Aggressive B-cell lymphomas and gene expression profiling – towards individualized therapy ?

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NEW WHO CLASSIFICATION 2008

Diffuse large B-cell lymphoma

1. DLBCL, not otherwise specified (NOS)
2. T-cell/histiocyte-rich large B-cell lymphoma
3. DLBCL of the CNS
4. Cutaneous DLBCL, leg type
5. EBV-positive DLBCL of the elderly
6. Mediastinal large B-cell lymphoma
7. ALK positive large B-cell lymphoma
8. DLBCL associated with chronic inflammation
9. Intravascular large B-cell lymphoma
10. Plasmablastic lymphoma

TOPICS

1. Germinal center B-like DLBCL vs.
   Activated B-like DLBCL
   - biology,prognosis,diagnostics

2. Genetics
   - prognosis, diagnostics

3. Grey zone between DLBCL and Burkitt lymphoma
   - prognosis, diagnostics

4. EBV-association
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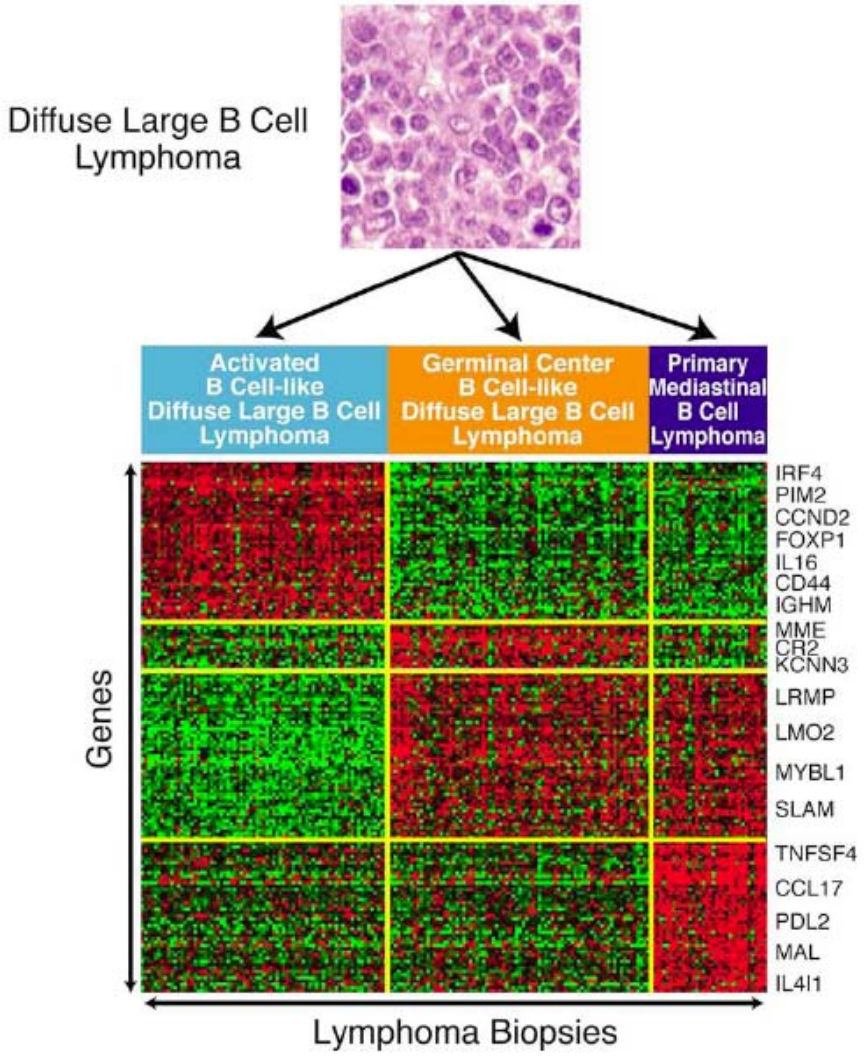
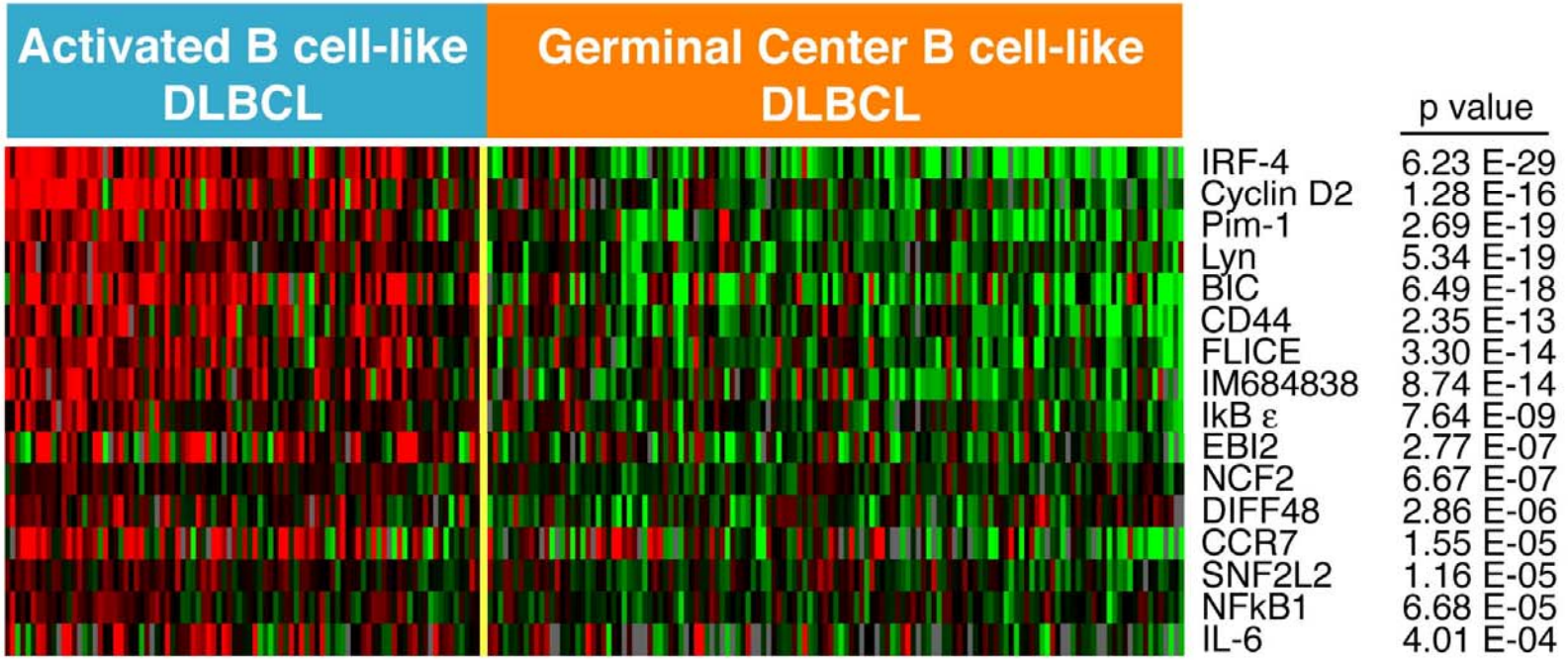
DLBCL - Gene Expression Profiling

