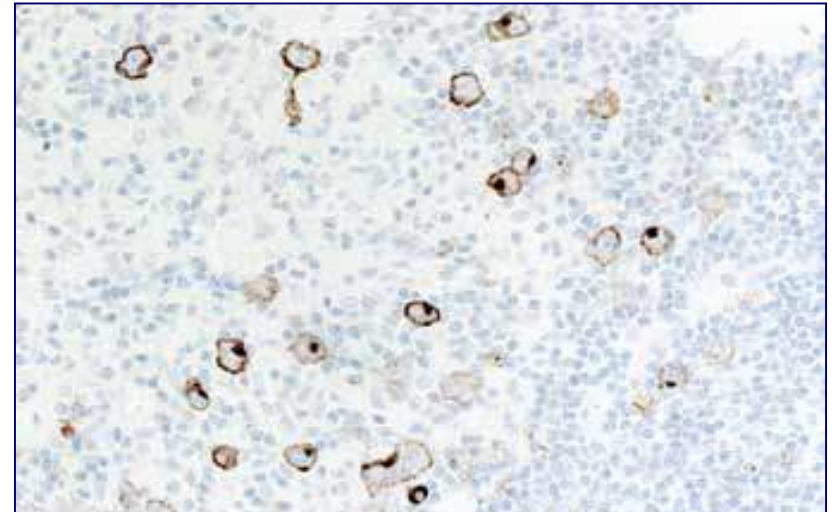


Reactive LN: activated B-cells

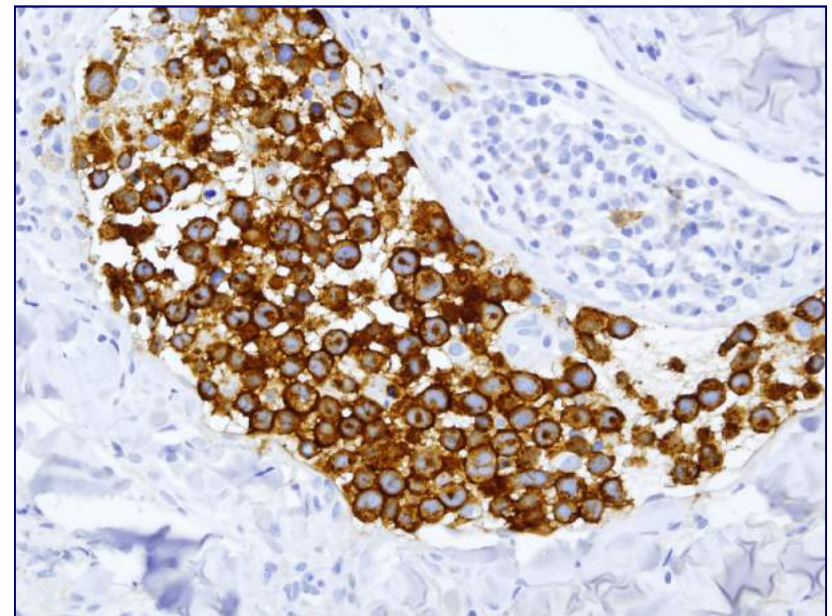
CD30 in lymphoma

"CD30+ lymphoproliferations":

- Primary skin anaplastic large cell lymphoma (ALCL)
- Systemic ALCL
- Lymphomatoid papulosis
- Mycosis fungoides transformation
- Hodgkin lymphoma
 - HRS cells in classical types
 - Popcorn cells in HL-LP: 0% -10%
- Ca. 30% of other T-cell NHL
- Ca. 20% DLBCL
- Target for Brentuximab



Hodgkins lymphoma: CD30



ALCL – sinus pattern CD30

IHC for Hodgkins Lymphoma

Add to basic panel:

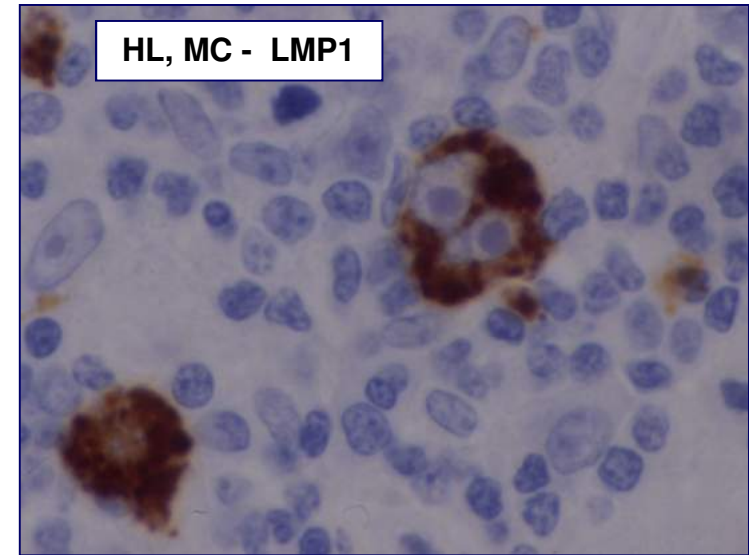
- **PAX-5 (ALCL?)**
- **BCL-6, CD57, BOB-1, OCT-2 (HL, LP?)**
- **ALK (ALCL?)**
- **EBV**
- **(CD15)**

HL vs ALCL: Immunophenotype

	HL	ALK - pos T/null - ALC	ALK - neg T/null - ALC
ALK	-	+	-
EBV	> 40 %	-	-
CD30	+	+	+
CD15	ca. 90 %	< 5 %	- / +
EMA	-	ca. 50 %	ca. 50 %
 PAX5	> 80 %	-	-
CD20	ca. 25 %	-	-
CD3	ca. 2 %	+ / -	+ / -
CD45	-	ca. 50 %	ca. 50 %
CD43	-	most +	most +
Granzyme/ perforin	10 – 20 %	ca. 90 %	ca. 70 %
TCR genes	G	R	R
Ig genes	R (single cell)	G	G

Secondary stain: EBV

- **Most viral antigens not relevant**
- **Latent membrane protein 1**
 - Normal primary infection (IM)
 - Latency patterns II and III
 - HRS-cell-like morphology
- **EBNA2**
 - Nuclear reaction
 - Normal primary infection (IM)
- **In lymphoma:**
 - **Hodgkin lymphoma:**
 - **Classical types: 25% - 50% positive in HRS cells: LMP1+ EBNA2-**
 - **HL-LP: L&H/Popcorn cells negative**
 - **EBV+ immunodefect associated lymphomas**
 - Variable (diagnostically useful) latency patterns
 - **Sporadic B-NHL**
 - Ca. 5% (EBV+ DLBCL, NOS)
 - **T cell lymphomas**
 - Variably positive (5% - 100% depending on type)
 - ALCL are negative

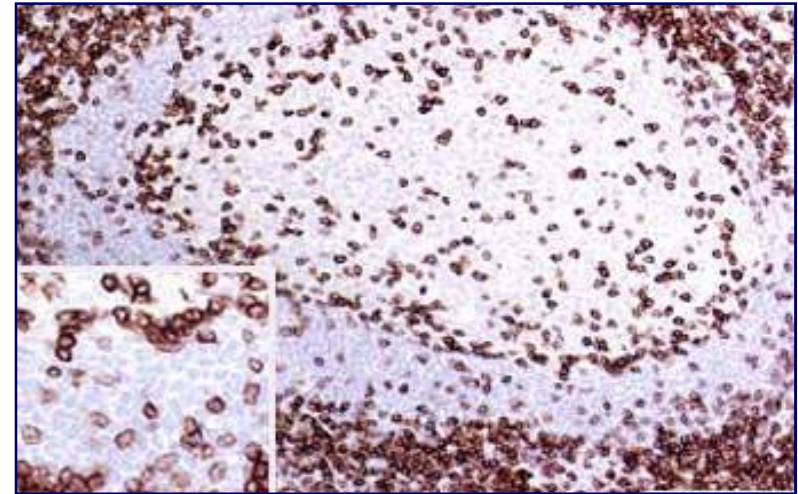


T-cell lymphoma: immunophenotype

Complex!

