Haematolymphoid neoplasms: IHC for diagnositic use

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Haematolymphoid Neoplasms:

What are they?

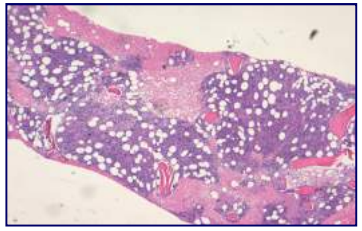
Haematolymphoid Neoplasms:
Leukaemia *vs* Lymphoma

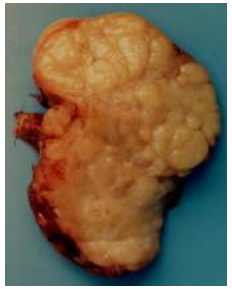
CLONAL LYMPHOID MALIGNANCIES

Bone marrow

Blood

- **Lymph node**
- **Extranodal site**





Leukaemia

Lymphoma

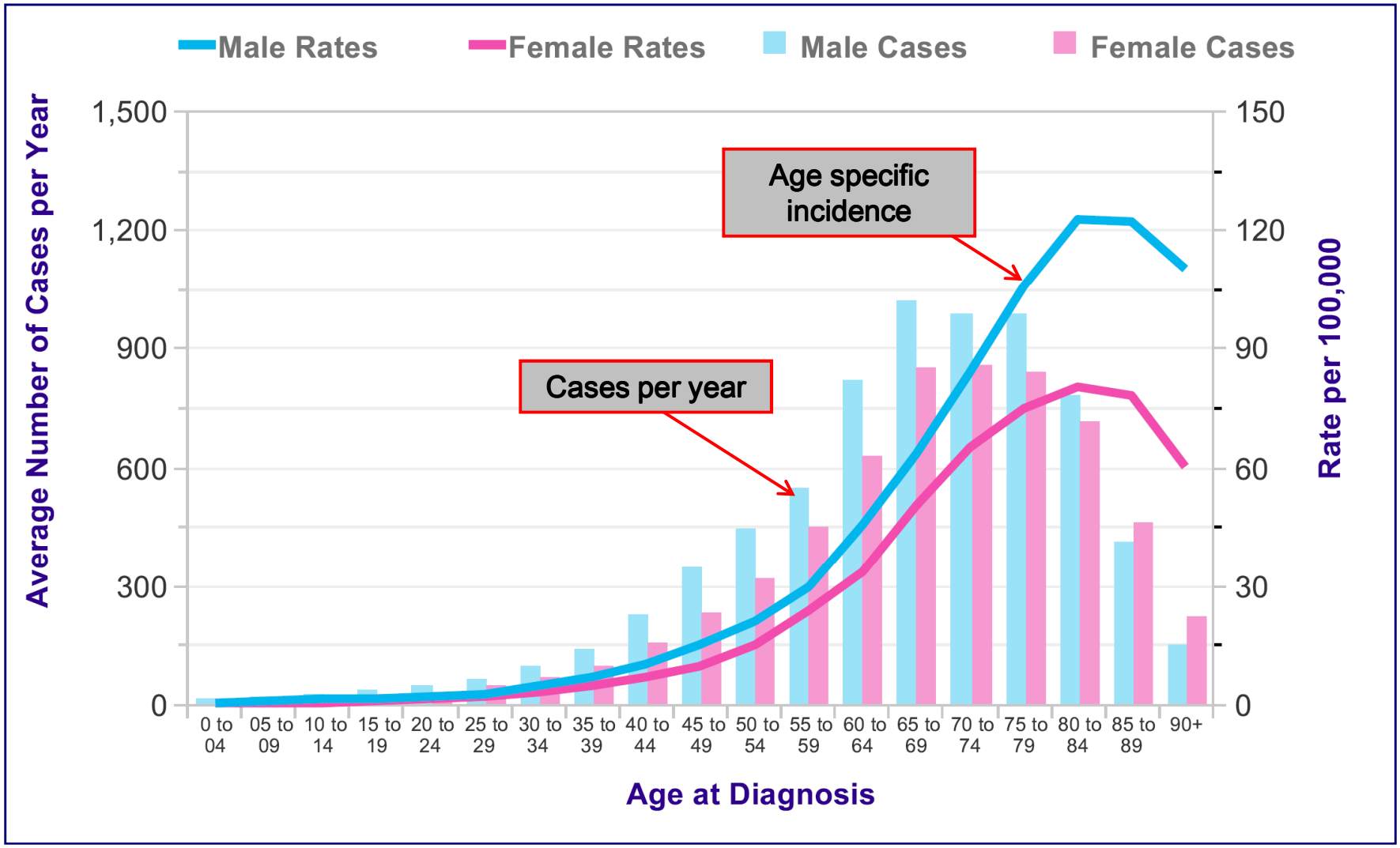
Haematolymphoid Neoplasms:

How common are they?

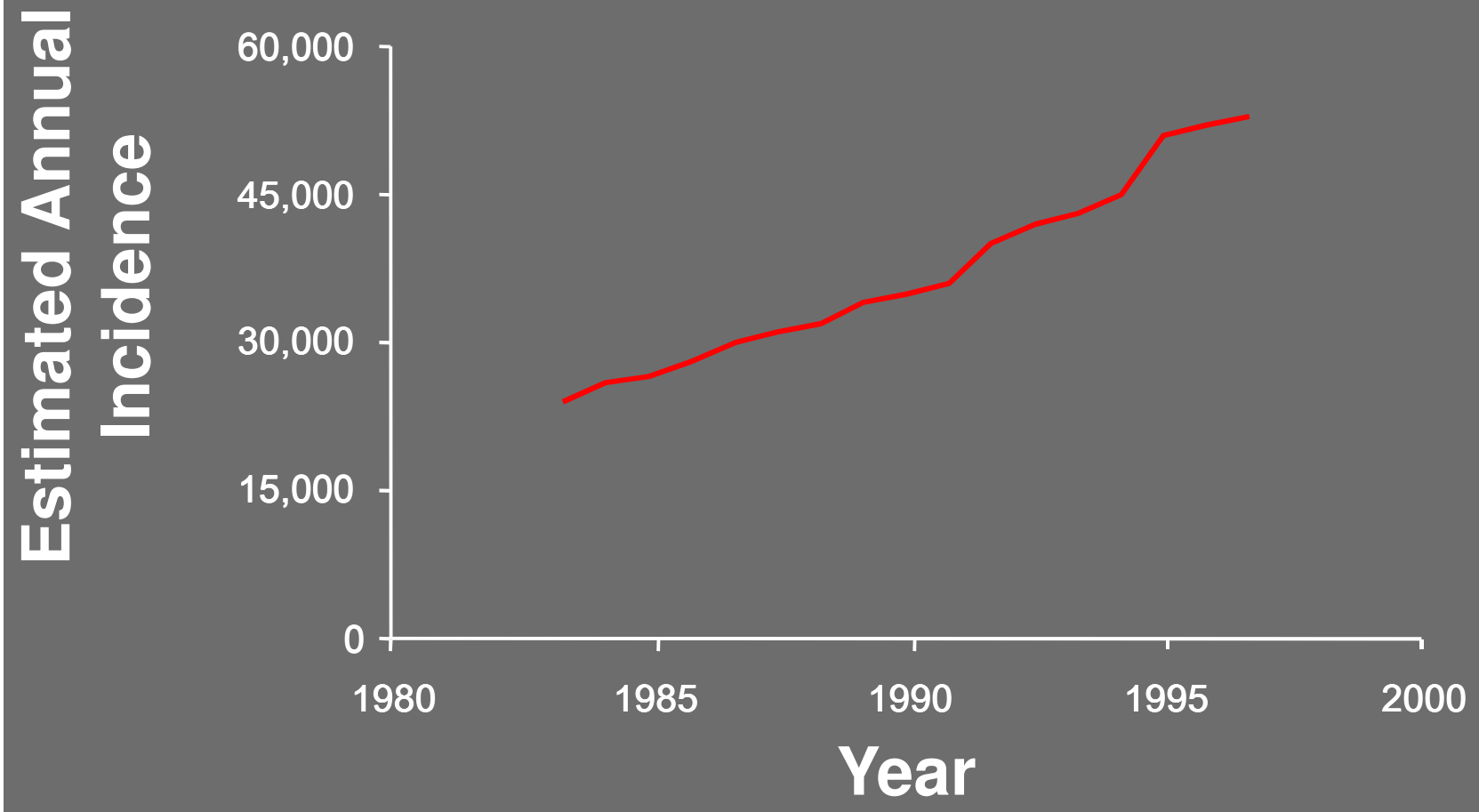
Malignant lymphoproliferative diseases

- **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-Hodgkin lymphoma (UK)



Estimated Incidence of NHL in the USA



Malignant lymphoproliferative diseases

What causes them?

Largely unkown.....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**

Haematolymphoid Neoplasms:

What causes them?

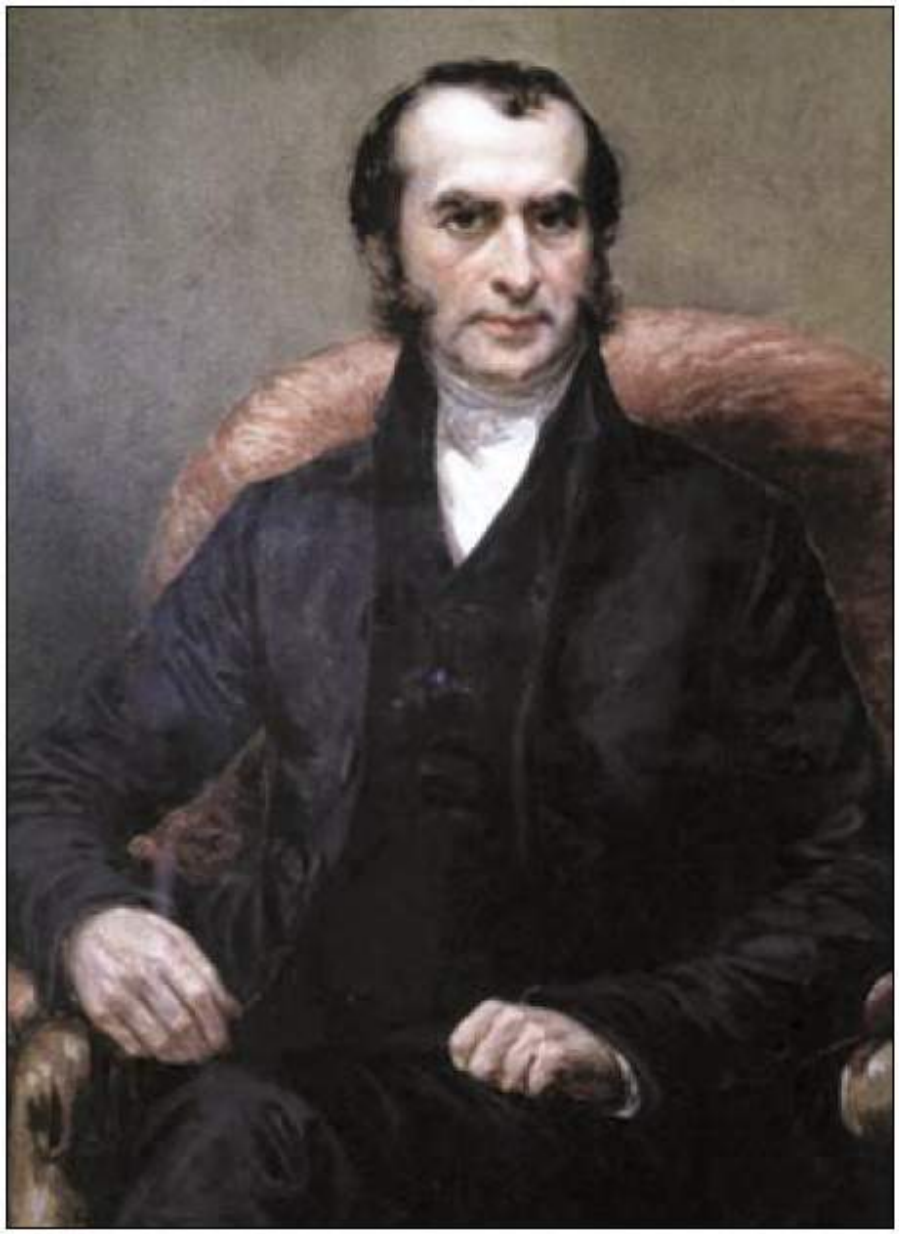
HEALTH • CALIFORNIA

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

Haematolymphoid Neoplasms:

How are they classified?

Thomas Hodgkin 1798-1866



ON SOME

MORBID APPEARANCES

OF

THE ABSORBENT GLANDS

AND

SPLEEN.

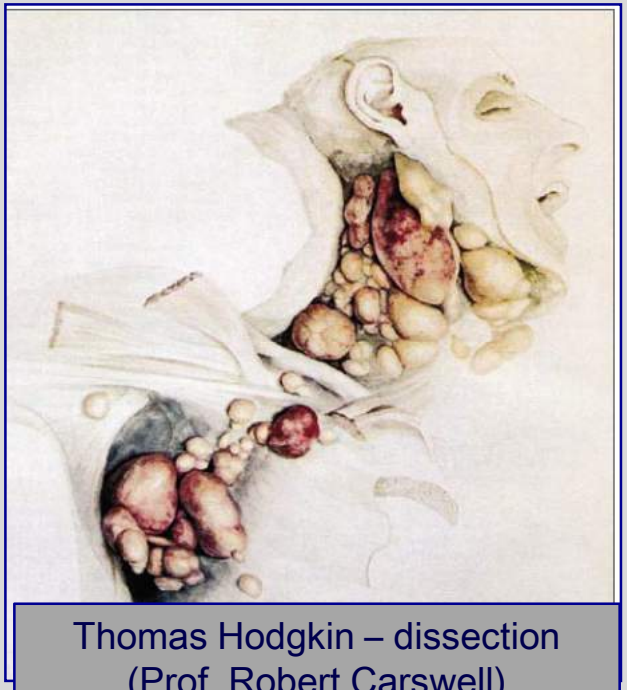
BY DR. HODGKIN.

PRESENTED

BY DR. R. LEE.

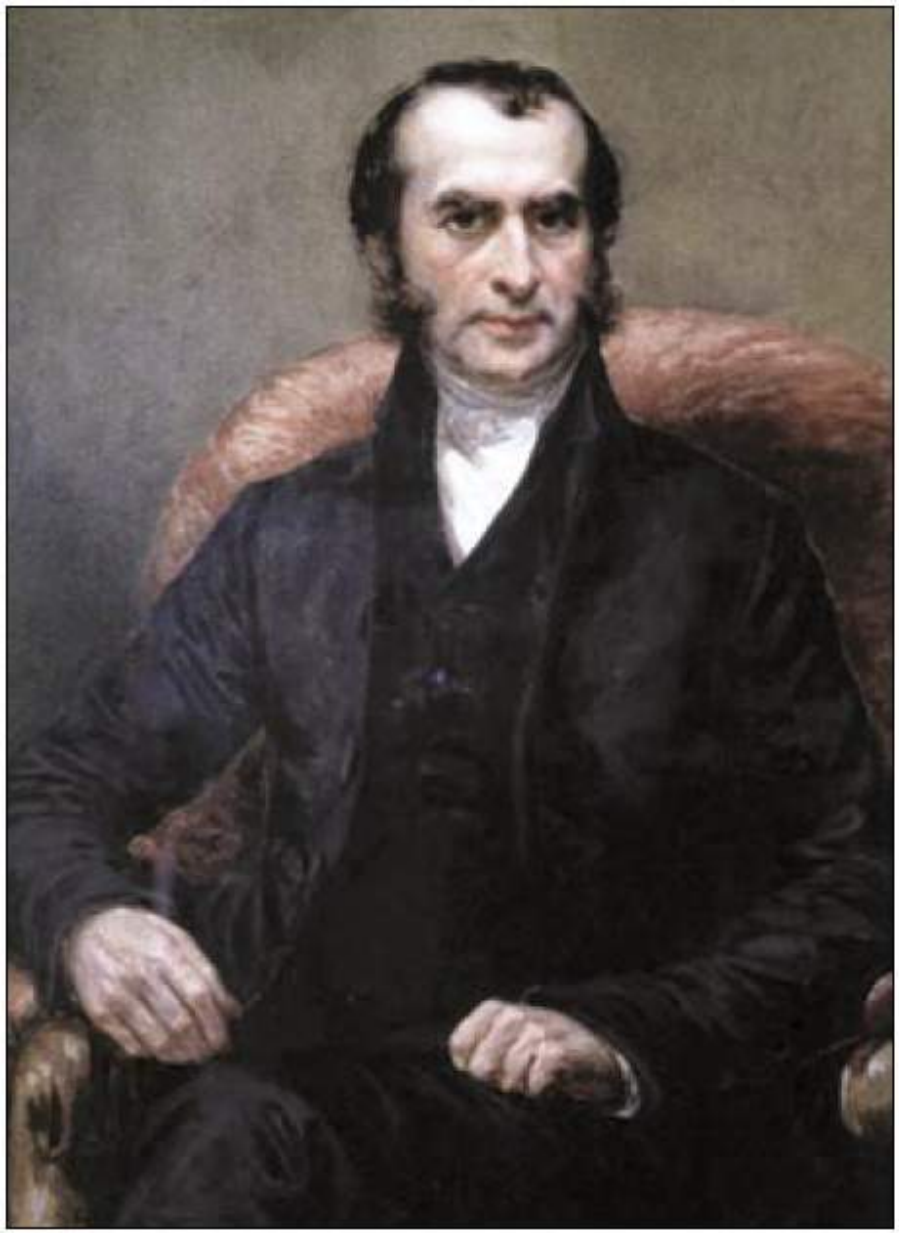
READ JANUARY 10TH AND 24TH, 1832.

