

Haematolymphoid neoplasms: IHC for diagnostic use

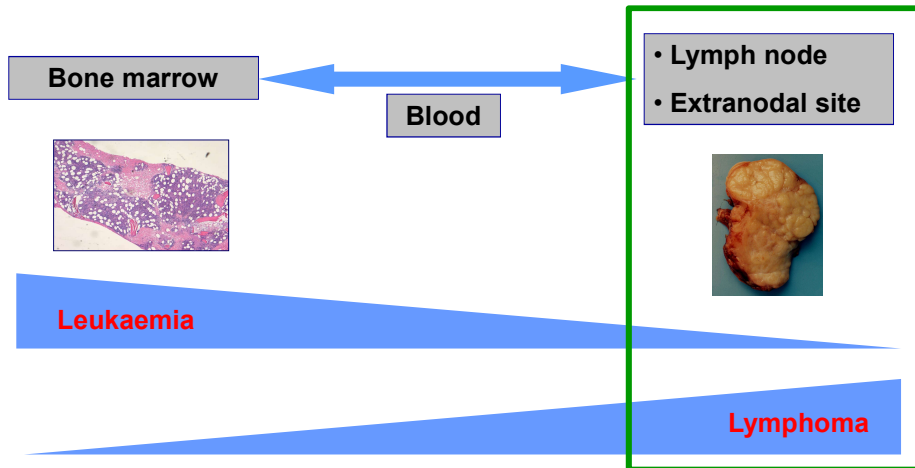
**Stephen Hamilton-Dutoit
Institute of Pathology
Aarhus University Hospital**

Haematolymphoid Neoplasms:

What are they?

Haematolymphoid Neoplasms: Leukaemia vs Lymphoma

CLONAL LYMPHOID MALIGNANCIES



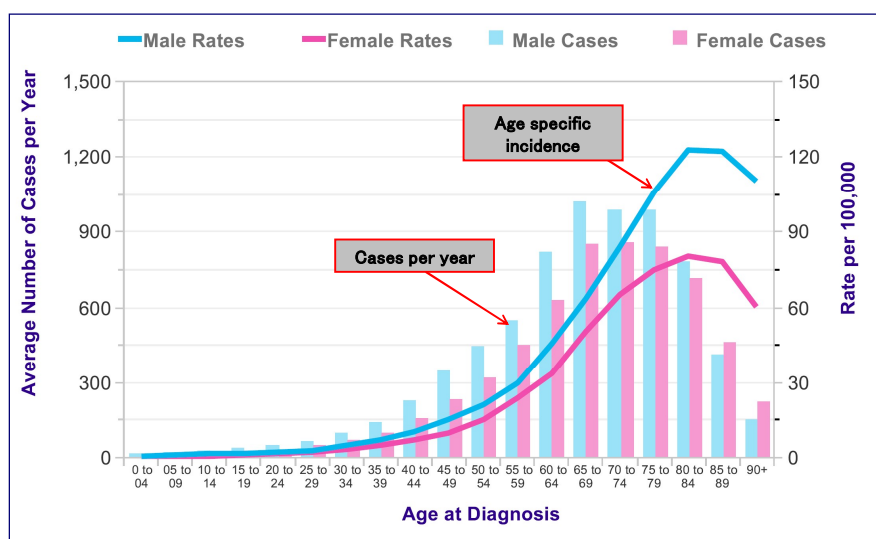
Haematolymphoid Neoplasms:

How common are they?

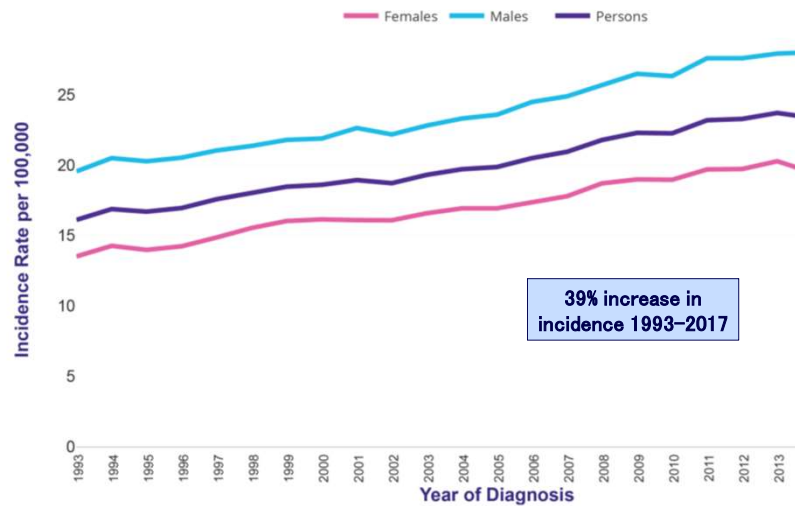
Malignant lymphoproliferative diseases

- **Malignant lymphoma**
- **Leukaemia**
 - Acute lymphoblastic leukaemia
 - Chronic lymphocytic leukaemia (CLL)
- **Ca. 1,600 per year in DK**
- **> 1,300,000 per year in the world (?)**
- **5th commonest cancer globally**

Age & sex: Non-Hodgkin lymphoma (UK)



Non-Hodgkin lymphoma (NHL) incidence trends over time (UK)



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

Haematolymphoid Neoplasms:

What causes them?

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



Glyphosate-based herbicides

Haematolymphoid Neoplasms:

How are they classified?

Thomas Hodgkin 1798–1866



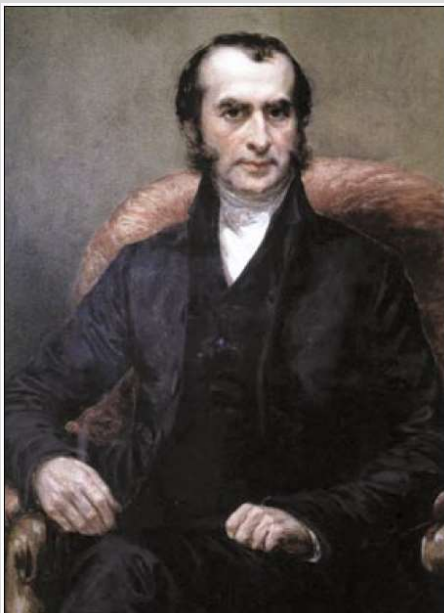
ON SOME
MORBID APPEARANCES
OF
THE ABSORBENT GLANDS
AND
SPLEEN.
BY DR. HODGKIN.
PRESENTED
BY DR. R. LEE.
READ JANUARY 10TH AND 24TH, 1832.

