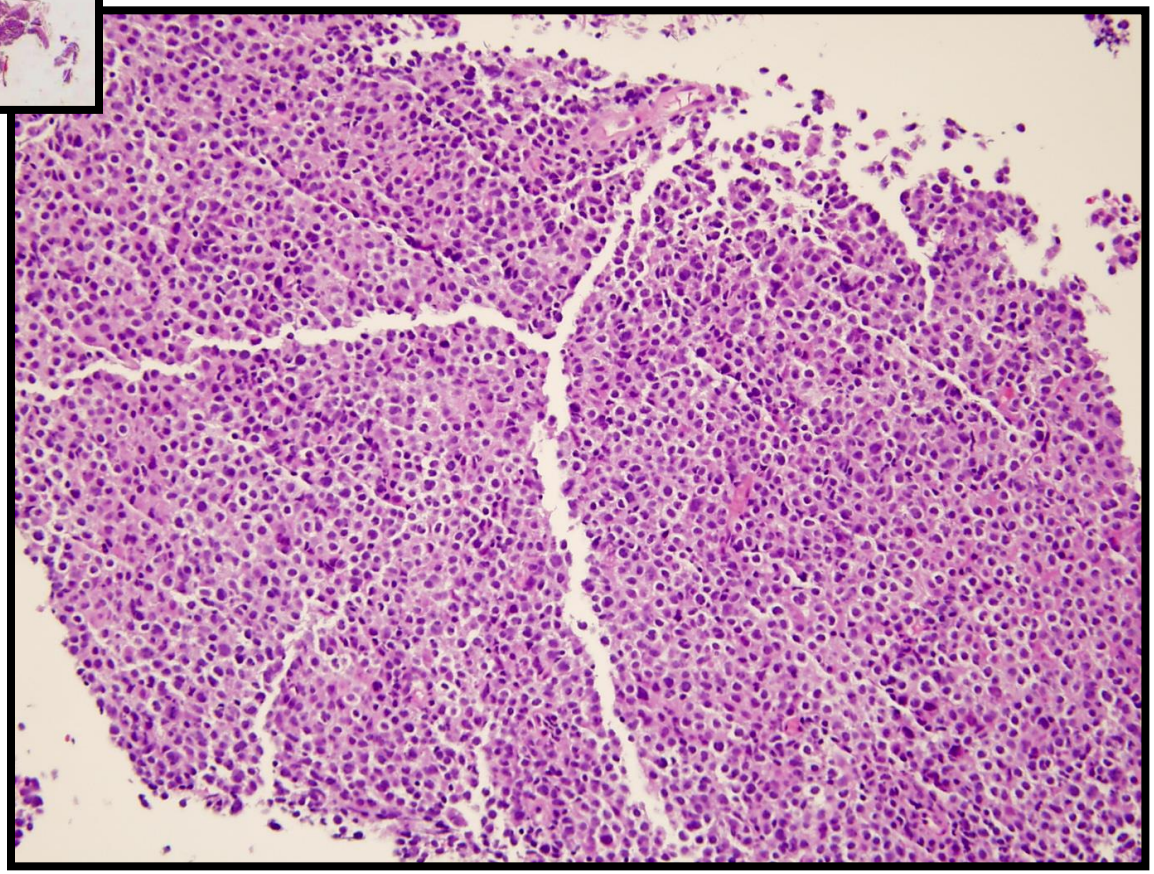
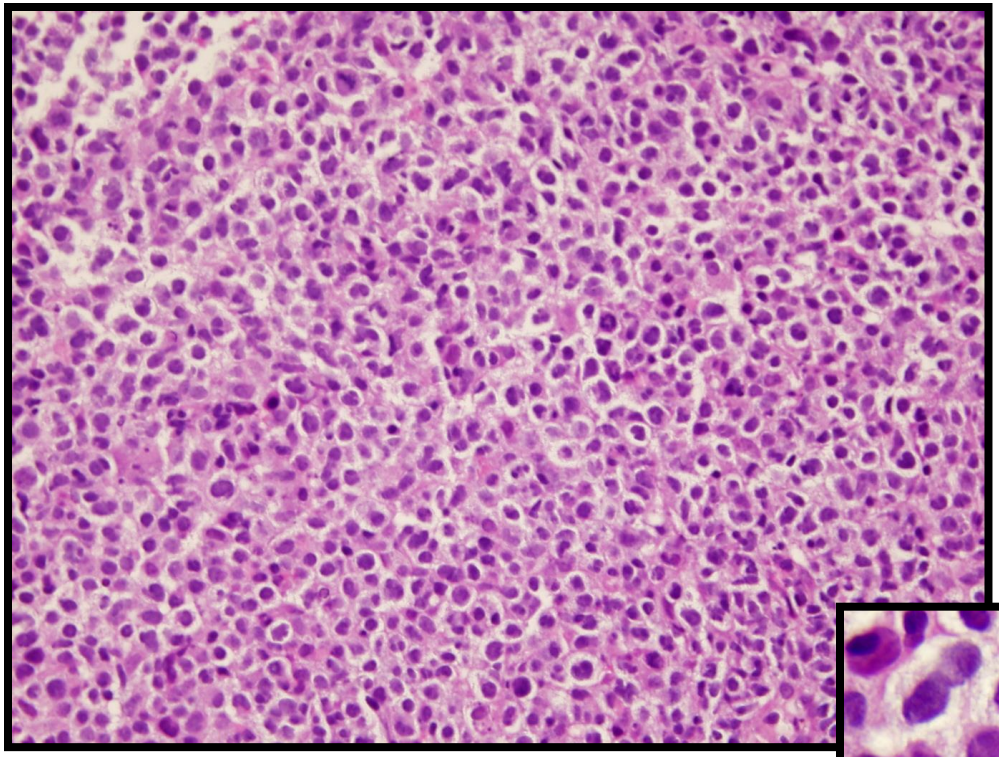
CASE 4

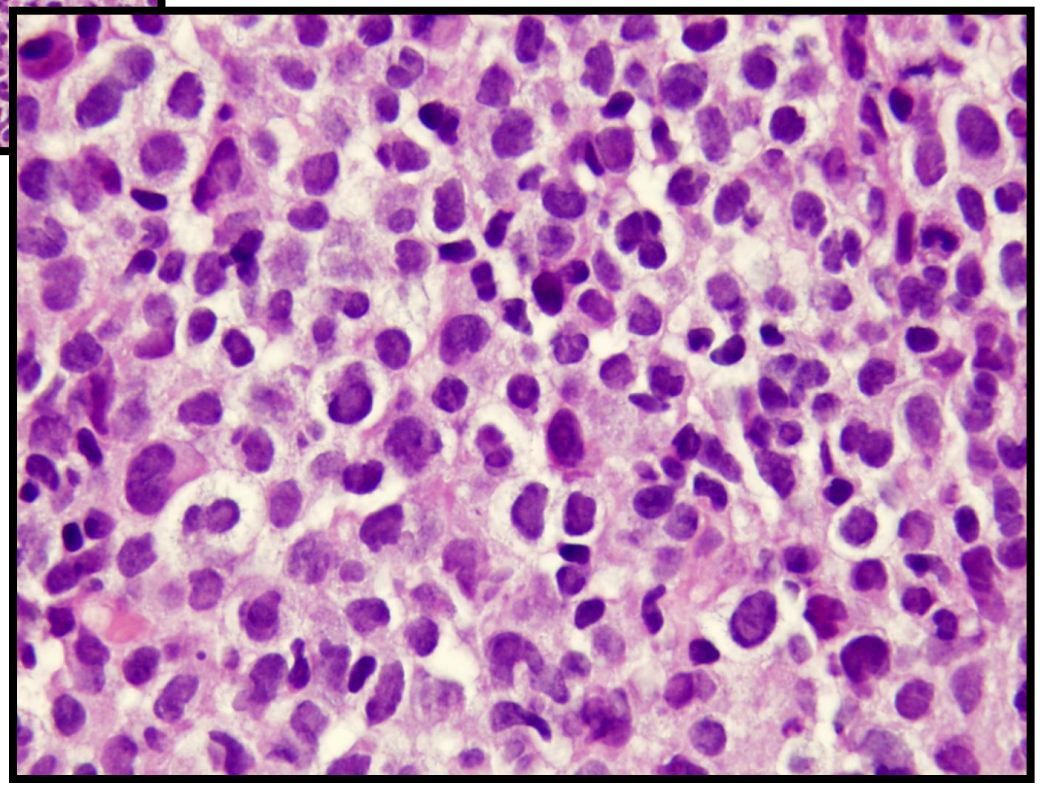
A 70-year-old woman with a history of diabetes, hypertension and kidney failure presented with acute onset of fatigue and dizziness. Physical examination showed B-type symptoms. Laboratory evaluation showed hypercalcemia. PET/CT showed lymphadenopathy, splenomegaly and bone lesions. This is a right axillary lymph node needle biopsy.