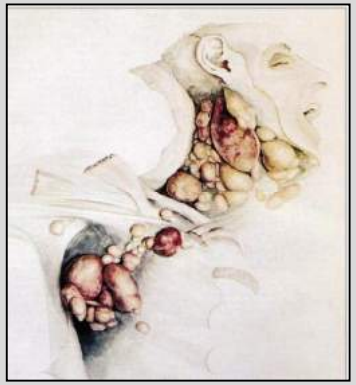
THE morbid alterations of structure which I am about to describe are probably familiar to many



Thomas Hodgkin – dissection
(Prof. Robert Carswell)

Thomas Hodgkin 1798-1866





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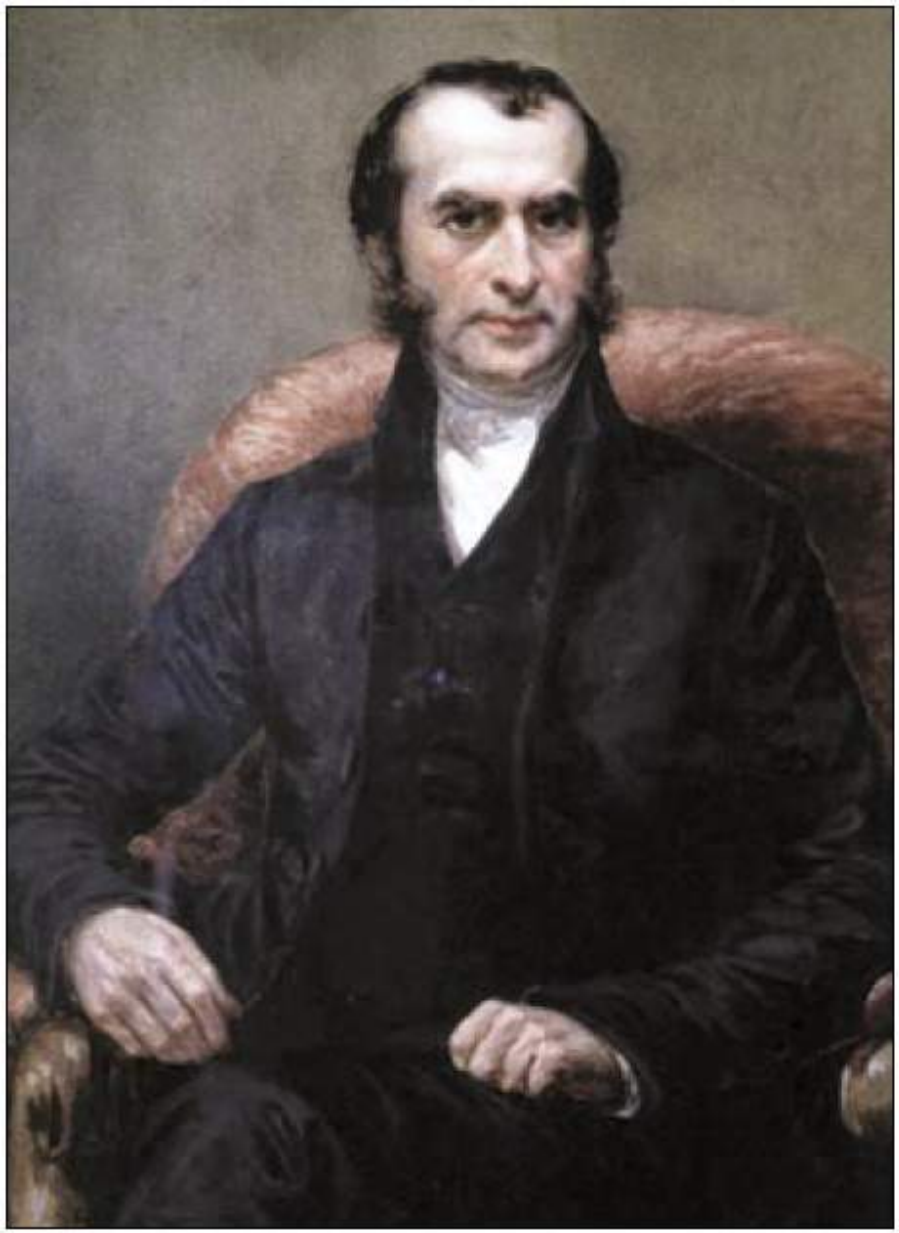
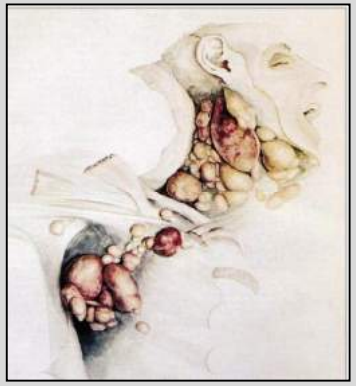
Hodgkin's original case: abdominal nodes

Gordon Museum,
King's College London

Thomas Hodgkin 1798-1866





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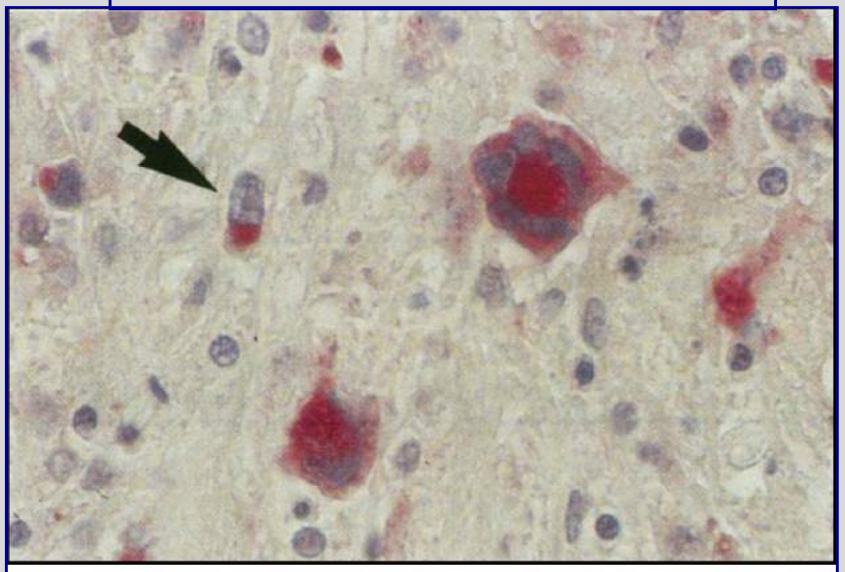
Hodgkin's original case: CD15 (1991)

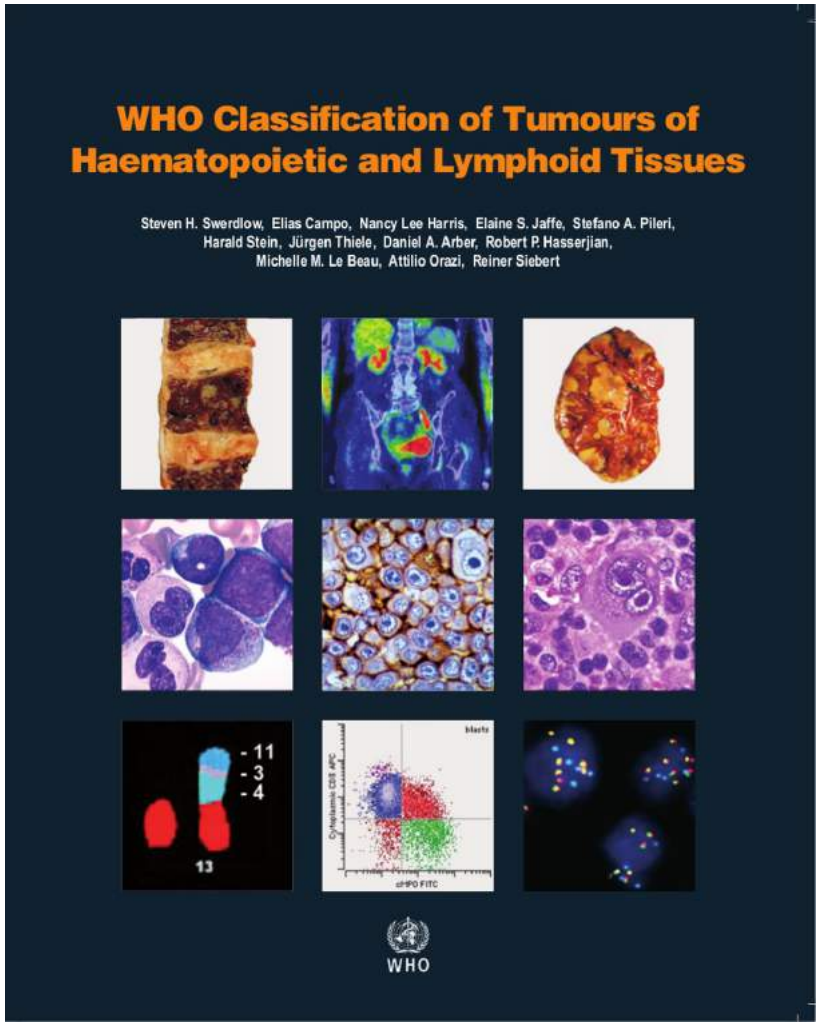


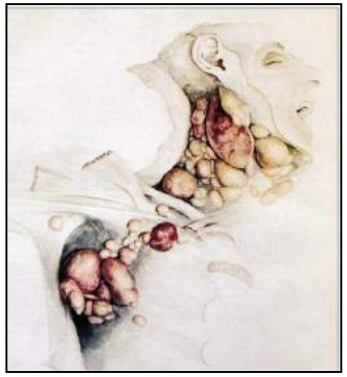
Paraffin-embedded tissue of one of the original cases byThomas Hodgkin immunostained with CD15 (David Mason)

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017

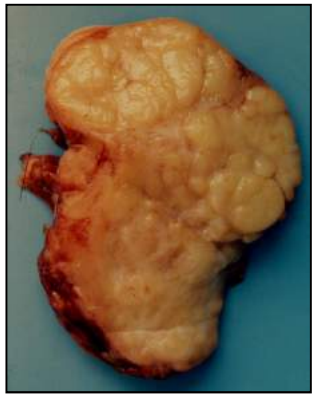
- 70s – 80s: Kiel classification
  - B vs T cells: IHC!!
- 90s: REAL classification
- WHO (latest …..2017)
  - ”Real” disease entities

  - 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. Jaffe[11]

[1]Division of Hematopathology, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Department of Pathology, Hospital Clinic, University of Barcelona, August Pi i Sunyer Biomedical Research Institute, Barcelona, Spain; [3]Haematopathology Unit, European Institute of Oncology, Milan, and Department of Experimental, Diagnostic and Specialty Medicine, Bologna University Medical School, Bologna, Italy; [4]Department of Pathology, Harvard Medical School and Massachusetts General Hospital, Boston, MA; [5]Pathodiagnostik, Berlin, Germany; [6]Institute of Human Genetics, Christian Albrechts University Kiel, Kiel, Germany; [7]Division of Oncology, Department of Medicine, Stanford University, Stanford, CA; [8]Department of Medical Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [9]Department of Hematology, Hospices Civils de Lyon, and Université Claude Bernard Lyon-1, Lyon, France; [10]Department of Medicine, Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY; and [11]Hematopathology 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 gets longer & longer!**
- **> 100 lymphoma entities**

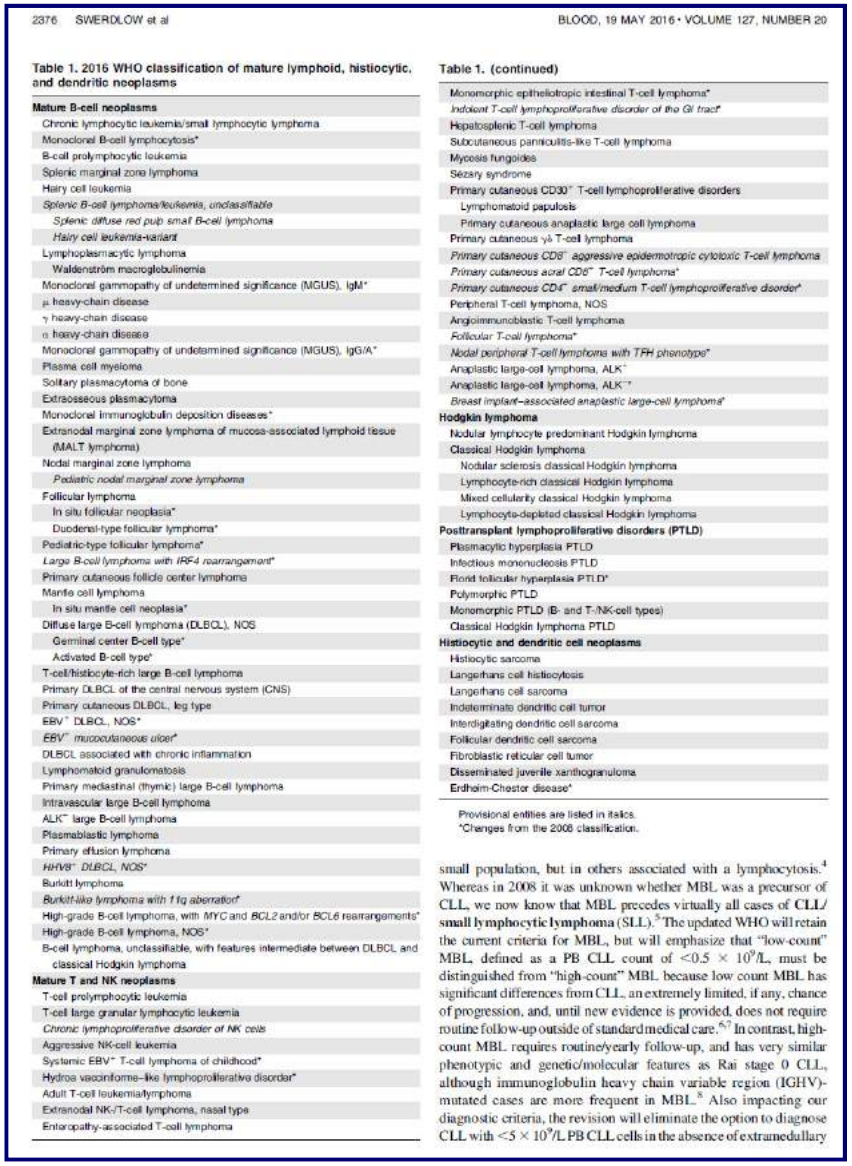


Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
γ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
*Large B-cell lymphoma with IRF4 rearrangement**
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV+ DLBCL, NOS*
*EBV+ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK+ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8+ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
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*
Hydroa vacciniforme–like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma

Table 1. (continued)

Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract**
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30+ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8+ T-cell lymphoma**
*Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large-cell lymphoma, ALK+
Anaplastic large-cell lymphoma, ALK−*
*Breast implant–associated anaplastic large-cell lymphoma**
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

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.[5,7] In contrast, high-count MBL requires routine/yearly 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

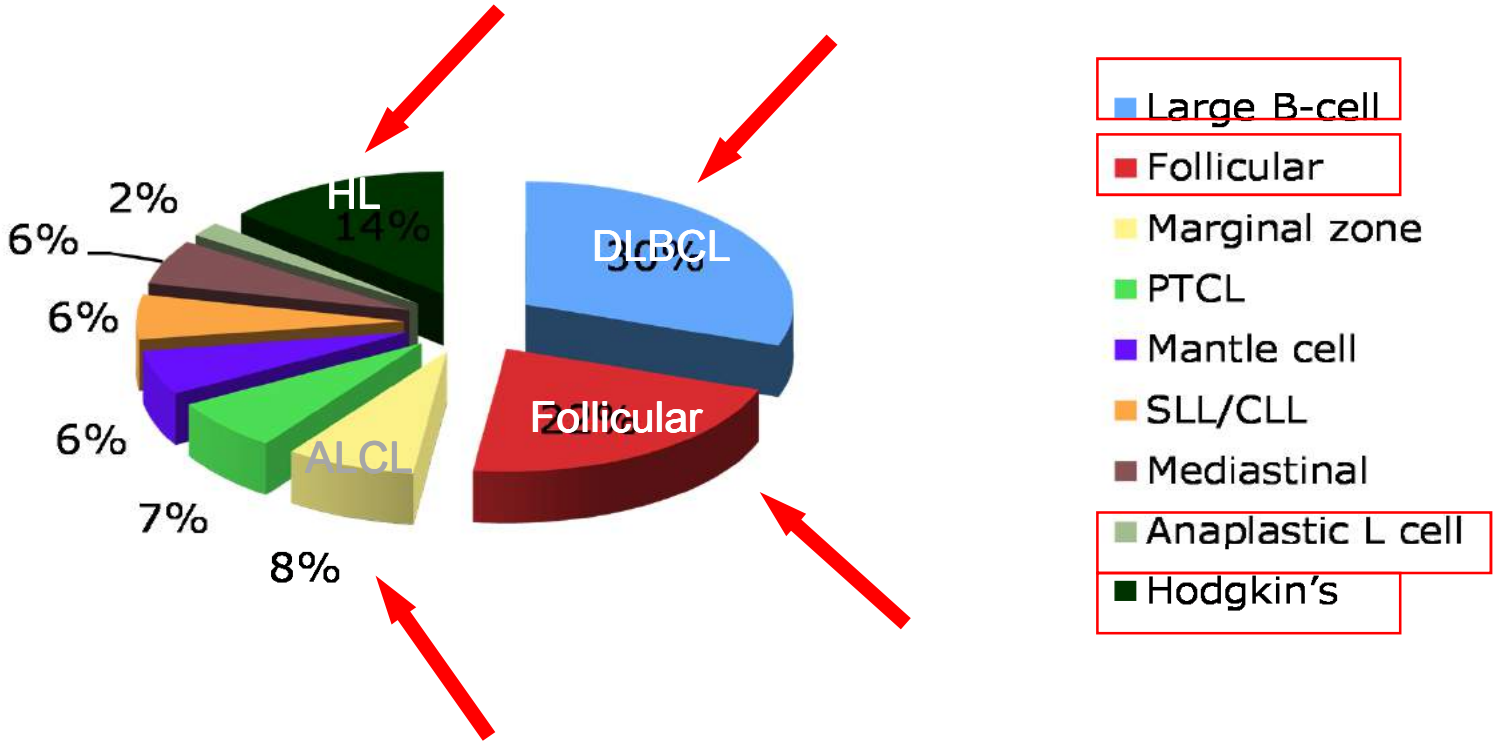
Major immunophenotypic changes:

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*"**

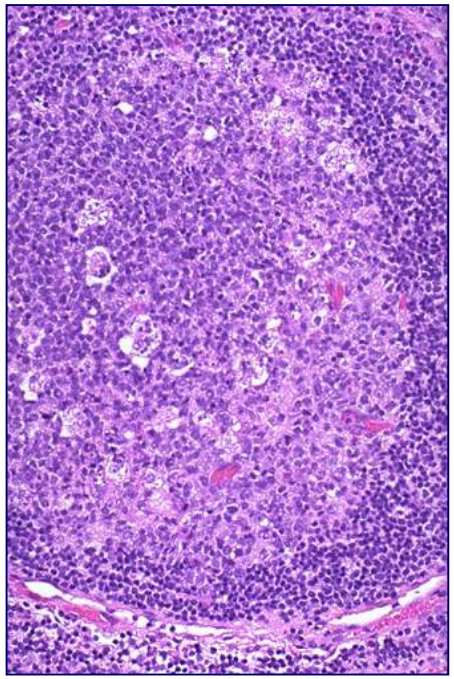
Lymphoma frequencies

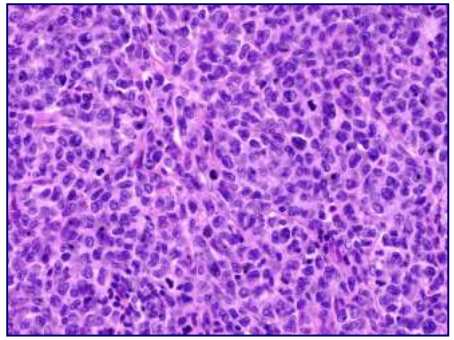


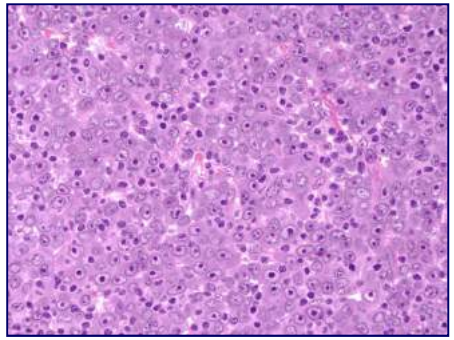
2002 SEER database. O'Connor

What is lymphoma?

