The young side of
LYMPHOMA

gli under 40 a confronto

Milano, 14-15 aprile 2023

Una nuova era per i DLBCL?
Classificazione molecolare o nanostring

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Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Abbvie						x	

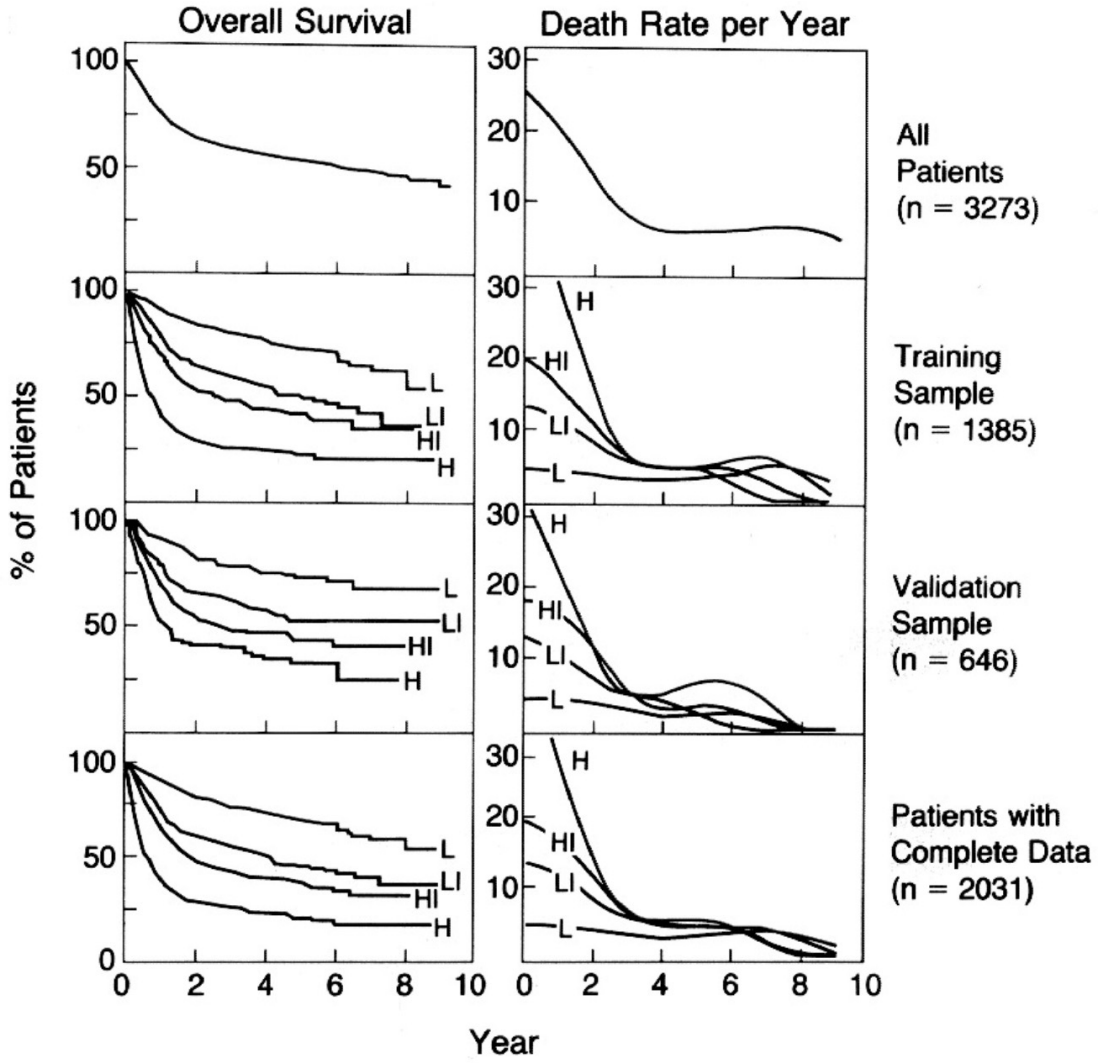
Agenda

- **Different molecular classification methods for large B cell lymphomas**
- Clinical trial dedicated to specific molecular subtypes
- Molecular classification on the liquid biopsy

Initial evidences of outcome heterogeneity in DLBCL

Factor	Relative Risk	P Value
All patients (n = 1385)		
Age (≤60 vs. >60)	1.96	<0.001
Serum LDH (≤1× normal vs. >1× normal)	1.85	<0.001
Performance status (0 or 1 vs. 2–4)	1.80	<0.001
Stage (I or II vs. III or IV)	1.47	<0.001
Extranodal involvement (≤1 site vs. >1 site)*	1.48	<0.001
Patients ≤60 years old (n = 885)		
Stage (I or II vs. III or IV)	2.17	<0.001
Serum LDH (≤1× normal vs. >1× normal)	1.95	<0.001
Performance status (0 or 1 vs. 2–4)	1.81	<0.001

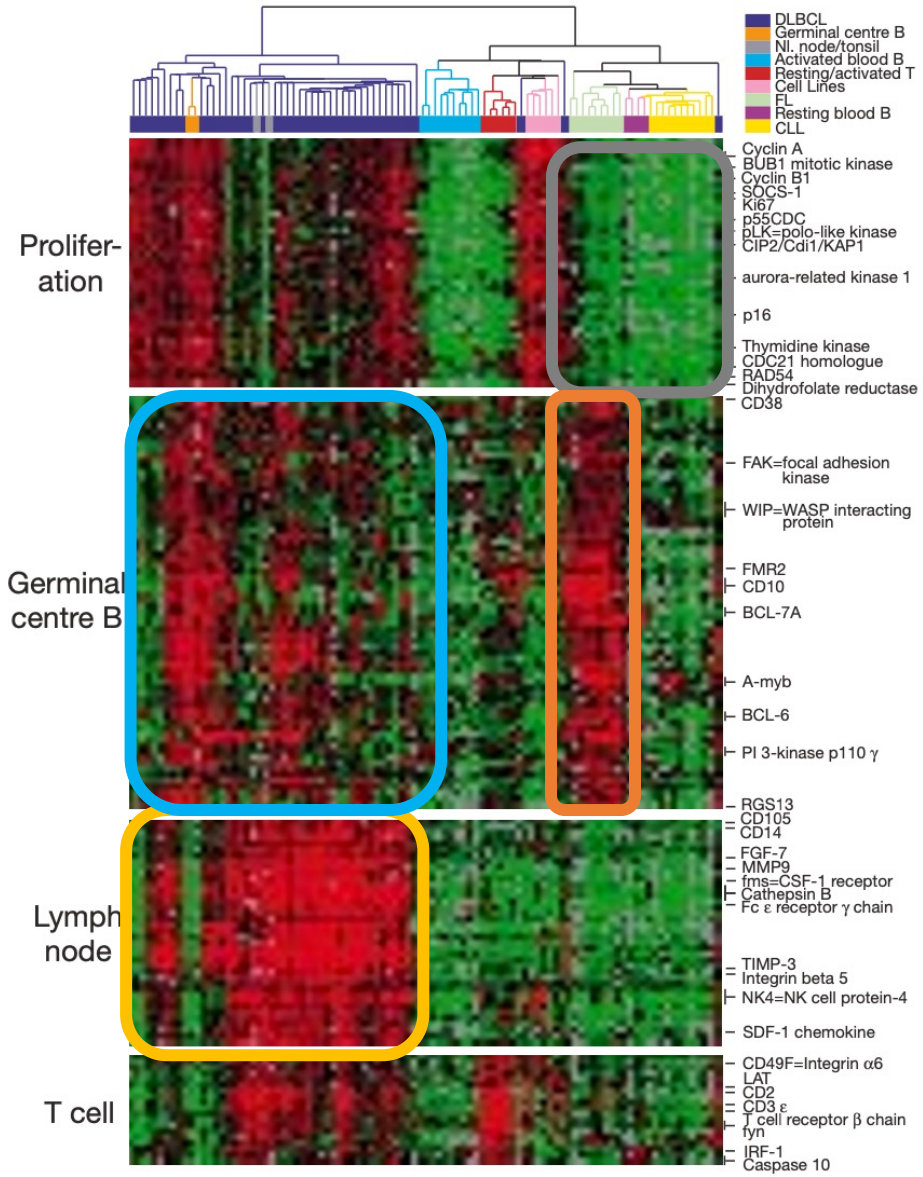
*This was the only factor that did not retain independent prognostic significance in patients ≤60 years old (≤1 site vs. >1 site: relative risk, 1.20; P = 0.134).



…the biologic heterogeneity of this disease may be better understood

The International Non-Hodgkin's Lymphoma Prognostic Factors Project., *NEJM*. 1993.

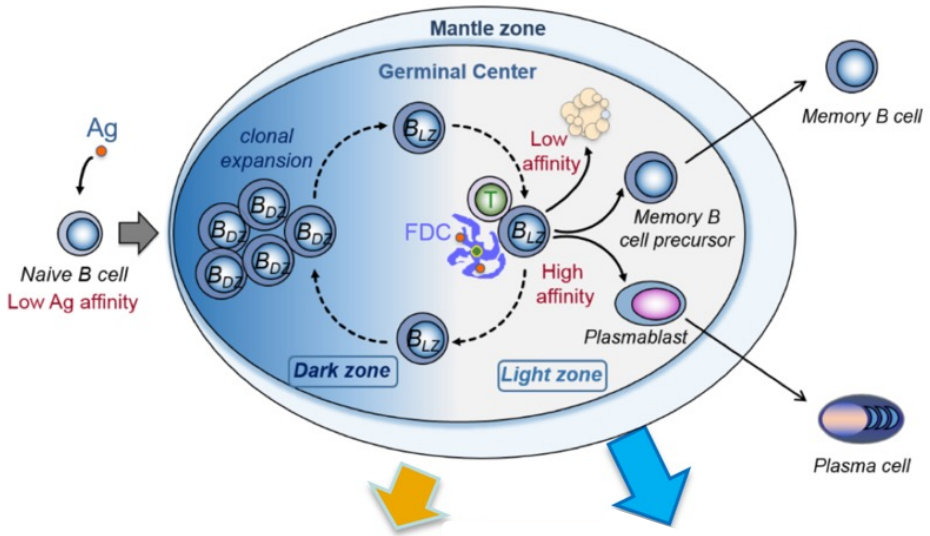
Distinct gene expression signatures among B-cell neoplasms

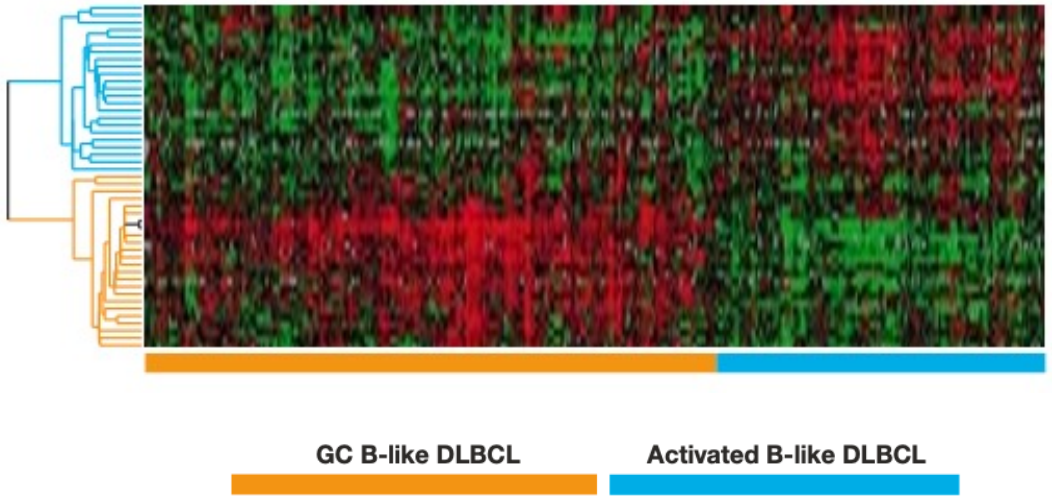


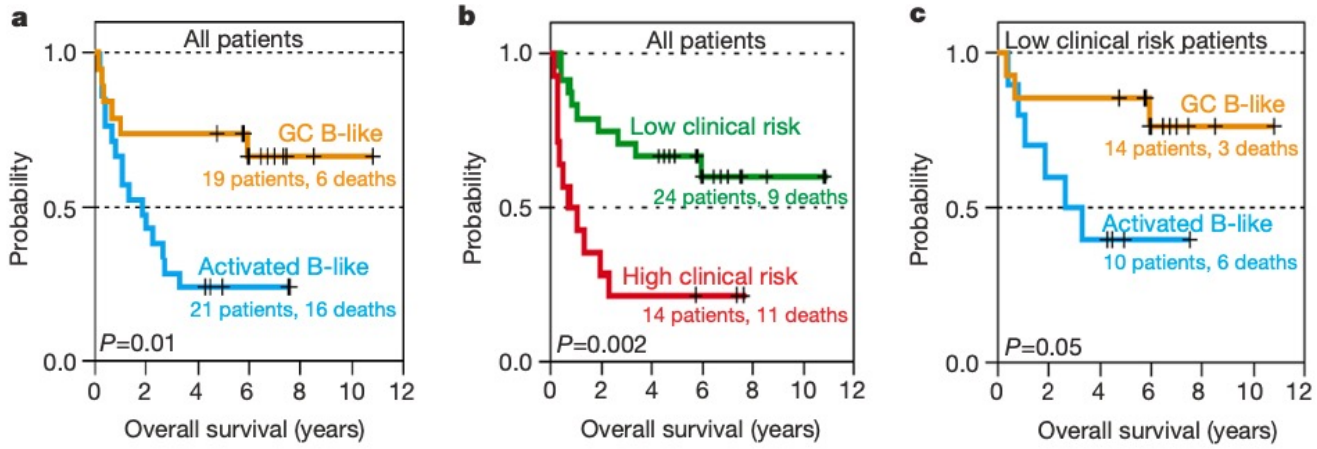
- CLL and FL present a GEP profile similar to resting B cell
- FL are similar to germinal centre B-cell
- DLBCL are distinct from CLL and FL and are enriched of ”lymph node” transcriptomic features
- Genes that distinguished germinal centre B cells from other stages in B-cell ontogeny were also differentially expressed among DLBCLs suggesting that B-cell differentiation genes may also be used to sub- divide DLBCL

Alizadeh *et al., Nature.* 2000.

