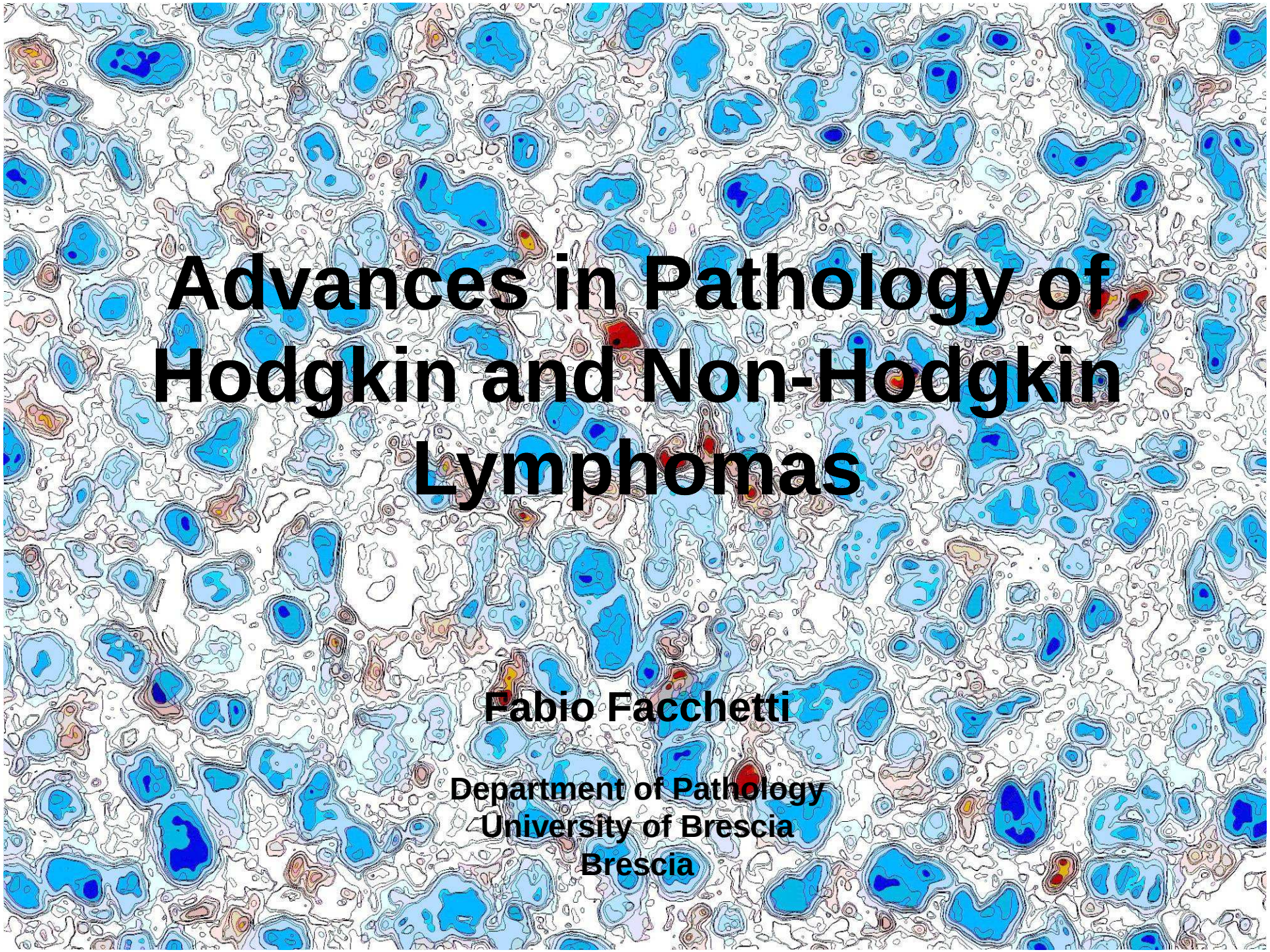
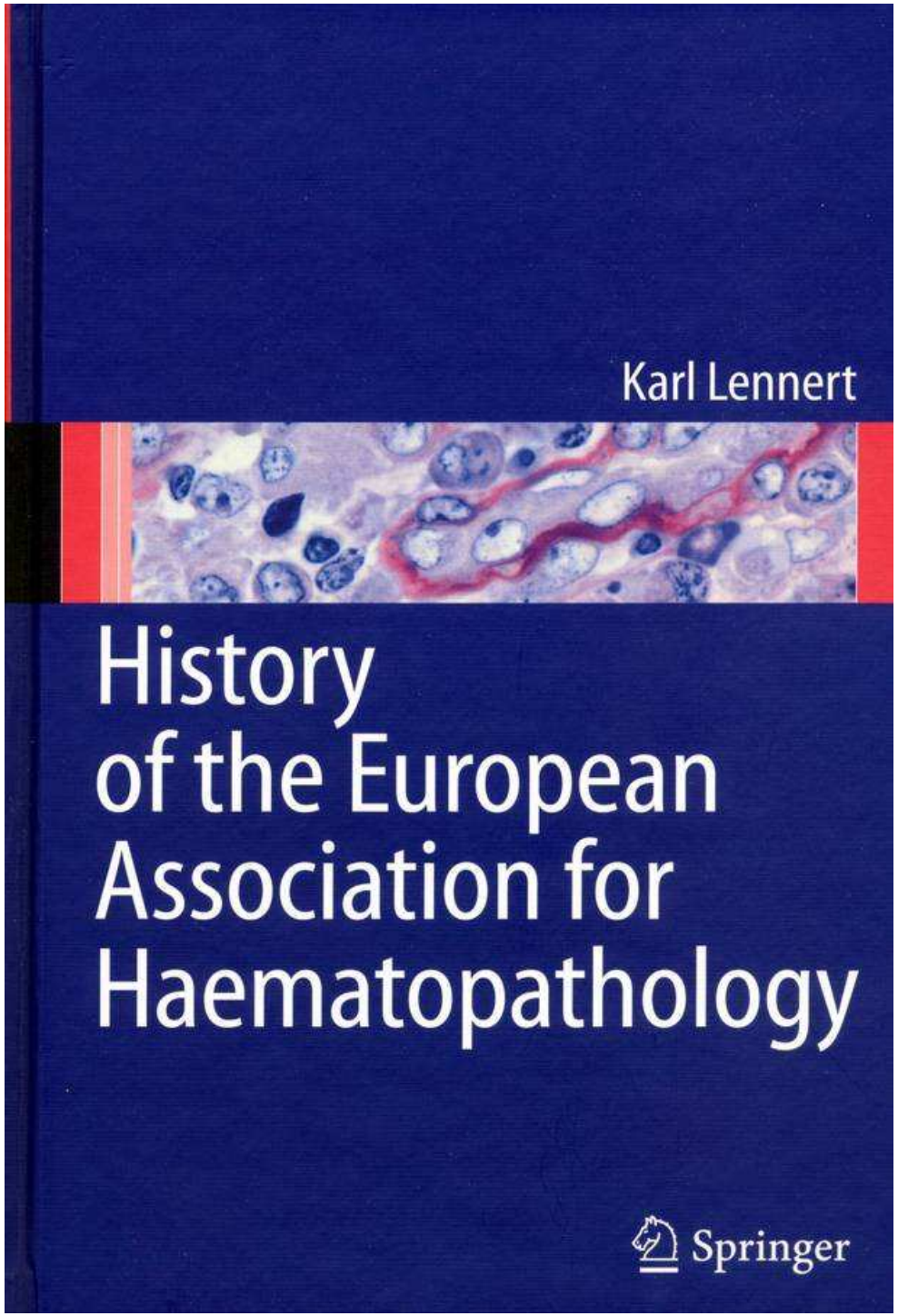
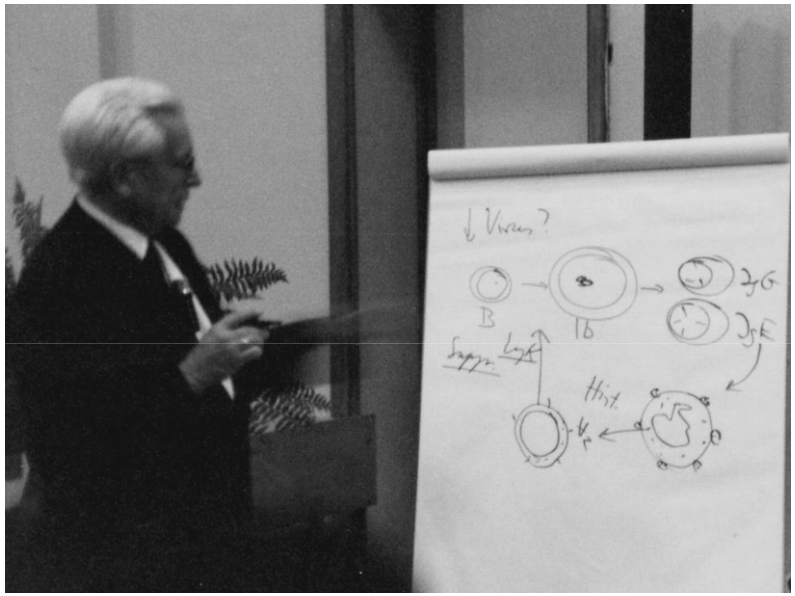


Advances in Pathology of Hodgkin and Non-Hodgkin Lymphomas

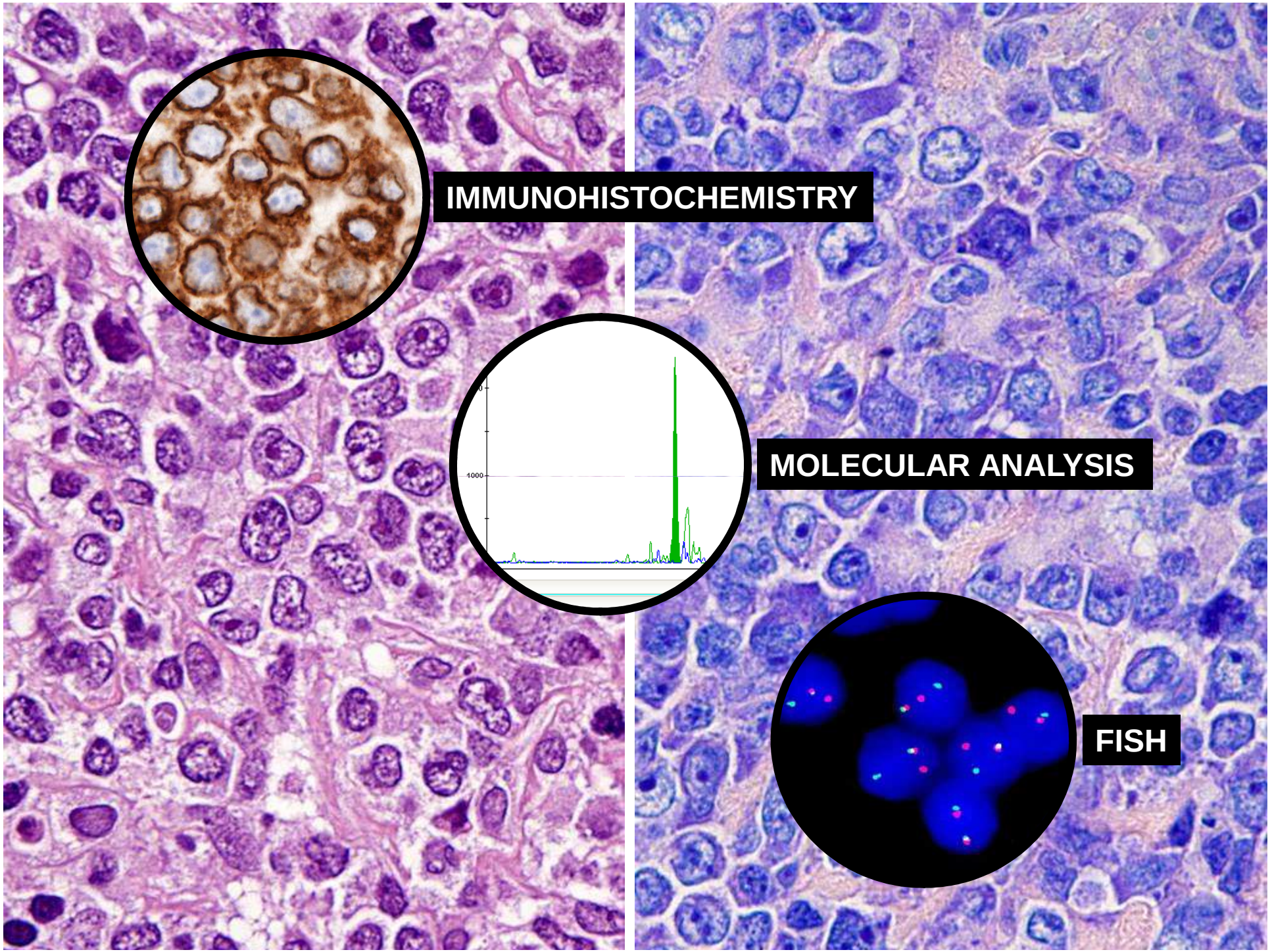
Fabio Facchetti

Department of Pathology
University of Brescia
Brescia

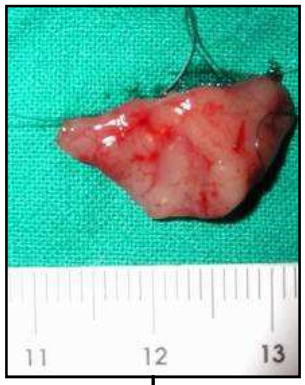




Rome, 3-6 october 1982

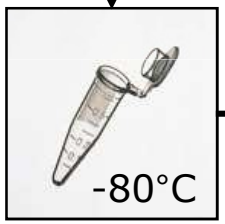

IMMUNOHISTOCHEMISTRY
MOLECULAR ANALYSIS
FISH

High sensitivity PCR protocols for BCR and TCR rearrangements



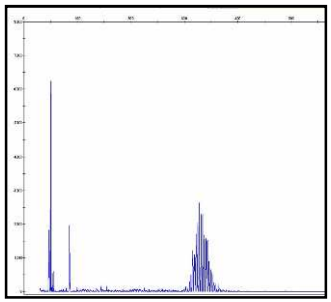
Fix

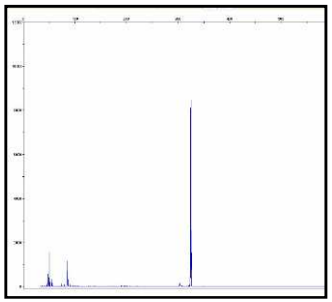
Frozen



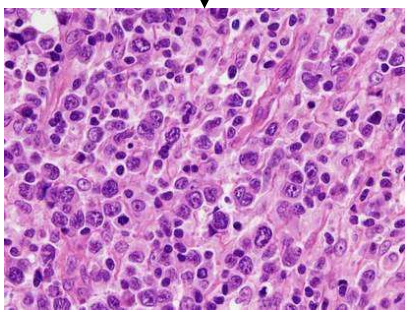
DNA EXTRACTION

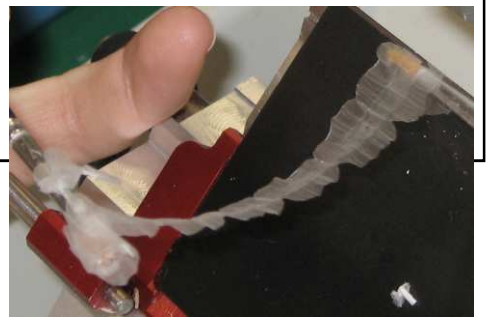
PCR and Gene-scan analysis





polyclonal **monoclonal**





BIOMED-2 rearrangement protocols

- Highly effective and sensitive (0.1%-5% tumor cells) for Ig and TCR rearrangements

