From Cell of Origin to Mutational Landscape

Laura Hilton, PhD

BC Cancer Centre for Lymphoid Cancer

IUCLS 2023

BC CANCER

Provincial Health Services Authority

Disclosures

Dr. Hilton

Nothing to disclose

Dr. Scott

Consulting: Abbvie, AstraZeneca, Incyte, Janssen

Research funding: Janssen, Roche/Genentech

Patents: named inventor on patents related to using gene expression to identify subtypes of aggressive B-cell lymphomas – one of which is licensed to NanoString Technologies

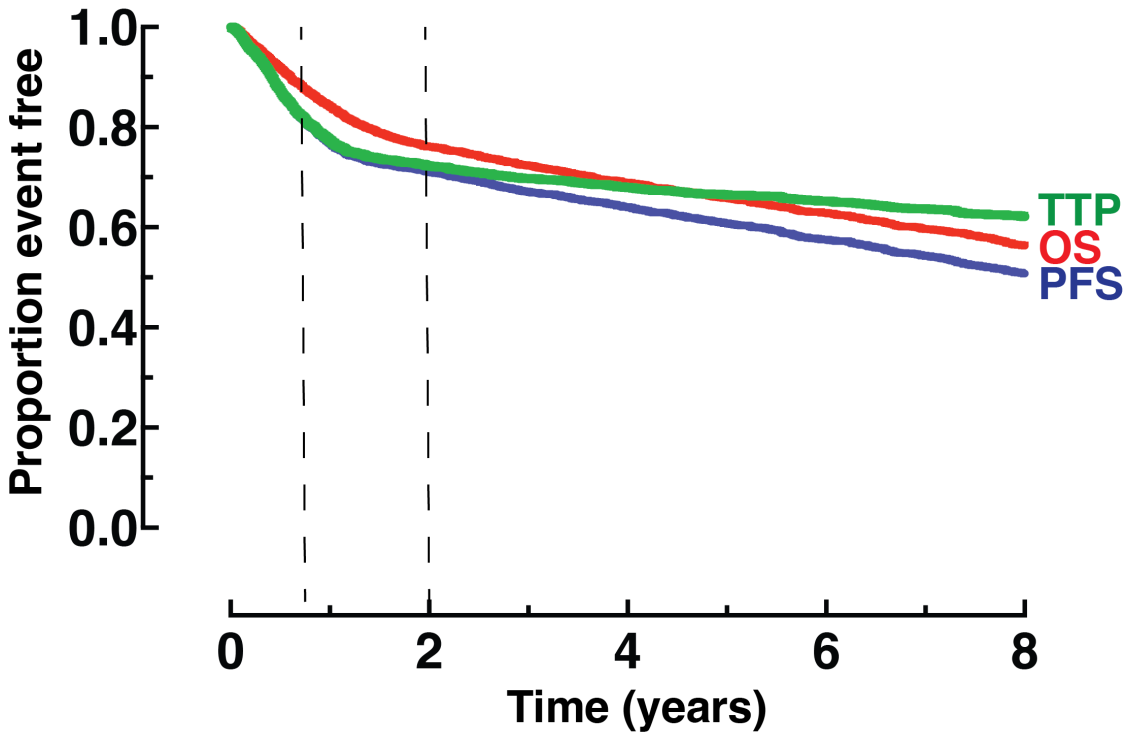
Off-label medications: discussion of targeted agents in the treatment of diffuse large B-cell lymphoma

Objectives

- Present the history of gene expression- and genetics-based classification of DLBCL

- Discuss the application of molecular classification to precision medicine in DLBCL

DLBCL – the clinical problem



BC Cancer R-CHOP treated patients
2001-2020 n = 3264

- ~70% of patients experience long-term remission with R-CHOP
- Upfront treatment (R-CHOP) has not changed in over 2 decades
- Despite a growing arsenal of treatments at relapse, outcomes are still poor

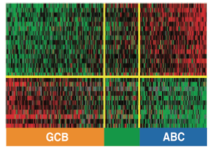
Major improvements will require a better understanding of the disease

Classification – the ideal

- **Sizeable homogeneous groups defined by strong shared (preferably targetable) biology**
- **Places all (or most) tumors into a category**
- **Can be widely (or universally) integrated into diagnostic workflows**
  - **Performed on routinely available materials**
  - **Appropriate turn-around-time to guide management**

Associations to outcomes with current therapy is not a requirement

Timeline of molecular classification in DLBCL



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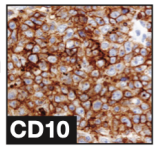

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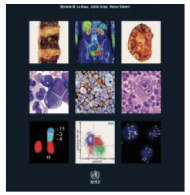

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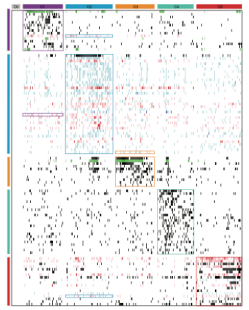

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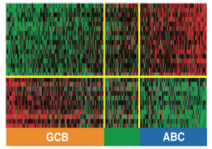

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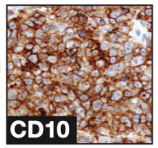

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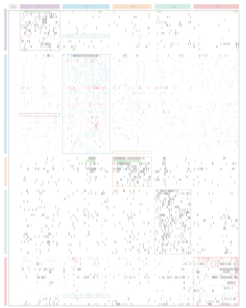

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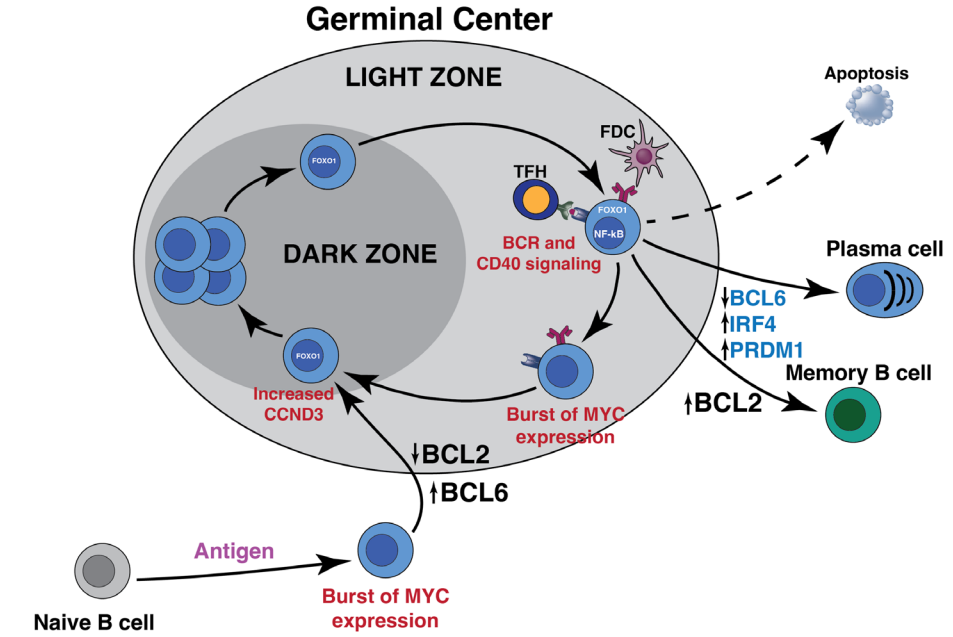
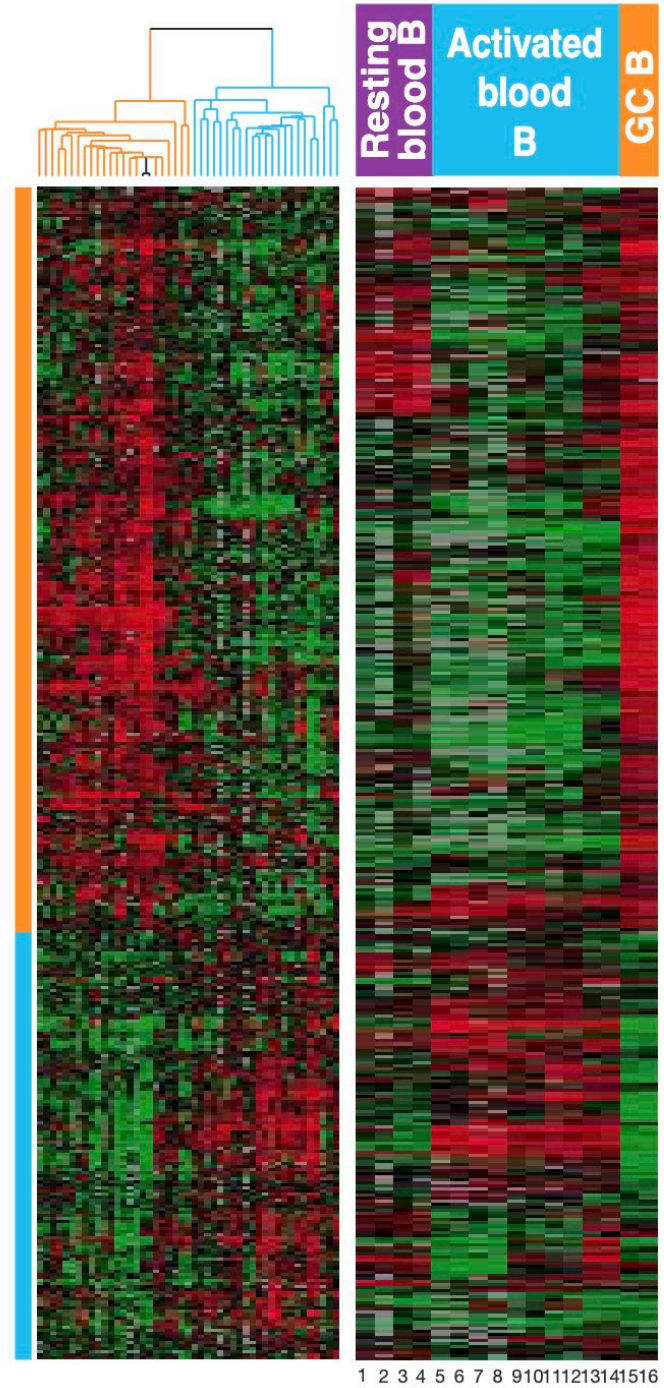


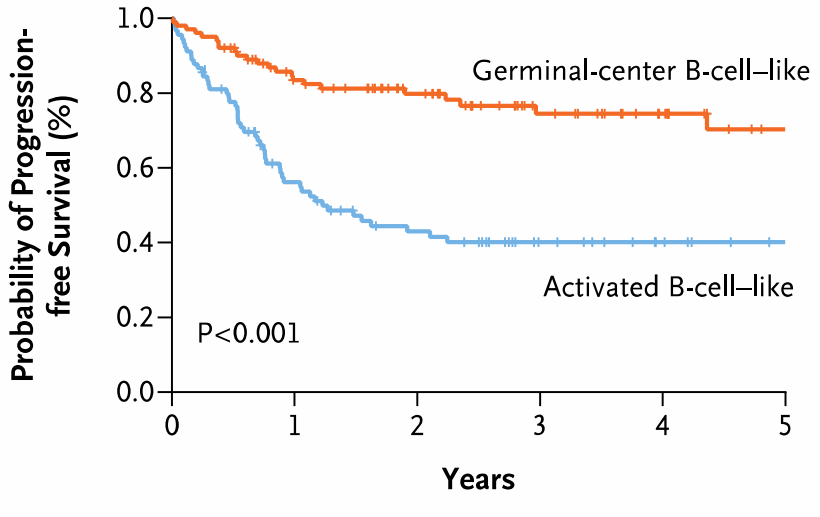
Alizadeh et al Nature 2000

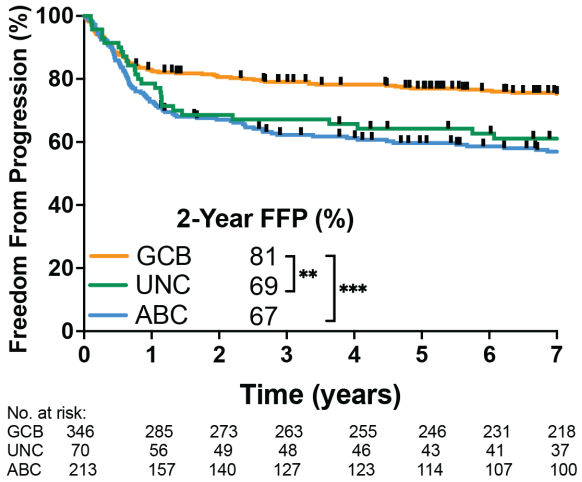


Cell-of-origin – the Foundation

- Comparison of gene expression of tumors with B-cells at different stages of differentiation
- Clustering approach producing binary groups – GCB v ABC
- Defined patient groups with distinct outcomes following CHOP and then R-CHOP

R-CHOP





BC Cancer 2005-10

Alizadeh et al Nature 2000 *Rosenwald et al N Eng J Med 2002* *Lenz et al N Eng J Med 2008*

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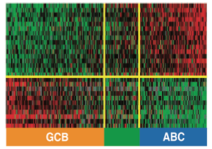

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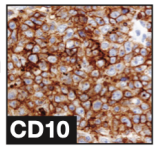

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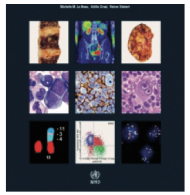

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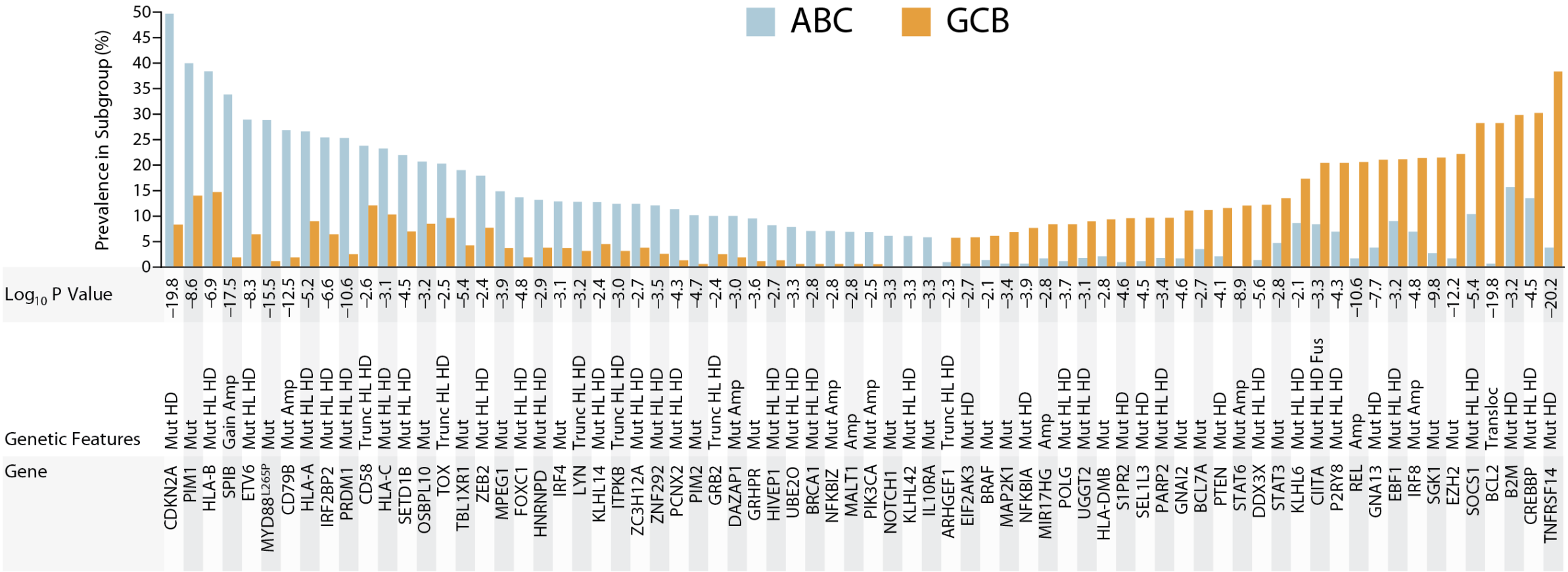
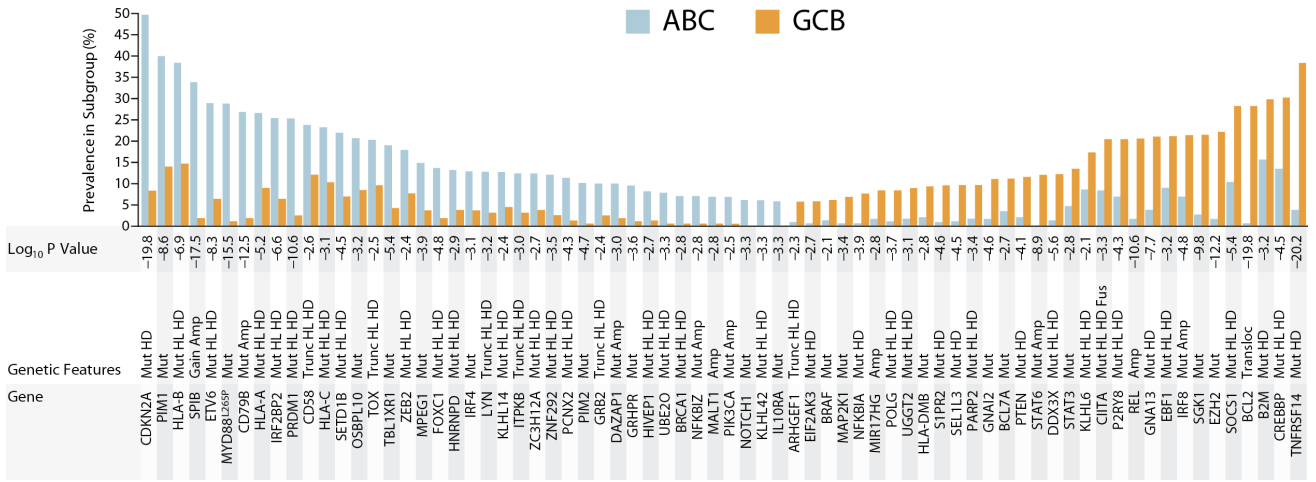
Cell-of-origin – distinct mutational landscapes