Relationship of DLBCL subgroups to normal B-lymphocyte differentiation and activation



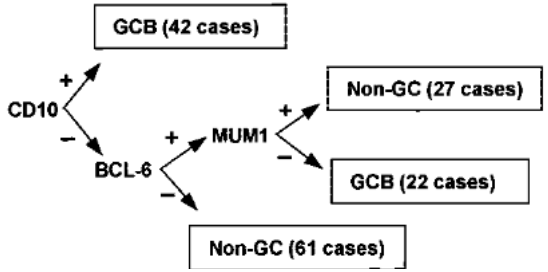


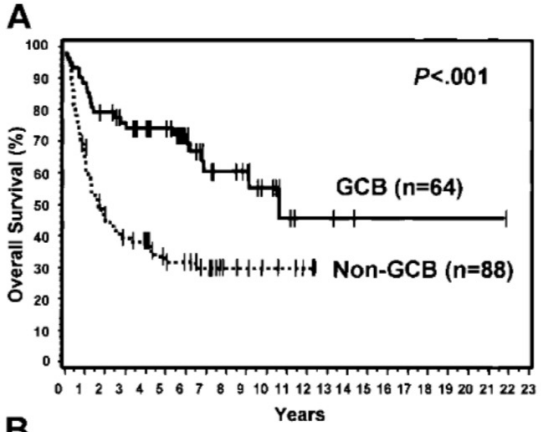


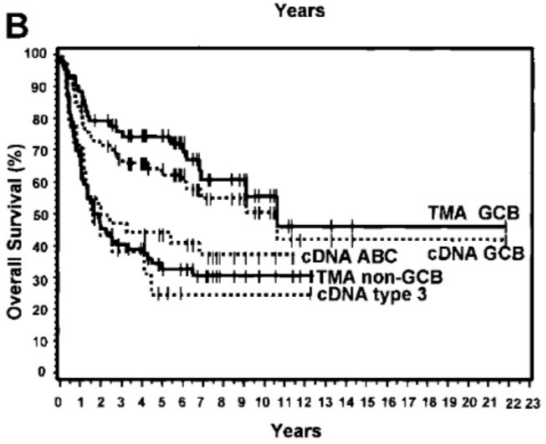
Alizadeh *et al., Nature.* 2000.

Simplified methods for COO for routine clinical practice

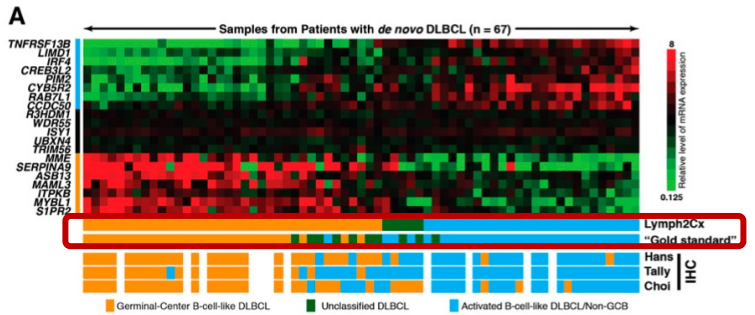
Hans algorithm

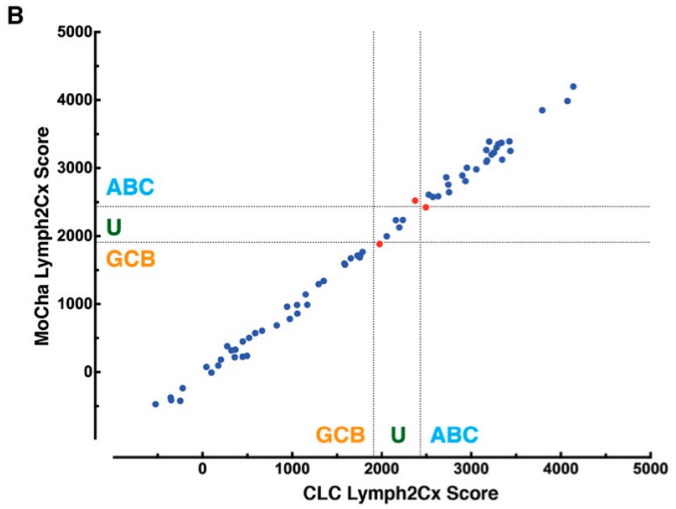


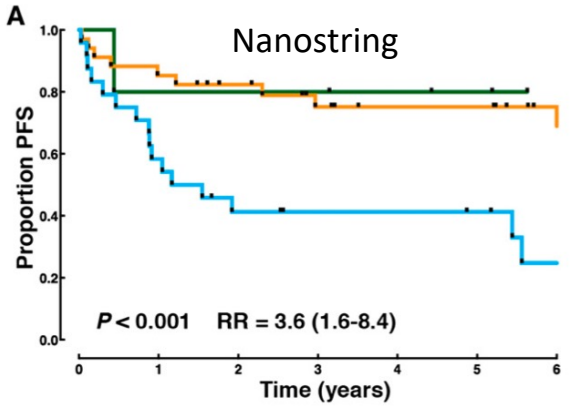


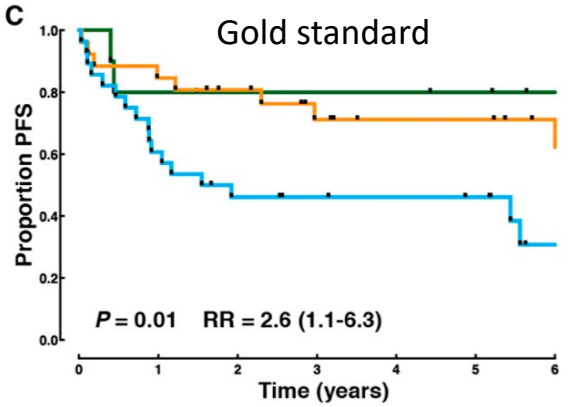


Nanostring/Lymph2Cx assay





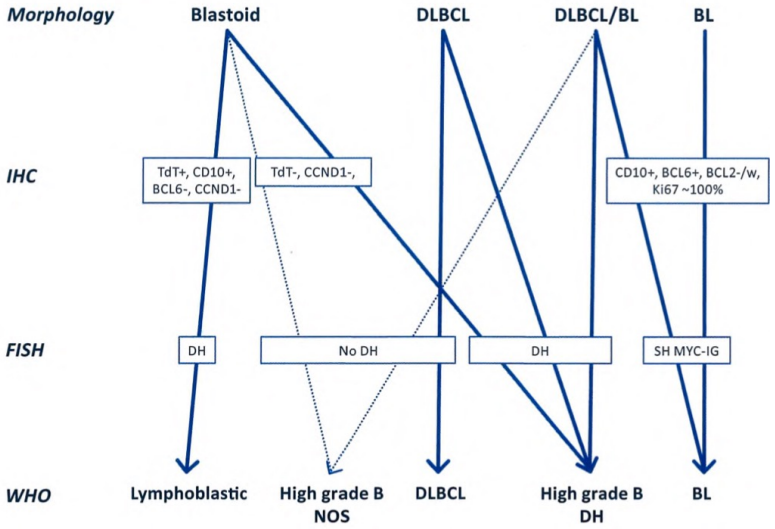


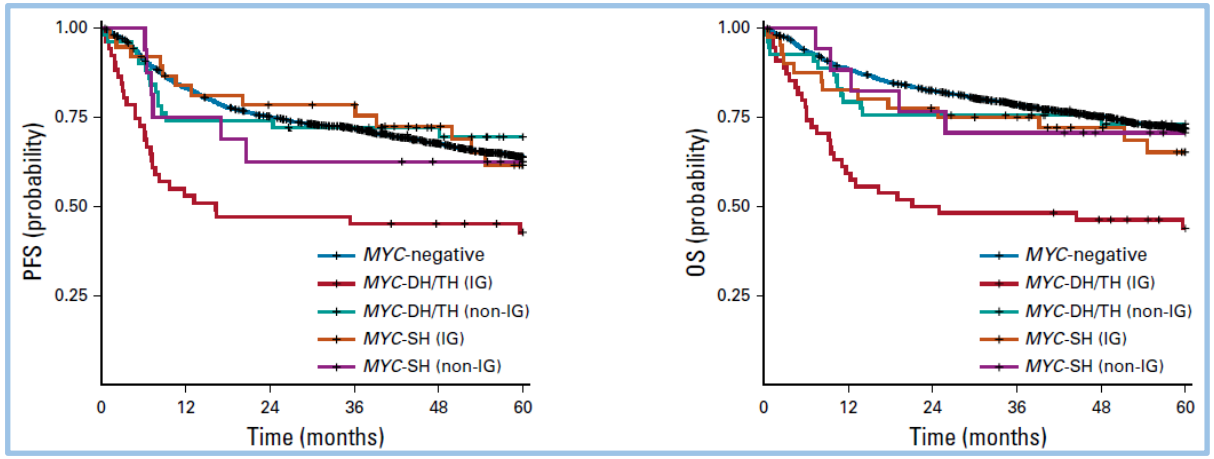


Germinal-Center B-cell-like DLBCL Unclassified DLBCL Activated B-cell-like DLBCL

Hans *et al., Blood.* 2004; Scott *et al., Blood*. 2014.

Chromosomal translocations of MYC, BCL2 and BCL6 identify high-grade B-cell lymphoma





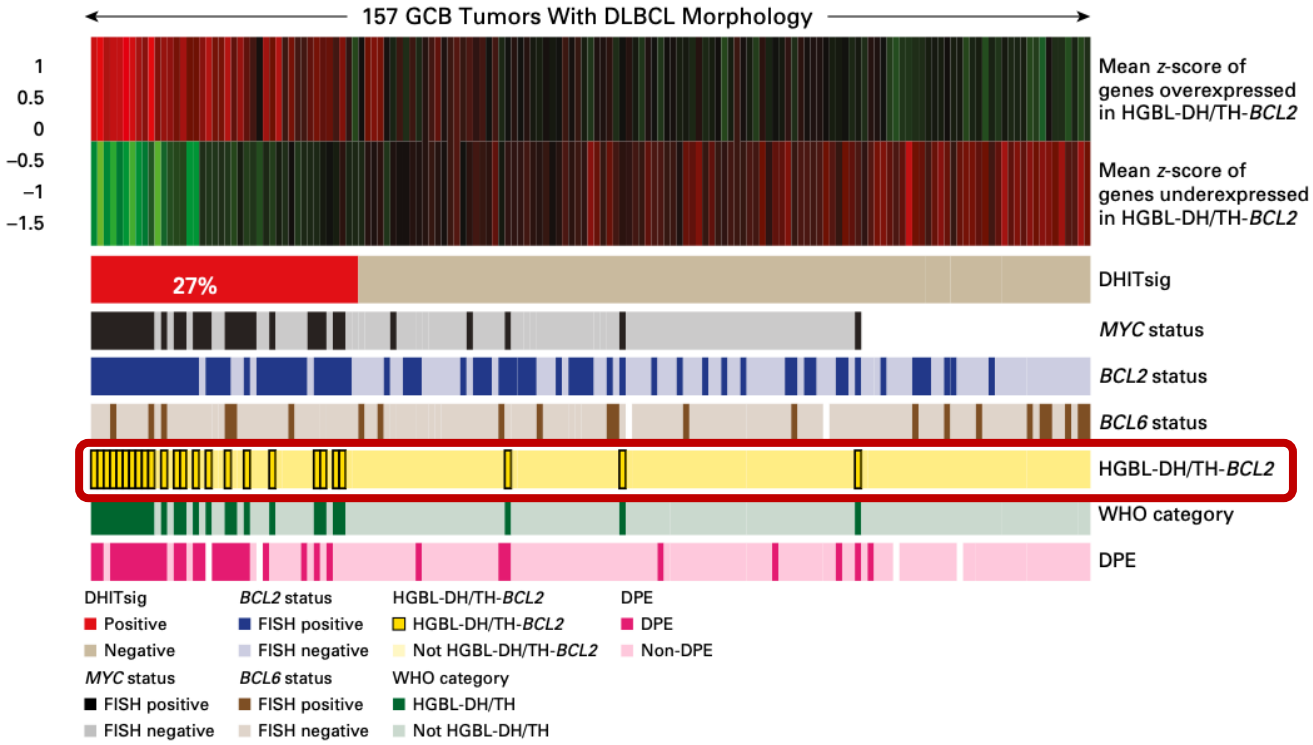
	PFS		OS	
Model	HR (95% CI)	*P*	HR (95% CI)	*P*
Model 2				
MYC-negative	1		1	
MYC-DH/TH (IG) before 24 months	2.43 (1.65 to 3.58)	**< .001**	3.04 (2.05 to 4.60)	**< .001**
MYC-DH/TH (IG) after 24 months	0.45 (0.11 to 1.81)	.26	0.71 (0.23 to 2.21)	.55
Other*	1.04 (0.74 to 1.48)	.91	1.24 (0.87 to 1.77)	.24
IPI low	1		1	
IPI high	2.52 (2.18 to 2.91)	**< .001**	2.82 (2.40 to 3.32)	**< .001**

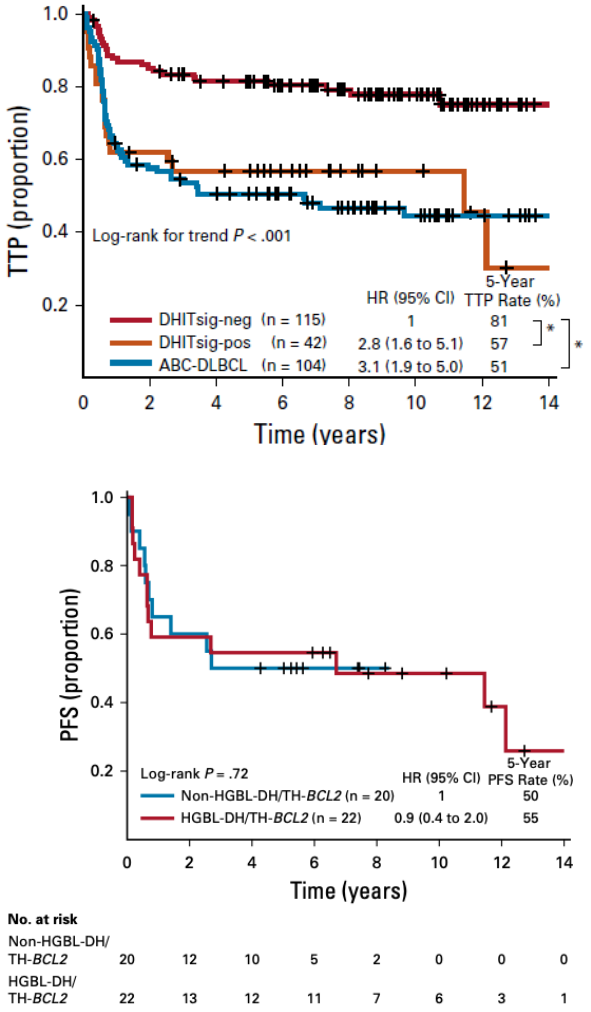
Among patients with *MYC*-R, only those with *MYC*-DH/TH in which *MYC* was rearranged with an *IG* partner demonstrated inferior outcome

Patients with *MYC*-SH (either *IG* or non-*IG*) and those with *MYC*-DH/TH non-*IG* had an outcome comparable with those with DLBCL without *MYC*-R

