gmds LEIPZIG 2006
Biologie maligner Lymphome

Molekulare Charakterisierung der aggressiven B-Zell-Lymphome

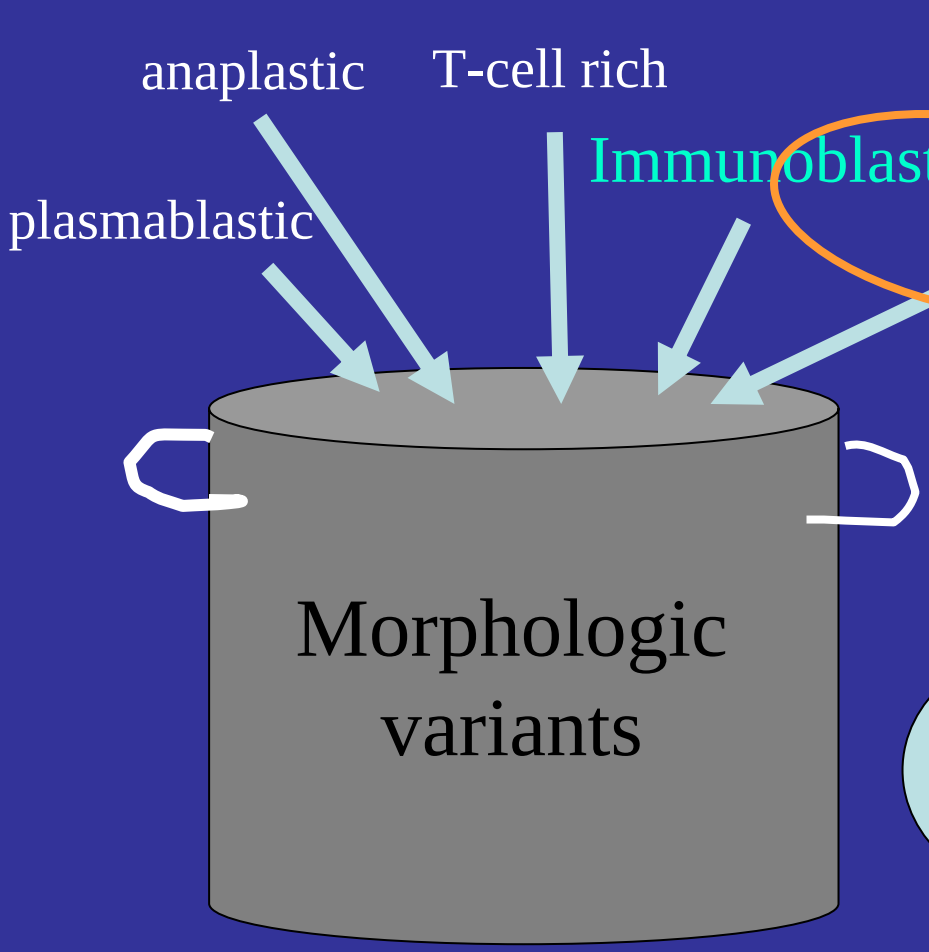
Harald Stein
Für den Forschungsverbund der Deutschen Krebshilfe
„Molekulare Mechanismen bei malignen Lymphomen“

CHARITÉ CAMPUS BENJAMIN FRANKLIN

Berlin

Mature Aggressive B-NHL of the WHO Classification

Diffuse large B-cell lymphoma (DLBCL)

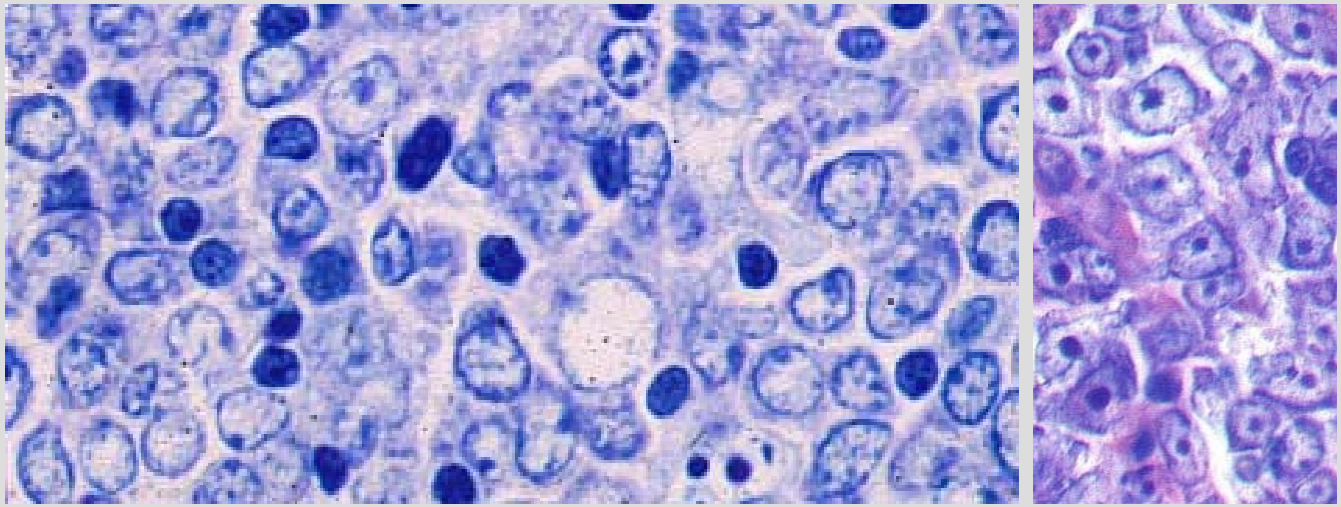


Burkitt lymphoma

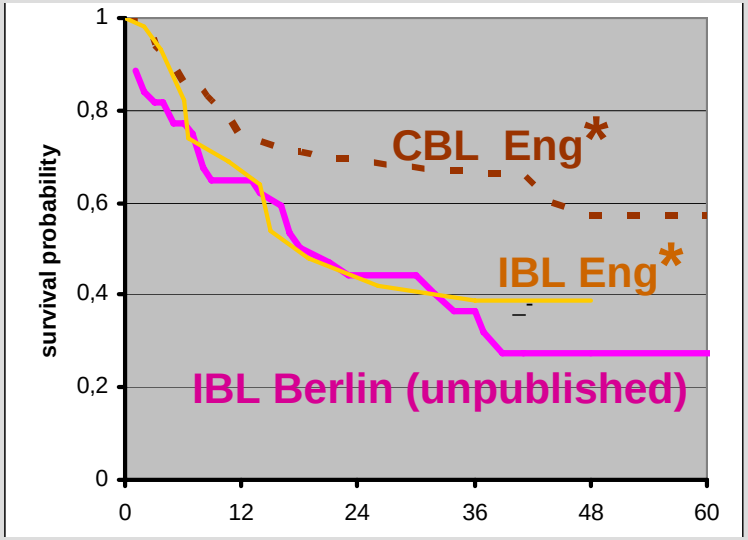
DLBCL:
Subtypes/Entities

- mediastinal
- intravascular
- effusion

Morphological subtyping of DLBCLs according to Lennert 1978

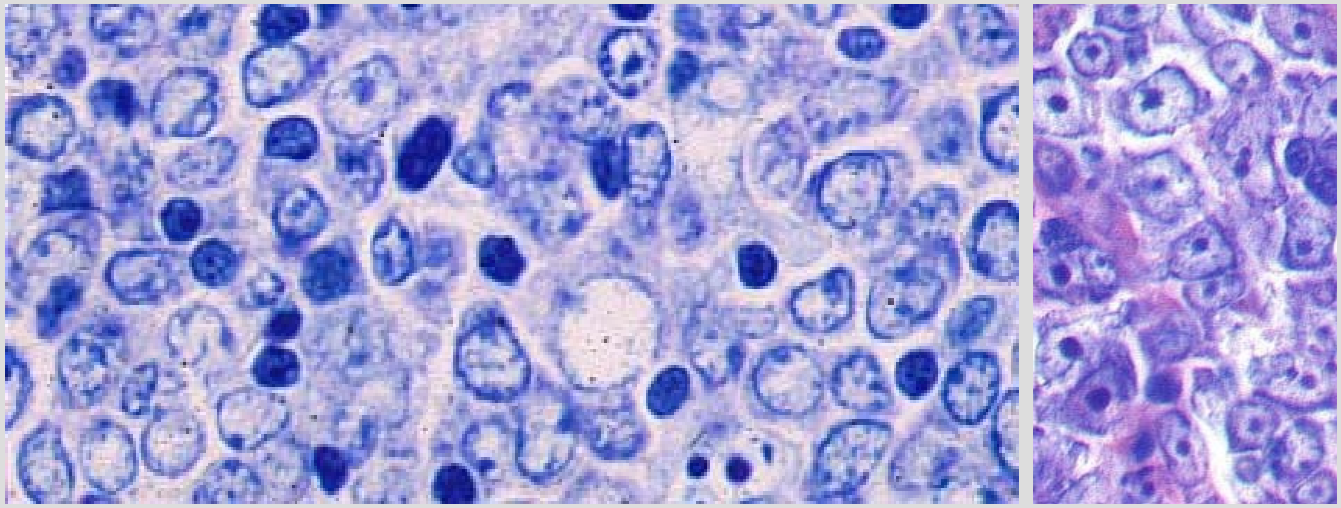


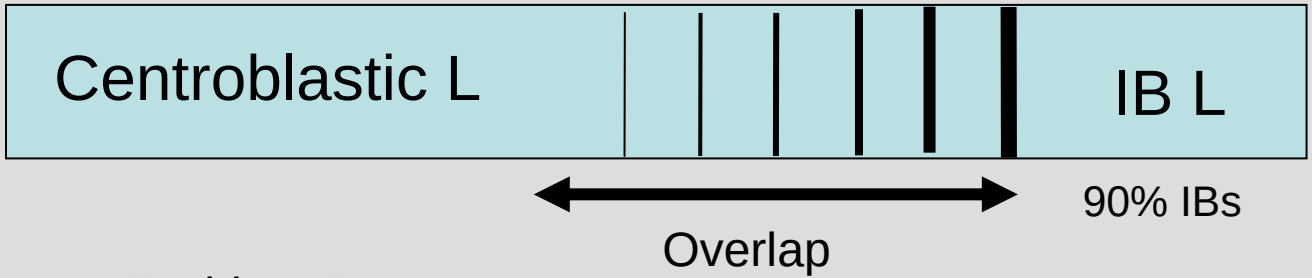
Centroblastic	< 90% IB-like cells	IB	>90% IB-like cells