Schmitz et al N Eng J Med 2018

Cell-of-origin – distinct (targetable) biology

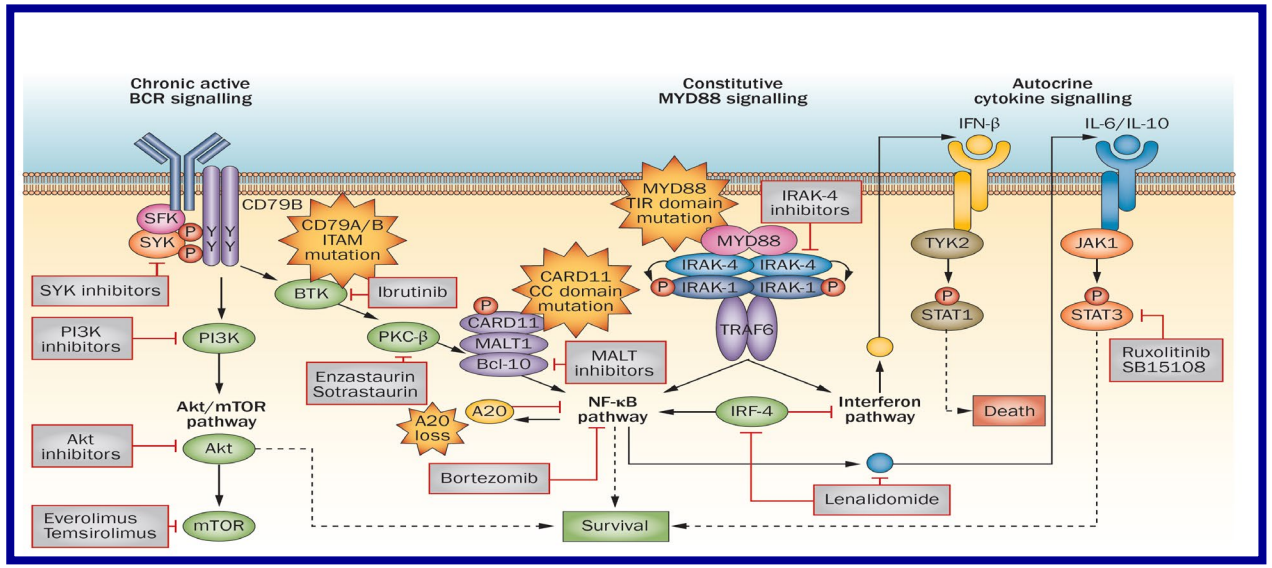


	ABC-DLBCL	GCB-DLBCL
Differentiation Block	Up to plasmablast stage - *BCL6, PRDM1*	Up to the light zone stage - Epigenetic
Proliferation	Chronic Active BCR Signaling	Toncogenic BCR Signaling
Survival	*BCL2* Amplification	*BCL2* Rearrangement

Schmitz et al N Eng J Med 2018

Distinct Signaling Pathways According to Cell-of-Origin & Potential Agents

ABC



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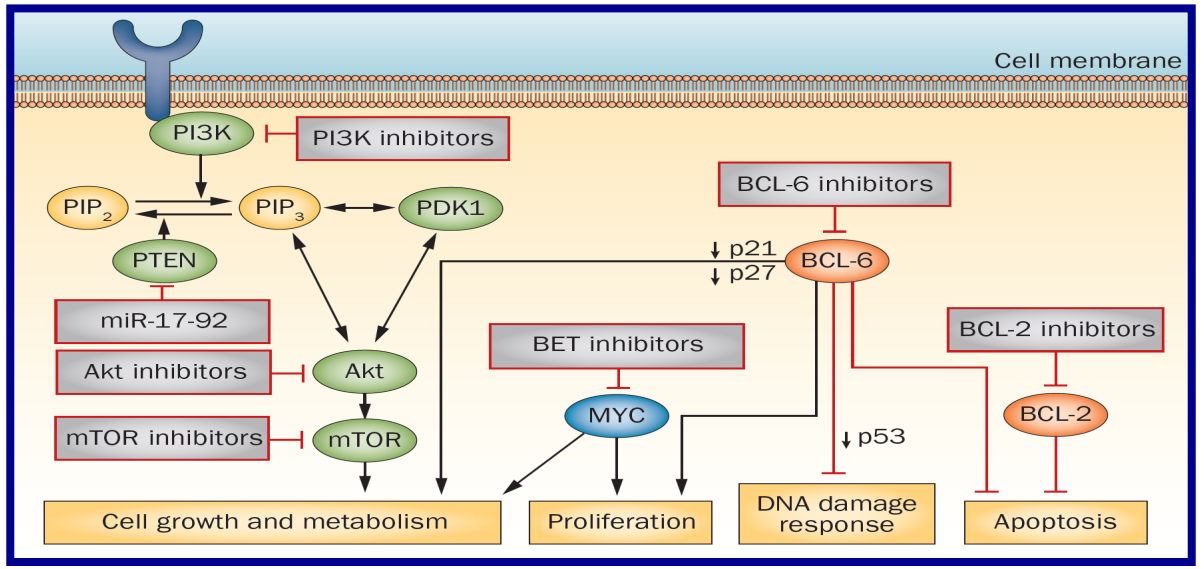

Roschewski, et al Nat Rev Clin Oncol 2014

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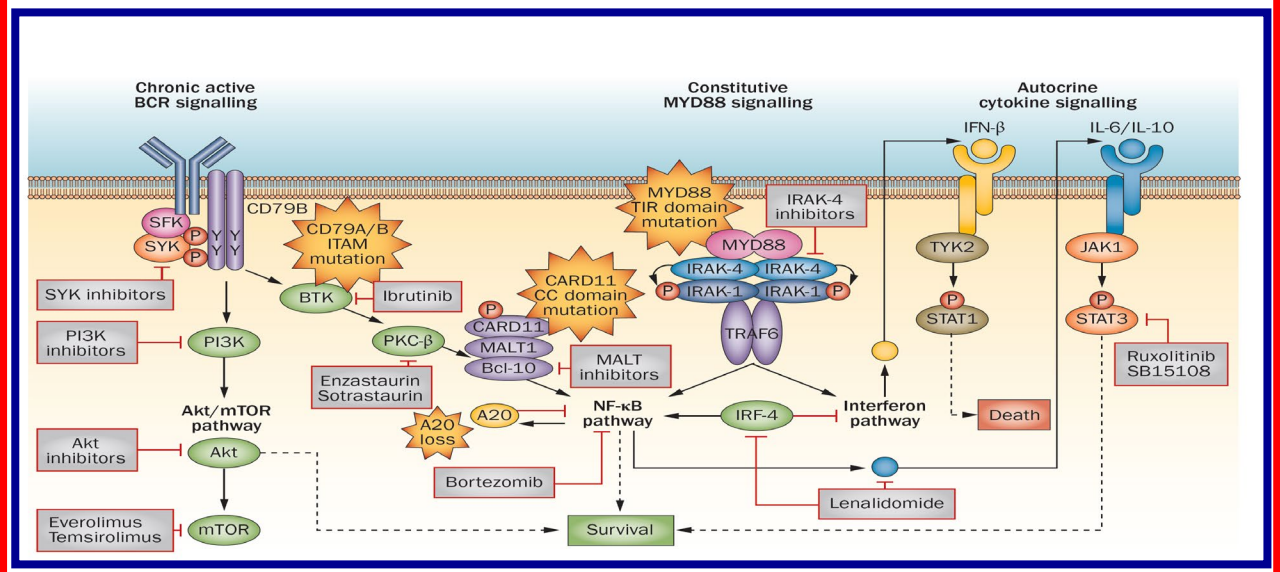

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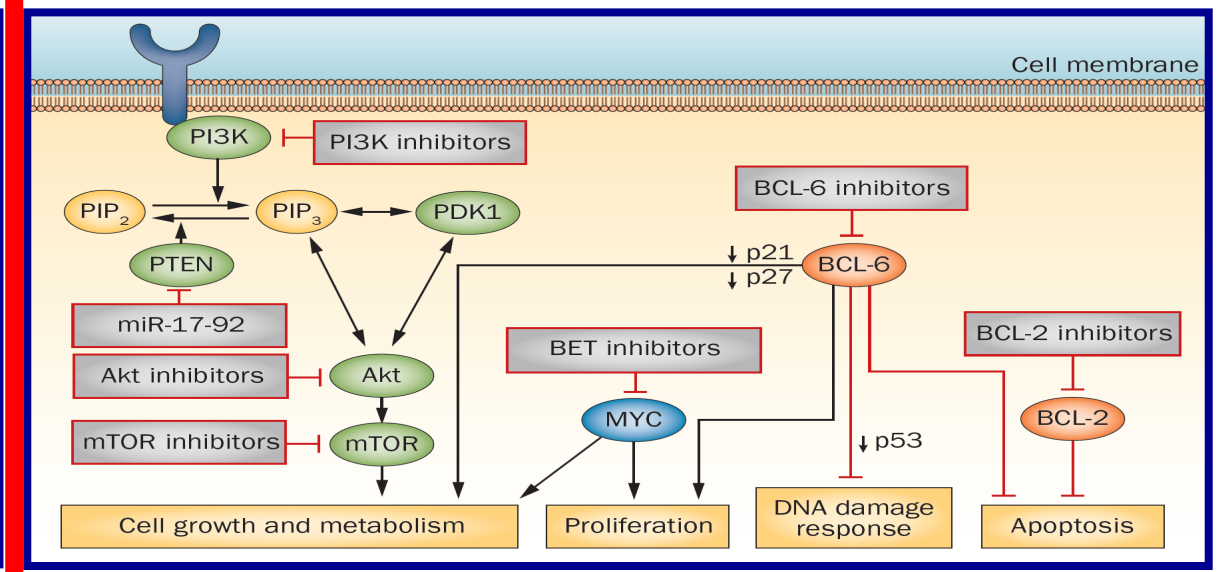
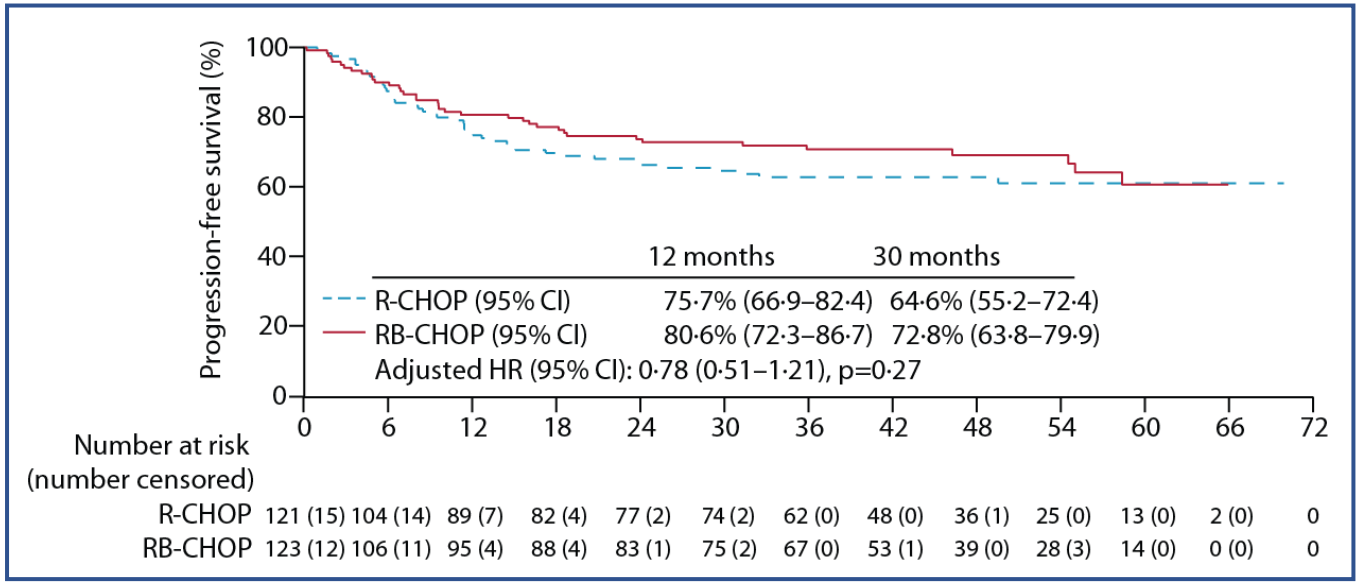
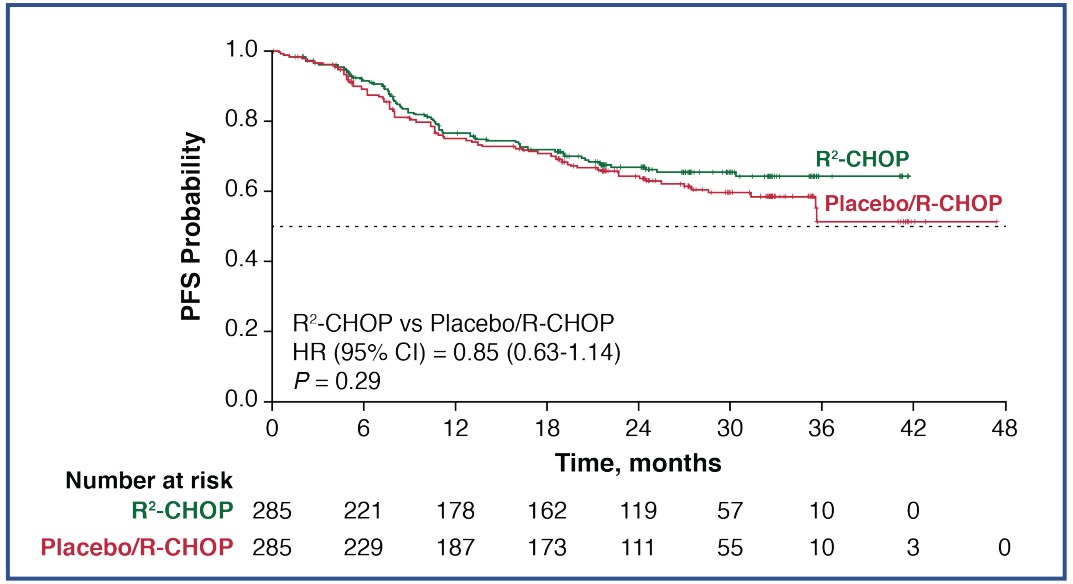


Trials have focused on the poor prognosis ABC-DLBCL

Roschewski, et al Nat Rev Clin Oncol 2014

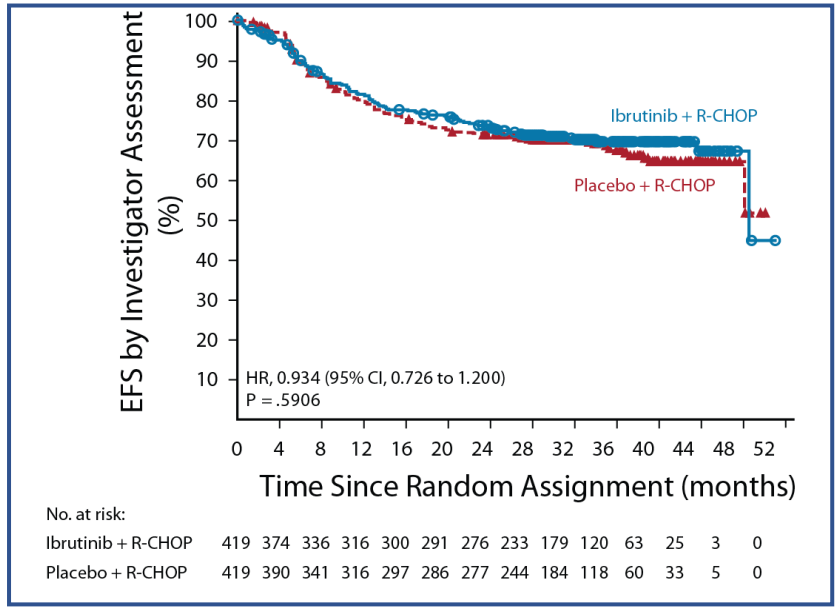
R-CHOP-X – moving beyond R-CHOP?





