

The use of molecular profiling for patient selection David Scott

BC Cancer and University of British Columbia

Florence, March 20-21, 2025

Hotel Brunelleschi

President: P.L. Zinzani



Disclosures

Disclosures of David Scott

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Abbvie			Х				
AstraZeneca			Х				
GenMab			Х				
Kite/Gilead			Х				
Roche/Genentech	х		Х				
Veracyte			Х				

Named inventor on patents describing the use of gene expression profiling to subtype aggressive B-cell lymphomas, one of which is licensed to nanoString Technologies

Outline

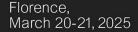
- Current classifications of aggressive B-cell lymphoma
- **Treatment selection "today":** (what we do at BC Cancer)
- High-grade B-cell lymphoma with MYC and BCL2
 rearrangement
- Cell-of-origin

9th POSTGRADUATE

Dark zone lymphoma

Molecular profiling for patient selection for clinical trials:

- Genetics-based subtypes
- Opportunities and challenges

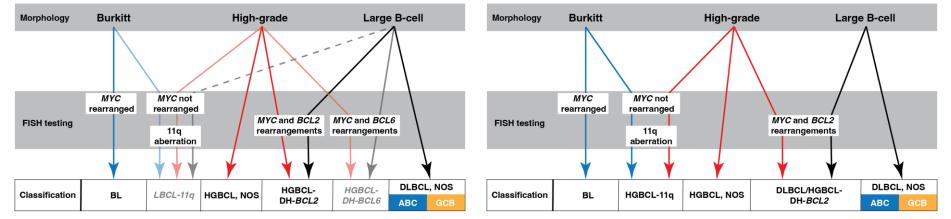


Aggressive B-cell lymphoma classification

International Consensus Classification

9th POSTGRADUATE

WHO HAEM5



Campo et al Blood 2022

Alaggio et al Leukemia 2022

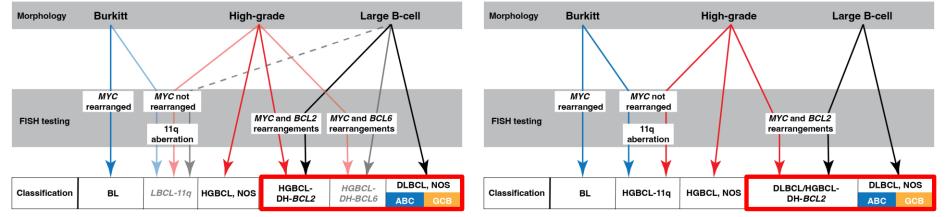


Aggressive B-cell lymphoma classification

International Consensus Classification

9th POSTGRADUATE

WHO HAEM5



Classifications are largely concordant with the exception of aggressive tumors with *MYC* and *BCL6* rearrangements

Campo et al Blood 2022

Alaggio et al Leukemia 2022



WHO HAEM5

Aggressive B-cell lymphoma classification

International Consensus Classification

9th POSTGRADUATE

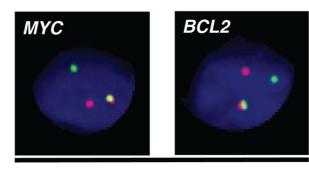
Morphology Burkitt **High-grade** Large B-cell Morphology Burkitt **High-grade** Large B-cell MYC MYC not MYC MYC not rearranged rearranged rearranged rearranged MYC and BCL2 MYC and BCL6 MYC and BCL2 **FISH** testing FISH testing rearrangements rearrangements 11a 11a rearrangements aberration aberration DLBCL, NOS DLBCL, NOS HGBCL-HGBCL-DLBCL/HGBCL-Classification BL LBCL-11a HGBCL. NOS Classification BL HGBCL-11a HGBCL, NOS DH-BCL2 DH-BCL6 DH-BCL2 ABC ABC

Classifications are largely concordant with the exception of aggressive tumours with *MYC* and *BCL6* rearrangements

Campo et al Blood 2022

Alaggio et al Leukemia 2022

High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements – HGBCL–DH–*BCL2*

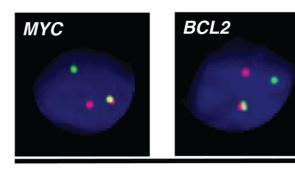


9th POSTGRADUATE

Detected using FISH or (rarely) karyotype Translocations of both *MYC* and *BCL2* Can also harbor a *BCL6* rearrangement NOT copy number gains NOT dual protein expression of MYC and BCL2

Campo et al Blood 2022 Alaggio et al Leukemia 2022 Collinge et al Blood 2022

High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements – HGBCL–DH–*BCL2*



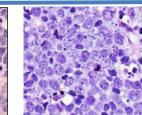
9th POSTGRADUATE

Detected using FISH or (rarely) karyotype Translocations of both *MYC* and *BCL2* Can also harbor a *BCL6* rearrangement NOT copy number gains NOT dual protein expression of MYC and BCL2

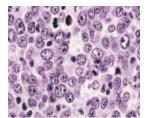
MORPHOLOGY – "High-grade"

High-grade





Intermediate between DLBCL and Burkitt "BCLU"