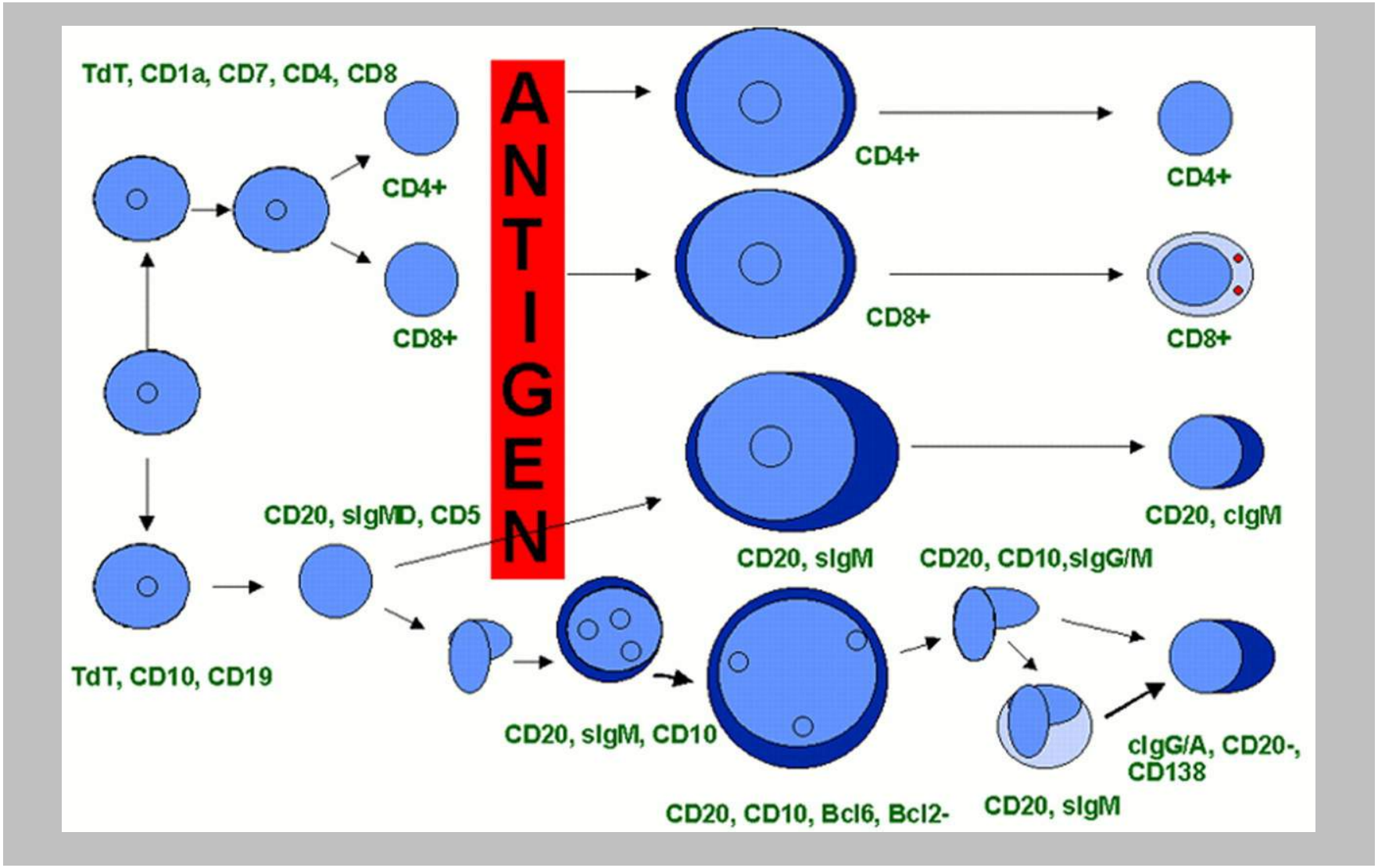
- **Clonal malignancy**
  - **→ mutational events cause cells to freeze at a single stage of normal lymphocyte differentiation**
- **Morphology, immunophenotype & molecular features:**
  - **mirror stages of normal lymphocyte development**



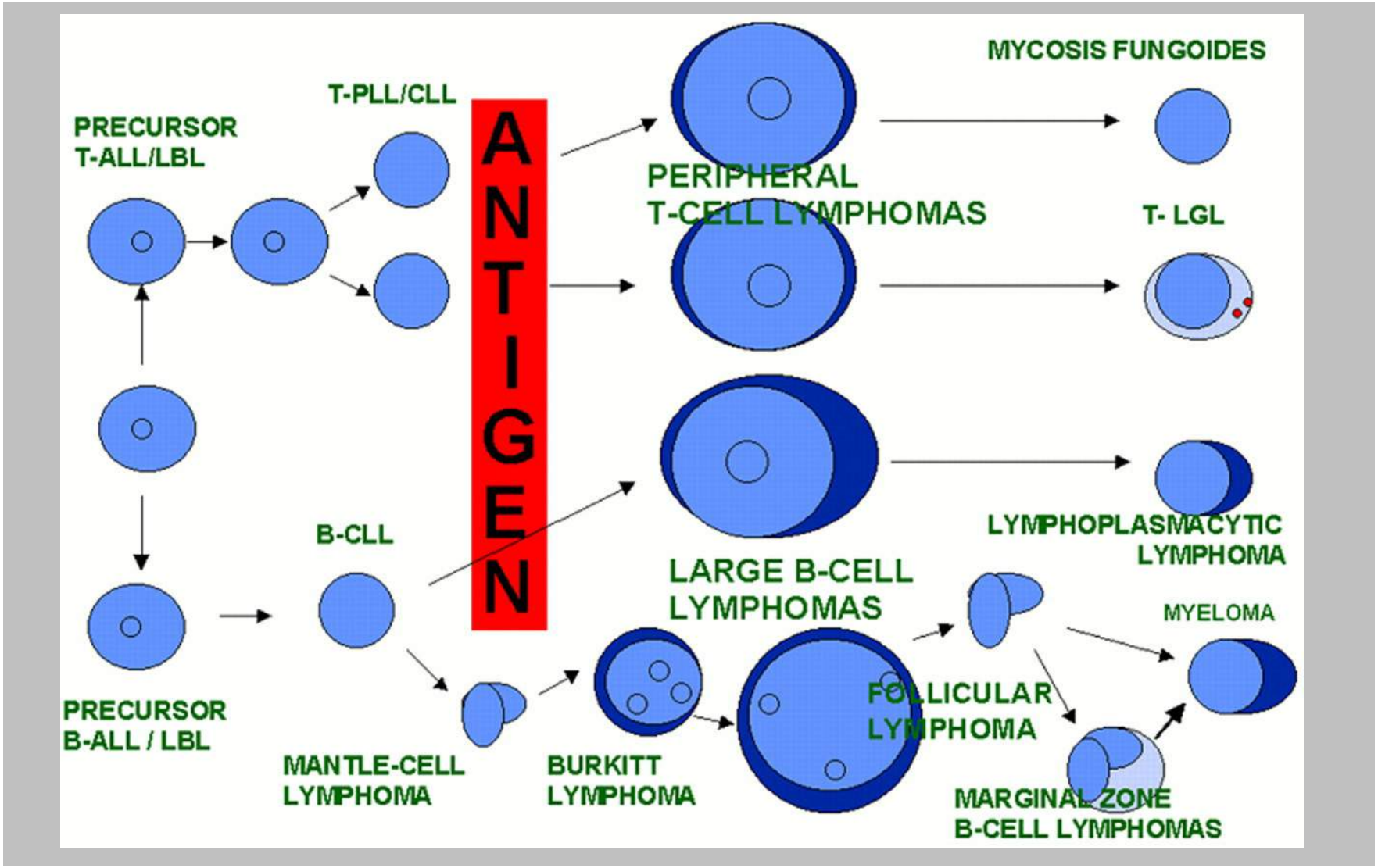




T and B-cell differentiation:
Stage-specific surface antigen expression

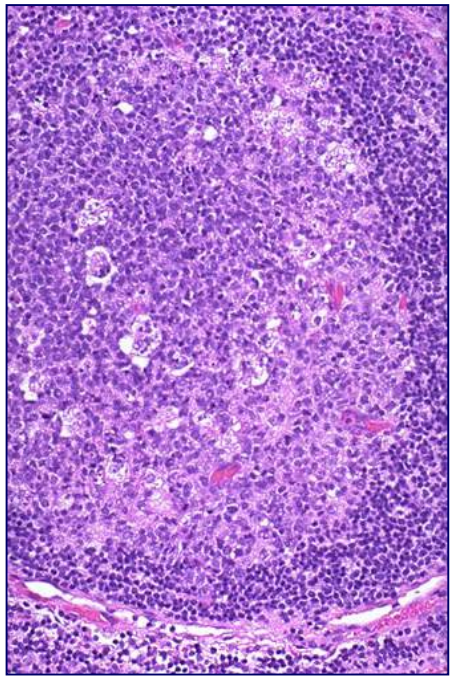


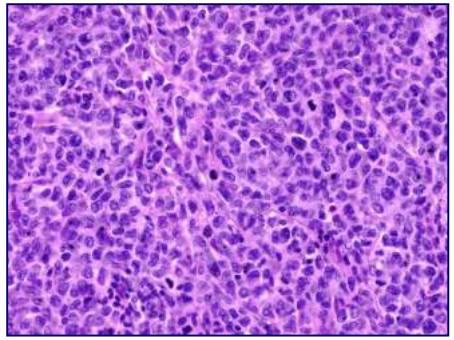
Lymphoid neoplasms:
Correlation with normal T or B-cell differentiation

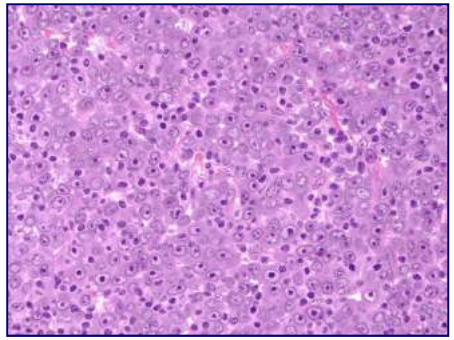


What is lymphoma?

- 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**





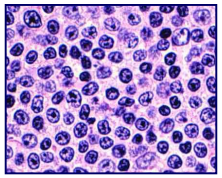
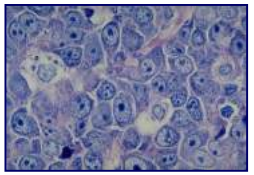


Lymphoma & Leukaemia diagnosis

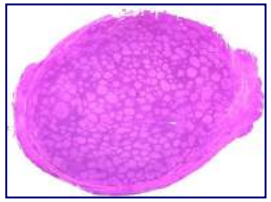
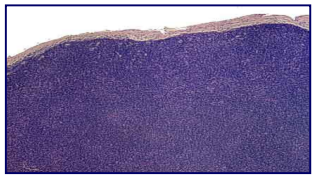
- Clinical features
- **Morphology**
- Immunophenotype
- Molecular diagnosis

Lymphoma differential diagnosis

- **Assess morphology:**
  - **cell size**

  - **architecture**

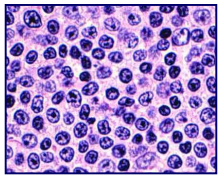
But...that's not enough!

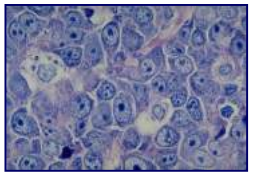
Lymphoma & Leukaemia diagnosis

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

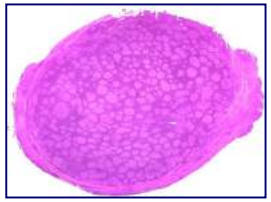
Lymphoma differential diagnosis

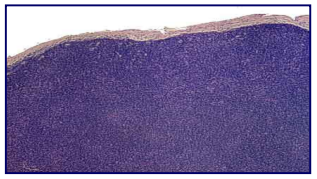
- **Assess morphology:**
  - **cell size**



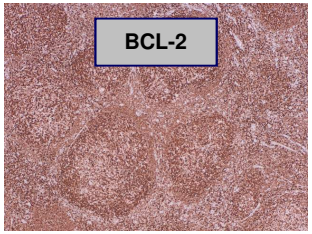


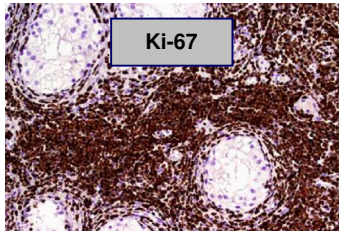
  - **architecture**

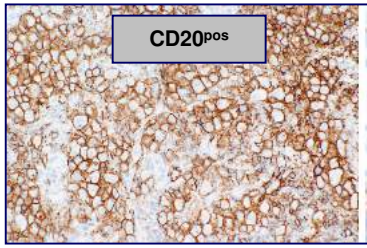


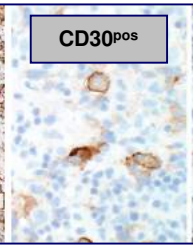


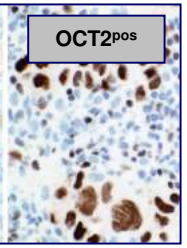
- **Select appropriate immune panel(s)**

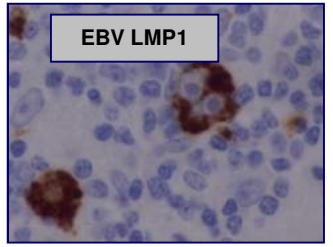






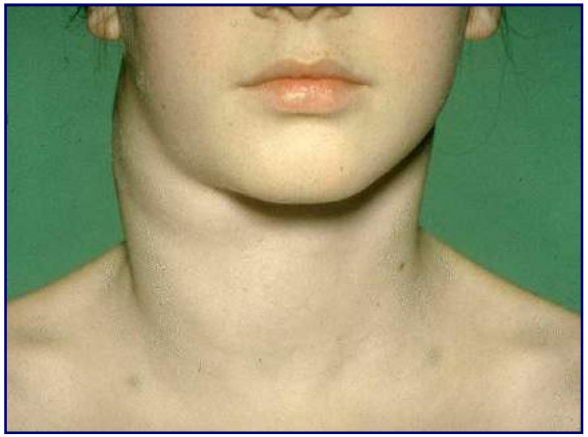






Enlarged lymph node

Is it malignant?



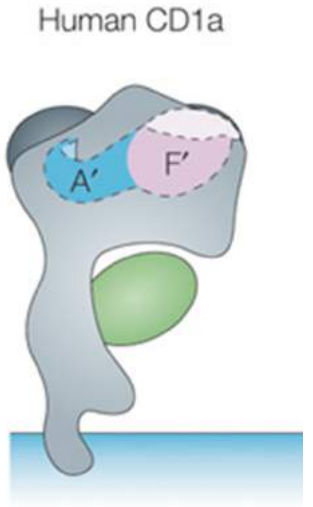
- Emphasis on lymphoma classification
- Reactive *vs* malignant
  - often more challenging diagnosis
- Use IHC to evaluate lymphoid tissue cytology and architecture
- Correlate immunophenotype with disease entity

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

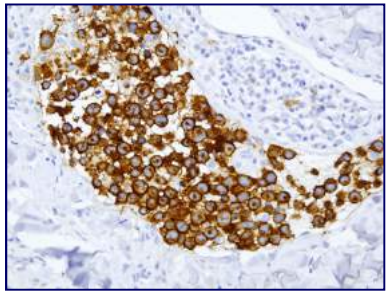
...and many more!

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

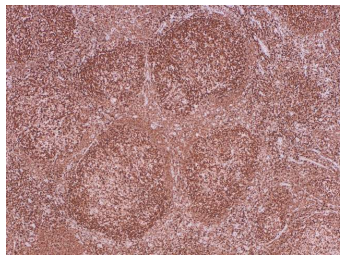
IHC Dogma



(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

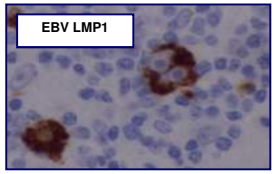
Diagnostic Applications of IHC 1



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



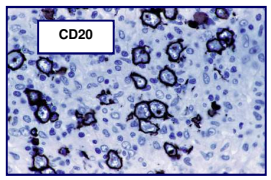
- T-cell lymphoma *vs* B-cell lymphoma
- T-cell lymphoma *vs* T-zone hyperplasia
- Hodgkin lymphoma *vs* NHL
- Hodgkin lymphoma
  - NLPHL *vs* classical HL
- Lymphoblastic vs. Myeloblastic vs. Burkitt
- Undifferentiated malignant tumor
- Lymphoma prognosis
  - e.g. Ki-67; ALK; c-myc
- Targeted therapy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

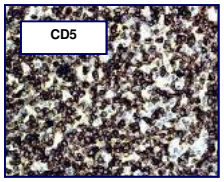
Useful antigens in haematopathology

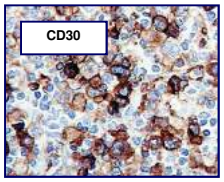
- CD45
- B-cell ‘specific’
  - CD19
  - CD20
  - CD79α
  - Pax-5
  - OCT-2 / BOB1
  - Ig
- T-cell ‘specific’
  - CD3
  - CD5
  - CD2
  - CD7
  - CD1a
  - CD4
  - CD8
  - PD-1/CXCL-13 (TFH)

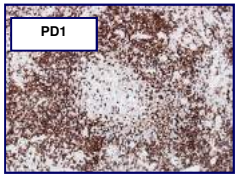
- Other
  - CD30
  - CD10
  - Bcl-2
  - Bcl-6
  - ALK
  - c-myc
  - CD21
  - CD23
  - CD15
  - TdT
  - Cyclin-D1
  - SOX-11
  - CD56
  - TIA-1, granzyme, perforin
  - PDL-1

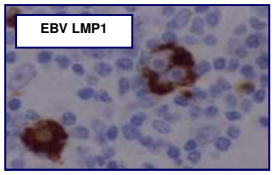
- Other
  - EBV
    - LMP1
    - EBNA2
    - (EBER)
  - CD56
  - CD57
  - EMA
  - S100
  - CD68
  - CD163









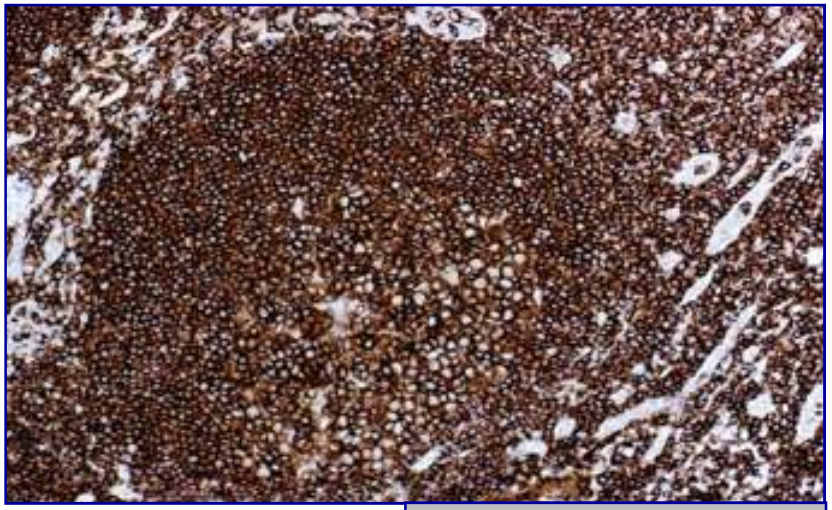


Basic IHC panel for lymphoma diagnosis

- CD45
- CD20
- CD79α
- (PAX-5)
- (kappa/lambda)
- CD3
- CD5
- CD30
- CD43
- Bcl-2
- Bcl-6
- CD23 (CD21)
- Cyclin-D1
- Ki-67