Basic stain: CD3

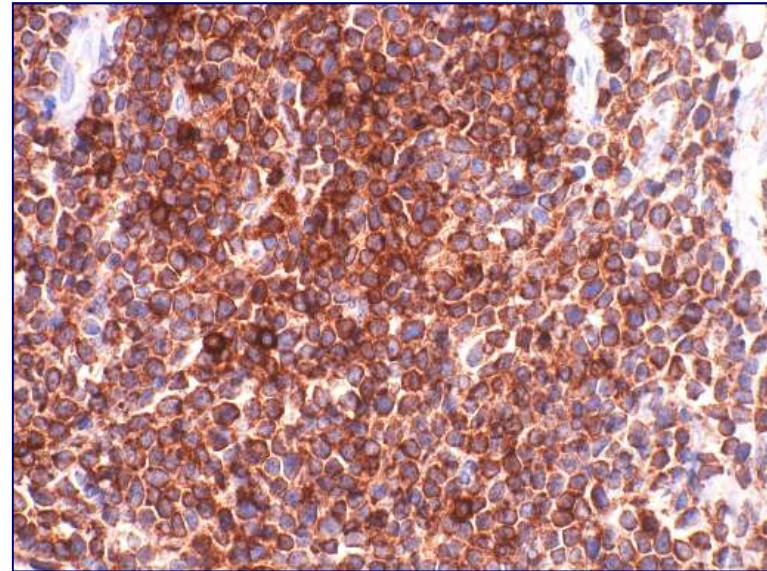
- transmembrane molecule
- Ig superfamily
- part of T-cell receptor
- most specific T-cell marker
- pan-T cell marker
 - thymocytes: cyt. → membrane
 - most post-thymic T-cells
 - activated NK-celler



Reactive LN: CD3

CD3 in lymphoma

- **>90% peripheral TCLs**
- **Primitive precursor T-LB in cytoplasm**
- **B-cell lymphomas negative**
- **Hodgkin lymphoma negative**
- **(NK-lymfomer: cyt. expression)**



- **Precursor T-LB**
- **CD3-cyt**

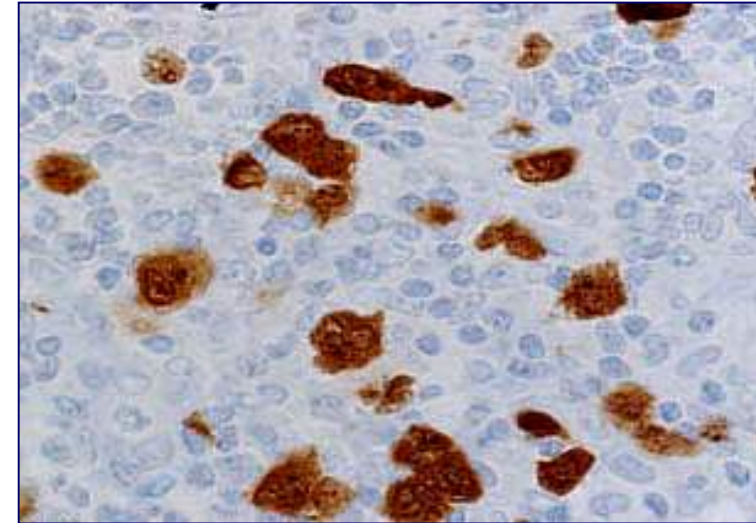
IHC for PTL

Add to basic panel:

- **CD1a**
- **CD2**
- **CD4**
- **CD7**
- **CD8**
- **CD3epsilon, TdT, CD43**
 - T-LB?
- **CD10, CD21, CD23, PD-1**
 - AILD?
- **CD56, CD57, perforin, granzyme B, TIA-1**
 - NK/NK-like?
- **EBV**

Secondary stain: Anaplastic lymphoma kinase (ALK, CD246)

- Normal tissues only in CNS
- In neoplasia:
 - ALCL with t(2;5) or other translocation
 - positive prognostic factor
 - cellular localisation varies with partner gene
 - ALK-ve B-cell NHL (rare)
 - Negative in primary cutaneous ALCL



