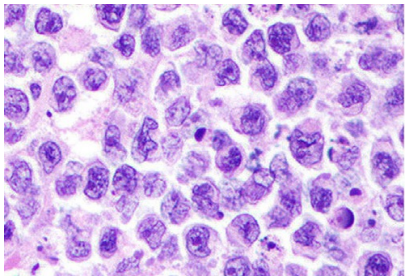
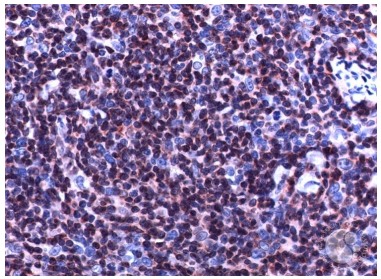
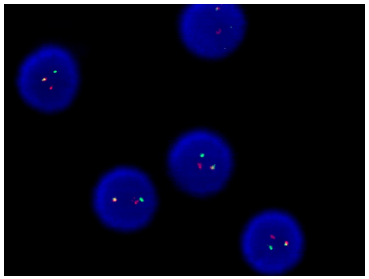
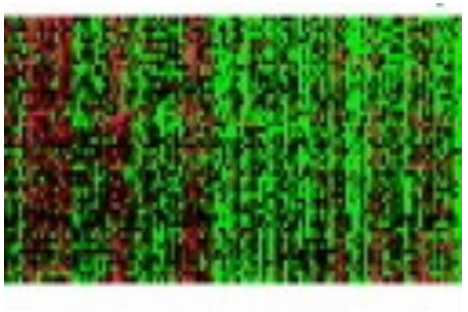
UNIVERSITY OF CAMBRIDGE
Department of Haematology

wellcome-MRC cambridge stem cell institute

Molecular profiling in DLBCL

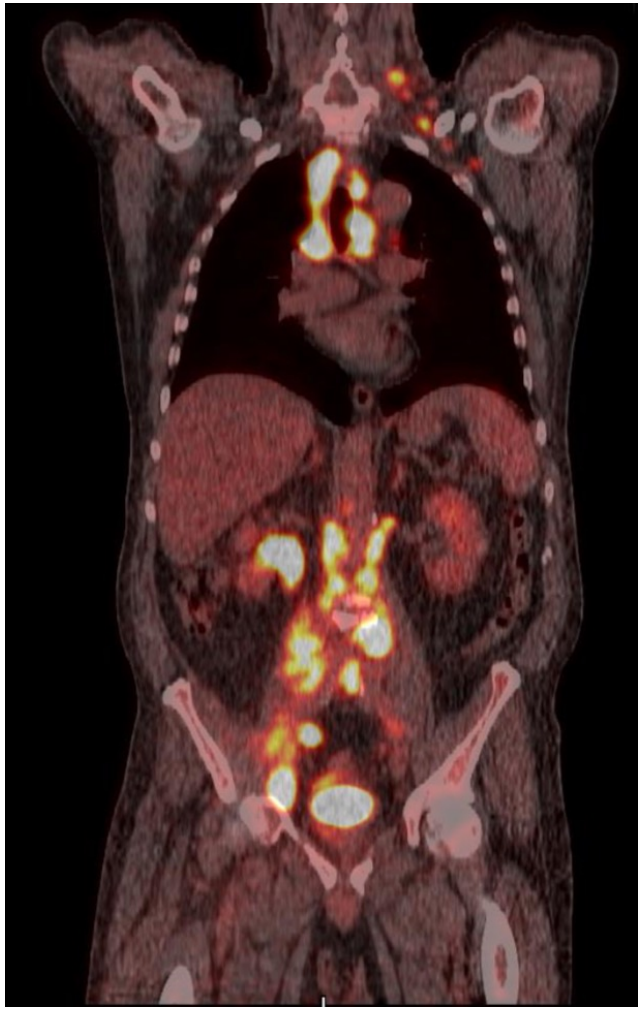
Do we need NGS in lymphoma practice?

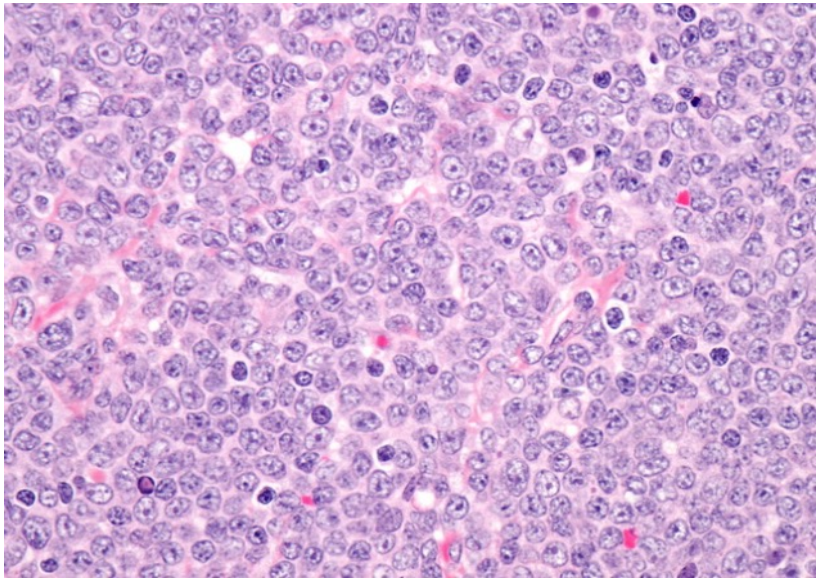





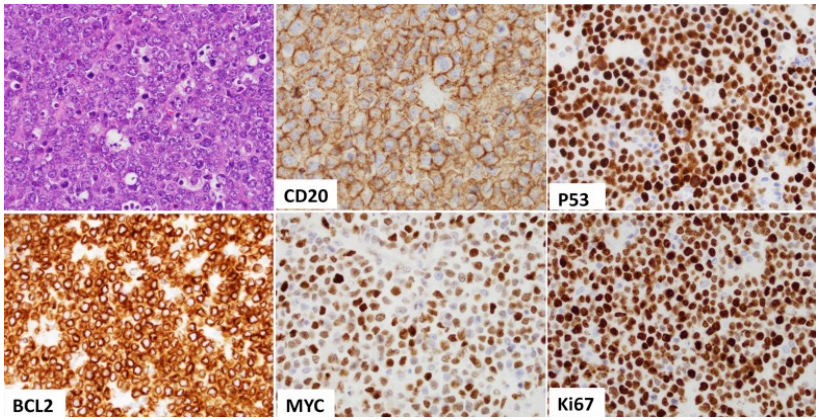
Dan Hodson MD PhD

CRUK Senior Cancer Research Fellow
Honorary Consultant Haematologist
Wellcome MRC Cambridge Stem Cell Institute
University of Cambridge, UK

Diffuse Large B cell Lymphoma (DLBCL)







Epidemiology
- Commonest NHL
- 6-8 per 100,000 per year

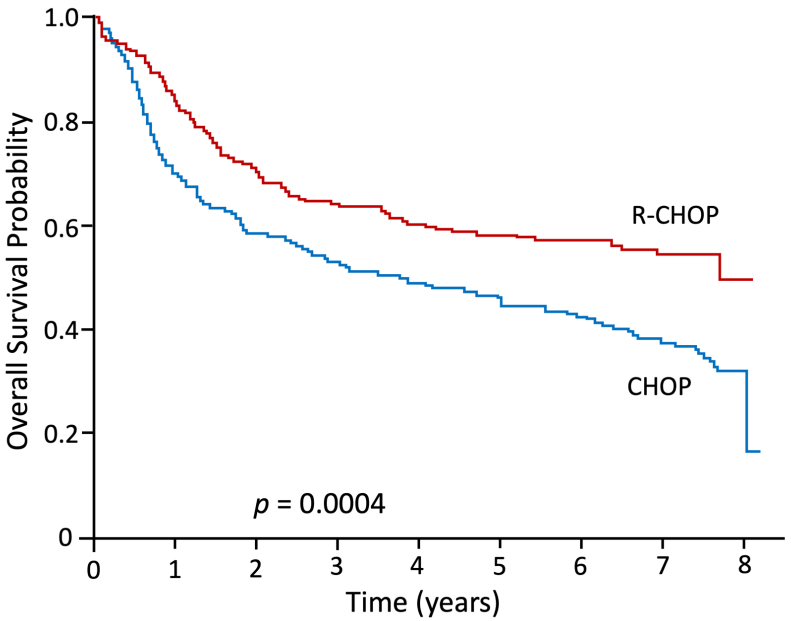
Presentation
- Rapidly enlarging nodal and extranodal tumors

Diagnosis requires biopsy
- Histology
- Immunohistochemistry
- COO assignment
- FISH

Staging
- PET-CT

First-line treatment
- R-CHOP

DLBCL – improving *first-line* therapy



CHOP Chemotherapy plus Rituximab Compared with CHOP Alone in Elderly Patients with Diffuse Large-B-Cell Lymphoma

Bertrand Coiffier, M.D., Eric Lepage, M.D., Ph.D., Josette Brière, M.D., Raoul Herbrecht, M.D., Hervé Tilly, M.D., Reda Bouabdallah, M.D., Pierre Morel, M.D., Eric Van Den Neste, M.D., Gilles Salles, M.D., Ph.D., Philippe Gaulard, M.D., Felix Reyes, M.D., Pierre Lederlin, Pierre Lederlin, Ph.D., et al.

Published **2002**

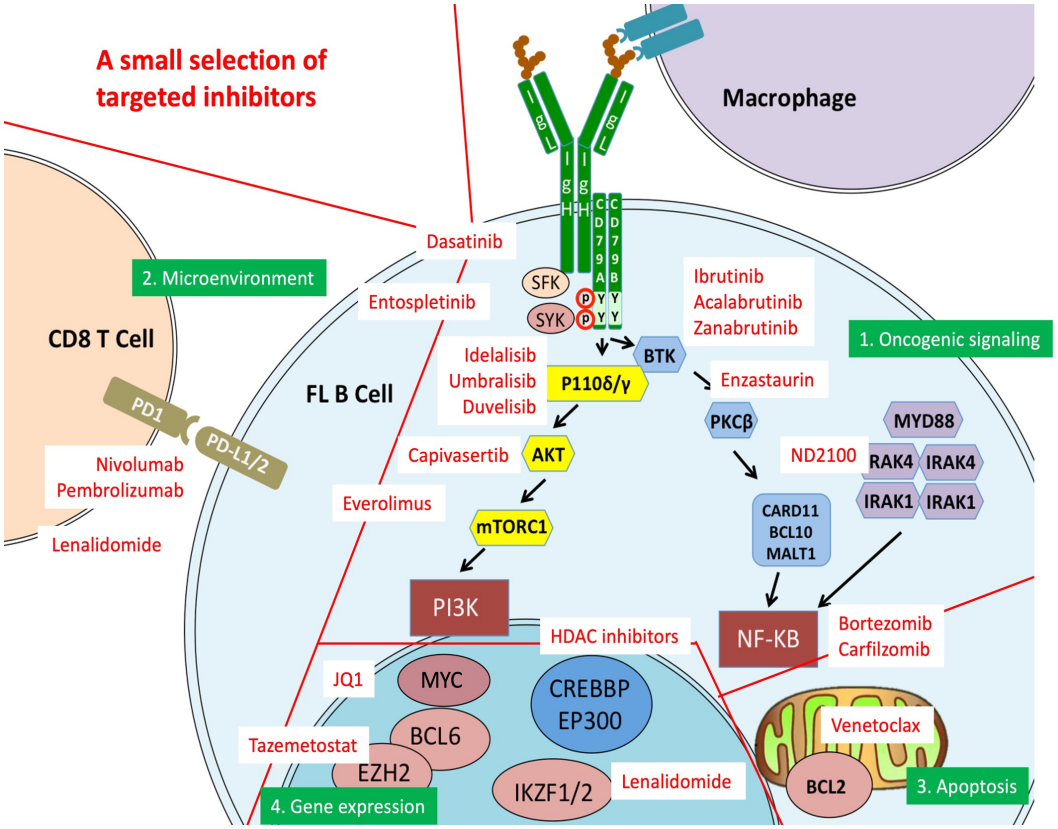
Post R-CHOP - Major 1st line DLBCL trials

- Chemo intensification
- *Promising* targeted drugs

Study	Date	Author	Journal	Enrolled	Prim endpoint met
ACVBP-R	2011	Recher C	Lancet	380	Debatable
R-CHOEP-14 / MegaCHOEP-R	2012	Schmitz N	Lancet Oncol	275	No
R-CHOP14	2013	Delarue R	Lancet Oncol	602	No
R-CHOP14	2013	Cunningham D	Lancet	1,080	No
ASCT	2013	Stiff PJ	NEJM	397	PFS but not OS
Bevacizumab + RCHOP	2014	Seymour J	Haematologica	787	No
R-CHOP + R-Maint	2015	Jaeger U	Haematologica	662	No
Enzastaurin maintenance	2016	Crump M	JCO	758	No
Dose dense RCHOP / ASCT	2017	Chiappella A	Lancet Oncol	412	PFS but no OS
G-CHOP (GOYA)	2017	Vitolo U	JCO	1,418	No
Lena Maintenance	2017	Thieblemont C	JCO	784	PFS but not OS
Everolimus adjuvant	2018	Witzig TE	Ann Oncol	742	No
DA-EPOCH-R	2019	Bartlett NL	JCO	524	No
Bortezomib-R-CHOP	2019	Davies A	Lancet Oncol	1,128	No
Ibrutinib RCHOP (Phoenix)	2019	Younes A	JCO	838	No
Lena-R-CHOP (Robust)	2021	Nowakowski G	JCO	570	No
Pola-R-CHP (Polarix)	2021	Tilly H	NEJM	879	PFS but no OS

>12,000 patients enrolled. But no change to standard of care 1st line therapy.