REMoDL-B:
R-CHOP ± bortezomib
Davies et al Lancet Oncol 2019
COO by gene expression

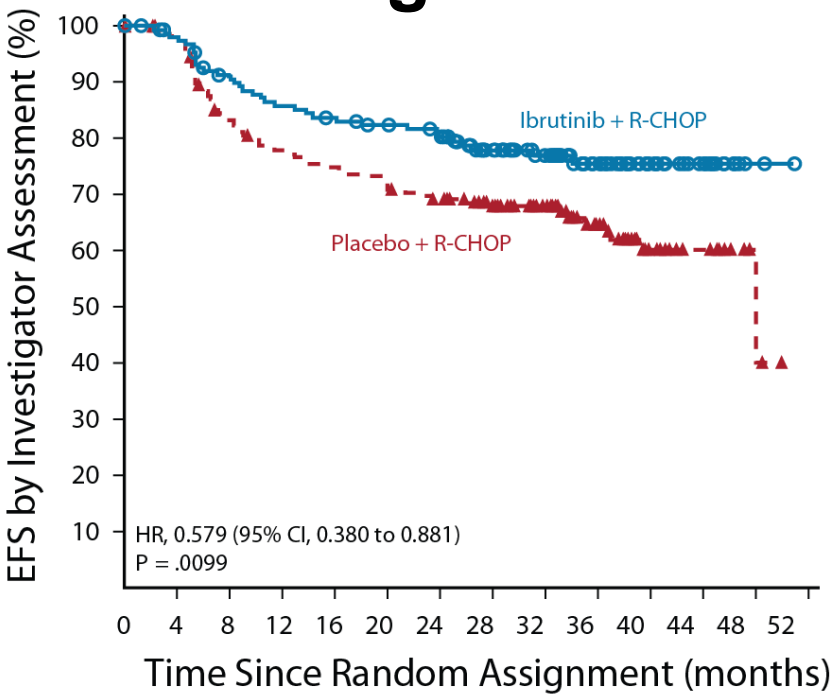
PHOENIX:
R-CHOP ± ibrutinib
Younes et al J Clin Oncol 2019
COO by Hans IHC



ROBUST:
R-CHOP ± lenalidomide
Vitolo et al ICML 2019
COO by gene expression

Subgroup analysis of PHOENIX

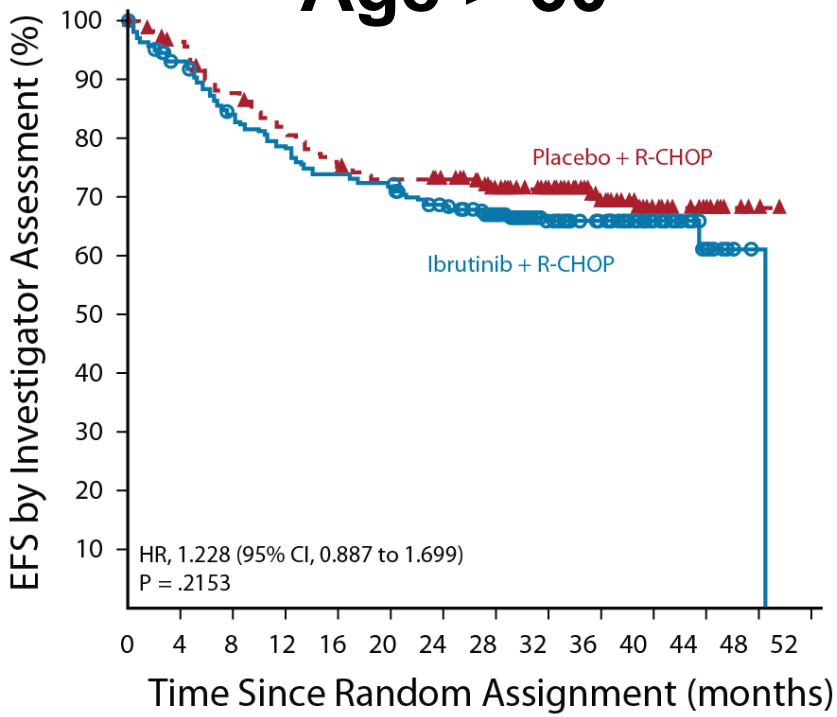
Age < 60



No. at risk:

Ibrutinib + R-CHOP	156	146	133	125	121	117	113	93	72	44	27	13	2	0
Placebo + R-CHOP	186	177	148	137	132	127	120	104	78	52	24	16	3	0

Age > 60



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Placebo + R-CHOP	233	213	193	179	165	159	157	140	106	66	36	17	2	0

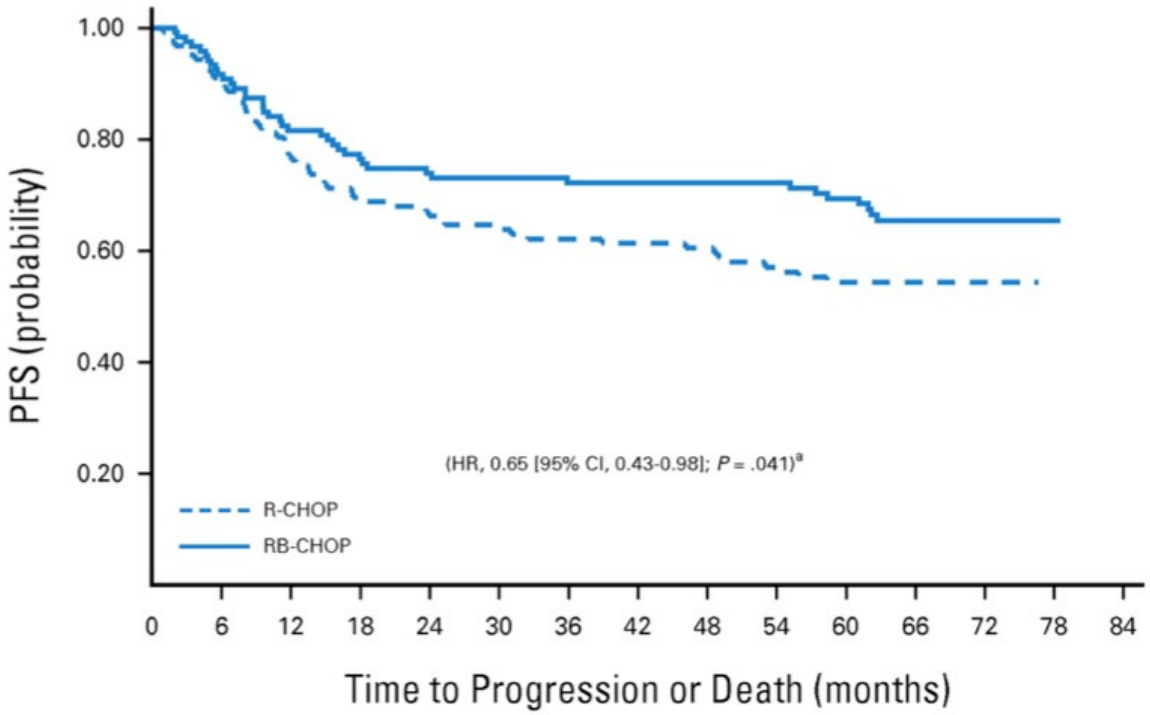
POST HOC Subgroup analysis that requires confirmation

One explanation put forward for this interaction is the effect of age on toxicity

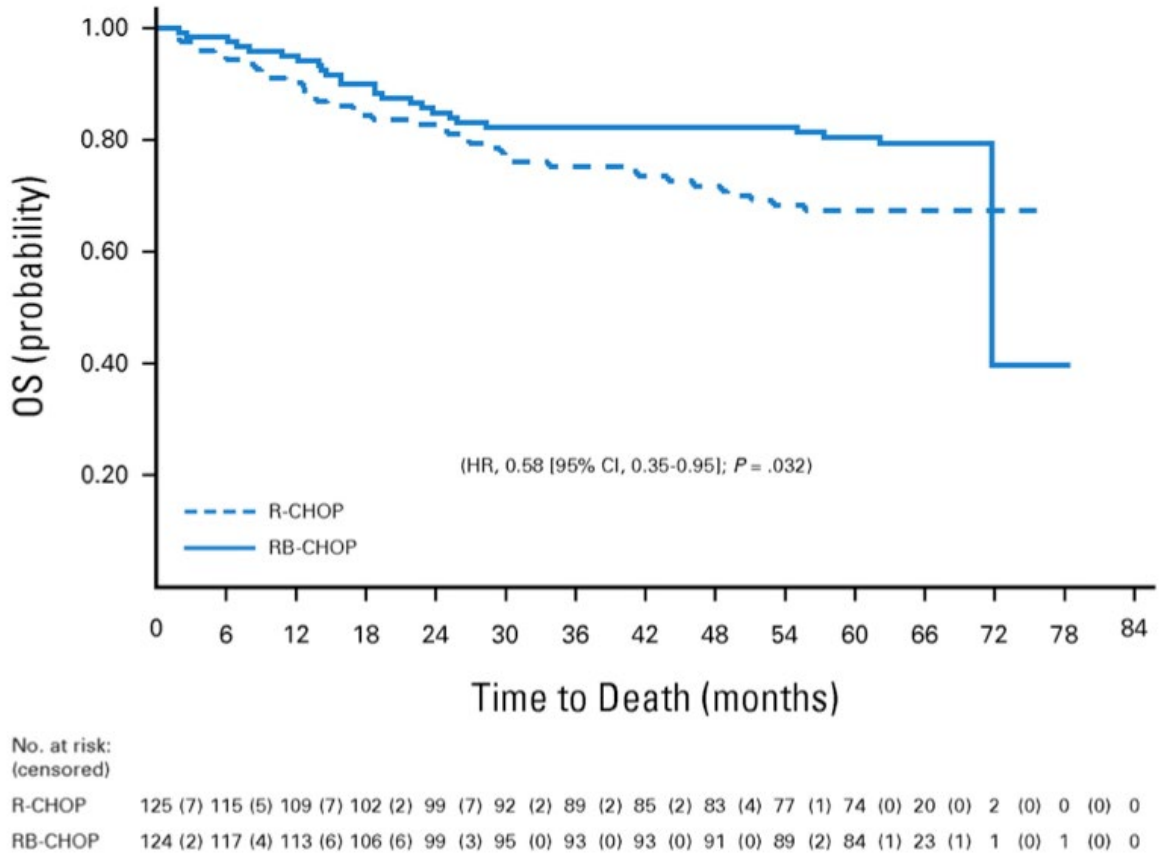
Younes et al J Clin Oncol 2019

Adding bortezomib to R-CHOP improves PFS and OS – update of REMoDL-B

Progression-free survival

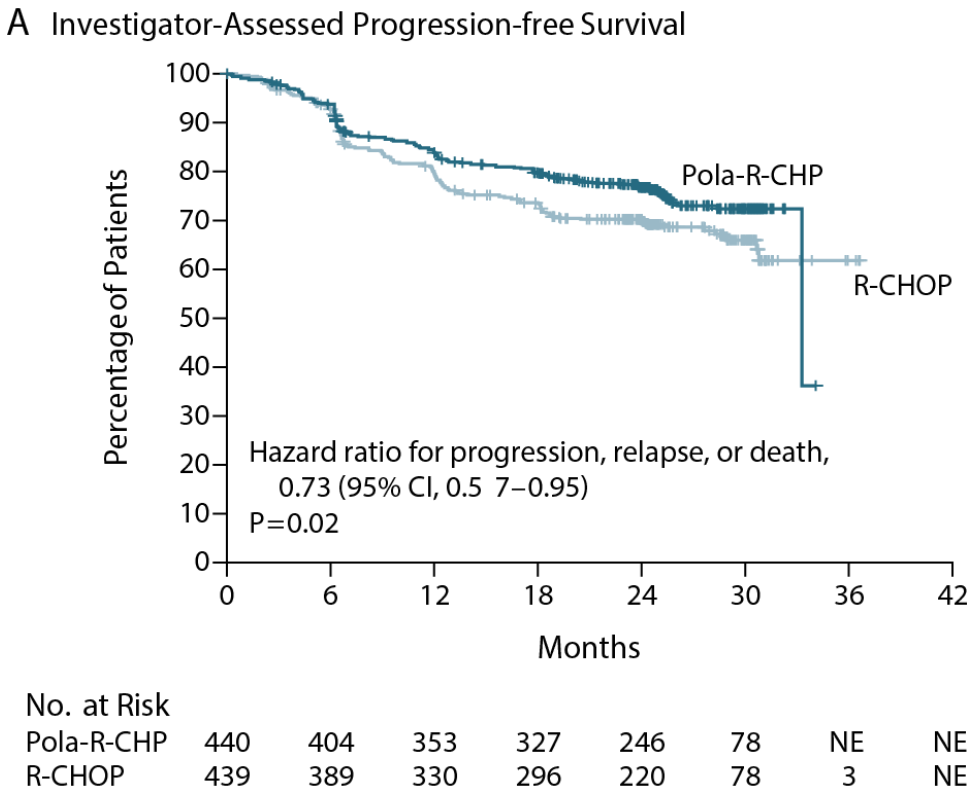


Overall survival



Davies et al J Clin Oncol 2023

Subgroup analysis of POLARIX: R-CHOP vs R-CHP plus polatuzumab vedotin



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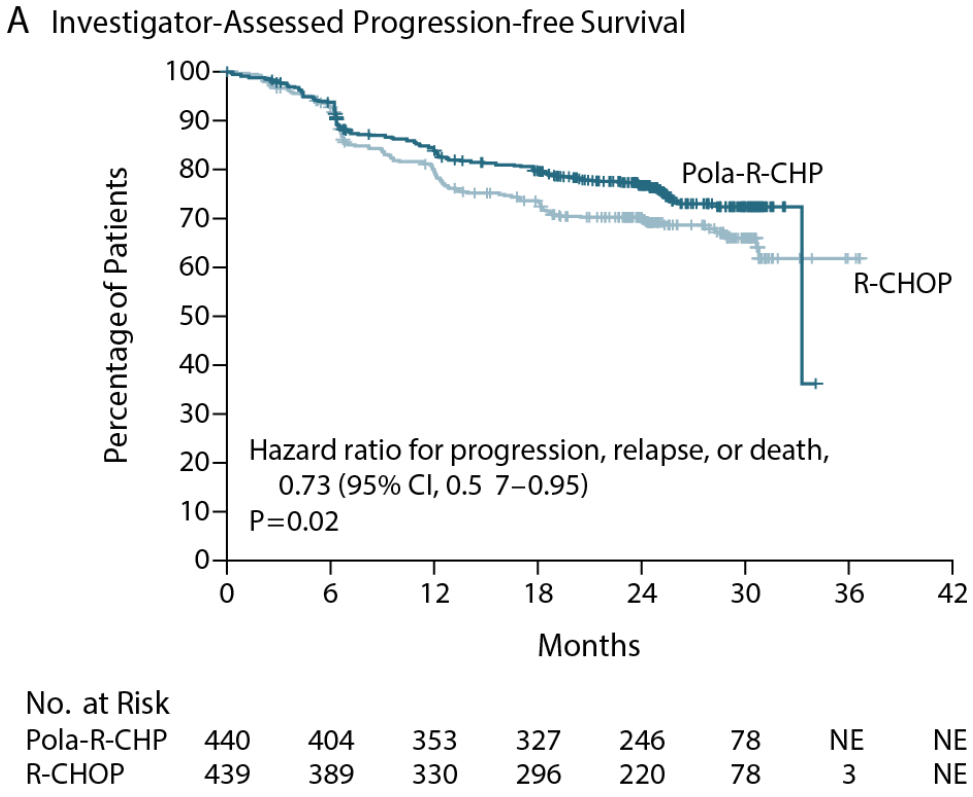

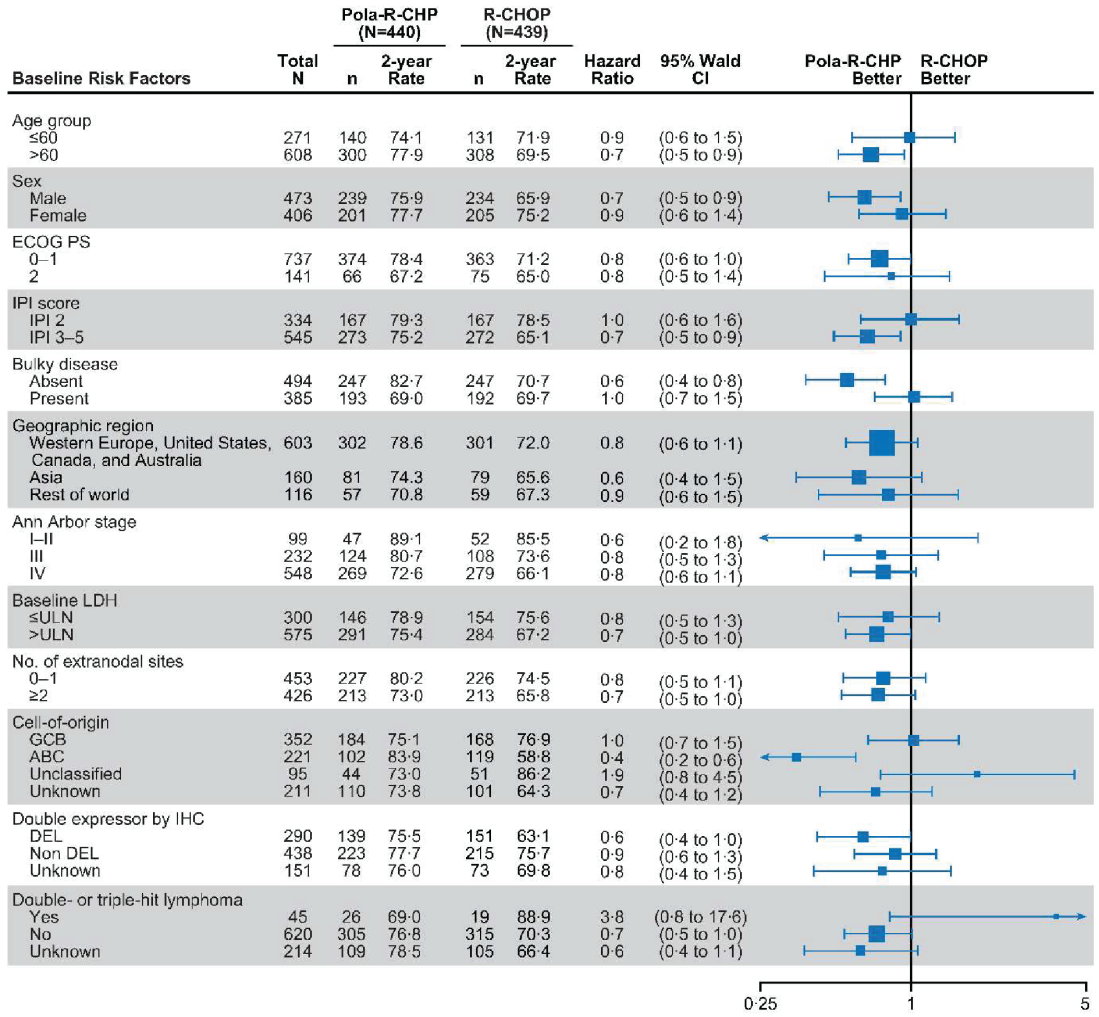

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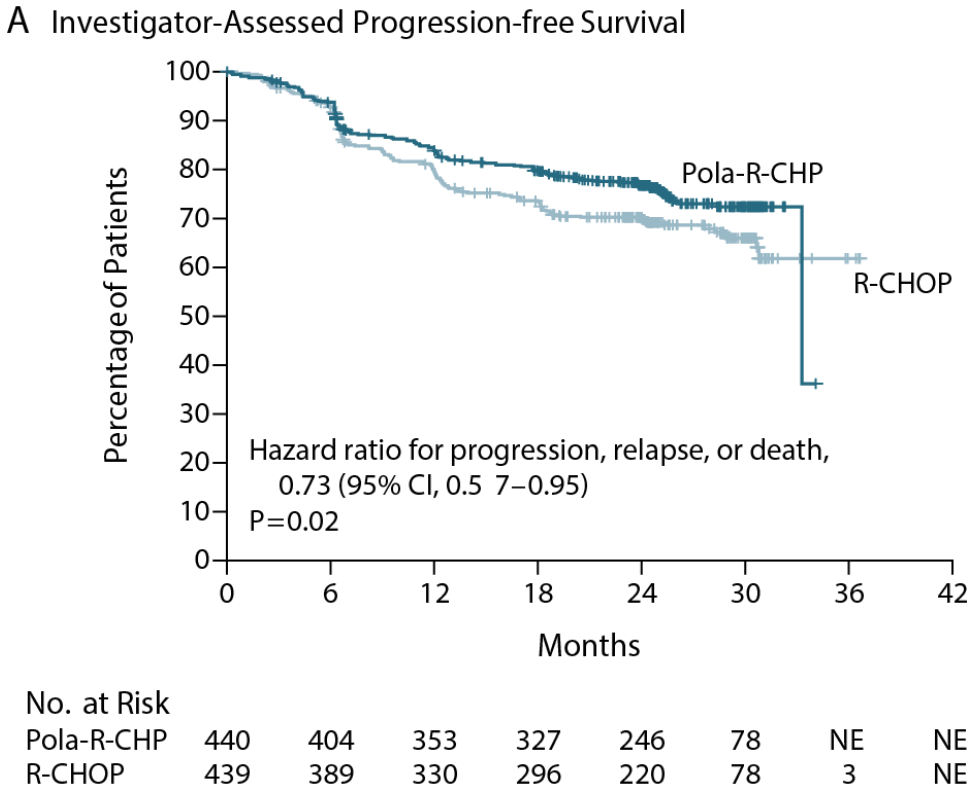