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



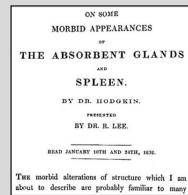
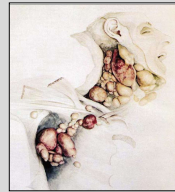
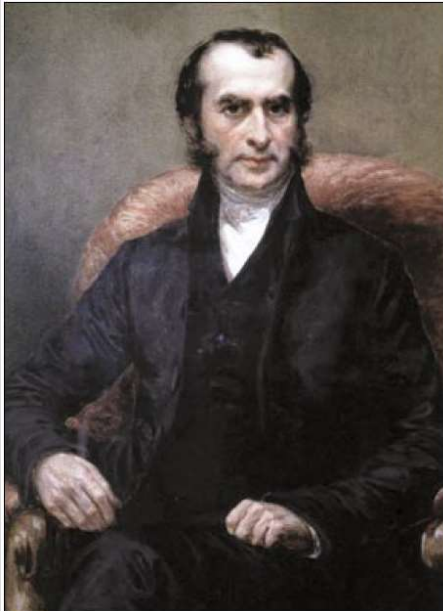
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Hodgkin's original case:
abdominal nodes

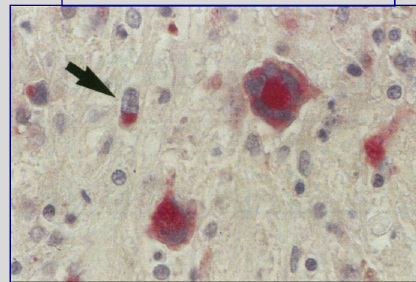


Gordon Museum,
King's College London

Thomas Hodgkin 1798–1866



Hodgkin's original case: CD15 (1991)

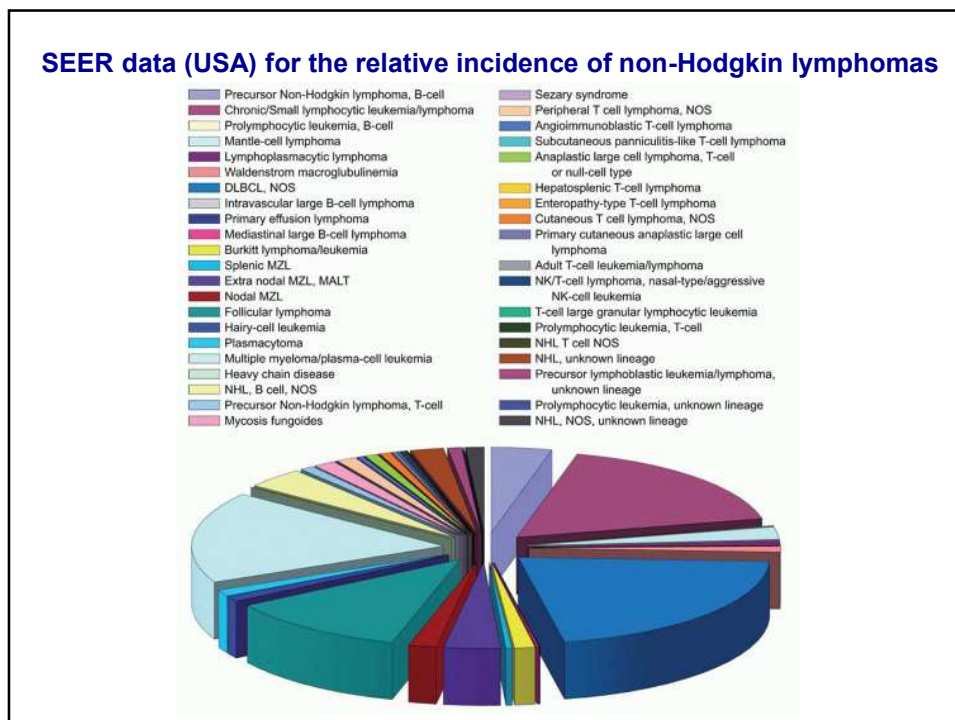
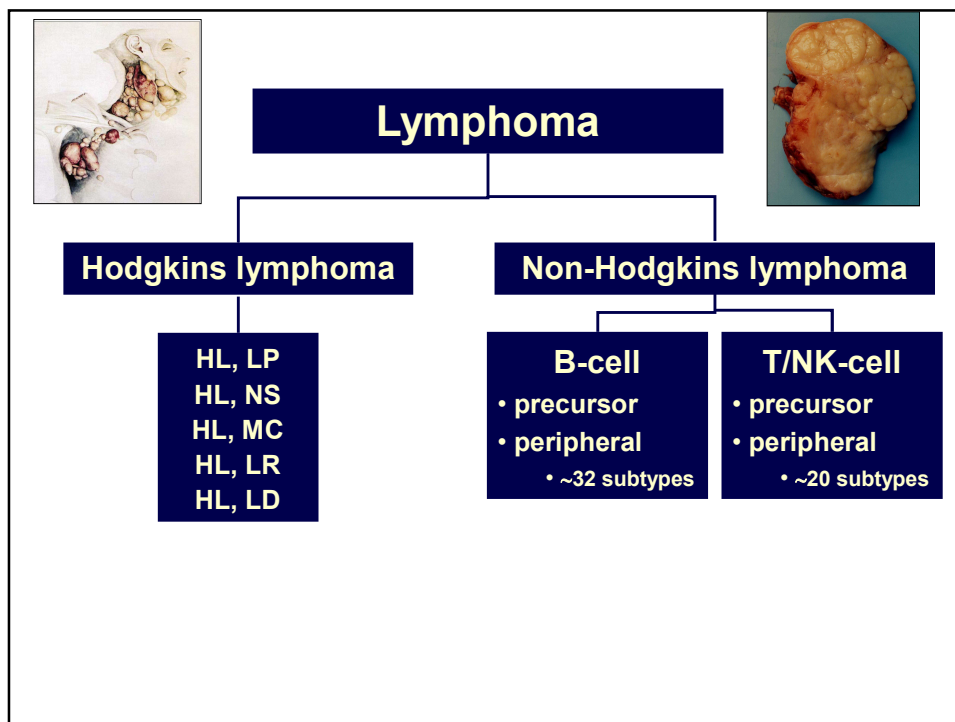


Paraffin-embedded tissue of one of the original cases by Thomas Hodgkin immunostained with CD15 (David Mason)

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





Review Series

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

¹Division of Hematopathology, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Department of Pathology, Hospital Clinic, University of Barcelona, August 11 Sunyer Biomedical Research Institute, Barcelona, Spain; ³Hæmopathology 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

