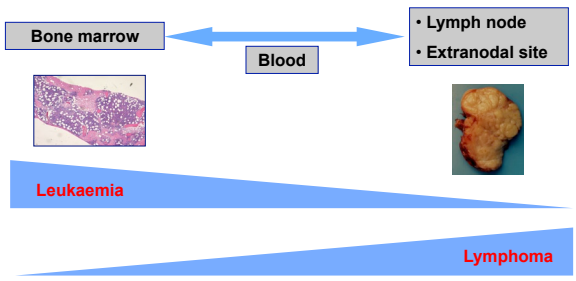
Immunohistochemical classification of haematolymphoid tumours

Stephen Hamilton-Dutoit
Institute of Pathology
Aarhus University Hospital

Haematolymphoid Neoplasias: Leukaemia *vs* Lymphoma

CLONAL MALIGNANCIES

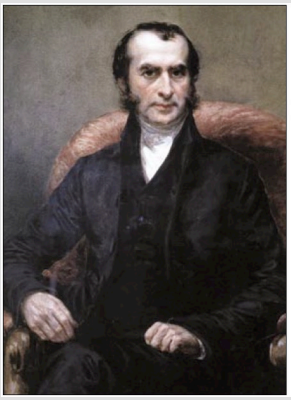


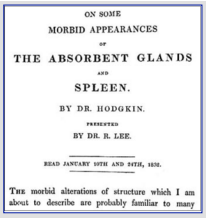
Malignant lymphoproliferative diseases

- Malignant lymphoma
- Leukaemia
  - Acute lymphoblastic leukaemia
  - chronic lymphocytic leukaemia (CLL)
- Ca. 1600 per year in DK
- Ca. 800 000 per year in the world

Classification!

Thomas Hodgkin 1798-1866

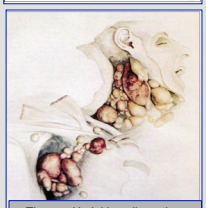




ON SOME
MORBID APPEARANCES
OF
THE ABSORBENT GLANDS
AND
SPLEEN.
BY DR. HODGKIN.
PRESENTED
BY DR. R. LEE.

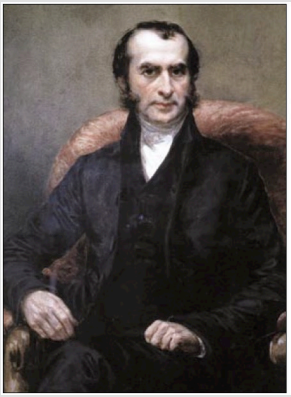
READ JANUARY 10TH AND 24TH, 1832.

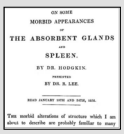
THE morbid alterations of structure which I am about to describe are probably familiar to many



Thomas Hodgkin – dissection
(Prof. Robert Carswell)

Thomas Hodgkin 1798-1866

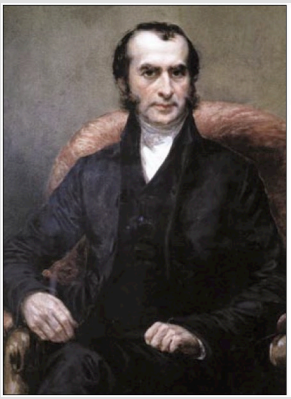


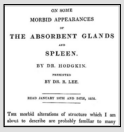


Hodgkin's original case:
abdominal nodes

Gordon Museum,
King's College London

Thomas Hodgkin 1798-1866





ON SOME
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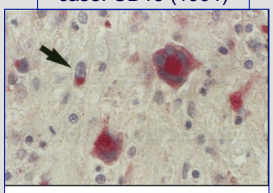
OF
THE ABSORBENT GLANDS
AND
SPLEEN.
BY DR. HODGKIN.
COMMUNICATED
BY DR. S. LEE.