Engelhardt et al Blood 1997

Morphological subtyping of DLBCLs





Problem 1: Mixture of CB-like and IB-like cells

Problem 2: DLBCLs with >90% IB-like cells are rare (< 10% of DLBCL)

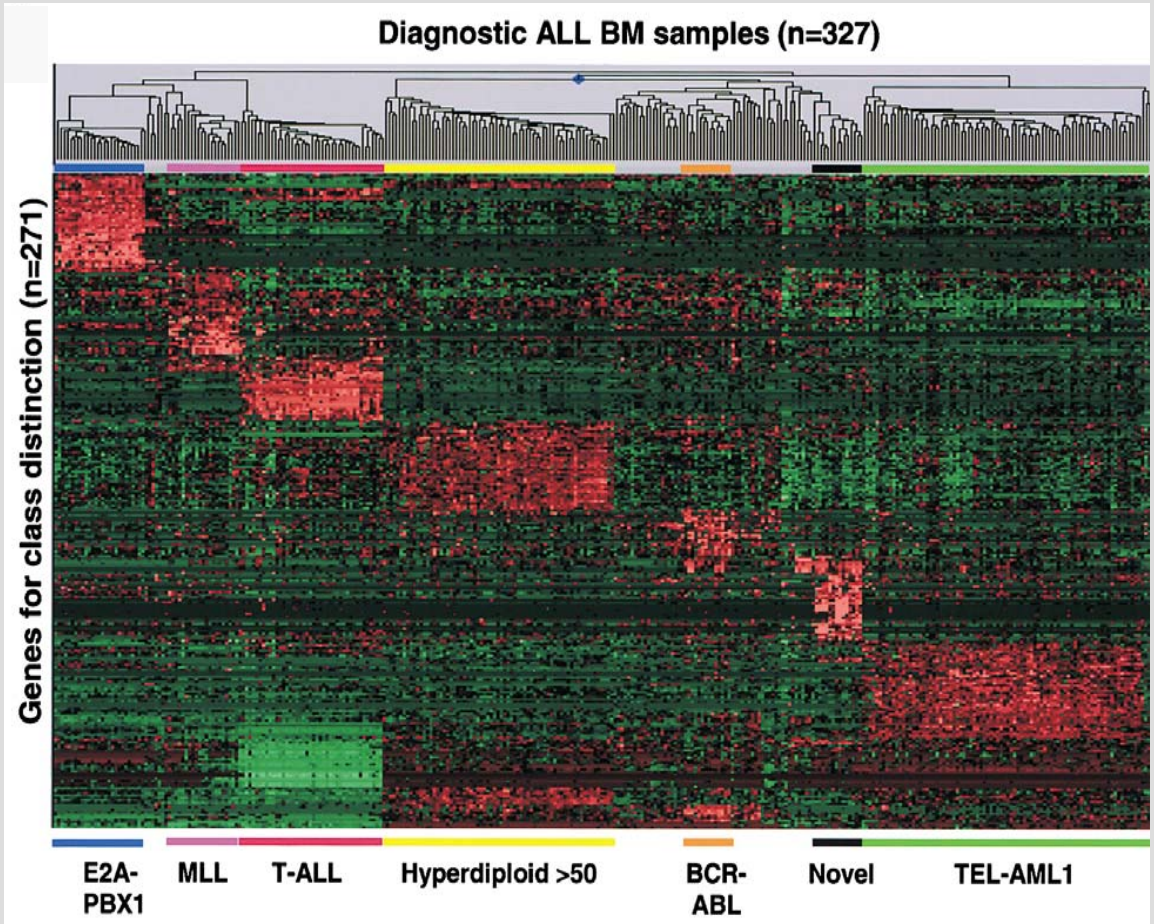
Problem 3: Internationally the distinction between CBL and IBL was not convincingly reproducible

Consequence: in the REAL/WHO classification
the CBL, IBL and other variants were subsumed under the term DLBCL

GE profiles of pediatric ALL allowed a precise d*iagnosis without cytogenetics and the identification of a new ALL-disease**

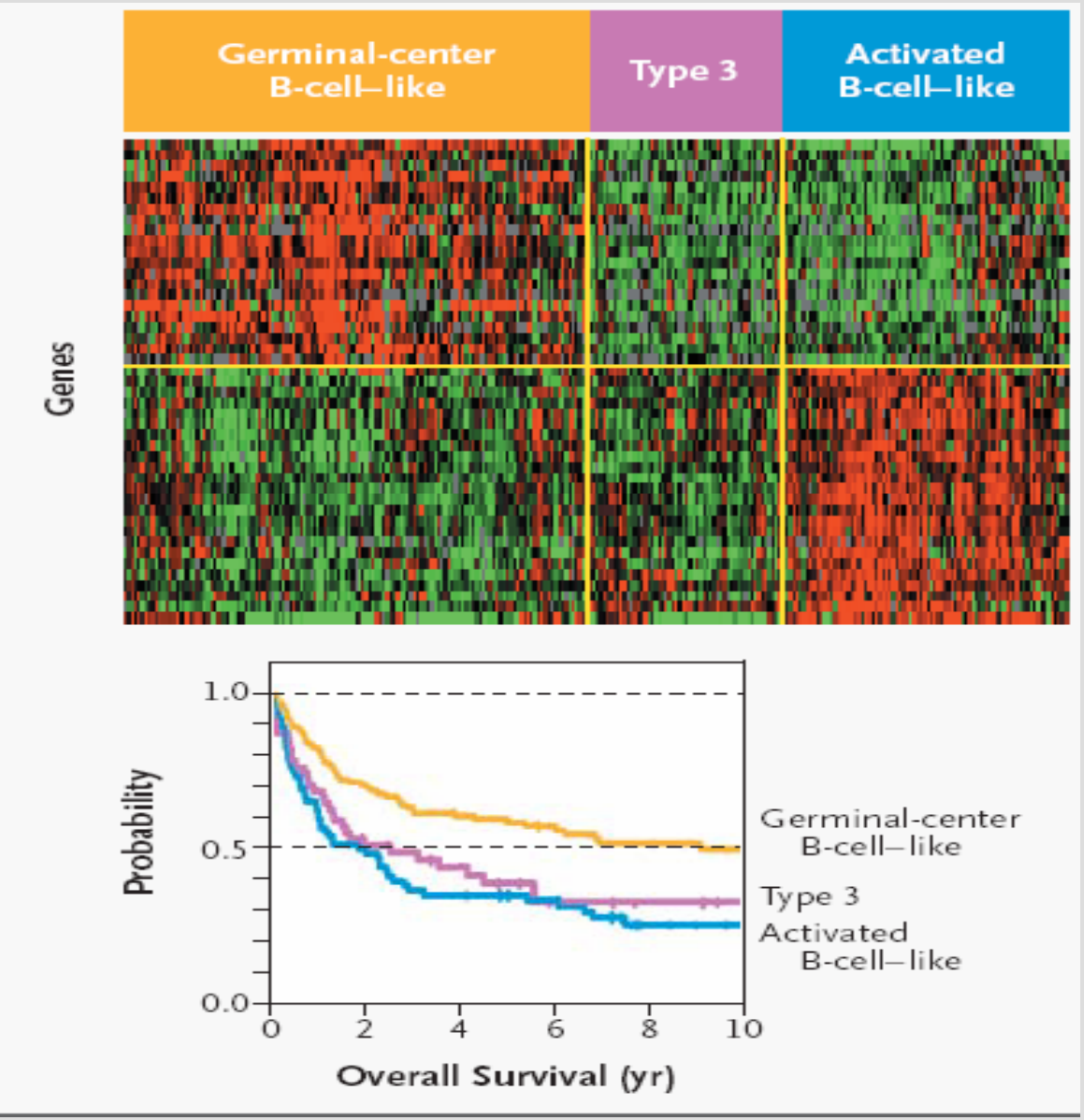
Cytogenetic Classification of ALL

t(1;19)(q23;p13.3)	*E2A-PBX1*
t(4;11)(q21;q23)	*MLL-AF4*
T-ALL	
Hyperdiploid >50	
t(9;22)(q34;q11.2)	*BCR-ABL*
t(12;21)(p13;q22)	*TEL-AML1*



* Downing's group: Cancer Cell 2002

Due to the sub-classification problems by morphology Rosenwald et al applied a new approach: Transcriptional profiling using an own designed Gene Array Chip called ***LymphoChip***



An ABC/GCB signature was established called „Wright“-classifier

Impressed & disapppointed: The German lymphoma reference pathologists expected more than two subgroups among DLBCL

Rosenwald et al
NEJM 2002 (Staudt-Gruppe)

2002: Founding a Germany-wide multi-center lymphoma network project for the molecular profiling of aggressive B-NHL

Aims:

- to identify new disease entities and/or prognostic subgroups within DLBCL,
- to better define Burkitt lymphoma and
- to find genes related to pathogenesis and prognosis within aggressive lymphomas

