Molecular Tools in the Diagnosis of Lymphoma

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Learning Objectives

- To be familiar with common algorithms that incorporate FISH testing in the work up of diffuse large B-cell lymphoma
- To understand when it is appropriate to use molecular clonality testing in the work up and diagnosis of lymphoma
- To be familiar with the limitations and “pitfalls” of clonality testing

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

Steven H. Swerdlow,[1] Elias Campo,[2] Stefano A. Pileri,[3] Nancy Lee Harris,[4] Harald Stein,[5] Reiner Siebert,[6] Ranjana Advani,[7] Michele Ghielmini,[8] Gilles A. Salles,[9] Andrew D. Zelenetz,[10] and Elaine S. Jaffe[11]

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
γ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
*Large B-cell lymphoma with IRF4 rearrangement**
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
*EBV$^+$ mucocutaneous ulcer**
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
Primary effusion lymphoma
*HHV8$^+$ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma
Mature T and NK 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 lymphoma of childhood*
Hydroa vacciniforme–like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract**
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 γδ T-cell lymphoma
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8$^+$ T-cell lymphoma**
*Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large-cell lymphoma, ALK$^+$
Anaplastic large-cell lymphoma, ALK$^-$*
*Breast implant–associated anaplastic large-cell lymphoma**
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

Provisional entities are listed in italics.
*Changes from the 2008 classification.

Modifications to DLBCL category

- DLBCL
  - Germinal center B-cell type
  - Activated B-cell type
- TCHRLBCL
- Primary CNS
- Primary cutaneous DLBCL, leg type
- EBV+ DLBCL, NOS
- EBV+ mucocutaneous ulcer
- Intravascular LBCL
- High grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements
- High grade B-cell lymphoma, NOS
- ALK+ large B-cell lymphoma
- HHV8+ DLBCL, NOS
- Large B-cell lymphoma with IRF4 rearrangement

Diffuse Large B-cell Lymphoma

- How do you work this up?
- What is sufficient?

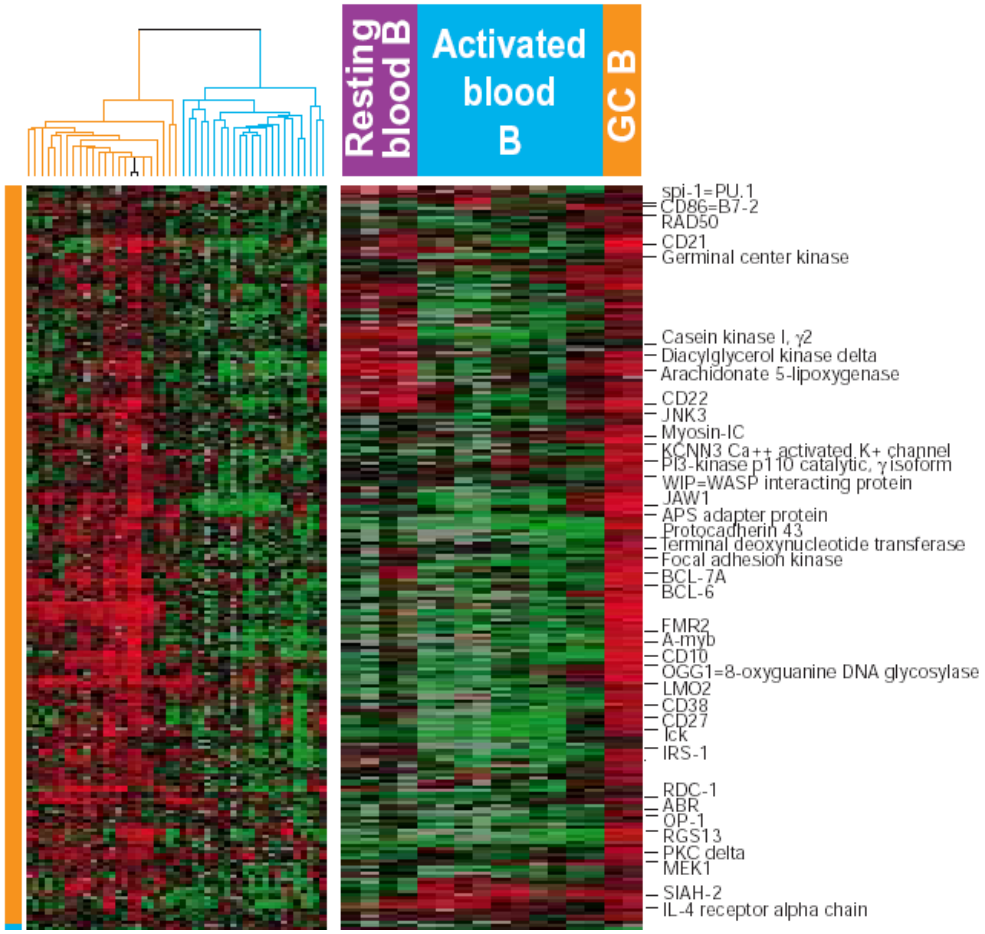
Diffuse Large B-cell Lymphoma Ancillary Testing

- Ancillary testing for sub-classification and/or prognostic information
  - GC vs. non-GC subtyping
  - FISH for *MYC*, *BCL2*, *BCL6*
  - Immunohistochemistry for MYC, BCL2
  - ISH for EBV (EBER)


POLICE BOX
POLICE BOX

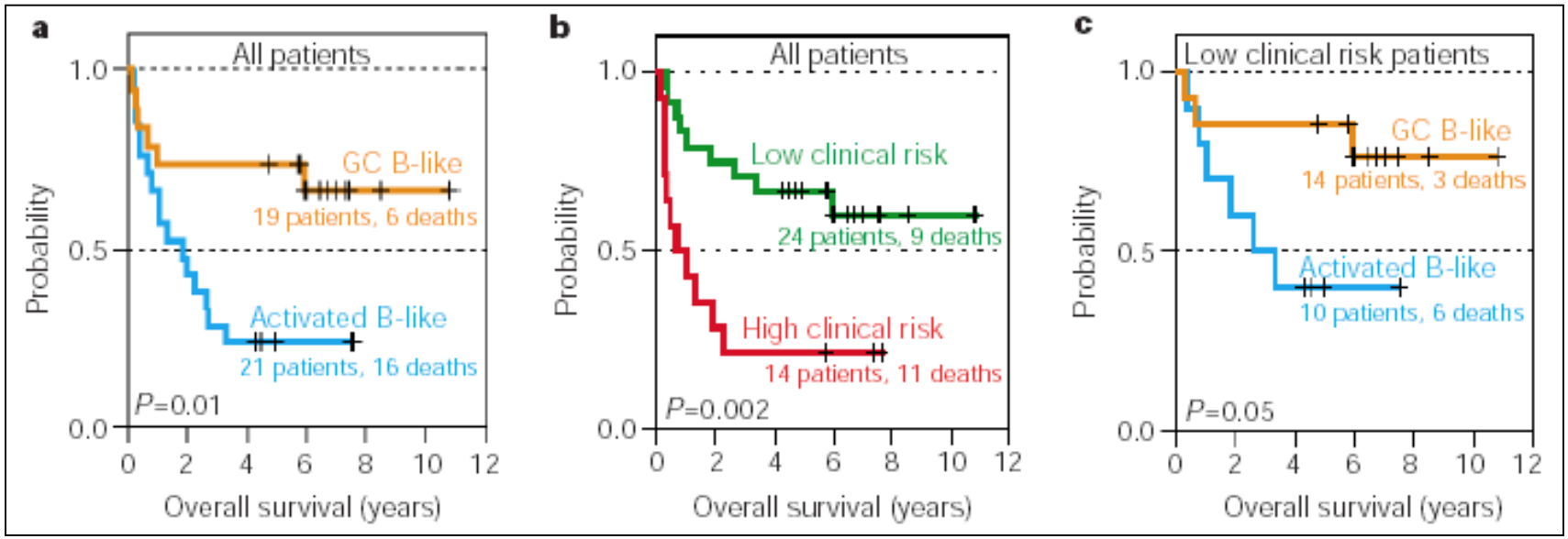
Microarray analysis identified two distinct gene expression patterns in DLBCL

- Germinal center B-cell (GC) group
- Activated B-cell (ABC) group
- 50-60% of adult DLBCL are GC



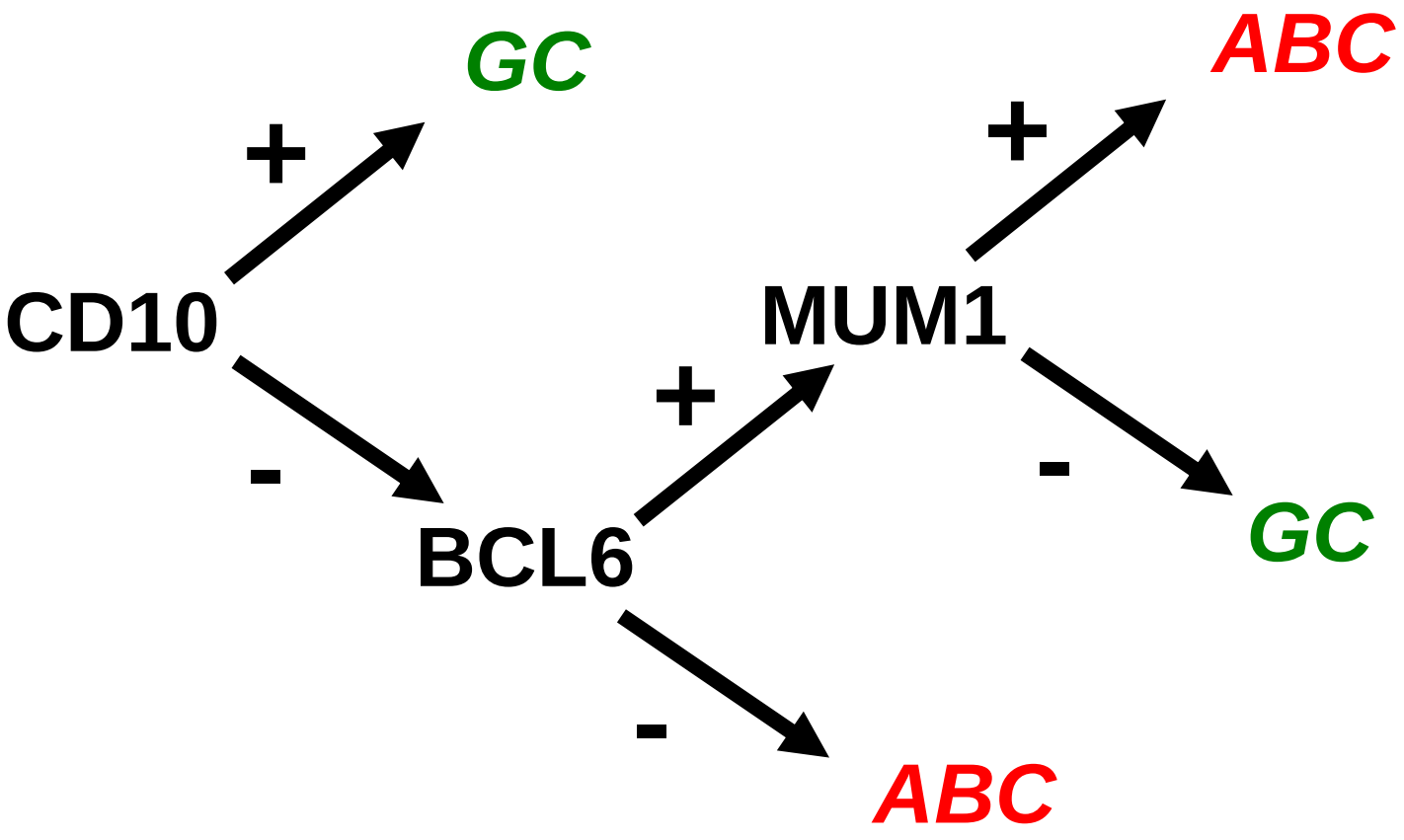
Alizadeh *et al.*, Nature 403:503, 2000

GC gene expression profiles were associated with a better overall survival, independent of IPI



Alizadeh *et al.*, Nature 403:503, 2000

IHC Subtypes of DLBCL



Hans *et al.*, Blood, 2004

2011: R-CHOP Era
Outcome of DLBCL according to **molecular** subtype (GCB vs ABC).

blood
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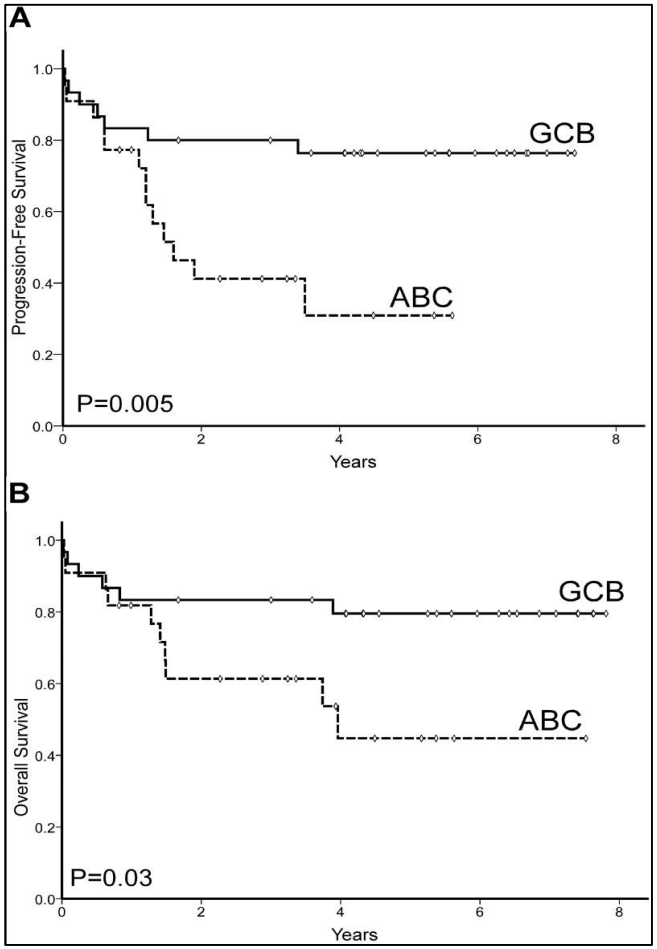
Progression-free survival

Overall survival



Patients treated with R-CHOP (n=52)

Gutiérrez-García G et al. Blood 2011;117:4836-4843

blood
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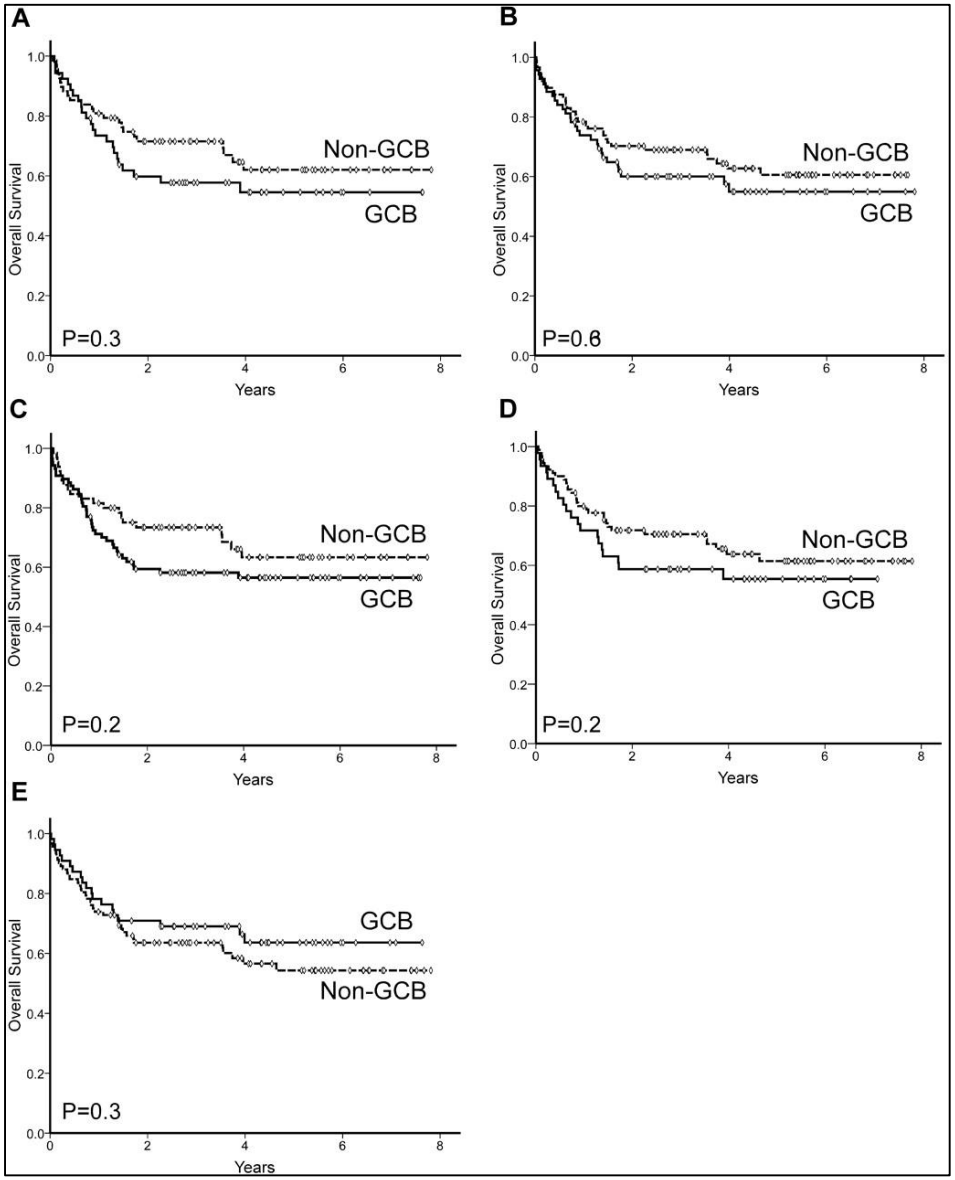
Outcome of 157 DLBCL patients according to GCB vs non-GCB profile as assessed by 5 immunohistochemistry algorithms.



IHC Algorithms:
A: Colomo
B: Hans
C: Muris
D: Choi
E: Tally

Overall survival

Patients treated with R-CHOP

Gutiérrez-García G et al. Blood 2011;117:4836-4843

Cell of Origin Subtyping in DLBCL

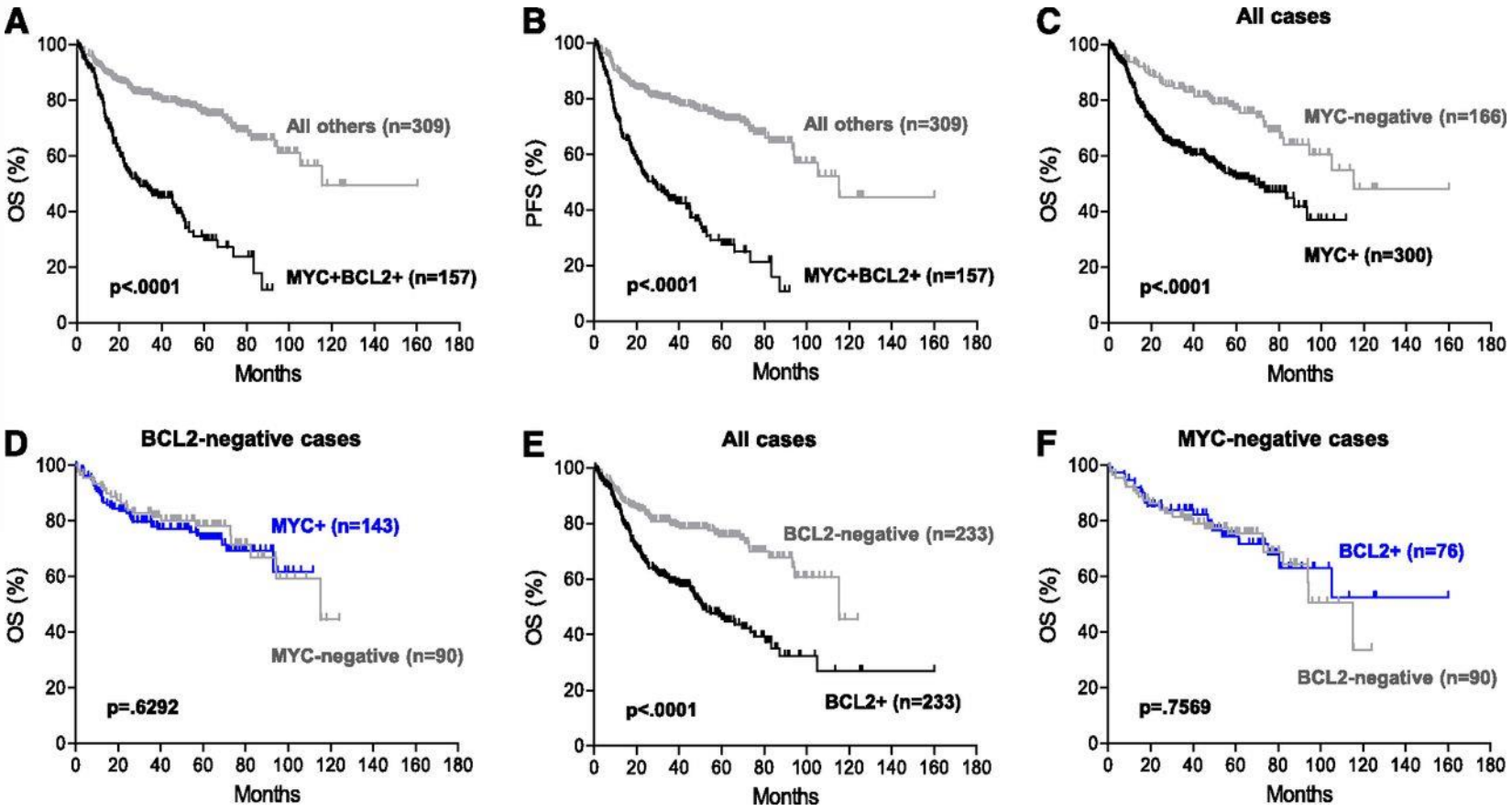
- Difference in prognosis is smaller in patients treated with R-CHOP than CHOP.
- Gene expression profiling can still segregate these groups.
- Immunophenotyping approaches cannot reliably separate groups with distinct prognoses.
- Testing may have emerging role for guiding targeted therapy.