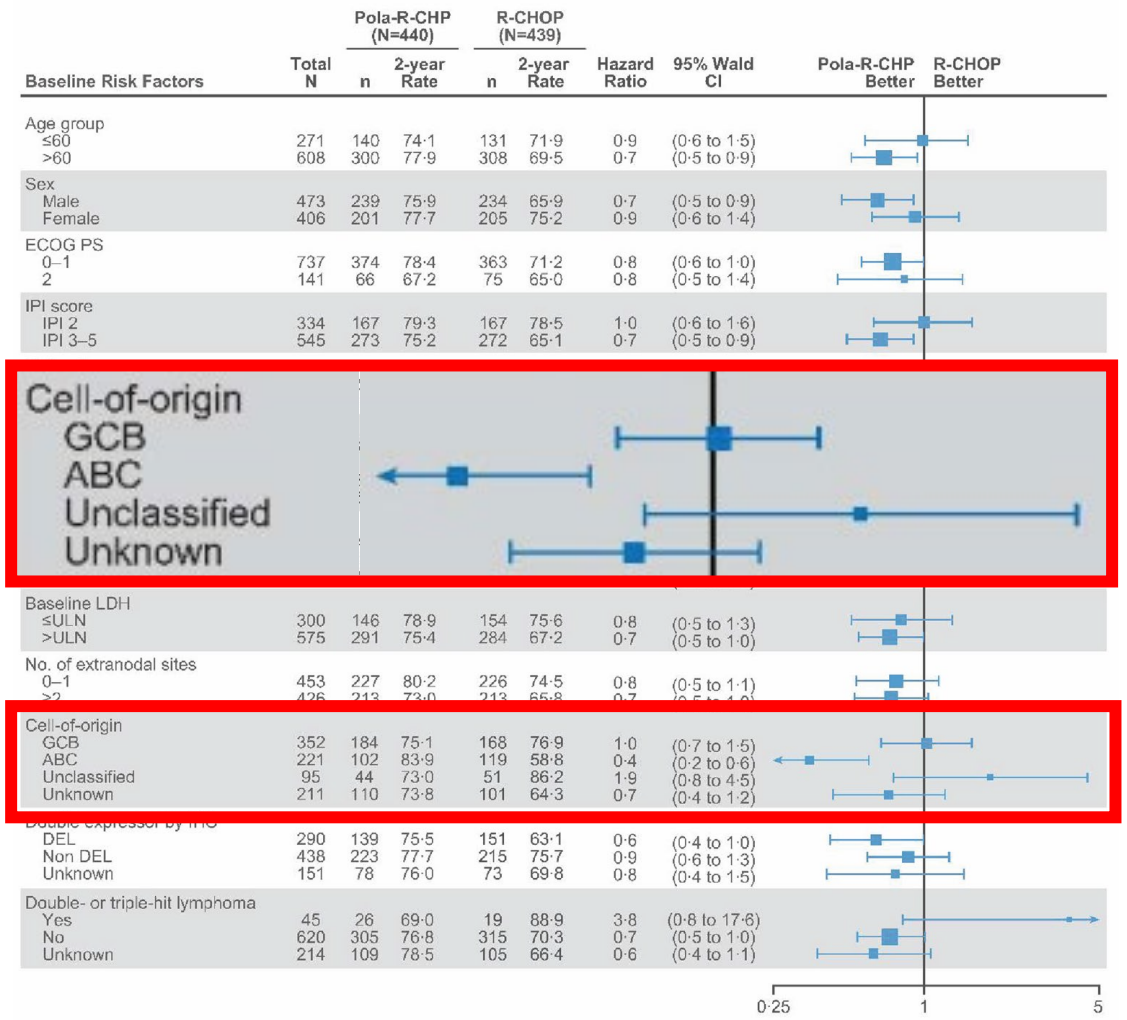

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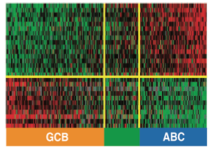

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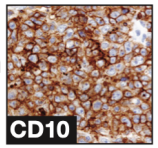

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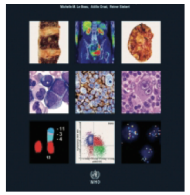

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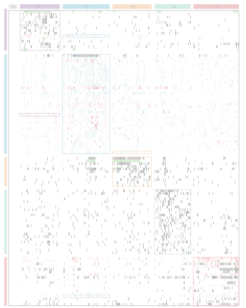

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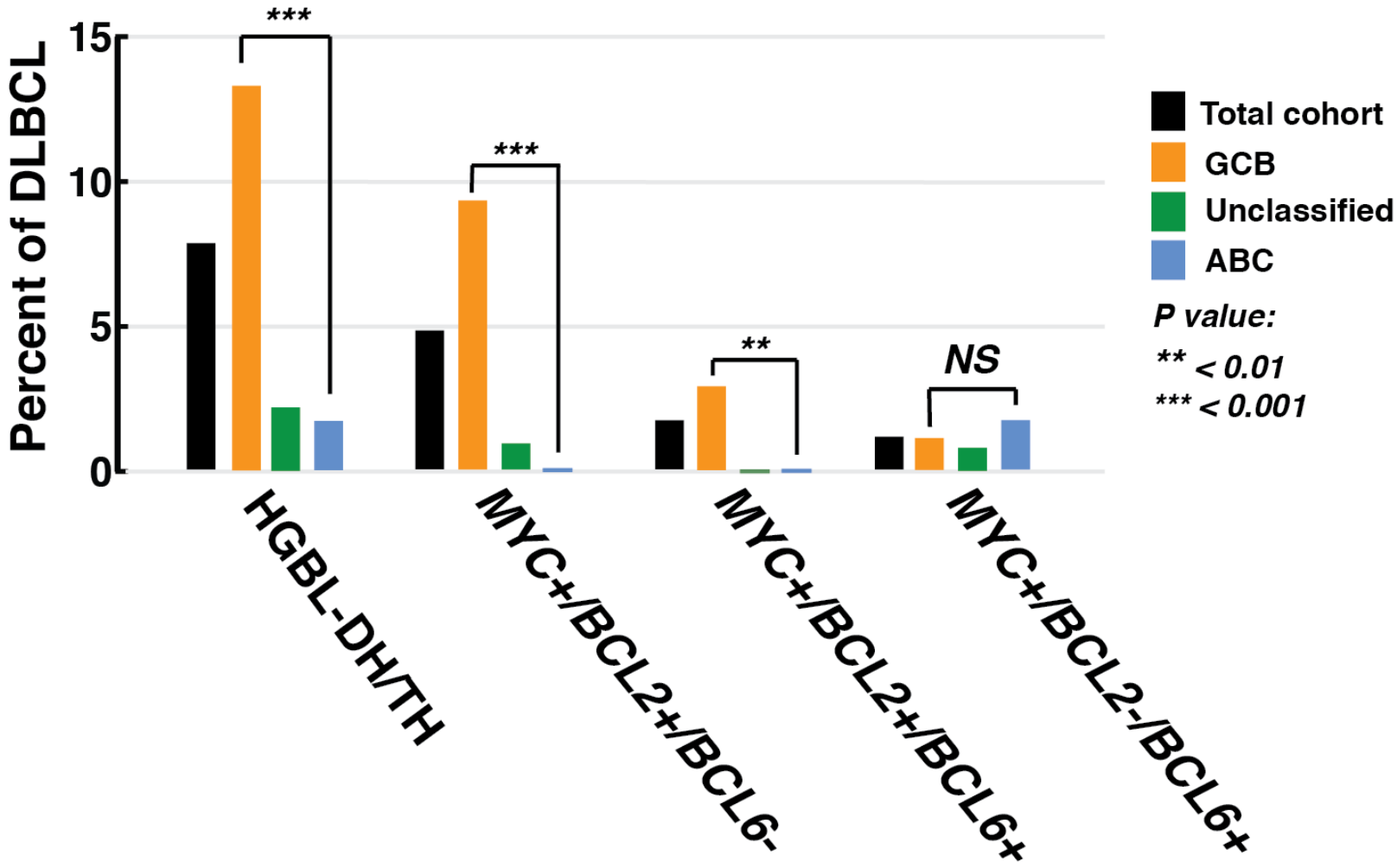

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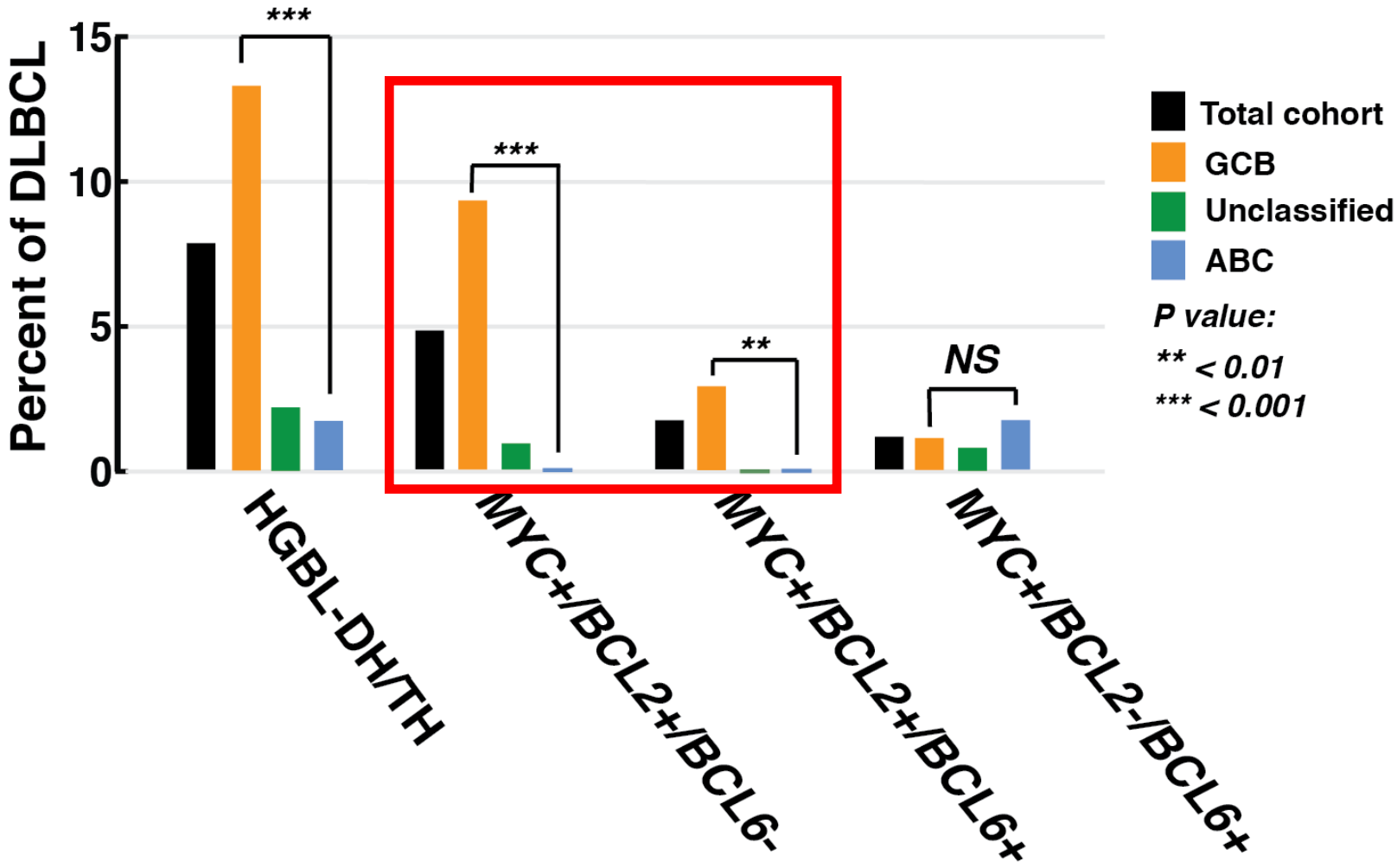

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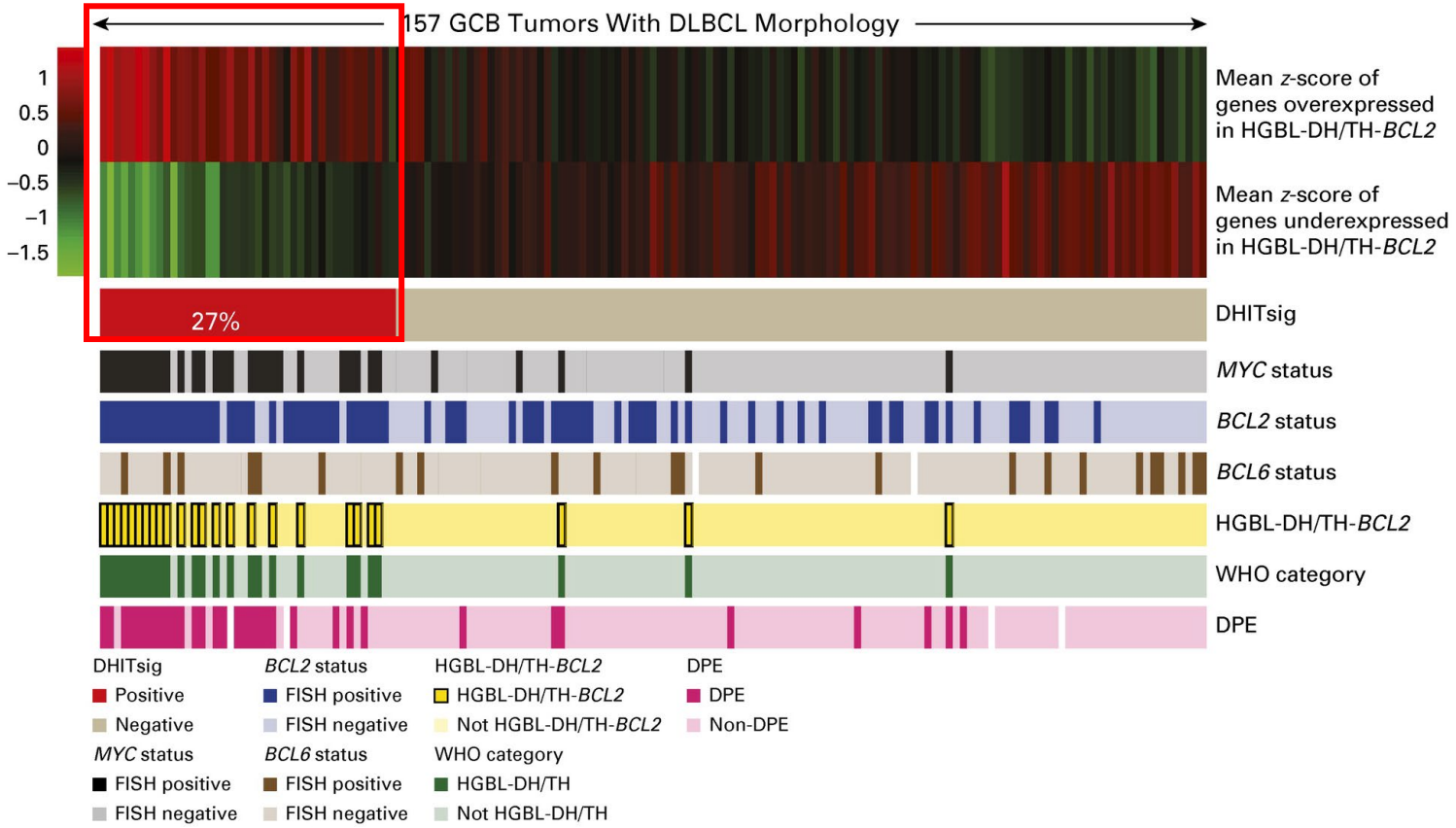