Rosenwald *et al., J Clin Oncol*. 2019

Double hit singnature (DHITsig) identify high risk DLBCL





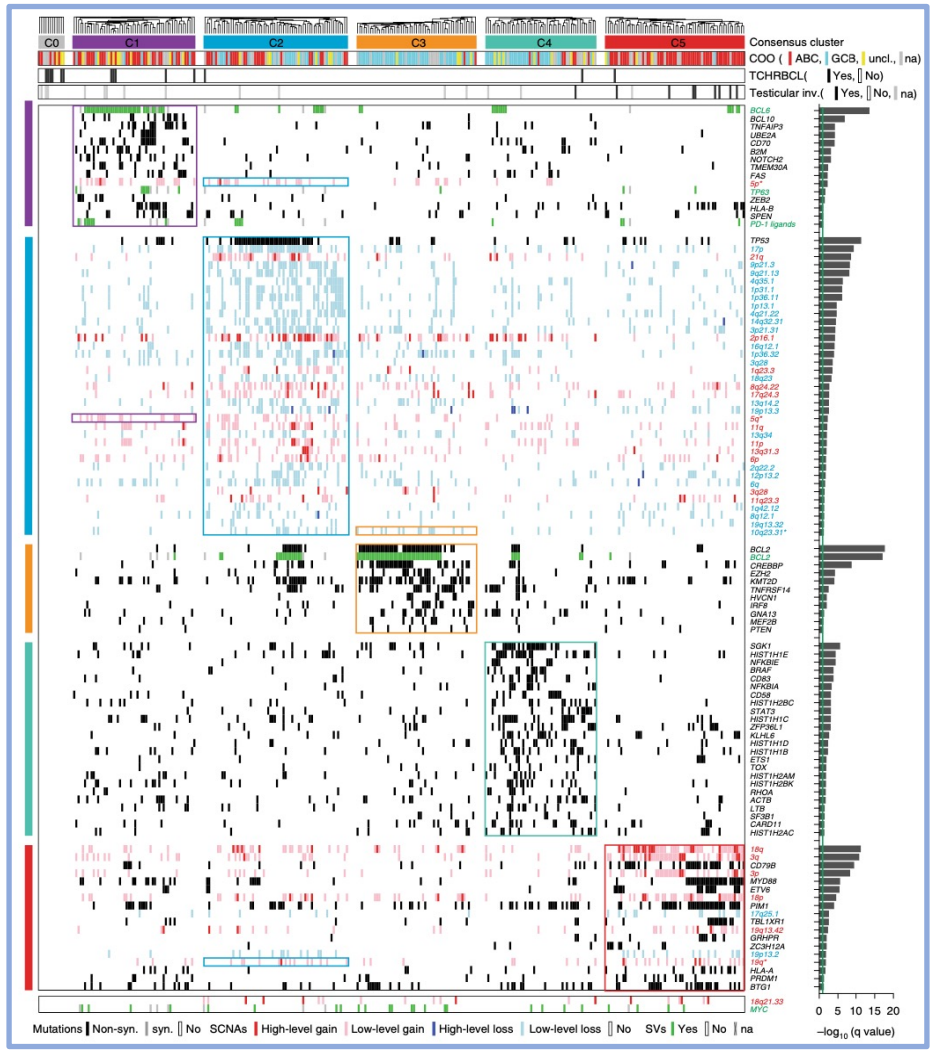
DHITsig is a panel of **104 genes tested by RNAseq** that are significantly differentially expressed between **HGBL-DH/TH-*BCL2*** and **other GCB-DLBCL**

27% of GCB-DLBCL was assigned to **DHITsig-positive group,** with **only one-half harboring MYC and BCL2 rearrangement**

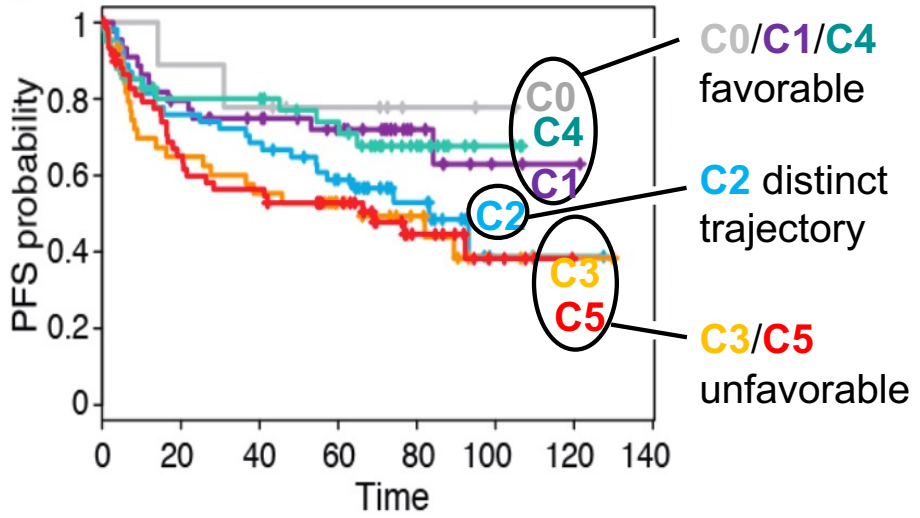
DHITsig-positive without HGBL-DH patients had superimposable outcomes after R-CHOP compared to HGBL-DH/TH-*BCL2* status

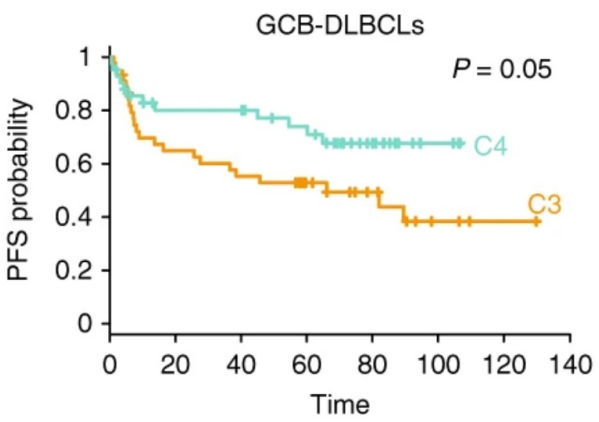
Ennishi *et al., J Clin Oncol*. 2018

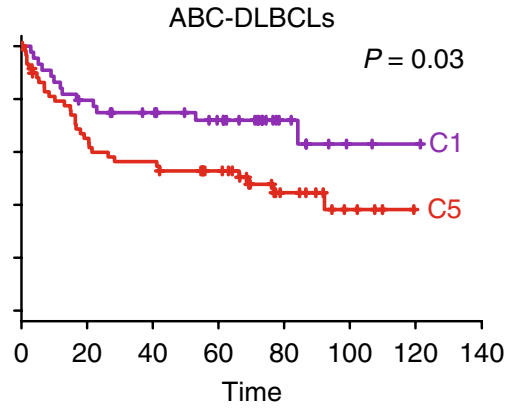
Molecular cluster on lymph node biopsy: the Harvard classification