Case 4

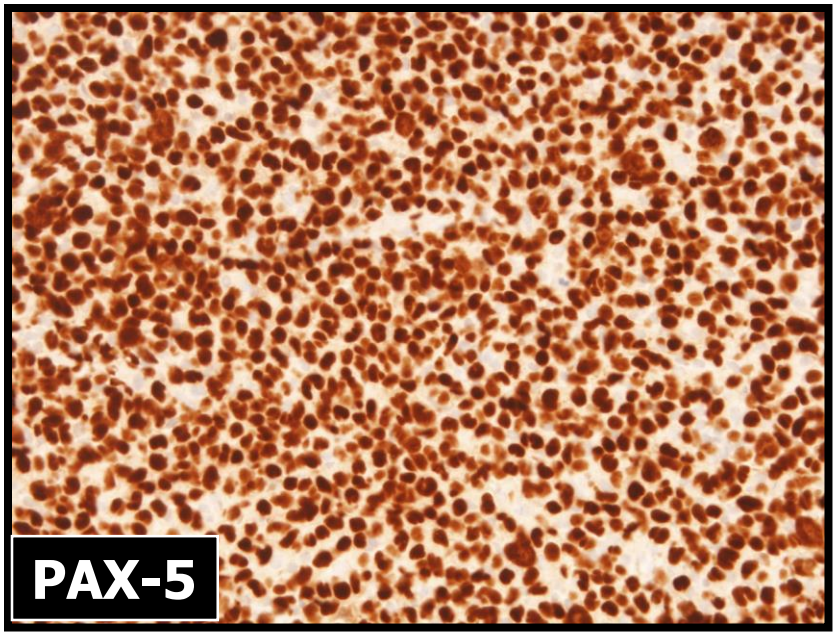


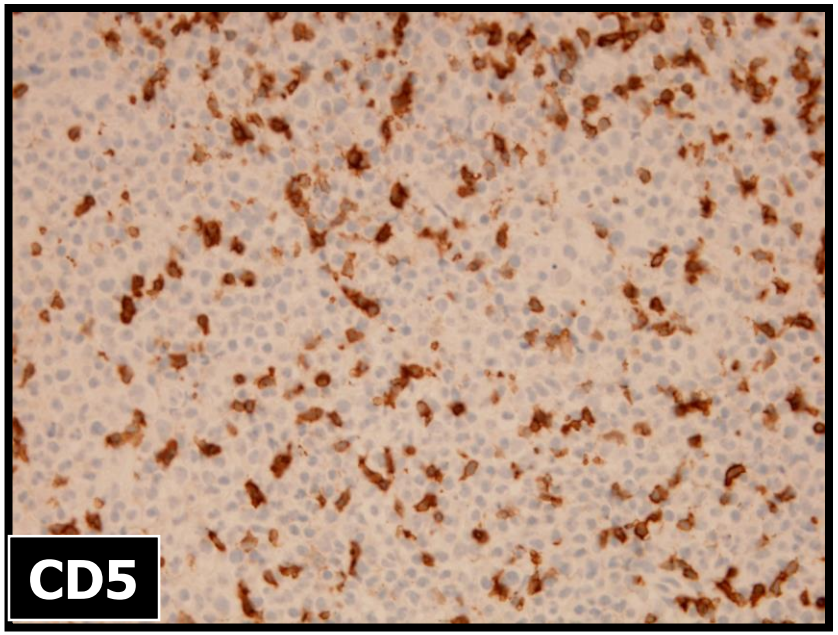
Case 4

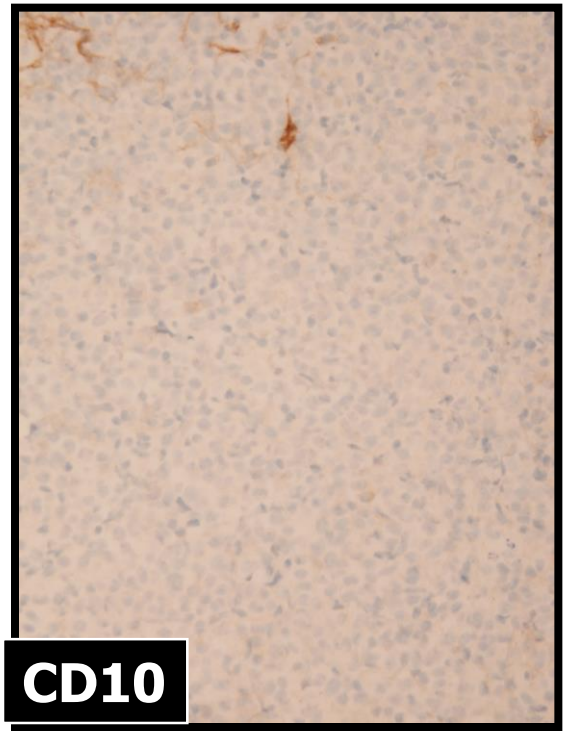


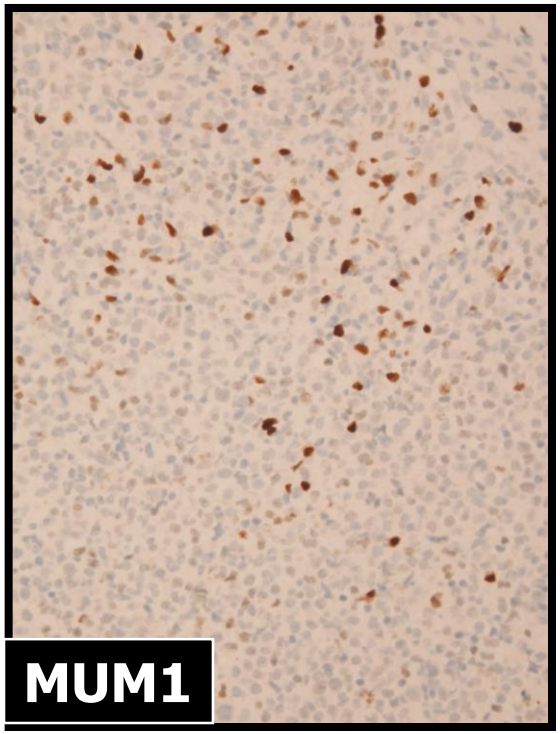


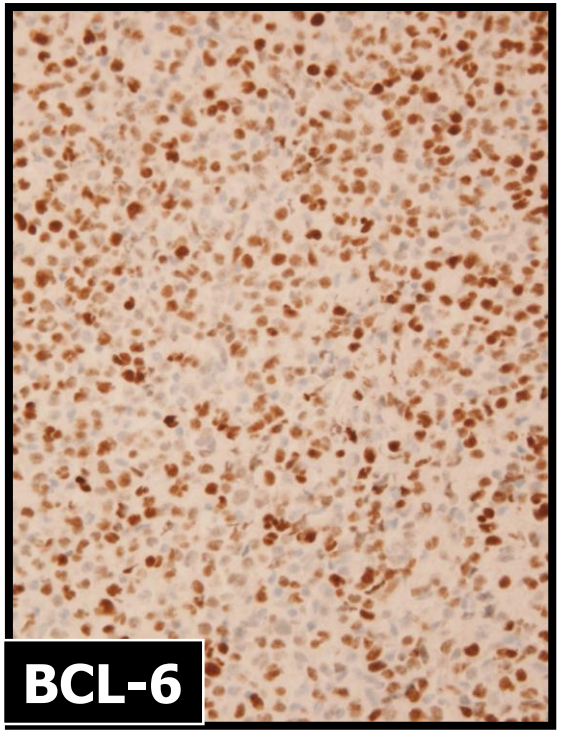
Case 4

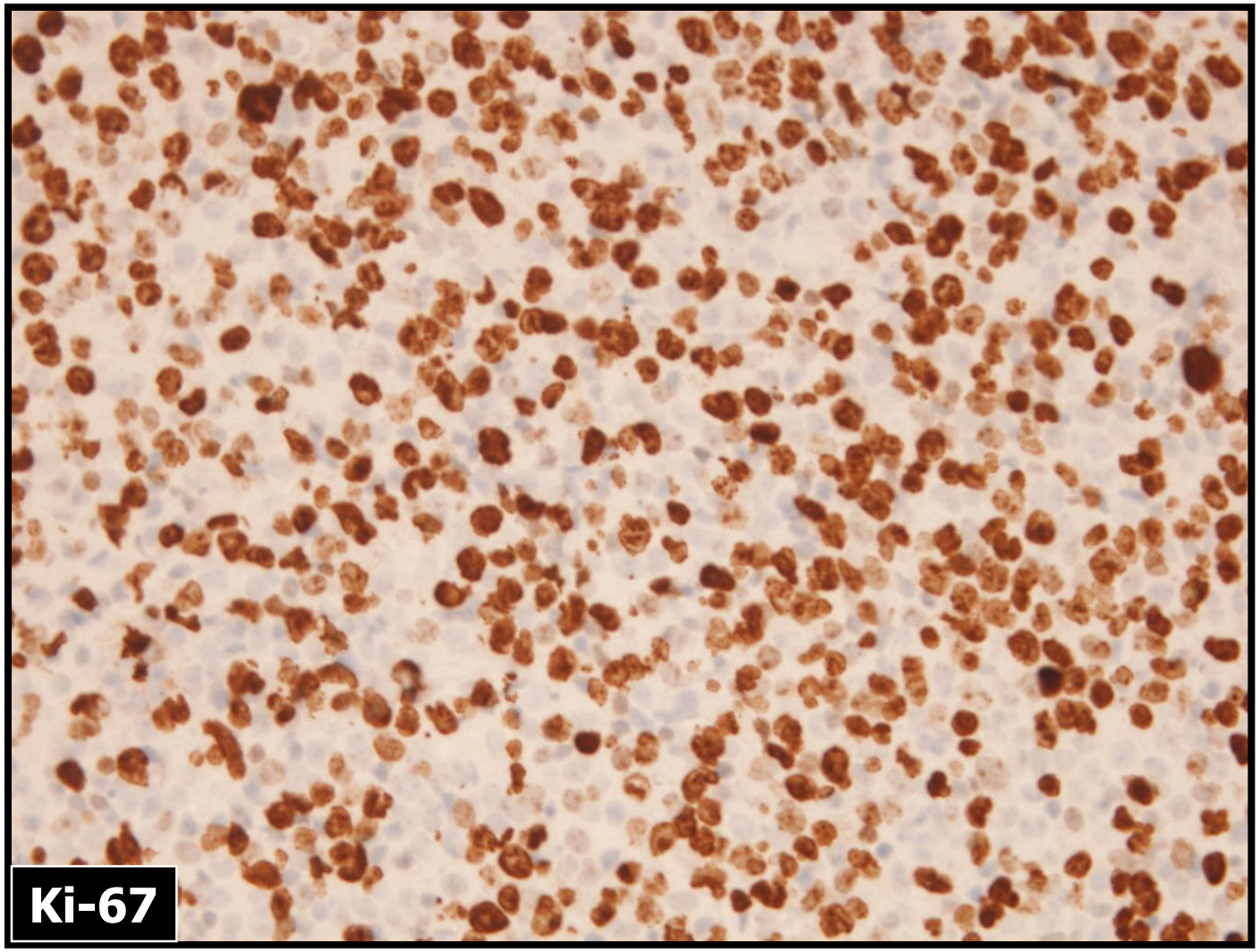




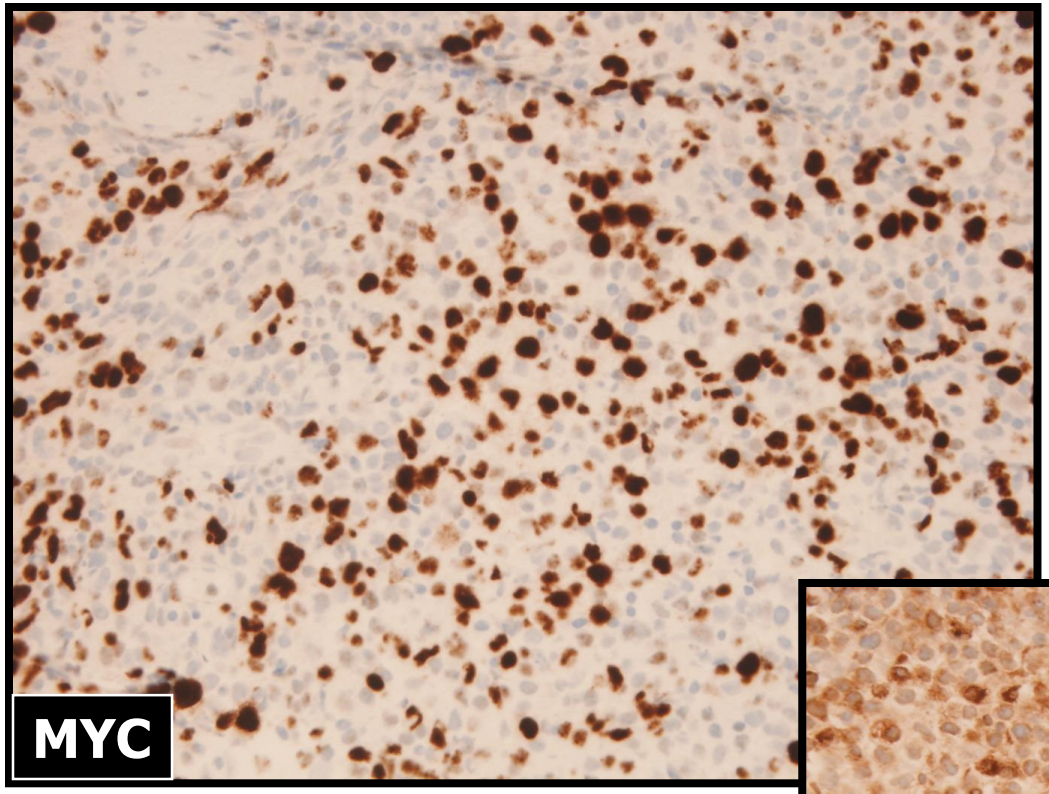


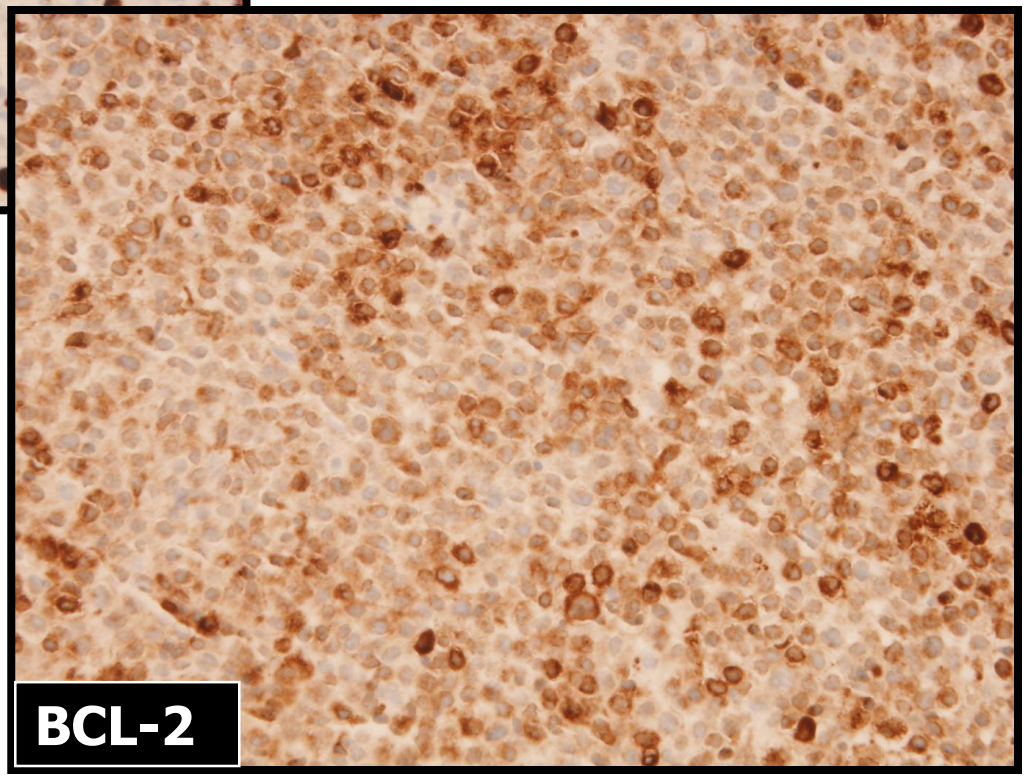





Ki-67

Case 4





DIAGNOSIS (CASE 4)

Diffuse large B-cell lymphoma

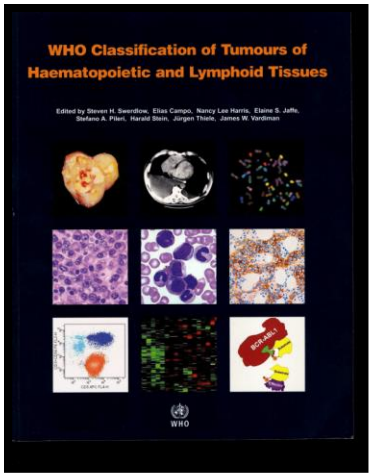
The neoplasm also has:

Germinal center B-cell immunophenotype
Double expressor immunophenotype
Ki-67 ~70%
***MYC* not rearranged**
***BCL6* rearranged**
***BCL2* not rearranged**

Diffuse Large B-cell Lymphoma

Definition

DLBCL is a neoplasm with a diffuse growth pattern composed of medium or large B lymphoid cells with nuclear size equal to or exceeding normal macrophage nuclei, or more than twice the size of normal lymphocyte nuclei



2017 WHO book, p. 291

WHO Classification of Diffuse Large B-cell Lymphoma (2017)

Diffuse large B-cell lymphoma, NOS

GCB versus ABC/non-GCB
CD5

Other lymphomas of large B-cells

T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system
Primary cutaneous DLBCL, leg-type
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
EBV+ diffuse large B-cell lymphoma
ALK+ large B-cell lymphoma
Plasmablastic lymphoma
HHV8+ lymphoproliferative disorders
Primary effusion lymphoma

Borderline cases

High-grade B-cell lymphoma (NOS versus double hit)
B-cell lymphoma, unclassifiable, intermediate between DLBCL & CHL