ALK-positive ALCL

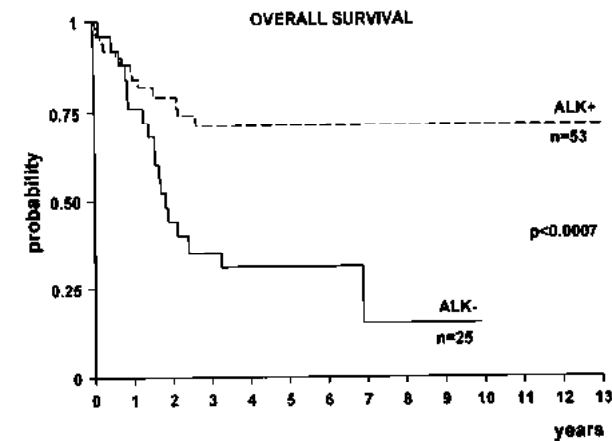
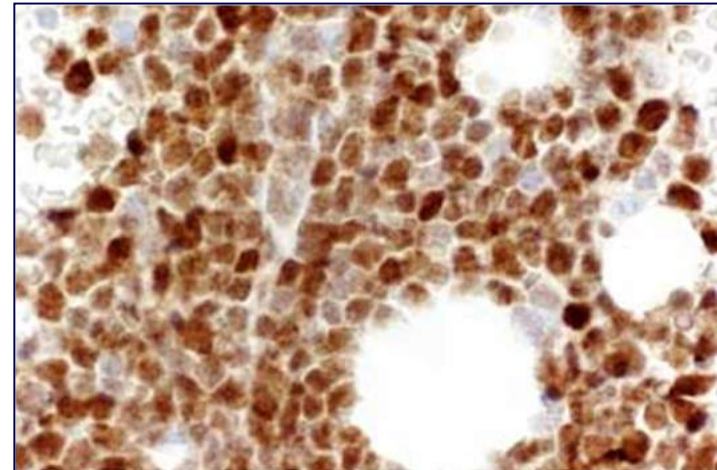


Fig 4. Overall survival of ALK⁺ versus ALK⁻ lymphoma.

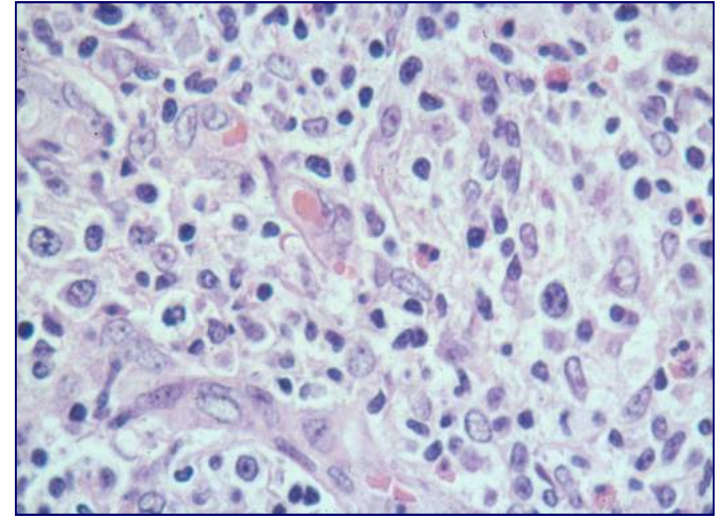
Secondary stain: Terminal deoxynucleotidyl transferase (TdT)

- **Nuclear protein involved in DNA synthesis**
- **Normal expression:**
 - early thymocytes
 - pre-B and pre-pre-B cells
- **In lymphomas:**
 - stem cell leukaemias
 - most (>90%) precursor LBs
 - negative in most peripheral TCLs
 - some AMLs (up to 20%)

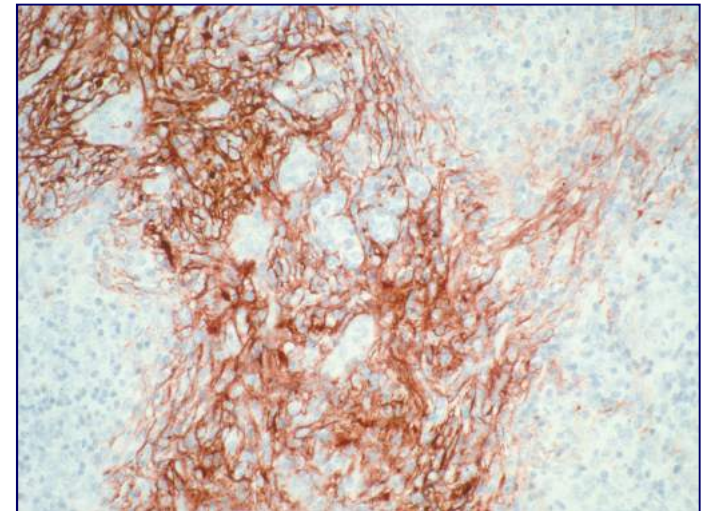


Basic stain: CD21

- **Membrane glycoprotein**
- **Normal:**
 - Mature B cells
 - mantle zone & marginal zone B cells
 - Lost on B-cell activation
 - Follicular dendritic reticulum cells – in GCs
- **C3d/EBV receptor**
- **In lymphomas:**
 - most follicular lymphomas
 - some other B-cell NHL
 - FDC network in GC-derived tumours
 - MCL, HL, AILD