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

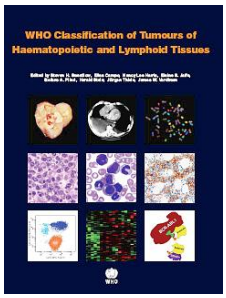
Hodgkin's original case: CD15 (1991)



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

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2008

- 70s – 80s: Kiel classification
  - B vs T cells: IHC!!
- 90s: REAL classification
- WHO (2008…..2016?)
  - "Real" disease entities
  - Clinical features
  - Morphology
  - Immunophenotype
  - Molecular genetics



Lymphoma

Hodgkins lymphoma

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

Non-Hodgkins lymphoma

B-cell
- precursor
- peripheral
  - ~32 subtypes

T/NK-cell
- precursor
- peripheral
  - ~20 subtypes

WHO Classification: B-cell Lymphoma

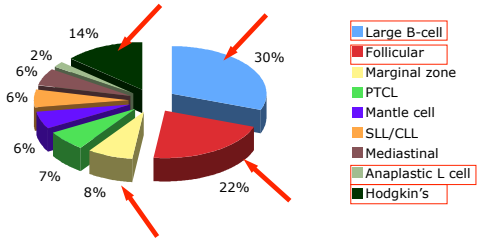
Table 3. WHO classification of the mature B-cell, T-cell, and NK-cell neoplasms (2008)

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic lymphoma/leukemia, unclassifiable*
Splenic diffuse red pulp small B-cell lymphoma*
Hairy cell leukemia-variant*
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Heavy chain diseases
Alpha heavy chain disease
Gamma heavy chain disease
Mu heavy chain disease
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma*
Follicular lymphoma
Pediatric follicular lymphoma*
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma (DLBCL), NOS
T-cell/histiocyte rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV+ DLBCL of the elderly*
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK+ large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

WHO Classification: T-cell & NK-cell neoplasms

Mature T-cell and NK-cell neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells*
Aggressive NK cell leukemia
Systemic EBV+ T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Adult T-cell leukemia/lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30+ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma*
Primary cutaneous CD4+ small/medium T-cell lymphoma*
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, *ALK*+
Anaplastic large cell lymphoma, ALK- *

Lymphoma frequencies

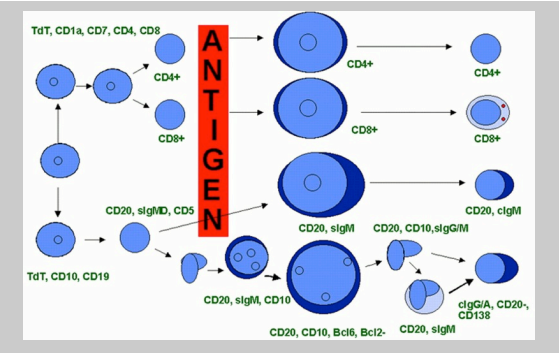


2002 SEER database. O'Connor

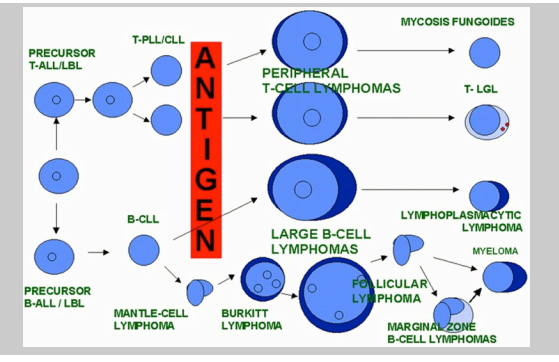
What is lymphoma?

- 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

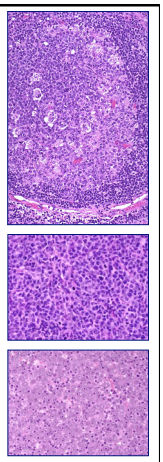
T and B-cell differentiation: Stage-specific surface antigen expression



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

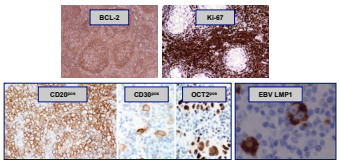
- Clinical features
- Morphology
- **Immunophenotype**
- Molecular diagnosis

Lymphoma differential diagnosis

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

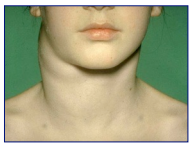
  - **architecture**

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



Enlarged lymph node

Is it malignant?

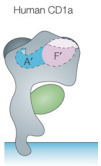


- Emphasis on lymphoma classification
- Reactive *vs* malignant
  - often more challenging diagnosis
- Use IHC to evaluate lymphoid tissue cytology and architecture
- Correlate immunophenotype with disease entity

International recommendations for lymphoma diagnostics

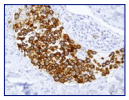
Danish lymphoma group	http://www.lymphoma.dk/index.php?id=56,0,0,1,0,0 See Lymfomdiagnostik¨
UK: RCPath / BCSH	http://www.rcpath.org/publications-media/publications/datasets/lymphoma

What are CD numbers?