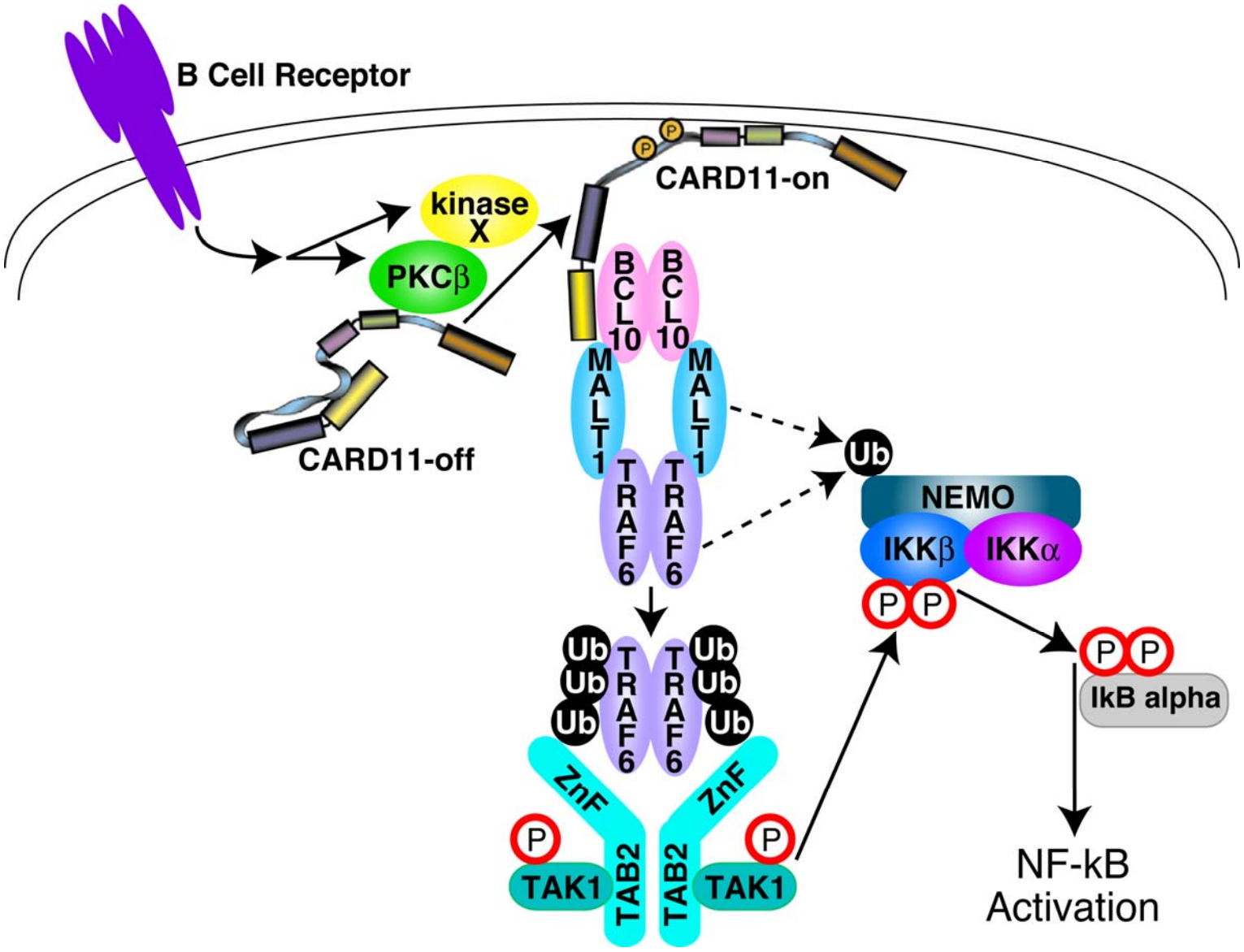


NFκB Activation in ABC DLBCL



Intrinsic biological program or pathogenetic event?

CARD11 activates the NF-κB Pathway



CARD11 mutations in DLBCL

Summary:

- CARD11 mutations are acquired and tumor-specific
- CARD11 mutations occur predominantly in the ABC DLBCL subtype
- CARD11 mutations constitutively activate the NF-κB pathway
- CARD11 mutations are required for survival in ABC DLBCL

Ngo et al., Nature 2006
Lenz et al., Science, 2008

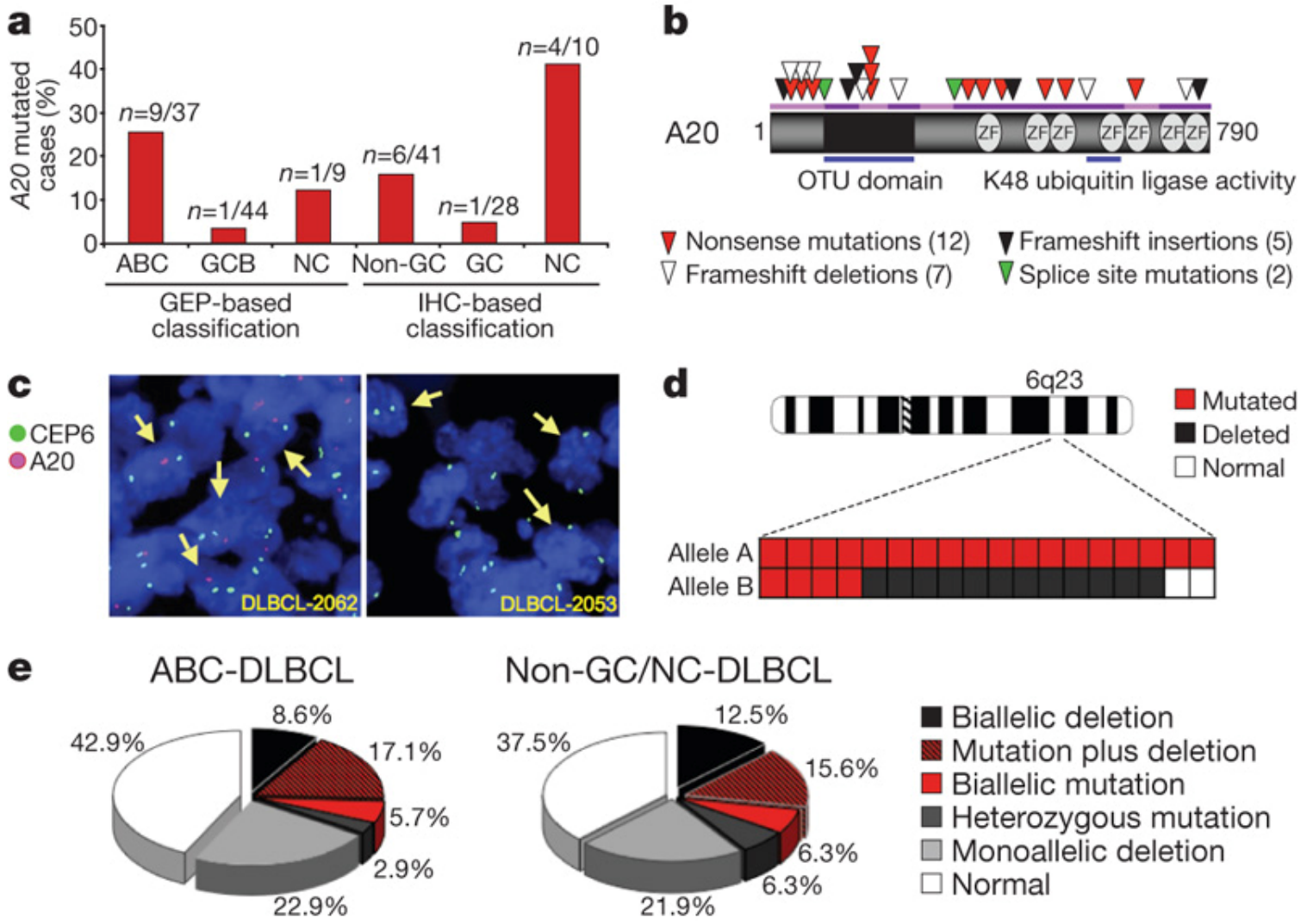
Mutations of multiple genes deregulate NFkB in DLBCL

A20 (TNFAIP3):	24% of ABC DLBCL
CARD11:	11% of ABC DLBCL
RANK:	8% of ABC DLBCL
TRAF5:	5% of ABC DLBCL
TRAF2:	3% of ABC DLBCL
MAP3K7(TAK1):	5% of ABC DLBCL

Conclusion: More than 50% of ABC DLBCL carry mutations in positive or negative regulators of NFkB!

Compagno et al., Nature 2009

Mutations and deletions of the *A20* gene in ABC-DLBCL



Compagno et al., Nature 2009

Is the distinction of GCB/ABC DLBCL still prognostically relevant in the R-CHOP era?

Investigated samples:

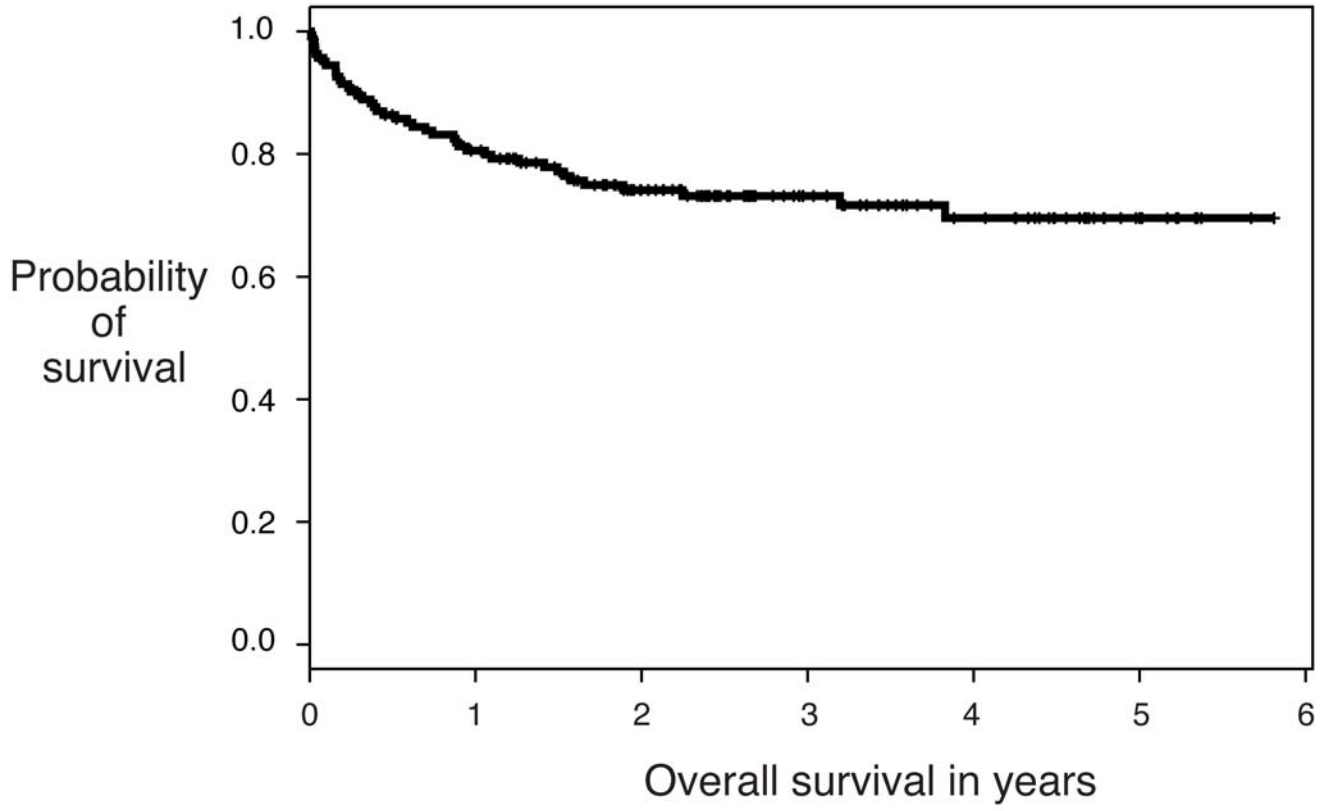
- 176 *de novo* DLBCL patient samples
- Biopsy obtained prior to treatment
- All patients received R-CHOP-like chemotherapy