- Highly effective on fresh-frozen material; on paraffin material if DNA fragments >300 bp are available for amplification

Br J Haematol 2007

B

Diagnosis	No. of clonal specimens/ No. of specimens tested (%)
Diagnostic specimens with no evidence of B-cell neoplasms	0/70 (0)
Mature B-cell neoplasms	
MALT lymphoma	31/32 (97)
Follicular lymphoma	30/32 (94)
Diffuse large B-cell lymphoma	25/26 (96)
Plasma cell neoplasms	19/20 (95)
Small lymphocytic lymphoma/ chronic lymphocytic leukaemia	10/10 (100)
Primary cutaneous follicle centre lymphoma	5/5 (100)
Mantle cell lymphoma	4/4 (100)
Burkitt lymphoma	3/3 (100)
Splenic marginal zone lymphoma	2/2 (100)
Primary effusion lymphoma	1/1 (100)
B-cell non-Hodgkin lymphoma – unclassified	17/18 (94)
Subtotal	147/153 (96)

T

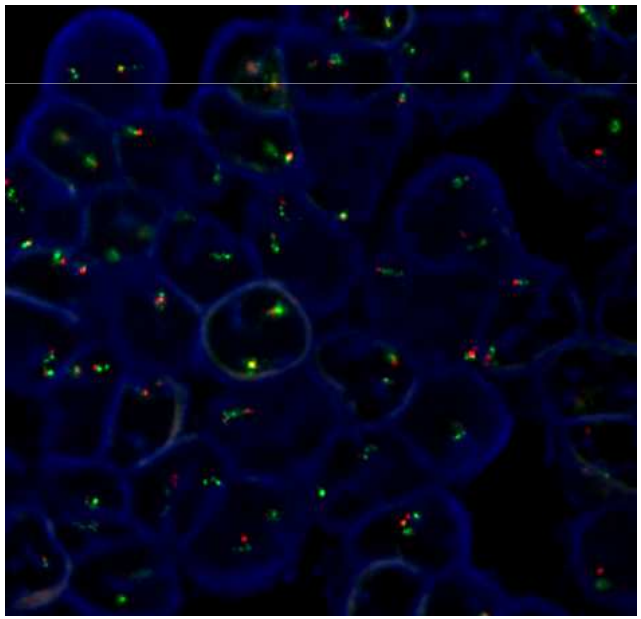
Diagnosis	No. of clonal specimens/ No. of specimens tested (%)
Diagnostic specimens with no evidence of T-cell malignancy	3/41 (7)
Mature T-cell neoplasms	
T-cell large granular lymphocytic leukaemia	8/8 (100)
Mycosis fungoides/Sezary syndrome	7/7 (100)
Anaplastic large cell lymphoma	5/5 (100)
Primary cutaneous $CD30^+$ T-cell LPD	4/4 (100)
Enteropathy-type T-cell lymphoma	3/3 (100)
Angioimmunoblastic T-cell lymphoma	3/3 (100)
T-cell prolymphocytic leukaemia	2/2 (100)
Hepatosplenic T-cell leukaemia/lymphoma	2/2 (100)
Peripheral T-cell lymphoma, unspecified	22/23 (96)
Subtotal	56/57 (98)
Staging/follow-up bone marrow specimens with no morphological/phenotypic evidence of T-cell LPD	2/8 (25)
Other diseases	
Coeliac disease	6*/18 (33)
Common variable immunodeficiency	2/2 (100)
Aggressive NK cell leukaemia	0/2
$CD4^+/CD56^+$ haematodermic neoplasm (blastic NK-cell lymphoma)	0/1
B-cell LPD	2†/15 (13)
Total	71/144 (49)

Examples of published reports of FISH labelling of Paraffin-embedded tisue sections for the detection of Lymphoma-related chromosomal abnormalities		
Lymphoma category	**Chromosomal aberration(s)**	**Genes**
Follicular L.	t(14;18)(q32;q21) +3	*JGH/BCL2*
Mantle cell L.	t(11;14)(q13;q32)	*CCND1/IGH*
MALT Lymphoma	t(11;18)(q21;q21) t(14;18) t(1;14) +3, +7, +12, +18	*AP12/MLT1* *IGH/MALT1* *BCL10/IGH*
Lymphoplasmacytic L.	t(9;14)(p13;q32)	*PAX5/IGH*
Diffuse Large B-cell Lymphoma	t(8;14)(q24;q32) and var. t(14;18)(q32;q21)	*MYC/IGH* *IGH/BCL2,* and *BCL6*
Burkitt Lymphoma	t(8;14)(q24;q32) t(2;8)(p12;p24) t(8;22)(p24;q11)	*MYC/IGH* *MYC/IGK* *MYC/IGL*
Anaplastic Large Cell Lymphoma	t(2;5)(p23;q35) t(1;2) t(2;3)	*ALK/NPM* *TPM3/ALK* *TGF/ALK*

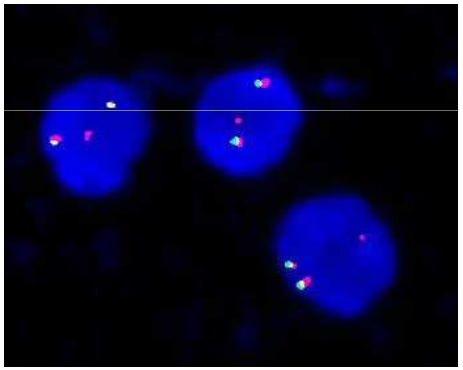
FISH ANALYSIS IN HEMATOPATHOLOGY

FRESH MATERIAL

Imprints

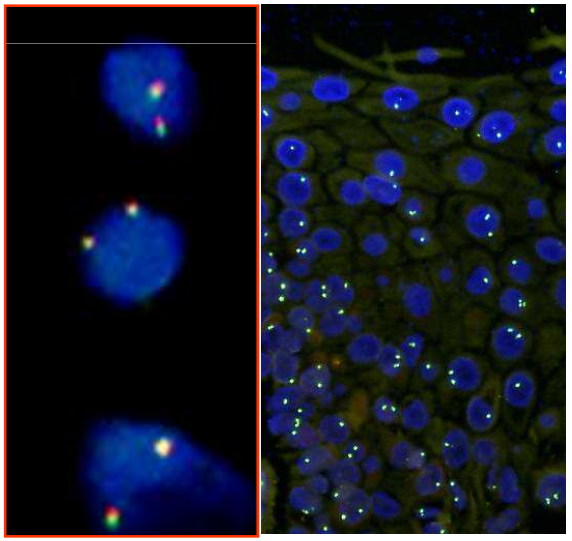


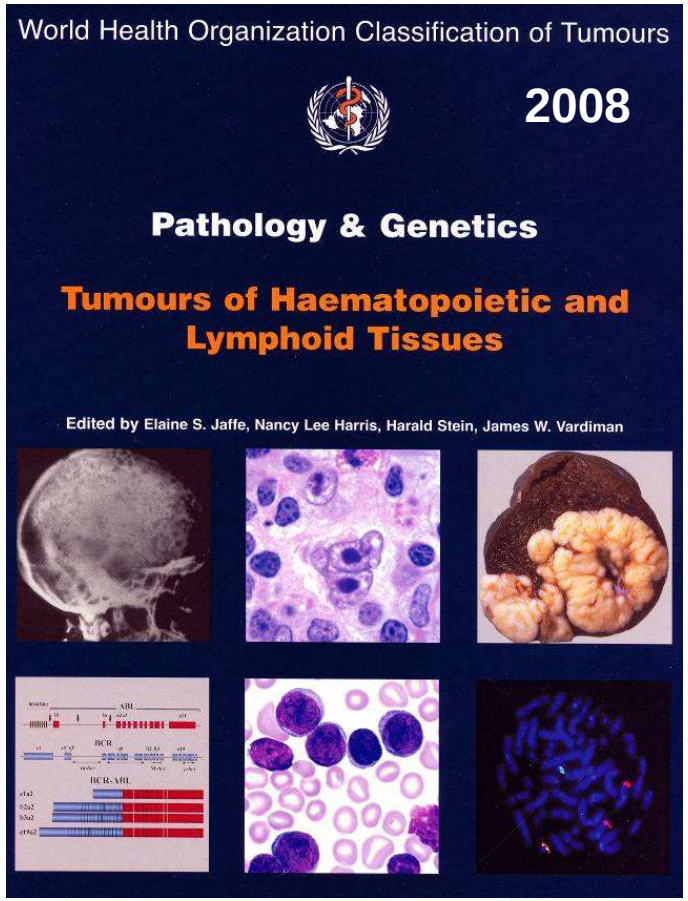
SMEARS FNA



PARAFFIN BLOCKS

Extracted nuclei	Sections





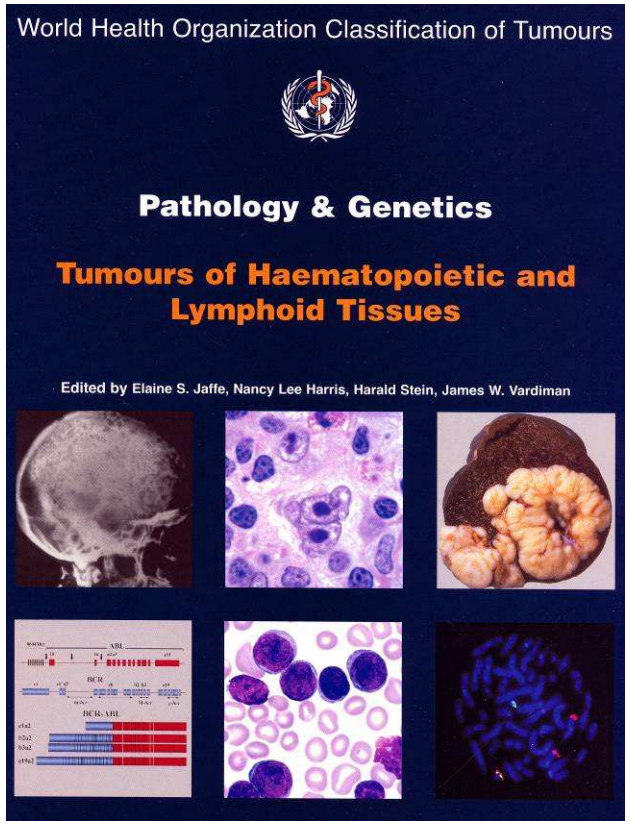
Lymphoid neoplasms
57 variants

Myeloid neoplasms
35 variants

WHO 2008

Comments on:

- **Diffuse Large B-Cell Lymphoma**
- **Grey-zone between Hodgkin Lymphoma and Non-Hodgkin B-cell Lymphomas**
- **Follicular Lymphoma**

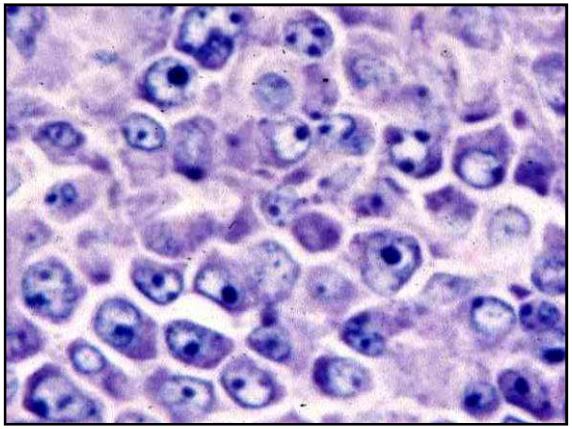


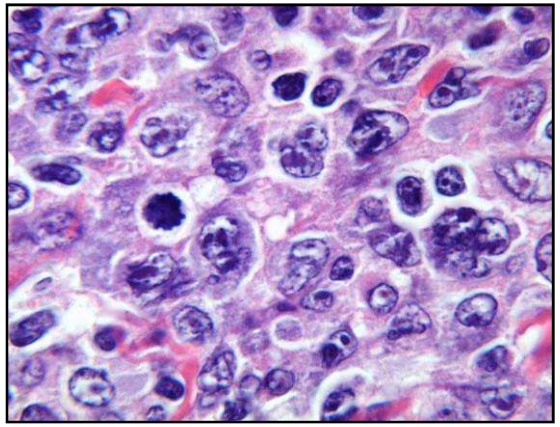
- **Diffuse Large B-Cell Lymphoma**
- **Lymphoma of large B cells, with diffuse growth pattern**
- **25-30% of all lymphomas in western countries**
- **Various morphological variants, molecular and immunophenotypical subgroups and distinct disease entities**
- **The vast majority, however, do not have distinguishable features and are defined as to DLBCL-NOS (not otherwise specified)**
- **Nodal or (40%) extranodal presentation**

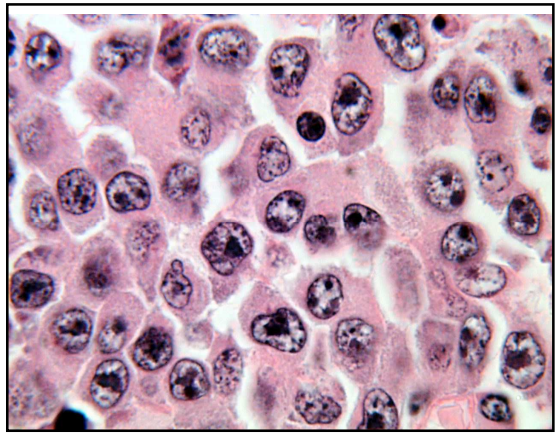
Diffuse Large B-cell Lymphoma, NOS

Heterogeneous category for:

- **Histology and Phenotype**
- **Genetic alterations**
- **Response to treatment and prognosis**

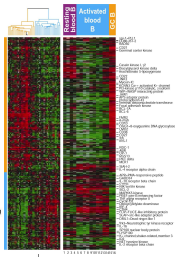






Nature 2000; 4051: 503

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling



Ash A. Alizadeh[1,2], Michael B. Eisen[2,3,4], R. Eric Davis[5], Chi Ma[5], Izidore S. Lossos[6], Andreas Rosenwald[5], Jennifer C. Boldrick[1], Hajeer Sabet[5], Truc Tran[5], Xin Yu[5], John I. Powell[7], Liming Yang[7], Gerald E. Marti[8], Troy Moore[9], James Hudson Jr[9], Lisheng Lu[10], David B. Lewis[10], Robert Tibshirani[11], Gavin Sherlock[4], Wing C. Chan[12], Timothy C. Greiner[12], Dennis D. Weisenburger[12], James O. Armitage[13], Roger Warnke[14], Ronald Levy[6], Wyndham Wilson[15], Michael R. Grever[16], John C. Byrd[17], David Botstein[4], Patrick O. Brown[1,18] & Louis M. Staudt[5]

Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning

NATURE MEDICINE • VOLUME 8 • NUMBER 1 • JANUARY 2002

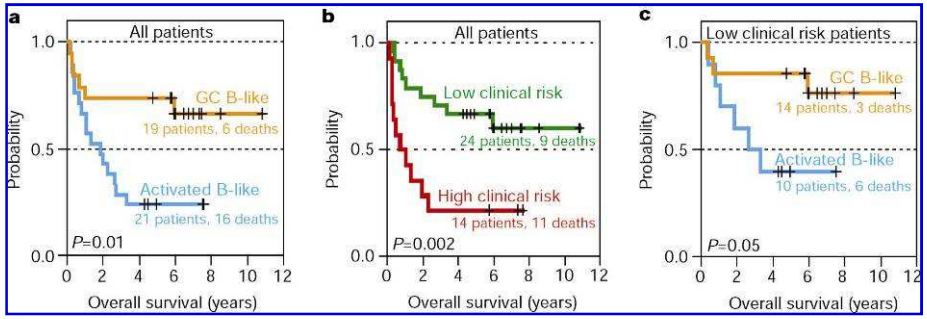
Margaret A. Shipp[1], Ken N. Ross[2], Pablo Tamayo[2], Andrew P. Weng[3], Jeffery L. Kutok[3], Ricardo C.T. Aguiar[1], Michelle Gaasenbeek[2], Michael Angelo[2], Michael Reich[2], Geraldine S. Pinkus[3], Tane S. Ray[6], Margaret A. Koval[1], Kim W. Last[4], Andrew Norton[5], T. Andrew Lister[4], Jill Mesirov[2], Donna S. Neuberg[1], Eric S. Lander[2,7], Jon C. Aster[3] & Todd R. Golub[1,2]

The New England Journal of Medicine

VOLUME 346 June 20, 2002 NUMBER 25

THE USE OF MOLECULAR PROFILING TO PREDICT SURVIVAL AFTER CHEMOTHERAPY FOR DIFFUSE LARGE-B-CELL LYMPHOMA

Andreas Rosenwald, M.D., George Wright, Ph.D., Wing C. Chan, M.D., Joseph M. Connors, M.D., Elias Campo, M.D., Richard I. Fisher, M.D., Randy D. Gascoyne, M.D., H. Konrad Muller-Hermelink, M.D., Erlend B. Smeland, M.D., Ph.D., and Louis M. Staudt, M.D., Ph.D., for the Lymphoma/Leukemia Molecular Profiling Project

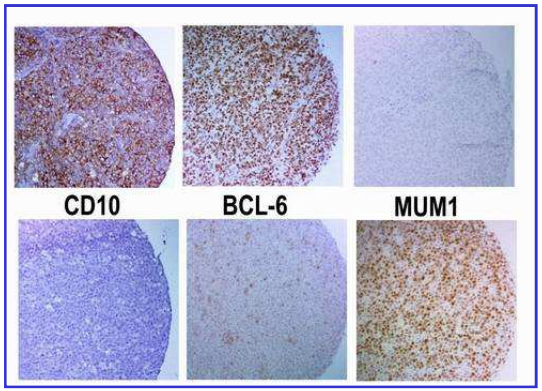


NEOPLASIA

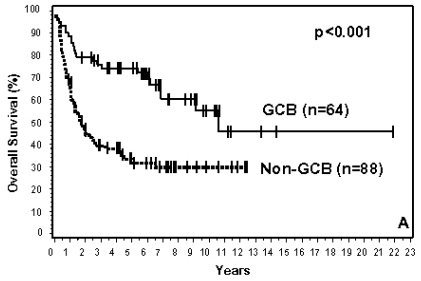
BLOOD, 1 JANUARY 2004 • VOLUME 103, NUMBER 1

Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray

Christine P. Hans, Dennis D. Weisenburger, Timothy C. Greiner, Randy D. Gascoyne, Jan Delabie, German Ott, H. Konrad Müller-Hermelink, Elias Campo, Rita M. Braziel, Elaine S. Jaffe, Zenggang Pan, Pedro Farinha, Lynette M. Smith, Brunangelo Falini, Alison H. Banham, Andreas Rosenwald, Louis M. Staudt, Joseph M. Connors, James O. Armitage, and Wing C. Chan



Hans CP, Blood 2004	CD10	BCL6	MUM1
GC			
Activated Non-GC			



Hans et al. (Blood 2004)

- Positive predictive value
  - GCB-like 87%
  - ABC-like 73%
- Sensitivity
  - GCB-like 71%
  - ABC-like 88%
- Mis-classification
  - 30/152 20%

AUTHOR	GCB-like (%)	ABC-like (%)
Rosenwald (NEJM 2002)	61	39
Natkunam (Blood 2005)	50	50
Munis (Blood 2005)	37	63
Pasqualucci (JEM 2006)	52	48
Chen (Blood 2006)	62	38
De Paepe (JCO 2005)	49	51

VOLUME 25 · NUMBER 7 · MARCH 1 2007

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Immunohistochemical Prognostic Markers in Diffuse Large B-Cell Lymphoma: Validation of Tissue Microarray As a Prerequisite for Broad Clinical Applications—A Study From the Lunenburg Lymphoma Biomarker Consortium

Daphne de Jong, Andreas Rosenwald, Mukesh Chhanabhai, Philippe Gaulard, Wolfram Klapper, Abigail Lee, Birgitta Sander, Christoph Thorns, Elias Campo, Thierry Molina, Andrew Norton, Anton Hagenbeek, Sandra Horning, Andrew Lister, John Raemaekers, Randy D. Gascoyne, Gilles Salles, and Edie Weller

- **TISSUE MICROARRAY from 36 PATIENTS with DLBCL**
- **8 LABS, 8 ANTIBODIES (CD5, CD10, CD20, BCL2, BCL6, MUM1, MIB1, HLADR)**

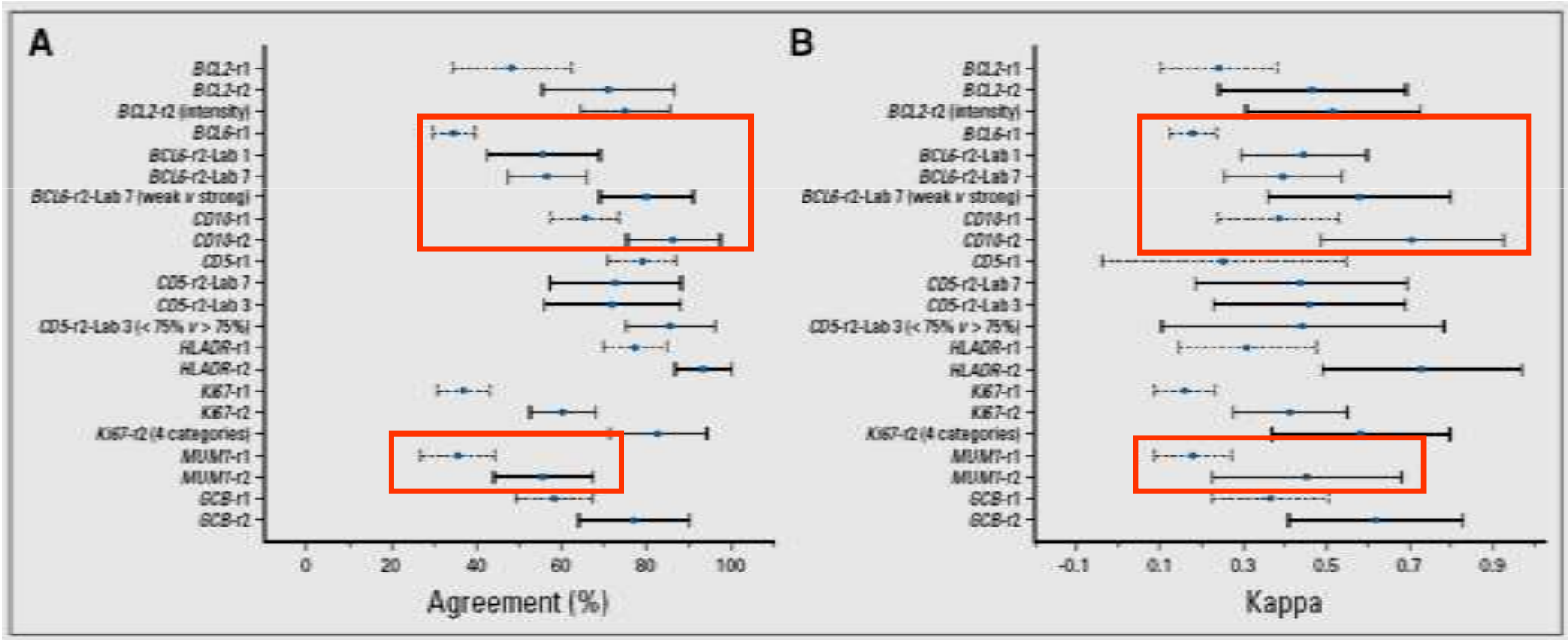


Fig 1. Percent agreement (A) and generalized κ statistic (B) and the 95% bootstrap percentile CIs from the first round (denoted by r1 in the labels and [– – –] in the figure) and the second round (denoted by r2 in the labels and [——] in the figure).

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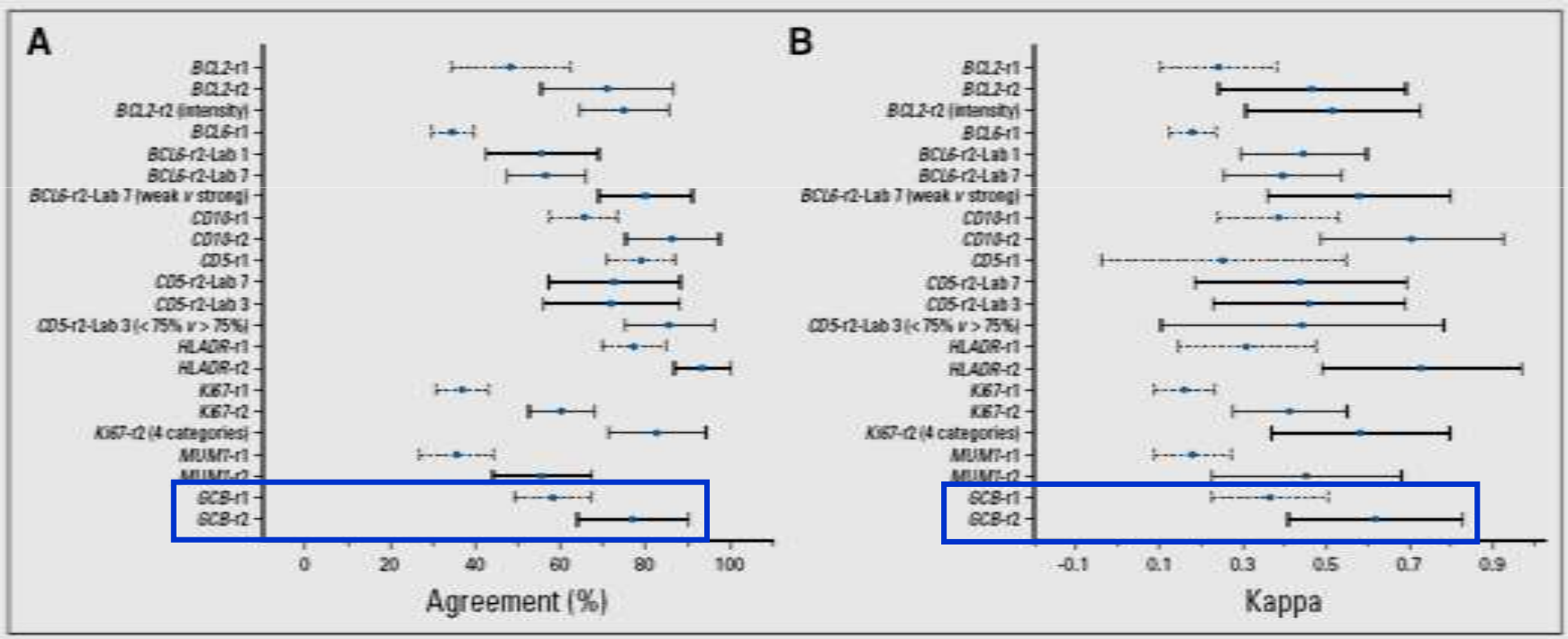

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- **8 LABS, 8 ANTIBODIES (CD5, CD10, CD20, BCL2, BCL6, MUM1, MIB1, HLADR)**

Conclusion

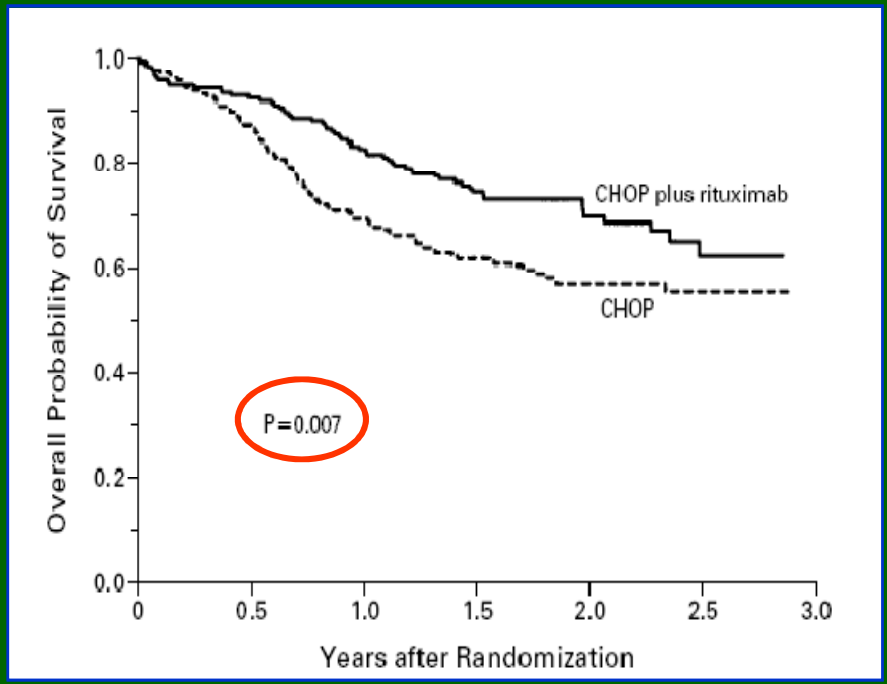
This study shows that semiquantitative immunohistochemistry for subclassification of DLBCL is feasible and reproducible, but exhibits varying rates of concordance for different markers. These findings may explain the wide variation of biomarker prognostic impact reported in the literature. Harmonization of techniques and centralized consensus review appears mandatory when using immunohistochemical biomarkers for treatment stratification.

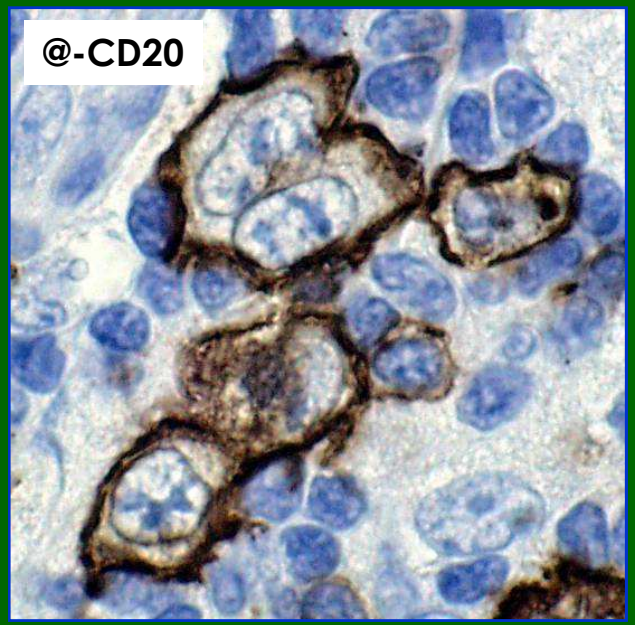
CHOP PLUS RITUXIMAB VS. CHOP ALONE IN ELDERLY PATIENTS WITH DIFFUSE LARGE-B-CELL LYMPHOMA

CHOP CHEMOTHERAPY PLUS RITUXIMAB COMPARED WITH CHOP ALONE IN ELDERLY PATIENTS WITH DIFFUSE LARGE-B-CELL LYMPHOMA

BERTRAND COIFFIER, M.D., ERIC LEPAGE, M.D., PH.D., JOSETTE BRIÈRE, M.D., RAOUL HERBRECHT, M.D., HERVÉ TILLY, M.D., REDA BOUABDALLAH, M.D., PIERRE MOREL, M.D., ERIC VAN DEN NESTE, M.D., GILLES SALLES, M.D., PH.D., PHILIPPE GAULARD, M.D., FELIX REYES, M.D., AND CHRISTIAN GISSELBRECHT, M.D.