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

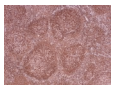
IHC Dogma



(also applies in diagnostic haematopathology)

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

Diagnostic Applications of IHC 1



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

Diagnostic Applications of IHC 2

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

Useful antigens in haematopathology

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

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

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



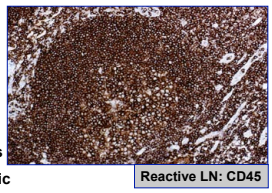


Basic IHC panel for lymphoma diagnosis

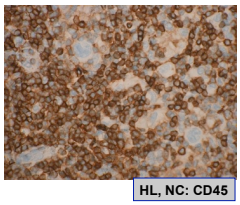
- CD45
- CD20
- CD79α
- (PAX-5)
- kappa/lambda
- CD3
- CD5
- CD30
- CD43
- Bcl-2
- Bcl-6
- CD23 (CD21)
- Cyclin-D1
- Ki-67

Basic stains: CD45

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


Reactive LN: CD45

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


HL, NC: CD45

Basic stain: Immunoglobulin

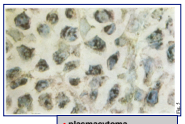
J. clin. Path., 1974, **27**, 14-20

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

C. R. TAYLOR AND J. BURNS

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

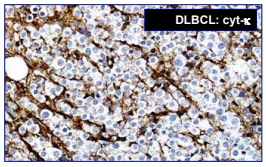
- IHC-Ig
  - first protocol for IHC in FFPE
  - still one of the hardest to perform & evaluate!

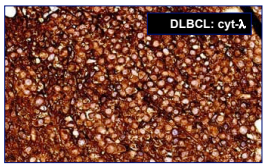


- plasmacytoma
- monoclonal Ig-kappa

Basic stains: Immunoglobulin

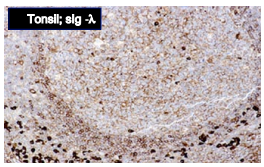
- B-cell specific
- Normal κ:λ ratio ca. 3-4:1
- Monotypic Ig restriction
  - Suggests clonality
  - >10:1 or < 0.2:1 = restriction
- Cytoplasmic Ig easily shown
- In lymphomas:
  - Cy Ig:
    - lymphoplasmacytic; myeloma; MZL; DLBCL, FL
  - Surface Ig

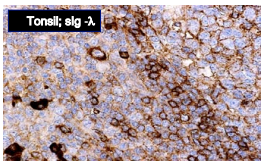




Basic stains: Immunoglobulin

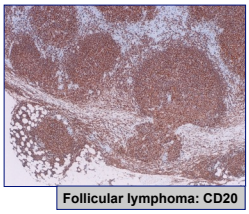
- Surface Ig
  - B-NHL clonality
  - Requires sensitive, optimised technique
  - Interpretation difficult (serum Ig)





Basic stains: CD20

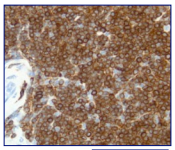
- Many B-cell neoplasms
- Negative in:
  - early precursor B-LB
  - plasma cell neoplasms
- Negative in T-cell lymphomas
  - rare cases positive
- Hodgkins lymphoma
  - HL-LP: 90% positive
  - Other types – variably positive (10% - 30%; not all HRS cells)
- Predictive marker for Rituximab therapy



Follicular lymphoma: CD20

Basic stains: CD79α

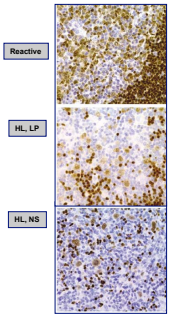
- Fairly specific, sensitive B-cell marker
- Normal (wide B-cell expression):
  - pre-B cell to plasma cell
- Lymphomas:
  - majority B-cell leukaemias and lymphomas
  - 50% myelomas
  - 10%+ T-LBs positive
  - rare in mature T-cell NHL
  - Hodgkin lymphoma:
    - L&H/popcorn cells positive
    - HRS cells in classical HL ca. 20% cases positive



B-CLL: CD79

Basic stains: Pax-5 (BSAP)

- Most specific B-cell marker available
- B-cell nuclear transcription factor
- Normal – many B cells
- Lymphomas:
  - nearly all B-cell NHLs
  - Hodgkins: HRS cells and variants positive in most cases
  - plasma cell neoplasms negative
  - peripheral TCLs negative
  - some pre-T-LB positive
  - some AML positive