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)

- It gets longer & longer!
- > 100 lymphoma entities

[illegible]

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*"

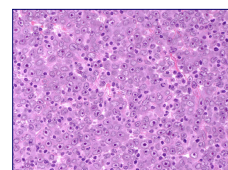
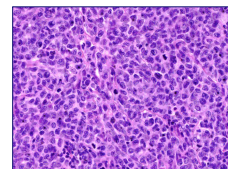
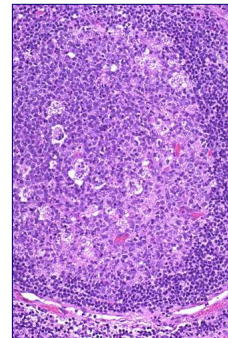
Lymphoma frequencies



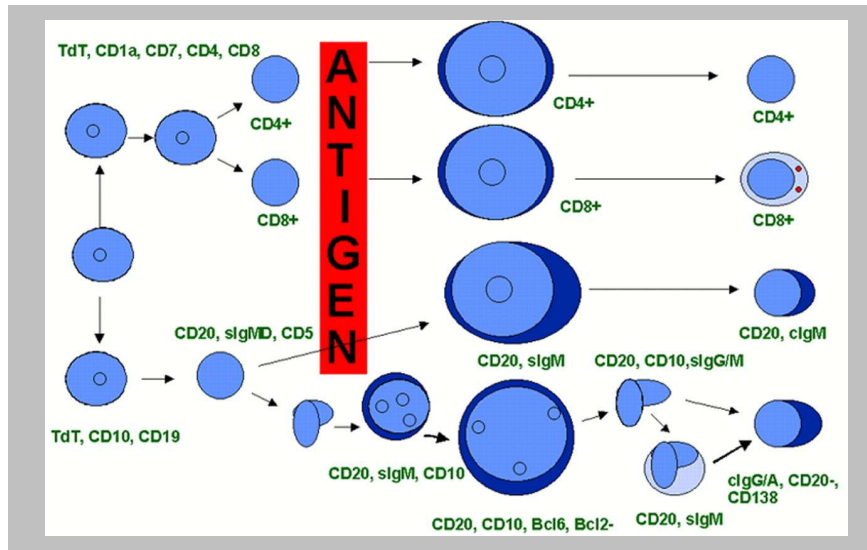
2002 SEER database. O'Connor

What is lymphoma?

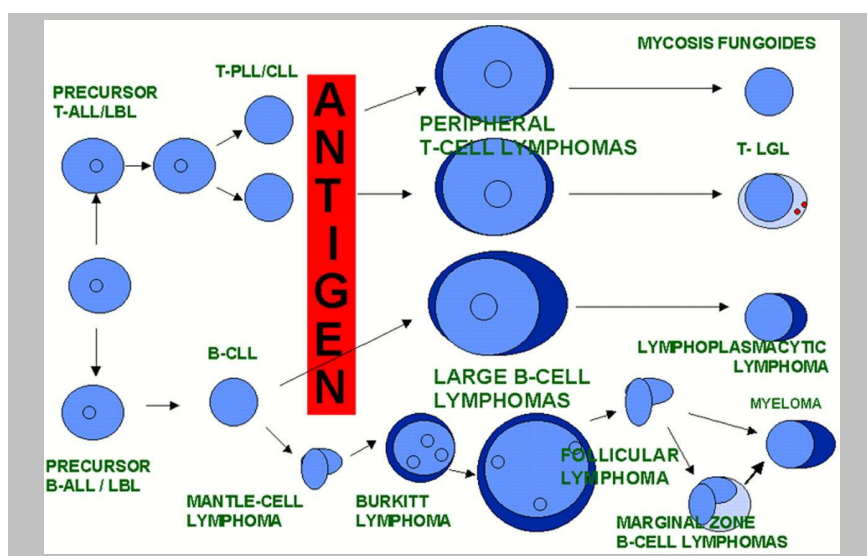
- **Clonal malignancy**
 - → mutational events cause cells to freeze at a single stage of normal lymphocyte differentiation
- **Morphology, immunophenotype & molecular features:**
 - mirror stages of normal lymphocyte development



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

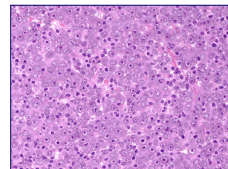
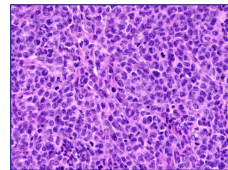
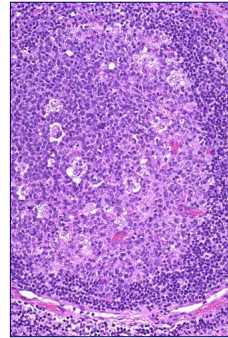


Lymphoid neoplasms: Correlation with normal T or B-cell differentiation



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



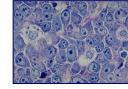
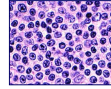
Lymphoma & Leukaemia diagnosis

- Clinical features
- Morphology
- Immunophenotype
- Molecular diagnosis

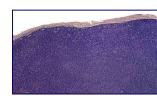
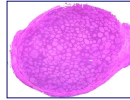
Lymphoma differential diagnosis

- **Assess morphology:**

- cell size



- architecture



But...that's not enough!

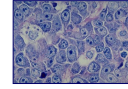
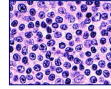
Lymphoma & Leukaemia diagnosis

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

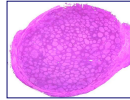
Lymphoma differential diagnosis

- Assess morphology:

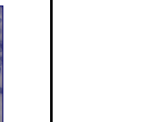
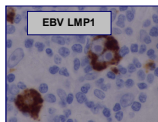
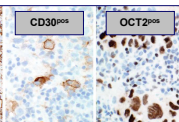
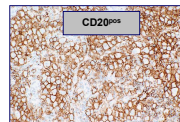
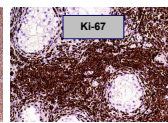
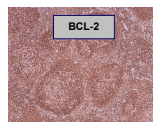
- cell size



- architecture

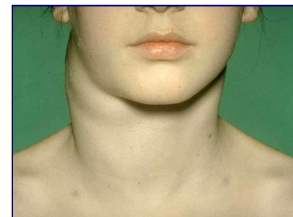


- Select appropriate immune panel(s)



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"

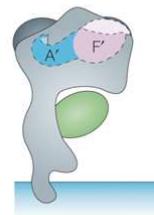
UK: RCPaTh / BCSH

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

...and many more!