Predictive for Outcome

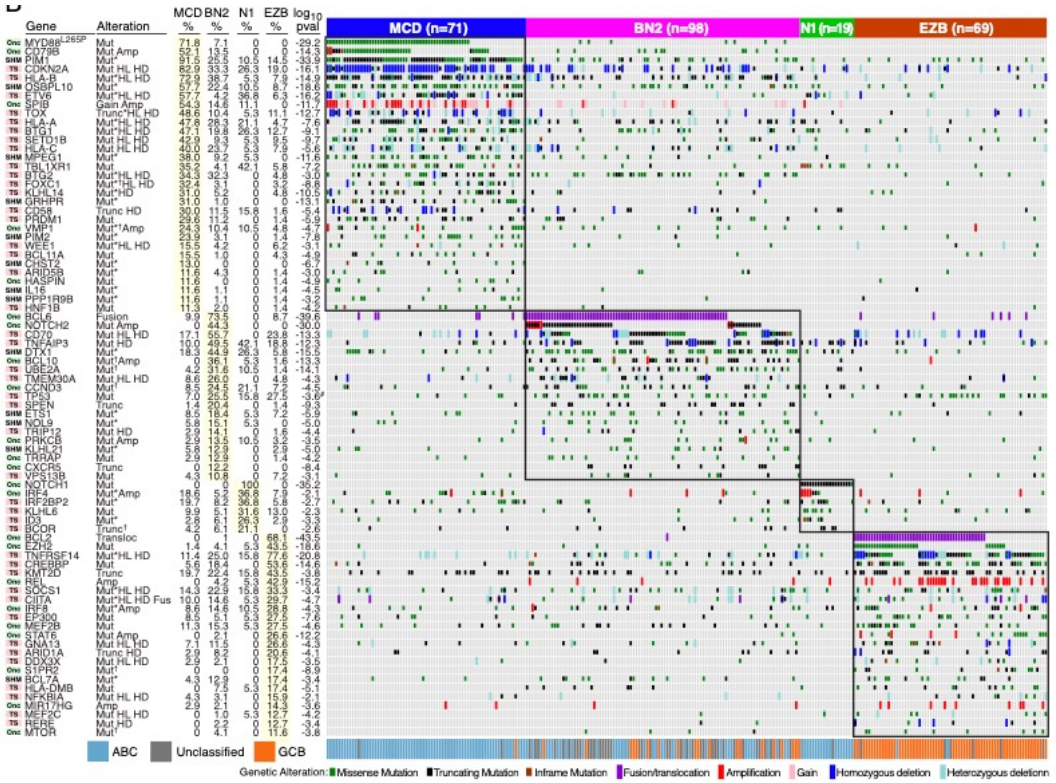


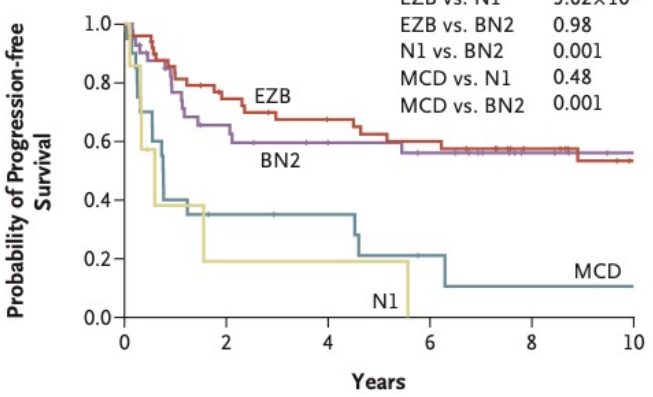




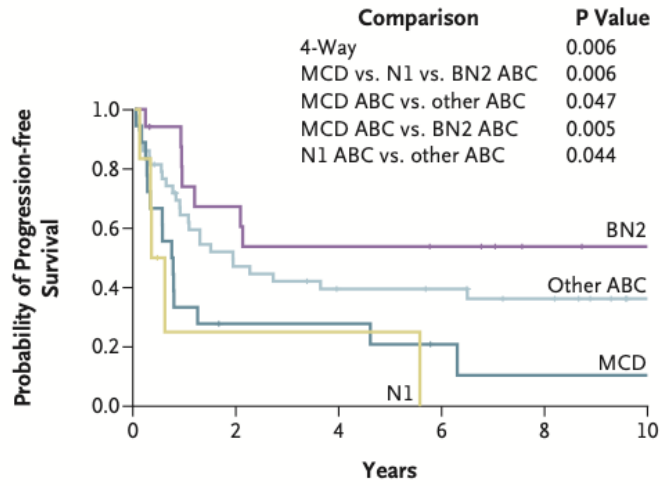
Chapuy *et al., Nat Med*. 2018

Molecular cluster on lymph node biopsy: the NCI classification

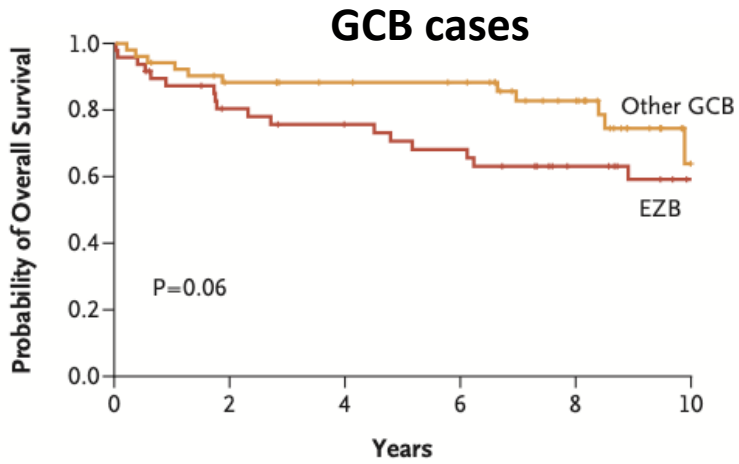




ABC cases

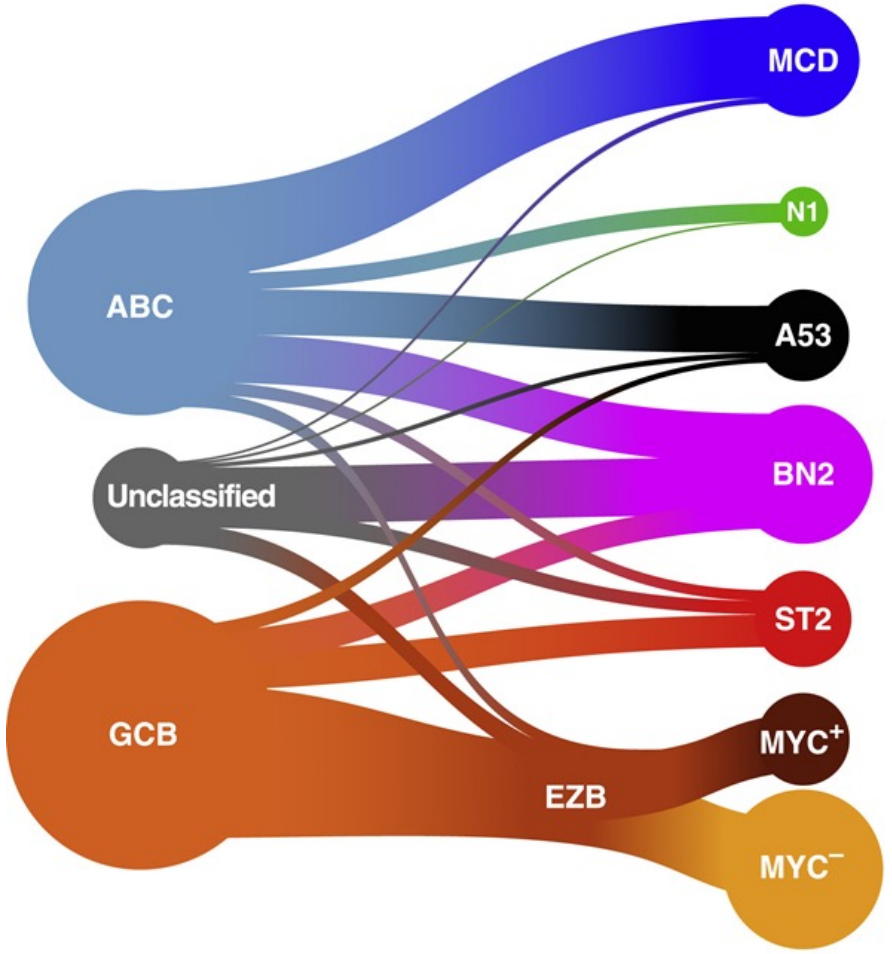


GCB cases



Schmitz *et al., NEJM*. 2018

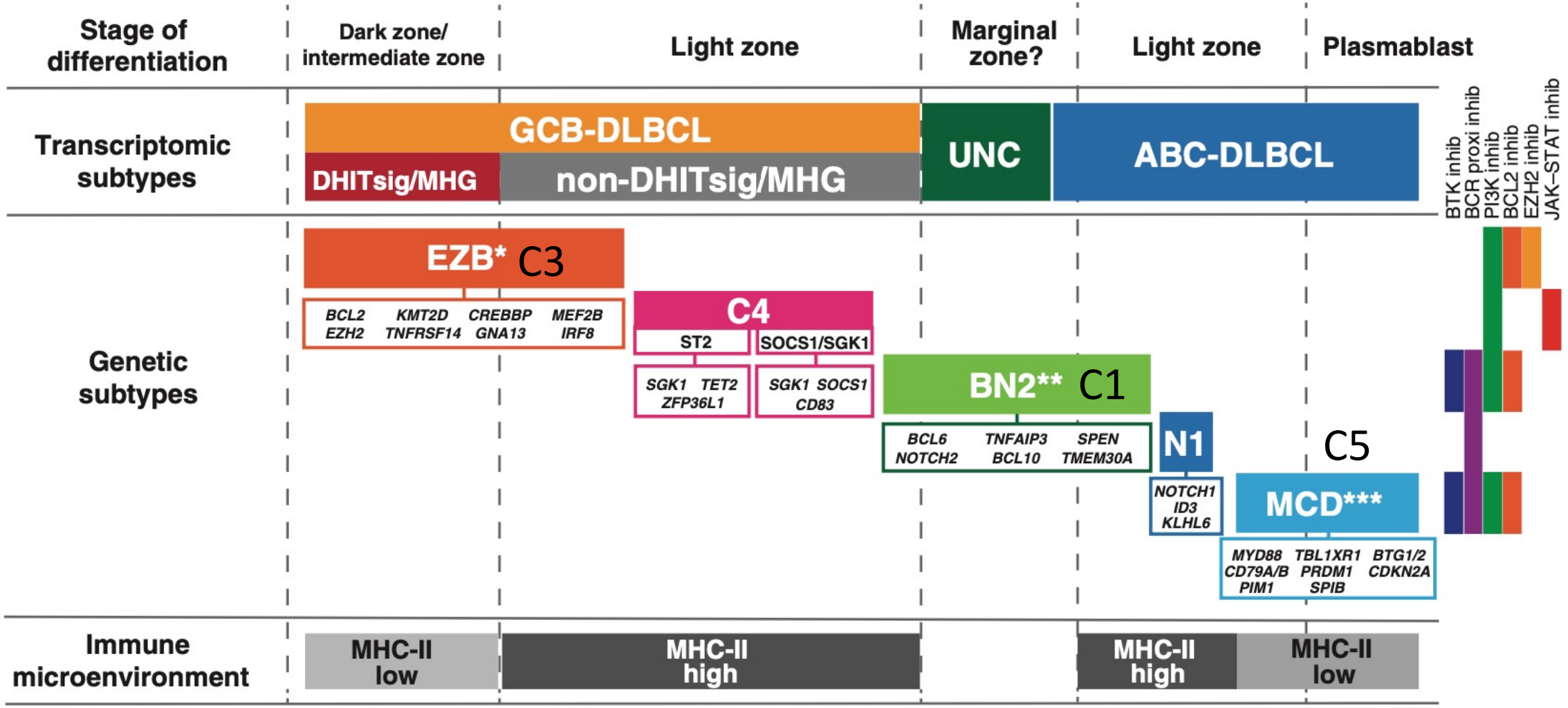
LymphGen Tool



Approximately 40% of patients remained unclassified

Wilson *et al., Cancer Cell*. 2021.

Comparison between molecular classification



Ennishi *et al., Cancer Discov*. 2020.

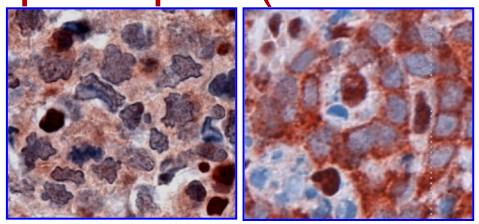
Agenda

- Different molecular classification methods for large B cell lymphomas
- **Clinical trial dedicated to specific molecular subtypes**
- Molecular classification on the liquid biopsy

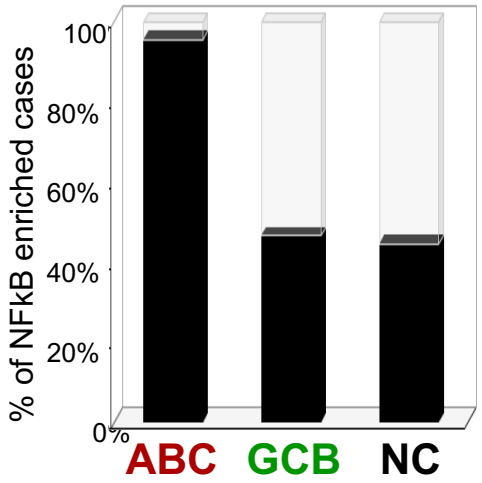
The NF-kB pathway in ABC DLBCL

100% ABC-DLBCL have NF-kB activation

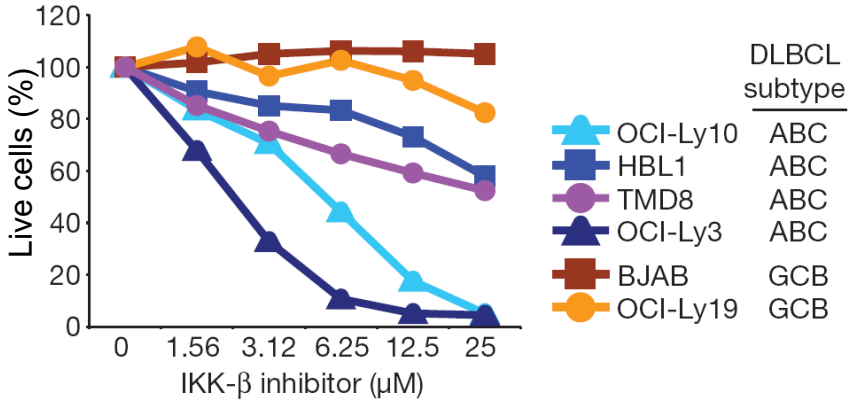
p105/p50 (classical)

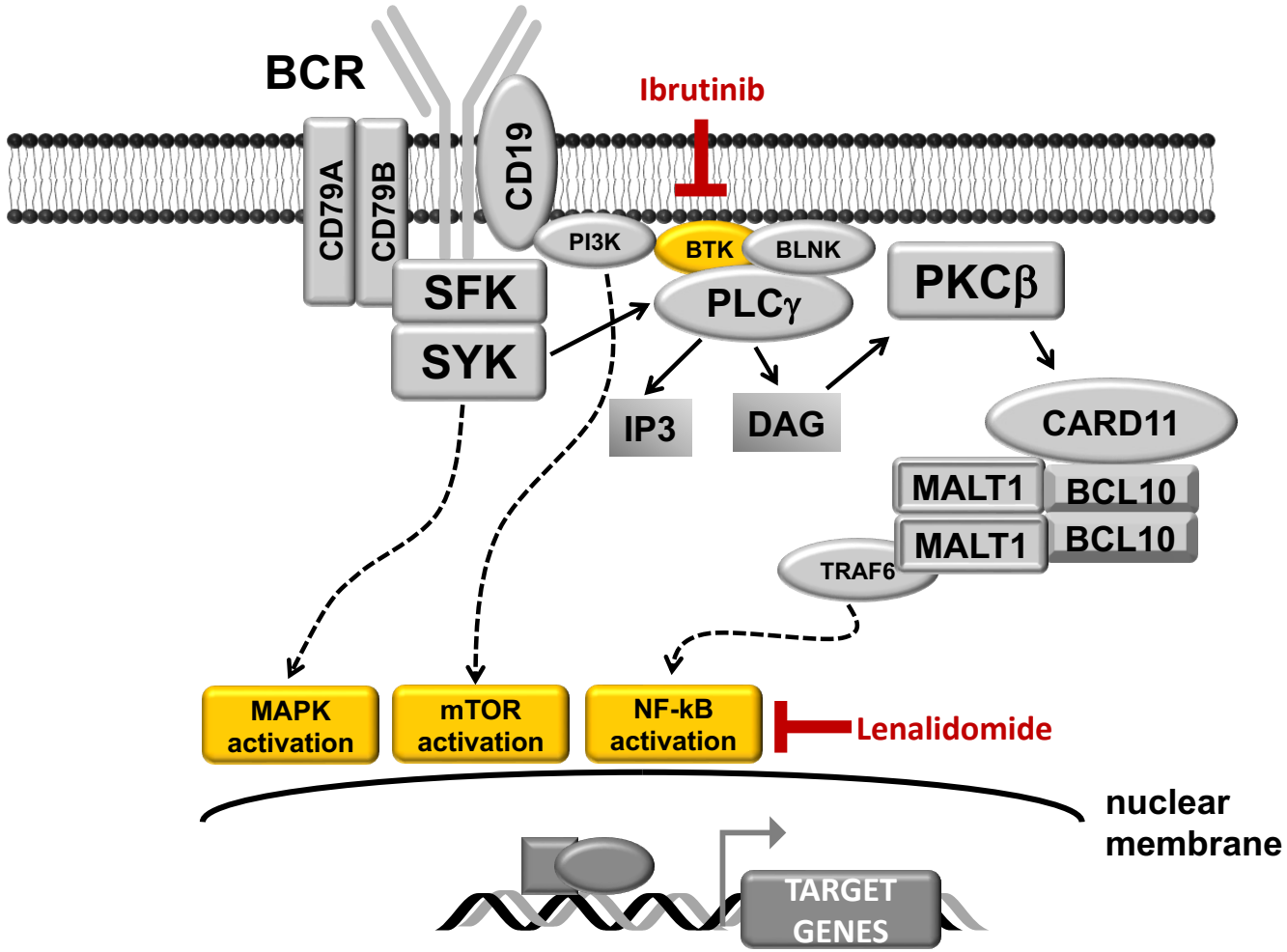


nuclear cytoplasmic



NF-kB inhibition is lethal for ABC DLBCL

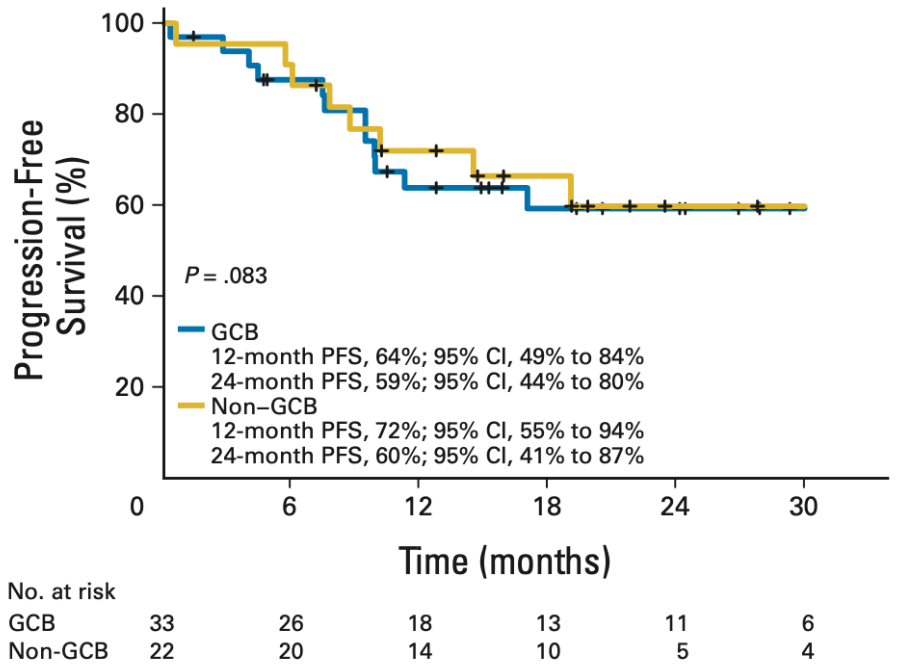




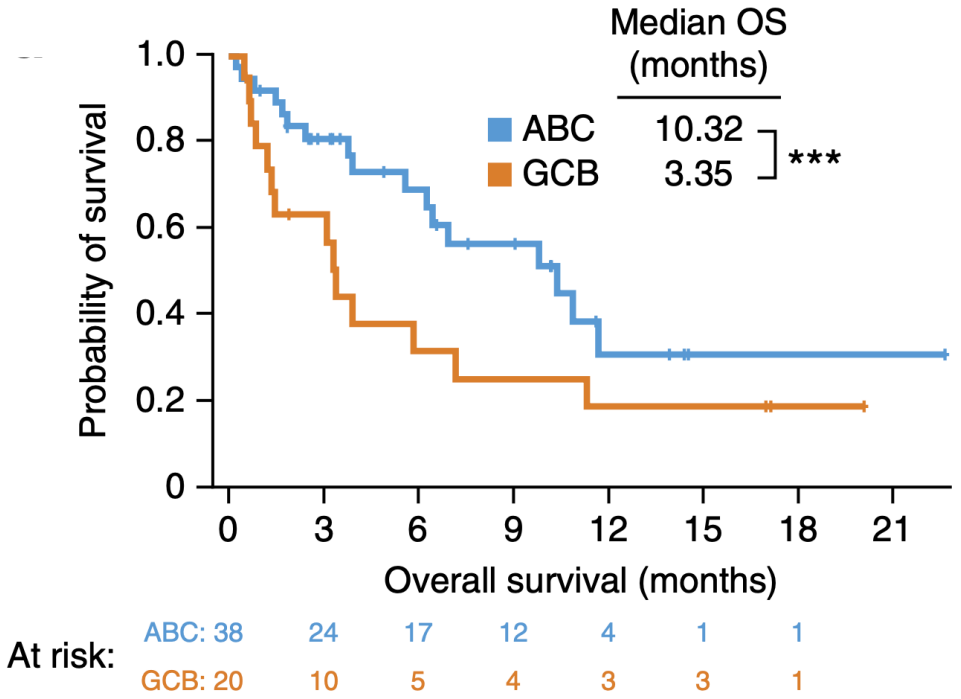
Lenz et al. NEJM, 2008
Compagno et al, Nature 2009
Davis et al, Nature 2010

Lenalidomide and ibrutinib are active in ABC DLBCL: evidences from phase Ib/II trials

Phase II trial in newly diagnosed DLBCL
Lenalidomide + R-CHOP



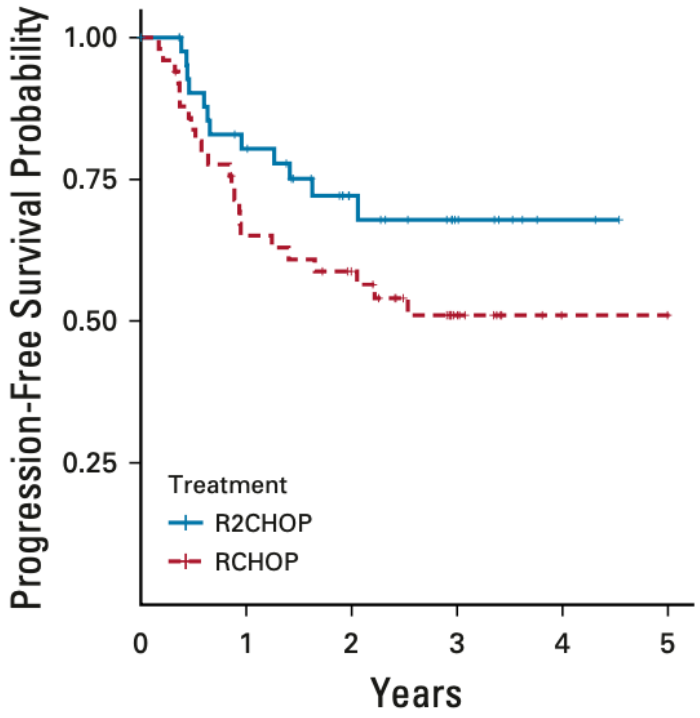
Phase Ib/II trial in R/R DLBCL
Ibrutinib