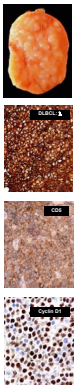
Reactive

HL, LP

HL, NS

Usual staining pattern of B-cell neoplasms

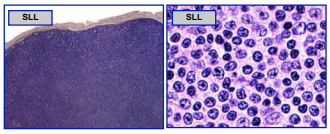
	CD20	CD79	CD5	CD23	CD10	CD30	CD15	CyclinD1
Precursor B-cell neoplasms								
Precursor B-lymphoblastic leukaemia/lymphoma	–	+/–	–	–	+	–	–	–
Mature B-cell neoplasms								
B-cell chronic lymphocytic leukaemia/lymphoma	+	+	+	+	–	–	–	–
B-cell prolymphocytic leukaemia	+	+	–	+/–	–	–	–	–/+
Lymphoplasmacytic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



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

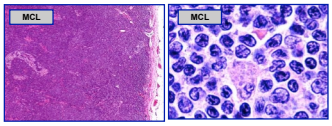
Small cell B-Cell lymphomas: Differential Diagnosis

Small lymphocytic NHL

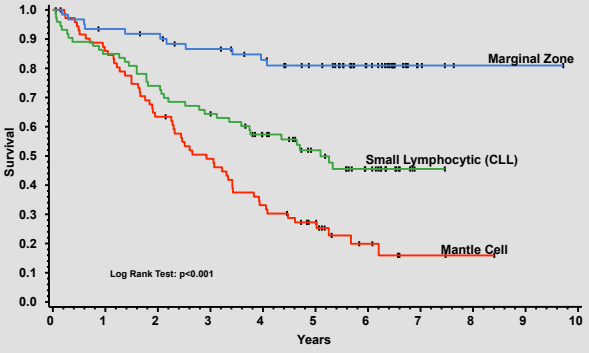


?

Mantle cell NHL



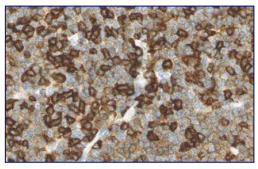
Small B-Cell Lymphomas: Overall Survival



Armitage et al, 1997

Basic stains: CD5

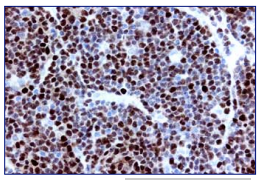
- Modulates T & B cell signalling
- Pan-T cell marker
  - 95% thymocytes
  - 100% post-thymic T-cells
  - ↑ expression with maturity
- Minor population normal B-cells:
  - ca. 10%+ peripheral B-cells
  - ↑ in autoimmunity
- Lymphomas:
  - 90% T-cell neoplasias
  - B-cell NHL
    - B-CLL / SLL (90%)
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



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

Basic stains: Cyclin D1

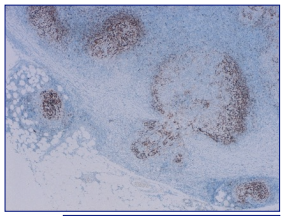
- cyclin family
  - control cell cycle
- normal proliferating cells, e.g. basal epidermal cells positive
- variable clone sensitivity
- *Bcl-1* gene product at 11q13
- upregulated in cells with t(11;14)
- >90% MCLs positive (nuclear)
- 15% myelomas positive (nuclear)



Mantle cell NHL: cyclin-D1

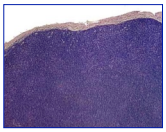
Basic stains: CD23

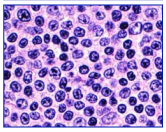
- Normal:
  - activated germinal centre cells
  - some mantle zone lymphocytes
  - some mature B cells
  - follicular dendritic reticulum cells
  - T-cells, etc.
- In lymphomas:
  - some small cell B-cell NHL
    - SLL/CLL
    - negative in MCL, pre-LB, TCLs

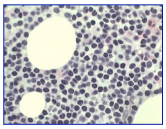


Follicular lymphoma: CD23; FDCs

B-cell Small Lymphocytic Lymphoma (CLL)



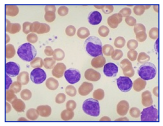


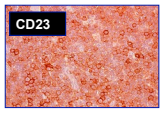


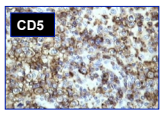
Morphology
- small lymphocytes
- proliferation centres