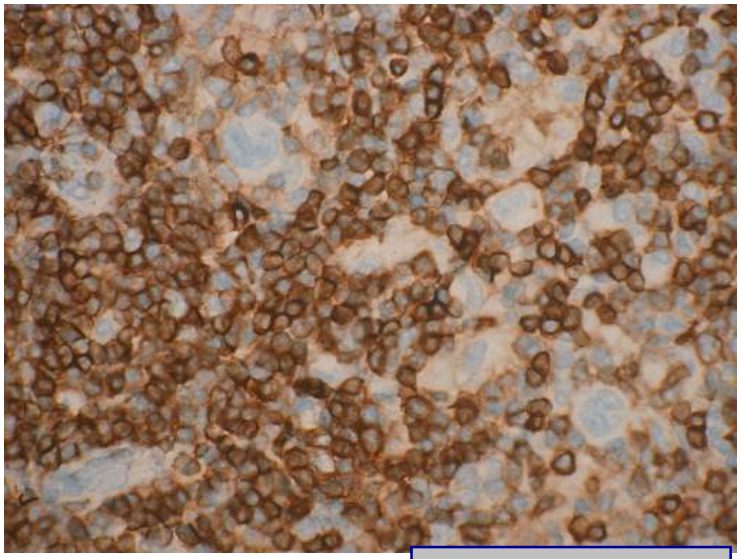
Basic stains: CD45

- 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

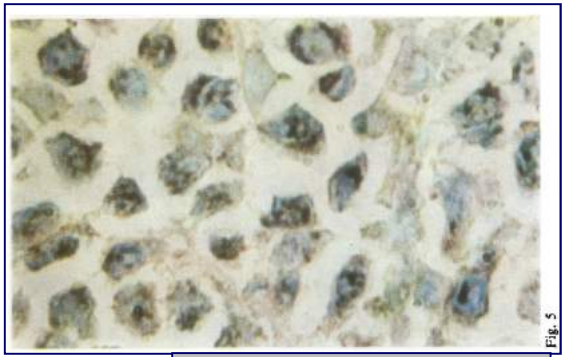
J. clin. Path., 1974, **27**, 14-20

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

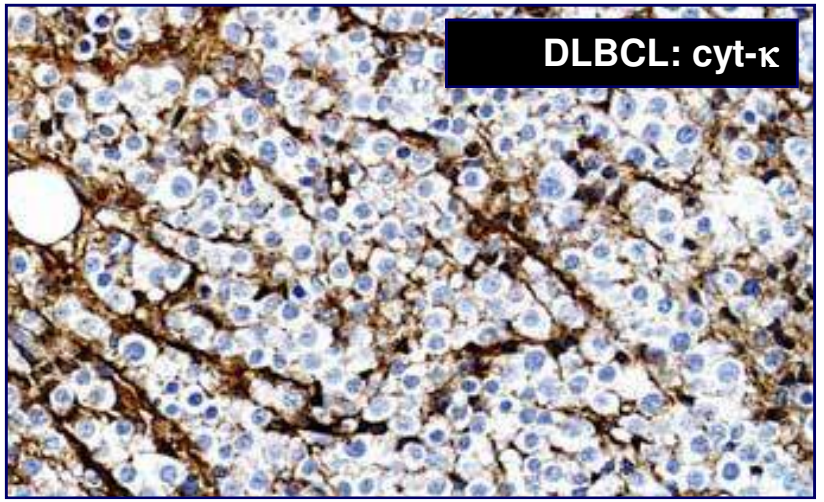
- **IHC-Ig**
  - **first protocol for IHC in FFPE**
  - **still one of the hardest to perform & evaluate!**

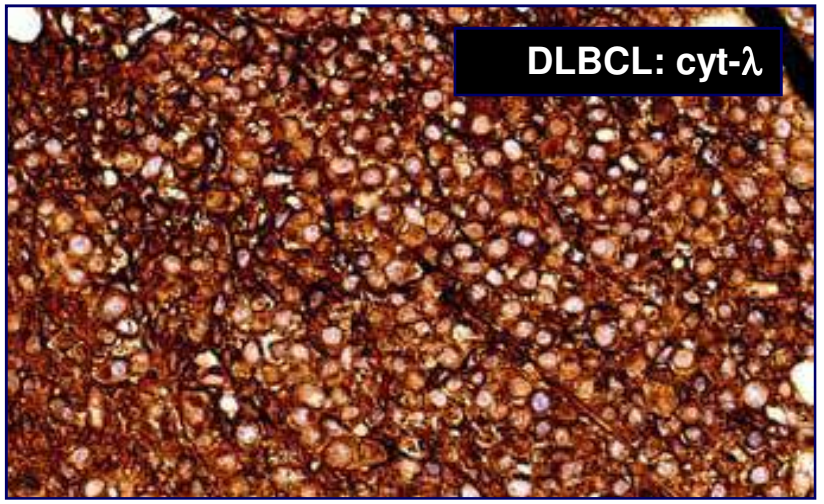


- **plasmacytoma**
- **monoclonal Ig-kappa**

Basic stains: Immunoglobulin

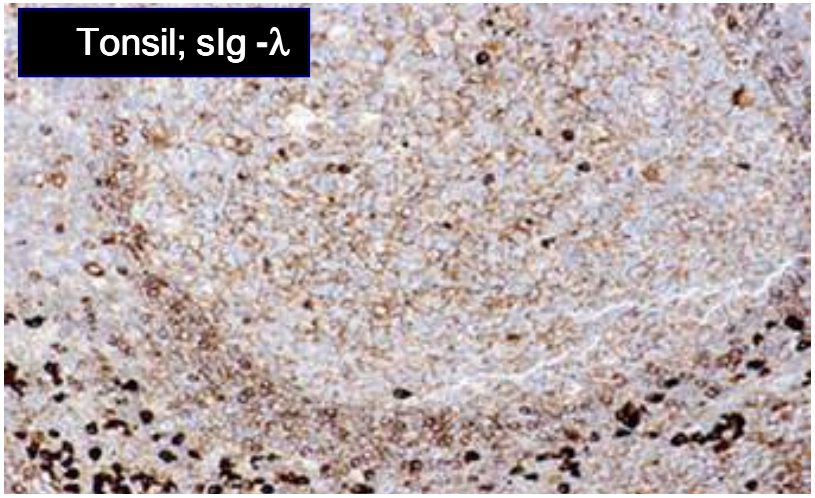
- B-cell specific
- Normal κ:λ ratio ca. 3-4:1
- Monotypic Ig restriction
  - Suggests clonality
  - >10:1 or < 0.2:1 = restriction
- Cytoplasmic Ig easily shown
- In lymphomas:
  - Cy Ig:
    - lymphoplasmacytic; myeloma; MZL; DLBCL, FL
  - Surface Ig

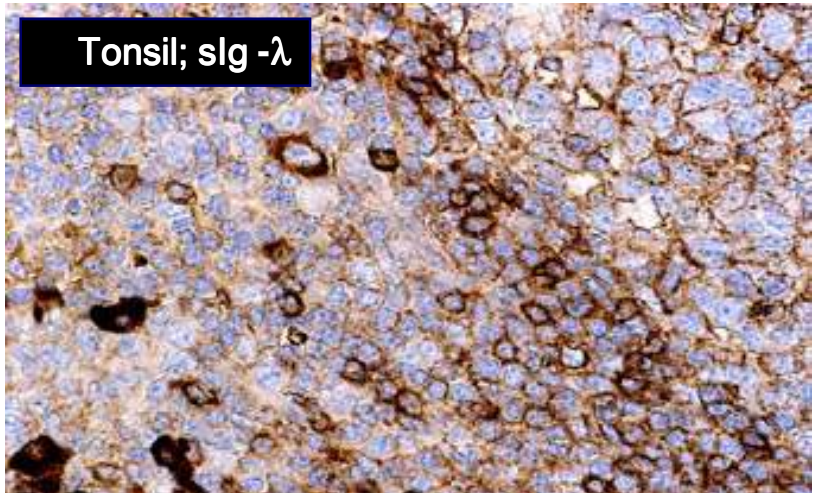




Basic stains: Immunoglobulin

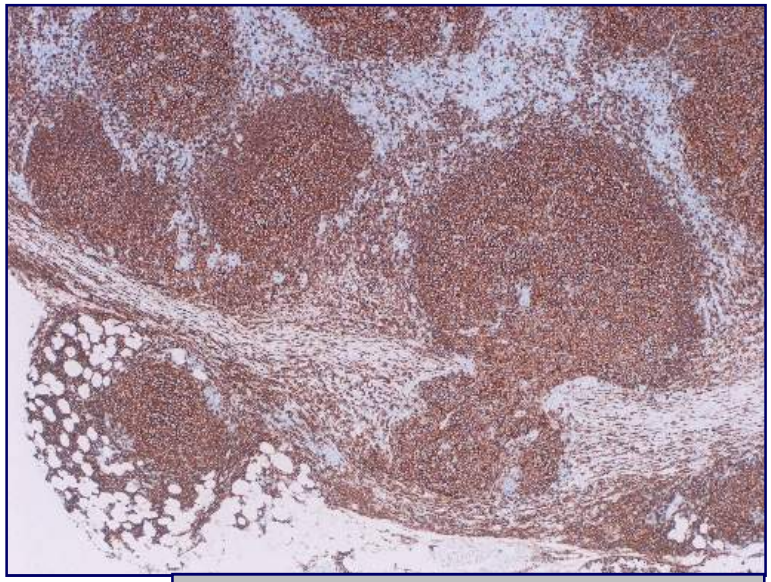
- **Surface Ig**
  - **B-NHL clonality**
  - **Requires sensitive, optimised technique**
  - **Interpretation difficult (serum Ig)**





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
  - may be aberrantly lost after treatment with Rituximab



Follicular lymphoma: CD20

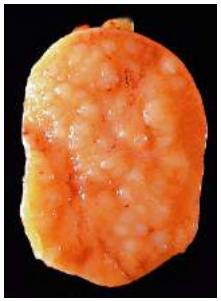
Usual staining pattern of B-cell neoplasms

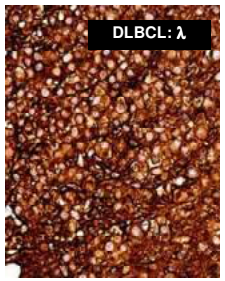
	CD20	CD79	CD5	CD23	CD10	CD30	CD15	CyclinD1
Precursor B-cell neoplasms								
Precursor B-lymphoblastic leukaemia/lymphoma	–	+/–	–	–	+	–	–	–
Mature B-cell neoplasms								
B-cell chronic lymphocytic leukaemia/lymphoma	+	+	+	+	–	–	–	–
B-cell prolymphocytic leukaemia	+	+	–	+/–	–	–	–	–/+
Lymphoplasmacytic 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	+	+	–	–	+	–	–	

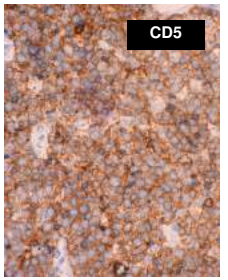
Key

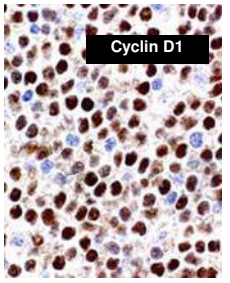
+/– The lymphoma cells are commonly but not always positive

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



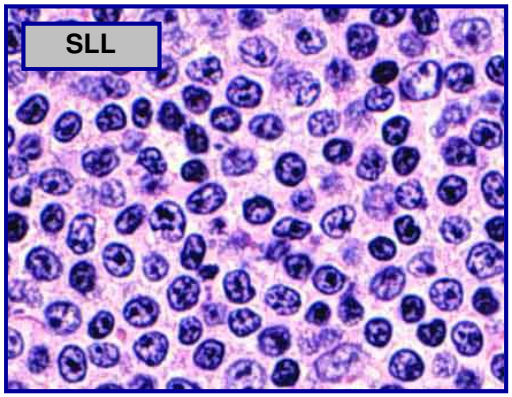






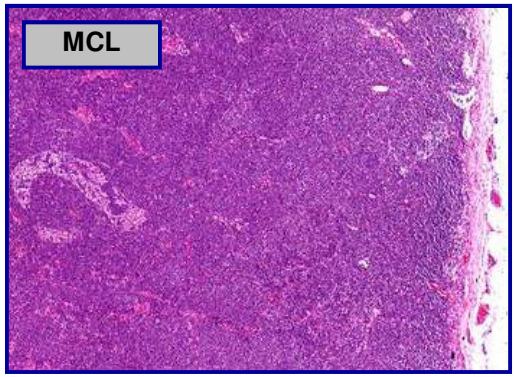
Small cell B-Cell lymphomas: Differential Diagnosis

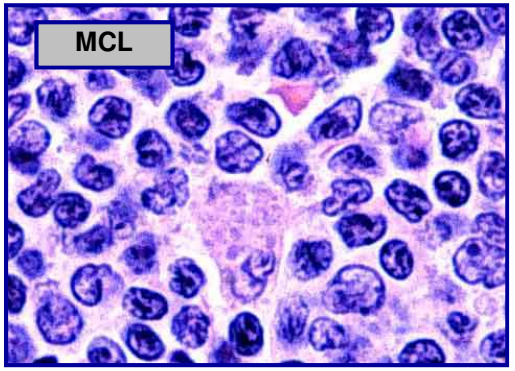
Small lymphocytic NHL



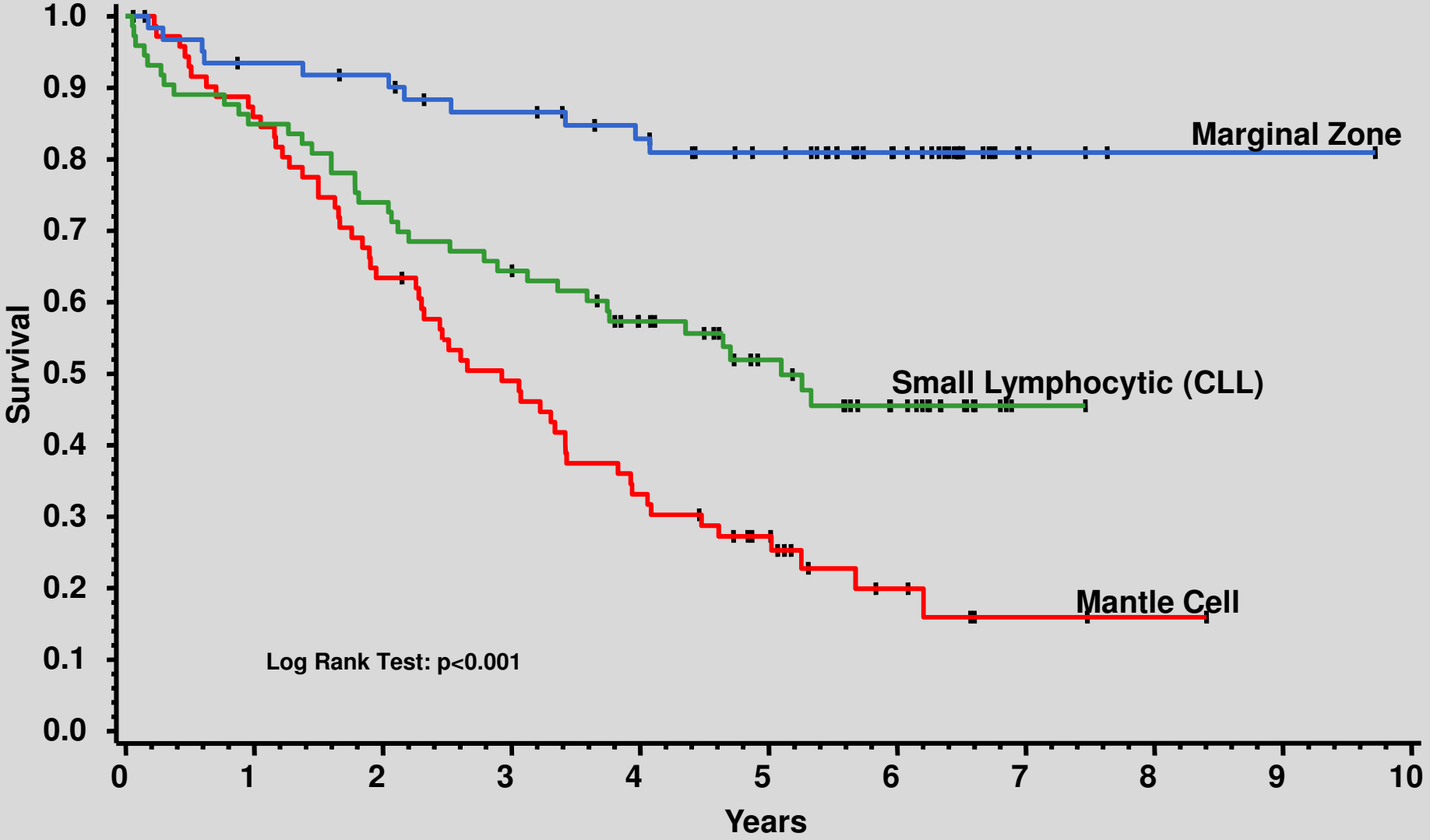
?

