What is the cellular pathologist's role in molecular diagnostics for lymphoma?

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London

Molecular diagnostics

- **Diagnostic tests – Benign or malignant**
- **Diagnostic tests – to assign a specific diagnosis within the current WHO classification system**
- **Biomarkers – predict disease behaviour, identify therapeutic targets, disease stratification, personalised medicine**
- **Rarely molecular monitoring of disease – response and early recurrence**

Cellular pathologist's role in molecular diagnostics for lymphoma - Diagnostic markers

- Sample quality
- Choice and request of a molecular test
- Interaction with clinical/biomedical scientists
- Result interpretation, integration and clinical context

Sample quality

- Though fresh tissue is preferred, paraffin embedded tissue is more practical
- Optimal fixation across the entire specimen
- Fixation in buffered formalin
- Avoid over-fixation
- Adequate representation of the abnormal population in the sample

Request of a ‘diagnostic’ molecular test

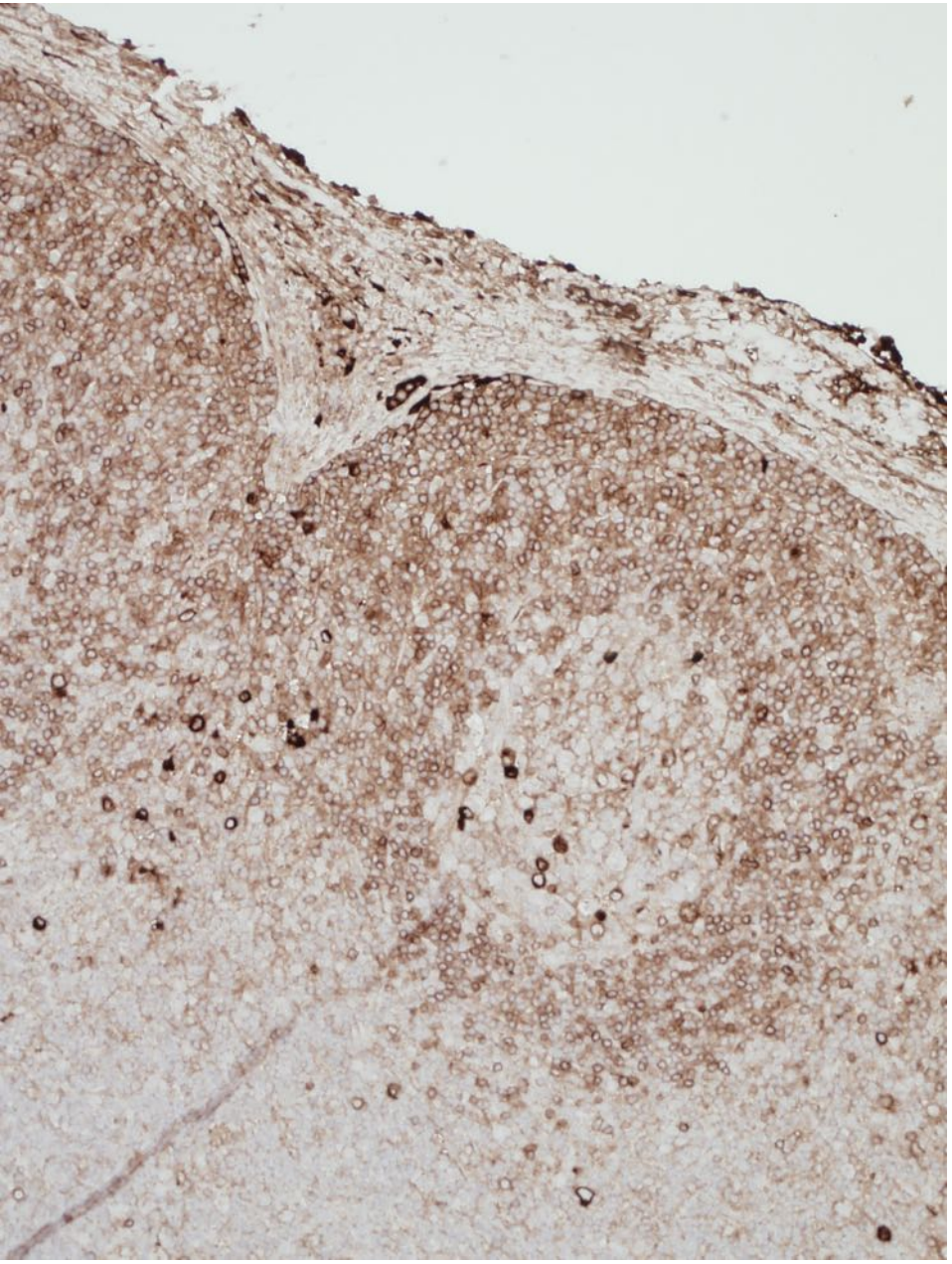
- **Should not be part of a general panel of investigations**
- **Should be requested by an expert haematopatholgist following morphological and immunohistochemical / immunophenotypic work-up**
- **Under the current scenario <20% of the lymphoid lesions require a molecular test**

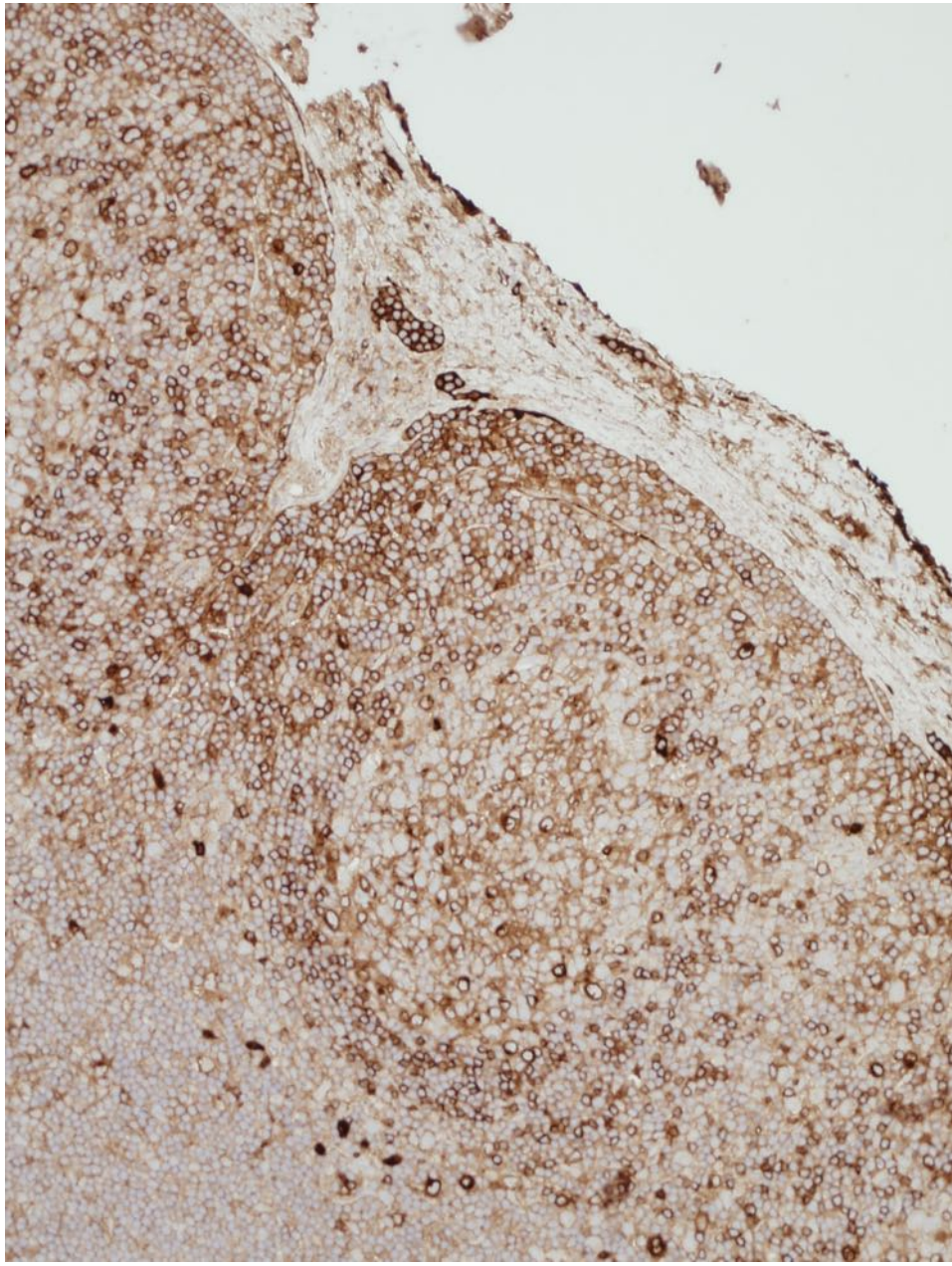
Request of a ‘diagnostic’ molecular test

- **A molecular test should only be requested when the result clearly impacts on final diagnosis**

- **Reactive lymphoid lesions: <10% show monoclonal rearrangements of IG/TCR genes, and ~15% show oligoclonal rearrangements of IG/TCR genes without an apparent explanation.**

- **Good quality light chain immunostains and application of flow cytometry reduces the requirement of *IG* gene rearrangement studies.**