MYC and BCL2 Rearrangements and Protein Expression: Inform Prognosis and Guide Therapy

- Diffuse large B-cell lymphoma, NOS
- Double-expresser (DE) DLBCL, NOS
  - Expresses MYC (>40%) and BCL2 (>50%) protein
  - Poor prognosis
- High grade B-cell lymphoma double hit (HGBL-DH), 4-6% of DLBCL.
  - MYC/BCL2, 80% (includes 20% triple hit).
  - MYC/BCL6, 20%.

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

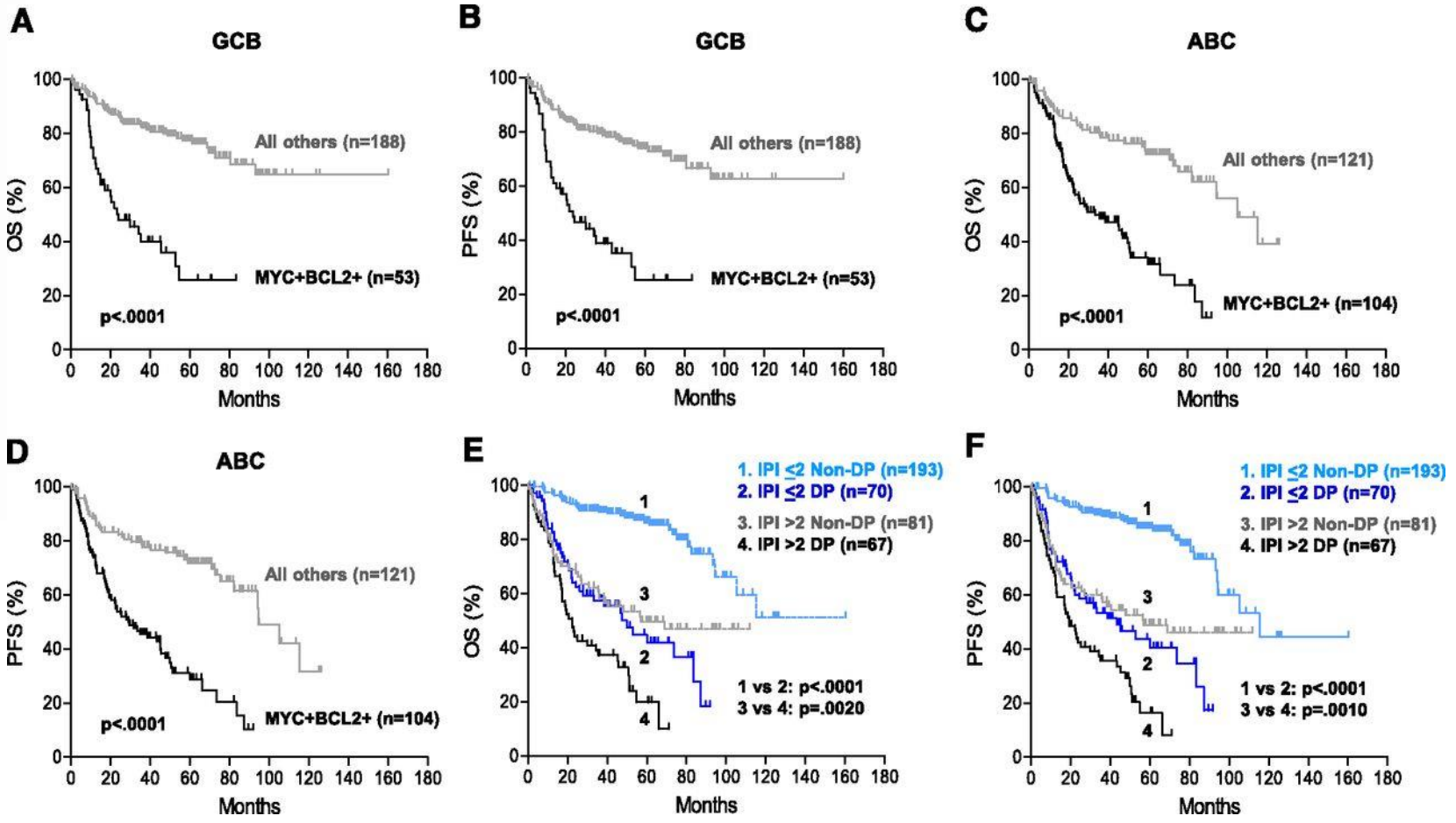
Prognostic impact of MYC/BCL2 coexpression in DLBCL. (A-B) OS (A) and PFS (B) of patients with DLBCL with MYC/BCL2 coexpression (MYC+BCL2+) in the training set.



Hu S et al. Blood 2013;121:4021-4031

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Prognostic impact of MYC/BCL2 coexpression in DLBCL risk-stratified according to clinicopathologic parameters.



Hu S et al. Blood 2013;121:4021-4031

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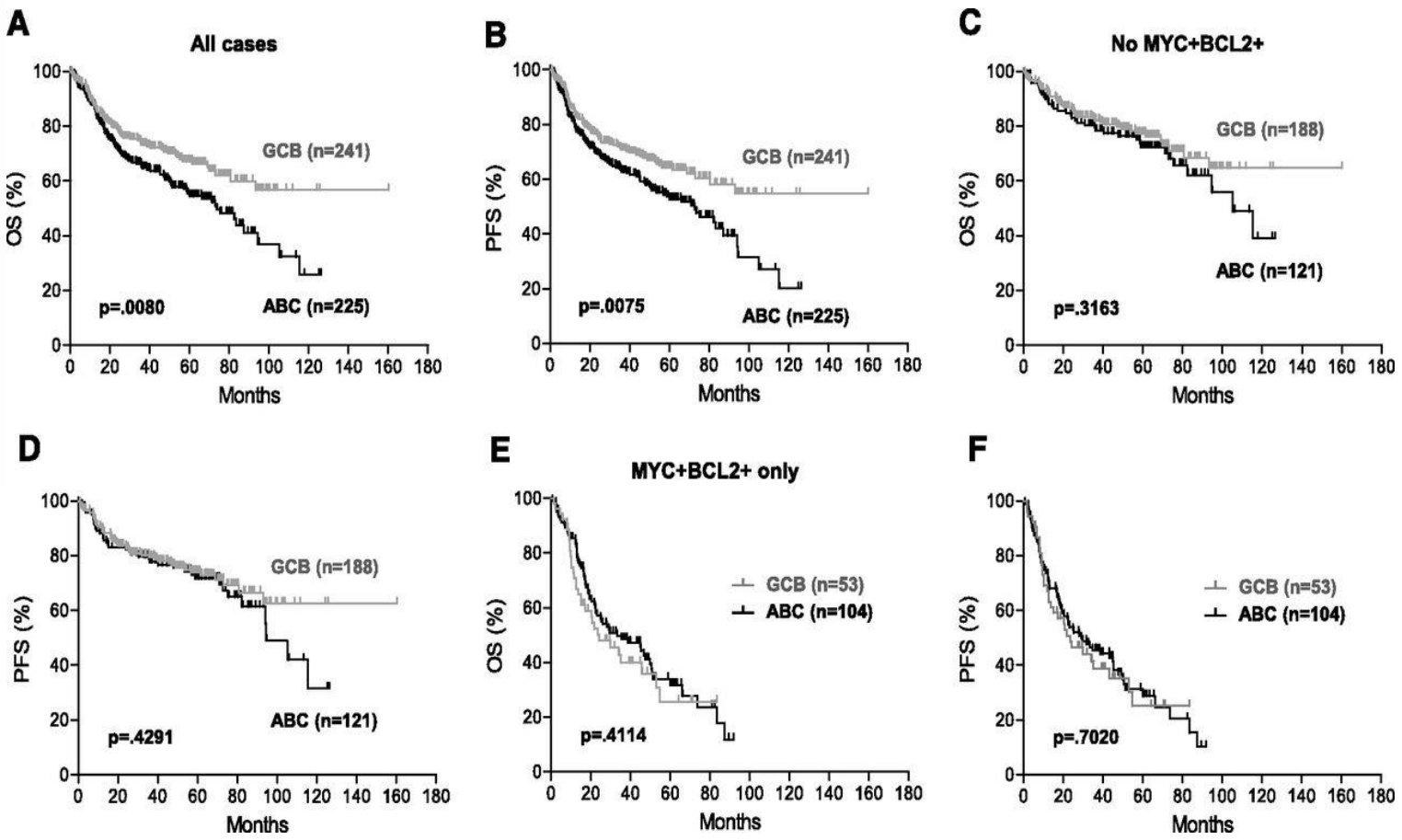
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

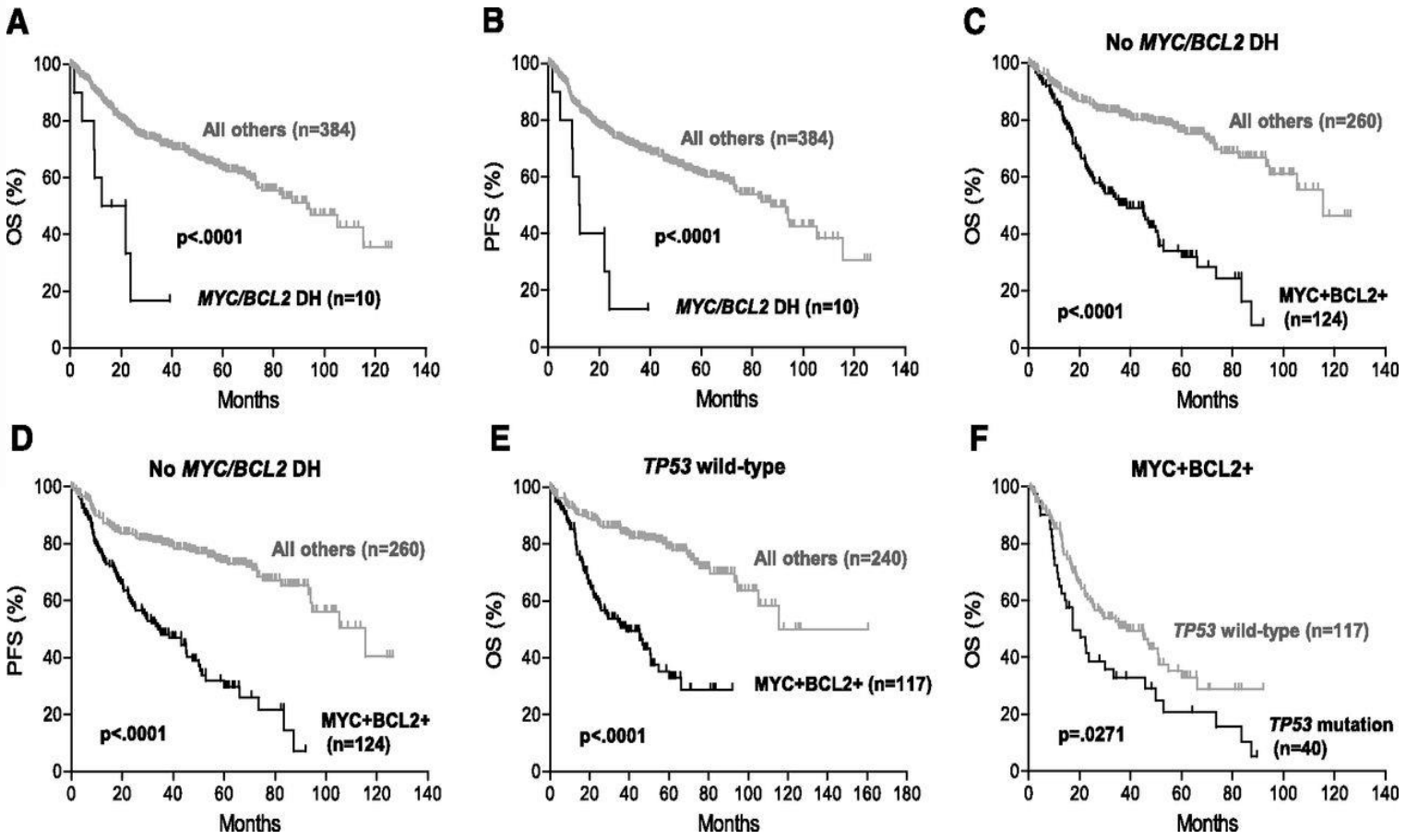
MYC/BCL2 coexpression contributes to the inferior prognosis of ABC-DLBCL.



Hu S et al. Blood 2013;121:4021-4031

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Prognostic impact of MYC/BCL2 coexpression in DLBCL is independent of MYC/BCL2 corearrangement and TP53 mutation status.



Hu S et al. Blood 2013;121:4021-4031

Key Points from Hu *et al.*

- MYC/BCL2 protein co-expression is found in ~30% of de novo DLBCL.
- These patients have a poor clinical outcome with a 5-year OS and PFS of <30%.
- MYC/BCL2 co-expression correlates with ABC subtype, so the latter is NOT an independent negative prognostic factor.
- MYC/BCL2 co-expression is a negative prognostic factor *independent* of MYC/BLC2 double hit.
- MYC/BCL2 co-rearranged (double hit) DLBCLs are rare (10/394 cases); 8/10 had MYC/BCL2 protein co-expression.

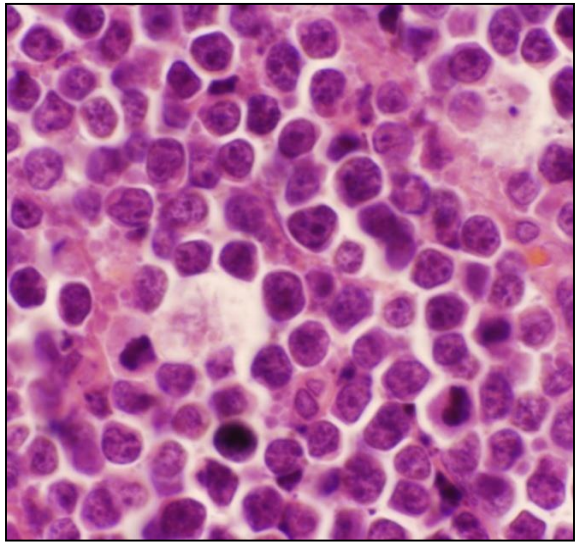
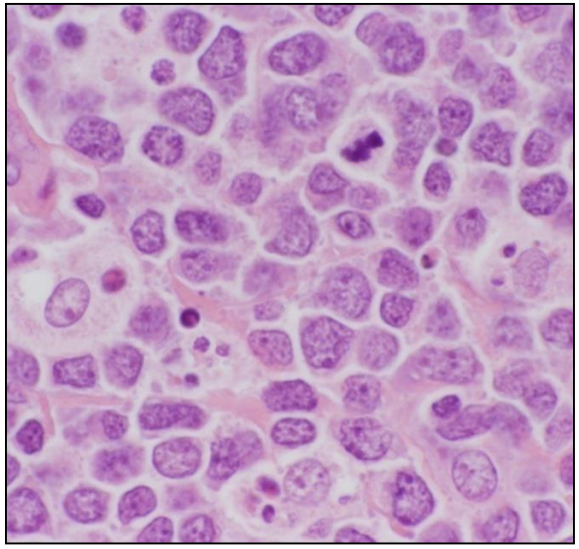
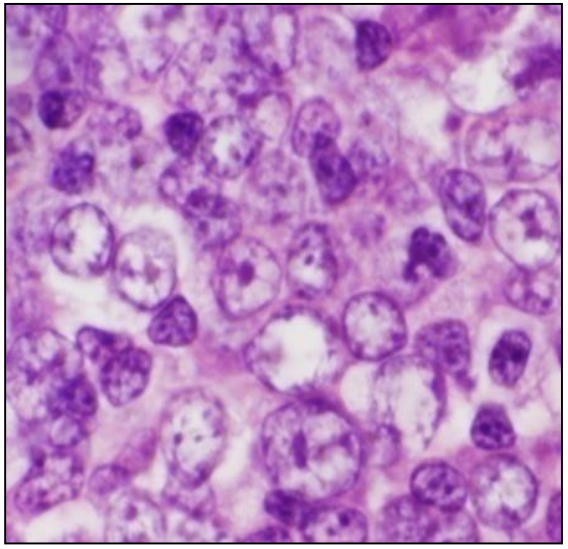
MYC/BCL2 Co-Expression Contributes to Inferior Prognosis of ABC subtype

- Presence of MYC/BCL2 co-expression was significantly correlated with the ABC subtype.
- After excluding patients with MYC/BCL2 co-expression, the prognosis of patients with ABC subtype was similar to that of GCB subtype.

Hu *et al.*, Blood 121:4021-31, 2013.

"Double Hit" Lymphoma

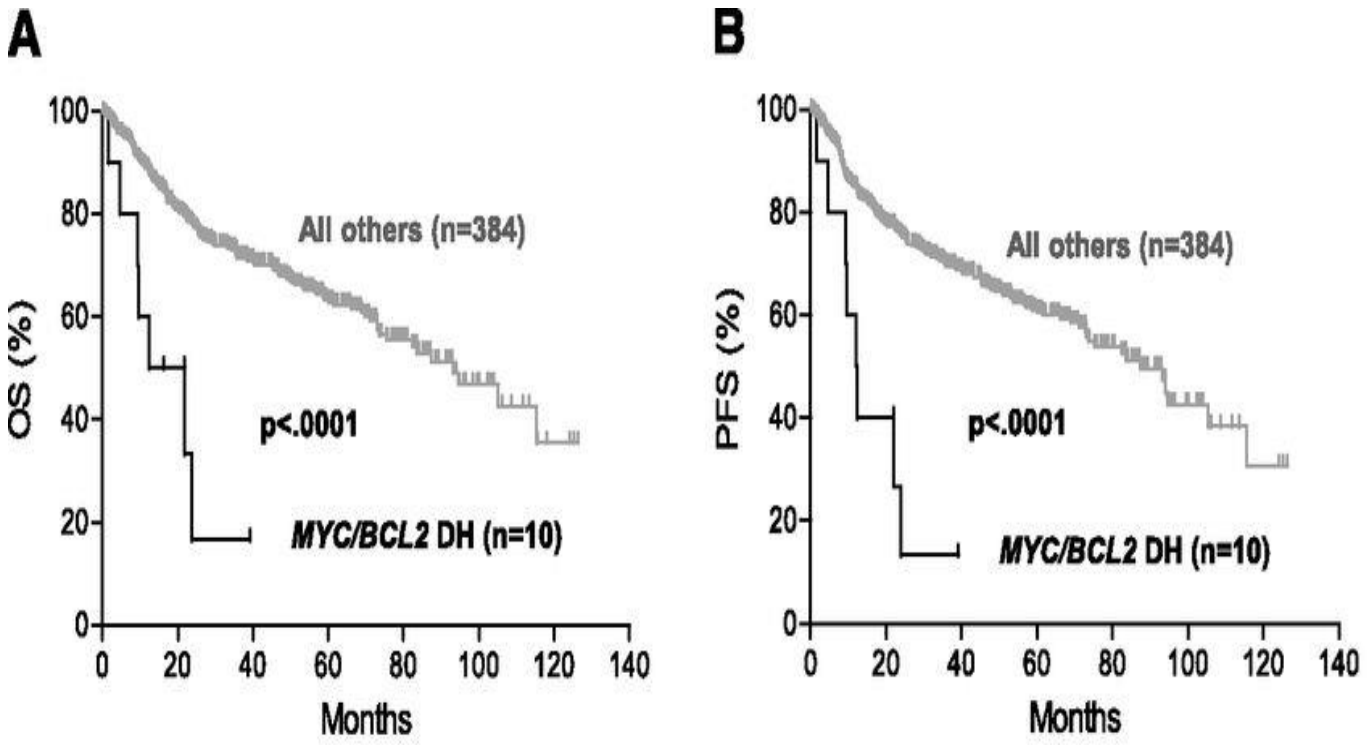
- Have two of these three genetic abnormalities
  - *MYC*
  - *BCL2*
  - *BCL6*
- Morphology may appear to be DLBCL or may have features that overlap with Burkitt lymphoma
- Aggressive clinical behavior—may require different therapy than DLBCL.

High-Grade B-cell Lymphoma with MYC and BCL2 and/or BCL6 Rearrangements (WHO 2016)

- Aggressive presentation, often disseminated (PB, BM, CSF).
- Can resemble BL with increased pleomorphism and/or atypical immunophenotype or genetic features.
- MYC complex karyotype is common.