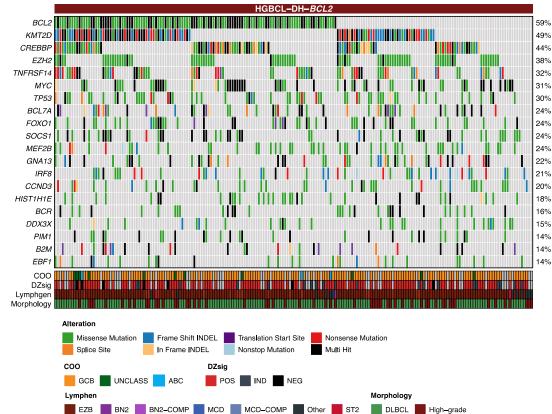


Diffuse large B-cell lymphoma

NOT follicular lymphoma

Campo et al Blood 2022 Alaggio et al Leukemia 2022 Collinge et al Blood 2022

HGBCL-DH-BCL2: mutational landscape

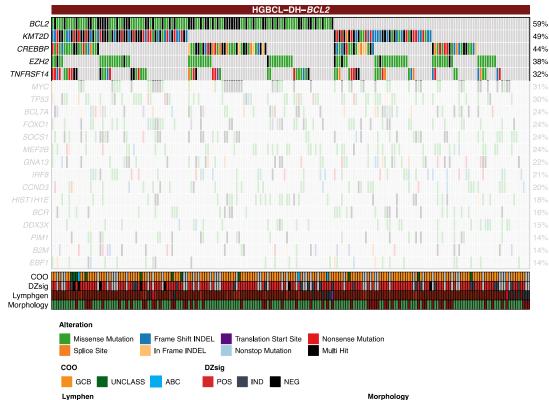


9th POSTGRADUATE

Cucco et al Leukemia 2020 Collinge et al ICML 2021



HGBCL-DH-BCL2: mutational landscape



MCD MCD-COMP Other

ST2

DLBCL

High-grade

9th POSTGRADUATE

BN2

BN2-COMP

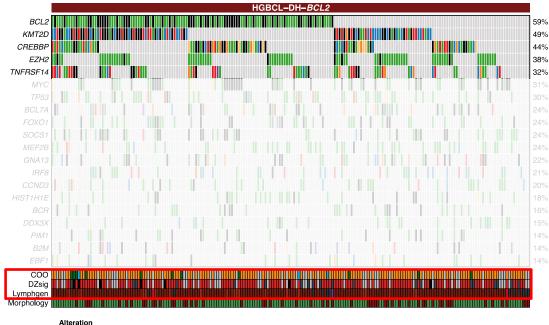
EZB

 Most recurrently mutated genes are shared with follicular lymphoma suggesting a common precursor cell population

> *Cucco et al Leukemia 2020 Collinge et al ICML 2021*



HGBCL-DH-BCL2: mutational landscape



9th POSTGRADUATE

Missense Mutation Frame Shift INDEL Translation Start Site Nonsense Mutation Splice Site In Frame INDEL Nonstop Mutation Multi Hit coo DZsia UNCLASS ABC IND GCB POS NEG Lymphen Morphology MCD MCD-COMP Other EZB BN2 BN2-COMP ST2 DLBCL High-grade

- Most recurrently mutated genes are shared with follicular lymphoma suggesting a common precursor cell population
- Almost all are GCB, express the "dark zone" signature and are EZB genetic subgroup

Cucco et al Leukemia 2020 Collinge et al ICML 2021



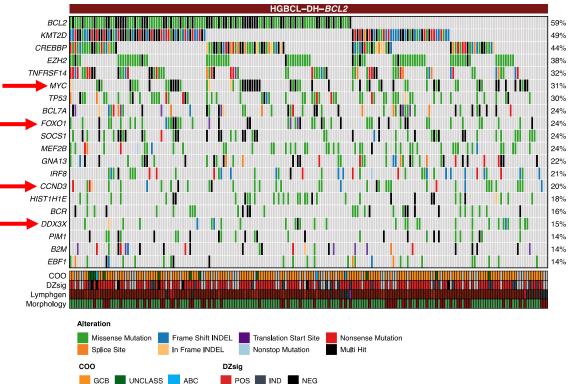
HGBCL-DH-BCL2: mutational landscape

Morphology

DLBCL

High-grade

ST2



BN2-COMP MCD MCD-COMP Other

9th POSTGRADUATE

Lymphen

BN2

- Most recurrently mutated genes are shared with follicular lymphoma suggesting a common precursor cell population
 - Almost all are GCB, express the "dark zone" signature and are EZB genetic subgroup
- Shared mutations with Burkitt lymphoma – genes that regulate the dark zone of the germinal centre





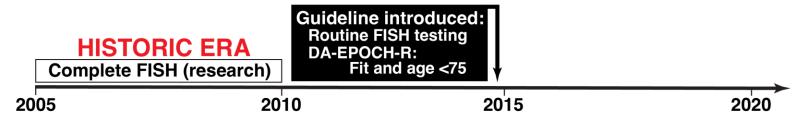
HGBCL-DH-*BCL2* – why does it matter? Intensification is associated with better outcomes

Petrich et al Blood 2014

Goyal et al Haematologica 2023



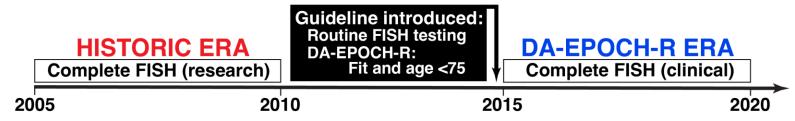
HGBCL-DH-BCL2 – why does it matter? Intensification is associated with better outcomes