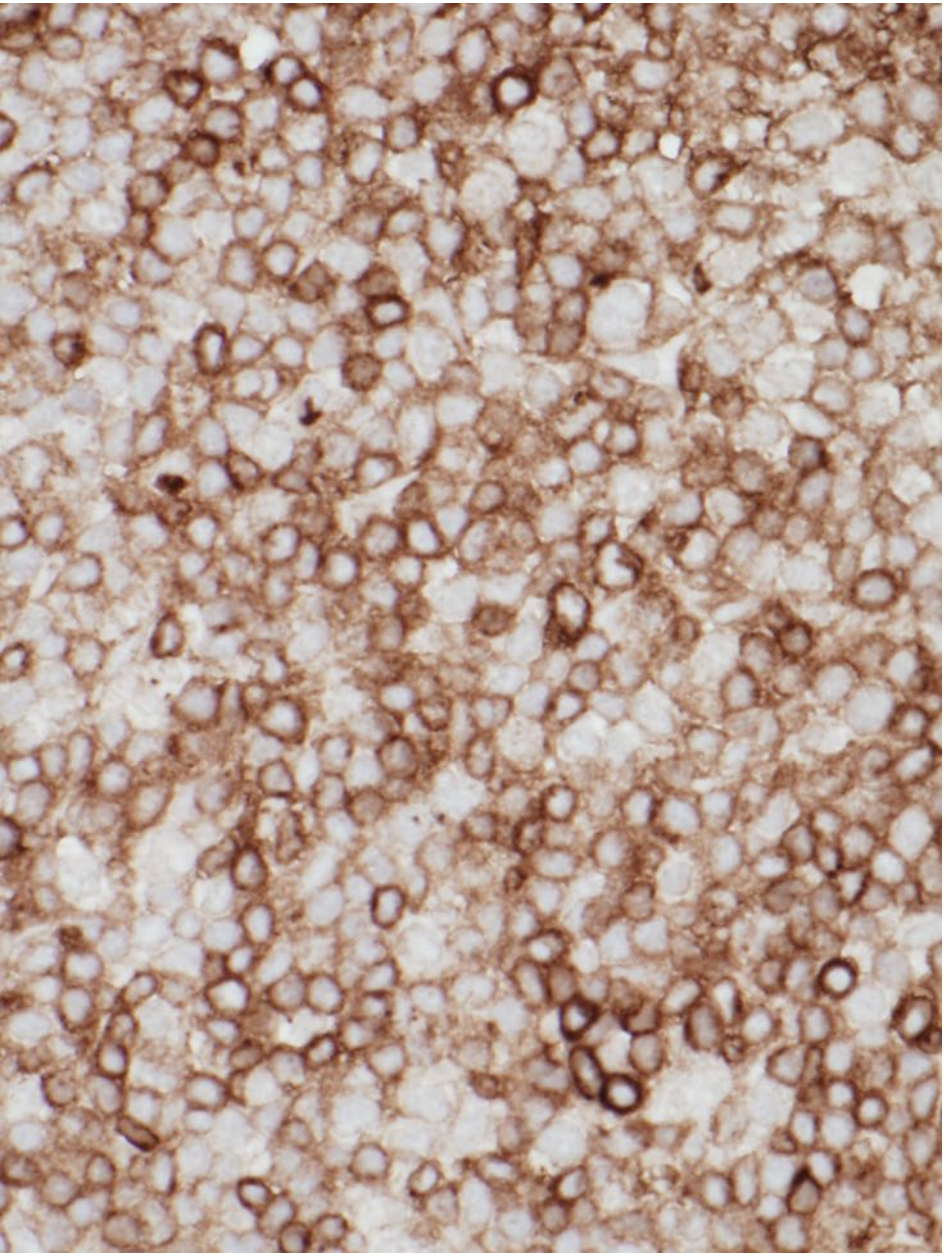


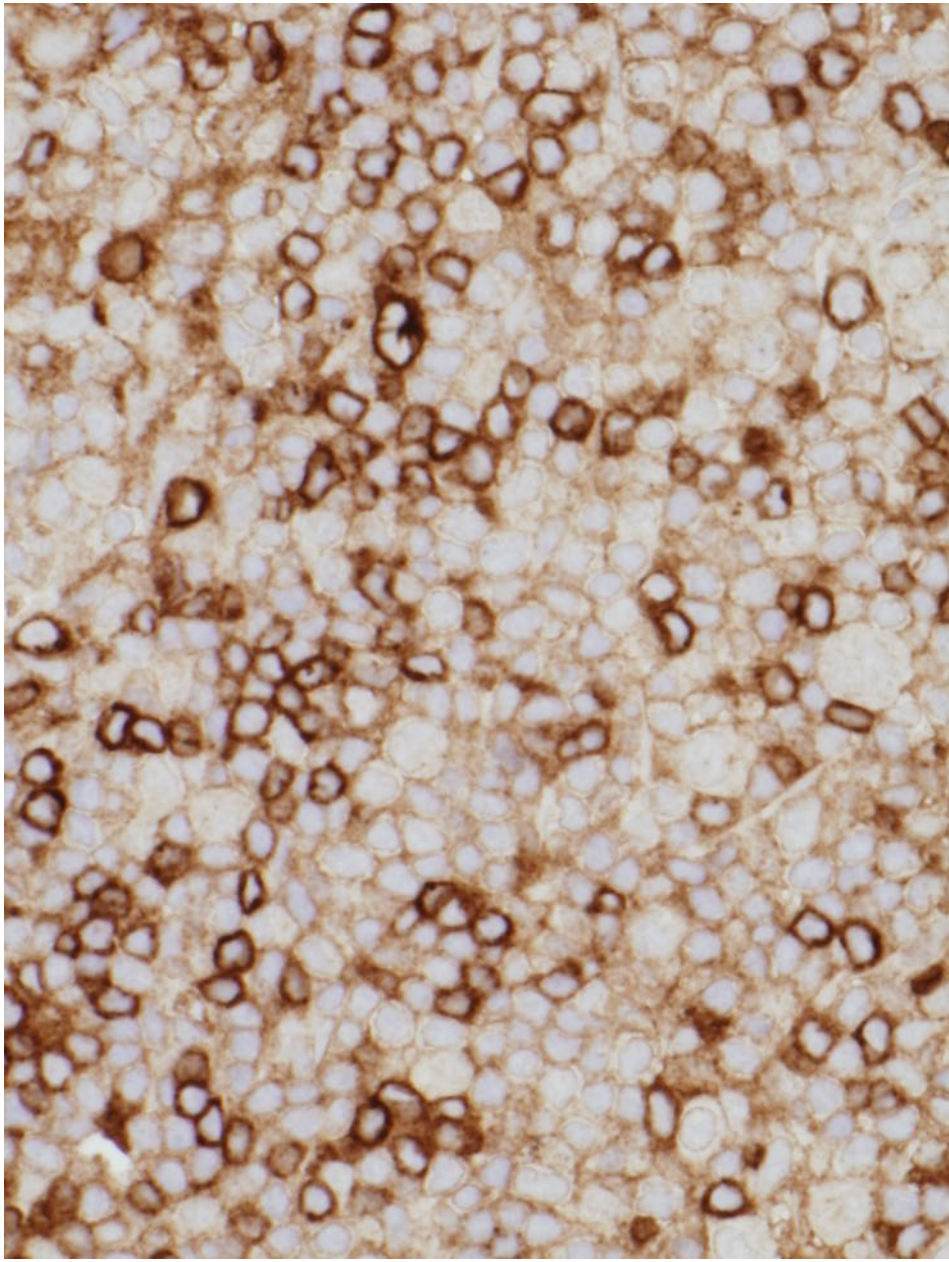


Kappa

Polytypic

Lambda

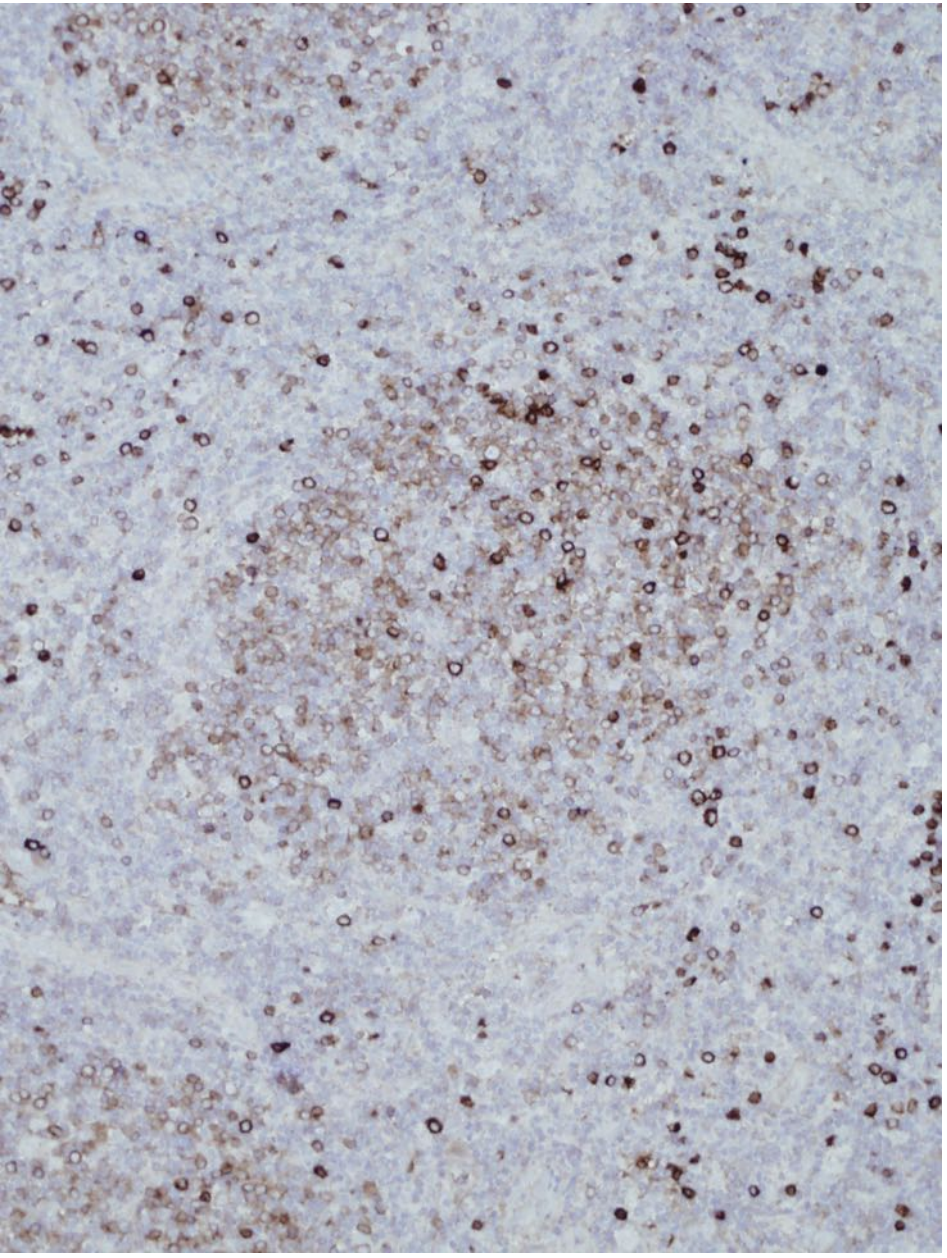


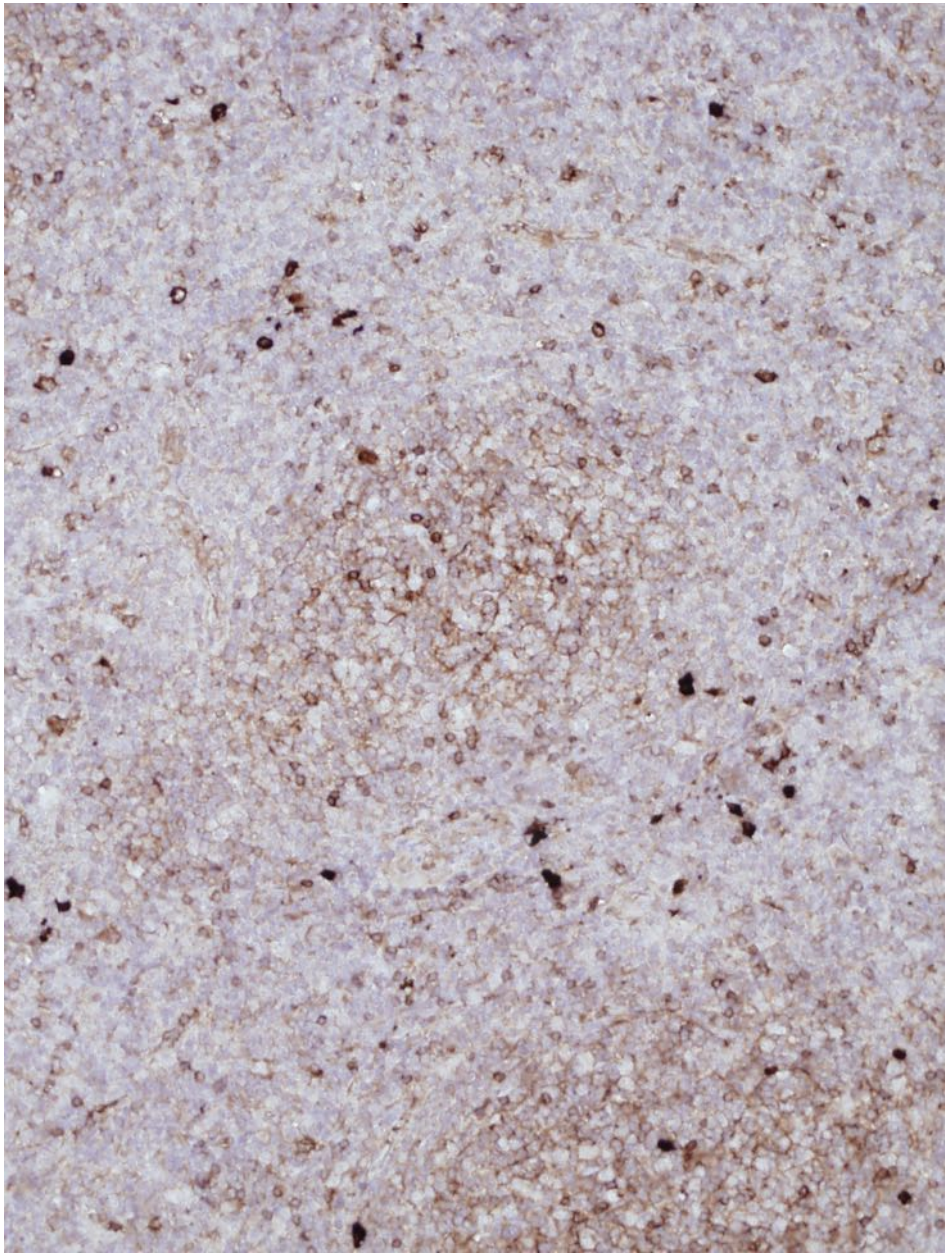


Kappa

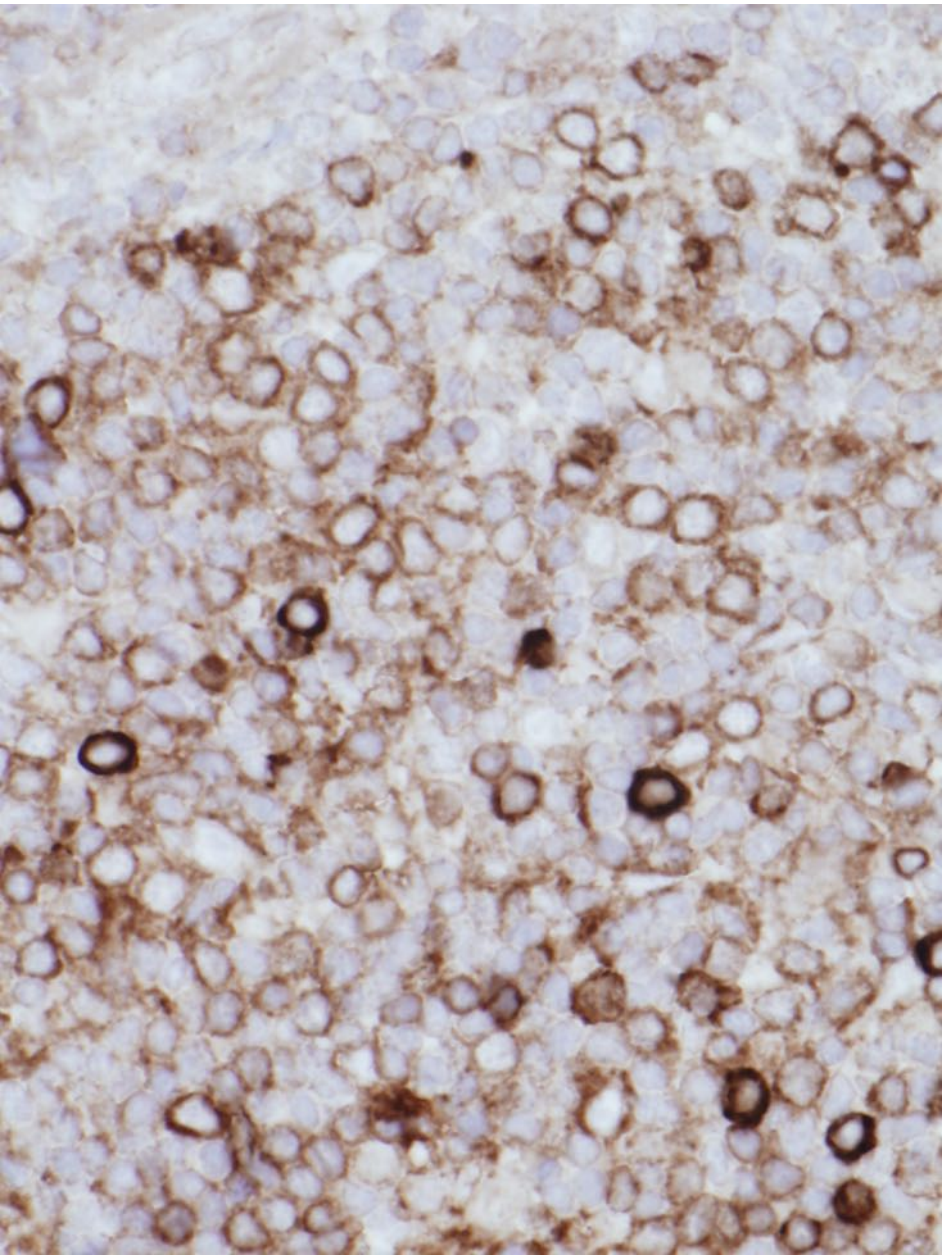
Polytypic

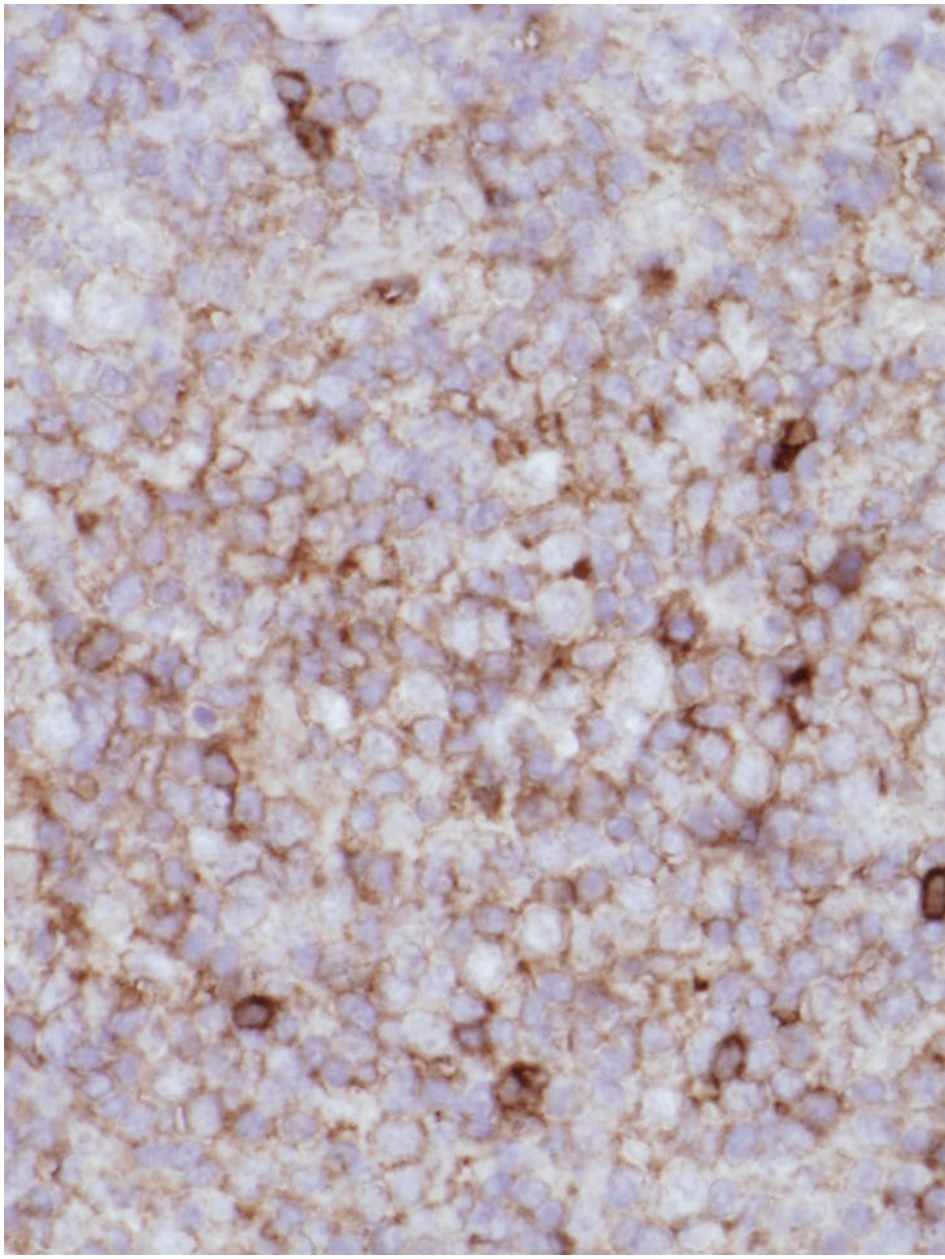
Lambda



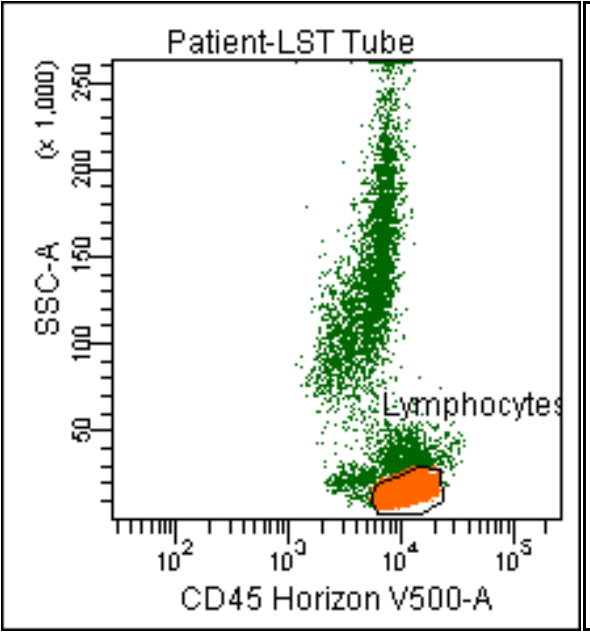


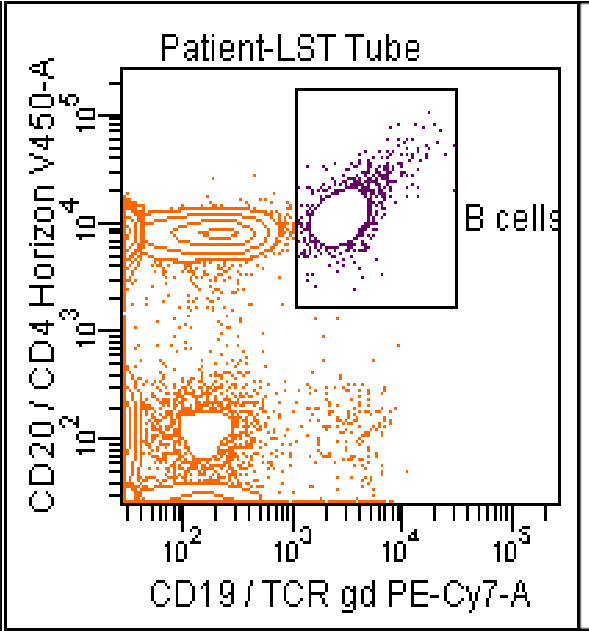
Kappa **Monotypic** **Lambda**

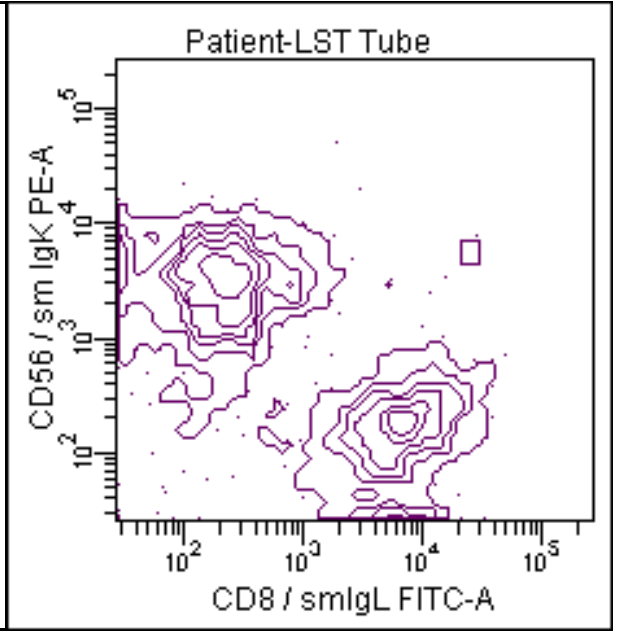


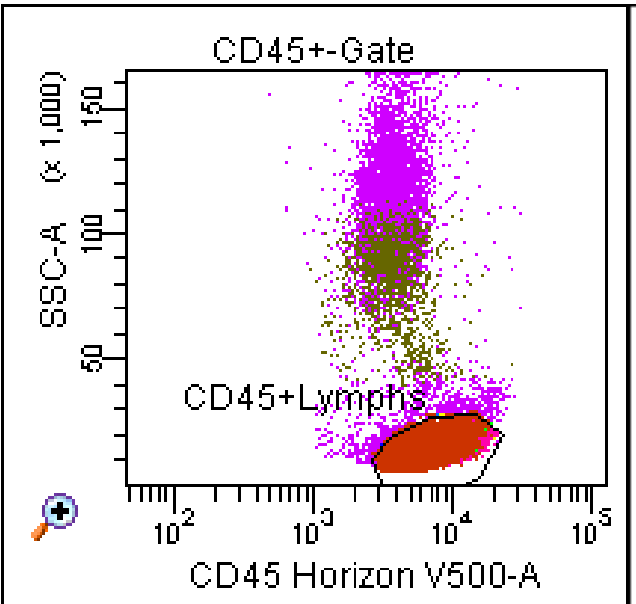


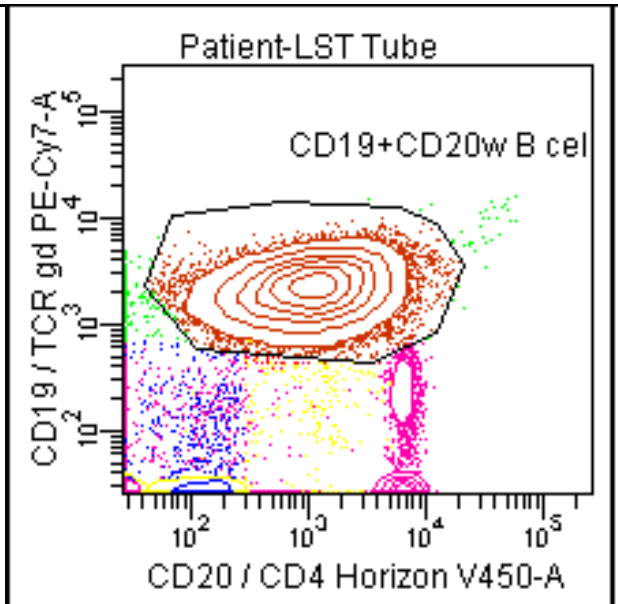
Kappa **Monotypic** **Lambda**

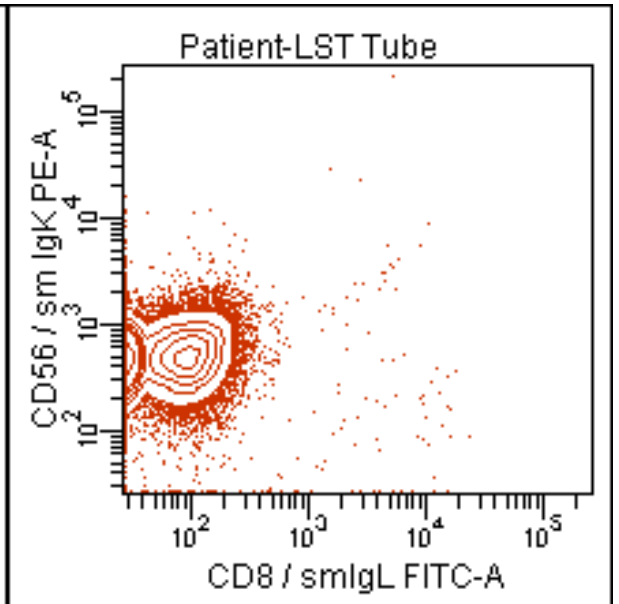

Patient-LST Tube
SSC-A (x 1,000)
Lymphocytes
CD45 Horizon V500-A


Patient-LST Tube
CD20 / CD4 Horizon V450-A