Diffuse Large B-cell Lymphoma
R-CHOP is Standard Frontline Therapy

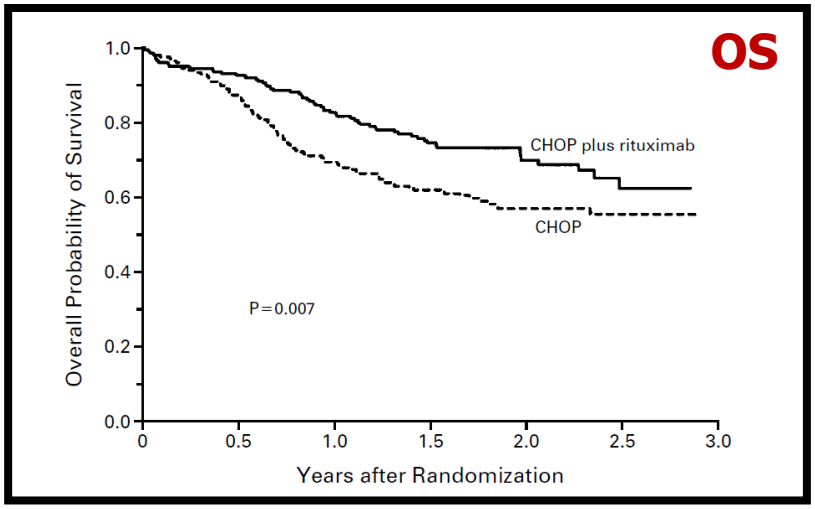
Rituximab
Cyclophosphamide
Hydroxydaunorubicin/Adriamycin
Oncovin/vincristine
Prednisone

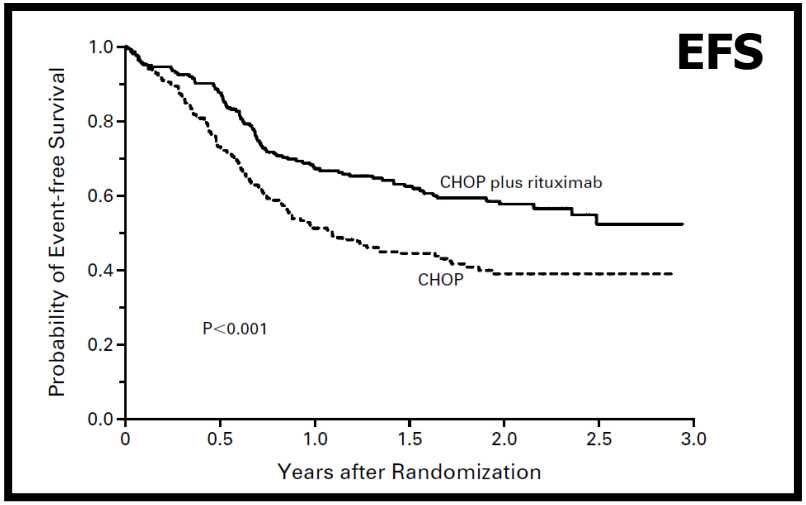
Bertrand Coiffier, MD

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 346: 235, 2002





Diffuse Large B-cell Lymphoma NOS

Clinical Findings

Median age	64 y (wide range)
Male	55%
Stage I-II	54%
III-IV	46%
B symptoms	33%
BM involved	16%
IPI 0-1	35%
2-3	46%
4-5	19%

Nebraska NHL Classification Project

Diffuse Large B-cell Lymphoma
International Prognostic Index

Age	≤ 60 vs. >60 years
Performance status	0-1 vs. 2-4
LDH	Normal vs elevated
Extranodal sites	≤ 1 vs >1 site
Stage	I-II vs III-IV

N Engl J Med 329: 987, 1993

An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era

Zheng Zhou,[1] Laurie H. Sehn,[2] Alfred W. Rademaker,[1] Leo I. Gordon,[1] Ann S. LaCasce,[3] Allison Crosby-Thompson,[3] Ann Vanderplas,[4] Andrew D. Zelenetz,[5] Gregory A. Abel,[3] Maria A. Rodriguez,[6] Auayporn Nademanee,[7] Mark S. Kaminski,[8] Myron S. Czuczman,[9] Michael Millenson,[10] Joyce Niland,[4] Randy D. Gascoyne,[2] Joseph M. Connors,[2] Jonathan W. Friedberg,[11] and Jane N. Winter[1]

Table 3. The NCCN-IPI

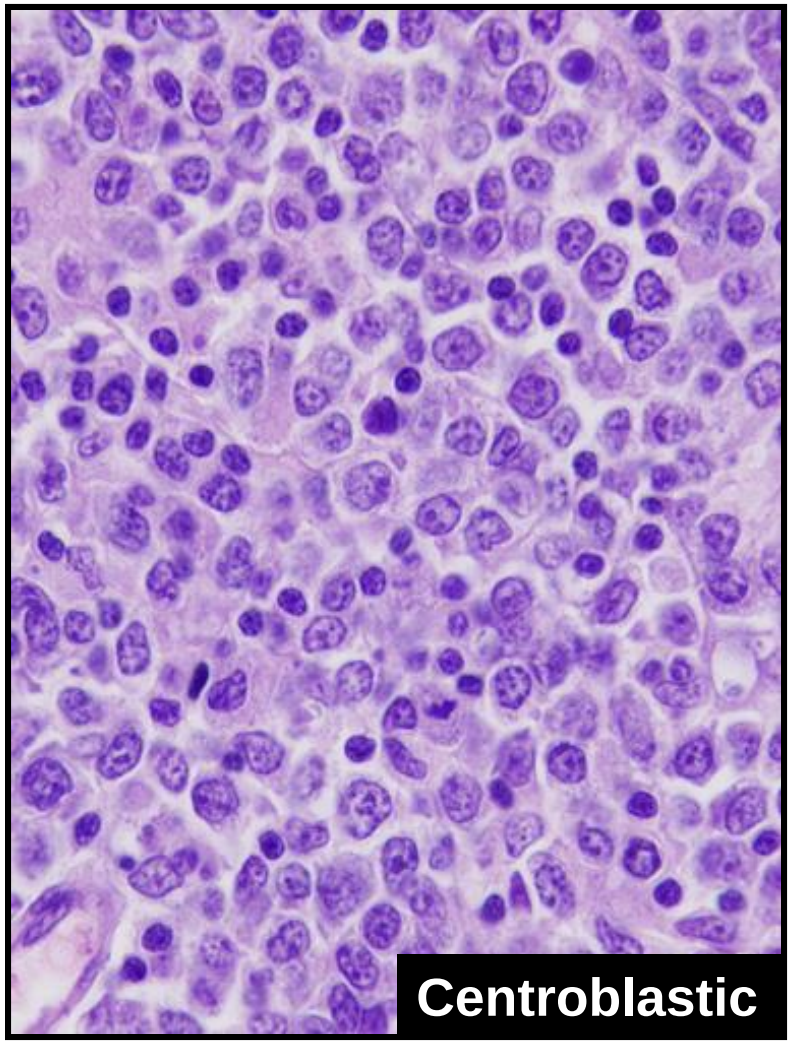
NCCN-IPI	Score
Age, y	
>40 to ≤60	1
>60 to ≤75	2
>75	3
LDH, normalized	
>1 to ≤3	1
>3	2
Ann Arbor stage III-IV	1
Extranodal disease*	1
Performance status ≥2	1

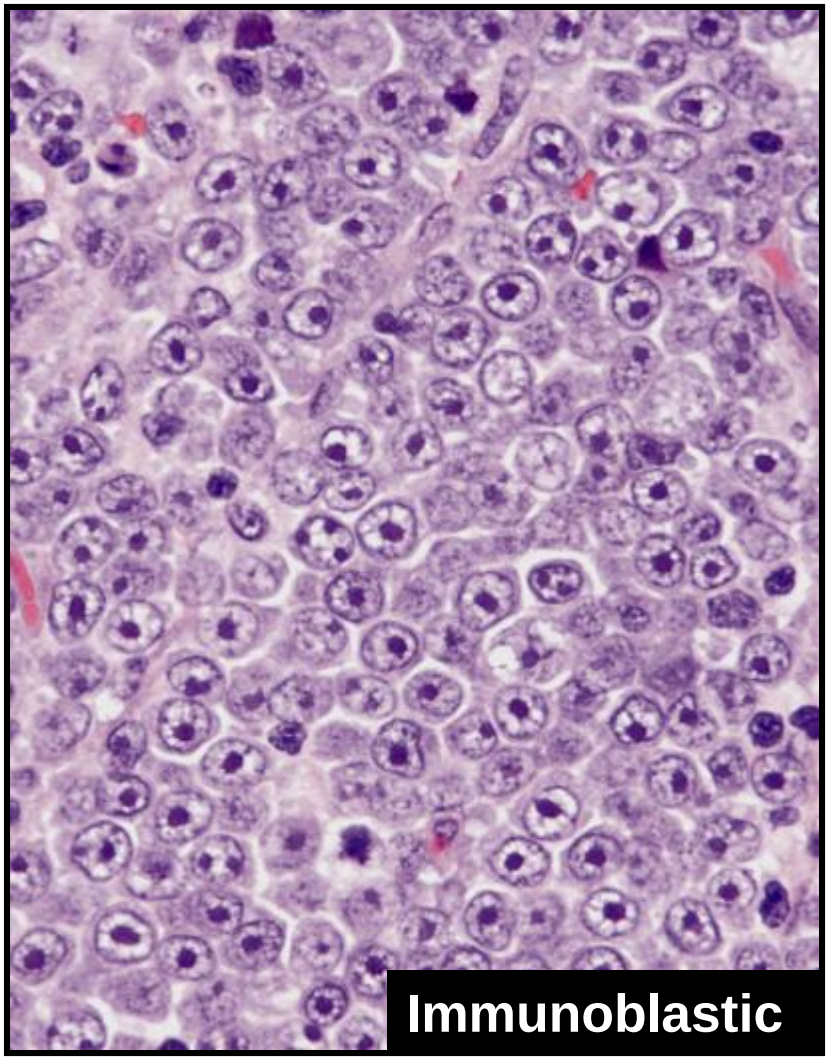
*Disease in bone marrow, CNS, liver/GI tract, or lung.

