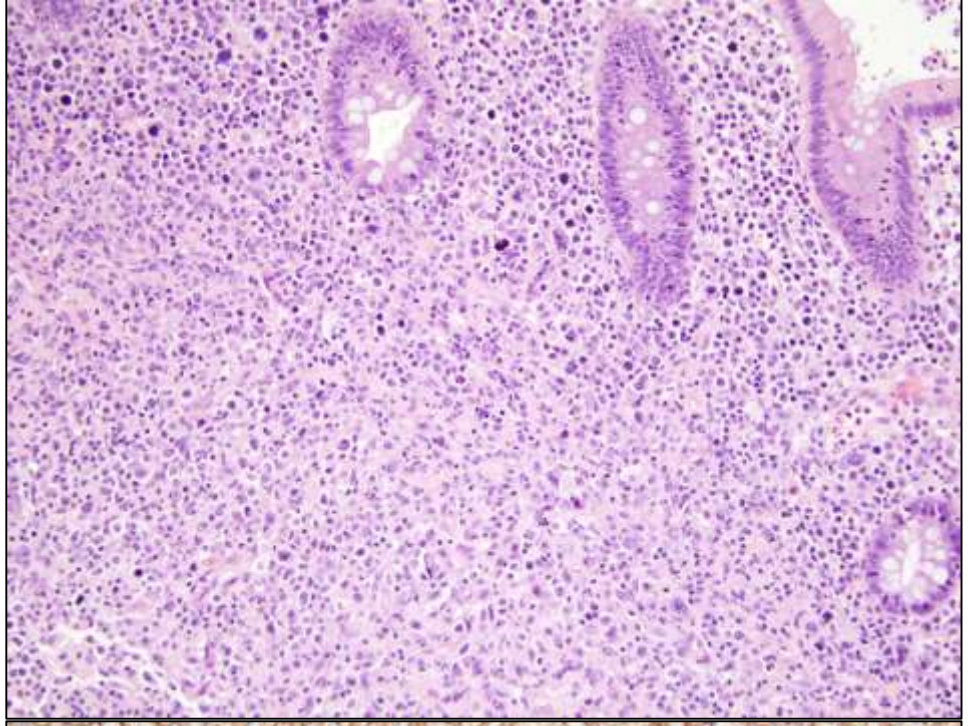
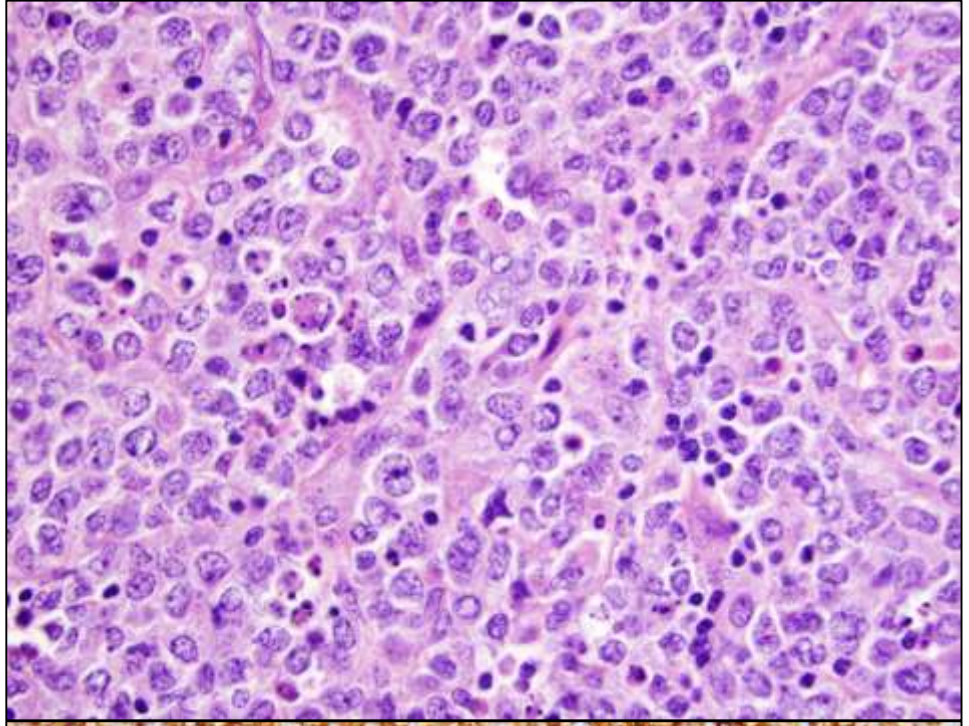
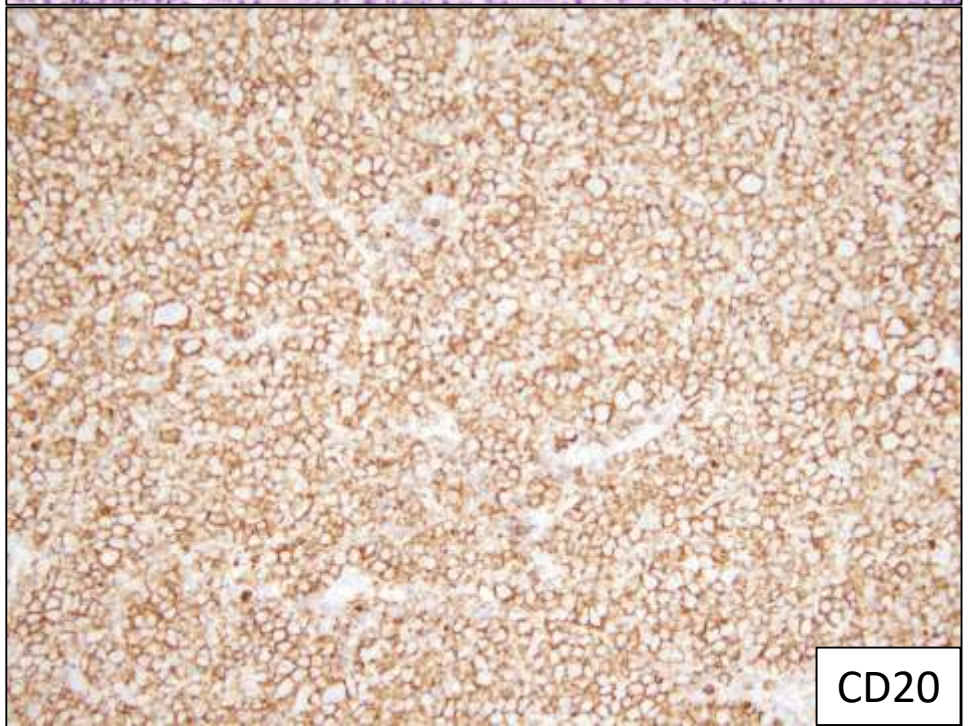
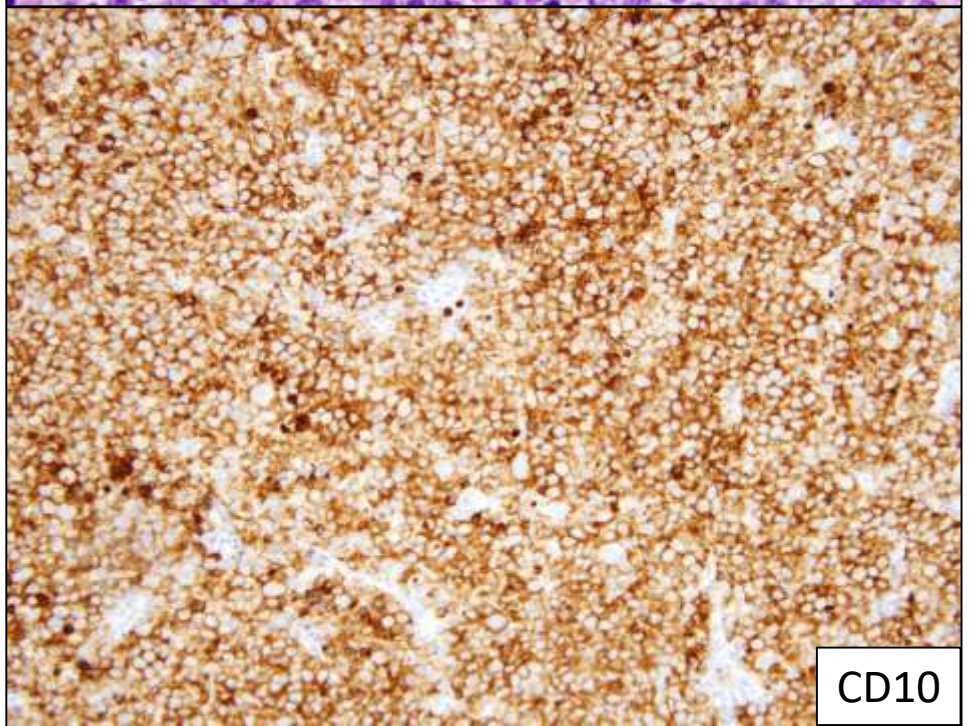
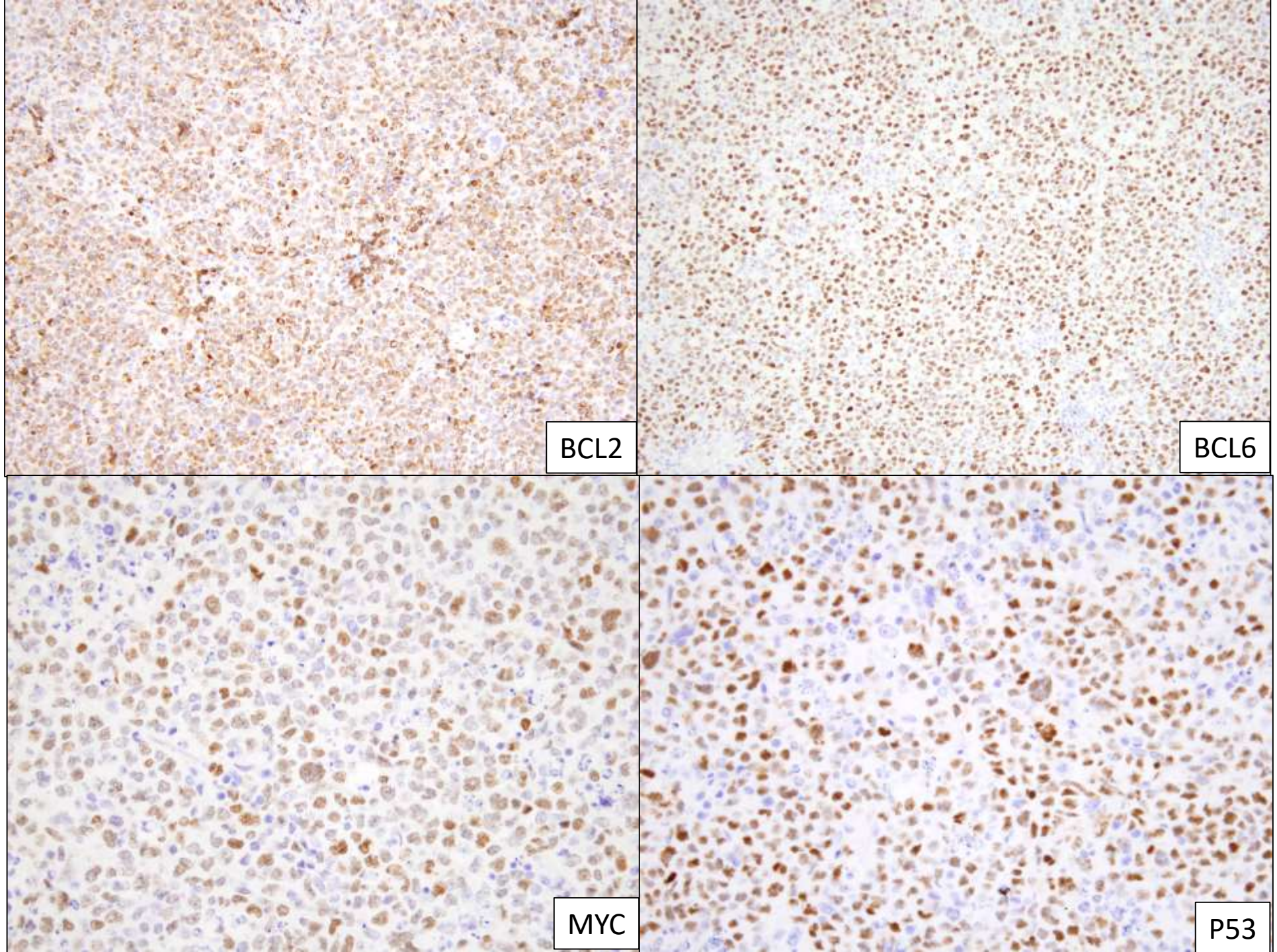
- 63 year old female presented with 2 month history of gastric pain
- EGD was performed and a large, partly ulcerated mass was biopsied