B cells
CD19 / TCR gd PE-Cy7-A


Patient-LST Tube
CD56 / sm IgK PE-A
CD8 / smIgL FITC-A


CD45+-Gate
SSC-A (x 1,000)
CD45+Lymphs
CD45 Horizon V500-A


Patient-LST Tube
CD19+CD20w B cel
CD19 / TCR gd PE-Cy7-A
CD20 / CD4 Horizon V450-A


Patient-LST Tube
CD56 / sm IgK PE-A
CD8 / smIgL FITC-A

Choice of ‘diagnostic’ molecular tests

- **FISH based tests investigating translocations (also provide information on copy number changes)**
- **Clonality tests based on clonal rearrangements of antigen receptor genes**
- **Mutation analysis**

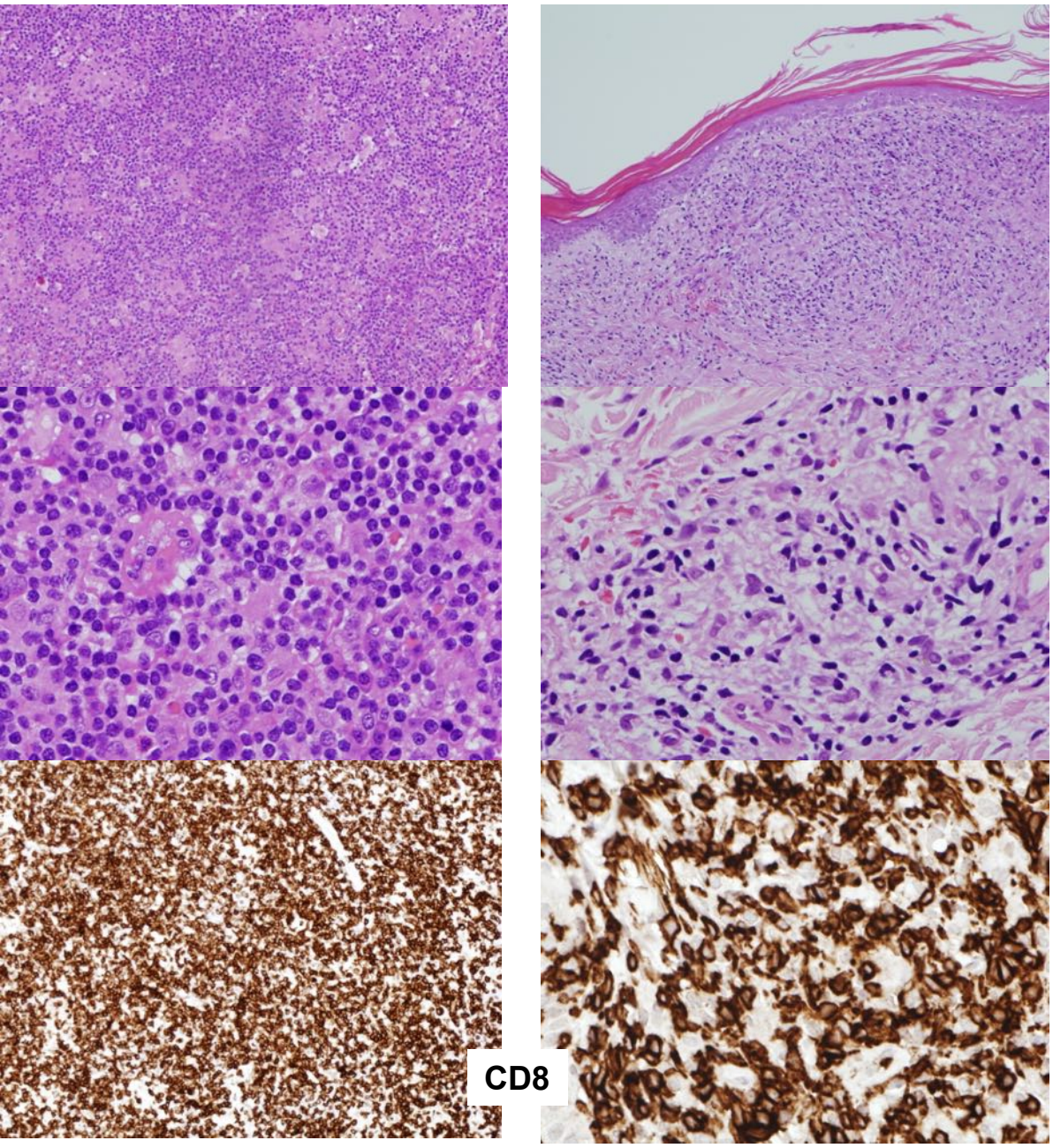
In lymphomas associated with specific chromosomal translocations, interphase-FISH is preferable over antigen receptor gene rearrangement analysis.