What are CD numbers?

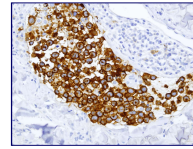
Human CD1a



- 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

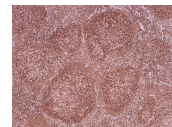
IHC Dogma

(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

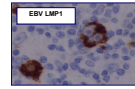
Diagnostic Applications of IHC 1



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



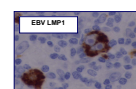
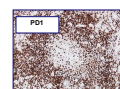
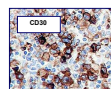
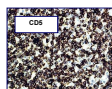
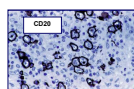
- T-cell lymphoma vs B-cell lymphoma
- T-cell lymphoma vs T-zone hyperplasia
- Hodgkin lymphoma vs NHL
- Hodgkin lymphoma
 - NLPHL vs classical HL
- Lymphoblastic vs. Myeloblastic vs. Burkitt
- Undifferentiated malignant tumor
- Lymphoma prognosis
 - e.g. Ki-67; ALK; c-myc
- Targeted therapy
 - e.g. 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
 - PDL-1

- Other
 - EBV
 - LMP1
 - EBNA2
 - (EBER)
 - CD56
 - CD57
 - EMA
 - S100
 - CD68
 - CD163
 - CD123

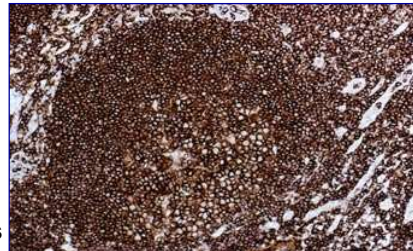


Basic IHC panel for lymphoma diagnosis

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

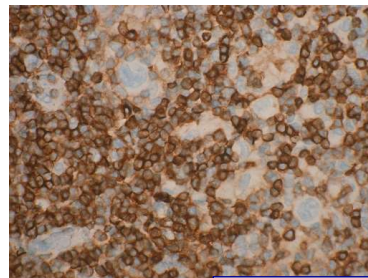
Basic stains: CD45

- Membrane glycoprotein family
- Positive in all (?) haemopoietic 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

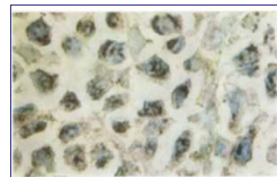
J. clin. Path., 1974, **27**, 14-20

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

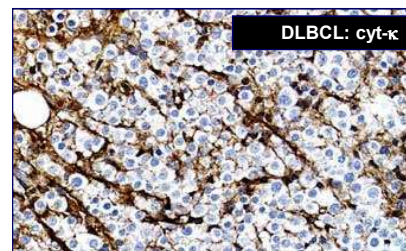
- IHC-Ig
 - first protocol for IHC in FFPE
 - still one of the hardest to perform & evaluate!



• plasmacytoma
• monoclonal Ig-kappa

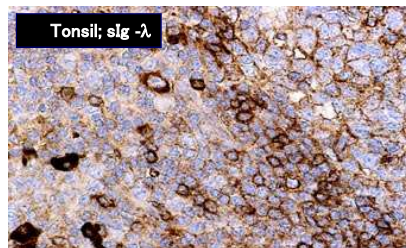
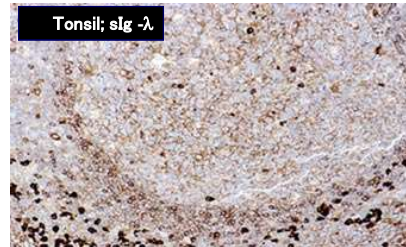
Basic stains: Immunoglobulin

- 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



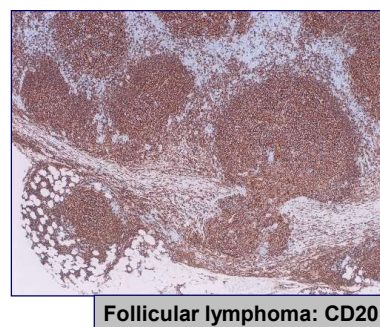
Basic stains: Immunoglobulin

- **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**
 - may be aberrantly lost after treatment with Rituximab



Follicular lymphoma: CD20

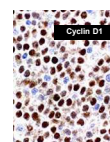
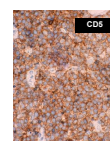
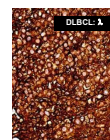
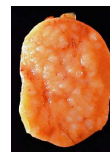
Usual staining pattern of B-cell neoplasms

	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

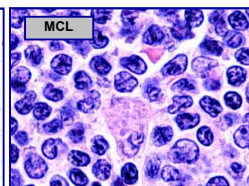
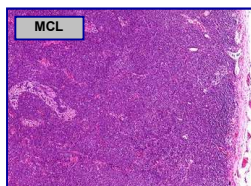
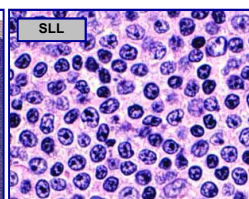


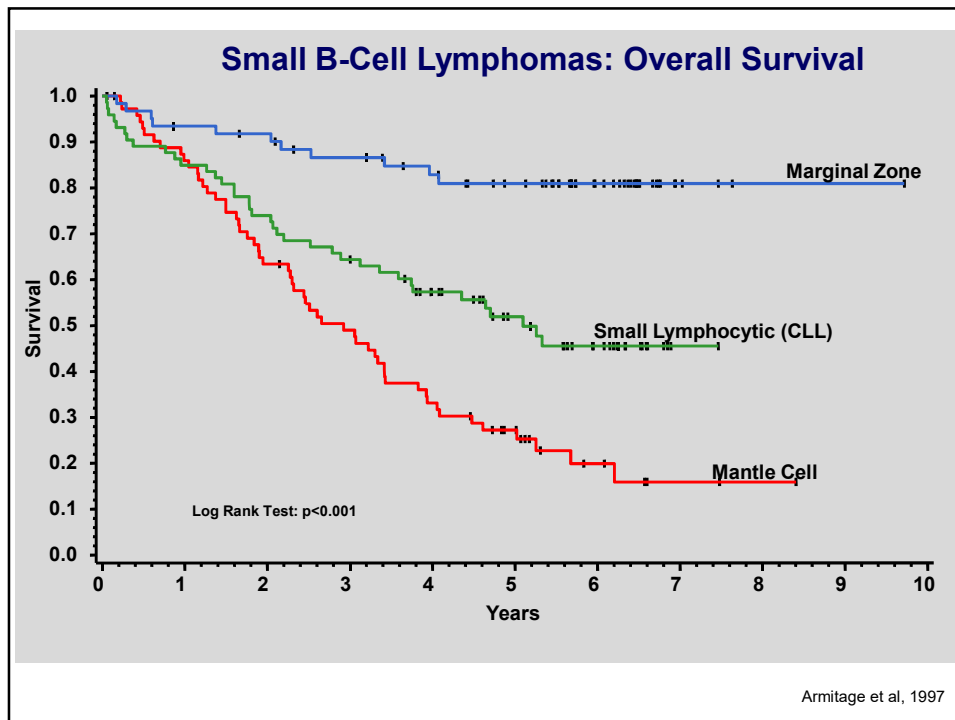
Small cell B-Cell lymphomas: Differential Diagnosis