What is the barrier to progress?



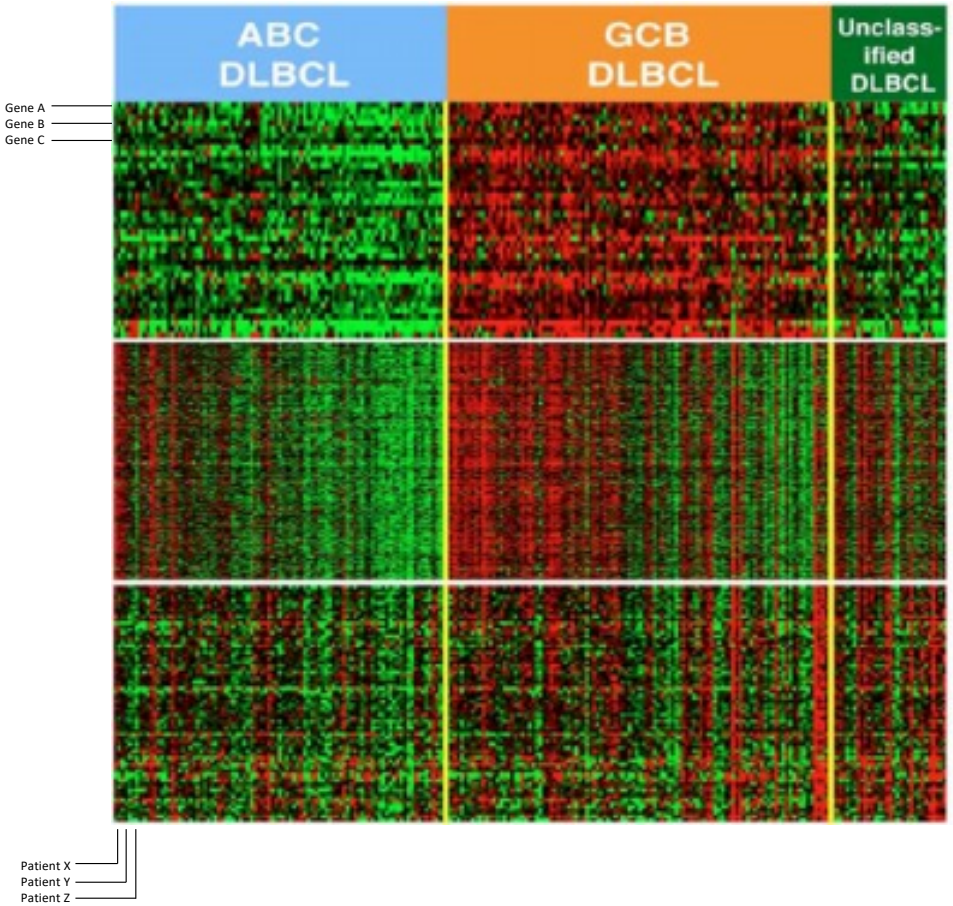
What is the barrier to progress?

Biologically-targeted therapies need to be targeted at the biology

There are no "biology-agnostic" therapies. There are only "biology-agnostic" trials.
(Examples – CAR-T / Pola)

At the biological level, DLBCL comprises multiple different molecular diseases

20 Years of Cell of Origin (COO)



Gene expression / transcriptional profiling
(Microarray / RNA-Sequencing)

Two dominant clusters based on pattern of mRNA abundance.

- Little practical benefit in the clinic
- Invaluable as a framework to dissect the biology

2000 – Described Alizadeh / Staudt

- Technical challenges for clinical labs
- Oversimplistic proxy assays limited accuracy
- Confusion prognostic vs biological implications

2017 – Incorporated into WHO classification

2018 – Genetic classifications proposed

DLBCL - genetic classification

ORIGINAL ARTICLE

574 cases

Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma

R. Schmitz, G.W. Wright, D.W. Huang, C.A. Johnson, J.D. Phelan, J.Q. Wang, S. Roulland, M. Kasbekar, R.M. Young, A.L. Shaffer, D.J. Hodson, W. Xiao, X. Yu, Y. Yang, H. Zhao, W. Xu, X. Liu, B. Zhou, W. Du, W.C. Chan, E.S. Jaffe, R.D. Gascoyne, J.M. Connors, E. Campo, A. Lopez-Guillermo, A. Rosenwald, G. Ott, J. Delabie, L.M. Rimsza, K. Tay Kuang Wei, A.D. Zelenetz, J.P. Leonard, N.L. Bartlett, B. Tran, J. Shetty, Y. Zhao, D.R. Soppet, S. Pittaluga, W.H. Wilson, and L.M. Staudt

nature medicine

304 cases

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

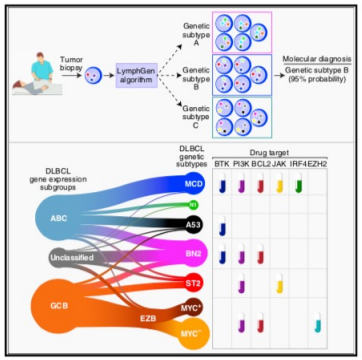
Bjoern Chapuy[1,2,18], Chip Stewart[3,18], Andrew J. Dunford[3,18], Jaegil Kim[3], Atanas Kamburov[3], Robert A. Redd[4], Mike S. Lawrence[2,3,5], Margaretha G. M. Roemer[1], Amy J. Li[6], Marita Ziepert[7], Annette M. Staiger[8,9], Jeremiah A. Wala[3], Matthew D. Ducar[10], Ignaty Leshchiner[3], Ester Rheinbay[3], Amaro Taylor-Weiner[3], Caroline A. Coughlin[1], Julian M. Hess[3], Chandra S. Pedamallu[3], Dimitri Livitz[3], Daniel Rosebrock[3], Mara Rosenberg[3], Adam A. Tracy[3], Heike Horn[8], Paul van Hummelen[10], Andrew L. Feldman[11], Brian K. Link[12], Anne J. Novak[11], James R. Cerhan[11], Thomas M. Habermann[11], Reiner Siebert[13], Andreas Rosenwald[14], Aaron R. Thorner[10], Matthew L. Meyerson[2,3], Todd R. Golub[2,3], Rameen Beroukhim[2,3], Gerald G. Wulf[15], German Ott[9], Scott J. Rodig[2,16], Stefano Monti[6], Donna S. Neuberg[2,4], Markus Loeffler[7], Michael Pfreundschuh[17], Lorenz Trümper[15], Gad Getz[2,3,5,19*] and Margaret A. Shipp[1,2,19*]

Cancer Cell

Pooled Reanalysis

A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications

Graphical Abstract



Authors

George W. Wright, Da Wei Huang, James D. Phelan, ..., Wyndham H. Wilson, David W. Scott, Louis M. Staudt

Correspondence

lstaudt@mail.nih.gov

In Brief

Wright et al. identify seven genetic subtypes of diffuse large B cell lymphoma (DLBCL) with distinct outcomes and therapeutic vulnerabilities. The LymphGen probabilistic classification tool that can classify a DLBCL biopsy into the genetic subtypes is developed, which could be used for precision medicine trials.

blood

Regular Article

928 cases

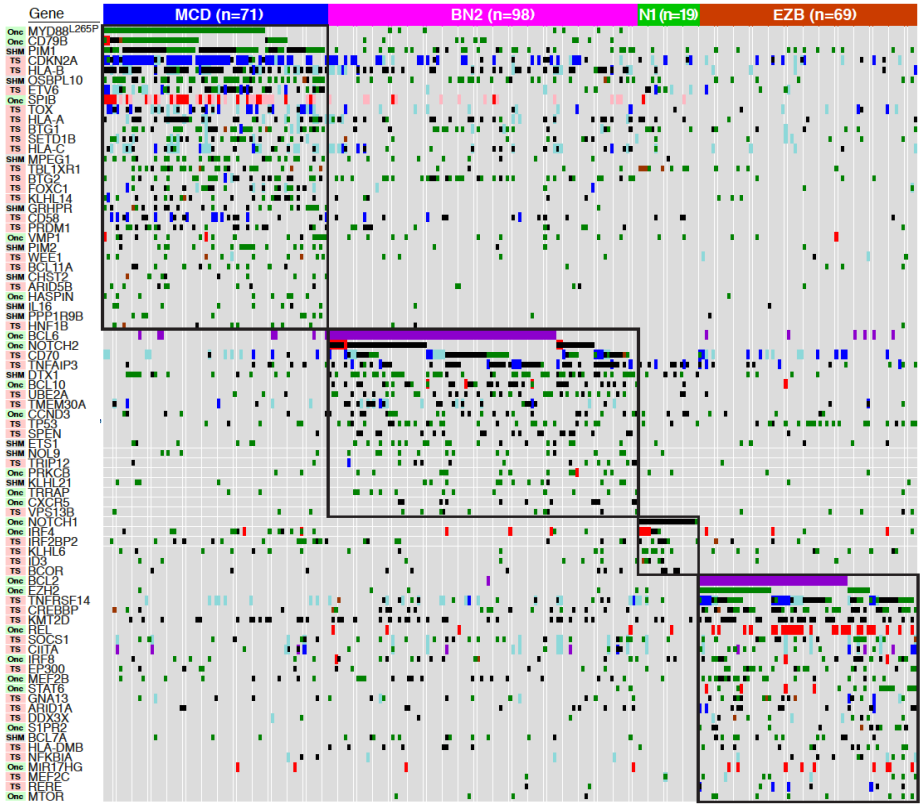
LYMPHOID NEOPLASIA

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

Stuart E. Lacy,[1,*] Sharon L. Barrans,[2,*] Philip A. Beer,[3,*] Daniel Painter,[1] Alexandra G. Smith,[1] Eve Roman,[1] Susanna L. Cooke,[4] Camilo Ruiz,[3] Paul Glover,[2] Suzan J. L. Van Hoppe,[2] Nichola Webster,[2] Peter J. Campbell,[3] Reuben M. Tooze,[5] Russell Patmore,[6] Cathy Burton,[2,†] Simon Crouch,[1,†] and Daniel J. Hodson[7,†]

