Eppur si muove...

La terapia nel MONDO LINFOMI

Linfoma diffuso a grandi cellule B: ottimizzazione diagnostica

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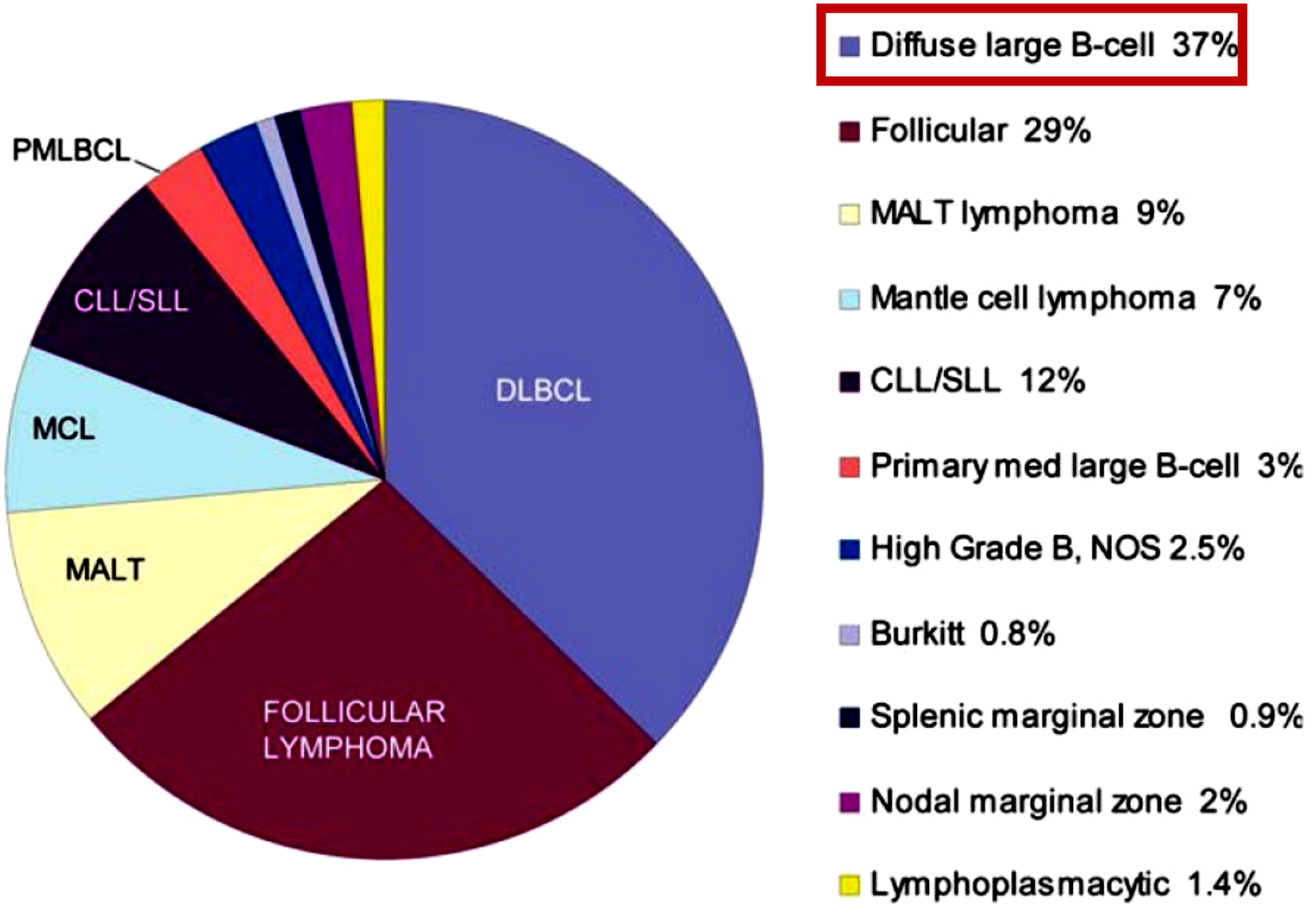
Catania 11 Luglio 2022

Disclosures of Enrico Derenzini

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
ADC-Therapeutics	Y	N	N	N	N	N	N
Roche	N	N	N	N	N	Y	N
BeiGene	N	N	N	N	N	Y	N
Astra-Zeneca	N	N	Y	N	N	Y	N
Takeda	Y	N	N	N	N	Y	N
Abbvie	N	N	Y	N	N	Y	N
Y= Yes							
N= No							


PMLBCL
CLL/SLL
MCL
MALT
DLBCL
FOLLICULAR LYMPHOMA
Diffuse large B-cell 37%
Follicular 29%
MALT lymphoma 9%
Mantle cell lymphoma 7%
CLL/SLL 12%
Primary med large B-cell 3%
High Grade B, NOS 2.5%
Burkitt 0.8%
Splenic marginal zone 0.9%
Nodal marginal zone 2%
Lymphoplasmacytic 1.4%

Diffuse large B-cell lymphoma: variants, subgroups and subtypes/entities

Diffuse large B-cell lymphoma, noth otherwise specified (NOS)

- Common morphologic variants
  - Centroblastic
  - Immunoblastic
  - Anaplastic
  - Other rare variants
- Molecular subgroups
  - Germinal centre B-cell-like (GCB)
  - Activated B-cell-like (ABC)

Diffuse large B-cell lymphoma subtypes

- T-cell/histiocyte-rich large B-cell lymphoma
- Primary DLBCL of the CNS
- Primary cutaneous DLBCL, leg type
- EBV-positive DLBCL, NOS
- *Large B-cell lymphoma with IRF4 rearrangements**

Other lymphomas of large B-cells

- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- ALK-positive large B-cell lymphoma
- Plasmablastic lymphoma
- HHV-8-positive DLBCL, NOS
- Primary effusion lymphoma

New provisional categories

- High grade B-cell lymphoma
  - High grade B-cell lymphoma, with *BCL2* and/or *BCL6* and *MYC* rearrangements
  - High grade B-cell lymphoma, NOS

Diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS)
  Germinal center B-cell subtype
  Activated B-cell subtype
Large B-cell lymphoma with 11q aberration[*] ⟸
Nodular lymphocyte predominant B-cell lymphoma[*] ⟸
T cell/histiocyte-rich large B-cell lymphoma

Primary DLBCL of the central nervous system
Primary DLBCL of the testis[*] ⟸
Primary cutaneous DLBCL, leg type
Intravascular large B-cell lymphoma
HHV-8 and EBV-negative primary effusion-based lymphoma[*] ⟸

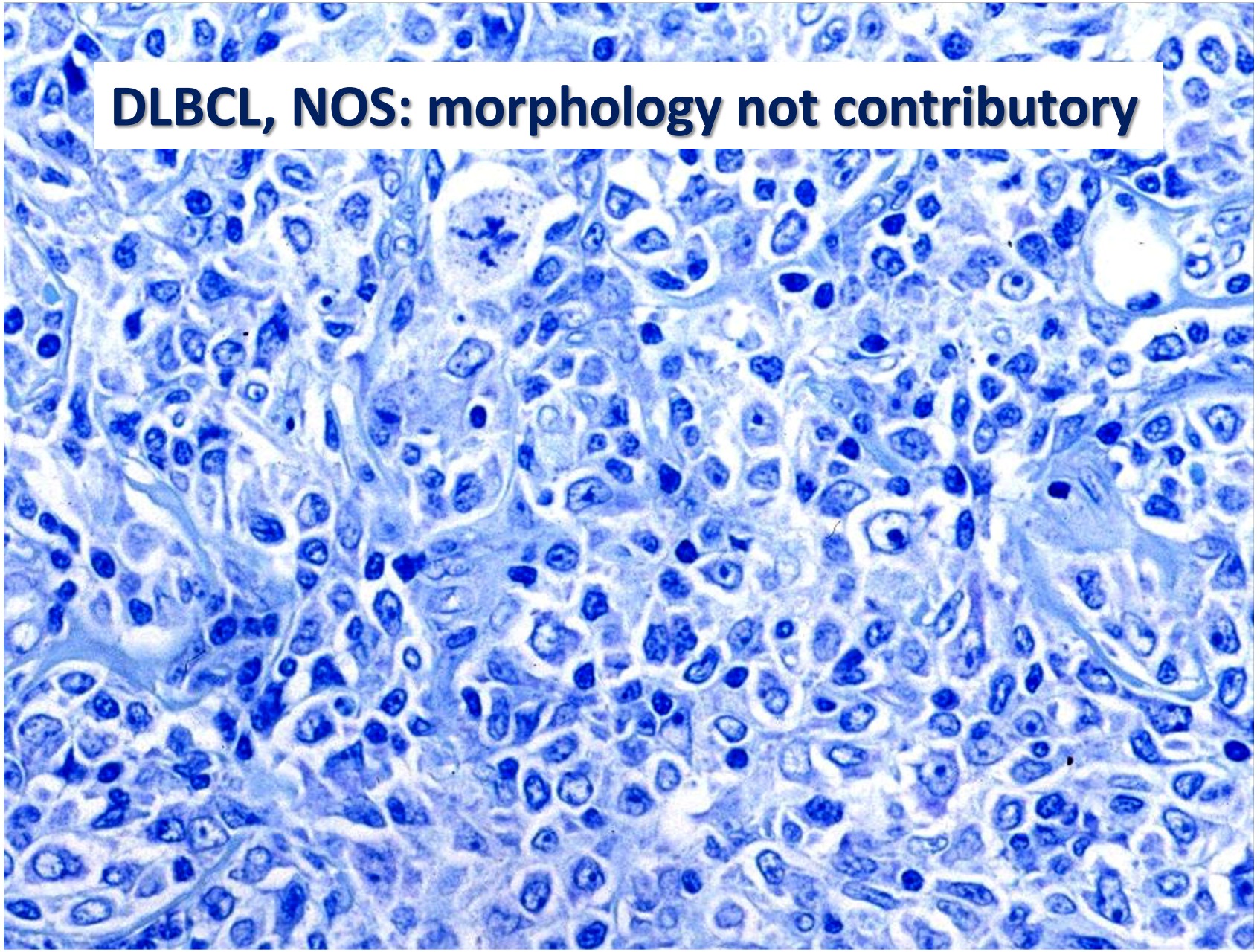
EBV-positive mucocutaneous ulcer[*] ⟸
EBV-positive DLBCL, NOS
DLBCL associated with chronic inflammation
  Fibrin-associated DLBCL
Lymphomatoid granulomatosis
EBV-positive polymorphic B-cell lymphoproliferative disorder, NOS[*] ⟸

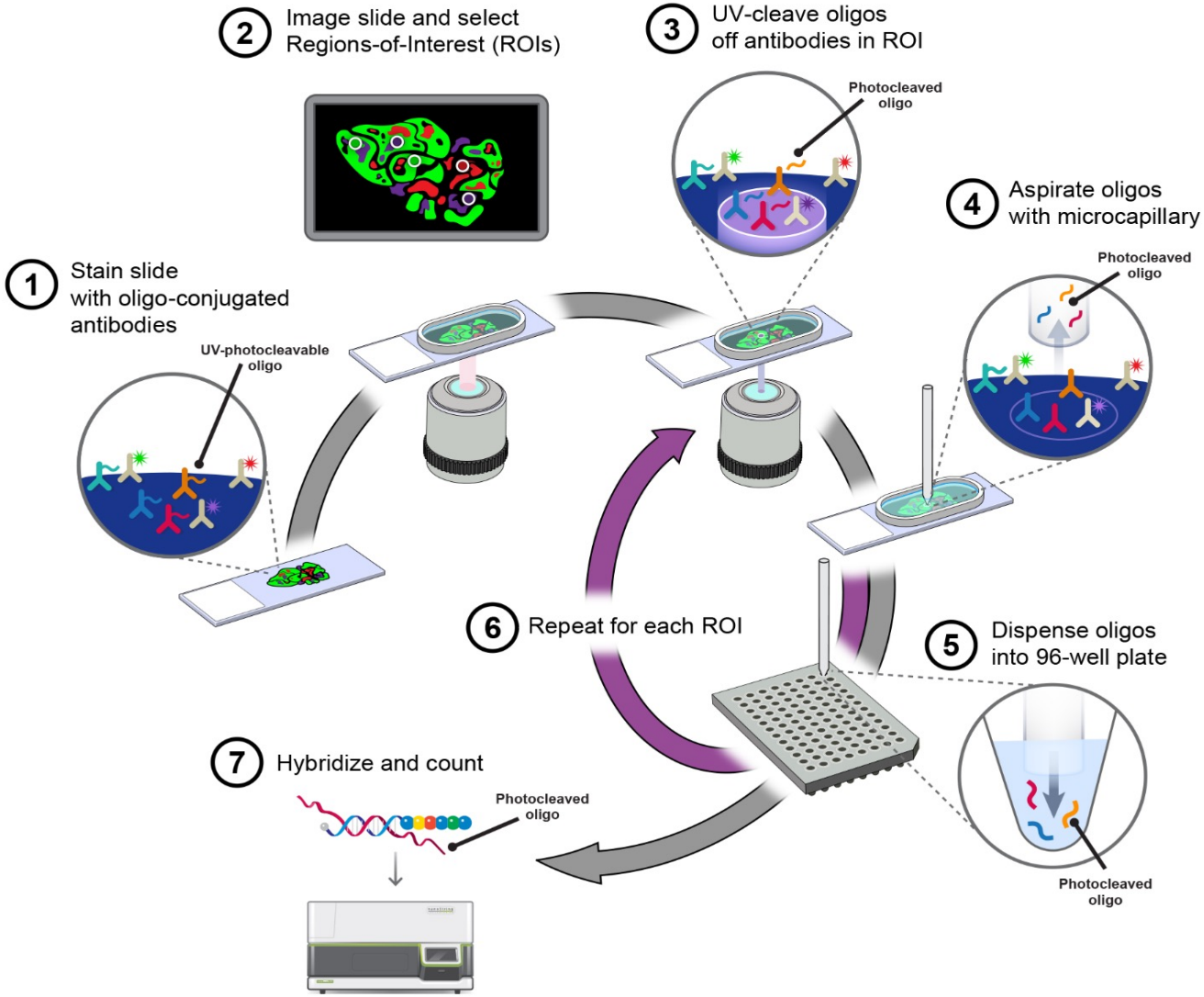
ALK-positive large B-cell lymphoma
Plasmablastic lymphoma
HHV8-associated lymphoproliferative disorders
  Multicentric Castleman disease
  HHV8-positive germinotropic lymphoproliferative disorder
  HHV8-positive DLBCL, NOS
  Primary effusion lymphoma

Burkitt lymphoma
High-grade B-cell lymphoma, with *MYC* and *BCL2* rearrangements[*] ⟸
High-grade B-cell lymphoma with MYC and BCL6 rearrangements[*] ⟸
High-grade B-cell lymphoma, NOS

Primary mediastinal large B-cell lymphoma
Mediastinal gray-zone lymphoma[*]

DLBCL, NOS: morphology not contributory




1 Stain slide with oligo-conjugated antibodies
UV-photocleavable oligo
2 Image slide and select Regions-of-Interest (ROIs)
3 UV-cleave oligos off antibodies in ROI
Photocleaved oligo
4 Aspirate oligos with microcapillary
Photocleaved oligo
5 Dispense oligos into 96-well plate
Photocleaved oligo
6 Repeat for each ROI
7 Hybridize and count
Photocleaved oligo

Gene Expression Profiling

CELL OF ORIGIN

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

Affimetrix
Fresh Frozen Tissue
Hundreds genes

Alizadeh et al. Nature 2000

Intrinsic limitation:

need for fresh or frozen tissue, available in only a few patients!

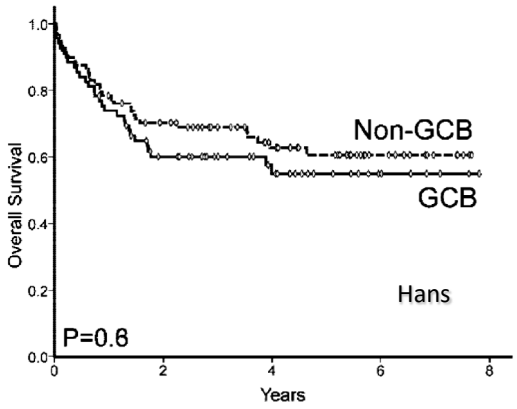
Ideally, tool to apply to FFPE samples!