Small lymphocytic NHL



Mantle cell NHL





B-cell Small Lymphocytic Lymphoma (CLL)

Morphology

- small lymphocytes
- proliferation centres

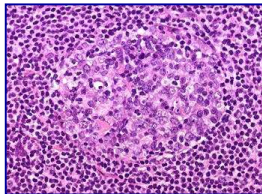
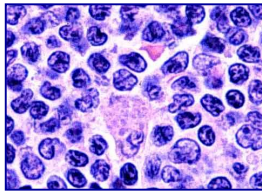
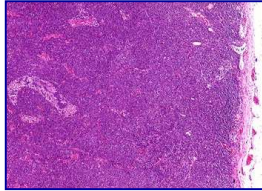
Immunology

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

CD23

CD5

Mantle Cell Lymphoma

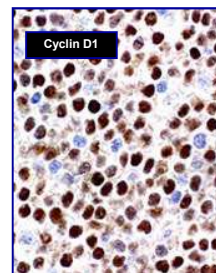
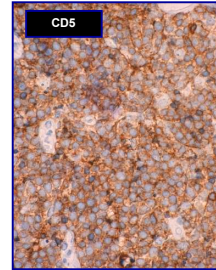


Morphology

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

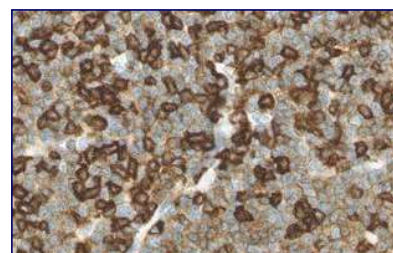
Immunology

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



Basic stains: CD5

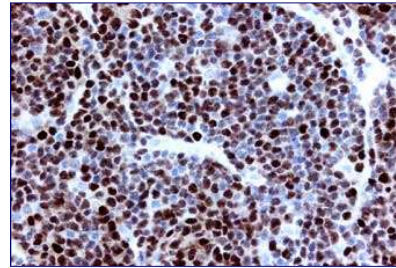
- Modulates T & B cell signalling
- Pan-T cell marker
 - 95% thymocytes
 - 100% post-thymic T-cells
 - ↑ expression with maturity
- Minor population normal B-cells:
 - ca. 10%+ peripheral B-cells
 - ↑ in autoimmunity
- Lymphomas:
 - 90% T-cell neoplasias
 - B-cell NHL
 - B-CLL / SLL (90%)
 - Mantle cell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

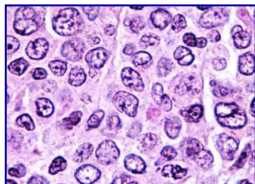
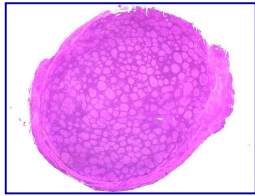


Mantle cell NHL: cyclin-D1

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

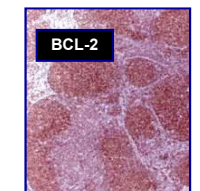
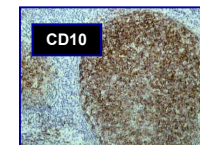
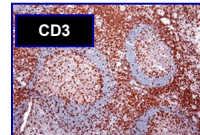
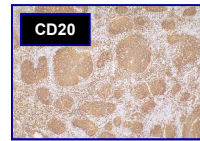


Morphology

- germinal centre cells
- CBs & CCs
- follicular

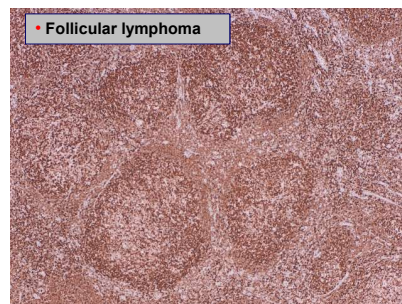
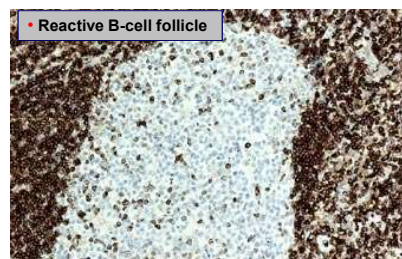
Immunology

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



Basic stain: bcl-2

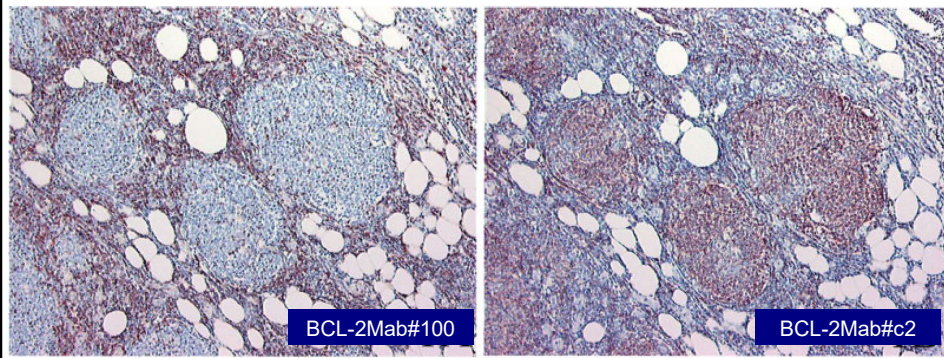
- Apoptosis inhibitor
- Nuclear and cytoplasmic stain
- Normal:
 - Mature B- and T-cells
 - Negative in cortical thymocytes and germinal centre cells
- In lymphoma:
 - Positive in most peripheral B-NHL and T-NHL
 - Negative in BL
 - Associated with, but not specific for t (14;18)
 - Positive in neoplastic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