DLBCL - genetic classification

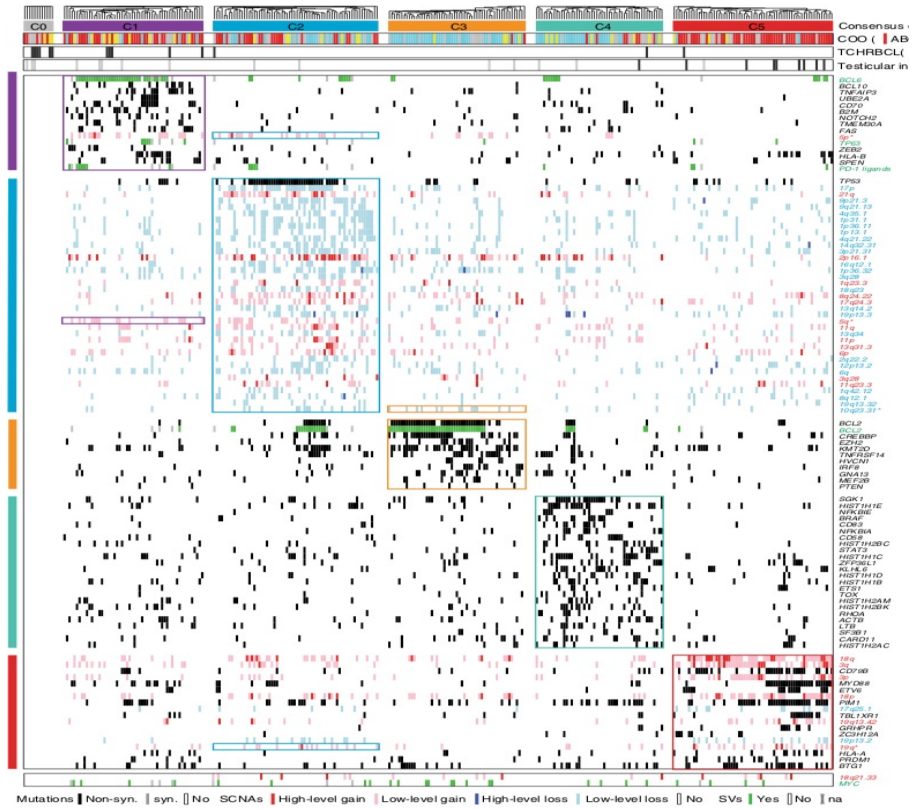
Staudt & Co; NCI; n= 574



4 subtypes, 47% classified

Named by acronym

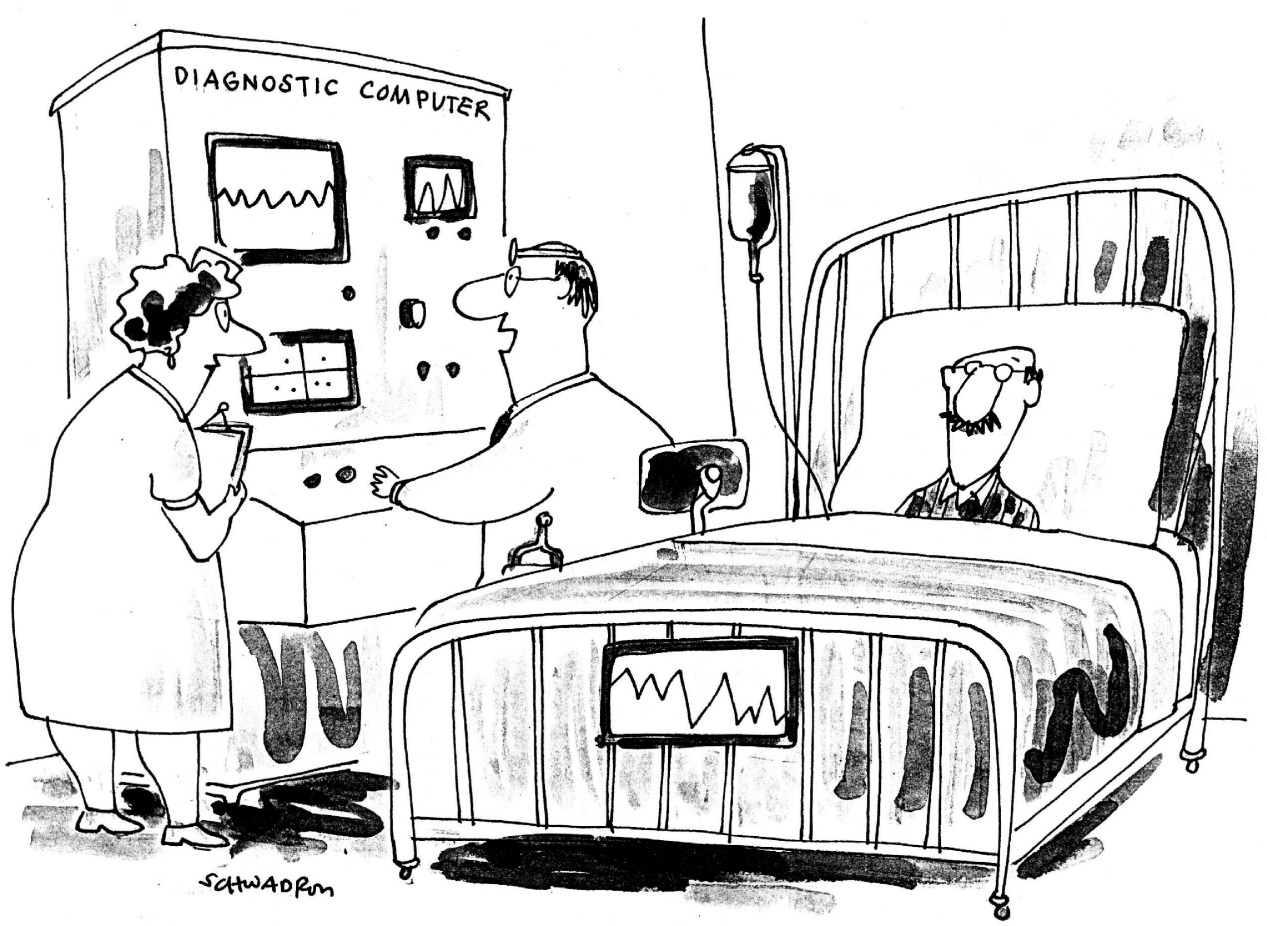
Shipp & Co; Harvard; n= 304



5 subtypes, 96% classified

Named C1-5

DLBCL Molecular Subtypes – *why should we care*?



DLBCL Molecular Subtypes – *questions needing balanced answers*

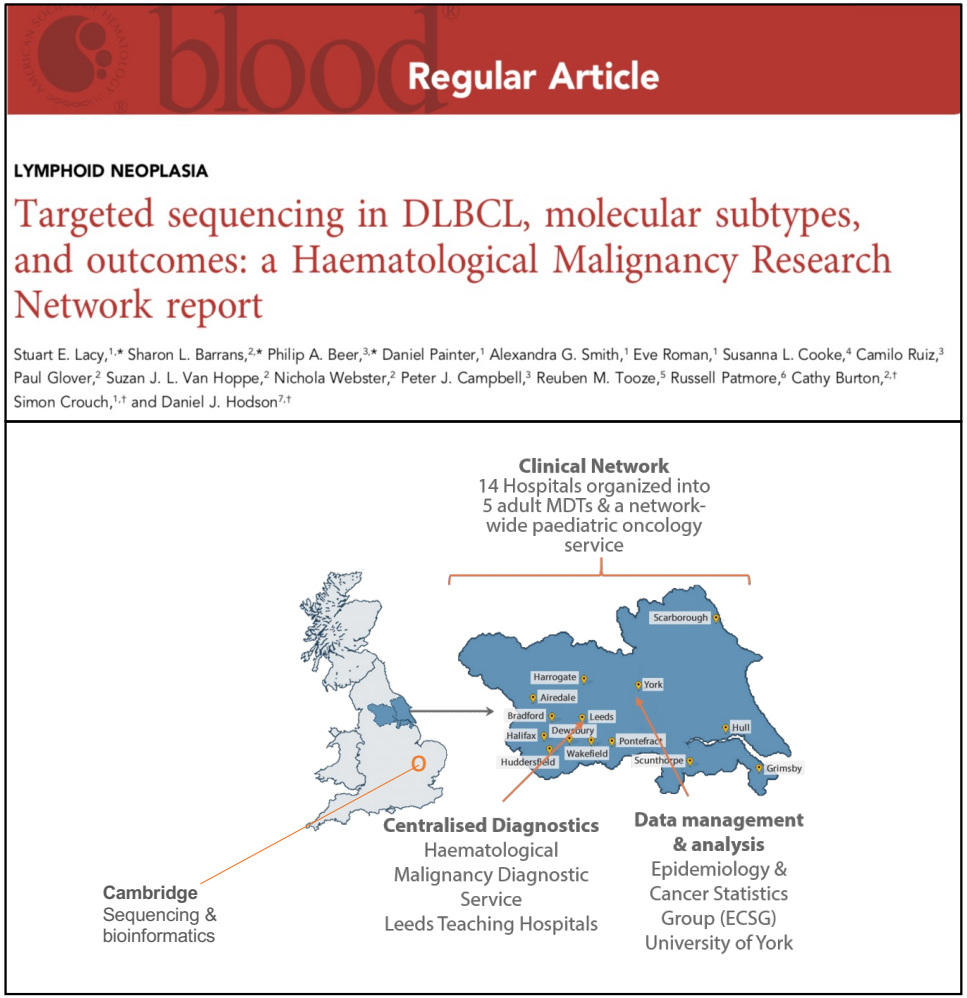
1. What are the subtypes and why do we believe they are real?

2. Do the classification systems agree at the individual patient level?

3. Do they tell us about prognosis?

4. Do they tell us how to treat?

DLBCL Genetic subtypes; the UK experience



blood Regular Article

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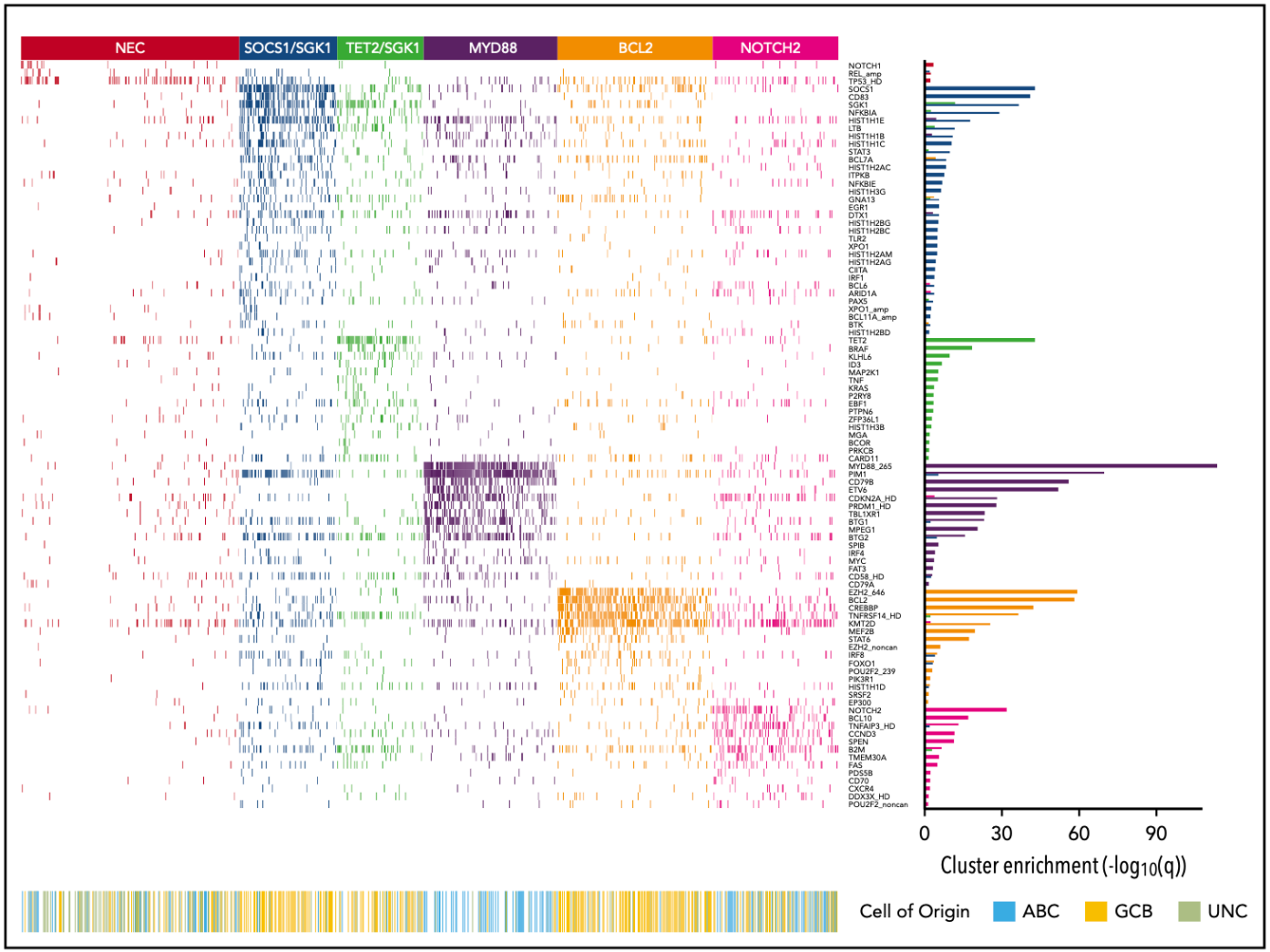
- **UK HMRN Registry**
  - Enrolls EVERY new haem cancer diagnosis
  - Avoids clinical trial or pathology referral bias.
  - Clinical outcome data for every patient
- DNA extracted from **FFPE biopsy**
- Targeted sequencing (293 genes - limited copy number data available)
- Sequenced **928 cases of 1st line DLBCL**
- Bernoulli mixture modelling to identify clusters of tumours with the greatest genetic similarity.
- **This is a different and independent strategy to identify genetic subtypes.**
- Did we reach same or different conclusions?