Alduaij et al In preparation



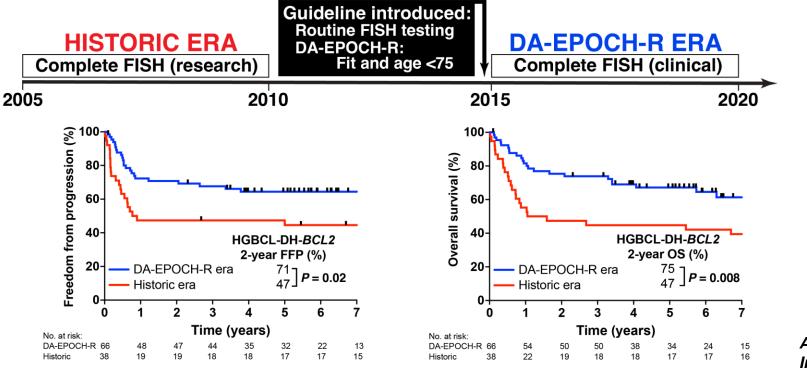
HGBCL-DH-*BCL2* – why does it matter? Intensification is associated with better outcomes



Alduaij et al In preparation



HGBCL-DH-*BCL2* – why does it matter? Intensification is associated with better outcomes



Alduaij et al In preparation



Aggressive B-cell lymphoma classification

International Consensus Classification

High-grade

MYC and BCL2

HGBCL-

MYC and BCL6

HGBCL-

rearrangements rearrangements

9th POSTGRADUATE

Burkitt

MYC

rearranged

BL

MYC not

rearranged

11a

aberration

LBCL-11a

HGBCL. NOS

Morphology

FISH testing

Classification

Large B-cell Morphology Burkitt High-grade Large B-cell

MYC not

rearranged

11a

aberration

HGBCL-11a

HGBCL, NOS

MYC

rearranged

BL



DLBCL. NOS

Campo et al Blood 2022

FISH testing

Classification

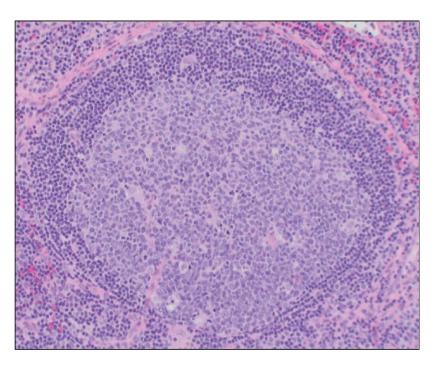
Alaggio et al Leukemia 2022

DLBCL. NOS

MYC and BCL2

rearrangements

DLBCL/HGBCL



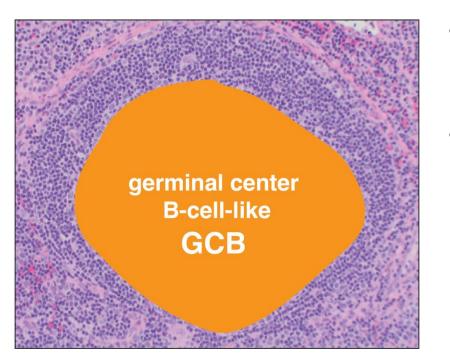
Comparison of gene expression of tumors with B cells at different stages of differentiation

Alizadeh et al Nature 2000

9th POSTGRADUATE

Rosenwald et al N Eng J Med 2002

Lenz et al N Eng J Med 2008



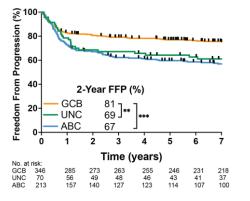
- Comparison of gene expression of tumors with B cells at different stages of differentiation
- Clustering approach producing binary groups – tumors that "look like" (phenocopy) germinal center B cells (GCB) vs those that don't (ABC)

Alizadeh et al Nature 2000

9th POSTGRADUATE

Rosenwald et al N Eng J Med 2002

Lenz et al N Eng J Med 2008

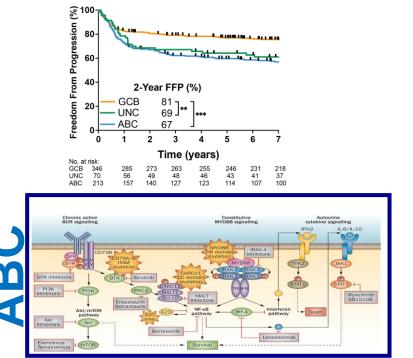


- Comparison of gene expression of tumors with B cells at different stages of differentiation
- Clustering approach producing binary groups – tumors that "look like" (phenocopy) germinal center B cells (GCB) vs those that don't (ABC)
- Different outcomes following R-CHOP

9th POSTGRADUATE

Rosenwald et al N Eng J Med 2002

Lenz et al N Eng J Med 2008



- Comparison of gene expression of tumors with B cells at different stages of differentiation
- Clustering approach producing binary groups tumors that "look like"
 (phenocopy) germinal center B cells
 (GCB) vs those that don't (ABC)
- Different outcomes following R-CHOP
- Distinct mutational landscapes and underlying biology