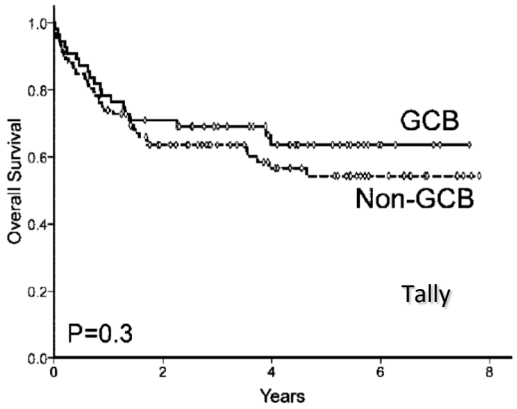
Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy

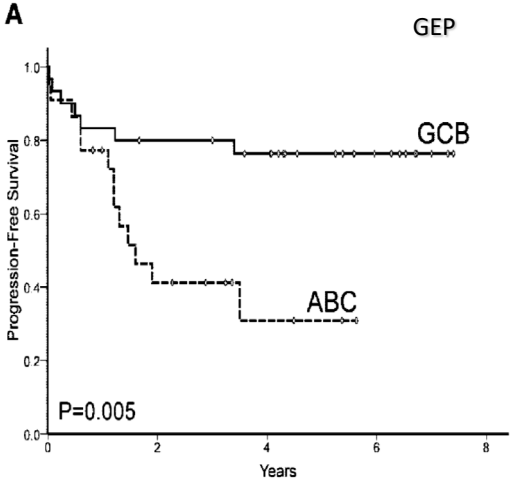
*Gonzalo Gutiérrez-García,[1] *Teresa Cardesa-Salzmann,[1] Fina Climent,[2] Eva González-Barca,[2] Santiago Mercadal,[2] José L. Mate,[3] Juan M. Sancho,[3] Leonor Arenillas,[4] Sergi Serrano,[4] Lourdes Escoda,[5] Salomé Martínez,[5] Alexandra Valera,[1] Antonio Martínez,[1] Pedro Jares,[1] Magdalena Pinyol,[1] Adriana García-Herrera,[1] Alejandra Martínez-Trillos,[1] Eva Giné,[1] Neus Villamor,[1] Elías Campo,[1] Luis Colomo,[1] and Armando López-Guillermo,[1] for the Grup per l'Estudi dels Limfomes de Catalunya I Balears (GELCAB)

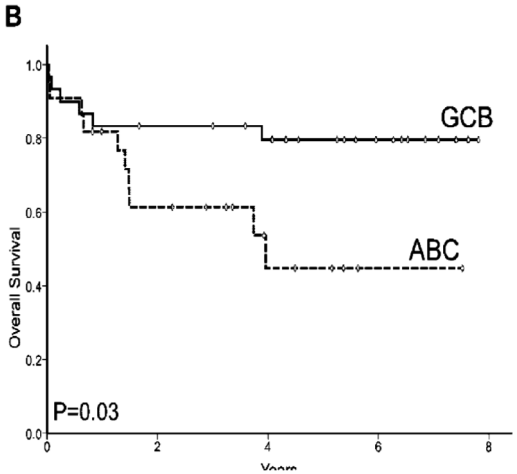
[1]Departments of Hematology and Pathology, Hospital Clínic, University of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [2]Hospital Duran i Reynals, Hospitalet de Llobregat, Spain; [3]Hospital Germans Trias I Pujol, Badalona, Spain; [4]Hospital del Mar, Barcelona, Spain; and [5]Hospital Joan XXIII, Tarragona, Spain

Diffuse large B-cell lymphomas (DLBCLs) can be divided into germinal-center B cell–like (GCB) and activated-B cell–like (ABC) subtypes by gene-expression profiling (GEP), with the latter showing a poorer outcome. Although this classification can be mimicked by different immunostaining algorithms, their reliability is the object of controversy. We constructed tissue microarrays with samples of 157 DLBCL patients homogeneously treated with immunochemotherapy to apply the following algorithms: Colomo (MUM1/IRF4, CD10, and BCL6 antigens), Hans (CD10, BCL6, and MUM1/IRF4), Muris (CD10 and MUM1/IRF4 plus BCL2), Choi (GCET1, MUM1/IRF4, CD10, FOXP1, and BCL6), and Tally (CD10, GCET1, MUM1/IRF4, FOXP1, and LMO2). GEP information was available in 62 cases. The proportion of misclassified cases by immunohistochemistry compared with GEP was higher when defining the GCB subset: 41%, 48%, 30%, 60%, and 40% for Colomo, Hans, Muris, Choi, and Tally, respectively. Whereas the GEP groups showed significantly different 5-year progression-free survival (76% vs 31% for GCB and activated DLBCL) and overall survival (80% vs 45%), none of the immunostaining algorithms was able to retain the prognostic impact of the groups (GCB vs non-GCB). In conclusion, stratification based on immunostaining algorithms should be used with caution in guiding therapy, even in clinical trials. (*Blood*. 2011;117(18):4836-4843)



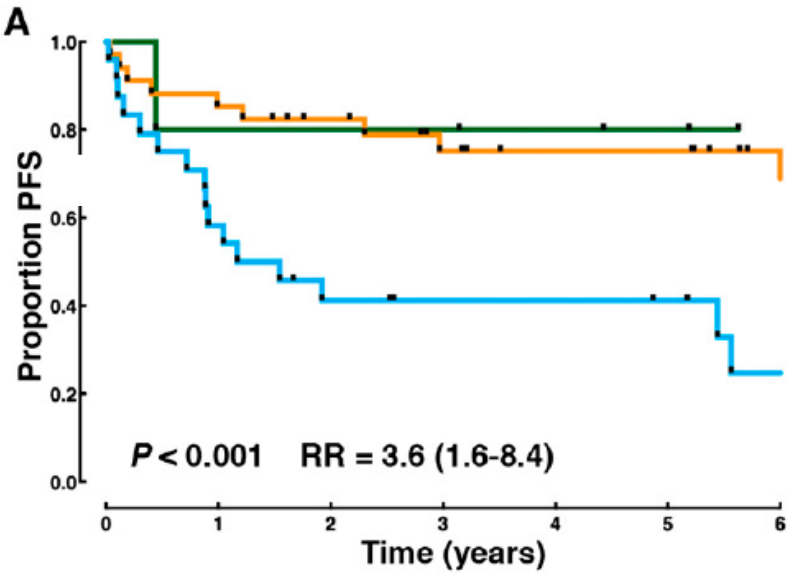


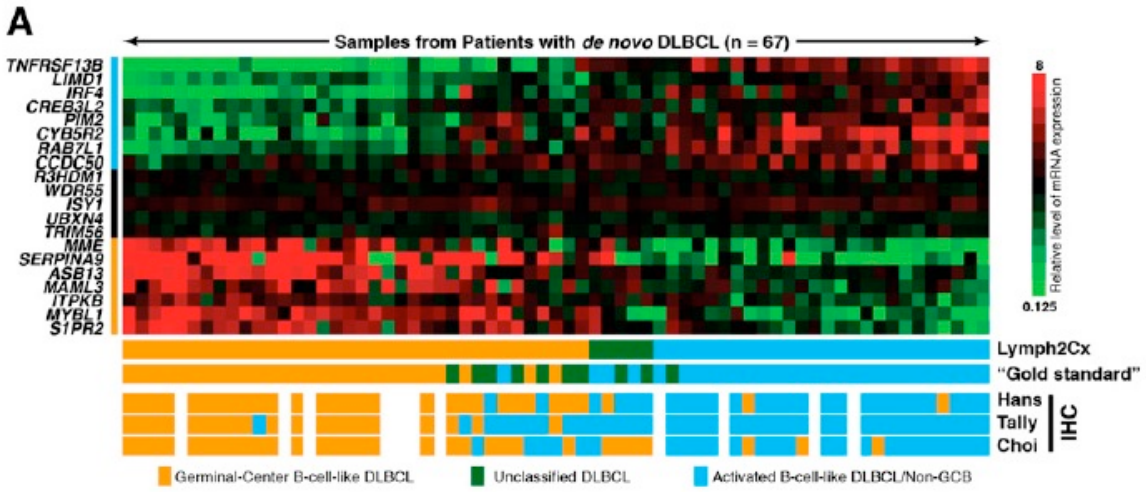




Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue

- A 20-gene gene expression-based assay accurately and robustly assigns COO subtypes of DLBCL using formalin-fixed paraffin-embedded tissue.

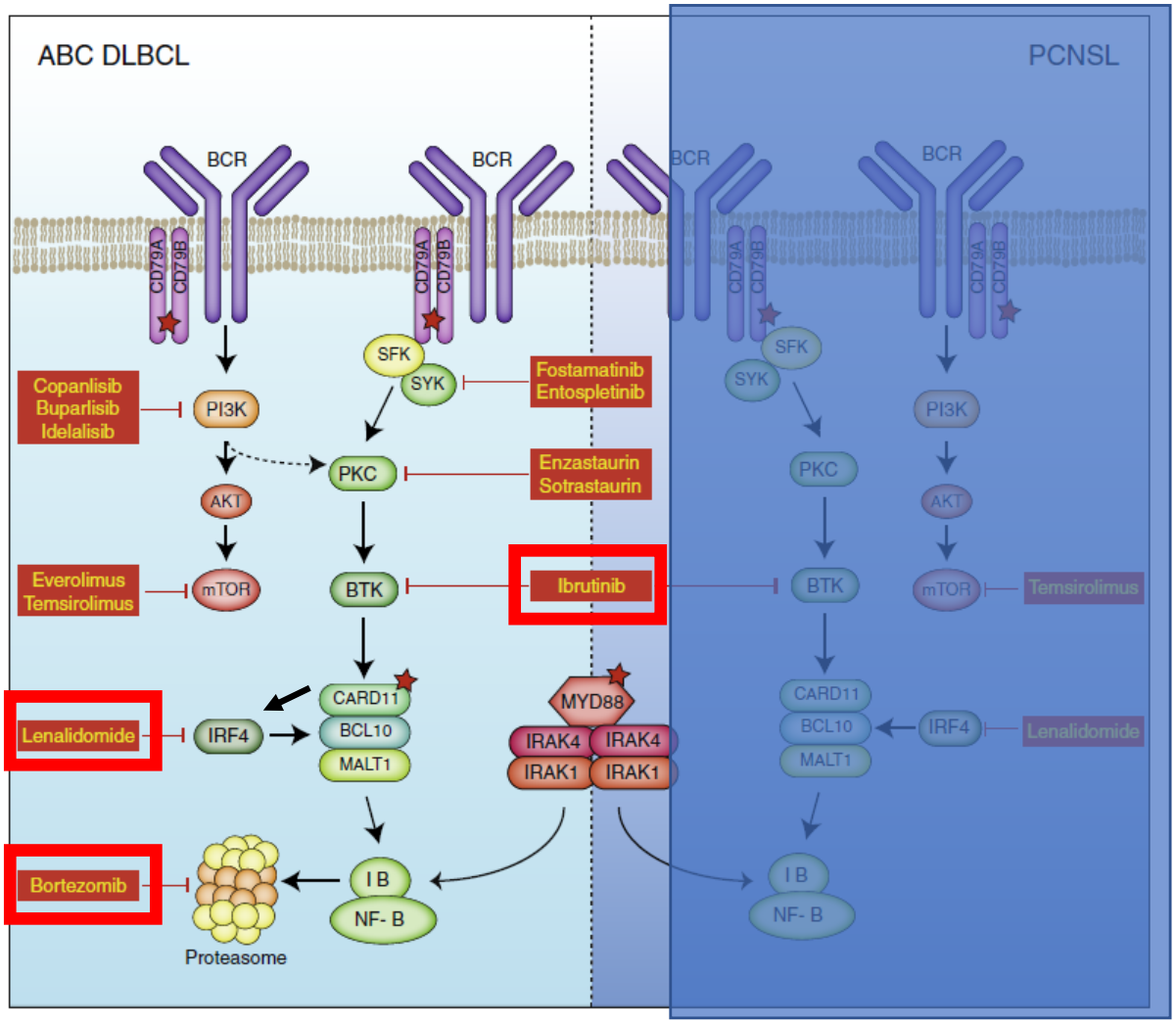




NanoString
FFPE Tissue
20 genes

Scott DW et al. Blood 2014

THERAPEUTIC TARGETS AND VULNERABILITIES OF ABC-DERIVED DLBCL



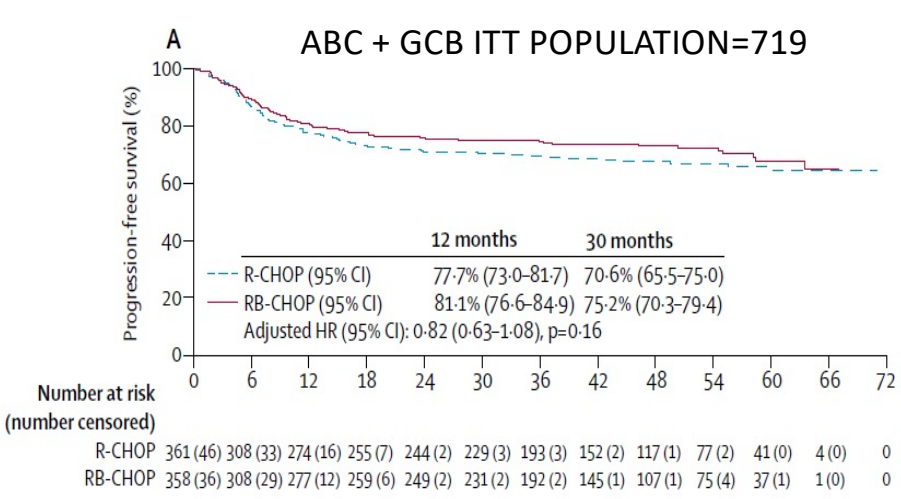
Dunleavy et al. Cancer Treat Rev 2018

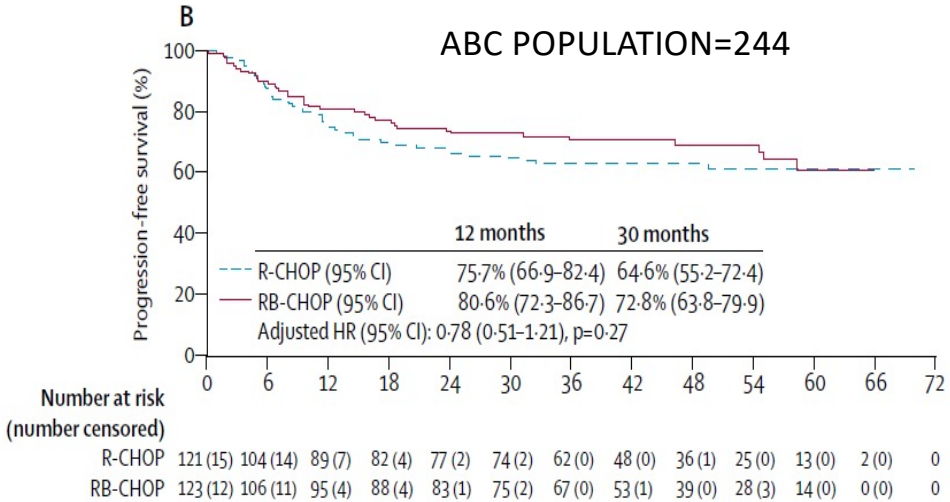
REMoDL-B

Gene-expression profiling of bortezomib added to standard chemoimmunotherapy for diffuse large B-cell lymphoma (REMoDL-B): an open-label, randomised, phase 3 trial

Andrew Davies, Thomas E Cummin, Sharon Barrans, Tom Maishman, Christoph Mamot, Urban Novak, Josh Caddy, Louise Stanton, Shamim Kazmi-Stokes, Andrew McMillan, Paul Fields, Christopher Pocock, Graham P Collins, Richard Stephens, Francesco Cucco, Alexandra Clipson, Chulin Sha, Reuben Tooze, Matthew A Care, Gareth Griffiths, Ming-Qing Du, David R Westhead, Catherine Burton, Peter W M Johnson

Interpretation This is the first large-scale study in diffuse large B-cell lymphoma to use real-time molecular characterisation for prospective stratification, randomisation, and subsequent analysis of biologically distinct subgroups of patients. The addition of bortezomib did not improve progression-free survival.

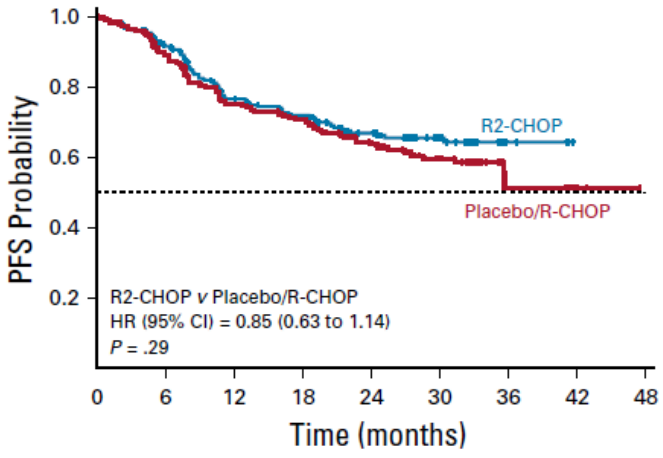




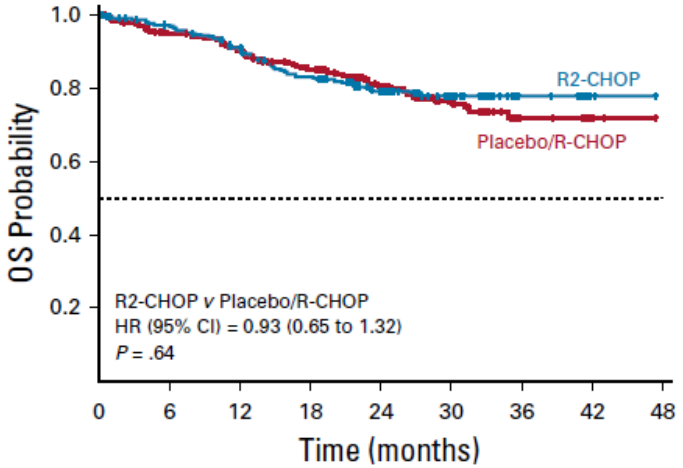
Davies et al. Lancet Oncol 2019

ROBUST

ROBUST: A Phase III Study of Lenalidomide Plu R-CHOP Versus Placebo Plus R-CHOP in Previously Untreated Patients With ABC-Type Diffuse Large B-Cell Lymphoma



No. at risk:									
R2-CHOP	285	221	178	162	119	57	10	0	
Placebo/R-CHOP	285	229	187	173	111	55	10	3	0

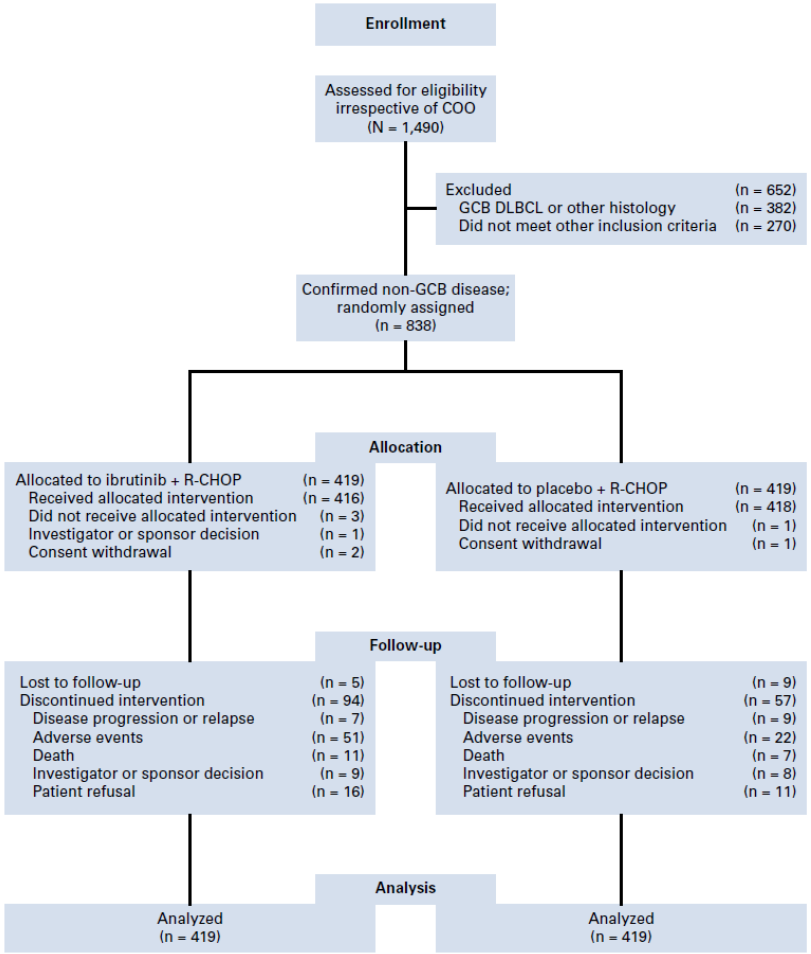


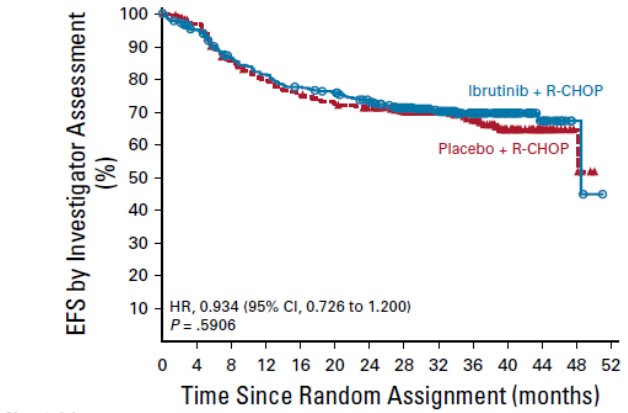
No. at risk:									
R2-CHOP	285	269	248	224	165	83	18	2	0
Placebo/R-CHOP	285	260	245	226	162	77	15	3	0

Nowakowsky et al. J Clin Oncol 2021

PHOENIX

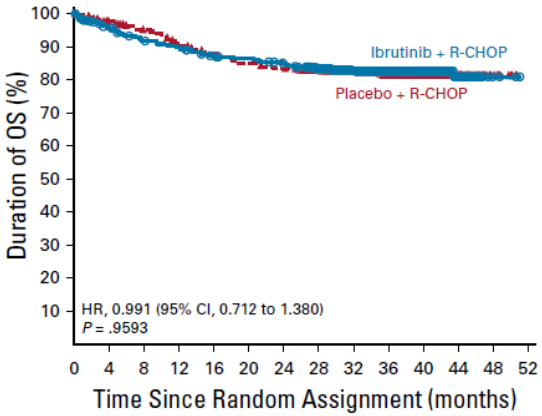
Randomized Phase III Trial of Ibrutinib and Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Non–Germinal Center B-Cell Diffuse Large B-Cell Lymphoma





No. at risk:

Ibrutinib + R-CHOP	419	374	336	316	300	291	276	233	179	120	63	25	3	0
Placebo + R-CHOP	419	390	341	316	297	286	277	244	184	118	60	33	5	0



No. at risk:

Ibrutinib + R-CHOP	419	384	365	356	342	337	328	309	236	159	100	38	4	0
Placebo + R-CHOP	419	400	382	363	347	335	329	301	237	157	99	51	12	0

Younes et al. J Clin Oncol 2019

WRONG THERAPY OR WRONG SIGNATURE?