DLBCL Genetic subtypes; the UK experience



Genetic subtypes closely recapitulate the **main findings** of NCI and Harvard classifications.

Six genetic clusters – named by gene.

All clusters can be mapped to an NCI and/or Harvard cluster.

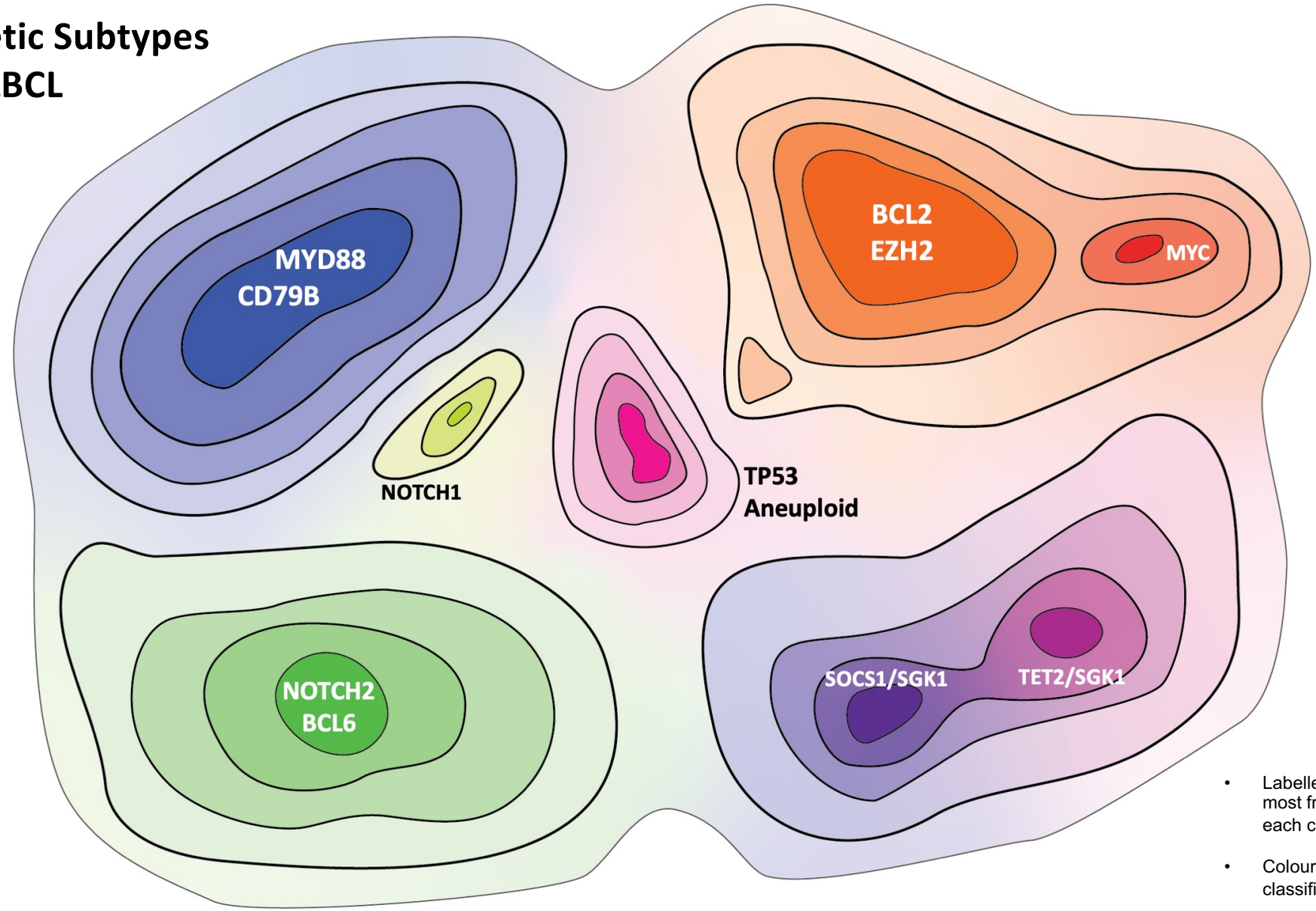
Remarkable level of consensus

Shared genetic features with other lymphoma subtypes - eg:

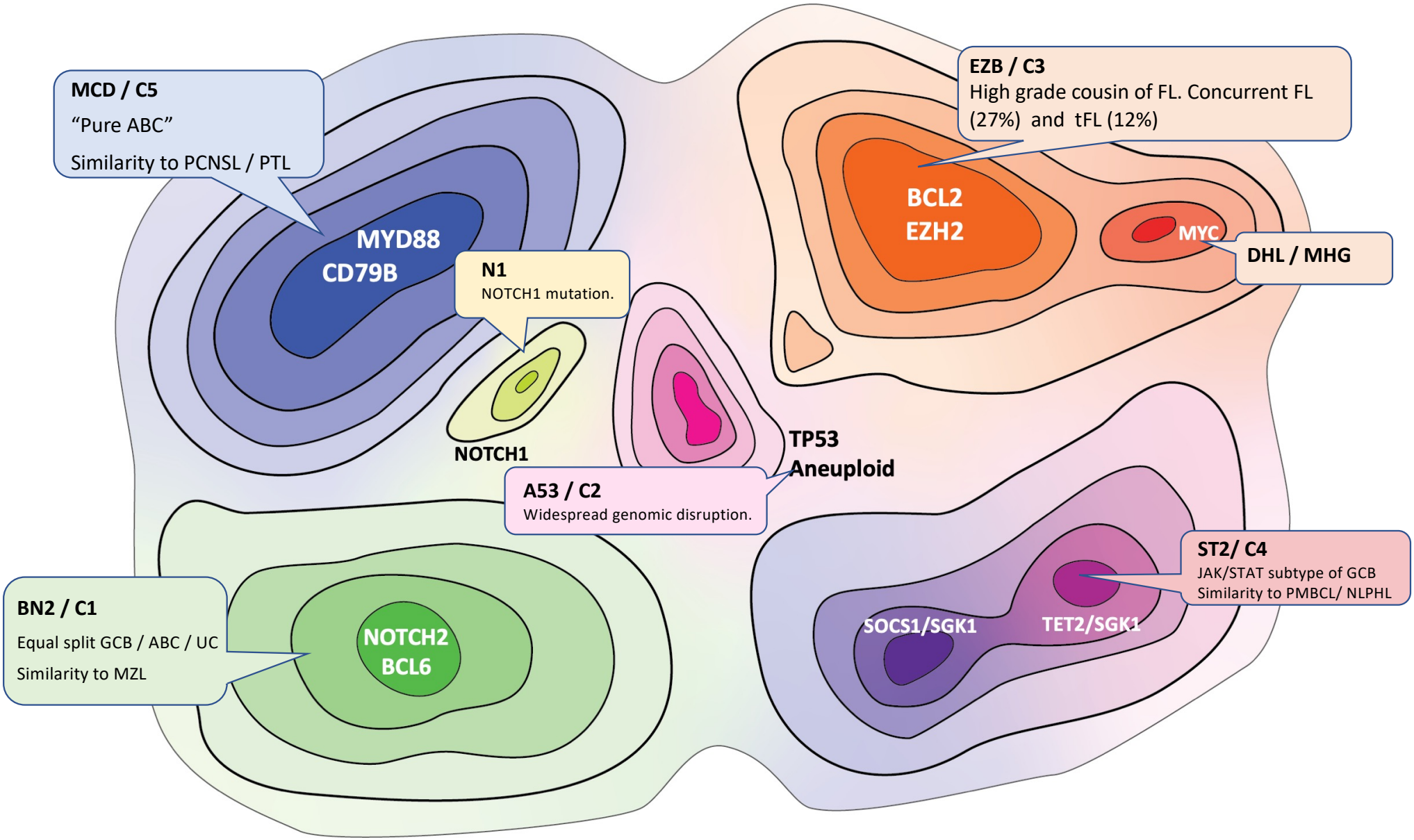
- FL
- PCNSL/PTL/PBL
- PMBCL
- MZL
- DHL

Blurring of boundaries of clinical and biological classifications.

Genetic Subtypes of DLBCL



- Labelled genes are those most frequently mutant in each cluster.
- Colour density reflects classification confidence


MCD / C5
"Pure ABC"
Similarity to PCNSL / PTL
MYD88
CD79B
N1
NOTCH1 mutation.
NOTCH1
EZB / C3
High grade cousin of FL. Concurrent FL (27%) and tFL (12%)
BCL2
EZH2
MYC
DHL / MHG
TP53
Aneuploid
A53 / C2
Widespread genomic disruption.
BN2 / C1
Equal split GCB / ABC / UC
Similarity to MZL
NOTCH2
BCL6
SOCS1/SGK1
TET2/SGK1
ST2/ C4
JAK/STAT subtype of GCB
Similarity to PMBCL/ NLPHL

DLBCL Molecular Subtypes – *questions needing balanced answers*

1. What are the subtypes and why do we believe they are real?

Remarkable consensus between independent studies
Gene expression further supports a biological basis.