N Engl J Med, Vol. 346, No. 4 · January 24, 2002





R-CHOP: standard therapy for DLBCL

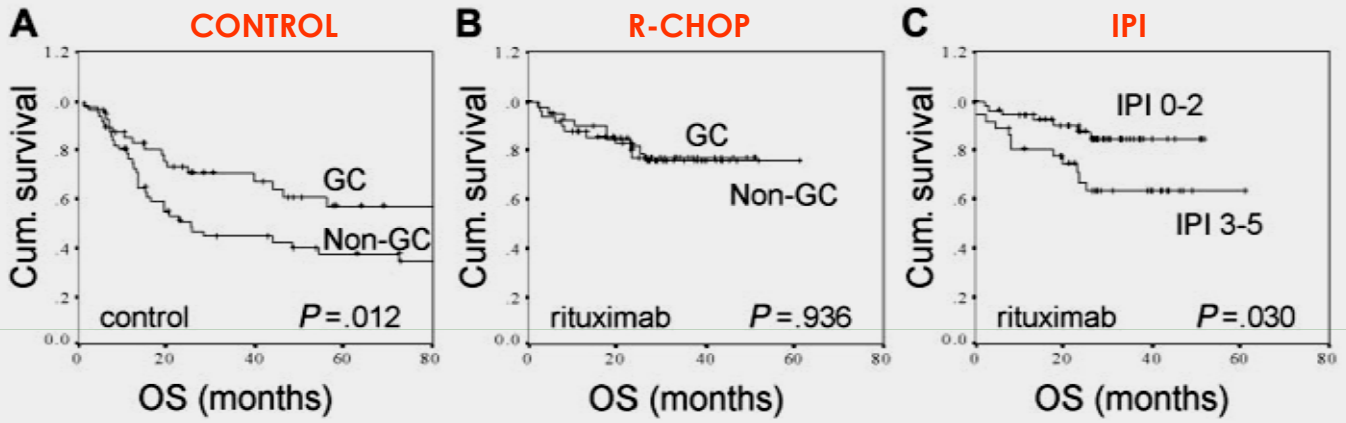
- **Patients >60 yrs**
  - **GELA**
  - **ECOG/CALGB 4494**
  - **HOVON, RICOVER-60**
- **Patients <60 yrs**
  - **MINT (low-risk pts)**

BLOOD, 1 JUNE 2007 • VOLUME 109, NUMBER 11

Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy

Heidi Nyman,[1,2] Magdalena Adde,[3] Marja-Liisa Karjalainen-Lindsberg,[4] Minna Taskinen,[1,2] Mattias Berglund,[3] Rose-Marie Amini,[5] Carl Blomqvist,[1,3] Gunilla Enblad,[3] and Sirpa Leppä[1,2]

[1]Department of Oncology, Helsinki University Central Hospital, Finland; [2]Molecular Cancer Biology Research Program, Biomedicum Helsinki, University of Helsinki, Finland; [3]Department of Oncology, Uppsala University Hospital, Sweden; [4]Department of Pathology, Haartman Institute, University of Helsinki, Finland; [5]Department of Genetics and Pathology, University of Uppsala, Sweden



R-CHOP eliminates the prognostic value of immunohistochemically defined GC- and non-GC phenotypes in DLBCL.

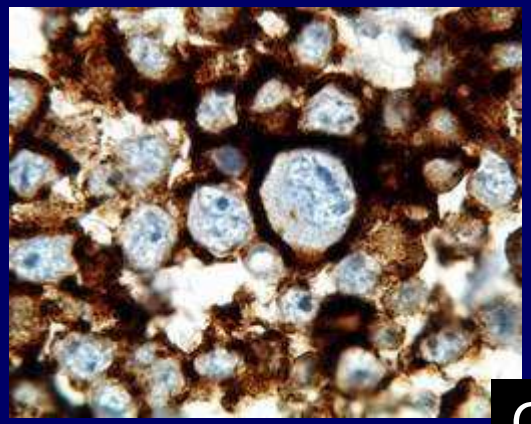
WHO 2008

“The immunophenotypic subgrouping (GCB vs. non-GCB) does not currently determine therapy”.

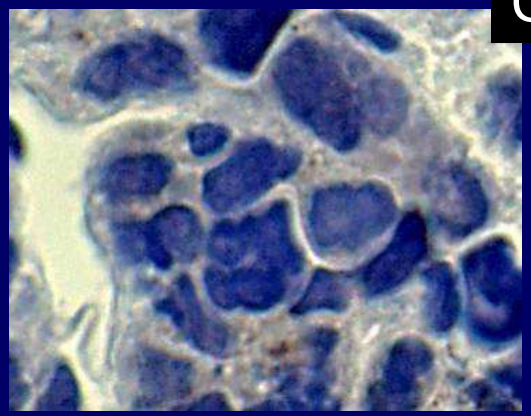
“The use of immunohistochemical panels to assign prognostic groups does not currently have a role in routine clinical practice”.

What to do at present time?

1. **Diagnose DLBCL-NOS**
2. **Indicate the degree of CD20 expression**



CD20



Diffuse Large B-Cell Lymphoma(s)

DLBCL NOS

Clinico-pathological variants

Special morphology and phenotype

- T-rich/Histiocyte-rich DLBCL
- ALK-positive DLBCL
- Plasmablastic lymphoma

EBV+

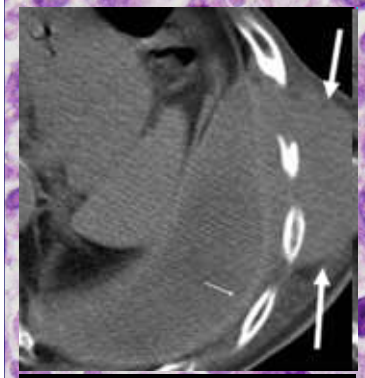
- Lymphomatoid granulomatosis
- DLBCL associate with Chronic inflammation (including pneumotorax associated lymphoma/PAL)
- DLBCL EBV+ of the eldery

Sites

- Primary DLBCL of the Central Nervous System
- Primary cutaneous DLBCL (“leg-type”)
- Primary mediastinal (thymic) DLBCL
- Intravascular DLBCL

HHV8+

- DLBCL arising in HHV8-associated multicentric Castleman disease
- Primary Effusion Lymphoma



Pleural mass in a patient treated for TBC 40 years before

Male, 63 yr, malaise and progressive anemia

EBV in situ hybridization

CD20

Diffuse Large B-Cell Lymphoma

Clinico-pathological variants

Special morphology and phenotype

- T-rich/Histiocyte-rich DLBCL
- ALK-positive DLBCL
- Plasmablastic lymphoma

EBV+

- Lymphomatoid granulomatosis
- DLBCL associate with Chronic inflammation (including pneumotorax associated lymphoma/PAL)
- DLBCL EBV+ of the eldery

Sites

- Primary DLBCL of the Central Nervous System
- Primary cutaneous DLBCL ("leg-type")
- Primary mediastinal (thymic) DLBCL
- Intravascular DLBCL

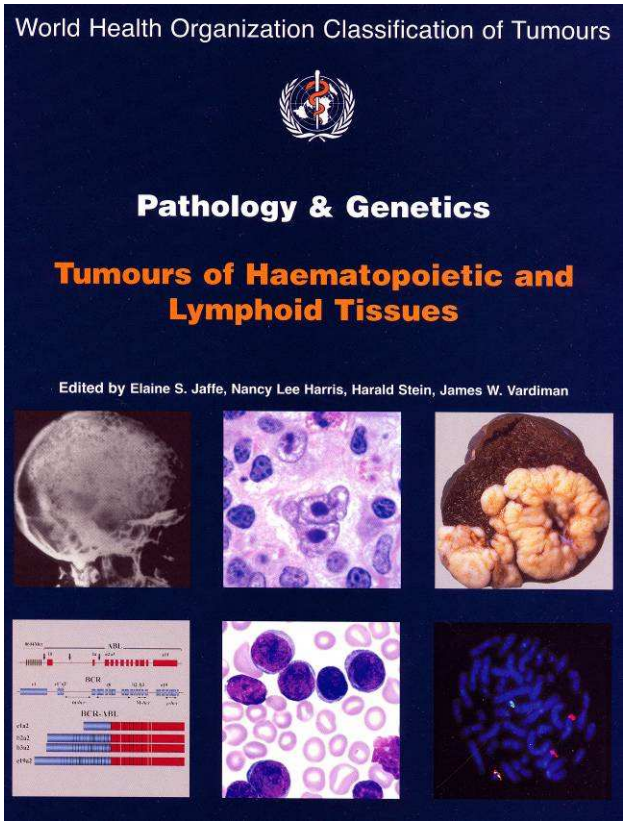
HHV8+

- DLBCL arising in HHV8-associated multicentric Castleman disease
- Primary Effusion Lymphoma

DLBCL-NOS

5 yr survival: 15-60%, significantly improved upon R-CHOP

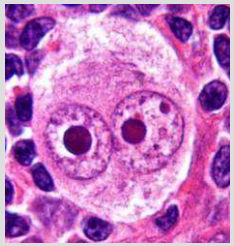
Very aggressive



WHO 2008

Comments on:

- **Diffuse Large B-Cell Lymphoma**
- **Grey-zone between Hodgkin Lymphoma and Non-Hodgkin B-cell Lymphomas**
- **Follicular Lymphoma**



Sternberg C.

Reed D.

Hodgkin's Disease

Hodgkin's Lymphoma

REAL WHO

1898 1902

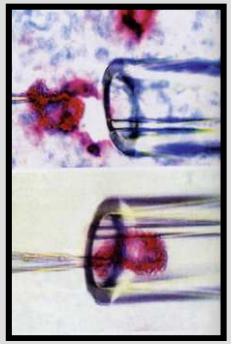
1832 1865 1947 1966 1994 2001-8

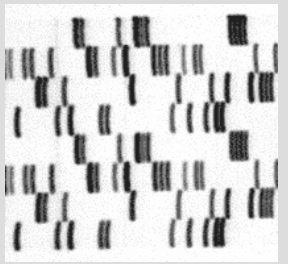
Jackson-Parker

Lukes-Butler
Rye conference

Kuppers R, et al.

HL is a lymphoid neoplasm of B-cell origin





HODGKIN LYMPHOMA

•Classical (95%)

B-cell lymphoma with derangement of B-cell programming, resulting in defective B-cell antigen expression and positivity for CD30 (and CD15)

•Lymphocyte predominance (5%)

B-cell lymphoma with preserved B-cell programming, normal B-cell antigen expression, and negativity for CD30 (and CD15)

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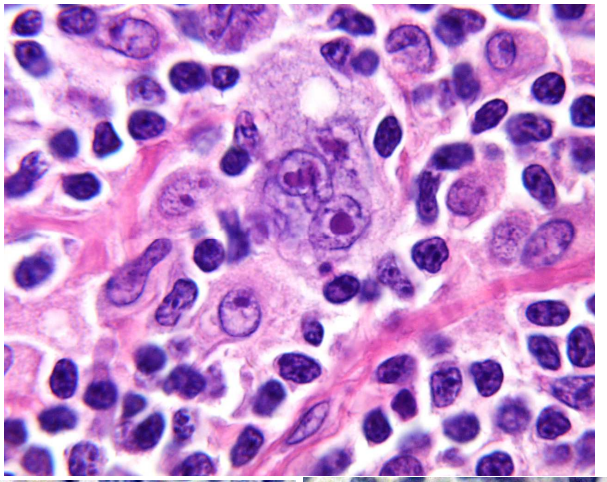
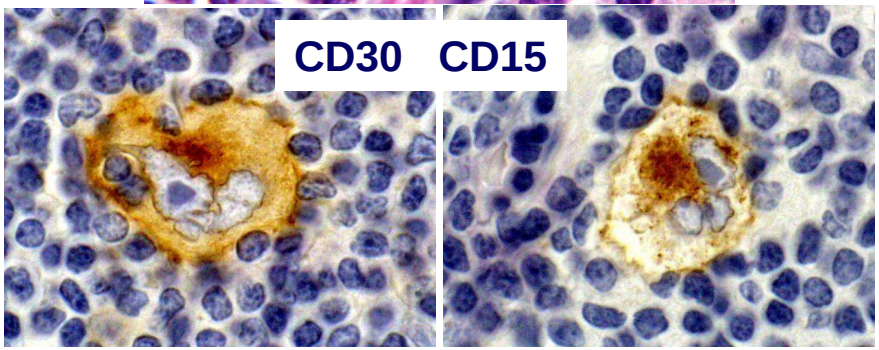
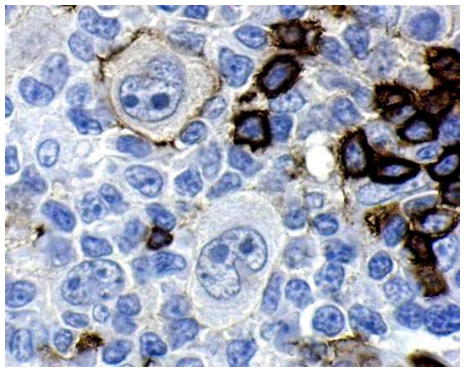
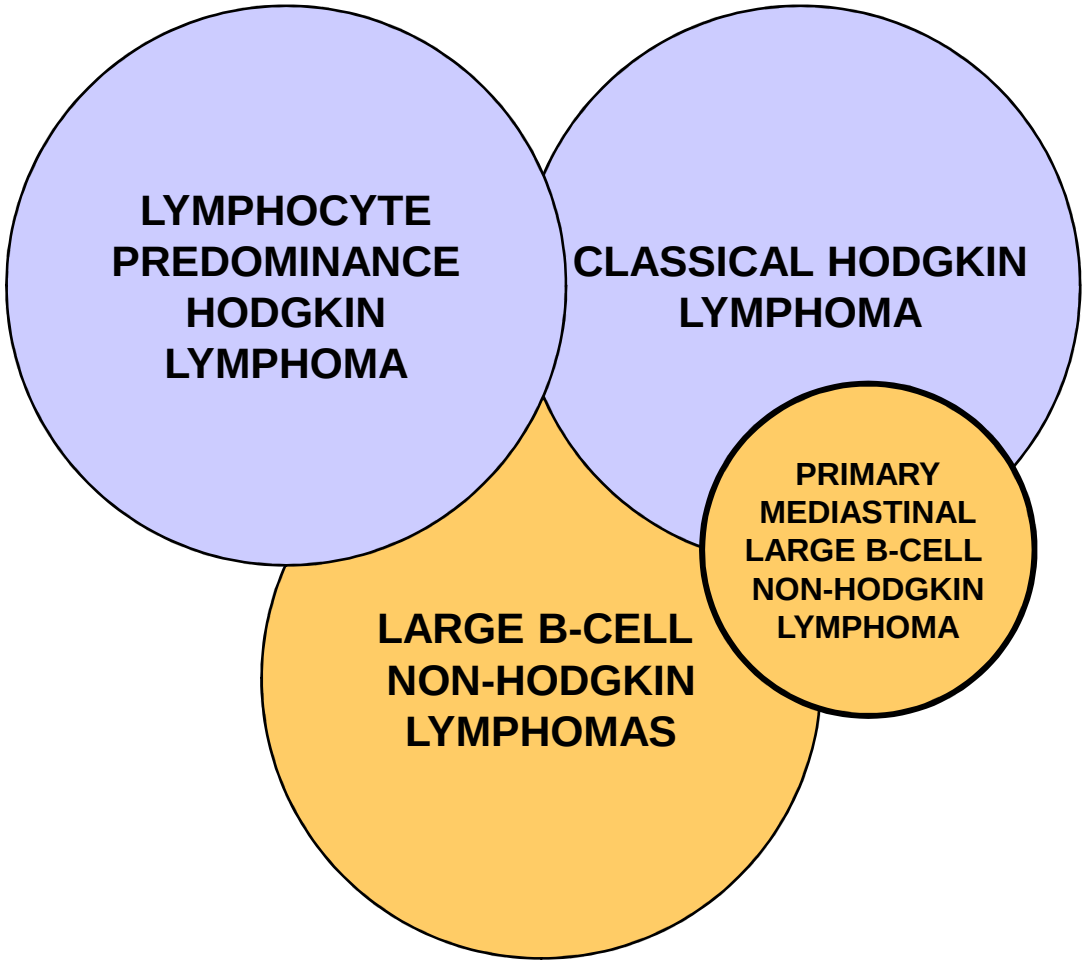