Immunology

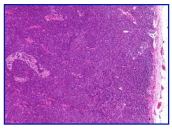
surface IgMD weak	
CD19, 20, 79a	+
CD5	+
CD23	+
CD10, CycD1	-

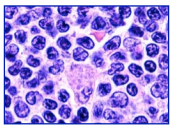


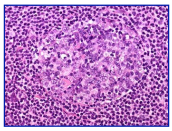




Mantle Cell Lymphoma



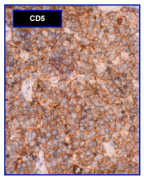


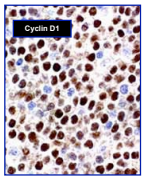


Morphology
- small-medium lymphocytes
- cleaved / irregular
- blastoid variant
- nodular / mantle / diffuse

Immunology

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

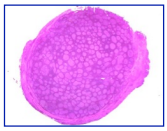


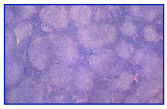


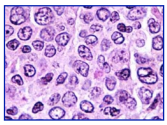
Immunophenotype: Small B-Cell Lymphomas

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

Follicular Lymphoma



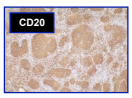


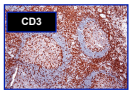


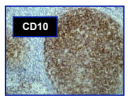
Morphology
- germinal centre cells
- CBs & CCs
- follicular

Immunology

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

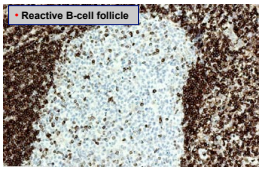




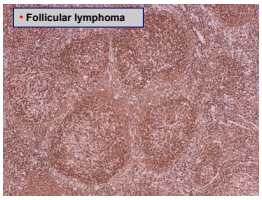


Basic stain: bcl-2

- Apoptosis inhibitor
- Nuclear and cytoplasmic stain
- Normal:
  - Mature B- and T-cells
  - Negative in cortical thymocytes and germinal centre cells



- In lymphoma:
  - Positive in most peripheral B-NHL and T-NHL
  - Negative in BL
  - Associated with, but not specific for t (14;18)
  - Positive in neoplastic germinal centres
  - Often negative in skin lymphoma
  - Ca 10% of follicular lyphomas re bcl-2 negative

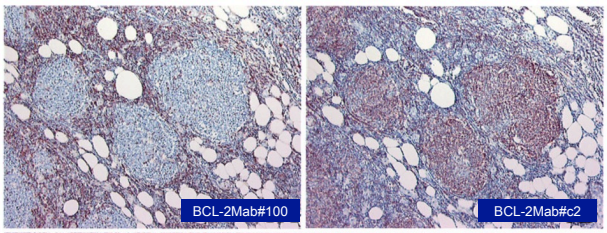


Journal of Pathology
J Pathol 2005; **205**: 329–335
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1689

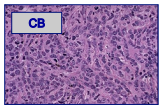
Original Paper

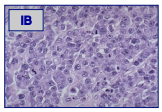
Lack of Bcl-2 expression in follicular lymphoma may be caused by mutations in the *BCL2* gene or by absence of the t(14;18) translocation

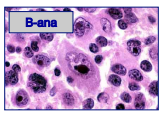
Margit Schraders,[1]* Daphne de Jong,[2] Philip Kluin,[3] Patricia Groenen[1] and Han van Krieken[1]



Diffuse Large B-cell Lymphoma



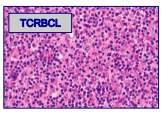


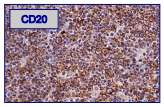


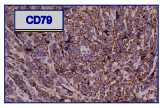
Morphology
- large cells
- nucleoli
- diffuse

Immunology

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

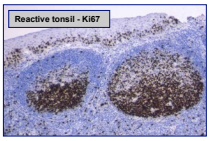


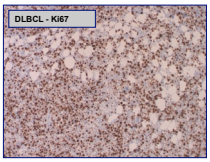


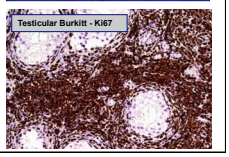


Basic stain: Ki- 67

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

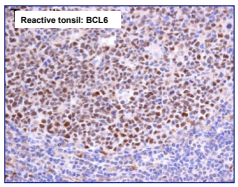






Basic stain: Bcl-6

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

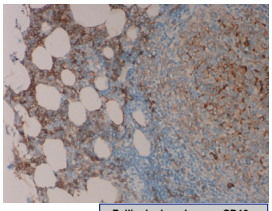


IHC for DLBCL
Add to basic panel:

- CD10
- CD138
- MUM1

Secondary stain: CD10

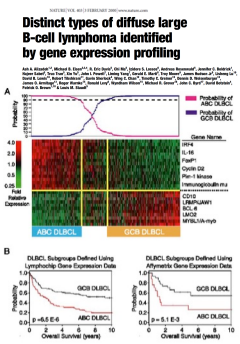
- >90% precursor B-LB (membrane & paranuclear stain)
- ca. 25% precursor T-LB
- Burkitt lymphoma
- Follicular lymphoma
  - Interfollicular CD10+ cells suggets lymphoma
- Some DLBCL
  - 'Cell of origin' algorithm in DLBCL
  - GCB *vs* ABC



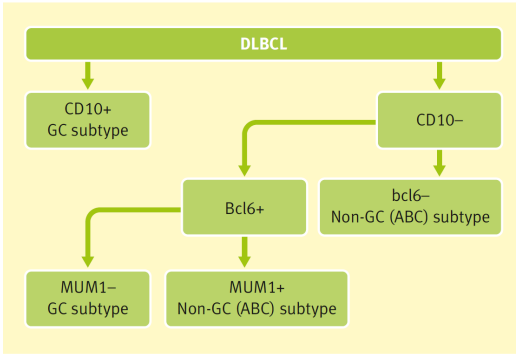
- Follicular lymphoma – CD10
- Interfollicular tumour cells

Large B-cell Lymphomas Molecular Variants

- Gene profiling identified 2 types of DLBCL
  - Germinal Centre B-cell
  - Activated B-cell
- 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

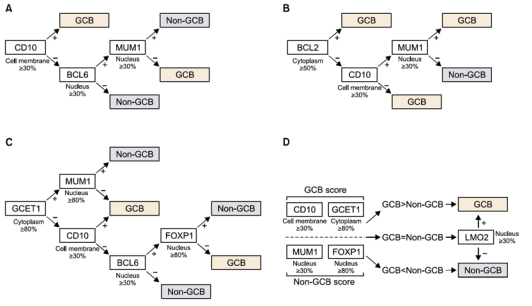
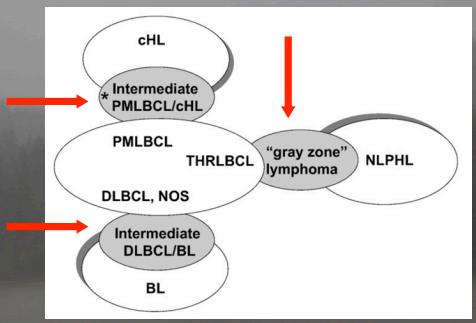


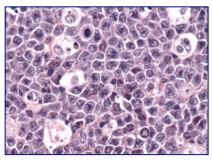
Fig. 2. Summary of the (A) Hans, (B) Muris, (C) Choi, and (D) Tally algorithms, and criteria for a positive signal for individual immunohistochemical markers (below or to the right of the white-filled box). Note that the positive criterion for MUM1/IRF4 in the Choi algorithm (more than 80%) is different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

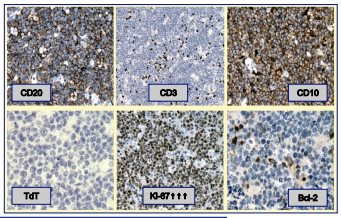
Gray zone (bordeline) B-cell lymphomas



Immunophenotyping in Gray zone B-NHL

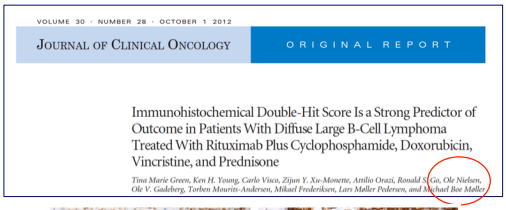
	CD20	CD79a	CD5	CD10	CD23	Ki67	TdT	bcl-2	CyclinD1
Diffuse large B	+	+	–/+	–/+	–	<90%	–	+/–	–
Burkitt	+	+	–	+	–	>95%	–	–	–
Blastic mantle cell	+	+	+	–	–	<90%	–	+/–	+
B lymphoblastic	+	+	–	+	–	<90%	+	+/–	–
Blastic myeloma	–	+	–	–	–	<90%	–	+/–	–/+





