

Haematolymphoid neoplasms: IHC for diagnostic use

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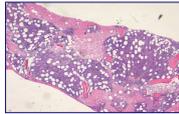
Haematolymphoid Neoplasms:

What are they?

Haematolymphoid Neoplasms: Leukaemia vs Lymphoma

CLONAL LYMPHOID MALIGNANCIES

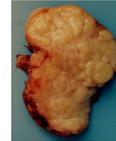
Bone marrow



Leukaemia

Blood

- Lymph node
- Extranodal site



Lymphoma

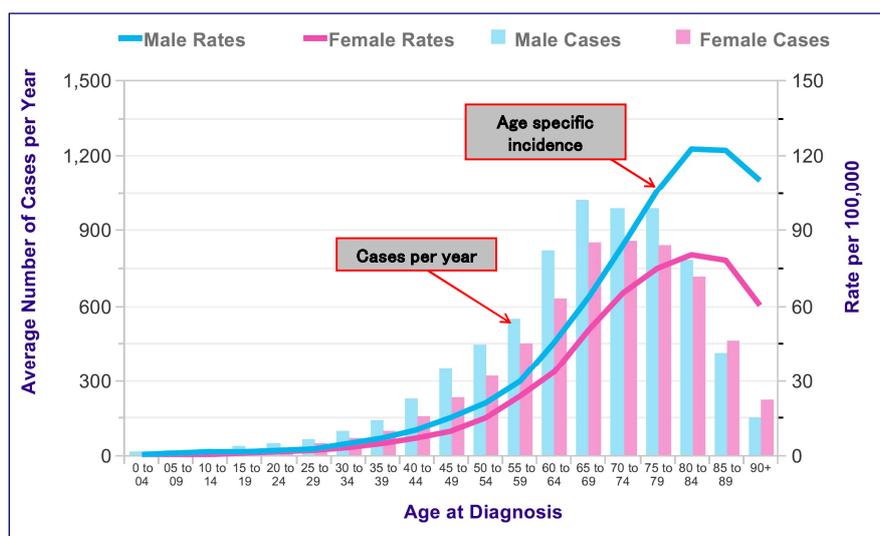
Haematolymphoid Neoplasms:

How common are they?

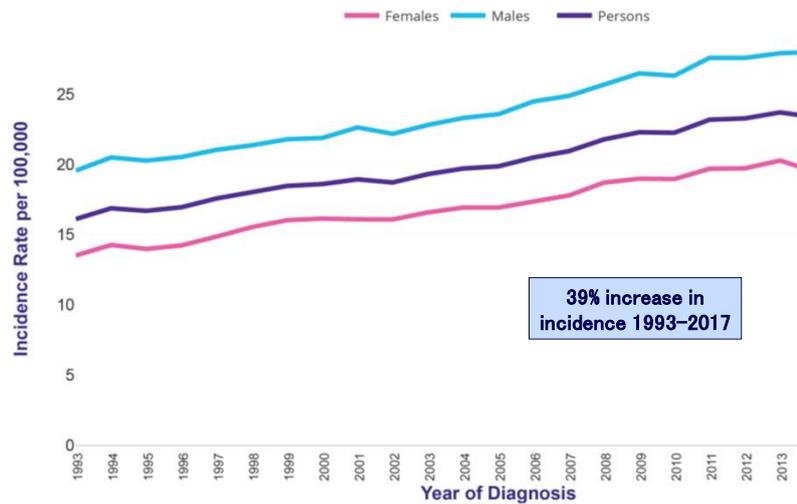
Malignant lymphoproliferative diseases

- Malignant lymphoma
- Leukaemia
 - Acute lymphoblastic leukaemia
 - Chronic lymphocytic leukaemia (CLL)
- Ca. 1,600 per year in DK
- > 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

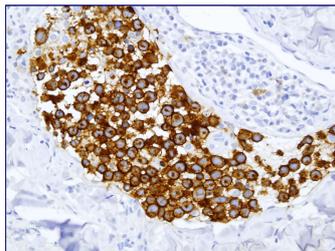


Glyphosate-based herbicides

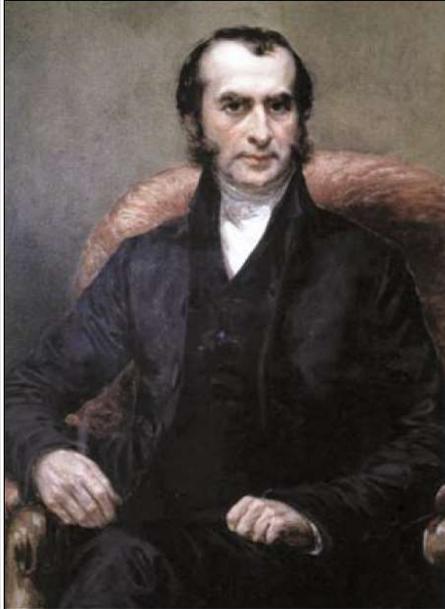
Haematolymphoid Neoplasms:

How are they classified?

IHC is essential in all cases!



Thomas Hodgkin 1798-1866



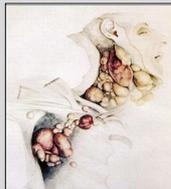
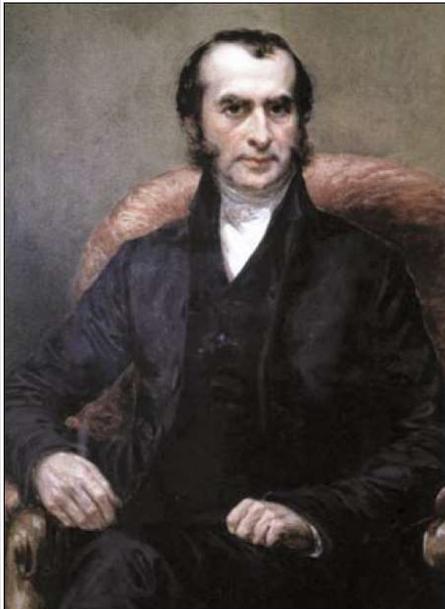
ON SOME
MORBID APPEARANCES
OF
THE ABSORBENT GLANDS
AND
SPLEEN.
BY DR. HODGKIN.
PRESENTED
BY DR. R. LEE.
READ JANUARY 10TH AND 24TH, 1832.

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



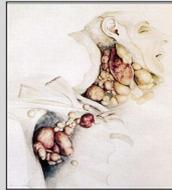
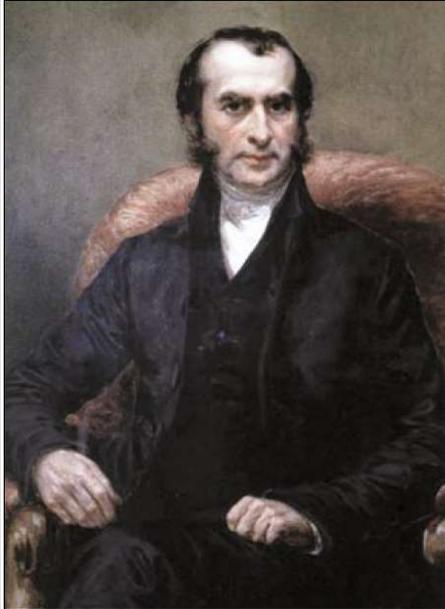
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The morbid alterations of structure which I am about to describe are probably familiar to many