Blood 123: 837, 2014

Diffuse Large B-cell Lymphoma NOS

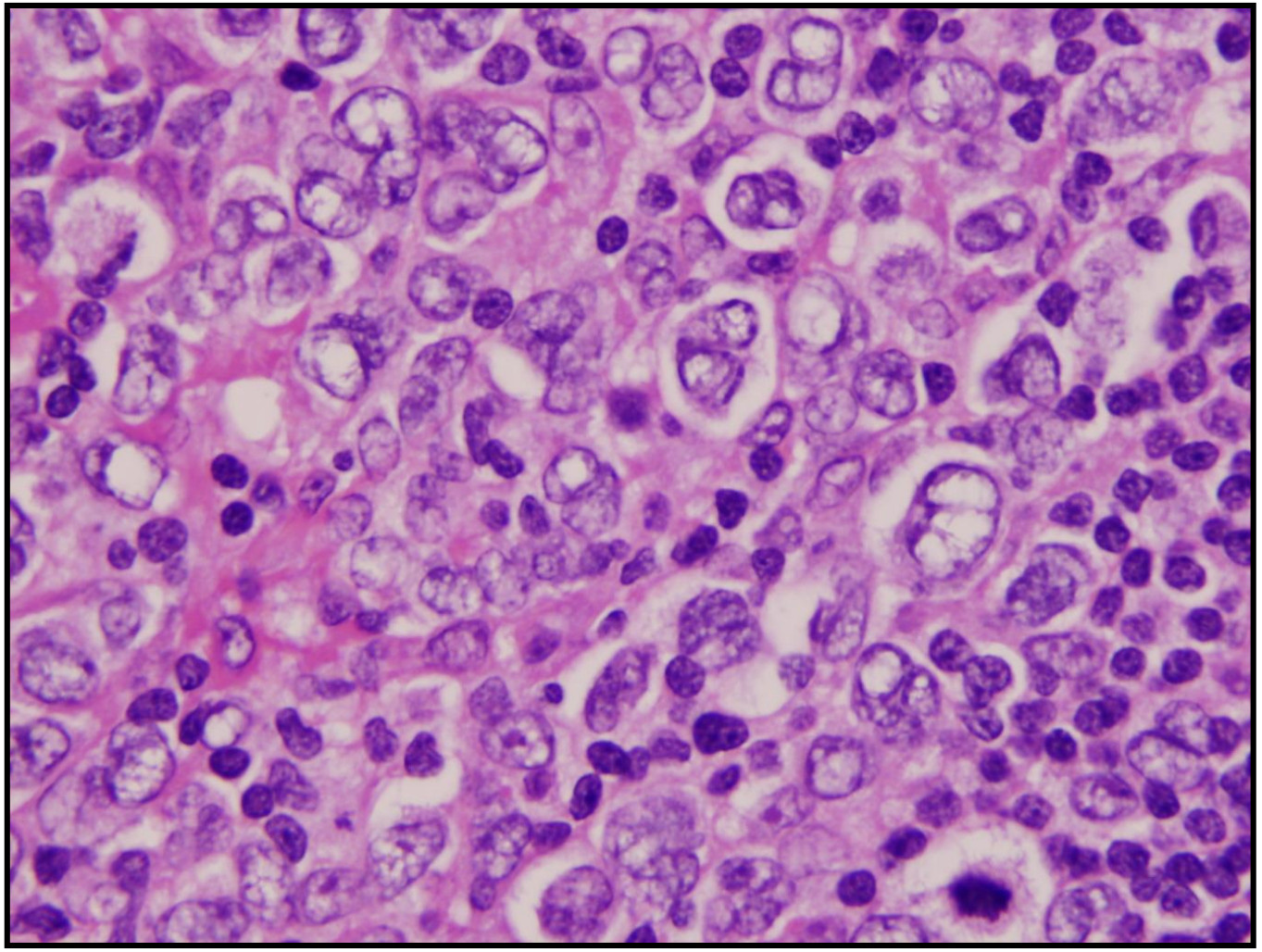
Morphologic Variants





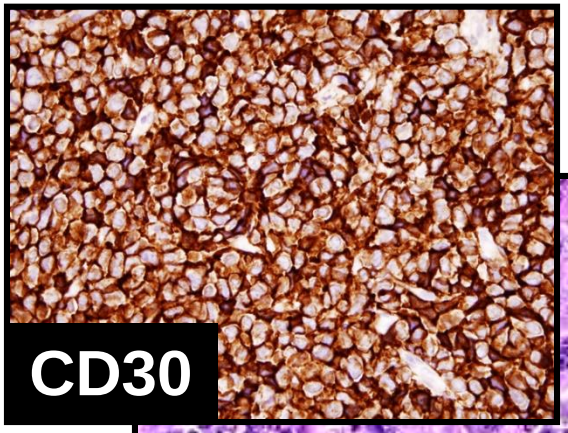
Diffuse Large B-cell Lymphoma NOS

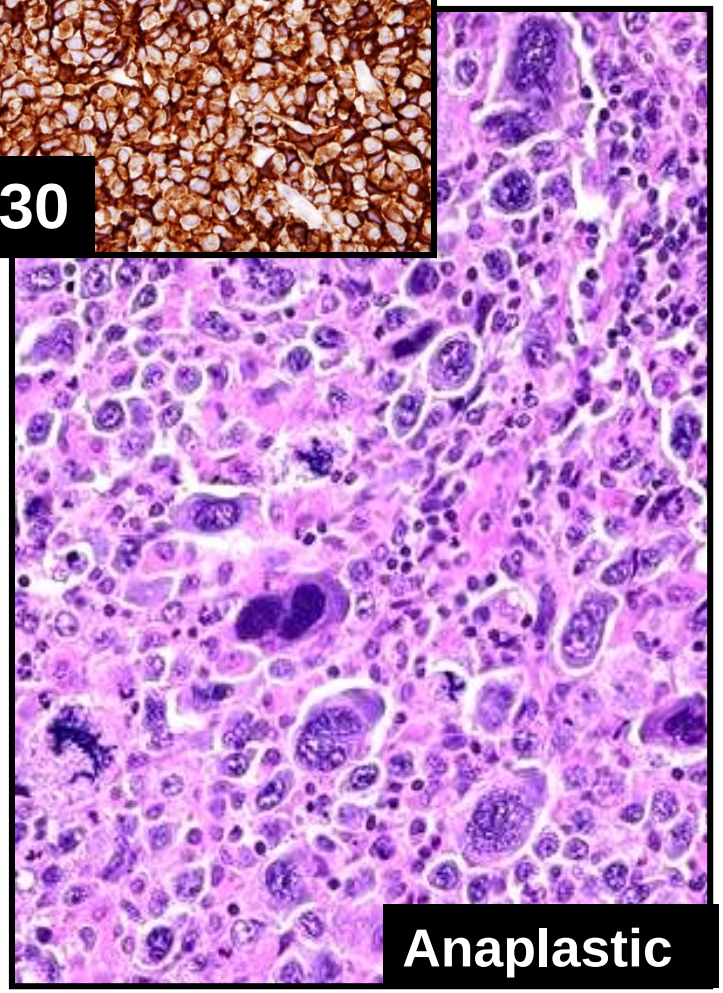
Multilobated Variant

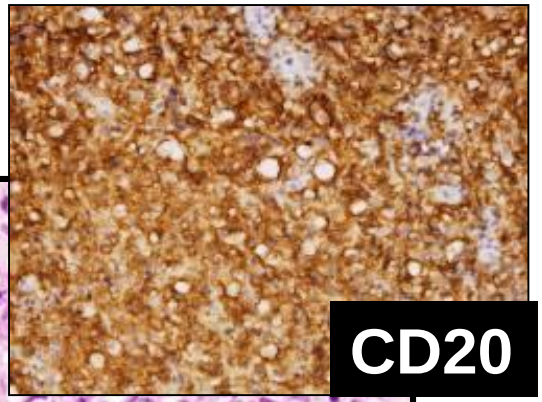


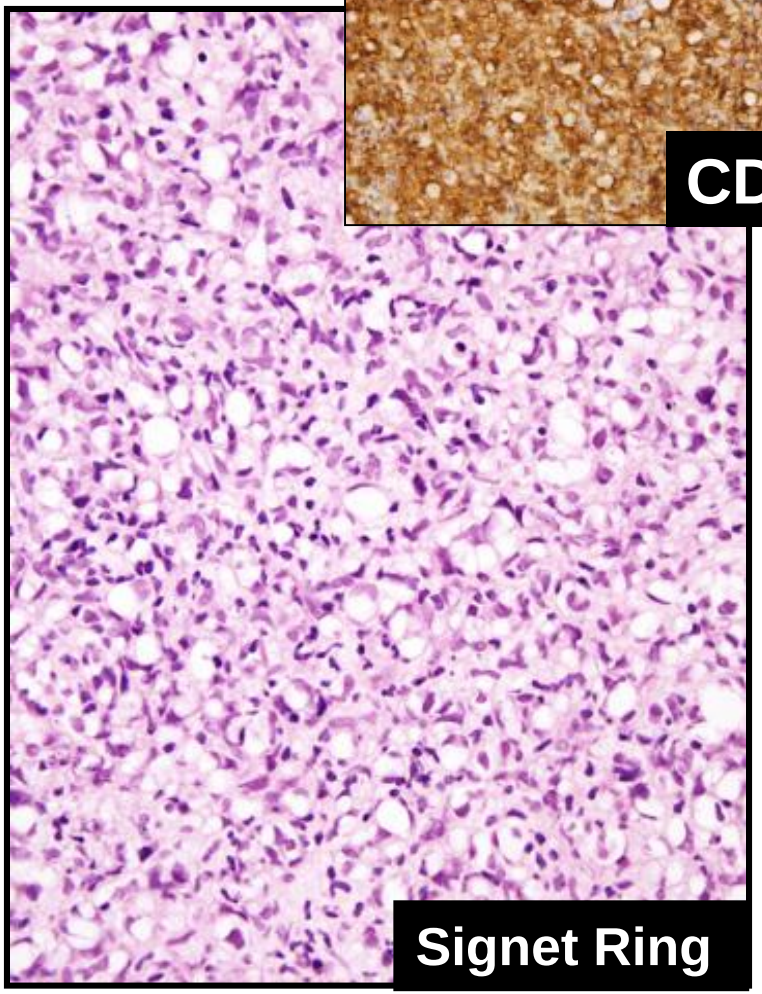
Diffuse Large B-cell Lymphoma NOS

Morphologic Variants



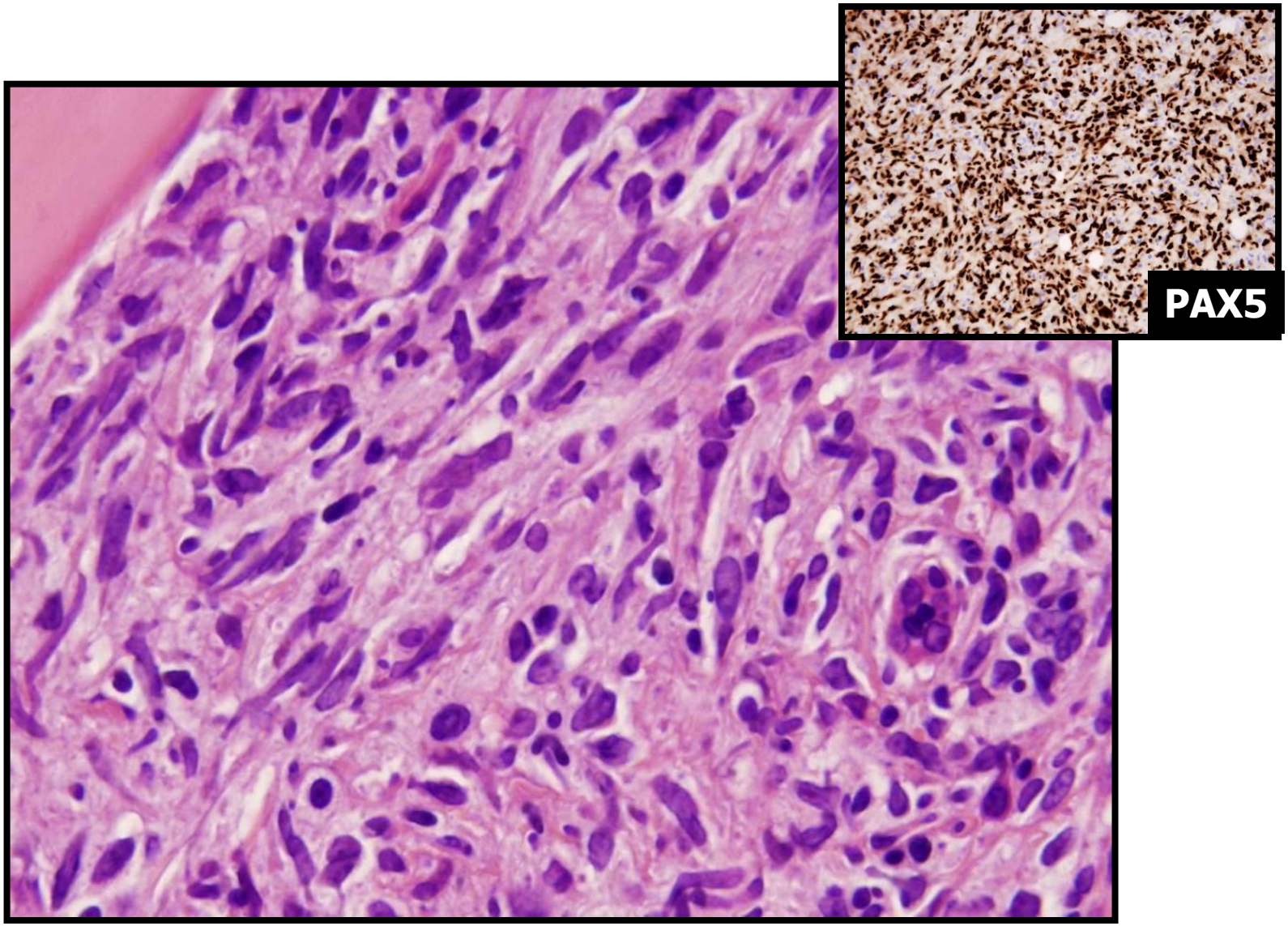






Diffuse Large B-cell Lymphoma NOS

Spindle Cell Variant



Diffuse Large B-cell Lymphoma NOS

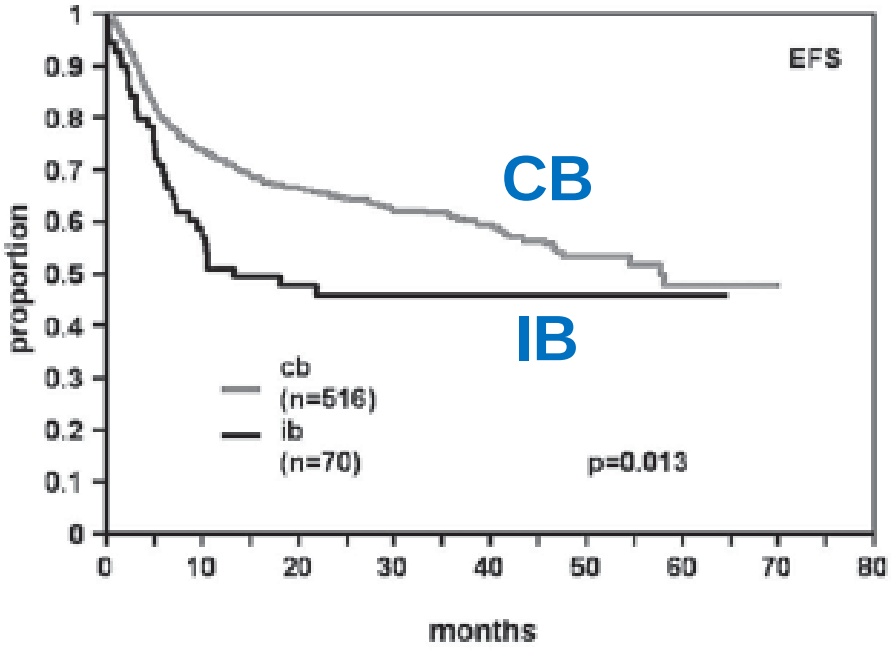
Morphologic Variants

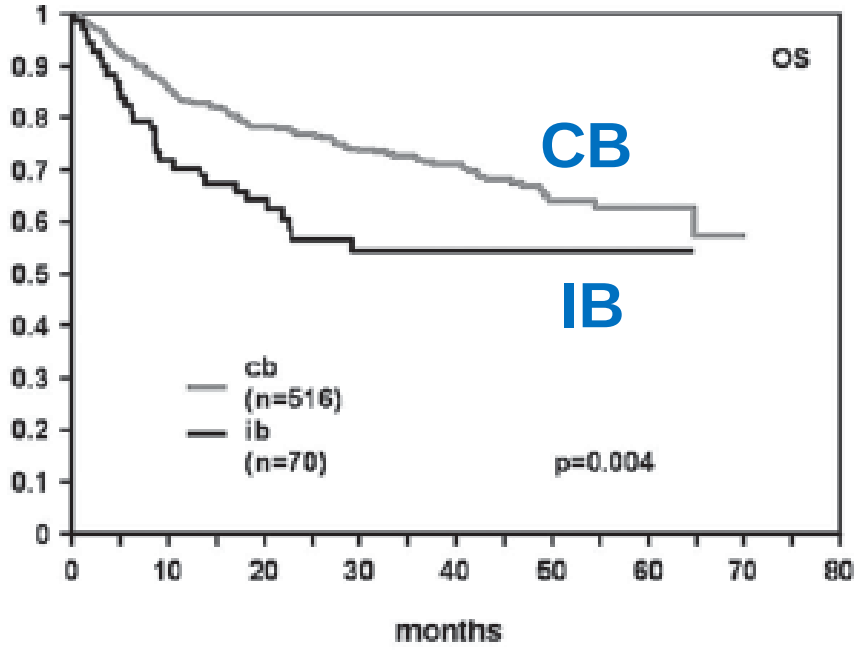
Common	Rare
Centroblastic (~80%)	Sinusoidal
Immunoblastic (~10%)	Spindled
Multilobated (<5%)	Myxoid
Anaplastic (<5%)	Signet Ring
	Rosettes

Does morphology correlate with prognosis ?