Gene targets for clonality analysis

Gene	Value
IGH	+++
IGK	+++
IGK del	+++
IGL	+
TCRG	+++
TCRB	++

Antigen receptor gene rearrangement studies

Histological pattern	Diagnostic suspicion	Test
Expansion of interfollicular T-cell areas	Early phase of angioimmunoblastic T-cell lymphoma	T-cell and B-cell clonality
Angioimmunoblastic T cell lymphoma with large B cells without demonstrable light chain restriction	Clonal large B cell expansion or evolving DLBCL in the context of angioimmunoblastic T-cell lymphoma	B-cell clonality
Medium and large T-cell expansion inside B-cell follicles	Peripheral T-cell lymphoma NOS, follicular variant	T-cell clonality
Paracortical expansion in a lymph node with mycosis fungoides	LN involvement by mycosis fungoides	T-cell clonality
T cell infiltrates in skin suspicious but not diagnostic of lymphoma	Mycosis fungoides and other cutaneous T cell lymphomas	T-cell clonality
Low-density lymphoid infiltrates in HTLV1 positive patients	Adult T cell leukaemia/lymphoma	T-cell clonality
Coeliac disease with downregulation of CD8 and clinical refractoriness	Refractory coeliac disease and Enteropathy associated T cell lymphoma in-situ	T-cell clonality
HRS cells with background atypical T cells	Classical Hodgkin lymphoma vs. T cell lymphoma	T-cell clonality



60Y Male
Skin lesions,
Lymphadenopathy
& renal failure

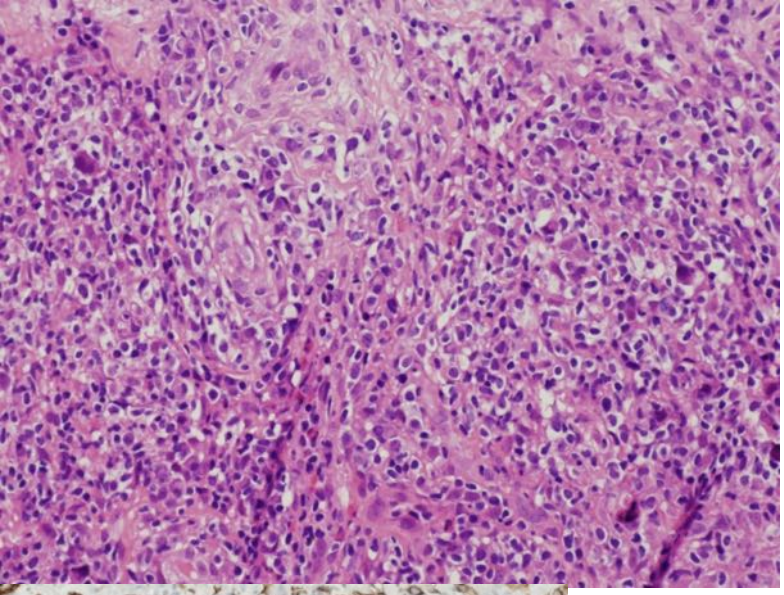
Diagnosis
Peripheral T cell lymphoma, NOS; lymphoepithelioid var. (Lennert's lymphoma)

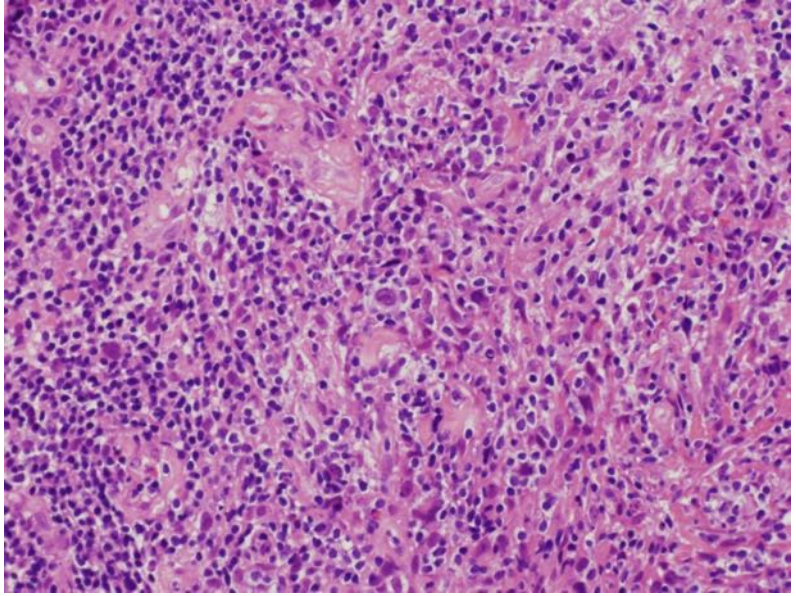
Immunophenotype:

Positive: CD2, CD3, CD5, CD7, CD8

Negative: CD4, PD1, CD30 & B cell markers

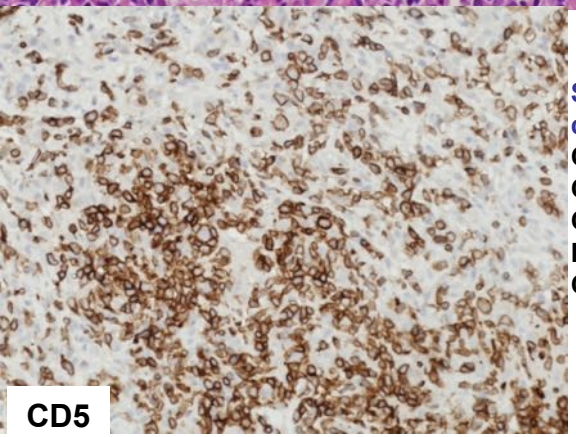
***TCRG* rearrangements studies:**
Identical clonal products from skin, LN and renal biopsies





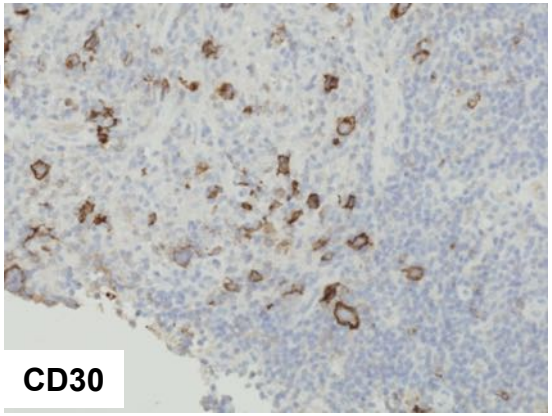
60Y Male
Lymphadenopathy & Splenomegaly

Diagnosis
Classical Hodgkin lymphoma



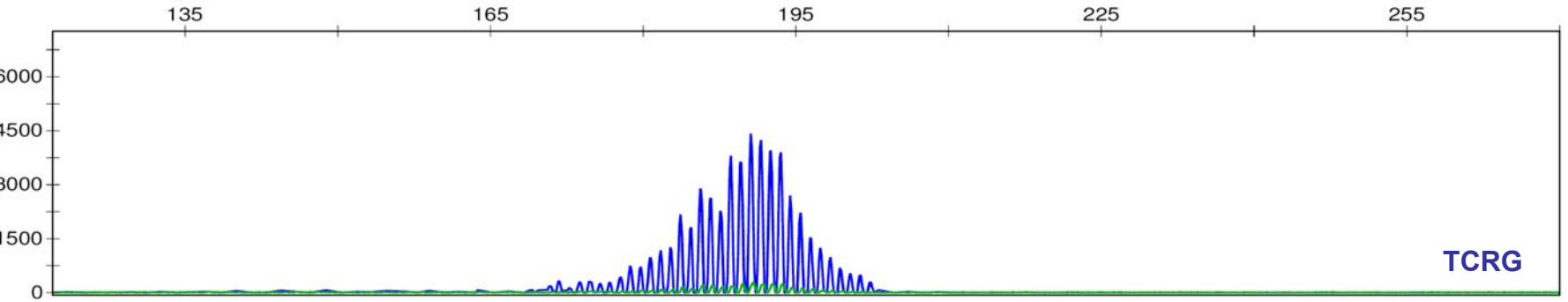
CD5

Smaller lymphoid cells:
CD3+
CD2+
CD4+
PD1+
CD10 – occ.



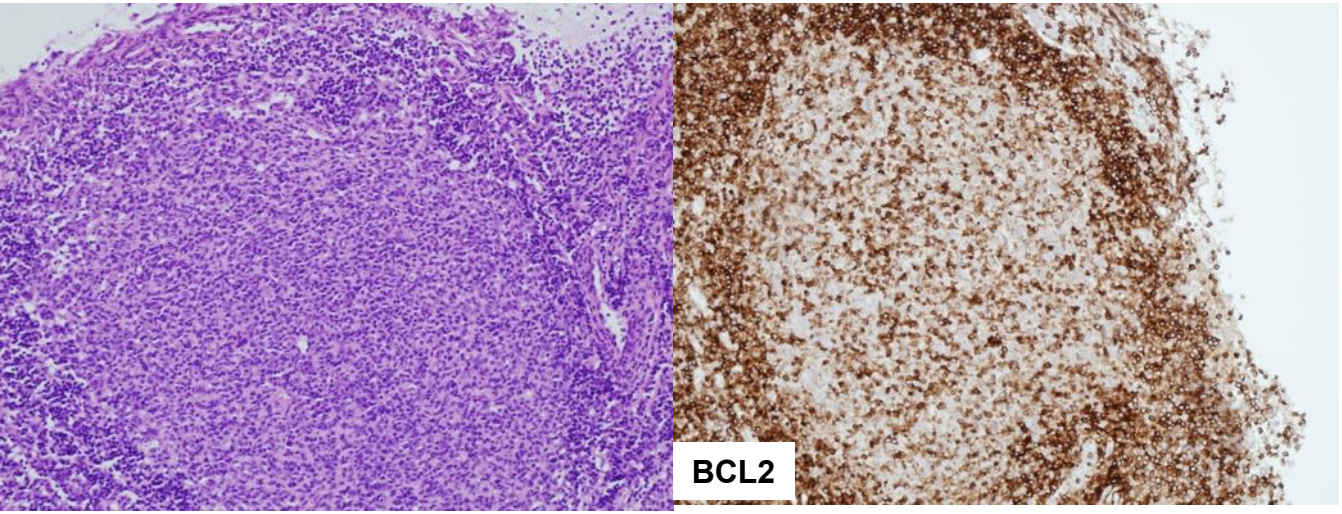
CD30

Larger lymphoid cells:
CD30+
CD15+
Pax5w
EBER-
CD20-



Antigen receptor gene rearrangement studies

Histological pattern	Diagnostic suspicion	Test
Marginal zone expansion in a lymph node, spleen, or an extranodal sample without demonstration of light chain restriction	Marginal zone lymphoma	B-cell clonality
Suspicion of mantle cell lymphoma but overfixed with negative cyclin D1 staining of internal positive control, and failed FISH	Mantle cell lymphoma	B-cell clonality
BCL2 negative follicles in a sample suspicious of follicular lymphoma, and with negative FISH results	Follicular lymphoma	B-cell clonality
Multicentric Castleman's disease with a high density of HHV8+ cells in the mantle zone	'Micro-lymphoma'	B-cell clonality



BCL2

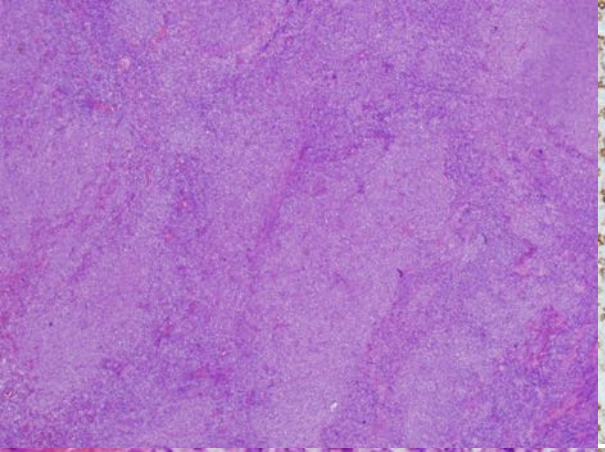
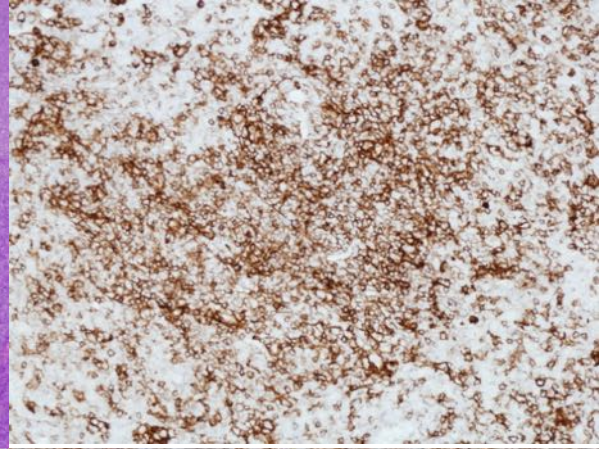
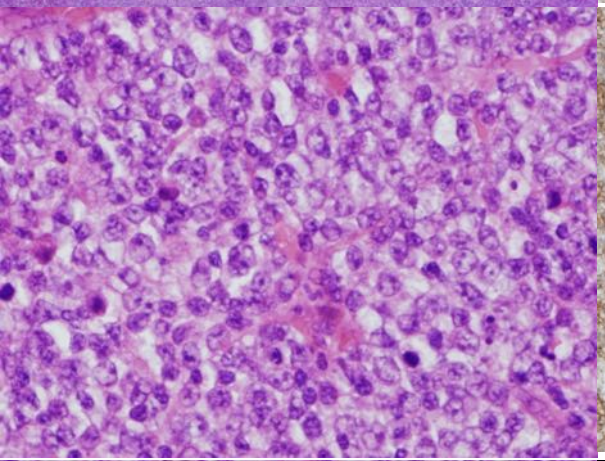
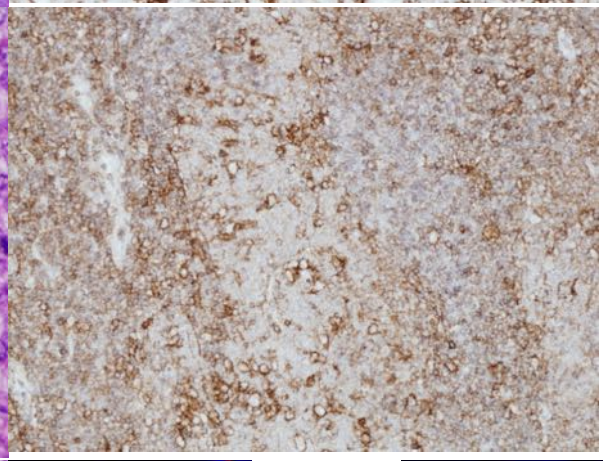
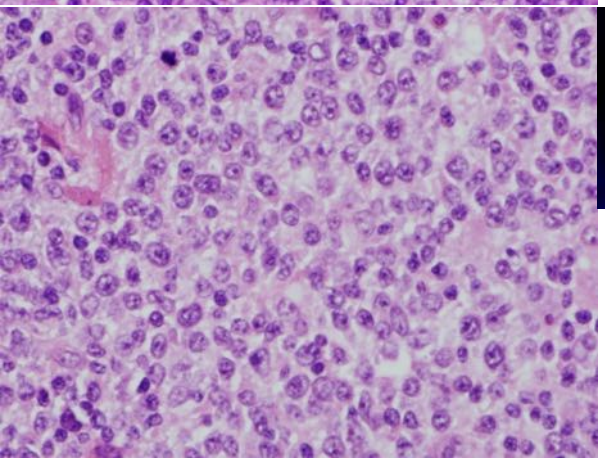
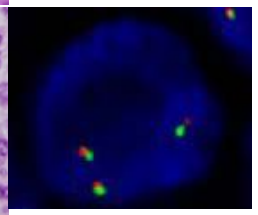
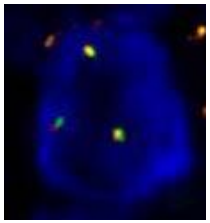
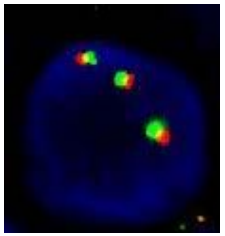
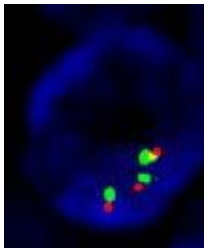
20Y Male
Right groin LN

Diagnosis
Follicular lymphoma, gr. 1

***IGH & IGK* rearrangements studies:**
Identical clonal products from needle core and excision biopsies

Interphase FISH studies as ‘diagnostic’ tests

Histological pattern	Diagnostic suspicion	Test
Marginal zone expansion in an extranodal sample without demonstration of light chain restriction	Marginal zone lymphoma	*MLT1* *BCL10*
BCL2 negative follicles in a sample suspicious of follicular lymphoma	Follicular lymphoma	*BCL2* *BCL6*
Extensive follicular colonisation	Distinction of follicular lymphoma and marginal zone lymphoma with follicular colonisation	*BCL2* *BCL6*
Suspicion of mantle cell lymphoma but overfixed with negative cyclin D1 staining of internal positive control	Mantle cell lymphoma	*CCND1*
Diagnosis of Burkitt lymphoma unresolved with morphology and immunohistochemistry	Burkitt lymphoma or a ‘grey’ zone lymphoma / double-hit lymphoma	*MYC* *BCL2* *BCL6* *IG*
Diffuse large B cell lymphoma with cyclin D1 expression	Distinction of DLBCL from Blastoid MCL	*CCND1*
CD5+ small B cell lymphomas with features not characteristic of CLL, MCL or MZL	CD5+ lymphoproliferative disorder associated with t(14;19) *BCL3-IGH*	*BCL3*

60Y Male
Rapid growth of left tonsil

Diagnosis
Follicular lymphoma gr. 2-3a with marginal zone diff.