27% of GCB-DLBCLs express a signature associated with DH-*BCL2*

Ennishi et al J Clin Oncol 2019

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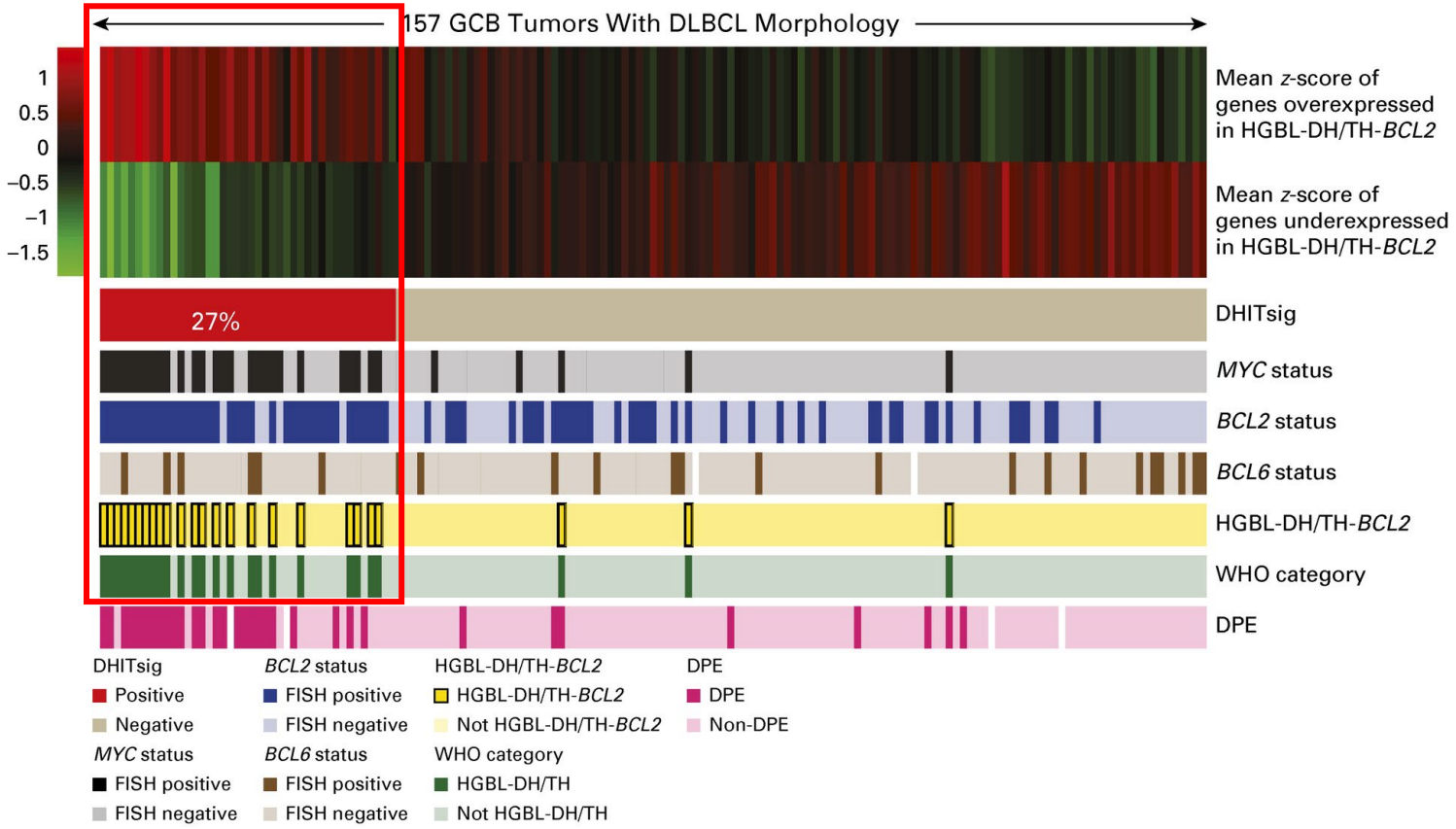

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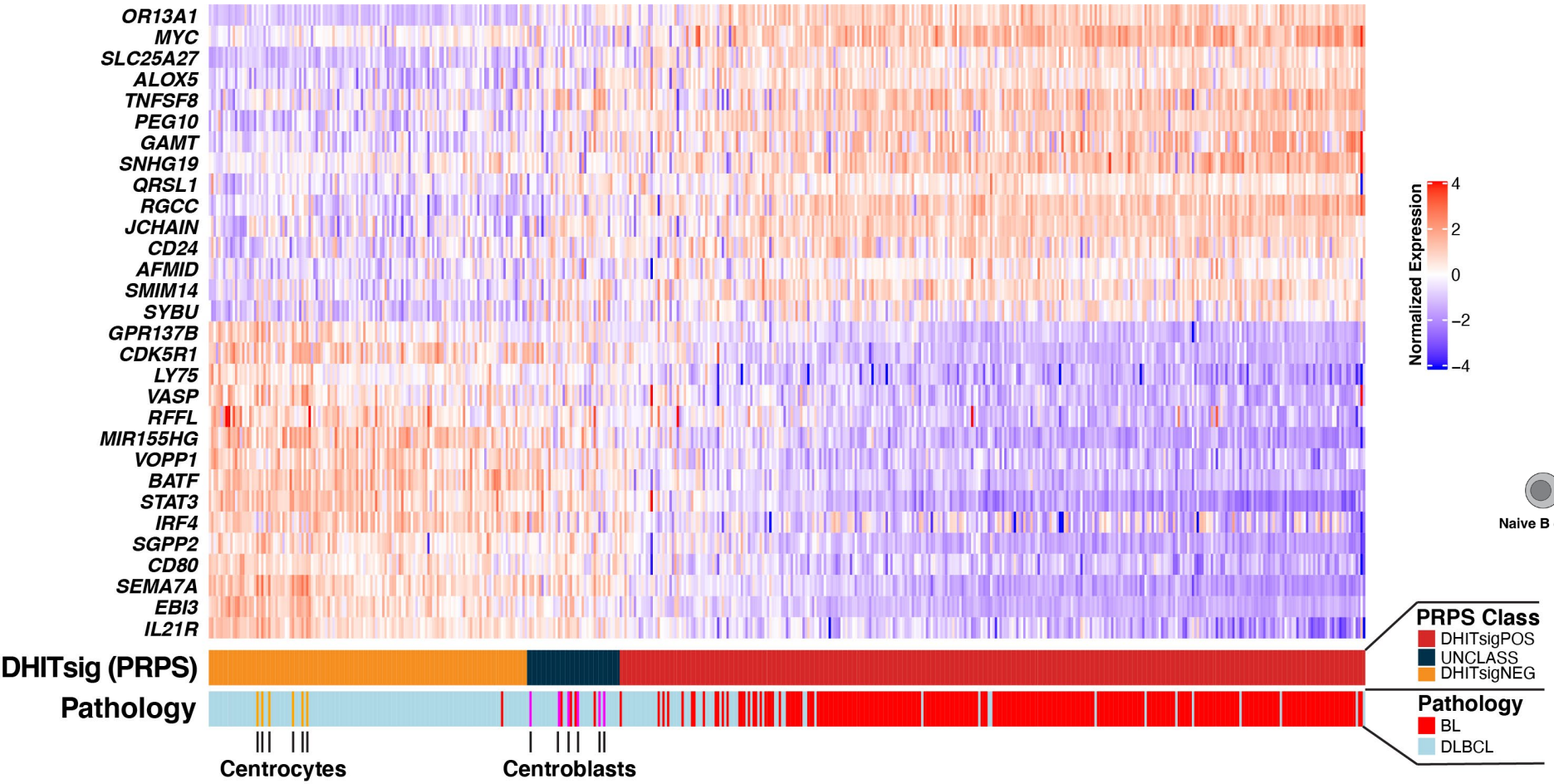
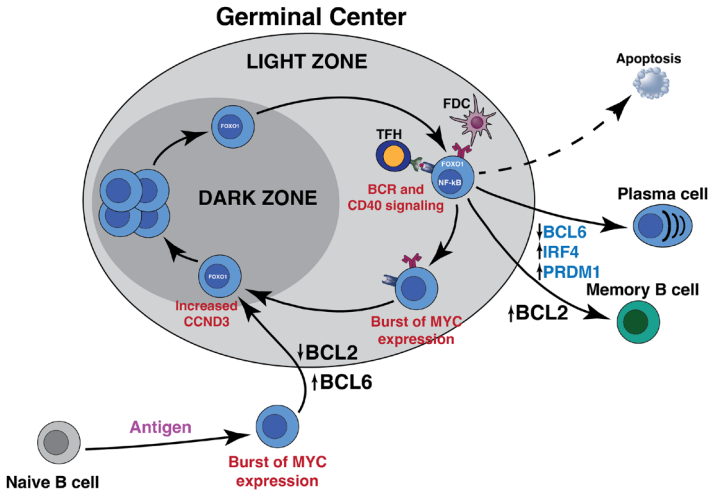
Only half of these have both *MYC* and *BCL2* rearrangements

Ennishi et al J Clin Oncol 2019

“Double hit signature” is a misnomer – renamed the “dark zone signature”



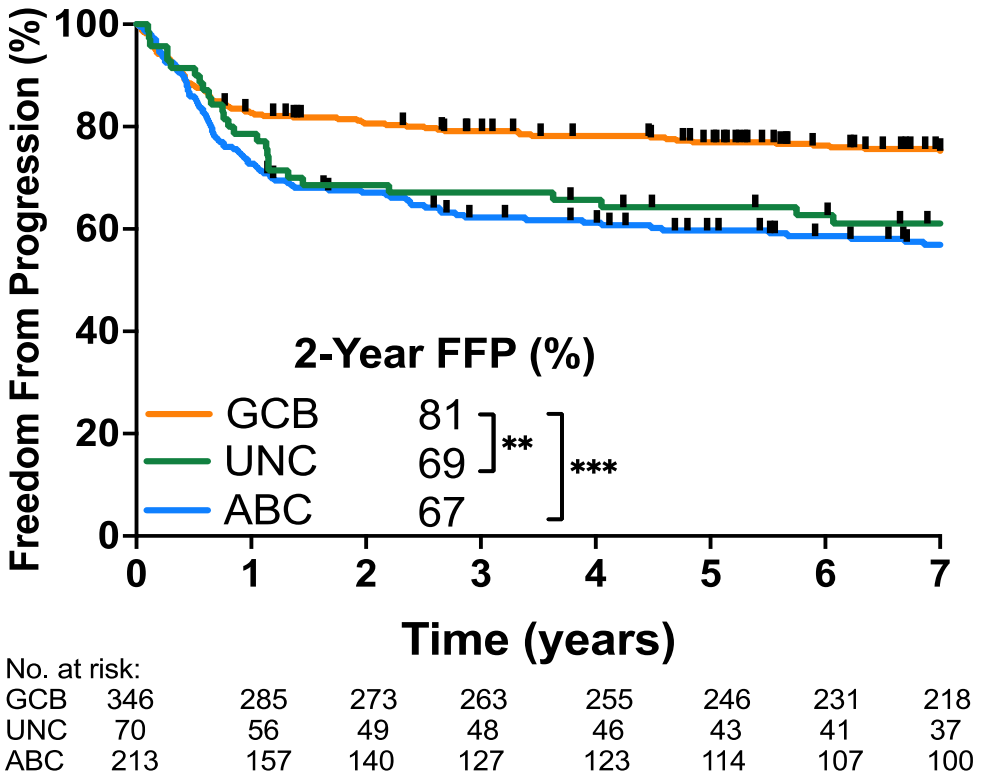


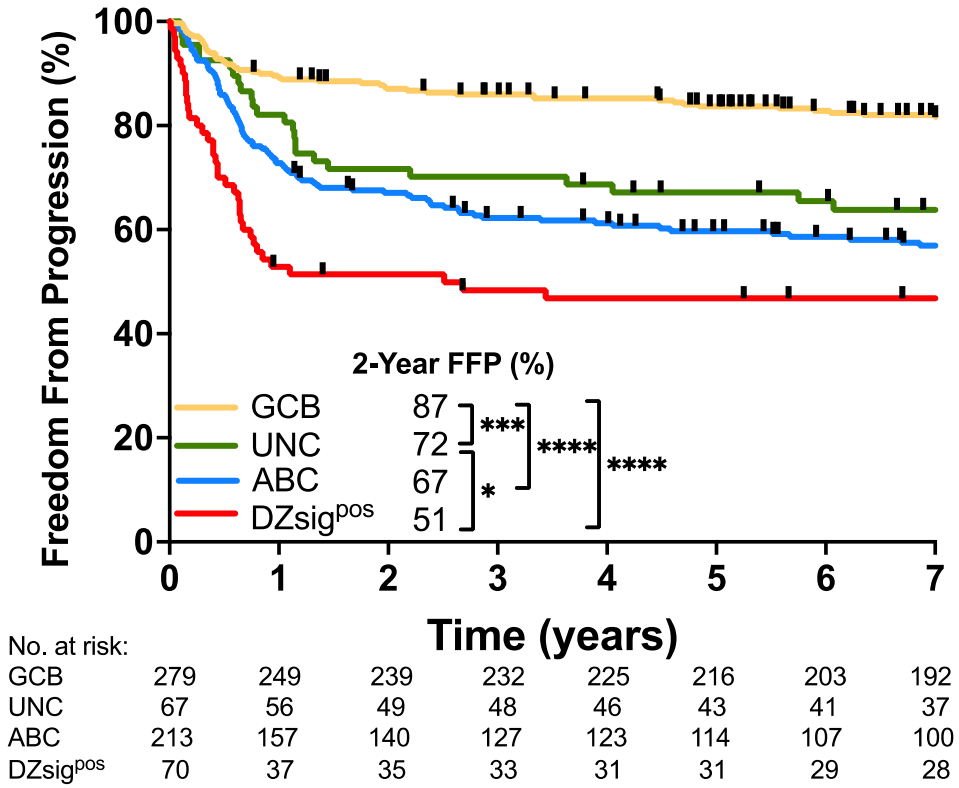
Alduaij, Collinge et al Blood 2022

Grande et al Blood 2019

Ennishi et al J Clin Oncol 2019

DZsig+ DLBCL: poor prognosis

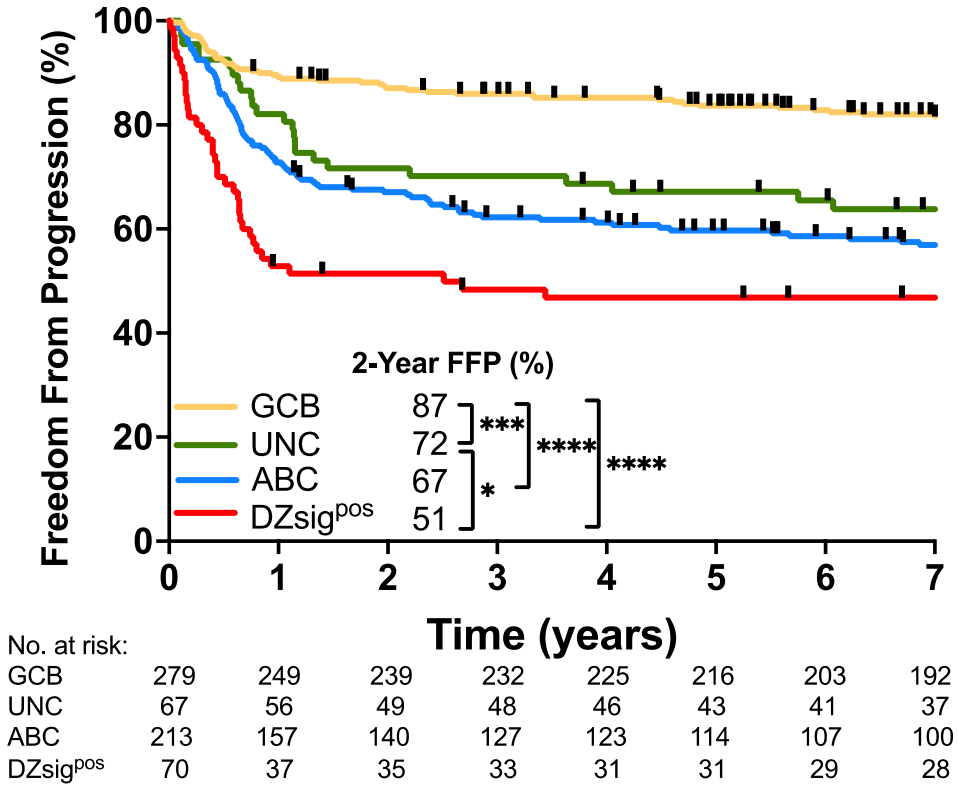


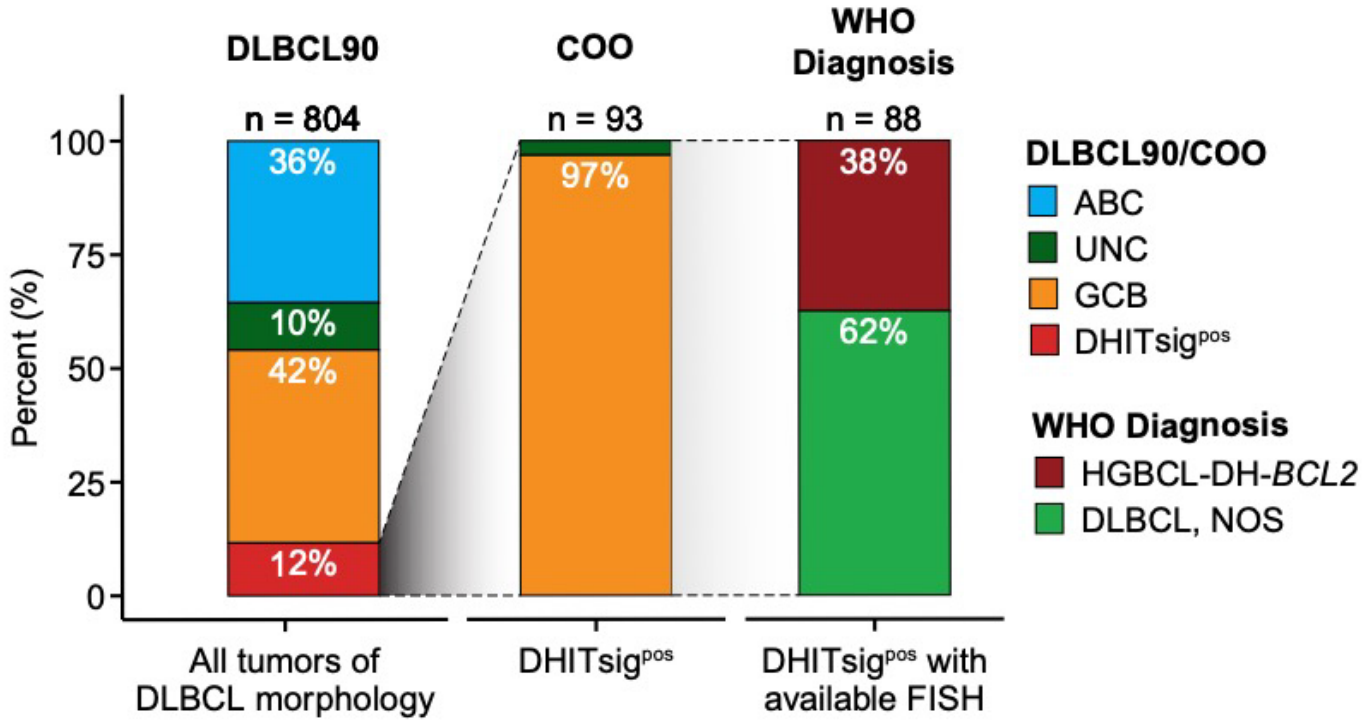


DZsig+ identifies the poorest prognosis group
Removing these tumours from GCB-DLBCL leaves a patient group with excellent outcomes following R-CHOP