Methods:

- Gene expression performed on Affymetrix U133 plus 2.0 arrays
- Classification by gene expression profiling:
  - 78 GCB DLBCL
  - 76 ABC DLBCL
  - 22 unclassified DLBCL

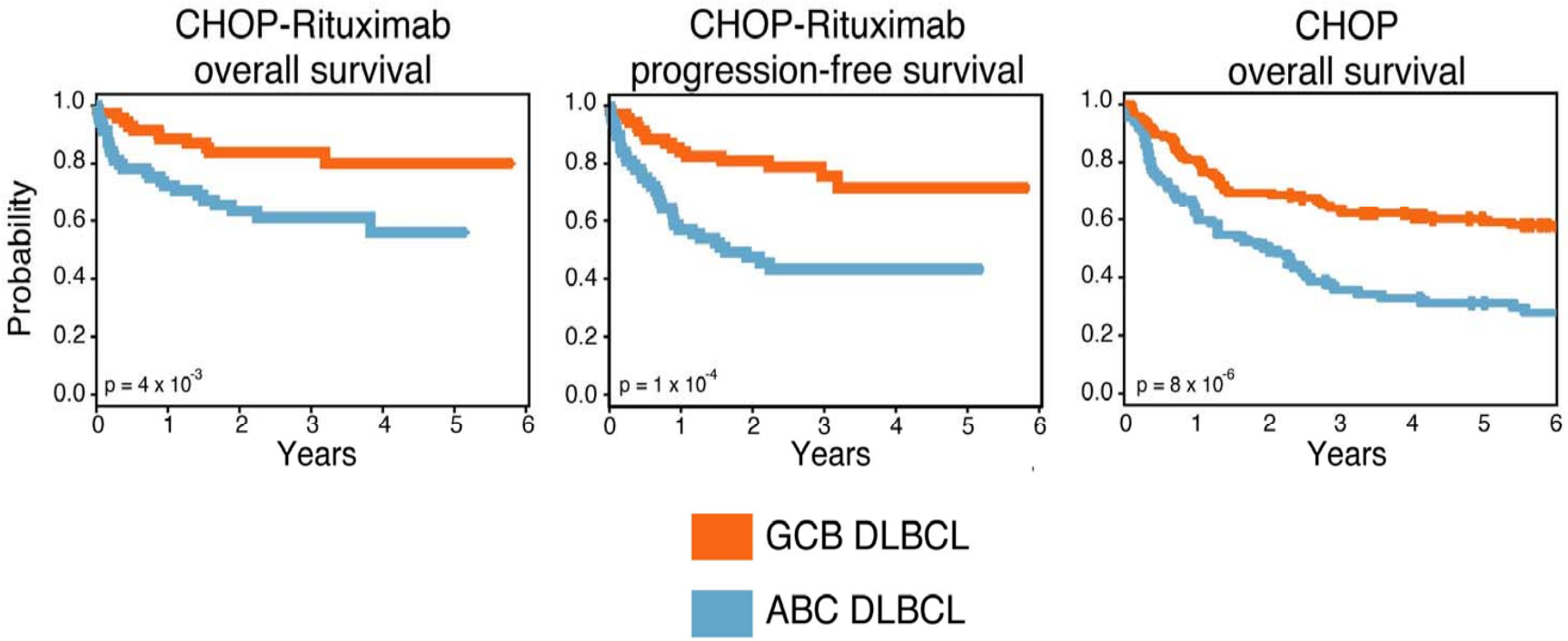
Lenz et al., NEJM, 2008

Overall survival following R-CHOP-like chemotherapy in DLBCL



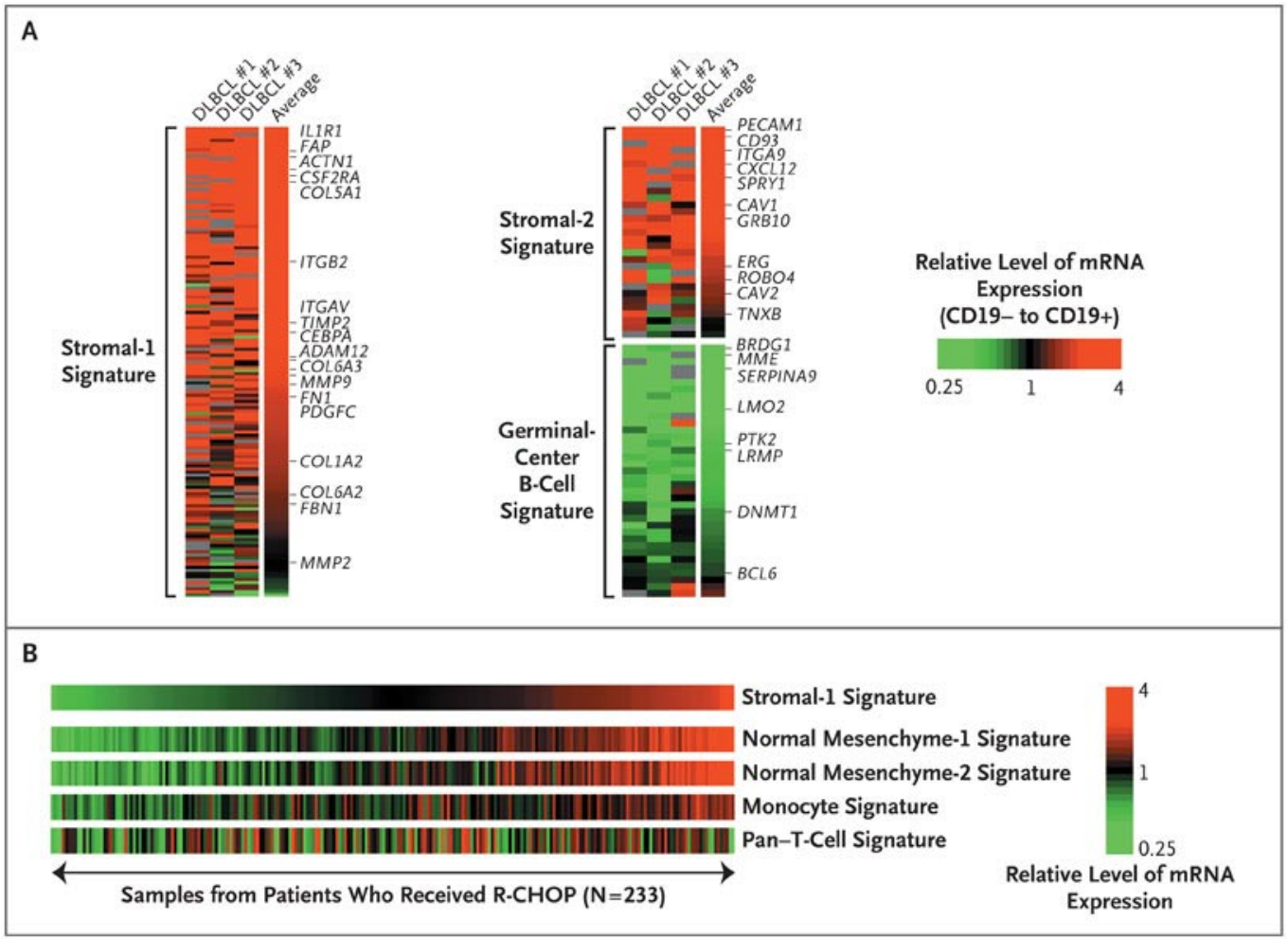
Lenz et al., NEJM, 2008

The Distinction Between the GCB and ABC Subtypes of DLBCL Retains Prognostic Significance with CHOP-Rituximab Therapy