Immunophenotype:

Positive: CD20, CD79a, BCL6, BCL2, MUM1, IgM, IgD, CD38 & CD44

Negative: CD5, CD10, CD23 Cyclin D1 ……

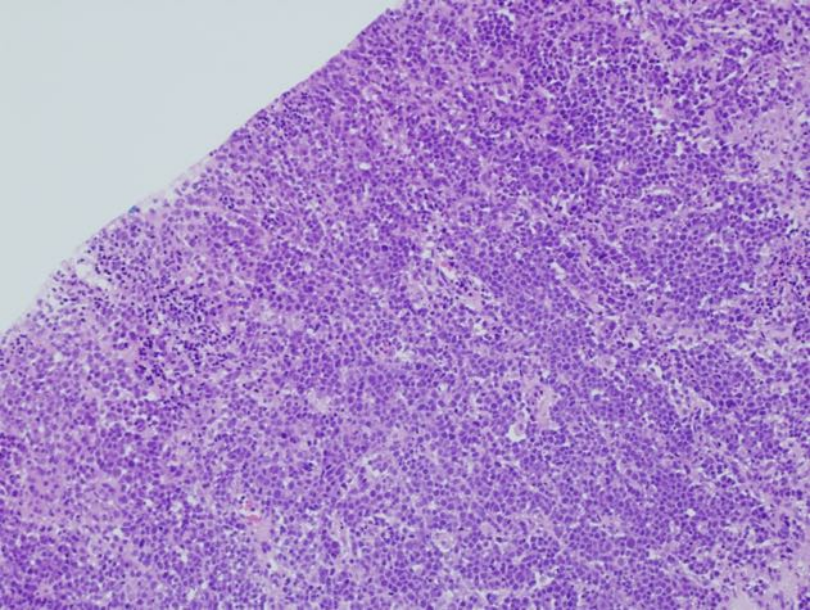
BCL6

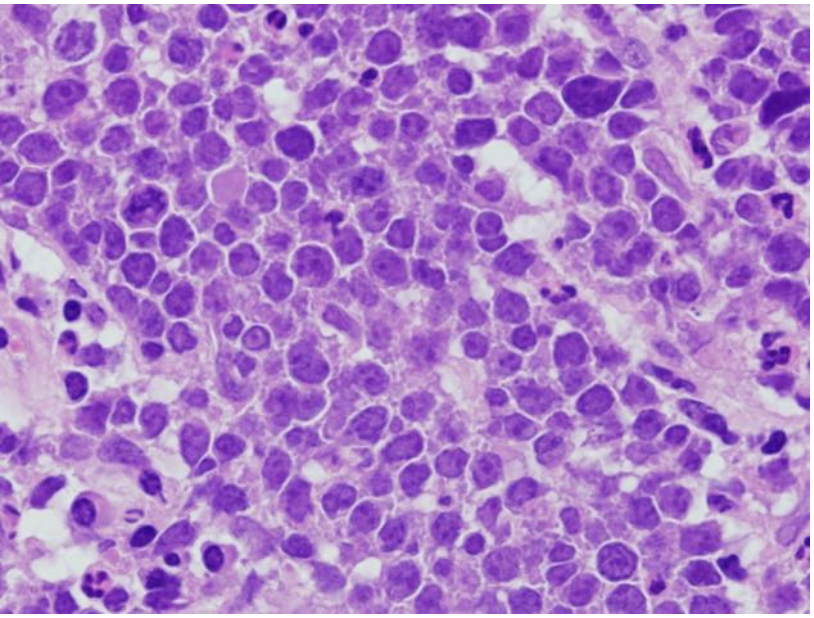
BCL2

FISH:

Additional copies of *BCL2* and *BCL6*; no rearrangement

No rearrangement of *IGH*





70Y Male
Splenomegaly & multiple left large axillary LNs

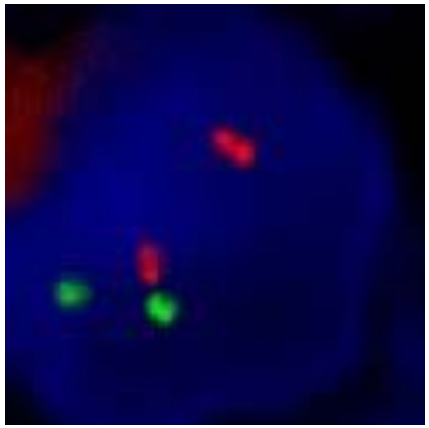
Diagnosis: DLBCL

Immunophenotype:

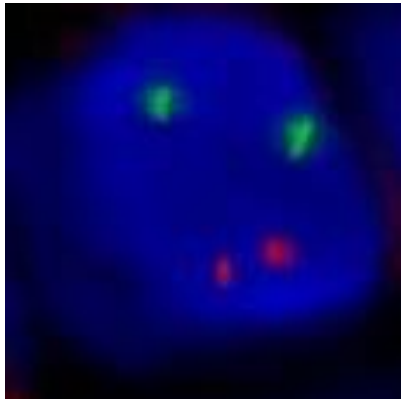
Positive: CD20, CD10, BCL6, BCL2, MUM1

Ki-67>90%

Negative: CD5, Cyclin D1, EBER TdT



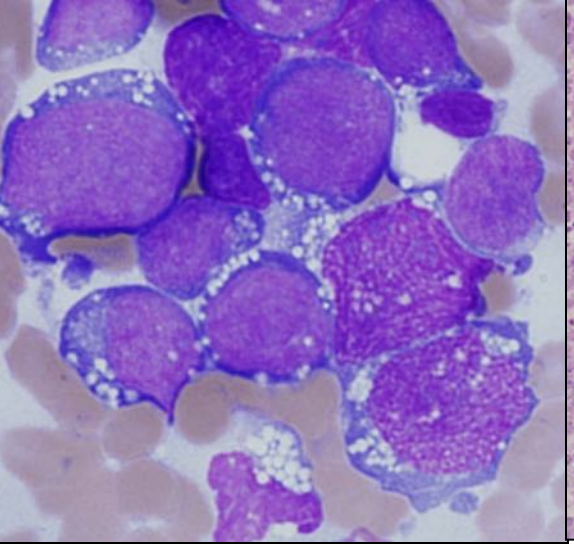
BCL6

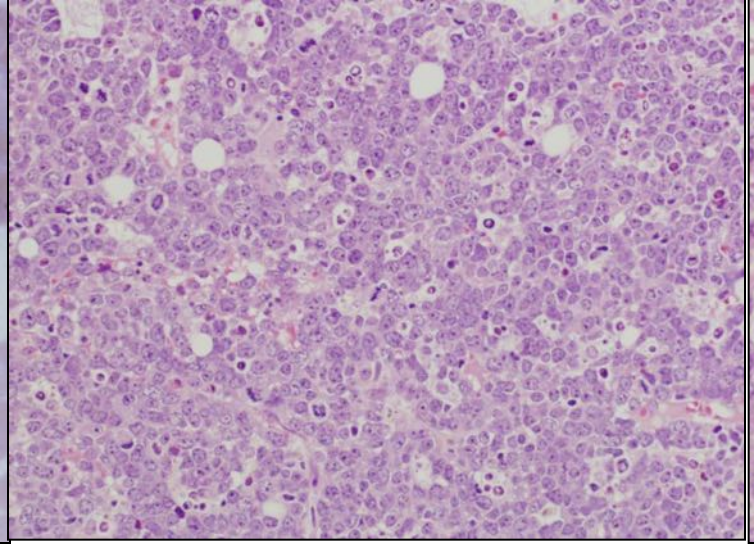


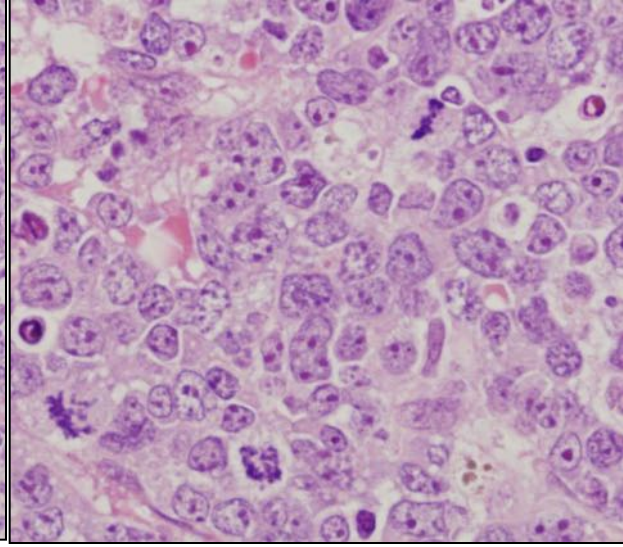
FISH:

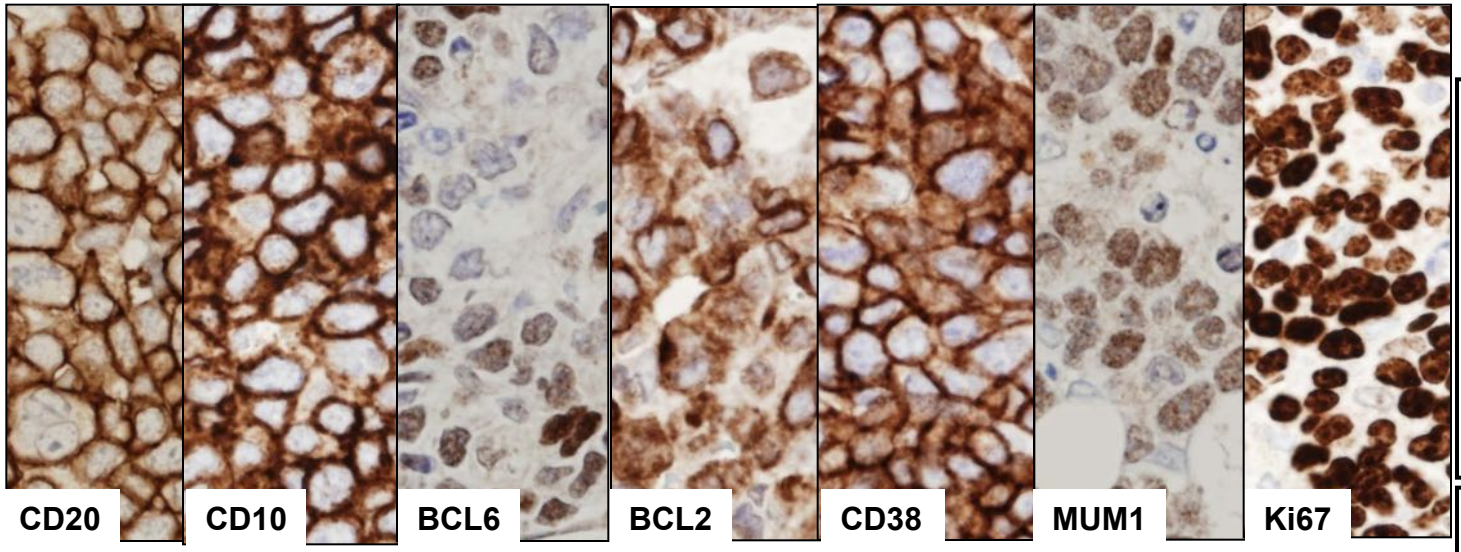
Two copies of rearranged *BCL6*; No normal BCL6

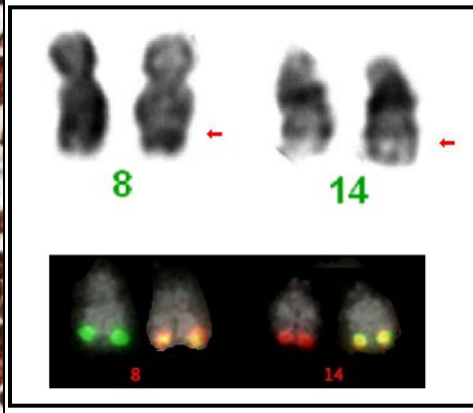
No rearrangement of *BCL2* or *MYC*

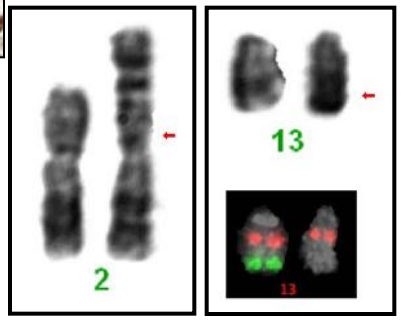












B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

t(8;14)(q24;32); der(2)t(2;7)(p1?3;q?11.2); add(13)(q34)

Interphase FISH studies as ‘diagnostic’ tests

Morphology / immunophenotype	Diagnostic suspicion	Test
Differential diagnosis of splenic marginal zone lymphoma, hairy cell leukaemia and other B cell lymphomas	Splenic marginal zone lymphoma	Del 7q31-32
CD4+ T cell lymphocytosis with cells having features of prolymphocytes	T-cell prolymphocytic leukaemia	t(14;14)(q11; q32)
Features of hepatosplenic T cell lymphoma	Hepatosplenic T cell lymphoma	iso7q

Mutation analysis as ‘diagnostic’ tests

- ***MYD88*** **mutation in lymphoplasmacytic lymphoma**

- ***BRAF*** **mutation in hairy cell leukaemia**

Molecular tests – prognostic markers in current clinical practice

- ***IGVH*** **mutation in CLL and other small B cell lymphomas**

- ***TP53*** **mutation**

FISH tests – prognostic markers in current clinical practice

- ***TP53*** **deletion**
- ***API2-MLT1*** **translocation in gastric MALT lymphoma**
- **CLL: 13q- (good prognosis)**
  +12, 11q-, 17p- (poor prognosis)

Interaction with clinical/biomedical scientists – pre-analytical

- **Mark the most involved area on the section for FISH analysis – saves reagents and time!**

- **Mention the content of B cells, T cells or presumed neoplastic cells for clonality tests – beware of pseudoclonality due to low-levels of specific template**

- **Mention the provisional histological diagnosis for clonality tests -**

  -somatic hypermutation process can hamper primer binding and result in false negative test results

  - florid reactive process may show oligoclonality/ monoclonality.