Hodgkin's original case:
abdominal nodes



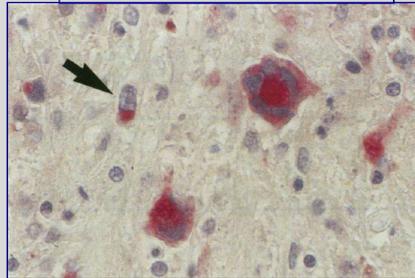
Gordon Museum,
King's College London

Thomas Hodgkin 1798-1866



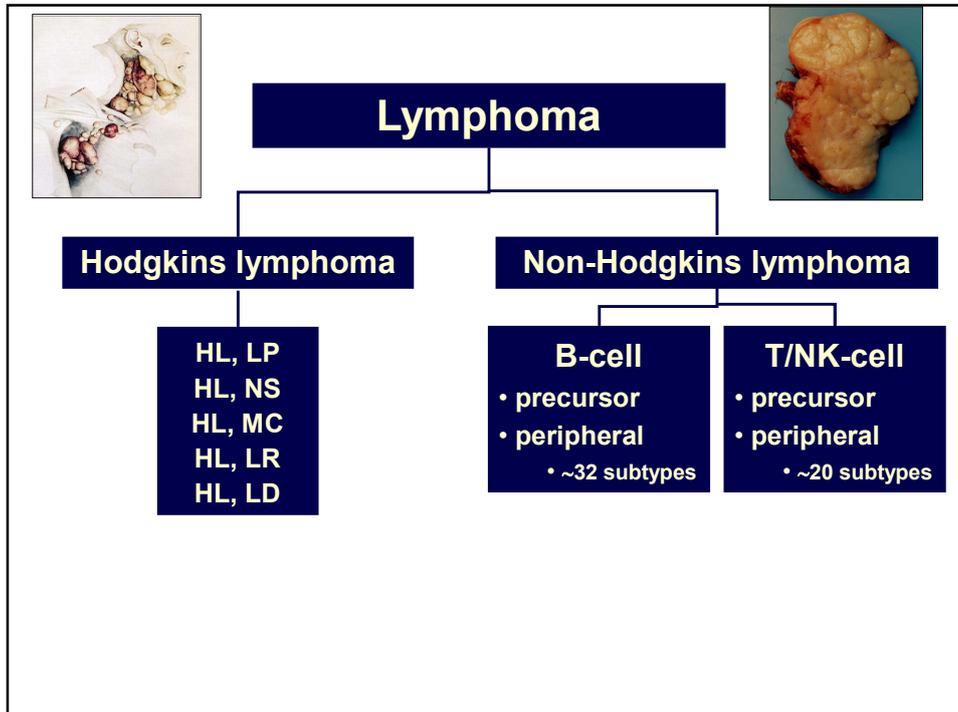
ON SOME
MORBID APPEARANCES
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THE ABSORBENT GLANDS
AND
SPLEEN.
BY DR. HODGKIN.
PRESENTED
BY DR. R. LEE.
READ JANUARY 1816 AND 1818, 1821.
THE MORBID ALTERATIONS OF STRUCTURE WHICH I AM
ABOUT TO DESCRIBE ARE PROBABLY FAMILIAR TO MANY

Hodgkin's original
case: CD15 (1991)



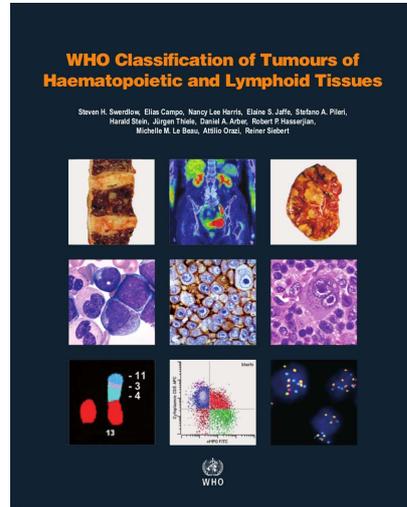
Paraffin-embedded tissue of one of the original cases by Thomas Hodgkin immunostained with CD15 (David Mason)

King's College London



Lymphoma Classification

- Previously many different classifications
- Then one widely accepted classification – 25 year consensus (WHO – 2016) 😊



Lymphoma Classification

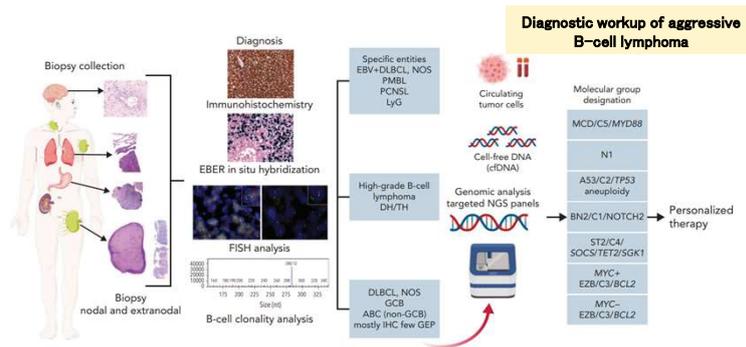
- Previously many different classifications
- Then one widely accepted classification – 25 year consensus (WHO – 2016) 😊
- Now again, two parallel classifications ☹️:
 - New WHO (5th. ed. 2022)
 - International Consensus Classification – ICC (SH/EAHP-sponsored) - 2022



Lymphoma Classification

- Disease entities based on:

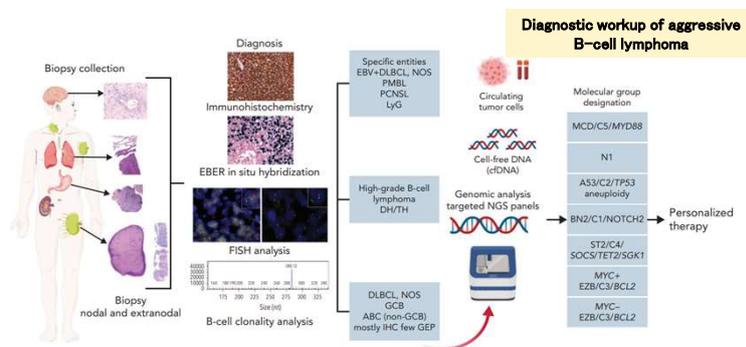
- Clinical features
- Morphology
- Immunophenotype
- Molecular genetics



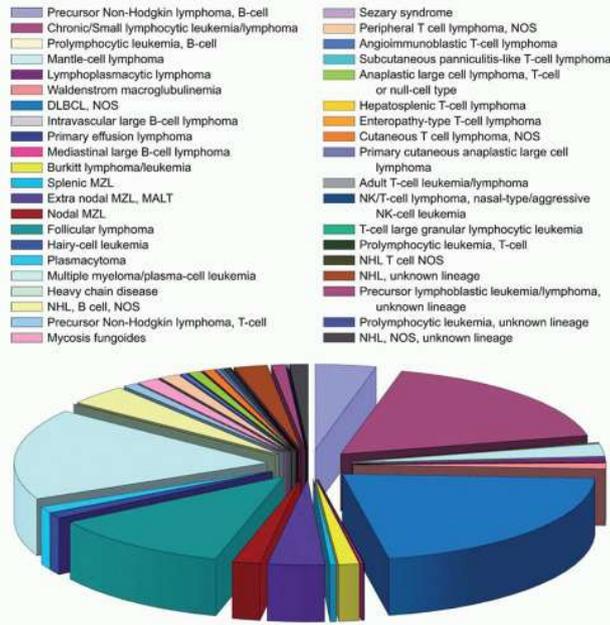
Lymphoma Classification

- Disease entities based on:

- Clinical features
- Morphology
- Immunophenotype
- Molecular genetics



SEER data (USA) for the relative incidence of non-Hodgkin lymphomas



International Consensus Classification of Lymphomas – 2022



The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee

Elias Campo,¹ Elaine S. Jaffe,² James R. Cook,³ Leticia Quintanilla-Martinez,⁴ Steven H. Swerdlow,⁵ Kenneth C. Anderson,⁶ Pierre Brousset,⁷ Lorenzo Cerroni,⁸ Laurence de Leval,⁹ Stefan Dirnhofer,¹⁰ Ahmet Dogan,¹¹ Andrew L. Feldman,¹² Falko Fend,⁴ Jonathan W. Friedberg,¹³ Philippe Gaulard,^{14,15} Paolo Ghia,¹⁶ Steven M. Horwitz,¹⁷ Rebecca L. King,¹² Gilles Salles,¹⁷ Jesus San-Miguel,¹⁸ John F. Seymour,¹⁹ Steven P. Treon,⁶ Julie M. Vose,²⁰ Emanuele Zucca,²¹ Ranjana Advani,²² Stephen Ansell,²³ Wing-Yan Au,²⁴ Carlos Barrionuevo,²⁵ Leif Bergsagel,²⁶ Wing C. Chan,²⁷ Jeffrey I. Cohen,²⁸ Francesco d'Amore,²⁹ Andrew Davies,²⁰ Brunangelo Falini,³¹ Irene M. Ghobrial,^{6,32} John R. Goodlad,³³ John G. Gribben,³⁴ Eric D. Hsi,³⁵ Brad S. Kahl,³⁶ Won-Seog Kim,³⁷ Shaji Kumar,²³ Ann S. LaCasce,⁶ Camille Laurent,⁷ Georg Lenz,³⁸ John P. Leonard,³⁹ Michael P. Link,⁴⁰ Armando Lopez-Guillermo,⁴¹ Maria Victoria Mateos,⁴² Elizabeth Macintyre,⁴³ Ari M. Melnick,⁴⁴ Franck Morschhauser,⁴⁵ Shigeo Nakamura,⁴⁶ Marina Narbaitz,⁴⁷ Astrid Pavlovsky,⁴⁸ Stefano A. Pileri,⁴⁹ Miguel Priis,⁵⁰ Barbara Pro,⁵¹ Vincent Rajkumar,¹² Steven T. Rosen,⁵² Birgitta Sander,⁵³ Laurie Sehn,⁵⁴ Margaret A. Shipp,⁶ Sonali M. Smith,⁵⁵ Louis M. Staudt,⁵⁶ Catherine Thieblemont,^{57,58} Thomas Tousseyn,⁵⁹ Wyndham H. Wilson,⁵⁶ Tadashi Yoshino,⁶⁰ Pier-Luigi Zinzani,⁶¹ Martin Dreyling,⁶² David W. Scott,⁵⁴ Jane N. Winter,⁶³ and Andrew D. Zelenetz^{17,64}

ICC B-cell Lymphomas – 2022

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

Table 1. International Consensus Classification of mature lymphoid and histiocytic/dendritic cell neoplasms

Mature B-cell neoplasms

Chronic lymphocytic leukemia/small lymphocytic lymphoma
 Monoclonal B-cell lymphocytosis
 Chronic lymphocytic leukemia type
 Non-chronic lymphocytic leukemia type
 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
 Immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (MGUS)
 IgM MGUS, plasma cell type*
 IgM MGUS, not otherwise specified (NOS)*
 Primary cold agglutinin disease*
 Heavy chain disease
 κ heavy chain disease
 Gamma heavy chain disease
 Alpha heavy chain disease
 Plasma cell neoplasms
 Non-IgM MGUS
 Multiple myeloma (plasma cell myeloma)†
 Multiple myeloma, NOS
 Multiple myeloma with recurrent genetic abnormality
 Multiple myeloma with CD138 family translocation
 Multiple myeloma with MAF family translocation
 Multiple myeloma with KND2 translocation
 Multiple myeloma with hyperdiploidy
 Solitary plasmacytoma of bone
 Extramedullary plasmacytoma
 Monoclonal Ig deposition disease†
 Ig light chain amyloidosis (AL)
 Localized AL amyloidosis*
 Light chain and heavy chain deposition disease
 Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) lymphoma†
 Primary cutaneous marginal zone lymphoproliferative disorder*
 Nodal marginal zone lymphoma
 Pediatric nodal marginal zone lymphoma
 Follicular lymphoma
 In situ follicular neoplasia
 Double-hit type follicular lymphoma
 BCL2B-negative, CD23 positive follicle center lymphoma
 Primary cutaneous follicle center lymphoma
 Pediatric-type follicular lymphoma

Table 1. (continued)

Traumatic follicular lymphoma*
 Large B-cell lymphoma with IRF4 rearrangement*
 Mantle cell lymphoma
 In situ mantle cell neoplasia
 Leukemic non-nodal mantle cell lymphoma
 Diffuse large B-cell lymphoma, NOS
 Germinal center B-cell subtype
 Activated B-cell subtype
 Large B-cell lymphoma with 11q aberration*
 Nodular lymphocyte predominant B-cell lymphoma*
 T cell/histiocyte-rich large B-cell lymphoma
 Primary diffuse large B-cell lymphoma of the central nervous system
 Primary diffuse large B-cell lymphoma of the testis*
 Primary cutaneous diffuse large B-cell lymphoma, leg type
 Intravascular large B-cell lymphoma
 HHV-8 and Epstein-Barr virus-negative primary effusion-based lymphoma*
 Epstein-Barr virus-positive mucocutaneous ulcer*
 Epstein-Barr virus-positive diffuse large B-cell lymphoma, NOS
 Diffuse large B-cell lymphoma associated with chronic inflammation
 Fibrin-associated diffuse large B-cell lymphoma
 Lymphomatoid granulomatosis
 Epstein-Barr virus-positive polymorphic B-cell lymphoproliferative disorder, NOS†
 ALK-positive large B-cell lymphoma
 Plasmablastic lymphoma
 HHV-8-associated lymphoproliferative disorders
 Multicentric Castlemann disease
 HHV-8-positive germinal-center lymphoproliferative disorder
 HHV-8-positive diffuse large B-cell lymphoma, NOS
 Primary effusion lymphoma
 Burkitt lymphoma
 High-grade B-cell lymphoma with MYC and BCL2 rearrangement*
 High-grade B-cell lymphoma with MYC and BCL6
 Rearrangement*
 High-grade B-cell lymphoma, NOS
 Medullary large B-cell lymphoma*
 Medullary large B-cell lymphoma*

Classical Hodgkin lymphoma

Nodular sclerosis classical Hodgkin lymphoma
 Lymphocyte-rich classical Hodgkin lymphoma
 Mixed cellularity classical Hodgkin lymphoma
 Lymphocyte-depleted classical Hodgkin lymphoma

Mature T-cell and NK-cell neoplasms

T-cell prolymphocytic leukemia
 T-cell large granular lymphocytic leukemia

Abbreviations: NOS, not otherwise specified; *WHO, 5th edition; †WHO, 4th edition.
 *Compared to the 2016 WHO classification.
 †These lesions are classified according to the lymphoma to which they correspond.

INTERNATIONAL CONSENSUS CLASSIFICATION Blood | 15 SEPTEMBER 2022 | VOLUME 140 NUMBER 11 | 3231



Why does it all have to be so complicated!?

Because of "Personalized Medicine" –

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

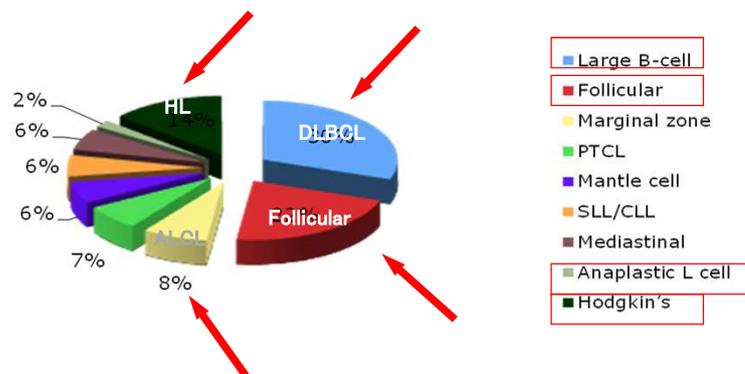
Updated Lymphoma Classifications – 2022

Recent 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*”

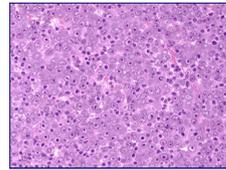
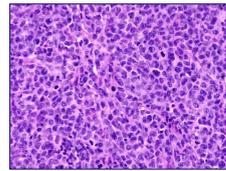
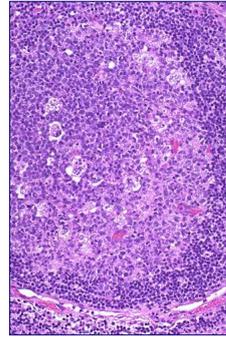
Lymphoma frequencies