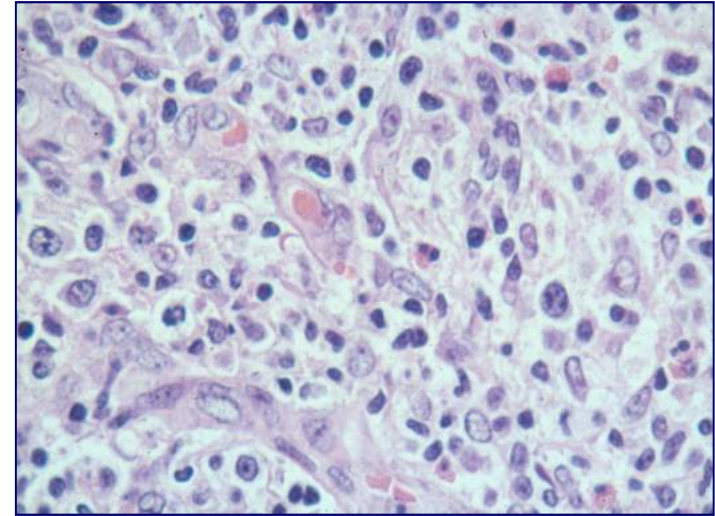
• AILD-T-cell lymphoma



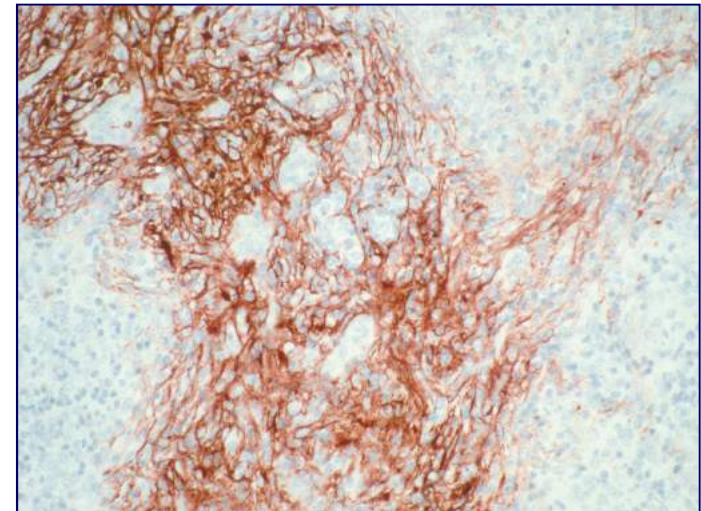
• AILD-T-cell lymphoma: CD21

Basic stain: CD21

- **Membrane glycoprotein**
- **Normal:**
 - Mature B cells
 - mantle zone & marginal zone B cells
 - Lost on B-cell activation
 - Follicular dendritic reticulum cells – in GCs
- **C3d/EBV receptor**
- **In lymphomas:**
 - most follicular lymphomas
 - some other B-cell NHL
 - FDC network in GC-derived tumours
 - MCL, HL, **AILD**



• AILD-T-cell lymphoma



• AILD-T-cell lymphoma: CD21

Nodal PTCL - immunophenotype

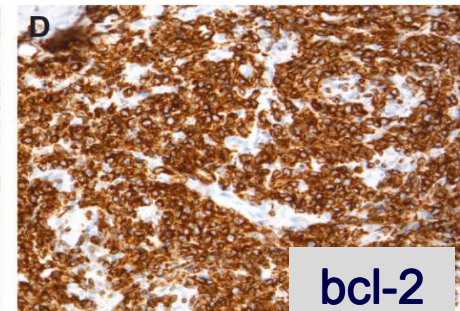
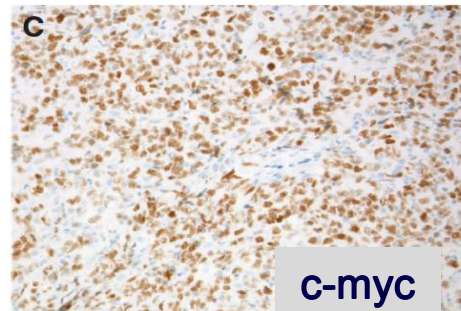
	PTCL, NOS	AITL	ALCL ALK+	ALCL ALK-	ATLL	MF	T-PLL	EATL
CD2	+	+	-/+	-/+	+	+	+	+
CD3	+	+	-/+	-/+	+	+	+	+
CD4	+/-	+	-/+	-/+	+	+	+/-	-
CD5	+/-	+	-	-	+	+	+	-
CD7	+/-	-/+	-	-	-	-	+	-
CD8	-/+	-	-	-	-	-	-/+	-/+
CD10	-	+/-	-	-	-	-	-	-
CD25	-/+	-	+	+	+	-/+	-	-/+
CD30	-/+	-	+	+	-/+	-/+	-	-/+
CD45RO	+	+	+	+	+	+	+	+
CD56	-/+	-	-/+	-	-	-	-	-/+
ALK	-	-	+	-	-	-	-	-
CXCL13	-	+/-	-	-	-	-	-	-
PD1	-/+	+	-	-	-	-	-	-
TCR-β	+/-	+	-	-	+	+	+	+/-
FOXP3	-/+	-	-	-	+/-	+	-/+	-
TCL1	-	-	-	-	-	-	+	-
TIA-1	-/+	-	+/-	+/-	-	-	-	+
GranB	-/+	-	+/-	+/-	-	-	-	+