Immunoblastic morphology but not the immunohistochemical GCB/nonGCB classifier predicts outcome in diffuse large B-cell lymphoma in the RICOVER-60 trial of the DSHNHL

German Ott,[1,2] Marita Ziepert,[3] Wolfram Klapper,[4] Heike Horn,[2] Monika Szczepanowski,[4] Heinz-Wolfram Bernd,[5] Christoph Thorns,[5] Alfred C. Feller,[5] Dido Lenze,[6] Michael Hummel,[6] Harald Stein,[6] Hans-Konrad Müller-Hermelink,[1] Matthias Frank,[7] Martin-Leo Hansmann,[7] Thomas F. E. Barth,[8] Peter Möller,[8] Sergio Cogliatti,[9] Michael Pfreundschuh,[10] Norbert Schmitz,[11] Lorenz Trümper,[12] Markus Loeffler,[3] and Andreas Rosenwald[1]





Blood 116: 4916, 2010

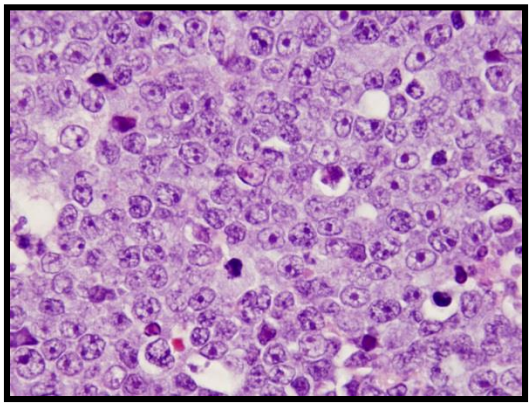
Diffuse Large B-cell Lymphomas of Immunoblastic Type Are a Major Reservoir for *MYC-IGH* Translocations

Heike Horn, PhD, Annette M. Staiger, MSc,* Matthias Vöhringer, MD,† Ulrich Hay, MD,‡ Elias Campo, MD,§ Andreas Rosenwald, MD,‖ German Ott, MD,* and M. Michaela Ott, MD¶*

The authors assessed 107 DLBCL using FISH with MYC breakapart and *MYC-IGH* fusion probes

***MYC* translocations detected in**

13 / 39 (33%) immunoblastic
5 / 68 (7%) centroblastic



All immunoblastic DLBCL with *MYC* translocations had *MYC-IGH* fusions

Am J Surg Pathol 39: 61, 2015

Immunophenotyping of DLBCL
What Is The Purpose ?

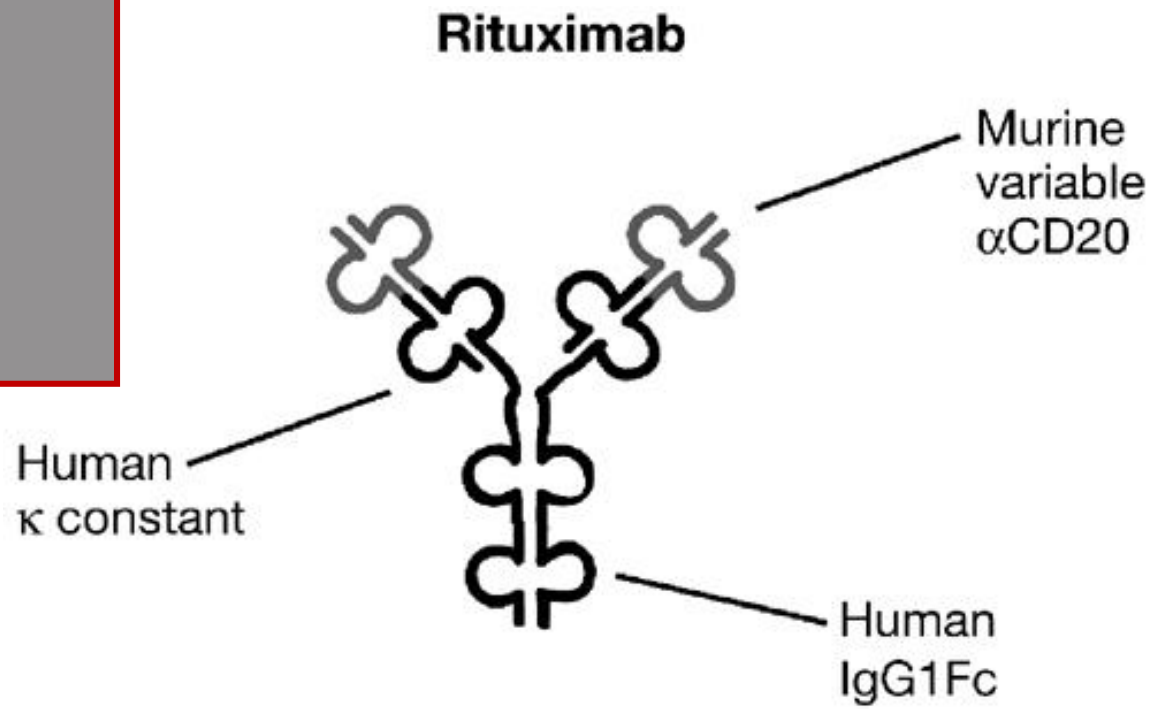
In the past

- Diagnosis

Currently

- Diagnosis
- Prognosis
- Identifying targets for therapy

Monoclonal Antibodies are Being Added to Standard Therapy



CD20 is used for diagnosis and is a therapeutic target

Potential Targets Assessable by IHC

Target	Drug	Pathway
CD19	Tafasitamab	B-cell receptor signaling
CD30	Brentuximab vedotin	NF-κB
CD38	Daratumumab	Cell migration, adhesion, signaling
CD79A	Polatuzumab vedotin	B-cell receptor signaling
BTK	Ibrutinib	B-cell receptor signaling
XPO1	Selinexor	Selective inhibitor of nuclear export
BRAF, MEK	Vemurafinib, cobimetinib	MAP kinase
BCL-2	Venetoclax	Apoptosis
PD-L1/L2	Nivolumab, others	Checkpoint inhibitors

Common Translocations in DLBCL

t(3;14)(q27;q32); ***BCL6::IGH*** **~25%**

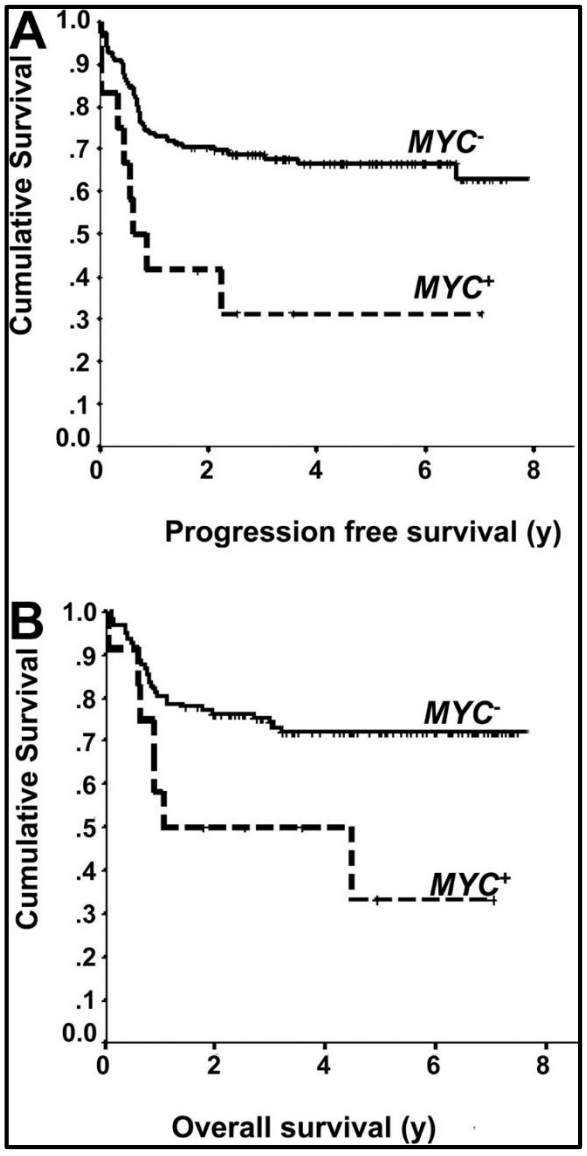
BCL6 also partners with other genes

t(14;18)(q32;q21); ***IGH::BCL2*** **~20%**

t(8;14)(q24;q32); ***MYC::IGH*** **~10%**

MYC also partners with other genes

MYC Rearrangment is Prognostic in DLBCL



t(8;14)(q24;q32) - *IGH* (80%)
t(8;22)(q24;q11) - *IGλ* (15%)
t(2;8)(p11;q24) - *IGκ* (5%)

Diagnostic tests

Conventional cytogenetics

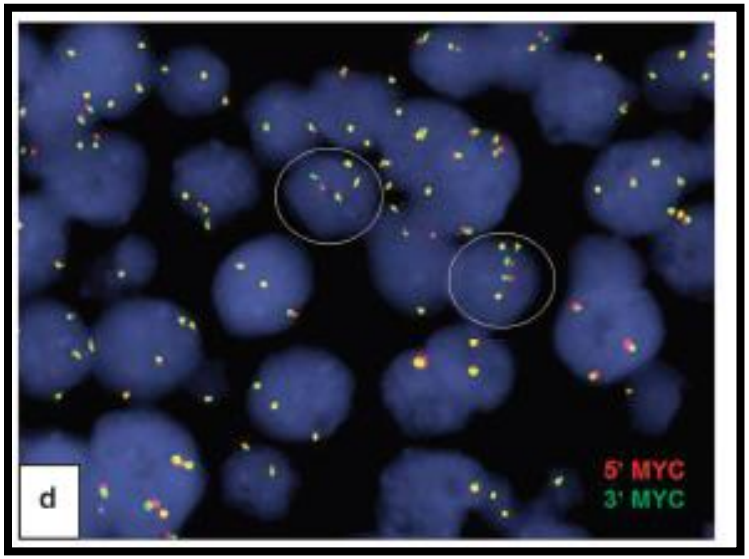
- **Need viable cells**

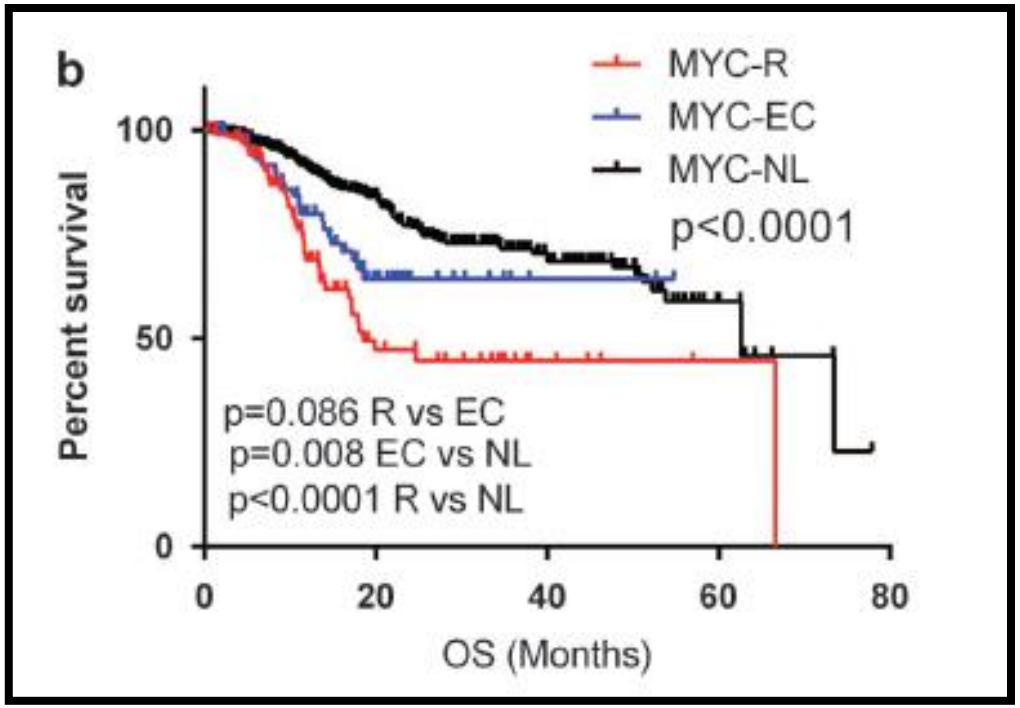
FISH

- ***IGH* and *MYC* probes**
- ***MYC* breakapart probe**

Blood 114:3533, 2009

MYC Extra Copies by FISH Predict Poorer Prognosis in DLBCL





Andres Quesada, MD

Increased *MYC* copy number is an independent prognostic factor in patients with diffuse large B-cell lymphoma

Andrés E Quesada[1], L Jeffrey Medeiros[1], Parth A Desai[1], Pei Lin[1], Jason R Westin[2], Huda M Hawsawi[1], Peng Wei[3], Guilin Tang[1], Adam C Seegmiller[4], Nishitha M Reddy[5], C Cameron Yin[1], Wei Wang[1], Jie Xu[1], Roberto N Miranda[1], Zhuang Zuo[1] and Shaoying Li[1]