MYC/BCL2 Double Hit Lymphomas Have a Poor Prognosis



Hu et al. Blood 2013;121:4021-4031

Prognostic Impact of Single Hits

Entire cohort

GCB Subtype

ABC Subtype

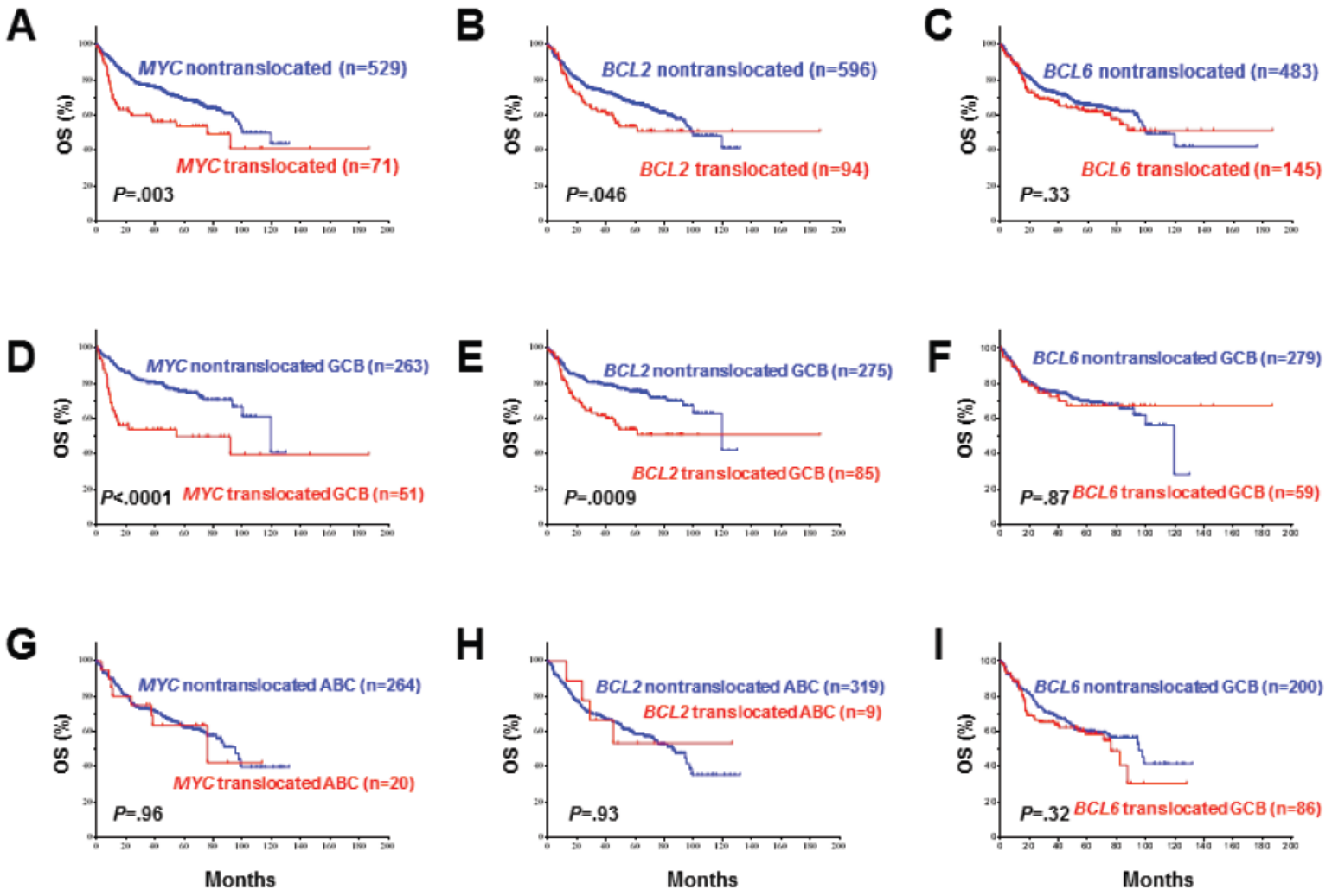


Figure 1: Univariate analysis for patients with DLBCL with *MYC*, *BCL2*, and *BCL6* rearrangemnts in the overall-, GCB, and ABC groups. **A.-B., D.-E, G.-H.** *MYC* and *BCL2* rearrangements correlated with significantly poorer overall survival in overall and GCB- but not ABC-DLBCL. **C., F., I.** *BCL6* translocation did not correlate with poorer overall survival.

Ye Oncotarget 2015

Prognostic Impact of Double Hits

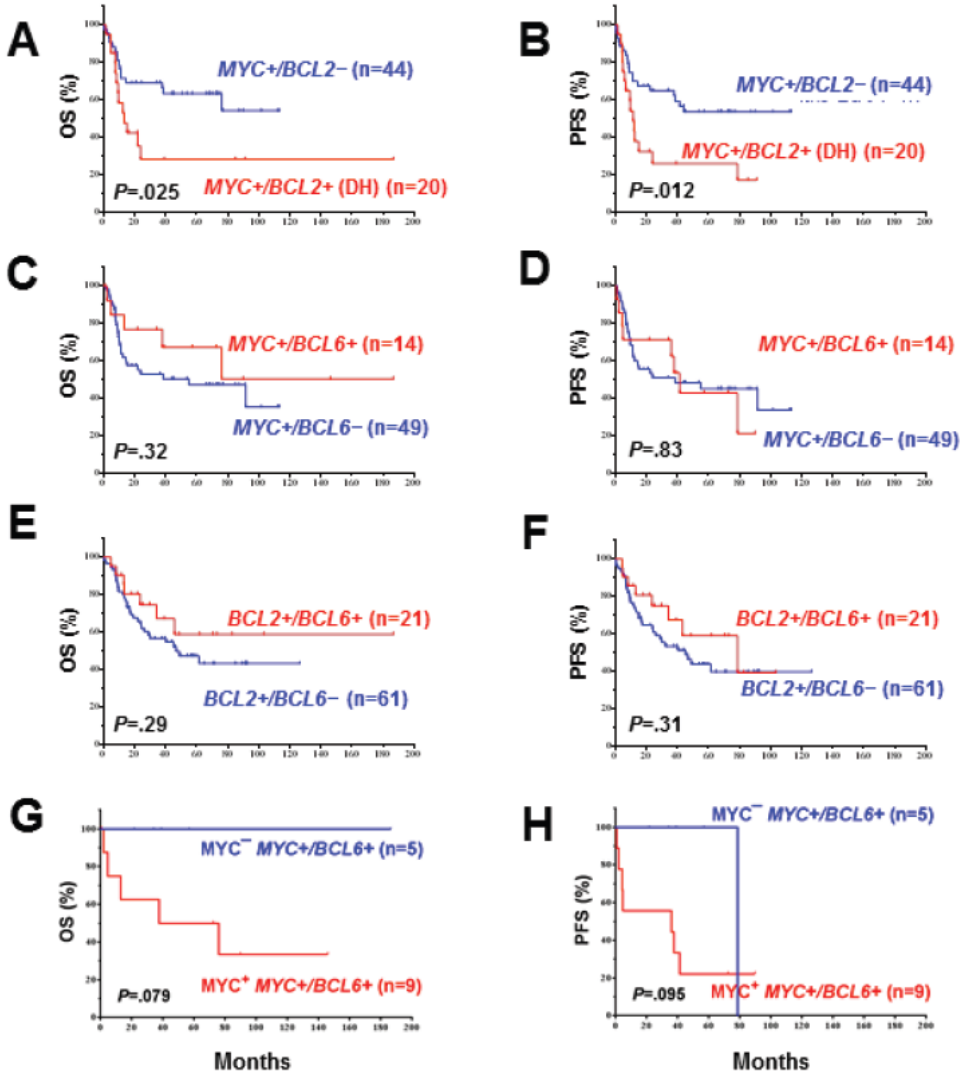


Figure 2: A.-B. The prognostic significance of *MYC* rearrangements in DLBCL depends on *BCL2* rearrangement. **C.-D.** *BCL6* rearrangement had no additive effect to *MYC* rearrangements. **E.-F.** *BCL6* translocation had no additive effect to BCL2 rearrangements. **G.-H.** MYC expression levels appeared to impact the survival of *MYC+/BCL6+* rearranged DLBCL with marginal *P* values probably due to the small case numbers.

Ye Oncotarget 2015

Only *MYC/BCL2* Pts. Show Worse Survival

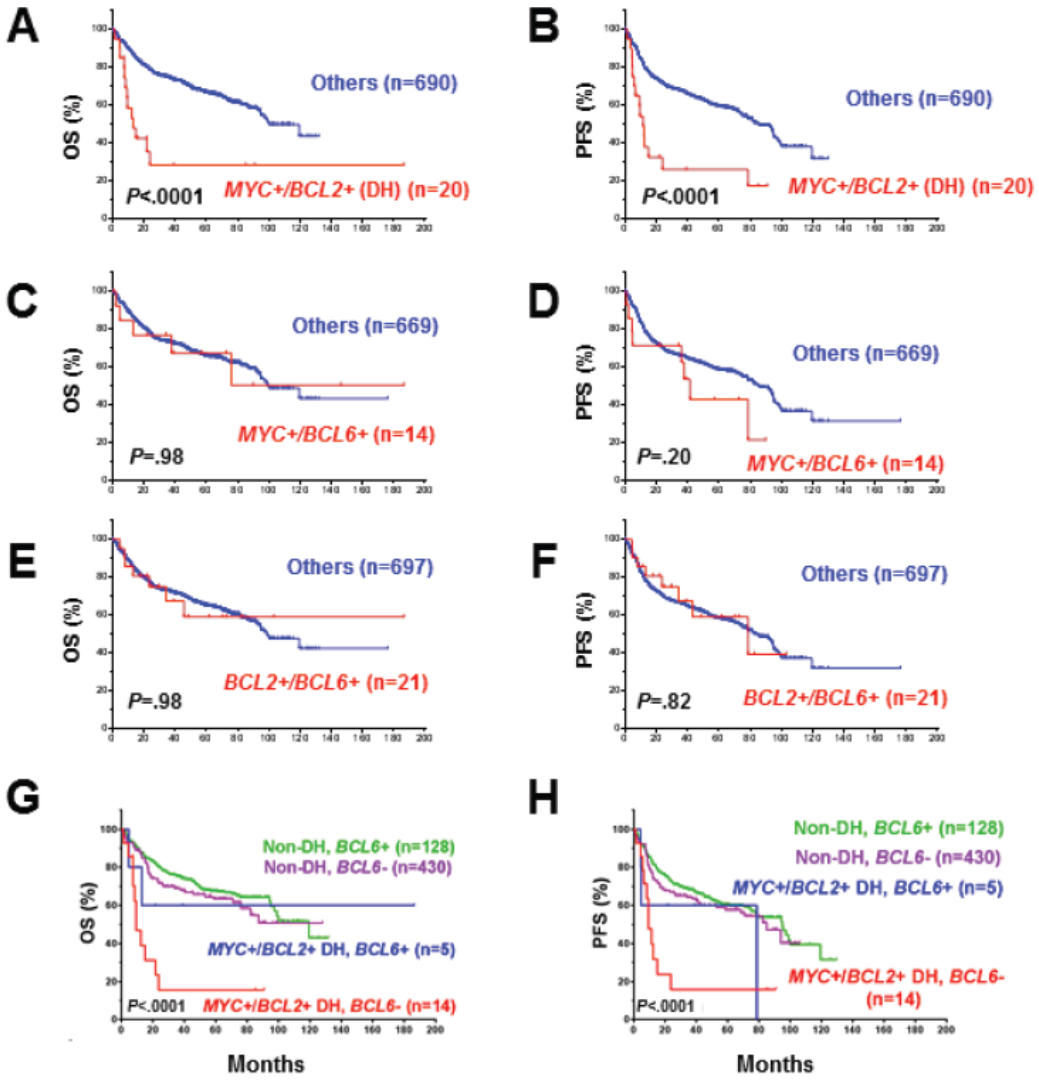


Figure 3: A.-B. Concurrent *MYC/BCL2* rearrangements correlated with significant poorer overall survival. **C.-D.** Concurrent *MYC+/BCL6+* rearrangements did not correlate with poorer overall survival. **E.-F.** Concurrent *BCL2+/BCL6+* rearrangements did not correlate with poorer overall survival. **G.-H.** BCL6 attenuated the adverse prognostic impact of *MYC+/BCL2+* double-hit lymphoma.

Ye Oncotarget 2015

Re-thinking Double Hits

- *MYC/BCL6* DHLs do not have a worse prognosis and should not be grouped with or treated as *MYC/BCL2* DHLs.
- *MYC/BCL6* DHLs do not have a different gene expression profile.
  - *BCL6* partners and expression levels vary.
  - 36% of *MYC/BCL6* have low MYC expression.

Incidence of Double Hits

Translocation	Incidence (%)
MYC	11.8
BCL2	13.6
BCL6	23.1
MYC / BCL2	2.8
MYC / BCL6	2.0
BCL2 / BCL6	2.9

- *MYC* and *BCL2* more common in GCB.
- *BCL6* more common in ABC.
- *MYC/BCL2* almost all in GCB (19/20).
- *MYC/BCL6* in GCB and ABC.

Ye *et. al*, Oncotarget 7(3):2401-2416, 2015.

DLBCL Prognostic Testing Strategy

De novo DLBCL (excludes transformation, relapse, PTLD unless specifically requested by clinician)

DLBCL, NOS

↓

MYC and BCL2 Immunohistochemistry;
CD10, BCL6, and MUM1 for Hans COO;

MYC FISH
If pos., *BCL2, BCL6*

Clinical and/or morphology suggests DLBCL subtype:

- TCHRBCL
- EBV+ DLBCL of elderly
- Primary mediastinal (PMBL)
- Primary CNS
- Primary cutaneous leg type

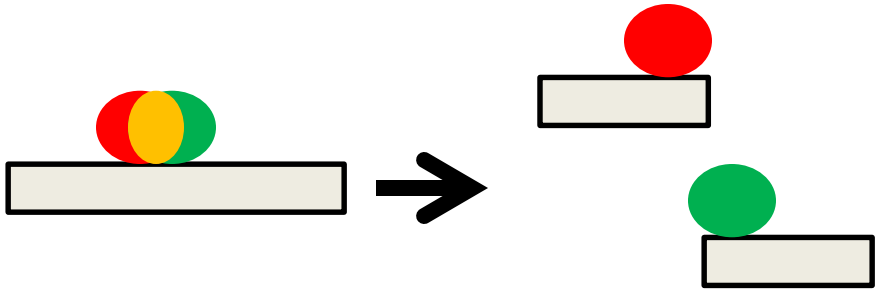
↓

Testing not indicated

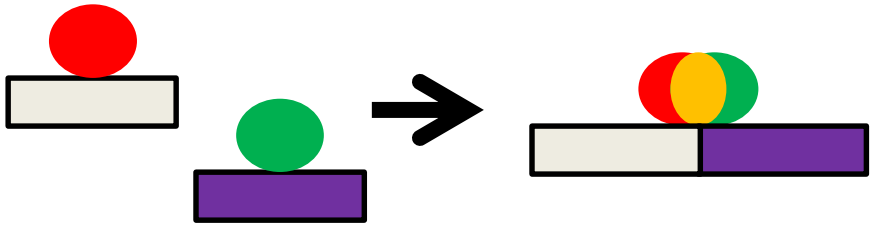
FISH: Fluorescence *in situ* Hybridization

- Detection of specific, defined abnormalities
- Relatively rapid turn-around (24-48 hrs)
- May be performed on fresh or paraffin-embedded tissues

- Break-apart probes:
  - *Separation* of the signals is abnormal.

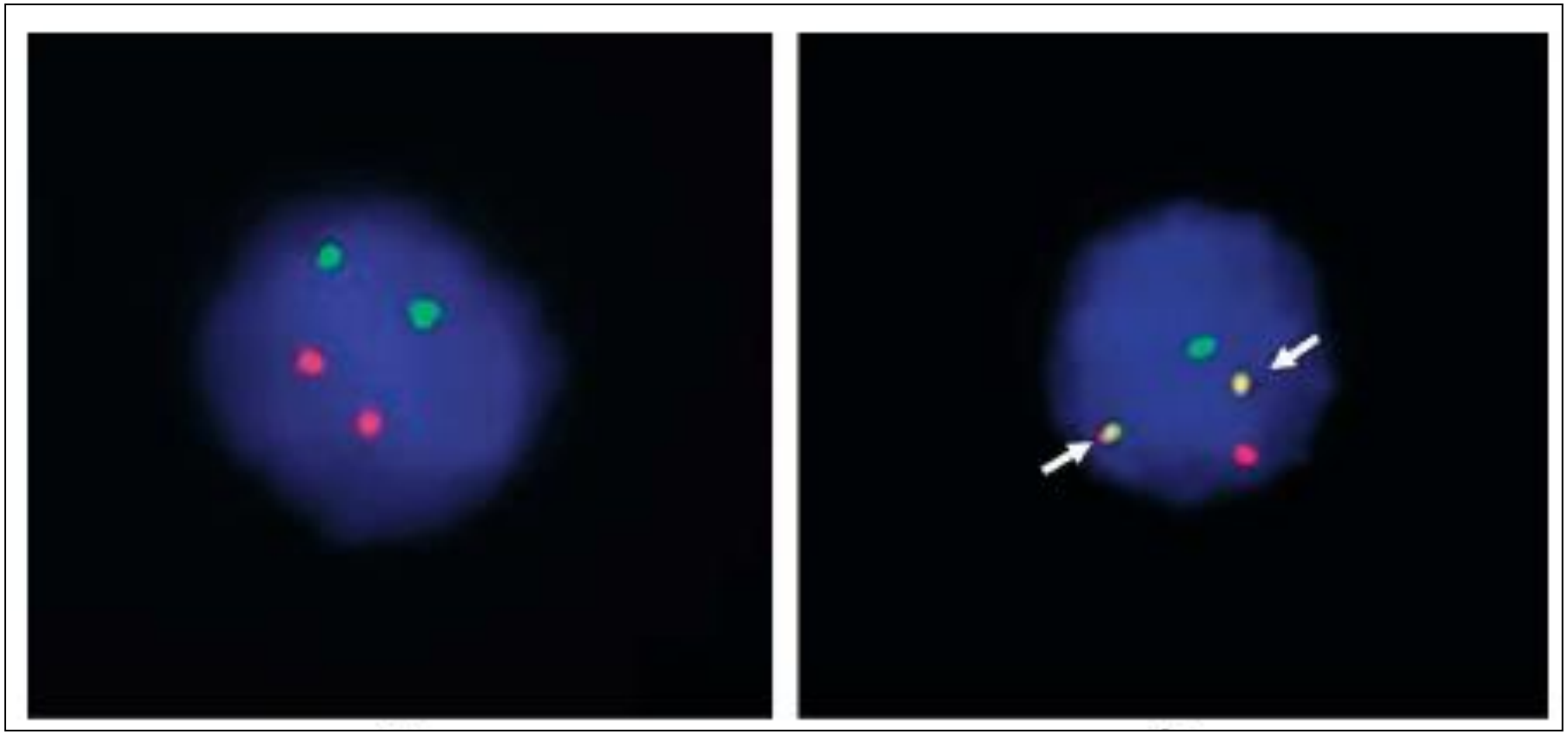


- Fusion probes:
  - *Fusion* of probe signals is abnormal.



FISH for t(14;18) *IGH/BCL2*

IGH/BCL2 fusion probe.