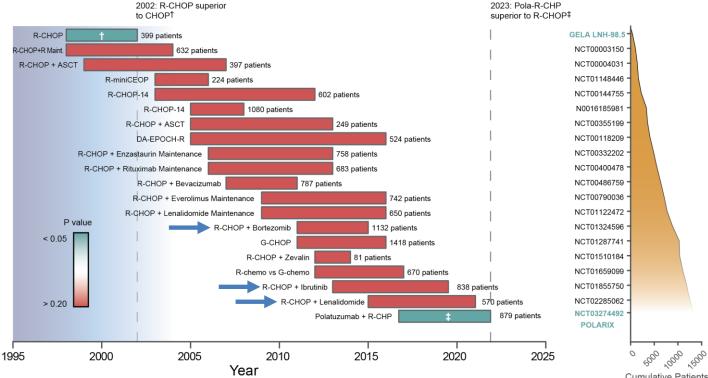
Alduaij, Collinge et al Blood 2023 Alizadeh et al Nature 2000

9th POSTGRADUATE

Rosenwald et al N Eng J Med 2002

Roschewski et al Nat Rev Clin Oncol 2014 Lenz et al N Eng J Med 2008

Phase III RCTs moving beyond R-CHOP

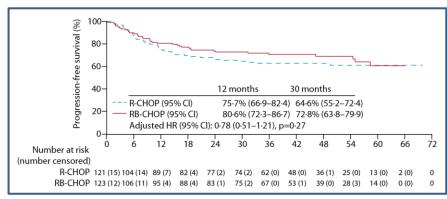


Cumulative Patients Enrolled in Ph3 RCTs

Palmer et al N Engl J Med 2023

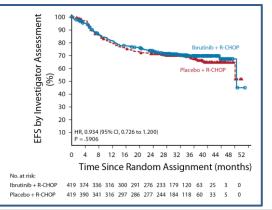
9th POSTGRADUATE

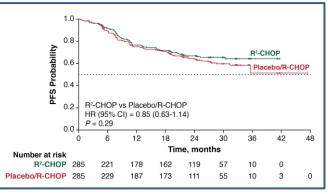
Is cell-of-origin now a historic footnote?



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

9th POSTGRADUATE

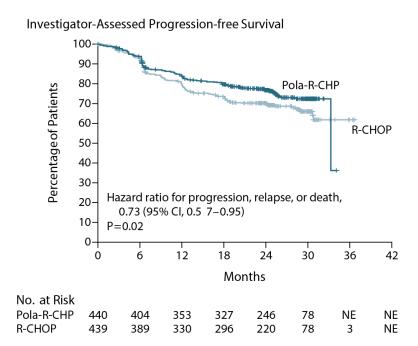




ROBUST: R-CHOP ± lenalidomide Vitolo et al ICML 2019 COO by gene expression

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

Is cell-of-origin now a historic footnote? POLARIX: polatuzumab vedotin-R-CHP vs R-CHOP



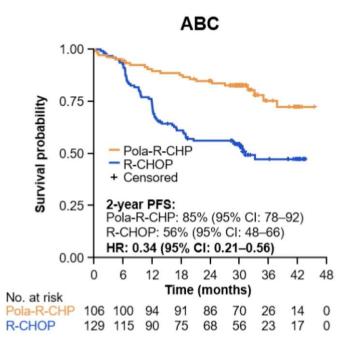
9th POSTGRADUATE

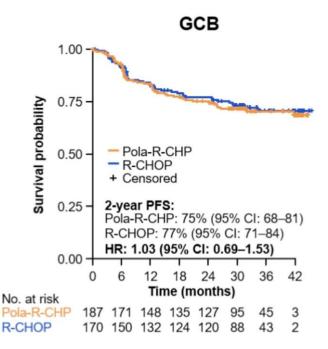
			a-R-CHP I=440)		-CHOP (=439)				
Baseline Risk Factors	Total N	n	2-year Rate	n	2-year Rate	Hazard Ratio	95% Wald Cl	Pola-R-CHP Better	R-CHOP Better
Age group ≤60 >60	271 608	140 300	74·1 77·9	131 308	71-9 69-5	0-9 0-7	(0-6 to 1-5) (0-5 to 0-9)		i
Sex Male Female	473 406	239 201	75·9 77·7	234 205	65-9 75-2	0-7 0-9	(0·5 to 0·9) (0·6 to 1·4)		
ECOG PS 0-1 2	737 141	374 66	78·4 67·2	363 75	71-2 65-0	0-8 0-8	(0.6 to 1.0) (0.5 to 1.4)	, 	
IPI score IPI 2 IPI 3–5	334 545	167 273	79·3 75·2	167 272	78-5 65-1	1·0 0·7	(0.6 to 1.6) (0.5 to 0.9)		
Bulky disease Absent Present	494 385	247 193	82·7 69·0	247 192	70-7 69-7	0·6 1·0	(0.4 to 0.8) (0.7 to 1.5)		
Geographic region Western Europe, United States, Canada, and Australia	603	302	78.6	301	72.0	0.8	(0-6 to 1-1)		4
Asia Rest of world	160 116	81 57	74.3 70.8	79 59	65.6 67.3	0.6	(0.4 to 1.5) (0.6 to 1.5)		4
Ann Arbor stage I–II III IV	99 232 548	47 124 269	89·1 80·7 72·6	52 108 279	85-5 73-6 66-1	0.6	(0.2 to 1.8) (0.5 to 1.3) (0.6 to 1.1)		
Baseline LDH ≤ULN >ULN	300 575	146 291	78-9 75-4	154 284	75-6 67-2	0·8 0·7	(0.5 to 1.3) (0.5 to 1.0)		-
No. of extranodal sites 0-1	453	227	80.2	226	74-5	0-8	(0·5 to 1·1)		4
Cell-of-origin GCB ABC Unclassified Unknown	352 221 95 211	184 102 44 110	75-1 83-9 73-0 73-8	168 119 51 101	76-9 58-8 86-2 64-3	1·0 0·4 1·9 0·7	(0.7 to 1.5) (0.2 to 0.6) (0.8 to 4.5) (0.4 to 1.2)		
Det DEL Non DEL Unknown	290 438 151	139 223 78	75-5 77-7 76-0	151 215 73	63·1 75·7 69·8	0-6 0-9 0-8	(0·4 to 1·0) (0·6 to 1·3) (0·4 to 1·5)		
Double- or triple-hit lymphoma Yes No Unknown	45 620 214	26 305 109	69·0 76·8 78·5	19 315 105	88-9 70-3 66-4	3·8 0·7 0·6	(0.8 to 17-6) (0.5 to 1.0) (0.4 to 1.1)		+
							(0.25	1