Mantle cell NHL



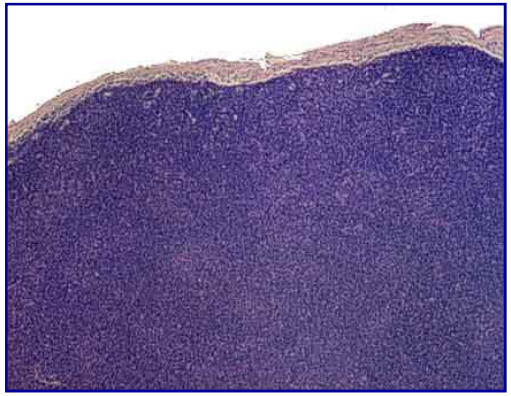


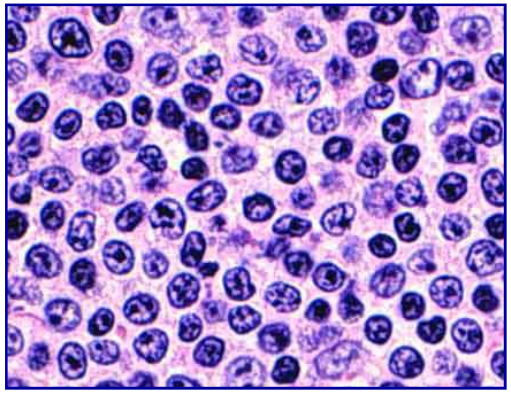
Small B-Cell Lymphomas: Overall Survival

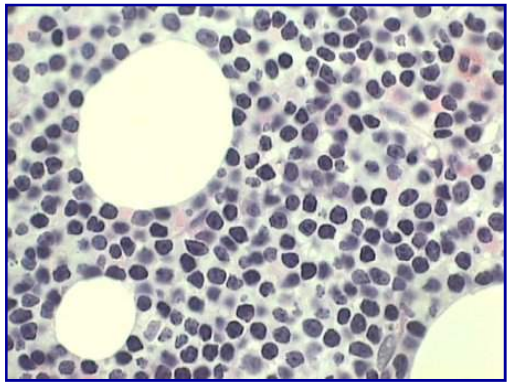


Armitage et al, 1997

B-cell Small Lymphocytic Lymphoma (CLL)





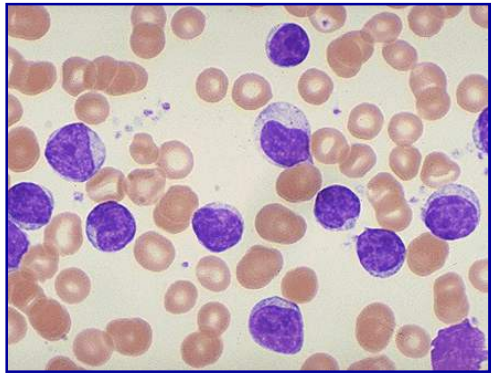


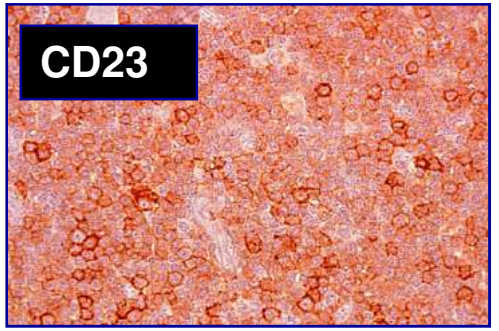
Morphology

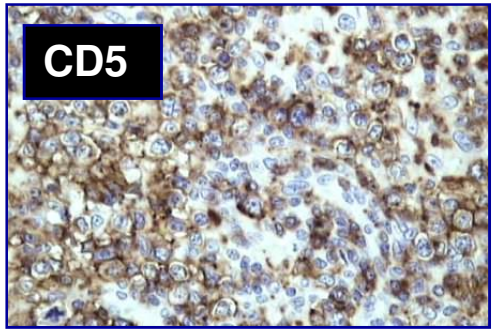
- small lymphocytes
- proliferation centres

Immunology

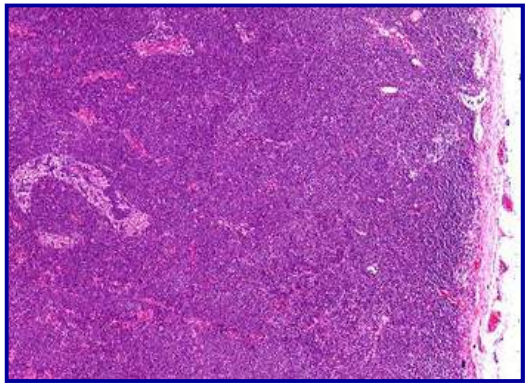
• surface IgMD weak	
• CD19, 20, 79a	+
• CD5	+
• CD23	+
• CD10, CycD1	-

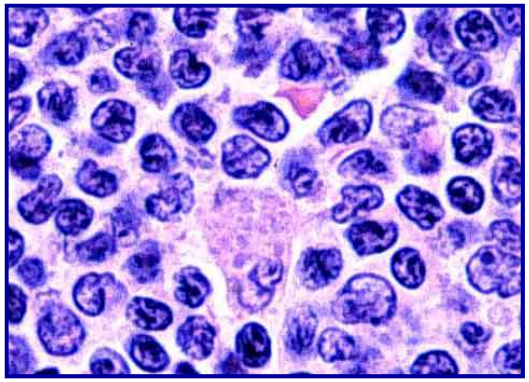


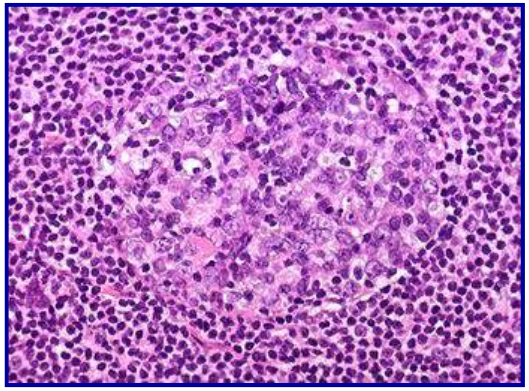




Mantle Cell Lymphoma





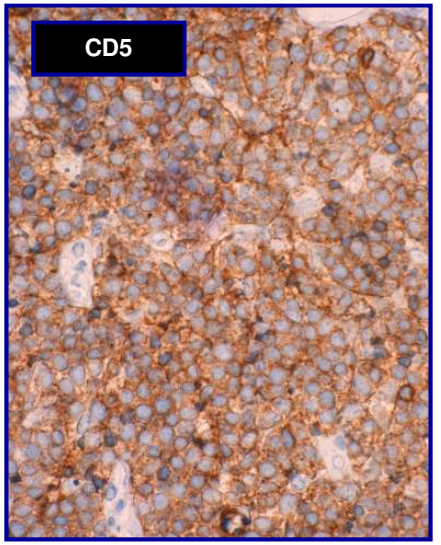


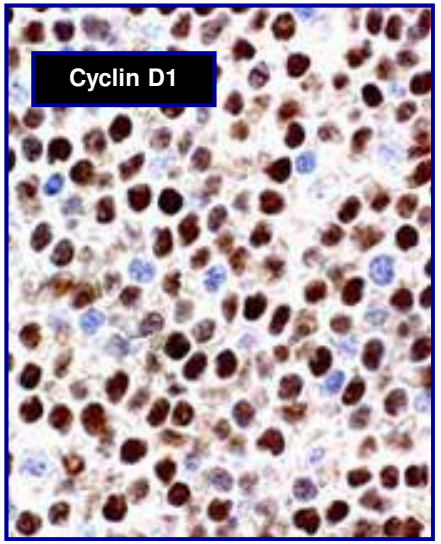
Morphology

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

Immunology

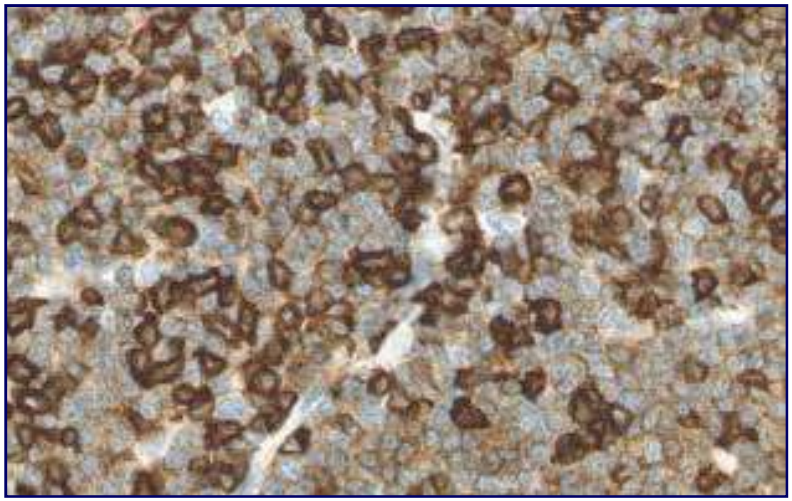
• surface Ig	+
• CD19, 20, 22, 79a	+
• CD5	+
• CD23	-
• Cyclin D1	+
• CD10	-





Basic stains: CD5

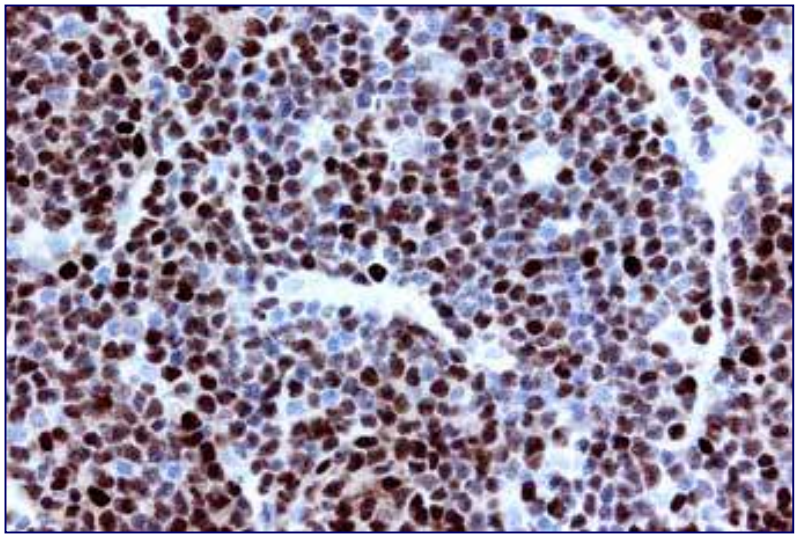
- Modulates T & B cell signalling
- Pan-T cell marker
  - 95% thymocytes
  - 100% post-thymic T-cells
  - ↑ expression with maturity
- Minor population normal B-cells:
  - ca. 10%+ peripheral B-cells
  - ↑ in autoimmunity
- Lymphomas:
  - 90% T-cell neoplasias
  - B-cell NHL
    - B-CLL / SLL (90%)
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

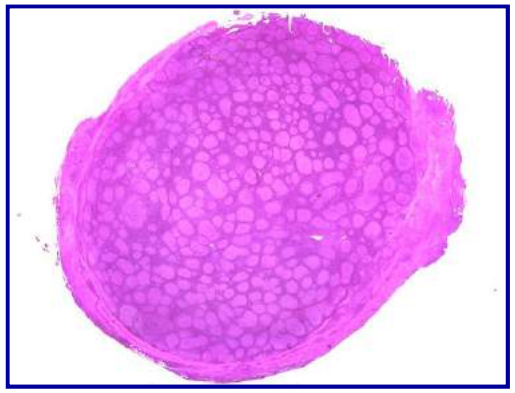


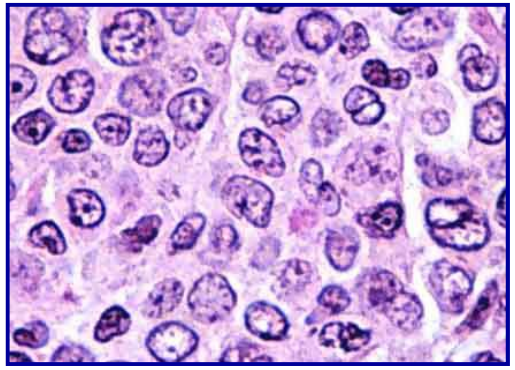
Mantle cell NHL: cyclin-D1

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



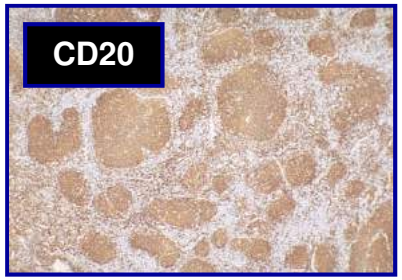


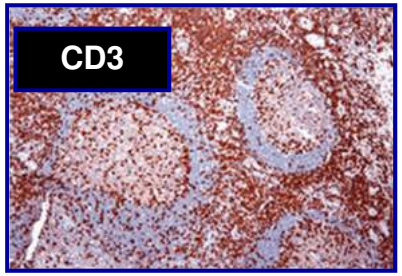
Morphology

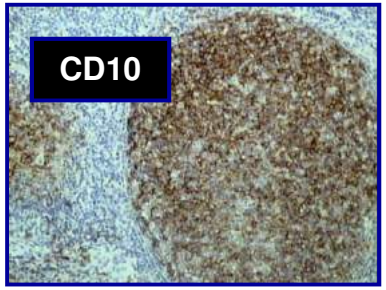
- germinal centre cells
- CBs & CCs
- follicular

Immunology

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

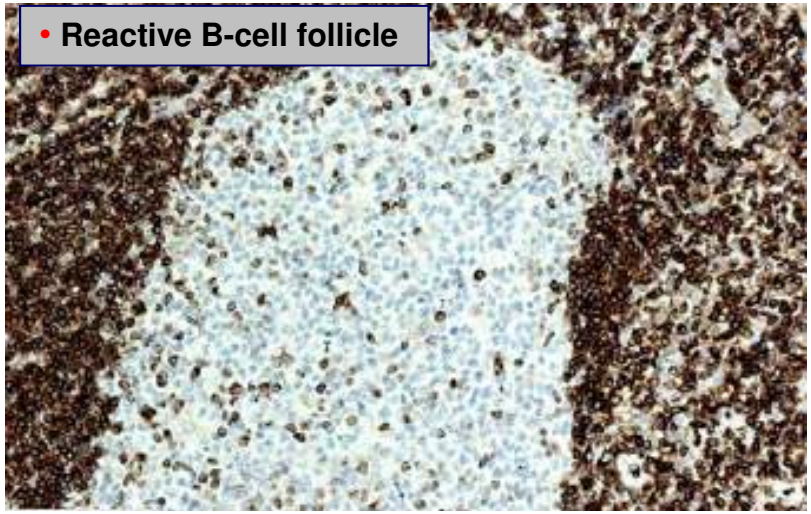




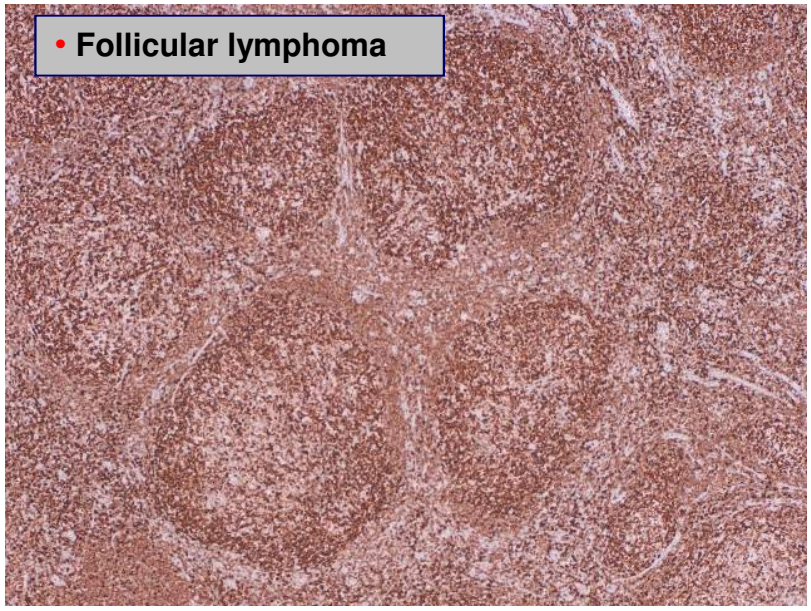


Basic stain: bcl-2

- 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 lyphomas re bcl-2 negative

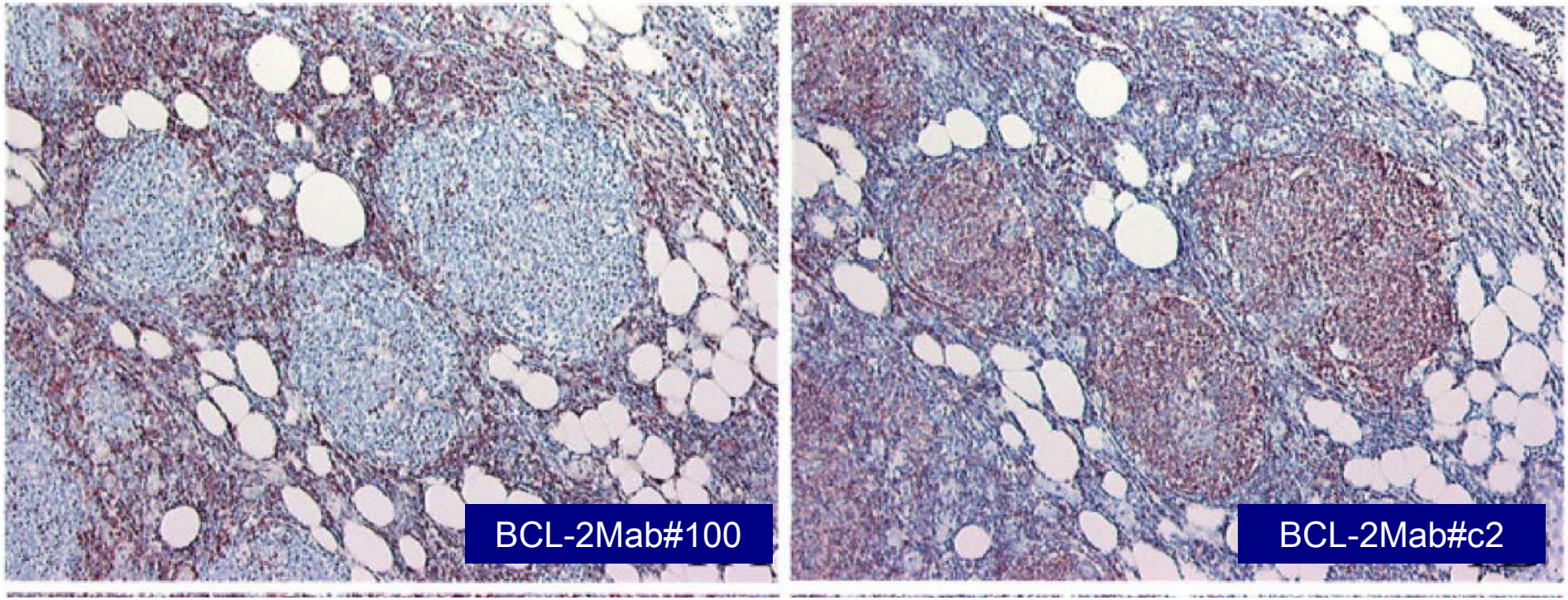


Journal of Pathology
J Pathol 2005; **205**: 329–335
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1689

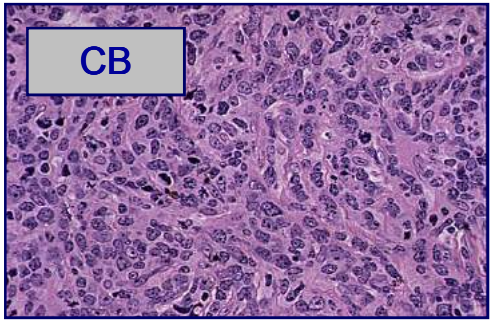
Original Paper

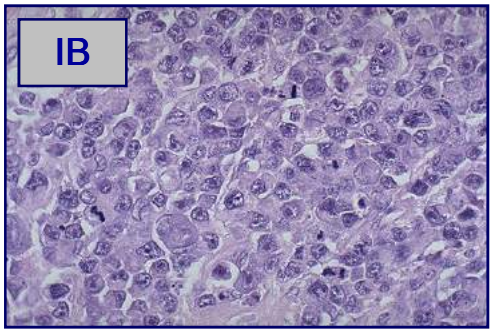
Lack of Bcl-2 expression in follicular lymphoma may be caused by mutations in the *BCL2* gene or by absence of the t(14;18) translocation

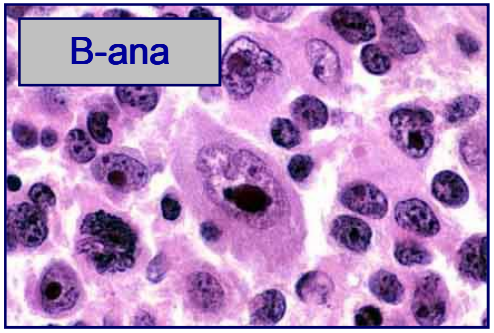
Margit Schraders,[1]* Daphne de Jong,[2] Philip Kluin,[3] Patricia Groenen[1] and Han van Krieken[1]