- **Ideal for cellular pathologists involved in haematopatholgy and staff in involved in molecular pathology to be located in the same laboratory or work area**

Interaction with clinical/biomedical scientists – post-analytical

- **Get involved in fluorescent microscopy in cases posing difficulties in interpretation of FISH results – most cases are straight forward.**

- **Closer interaction with biomedical/clinical scientists is preferred for reporting of antigen receptor gene rearrangements.**

- **Involve biomedical/clinical scientists in integrated reporting.**

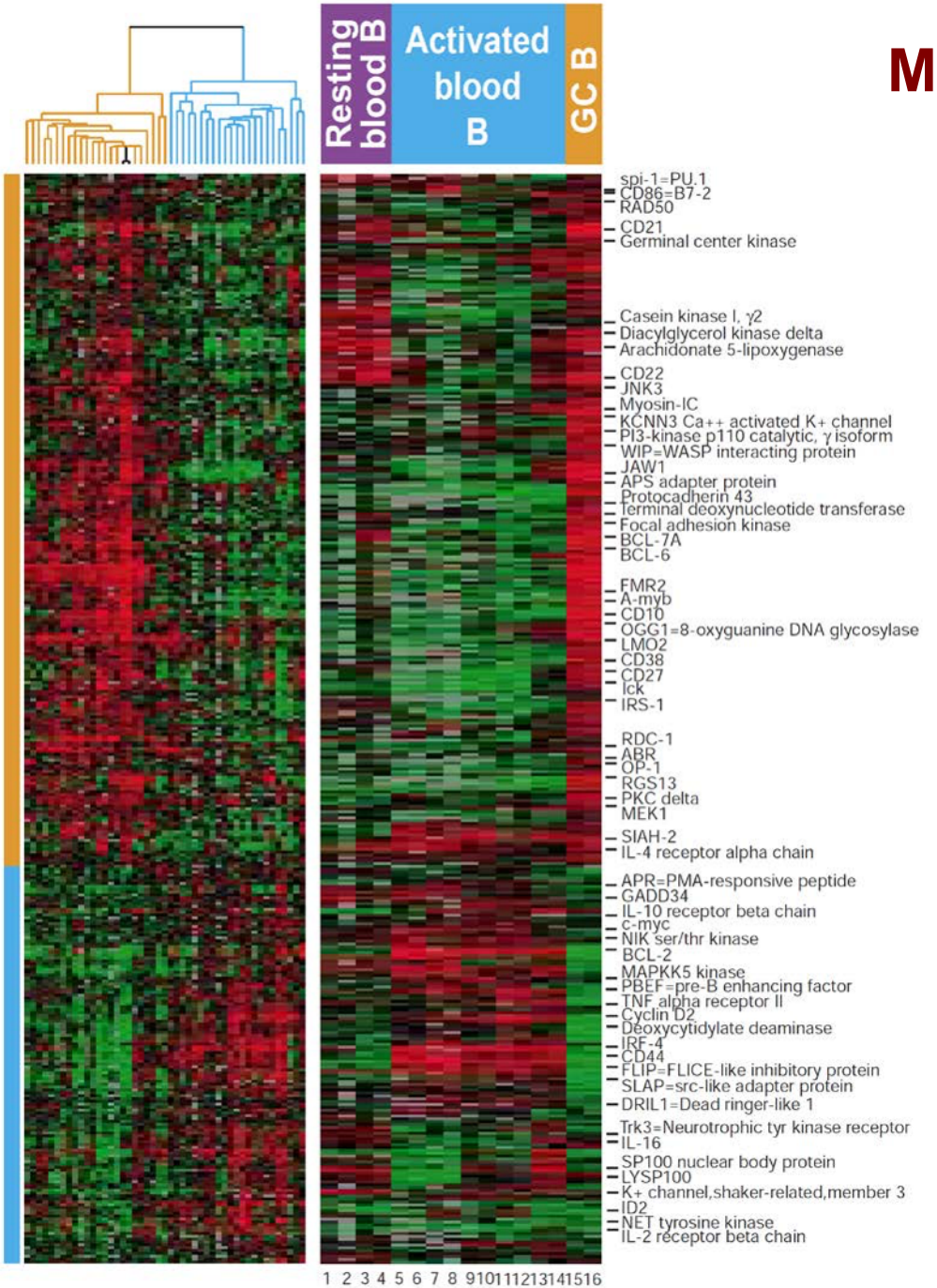
False positive results commonly encountered with antigen receptor gene rearrangement studies

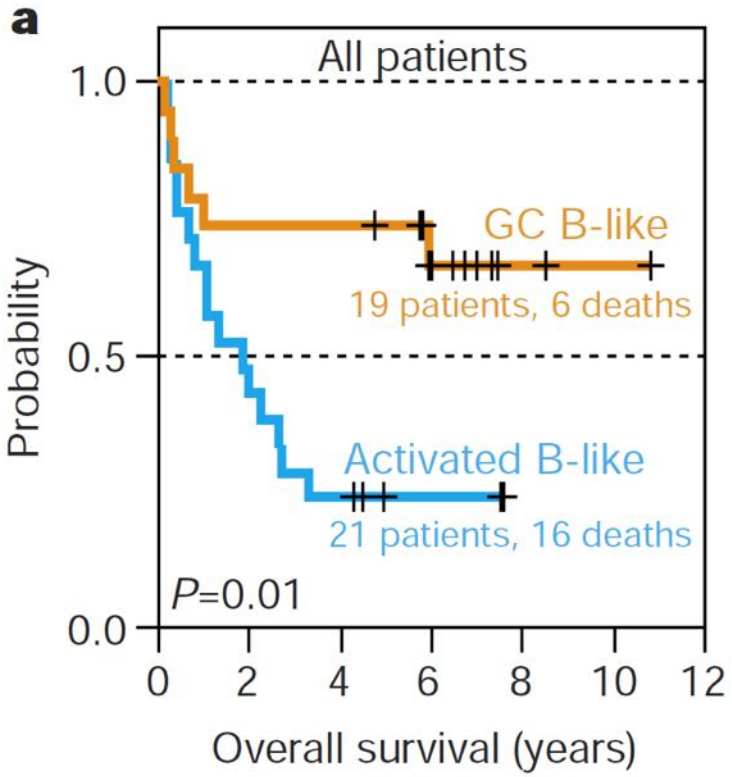
- Contamination
- Pseudoclonality (small biopsies)
- Reactive / inflammatory pathology: H.pylori gastritis; Hepatitis; viral infections; Sjögren's syndrome, Rheumatoid arthritis
- Canonical TCRγ
- Immune reconstitution following BMT
- Immune response to tumour
- Clonal lymphoid infiltrates in skin

False negative results commonly encountered with antigen receptor gene rearrangement studies

- **Sample issues: representativeness, fixation issues, degradation of DNA**
- **Technical: Not using the complete panel of primers**
- **Precursor B cell expansions:**
  - **Partial DJ rearrangements**
  - **Oligoclonal (1/3 of B-ALL)**
  - **Ongoing rearrangements at relapse**
- **Germinal centre and post-germinal centre expansions:**
  - **Somatic hypermutations**
  - **IgH deletion**

Molecular subtyping of DLBCL



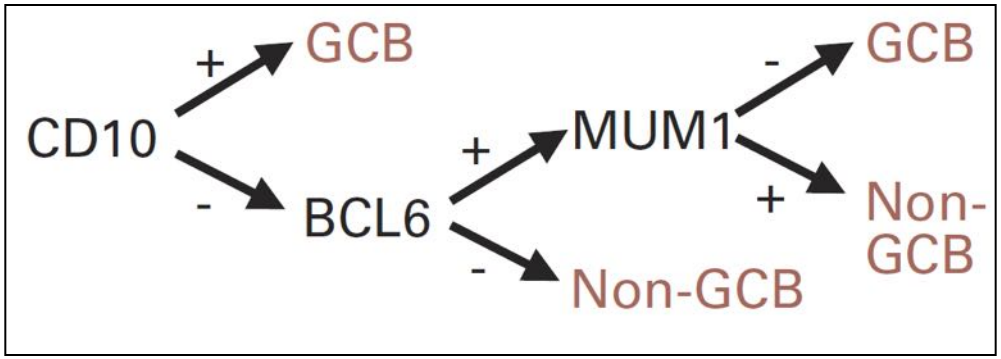


Alizadeh AA. Nature. 2000 Feb 3;403(6769):503-11

DLBCL molecular subtypes

Immunohistochemistry based algorithms show concordance with GEP
All the algorithms tested showed significant difference in survival

Hans et al , 2004

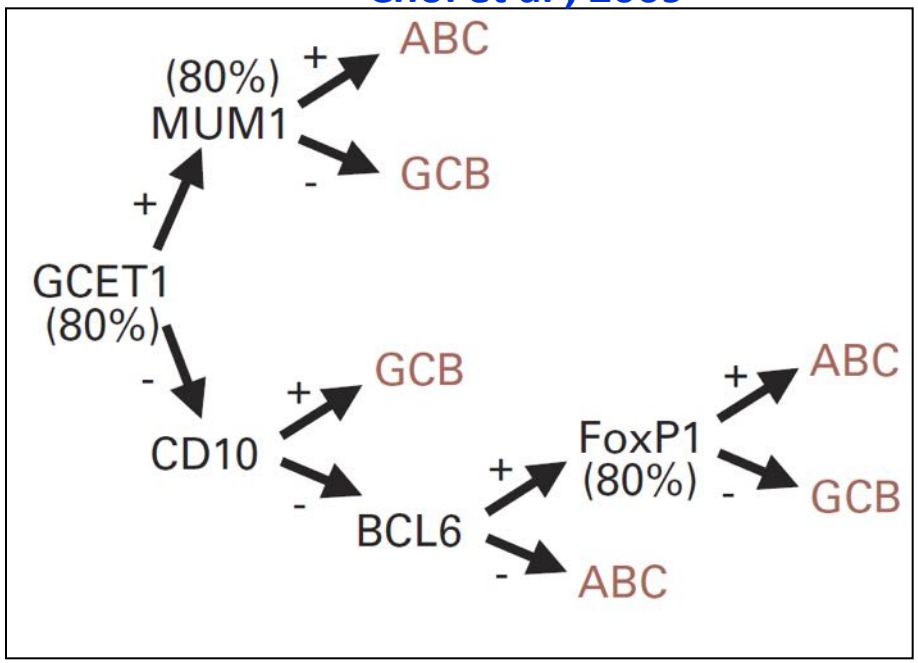


Natkunam et al , 2008

LMO2 ≥ 30% → GCB

LMO2 < 30% → ABC

Choi et al , 2009



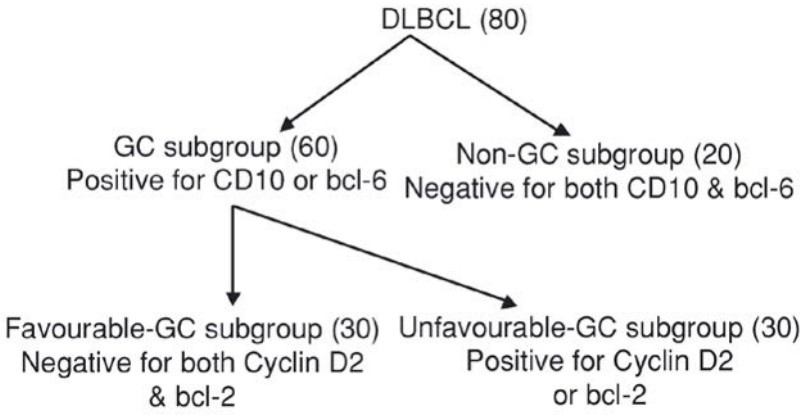
Meyer et al , 2011

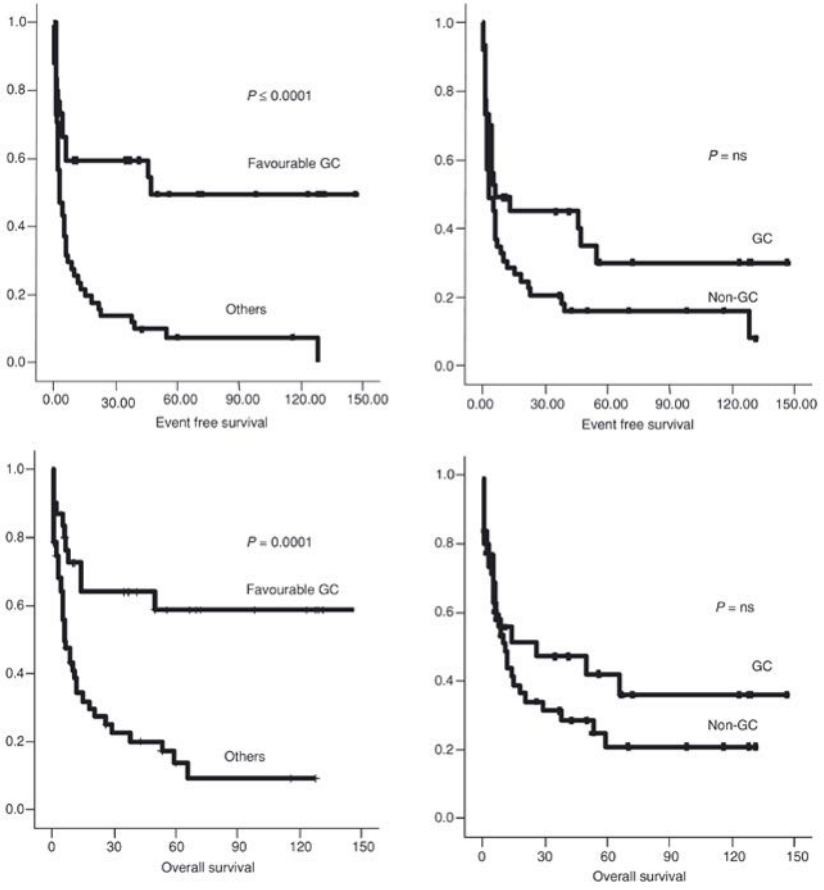
GCB	ABC	Score
CD10 (+ or -)	Mum1 (+ or -)	GCB > ABC
GCET1 (+ or -)	FoxP1 (+ or -)	or
Score (0, 1, 2)	Score (0, 1, 2)	ABC > GCB

If GCB Score = ABC Score:
- LMO2 ≥ 30% → GCB
- LMO2 < 30% → ABC

Meyer PN et al; J Clin Oncol.2011 Jan 10;29(2):200-7.

DLBCL – Molecular subtypes
Alternate algorithms



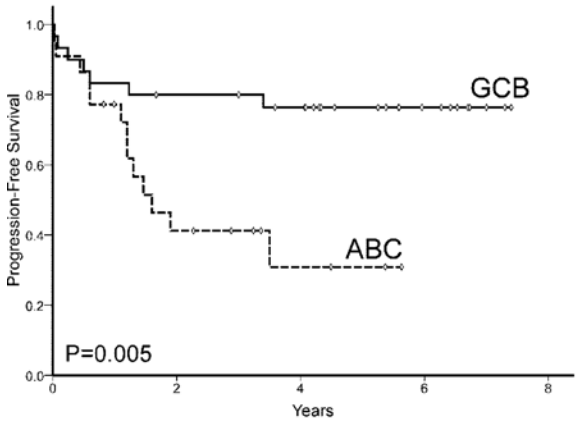


Amen F et al. Histopathology. 2007 Jul;51(1):70-9.