CD20


CD10


BCL2
BCL6
MYC
P53

- FISH studies were performed

- *IGH::MYC* was identified
- Reflex testing showed additional evidence of *IGH::BCL2*

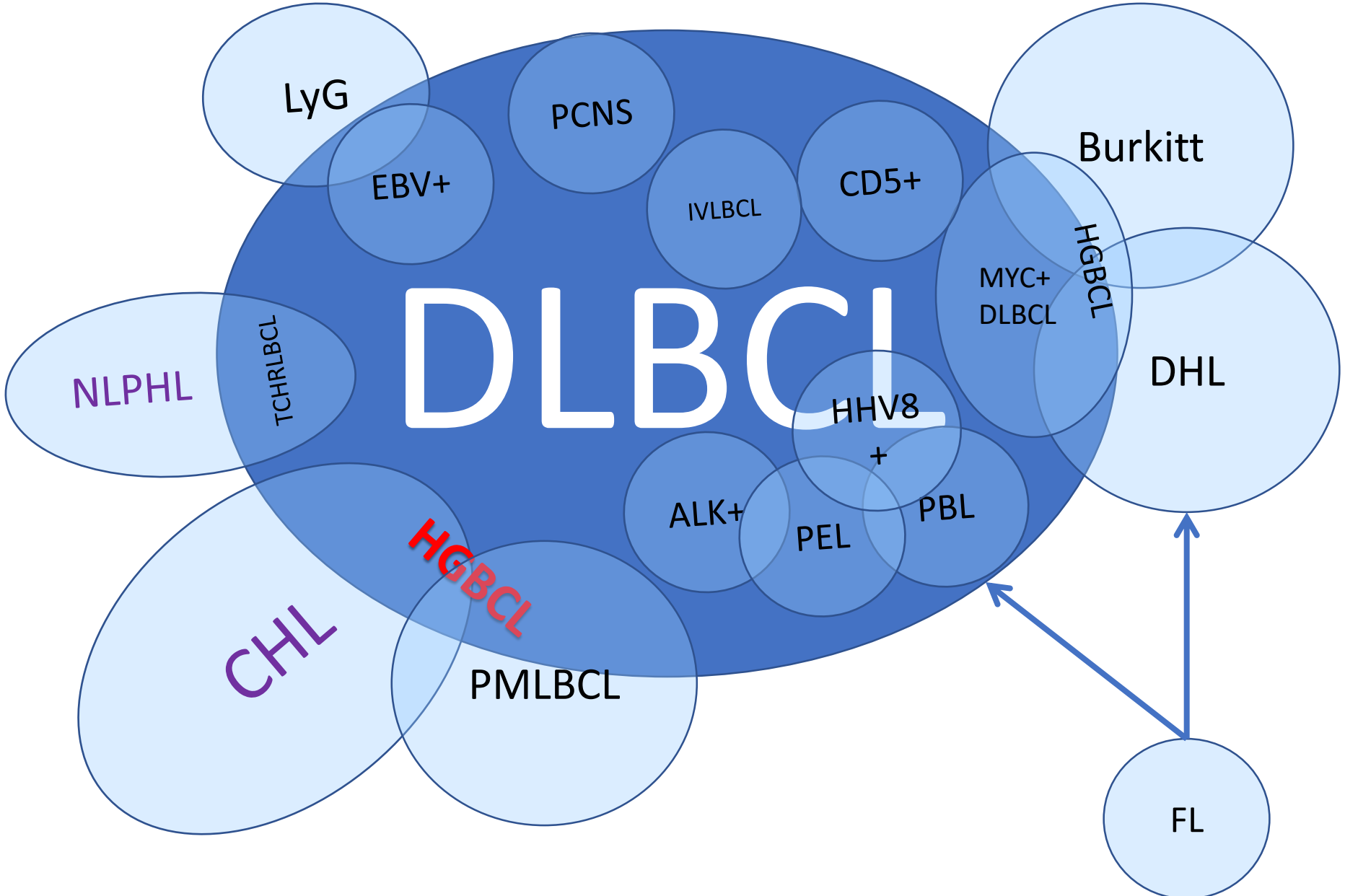
- *Diagnosis:* High grade B cell lymphoma with MYC and BCL2 rearrangements (double hit lymphoma)

Diagnostic evaluation in aggressive B cell lymphoma and related entities

Dennis P. O’Malley, MD

I apologize…

- I’ve become “that guy”
- I will be showing a lot of Kaplan-Meier survival curves
- I used to hate this in “pathology” lectures


LyG
EBV+
PCNS
IVLBCL
CD5+
Burkitt
MYC+
DLBCL
HGBCL
DHL
DLBCL
NLPHL
TCHRLBCL
HHV8
+
ALK+
PEL
PBL
HGBCL
CHL
PMLBCL
FL

Aggressive B cell lymphomas:

- What this lecture includes:
  - High grade B cell lymphoma, with BCL2 and/or BCL6 with MYC
  - High grade B cell lymphoma, NOS

- What this does not include (but there is some comparison and discussion):
  - DLBCL, NOS

Practice Guidelines in Oncology*
National Comprehensive Cancer Network®

- ESSENTIAL
  - Adequate immunophenotyping to establish diagnosis and GCB versus non-GCB origin
  - IHC Panel: CD20, CD3, CD5, CD10, CD45 (!?!), BCL2, BCL6, Ki67, MUM1, MYC
  - With or without flow cytometry: kappa/lambda, CD45, CD3, CD5, CD19, CD10, CD20
  - FISH for *MYC*; if positive FISH for *BCL2*, *BCL6* rearrangements

- USEFUL UNDER CERTAIN CIRCUMSTANCES
  - Additional IHC studies to establish subtype: cyclin D1, kappa/lambda, CD30, CD138, ALK, HHV8, SOX11, EBER

NB: It used to say “expert hematopathology review”!