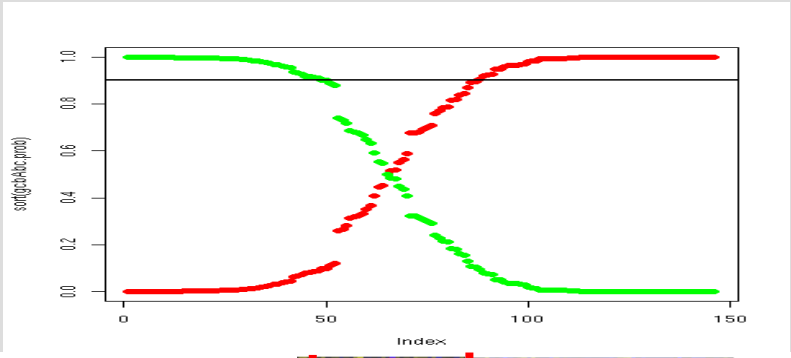
Approach: To study

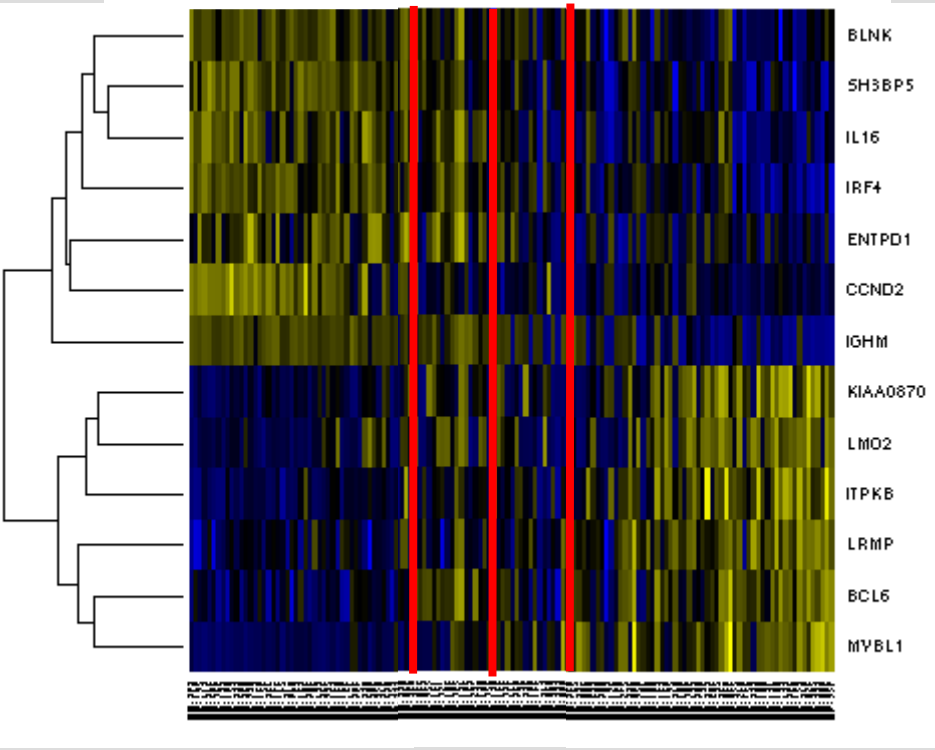
• DLBCL with a tumor cell content >70%	165 cases
• Classic and atypical Burkitt lymphomas	45 cases
• High grade B-NHL NOS	21 cases
	231 cases

by

• Morphologic panel review (6 expert pathologists)	231 cases
• Gene expression profiling (Affymetrix GeneChip 133A)	231 cases
• Cytogenetics:	
• FISH (t(14;18), MYC, BCL6, IgH, MALT1	224 cases
• Array (Matrix) CGH (genetic imbalances(	185 cases
• IgH and BCL6 mutations	145/139 cases
• Clinical features	140 cases

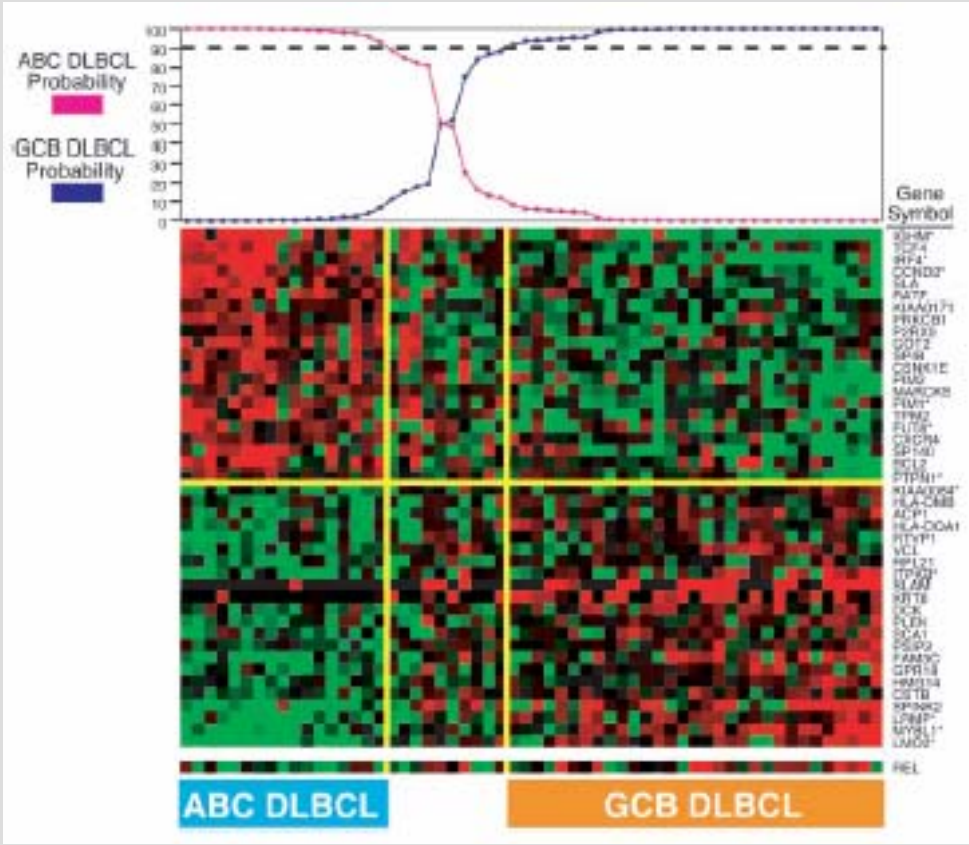
Application of the Wright-Classifier to the MMML-DLBCL cases