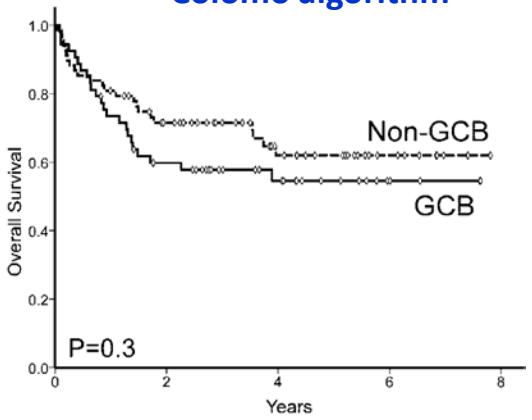
DLBCL molecular subtypes

Comparison of impact of immunohistochemistry-based algorithms & GEP-based classification on overall survival
62 patients on immuno-chemotherapy

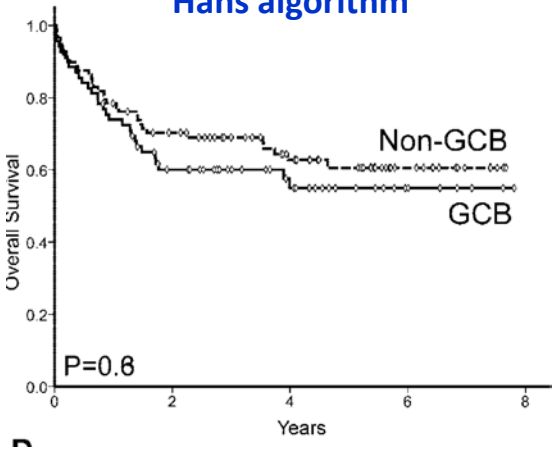
GEP



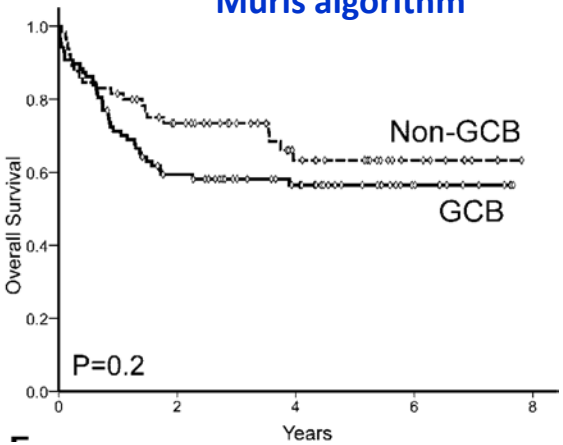
Colomo algorithm



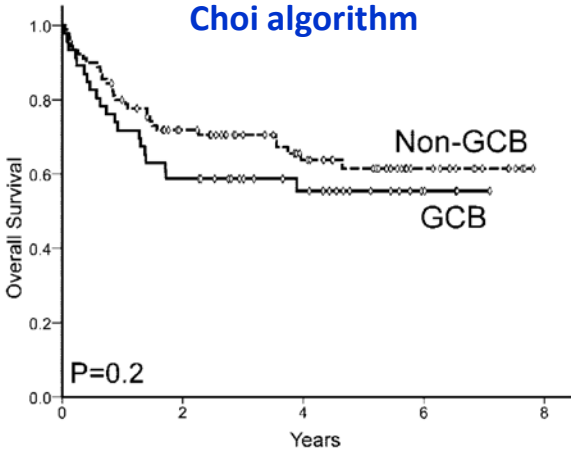
Hans algorithm



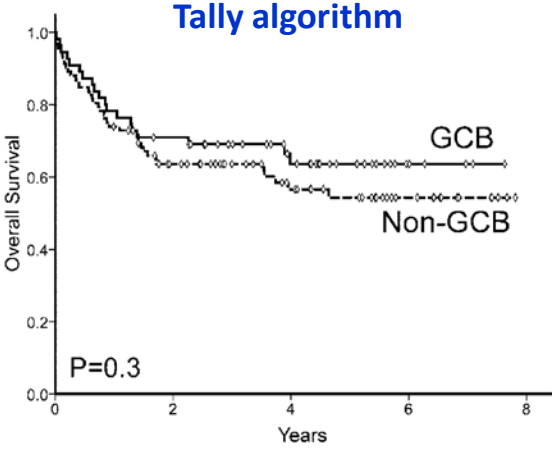
Muris algorithm



Choi algorithm



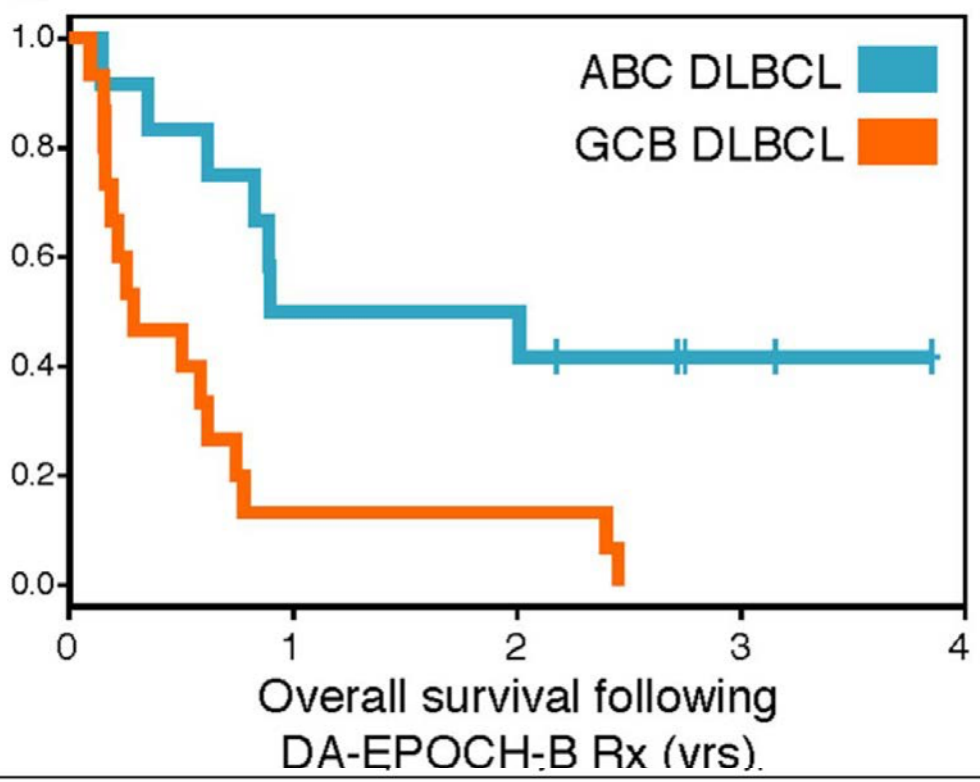
Tally algorithm



Misclassification of GEP-defined GCB by immunohistochemistry based algorithms: 30-60%

Gutiérrez-García G et al; Blood. 2011 May 5;117(18):4836-43.

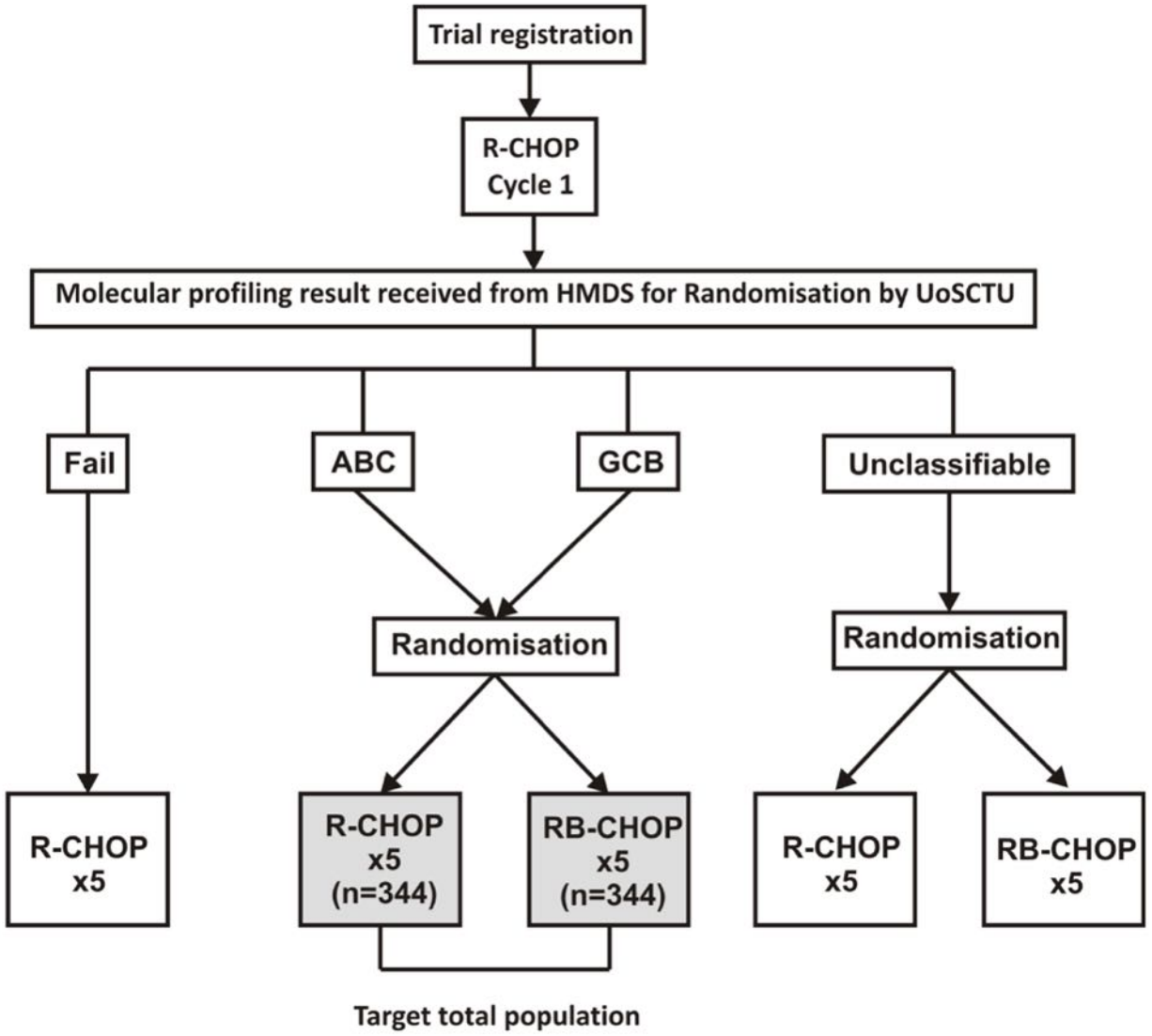
Impact of Bortezomib on molecular subsets of relapsed DLBCL



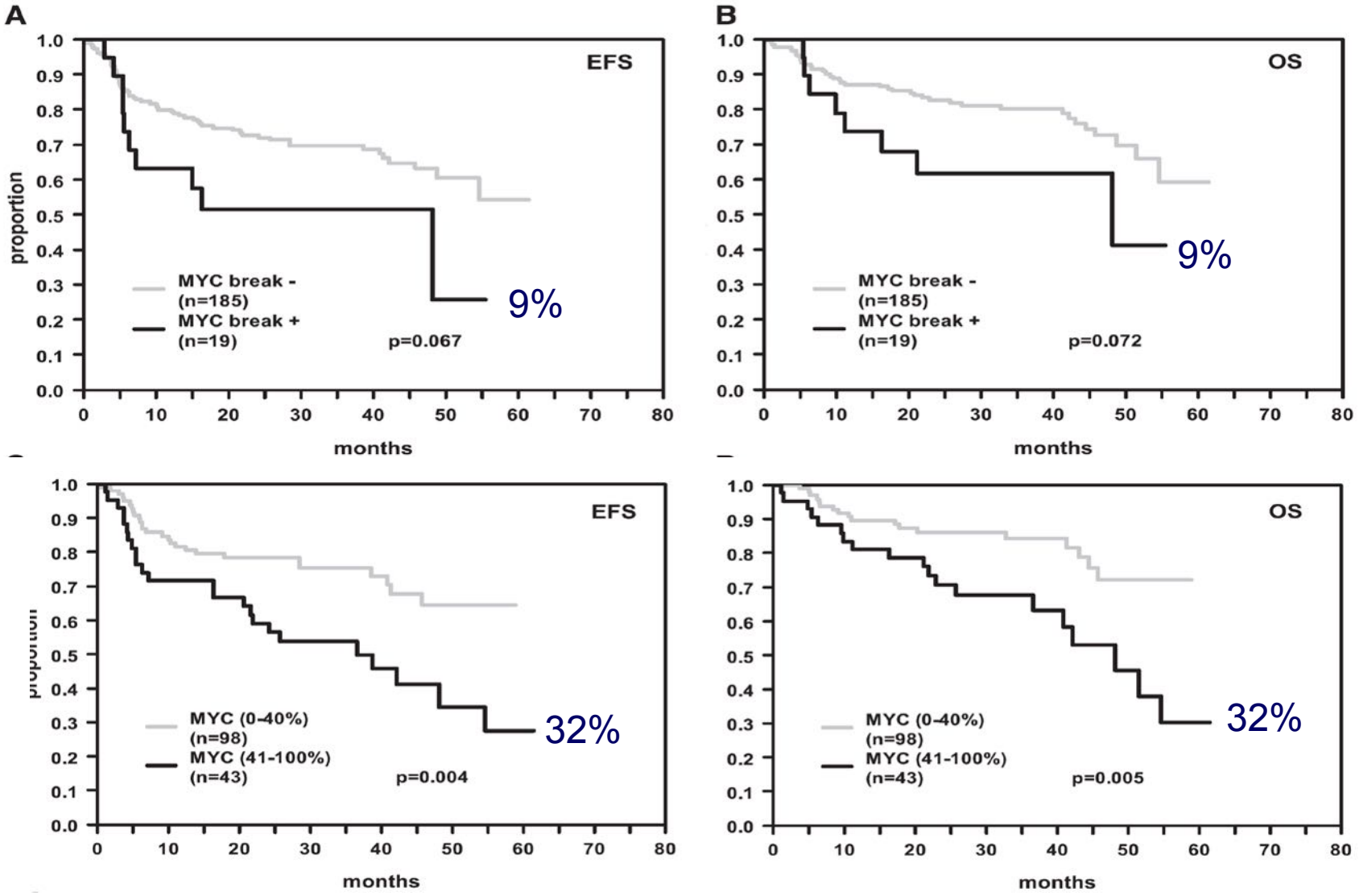
Treatment group	n (%)	Response, n (%)			P*
		Complete	Partial	None	
All patients	44	8 (18)	7 (16)	29 (66)	
DLBCL (de novo)†	31 (70)	7 (23)	6 (19)	18 (58)	.63
Molecular subtypes‡	27	6 (22)	6 (22)	15 (56)	
ABC DLBCL	12 (44)	5 (41.5)	5 (41.5)	2 (17)	
GCB DLBCL	15 (56)	1 (6.5)	1 (6.5)	13 (87)	< .001

Dunleavy K et al.Blood. 2009 Jun 11;113(24):6069-76.