Alduaij, Collinge et al Blood 2022

DZsig+ DLBCL: majority are not HGBCL-DH-*BCL2*

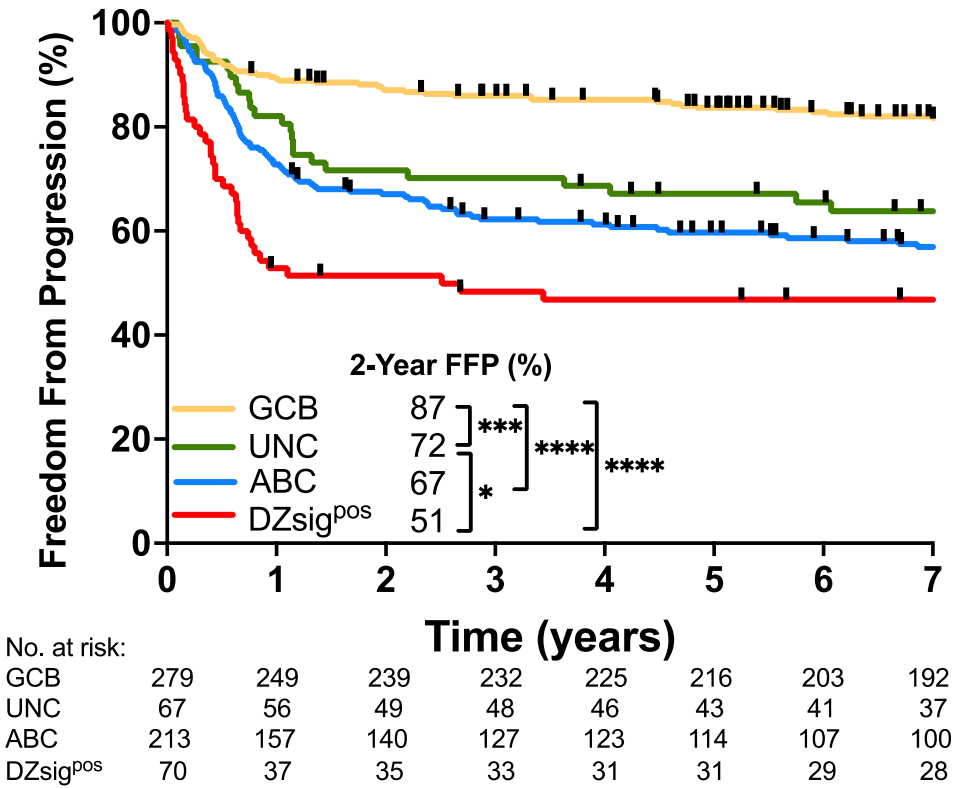




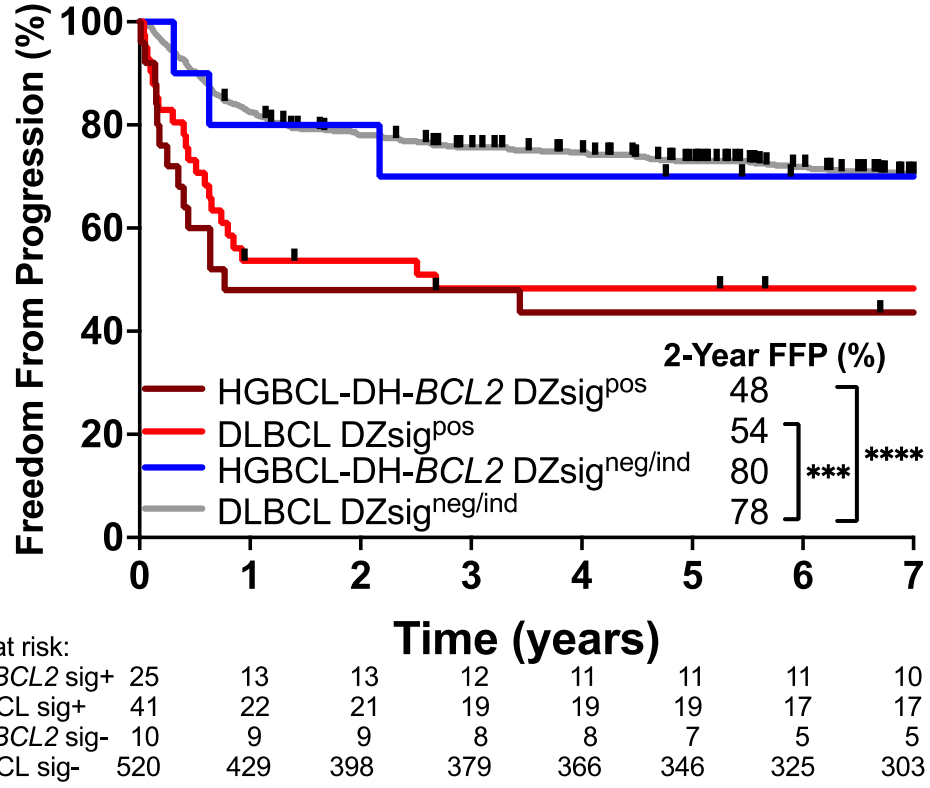
DZsig+ identifies the poorest prognosis group
Removing these tumours from GCB-DLBCL leaves a patient group with excellent outcomes following R-CHOP

Alduaij, Collinge et al Blood 2022

DZsig+ DLBCL: poor prognosis whether HGBCL-DH-*BCL2* or not



No. at risk:								
GCB	279	249	239	232	225	216	203	192
UNC	67	56	49	48	46	43	41	37
ABC	213	157	140	127	123	114	107	100
DZsig^{pos}	70	37	35	33	31	31	29	28

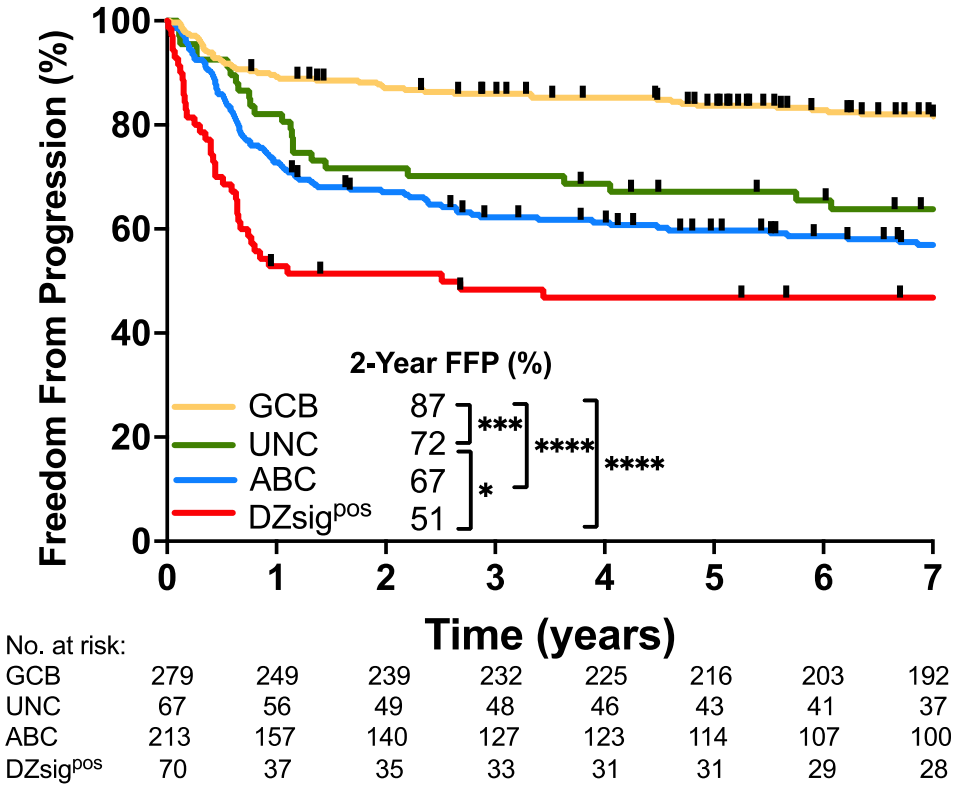


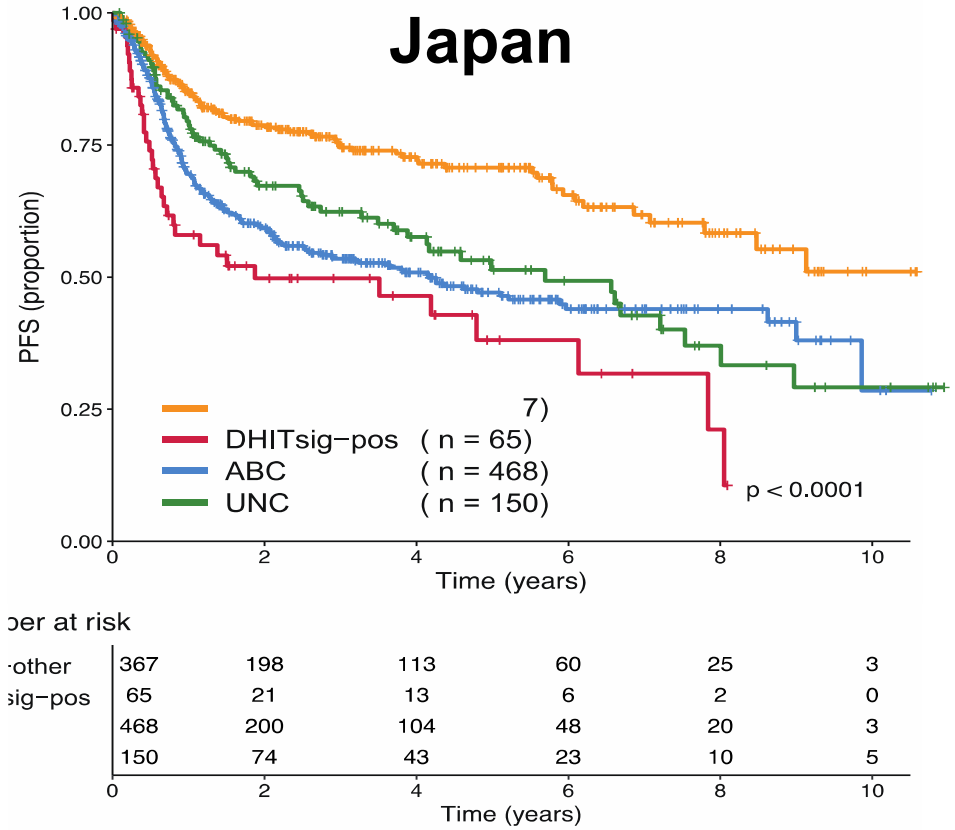
No. at risk:								
DH-*BCL2* sig+	25	13	13	12	11	11	11	10
DLBCL sig+	41	22	21	19	19	19	17	17
DH-*BCL2* sig-	10	9	9	8	8	7	5	5
DLBCL sig-	520	429	398	379	366	346	325	303

DZsig+ identifies the poorest prognosis group
Removing these tumours from GCB-DLBCL leaves a patient group with excellent outcomes following R-CHOP

Alduaij, Collinge et al Blood 2022

DZsig+ DLBCL: poor prognosis in diverse populations





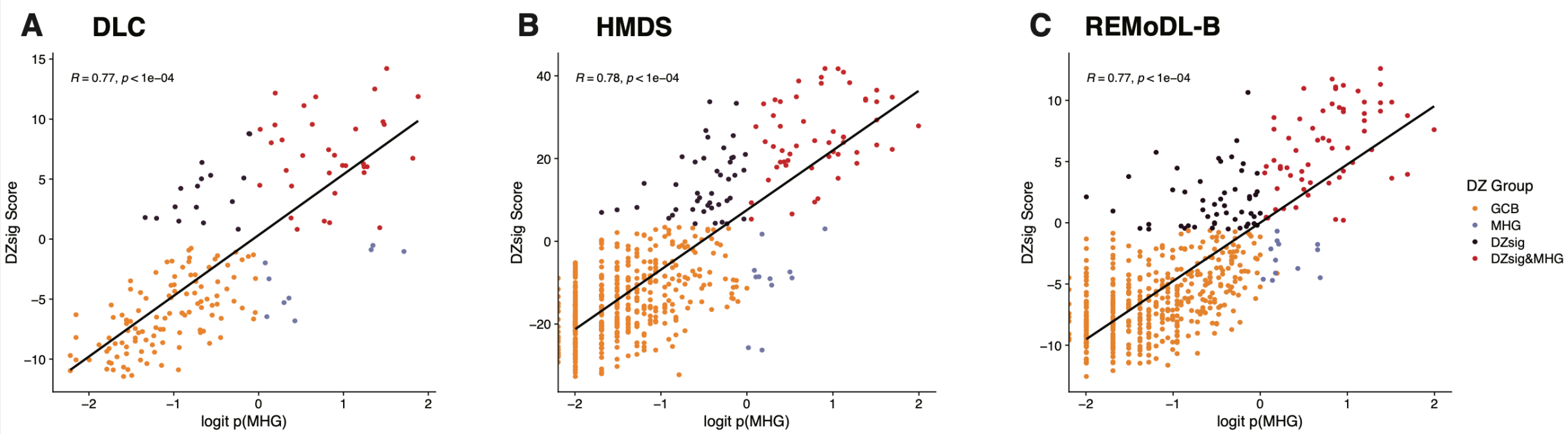
DZsig+ identifies the poorest prognosis group

Removing these tumours from GCB-DLBCL leaves a patient group with excellent outcomes following R-CHOP

Urata et al ASH 2022

Alduaij, Collinge et al Blood 2022

DZsig and MHG both identify dark zone DLBCL



DZsig: genes distinguishing HGBL-DH-*BCL2* from GCB-DLBCL

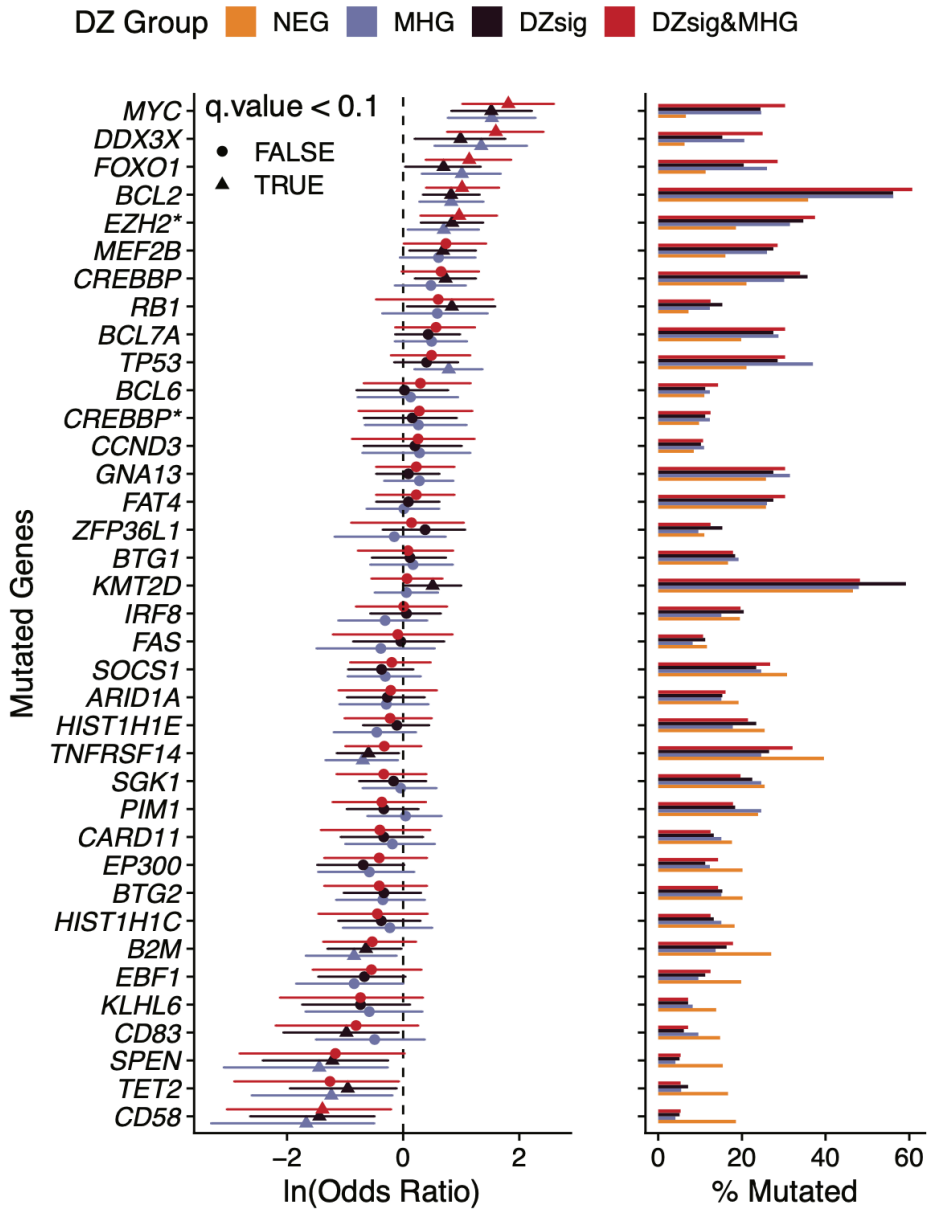
MHG: genes distinguishing Burkitt from DLBCL