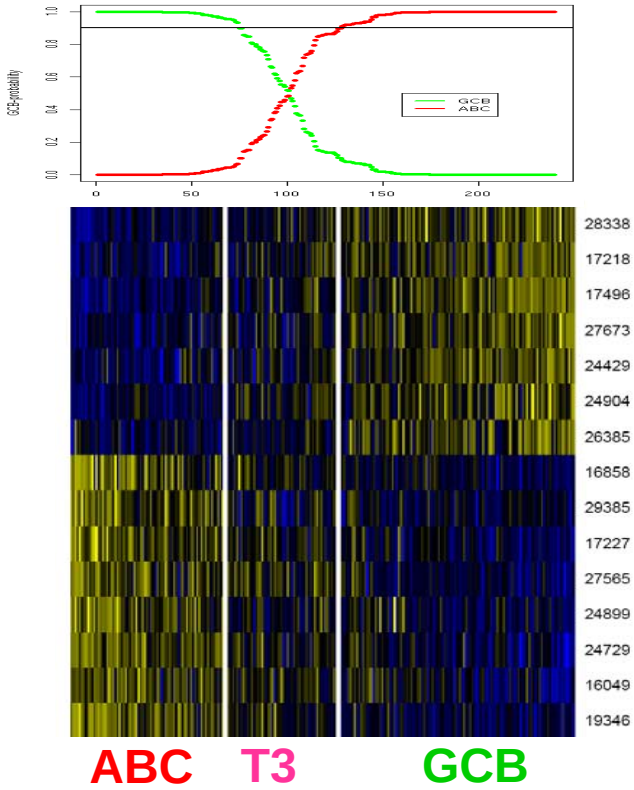
ABC GCB

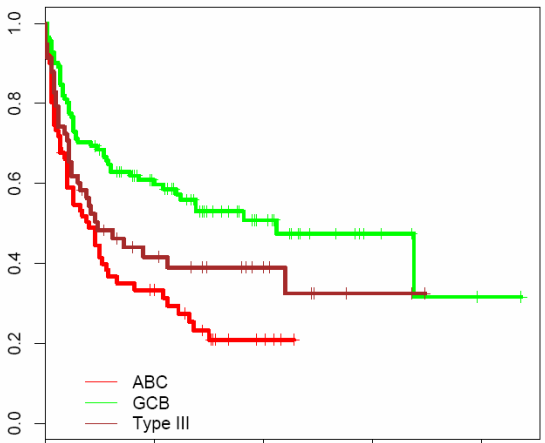
Wrigtht et al PNAS
LLMPP-Series



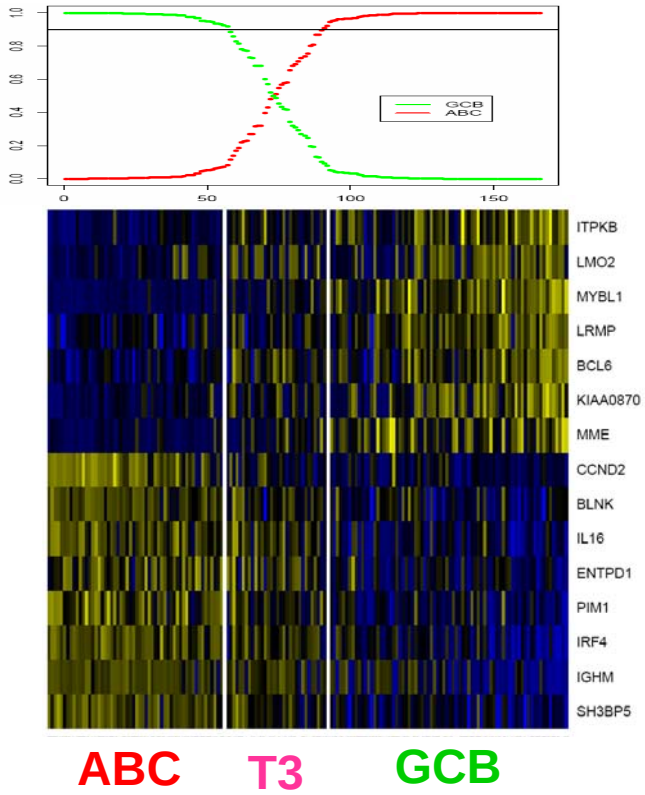
Type 3

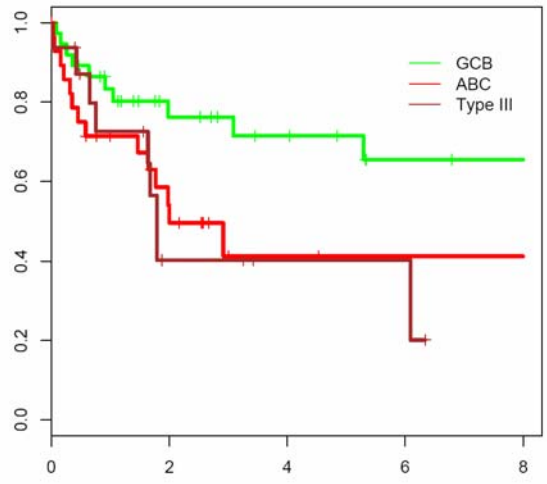
Rosenwald et al 2002
LymphoChip





Our study
Affymetrix GeneChip U133A

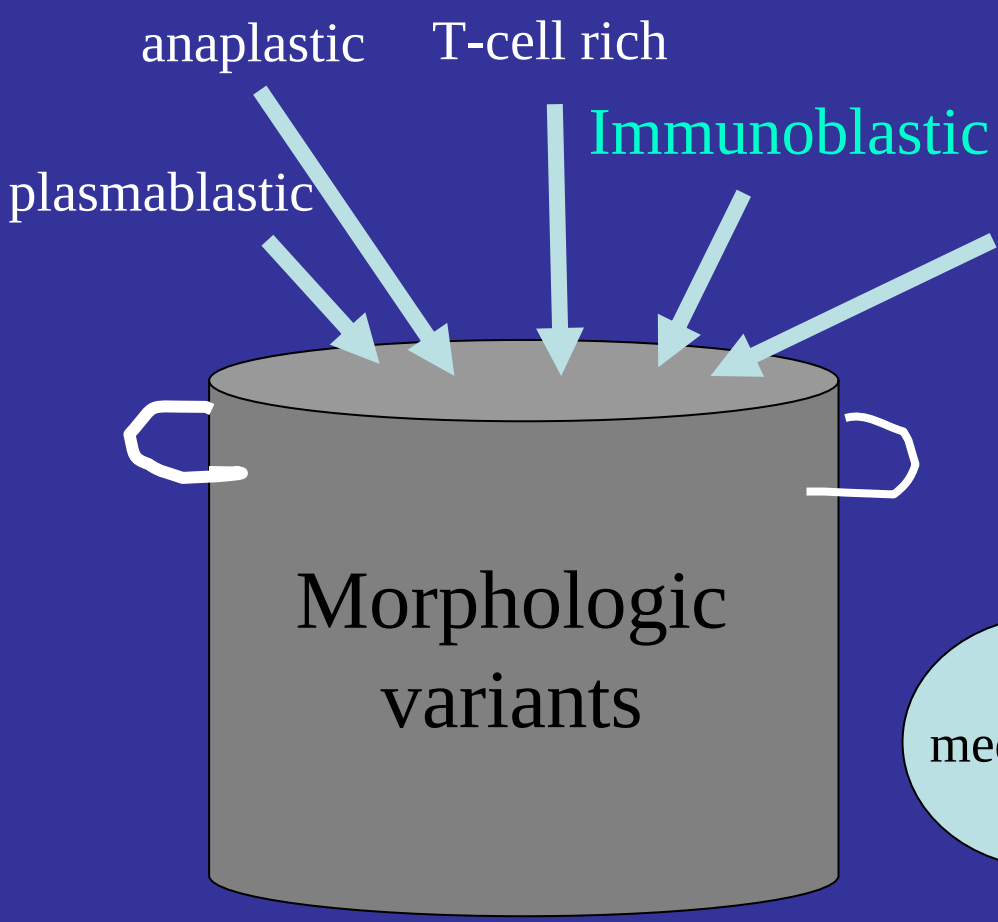




Mature Aggressive B-NHL of the WHO Classification

DLBCL

Burkitt lymphoma
Burkitt-like lymphoma



DLBCL:
Subtypes

mediastinal

intravascular

effusion

- Is the sporadic Burkitt lymphoma at the molecular level one (homogeneous) disease entity?

- Is the separation of the sporadic Burkitt lymphoma from DLBCL with molecular methods more reliable than on the basis of current WHO criteria?

Approach : Definition of a core-Burkitt group

based on the assumption that classical Burkitt lymphoma represents one homogeneous disease

Criteria of core-group:

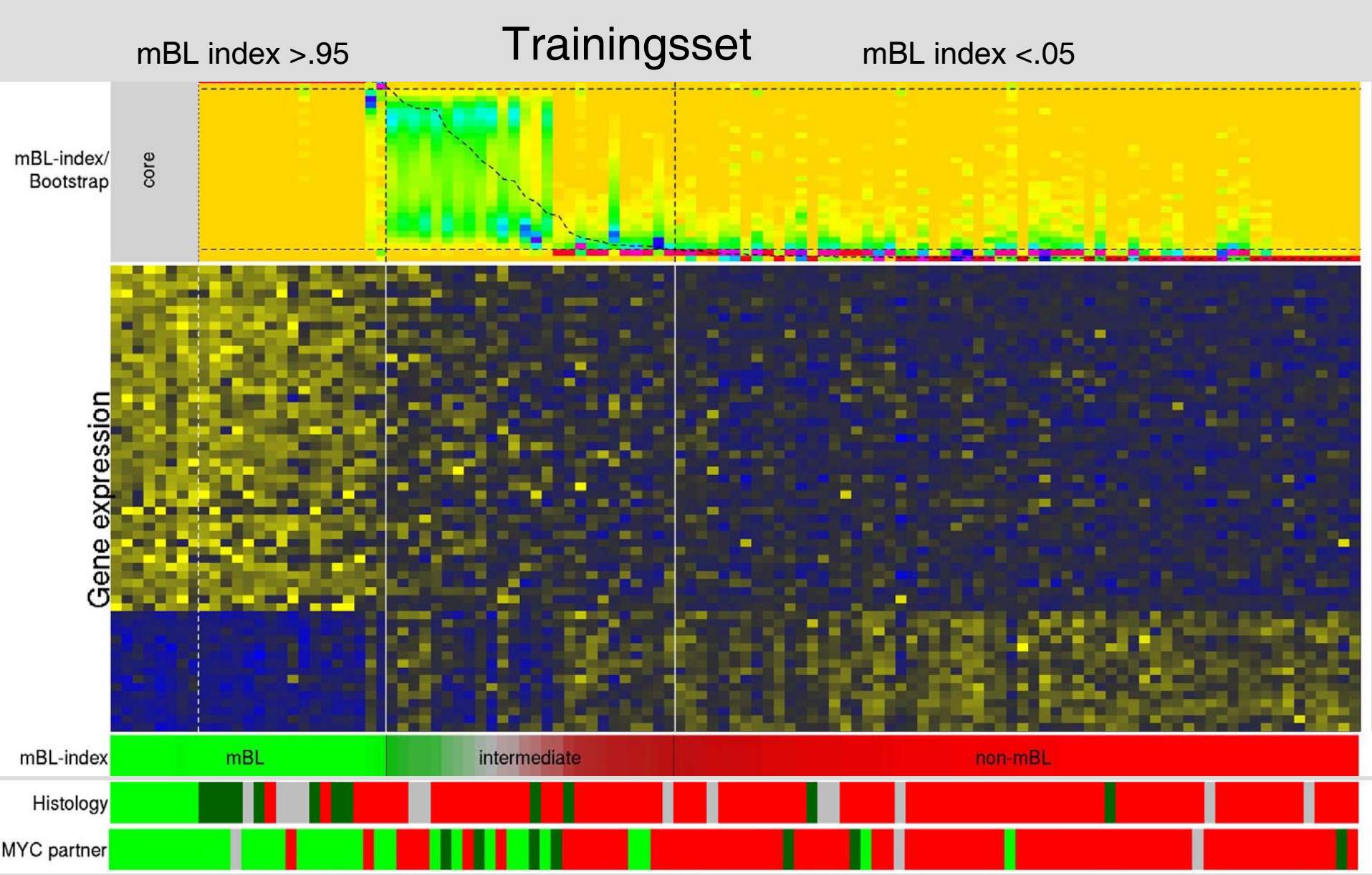
- consensus panel diagnosis: classical or atypical BL
- CD10 +
- BCL-6 +
- BCL-2 -
- CD5 -
- Ki-67 > 95%
- IG-MYC-break+