REMoDL-B study
Univ. of Southampton, UK
Hypothesis: Bortezomib improves survival in ABC-DLBCL subset

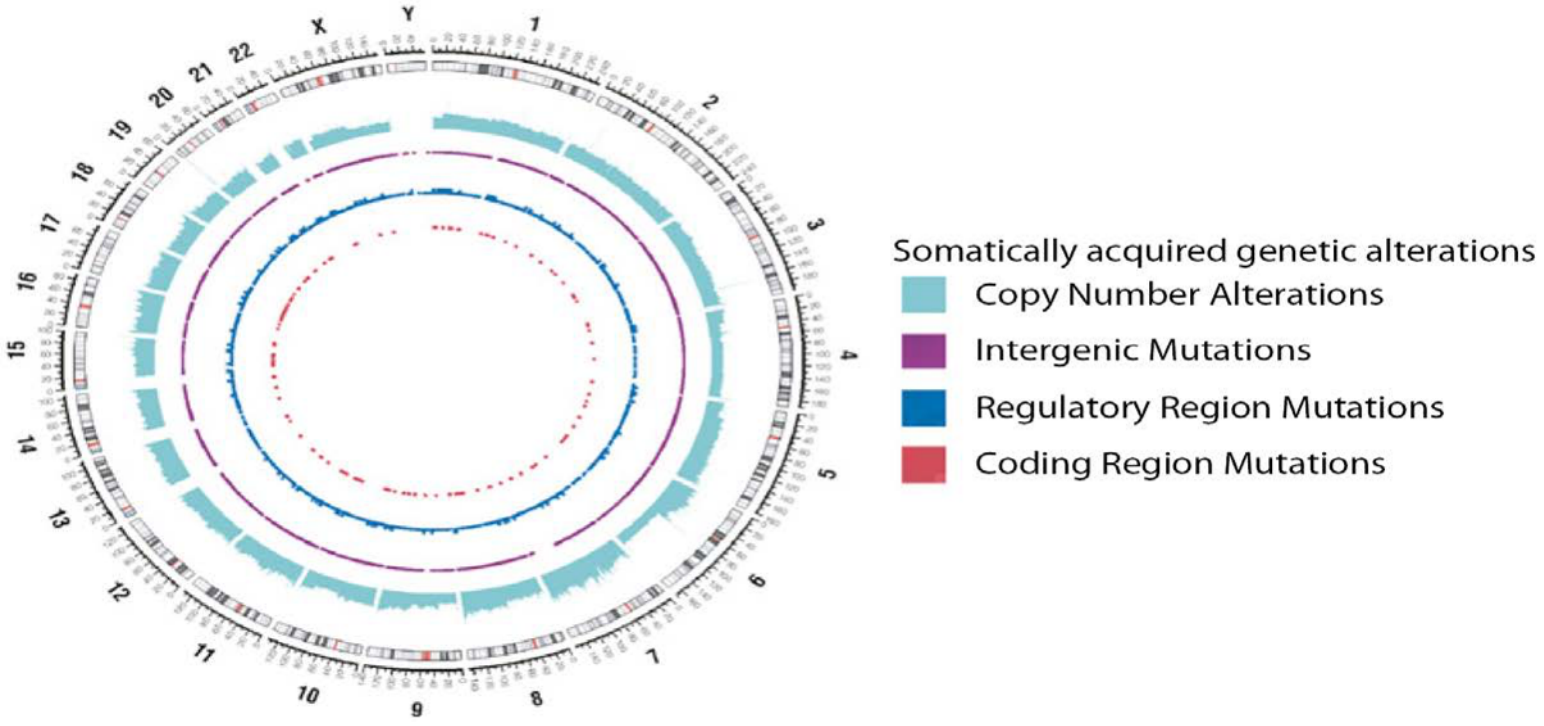


MYC translocation and protein expression in DLBCL



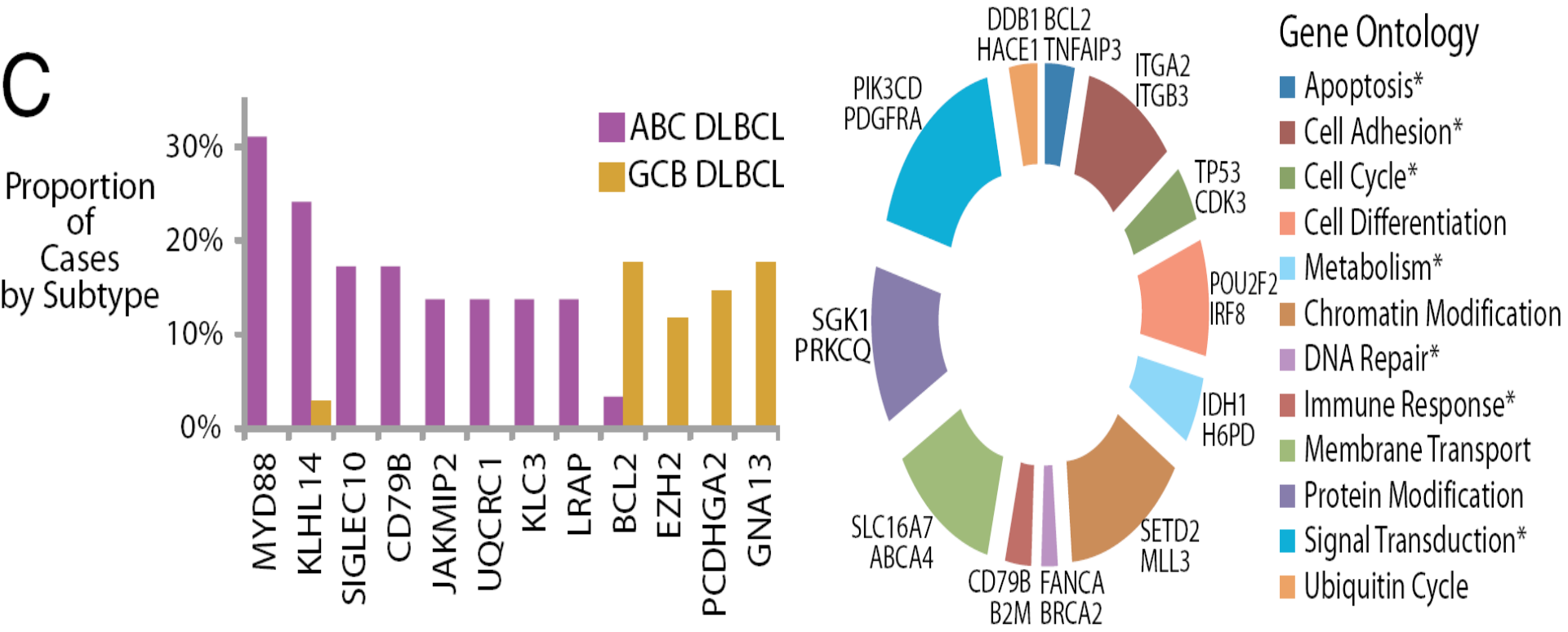
Horn et al, Blood. 2013;121(12):2253-2263

Genomic alterations in DLBCL



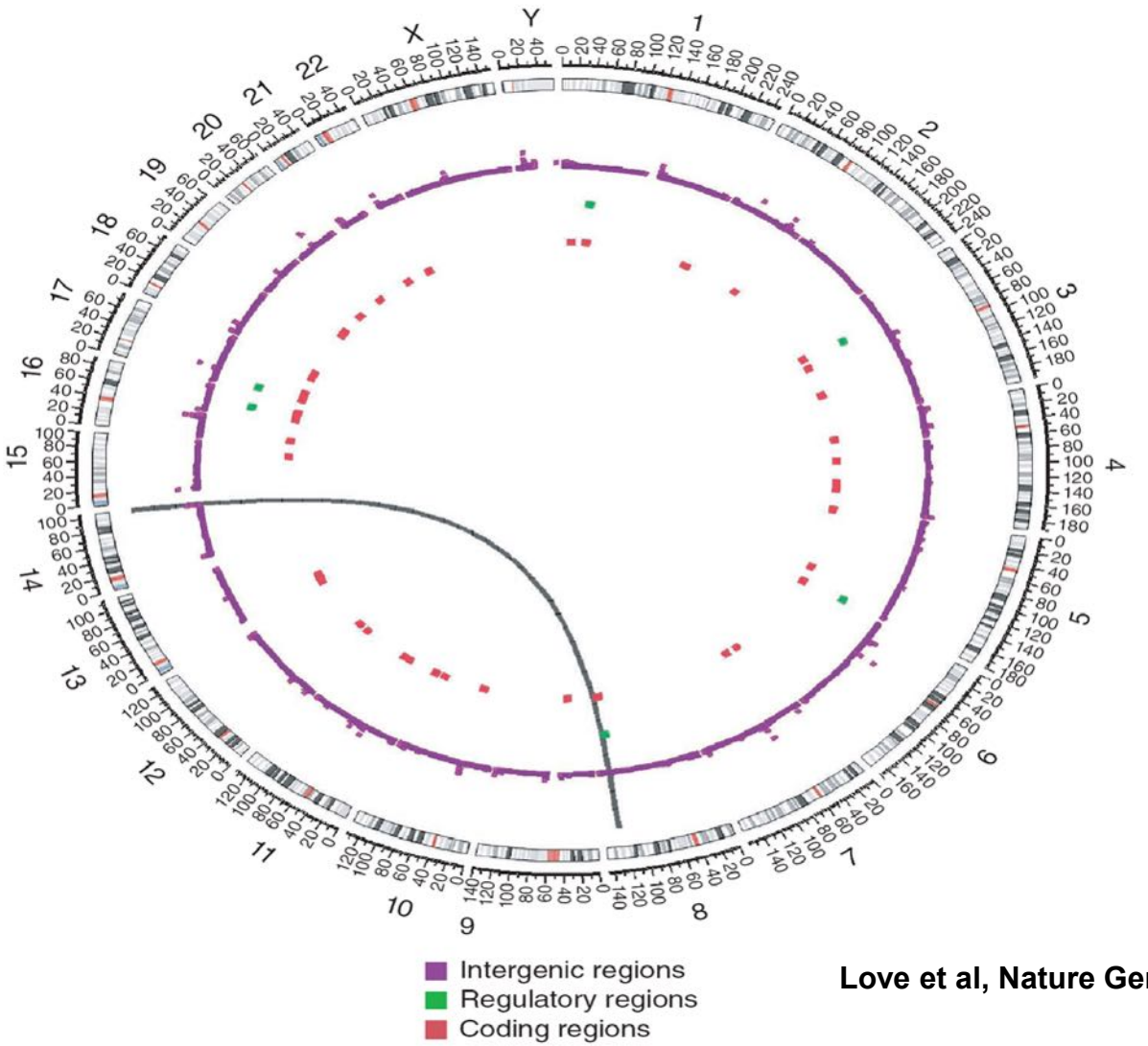
Zhang et al, PNAS 2012

Genomic alterations in DLBCL



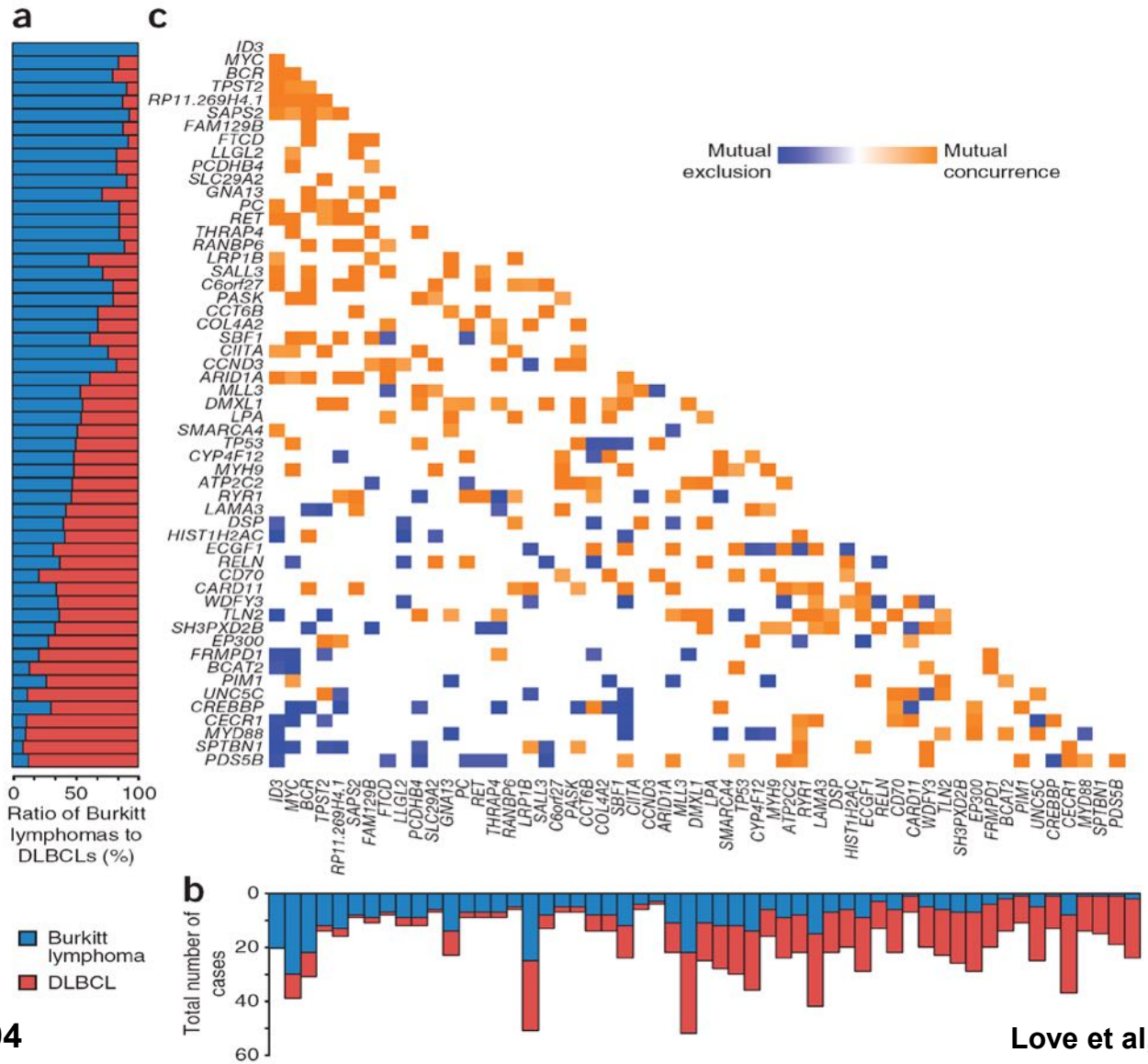
Zhang et al, PNAS 2012

Genomic alterations in Burkitt lymphoma



Love et al, Nature Genetics, 2012

Mutations in BL vs. DLBCL



BL:59; DLBCL:94

Love et al, Nature Genetics, 2012

Lymphoma diagnosis and work-up

- **Targeted NGS platforms for mutation based disease classification, prognostication/prediction and identification of drug-able targets.**

- **Immunohistochemistry based assays as surrogates for mutations?**

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