Nowakowski *et al., JCO.* 2015; Wilson *et al., Nat. Med*. 2015

Lenalidomide in ABC DLBCL: evidences from randomized phase II/III trials

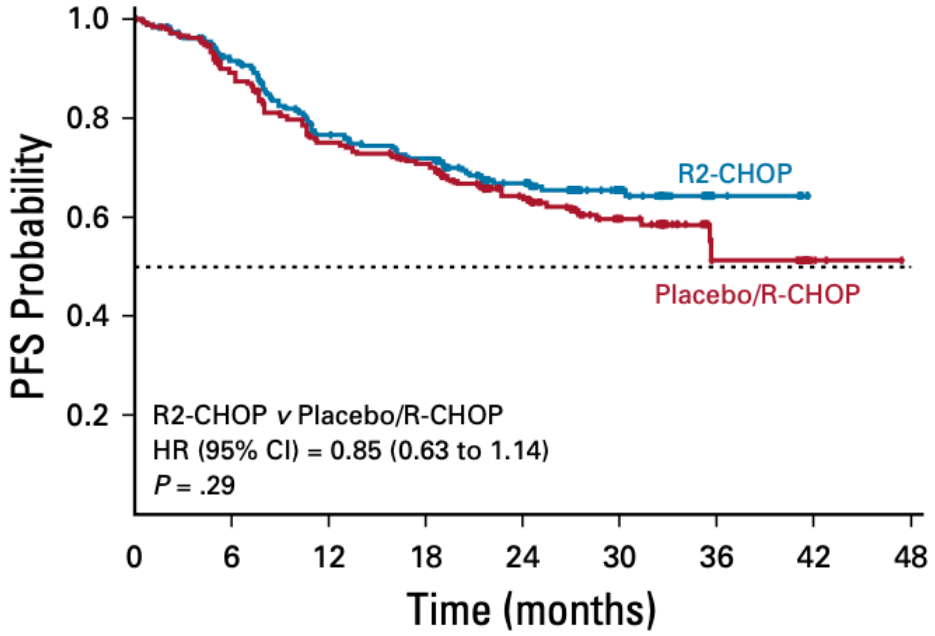
Phase II ECOG-ACRIN E1412



Number at risk						
R2CHOP	44	32	17	8	2	0
RCHOP	50	31	25	10	2	1

- Enrolled both ABC and GCB
- Median time from diagnosis to treatment: 31 days
- Lenalidomide 25 mg once daily on day 1-10

Phase III ROBUST trial



Number at Risk								
R2-CHOP	285	221	178	162	119	57	10	0
Placebo/R-CHOP	285	229	187	173	111	55	10	3	0

- Enrolled only ABC
- Median time from diagnosis to treatment: 21 days
- Lenalidomide 15 mg once daily on day 1-14

Nowakowski *et al., JCO.* 2021; Nowakowski *et al., JCO.* 2021

Ibrutinib in non-GC DLBCL: evidences from the randomized phase III Phoenix trial

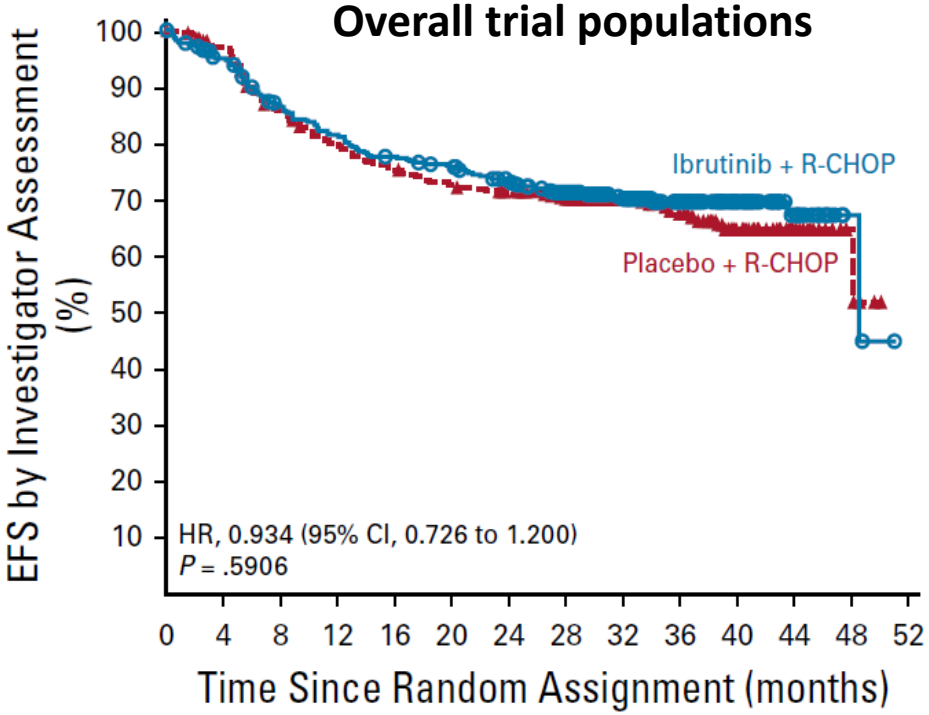
The phase III Phoenix trial
N=1490

R-CHOP + Ibrutinib

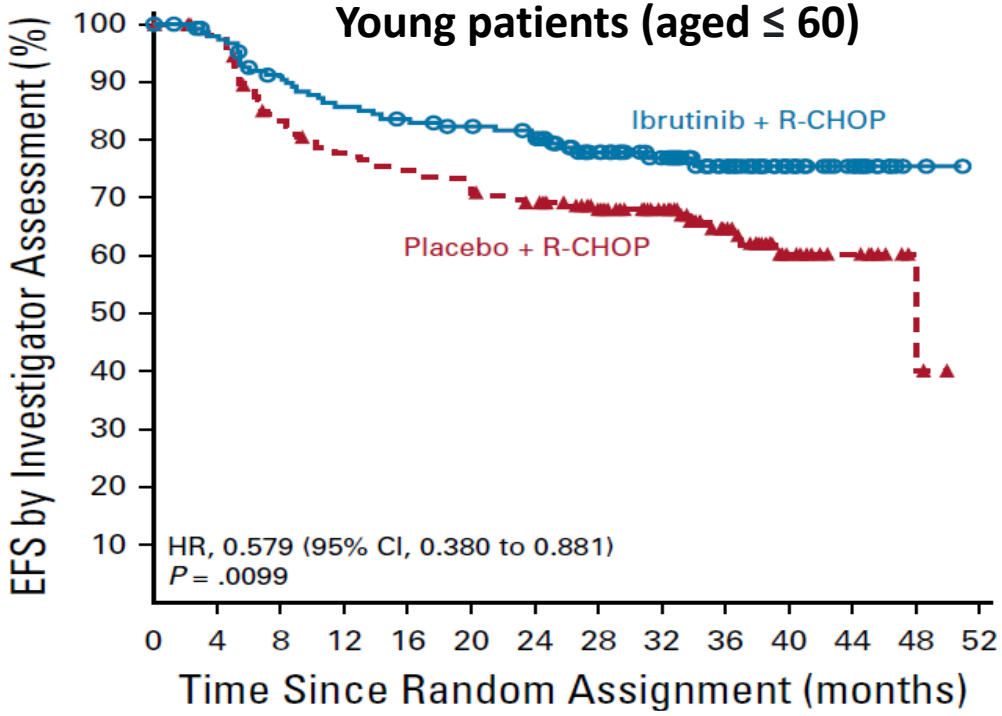
R-CHOP + Placebo

838 newly diagnosed non-GC DLBCL

Overall trial populations

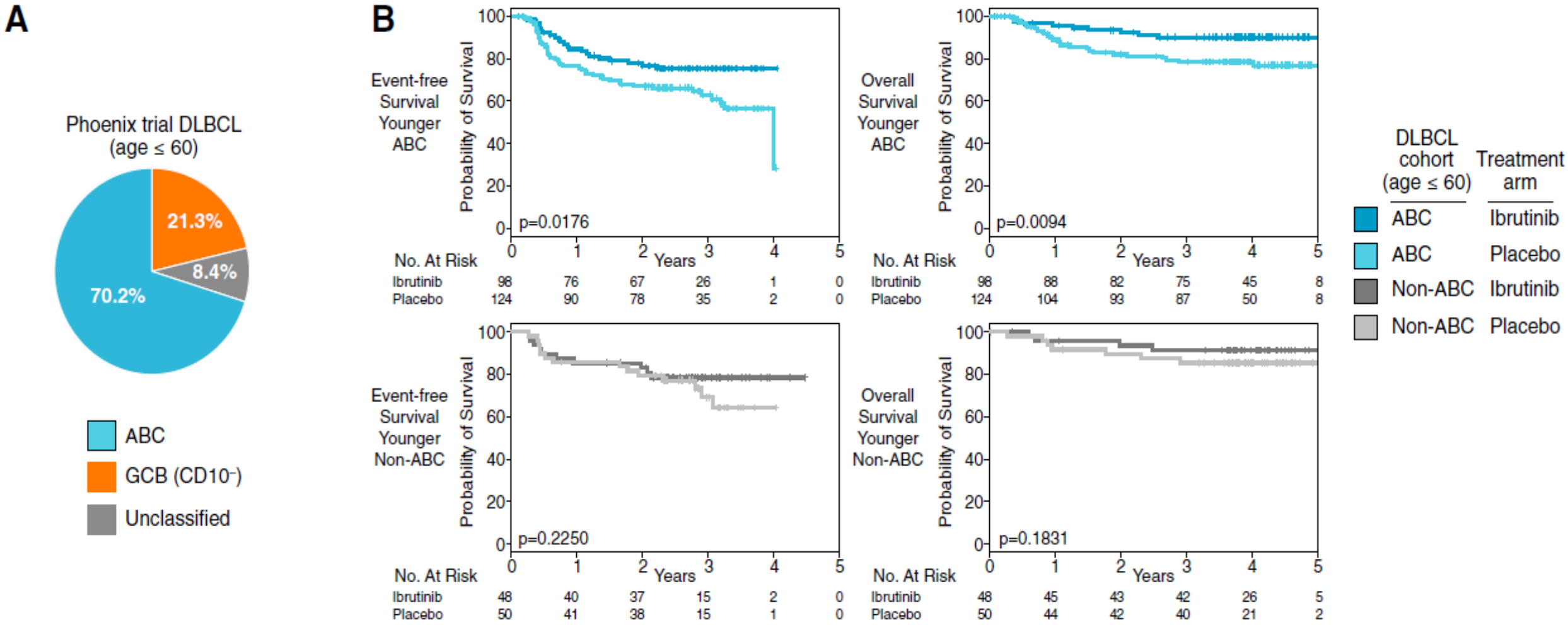


Young patients (aged ≤ 60)



Younes *et al., JCO*. 2019

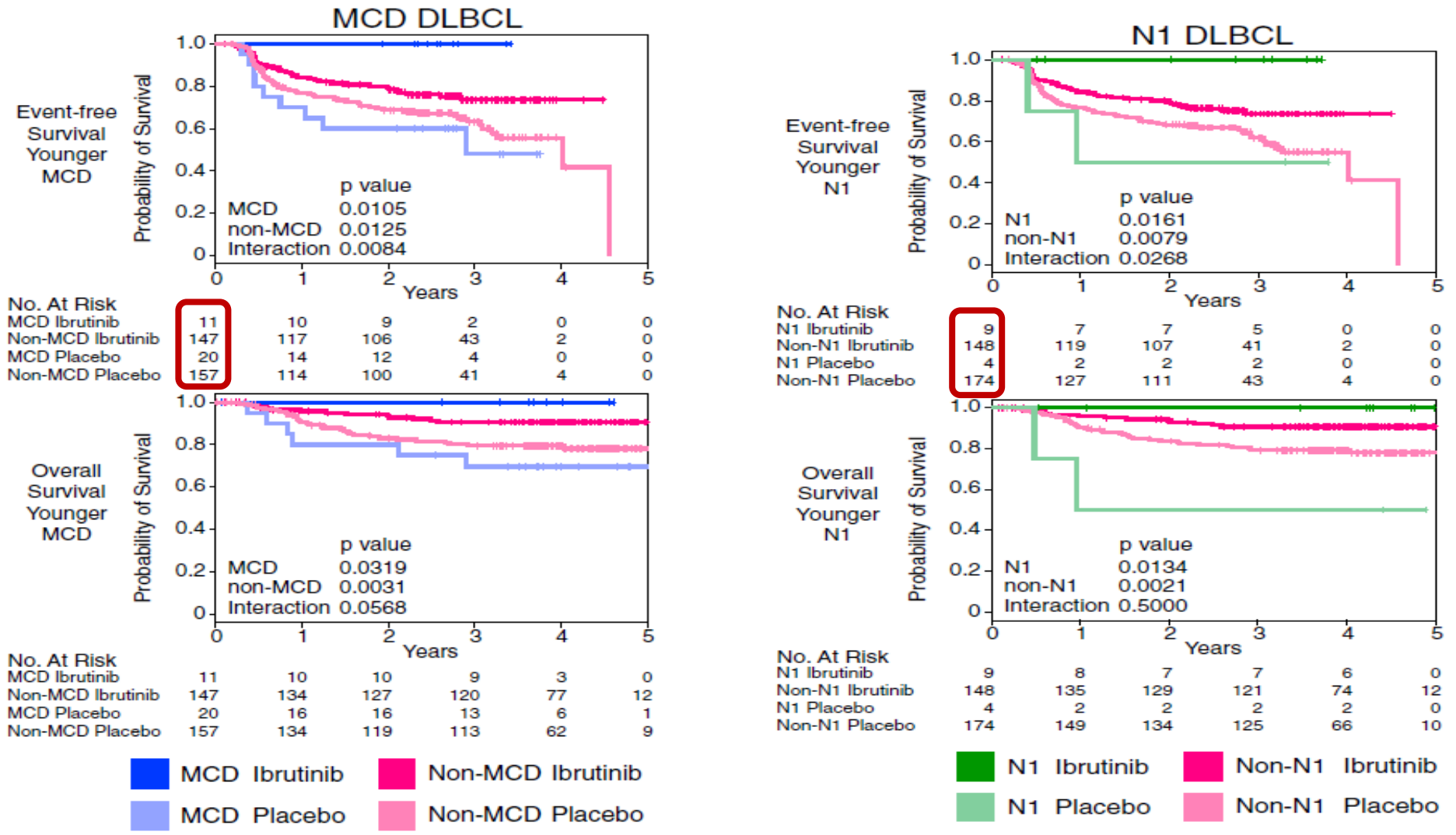
Nanostring reclassified a fraction of non-GC patients