Sha et al J Clin Oncol 2019

Ennishi et al J Clin Oncol 2019

Davies, Hilton et al Unpublished

DZsig and MHG both identify dark zone DLBCL

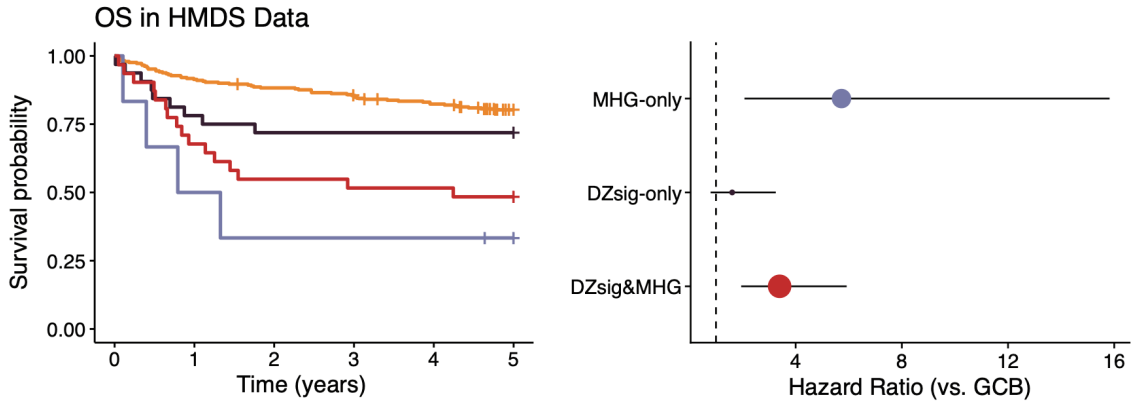


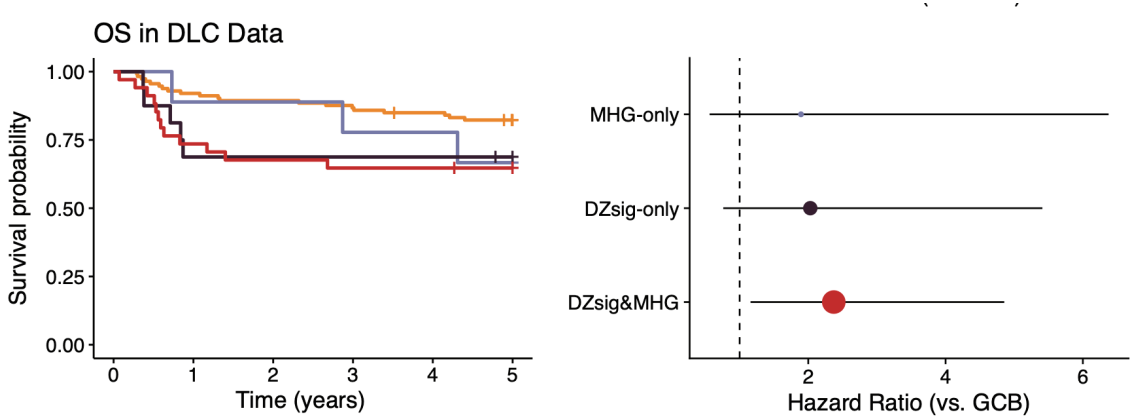
MHG and DZsig are enriched for mutations in many of the same genes relative to GCB-DLBCL

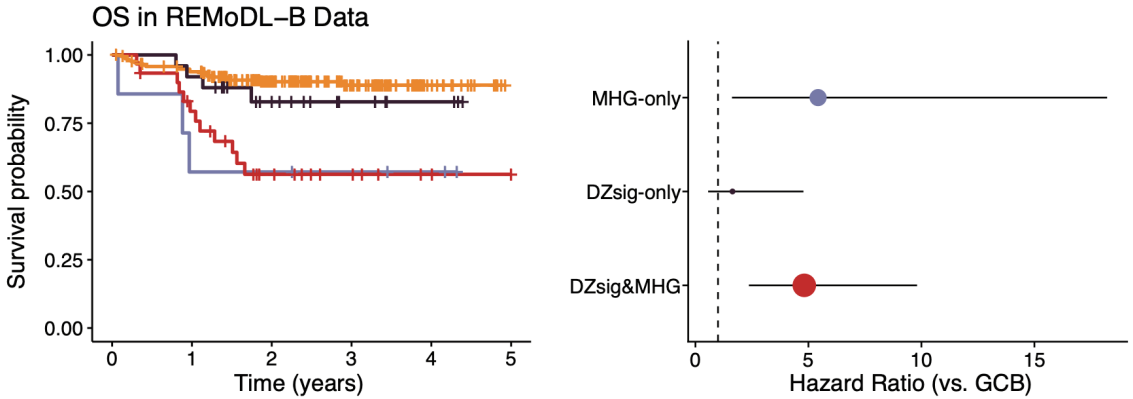
Ennishi et al JCO 2019
Sha et al JCO 2019

Davies, Hilton et al Unpublished

DZsig and MHG both identify dark zone DLBCL







The overlap of DZsig&MHG consistently identifies patients with poor outcomes relative to GCB-DLBCL

Sha et al JCO 2019

Ennishi et al JCO 2019

Davies, Hilton et al Unpublished

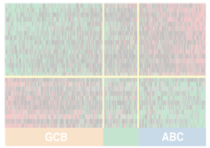
Dark zone lymphomas: COO Refined

- **The shared gene expression signature strengthens and validates HGBCL-DH-*BCL2* as the core of a true biological entity**

- **The "double hit signature" is a misnomer – it is a signature of a dysregulated dark zone cell-of-origin and is shared with Burkitt**

- **DZsig and MHG both identify GCB-DLBCL with a dark zone-like gene expression signature**

Timeline of molecular classification in DLBCL



Cell-of-origin discovery

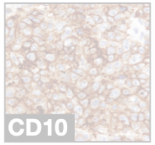
2000

Method for biopsy-by-biopsy COO assignment using gene expression

2003

IHC-based Hans algorithm developed

2004



First method for COO assignment using gene expression in FFPE

2007

COO by gene expression prognostic with R-CHOP

2008

Phase 3 RCTs initiated adding COO-specific agents to R-CHOP for upfront treatment:
REMoDL-B 2011
PHOENIX 2013
ROBUST 2015

2011

Lymph2Cx assay developed

2013

WHO 4th ed revised - COO assignment required for DLBCL, NOS

2016

Dark zone lymphomas - COO refined

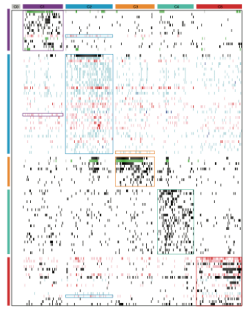
2019

2006

Achilles heel screens and targeted sequencing define key pathways in ABC-DLBCL

2011

Genome-wide sequencing defines the DLBCL mutational landscape



2018

Genetics-based subgroups of DLBCL described

2020

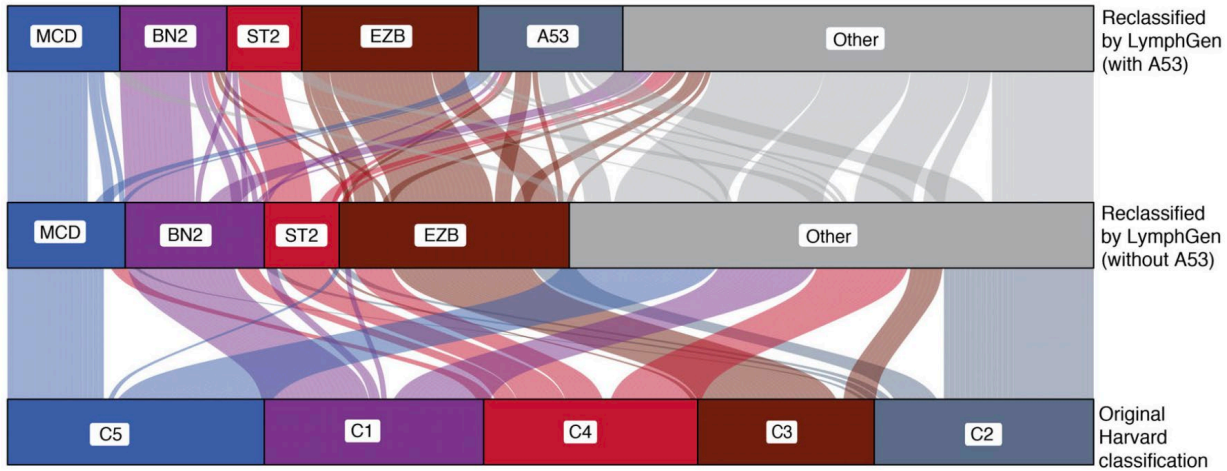
Method for biopsy-by-biopsy genetics-based subgroup assignment

Genetics-based subtypes of DLBCL

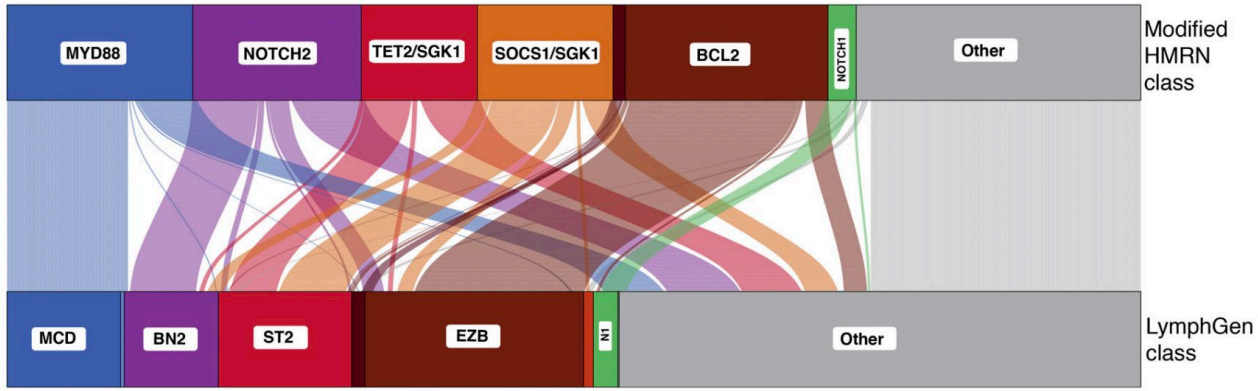
LymphGen

Schmitz et al N Engl J Med 2018
Wright et al Cancer Cell 2020

Harvard

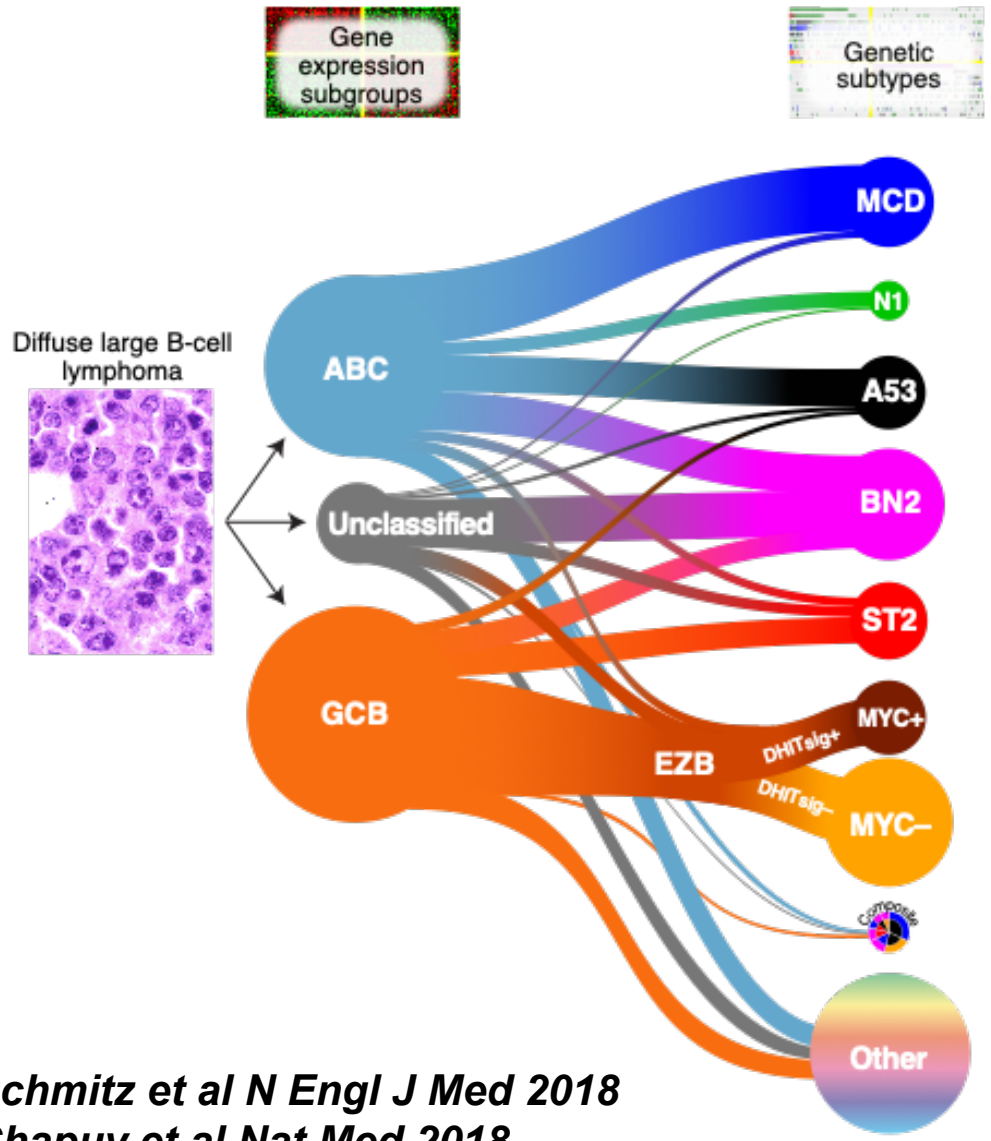


HMRN



Morin et al Brit J Haematol 2021
Chapuy et al Nat Med 2018
Lacy et al Blood 2020

Genetics-based subtypes of DLBCL

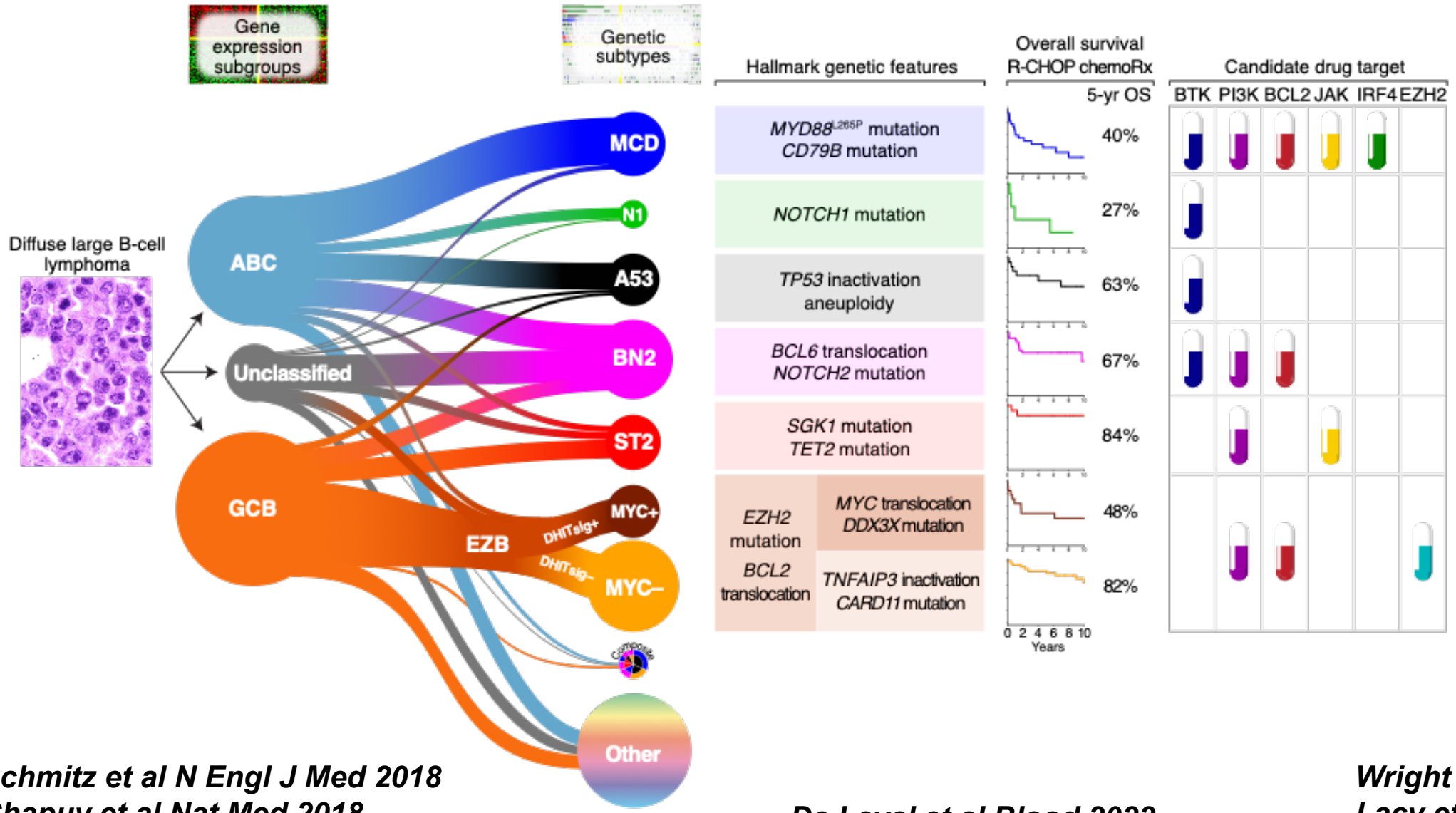


- Three groups have described similar (but not identical) groupings based on co-occurrence of selected genetic features
- LymphGen is currently the only system that can be applied on a biopsy-by-biopsy fashion
- Ongoing challenges with this probabilistic tool:
  - 5-10% are assigned to 2 or more groups
  - 37% are not assigned to any group with sufficient confidence
- How to resolve the 37% "Other" group?
  - Expand the features using whole genome sequencing
  - Add layers of gene expression, epigenetics and tumor microenvironment