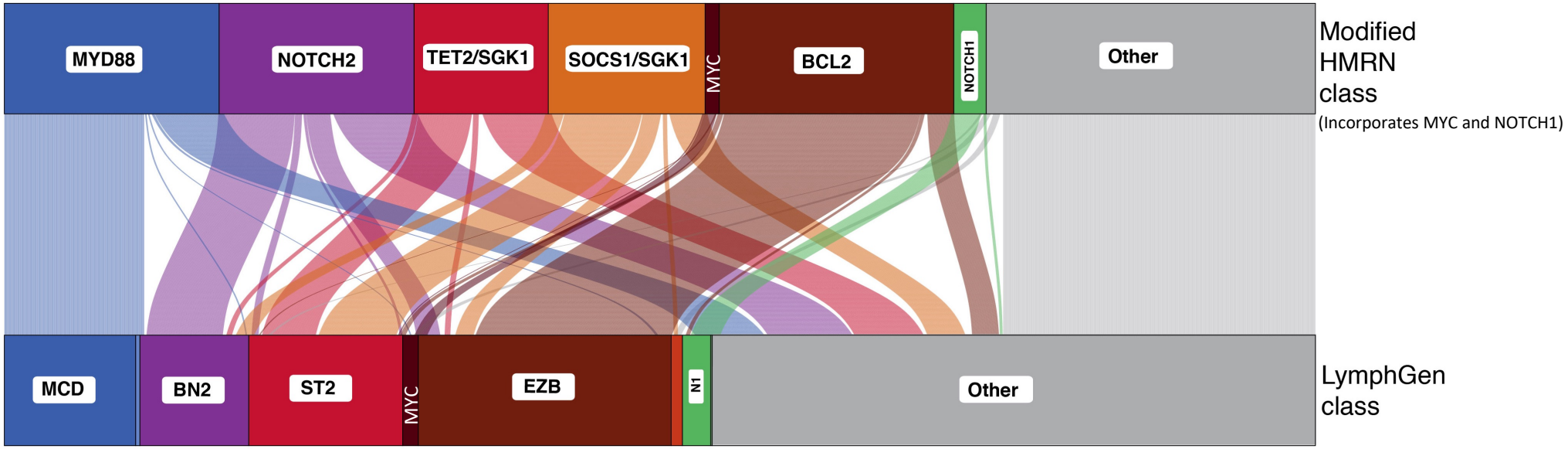
2. Do the classification systems agree at the individual patient level?

3. Do they tell us about prognosis?

4. Do they tell us how to treat?

Q2. Do classifiers agree at the individual patient level?

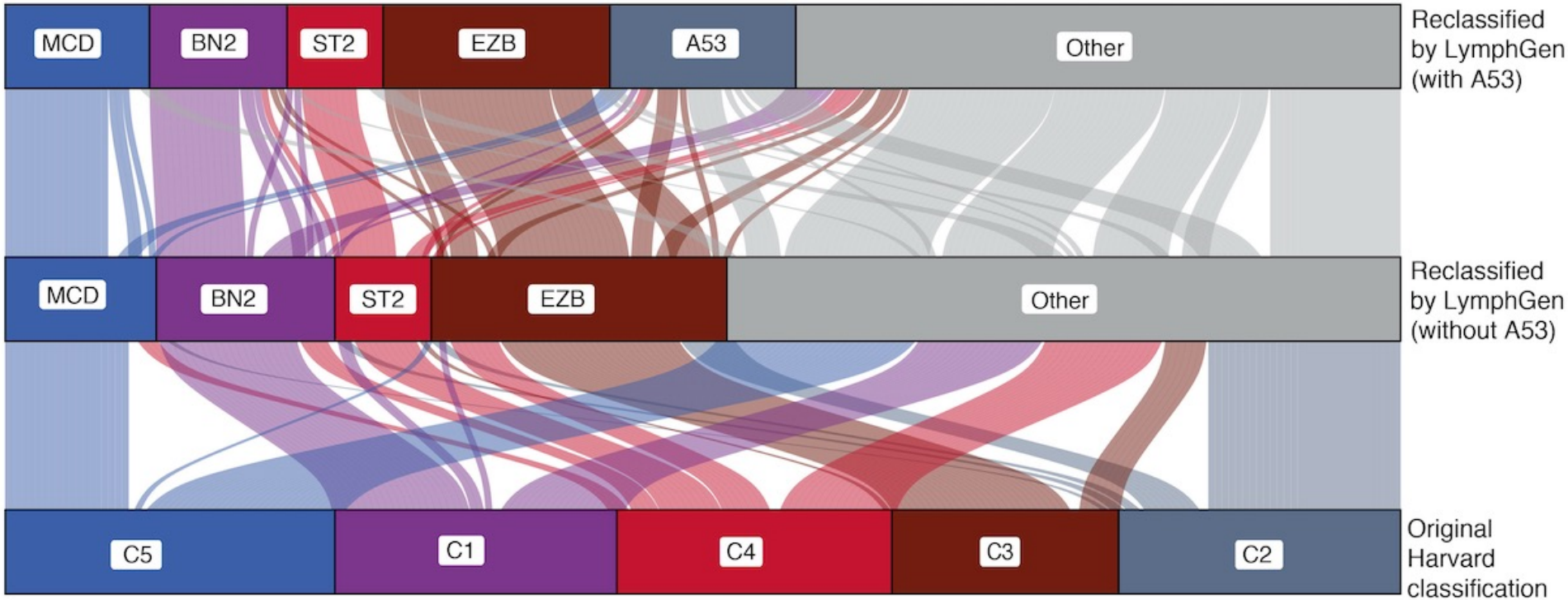
928 DLBCL cases classified by **HMRN** or NCI (**LymphGen**) classifiers



The greatest source of variability comes from whether a case is classified at all, rather than movement between classes

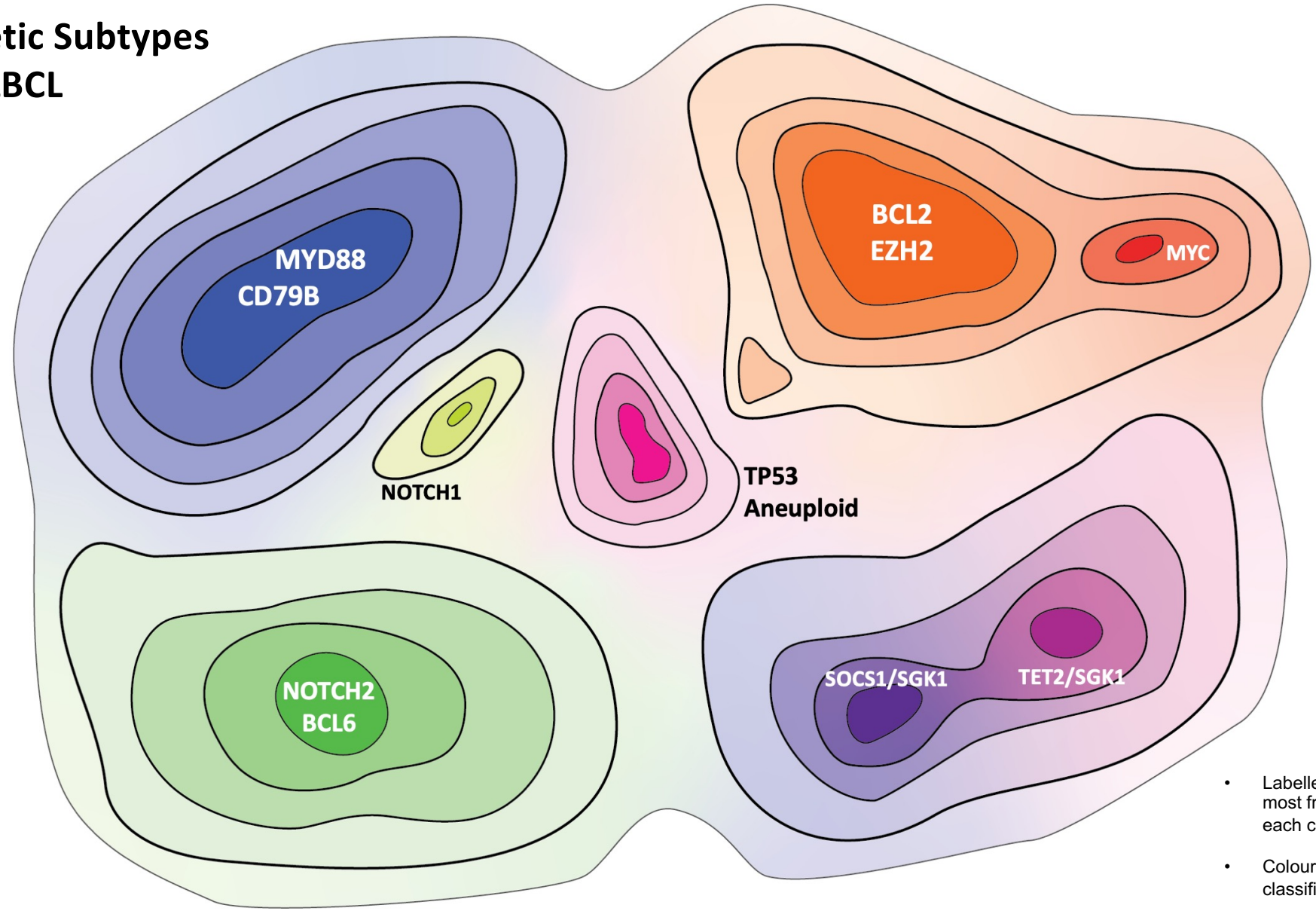
Do classifiers agree at the individual patient level?

Comparing LymphGen versus Harvard Classifiers.



- Harvard classifies 100% of cases – again variability is in the threshold to classify
- Both classifications agree on an Aneuploidy / TP53 subtype (A53 / C2). But there is little consensus on A53 at the individual patient level

Genetic Subtypes of DLBCL



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- Colour density reflects classification confidence

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1. What are the subtypes and why do we believe they are real?

   Remarkable consensus between independent studies
   Gene expression further supports a biological basis.

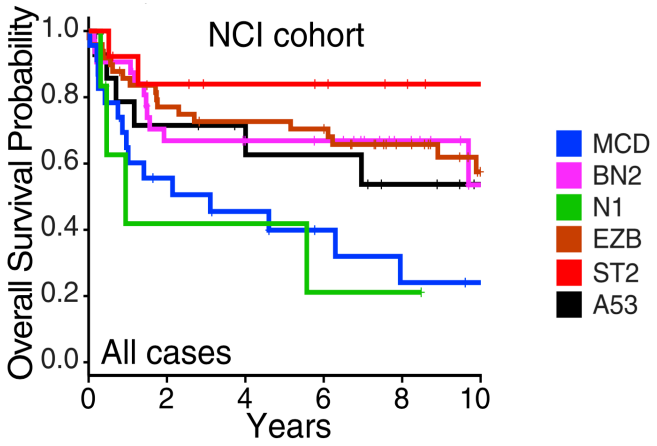
2. Do the classification systems agree at the individual patient level?

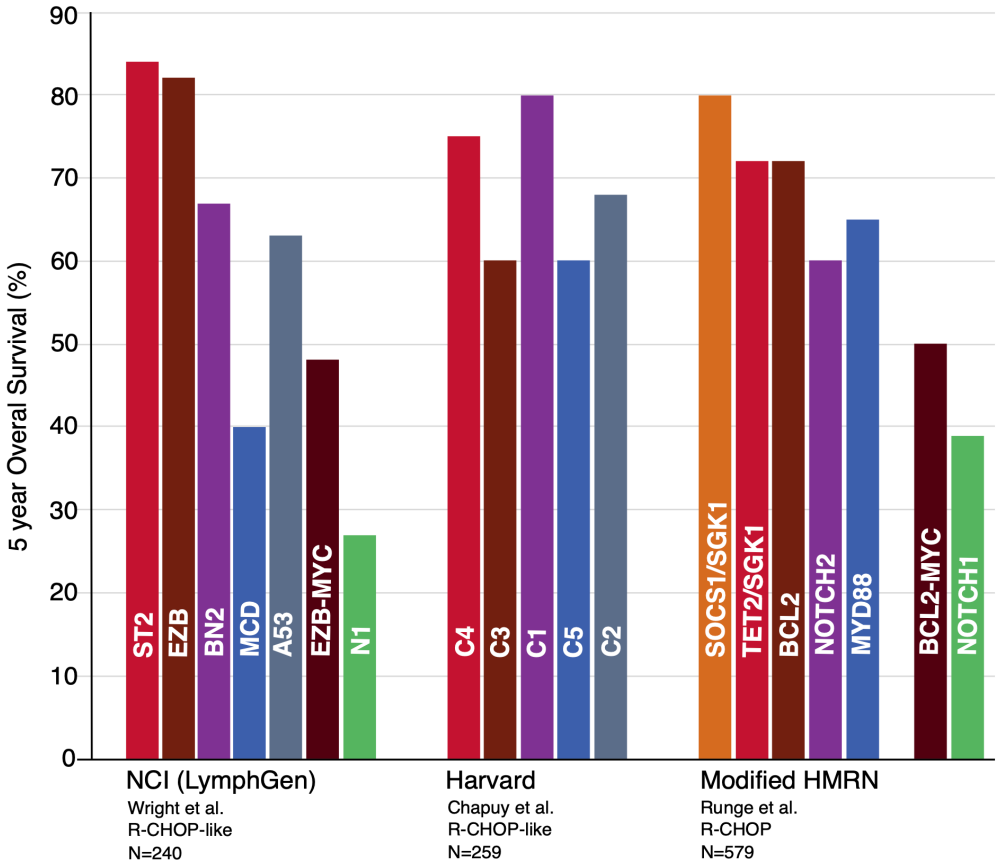
   Strong agreement for most clusters. Caveat A53.
   Main variability is threshold to classify (50% to 100%)

3. Do they tell us about prognosis?

4. Do they tell us how to treat?

Q3. Does genetic subtyping add useful prognostic information?