*v.2.2022

When to test for *MYC* translocation?

The WHO 2017 guidance is not all that clear:

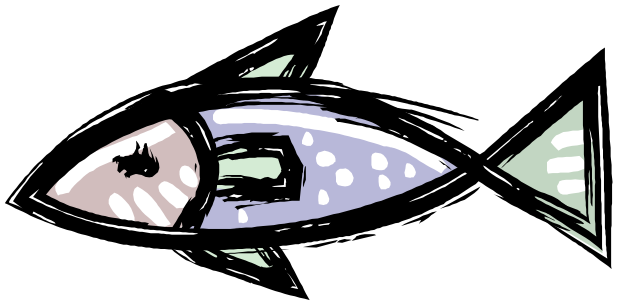
- "Because (DHL) cannot be distinguished from DLBCL, double hit status should be investigated in **all** DLBCL using cytogenetic or molecular cytogenetics studies"
- "Some pathologists prefer to look for DHL status only after IHC or other pre-selection"
- "MYC protein expression **cannot be used** for the selection of cases that should have cytogenetic or molecular/cytogenetic evaluation"

* Emphasis added by me

FISH

DLBCL
(or equivalent)

FISH PANEL



VERY BAD

Double Hit

IgH/BCL2 **+**
BCL6 b.a. **-**
IgH/MYC **+**

IgH/BCL2 **-**
BCL6 b.a. **+**
IgH/MYC **+**

IgH/BCL2 **+**
BCL6 b.a. **+**
IgH/MYC **+**

Triple Hit

BAD

IgH/BCL2 **-**
BCL6 b.a. **-**
IgH/MYC **+**

MYC translocation positive

IgH/BCL2 **-**
BCL6 b.a. **–**
MYC **amplification**

NOT ESPECIALLY BAD

IgH/BCL2 **+**
BCL6 b.a. **+**
IgH/MYC **-**

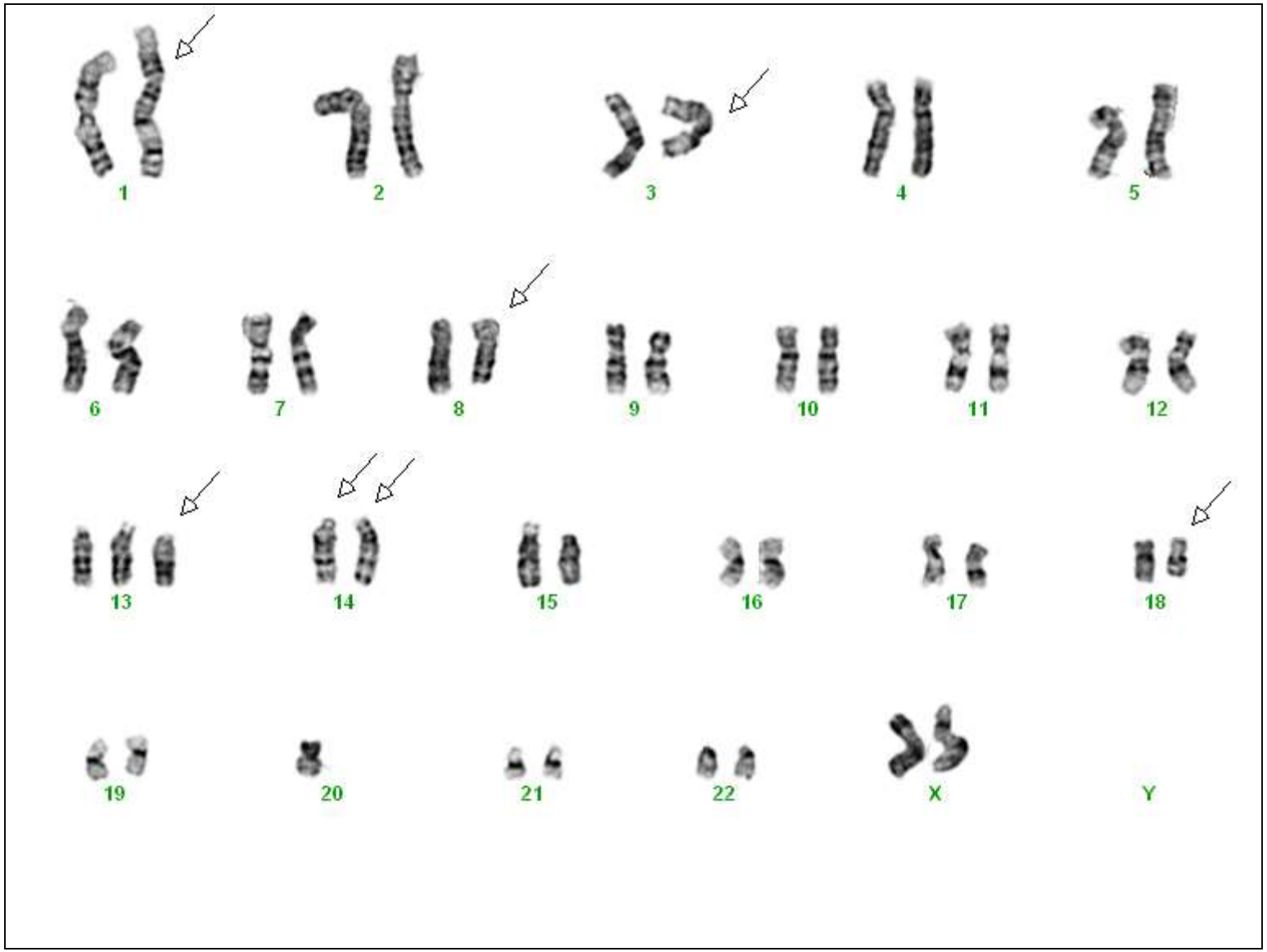
NOT SO BAD

IgH/BCL2 **+**
BCL6 b.a. **-**
IgH/MYC **-**

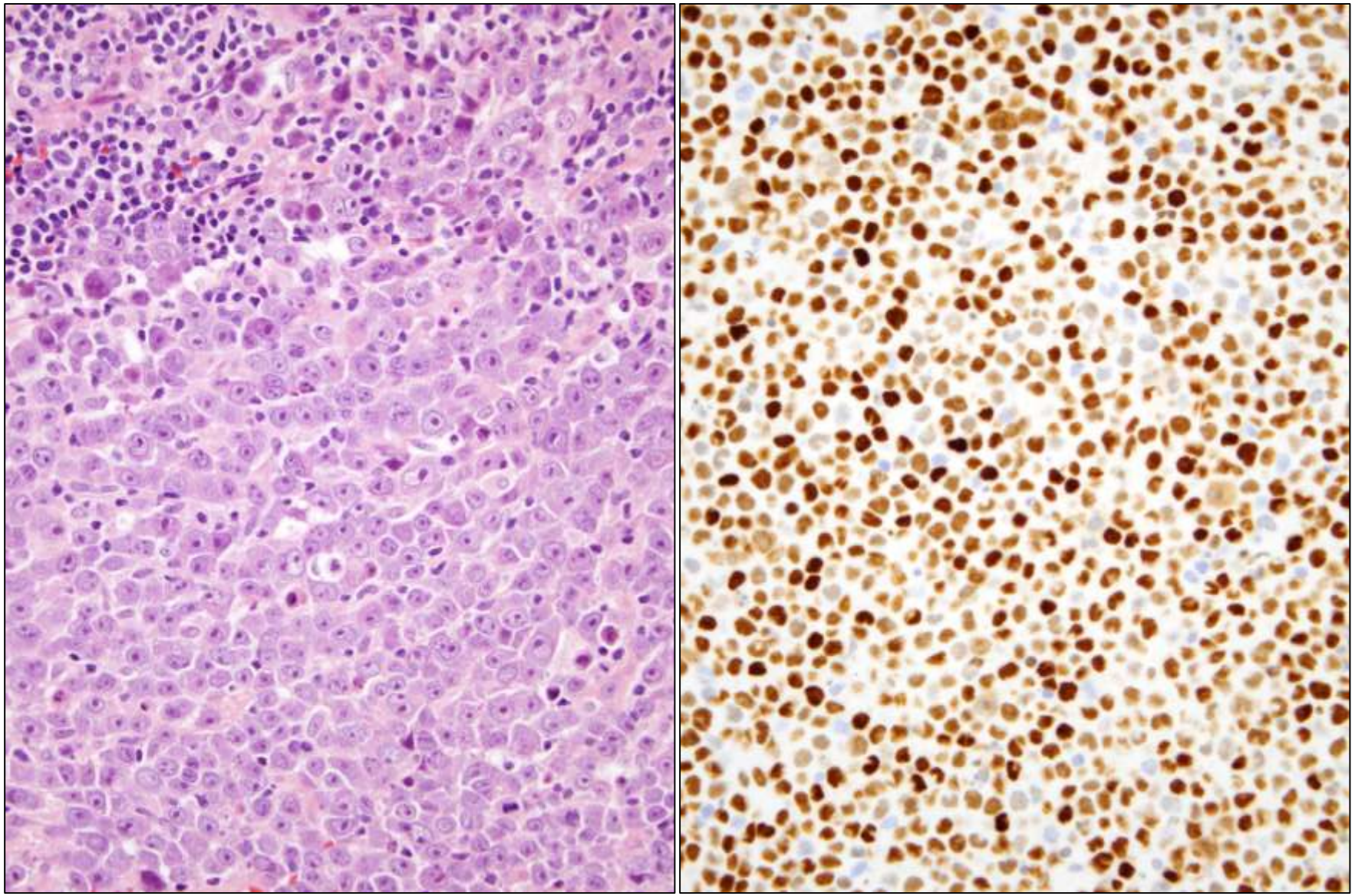
IgH/BCL2 **-**
BCL6 b.a. **+**
IgH/MYC **-**

IgH/BCL2 **-**
BCL6 b.a. **-**
IgH/MYC **-**

“DOUBLE HIT” LYMPHOMA KARYOTYPE



t(8;14) AND t(14;18), with some other abnormalities



H&E

MYC IHC

DLBCL with t*MYC*

MYC immunohistochemistry

- In DLBCL and related entities MYC is “always” expressed
- There is no “positive” or “negative” cut off
- As percentage increases the likelihood of translocation increases
  - In study I performed no translocations below 40%
- There is a *null* immunophenotype
  - Expression is zero (not normal)
  - There is a mutation that changes the epitope so it is not detectable by IHC