Schmitz et al N Engl J Med 2018
Chapuy et al Nat Med 2018

Wright et al Cancer Cell 2020
Lacy et al Blood 2020

Genetics-based subtypes of DLBCL

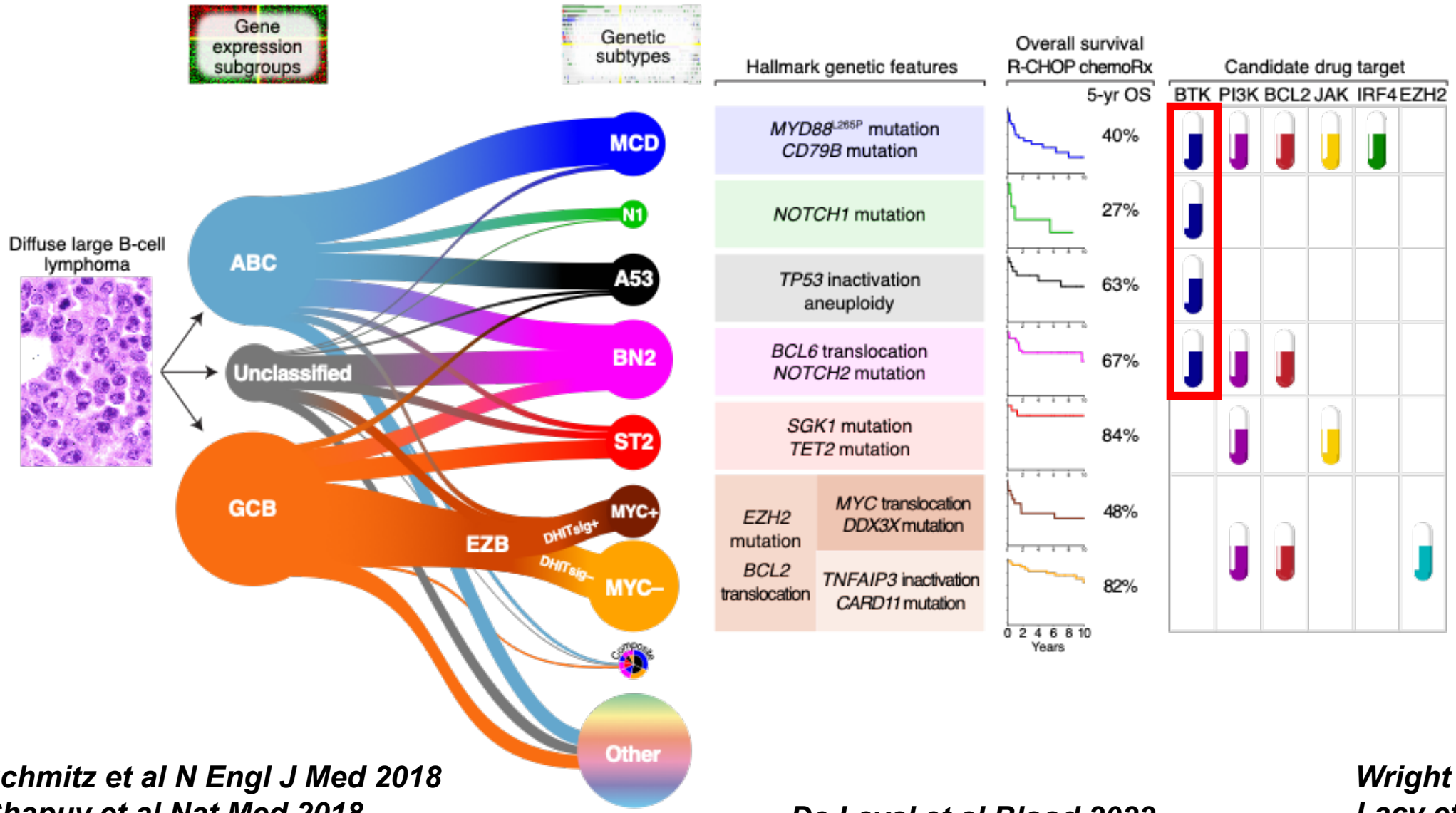


Schmitz et al N Engl J Med 2018
Chapuy et al Nat Med 2018

De Leval et al Blood 2022

Wright et al Cancer Cell 2020
Lacy et al Blood 2020

Genetics-based subtypes of DLBCL

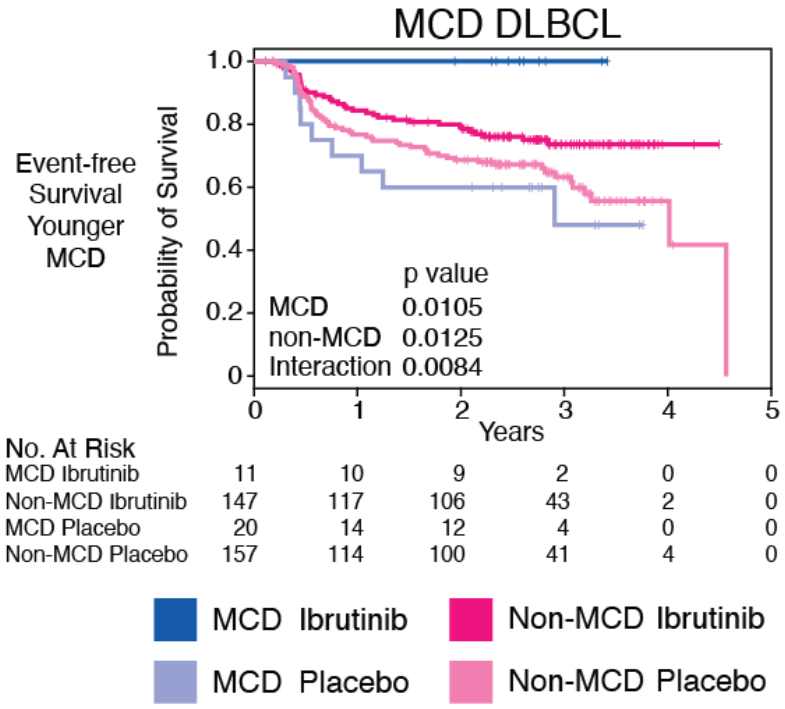


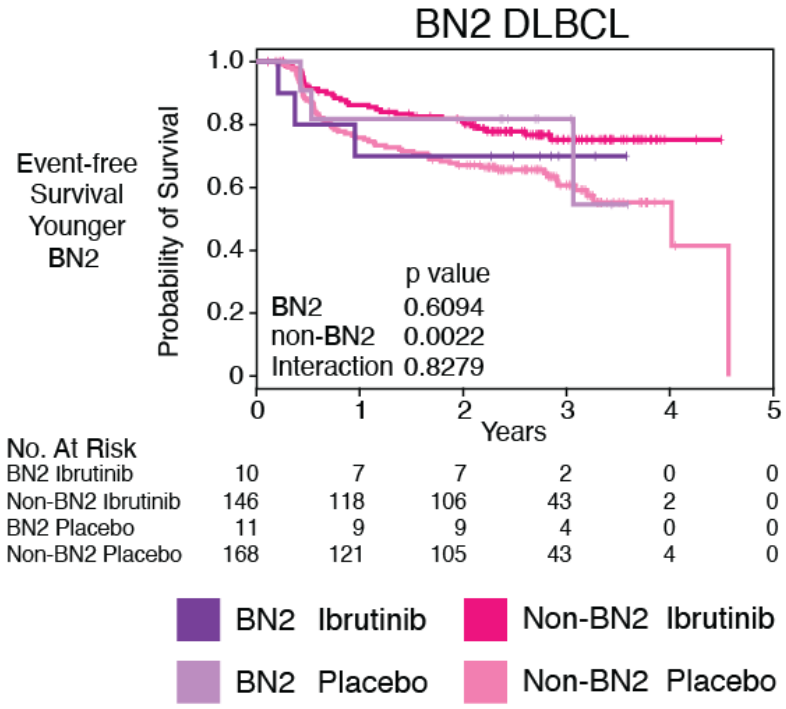
Schmitz et al N Engl J Med 2018
Chapuy et al Nat Med 2018

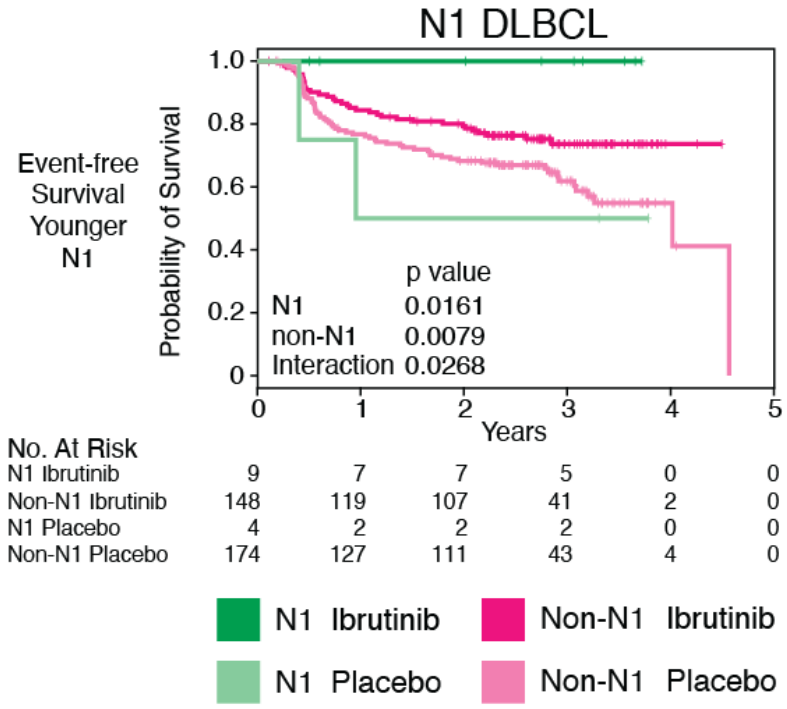
De Leval et al Blood 2022

Wright et al Cancer Cell 2020
Lacy et al Blood 2020

Genetics–based subtypes as a predictive biomarker – retrospective genomic analysis of PHOENIX







Wilson et al Cancer Cell 2021

Guidance-01: Randomized Phase 2 Trial of Genetic Subtype Guided Immunochemotherapy

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.

Untreated DLBCL
- Age 18-80
- IPI ≥ 2

→ R-CHOP×1 → R 1:1

Stratified by K-medoids algorithm (PAM) simulated genetic subtyping using targeted sequencing panel of 18 genes: *BTG1, CD70, CD79B, CREBBP, DTX1, EP300, EZH2, MPEG1, MTOR, MYD88, NOTCH1, NOTCH2, PIM1, STAT6, TBL1XR1, TNFAIP3, TNFRSF14,* and *TP53*

- MCD like: Ibrutinib+R-CHOP×5
- BN2 like: Ibrutinib+R-CHOP ×5
- N1 like: Lenalidomide+R-CHOP×5
- EZB like: Tucidinostat+R-CHOP×5
- TP53 mutated: Decitabine+R-CHOP×5
- Others: Lenalidomide+R-CHOP×5

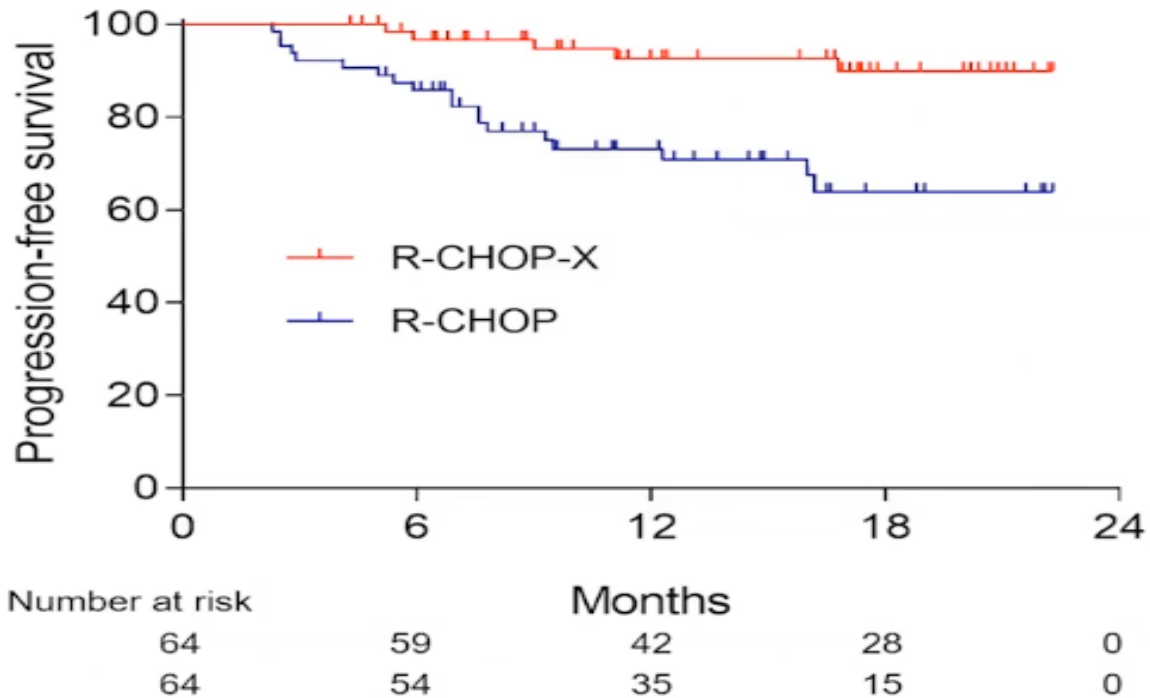
Ibrutinib[1]	420mg po qd
Lenalidomide[2]	25mg d1-10 po
Tucidinostat[3]	20mg d1, 4, 8, 11 po
Decitabine[4]	10 mg/m² d1-5
R-CHOP	Standard dose

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)

Zhang et al ICML 2021

Secondary Endpoint: PFS



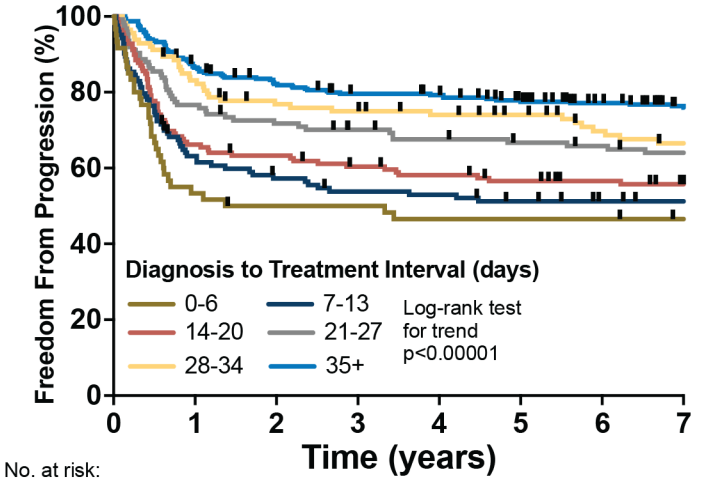
Median follow-up 16.1 months

	R-CHOP-X	R-CHOP
1-year PFS (95%CI)	93% (81%-97%)	73% (60%-83%)

Zhang et al ICML 2021

Challenges to implementing refined classifications

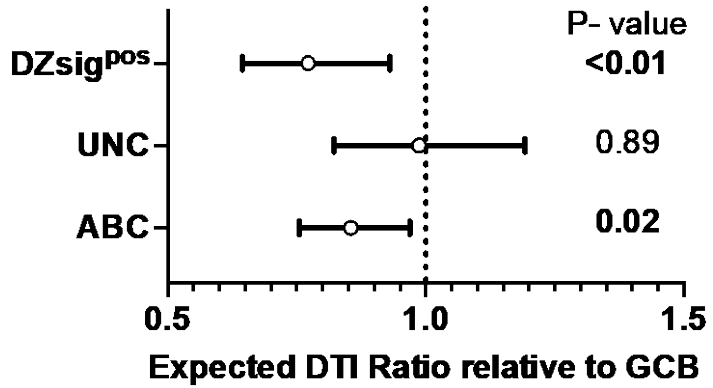
- Harmonization of the genetics-based classifications
- Settling on (and validating) an appropriate assay
- Turn-around-time



No. at risk:

0-6	60	33	30	30	28	28	28	26
7-13	123	76	68	63	62	58	54	52
14-20	139	93	88	82	78	74	70	65
21-27	124	96	89	84	81	77	75	72
28-34	113	94	84	82	78	74	66	62
35+	311	270	250	238	234	221	205	191

Maurer et al J Clin Oncol 2014



Impact of LymphGen on DTI is not known

Alduaij, Collinge et al Blood 2022

Challenges to implementing refined classifications

- Harmonization of the genetics-based classifications
- Settling on (and validating) an appropriate assay
- Turn-around-time
- Availability of tissue – small biopsies, bone marrow
  - Patients diagnosed with core needle biopsies have worse prognosis and are more likely to have inadequate tissue for molecular analyses
  - Patients where molecular analyses were not possible had shorter diagnosis-to-treatment interval
- US Intergroup trial based on LymphGen classes is in the late planning stage – will require a “test of the test” phase

Maurer et al J Clin Oncol 2014 *Desai et al Blood Adv 2022* *Alduaij, Collinge et al Blood 2022*

Concluding comments

- The phenotypic heterogeneity in DLBCL can be understood through the lens of dysregulation of normal B-cell differentiation
- While cell-of-origin has been foundational to our understanding of pathogenesis, this binary classification is not sufficiently granular to support precision medicine
- The genetics-based classifications are a very useful step forward and identify candidate drug targets that need to be tested in clinical trials
- Challenges going forward include defining assays with appropriate turn-around-time, reducing the unclassified group and developing trial designs that allow broad patient inclusion

Acknowledgements

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University of Wuerzburg

BLGSP

NIH

The Terry Fox Research Institute

MICHAEL SMITH FOUNDATION FOR HEALTH RESEARCH
BC's health research funding agency

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GenomeCanada

Genome BritishColumbia

Canadian Cancer Society
Société canadienne du cancer