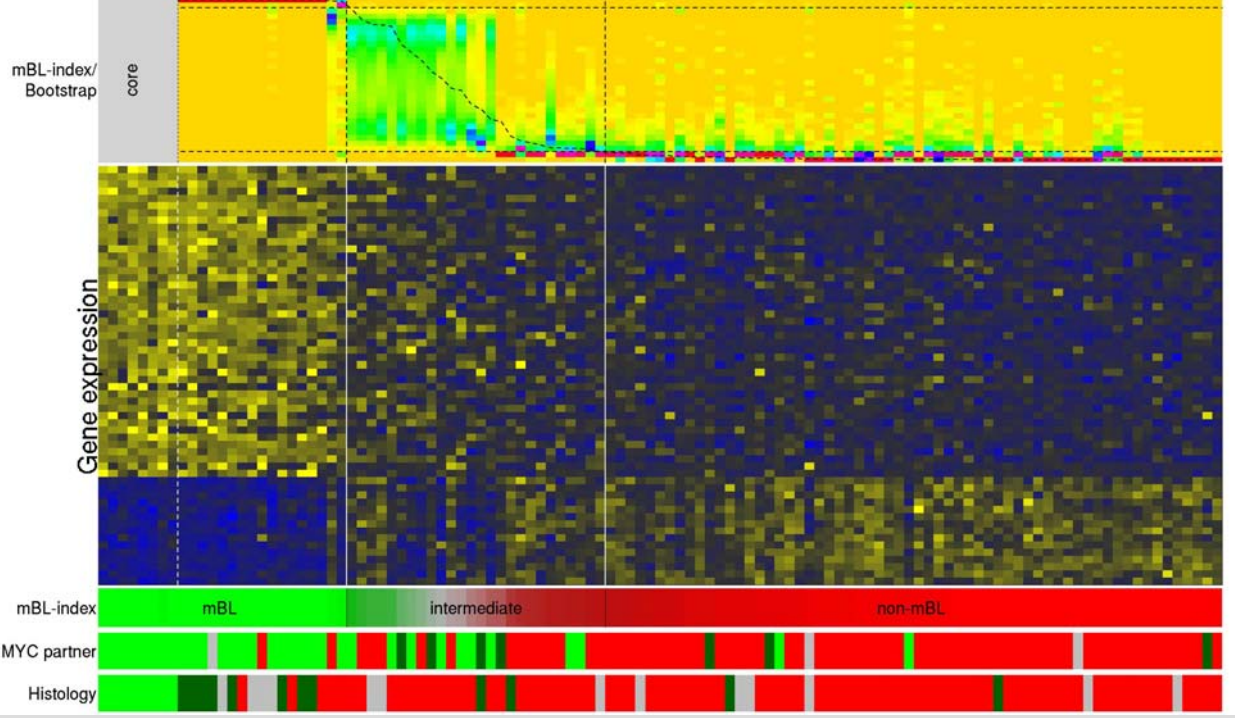
} 8 Fälle

Goal: Generation of BL-expression-signature

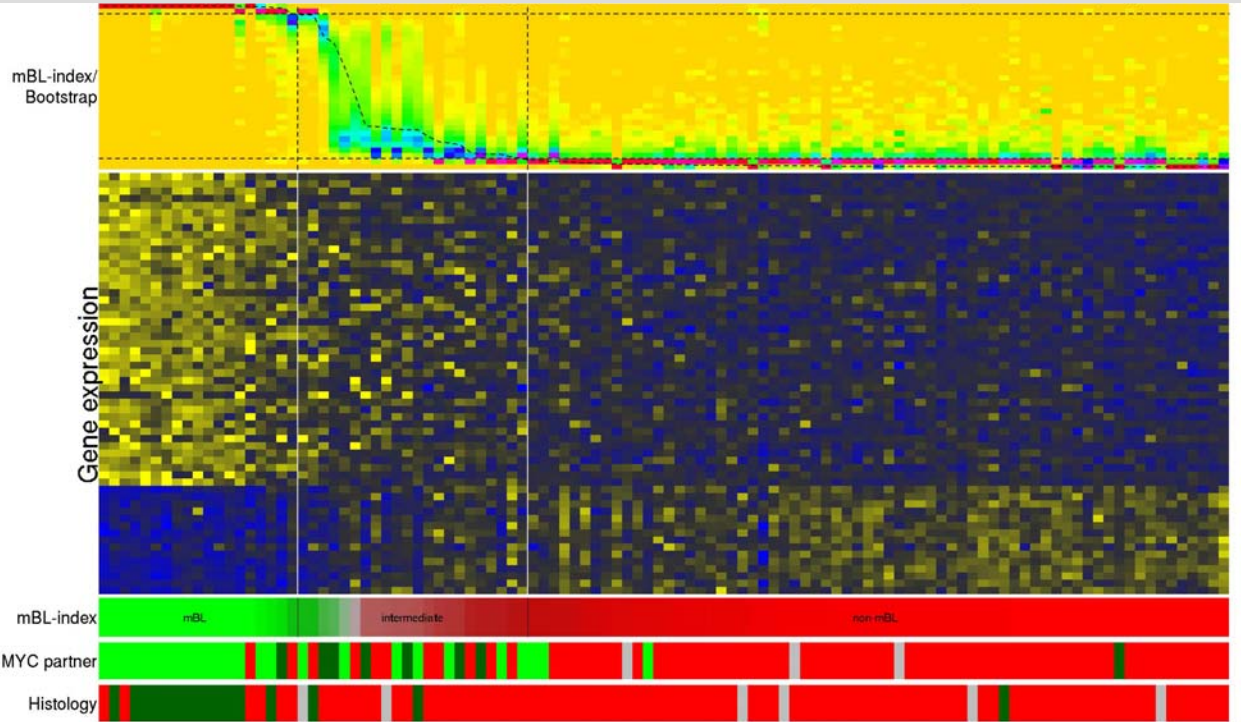
Novel bioinformatic approach:

Development of a "core group extension" method based on the core BL gene expression signature for the establishment of a molecular BL-index which can be used for the recognition of BL among a collection of aggressive B-NHL


mBL index >.95
Trainingsset
mBL index <.05
mBL-index/
Bootstrap
core
Gene expression
mBL-index
mBL
intermediate
non-mBL
Histology
MYC partner



Trainingsset



Testset

Correlation of the subgroups distinguished on the basis of the mBL signature with the „pure“ morphologic panel review diagnosis

Morphology	mBL	Intermediate	Non-mBL
Burkitt L., Core-Fälle	8	0	0
Atypical BL	21	**4**	**3**
DLBCL	**11**	39	115
B-NHL high	4	5	9
Total	**44**	**48**	**127**

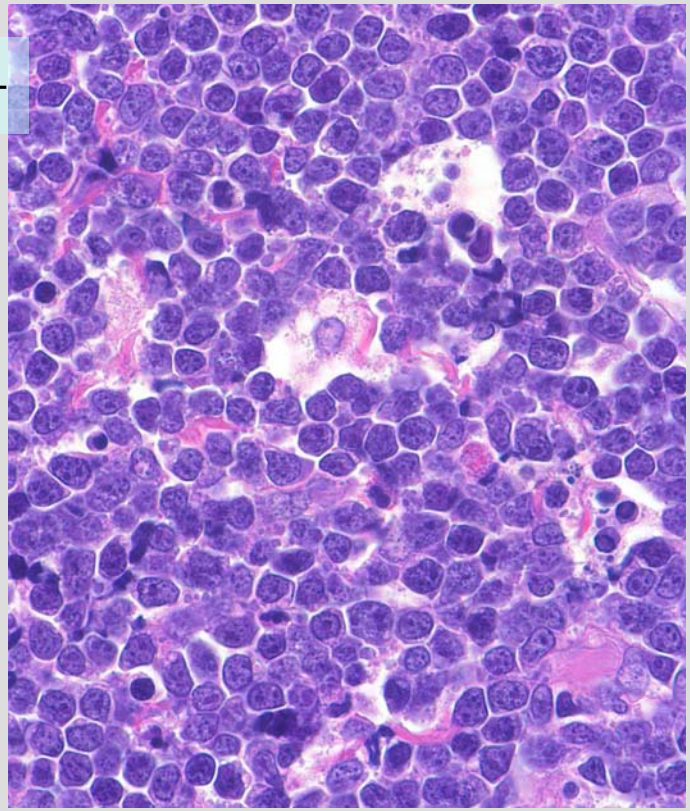
219

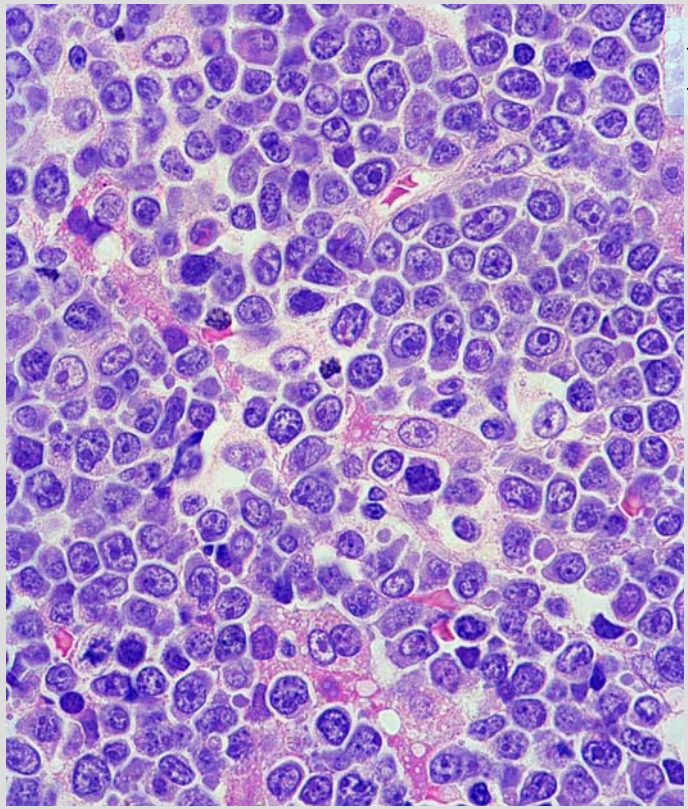
Morphologisches Spektrum der molekular definierten Burkitt Lymphome (mBL)