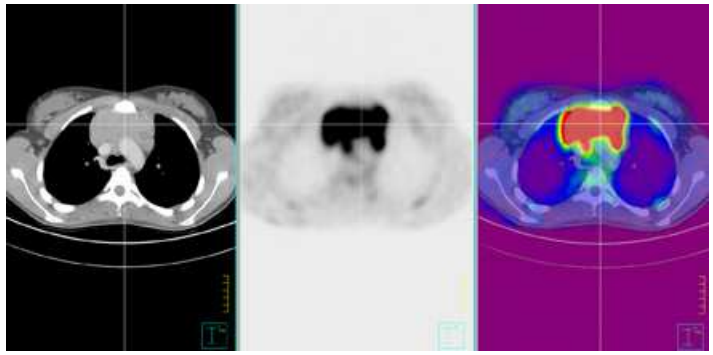
CD20

Grey-zones between Hodgkin and Non-Hodgkin Lymphomas



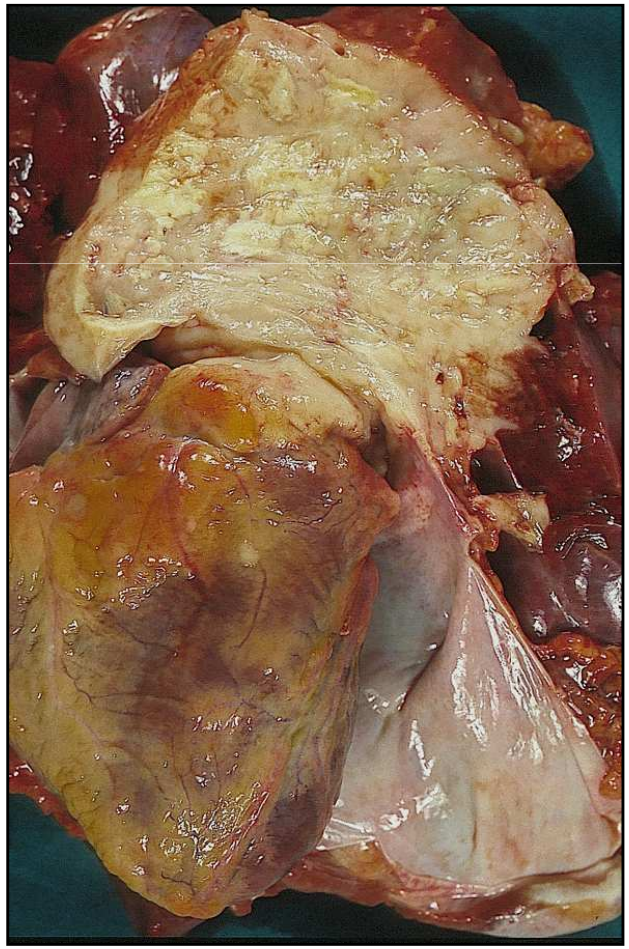
Mediastinal Large B-cell Lymphoma

- Young adults (mean: 32 ys)
- Predominantly females
- Bulky mediastinum
- Supraclavicular adenopathy
- Bone marrow involvement rare (3%)

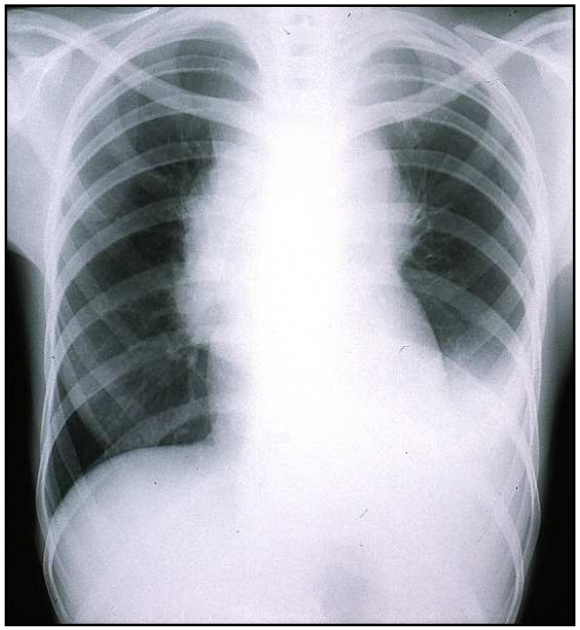


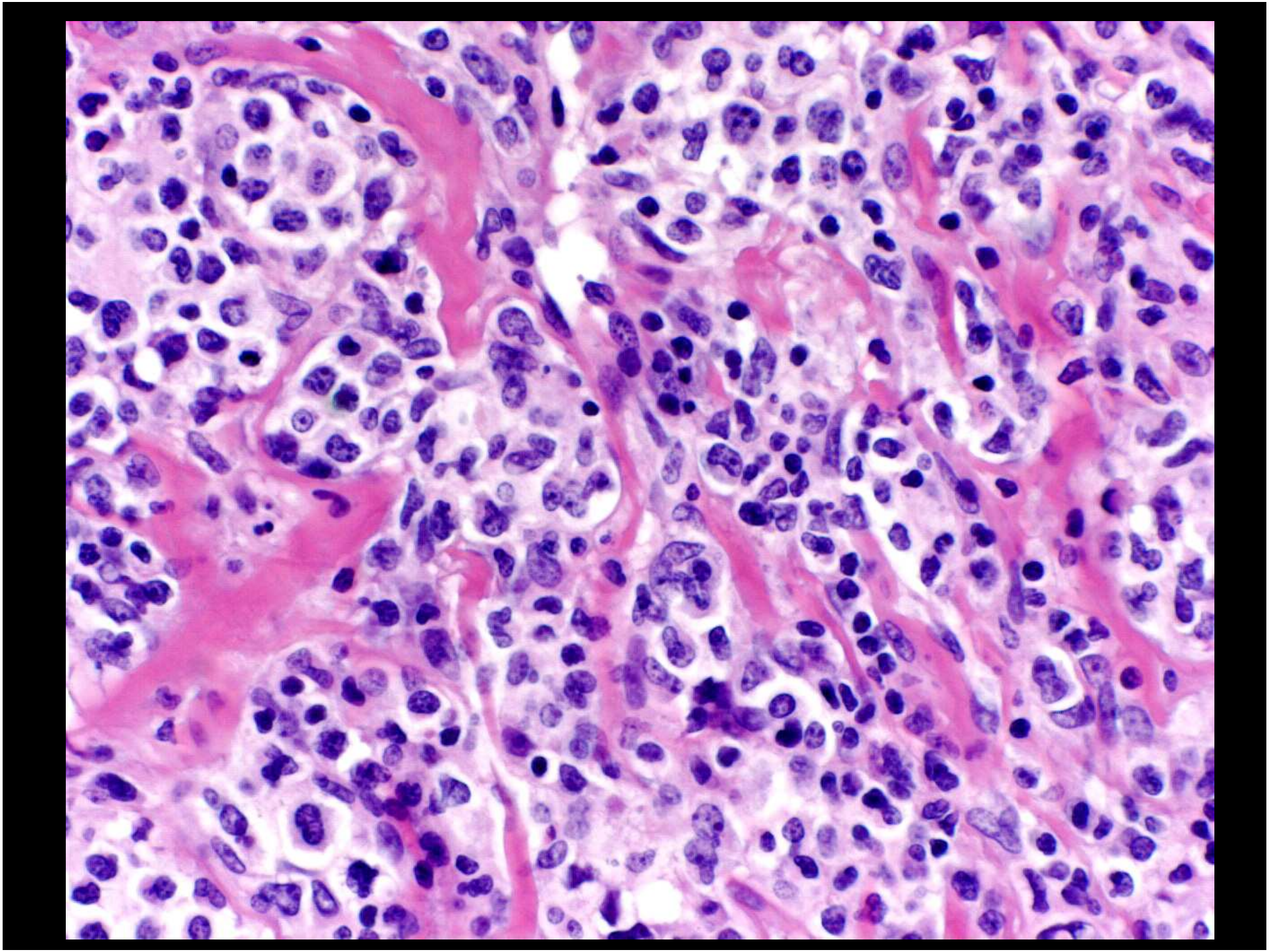
http://www.csmc.edu/

- Frequent extension to retroperitoneum (kidney, adrenal), liver, ovaries, CNS



- At presentation, not uncommon vena cava syndrome and adhesion to surrounding tissues/organs



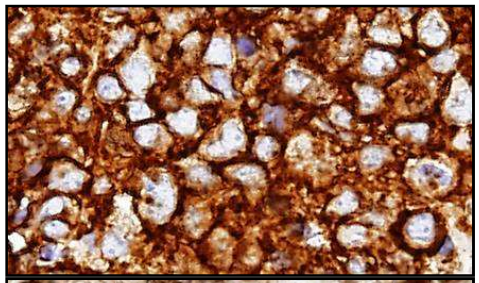


Mediastinal Large B-cell Lymphoma

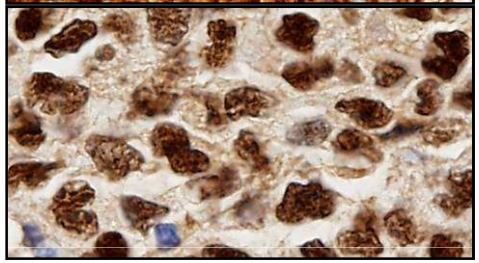
Phenotype

- **Strong and homogeneous expresssion of B-cell antigens (CD20, CD79a) and**
- **B-cell transcription factors (Oct2/Bob1; PU.1, Pax5)**
- **MAL+**

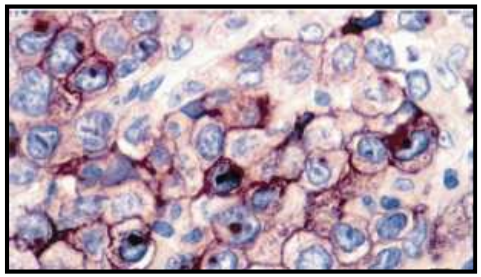
- **Lack of immunoglobulin & HLA-I**
- **CD23**
- ***CD30 can be expressed***



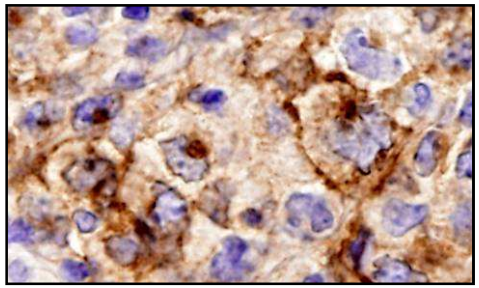
CD20



PAX5



MAL



CD30

HL-C and Med-LBCL

Gene expression profile analysis reveal many analogies between the two neoplasms

Rosenwald A, et al.

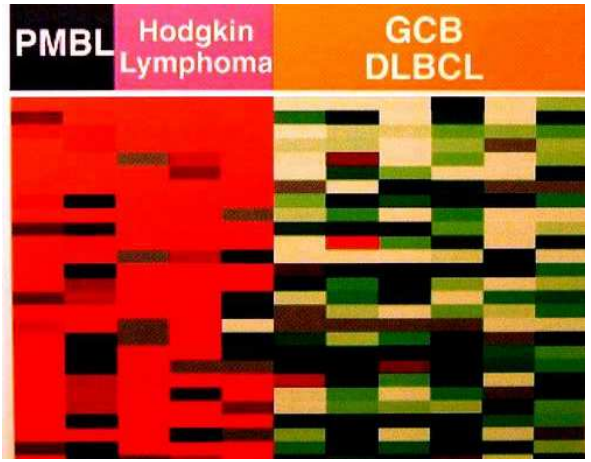
Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma.

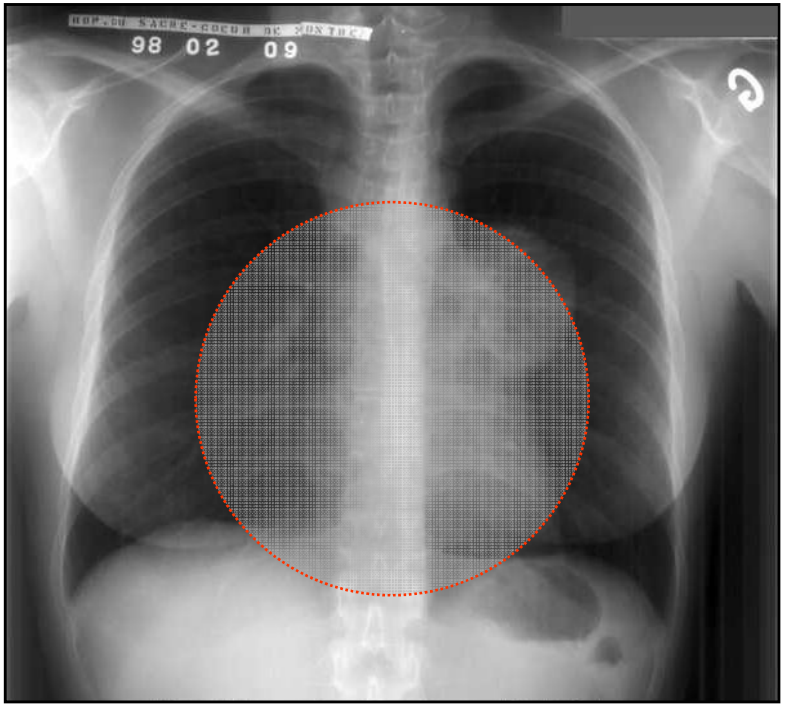
J Exp Med 2003;198:851-62.

Savage KJ, et al.

The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma.

Blood 2003;102:3871-9.