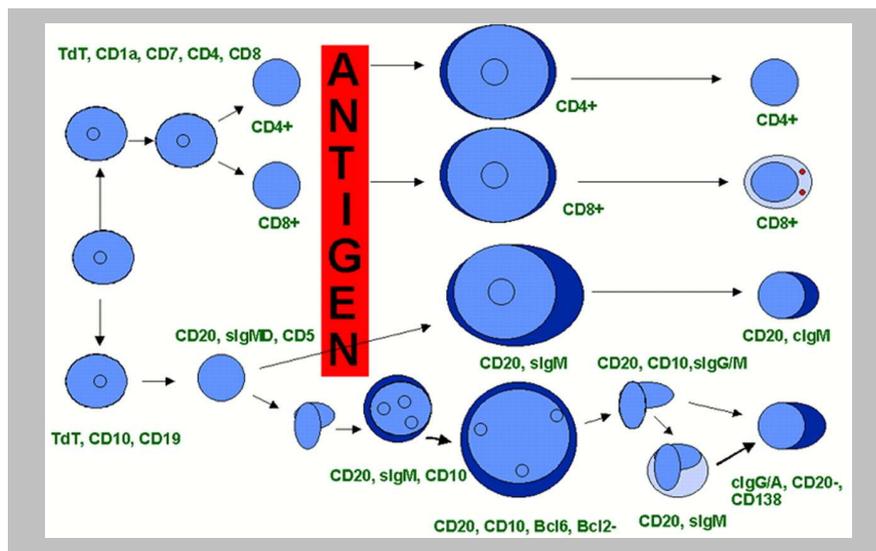
2002 SEER database. O'Connor

What is lymphoma?

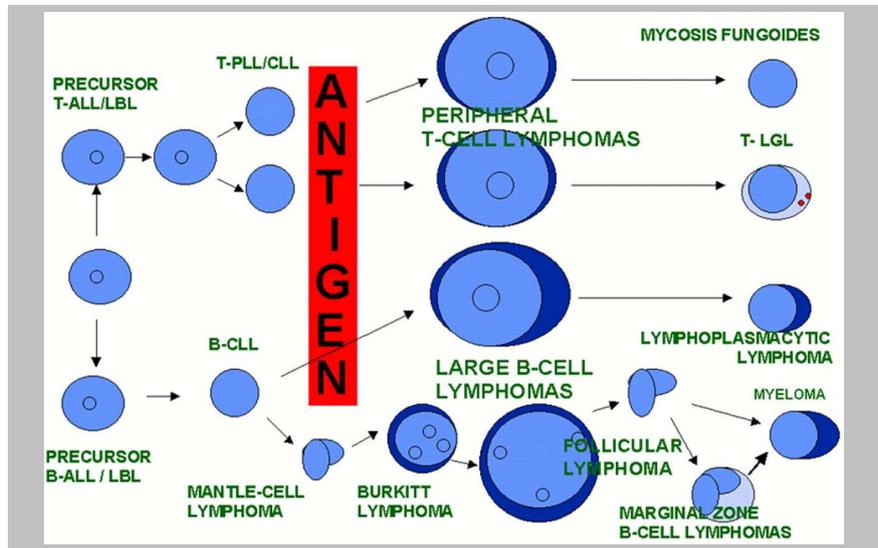
- Clonal malignancy
 - → mutational events cause cells to freeze at a single stage of normal lymphocyte differentiation
- Morphology, **immunophenotype** & molecular features:
 - mirror stages of normal lymphocyte development



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

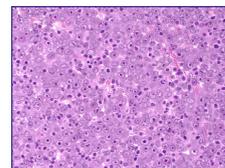
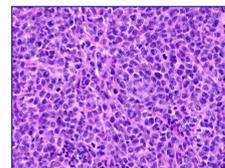
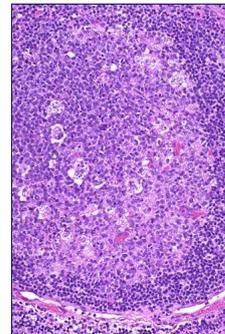


Lymphoid neoplasms: Correlation with normal T or B-cell differentiation



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



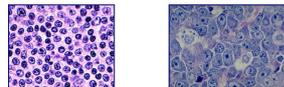
Lymphoma & Leukaemia diagnosis

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

Lymphoma differential diagnosis

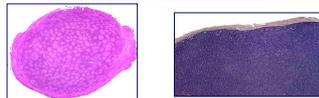
- Assess morphology:

- cell size



Small vs. Large

- architecture



Nodular vs. Diffuse

But...that's not enough!

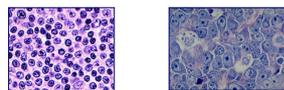
Lymphoma & Leukaemia diagnosis

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

Lymphoma differential diagnosis

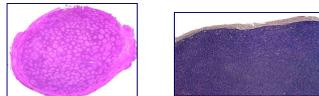
- Assess morphology:

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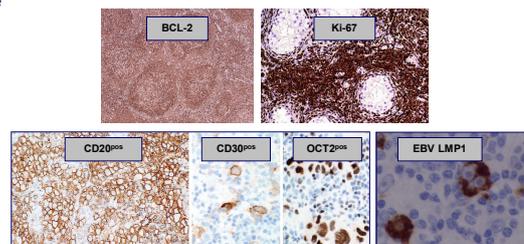
Small vs. Large

- architecture



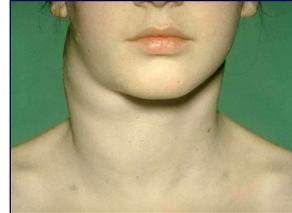
Nodular vs. Diffuse

- Select appropriate immune panel(s)



Enlarged lymph node

Is it malignant?



- **Emphasis on lymphoma classification**
- **Reactive vs malignant**
 - this is often a more challenging decision
- **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"

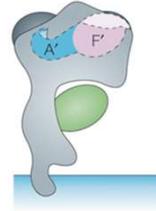
UK: RCPATH / BCSH

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

...and many more!

What are CD numbers?

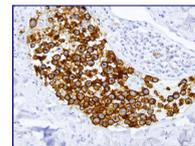
Human CD1a



- **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**

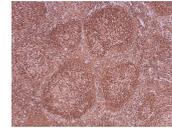
IHC Dogma

(also applies in diagnostic haematopathology)



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

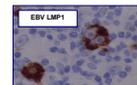
Diagnostic Applications of IHC 1



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



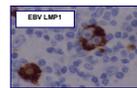
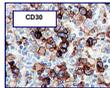
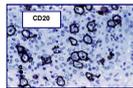
- T-cell lymphoma vs B-cell lymphoma
- T-cell lymphoma vs T-zone hyperplasia
- Hodgkin lymphoma vs NHL
- Hodgkin lymphoma
 - NLPHL vs classical HL
- Lymphoblastic vs. Myeloblastic vs. Burkitt
- Undifferentiated malignant tumor
- Lymphoma prognosis
 - e.g. Ki-67; ALK; c-myc
- Targeted therapy
 - e.g.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

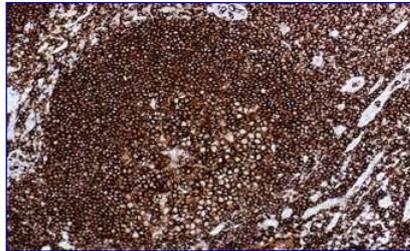


Basic IHC panel for lymphoma diagnosis

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

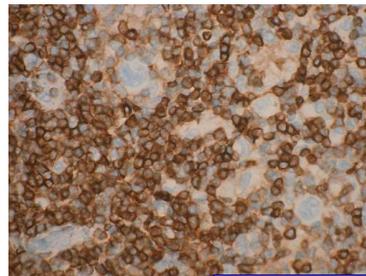
Basic stains: CD45

- Membrane glycoprotein family
- Positive in all (?) 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, MC: CD45

Basic stain: Immunoglobulin

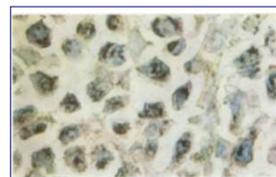
J. clin. Path., 1974, 27, 14-20

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

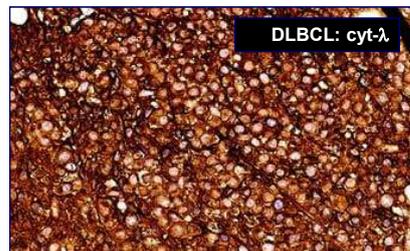
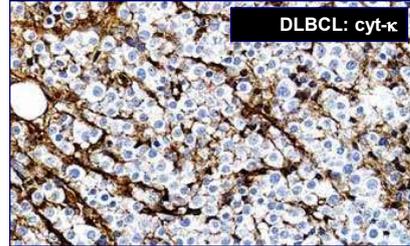
- IHC-Ig
 - first protocol for IHC in FFPE
 - still one of the hardest to perform & evaluate!