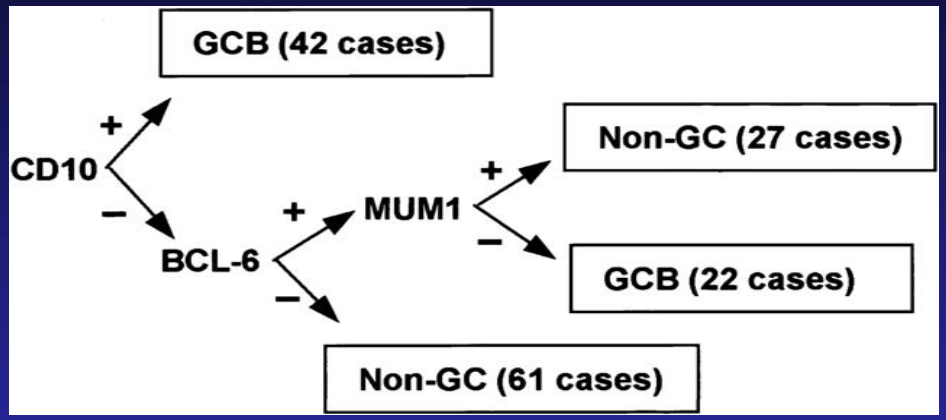
Lenz et al., NEJM, 2008

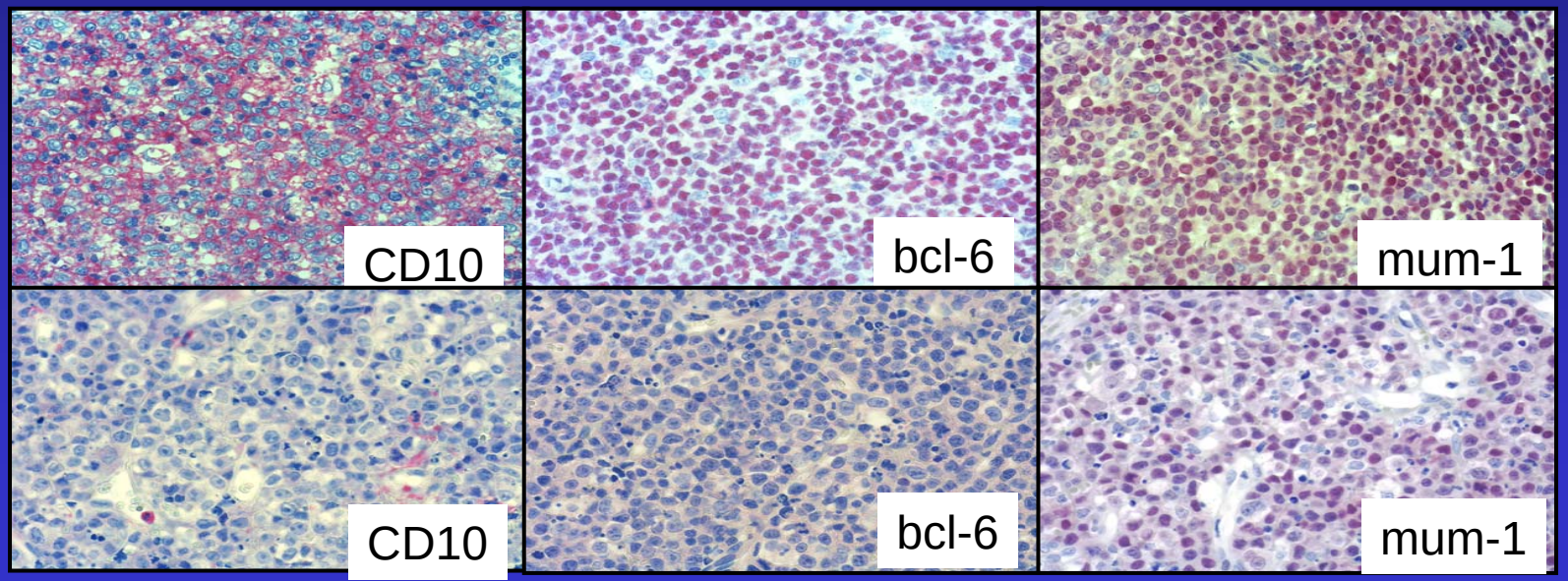
Stromal signatures in DLBCL



Lenz et al., NEJM, 2008

The Hans Classifier





Hans et al., Blood 2003

The Hans classifier – controversies in the CHOP and R-CHOP era

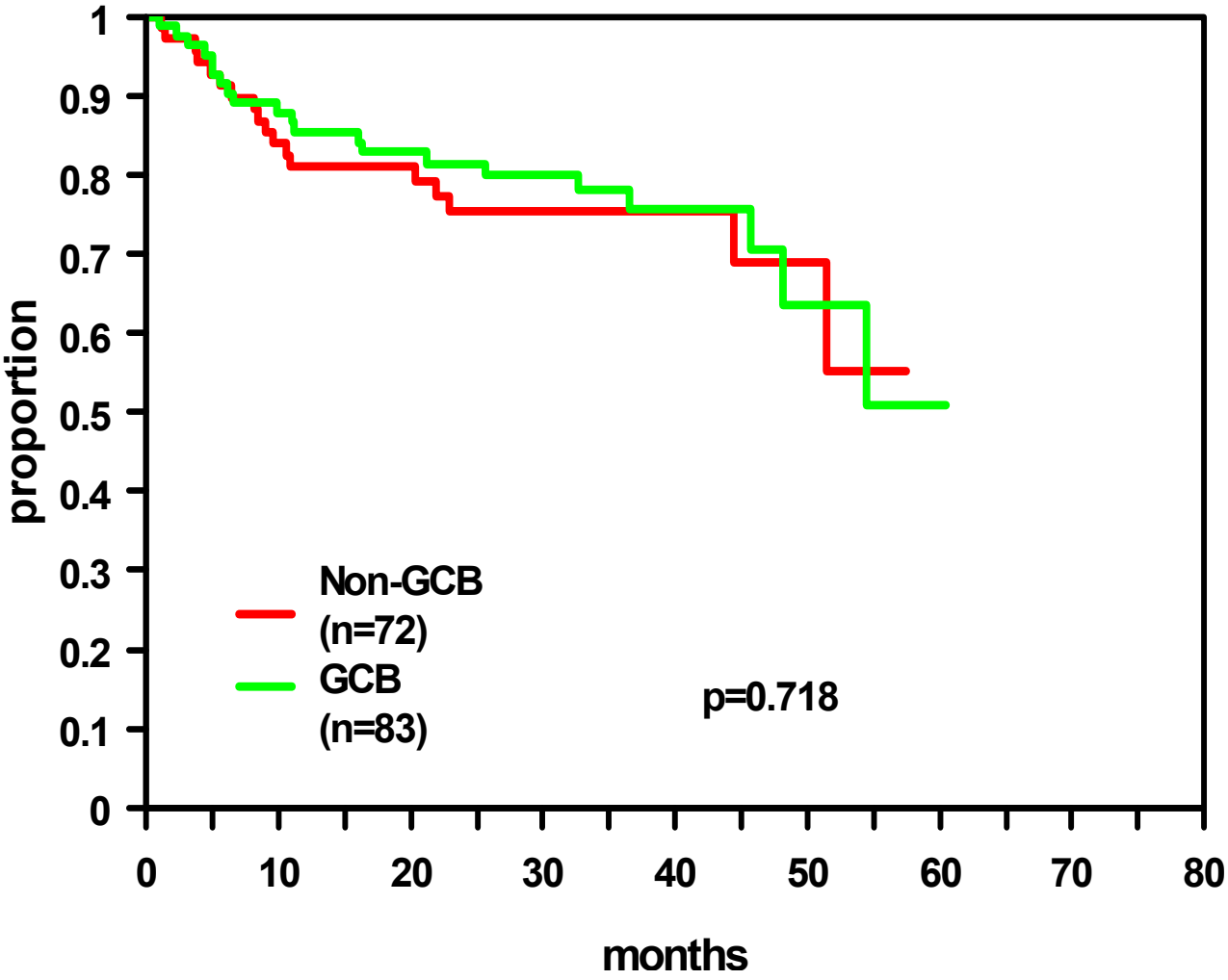
	Survival association
Hans et al., Blood 2003	yes
Berglund et al., Mod Pathol 2005	yes
Haarer et al., Arch Pathol Lab Med 2006	yes
Muris et al., J Pathol 2006	yes
Sjo et al., Eur J Haematol 2007	yes
van Imhoff et al., J Clin Oncol 2007	yes
Nyman et al., Blood 2007	yes
Amara et al., Mod Pathol 2008	yes
Fu et al., JCO, 2008 (R-CHOP)	yes
Colomo et al., Blood 2003	no
Nyman et al., Blood 2007 (R-CHOP)	no
De Paepe et al., J Clin Oncol 2005	no
Dupuis et al., Haematologica 2007	no
Natkunam et al., J Clin Oncol 2008	no
Veelken et al., Ann Oncol 2007	no
Amen et al., Histopathology 2007	no
Wilson et al., JCO 2008 (DE-EPOCH-R)	no
RICOVER 60, submitted	no

RICOVER 60 - Rituximab treated patients

(Pfreundschuh et al., Lancet Oncology 2008)