Targeted Digital Gene Expression Profiling

RefSeq NCBI	Gene	Length NCBI	Protein aa	
NM_002467.4	MYC	2379	454	
NM_000633.2	BCL2	6492	239	
NM_012452.2	TNFRSF13B	1377	293	
NM_014240.2	LIMD1	6284	676	
NM_001195286.1	IRF4	5329	451	*
NM_194071.3	CREB3L2	7471	520	*
NM_006875.3	PIM2	2234	311	
NM_001302826.1	CYB5R	1713	276	
NM_003929.2	RAB7L1	3324	203	
NM_174908.3	CCDC50	8421	306	
NM_015361.3	R3HDM1	4722	1099	
NM_017706.4	WDR55	2580	383	
NM_020701.3	ISY1	3778	285	
NM_014607.3	UBXN4	4018	508	
NM_030961.2	TRIM56	4723	755	
NM_000902.3	MME	5643	750	
NM_001284275.1	SERPINA9	1661	435	*
NM_024701.3	ASB13	2736	278	
NM_018717.4	MAML3	7086	1138	
NM_002221.3	ITPKB	6162	946	
NM_001080416.3	MYBL1	5192	752	
NM_004230.3	S1PR2	3589	353	
NM_020529.2	NFKBIA	1579	371	
NM_139276.2	STAT3	4978	770	
NM_000314.6	PTEN	8718	403	*
NM_006218.2	PKI3CA	3724	1068	

26-gene-panel for COO & key-genes Haematologica, 2020

50-gene-panel for microenvironment Ann Oncol, 2018

MF-related genes

DC-related genes

CD4⁺ T cell-related genes

STROMAL GENES

ACTA2	Actin, alpha 2, smooth muscle
AEBP1	AE binding protein 1
BGN	Biglycan
COL1A1	Collagen type I alpha 1
COL1A2	Collagen type I alpha 2
COL3A1	Collagen type III alpha 1
COL4A1	Collagen type IV alpha 1
COL5A2	Collagen type V alpha 2
COL6A3	Collagen type VI alpha 3
CTHRC1	Collagen triple helix repeat containing 1
CTSK	Cathepsin K
EGR1	Early growth response 1
FN1	Fibronectin 1
FSTL1	Follistatin like 1
GPNMB	Glycoprotein nmb
LAMB1	Laminin subunit beta 1
LUM	Lumican
MFAP2	Microfibrillar associated protein 2
MMP2	Matrix metallopeptidase 2
MRC2	Mannose receptor, C type 2
MXRA5	Matrix-Remodelling Associated 5
PCOLCE	Procollagen C-endopeptidase enhancer
PLOD2	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2
POSTN	Periostin, osteoblast specific factor
SPARC	Secreted protein acidic and cysteine rich
SULF1	Sulfatase 1
TGFBI	Transforming growth factor beta induced

IMMUNE GENES

ALCAM	Activated leukocyte cell adhesion molecule
AMICA1	Adhesion molecule, interacts with CXADR antigen 1
CD300LF	CD300 molecule-like family member F
COL4A2	Collagen, type IV, alpha 2
IGSF6	Immunoglobulin superfamily, member 6
MDFIC	MyoD Family Inhibitor Domain Containing
P2RY14	Purinergic receptor P2Y, G-protein coupled, 14
SLC29A3	Solute carrier family 29 (nucleoside transporters), member 3;
SLC2A3	Solute carrier family 2 (facilitated glucose transporter),
CTS2	Cathepsin 2
HS3ST3A1	Heparan Sulfate-Glucosamine 3-Sulfotransferase 3A1
PMPCB	Peptidase, Mitochondrial Processing Beta Subunit
RAB27A	RAB27A, Member RAS Oncogene Family
SMAD1	SMAD Family Member 1

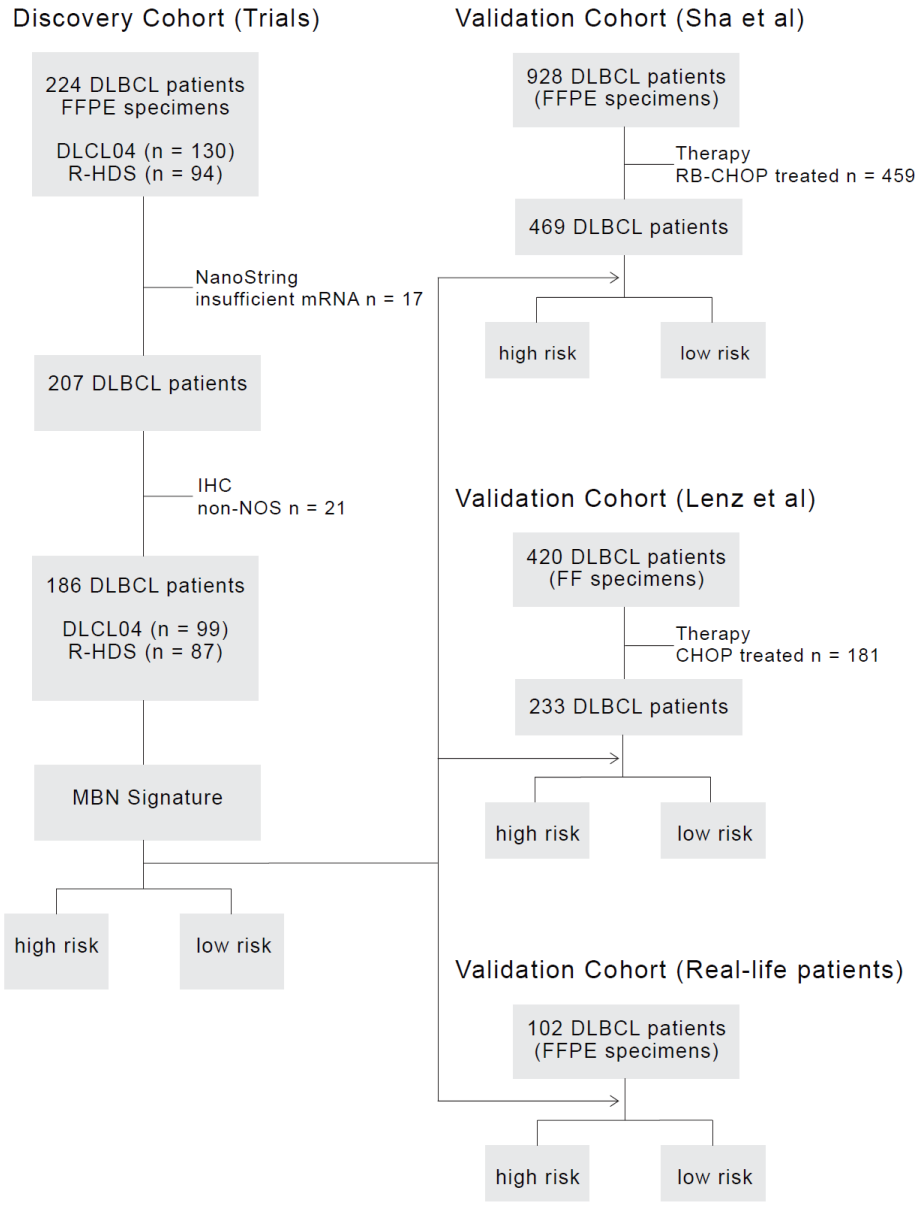
METHODS

A three-gene signature based on *MYC, BCL-2* and *NFKBIA* improves risk stratification in diffuse large B-cell lymphoma

Enrico Derenzini,[1,2] Saveria Mazzara,[3] Federica Melle,[3] Giovanna Motta,[3] Marco Fabbri,[3] Riccardo Bruna,[1] Claudio Agostinelli,[4] Alessandra Cesano,[5] Chiara Antonia Corsini,[6] Ning Chen,[5] Simona Righi,[4] Elena Sabattini,[4] Annalisa Chiappella,[7] Angelica Calleri,[3] Stefano Fiori,[3] Valentina Tabanelli,[3] Antonello Cabras,[8] Giancarlo Pruneri,[8] Umberto Vitolo,[9] Alessandro Massimo Gianni,[1] Alessandro Rambaldi,[10] Paolo Corradini,[7] Pier Luigi Zinzani,[11] Corrado Tarella[1,2] and Stefano Pileri[3]

4 independent cohorts

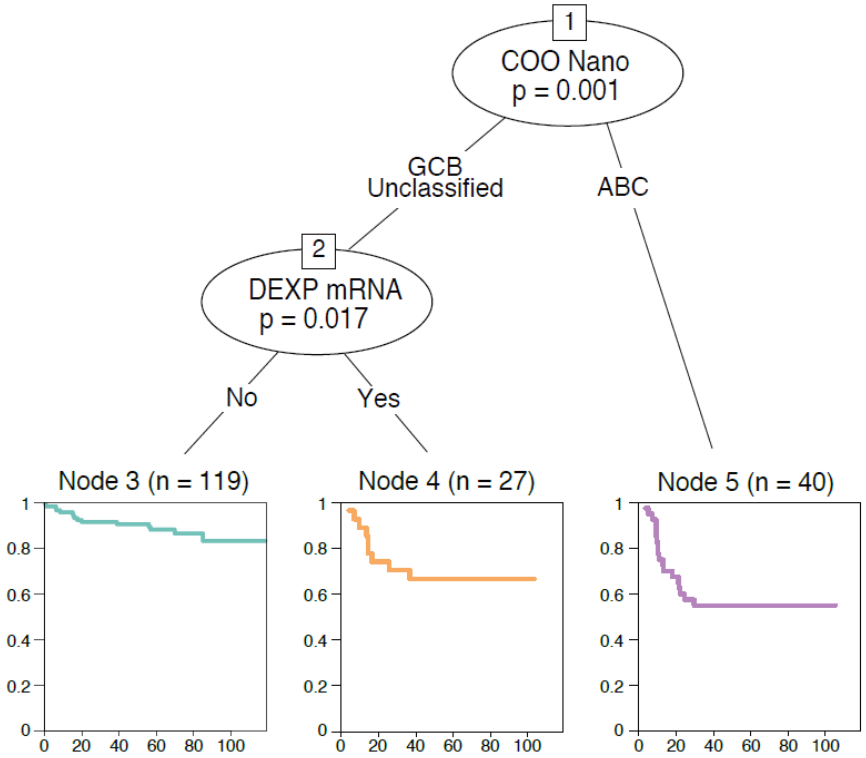
1449 patients

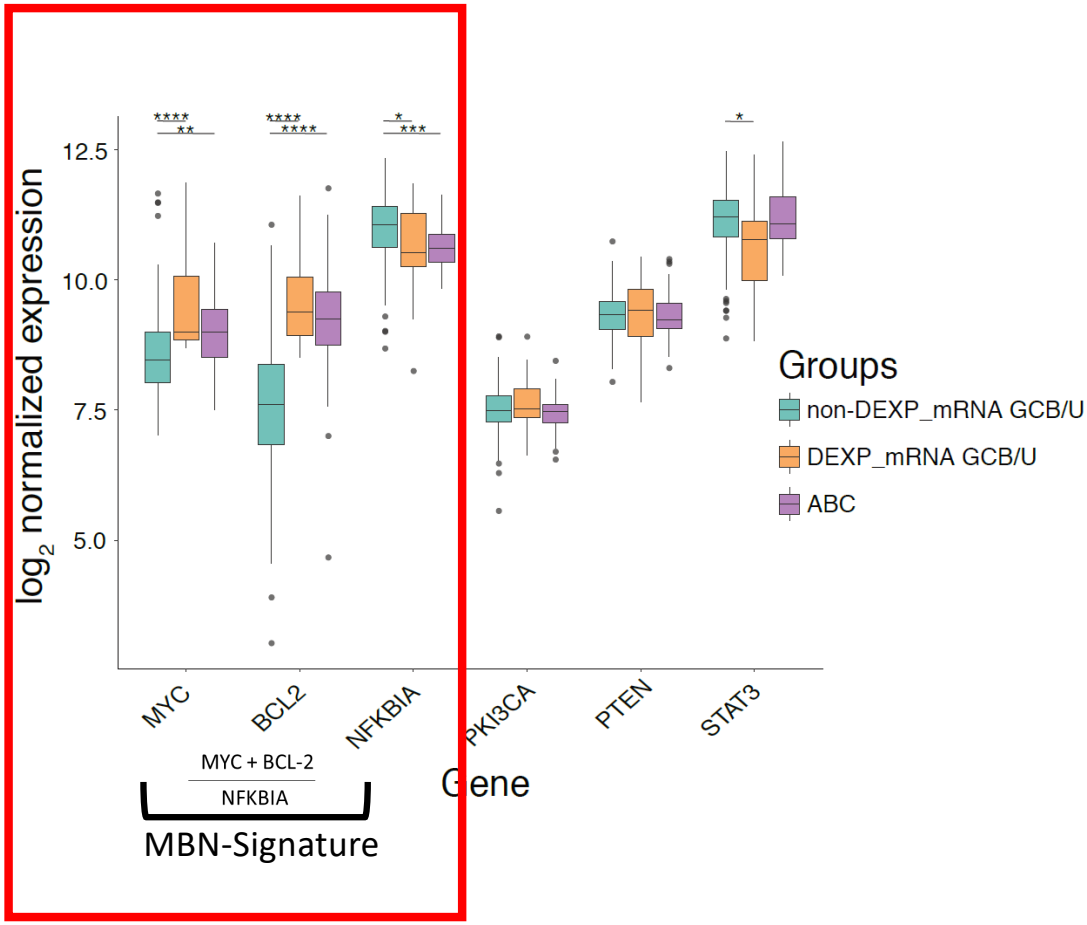


Derenzini et al. Haematologica 2020

RESULTS

Patient subgroups identified by recursive partitioning analysis integrating the COO with *MYC/BCL-2* status