Wilson *et al., Cancer Cell*. 2021

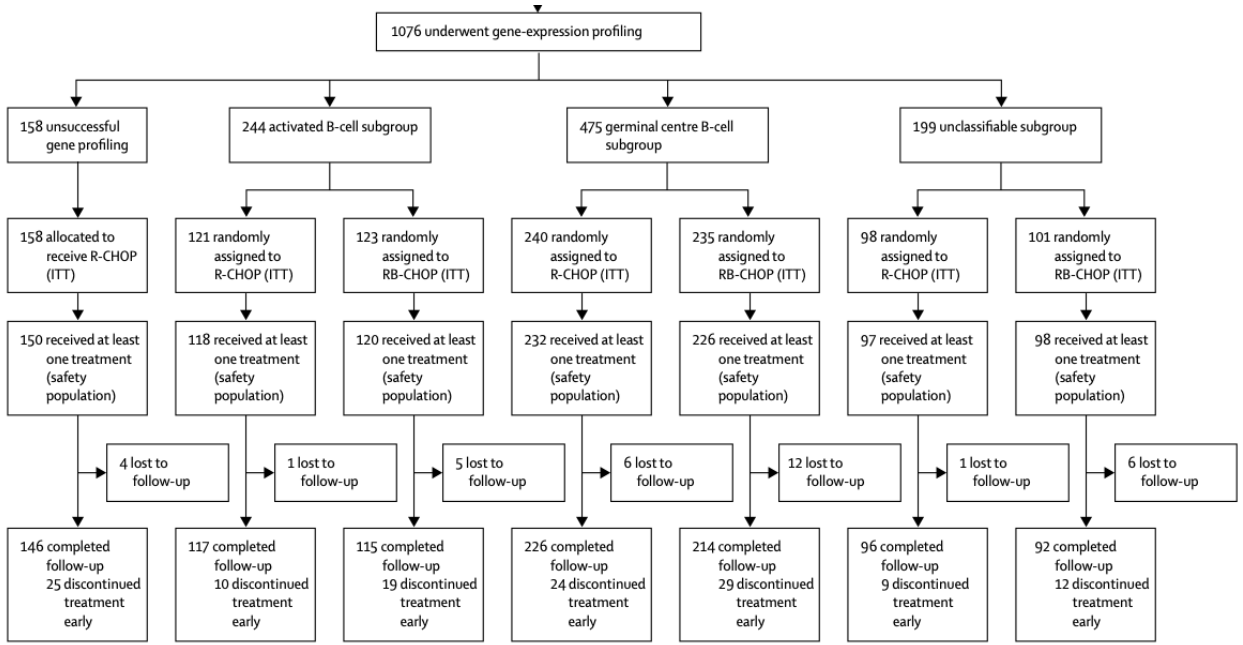
MCD and N1 patients benefit the most from ibrutinib: Sub/sub-group analysis…

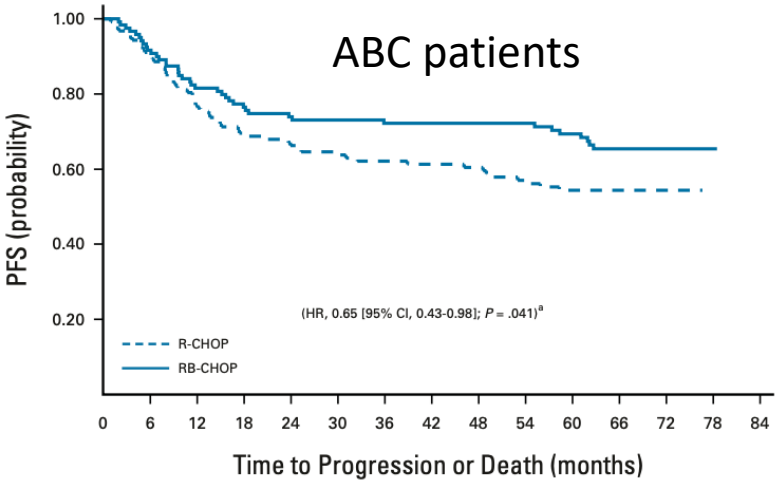
Patients aged ≤ 60 years

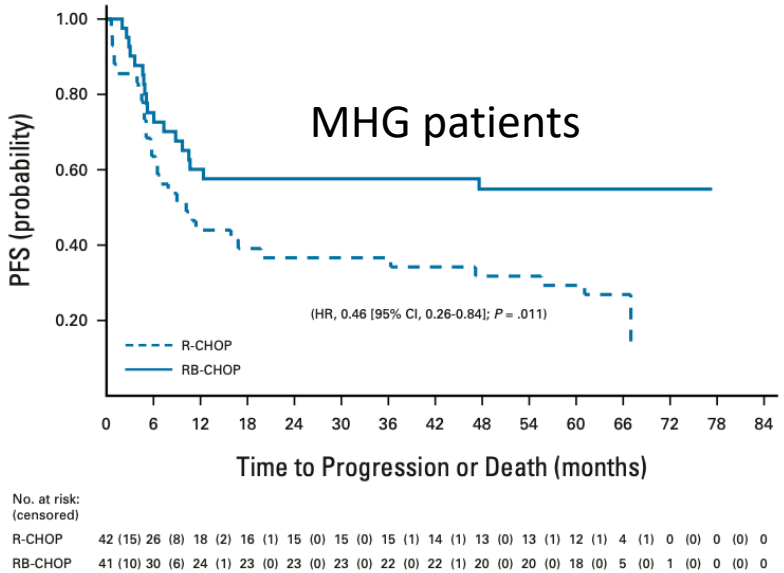


Wilson *et al., Cancer Cell*. 2021

ABC and MHG patients may benefit from R-CHOP + bortezomib: 5 years update of the REMoDL-B trial



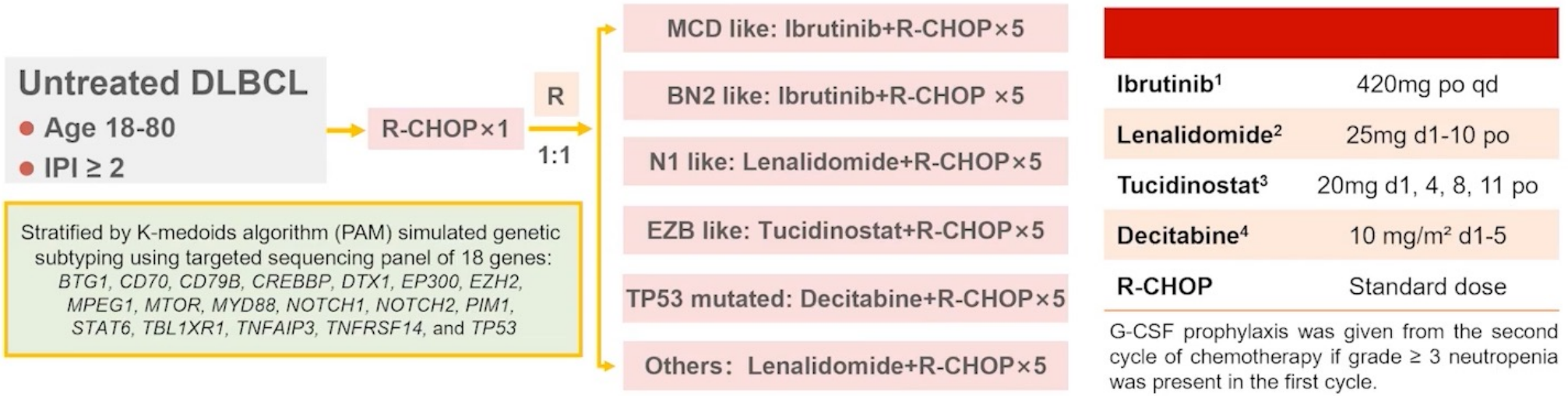




Davies *et al., Lancet Oncol*. 2019; Davies *et al., JCO.* 2023

Study Design (NCT04025593)

- The study started from **July, 2019**.
- All patients were treated with ONE cycle of standard R-CHOP immediately at diagnosis.
- Patients were randomly assigned 1:1 and stratified by genetic subtype.
- Using targeted sequencing and FISH for BCL2, MYC translocation and BCL6 fusion to classify patients into six genetic subtypes MCD like, BN2 like, N1 like, EZB like, according to **NEJM classification (2018)**, *TP53* mutation, and others.



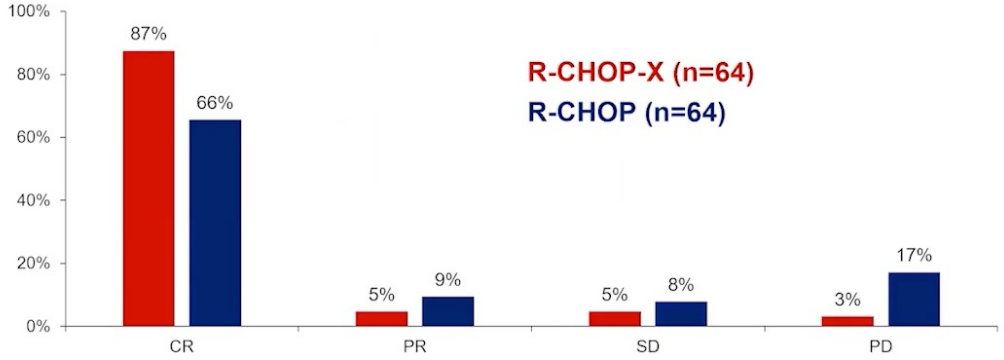
Ibrutinib[1]	420mg po qd
Lenalidomide[2]	25mg d1-10 po
Tucidinostat[3]	20mg d1, 4, 8, 11 po
Decitabine[4]	10 mg/m² d1-5
R-CHOP	Standard dose

G-CSF prophylaxis was given from the second cycle of chemotherapy if grade ≥ 3 neutropenia was present in the first cycle.

1. *Younes et al., J Clin Oncol* 2019. 2. *Nowakowski et al., J Clin Oncol* 2021. 3. *Zhang et al., Clin Epigenet* 2020. 4. *Zhang et al.*, ICML 2019 abstract (NCT02951728)

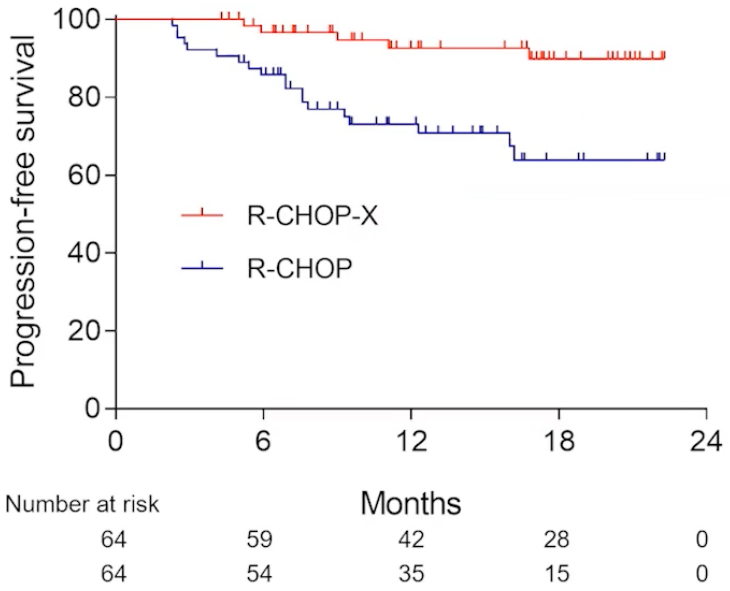
Zang *et al., ICML 2021* abstract 026.

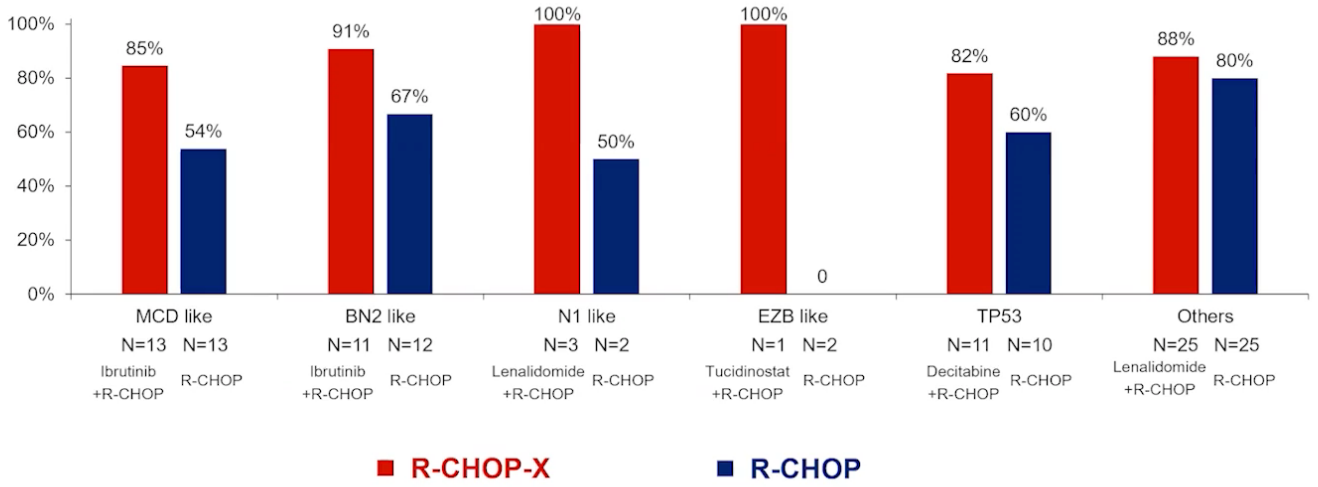
R-CHOP-X seems to improve outcome



	R-CHOP-X	R-CHOP	P value
CRR, % (95%CI)	87 (79-96)	66 (54-78)	0.003

- The study met the prespecified primary endpoint.