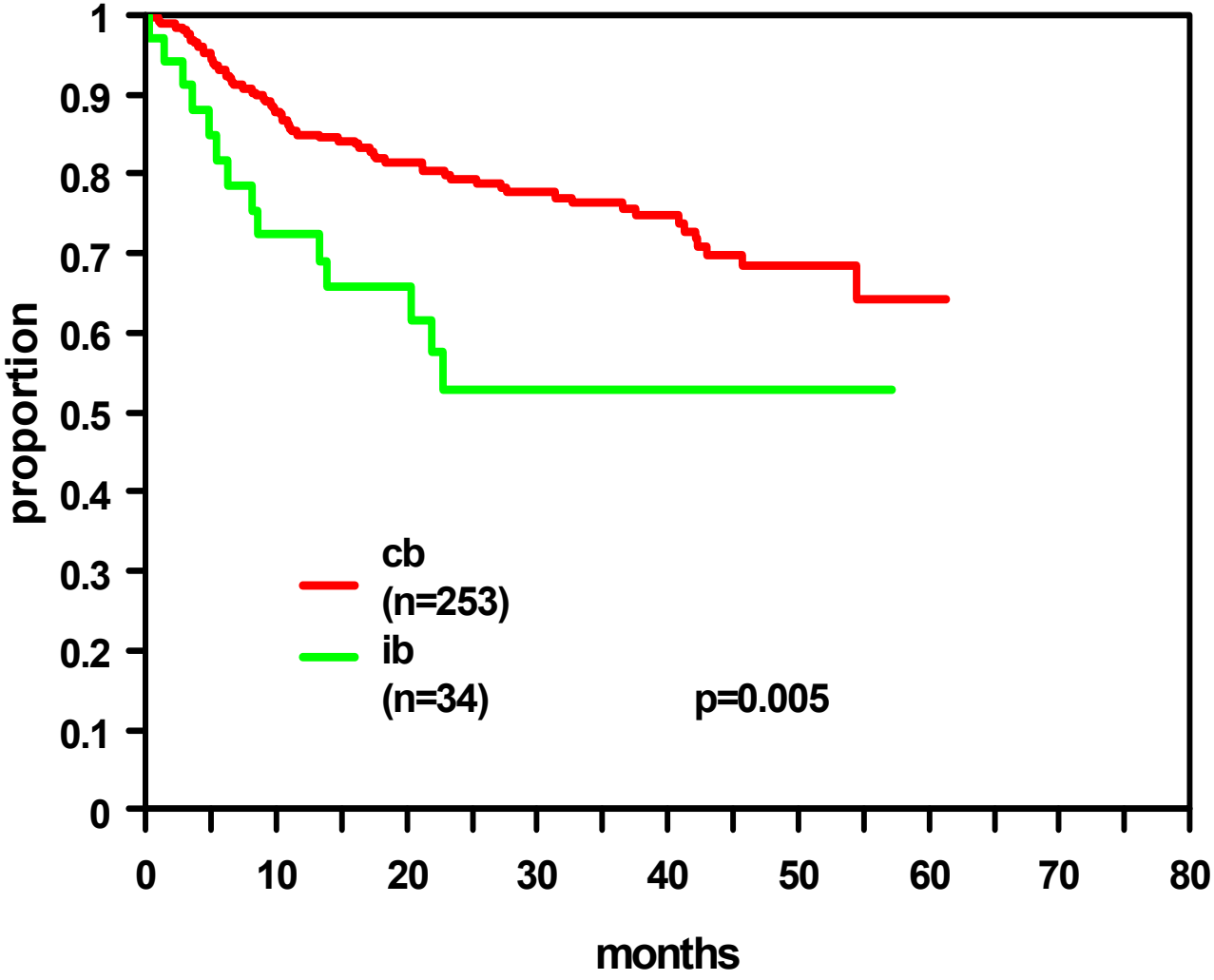
n=155

Overall Survival



RICOVER 60 - Rituximab treated patients

centroblastic vs. immunoblastic morphology (n=287)



RICOVER 60 - Rituximab treated patients

Multivariate analysis of OS

IPI factors and histological subtype (n=287)

parameters	rel. risk	95% CI	p-value
LDH > UNV	2.4	(1.4; 4.0)	0.001
ECOG > 1	2.2	(1.3; 3.8)	0.003
stage III/ IV	1.1	(0.6; 1.9)	0.701
extranodal disease > 1	1.7	(1.0; 3.1)	0.062
ib vs. cb	2.1	(1.1; 3.7)	0.017

RICOVER 60 Trial

1. Immunoblastic morphology predicts poor outcome in all patients and in patients treated with R

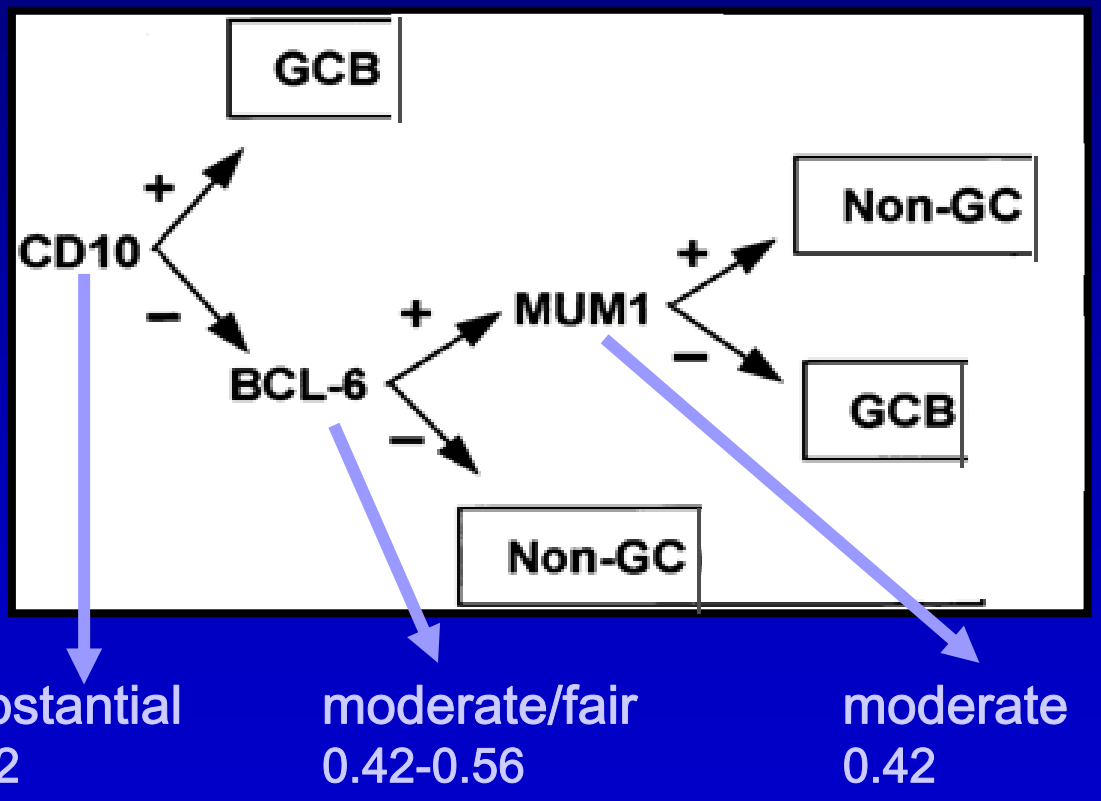
2. BCL2 expression is predictive in all patients, but not in patients treated with R

3. BCL6 expression is predictive in patients treated with R, but not in all patients

4. BCL2 and BCL6 have only modest impact in Cox models that include the IPI factors

5. The Hans classifier does not predict outcome

GCB versus non-GCB distinction on the basis of CD10, MUM-1 and bcl-6 (25% cut-off levels)



de Jong, JCO 2007

Improved GCB/ABC immunohistochemical classifiers?

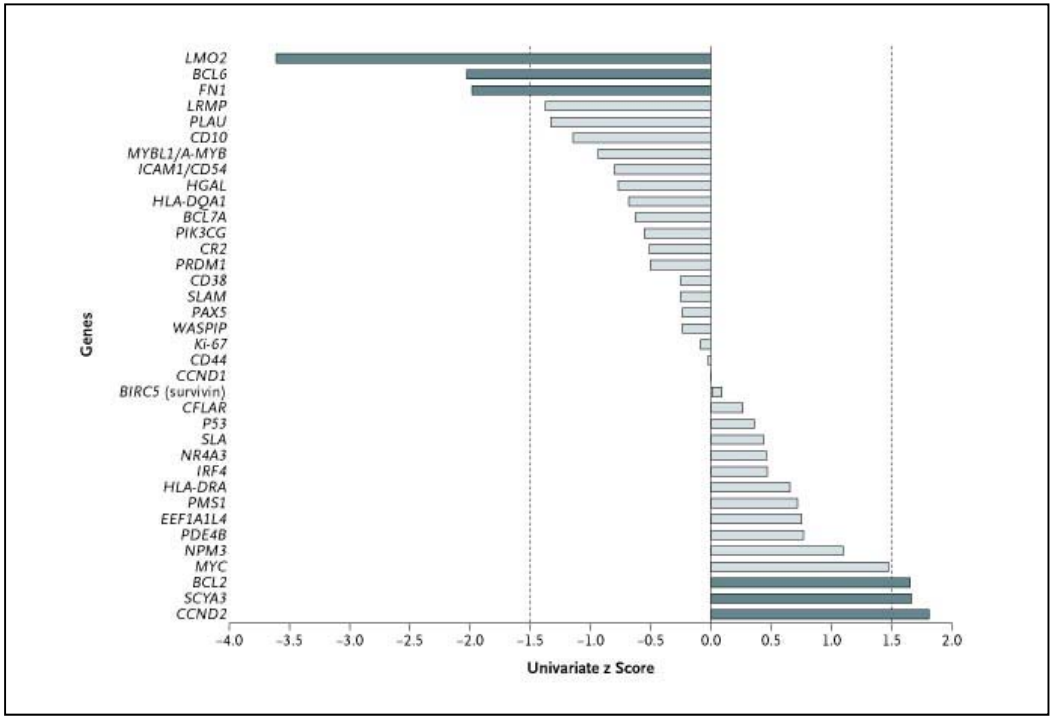
Improved Hans classifier (Choi et al., Clin Cancer Res, 2009)