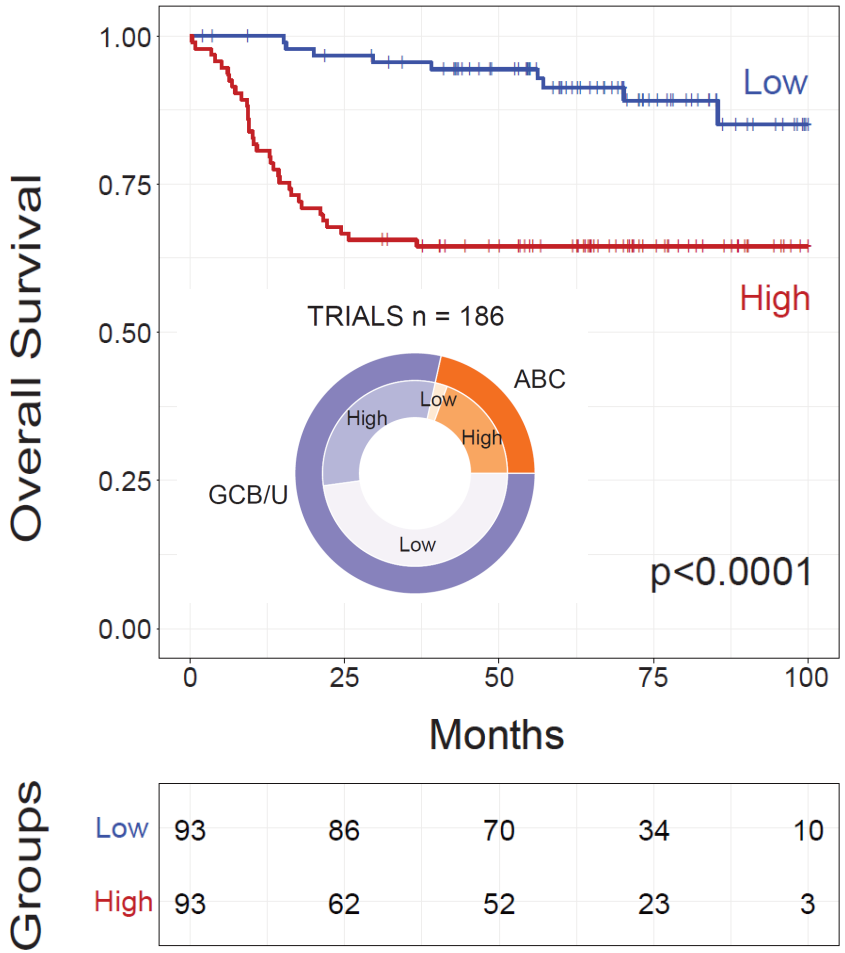


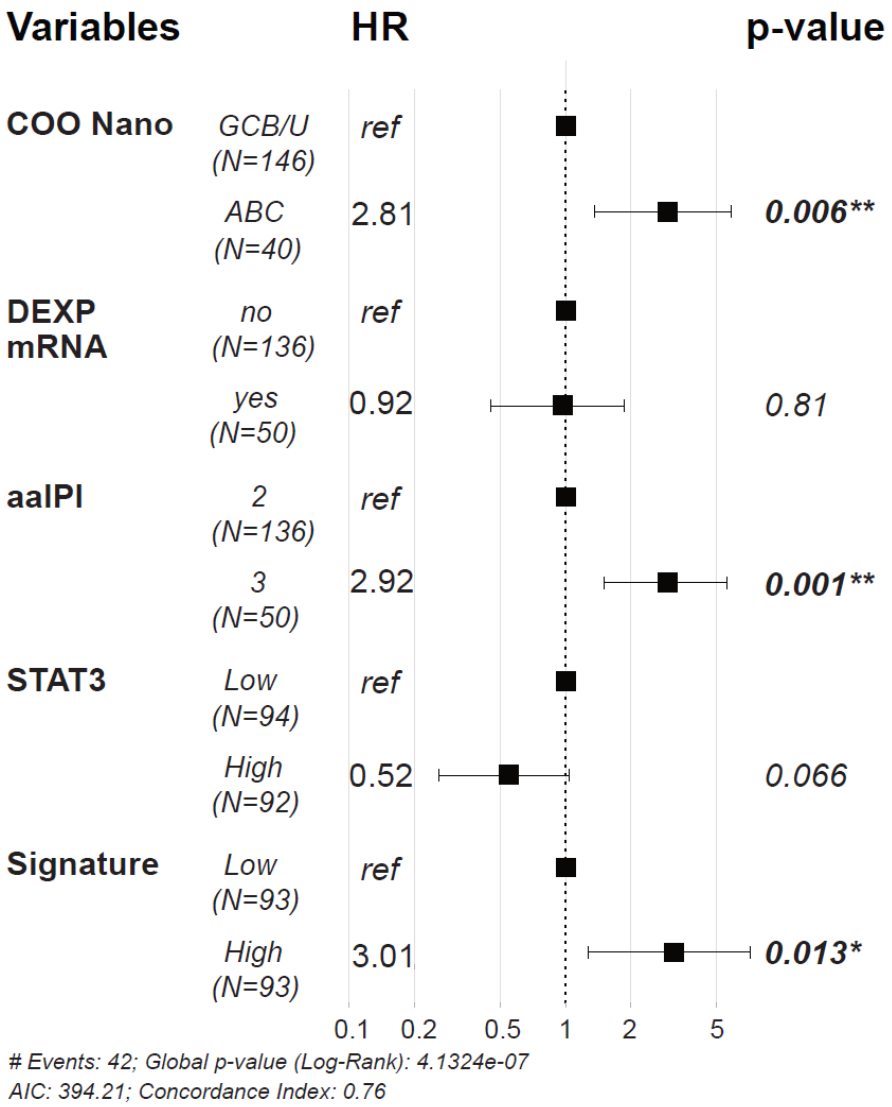


Derenzini et al. Haematologica 2020

RESULTS

MBN-Signature

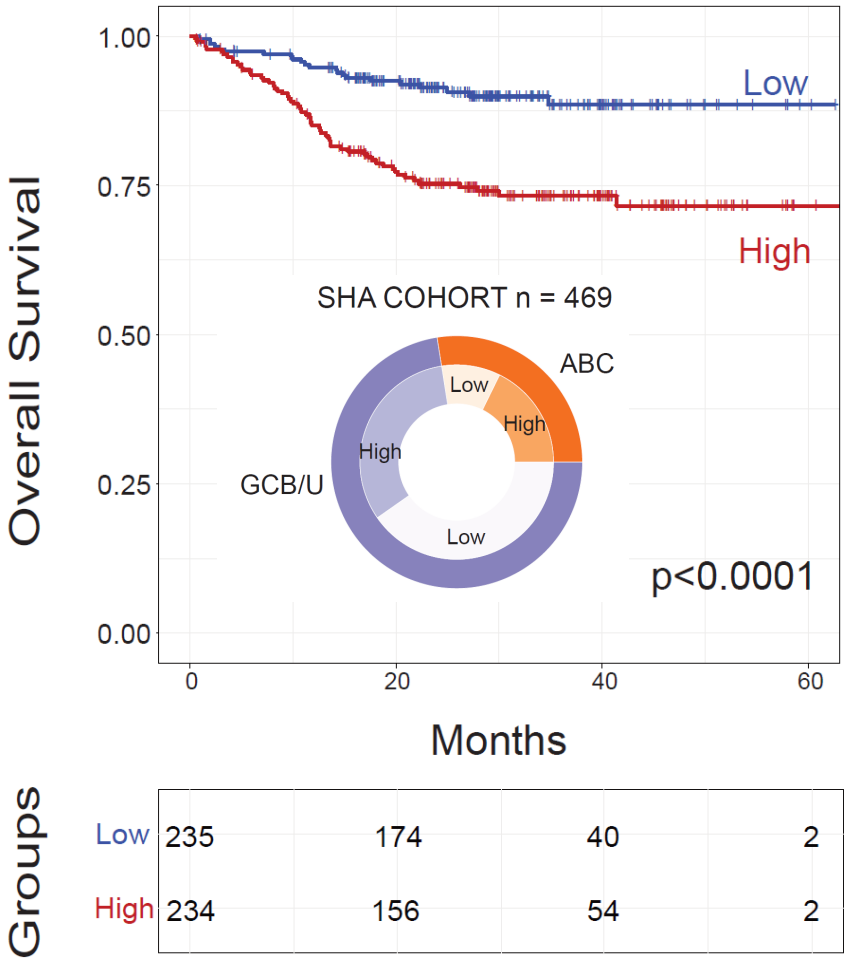


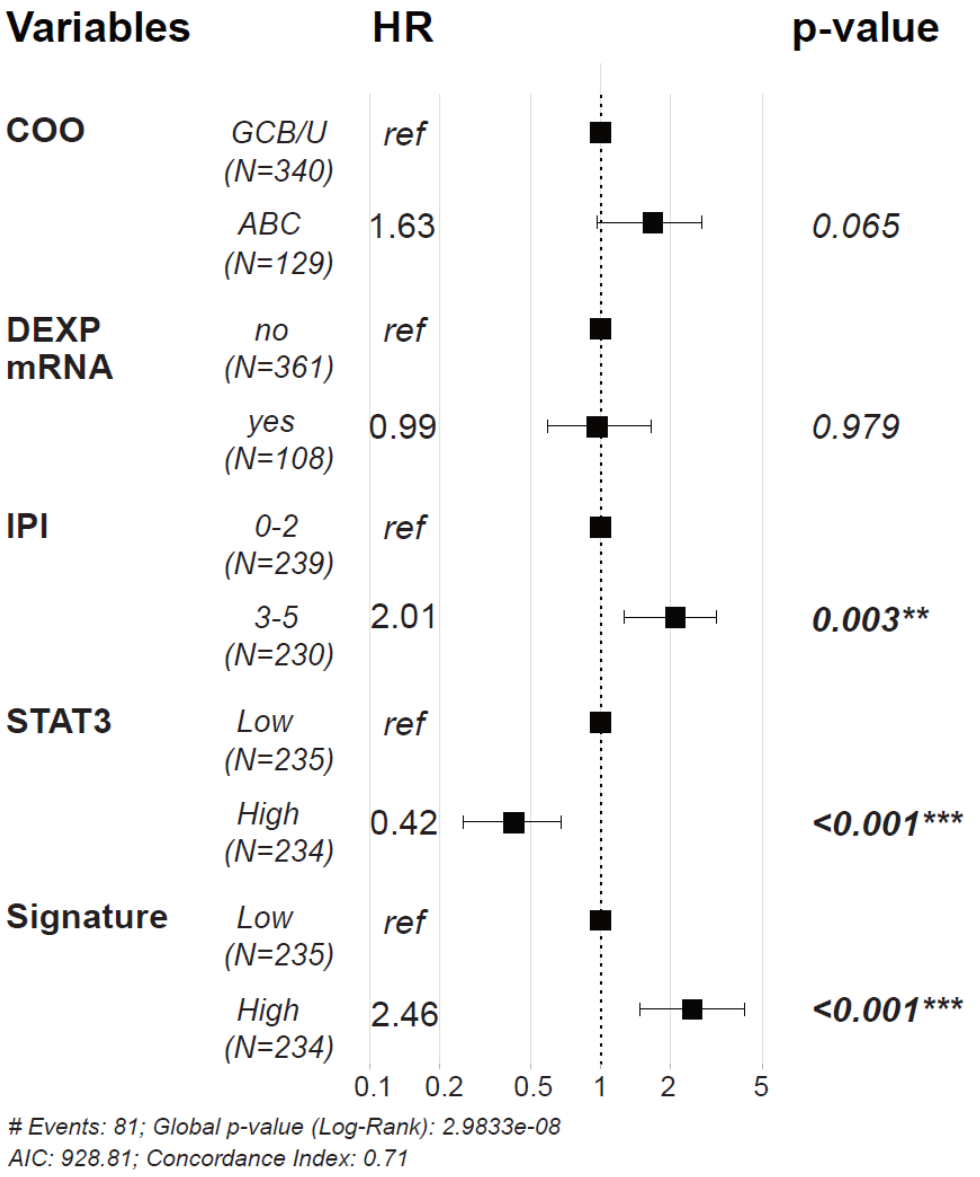


Derenzini et al. Haematologica 2020

MBN-Signature validation

RESULTS





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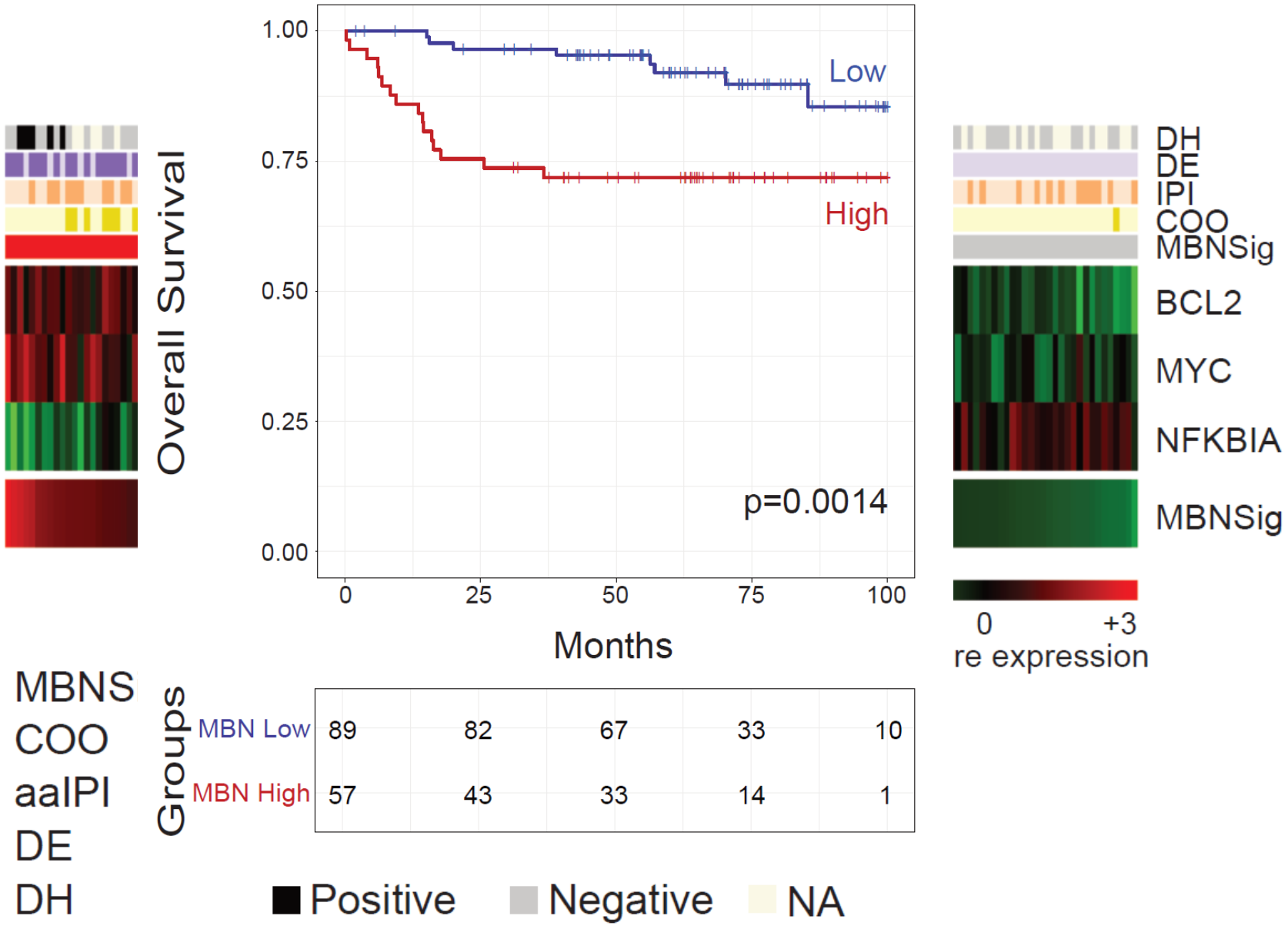
MBN-Signature in the GCB-subset

RESULTS

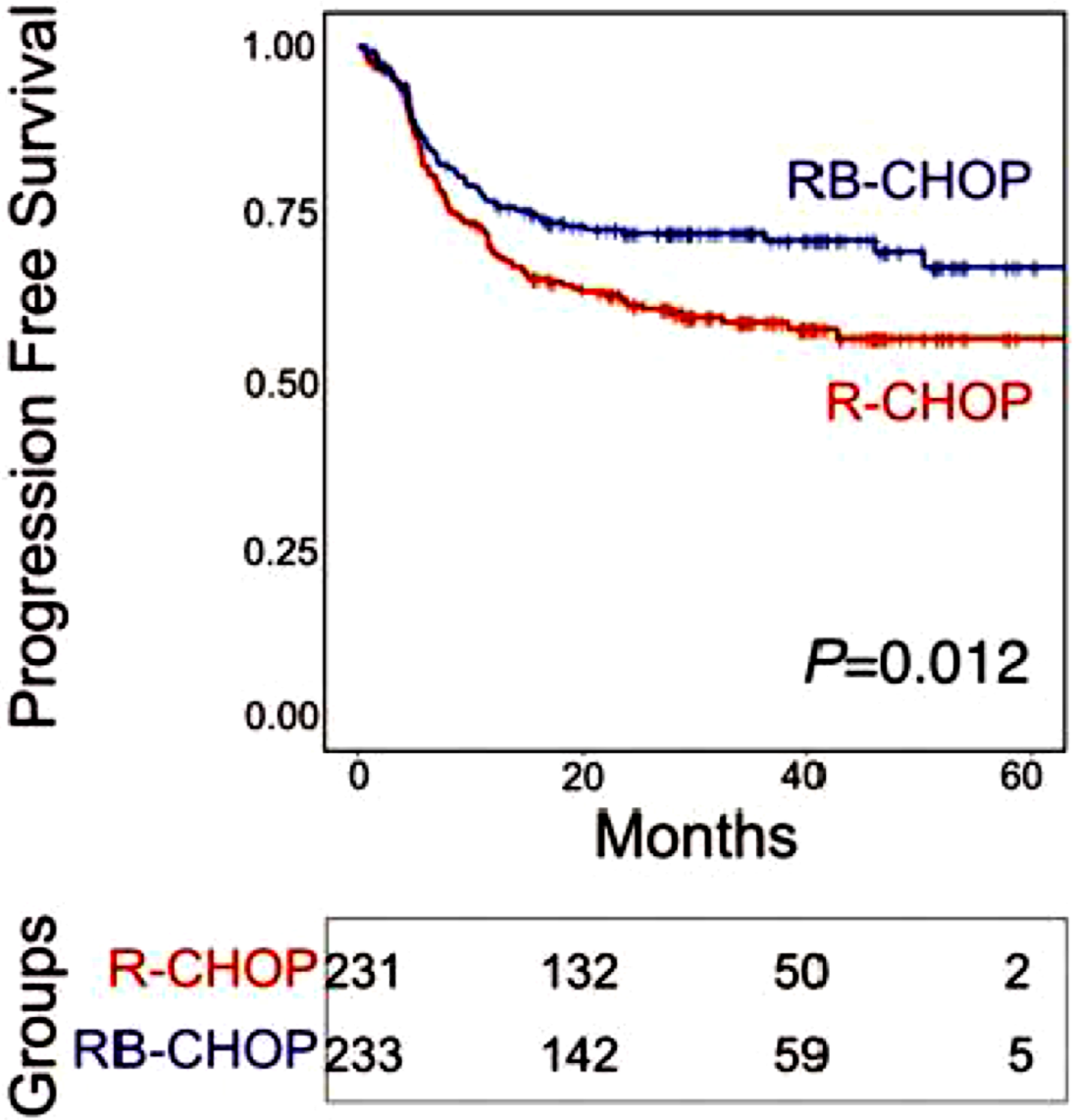


Derenzini et al. Haematologica 2020

RESULTS

PROMISING EFFICACY OF R-CHOP + BORTEZOMIB IN THE MBN-Sig HIGH SUSBET

Patients from Sha's series with high MBN



Derenzini et al. Haematologica 2020

Conclusions

- The MBN signature does implement the cell of origin (COO) determination.
- A high risk group (enriched in MYC/BCL-2 genetic aberrations) can be identified among GCB/U.
- Potential therapeutic implications.
- Applicable to all patients at low cost!!!

The NEW ENGLAND JOURNAL of MEDICINE

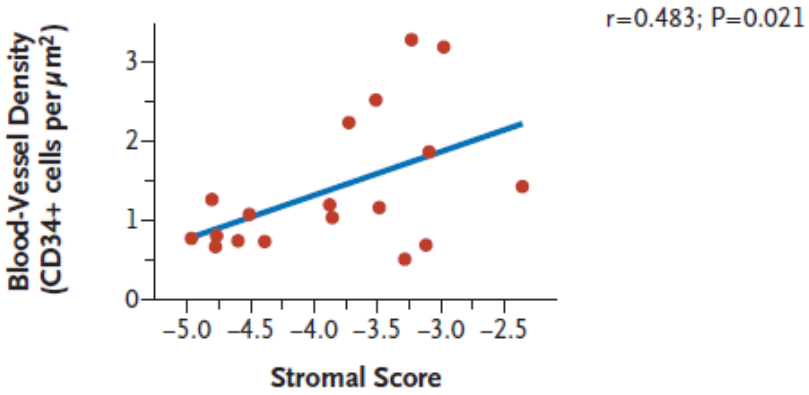
ESTABLISHED IN 1812 NOVEMBER 27, 2008 VOL. 359 NO. 22

Stromal Gene Signatures in Large-B-Cell Lymphomas

Affimetrix
Fresh Frozen Tissue
Hundreds genes

Lenz et al. N Eng J Med 2008

A multivariate model created from three gene-expression signatures — termed "germinal-center B-cell," "stromal-1," and "stromal-2" — predicted survival both in patients who received CHOP and patients who received R-CHOP. The prognostically favorable stromal-1 signature reflected extracellular-matrix deposition and histiocytic infiltration. By contrast, the prognostically unfavorable stromal-2 signature reflected tumor blood-vessel density.