Typisches Burkitt Lymphom
mit einem mBL Index >.95

Typisches DLBCL
mit einem mBL Index >.95

Ig-MYC+



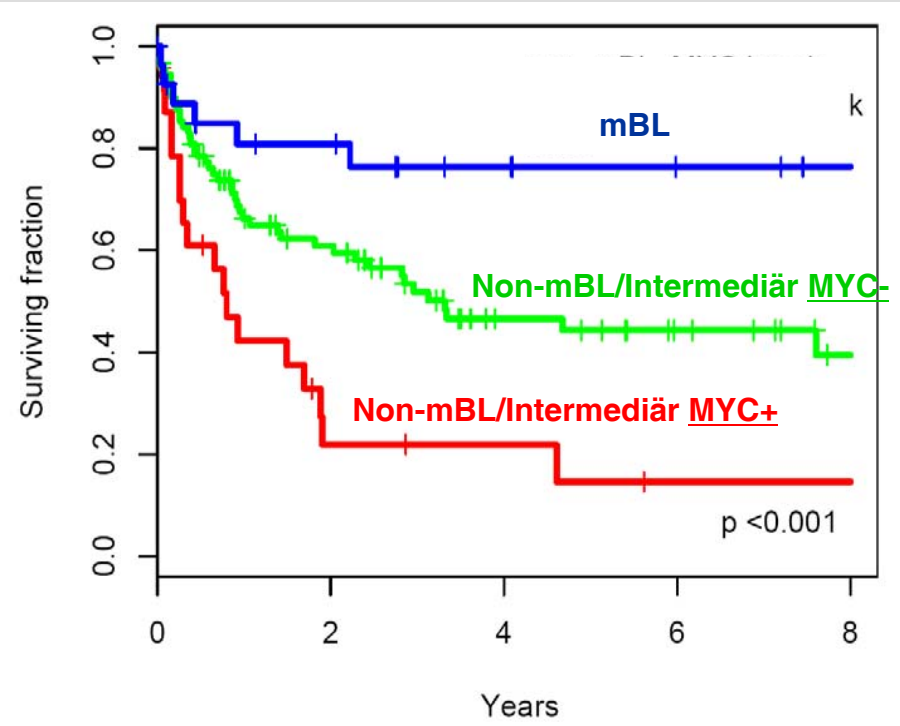


Ig-MYC+

Correlation of the subgroups distinguished on the basis of the mBL signature with immunohistology

Immunphenotype	mBL	Intermediate	Non-mBL
CD10	100%	57%	21%
BCL-6	100%	85%	77%
BCL-2	21%	83%	84%
Ki-67 Index	94%	75%	76%

Impact of MYC breaks on survival of molecular subgroup

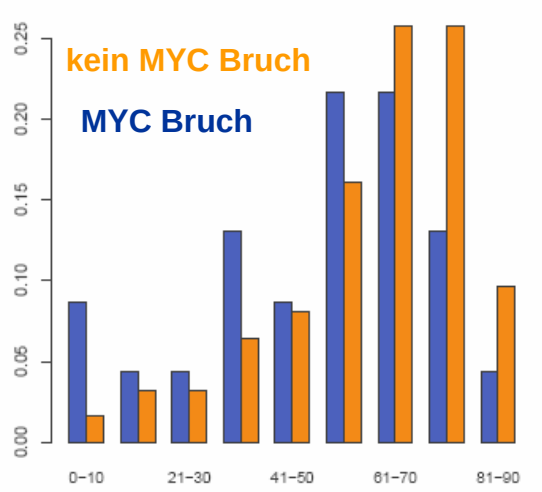


MYC break = favorable prognosis

MYC break = unfavorable prognosis

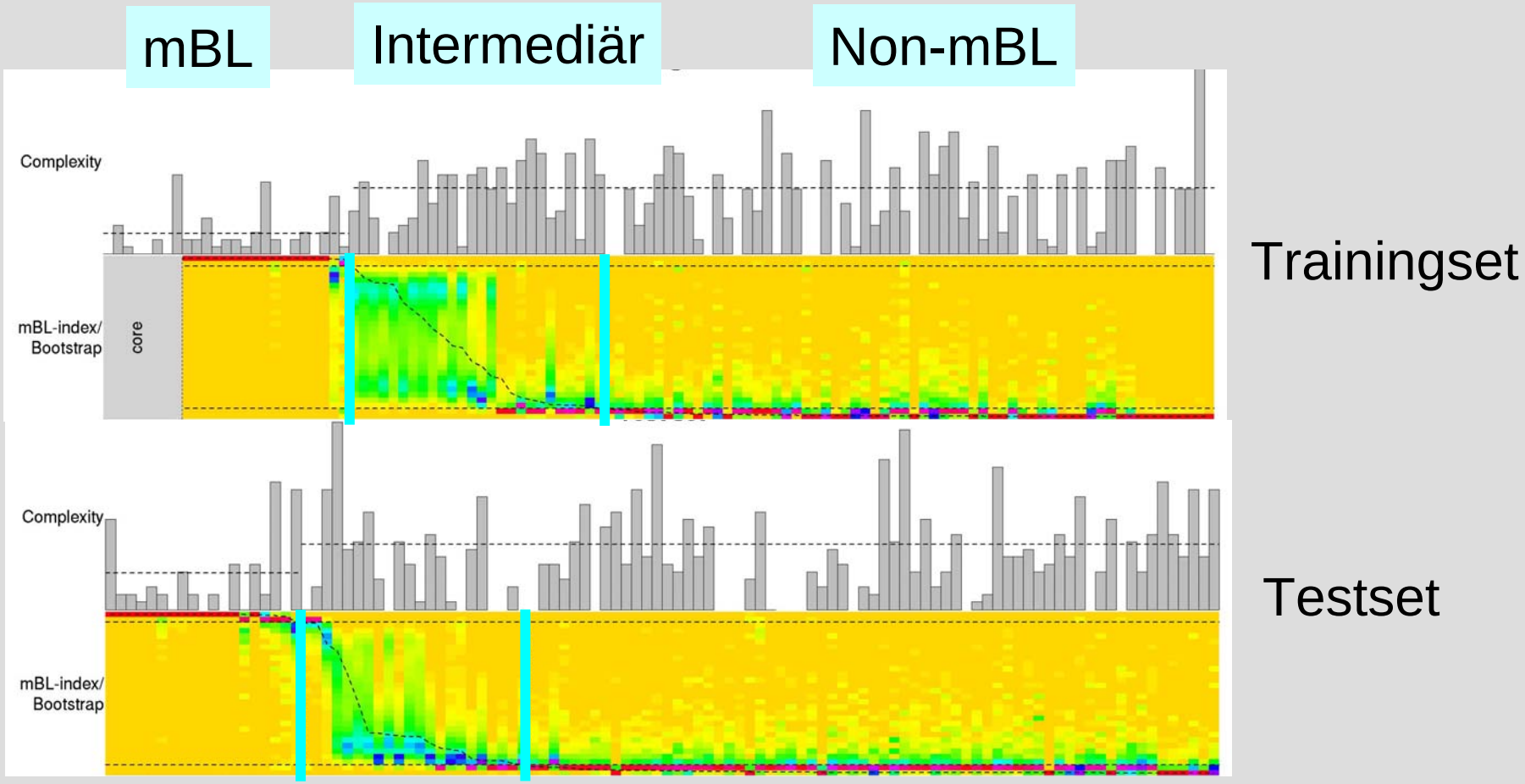
Definition of a novel prognostic subgroup in aggressive B-NHL or DLBCL

Age distribution

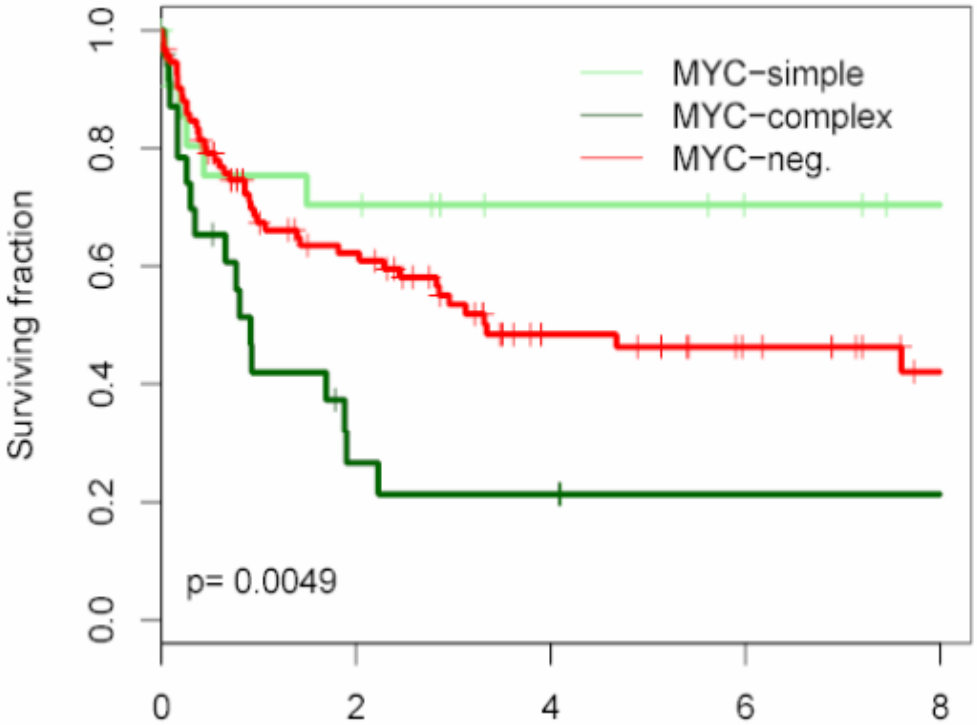


Genetic features	mBL	Intermediate	Non-mBL
Genetic Complexity	3,5	8,8	9,3
MYC break (total)	91%	54%	7%
IG-MYC-break	*91%*	*33%*	*4%*
NON-IG-MYC-break	*0%*	*21%*	*3%*