MYC versus P53

MYC

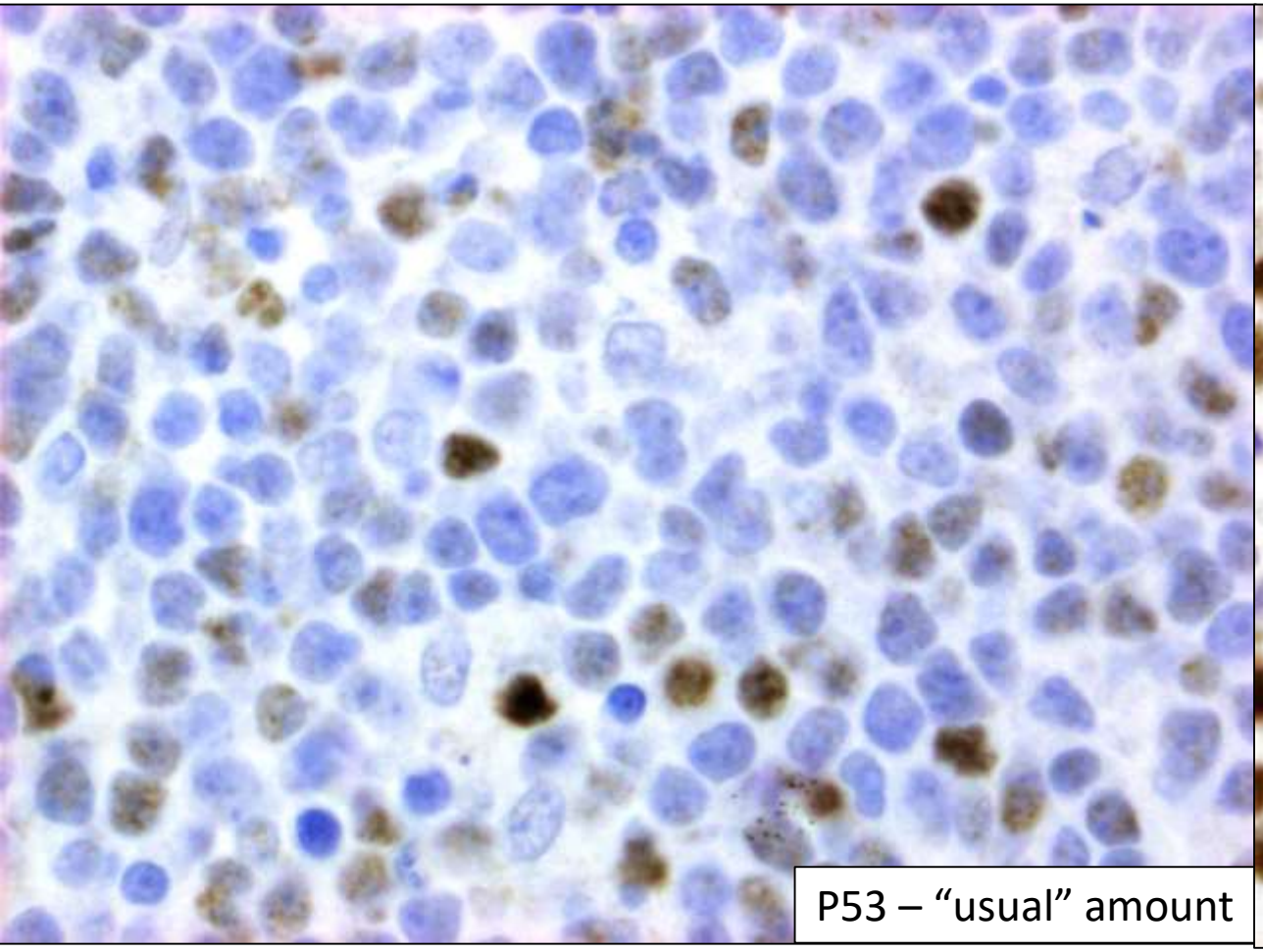
TP53
Tumor suppressor

P53 expression patterns

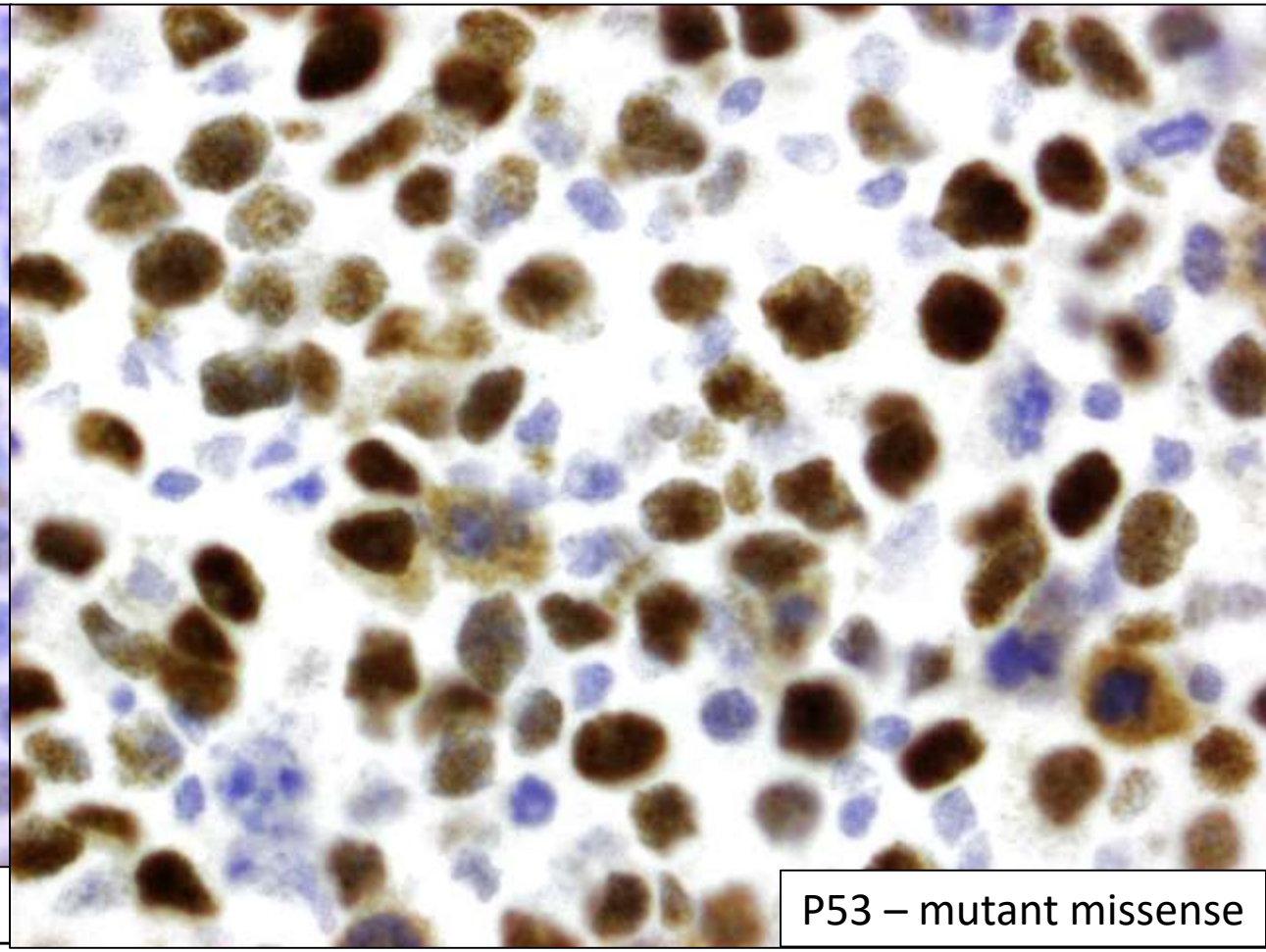
- P53 wt
- P53 increased
- P53 null (mut) – usually frameshift or nonsense
- P53 mut (missense)
- P53 cytoplasmic (mut) – usually frameshift or nonsense

MUST SEE LECTURE: https://isimm.org/education/isimm-webinars/pan-cancer-applications-of-p53-immunohistochemistry/

DLBCL



P53 – “usual” amount



P53 – mutant missense

WHO 2022 vs. ICC

- Here's what I know (subject to change)
  - WHO
    - Double hit/HGBCL will be limited to MYC with BCL2
    - MYC with BCL6 will be removed from double hit (reclassified or demoted?)
    - I think it is unlikely that *TP53* mutation will be addressed in a significant way
  - ICC
    - Who knows?

Molecular classification of aggressive lymphomas—past, present, future

Kirsty Wienand | Björn Chapuy

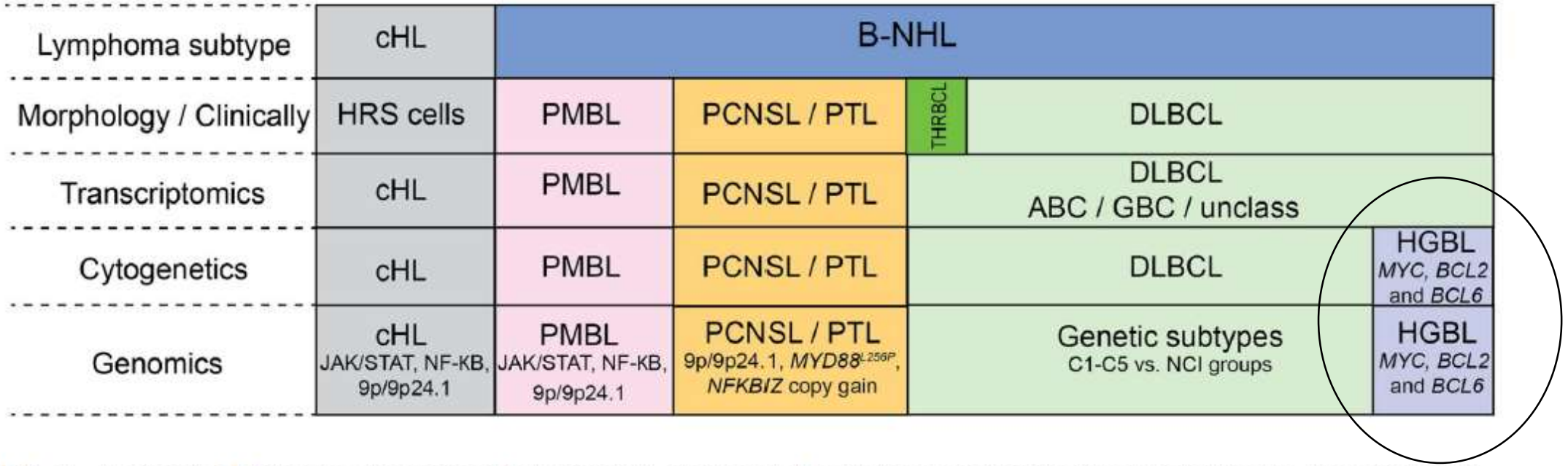
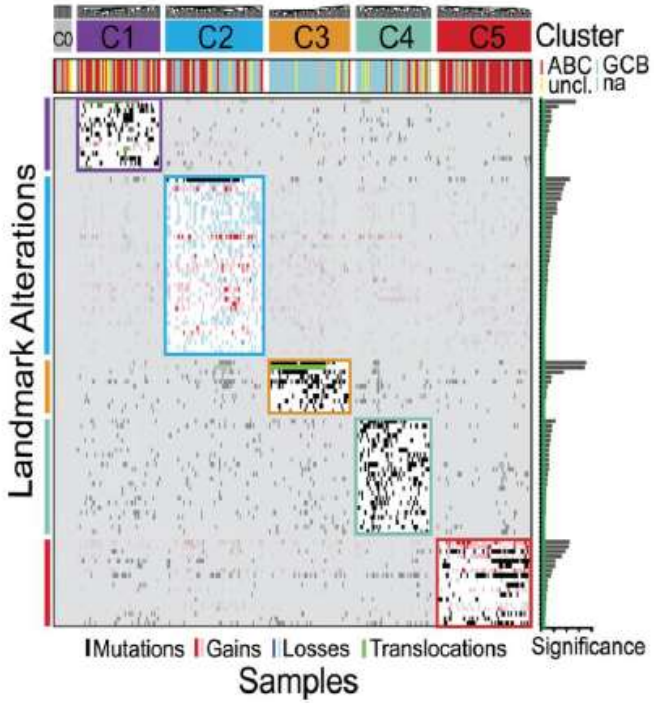