Diffuse Large B-cell Lymphoma





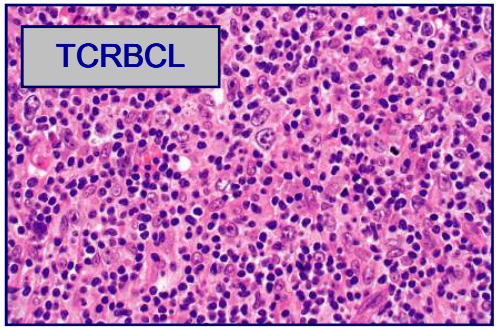


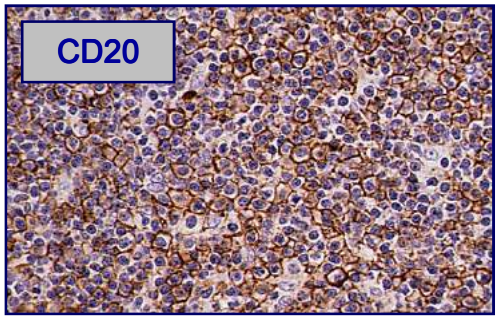
Morphology

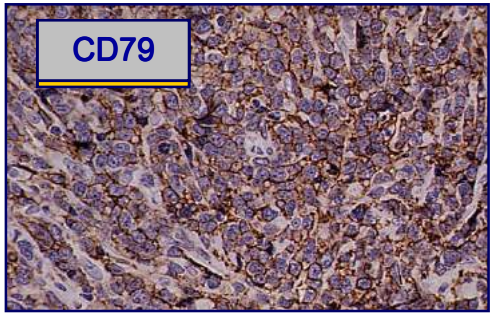
- large cells
- nucleoli
- diffuse

Immunology

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

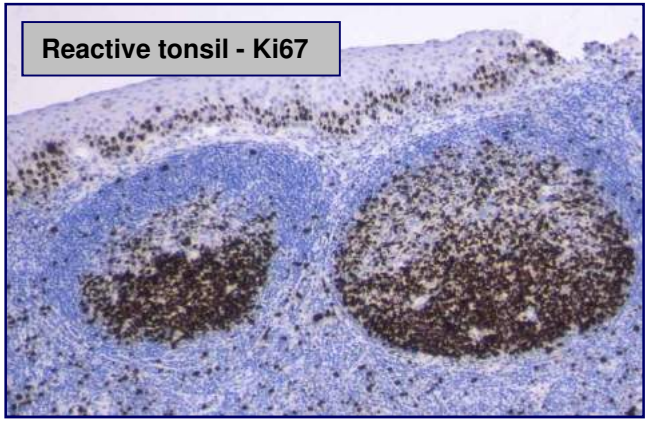


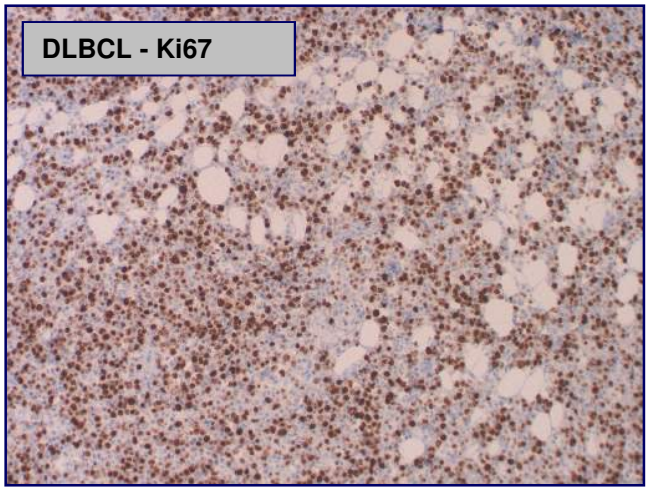


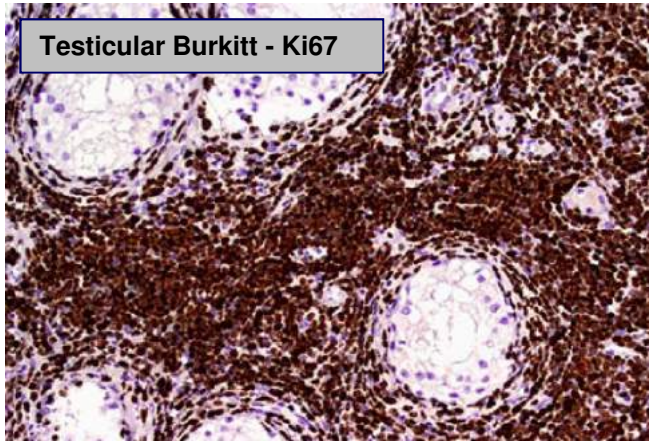


Basic stain: Ki- 67

- 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

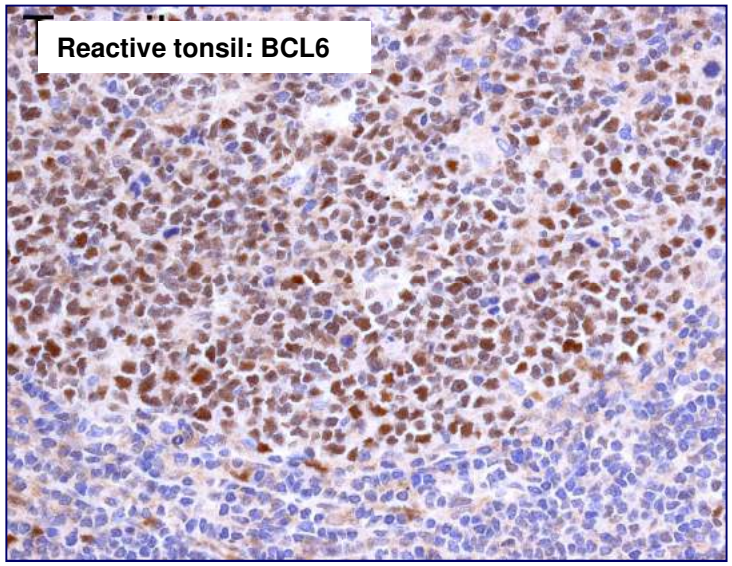






Basic stain: Bcl-6

- 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

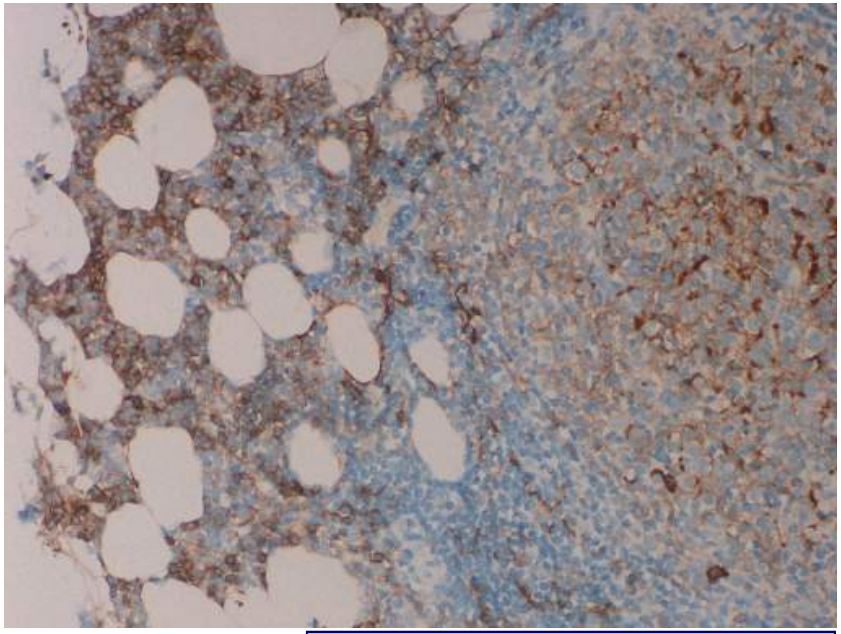


IHC for DLBCL
Add to basic panel:

- CD10
- CD138
- MUM1

Secondary stain: CD10

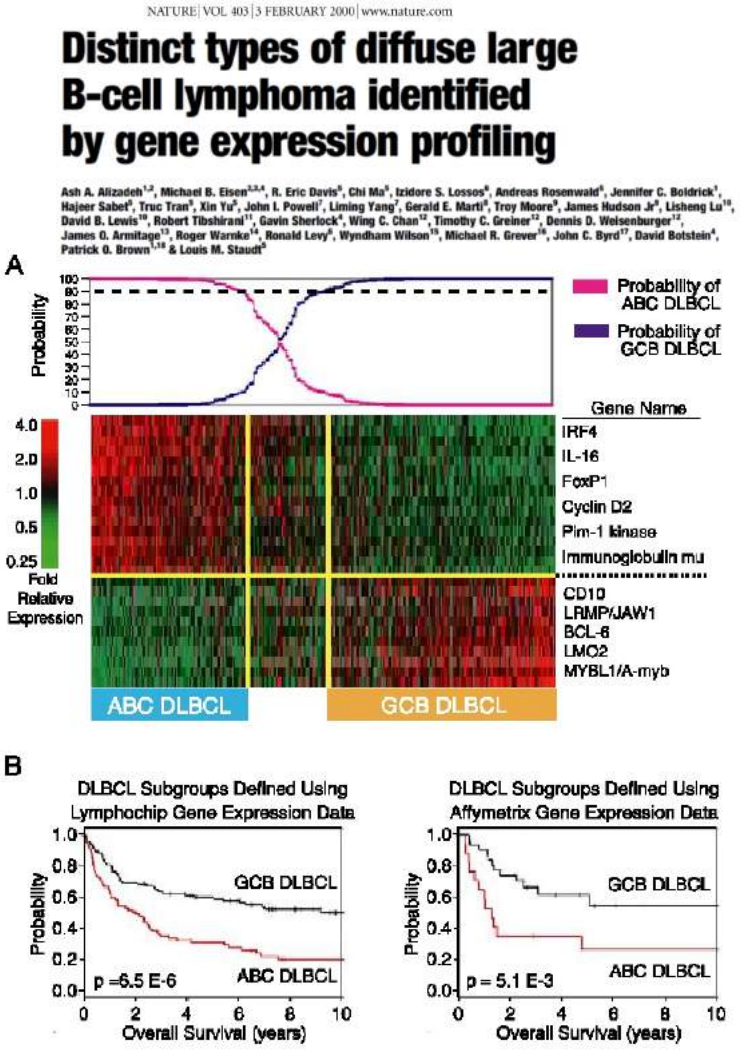
- >90% precursor B-LB (membrane & paranuclear stain)
- ca. 25% precursor T-LB
- Burkitt lymphoma
- Follicular lymphoma
  - Interfollicular CD10+ cells suggets lymphoma
- Some DLBCL
  - 'Cell of origin' algorithm in DLBCL
  - GCB *vs* ABC



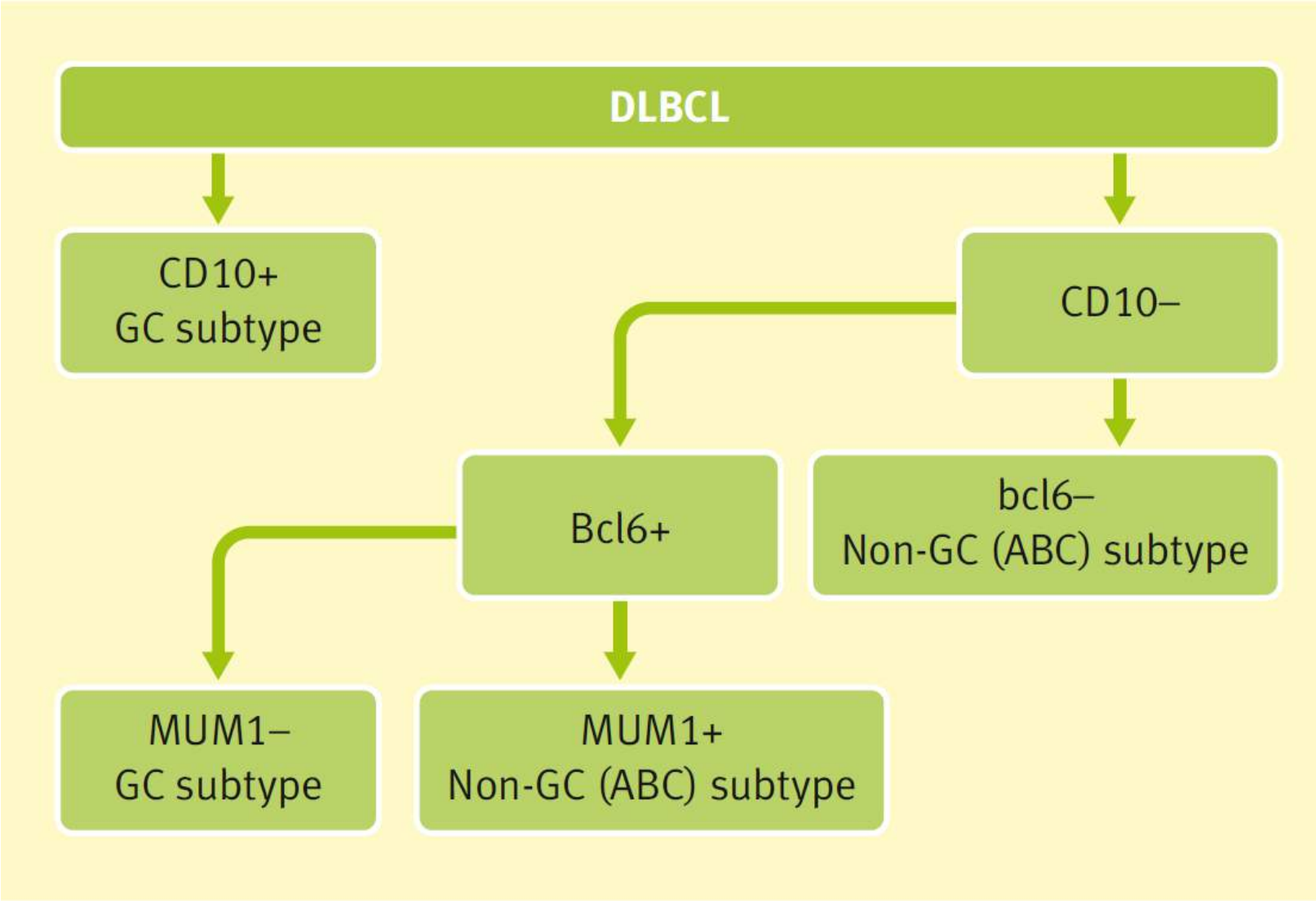
- Follicular lymphoma – CD10
- Interfollicular tumour cells

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

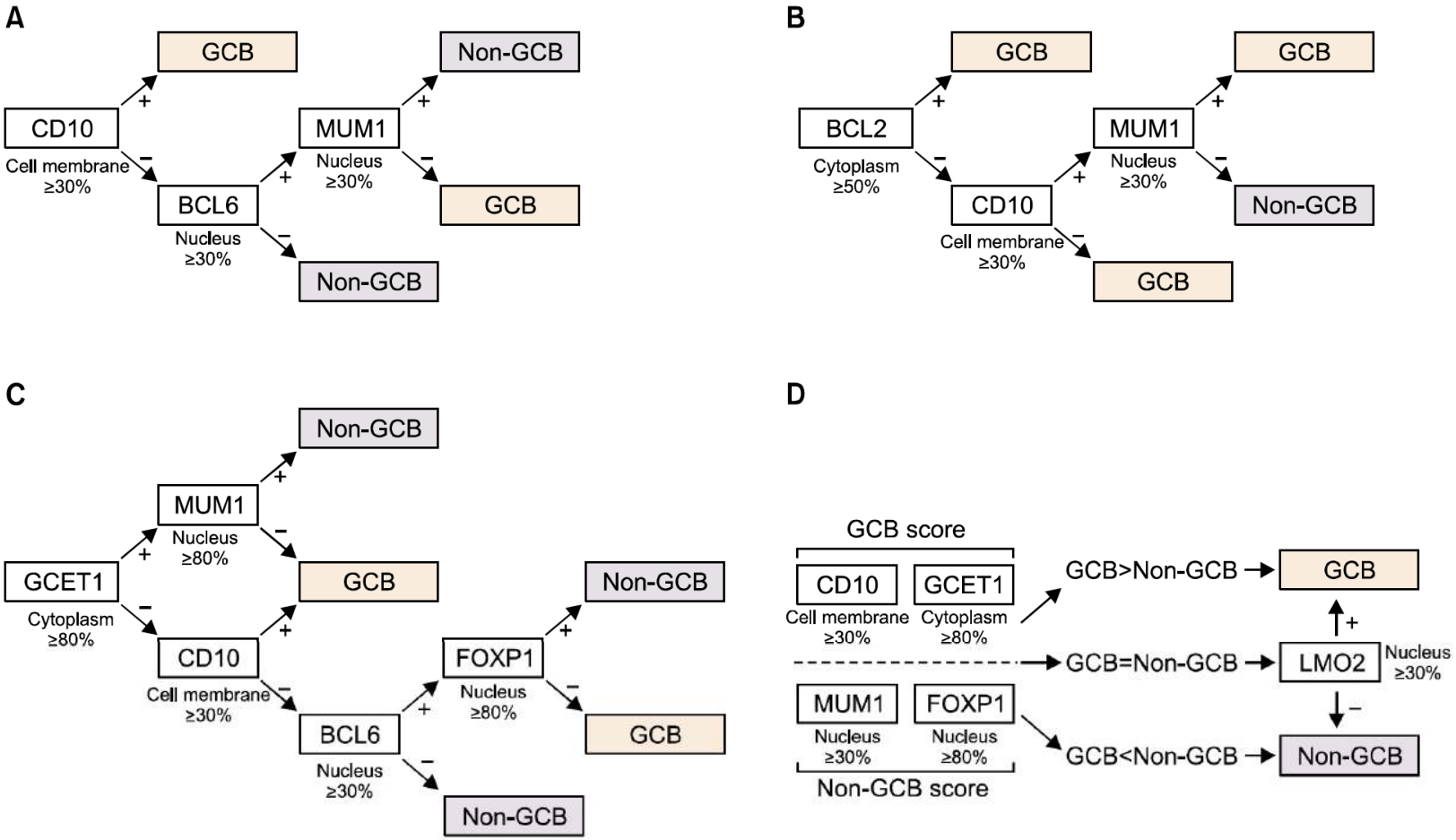
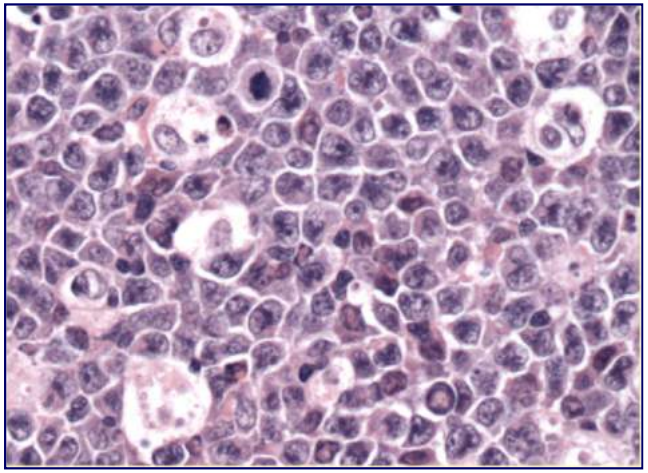