Tilly et al N Engl J Med 2022

Is cell-of-origin now a historic footnote?

POLARIX: polatuzumab vedotin-R-CHP vs R-CHOP





Morschhauser et al ASH Annual Meeting 2023

9th POSTGRADUATE

Is cell-of-origin now a historic footnote?

POLARIX: polatuzumab vedotin-R-CHP vs R-CHOP

							PFS								OS									
Baseline risk factors		Pola-R-CHP (n=440)		R-CHOP (n=439		HR	95% Wald	Pola-R-CHP	R-СНОР	Pola-R-CHP (n=440)		R-CHOP (n=439)		HR	95% Wald	Pola-R-CHP	R-CHOP							
Basenine ris		n	60-month (%)	n	60-month (%)		CI	better	better	n	60-month (%)	n	60-month (%)		CI	better	better							
All patients		440	64.9	439	59.1	0.78	0.62–0.97	ģ		440	82.3	439	79.5	0.85	0.63–1.16	н	4	_						
Age group	≤65	225	69.6	219	64.3	0.80	0.57–1.11	H	1	225	89.1	219	84.7	0.73	0.44–1.21	H								
Age group	>65	215	60.0	220	54.5	0.78	0.58–1.06	+		215	75.3	220	74.5	0.95	0.65–1.38		н	_						
Stratification –	2	167	67.2	167	68.3	0.91	0.61–1.36	H.	4	167	87.6	167	87.4	0.96	0.53–1.75	H								
	3–5	273	63.2	272	53.5	0.72	0.55–0.94			273	79.2	272	74.7	0.81	0.57–1.15	н	4	_						
Stratification – J	Absent	247	69.9	247	60.0	0.61	0.44–0.83	H		247	83.9	247	80.9	0.79	0.52–1.20	н	-							
	Present	193	58.5	192	57.9	1.02	0.73–1.41		н	193	80.3	192	77.9	0.92	0.60–1.43	F	н	_						
Baseline LDH	≤1xULN	146	65.3	154	64.8	0.83	0.55–1.23	⊢ •	-	146	88.7	154	87.9	0.85	0.45–1.61	н	-	•	are subgroup	se				
	>1xULN	291	64.3	284	55.7	0.77	0.59–1.01	H a r		291	79.0	284	74.9	0.85	0.60–1.19	н	ч	_						
No. of	0–1	227	68.1	226	64.2	0.78	0.56–1.09	⊢ ∎	н	227	83.7	226	81.9	0.86	0.56–1.34	н	4							
extranodal sites	≥2	213	61.2	213	53.8	0.78	0.58–1.06	⊢∎ <mark>-</mark>	1	213	80.9	213	77.1	0.85	0.56–1.28	н	н	_						
ſ	DLBCL	373	65.7	367	58.8	0.75	0.59–0.95	H		373	81.9	367	79.8	0.89	0.64–1.23	н	•							
NHL subtype	HGBL, DHL/THL	43	66.0	50	57.6	0.67	0.33–1.37		-	43	85.4	50	72.4	0.46	0.18–1.22	⊢	-							
	Other LBCL	24	49.7	22	70.3	1.86	0.69–5.04	H		24	83.3	22	90.9	1.93	0.35–10.52									
1	NanoString GCB	187	65.9	170	65.8	1.07	0.74–1.56		Η	187	82.9	170	82.3	0.99	0.60–1.61	F	-							
NanoString COO	NanoString ABC	106	72.5	129	45.8	0.38	0.24–0.59	H		106	84.6	129	69.9	0.49	0.28–0.88	H -	ł							
Nanostring COO	NanoString UNC	44	55.2	53	70.8	1.60	0.79–3.25	-		44	76.9	53	94.2	4.46	1.23–16.21		⊢ (
l	Unknown	103	60.2	87	59.7	0.83	0.51–1.33	H	1	103	81.3	87	79.0	0.80	0.42–1.51	н	-4							
Double	DEL	139	63.1	151	50.0	0.65	0.45–0.94	H		139	76.4	151	73.0	0.84	0.53–1.33	н	4							
expressor	Non DEL	223	66.6	215	64.7	0.89	0.64–1.24	l l	H	223	86.3	215	82.8	0.81	0.51–1.30	н	4							
by IHC	Unknown	78	63.7	73	63.5	0.84	0.48–1.47	H	4	78	81.6	73	84.1	1.18	0.53–2.59		- -1	_						
	1/100 1 100 1/100 1/100 Salles et al ASH Annual Meeting 2024																							