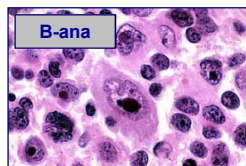
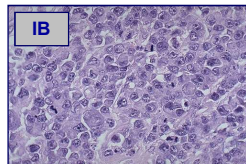
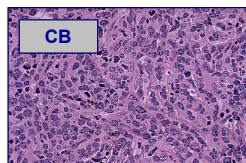
Original Paper

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

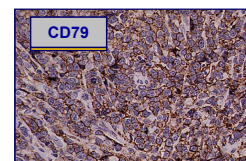
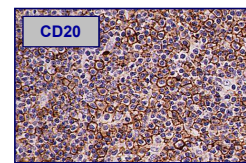
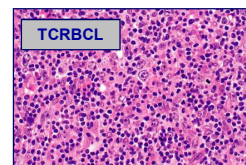


Morphology

- large cells
- nucleoli
- diffuse

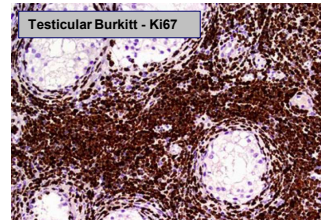
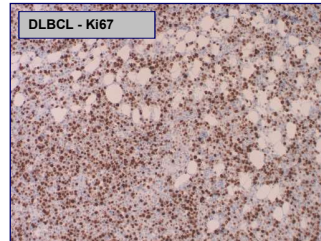
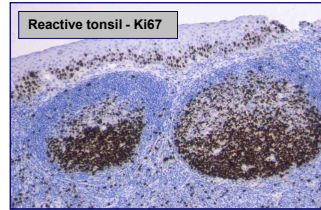
Immunology

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



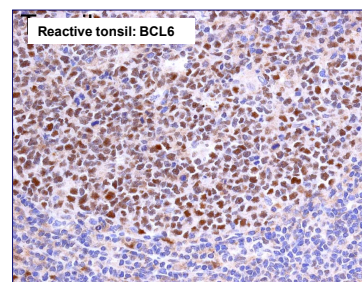
Basic stain: Ki- 67

- 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



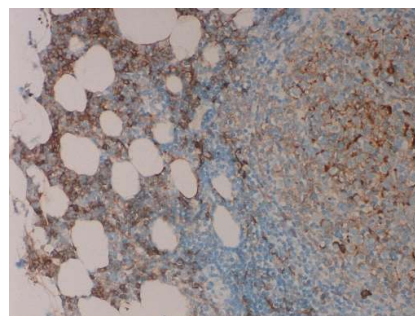
IHC for DLBCL

Add to basic panel:

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

Secondary stain: CD10

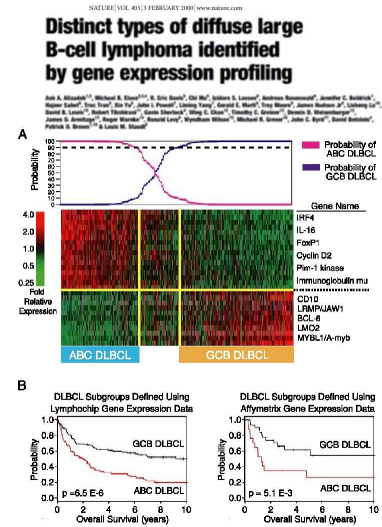
- **>90% precursor B-LB (membrane & paranuclear stain)**
- **ca. 25% precursor T-LB**
- **Burkitt lymphoma**
- **Follicular lymphoma**
 - Interfollicular CD10+ cells suggests lymphoma
- **Some DLBCL**
 - 'Cell of origin' algorithm in DLBCL
 - GCB vs ABC



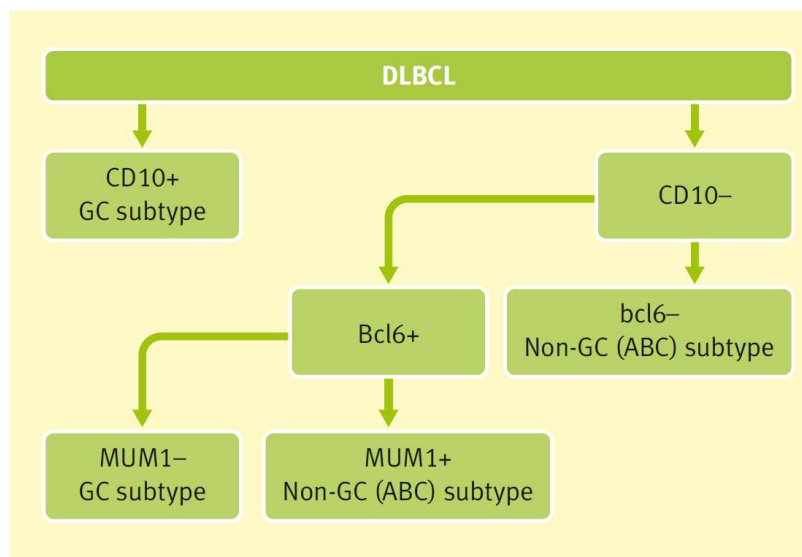
- **Follicular lymphoma – CD10**
- **Interfollicular tumour cells**

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

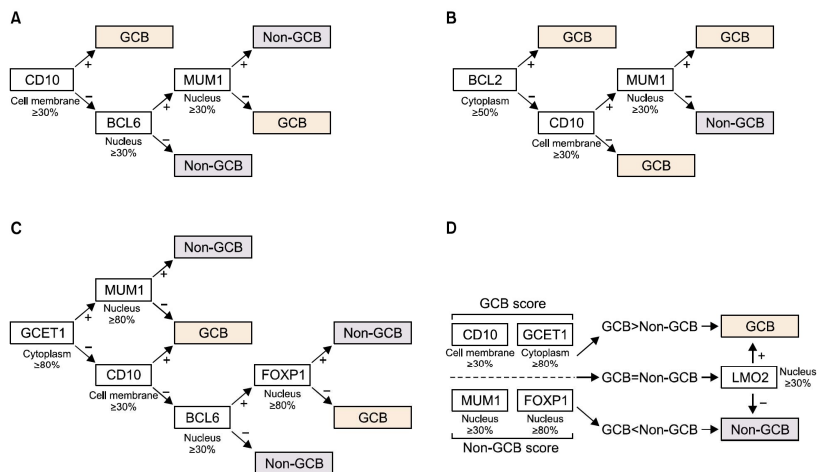
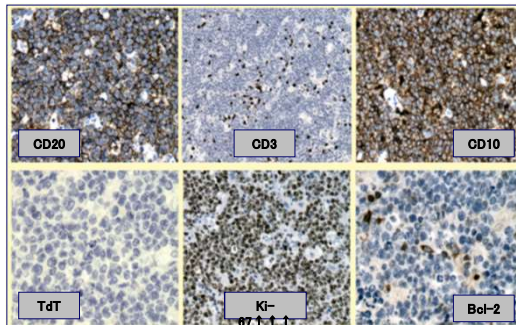
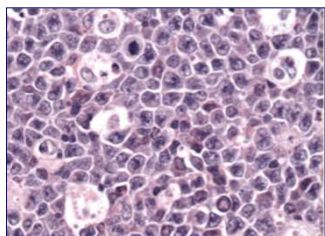