FIGURE 1 Aggressive lymphoma is classified into different entities and subtypes based on morphology/clinically, transcriptomic, cytogenetic, and genomic features

Molecular classification of aggressive lymphomas—past, present, future

Kirsty Wienand | Björn Chapuy

(A)



(B)

Genetic Subtype *DFCI*	Genetic Subtype *NCI*	Key Features	Transcriptional Subtype	Prognosis
C1	BN2	*BCL6* and NOTCH2- and NF-κB and immune escape pathway alterations; occulty transfomed MZL	ABC	Favorable
C2	(A53 / TP53)	Biallelic inactivation of *TP53*, 9p21.3/*CDKN2A*; genomic instablity	ABC/GCB independent	Steady rate of progression
C3	EZB	*BCL2* SVs, inactivating *PTEN* alterations and alterations of epigenetic enzymes	GCB	Unfavorable
C4	ST2	BCR/PI3K-, JAK/STAT-, RAS-pathway and histone alterations	GCB	Favorable
C5	MCD	*BCL2* copy gain, activating *MYD88* and *CD79B* mutations; extranodal tropism	ABC	Unfavorable

FIGURE 2 Discovery of genetic DLBCL subtypes (A) with associated genetic, transcriptomic and prognostic features (B). DLBCL, diffuse large B-cell lymphoma