• plasmacytoma
• monoclonal Ig-kappa

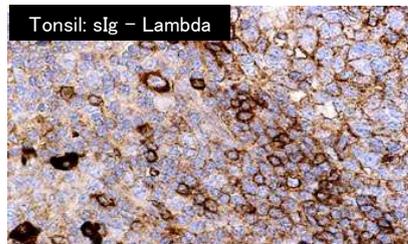
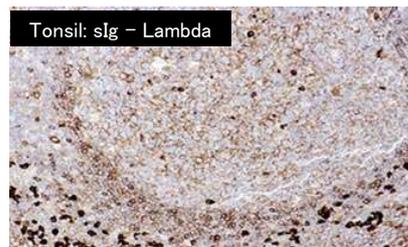
Basic stains: Immunoglobulin

- 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



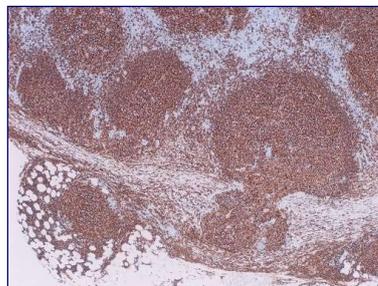
Basic stains: Immunoglobulin

- 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

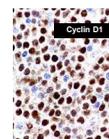
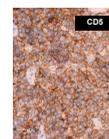


Follicular lymphoma: CD20

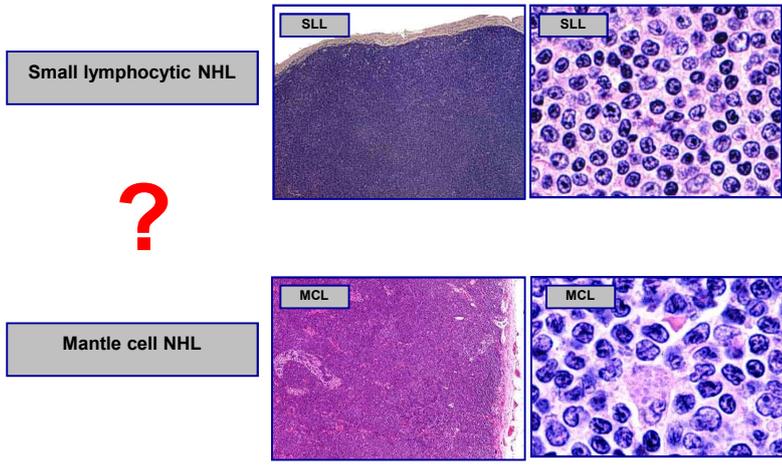
Usual staining pattern of B-cell neoplasms

	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	+	+	-	-	+	-	-	-

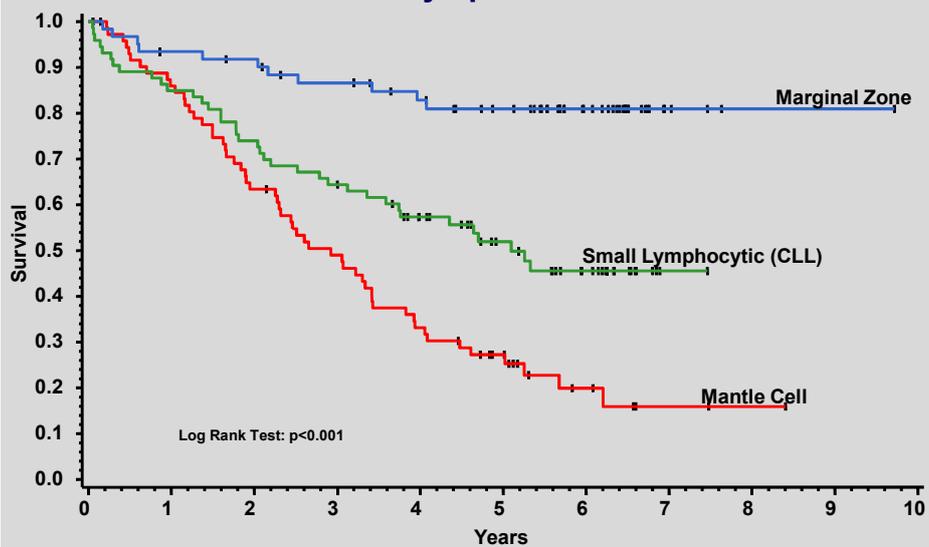
Key
 +/- The lymphoma cells are commonly but not always positive
 -/+ The lymphoma cells are usually but not always negative



Small cell B-Cell lymphomas: Differential Diagnosis

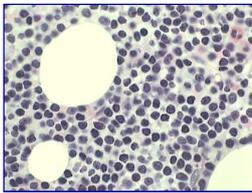
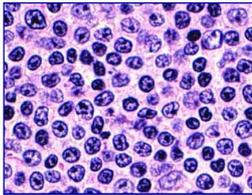
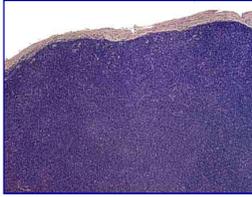


Small B-Cell Lymphomas: Overall Survival



Armitage et al, 1997

B-cell Small Lymphocytic Lymphoma (CLL)

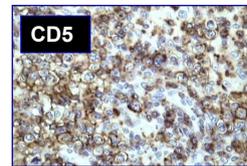
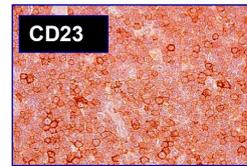
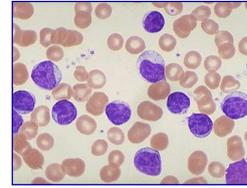


Morphology

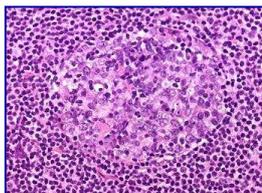
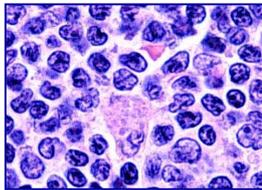
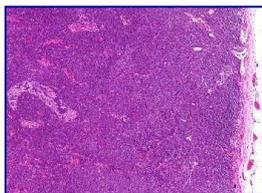
- small lymphocytes
- proliferation centres

Immunology

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



Mantle Cell Lymphoma

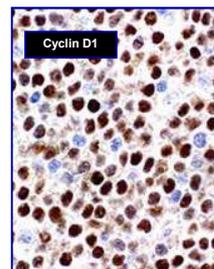
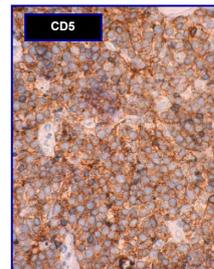