Fig. 2. Summary of the (A) Hans, (B) Muris, (C) Choi, and (D) Tally algorithms, and criteria for a positive signal for individual immunohistochemical markers (below or to the right of the white-filled box). Note that the positive criterion for MUM1/IRF4 in the Choi algorithm (more than 80%) is different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

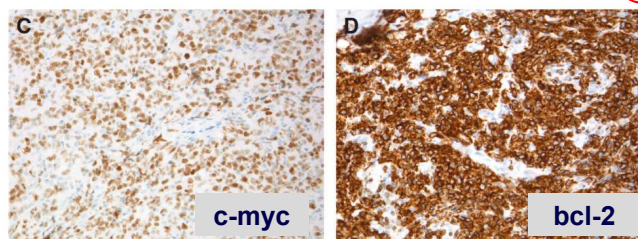
VOLUME 30 • NUMBER 28 • OCTOBER 1 2012

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, Atilio Orazi, Ronald S. Go, Ole Nielsen, Ole V. Gadeberg, Torben Mouris-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

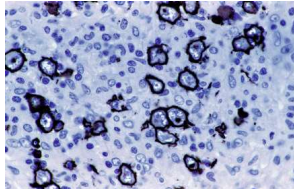
	CD20	CD79a	T-cell antigen	CD4 CD8	CD30	CD15	EMA
Nodular lymphocyte predominant HL	+	+	-	-	-/+	-	+
Classical HL	-/+	-/+	-	-	+	+	+
T-cell rich large B-cell lymphoma	+	+	-	-	-	-	-
Anaplastic large cell lymphoma	-	-	+/-	CD8>CD4> CD4&8 -ve	+	-	+

Key

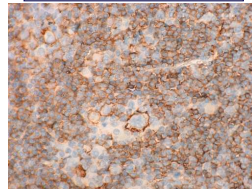
+/- The lymphoma cells are commonly but not always positive

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

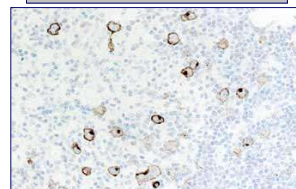
T-cell rich, B-cell lymphoma: CD20



Hodgkins lymphoma, LP: CD20

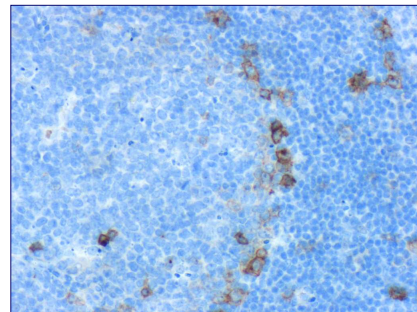


Classical Hodgkin lymphoma, MC: CD30



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

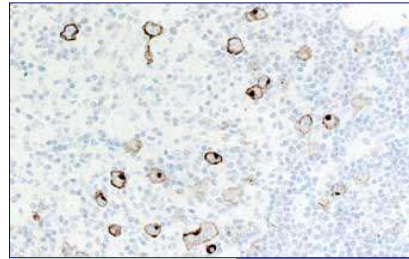


Reactive LN: activated B-cells

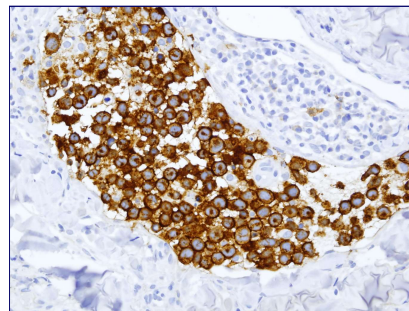
CD30 in lymphoma

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

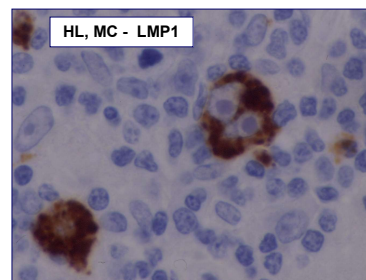
- HRS cells in cHL are:
 - CD30+ (>95%)
 - CD15+ (~70%)
 - CD20-/+ (~20%) PAX5 dim+ (> 95%)
 - CD45- (> 95%)
 - MUM1 (> 95%)
 - OCT2-/+ , BOB1-/+ (~30%) & BCL6-

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+ immunodeficient 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

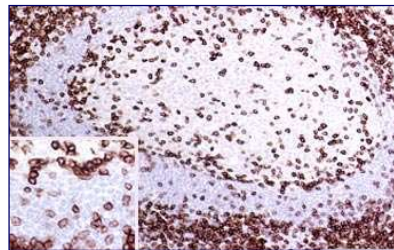


T-cell lymphoma: immunophenotype

Complex!

Basic stain: CD3

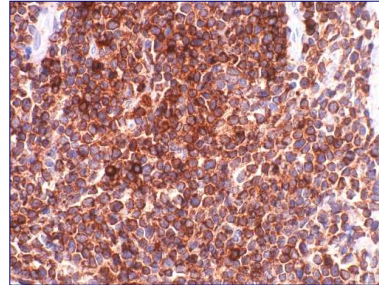
- transmembrane molecule
- Ig superfamily
- part of T-cell receptor
- most specific T-cell marker
- pan-T cell marker
 - thymocytes: cyt. → membrane
 - most post-thymic T-cells
 - activated NK-celler



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

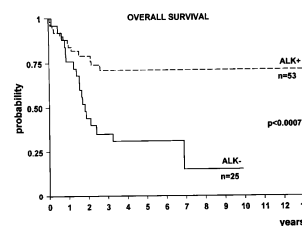
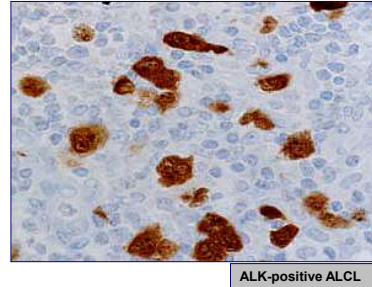
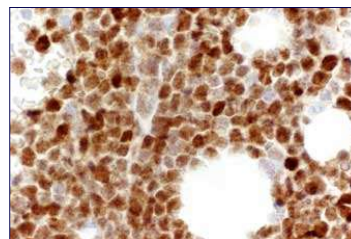