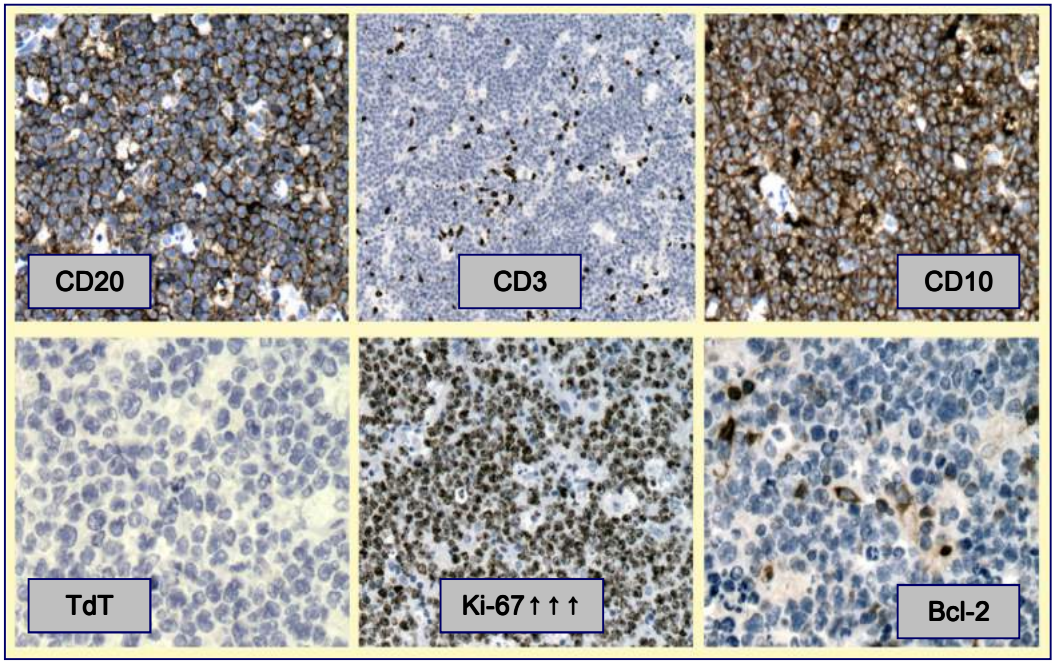
Fig. 2. Summary of the (A) Hans, (B) Muris, (C) Choi, and (D) Tally algorithms, and criteria for a positive signal for individual immunohistochemical markers (below or to the right of the white-filled box). Note that the positive criterion for MUM1/IRF4 in the Choi algorithm (more than 80%) is different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

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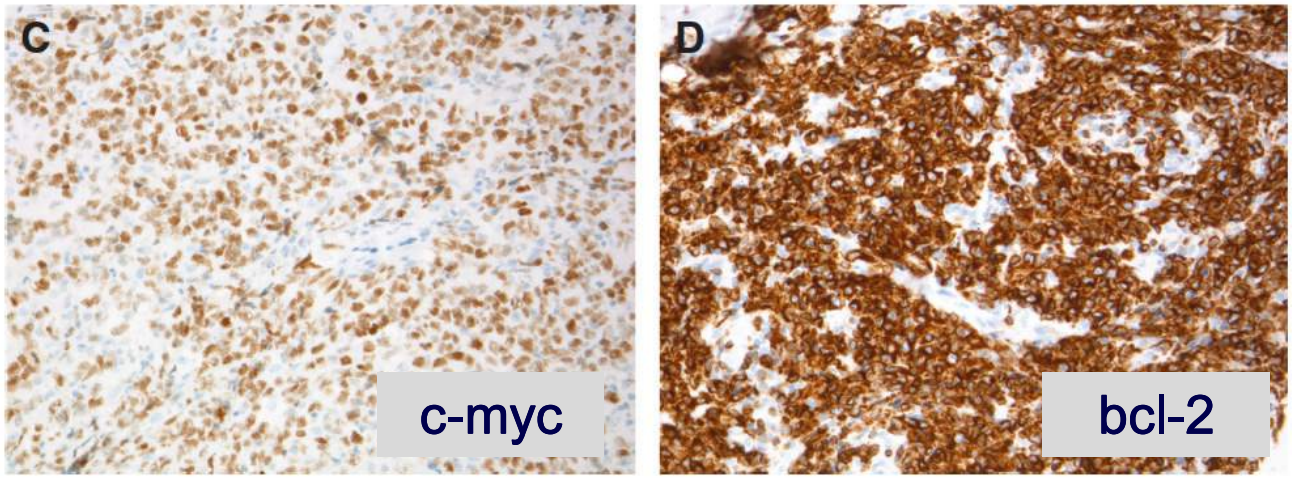
JOURNAL OF CLINICAL ONCOLOGY

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 – 20117

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

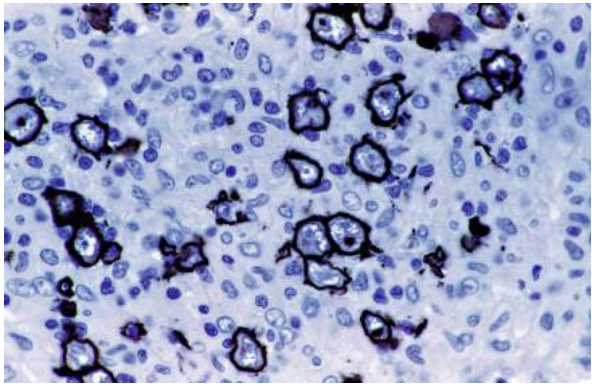
	CD20	CD79a	T-cell antigen	CD4 CD8	CD30	CD15	EMA
Nodular lymphocyte predominant HL	+	+	–	–	–/+	–	+
Classical HL	–/+	–/+	–	–	+	+	+
T-cell rich large B-cell lymphoma	+	+	–	–	–	–	–
Anaplastic large cell lymphoma	–	–	+/–	CD8>CD4> CD4&8 -ve	+	–	+

Key

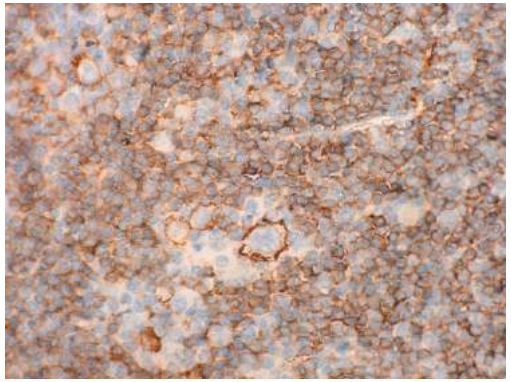
+/– The lymphoma cells are commonly but not always positive

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

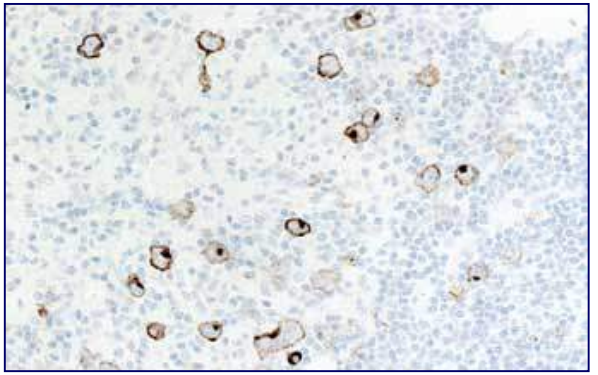
T-cell rich, B-cell lymphoma: CD20



Hodgkins lymphoma, LP: CD20

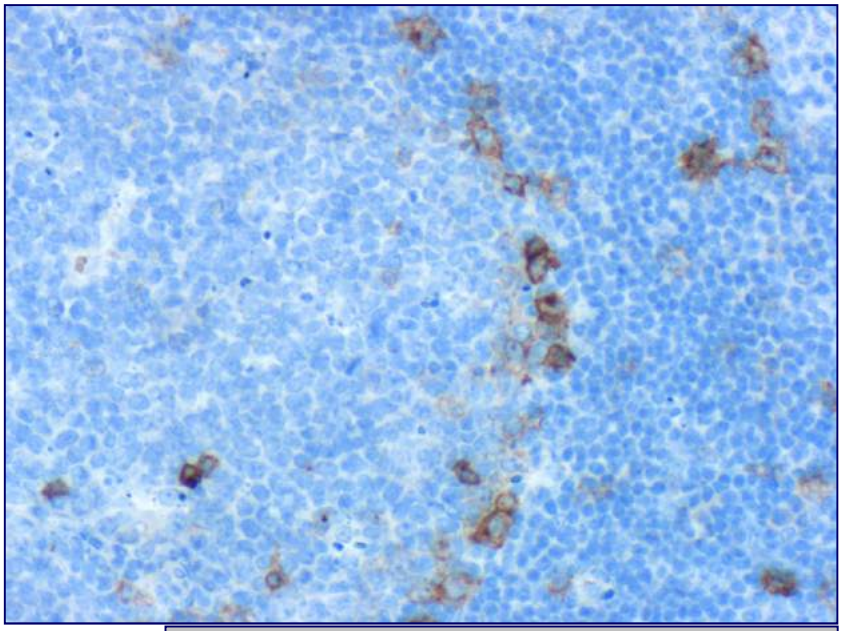


Classical Hodgkin lymphoma, MC: CD30



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

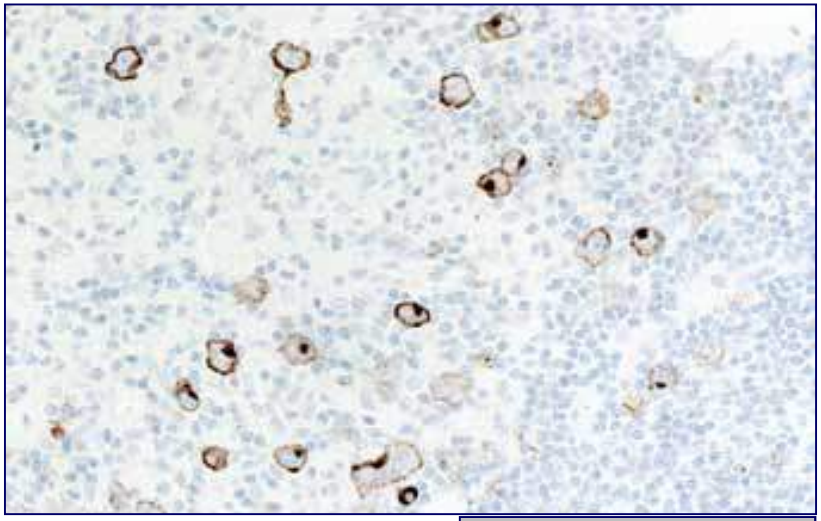


Reactive LN: activated B-cells

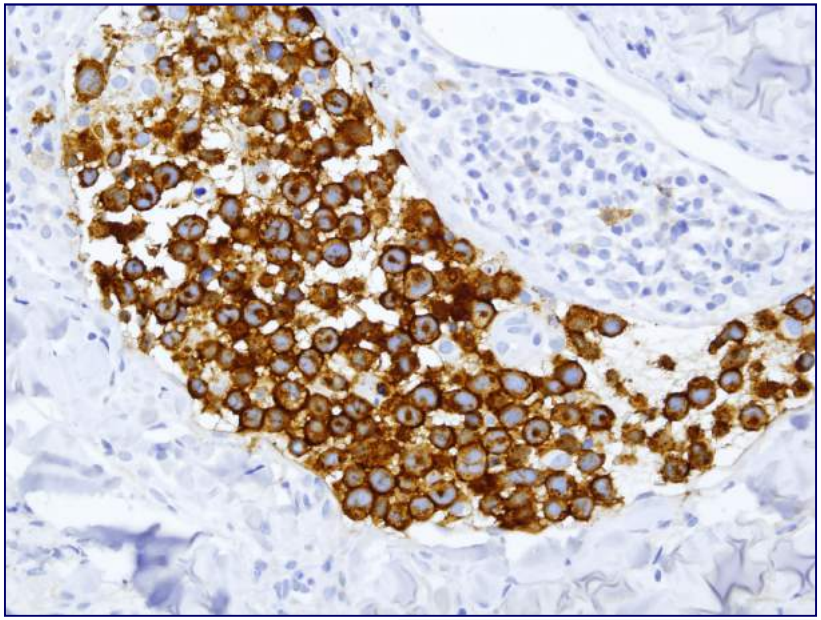
CD30 in lymphoma

”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:

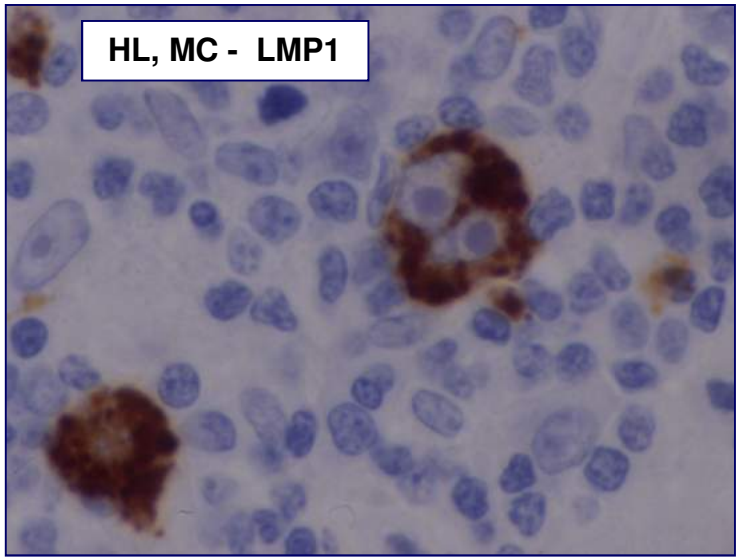
- PAX-5 (ALCL?)
- MUM1, BCL-6, CD57, BOB-1, OCT-2 (HL, LP?)
- ALK (ALCL?)
- EBV
- (CD15)

- HRS cells in cHL are:
  - CD30+ (>95%)
  - CD15+ (~70%)
  - CD20–/+ (~20%) PAX5 dim+ (> 95%)
  - CD45– (> 95%)
  - MUM1 (> 95%)
  - OCT2–/+, BOB1–/+ (~30%) & BCL6–

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



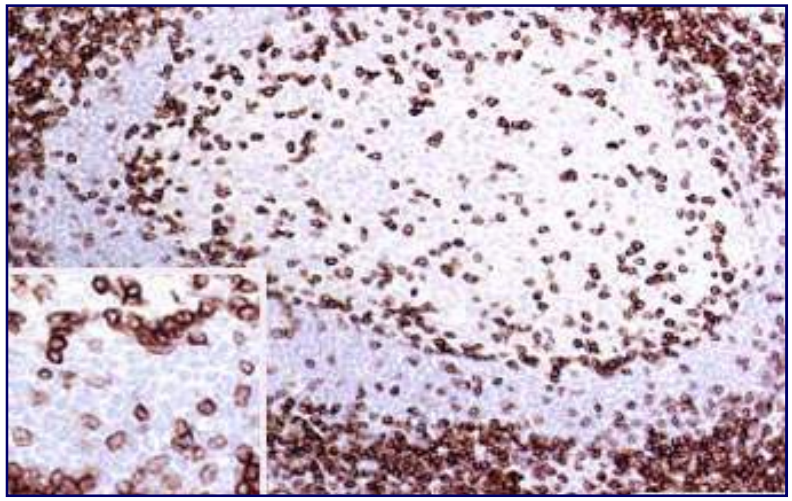
- Most viral antigens not relevant
- Latent membrane protein 1
  - Normal primary infection (IM)
  - Latency patterns II and III
  - HRS-cell-like morphology
- EBNA2
  - Nuclear reaction
  - Normal primary infection (IM)
- In lymphoma:
  - Hodgkin lymphoma:
    - Classical types: 25% - 50% positive in HRS cells: LMP1+ EBNA2-
    - HL-LP: L&H/Popcorn cells negative
  - EBV+ immundefect 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

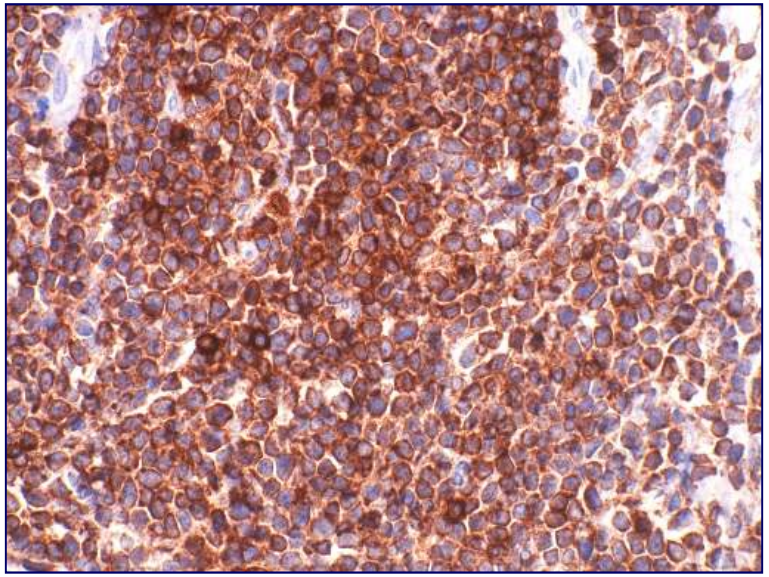
- transmembrane molecule
- Ig superfamily
- part of T-cell receptor
- most specific T-cell marker
- pan-T cell marker
  - thymocytes: cyt. → membrane
  - most post-thymic T-cells
  - activated NK-celler



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)



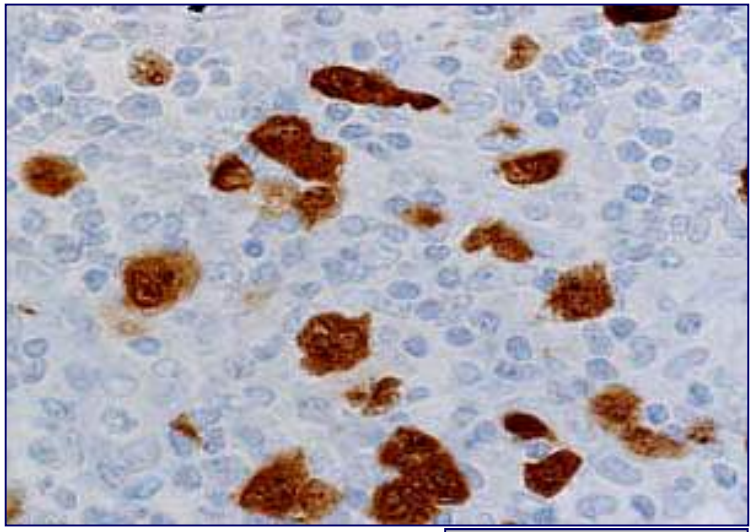
- Precursor T-LB
- CD3-cyt

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?
- PD1 (and other T-follicular helper cell markers)
- 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

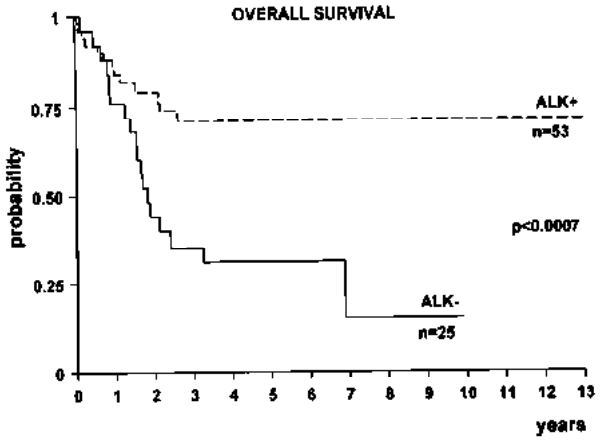
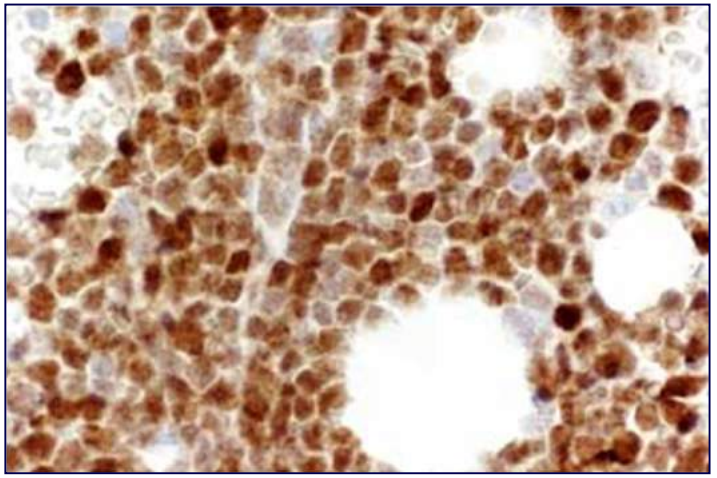


Fig 4. Overall survival of ALK⁺ versus ALK⁻ lymphoma.