- 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 B-NHL



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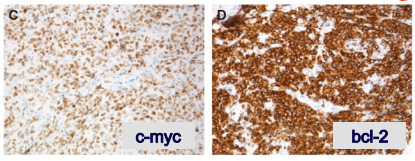
JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

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

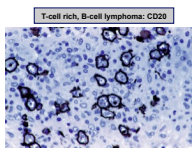
Tina Marie Green, Ken H. Young, Carlo Visco, Zijun Y. Xu-Monette, Attilio Orazi, Ronald S. Go, Ole Nielsen, Ole V. Gadeberg, Torben Mourits-Andersen, Mikael Frederiksen, Lars Møller Pedersen, and Michael Boe Møller

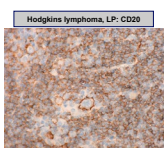


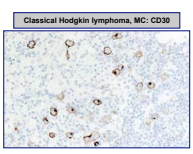
Hodgkins lymphoma: differential diagnosis

	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

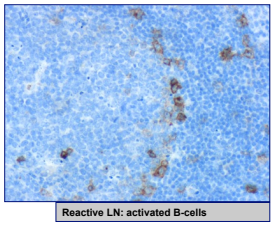






Basic stain: CD30

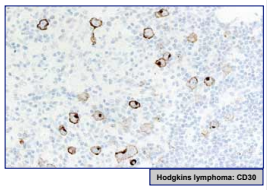
- TNF-R family
- 'Ki-1 antigen'
- Activation antigen
- Normal expression:
  - activated parafollicular immunoblasts
  - virally infected cells (EBV)
  - some clones stain plasma cells (Ber-H2)
- Pattern:
  - Membrane with dot-like Golgi



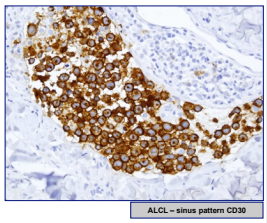
Reactive LN: activated B-cells

CD30 in lymphoma

- "CD30+ lymphoproliferations":
  - Py skin anaplastic large cell lymphoma
  - Systemic ALCL
  - Lymphomatoid papulosis
  - Mycosis fungoides transformation
  - Hodgkin lymphoma
    - HRS cells in classical types
    - Popcorn cells in HL-LP: 0% -10%
  - Ca. 30% of other T-cell NHL
  - Ca. 20% DLBCL
  - Target for Brentuximab



Hodgkins lymphoma: CD30



ALCL – sinus pattern CD30

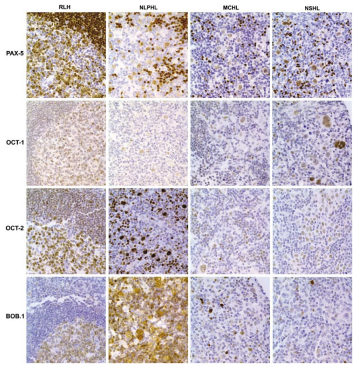
IHC for Hodgkins Lymphoma
Add to basic panel:

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

HL *vs* ALCL: Immunophenotype

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

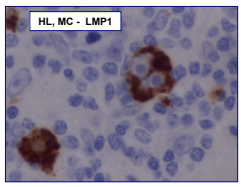
Expression of transcription factors Pax-5, Oct-1, Oct-2 and BOB.1 in Hodgkin's Lymphoma



McClune et. Al. Modern Pathology (2006) 19, 1010–1018

Secondary stain: EBV

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

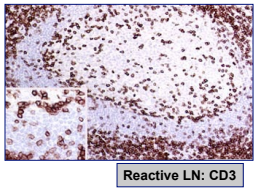


T-cell lymphoma: immunophenotype

Complex!

Basic stain: CD3

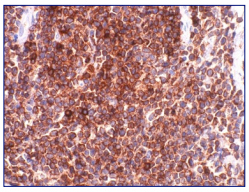
- transmembrane molecule
- Ig superfamily
- part of T-cell receptor
- most specific T-cell marker
- pan-T cell marker
  - thymocytes: cyt. → membrane
  - most post-thymic T-cells
  - activated NK-celler



Reactive LN: CD3

CD3 in lymphoma

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



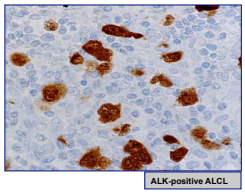
- Precursor T-LB
- CD3-cyt

IHC for PTL
Add to basic panel:

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

Secondary stain:
Anaplastic lymphoma kinase (ALK, CD246)

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



ALK-positive ALCL

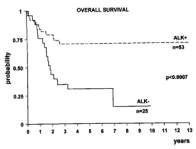
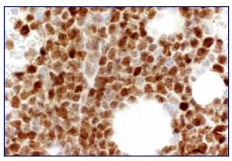


Fig 4. Overall survival of ALK$^+$ versus ALK$^-$ lymphoma.