+: Expressed, +/-: frequently expressed, -/+: expressed in a minority of cases, -: not expressed.

Oncogenes/ Tumor Suppressor Genes

Evaluation by Immunohistochemistry

- **Bcl-2: Follicular lymphoma, t(14;18)**
 - antigen expression not specific for translocation
- **Cyclin D1: Mantle cell lymphoma, t(11;14); myelomas (15%)**
- **p53: Progression in lymphomas, high grade lymphomas**
- **Bcl-6: Germinal center origin**
 - 'cell of origin' staining in DLBCL
- **c-myc**
 - Prognosis in DLBCL
 - 'double hit' & 'double-expressor' lymphomas (with Bcl-2)
- **ALK-1: ALCL; NPM/ALK (t2;5)**
- **CD99: Lymphoblastic, myeloblastic**



IHC for lymphoma vs other

Add to basic panel:

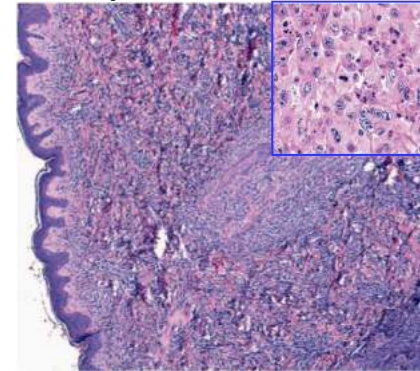
- **panCK**
- **S-100**
- **Melan-A**

IHC for lymphoid vs myeloid

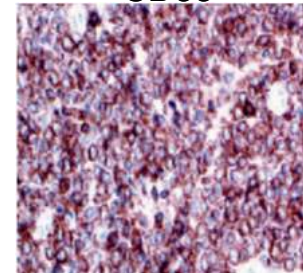
Add to basic panel

- **Myeloperoxidase**
- **CD43**
- **CD68**
- **CD163**
- **CD33**
- **(CD14, CD15, CD34, CD61, glycophorin C)**