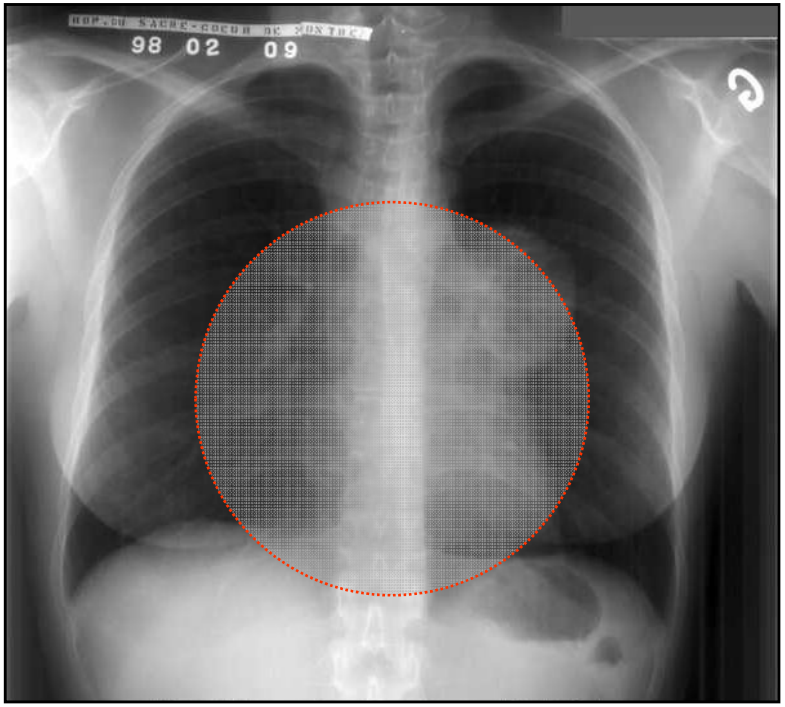


Hodgkin, classic, NS

Mediastinal LBCL

- **Combined lymphoma**

- **Sequential lymphomas**



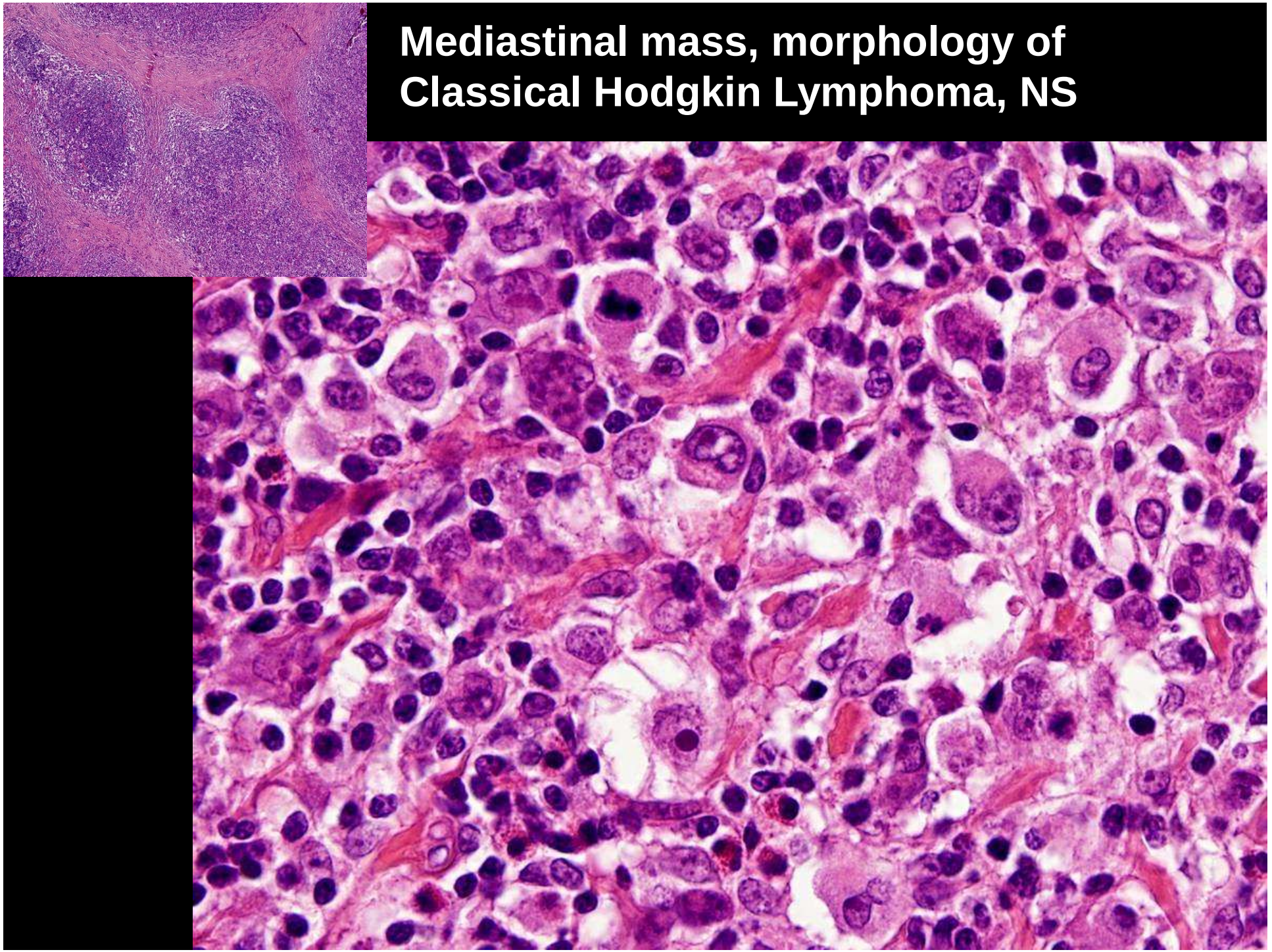
Hodgkin, classic, NS

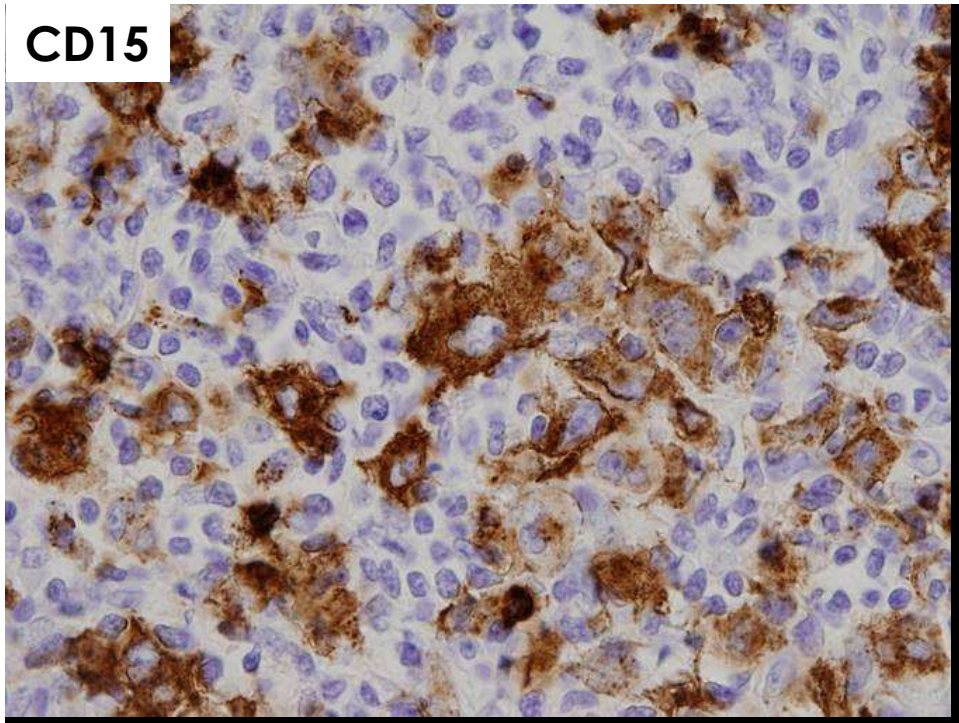
Mediastinal LBCL

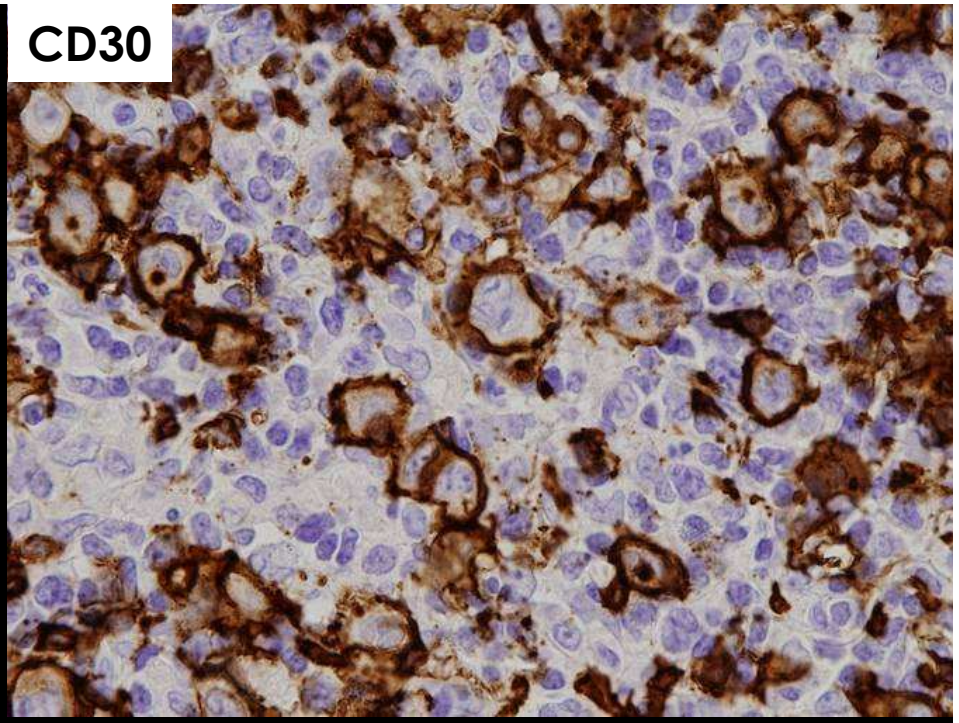
- Combined lymphoma
- Sequential lymphomas
- Mediastinal Gray zone lymphomas

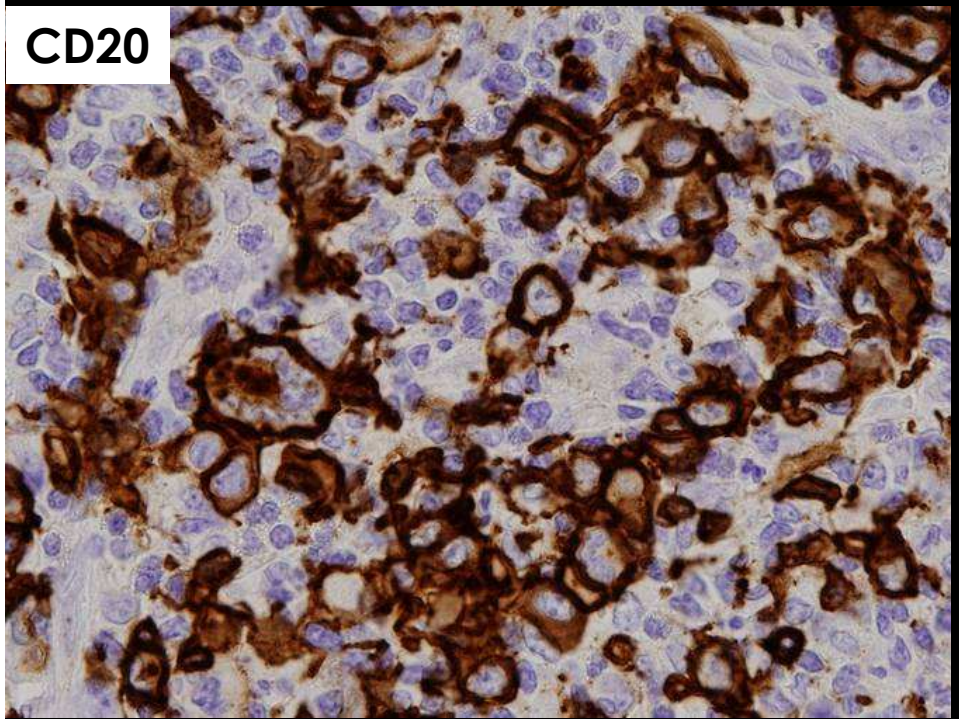
Mismatch between morphology and phenotype

Mediastinal mass, morphology of Classical Hodgkin Lymphoma, NS









Strong expression of CD15 and CD30, as in Classical HL,

but also strong expression fo CD20, as in PMDLBCL

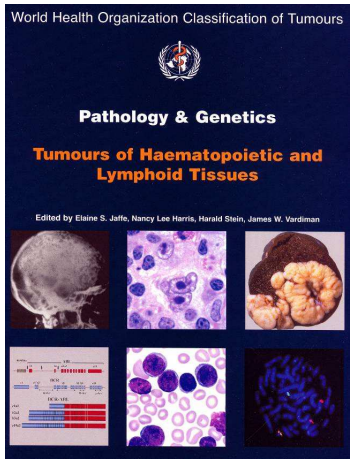
- **Predominantly males**
- **Aggressive tumors**

Although the small number of patients in this series makes it impossible to draw firm conclusions, it is worth mentioning that two cases initially diagnosed and treated with HL protocols failed to respond to therapy and finally died of the disease.