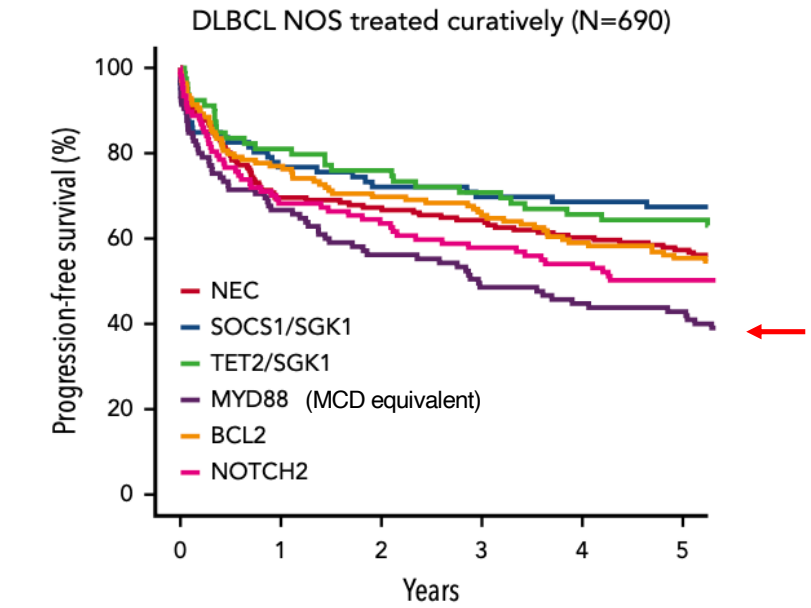


- Good survival of **ST2**;
- Poor survival of **N1** & **MCD**

- Patient mix is not equivalent across studies

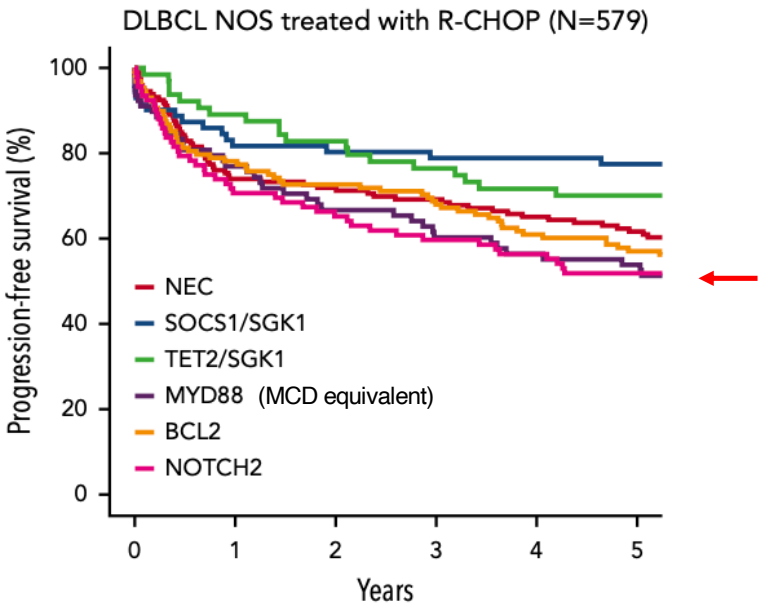
Prognosis in HMRN study – *importance of the patient cohort*

R-CHOP-like



NEC	171	119	115	110	103	98
SOCS1/SGK1	86	66	62	60	59	57
TET2/SGK1	79	64	59	55	51	50
MYD88	105	70	59	51	47	45
BCL2	139	107	97	91	82	77
NOTCH2	107	73	67	61	57	52

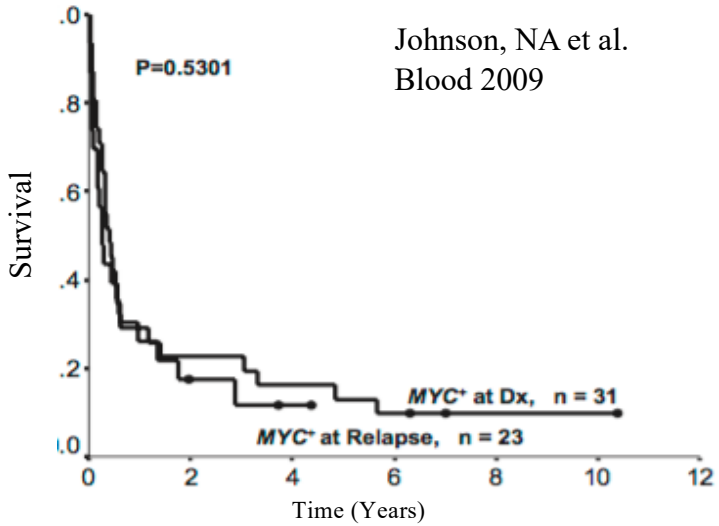
R-CHOP



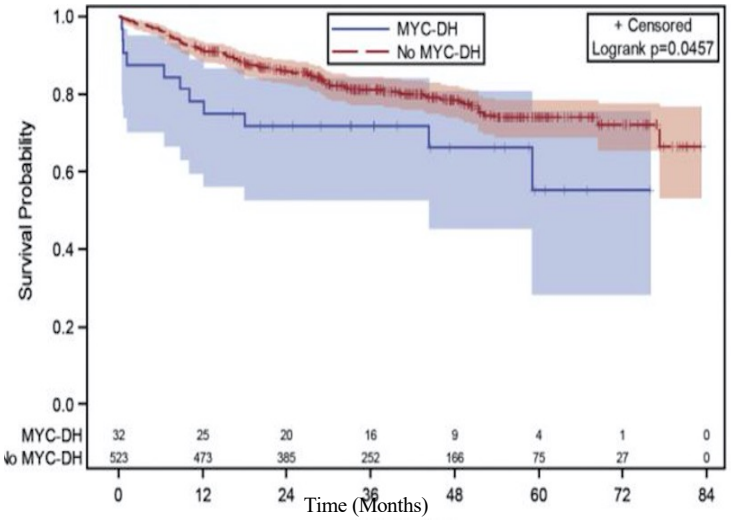
NEC	146	108	105	101	95	90
SOCS1/SGK1	71	58	57	56	56	54
TET2/SGK1	64	57	52	48	45	44
MYD88	78	60	52	47	44	42
BCL2	128	100	93	87	78	73
NOTCH2	92	65	59	54	51	46

Lacy….Hodson; Blood, 2020

Lessons from history – DHL as an example



Johnson, NA et al.
Blood 2009

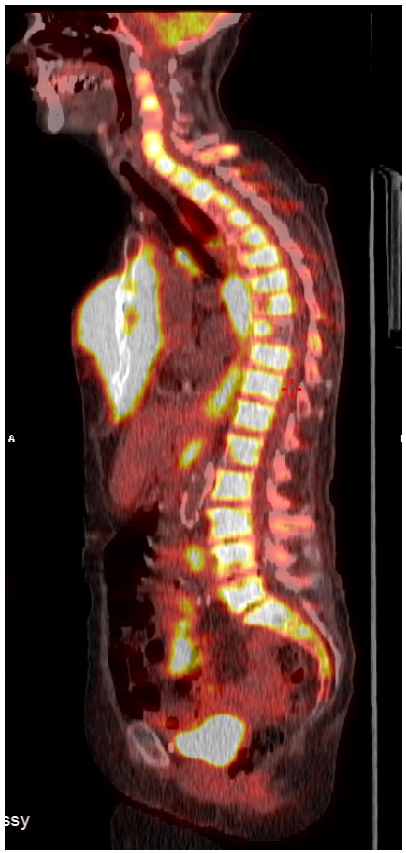


Copie-Bergman
Blood 2015

DLBCL patients enrolled in prospective GELA/LYSA studies

Selection Bias in case series – pathology v therapeutic

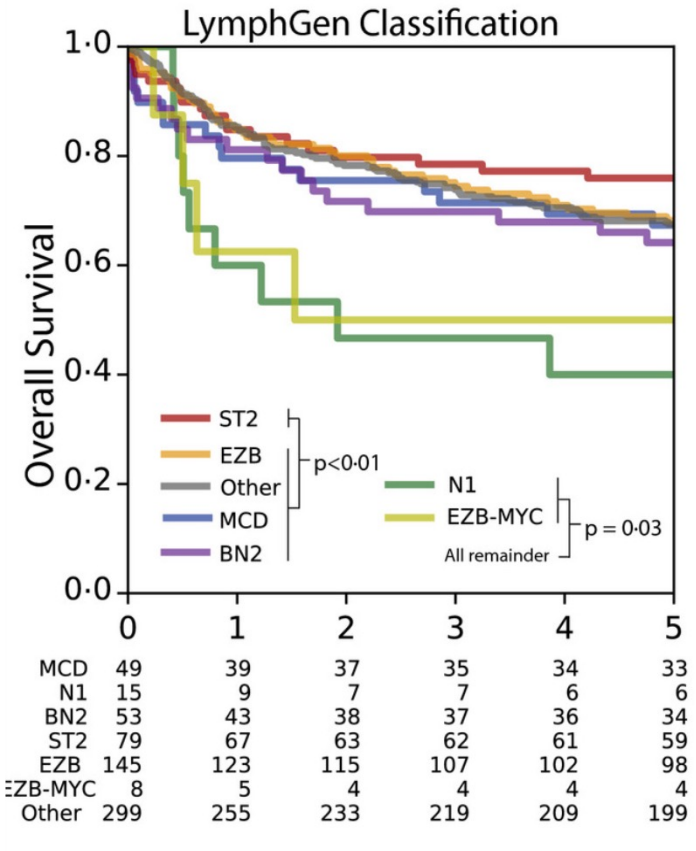
More likely to get additional pathology tests (FISH)

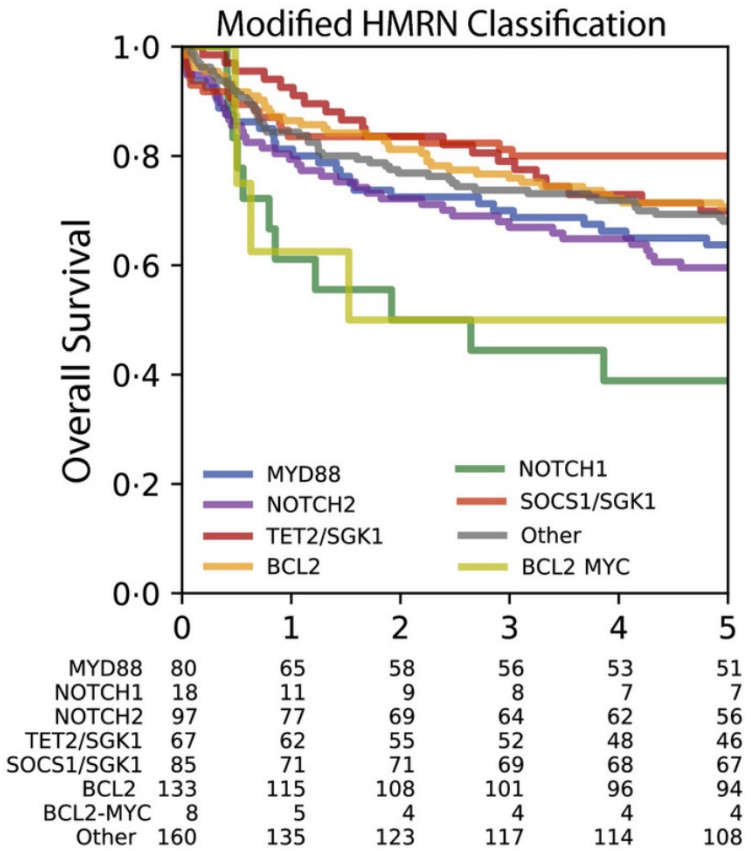


More likely to enter clinical trial

More likely to enter clinical trial and receive more aggressive therapy

Outcomes in unselected R-CHOP treated patients (HMRN cohort)





Data here shows 648 R-CHOP treated DLBCL diagnosed at HMRN , with sole inclusion criteria being availability of tumour DNA

Runge…Hodson;
BJ Haem, 2020

DLBCL Molecular Subtypes – *questions needing balanced answers*

1. What are the subtypes and why do we believe they are real?

   Remarkable agreement between independent studies
   Gene expression further supports a biological basis.