Myeloid Sarcoma

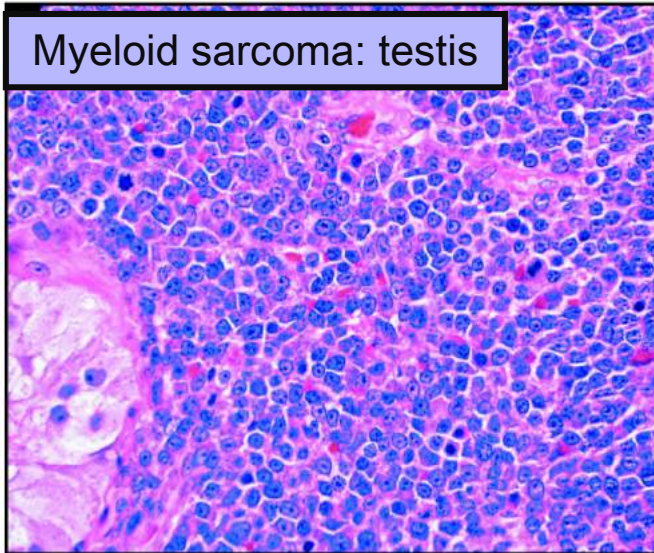


CD33

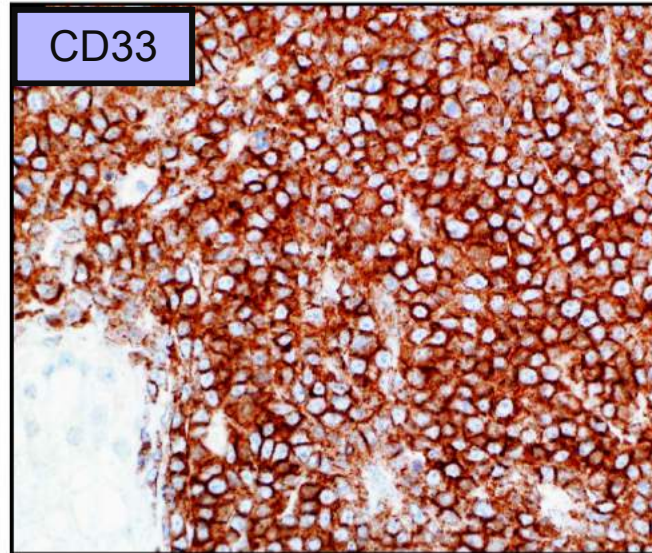


Myeloid sarcoma: testis

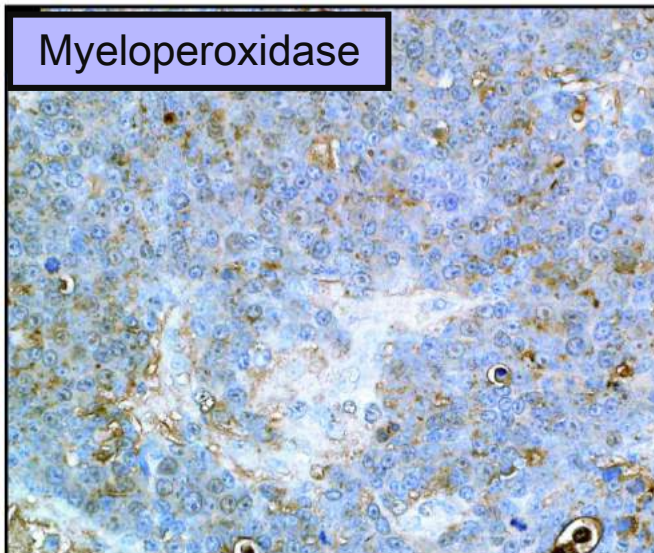
Myeloid sarcoma: testis



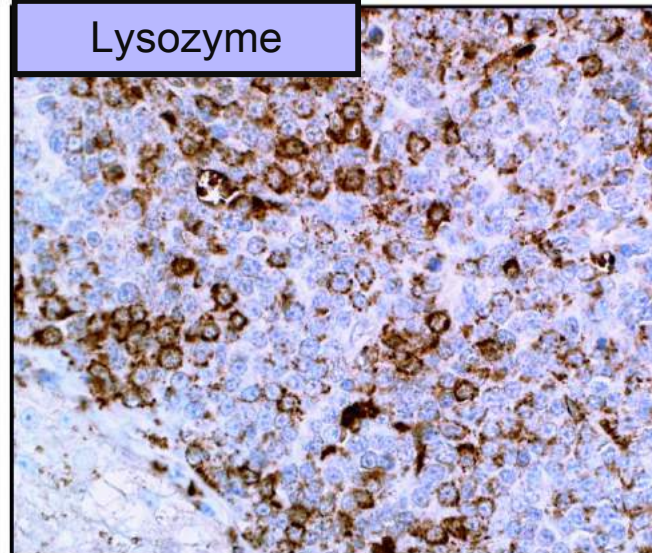
CD33



Myeloperoxidase



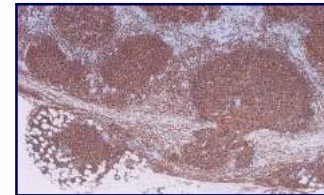
Lysozyme



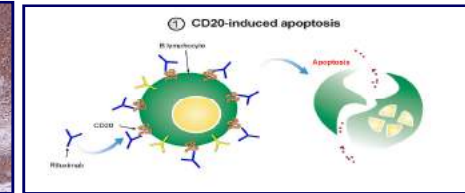
Targeted therapy

- **Rituximab (anti-CD20)**

- B-cell NHL

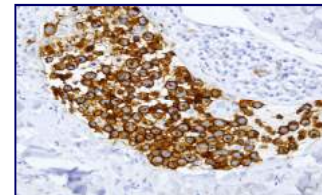


FL: CD20

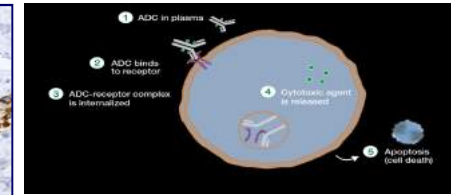


- **Brentuximab (anti-CD30)**

- HL
- ALCL
- CD30+ DLBCL

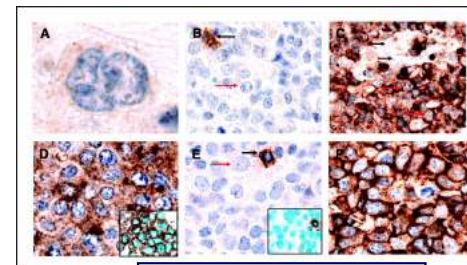


ALCL: CD30

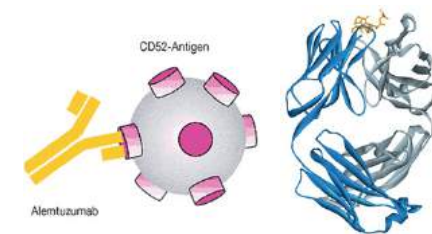


- **Alemtuzumab (anti-CD52)**