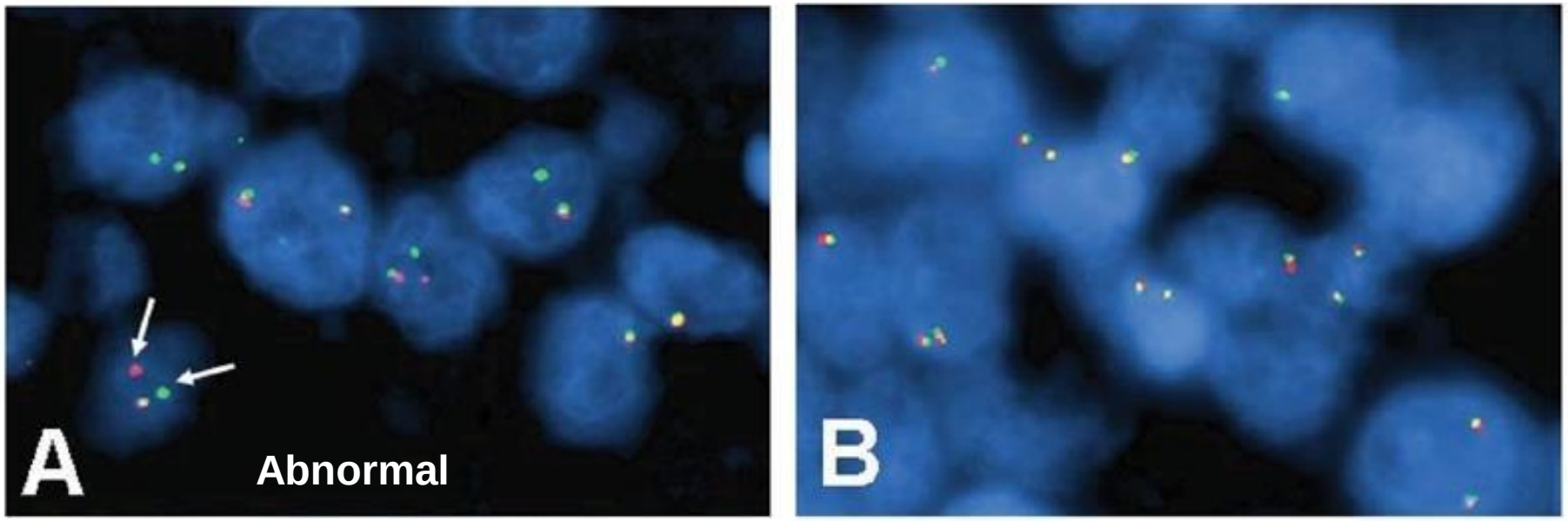


Normal

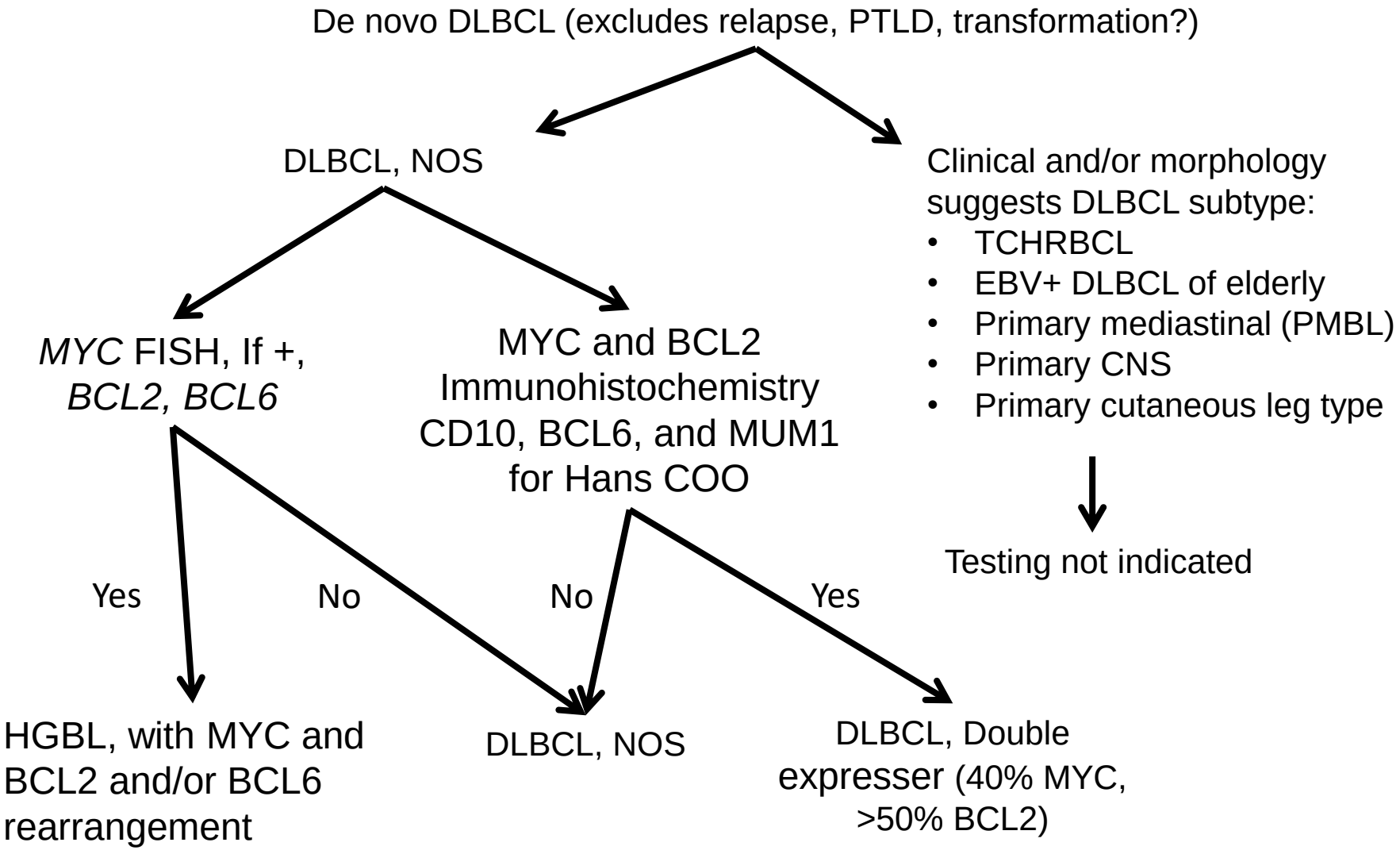
Abnormal

FISH for *MYC* Translocations

MYC break-apart probe



DLBCL Prognostic Testing Strategy



Challenge: Data Do Not Support the Current WHO Definitions

- *MYC/BCL6* DHLs do not have a worse prognosis and should not be grouped with or treated as *MYC/BCL2* DHLs.
- *MYC/BCL6* DHLs do not have a different gene expression profile.
  - *BCL6* partners and expression levels vary.
  - 36% of *MYC/BCL6* have low MYC expression.

DLBCL Conclusions

- Diagnosis of DLBCL requires only morphology and immunophenotype.
- Diagnosing or excluding the WHO 2016 category HGBL, with MYC+BCL2 +/- BCL6 rearrangement requires FISH.
- Best approach is evolving and lacks consensus at this time.
- Testing should be performed when results will affect patient care.


Clonality Testing

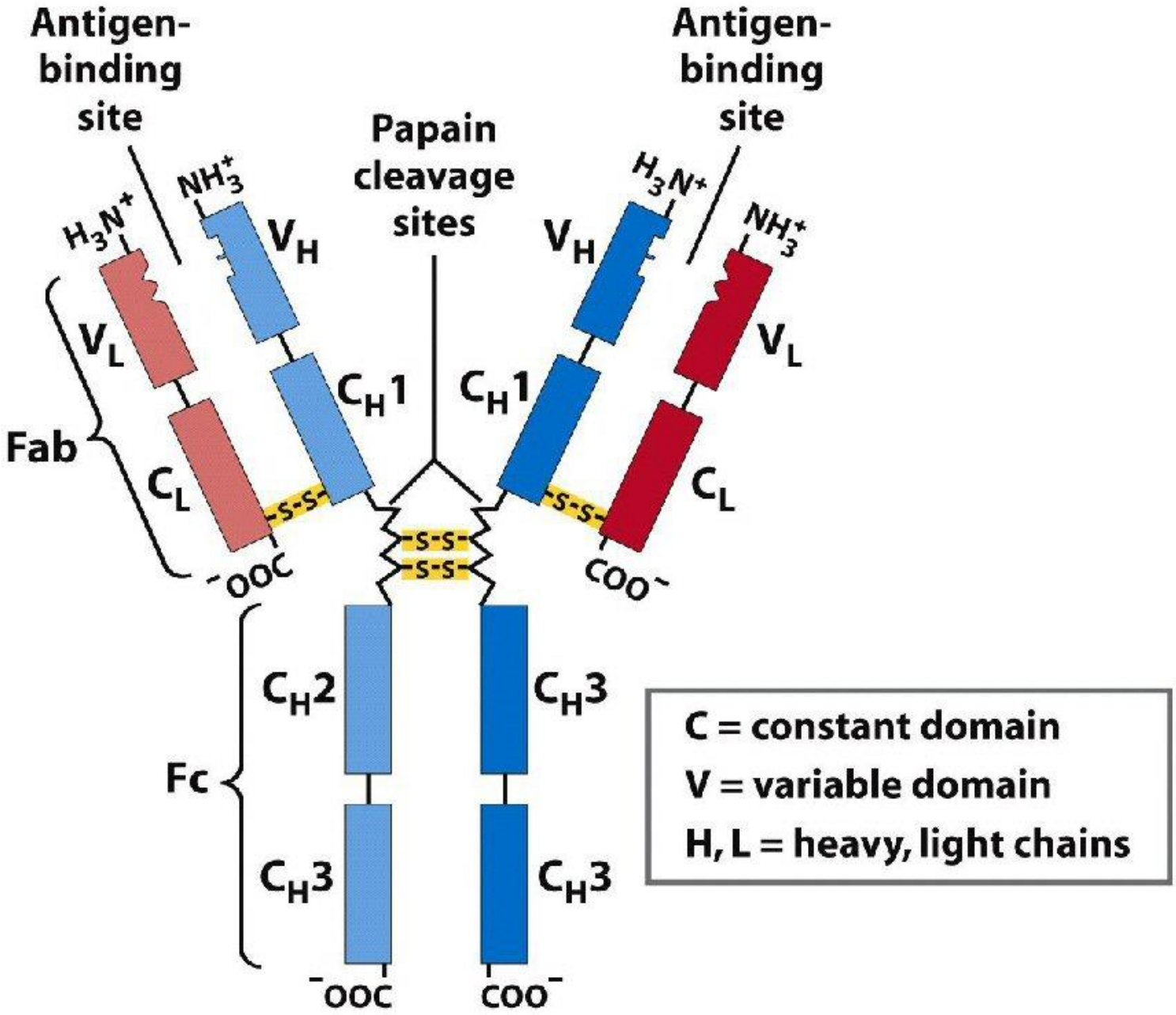


Figure 5-21a
Lehninger Principles of Biochemistry, Fifth Edition

Receptor diversity

- 100 million to 1 billion different receptor specificities in one individual
- Diversity is generated by
  - Different segments
  - Different combinations of segments
  - Junctional diversity during recombination
  - Somatic hypermutation

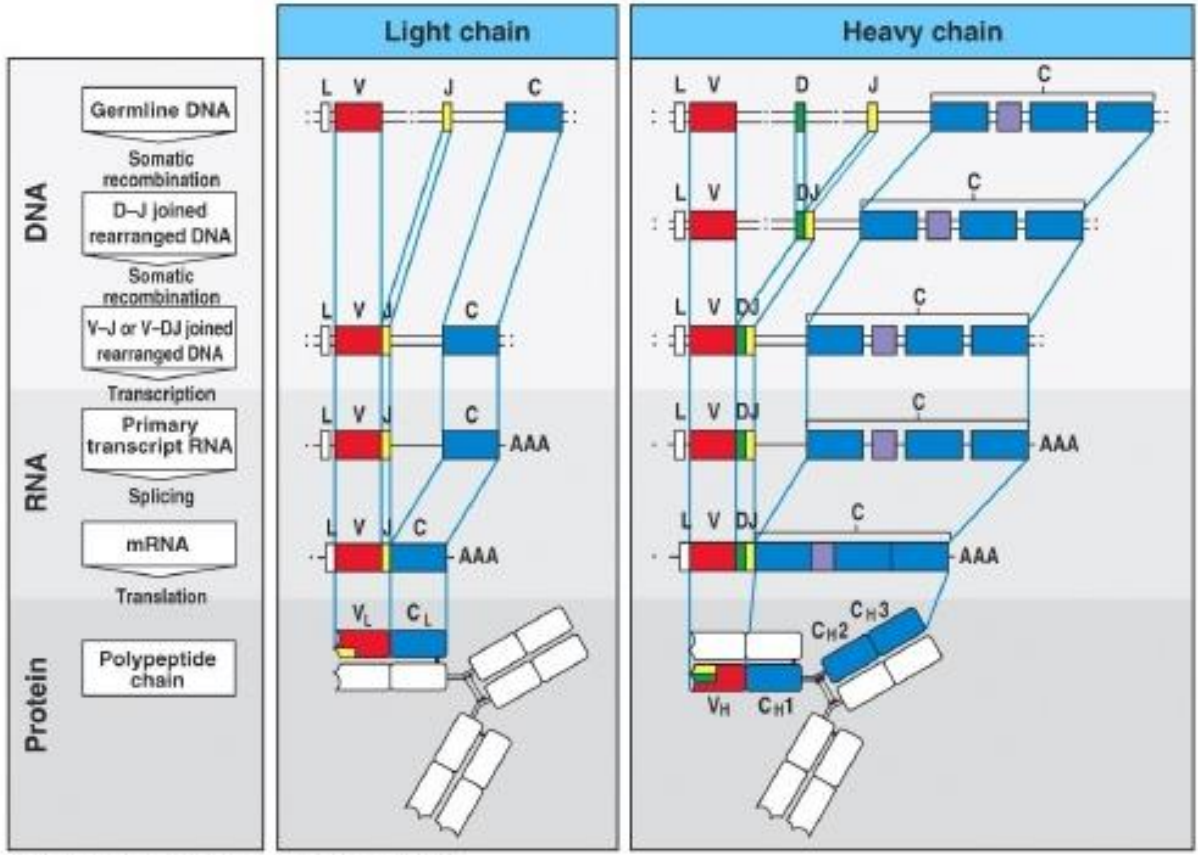
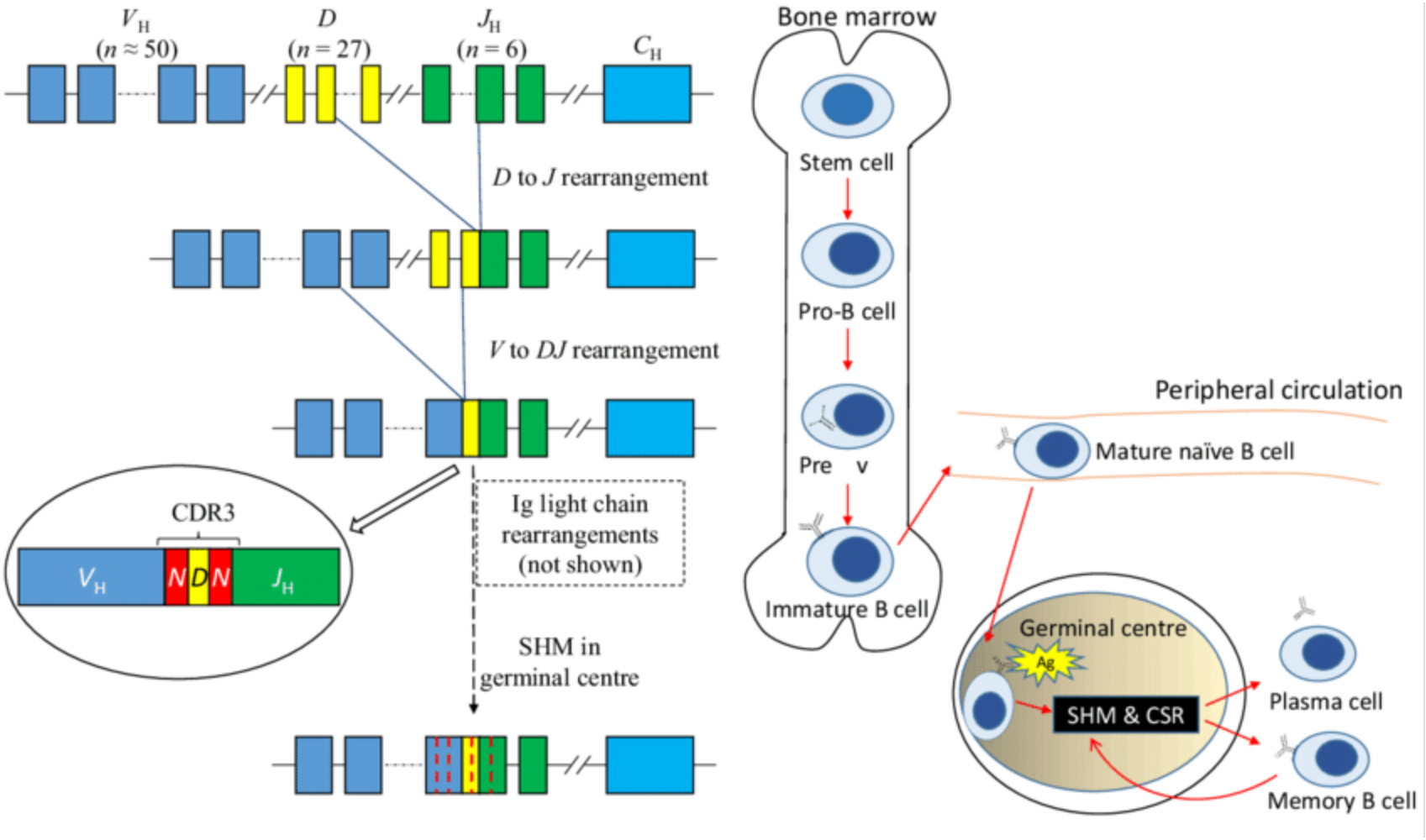


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)



BJH 2018; 181: 11-26.

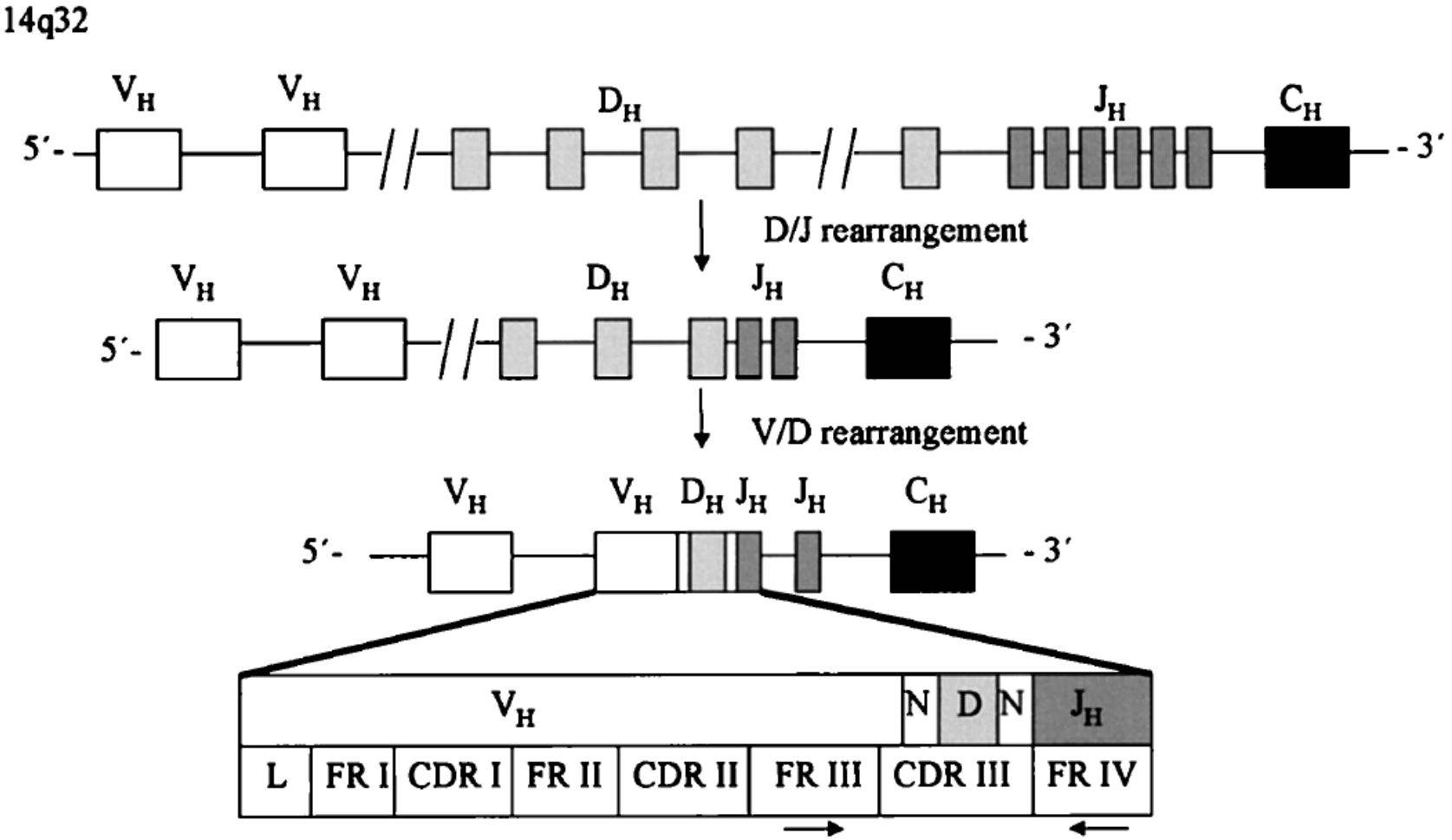
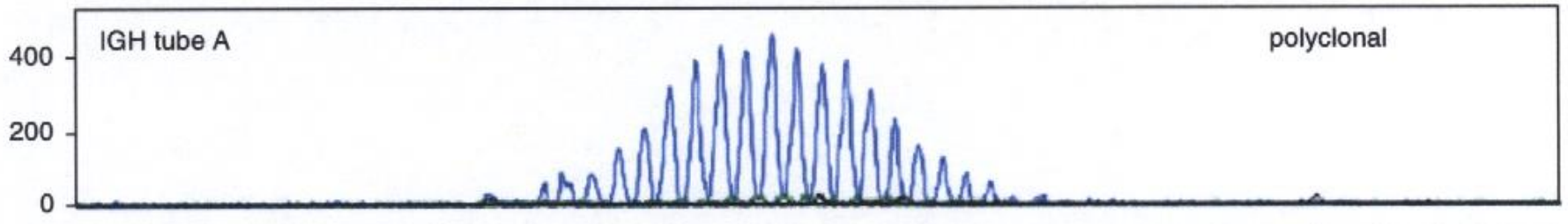
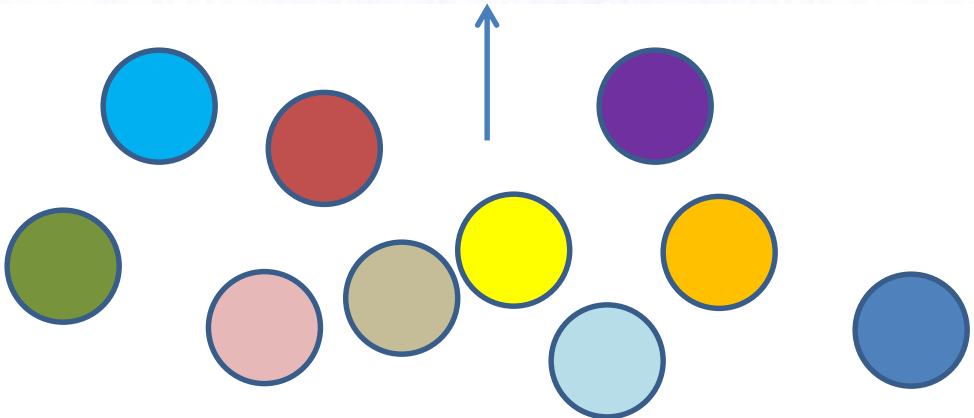


Figure 1. Immunoglobulin heavy chain gene rearrangement. Most PCR tests for this rearrangement use consensus primers directed against the framework three (FRIII) region and the heavy chain joining (J_H or FRIV) region of the rearranged product.