2. Do the classification systems agree at the individual patient level?

   Strong agreement for most clusters. Caveat A53.
   Main variability is threshold to classify (50% to 100%)

3. Do they tell us about prognosis?

   ST2 good. MYC or NOTCH1 very bad. MCD bad.
   Beware recruitment bias. Beware "R-CHOP-like".
   Prognosis is not the reason to do molecular subtyping

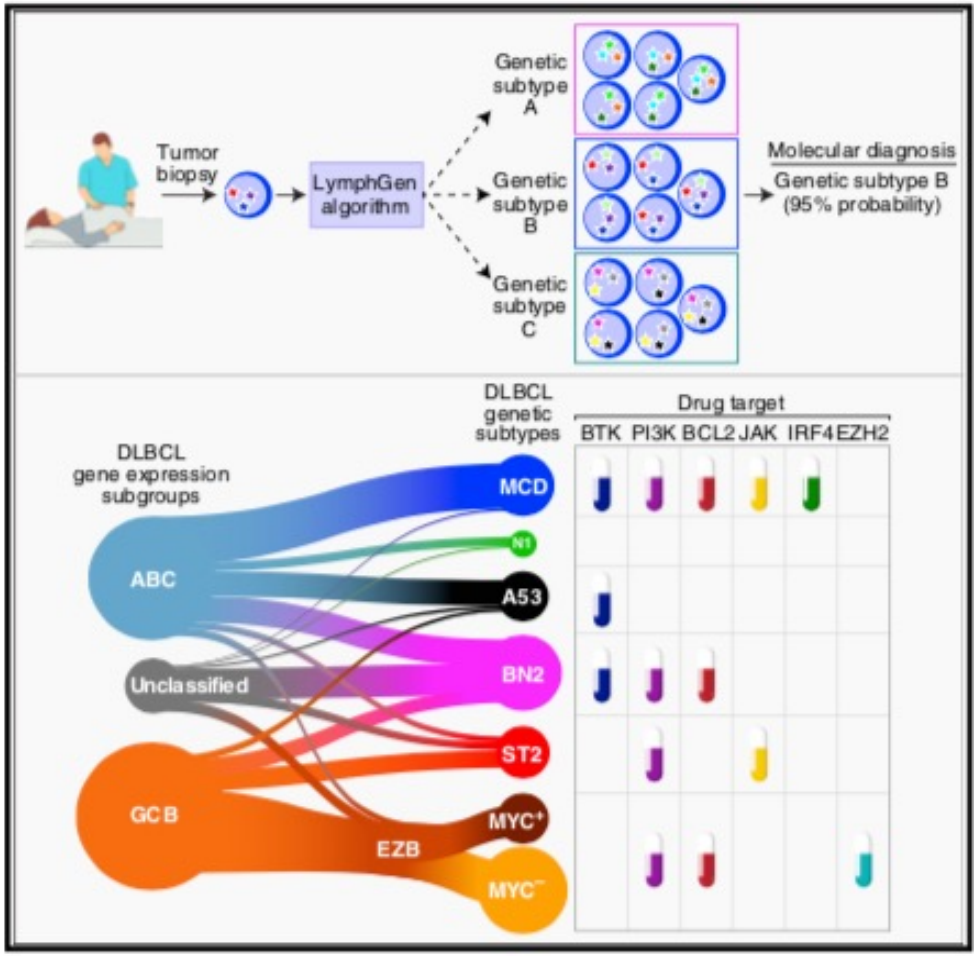
4. Do they tell us how to treat?

Does the classification allow us to select a targeted therapy?

Not yet.

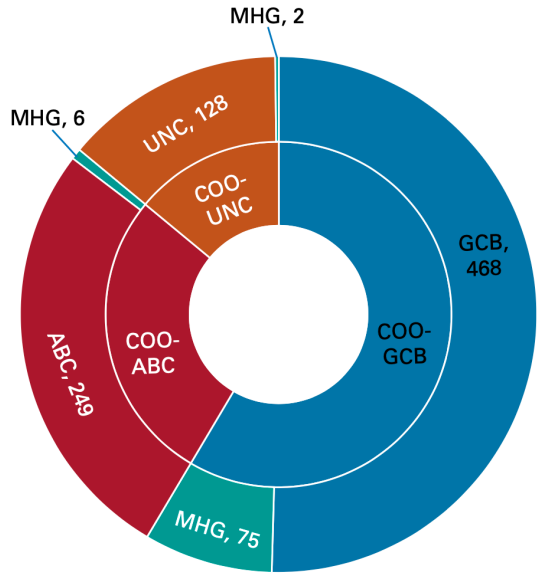
Not possible to predict accurately from existing data or **current** preclinical models.

But may allow us to design and interpret better trials

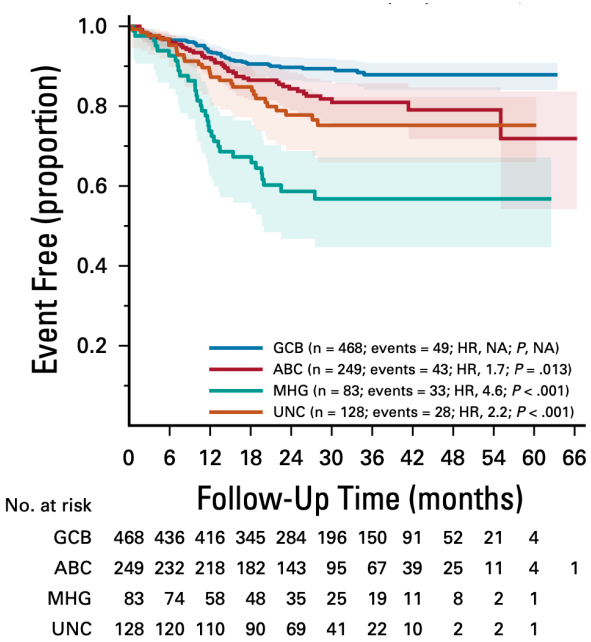


Subtype specific responses to Velcade in REMoDL-B study

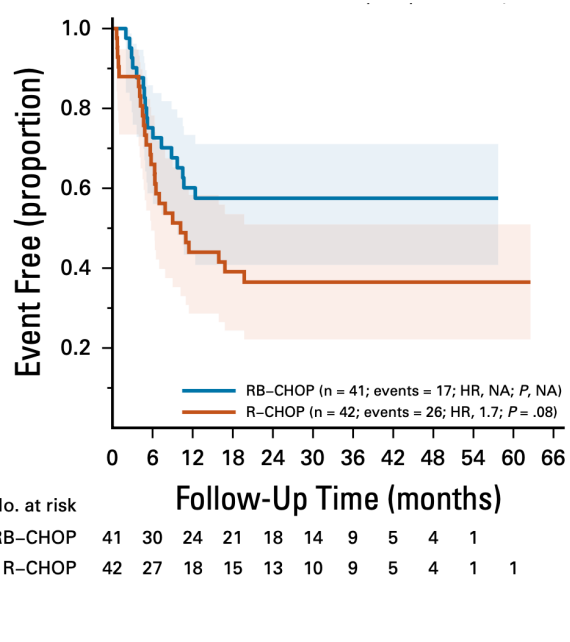
Molecular High Grade Lymphoma
DHSig in BC study
GCB subtype
Some but imperfect overlap with FISH



REMoDL-B by transcriptional subtype



Effect of Velcade in MHG cases



Applying molecular subtyping reveals subtype-specific responses in trials that otherwise appeared to have failed

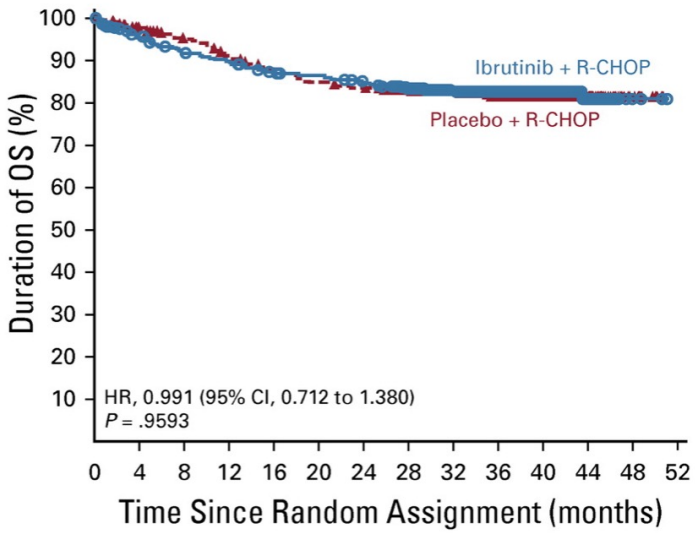
Sha, C et al; 2019 JCO – Molecular High Grade DLBCL (MHG)
Einishi et al; 2019 JCO – Double Hit Signature – (DHsig)

Results of the Phoenix Trial

original report

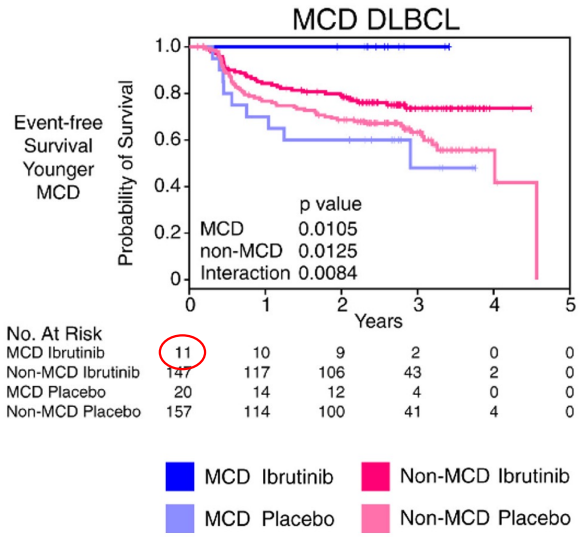
Randomized Phase III Trial of Ibrutinib and Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Non–Germinal Center B-Cell Diffuse Large B-Cell Lymphoma

Anas Younes, MD[1]; Laurie H. Sehn, MD[2]; Peter Johnson, MD[3]; Pier Luigi Zinzani, MD, PhD[4]; Xiaonan Hong, MD[5]; Jun Zhu, MD[6]; Caterina Patti, MD[7]; David Belada, MD, PhD[8,9]; Olga Samoilova, PhD[10]; Cheolwon Suh, MD, PhD[11]; Sirpa Leppä, MD[12,13]; Shinya Rai, MD, PhD[14]; Mehmet Turgut, MD, PhD[15]; Wojciech Jurczak, MD, PhD[16]; Matthew C. Cheung, MD[17]; Ronit Gurion, MD[18,19]; Su-Peng Yeh, MD[20]; Andres Lopez-Hernandez, MD[21]; Ulrich Dührsen, MD[22]; Catherine Thieblemont, MD, PhD[23,24]; Carlos Sergio Chiattone, MD, PhD[25]; Sriram Balasubramanian, PhD[26]; Jodi Carey, RN[27]; Grace Liu, PhD[28]; S. Martin Shreeve, MD, PhD[26]; Steven Sun, PhD[28]; Sen Hong Zhuang, MD, PhD[28]; Jessica Vermeulen, MD, PhD[29]; Louis M. Staudt, MD, PhD[30]; and Wyndham Wilson, MD, PhD[30]; on behalf of the PHOENIX investigators



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



Cancer Cell 2021

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

Reanalysis of results by genetic subtype

LymphGen genetic classifier

Ibrutinib appears more active in MCD and (surprisingly) N1.