Blood, Vol 93, No 8 (April 15), 1999: pp 2697-2706

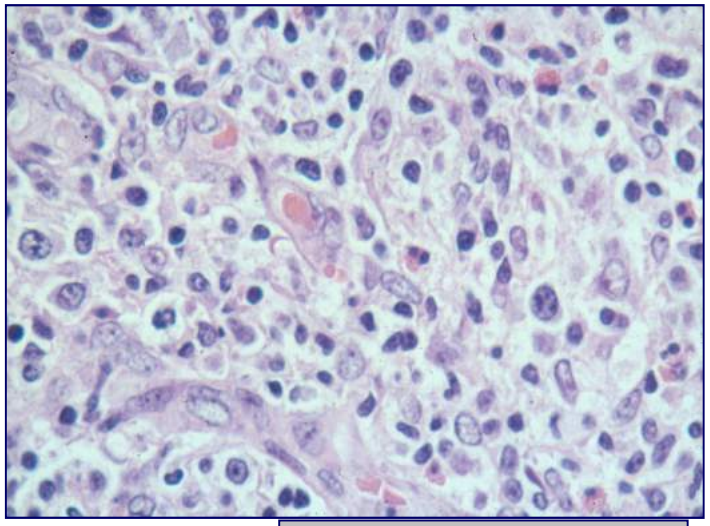
Secondary stain:
Terminal deoxynucleotidyl transferase (TdT)

- 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%)

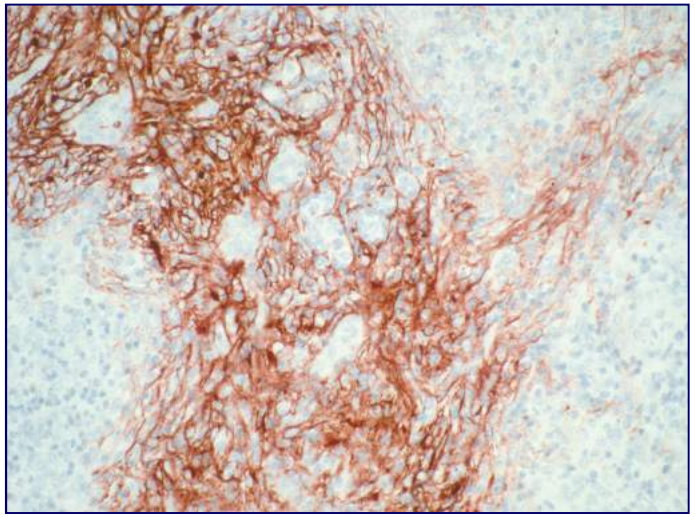


Basic stain: CD21

- 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
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- In lymphomas:
  - most follicular lymphomas
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  - FDC network in GC-derived tumours
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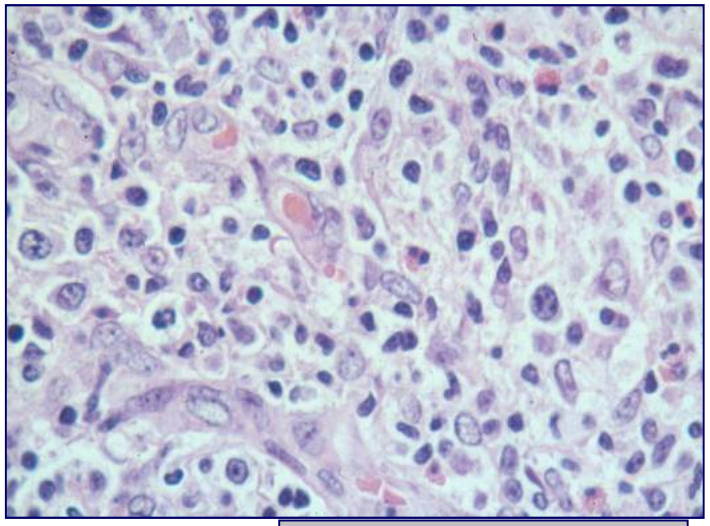
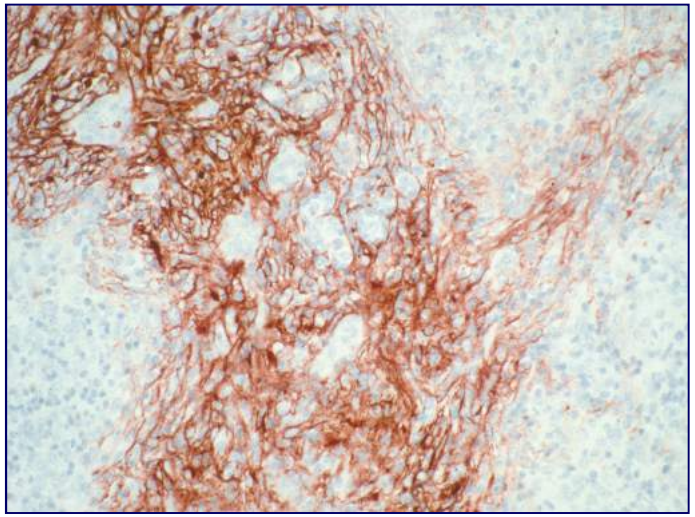


• AITL-T-cell lymphoma



• AITL-T-cell lymphoma: CD21

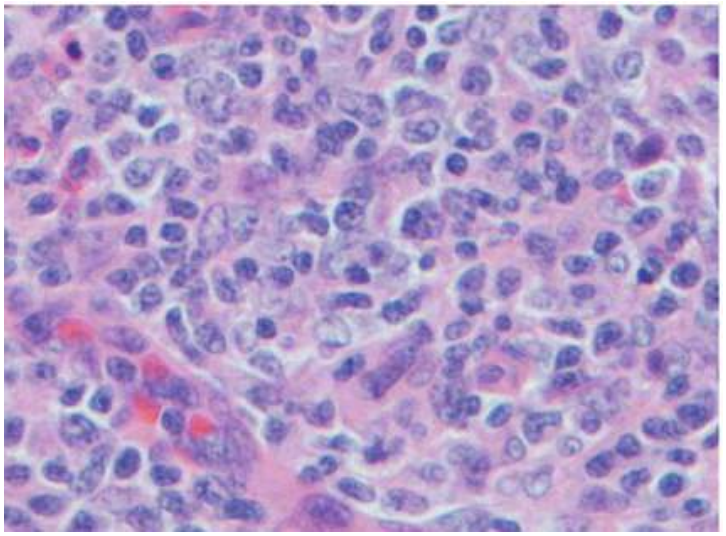
Nodal PTCL - immunophenotype

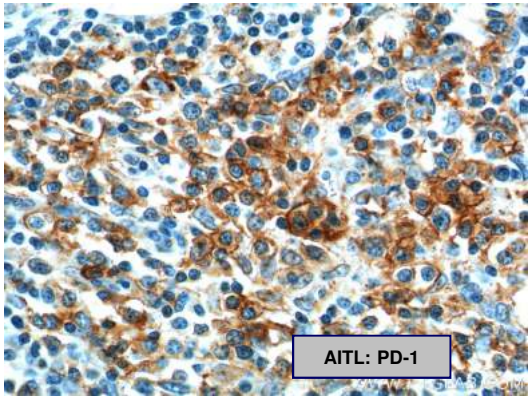
	PTCL, NOS	AITL	ALCL ALK+	ALCL ALK−	ATLL	MF	T-PLL	EATL
CD2	+	+	−/+	−/+	+	+	+	+
CD3	+	+	−/+	−/+	+	+	+	+
CD4	+/−	+	−/+	−/+	+	+	+/−	−
CD5	+/−	+	−	−	+	+	+	−
CD7	+/−	−/+	−	−	−	−	+	−
CD8	−/+	−	−	−	−	−	−/+	−/+
CD10	−	+/−	−	−	−	−	−	−
CD25	−/+	−	+	+	+	−/+	−	−/+
CD30	−/+	−	+	+	−/+	−/+	−	−/+
CD45RO	+	+	+	+	+	+	+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

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

T-cell lymphomas of TFH cell origin

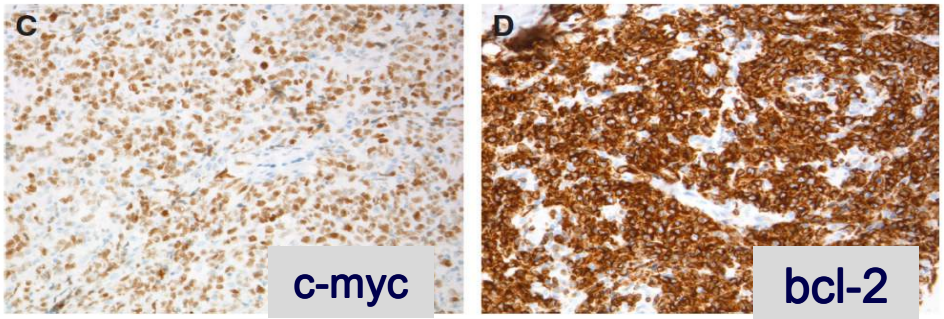
- TFH = T follicular helper cells
- Recently recognized
- Important subset of nodal PTCLs
  - e.g. AITL
- Express TFH-cell markers:
  - PD1 (CD279)
  - CD10
  - CXCL13
  - BCL6
  - ICOS





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

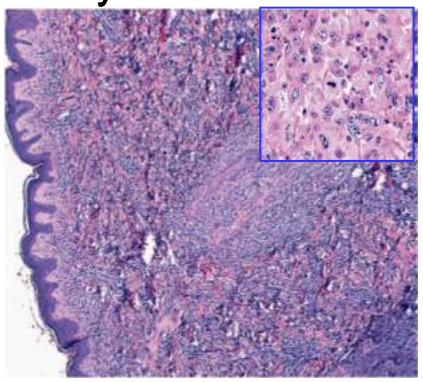
Add to basic panel:

- panCK
- S-100
- Melan-A

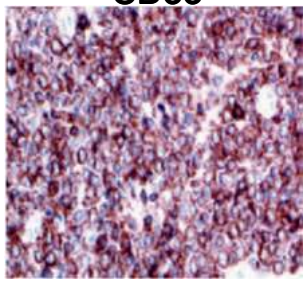
IHC for lymphoid vs myeloid
Add to basic panel

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

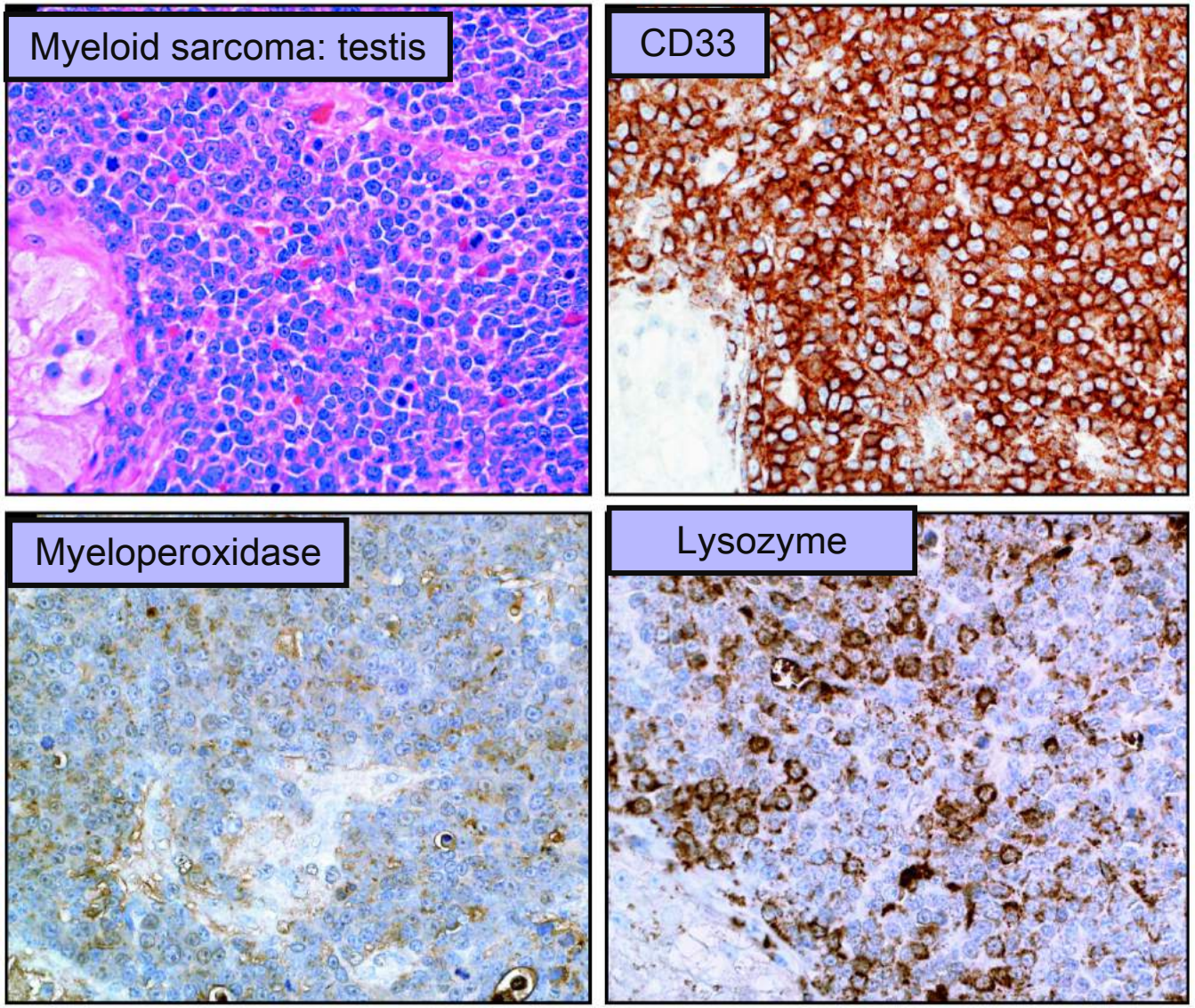
Myeloid Sarcoma



CD33

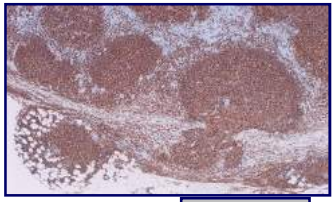


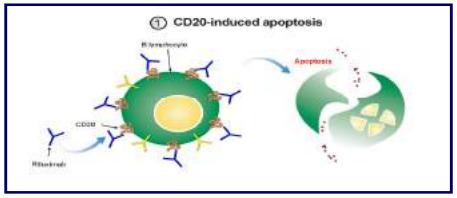
Myeloid sarcoma: testis



Targeted therapy

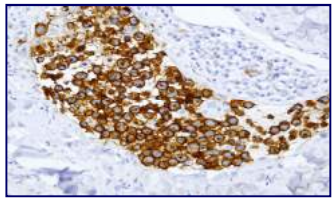
- **Rituximab (anti-CD20)**
  - **B-cell NHL**

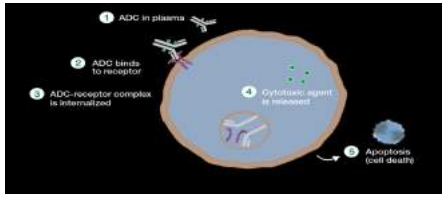




FL: CD20

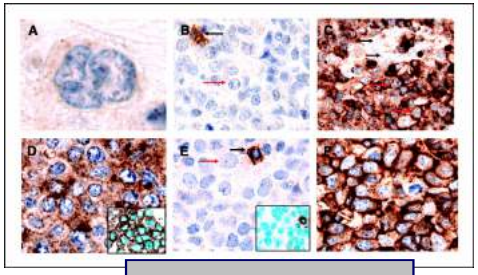
- **Brentuximab (anti-CD30)**
  - **HL**
  - **ALCL**
  - **CD30+ DLBCL**



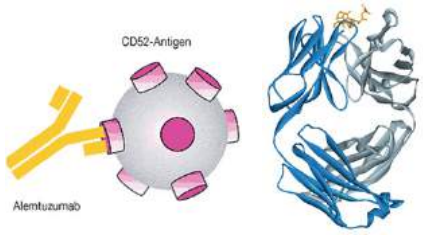


ALCL: CD30

- **Alemtuzumab (anti-CD52)**
  - **B-CLL**
  - **T-cell lymphoma**

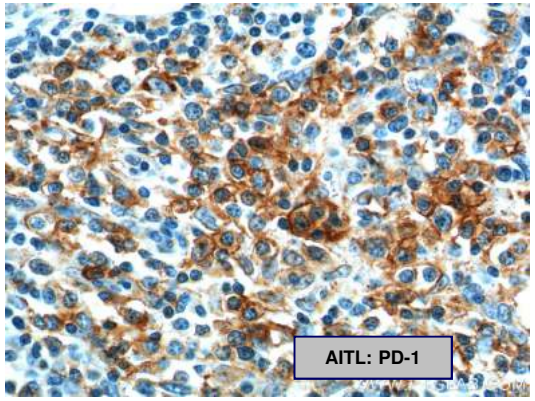


T-cell lymphomas: CD52

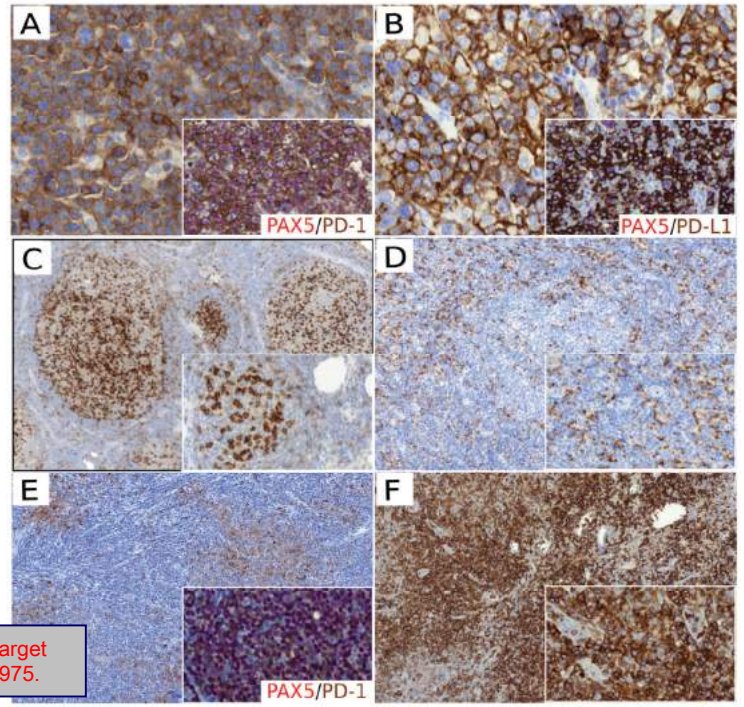


Immune checkpoint inhibitory therapy?

- **PD-1 AILD**



- **Hodgkin**
  - **PD-L1**
  - **PD-1**



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


Thanks!