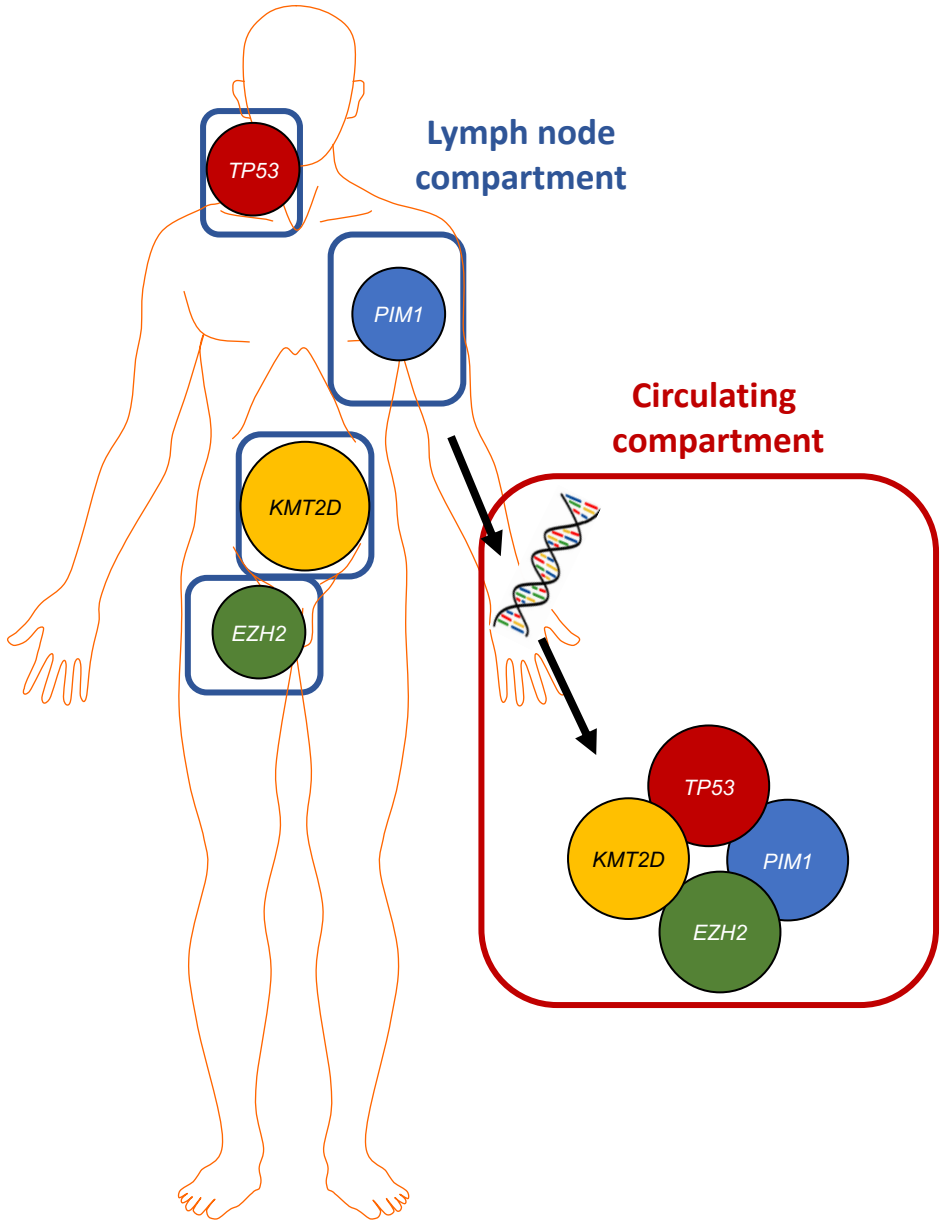


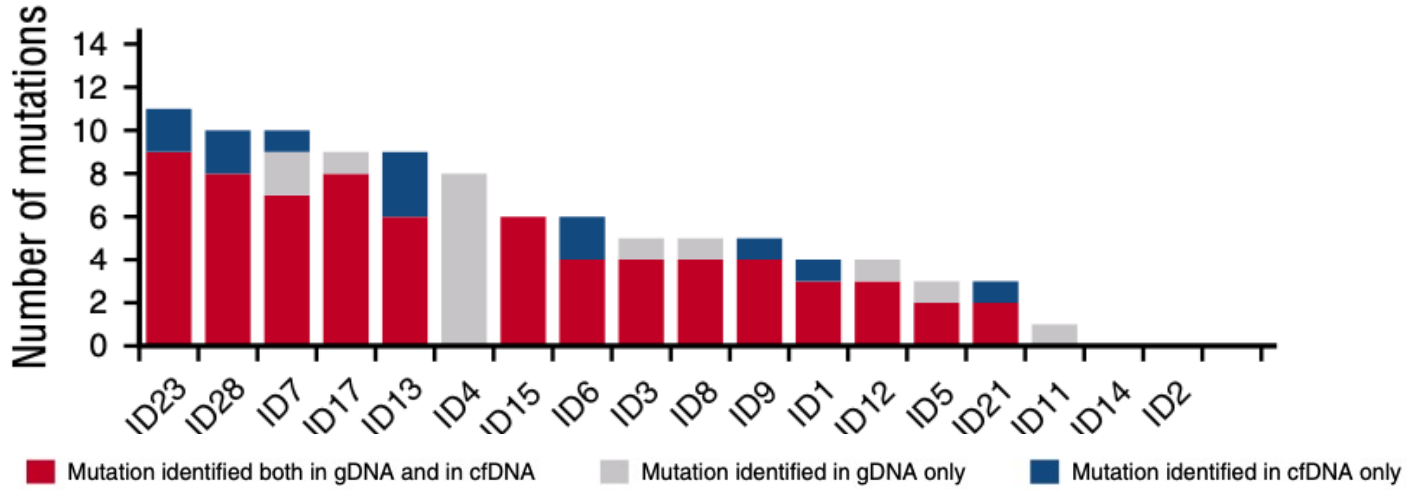
Zang *et al., ICML 2021* abstract 026.

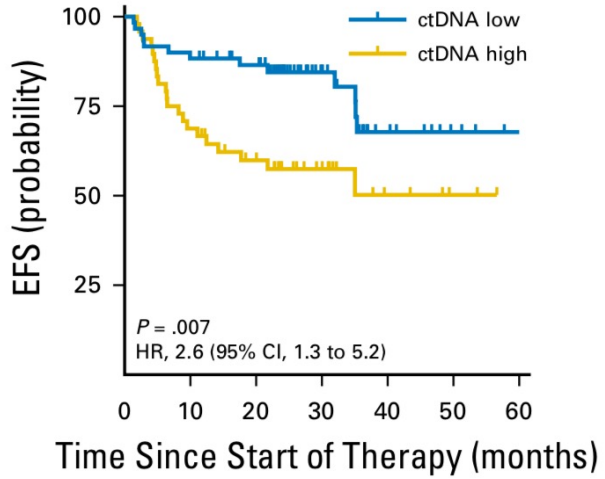
Agenda

- Different molecular classification methods for large B cell lymphomas
- Clinical trial dedicated to specific molecular subtypes
- **Molecular classification on the liquid biopsy**

ctDNA is a tool for DLBCL genotyping and for prognostic prediction

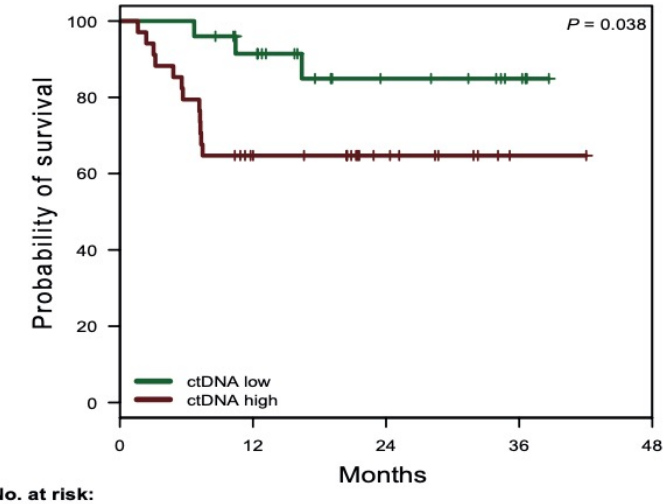






No. at risk:

ctDNA low	60	53	47	23	10	4	1
ctDNA high	48	33	25	13	5	2	0

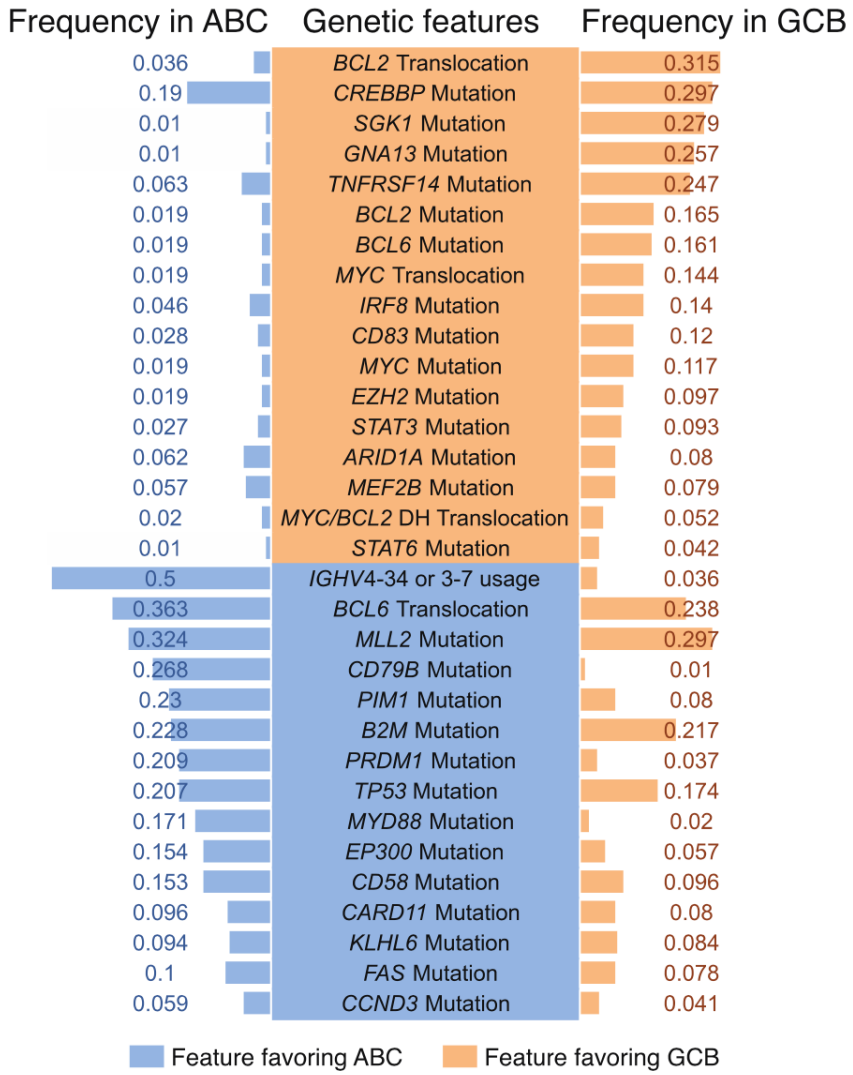


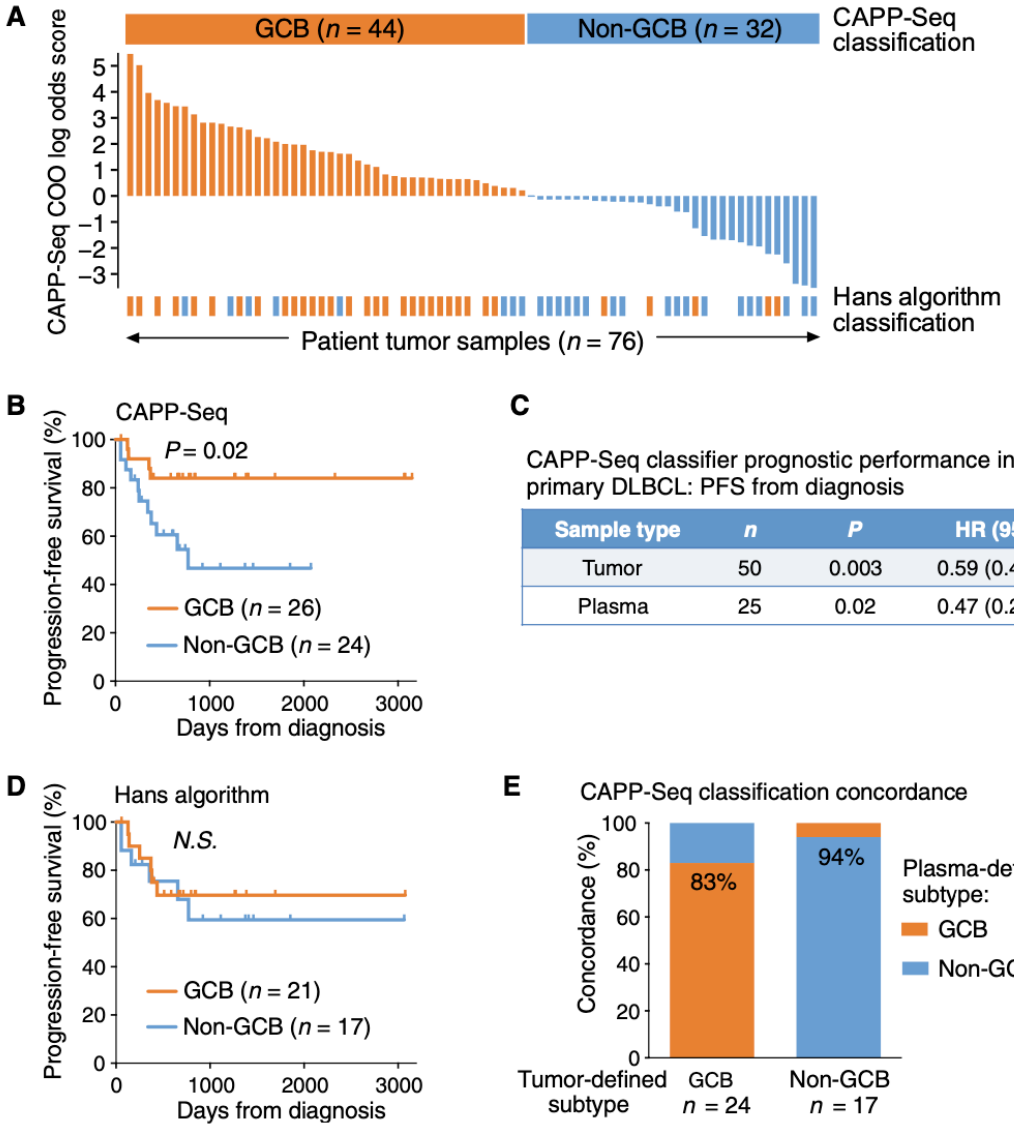
No. at risk:

ctDNA low	25	20	9	4	0
ctDNA high	34	18	9	1	0

Rossi *et al., Blood*. 2017; Kurtz *et al., JCO.* 2018; Rivas-Delgado *et al., Clin Cancer Res*. 2021

COO classification on the liquid biopsy





Scherer *et al., Sci Transl Med*. 2016

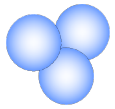
Experimental workflow

Università Piemonte Orientale

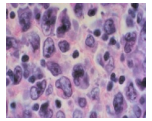
Sapienza University

77 newly diagnosed DLBCL patients

ctDNA from plasma



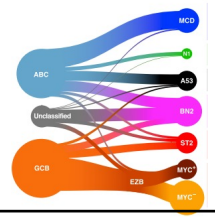
Peripheral blood cells



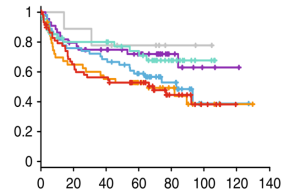
Tumor gDNA from LN (Fresh and FFPE)

Designed in collaboration with IEO

CAPP-Seq panel of genes recurrently mutated in mature B-cell malignancies



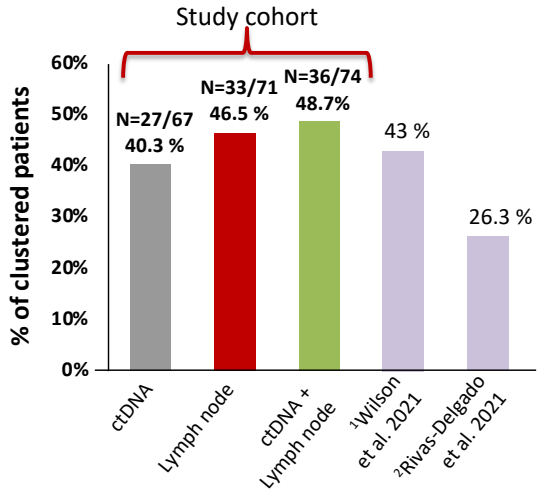
Cluster generation using the LymphGen tool[1] in LN vs ctDNA