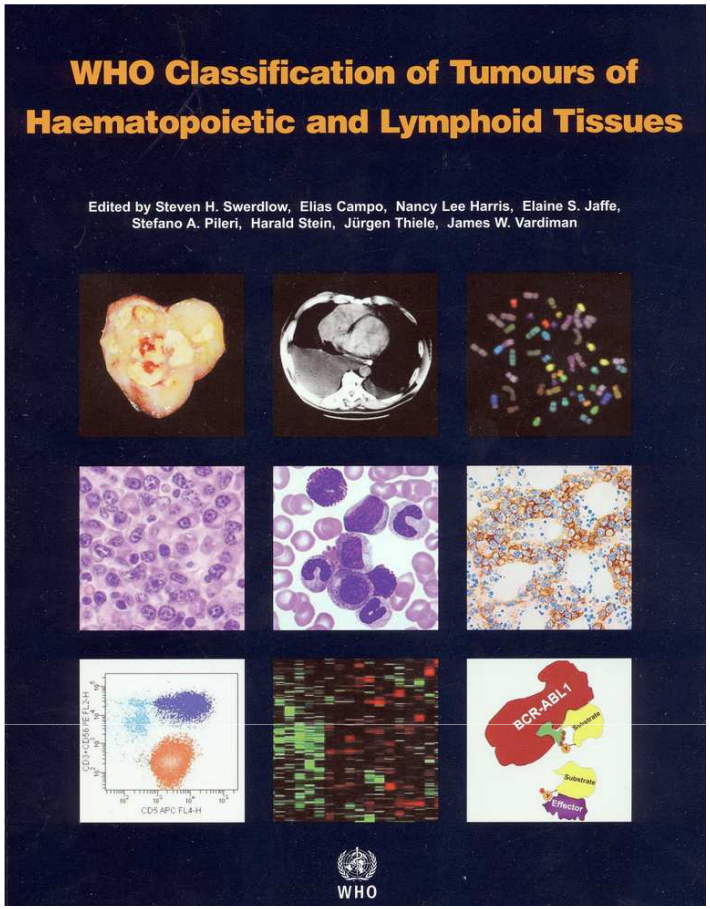
Histopathology, 2005 (47)

The existence of lymphomas with transitional features does not provide an answer regarding the optimal therapy for "gray zone lymphomas." Nevertheless, at the National Cancer Institute, such patients usually receive therapy appropriate for DLBCL, with the addition of Rituximab* for CD20-expressing lymphomas.[16,54,56]

Am J Surg Pathol 2005 (29)

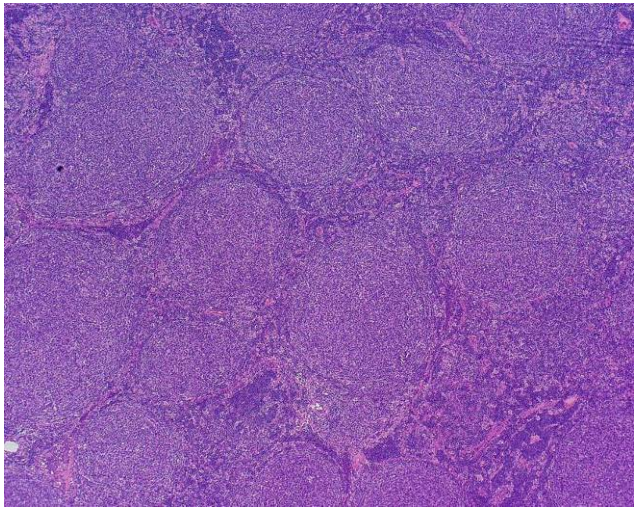


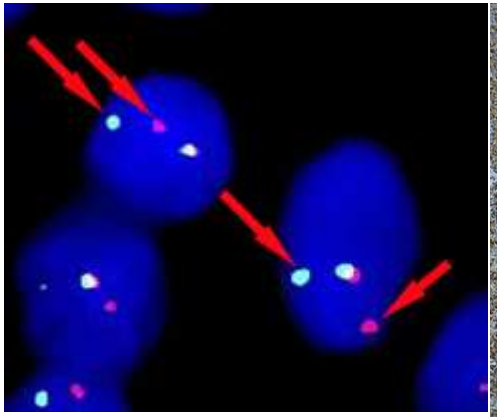
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin Lymphoma

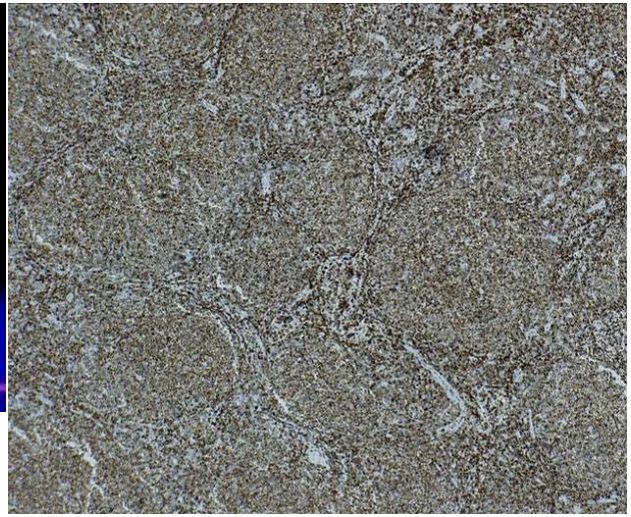


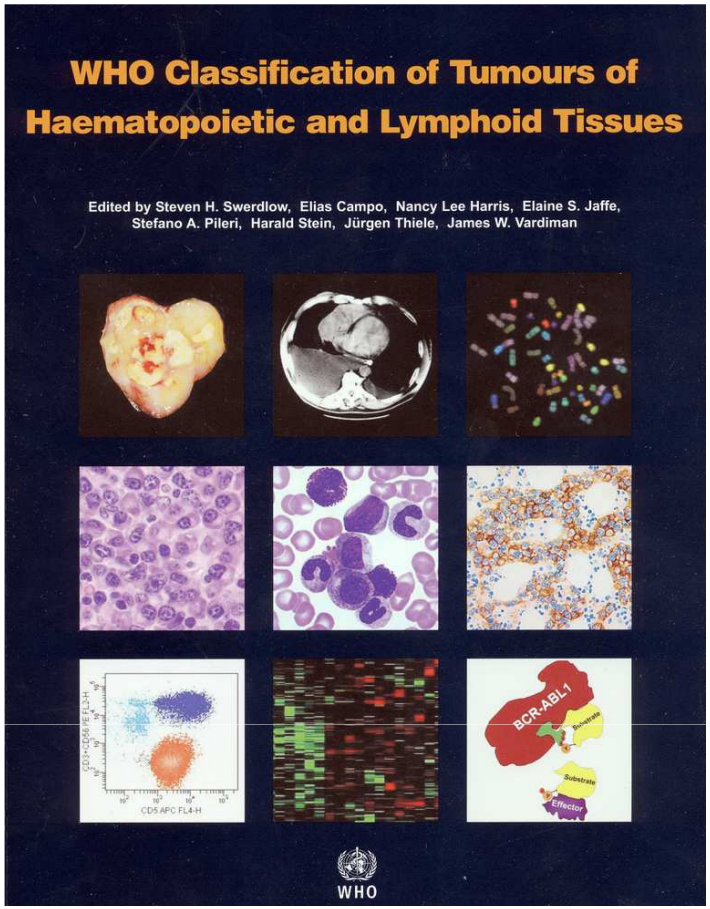
Follicular Lymphoma WHO 2008

- 20% of all lymphomas in western countries
- Tumor derived from germinal center cells of the secondary B follicles
- Nodular (follicular) growth pattern always recognizable
- Nodal disease, with frequent extranodal involvement (BM: 40-70%)
- t(14;18) translocation (→ 90% in low grades), with overexpression of the BCL2 protein



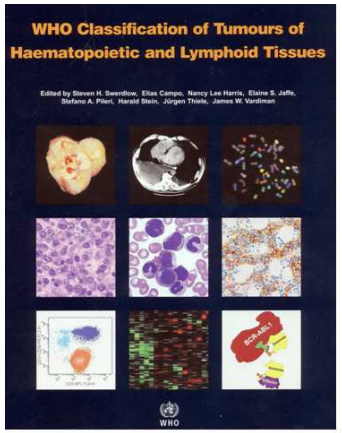






Follicular Lymphoma WHO 2008

- **Grading**
- **Definition of diffuse areas with "clinical relevance"**
- **Follicular lymphomas variants (with specific clinical features)**



Follicular Lymphoma WHO 2008

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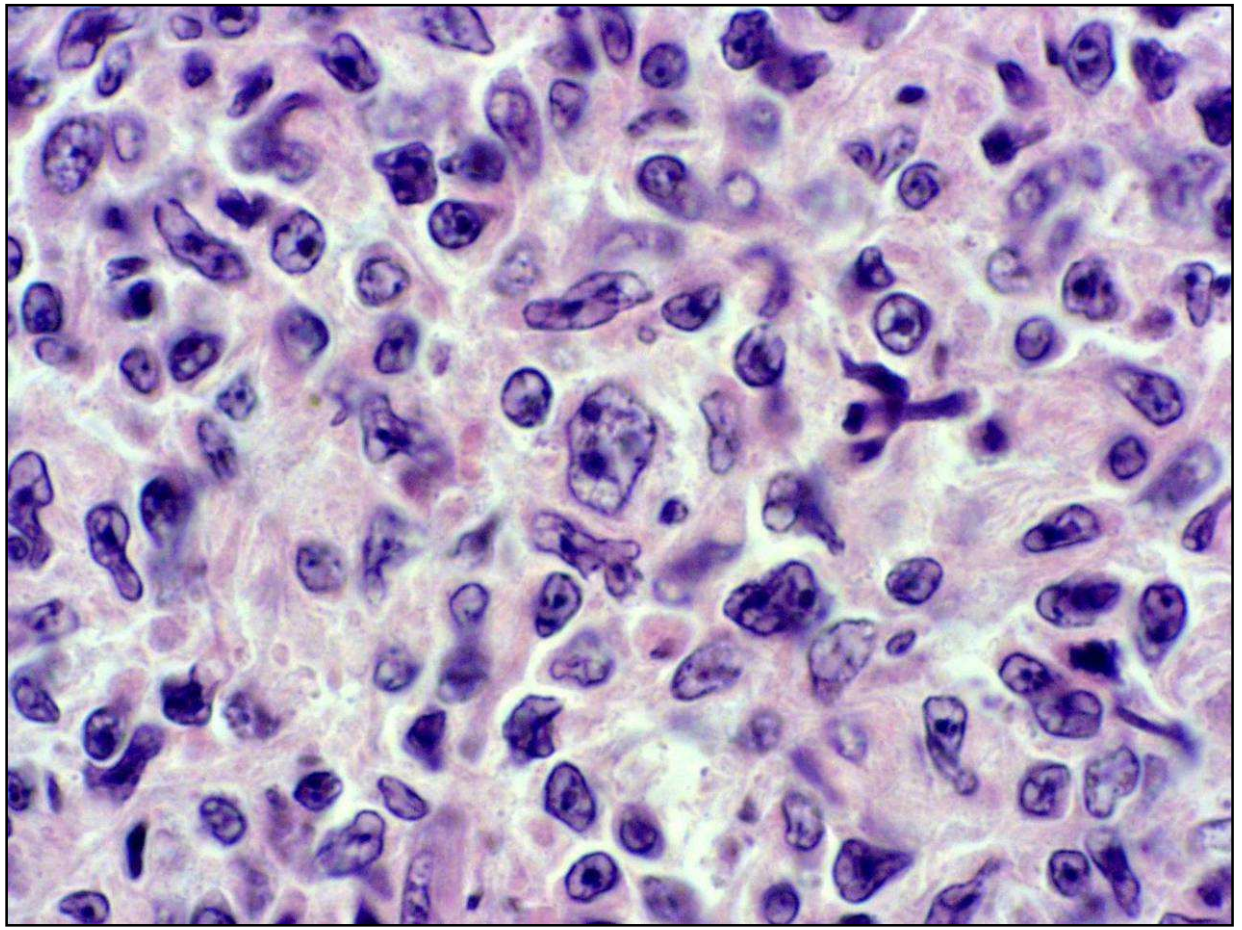
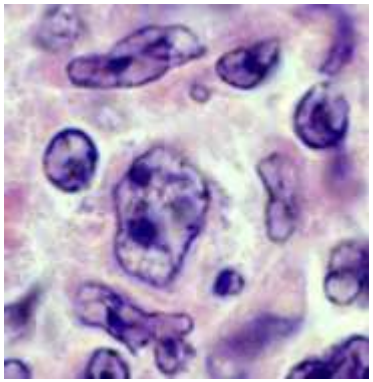
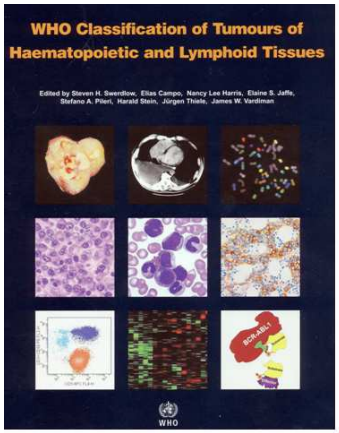
FL Grade (proportion of centrocytes and centroblast)

- relevant for prognosis
- used for therapy decision and often in trial design





N° centroblasts/10 HPF at random choice	WHO 2001	WHO 2008
<5	Grade 1	Low grade (1-2)
5-15	Grade 2	
>15 Centrocytes present	Grade 3a	Grade 3a
>15 Centrocytes absent	Grade 3b	Grade 3b



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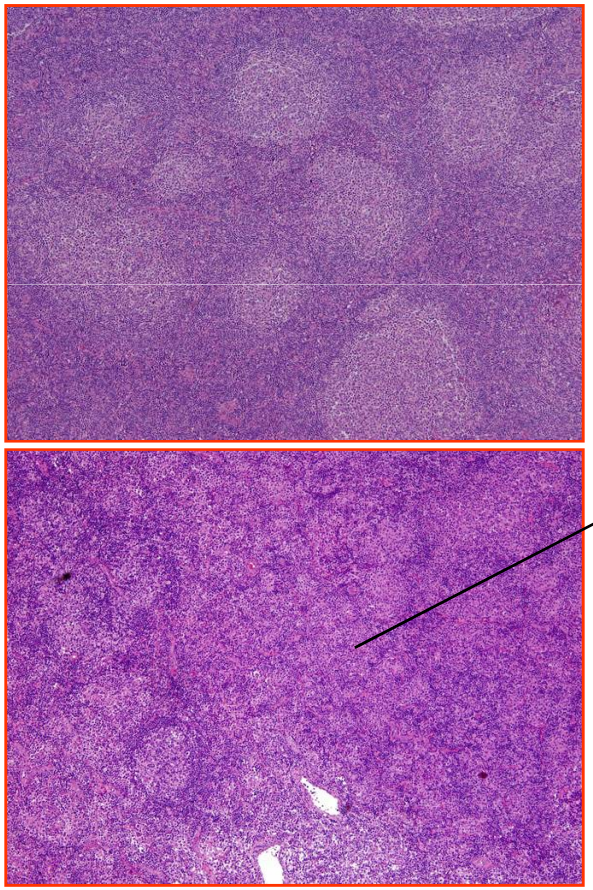
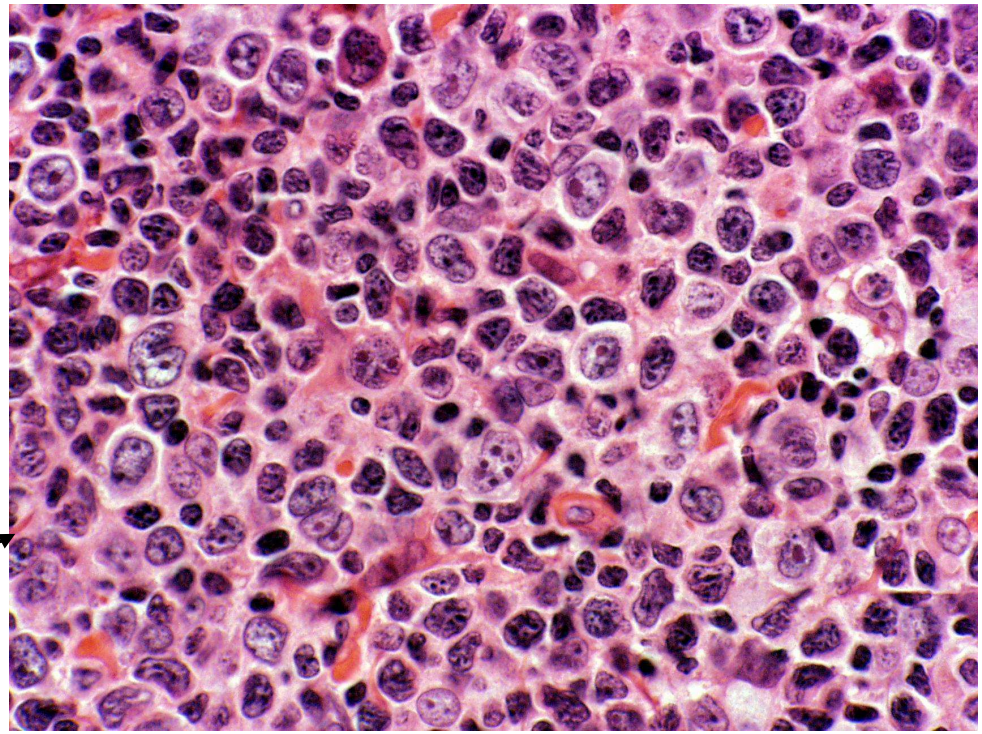
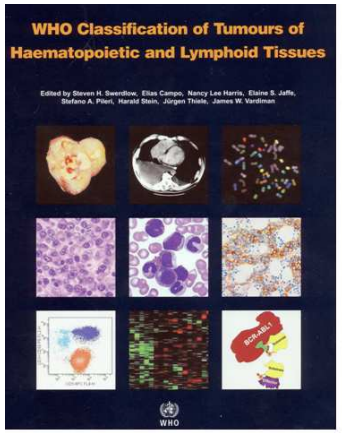
Areas with diffuse growth composed by 3a or 3b grade