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%)



Secondary stain: CD1a

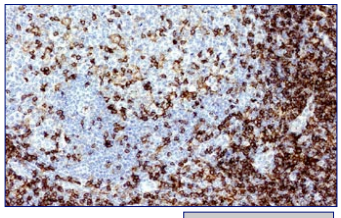
- T-cell marker
  - 70% cortical thymocytes
  - Peripheral T-cells neagtive
  - Langerhans cells / Interdigitating reticulum
- In lymphomas:
  - 50% precursor T-LB
  - Langerhans cell histiocytosis
  - Peripheral TCLs are negative in paraffin
- NB! May be positive in mediastinal biopsies from normal thymus or lymphocyte-rich thymoma

Secondary stain: CD7

- Early pan-T cell marker
  - 90% thymocytes
  - B-cells negative
  - ↑ NK cells negative
- In lymphoma:
  - nearly all T-LB +
  - many peripheral TCLs +
  - often lost in TCL
  - negative in B-NHL

Secondary stains: CD4 & CD8

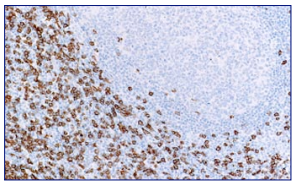
- CD4
  - Thymocytes
  - T-helper cells
  - Monocytes
  - Macrophages
  - Granulocytes



Normal tonsil: CD4

Secondary stains: CD4 & CD8

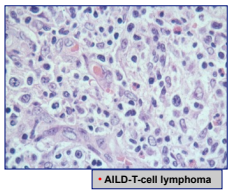
- CD8
  - T-cytotoxic/suppressor cells
  - NK cells
  - Intraepithelial lymphocytes CD8+
- Most lymphomas CD4+
- γδ TCL usually CD4- CD8-
- ALCL: CD4 > CD8
- Double negs & double pos:
  - aberrant = neoplastic?



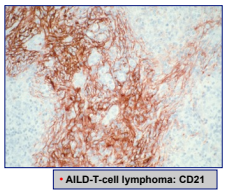
Normal tonsil: CD8

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

T-cell lymphoma: immunophenotype

Complex!

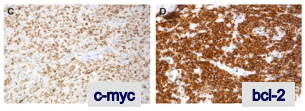
Nodal PTCL - immunophenotype

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

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

Oncogenes/ Tumor Suppressor Genes Evaluation by Immunohistochemistry

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

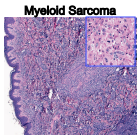


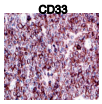
IHC for lymphoma vs other
Add to basic panel:

- panCK
- S-100
- Melan-A

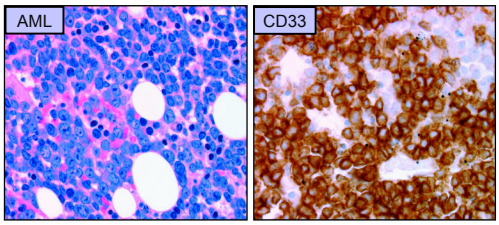
IHC for lymphoid vs myeloid
Add to basic panel

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

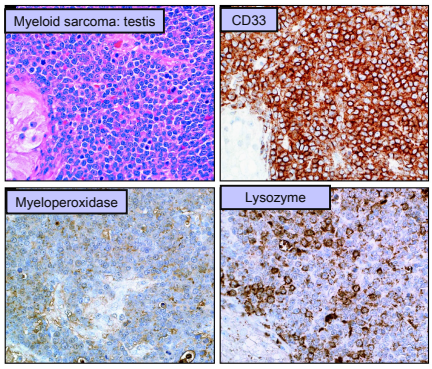




Acute myeloid leukaemia: CD33 (paraffin section):

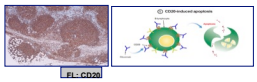


Myeloid sarcoma: testis

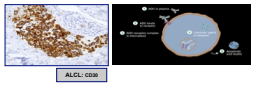


Targeted therapy

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



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



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