Mod Pathol 30: 1688, 2017

Diffuse Large B-cell Lymphoma

Gene Expression Profiling Using DNA Microarrays

Ash Alizadeh, MD, PhD

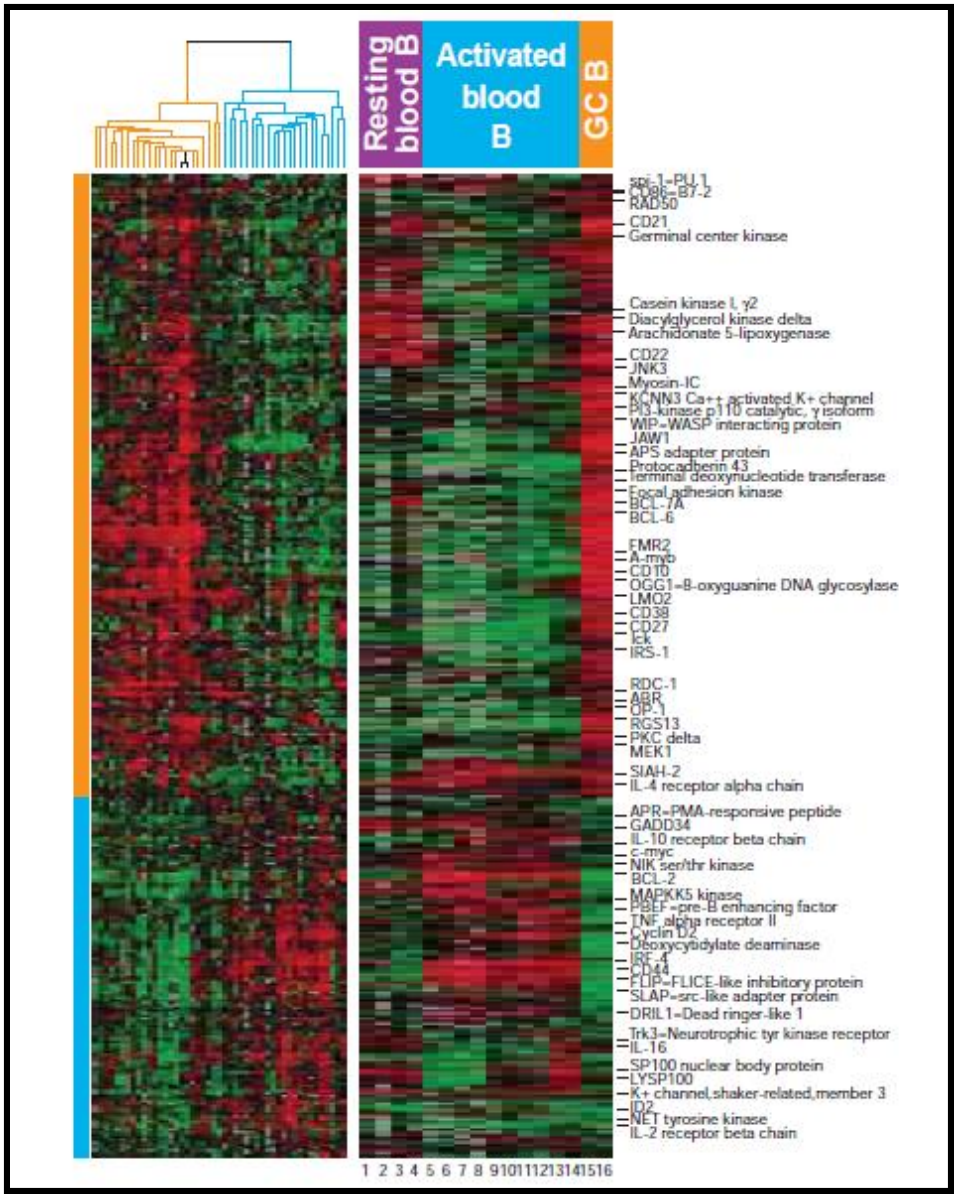
Lymphochip with 17,856 cDNA clones	
12,069	Germinal center B-cell genes
2,338	B-cell NHL genes
3,186	Activated lymphocyte genes

Louis Staudt, MD, PhD

Nature 403: 503, 2000

Diffuse Large B-cell Lymphoma

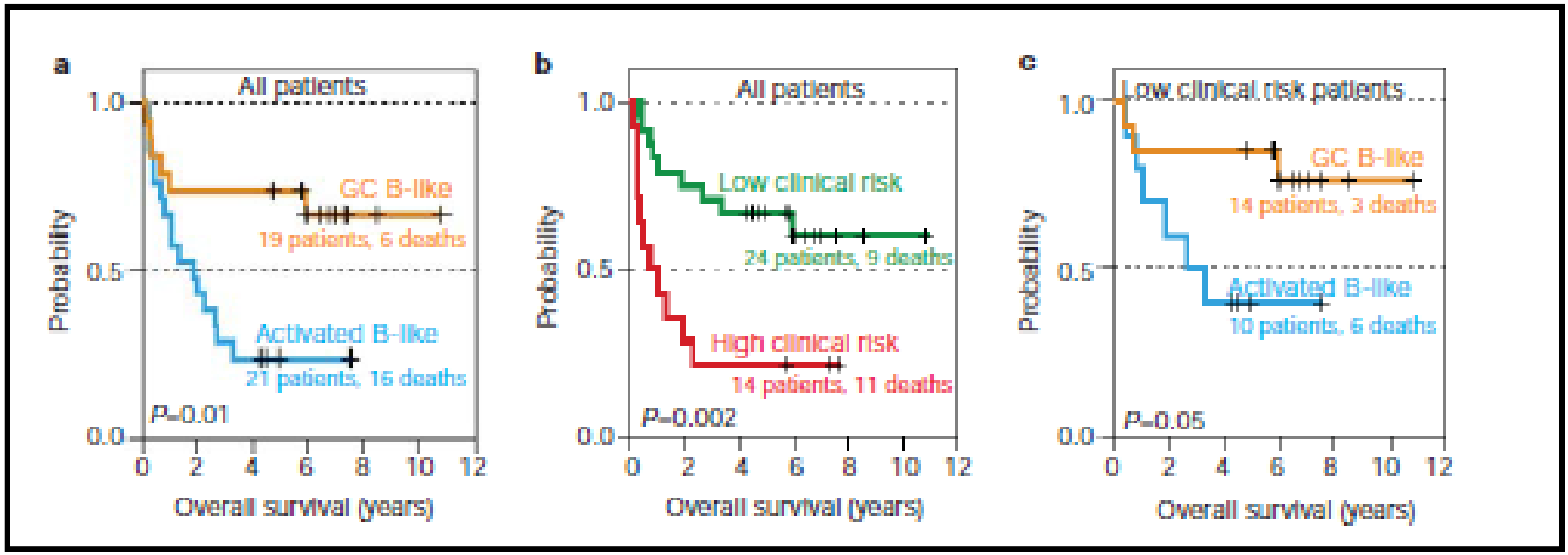
GCB
ABC



Nature 403: 503, 2000

Diffuse Large B-cell Lymphoma

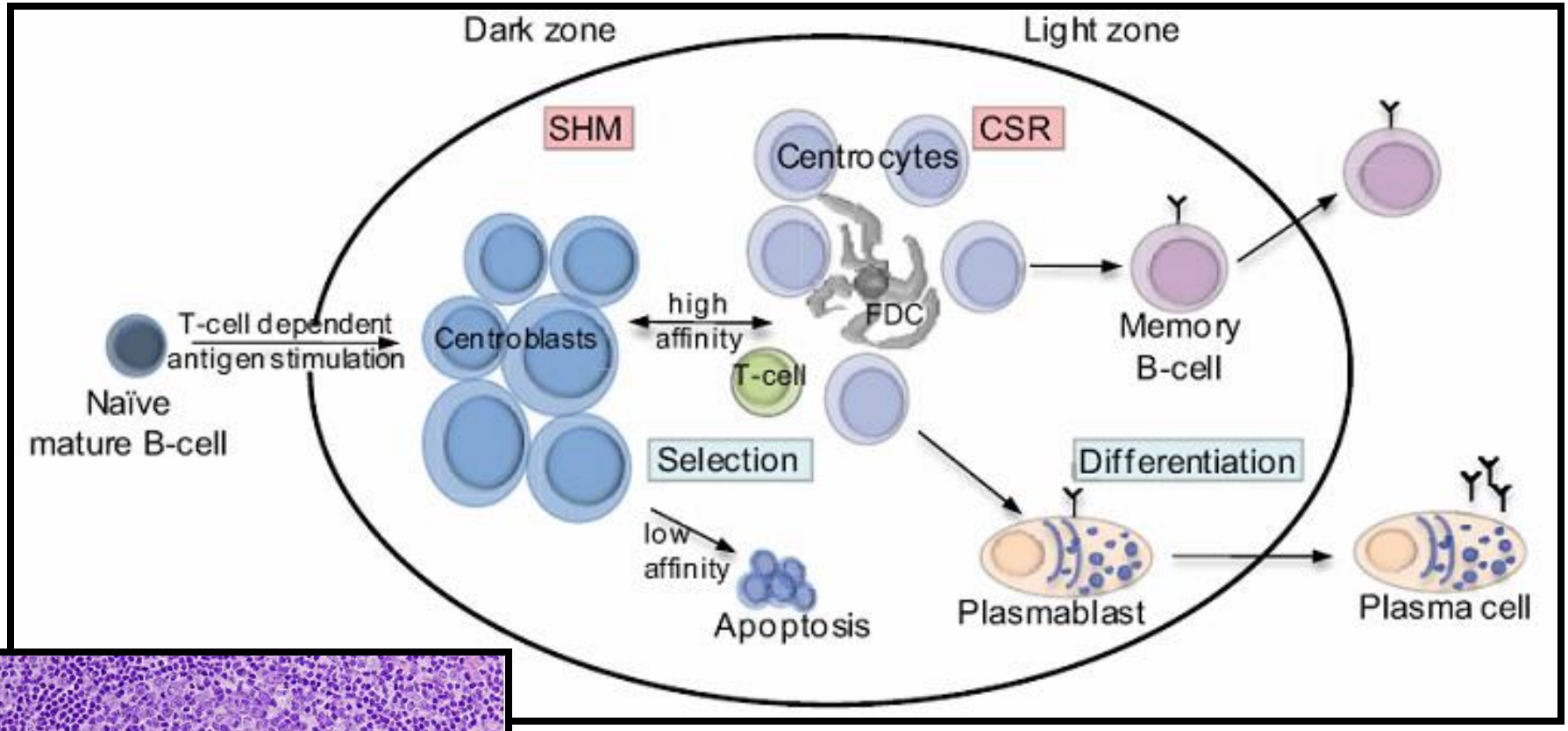
GEP Shows 2 Types that Predict Prognosis