Morphology

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

Immunology

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

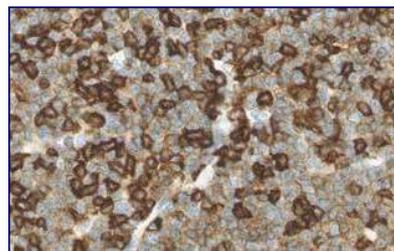


Immunophenotype: Small B-Cell Lymphomas

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

Basic stains: CD5

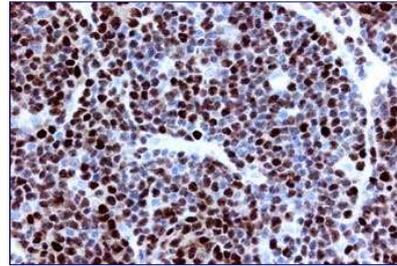
- Modulates T & B cell signalling
- Pan-T cell marker
 - 95% thymocytes
 - 100% post-thymic T-cells
 - ↑ expression with maturity
- Minor population normal B-cells:
 - ca. 10%+ peripheral B-cells
 - ↑ in autoimmunity
- Lymphomas:
 - 90% T-cell neoplasias
 - B-cell NHL
 - B-CLL / SLL (90%)
 - Mantle cell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



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

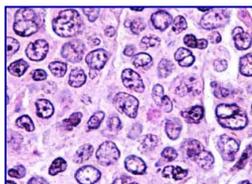
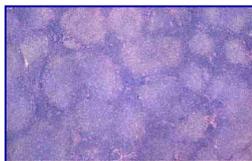
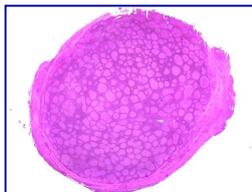
Basic stains: Cyclin D1

- 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

Follicular Lymphoma

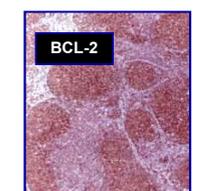
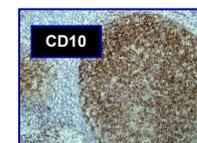
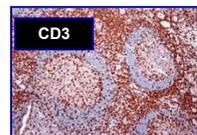
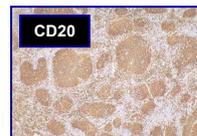


Morphology

- germinal centre cells
- CBs & CCs
- follicular

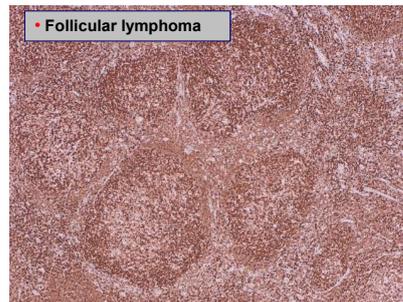
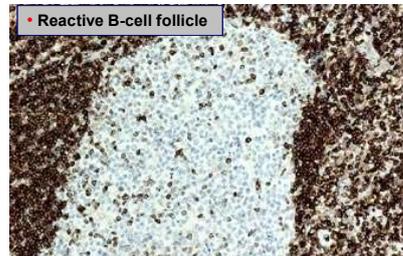
Immunology

- 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



Journal of Pathology

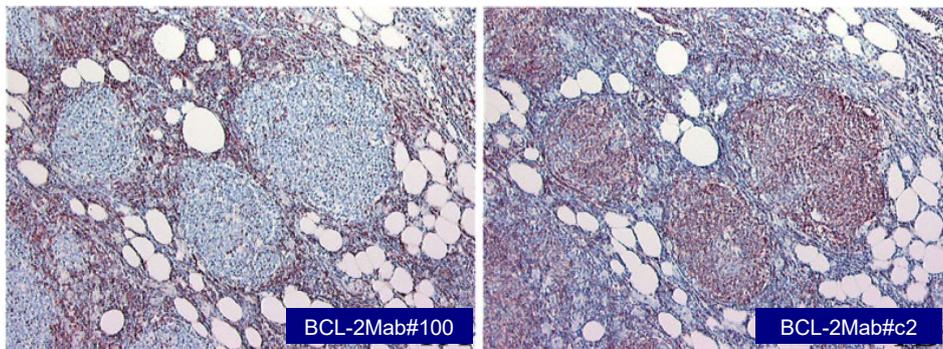
J Pathol 2005; 205: 329–335

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/path.1689

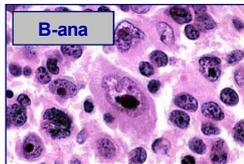
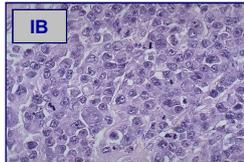
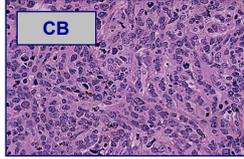
Original Paper

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 Groenen¹ and Han van Krieken¹



Diffuse Large B-cell Lymphoma

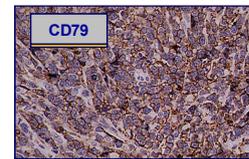
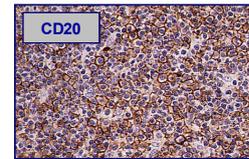
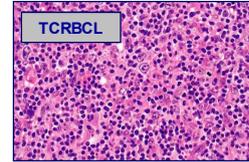


Morphology

- large cells
- nucleoli
- diffuse

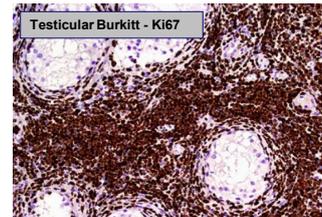
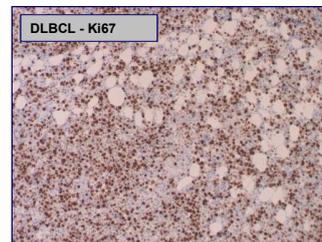
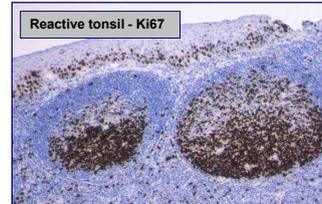
Immunology

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



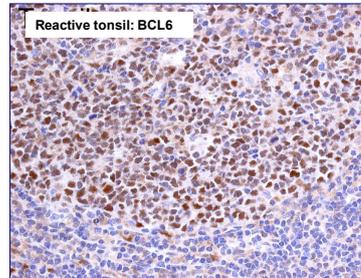
Basic stain: Ki- 67

- Nuclear protein
- Expressed in all cell cycle stages except G0
- In lymphomas:
 - Often (but not always) correlates to:
 - indolent vs. aggressive vs. 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:

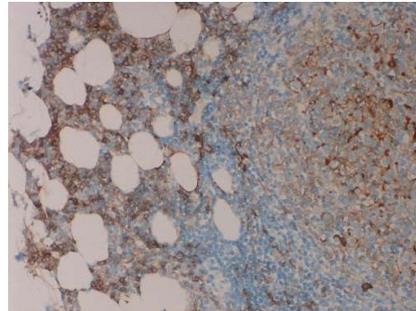
- CD10
- CD138
- MUM1

Basic IHC panel for lymphoma diagnosis

- CD45
- CD20
- CD79a
- PAX5
- Ki67
- CD3
- CD5
- CD10
- CD45
- Bcl-2
- Bcl-6
- CD20 (CD20)
- Cyclin D1
- MUM1

Secondary stain: CD10

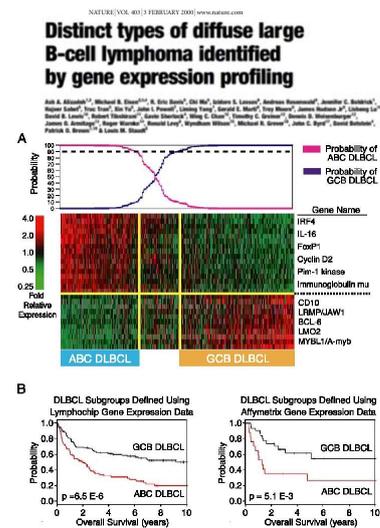
- >90% precursor B-LB (membrane & paranuclear stain)
- ca. 25% precursor T-LB
- Burkitt lymphoma
- Follicular lymphoma
 - Interfollicular CD10+ cells suggests lymphoma
- Some DLBCL
 - 'Cell of origin' algorithm in DLBCL
 - GCB vs ABC