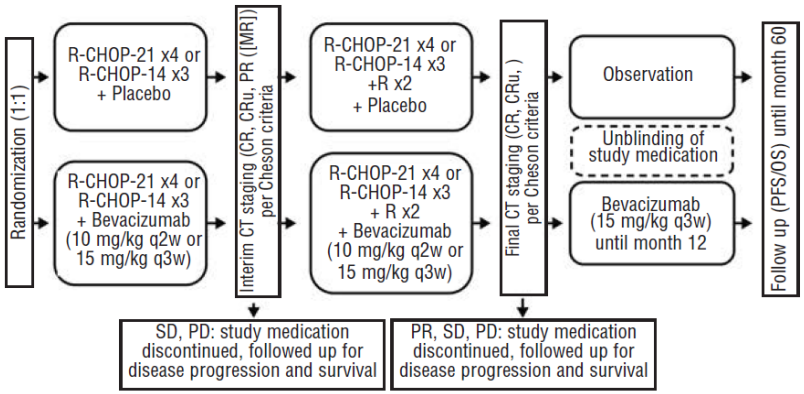


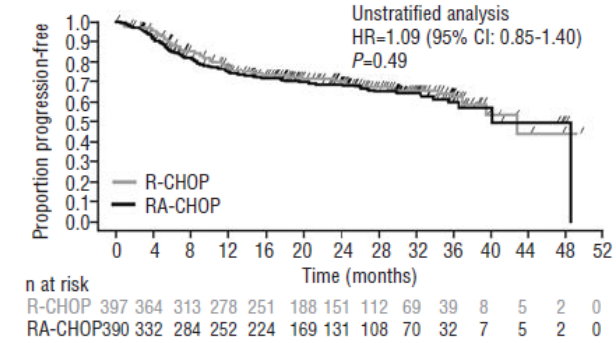
RATIONALE FOR TARGETING ANGIOGENESIS IN DLBCL

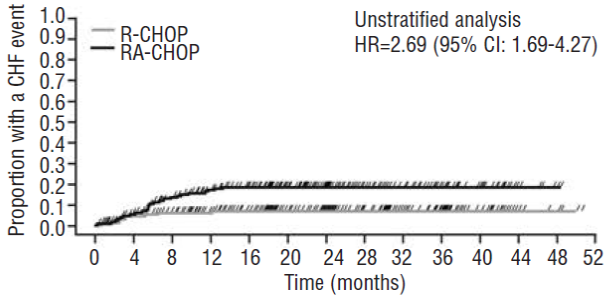
Lenz et al. N Eng J Med 2008

R-CHOP with or without bevacizumab in patients with previously untreated diffuse large B-cell lymphoma: final MAIN study outcomes

John F. Seymour,[1,2] Michael Pfreundschuh,[3] Marek Trněný,[4] Laurie H. Sehn,[5] John Catalano,[6] Eva Csinady,[7] Nicola Moore,[8] and Bertrand Coiffier;[9] on behalf of the MAIN Study Investigators





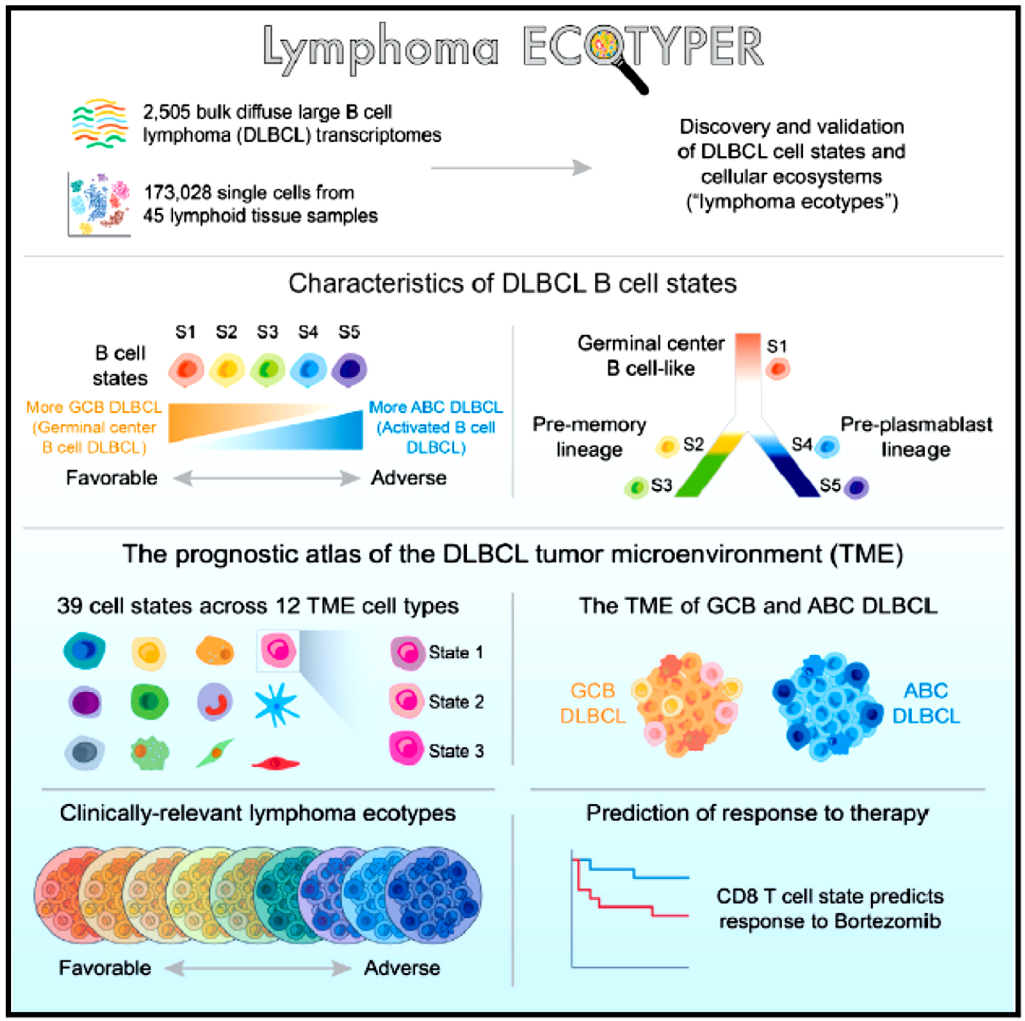


Seymour et al. Haematologica 2014

Cancer Cell

The landscape of tumor cell states and ecosystems in diffuse large B cell lymphoma

Graphical abstract



Authors

Chloé B. Steen, Bogdan A. Luca, Mohammad S. Esfahani, ..., Andrew J. Gentles, Aaron M. Newman, Ash A. Alizadeh

Correspondence

amnewman@stanford.edu (A.M.N.), arasha@stanford.edu (A.A.A.)

In brief

Steen et al. implement EcoTyper, a machine-learning approach for dissecting cellular heterogeneity in the most common blood cancer, diffuse large B cell lymphoma (DLBCL). Forty-four cell states spanning malignant cells and the microenvironment are defined, uncovering a rich landscape of cellular ecosystems that extend beyond traditional DLBCL classifications, revealing new opportunities for therapy selection.

ACCEPTED MANUSCRIPT

Dissection of DLBCL Microenvironment Provides a Gene Expression-Based Predictor of Survival Applicable to Formalin-Fixed Paraffin-Embedded Tissue

S Ciavarella, M C Vegliante, M Fabbri, S De Summa, F Melle, G Motta, V De Iuliis, G Opinto, A Enjuanes, S Rega, A Gulino, C Agostinelli, A Scattone, S Tommasi, A Mangia, F Mele, G Simone, A F Zito, G Ingravallo, U Vitolo, A Chiappella, C Tarella, A M Gianni, A Rambaldi, P L Zinzani, B Casadei, E Derenzini, G Loseto, A Pileri, V Tabanelli, S Fiori, A Rivas-Delgado, A López-Guillermo, T Venesio, A Sapino, E Campo, C Tripodo, A Guarini, S A Pileri ✉

Annals of Oncology, mdy450, https://doi.org/10.1093/annonc/mdy450

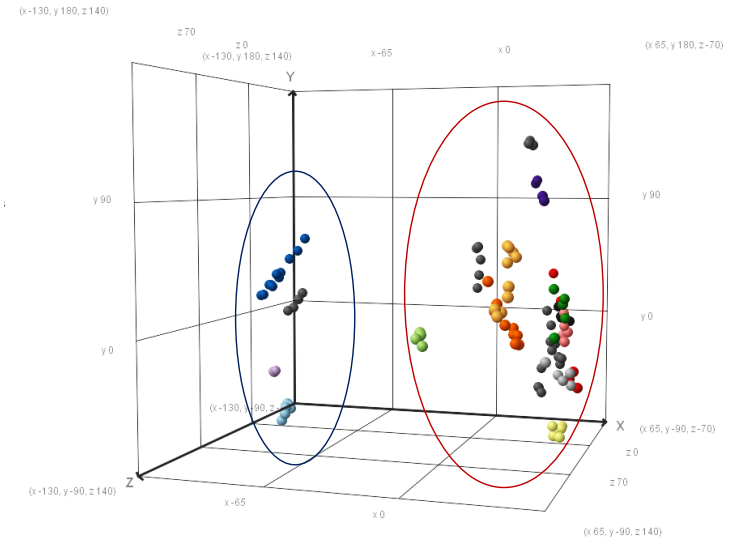
Published: 11 October 2018

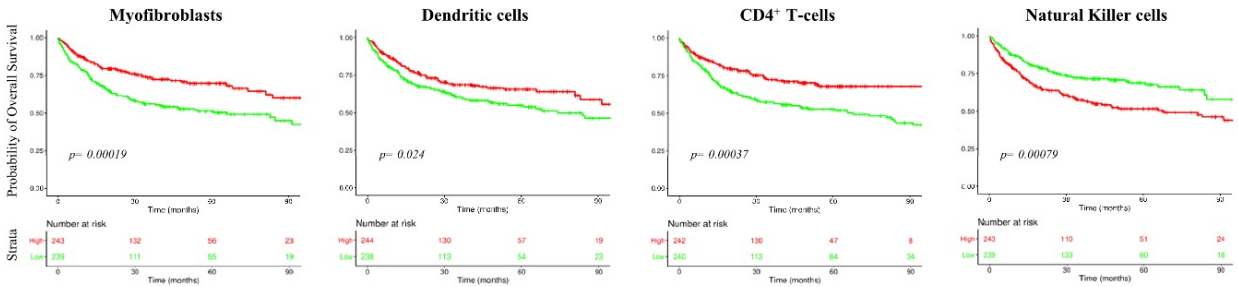
CIBERSORT analysis and selection of prognostic genes

A customized signature including 1,028 genes was generated to distinguish 17 cell types of both stromal and immune origin.

- Adipocites
- CD4-T cells
- CD8-T cells
- Dendritic cells
- Eosinophils
- Lymphatic endothelial cells
- Macrophages M2
- Memory_B_cells
- Monocytes
- Myofibroblasts
- NK_activated
- NK_resting
- Naive_B
- Neutrophils
- Pericytes
- Plasmacells
- Tgamma-delta

PCA





MF-related genes

ACTA2	Actin, alpha 2, smooth muscle
AEBP1	AE binding protein 1
BGN	Biglycan
COL1A1	Collagen type I alpha 1
COL1A2	Collagen type I alpha 2
COL3A1	Collagen type III alpha 1
COL4A1	Collagen type IV alpha 1
COL5A2	Collagen type V alpha 2
COL6A3	Collagen type VI alpha 3
CTHRC1	Collagen triple helix repeat containing 1
CTSK	Cathepsin K
EGR1	Early growth response 1
FN1	Fibronectin 1
FSTL1	Follistatin like 1
GPNMB	Glycoprotein nmb
LAMB1	Laminin subunit beta 1
LUM	Lumican
MFAP2	Microfibrillar associated protein 2
MMP2	Matrix metallopeptidase 2
MRC2	Mannose receptor, C type 2
MXRA5	Matrix-Remodelling Associated 5
PCOLCE	Procollagen C-endopeptidase enhancer
PLOD2	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2
POSTN	Periostin, osteoblast specific factor
SPARC	Secreted protein acidic and cysteine rich
SULF1	Sulfatase 1
TGFBI	Transforming growth factor beta induced

DC-related genes

ALCAM	Activated leukocyte cell adhesion molecule
AMICA1	Adhesion molecule, interacts with CXADR antigen 1
CD300LF	CD300 molecule-like family member F
COL4A2	Collagen, type IV, alpha 2
IGSF6	Immunoglobulin superfamily, member 6
MDFIC	MyoD Family Inhibitor Domain Containing
P2RY14	Purinergic receptor P2Y, G-protein coupled, 14
SLC29A3	Solute carrier family 29 (nucleoside transporters), member 3;
SLC2A3	Solute carrier family 2 (facilitated glucose transporter),

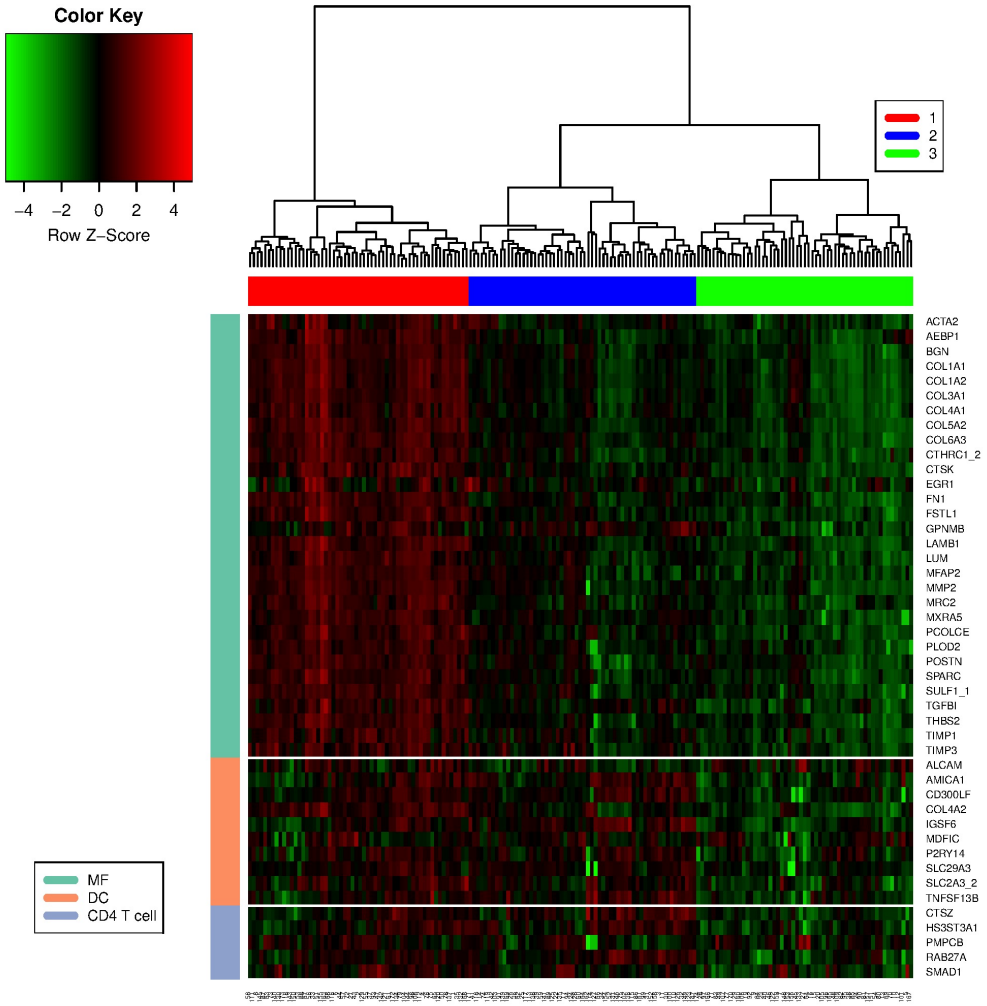
CD4⁺ T cell-related genes

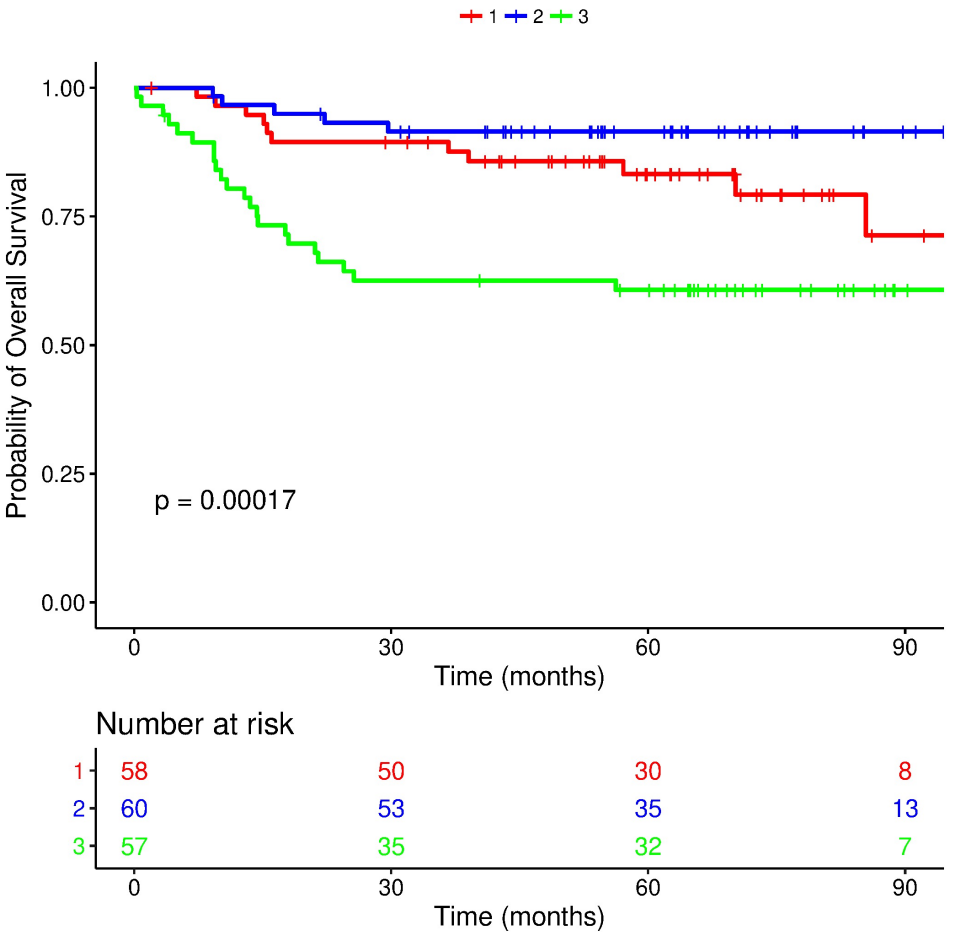
CTS2	Cathepsin Z
HS3ST3A1	Heparan Sulfate-Glucosamine 3-Sulfotransferase 3A1
PMPCB	Peptidase, Mitochondrial Processing Beta Subunit
RAB27A	RAB27A, Member RAS Oncogene Family
SMAD1	SMAD Family Member 1