9th POSTGRADUATE

Dark zone lymphomas: converging evidence

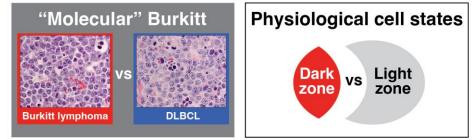
"Molecular" Burkitt

9th POSTGRADUATE

Dave et al N Engl J Med 2006 Hummel et al N Engl J Med 2006 Sha et al J Clin Oncol 2019

"Molecular high-grade" signature

Dark zone lymphomas: converging evidence



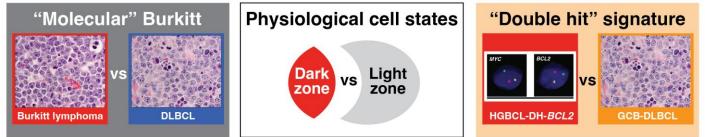
Dave et al N Engl J Med 2006 Hummel et al N Engl J Med 2006 Sha et al J Clin Oncol 2019

9th POSTGRADUATE

Victora et al Blood 2012 Dybkær et al J Clin Oncol 2015 Holmes et al J Exp Med 2020

"Molecular high-grade" signature

Dark zone lymphomas: converging evidence



Dave et al N Engl J Med 2006 Hummel et al N Engl J Med 2006 Sha et al J Clin Oncol 2019

9th POSTGRADUATE

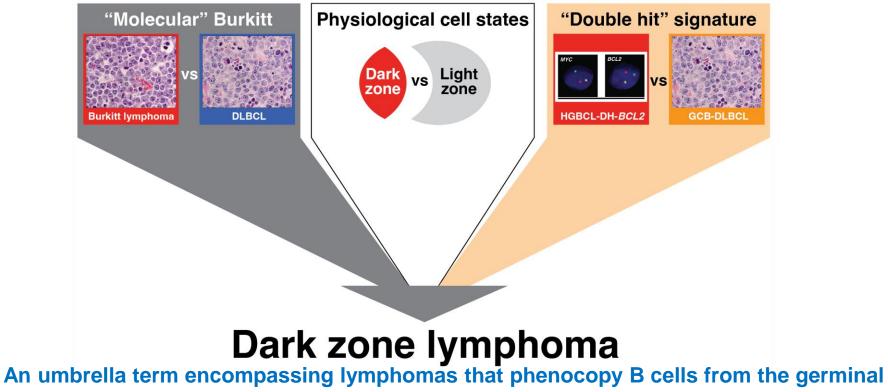
Victora et al Blood 2012 Dybkær et al J Clin Oncol 2015 Holmes et al J Exp Med 2020 Ennishi et al J Clin Oncol 2019 Alduaij et al Blood 2023

"Molecular high-grade" signature

"Dark zone" signature (DZsig)

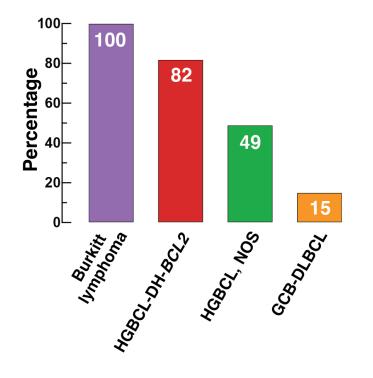
Dark zone lymphomas: converging evidence

9th POSTGRADUATE



center dark zone

Dark zone lymphomas



9th POSTGRADUATE

Defined ICC/WHO entities:

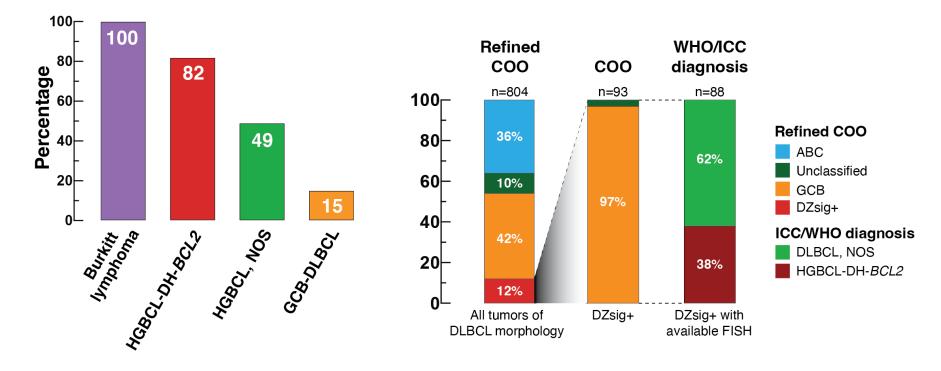
- All Burkitt lymphoma
- Most HGBCL-DH-BCL2 ("double hit")

Not otherwise specified groups:

- Half of high-grade B-cell lymphoma, NOS
- 15% of GCB-DLBCL, NOS

Dark zone lymphomas

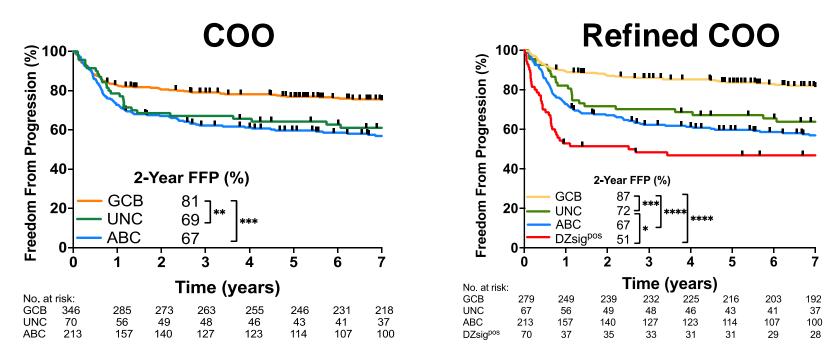
9th POSTGRADUATE



Alduaij, Collinge et al Blood 2023

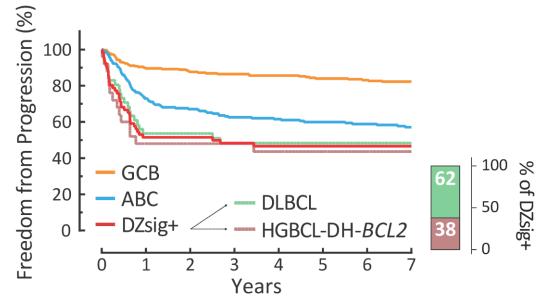
Dark zone lymphomas: outcomes

9th POSTGRADUATE



DZsig+ identifies the poorest prognosis group Removing these tumors from GCB-DLBCL leaves a patient group with excellent outcomes following R-CHOP Alduaij, Collinge et al Blood 2023

Dark zone lymphomas: poor outcomes whether HGBCL-DH-BCL2 or not



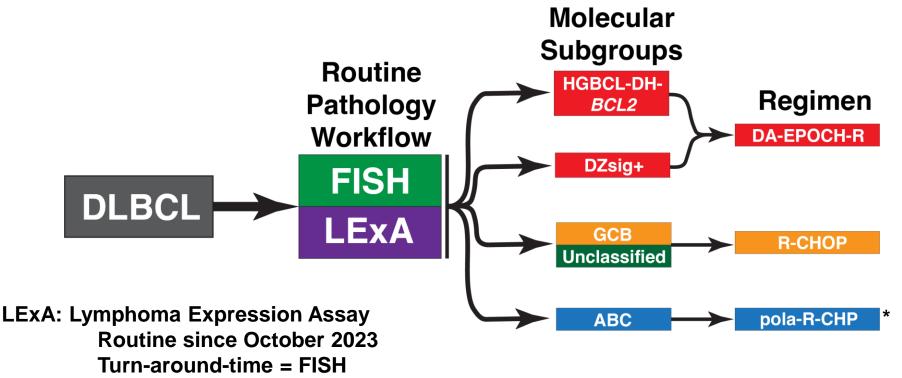
DZsig+ identifies the poorest prognosis group

9th POSTGRADUATE

Removing these tumors from GCB-DLBCL leaves a patient group with excellent outcomes following R-CHOP *Alduaij, Collinge et al Blood 2023*

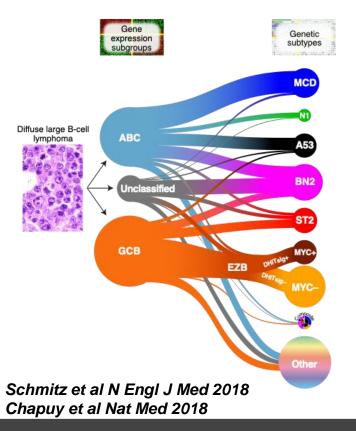
BC Cancer's treatment selection algorithm

9th POSTGRADUATE



*Awaiting Canadian funding decision

Genetics-based subtypes of DLBCL



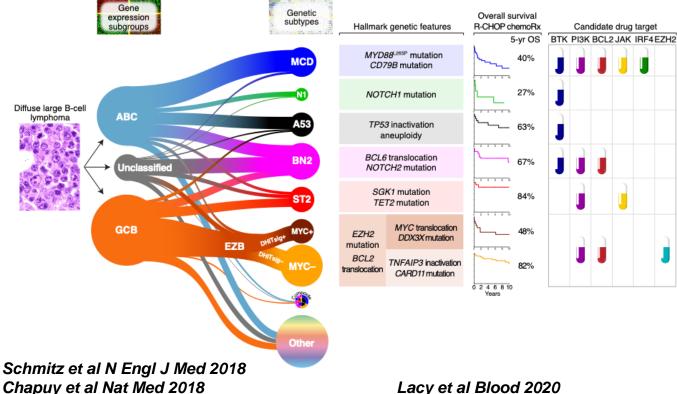
9th POSTGRADUATE

- Three groups have described similar (but not identical) groupings based on co-occurrence of selected genetic features
- Variable requirements for mutation, copy number and rearrangement data
- LymphGen was the only algorithm that could be applied on a biopsy-by-biopsy basis
 - ~35% of tumors that can not be assigned to a group ("Other")
- DLBclass has just been released (December 2024)
 - All tumors are assigned to a group (25% at low confidence)
 - Optimal performance needs copy number data