Aggressive B cell lymphoma: gene mutations

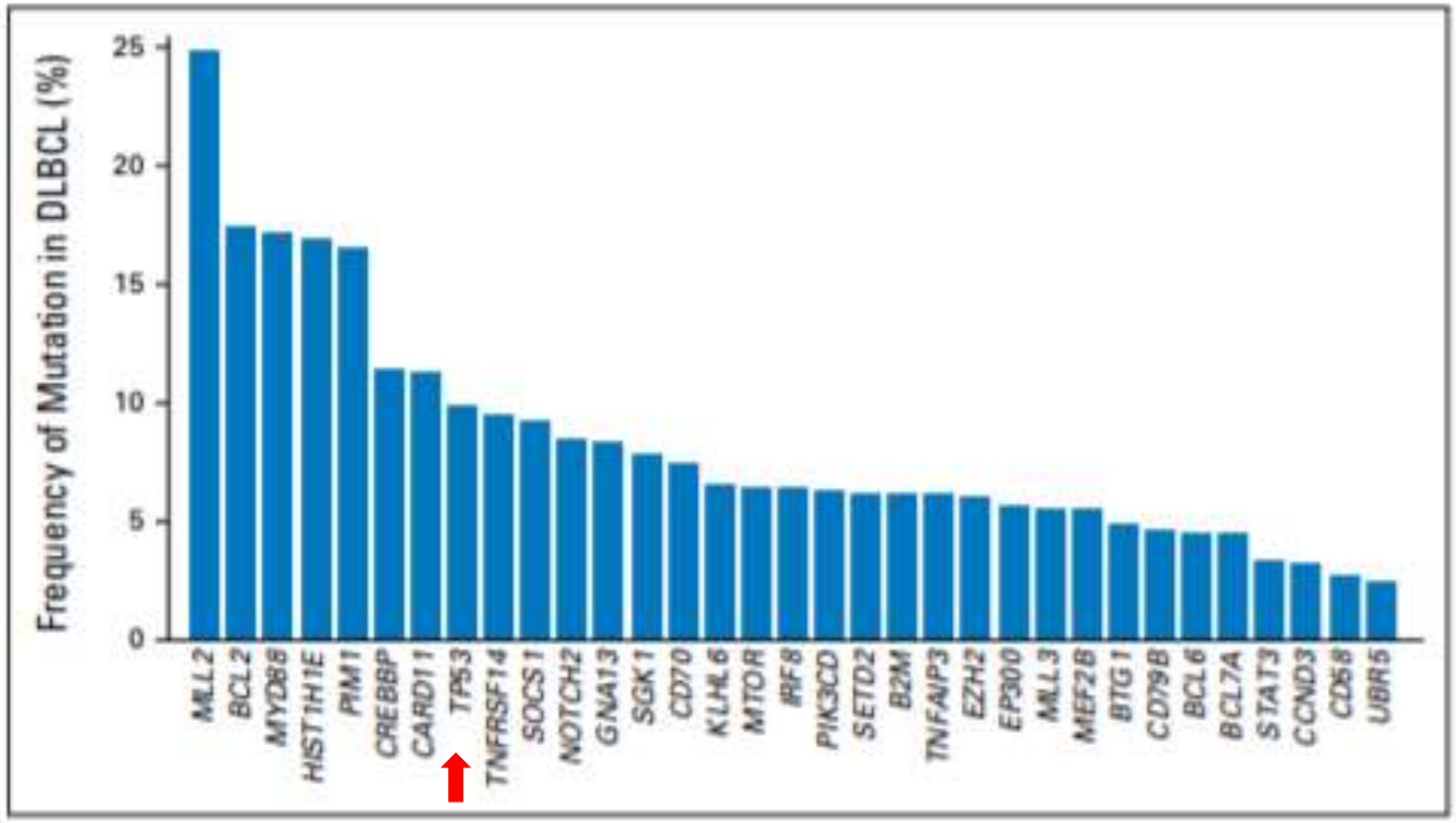
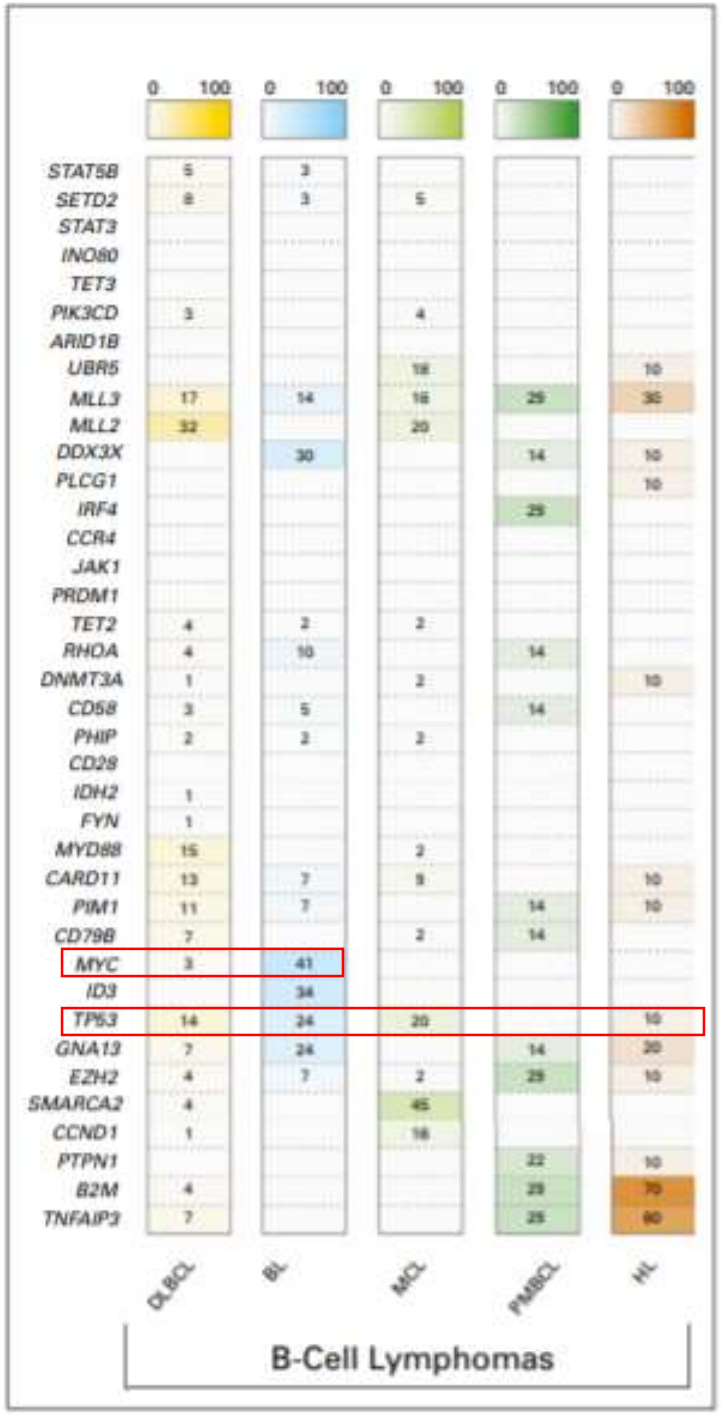


Fig 2. The most frequently mutated genes and their frequencies in diffuse large B-cell lymphoma (DLBCL).

VOLUME 35 · NUMBER 9 · MARCH 20, 2017
JOURNAL OF CLINICAL ONCOLOGY
REVIEW ARTICLE

Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma

Andrea B. Moffitt and Sandeep S. Dave



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REVIEW ARTICLE

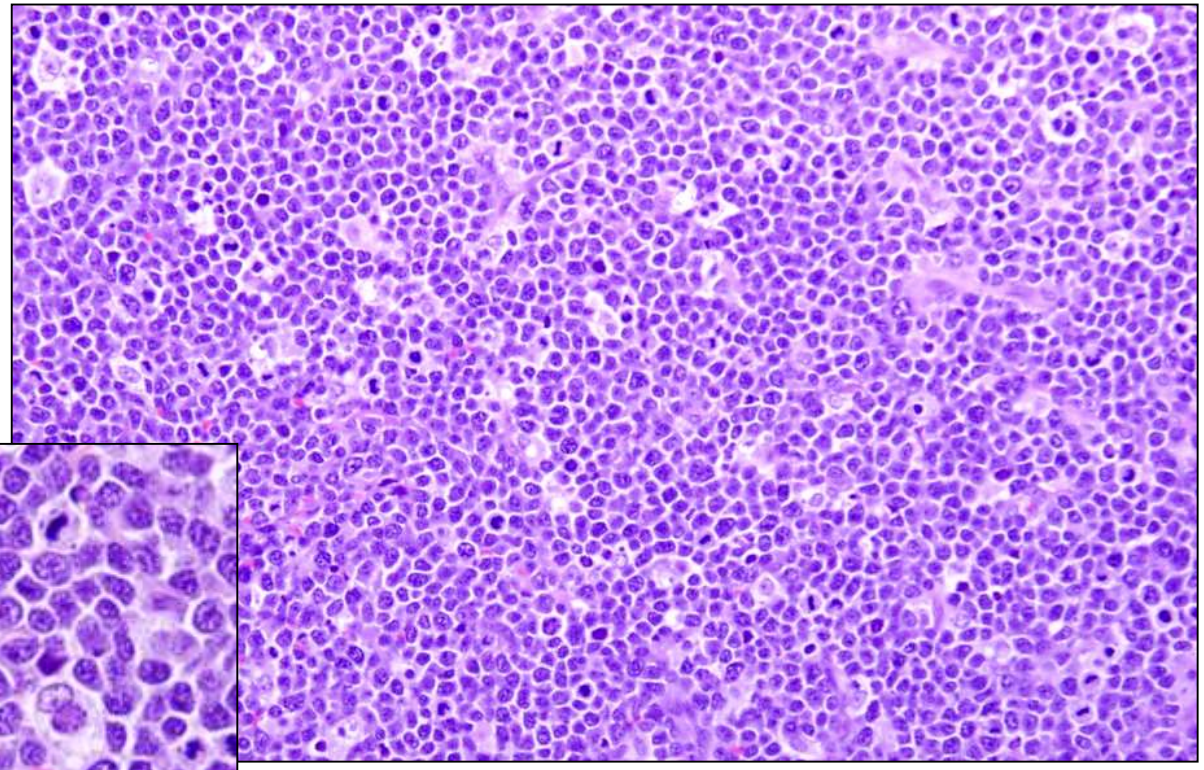
Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma

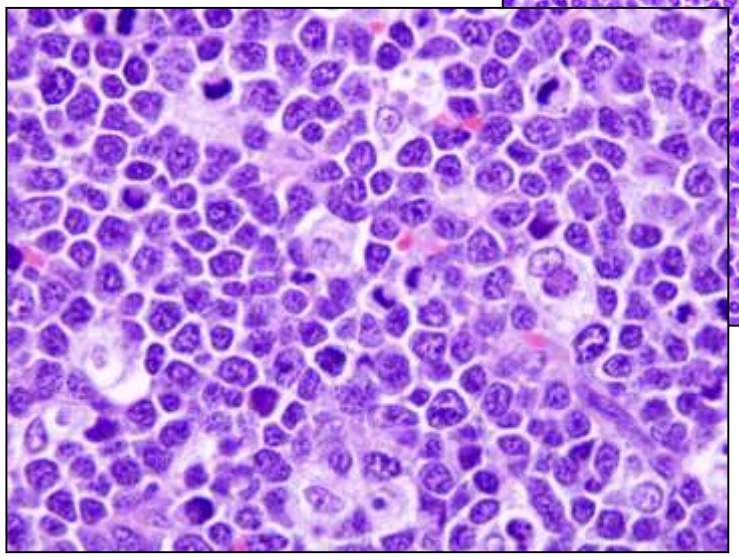
Andrea B. Moffitt and Sandeep S. Dave

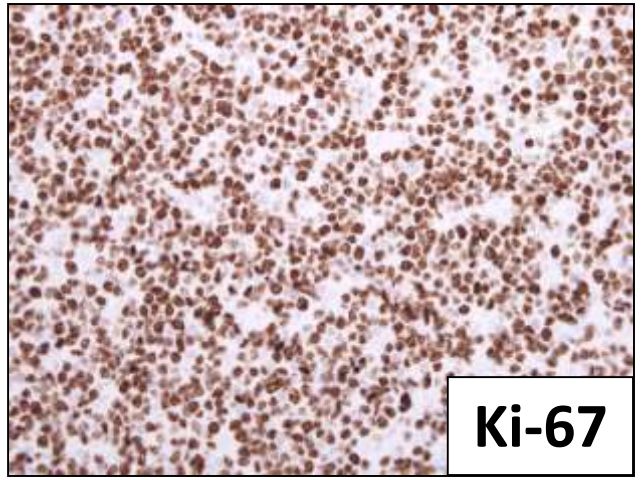
Comparison of Aggressive B cell Lymphomas

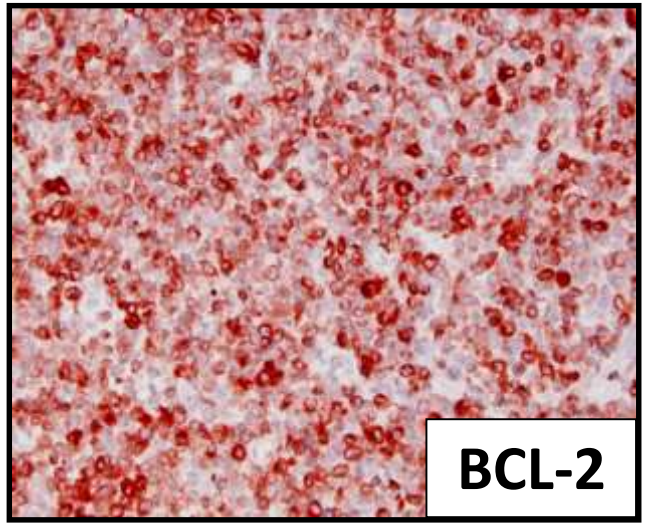
	Burkitt Lymphoma	DHL	DLBCL with *MYC* translocation
Morphology	**Usually distinctive, monomorphic, intermediate cells**	**Usually DLBCL**	**Usually large cells, pleomorphic**
IHC CD10/BCL6 BCL2 Ki67	 **+/+** **-** **100%**	 **Var/var** **+/-** **Var (usu >80-90%)**	 **Var/var** **+/-** **Var (usu >80-90%)**
MYC IHC	**100%**	**>70%**	**100%**
MYC FISH	**+**	**+**	**+**
IGH-BCL2 FISH or *BCL6* FISH	**-**	**+**	**-**
Genotype	**Simple**	**Complex**	**Complex**

High grade B cell lymphoma









What does the FISH show?

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

WHO 2017

AKA: double hit; triple hit

Histology: Burkitt, intermediate, or DLBCL

Immunophenotype:

GCB type B cell (usually): CD10+, BCL6+, **BCL2+**

Ki67 > 95% (usual, but range of 30-100%)

Cytogenetics:

t(14;18) *BCL2* rearrangement ~~or 3q27 *BCL6*~~

t(8;14) *MYC* rearrangement (or equivalent)

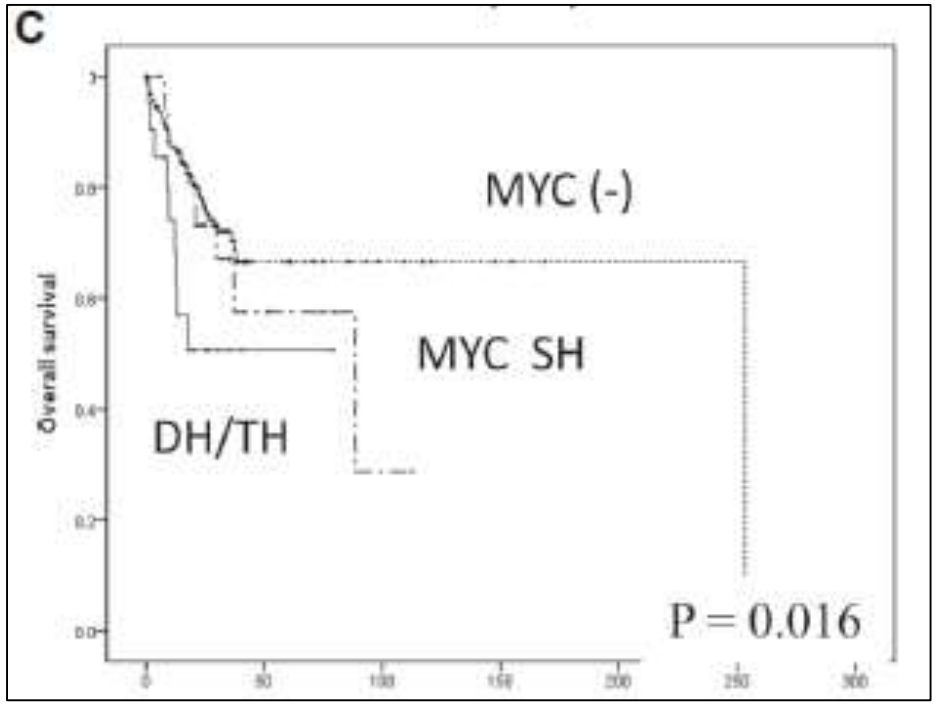
Prognosis:

Poor response to usual therapy (R-CHOP); survival < 1 year

HGBCL, with *MYC*, *BCL2* and/~~or~~ *BCL6* rearrangement

- Must have *MYC* translocation
  - Amplification does not qualify
- Must have *BCL2* ~~or *BCL6*~~ or both translocations
  - Double hit: (*MYC, BCL2* ~~or *BCL6*~~)
  - Triple hit: (*MYC, BCL2* and *BCL6*)
- Excluded: FL and B-ALL (with double or triple hit)

Overall survival of no MYC, MYC single hit, DHL/THL



Cho YA, Hyeon J, Lee H, Cho J, Kim SJ, Kim WS, Ko YH. MYC single-hit large B-cell lymphoma: clinicopathologic difference from MYC-negative large B-cell lymphoma and MYC double-hit/triple-hit lymphoma. Hum Pathol. 2021. PMID: 33771538.