45 GENE SIGNATURE

Ciavarella et al Ann Oncol 2018

DLCL04 and R-HDS0305

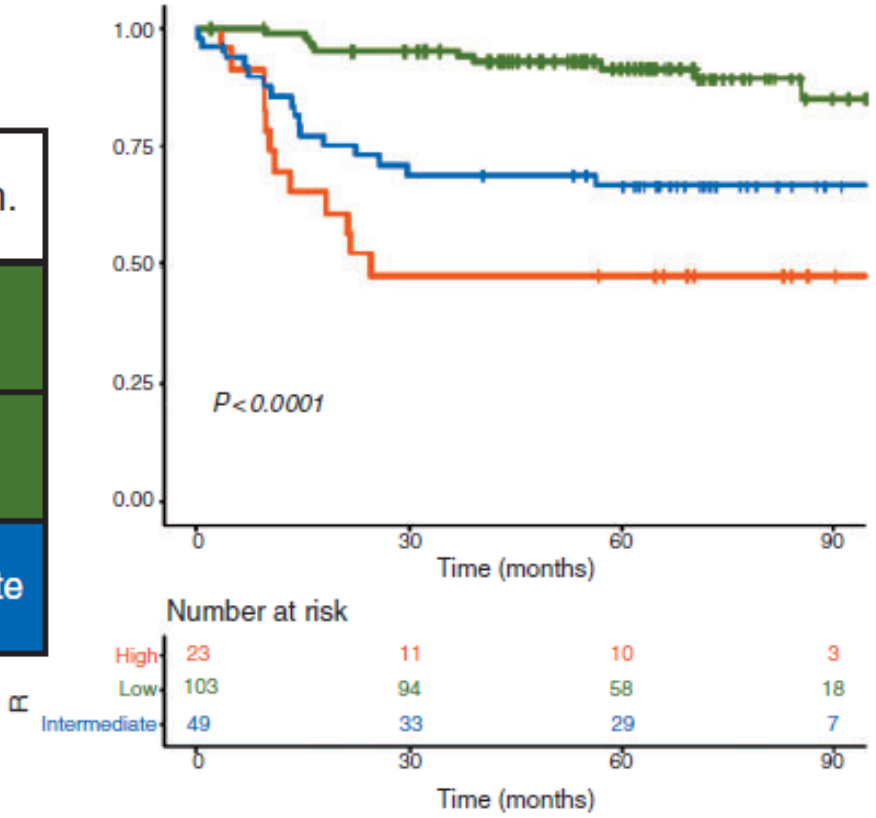




Ciavarella et al Ann Oncol 2018

Risk index

	ABC	GCB	undeterm.
Cluster 1	Intermediate	Low	Low
Cluster 2	Intermediate	Low	Low
Cluster 3	High	Intermediate	Intermediate



Ciavarella et al Ann Oncol 2018

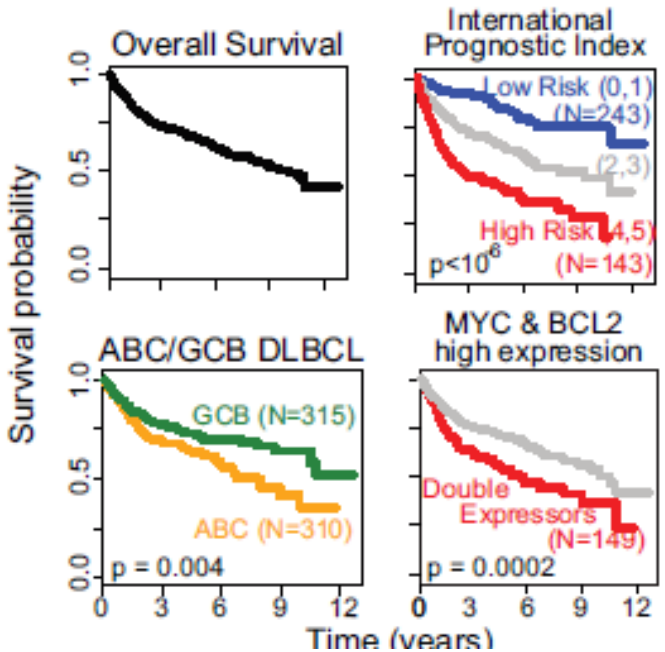
Next generation sequencing

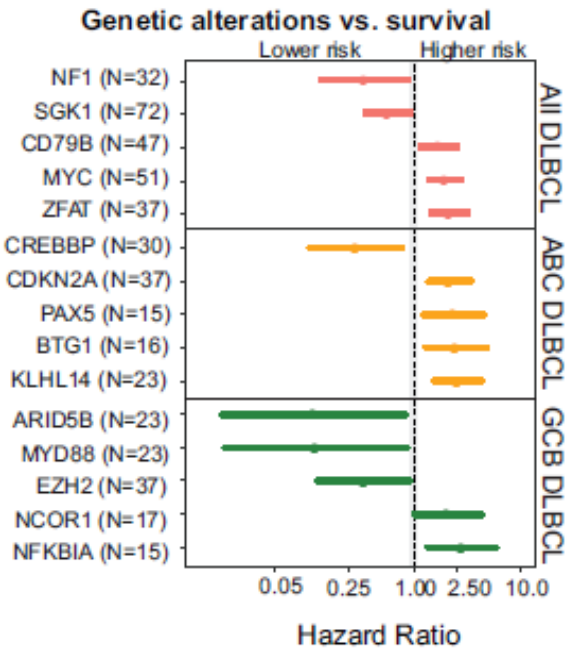
NGS

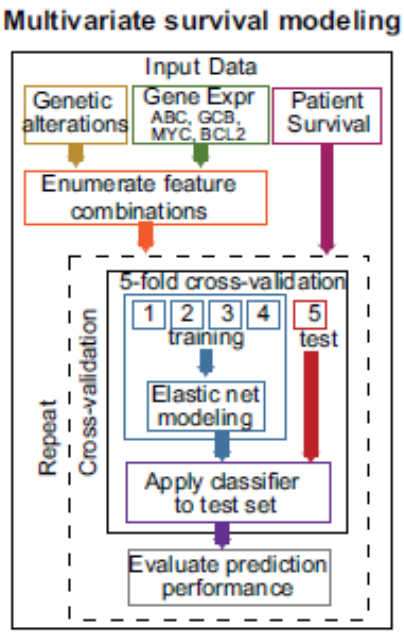
Resource

Cell

Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma

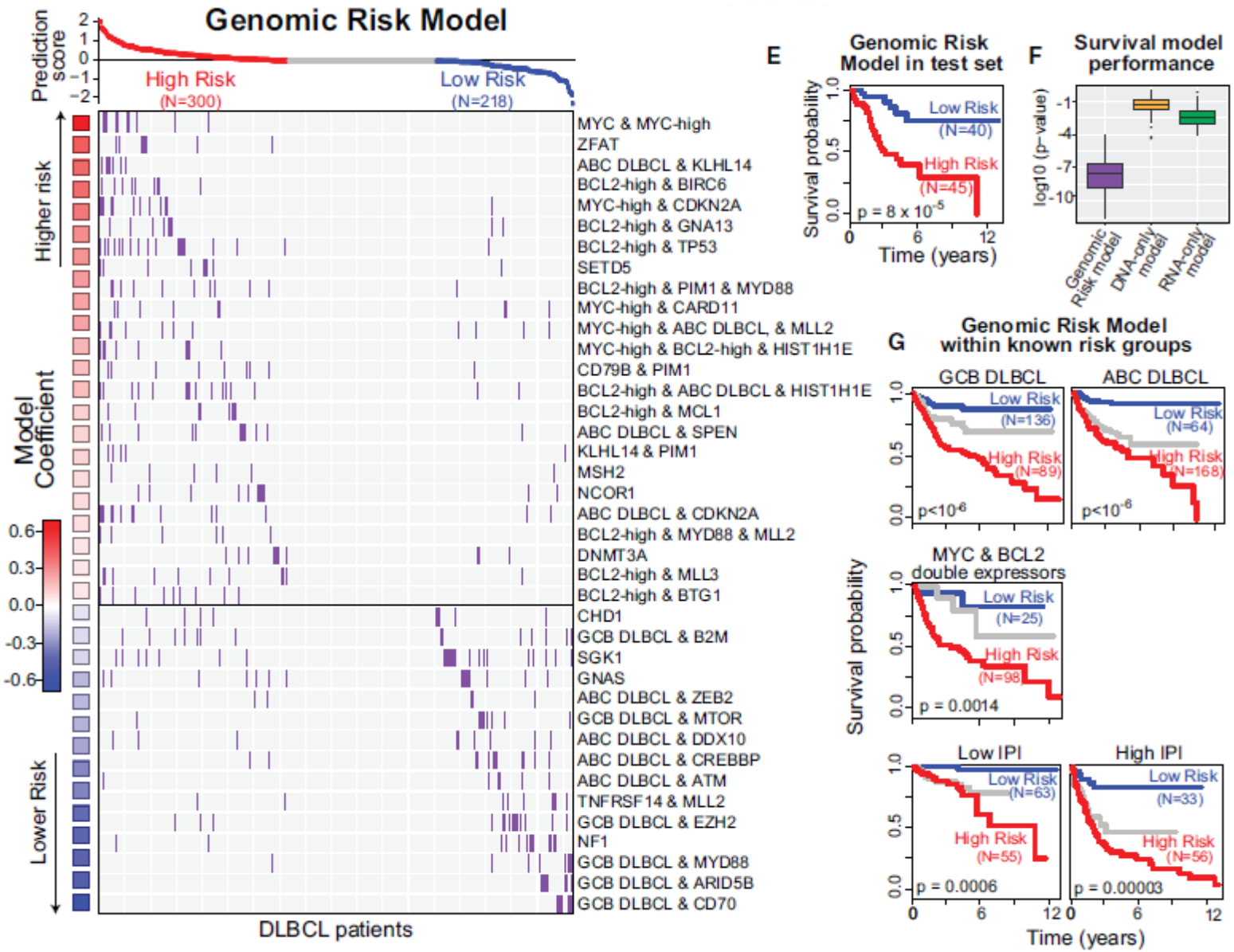






Reddy et al. Cell 2017

NGS



Reddy et al. Cell 2017

NGS

Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes

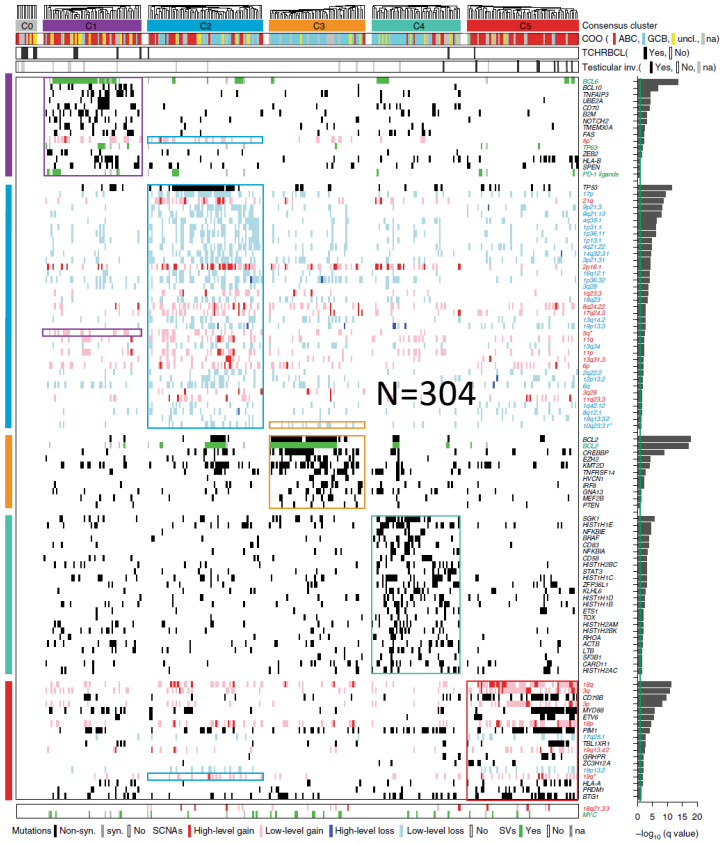
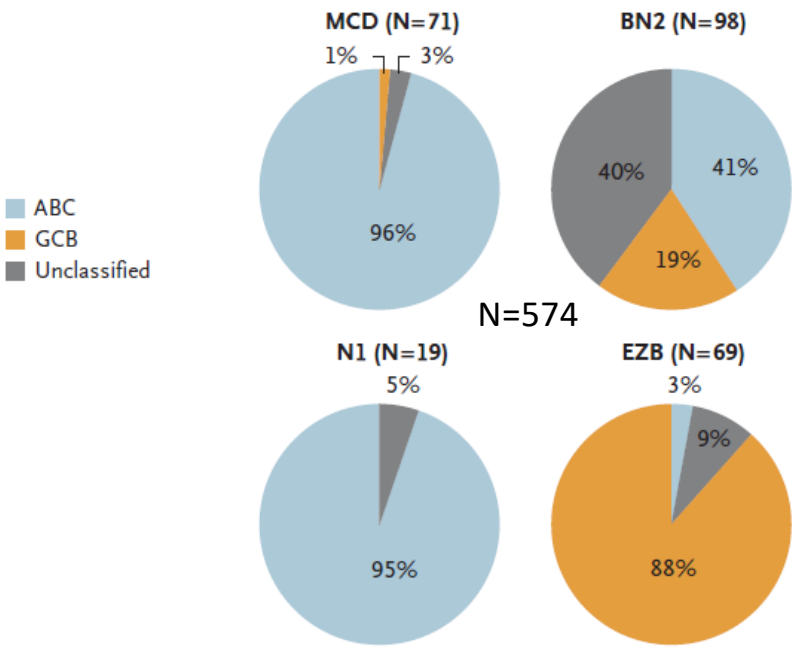


Fig. 5 | Identification of groups of tumors with coordinate genetic signatures. Non-negative matrix factorization consensus clustering was performed using all CCGs, SCNAs, and SVs in the 304 DLBCL samples (columns). Clusters C1–C5 with their associated landmark genetic alterations are visualized (boxed for each cluster). Samples without driver alterations are represented as cluster C0. Genetic alterations that were positively associated with each cluster were identified by a one-sided Fisher test and ranked by significance ($q < 0.1$, green line, bar graph to the right). Non-synonymous mutations, black; synonymous mutations, gray; single CN loss ($1.1 \leq CN \leq 1.6$ copies), cyan; double CN loss ($CN \leq 1.1$), blue; low-level CN gain (3.7 copies $\geq CN \geq 2.2$ copies), pink; high-grade CN gain ($CN \geq 3.7$ copies), red; chromosomal rearrangement, green; no alterations, white; gray crossed, not assessed. Header shows cluster association (C0, gray; C1, purple; C2, blue; C3, orange; C4, turquoise; C5, red), COO classification (ABC, red; GCB, cyan; unclassifiable, yellow; not assessed, gray), TCHRBCL cases (black, yes; white, no), and testicular involvement (black, yes; white, no; gray, na). Outcome-associated alterations that are not part of a specific cluster, SVs of *MYC*, and *18q21.33* copy gain are shown below.

Chapuy et al. Nature Med 2018

Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma



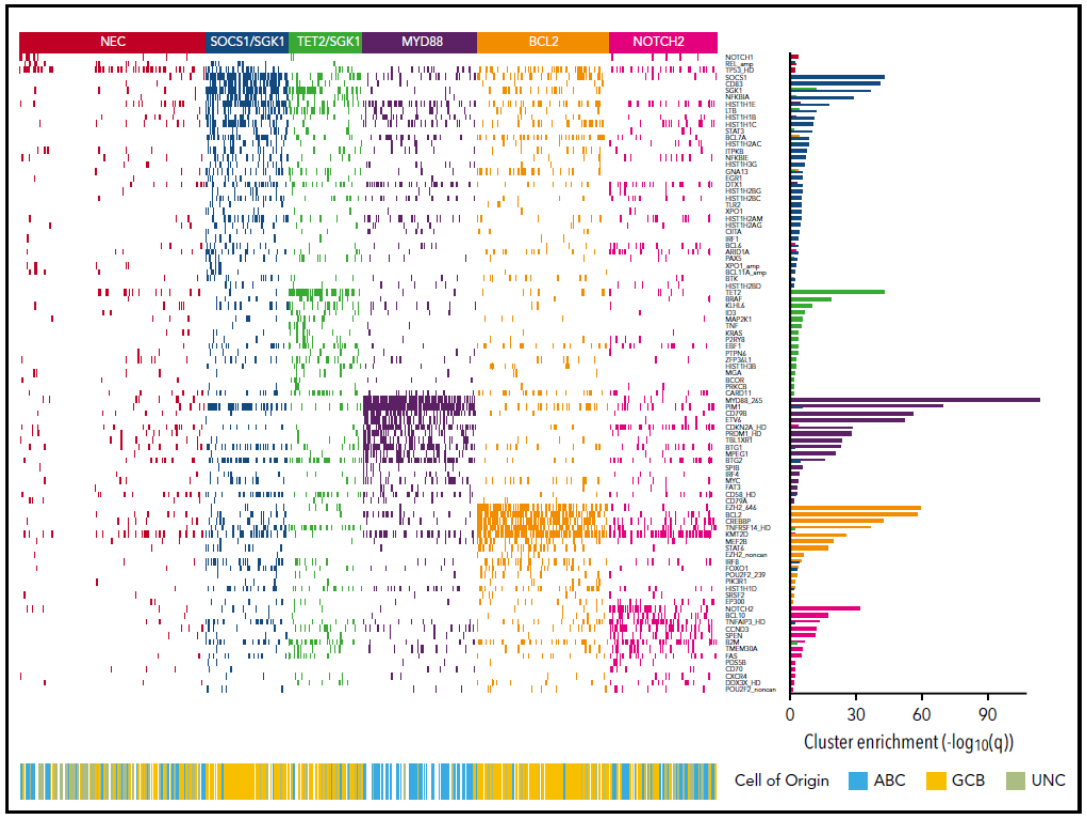
Schmitz et al. N Eng J Med 2018

NGS

LYMPHOID NEOPLASIA

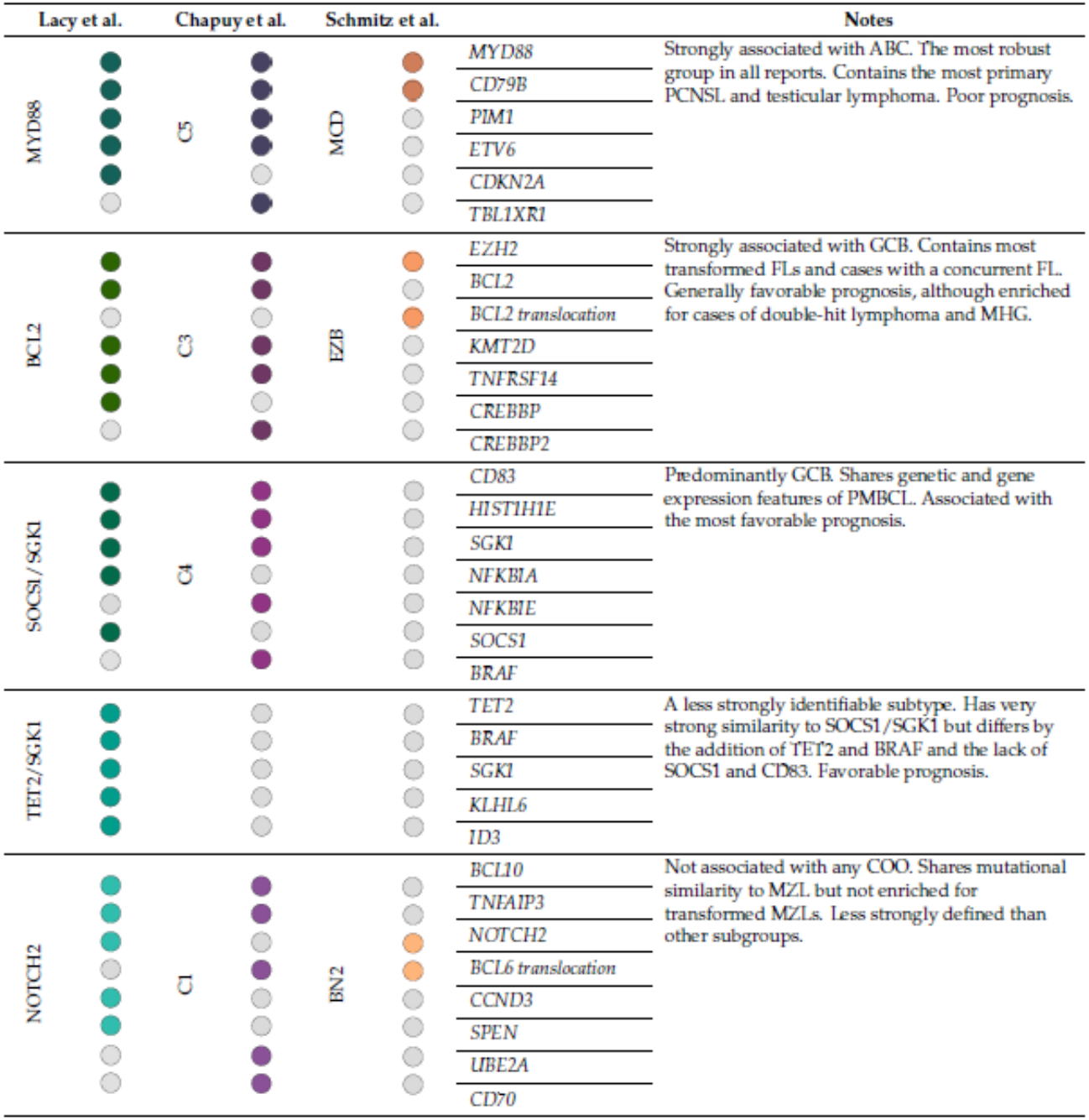
Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report

- Robust subtypes of DLBCL are identified by model-based clustering of genetic mutations in a large (n = 928) population-based cohort.
- With full follow-up data available for all sequenced patients, the prognostic significance of these subtypes is identified.