Numbers are **very** small
– (MCD-IB 11 out of 419)

Conclusions

1. Different genetic subgroups respond differently to biologically targeted drugs.
2. Responses are not always predictable from preclinical preclinical data (eg N1 response)

DLBCL Molecular Subtypes – *questions needing balanced answers*

1. What are the subtypes and why do we believe they are real?

   Remarkable agreement between independent studies
   Gene expression further supports a biological basis.

2. Do the classification systems agree at the individual patient level?

   Strong agreement for most clusters. Caveat A53.
   Main variability is threshold to classify (50% to 100%)

3. Do they tell us about prognosis?

   ST2 good. MYC or NOTCH1 very bad. MCD bad.
   Beware recruitment bias. Beware "R-CHOP-like".
   Prognosis is not the reason to do molecular subtyping

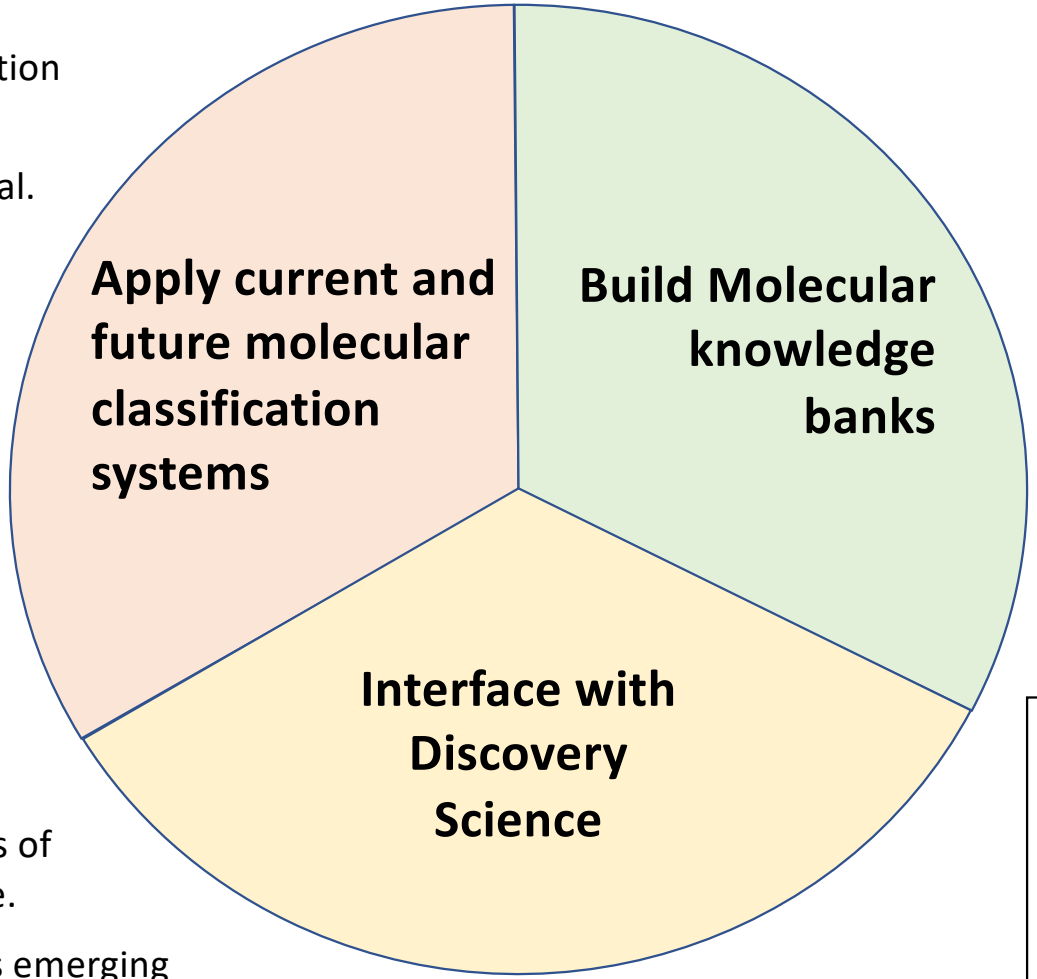
4. Do they tell us how to treat?

   Not yet. But will allow us to design and interpret clinical trials. When we look for subtype-specific responses we find them.

NGS to maximise the benefit of clinical trials?

Expect continued evolution and refinement

Future-proofing essential.



Accrue large sample numbers with paired clinical and molecular data to decipher prognostic and therapeutic implications. May need advanced analytical approaches.

Molecular determinants of response and resistance.

Test genetic hypotheses emerging from future discovery science

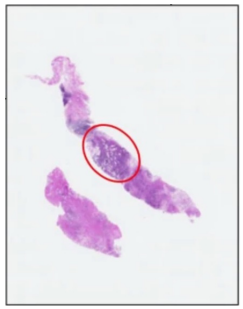
Trial requirements

- Full exome and transcriptome
- Paired with clinical data
- At time of trial publication

What about routine diagnostic labs?

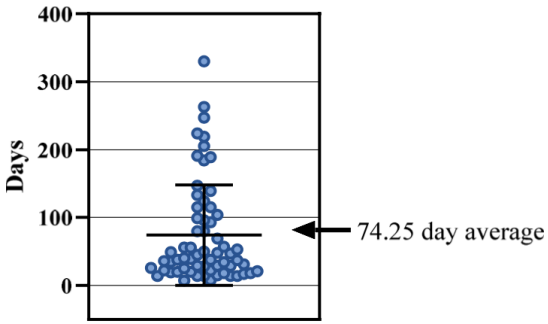
Sample related challenges

Sample size
All in same block
FFPE
ctDNA



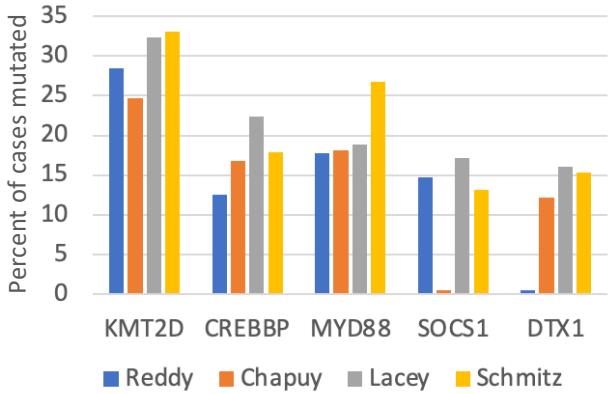
Logistic challenges

Sample routing
Batching
Patient consent
Cost



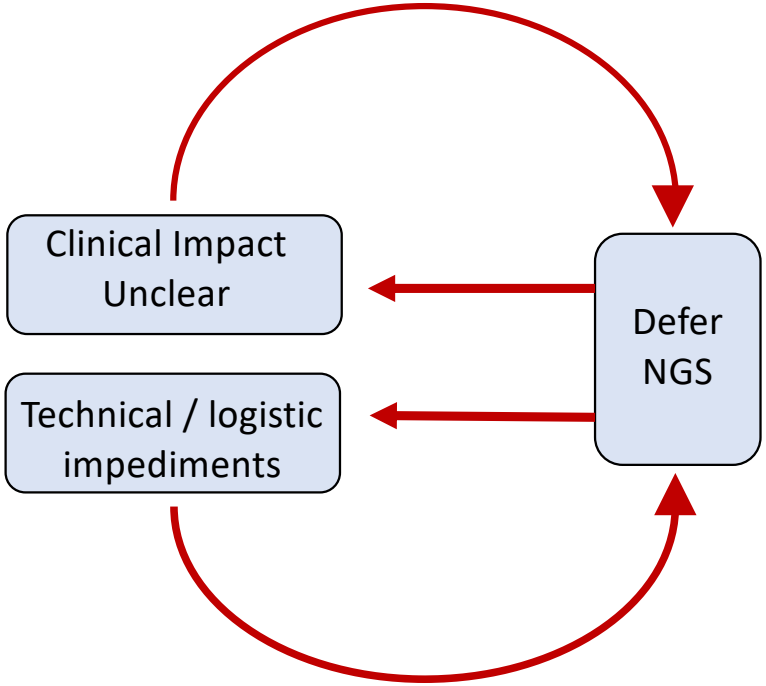
Technical challenges

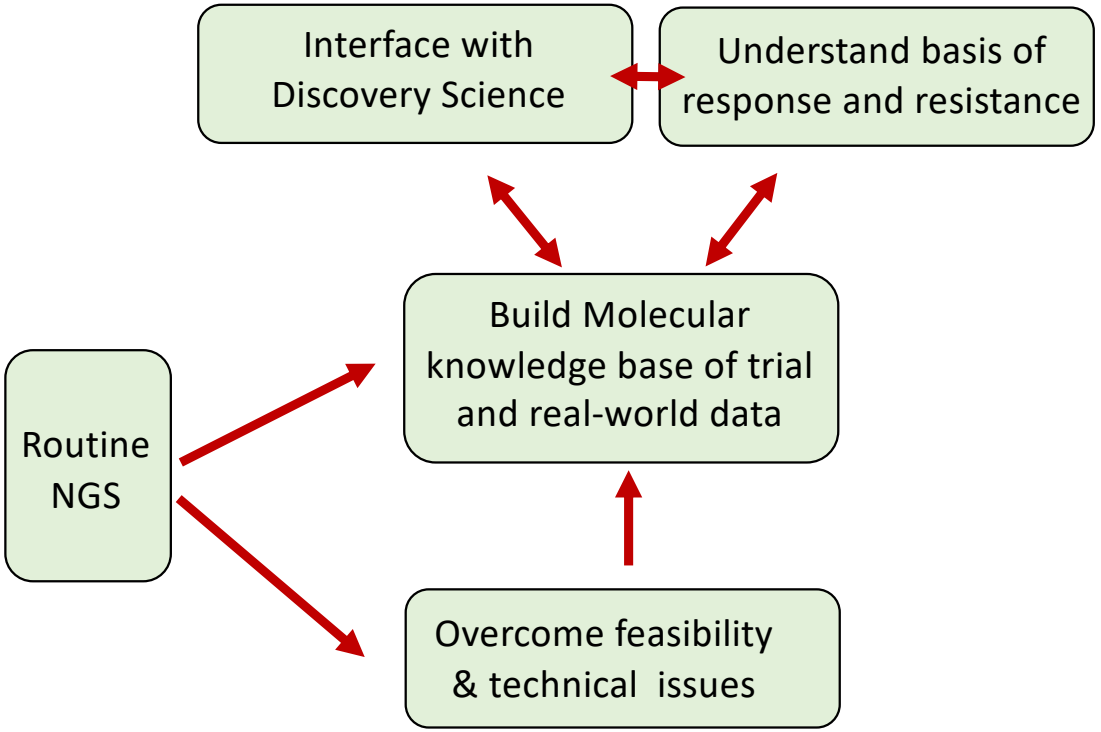
Sequencing platform
Variant calling pipelines
Standardisation
Quality management



- These problems will not be solved in academic studies – only by real world experience
- Reason to start now is to build the infrastructure. Or risk identifying subtype-specific therapies with no way to subtype

Breaking the Vicious Circle of NGS for DLBCL





Summary

DLBCL comprises distinct molecular diseases. Recognizing this heterogeneity will be essential to therapeutic progress in DLBCL

Remarkable consensus on genetic subtypes suggests this will be basis for resolving the heterogeneity.

Classifications will continue to evolve, but final answer will likely combine genetic and gene expression information.

Comprehensive genetic and transcriptomic profiling must be mandatory to maximize learning from DLBCL drug trials.

Role in routine clinical practice currently less clear, but now is the time to establish infrastructure for real time molecular profiling.