Wright et al Cancer Cell 2020 Chapuy et al Blood 2025

Lacy et al Blood 2020

Genetics-based subtypes of DLBCL



9th POSTGRADUATE

Wright et al Cancer Cell 2020 Chapuy et al Blood 2025

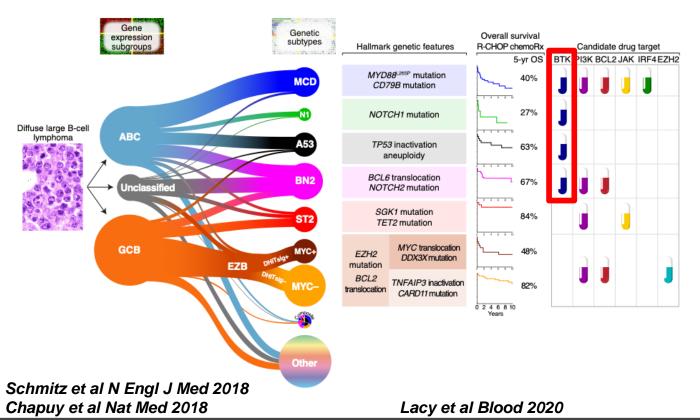
Florence.

March 20-21, 2025

Lacy et al Blood 2020



Genetics-based subtypes of DLBCL



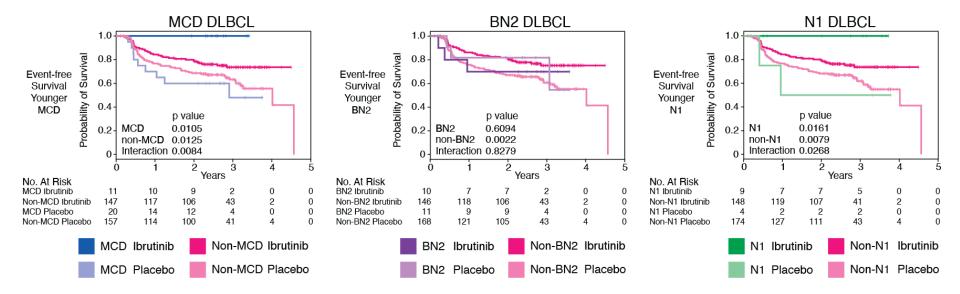
9th POSTGRADUATE

Wright et al Cancer Cell 2020 Chapuy et al Blood 2025



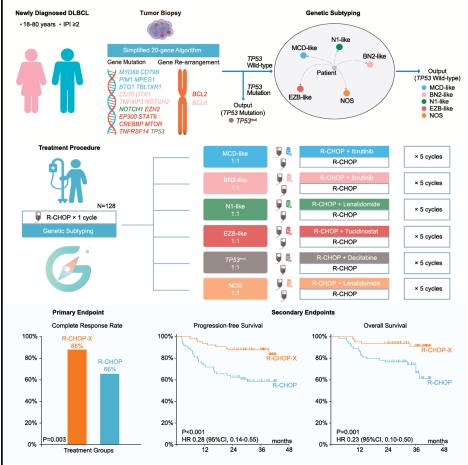
Genetics-based subtypes as a predictive biomarker – retrospective genomic analysis of the PHOENIX trial

9th POSTGRADUATE



Wilson et al Cancer Cell 2021

Guidance 1 – a model for trials of precision medicine based on geneticsbased subtypes



Zhang et al Cancer Cell 2023

9th POSTGRADUATE

Challenges to implementing refined classifications

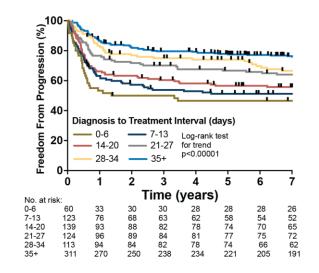
- Harmonization of the genetics-based classifications
- Settling on (and validating) an appropriate assay
- Turn-around-time

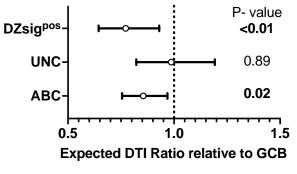
9th POSTGRADUATE

Challenges to implementing refined classifications

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

9th POSTGRADUATE





Impact of the genetics-based subtypes is not known

Maurer et al J Clin Oncol 2014

Florence.

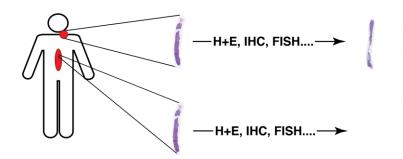
March 20-21, 2025

Challenges to implementing refined classifications

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

9th POSTGRADUATE

• Availability of tissue – small biopsies, bone marrow



- FFPE core needle biopsies are now the norm
- They are a suitable substrate for genomics assays if they are not "exhausted"
- Are we adequately sampling the tumor? This impacts our ability to detect mutational subclones

Maurer et al J Clin Oncol 2014

Alduaij, Collinge et al Blood 2023

Challenges to implementing refined classifications

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

9th POSTGRADUATE

- 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-totreatment interval
 - Characterization at relapse (particularly "late") is important as these can be *de novo* from a common precursor cell population
 - Circulating tumor DNA may be able to fill this gap
- US Intergroup trial based on genetics-based subtypes is in the late planning stage

Hilton et al J Clin Onocl 2023 Alduaij, Collinge et al Blood 2023

Maurer et al J Clin Oncol 2014

Desai et al Blood Adv 2022