Correlation of the mBL signature (*training* und *test set)* of aggressive B-NHL (n=220) with genetic complexity



Impact of MYC breaks on survival of aggressive B-NHL in relation of genetic complexitiy



MYC-simple:
- *IG-MYC* break,
- absence or only few chromosomale imbalances

MYC-negative

MYC-complex:
- *IG-MYC* or non-IG-MYC break,
- high number of chromosomal imbalances and/or translocations

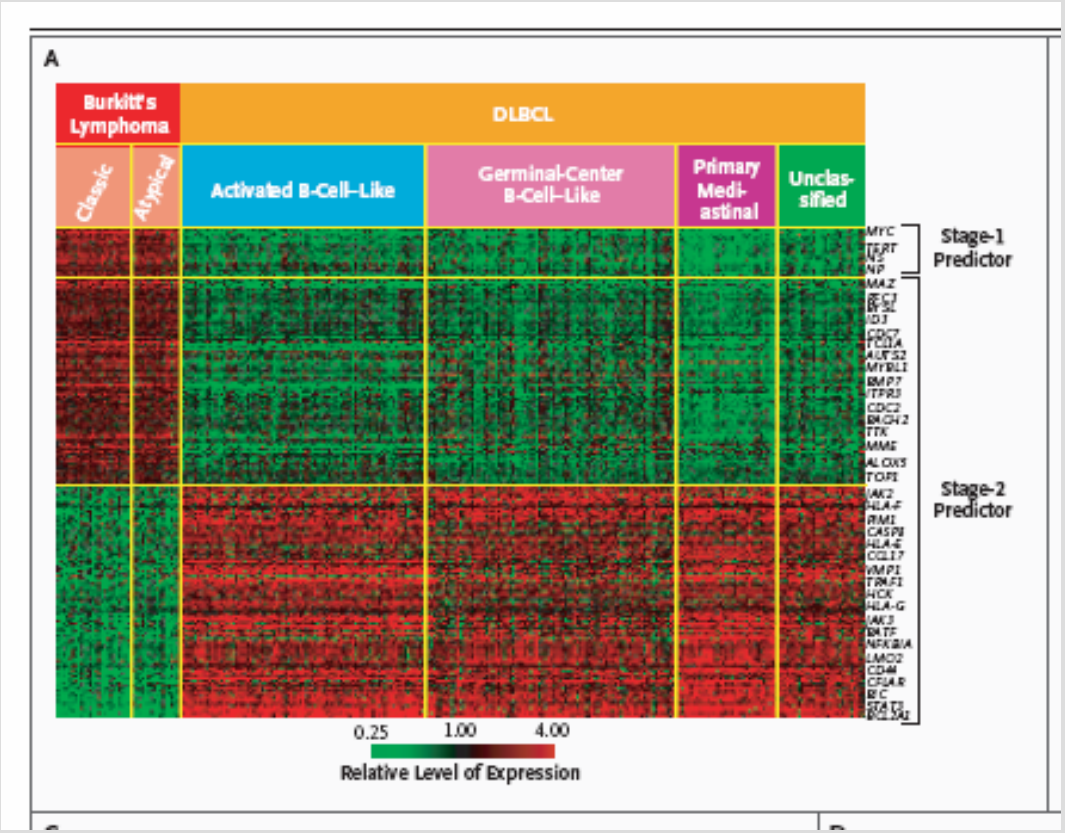
The NEW ENGLAND JOURNAL of MEDICINE

June 8, 2006

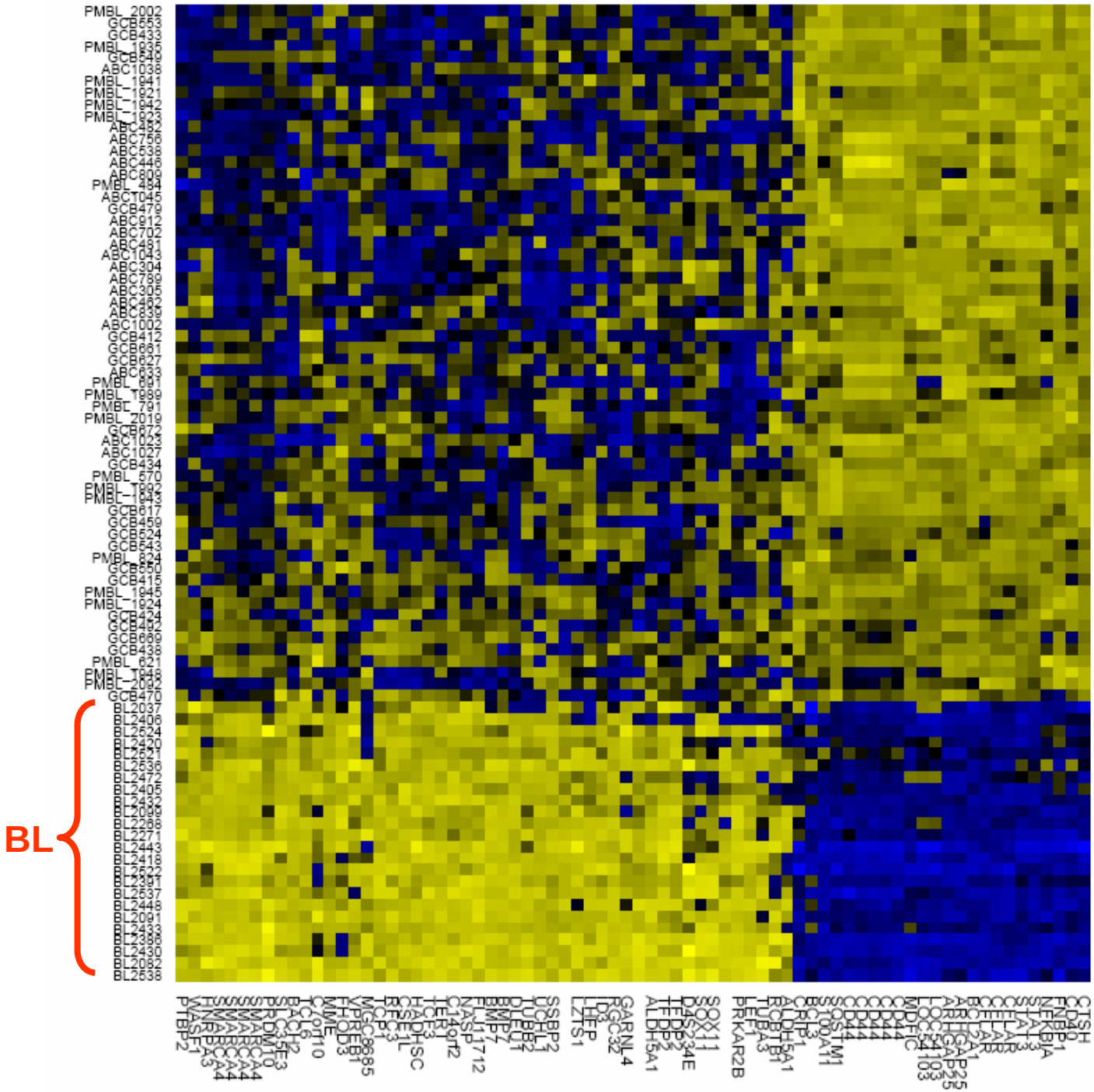
ORIGINAL ARTICLE

Molecular Diagnosis of Burkitt's Lymphoma

Sandeep S. Dave, M.D., Kai Fu, M.D., Ph.D., George W. Wright, Ph.D., Lloyd T. Lam, Ph.D., Philip Kluin, M.D., Evert-Jan Boerma, B.S., Timothy C. Greiner, M.D., Dennis D. Weisenburger, M.D., Andreas Rosenwald, M.D., German Ott, M.D., Hans-Konrad Müller-Hermelink, M.D., Randy D. Gascoyne, M.D.,



„Only specimens for which the diagnosis based on the histological review and molecular analysis of gene expression agreed are shown“



Application of the mBL signature to to the LLMPP (Dave et al) cases published in the NEJM 2006

Summary (I)

- The sporadic Burkitt lymphoma (BL) represents one single disease entity also at the molecular level.
- The generated BL signature clarifies and extends the WHO-criteria for the diagnosis of BL.
- The mBL signature identifies among the aggressive B-NHL two further subgroups: the non-mBL-group and an intermediate group.
- Clinically both groups significantly differ from the mBL group.
- The gene expression profile of the „intermediate“ cases is putatively caused by secondary genetic aberrations.

Summary (II)

- Aggressive B-NHL without a mBL-signature but with a MYC-break appears to represent novel subgroup with adverse prognosis.
- The MYC-break-positive aggressive B-NHL without a mBL-signature are associated with a high genetic complexity.
- The mBL gene expression signature cannot be applied in the daily diagnostic work. Therefore it is planned to establish on the basis of the the mBL signature a clinically applicable immuno-histological classifier.
- .

Verbundprojekt
der Deutschen Krebshilfe
"Molekulare Mechanismen
bei malignen Lymphomen"

Helfen. Forschen. Informieren.

Molecular Mechanisms in Malignant Lymphomas

Pathology (Chair: H. Stein)

W. Klapper, R. Parwaresch (Kiel), M.-L. Hansmann, M. (Frankfurt), W. Bernd, A. Feller (Lübeck), H.K. Müller-Hermelink, G. Ott, Andreas Rosenwald (Würzburg), P. Möller, T. Barth (Ulm), S. Cogliatti (St. Gallen), H. Stein (Berlin)

Gene Expression & Bioinformatics (Chair: H. Stein & M. Hummel)

M. Hummel, D. Lenze, H. Stein, (Berlin), S. Bentink, R. Spang (Berlin)

Genetics (Chair: R. Siebert)

L. Harder, R. Siebert (Kiel), H. Trautmann, C. Pott, M. Kneba (Kiel), S. Wessendorf, C. Schwänen, S. Stilgenbauer (Ulm), E.Murga, J. Dierlamm (Hamburg), E. Haralambieva, G. Ott (Würzburg), P. Lichter (Heidelberg), M. Bentz (Karlsruhe), P. Möller, T. Barth (Ulm); R. Küppers (Essen)

Clinical Groups (Chair: L. Trümper)

German High Grade Lymphoma Study Group, German Low Grade Lymphoma Study Group

Biometrics & Data Management (Chair: M. Löffler)

H. Berger, D. Hasenclever, U. Schönwiese, M. Löffler (Leipzig)

et al.