Muris algorithm: BCL2, CD10, MUM1/IRF4 (Muris et al., J Pathol 2006)

Modified activated B-like algorithm: MUM1/IRF4, FOXP1
(Nyman et al., Mod Pathol 2009)

Combined immunohistochemistry/FISH models

Quantitative RT-PCR: Outcome prediction in DLBCL using a 6 gene model



- 6 gene model predicts in CHOP-treated patients
- 6 gene model predicts in R-CHOP-treated patients
- 6 gene model can be applied in formalin-fixed tissue

The NEW ENGLAND JOURNAL of MEDICINE

Lossos, I. et al. N Engl J Med 2004
Malumbres et al., Blood 2008

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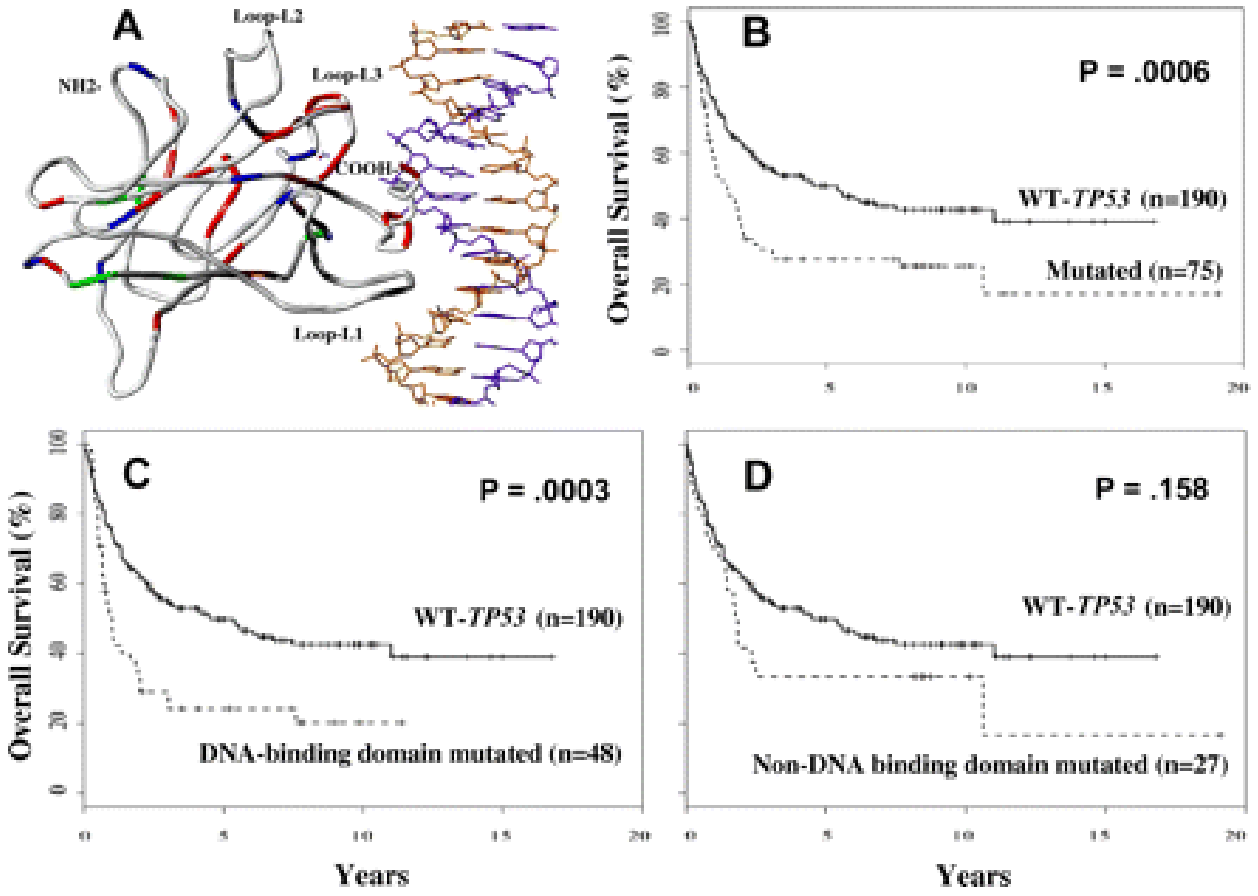
DLBCL – Genetic Alterations

1. Somatic hypermutation of oncogenes (*PIM1*, *MYC* etc.) 50%
2. *BCL6* promoter substitution (translocation) 30-40%
3. *TP53* inactivation 20%
4. *BCL2* translocation - t(14;18) 17%
5. Amplifications of *BCL2/REL/MYC* 20%
6. *MYC* translocation - t (8;14) 6%

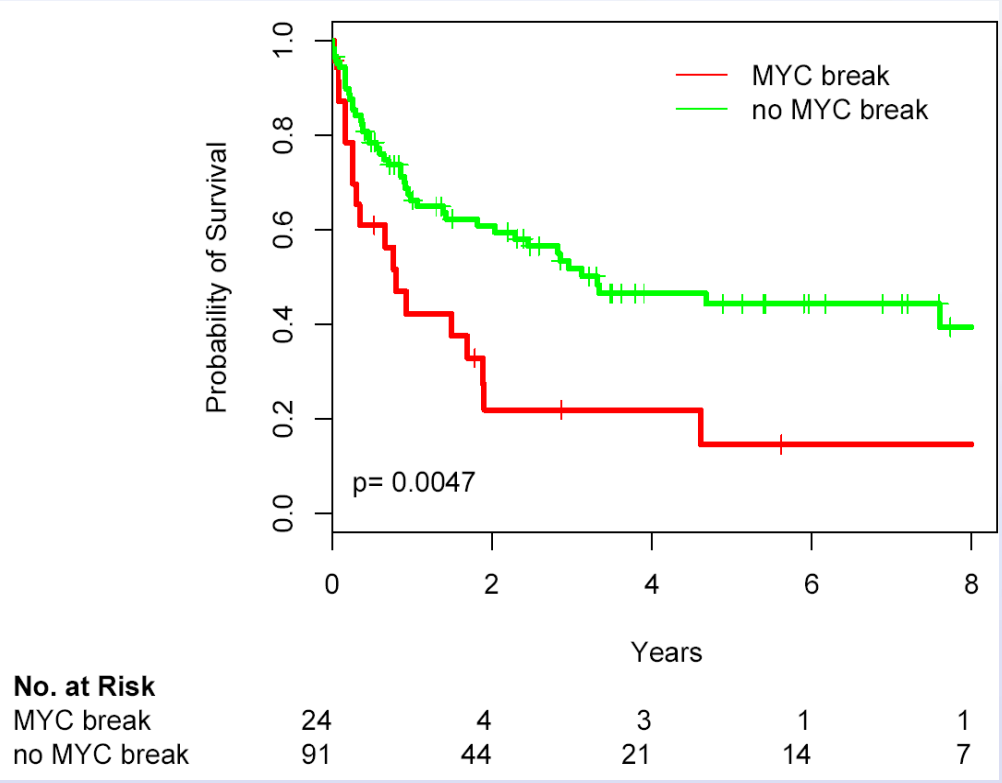
Clinical significance of *TP53* mutations in DLBCL

TP53 mutations in the DNA-binding domain are prognostically important!



Young, K. H. et al. Blood 2008;112:3088-3098

Clinical significance of the MYC-break in DLBCL



No. at Risk					
MYC break	24	4	3	1	1
no MYC break	91	44	21	14	7

Hummel et al., NEJM 2006
Klapper et al., Leukemia 2008
Niitsu et al., Cancer Sci 2008

Survival of patients with double hit lymphomas
(double hit = MYC plus BCL2 or BCL6 translocation)