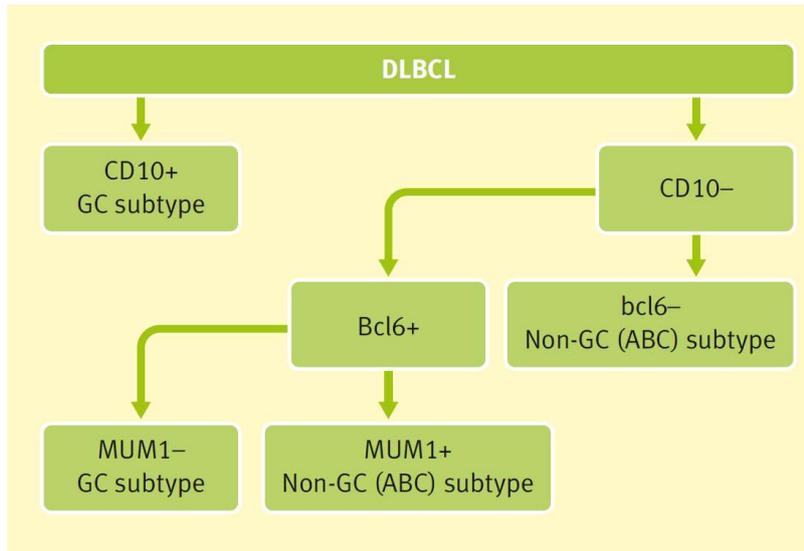
- Follicular lymphoma – CD10
- Interfollicular tumour cells

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**

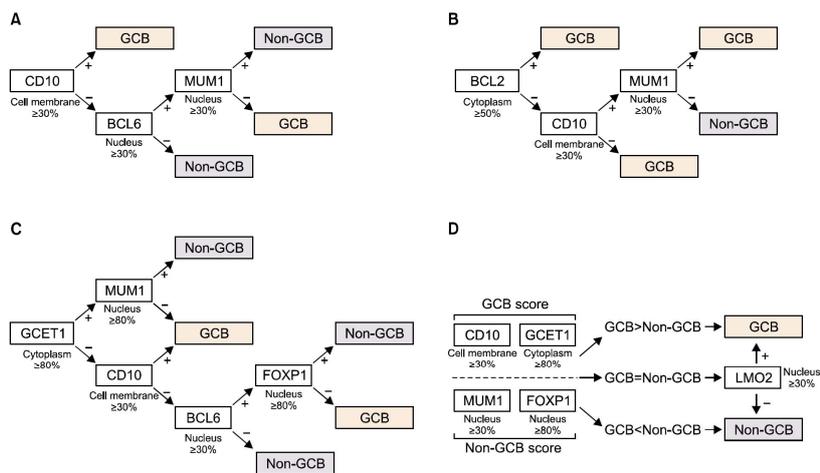
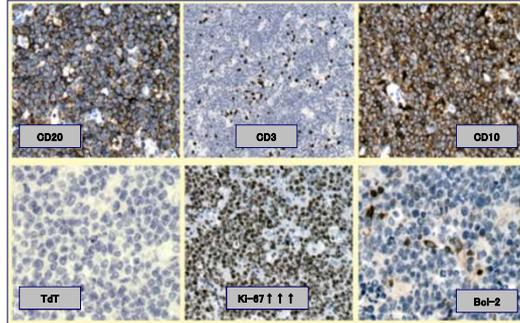
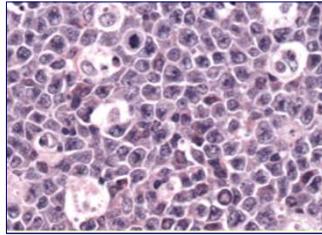


Fig. 2. Summary of the (A) Hans, (B) Muris, (C) Choi, and (D) Tally algorithms, and criteria for a positive signal for individual immunohistochemical markers (below or to the right of the white-filled box). Note that the positive criterion for MUM1/IRF4 in the Choi algorithm (more than 80%) is different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

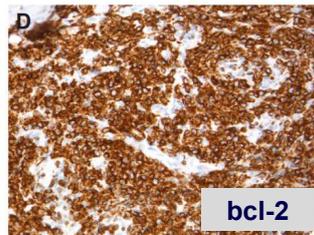
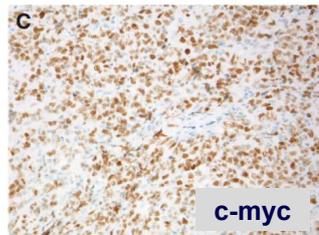
VOLUME 30 · NUMBER 28 · OCTOBER 1 2012

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 Mourits-Andersen, Mikael Frederiksen, Lars Møller Pedersen, and Michael Boe Møller



Updated Lymphoma Classifications – 2022

Recent 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*”

Hodgkin 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	+	-	+

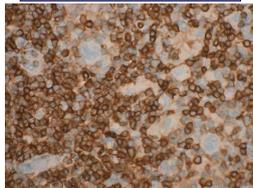
AKA: Nodular lymphocyte predominant B-cell lymphoma

Key

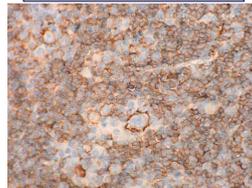
+/- The lymphoma cells are commonly but not always positive

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

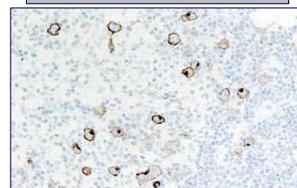
Hodgkin lymphoma, MC: CD45



Hodgkins lymphoma, LP: CD20

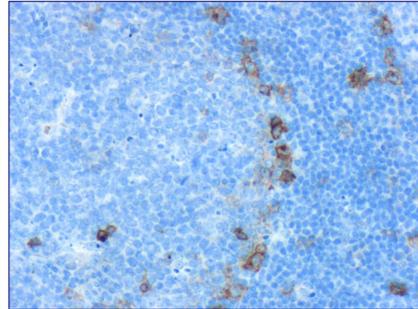


Classical Hodgkin lymphoma, MC: CD30



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

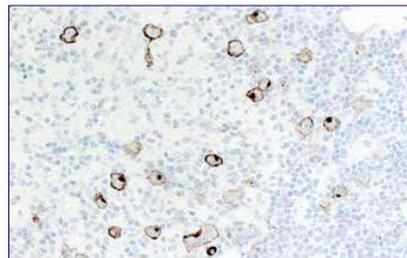


Reactive LN: activated B-cells

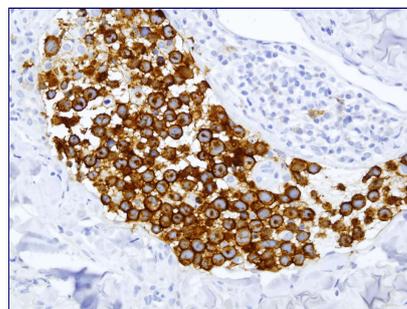
CD30 in lymphoma

"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



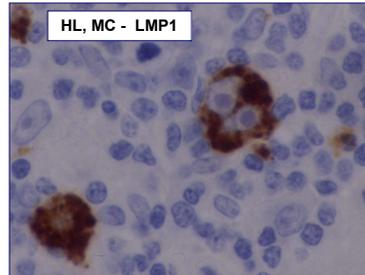
Hodgkins lymphoma: CD30



ALCL - sinus pattern CD30

Secondary stain: EBV

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

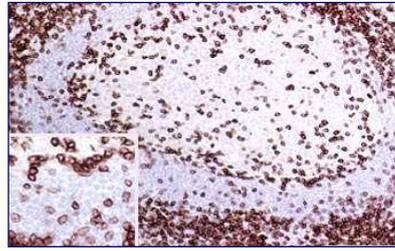


T-cell lymphoma: immunophenotype

Complex!