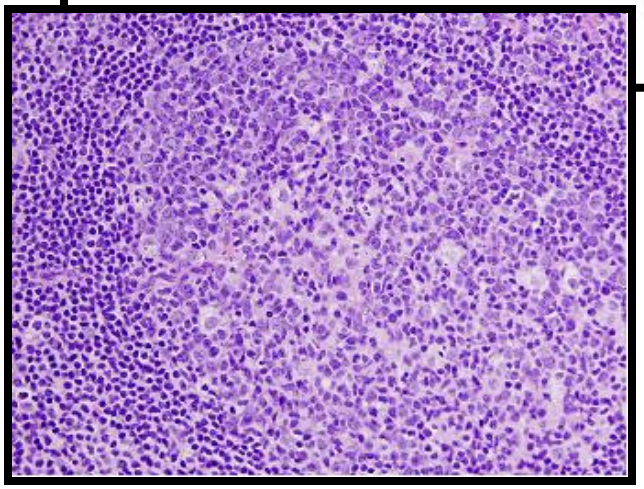


CHOP Therapy

Nature 403: 503, 2000

Germinal Center Reaction

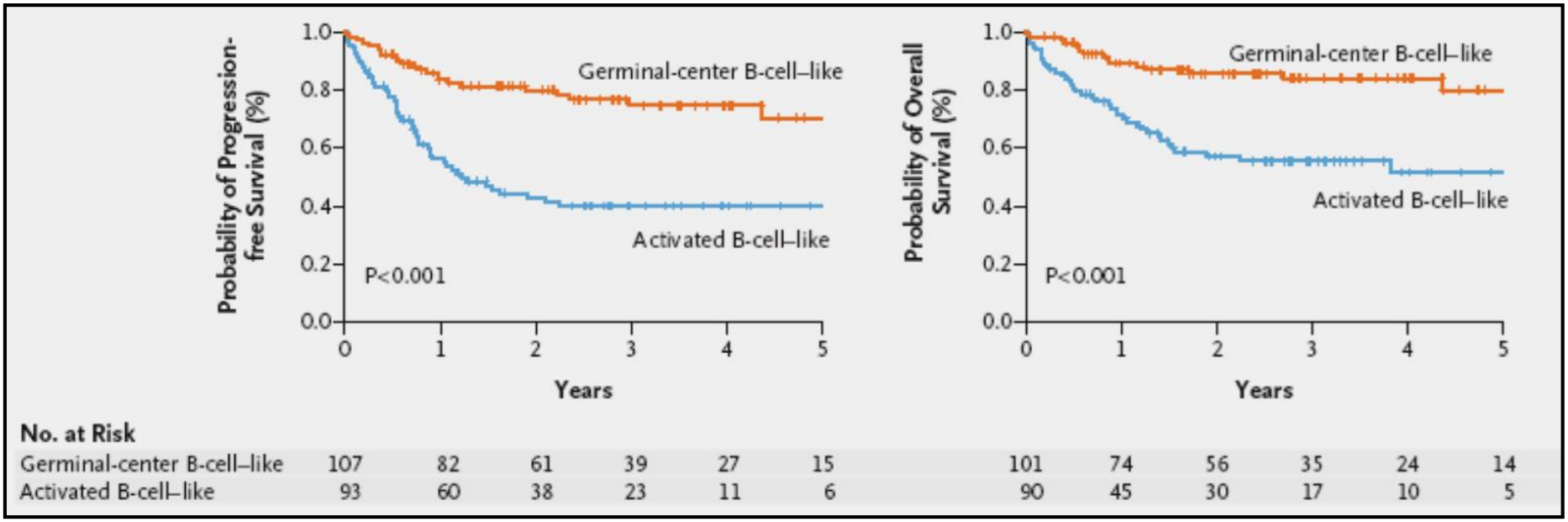




Sem Diagn Pathol 28: 167, 2011

Diffuse Large B-cell Lymphoma

GEP is Valid for R-CHOP Treated Patients



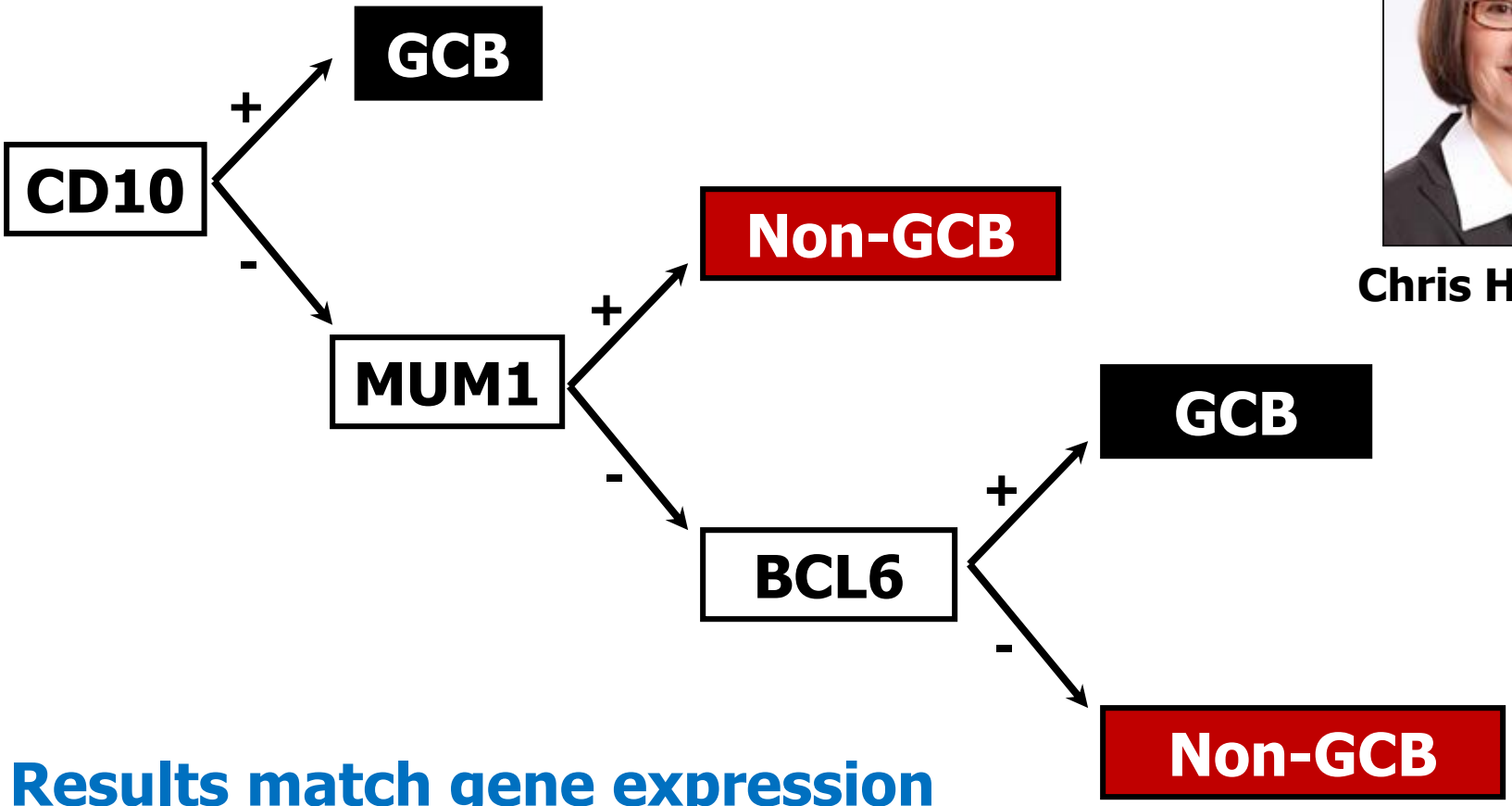
No. at Risk

Germinal-center B-cell–like	107	82	61	39	27	15	101	74	56	35	24	14
Activated B-cell–like	93	60	38	23	11	6	90	45	30	17	10	5

N Engl J Med 359: 2317, 2008

Can Immunohistochemistry be used as a Surrogate for GEP in DLBCL?

Chris Hans, MD



Results match gene expression profile in ~80% of cases

Blood 106: 275, 2004

VOLUME 35 · NUMBER 22 · AUGUST 1, 2017

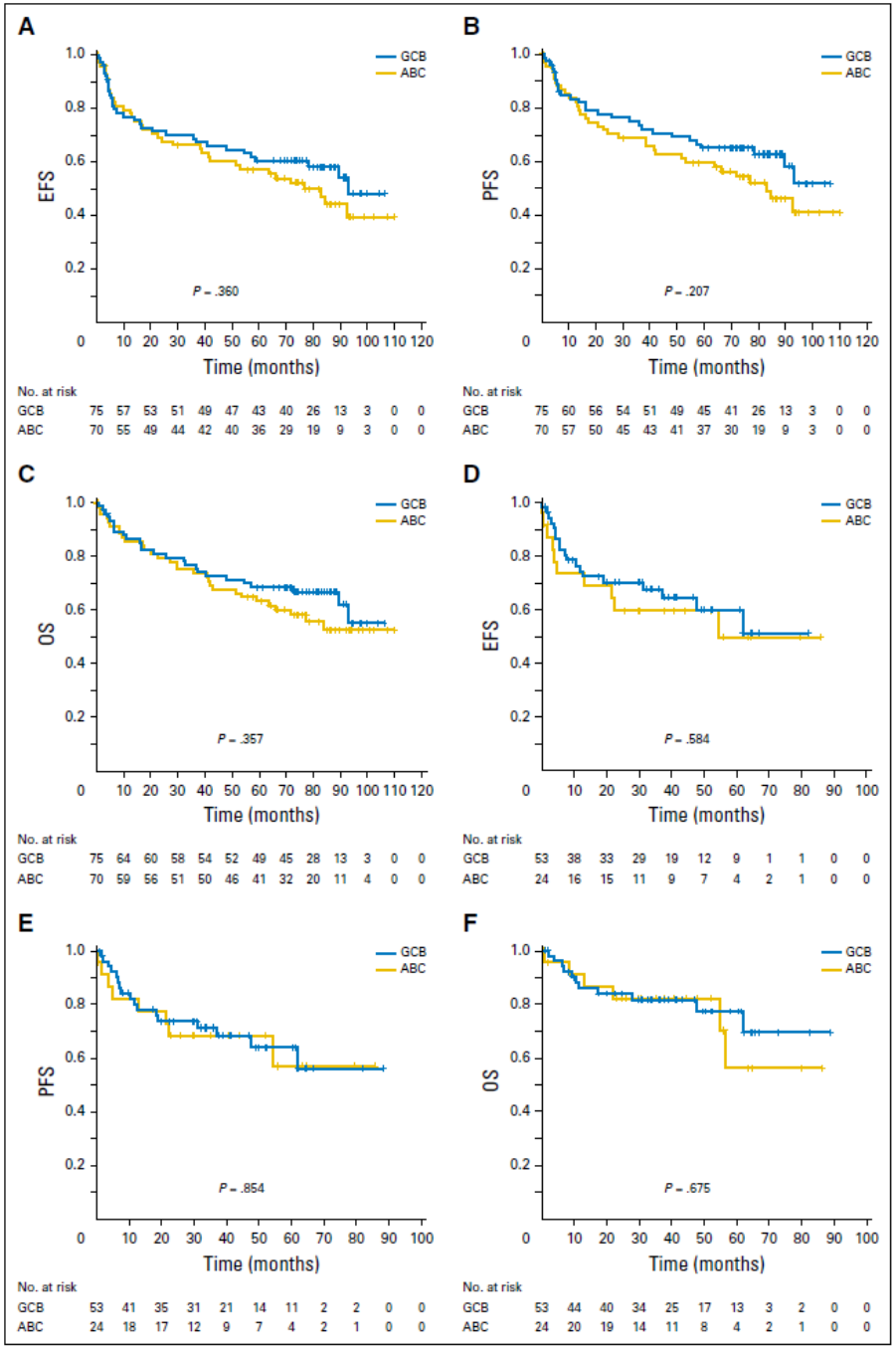
JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Clinical Impact of the Cell-of-Origin Classification and the *MYC*/*BCL2* Dual Expresser Status in Diffuse Large B-Cell Lymphoma Treated Within Prospective Clinical Trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group

Annette M. Staiger, Marita Ziepert, Heike Horn, David W. Scott, Thomas F.E. Barth, Heinz-Wolfram Bernd, Alfred C. Feller, Wolfram Klapper, Monika Szczepanowski, Michael Hummel, Harald Stein, Dido Lenze, Martin-Leo Hansmann, Sylvia Hartmann, Peter Möller, Sergio Cogliatti, Georg Lenz, Lorenz Trümper, Markus Löffler, Norbert Schmitz, Michael Pfreundschuh, Andreas Rosenwald, and German Ott for the German High-Grade Lymphoma Study Group

A,C,E. RICOVER-60 trial
B,D,F. R-MegaCHOEP trial

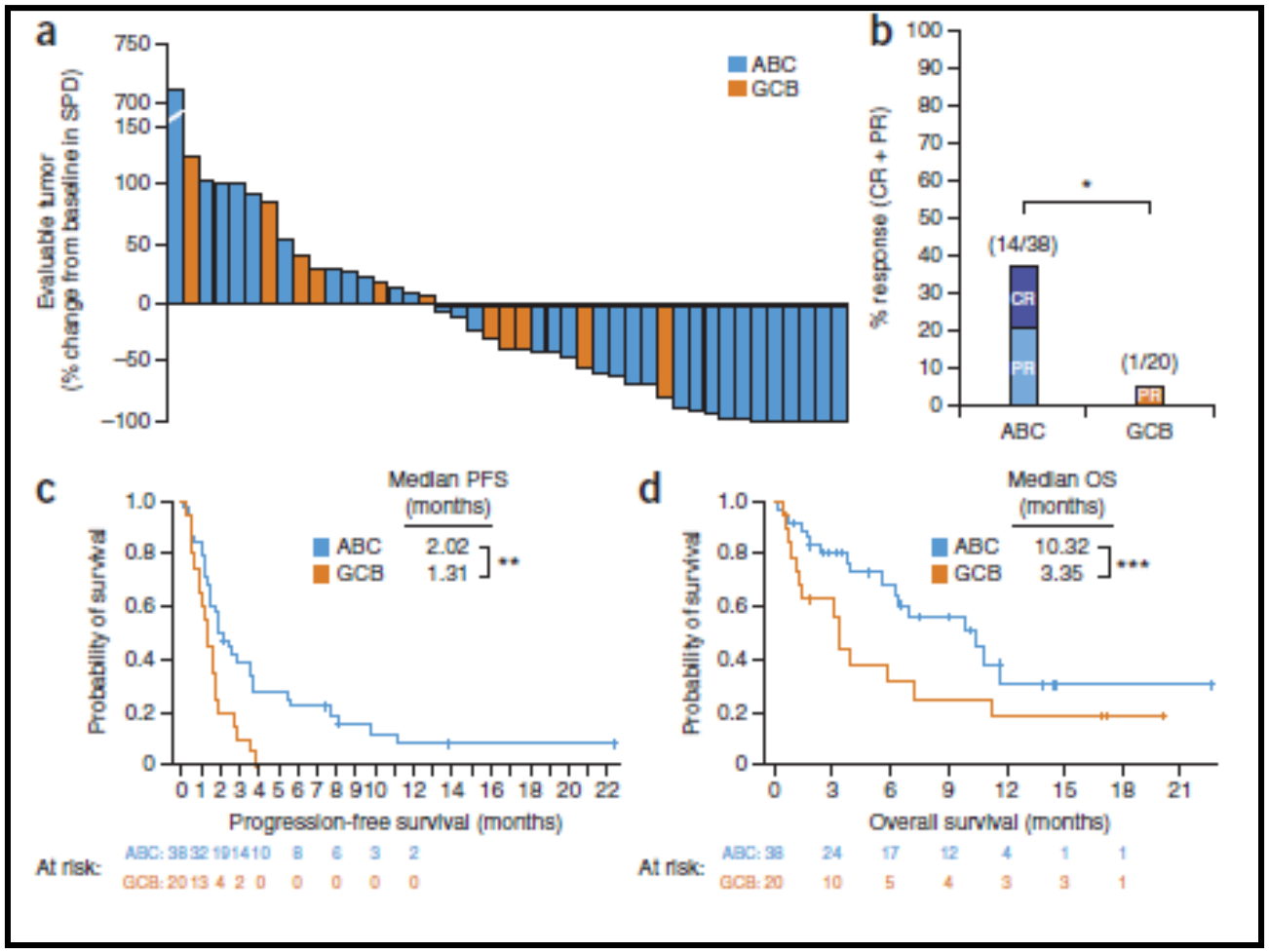
Cell-of-origin classification did not correlate with prognosis