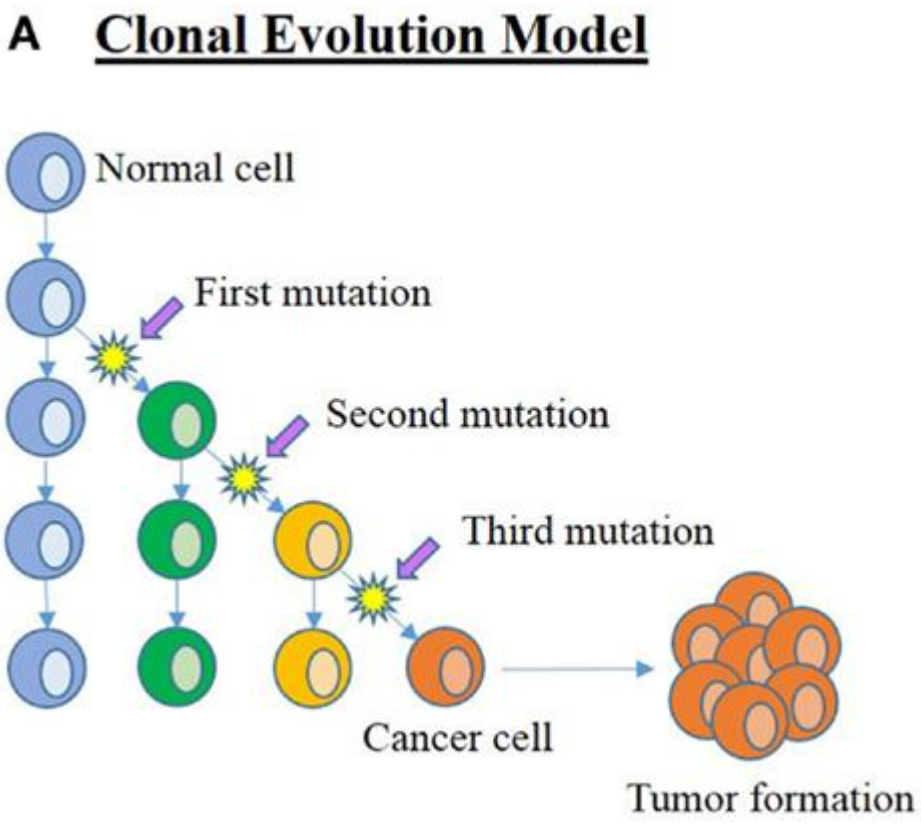
Arber, JMD 2000

Physiologic (“normal”) B-cell populations

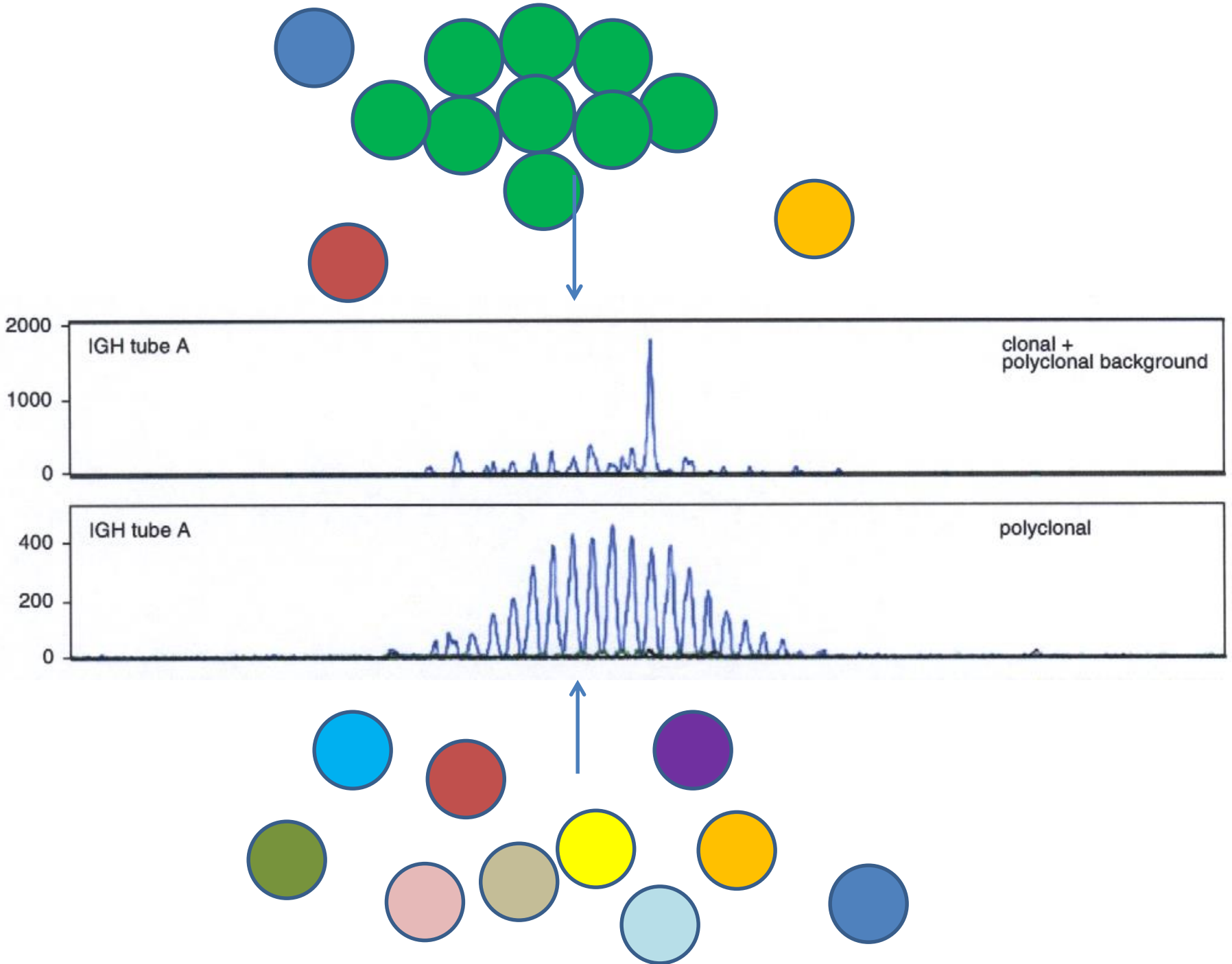




Assumption of Clonality in Cancer is Critical to Diagnostic Tools (Flow, Molecular)

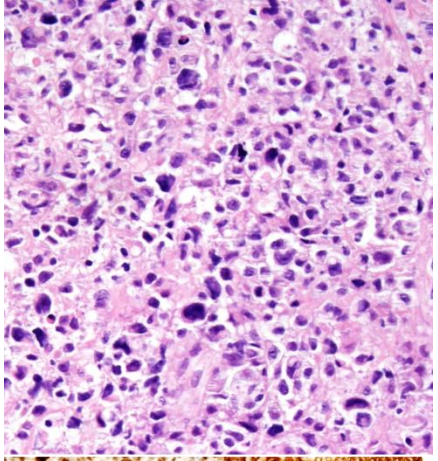


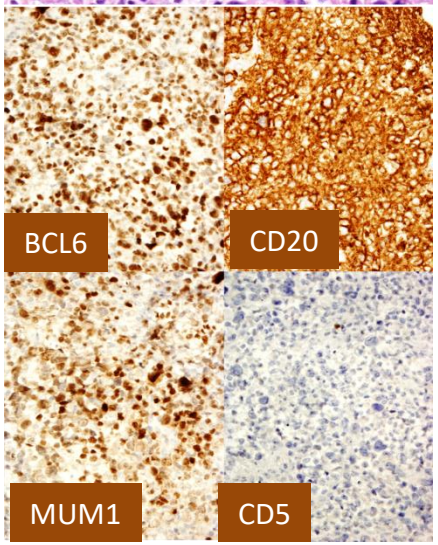
https://doi.org/10.3389/fsurg.2016.00021

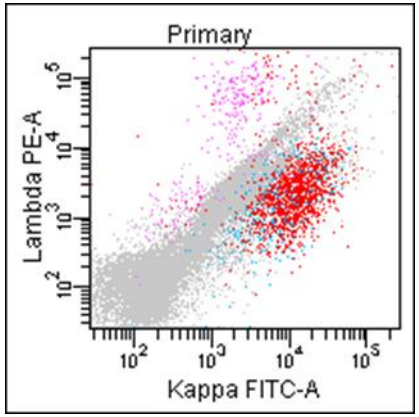

2000
1000
0
IGH tube A
clonal +
polyclonal background
400
200
0
IGH tube A
polyclonal

Lymphoma Diagnosis

- Morphology
- Immunohistochemistry
- Flow cytometry
  - This is enough! (Most of the time…)





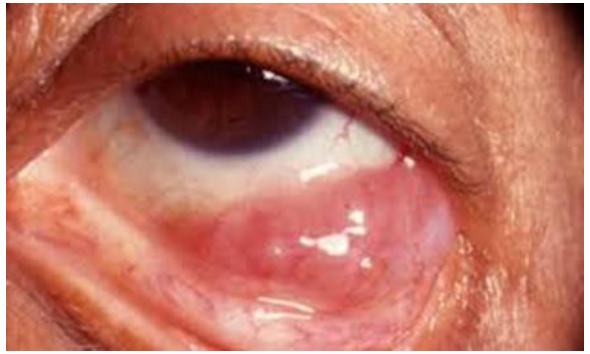


How should this test be used?

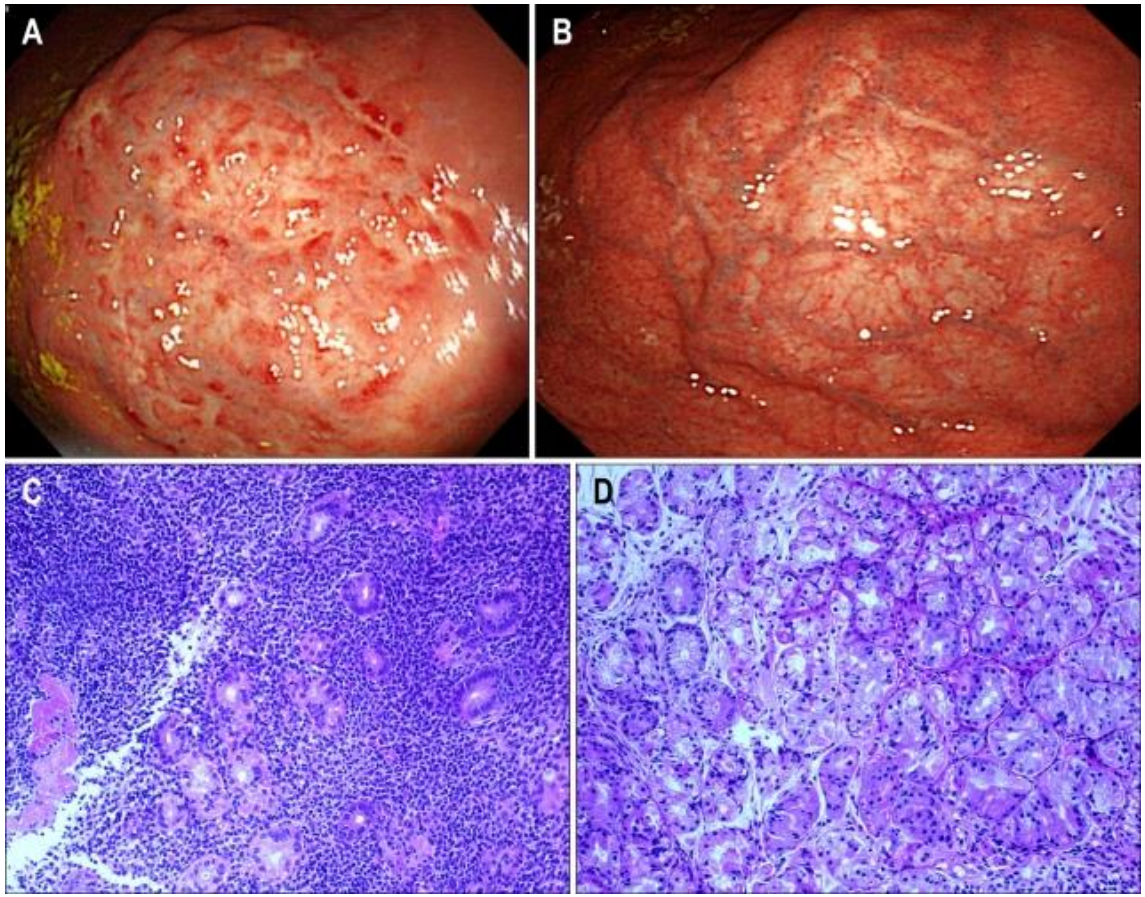
- Many/most diagnoses of lymphoma do NOT require molecular testing
  - Morphology and immunophenotype are sufficient
- Useful in difficult cases; usually where the differential diagnosis is an atypical reactive process
- Determining lineage (T vs. B)
  - Lineage infidelity
    - Much more common in immature neoplasms
    - Bagg A. J Mol Diagn. 2006 Sep; 8(4): 426–429.
- Comparing separate lesions (both spatially and chronologically)

MALT lymphoma

- Marginal zone (Mucosa associated lymphoid tissue) lymphoma
  - Low grade B-cell lymphoma
  - Some relationship to underlying chronic inflammation
  - Often in extranodal locations
    - Gastrointestinal (usually stomach)
    - Parotid gland, salivary glands, thyroid
    - Eye, lacrimal glands
    - Lung
    - Skin

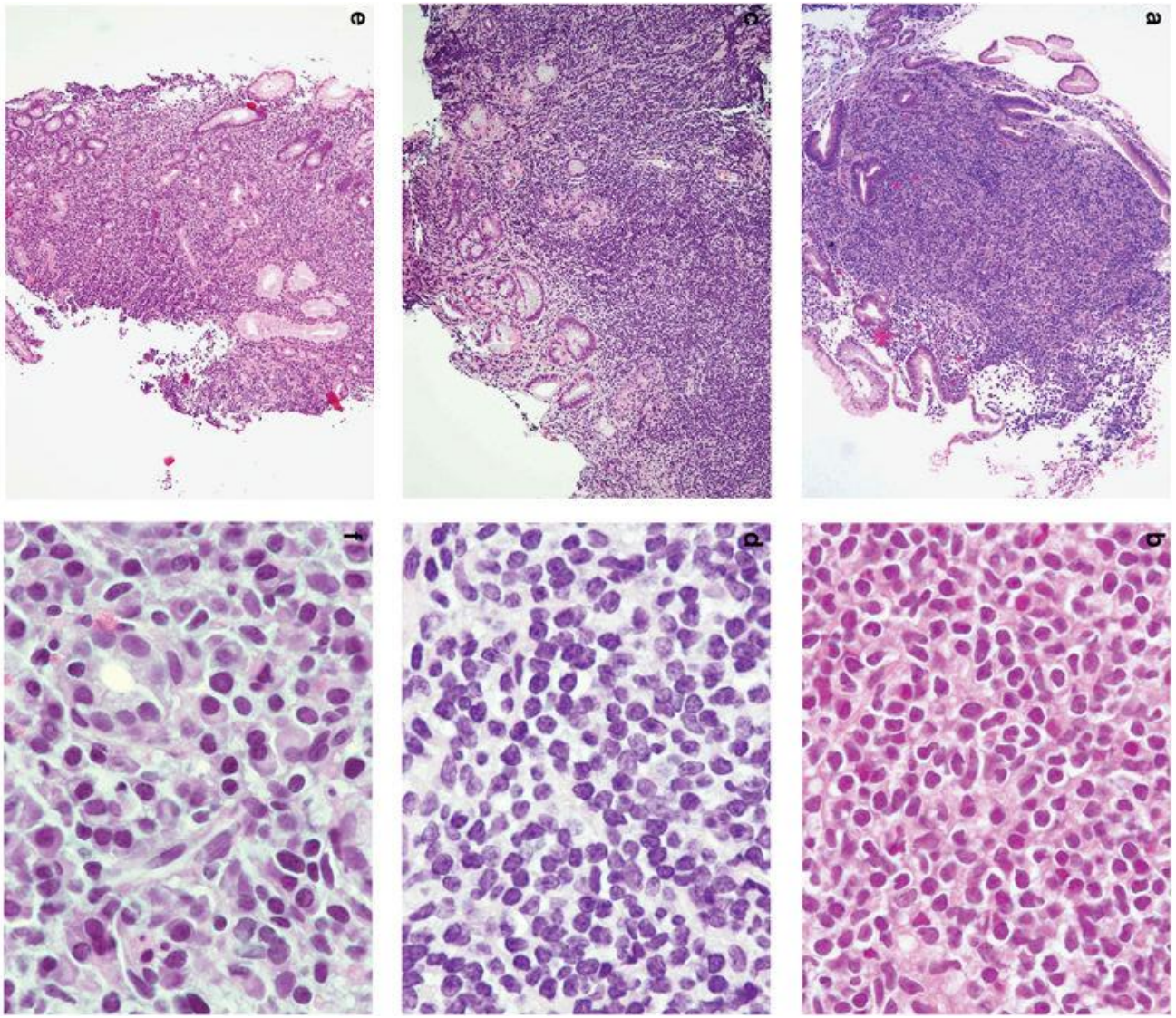


Emedicine.medscape.com



A&C – at presentation
B&D – after treatment

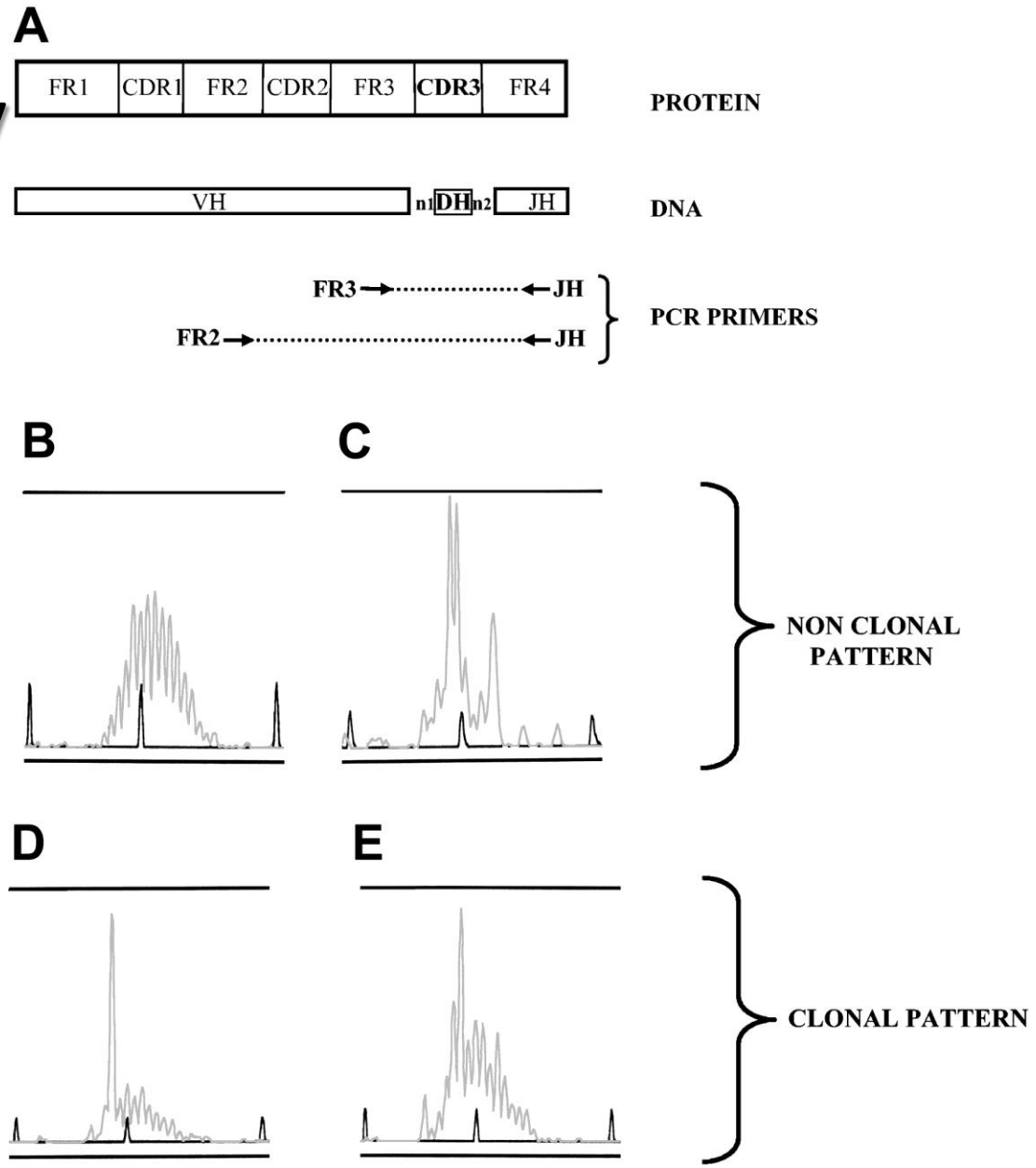
Suzuki, Hidekazu & Saito, Yoshimasa & Hibi, Toshifumi. (2009). Helicobacter pylori and Gastric Mucosa-associated Lymphoid Tissue (MALT) Lymphoma: Updated Review of Clinical Outcomes and the Molecular Pathogenesis. Gut and liver. 3. 81-7. 10.5009/gnl.2009.3.2.81.



Modern Pathology **volume22**, pages79–86 (2009)
doi:10.1038/modpathol.2008.155

- Gastric biopsies are usually small
- Extent of infiltration??