Lacy et al. Blood 2020

NGS



Lacy et al.	Chapuy et al.	Schmitz et al.		Notes
MYD88	C5	MCD	*MYD88* *CD79B* *PIM1* *ETV6* *CDKN2A* *TBL1XR1*	Strongly associated with ABC. The most robust group in all reports. Contains the most primary PCNSL and testicular lymphoma. Poor prognosis.
BCL2	C3	EZB	*EZH2* *BCL2* *BCL2 translocation* *KMT2D* *TNFRSF14* *CREBBP* *CREBBP2*	Strongly associated with GCB. Contains most transformed FLs and cases with a concurrent FL. Generally favorable prognosis, although enriched for cases of double-hit lymphoma and MHG.
SOCS1/SGK1	C4		*CD83* *HIST1H1E* *SGK1* *NFKBIA* *NFKBIE* *SOCS1* *BRAF*	Predominantly GCB. Shares genetic and gene expression features of PMBCL. Associated with the most favorable prognosis.
TET2/SGK1			*TET2* *BRAF* *SGK1* *KLHL6* *ID3*	A less strongly identifiable subtype. Has very strong similarity to SOCS1/SGK1 but differs by the addition of TET2 and BRAF and the lack of SOCS1 and CD83. Favorable prognosis.
NOTCH2	C1	BN2	*BCL10* *TNFAIP3* *NOTCH2* *BCL6 translocation* *CCND3* *SPEN* *UBE2A* *CD70*	Not associated with any COO. Shares mutational similarity to MZL but not enriched for transformed MZLs. Less strongly defined than other subgroups.

PARTIAL OVERLAP BETWEEN CLASSIFIERS

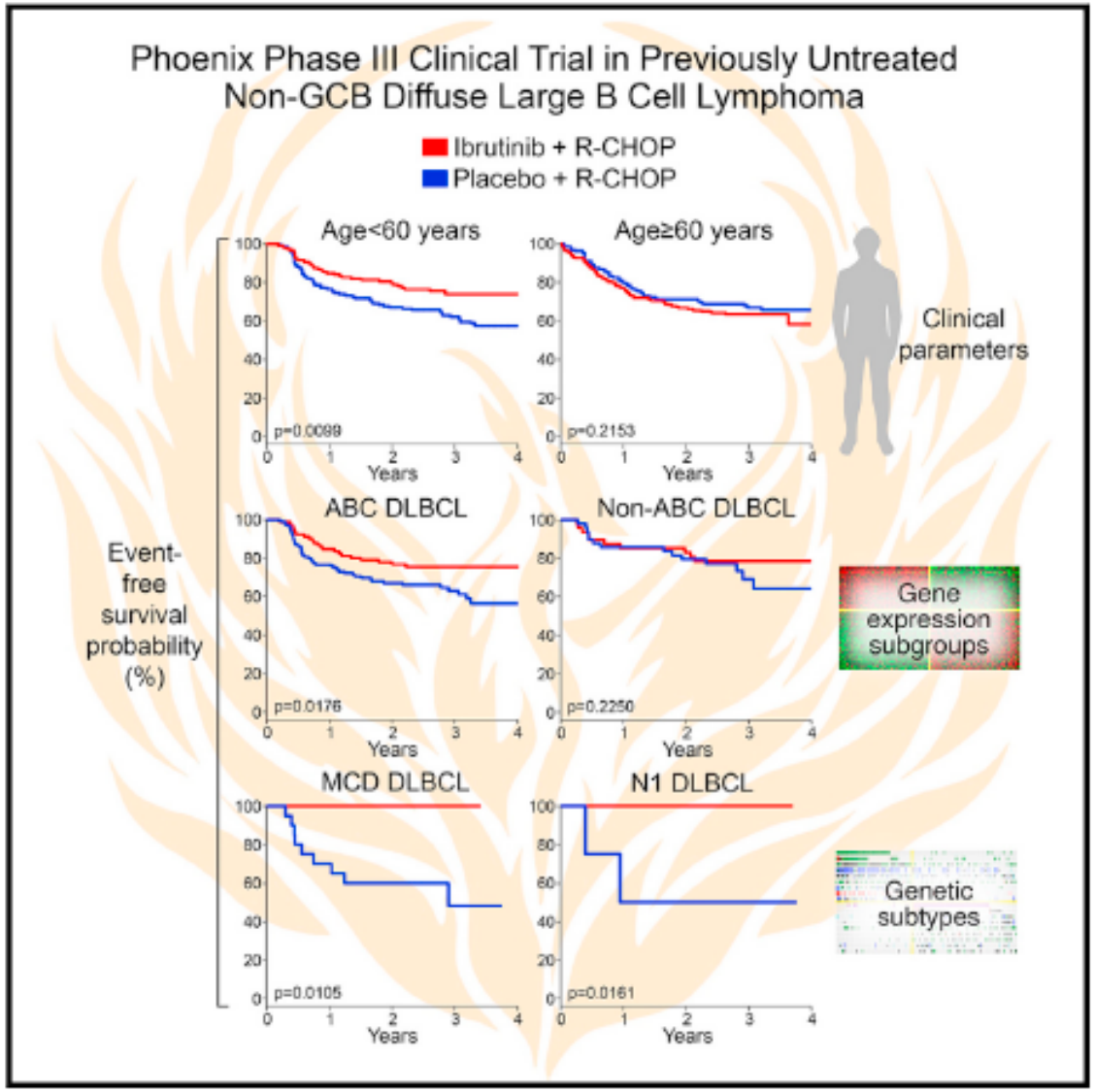
MULTIPLE TARGETS

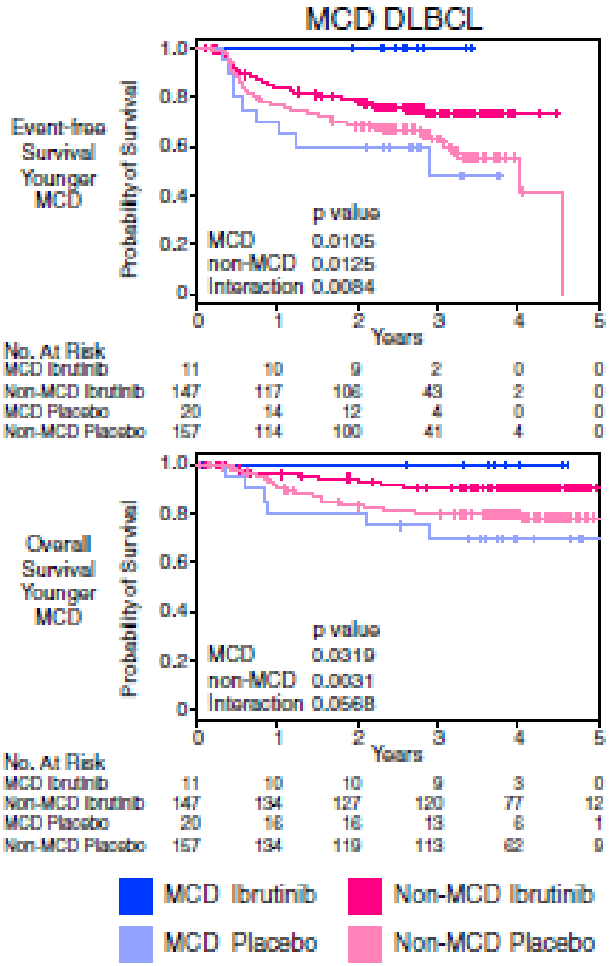
REAL-LIFE APPLICABILITY?

Pileri…Derenzini: Cells 2021

Cancer Cell

Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL



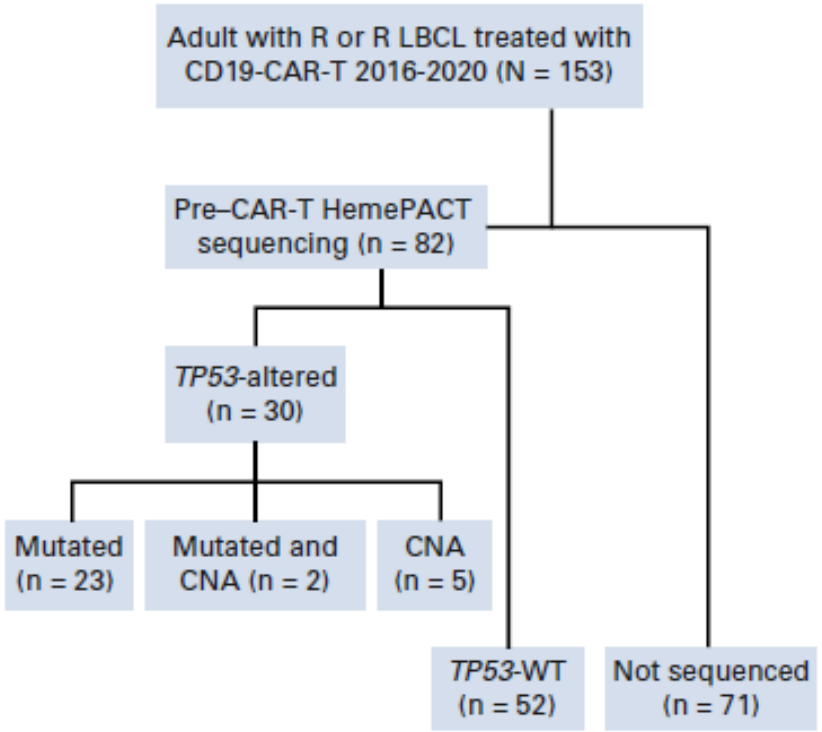


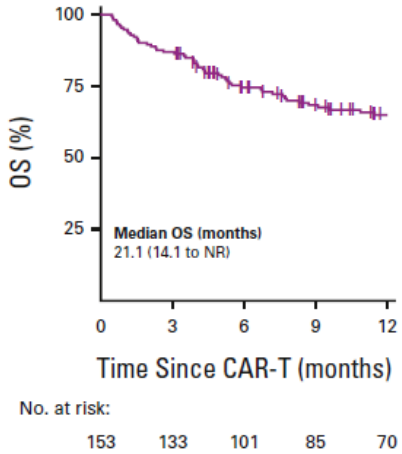
Wilson et al. Cancer Cell 2021

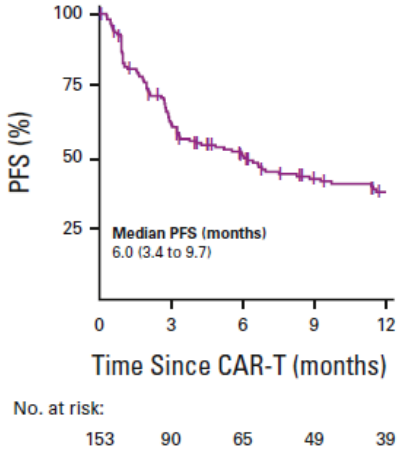
original reports

Impact of *TP53* Genomic Alterations in Large B-Cell Lymphoma Treated With CD19-Chimeric Antigen Receptor T-Cell Therapy

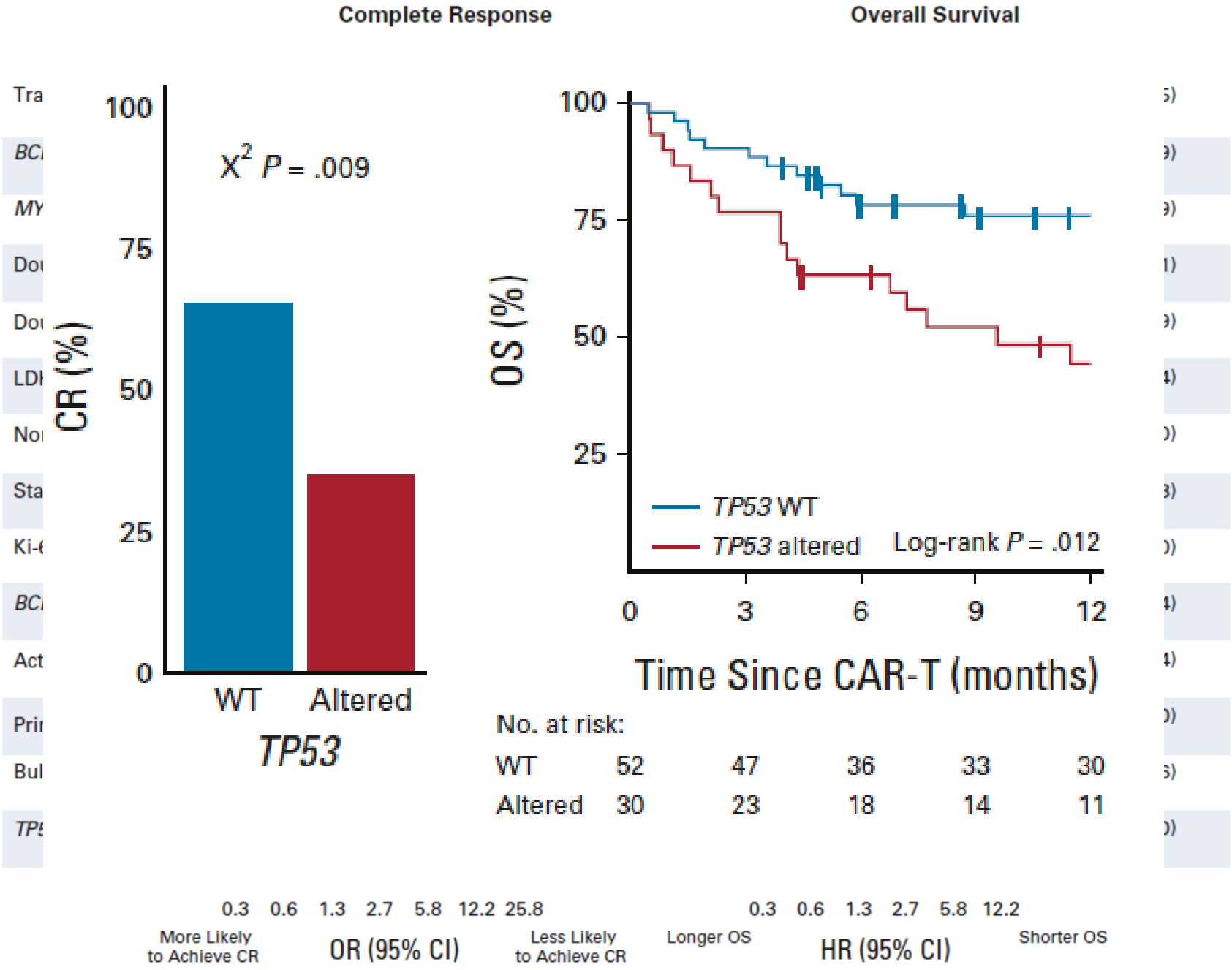
Roni Shouval, MD, PhD[1,2]; Ana Alarcon Tomas, MD[1,3]; Joshua A. Fein, MD[4]; Jessica R. Flynn, MSc[5]; Ettai Markovits, MD[6]; Shimrit Mayer, MSc[7]; Aishat Olaide Afuye, BA[1]; Anna Alperovich, MD[1]; Theodora Anagnostou, MD[1,8]; Michal J. Besser, PhD[6,9]; Connie Lee Batlevi, MD[2,10]; Parastoo B. Dahi, MD[1,2]; Sean M. Devlin, PhD[5]; Warren B. Fingrut, MD[1]; Sergio A. Giralt, MD[1,2]; Richard J. Lin, MD[1,2]; Gal Markel, MD, PhD[9,11]; Gilles Salles, MD[2,10]; Craig S. Sauter, MD[1,2]; Michael Scordo, MD[1,2]; Gunjan L. Shah, MD[1,2]; Nishi Shah, MD[1]; Ruth Scherz-Shouval, PhD[7]; Marcel van den Brink, MD, PhD[1,2]; Miguel-Angel Perales, MD[1,2]; and Maria Lia Palomba, MD[2,10]