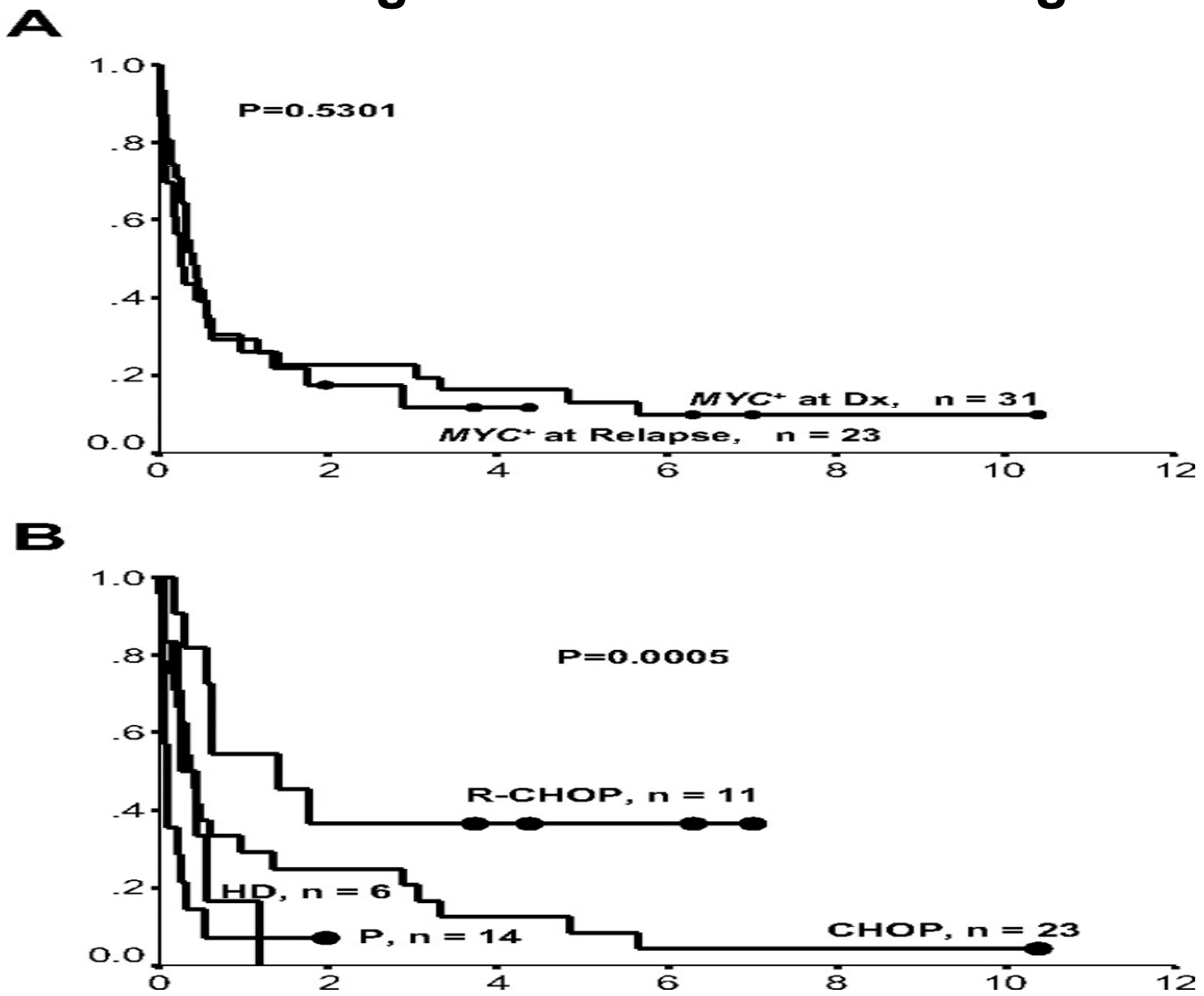
Table 1. Patients' clinical characteristics.

Cases	Age/Gender	IPI score	Extra-nodal sites	Prior history or concomitant low grade lymphoma	Therapy	Response	SCT	Survival (months)
1	59/M	3	BM, skin, CNS, blood	Yes, FL	CEEP, COPADM	PR	autologous (BEAM)	4
2	65/F	3	pleural effusion, CNS	no	CHOP, IVAM	CR		16
3	45/M	3	BM, lung	no	COPADM, CYVE	PR		8
4	50/F	3	BM	no	CEEP, DHAP	PR	autologous (BEAM)	7
5	62/F	2	BM, CNS, blood	no	COPADM, CYVE	PR		4
6	71/F	2	pleural effusion, BM	no	R-CHOP	PR		10
7	36/M	2	BM, liver, CNS	no	COPADM	PR	allogeneic (Bu/Cy)	4
8	69/F	3	BM, blood	no	R-CHOP	PR		5
9	56/M	3	BM, pleural effusion, CNS, stomach, blood	no	COPADM	CR	autologous (BEAM)	8
10	63/M	3	BM	Yes, FL	COPADM, CYVE	CR		4
11	48/M	3	BM, blood	no	R-CEEP	CR	allogeneic (TBI/Cy)	8
12	73/M	3	BM, blood	no	R-CHOP	CR		6
13	55/M	3	BM, CNS, blood	no	COPADM	Prog		3
14	59/M	3	BM, pleural effusion, peritoneal effusion, CNS, testis	no	R-CHOP	Prog		1
15	70/F	3	BM, CNS, pleural effusion	Yes, NOS	CHOP	Prog		1
16	72/M	3	BM, skin	Yes, NOS	steroids	Prog		1

BM,; bone marrow; CNS, central nervous system; PR, partial response, CR, complete response; SCT, stem cell transplantation; TBI, total body irradiation, Cy, cyclophosphamide; Bu: busulfan.

Le Gouill et al., Haematologica 2007

Survival curves of patients with BCL2+/MYC+ lymphomas according to the timing of MYC+ rearrangement and treatment regimen



Johnson, N. A. et al. Blood 2009;114:2273-2279

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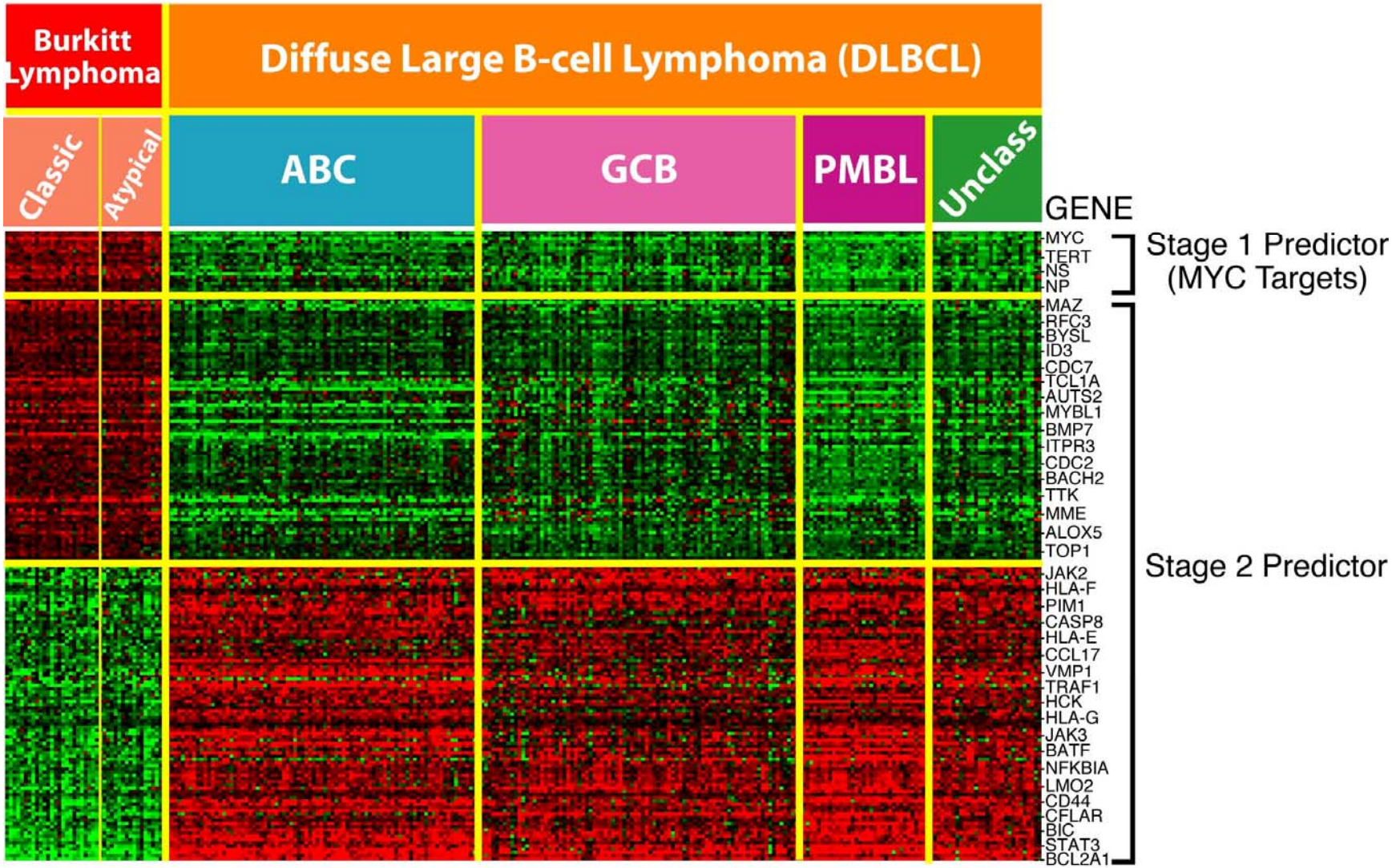
The new WHO classification:

BCLUWFIBDLBCLABL

Gene Expression Differentiates Burkitt Lymphoma from all Subgroups of Diffuse Large B Cell Lymphoma