B-cell Clonality Assay:

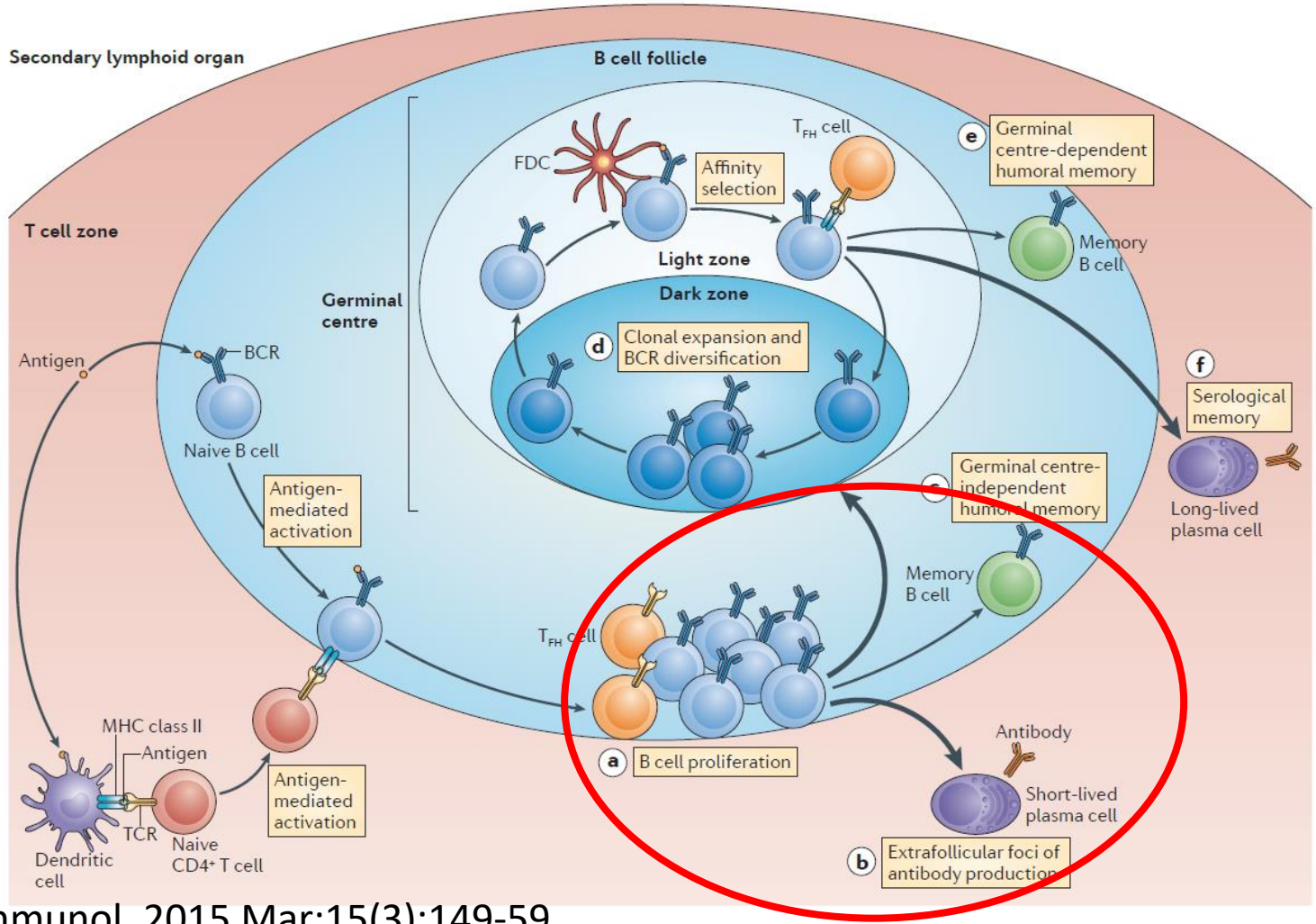


Simona Zompi et al. Blood 2004;103:3208-3215

Pitfalls of Clonality Testing

- Failed amplification
  - Low quantity
  - Poor quality (FFPE)
- Sampling
  - Pseudoclones
  - Wrong area
- False negatives
  - Somatic hypermutation (Follicular lymphoma)
  - Sampling wrong area
  - Clone too small; high reactive background
- "False positives"
  - Clonal proliferation in non-neoplastic processes

Clonal expansion as part of normal immune response



Nat Rev Immunol. 2015 Mar;15(3):149-59.

Pitfalls of Clonality Testing

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  - Sampling wrong area
  - Clone too small; high reactive background
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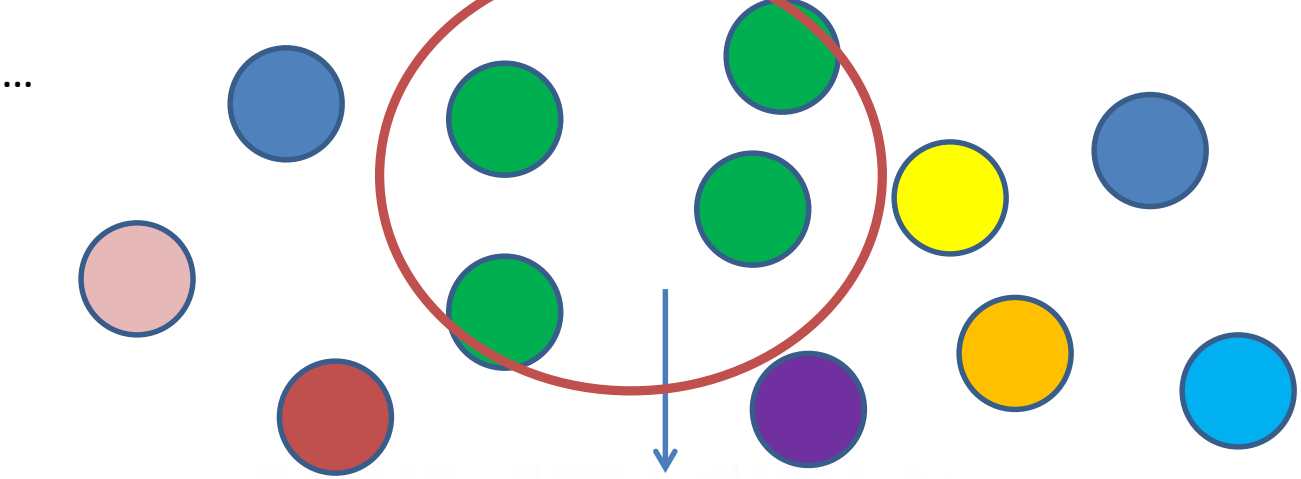
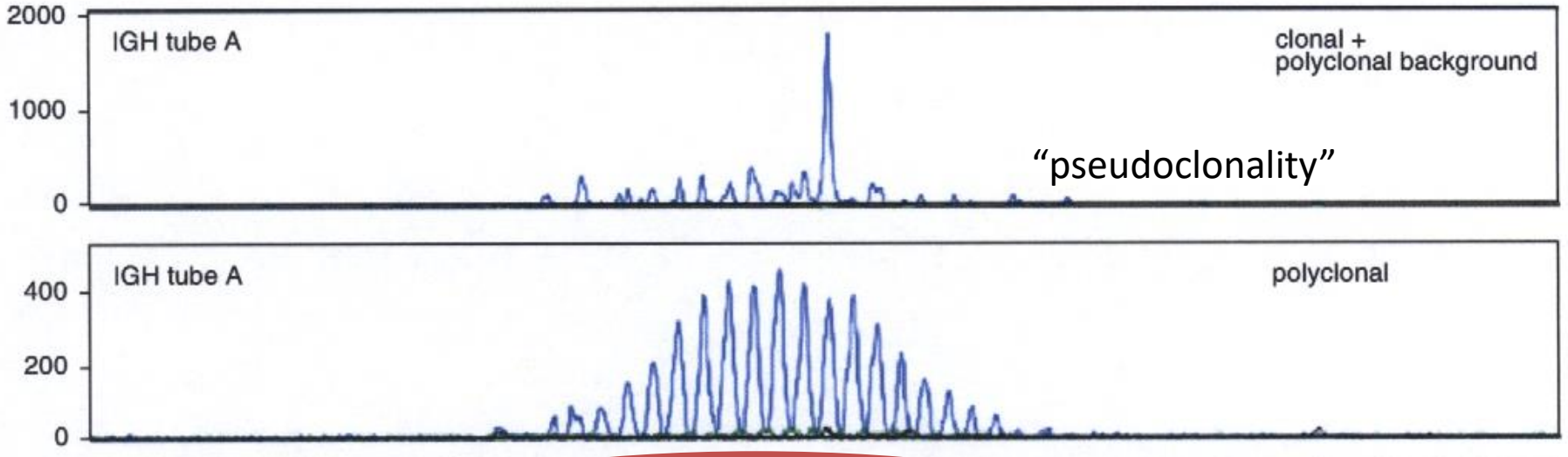
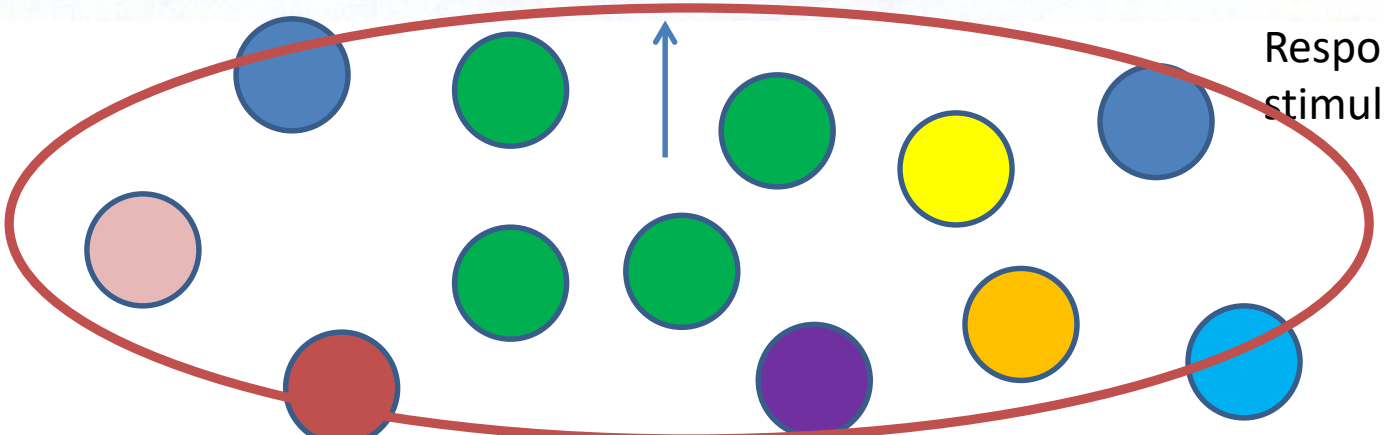
  - Clonal selection in non-neoplastic processes

Sampling…



Response to antigenic stimulation





Response to antigenic stimulation

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  - Clonal selection in non-neoplastic processes

Leukemia (2007) 21, 207–214

www.nature.com/leu

ORIGINAL ARTICLE

Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936

PAS Evans[1], Ch Pott[2], PJTA Groenen[3], G Salles[4], F Davi[5], F Berger[6], JF Garcia[7], JHJM van Krieken[3], S Pals[8], Ph Kluin[9], E Schuuring[9], M Spaargaren[8], E Boone[10], D González[11], B Martinez[12], R Villuendas[7], P Gameiro[13], TC Diss[14], K Mills[15], GJ Morgan[1], GI Carter[16], BJ Milner[17], D Pearson[18], M Hummel[19], W Jung[20], M Ott[21], D Canioni[22], K Beldjord[23], C Bastard[24], MH Delfau-Larue[25], JJM van Dongen[26], TJ Molina[27] and J Cabeçadas[28]

Table 3 The combined use of a three-tube *IGH* multiplex strategy to detect V_H–J_H rearrangements significantly improves clonality detection in mature B-cell malignancies

	V_H–J_H *FR1*	V_H–J_H *FR2*	V_H–J_H *FR3*	V_H–J_H *Total*
MCL ($n=54$)	100% 54/54	98% 53/54	96% 52/54	100% 54/54
B-CLL/SLL ($n=56$)	95% 53/56	91% 51/56	93% 52/56	100% 56/56
FL ($n=109$)	73% 80/109	76% 83/109	52% 57/109	84% 92/109
MZL (extranodal) ($n=31$)	68% 21/31	81% 25/31	61% 19/31	84% 26/31
MZL (nodal) ($n=10$)	90% 9/10	100% 10/10	90% 9/10	100% 10/10
MZL (total) ($n=41$)	73% 30/41	85% 35/41	68% 28/41	88% 36/41
DLBCL ($n=109$)	68% 74/109	61% 66/109	50% 55/109	79% 86/109
TOTAL ($n=369$)	79% 291/369	78% 288/369	66% 244/369	88% 324/369

Evans et al, Leukemia 2007

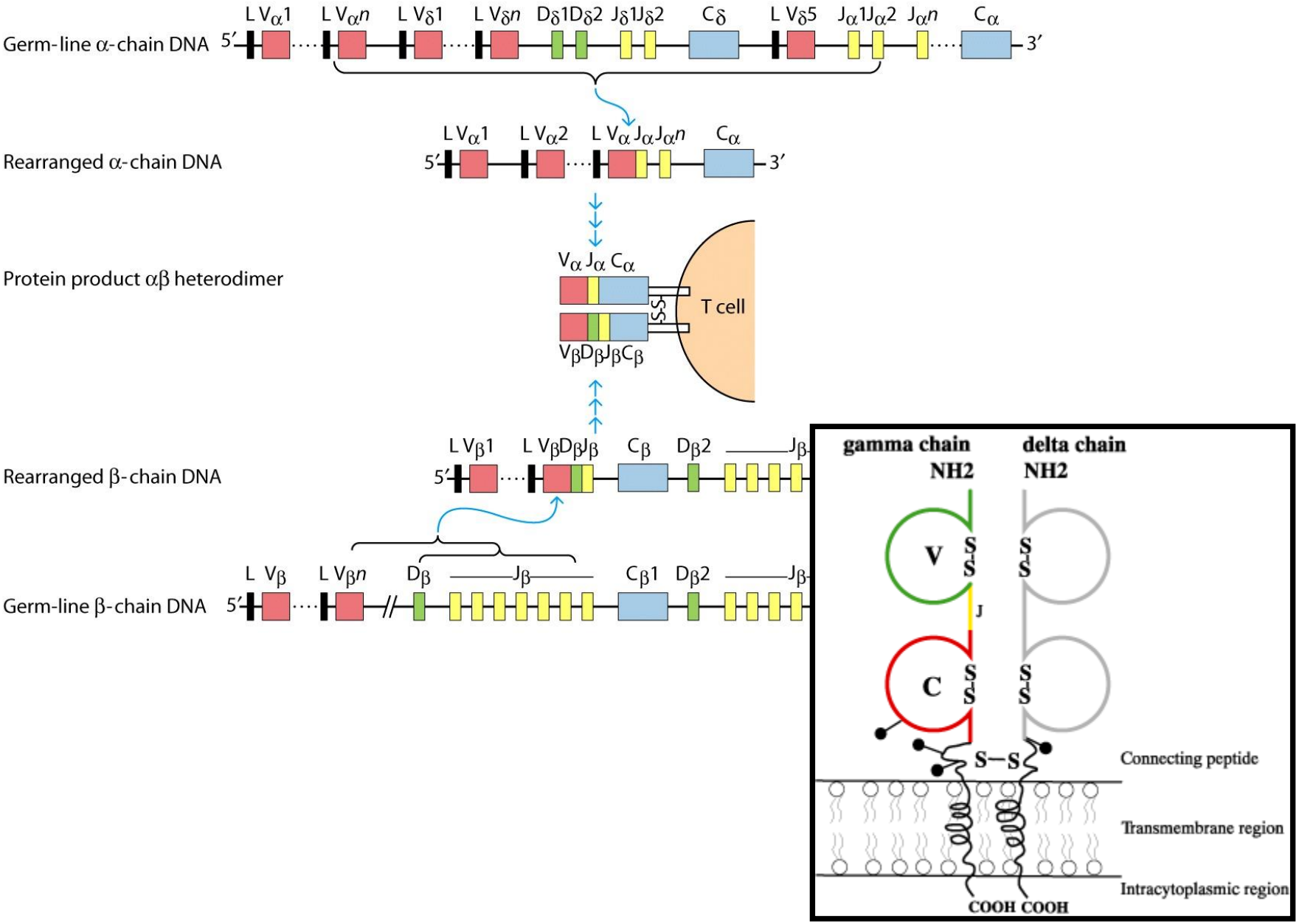
Table 2 The majority of mature B-cell malignancies can be identified by the use of three *IGH* (V_H–J_H) tubes and two *IGK* (V_κ–J_κ and Kde) tubes

	IGH (three V_H–J_H tubes: FR1, -2 and -3)[a]				*IGK (two tubes: V_κ–J_κ and Kde)*				*IGH (V_H–J_H) + IGK*			
	Total	*1*	*2*	*>2*	*Total*	*1*	*2*	*>2*	*Total*	*1*	*2*	*⩾3*
MCL ($n=54$)	100% 54/54	0% 0/54	0% 0/54	100% 54/54	100% 54/54	0% 0/54	27% 15/54	73% 39/54	100% 54/54	0% 0/54	0% 0/54	100% 54/54
B-CLL/SLL ($n=56$)	100% 56/56	2% 1/56	4% 2/56	94% 53/56	100% 56/56	0% 0/56	43% 24/56	57% 32/56	100% 56/56	0% 0/56	0% 0/56	100% 56/56
FL ($n=109$)	84% 92/109	10% 11/109	28% 30/109	47% 51/109	84% 92/109	32% 35/109	32% 35/109	20% 22/109	100% 109/109	9% 10/109	18% 20/109	73% 79/109
MZL ($n=41$)	87% 36/41	10% 4/41	17% 7/41	60% 25/41	83% 34/41	39% 16/41	20% 8/41	24% 10/41	97% 40/41b	12% 5/41	5% 2/41	80% 33/41
DLBCL ($n=109$)	79% 86/109	17% 19/109	22% 24/109	39% 43/109	80% 87/109	38% 41/109	34% 37/109	8% 9/109	96% 105/109b	18% 20/109	14% 15/109	64% 70/109
TOTAL ($n=369$)	88% 324/369	9% 34/369	17% 63/369	62% 227/369	88% 323/369	25% 92/369	32% 119/369	30% 112/369	98% 363/369	9% 34/369	10% 37/369	79% 292/369

Evans et al, Leukemia 2007

When to use T-cell clonality testing?

- There are MANY examples of clonal T-cell proliferations that are NOT neoplastic
  - Commonly skin, peripheral blood
  - Post transplant
  - Various immune responses
    - Inflammatory (Crohn's etc.)
    - Malignancy (CLL/SLL, etc.)
- Still can be very helpful in tissues (lymph node, etc.) that look like a T-cell lymphoma, but more evidence/support is needed.



Imgt.org

www.sbs.utexas.edu

T-cell receptor rearrangement

- TRD -> TRG -> TRB -> TRA
- This happens in all T-cells, regardless of αβ or γδ expression
- Thus, all αβ T-cells (the most common subset) will have identifiable (but not expressed) TRG rearrangements

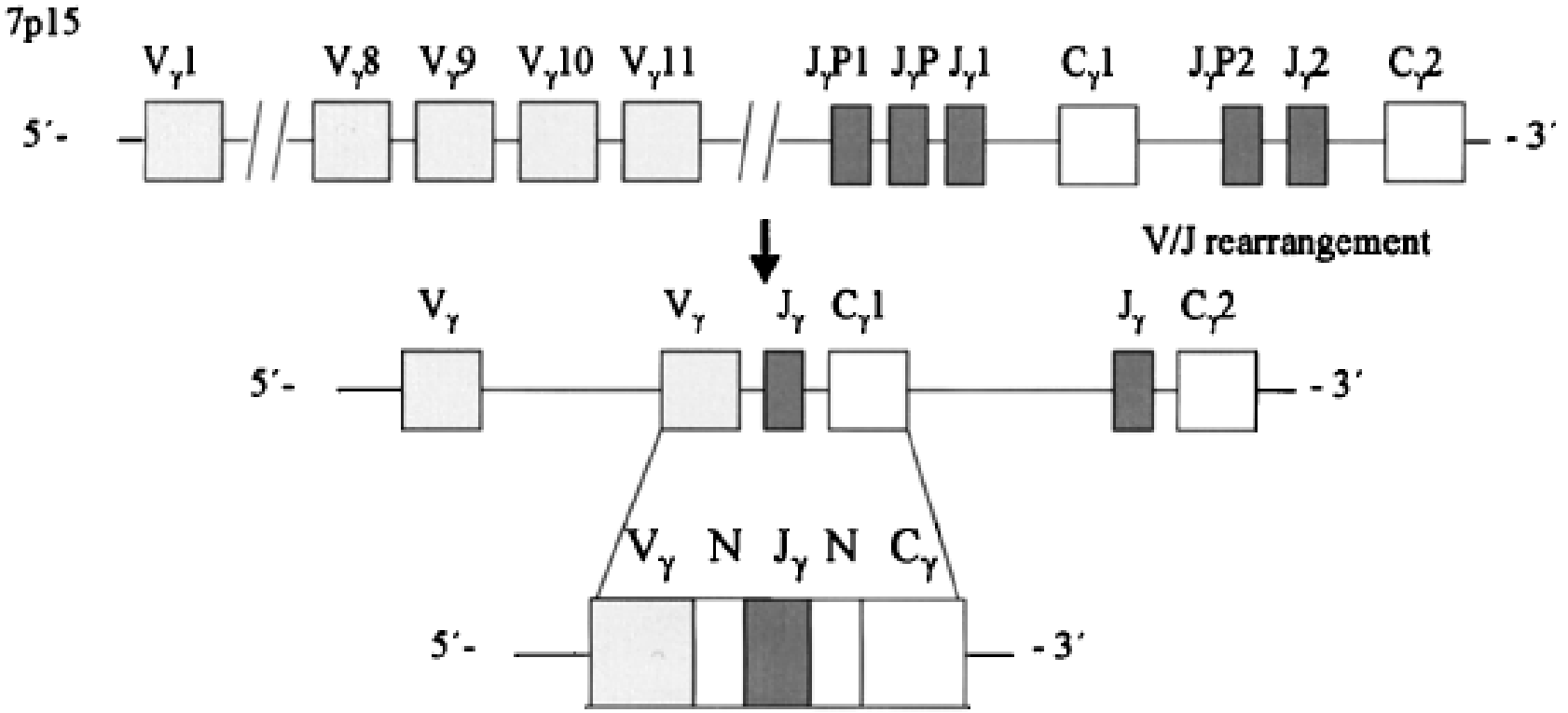


Figure 6. The T cell receptor γ chain locus on chromosome region 7p15 contains a limited number of variable and joining region genes that make it ideal for PCR amplification of the rearrangements.