Diffuse areas of grade 1-2 are unrelevant







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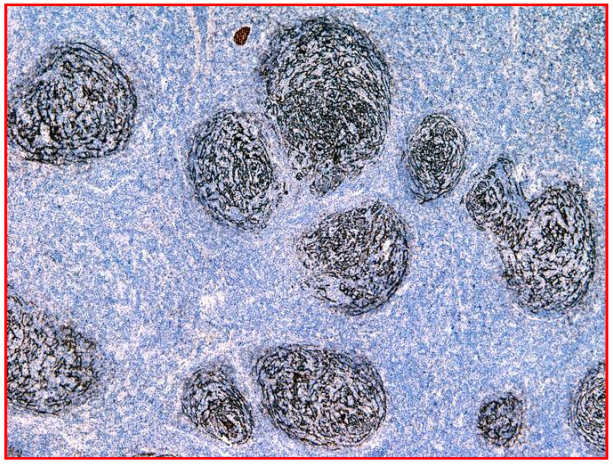
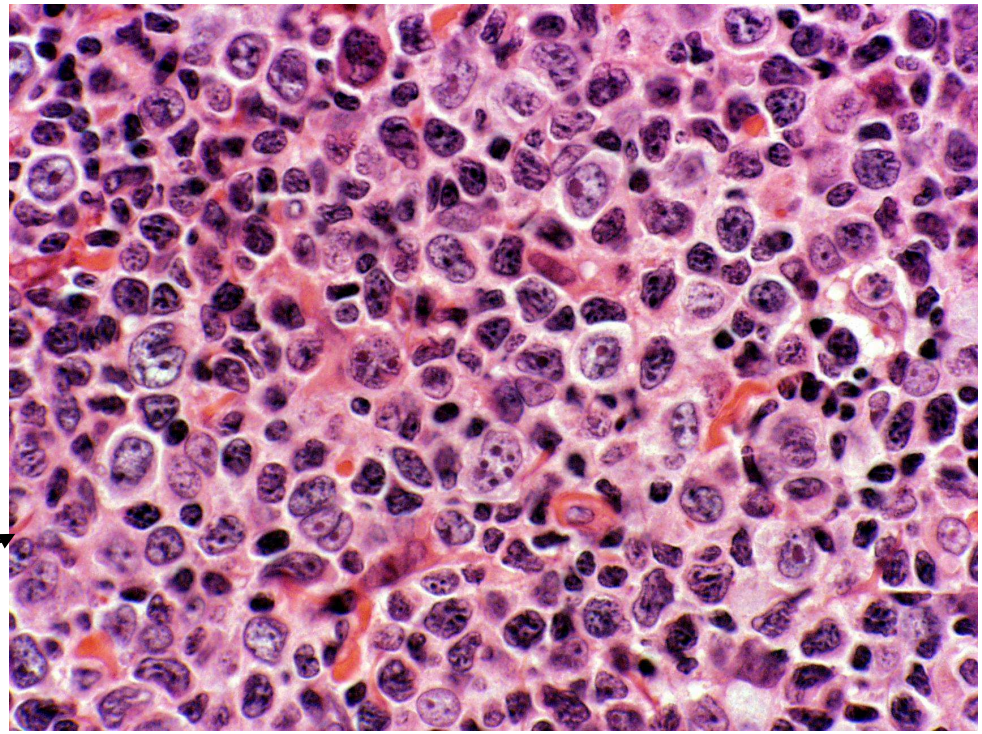
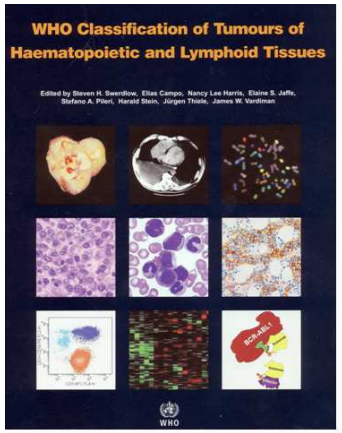




Lack of Follicular dendritic cells (CD21, CD23) is useful to identify these diffuse 3a-3b areas and to distinguish them from confluent nodules



Follicular Lymphoma WHO 2008

- **Grading**
- **Definition of diffuse areas with "clinical relevance"**
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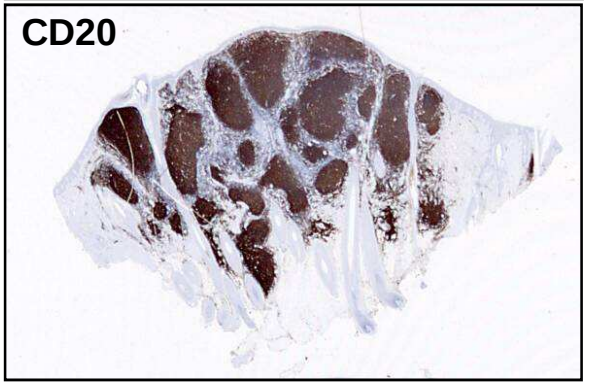
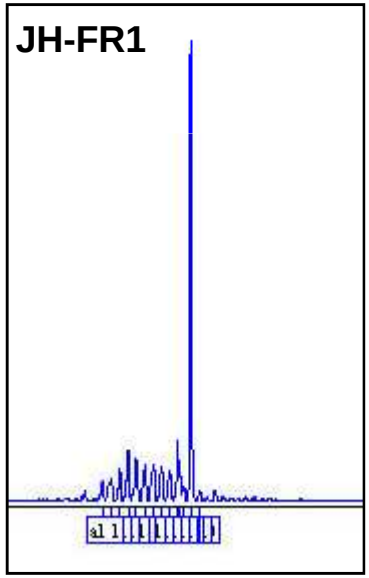
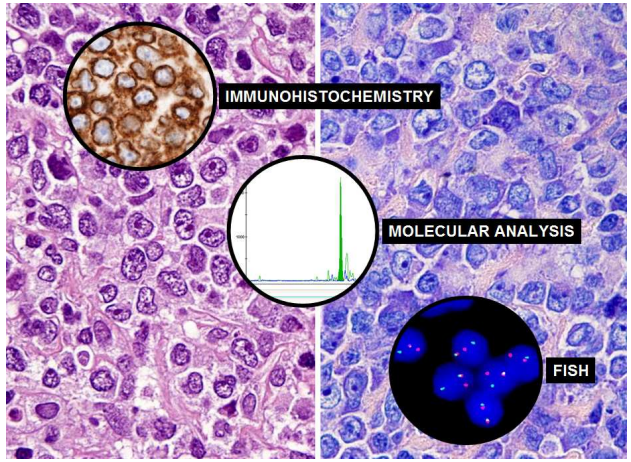
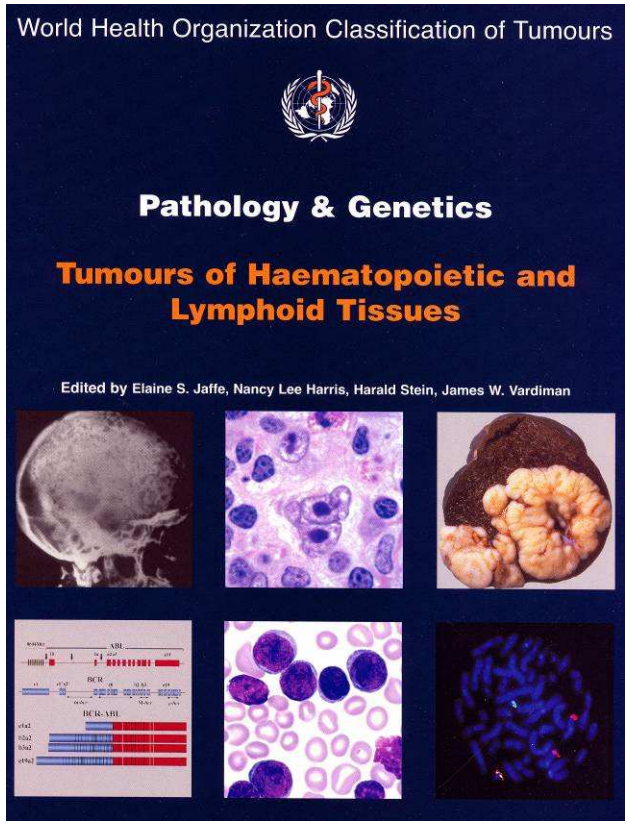
Follicular lymphoma variants			
	Grade	**t(14;18)**	**Evolution**
Paediatric FL	Often 3a-3b	No	Indolent
Testicular FL	Often 3a-3b	No	Indolent
Primary intestinal FL	As nodal	Yes	Indolent
Primary Cutaneous FL	Variable, with progression to 3a frequent	Rare	Indolent (recurrence, but rare extracutaneous spread) More aggressive if located to the lower extremity





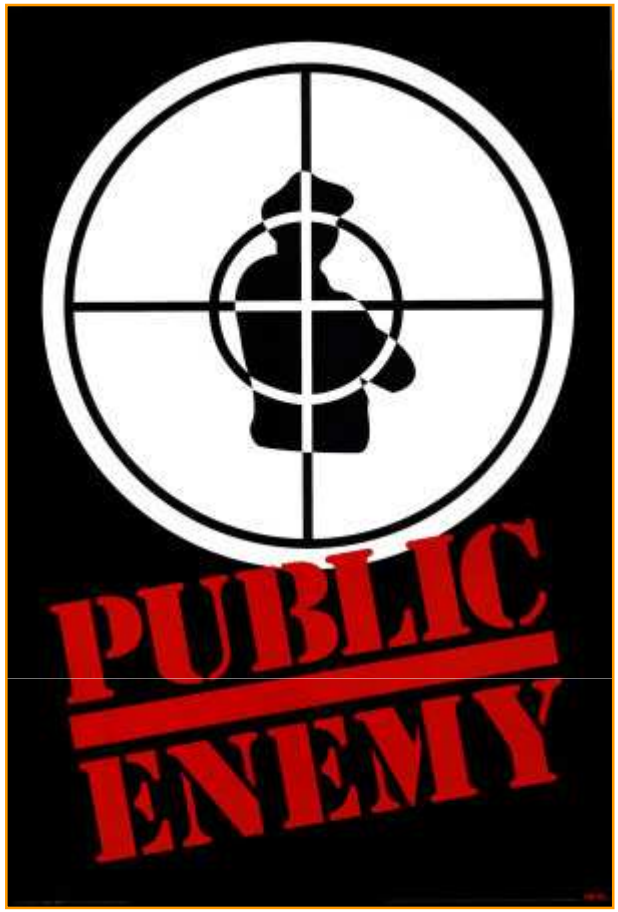
- **Trunk (back), scalp, forehead ("Crosti's Reticulohistiocytoma of the dorsum")**
- **Solitary or localized skin lesions**
- **Figurate plaques may precede tumourous lesions by months or years**
- **Recurrences proximate to original site**

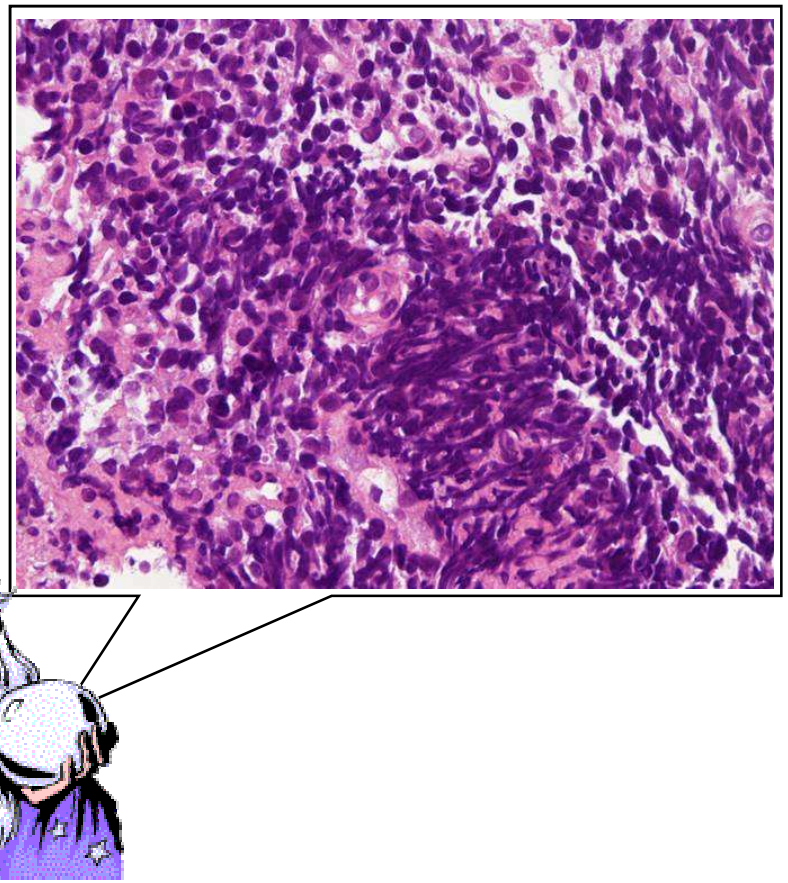


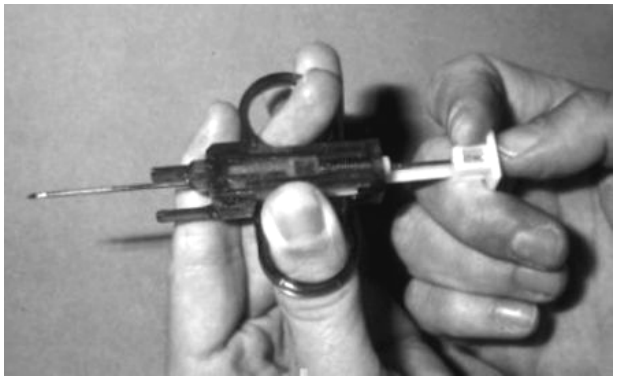


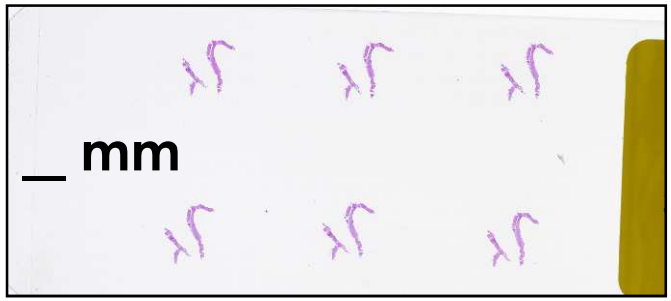
Diagnosis and appropriate classification of hemato-lymphoid neoplasms require excellent morphology and frequently the application of additional techniques

Pathological tissue preserved in tissue banks (fixed and frozen) will probably be used in the next future for the identification of bio-markers associated with response to new treatments


PUBLIC
ENEMY



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