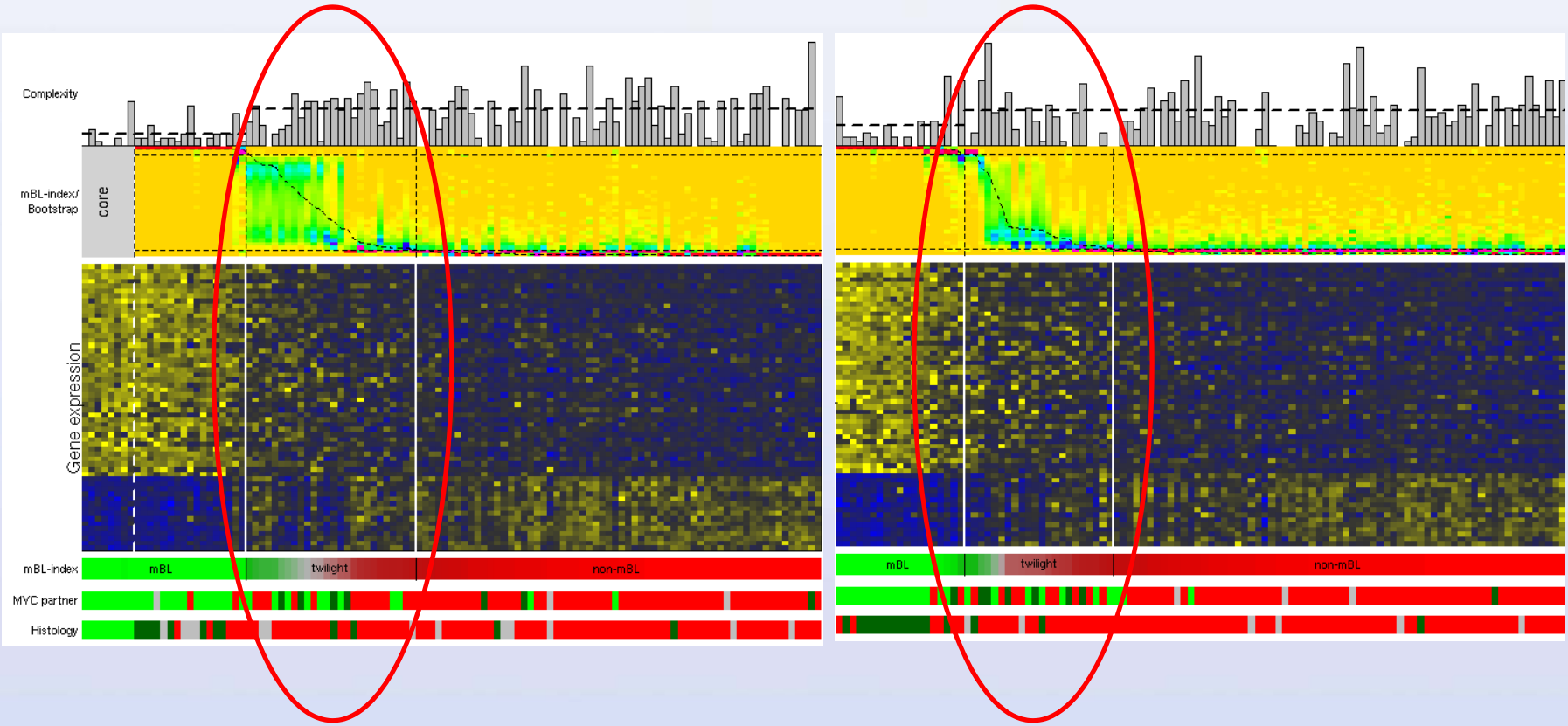


Dave et al., NEJM 2006

A molecular grey zone between DLBCL and BL

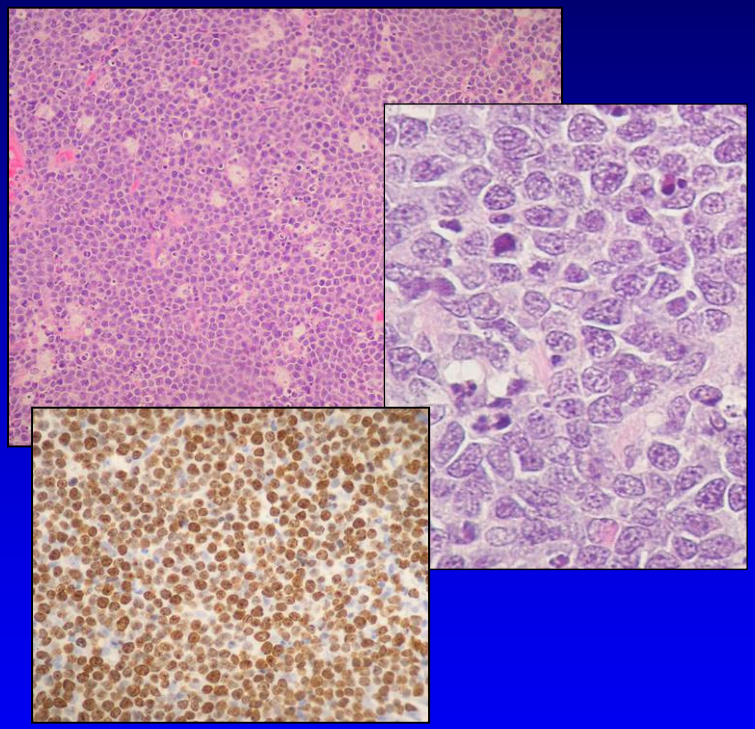
Training set

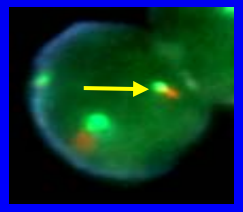
Test set



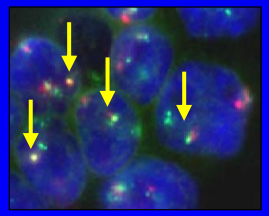
Hummel et al., NEJM 2006

B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and BL (BCLUWFIBDLBCLABL)





t(8;14)



t(14;18)

- Highly proliferative lymphomas with morphological and phenotypic features between Burkitt and DLBCL; occasionally leukemic
- A proportion of these cases carry double hit oncogenic events involving c-myc
- Clinically aggressive
- These cases should be recognized as a “practical category” but it is not clear they represent a specific disease entity

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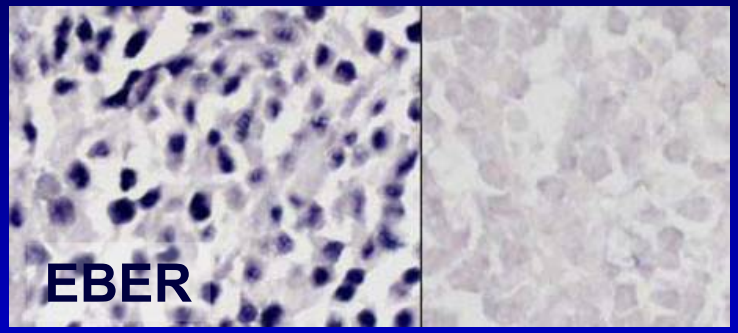
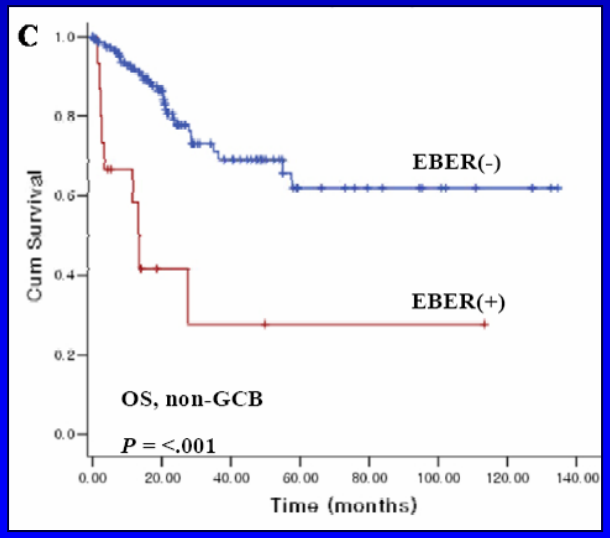
DLBCL and EBV





- **Secondary to other defined disorders**
  - Primary immune disorders, HIV
  - Post-transplant lymphoproliferative disorder
  - associated with chronic inflammation
  - Lymphomatoid granulomatosis
- **Primary (DLBCL of the elderly)**
  - Without any known immunodeficiency
  - age > 50 years
  - more advanced stage
  - more than one extranodal involvement
  - higher IPI risk group
  - B symptoms
  - poorer outcome to initial treatment

Park et al., Blood 2007

Summary / Role of Pathology

1. Fundamental biological differences between GCB/ABC DLBCL

   Mutations of multiple genes deregulate NFkB in ABC DLBCL – relevant therapeutic target!

2. Prognostic gene expression signatures from the CHOP era remain relevant in the R-CHOP era

3. Need to develop a diagnostically applicable test to distinguish GCB/ABC DLBCL for future clinical trials

   Immunohistochemical algorithms for DLBCL subtype distinction (e.g. Hans) remain controversial

Summary / Role of Pathology

4. Negative prognostic impact of MYC translocations

   More FISH in the diagnostic setting

5. Practical category in the new WHO: BCLUWFIBDLBCLABL

   ‘B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL’

   frequent (ca. 50%): ‘double hit’ lymphomas (MYC/BCL2)

6. EBV-associated DLBCL of the elderly

   age >50 years

   likely to be prognostically unfavorable

   implies increased EBER testing in diagnostic setting