- B-CLL
- T-cell lymphoma

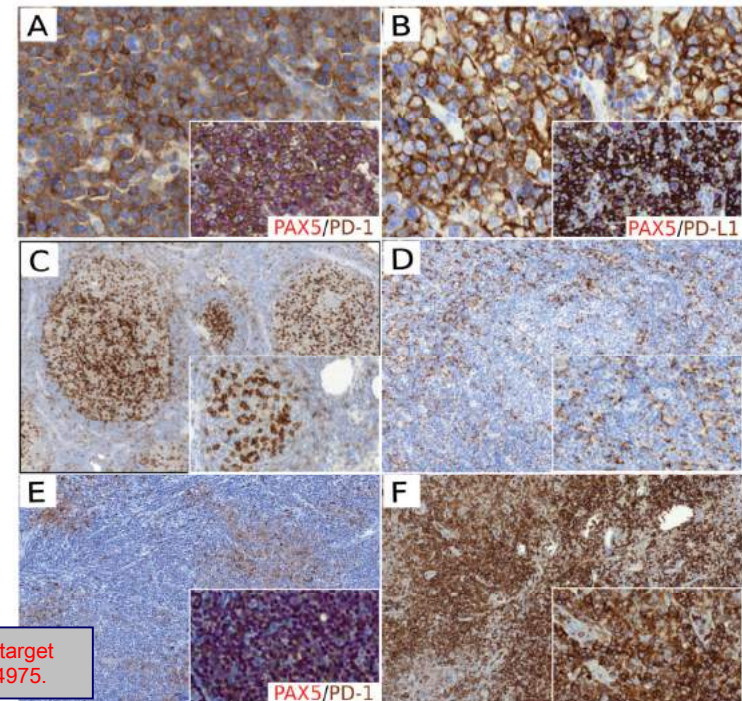
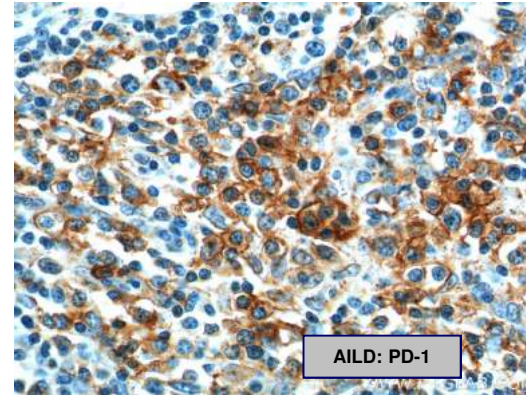


T-cell lymphomas: CD52



Immune checkpoint inhibitory therapy?

- PD-1 AILD
- Hodgkin
 - PD-L1
 - PD-1



Gravelle P, et al. Oncotarget
8.27 (2017): 44960–44975.

A black and white photograph of a large, curling ocean wave. The wave is dark and powerful, with a thick, white foam cresting over the top. The water below the wave is turbulent and white with foam. The sky is a light, hazy gray. The word "Thanks!" is written in a clean, white, sans-serif font, centered horizontally and slightly above the middle of the image, overlaid on the dark face of the wave.

Thanks!