Basic stain: CD3

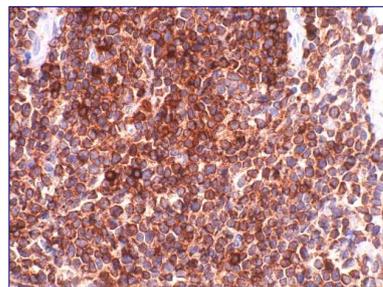
- transmembrane molecule
- Ig superfamily
- part of T-cell receptor
- most specific T-cell marker
- pan-T cell marker
 - thymocytes: cyt. → membrane
 - most post-thymic T-cells
 - activated NK-celler



Reactive LN: CD3

CD3 in lymphoma

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



• Precursor T-LB
• CD3-cyt

IHC for PTL

Add to basic panel:

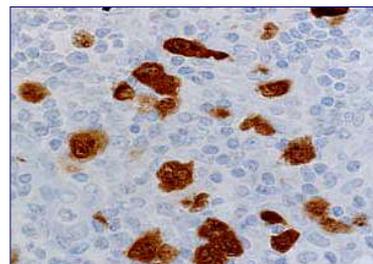
Basic IHC panel for lymphoma diagnosis

- CD45
- CD20
- CD79a
- PAX5
- (appa/lambda)
- CD3
- CD5
- CD30
- CD43
- Bcl-2
- Bcl-6
- CD20 (CD21)
- Cyclin-D1
- MALT

- 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

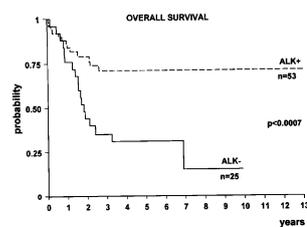
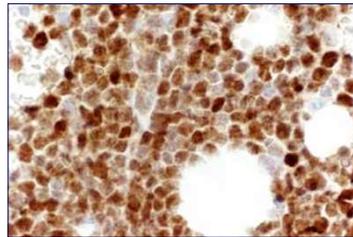


Fig 4. Overall survival of ALK⁻ versus ALK⁺ lymphoma.

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

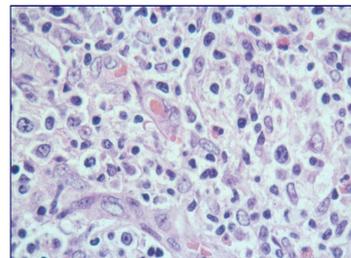
Secondary stain: Terminal deoxynucleotidyl transferase (TdT)

- 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%)

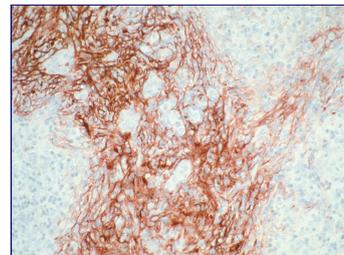


Basic stain: CD21

- 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



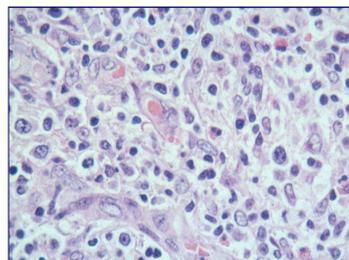
• AILD-T-cell lymphoma



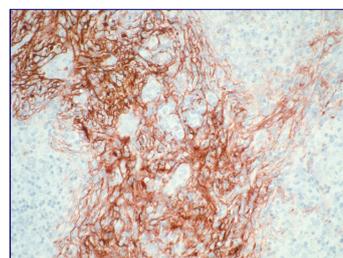
• AILD-T-cell lymphoma: CD21

Basic stain: CD21

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

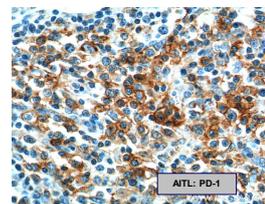
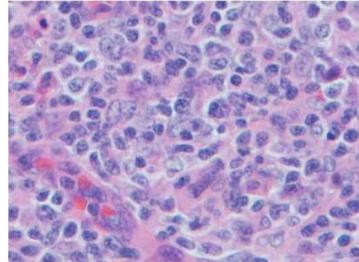
Nodal PTCL - immunophenotype

	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.

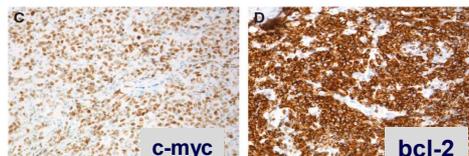
T-cell lymphomas of TFH cell origin

- 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

Basic IHC panel for lymphoma diagnosis	
-	CD45
-	CD20
-	CD79c
-	(PAX-5)
-	(Anaplastic)
-	CD3
-	CD5
-	CD30
-	CD43
-	Bcl-2
-	Bcl-6
-	CD23 (CD21)
-	Cytoker-D1
-	M-47

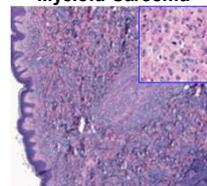
IHC for lymphoid vs myeloid

Add to basic panel

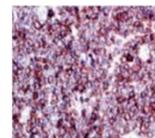
- Myeloperoxidase
- CD43
- CD68
- CD163
- CD33
- (CD14, CD15, CD34, CD61, glycophorin C)

Basic IHC panel for lymphoma diagnosis	
-	CD45
-	CD20
-	CD79c
-	(PAX-5)
-	(Anaplastic)
-	CD3
-	CD5
-	CD30
-	CD43
-	Bcl-2
-	Bcl-6
-	CD23 (CD21)
-	Cytoker-D1
-	M-47

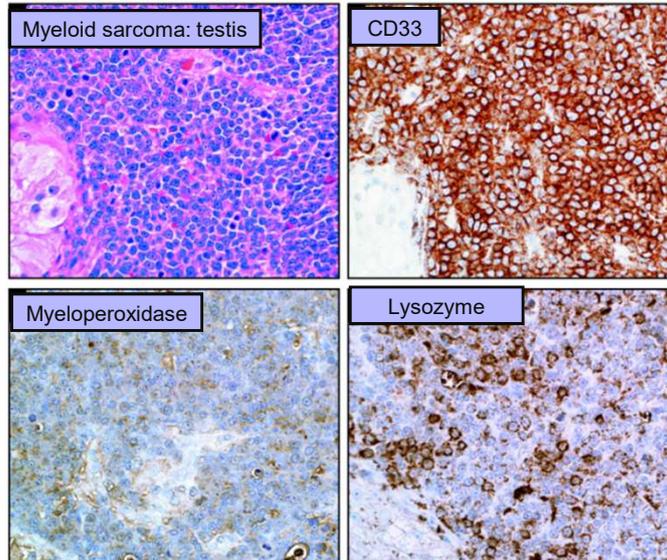
Myeloid Sarcoma



CD33



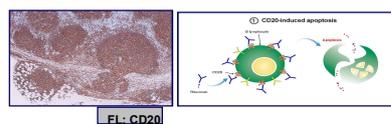
Myeloid sarcoma: testis



Targeted therapy

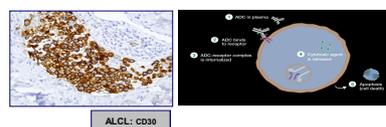
- **Rituximab (anti-CD20)**

- B-cell NHL



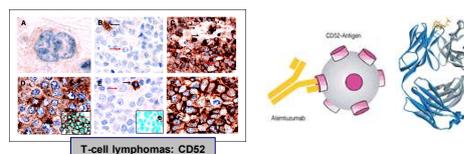
- **Brentuximab (anti-CD30)**

- HL
- ALCL
- CD30+ DLBCL



- **Alemtuzumab (anti-CD52)**

- B-CLL
- T-cell lymphoma



Immune checkpoint inhibitory therapy?

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