Arber, JMD 2000

Mycosis Fungoides – a common T-cell lymphoma of the skin

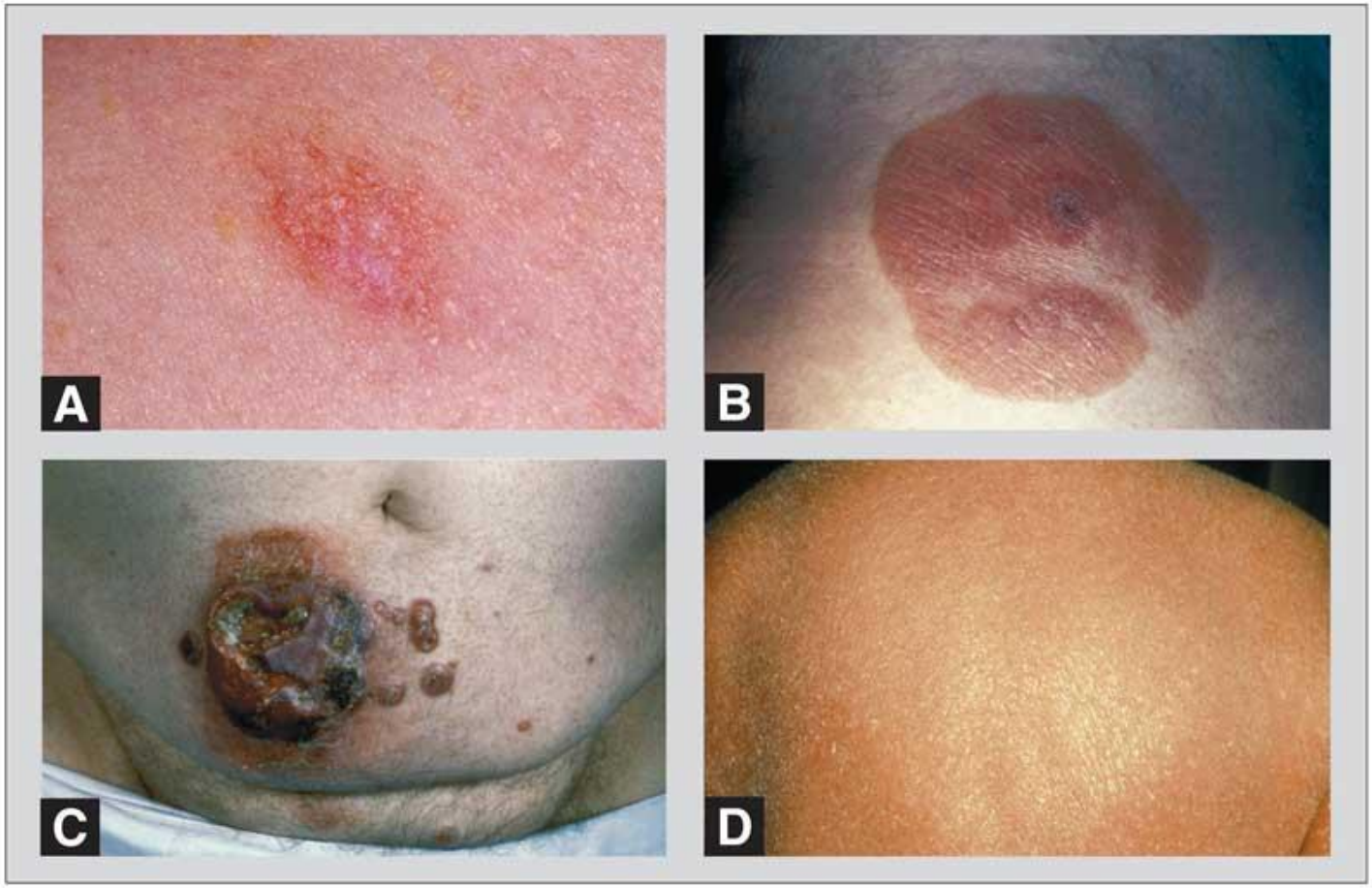
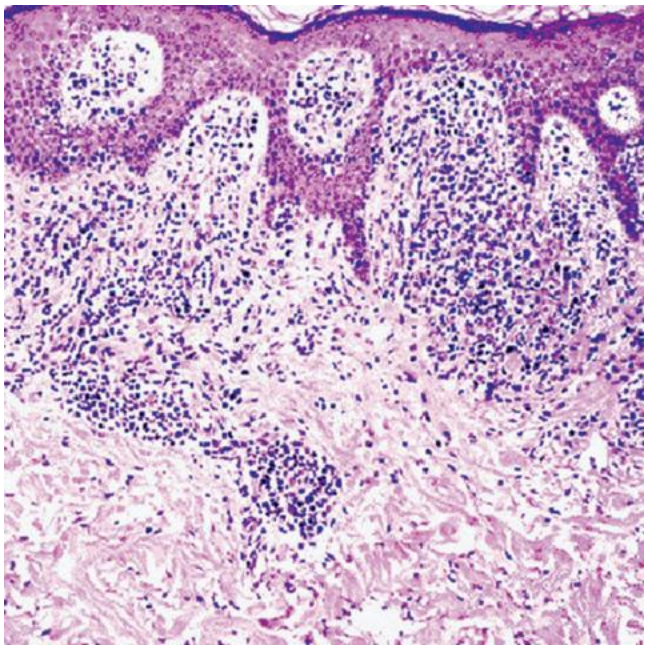
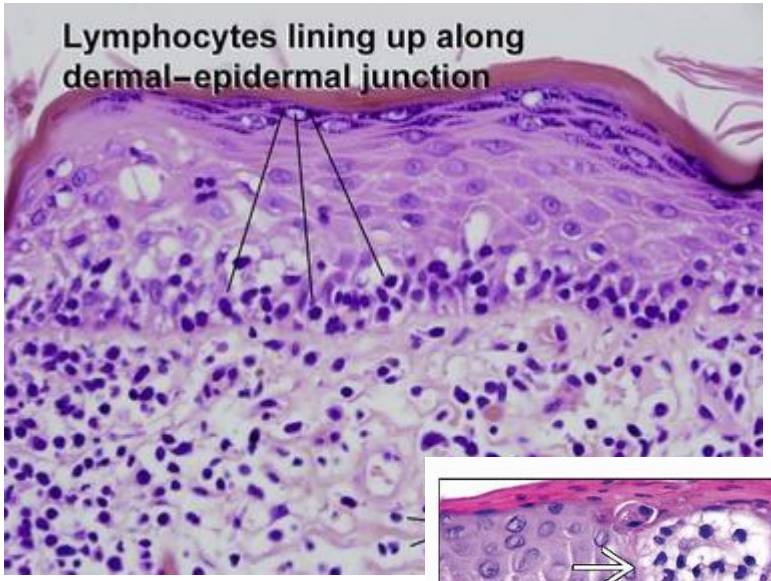


Figure 1: Clinical manifestations of mycosis fungoides—Image **(A)** shows typical early patch with erythema and mild scale; **(B)** shows a typical plaque, with raised, palpable borders, central clearing, and overlying scale; **(C)** shows a large tumor with necrosis and ulceration; and **(D)** shows generalized erythroderma. Reprinted with permission from Figure 1 in Smith B, Wilson L: *Oncology (Williston Park)* 17:1281-1288, 2003.[63]

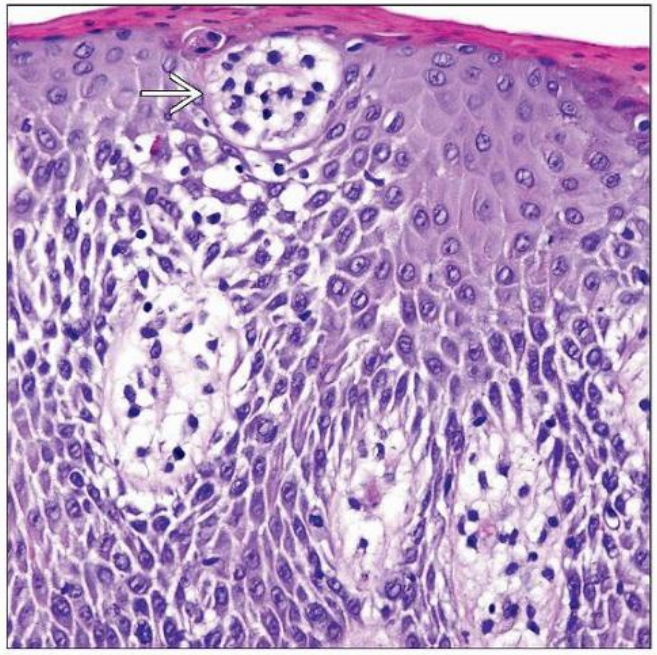
Mycosis fungoides



Pathologyoutlines.com

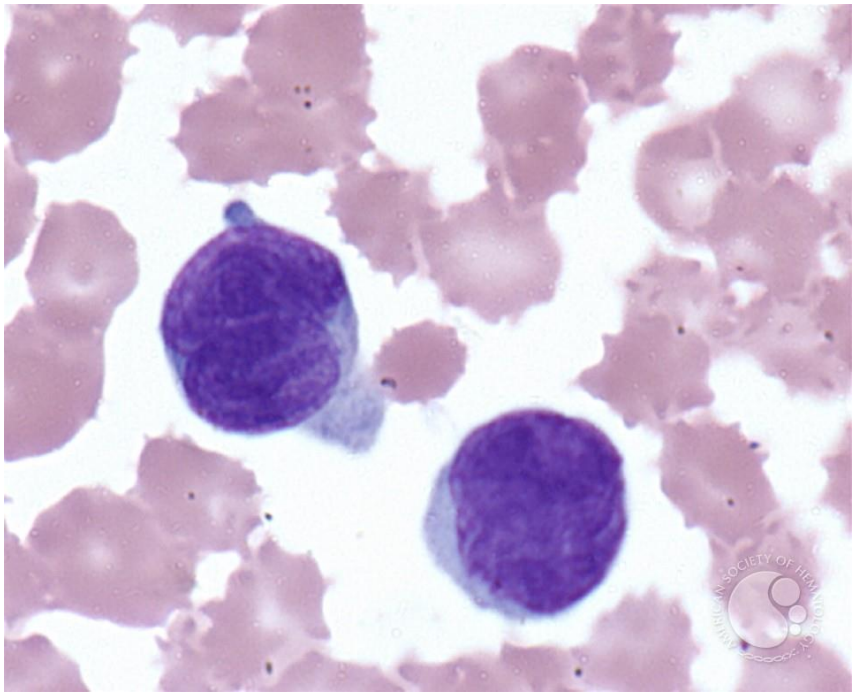


Clinicalgate.com



Pautrier microabscesses
(basicmedicalkey.com)

Sezary Syndrome – a type of T-cell lymphoma in blood and skin



Sezary cells – ASH image bank

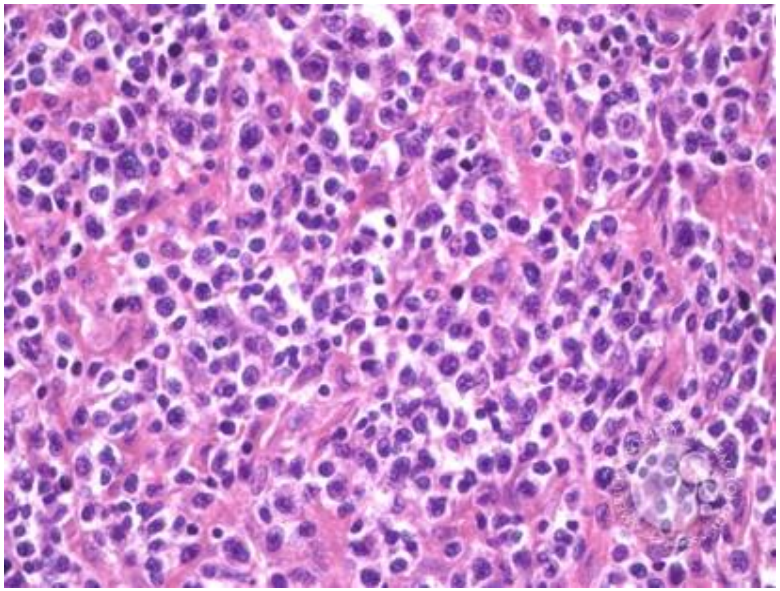
- Staging of mycosis fungoides and Sezary syndrome often involves evaluation of the peripheral blood for tumor cells, which may include TCR molecular studies if tumor cells are suspected by morphology.

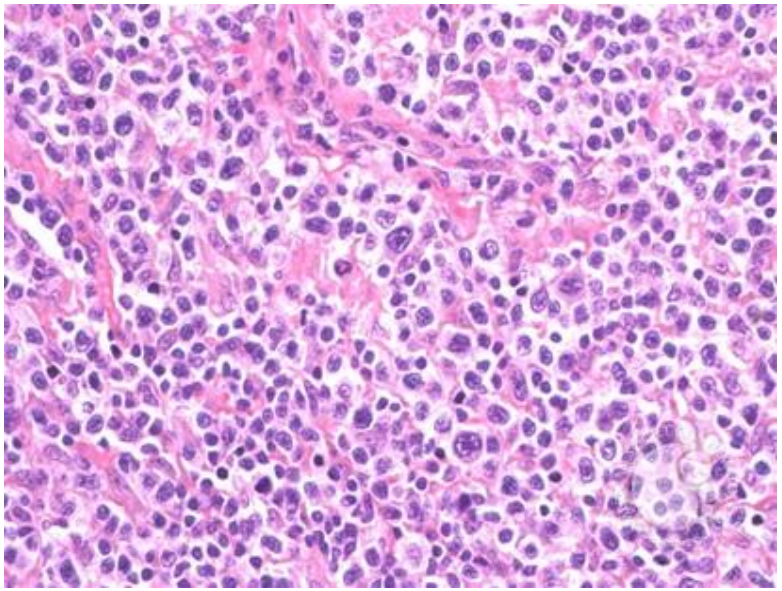
Table 4. ISCL/EORTC revision to the classification of mycosis fungoides and Sézary syndrome

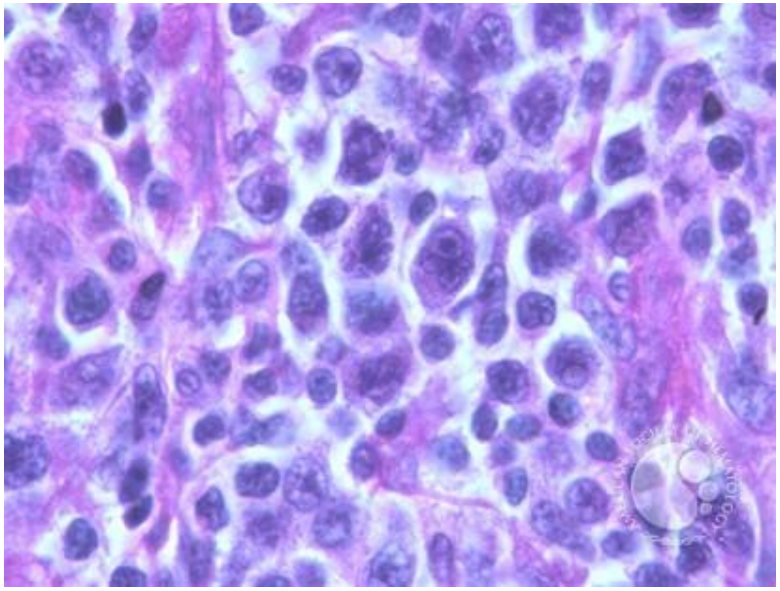
TNMB stages	
Skin	
T_1	Limited patches,* papules, and/or plaques† covering < 10% of the skin surface. May further stratify into T_{1a} (patch only) vs T_{1b} (plaque ± patch).
T_2	Patches, papules or plaques covering ≥ 10% of the skin surface. May further stratify into T_{2a} (patch only) vs T_{2b} (plaque ± patch).
T_3	One or more tumors‡ (≥ 1-cm diameter)
T_4	Confluence of erythema covering ≥ 80% body surface area
Node	
N_0	No clinically abnormal peripheral lymph nodes§; biopsy not required
N_1	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI LN_{0-2}
N_{1a}	Clone negative#
N_{1b}	Clone positive#
N_2	Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 2 or NCI LN_3
N_{2a}	Clone negative#
N_{2b}	Clone positive#
N_3	Clinically abnormal peripheral lymph nodes; histopathology Dutch grades 3-4 or NCI LN_4; clone positive or negative
N_x	Clinically abnormal peripheral lymph nodes; no histologic confirmation
Visceral	
M_0	No visceral organ involvement
M_1	Visceral involvement (must have pathology confirmation¶ and organ involved should be specified)
Blood	
B0	Absence of significant blood involvement: ≤ 5% of peripheral blood lymphocytes are atypical (Sézary) cells‖
B_{0a}	Clone negative#
B_{0b}	Clone positive#
B1	Low blood tumor burden: > 5% of peripheral blood lymphocytes are atypical (Sézary) cells but does not meet the criteria of B_2
B_{1a}	Clone negative#
B_{1b}	Clone positive#
B2	High blood tumor burden: ≥ 1000/μL Sézary cells‖ with positive clone#

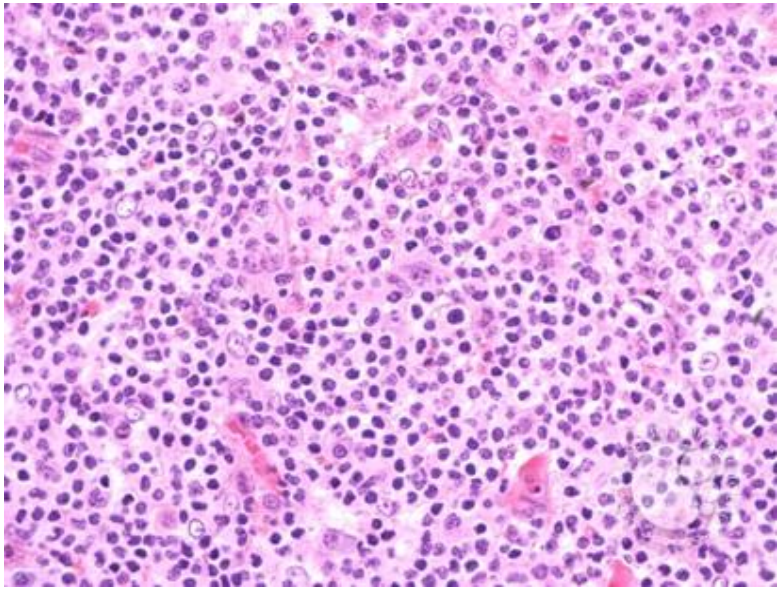
Olsen E et al. Blood 2007; 110: 1713-1722.