Fig 4. Overall survival of ALK+ versus ALK- lymphoma.
Blood, Vol 93, No 8 (April 15), 1999: pp 2697-2706

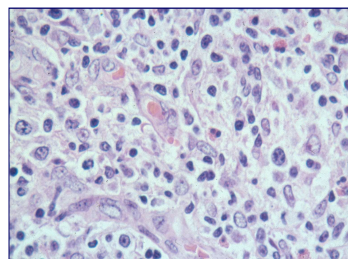
**Secondary stain:
Terminal deoxynucleotidyl transferase (TdT)**

- 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%)

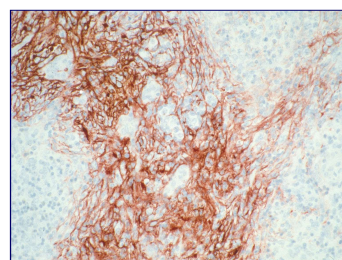


Basic stain: CD21

- 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



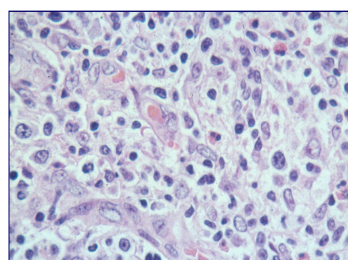
• AILD-T-cell lymphoma



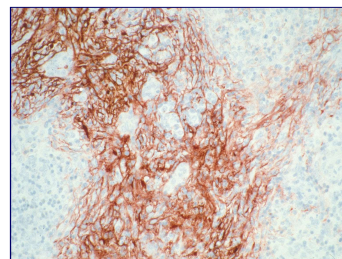
• AILD-T-cell lymphoma: CD21

Basic stain: CD21

- Membrane glycoprotein
- Normal:
 - Mature B cells
 - mantle zone & marginal zone B cells
 - 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**



• AITL-T-cell lymphoma



• AITL-T-cell lymphoma: CD21

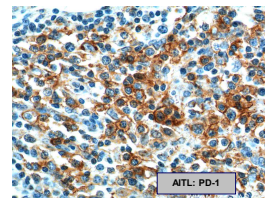
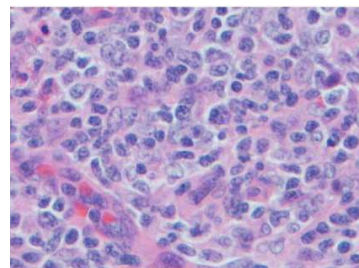
Nodal PTCL - immunophenotype

	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.

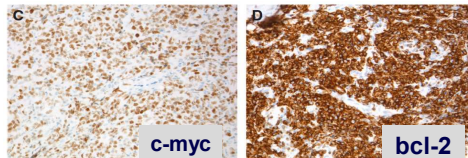
T-cell lymphomas of TFH cell origin

- 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**



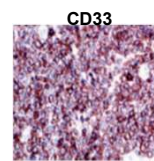
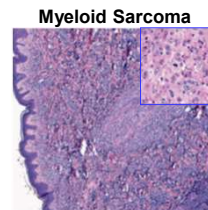
IHC for lymphoma vs other Add to basic panel:

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

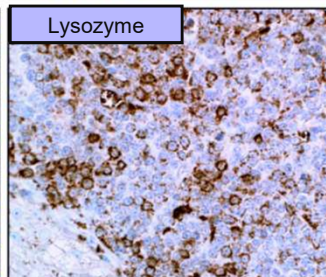
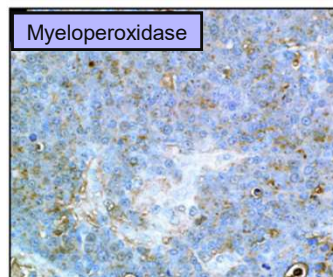
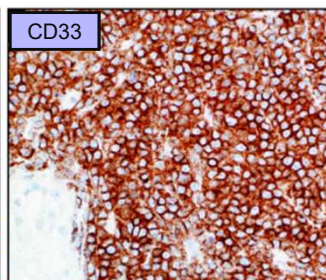
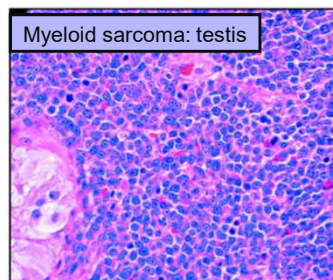
IHC for lymphoid vs myeloid

Add to basic panel

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



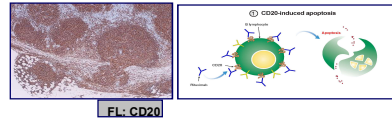
Myeloid sarcoma: testis



Targeted therapy

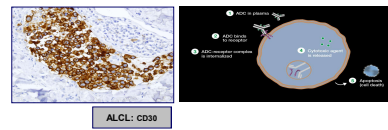
- **Rituximab (anti-CD20)**

- B-cell NHL



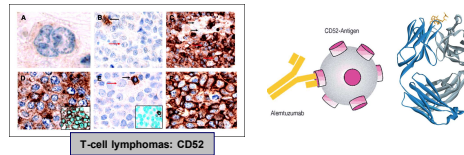
- **Brentuximab (anti-CD30)**

- HL
- ALCL
- CD30+ DLBCL



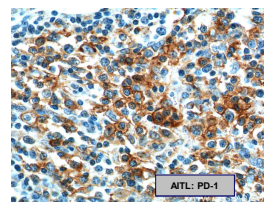
- **Alemtuzumab (anti-CD52)**

- B-CLL
- T-cell lymphoma



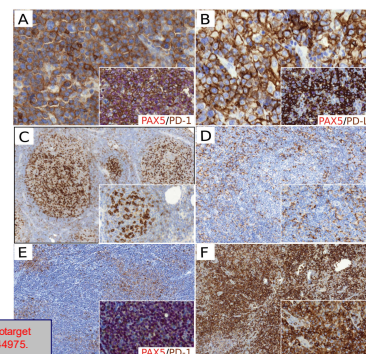
Immune checkpoint inhibitory therapy?

- **PD-1 AILD**



- **Hodgkin**

- PD-L1
- PD-1



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