J Clin Oncol 35:2515, 2017

R-CHOP+Ibrutinib for DLBCL

Impact of GCB versus ABC



Nat Med 21: 922, 2015

Mutations in Pathways Involved in DLBCL

B-cell receptor signaling

CD79A, CD79B, CARD11

Toll-like receptor signaling

NF-κB

MYD88

Lymphocyte differentiation

TNFAIP3/A20, TRAF3, BIRC3, IKKβ

DNA repair and transcriptional regulation

p53

Lymphocyte activation

STAT6, BCL10

DNA methylation

EZH2, MLL2

DNA acetylation

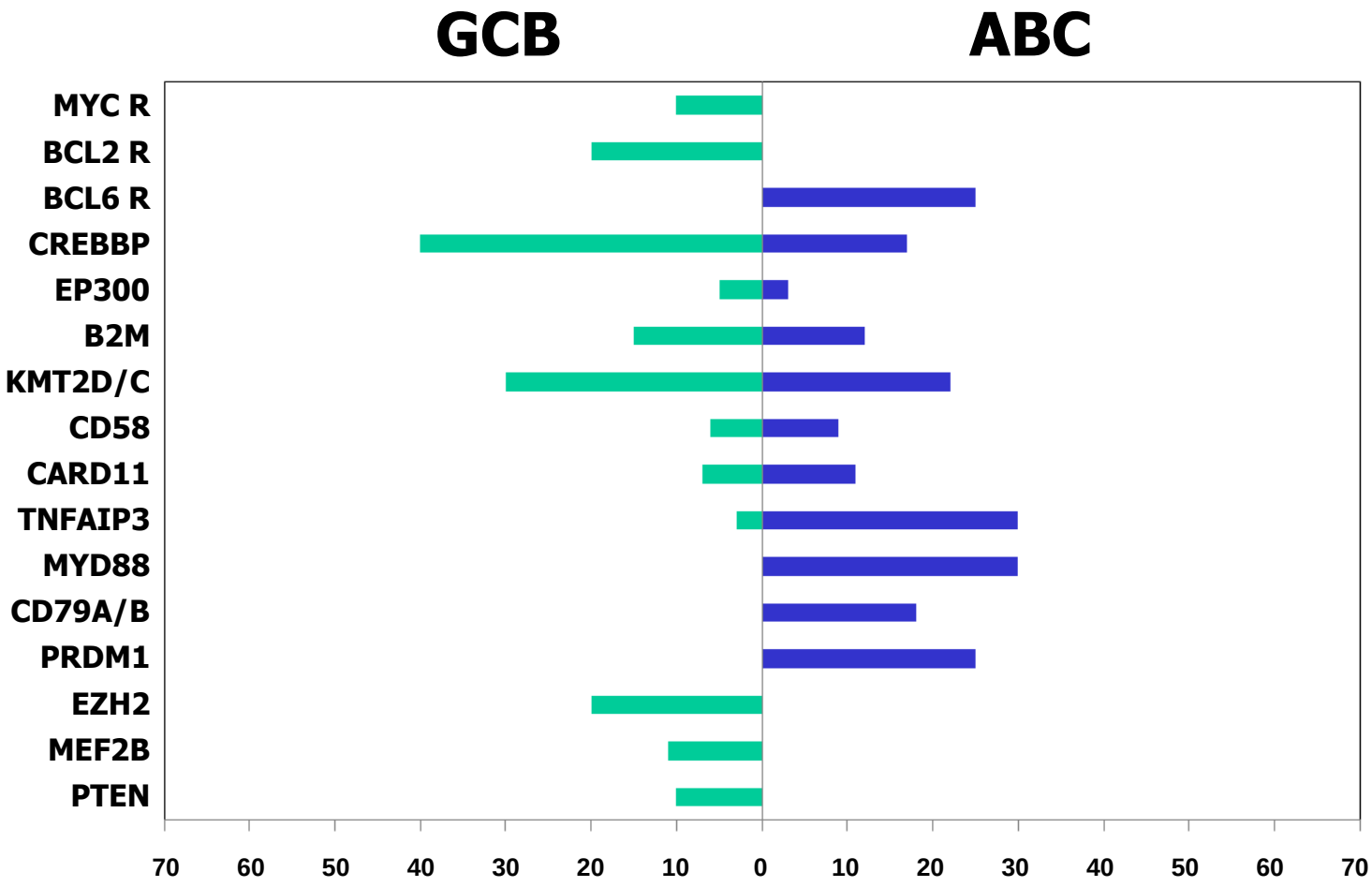
CREBBP, MEF2B

Immune surveillance

β2M, CD58

Diffuse Large B-cell Lymphoma, NOS

Rearrangements and mutations correlate with COO



Frequency of Mutations

Diffuse Large B-cell Lymphoma, NOS

3 Major Studies on Molecular Subgroups

Chapuy B, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med 2018; 24:679-690. (Harvard)

Wright GW, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. Cancer Cell 2020; 37:551-568. (LymphGen NIH)

Lacy SE, et al. Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. Blood 2020; 135:1759-1771.

Comparison of Three Systems

LymphGen	Modified HMRN	Harvard	Main gene mutations	COO	Outcome	Related Lymphoma
MCD	MYD88	C5	*MYD88^{L265P}, CD79B, PIM1*	ABC	Poor	Primary CNS Lymphoma, Primary Testicular Lymphoma
EZB	BCL2	C3	*BCL2, EZH2, CREBBP, KMT2D*	GCB	Good	Follicular Lymphoma
EZB-MYC+	BCL2-MYC				Poor	Double-hit Lymphoma
BN2	NOTCH2	C1	*NOTCH2, BCL10, SPEN, CD70, BCL6*	ABC, GCB, UC	Intermediate/ Good	Marginal Zone Lymphoma
ST2	TET2/SGK1	C4	*TET2, SGK1, KLHL6, BRAF, MAP2K1, KRAS*	GCB	Good	Nodular Lymphocyte Predominant Hodgkin Lymphoma
	SOCS1/SGK1		*SOCS1, SGK1, CD83, NFKBIA, HIST1H1E, STAT3*	GCB	Very Good	Primary Mediastinal B-Cell Lymphoma
N1	NOTCH1		*NOTCH1, ID3*	ABC	Poor	Chronic Lymphocytic Leukaemia
A53		C2	*TP53*, aneuploidy	Mixed	Intermediate	
Other	NEC	C0		ABC, GCB, UC	Intermediate	

Wright **Chapuy**

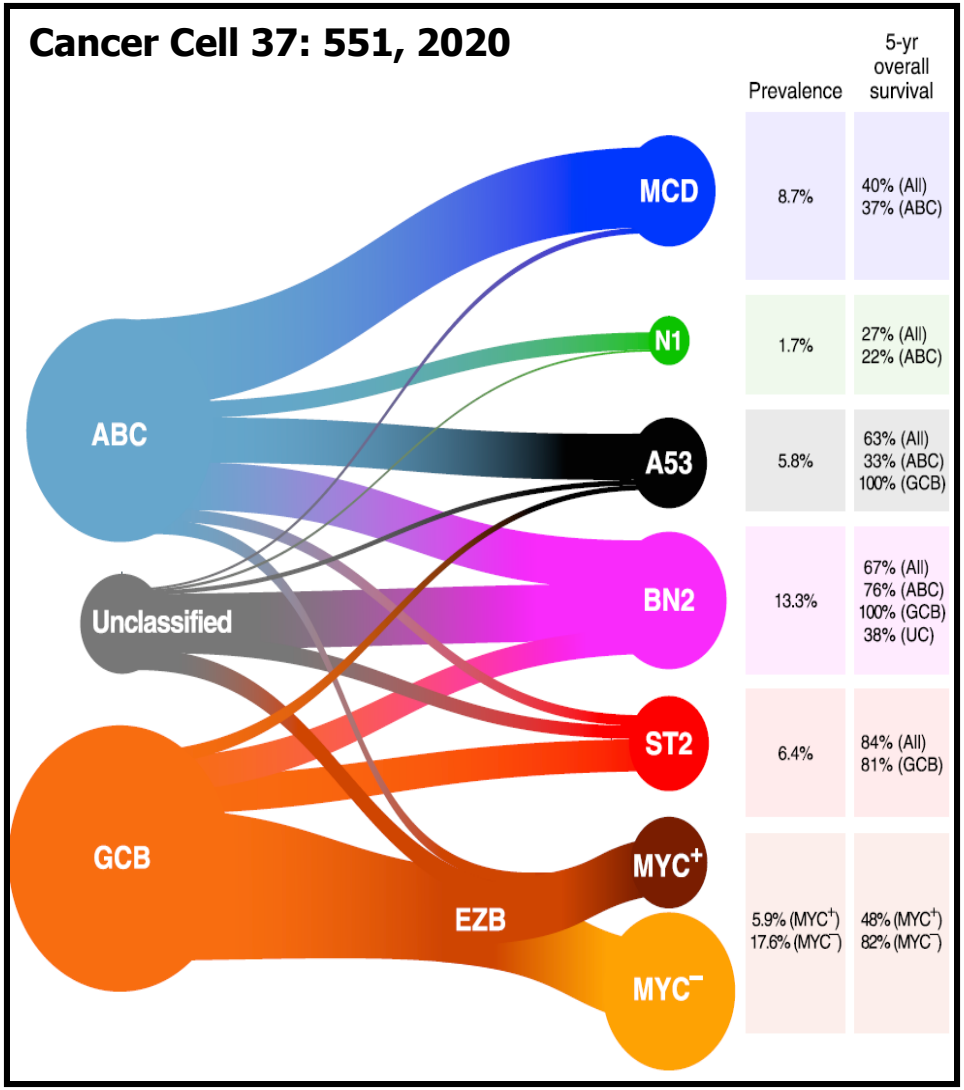
Courtesy of Daniel Hodson, MD
Cambridge, UK

Cancer Cell
Article

A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications

George W. Wright,[1] Da Wei Huang,[2] James D. Phelan,[2] Zana A. Coulibaly,[2] Sandrine Roulland,[2] Ryan M. Young,[2] James Q. Wang,[2] Roland Schmitz,[2] Ryan D. Morin,[3] Jeffrey Tang,[3] Aixiang Jiang,[3] Aleksander Bagaev,[4] Olga Plotnikova,[4] Nikita Kotlov,[4] Calvin A. Johnson,[5] Wyndham H. Wilson,[2] David W. Scott,[6] and Louis M. Staudt[2,7,*]

Cancer Cell 37: 551, 2020



LymphGen Classifier

DLBCL Subgroups

MCD
MYD88 **+** ***CD79B*** **mutations**

N1
NOTCH1 **pathway**

A53
Aneuploidy + TP53 mutations

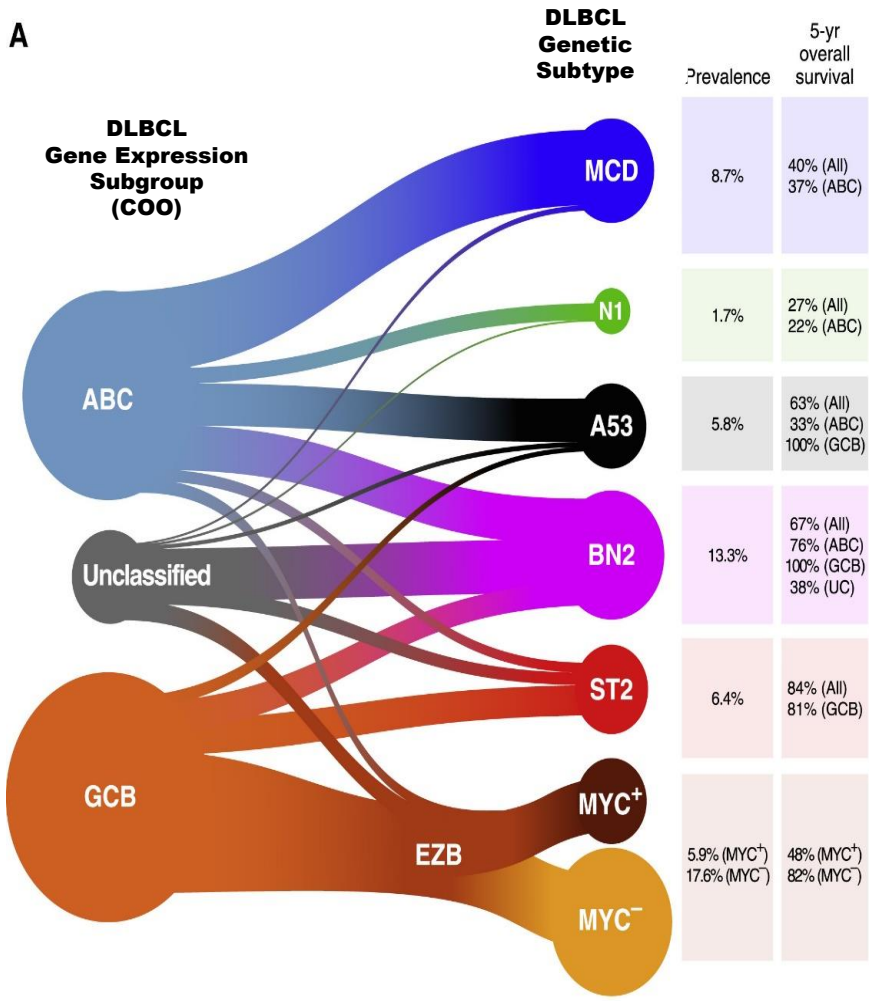
BN2
BCL6 **fusions +** ***NOTCH2*** **mutations**

ST2
SGK1 **and** ***TET2*** **mutations**

EZB
EZH2 **mutations +**
BCL2 **translocations**

DLBCL Genetic Subtypes

Implications for Pathogenesis and Therapy



Potential drug targets

MCD
BTK, PI3K, BCL2, JAK

N1

A53

BN2
BTK, PI3K, BCL2

ST2
PI3K, JAK

EZB
EZH2, PI3K, BCL2

Cancer Cell 37: 551, 2020

Take Home Points

The traditional cell-of-origin model (GCB vs ABC) is not sufficiently granular to predict prognosis or to plan therapy

For now, we will need to keep using this model, but only until a better, more practicable system becomes available

A new model may not lead to optimal therapy currently, but it will lead to design of clinical trials and evaluation of therapies

However, this new system needs to be practical