Peripheral T-cell lymphoma, NOS







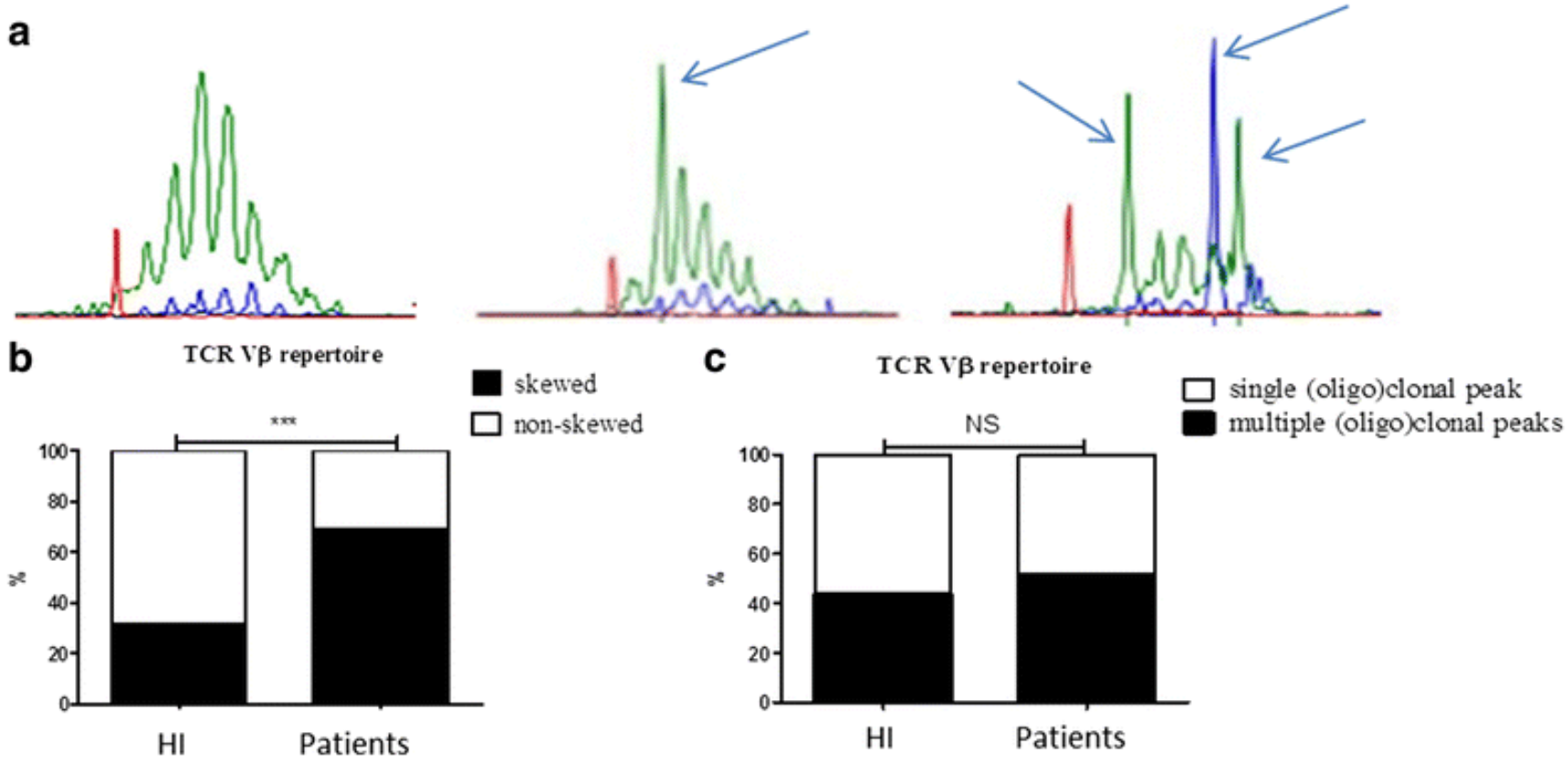


ASH Image Bank

Non-Neoplastic Clonal T-cells

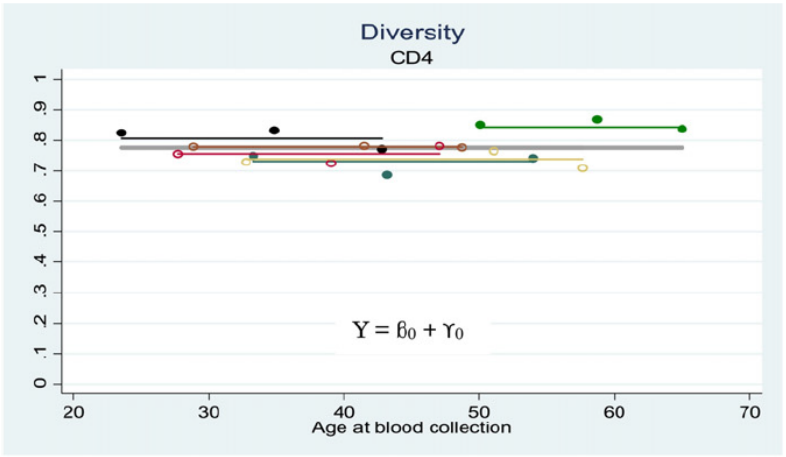
- There are MANY examples of clonal T-cell proliferations that are NOT neoplastic
  - Commonly skin, peripheral blood
  - Post transplant
  - Various immune responses
    - Inflammatory (Crohn's etc.)
    - Malignancy (CLL/SLL, etc.)

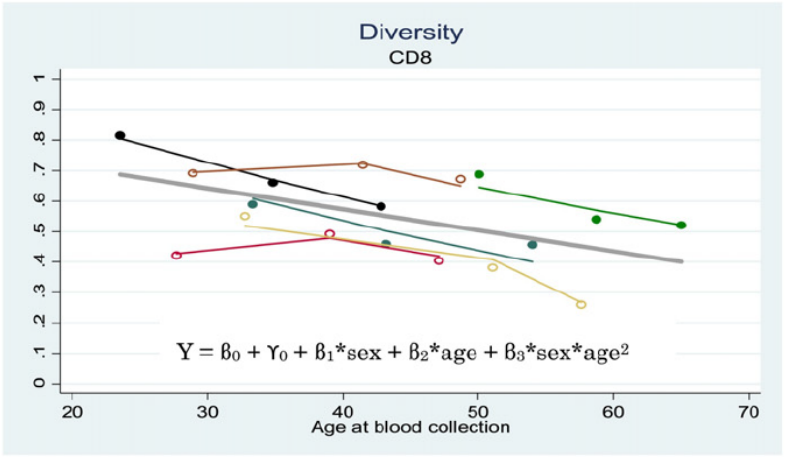
Example from ESRD patients – Peripheral blood T-cells

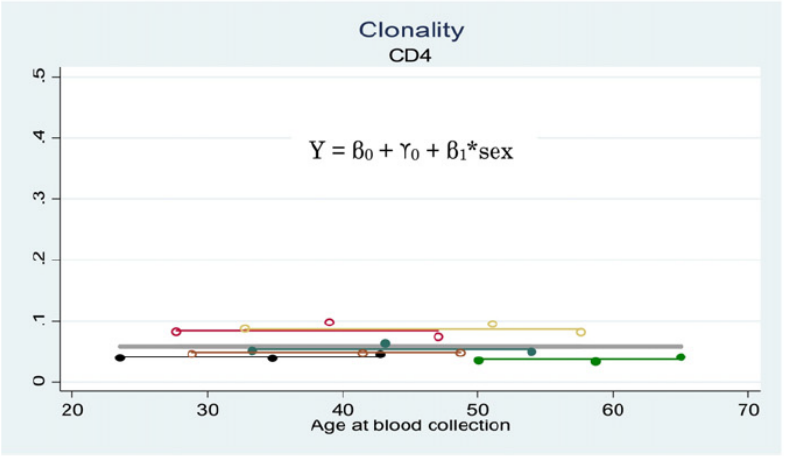


Huang et al. Immunity & Ageing 2015;12:28.

T-cell repertoire decreases with age







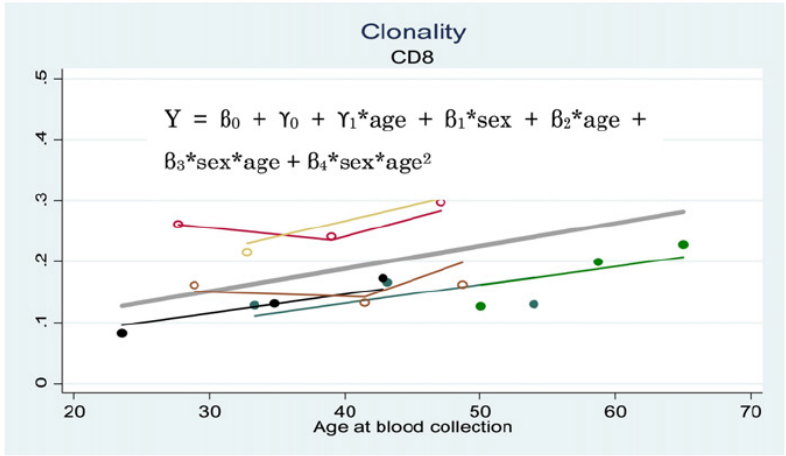


Fig. 1. CD4 and CD8 TCR diversity and clonality. Points are observed, repeated values: solid circles represent males (color coded in blue, green, and black); open circles represent females (color coded in brown, red, and tan). Solid lines connect the fitted values of the best-fitting models at the observed age points. The single gray line is the population-average trajectory, deviations from which reflect differences in overall level, slope, or both.

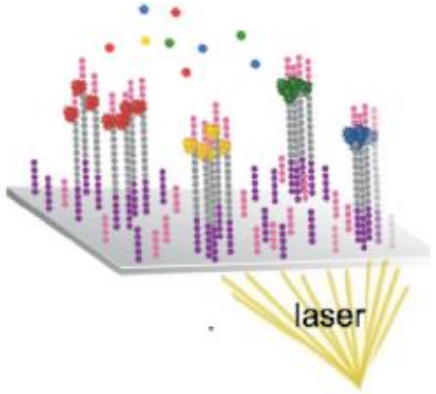
The future…

- Using NGS data for T-cell clonality
  - More powerful
  - Not just used for clonality, but can examine different types of T-cell immune responses in other non-hematologic malignancies
    - May alter therapy choices; immune checkpoint inhibitors
- The downside
  - Longer TAT
  - Higher cost
  - Clones may be readily identified and still does not solve the problem that clonality ≠ lymphoma!

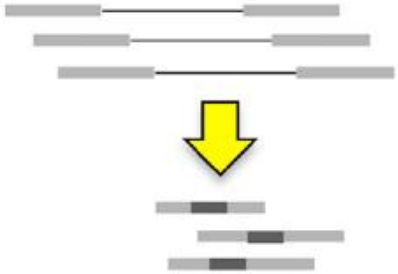
Multiplex PCR

Ligation of Illumina Adapters

MiSeq Sequencing



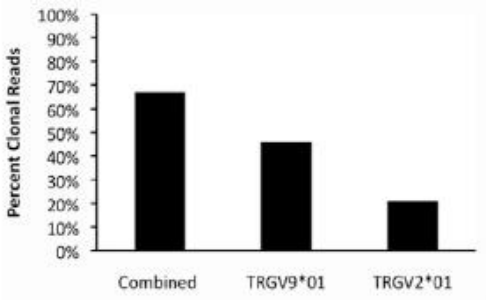
Combine Read Pairs



Count Reads

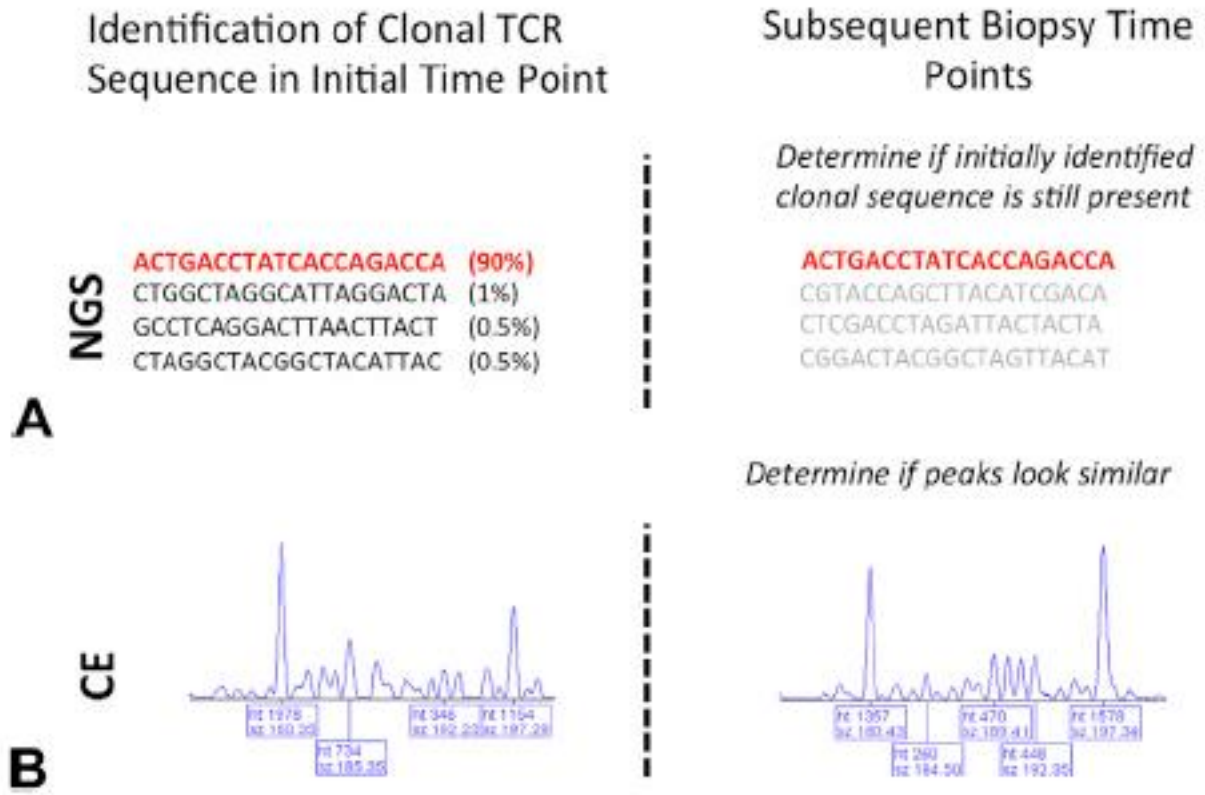
ACTGACCTATCACCAGACCA
ACTGACCTATCACCAGACCA
ACTGACCTATCACCAGACCA
3x
CTGGCTAGGCATTAGGACTA
GCCTCAGGACTTAACTTACT

Align Top Reads to IMGT Database



J Am Acad Dermatol 2015;73:228-36.

NGS in recurrence



J Am Acad Dermatol 2015;73:228-36.

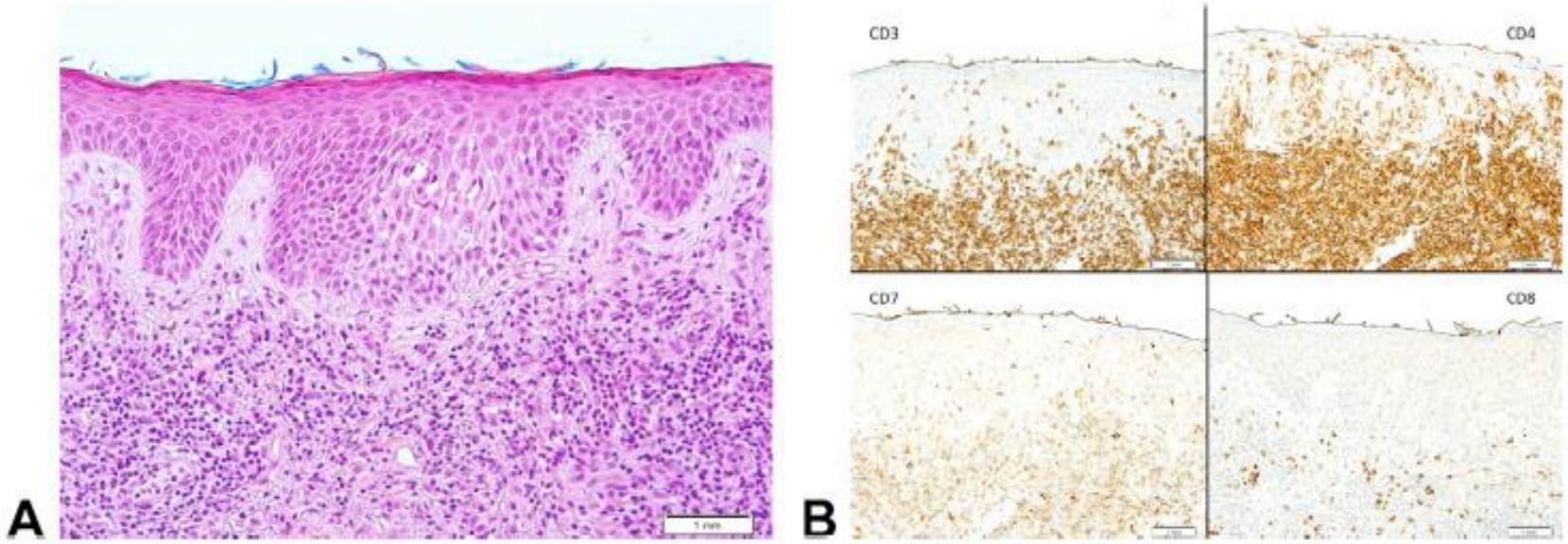
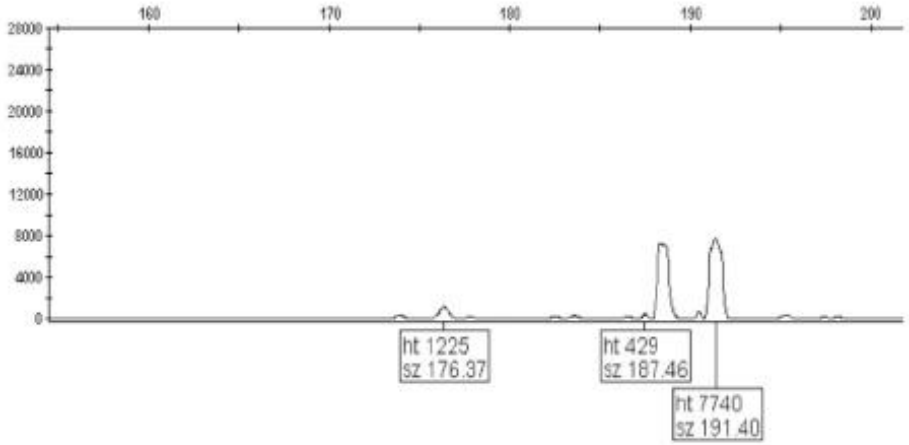


Fig 4. Mycosis fungoides. **A**, Representative skin biopsy specimen characterized by a lymphocytic infiltrate composed of small to medium atypical cerebriform cells demonstrating epidermotropism. Haloed cells are notable within the epidermis. (Hematoxylin-eosin stain; original magnification: ×20.) **B**, Lymphocytes were immunoreactive for CD2, CD3, and CD5, with reduced CD7 positivity. As CD4 also stains Langerhans cells in the epidermis, there are more $CD4^+$ cells than $CD3^+$ cells in the epidermis. Because of this, it is important to compare CD3 and CD8 when examining the epidermal compartment. The $CD3^+CD8^-$ cells likely correspond to $CD4^+$ T cells. CD4 expression was greater than that of CD8. (Immunohistochemistry, original magnification: ×20.)

J Am Acad Dermatol 2015;73:228-36.

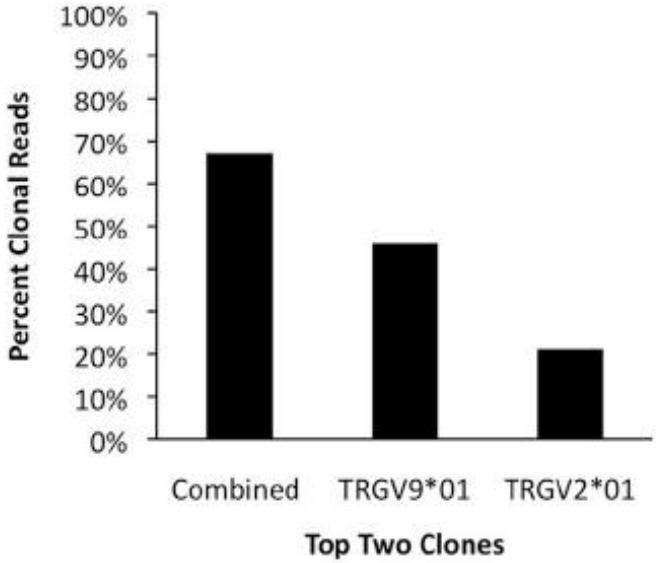
Case 1

CE



Clonal

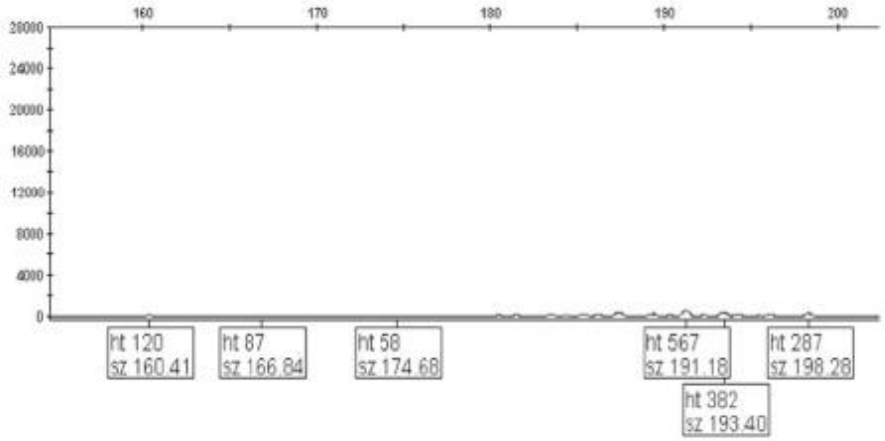
NGS



Clonal

Case 2

CE



Polyclonal

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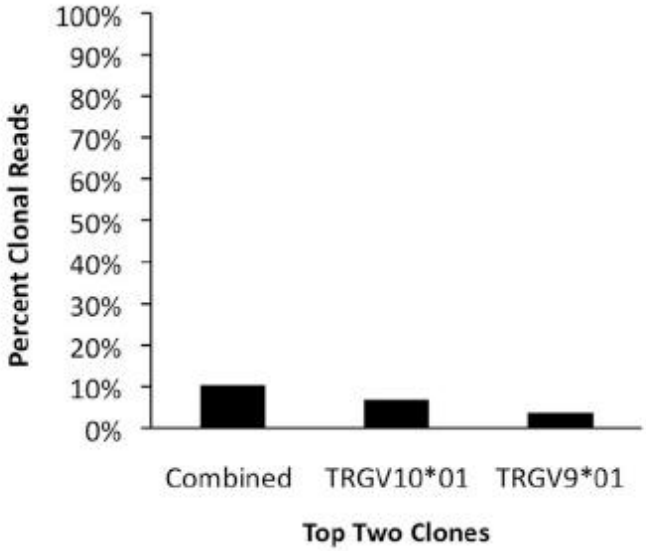

Clonal

Conclusions

- DLBCL work-up is constantly evolving but IHC and FISH are important for prognosis

- Molecular clonality assays can be very helpful if used in the right context, with an awareness of possible “pitfalls”.
  - Most importantly they should be combined with impression from all other studies and history