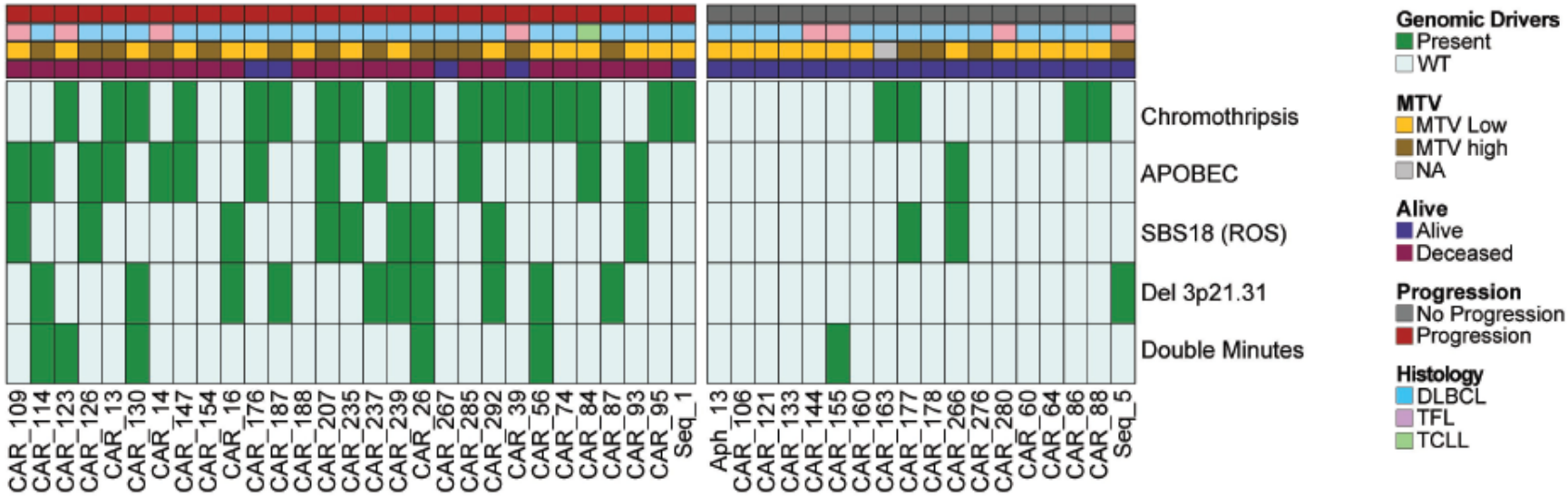


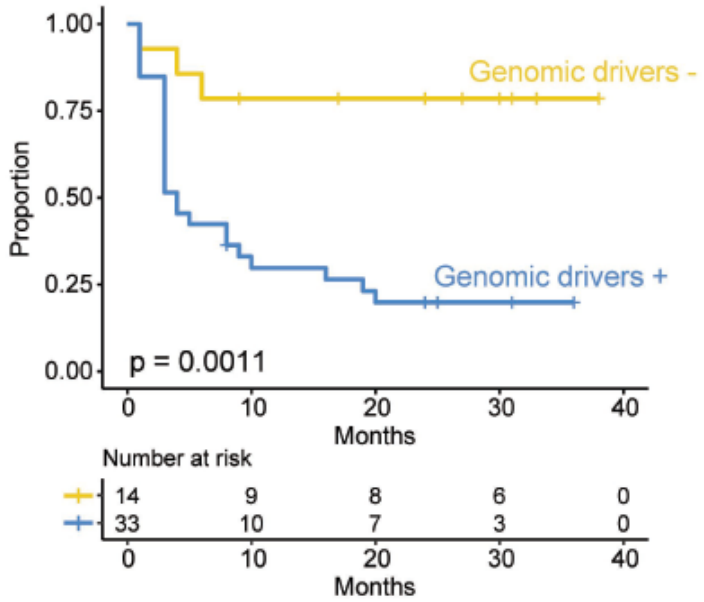
Shouval et al J Clin Oncol 2022

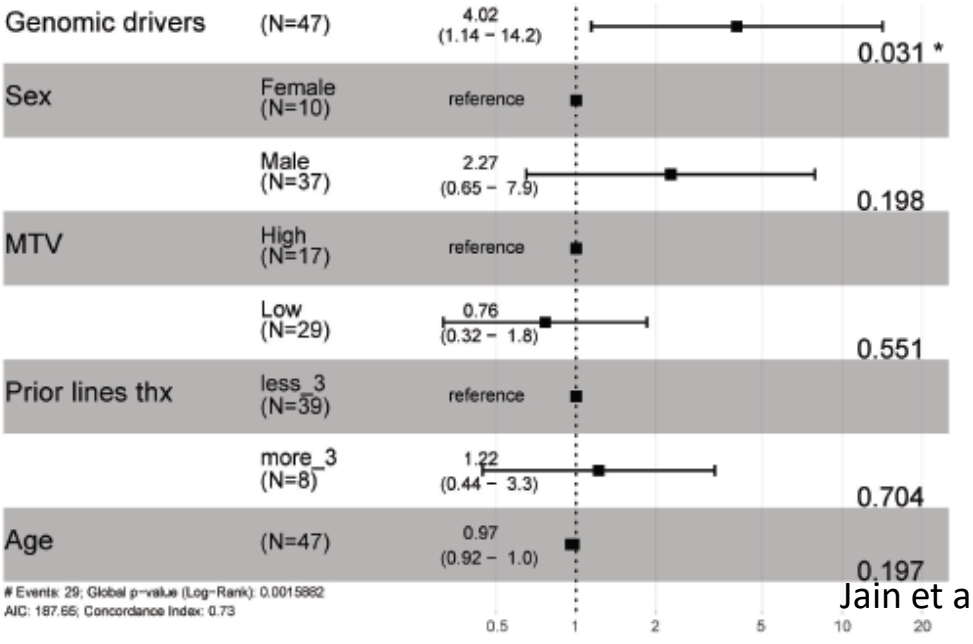

Complete Response
Overall Survival
100
75
50
25
0
CR (%)
X^2 P = .009
WT
Altered
TP53
OS (%)
TP53 WT
TP53 altered
Log-rank P = .012
0
3
6
9
12
Time Since CAR-T (months)
No. at risk:
WT 52 47 36 33 30
Altered 30 23 18 14 11
0.3 0.6 1.3 2.7 5.8 12.2 25.8
More Likely to Achieve CR
OR (95% CI)
Less Likely to Achieve CR
Longer OS
0.3 0.6 1.3 2.7 5.8 12.2
HR (95% CI)
Shorter OS

Whole-genome sequencing reveals complex genomic features underlying anti-CD19 CAR T-cell treatment failures in lymphoma

Tracking no: BLD-2021-015008R1





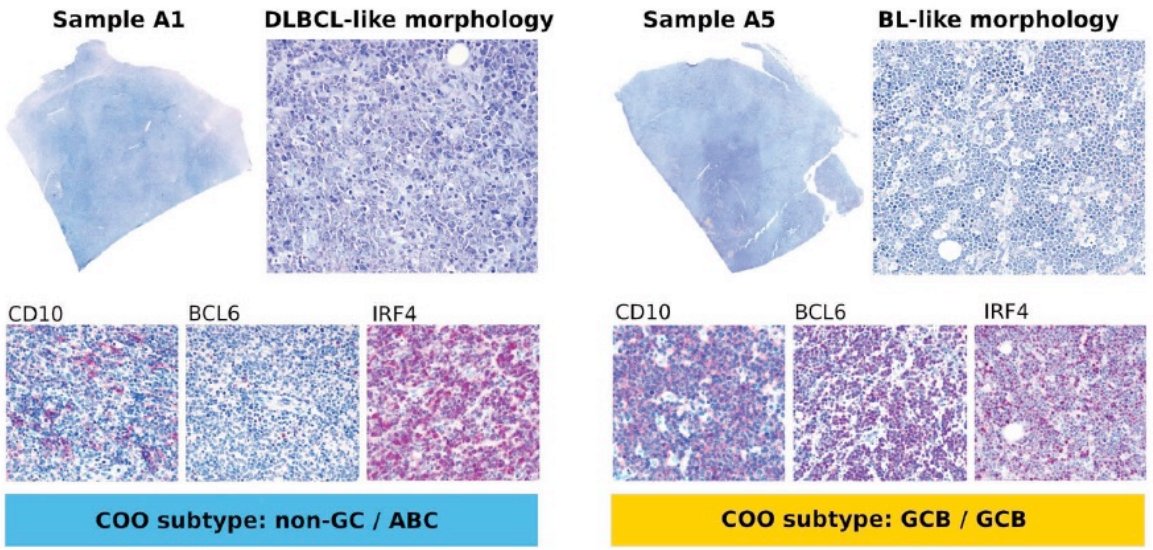


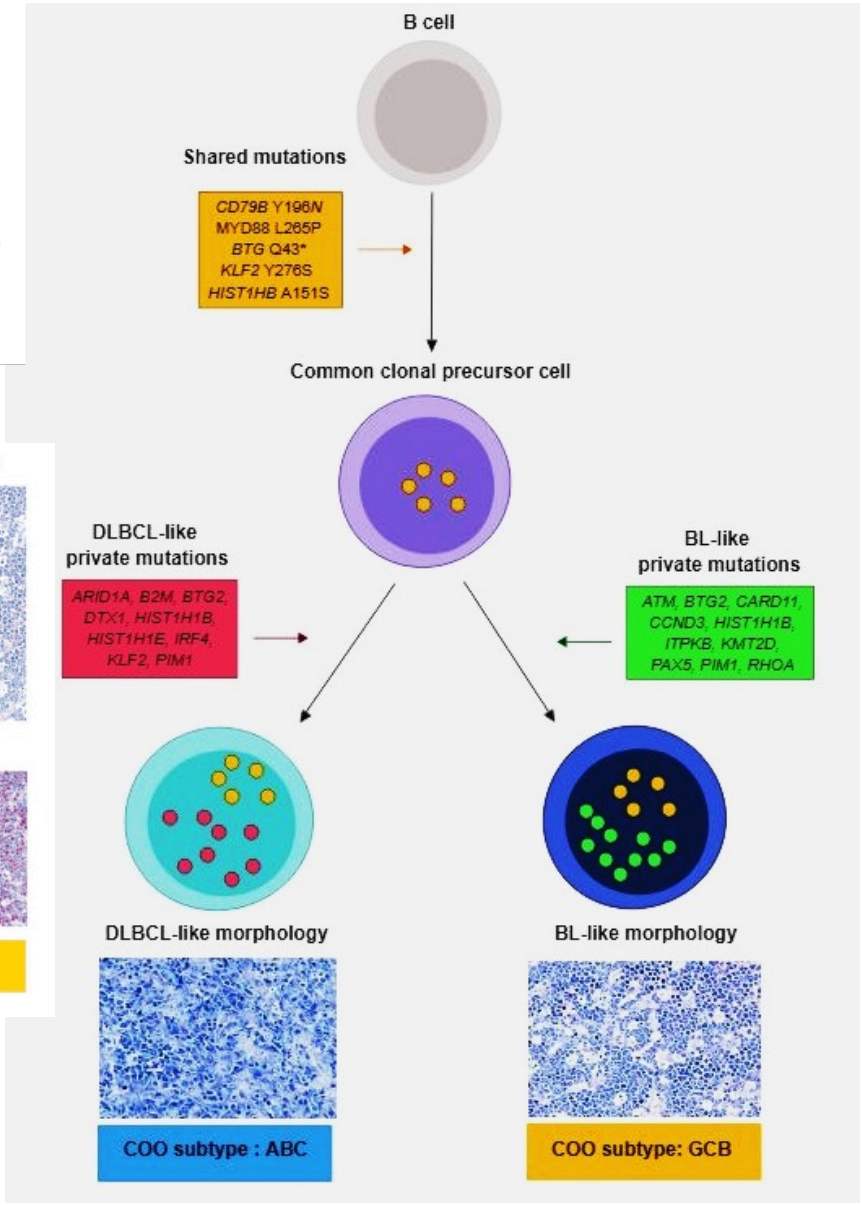
Jain et al Blood 2022

Evolutionary crossroads: morphological heterogeneity reflects divergent intra-clonal evolution in a case of high-grade B-cell lymphoma

by Valentina Tabanelli, Federica Melle, Giovanna Motta, Saveria Mazzara, Marco Fabbri, Chiara Corsini, Elvira Gerbino, Angelica Calleri, Maria Rosaria Sapienza, Ignazio Abbene, Viviana Stufano, Massimo Barberis, and Stefano A. Pileri

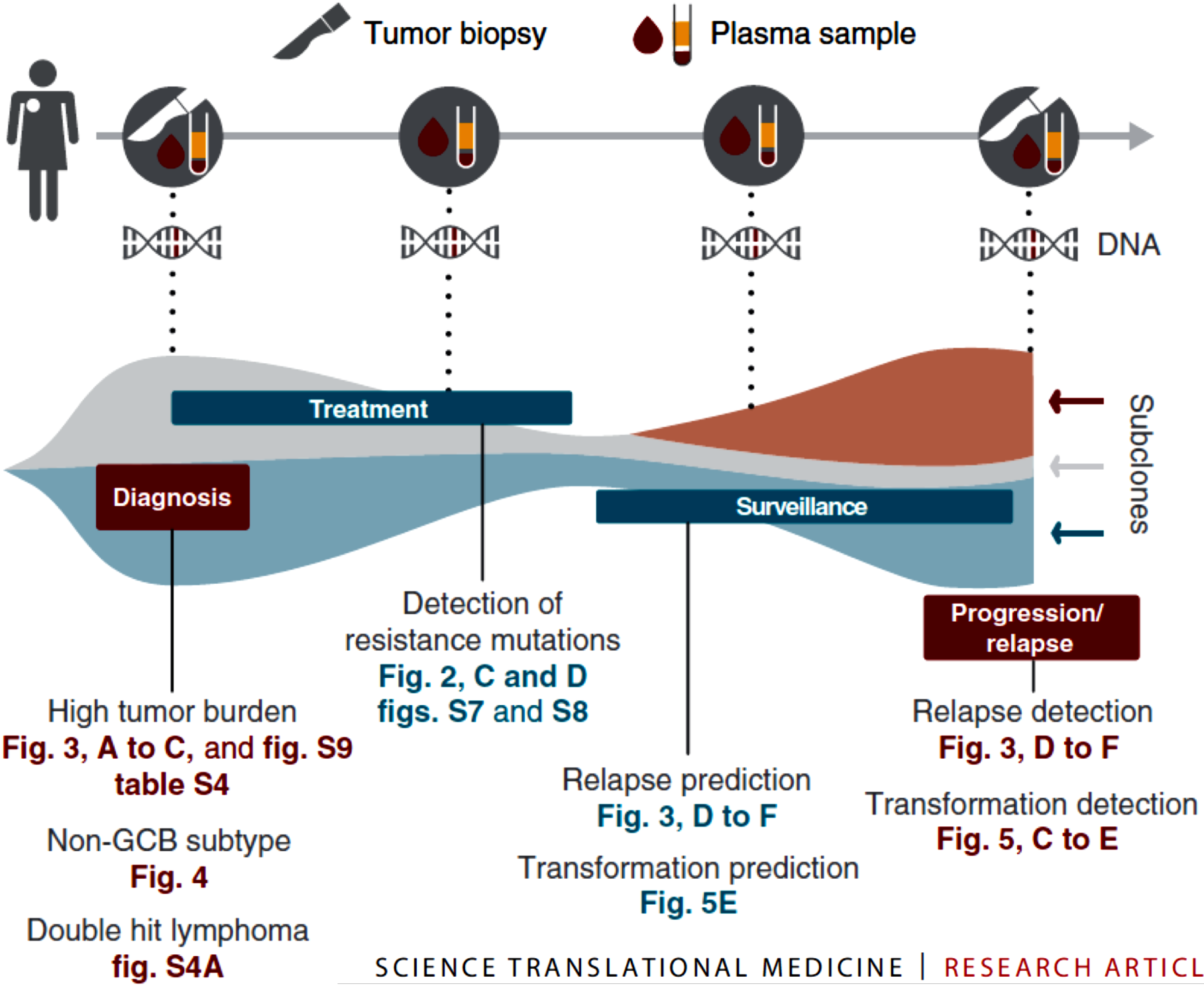
Haematologica 2020 [Epub ahead of print]





Tabanelli et al Haematologica 2020

Liquid Biopsy


Tumor biopsy
Plasma sample
DNA
Treatment
Diagnosis
Surveillance
Subclones
Progression/ relapse
Detection of resistance mutations
Fig. 2, C and D
figs. S7 and S8
High tumor burden
Fig. 3, A to C, and fig. S9
table S4
Non-GCB subtype
Fig. 4
Double hit lymphoma
fig. S4A
Relapse prediction
Fig. 3, D to F
Transformation prediction
Fig. 5E
Relapse detection
Fig. 3, D to F
Transformation detection
Fig. 5, C to E

Inferring gene expression from cell-free DNA fragmentation profiles

Mohammad Shahrokh Esfahani[1,2,3], Emily G. Hamilton[4], Mahya Mehrmohamadi[1,2], Barzin Y. Nabet[2,3], Stefan K. Alig[1], Daniel A. King[1], Chloé B. Steen[1,5,6], Charles W. Macaulay[1], Andre Schultz[3], Monica C. Nesselbush[4], Joanne Soo[1], Joseph G. Schroers-Martin[1,3], Binbin Chen[1], Michael S. Binkley[2], Henning Stehr[3], Jacob J. Chabon[2], Brian J. Sworder[1], Angela B-Y Hui[2], Matthew J. Frank[7], Everett J. Moding[2], Chih Long Liu[1], Aaron M. Newman[5,6], James M. Isbell[8], Charles M. Rudin[9], Bob T. Li[9], David M. Kurtz[1,3], Maximilian Diehn[2,3,5] ✉ and Ash A. Alizadeh[1,3,5] ✉

Profiling of circulating tumor DNA (ctDNA) in the bloodstream shows promise for noninvasive cancer detection. Chromatin fragmentation features have previously been explored to infer gene expression profiles from cell-free DNA (cfDNA), but current fragmentomic methods require high concentrations of tumor-derived DNA and provide limited resolution. Here we describe promoter fragmentation entropy as an epigenomic cfDNA feature that predicts RNA expression levels at individual genes. We developed 'epigenetic expression inference from cell-free DNA-sequencing' (EPIC-seq), a method that uses targeted sequencing of promoters of genes of interest. Profiling 329 blood samples from 201 patients with cancer and 87 healthy adults, we demonstrate classification of subtypes of lung carcinoma and diffuse large B cell lymphoma. Applying EPIC-seq to serial blood samples from patients treated with PD-(L)1 immune-checkpoint inhibitors, we show that gene expression profiles inferred by EPIC-seq are correlated with clinical response. Our results indicate that EPIC-seq could enable noninvasive, high-throughput tissue-of-origin characterization with diagnostic, prognostic and therapeutic potential.

PRACTICAL CONSIDERATIONS

MOLECULAR TECHNIQUE	DETECTED ALTERATION	THERAPEUTIC IMPLICATION
FISH	MYC*/BCL-2/BCL-6 REARR →	YES
	TP53 DEL →	CLINICAL TRIALS
T-GEP	COO →	CLINICAL TRIALS
T-NGS	GENOMIC CLASSIFIERS (TP53 MUT) →	CLINICAL TRIALS
LIQUID BX	GENOMIC CLASSIFIERS (TP53 MUT) MRD →	CLINICAL TRIALS

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