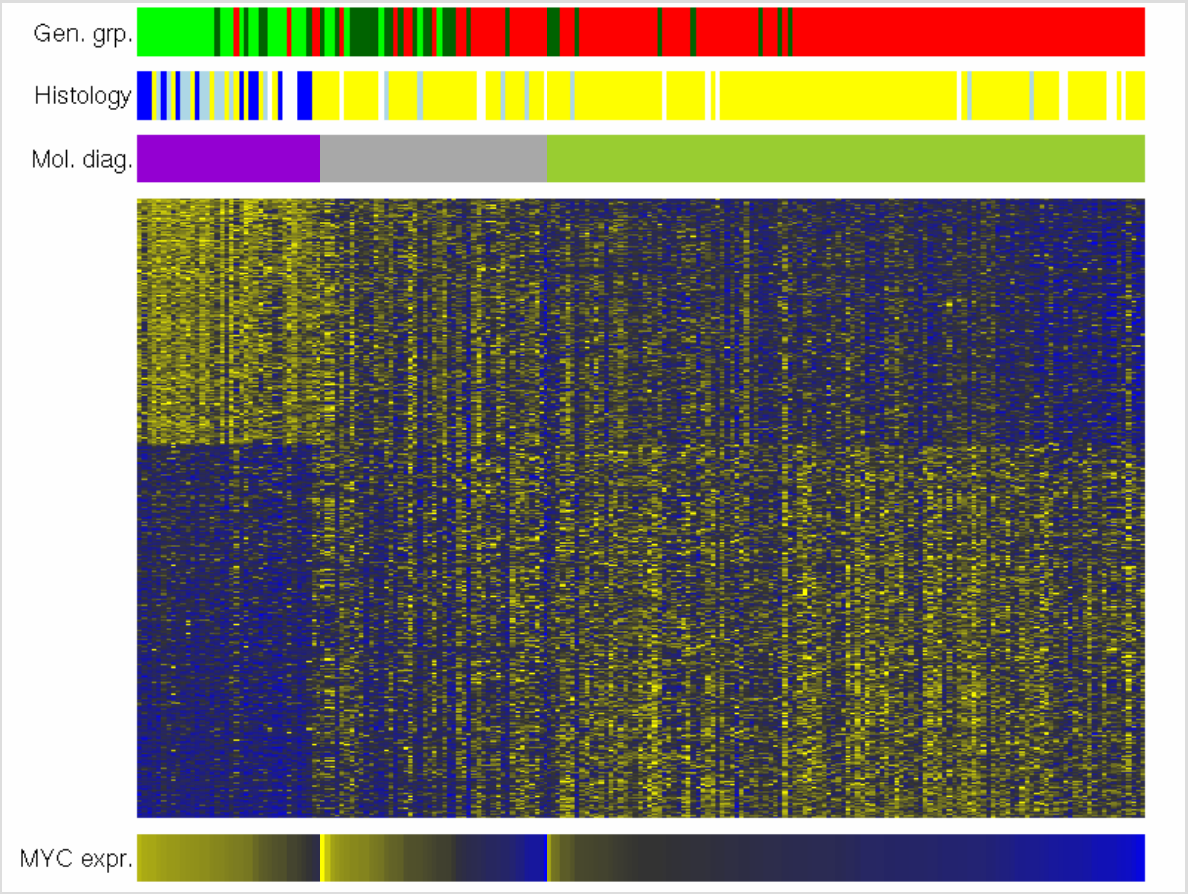
Thank you
for your attention

Comparison of MYC break effects on gene expression in the subgroups mBL, intermediate and non-mBL

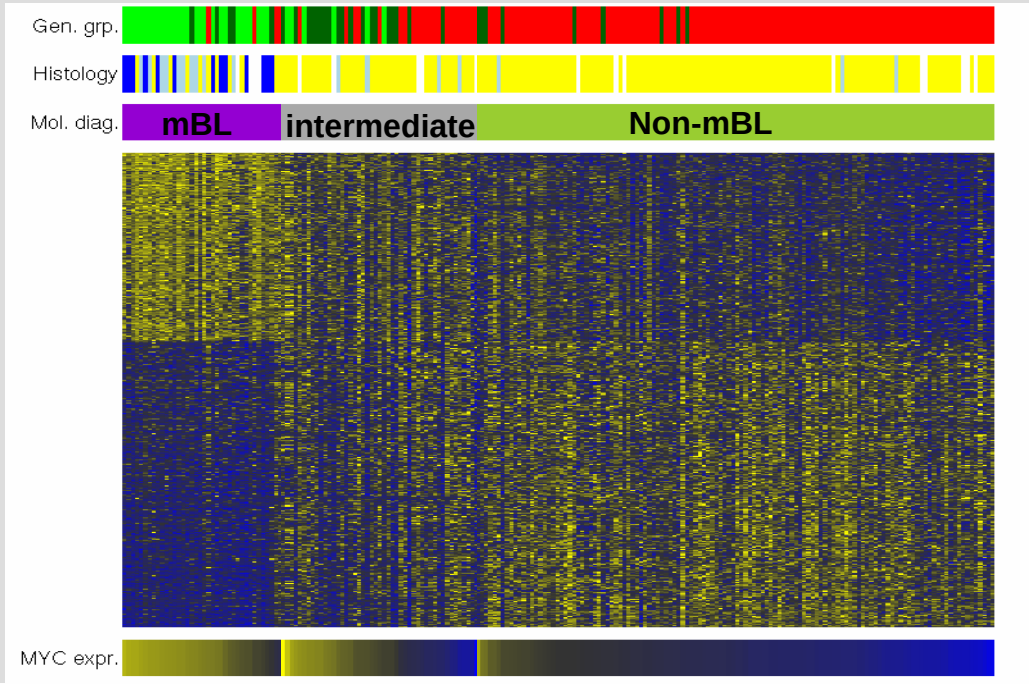
MYC status:
IG-MYC: light green
Non-IG-MYC: dark green
MYC-negative: red

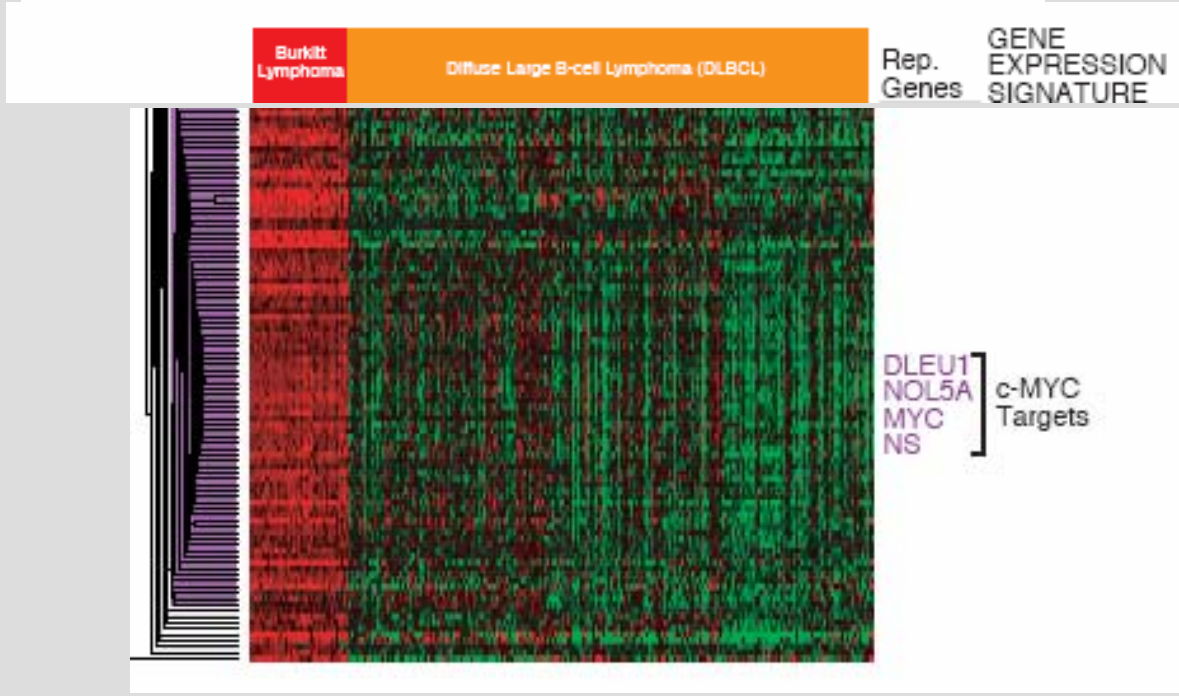
Review diagnosis:
Blue: Burkitt Lymphoma
Grey: BL-like Lymphom
white: B-NHL, high grade
Yellow: DLBCL

Molecular diagnosis:
Lila: mBL
Grey: Intermediate
Olive green: non-mBL



Bitte sortieren: mBL in MYC+ und negativ,
Intermediate cases into IG-MYC+, Non-IG-MYC and MYC-,
non-mBL into IG-MYC+, Non-IG-MYC and MYC-


Gen. grp.
Histology
Mol. diag.
mBL
intermediate
Non-mBL
MYC expr.


Burkitt Lymphoma
Diffuse Large B-cell Lymphoma (DLBCL)
Rep. Genes
GENE EXPRESSION SIGNATURE
DLEU1
NOL5A
MYC
NS
c-MYC Targets