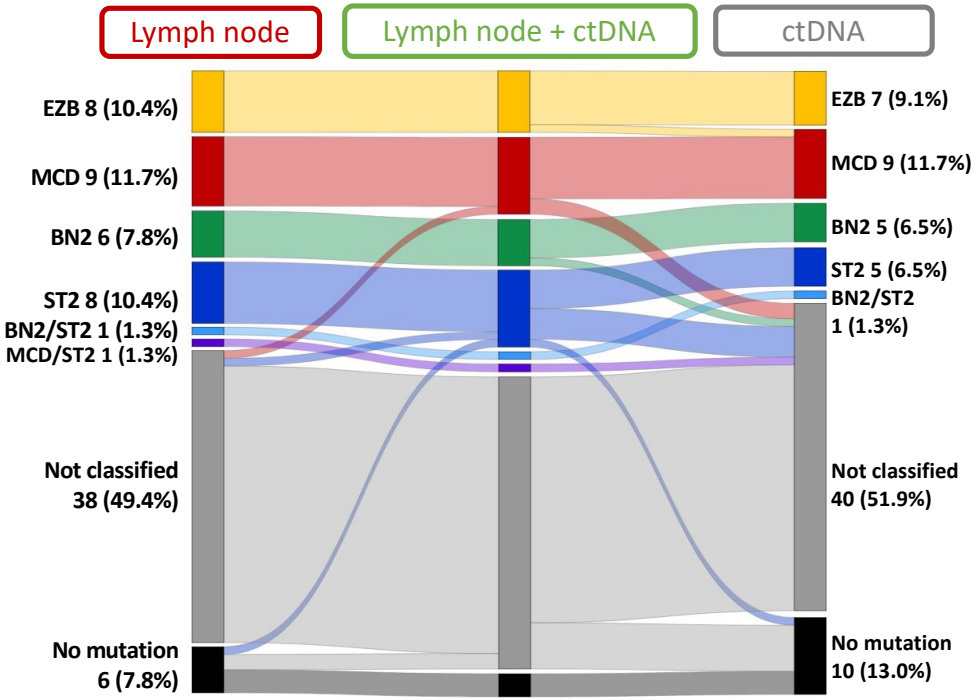


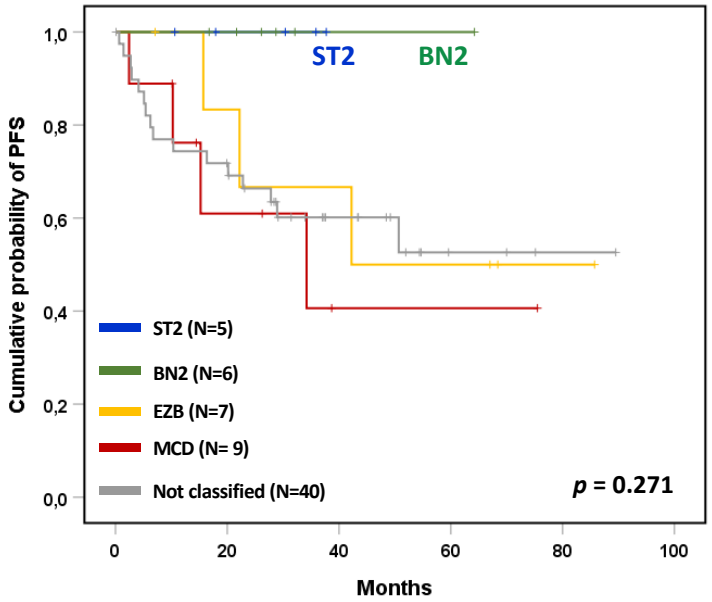
Stratify patients outcome

[1]Wright *et al., Cancer Cell.* 2020

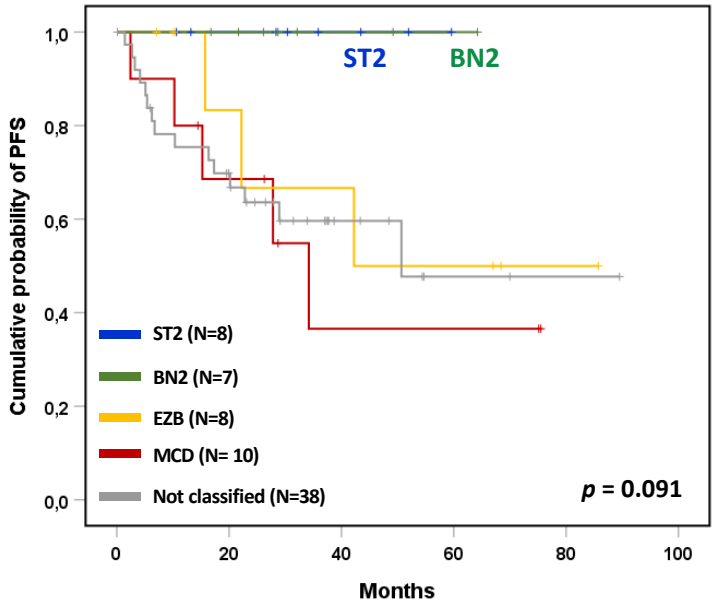
Clinical impact of molecular clusters and of ctDNA load







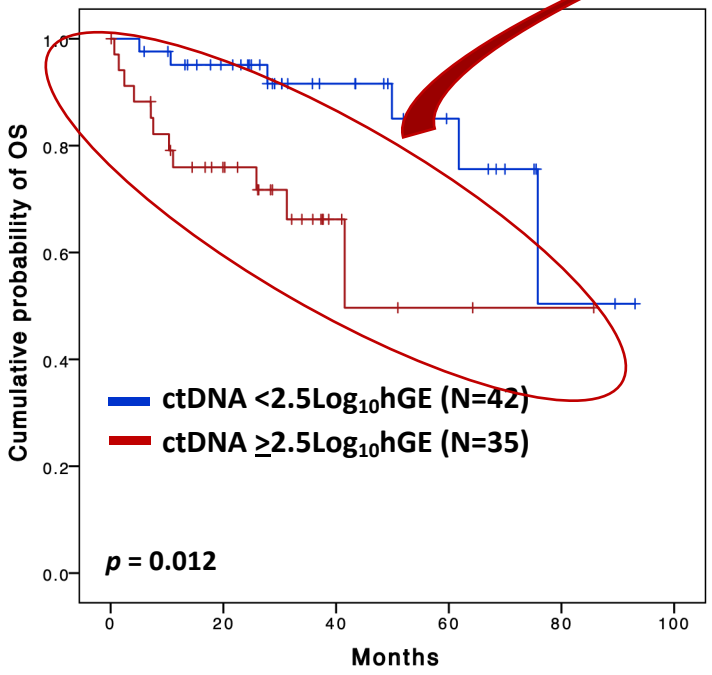
Cluster on ctDNA (N=67)



Cluster on lymph node (N=71)

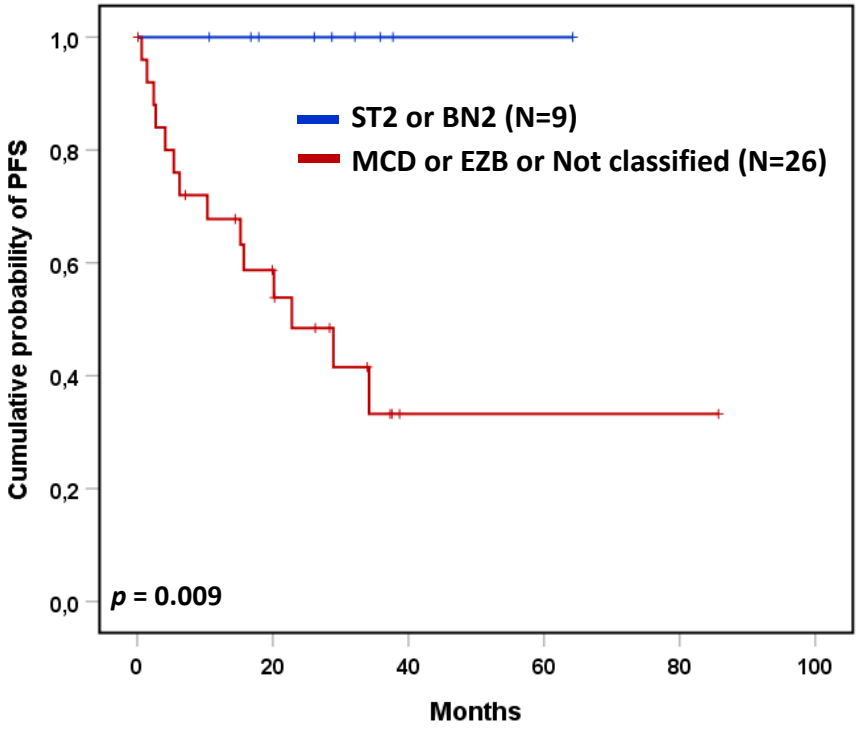
BN2/ST2 clusters predict outcome in patients with ctDNA levels >2.5 Log10 hGE

The baseline ctDNA cut-off of 2.5 Log_{10}hGE predicts survival

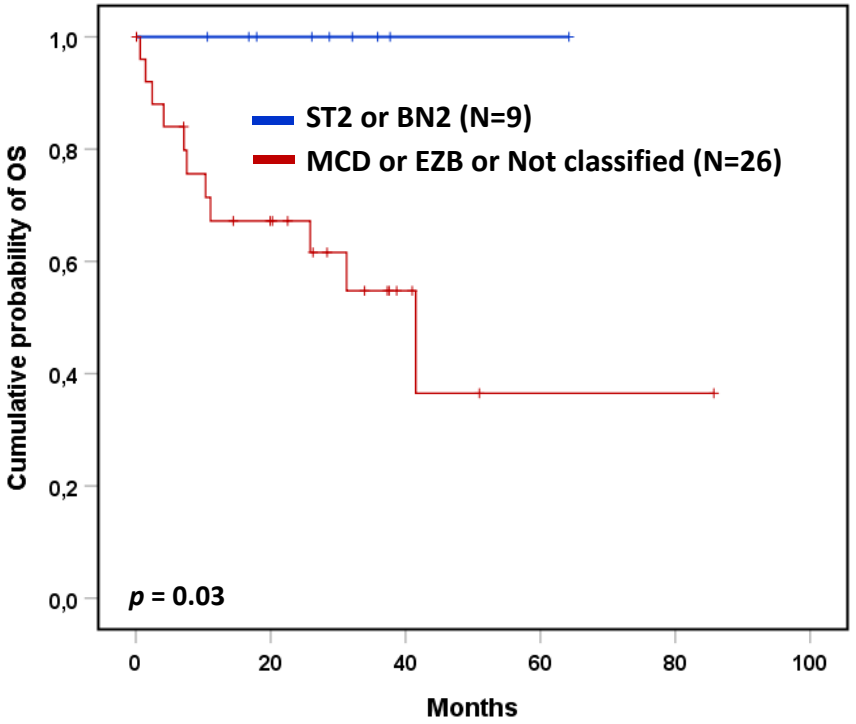


Molecular cluster

Progression-free survival



Overall survival

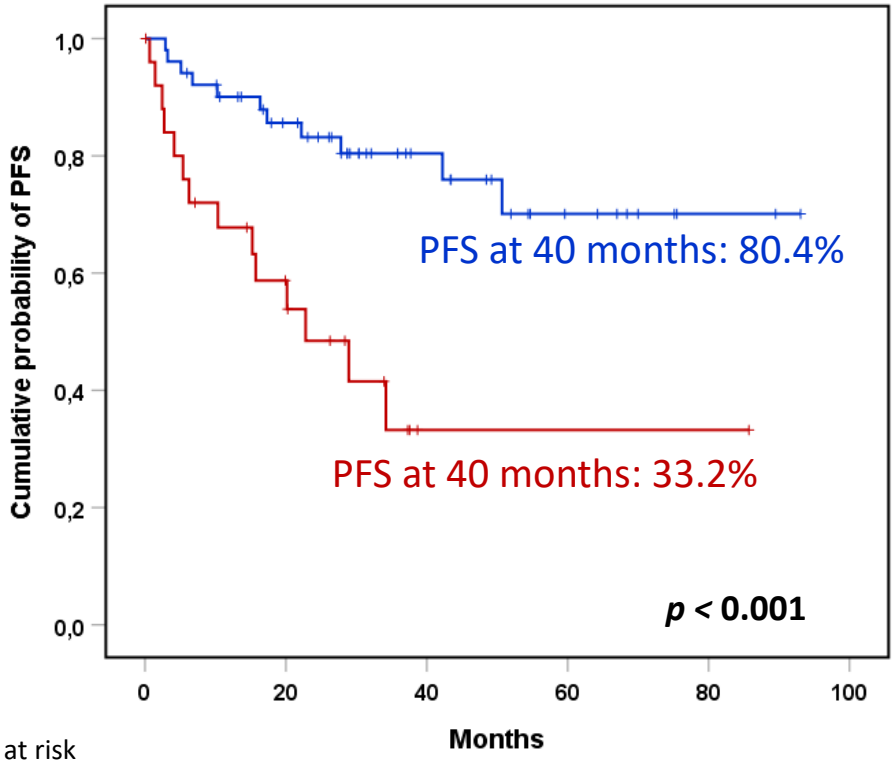


ctDNA load and molecular cluster improved patient stratification

ctDNA < 2.5 Log_{10}hGE and/or BN2/ST2 cluster

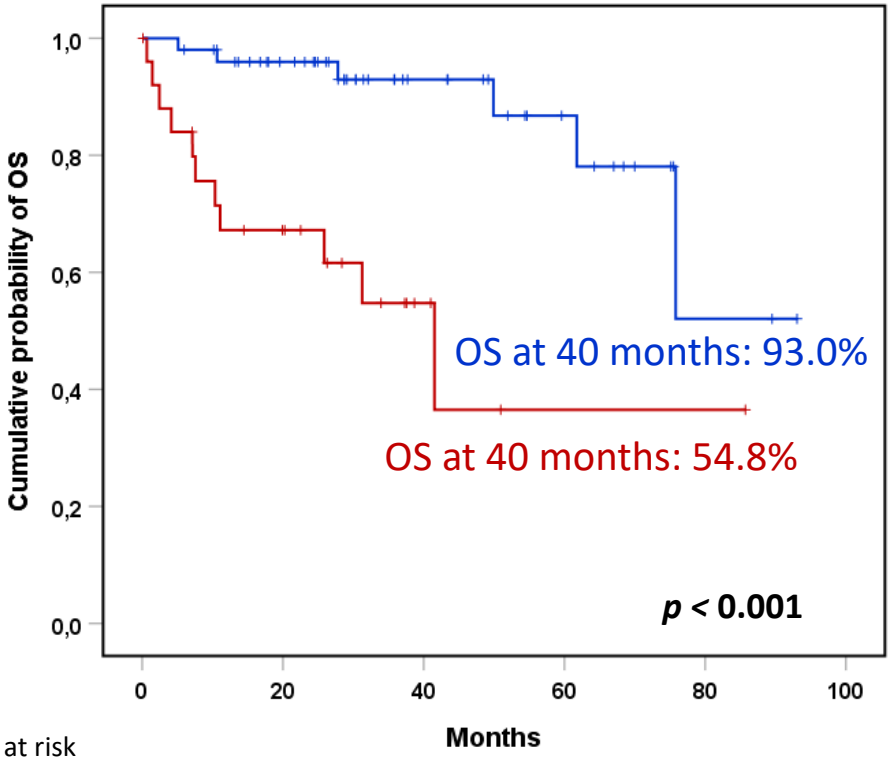
ctDNA > 2.5 Log_{10}hGE and no BN2/ST2 cluster

Progression-free survival



N. at risk						
	51	36	18	8	2	0
	26	12	1	1	1	0

Overall survival



N. at risk						
	51	39	19	10	2	0
	26	14	4	1	1	0

Conclusions

- The advances in genomic analysis significantly improved the knowledge of the biology of different molecular subgroups of Large B-cell Lymphomas

- Different clinical trials used targeted therapies trying to tackle unique vulnerabilities in each molecular subtypes without however improving outcome

- The step forward in the management of Large B-cell Lymphomas could be represented by clinical trials coupling baseline molecular features with the modulation of treatment intensity based on dynamic ctDNA monitoring