Somatic copy number gains in MYC, BCL2, and BCL6 identifies a subset of aggressive alternative–DH/TH DLBCL patients

Jordan E Krull [1 2], Kerstin Wenzl [2], Keenan T Hartert [2], Michelle K Manske [2], Vivekananda Sarangi [3], Matthew J Maurer [3], Melissa C Larson [3], Grzegorz S Nowakowski [2], Stephen M Ansell [2], Ellen McPhail [4], Thomas M Habermann [2], Brian K Link [5], Rebecca L King [4], James R Cerhan [3], Anne J Novak [6]

Affiliations + expand

PMID: 33168821 PMCID: PMC7652824 DOI: 10.1038/s41408-020-00382-3

Blue lines show “alternate” double hit
Red lines show usual double hit

Take home message: Copy number variation (CNV) matters in these genes

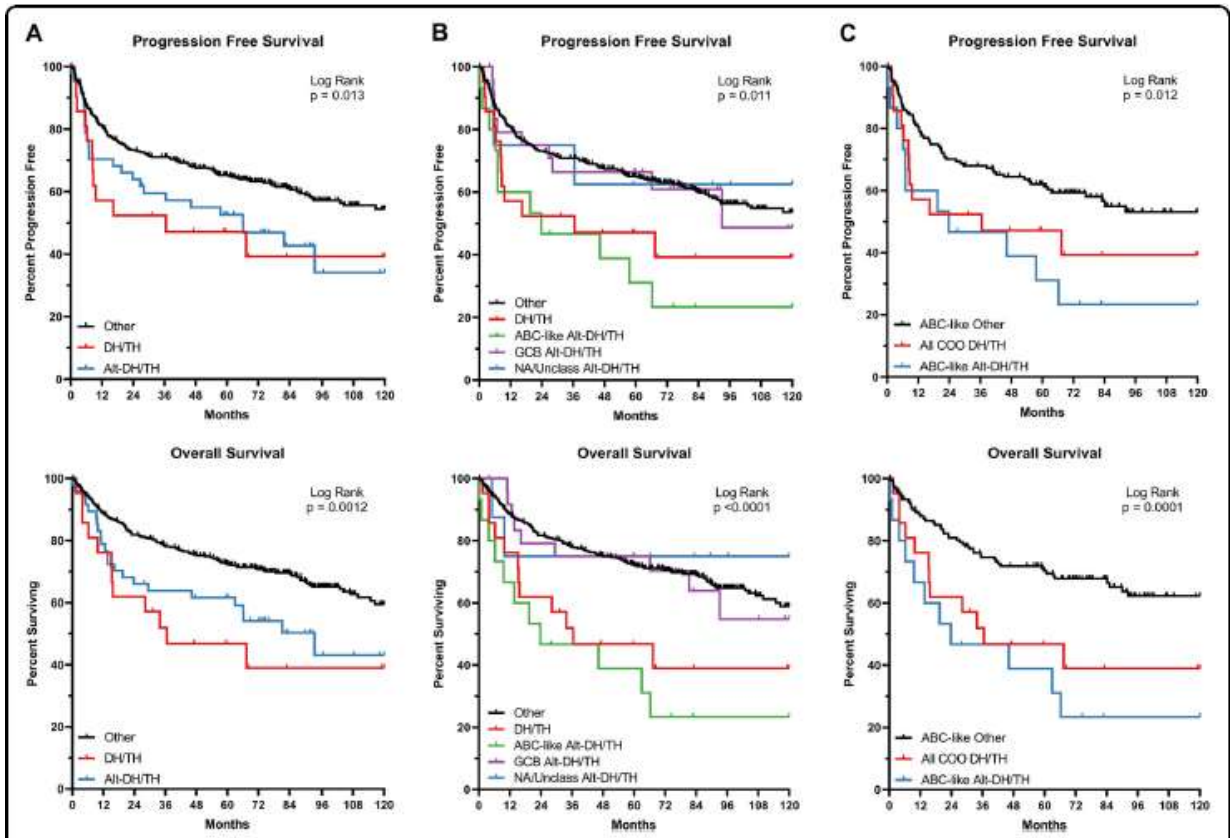


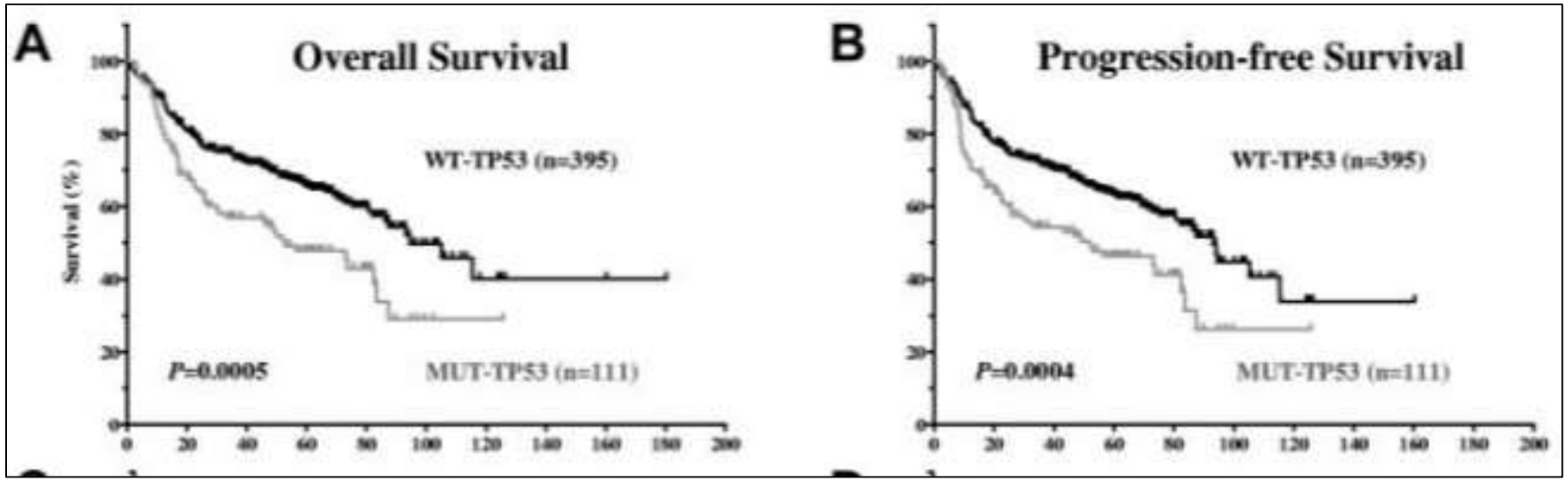
Fig. 2 Survival outcomes of Alt-DH/TH patients. Kaplan–Meier survival curves for event-free/progression-free (EFS/PFS) and overall survival (OS) of DH/TH, Alt-DH/TH, and Other (translocation positive + copy gain positive + no alteration patients). Progression-free survival is a combination of EFS from one study and PFS from another. **A** Progression-free survival ($p = 0.013$) and overall survival ($p = 0.0012$) of all Alt-DH/TH ($n = 51$), DH/TH ($n = 21$), and Other cases ($n = 410$) in the combined cohort. **B** Progression-free survival (EFS/PFS) ($p = 0.011$) and overall survival ($p < 0.001$) of Alt-DH/TH cases separated by COO class (ABC-like, $n = 15$; GCB, $n = 25$; NA/unclassifiable, $n = 8$), all DH/TH ($n = 21$), and all Other cases ($n = 410$). **C** Progression-free survival (EFS/PFS) ($p = 0.012$) and overall survival ($p = 0.001$) of ABC-like Alt-DH/TH ($n = 15$), DH/TH ($n = 21$), and ABC-like Other ($n = 151$) cases in the combined cohort. *p* Values listed on each graph were derived from a Log Rank test.

HGBCL, NOS

- Excludes DLBCL morphology (p. 335); **blastoid** OR intermediate between DLBCL and BL (p. 335)
- *MYC* translocation ALONE **does not qualify** for this diagnosis (e.g. DLBCL, NOS with *MYC* translocation)
- Double expressor **does not qualify** for this diagnosis (IHC MYC+, BCL2+)
- My understanding is cases "with *MYC* **amplification**, and another translocation (*BCL2, BCL6*) and Burkitt-like morphology" *OR*
- *MYC* translocation, *BCL2* amplification and Burkitt-like morphology *OR*
- Blastoid morphology
- This diagnosis should be **extraordinarily rare**

P53

Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study

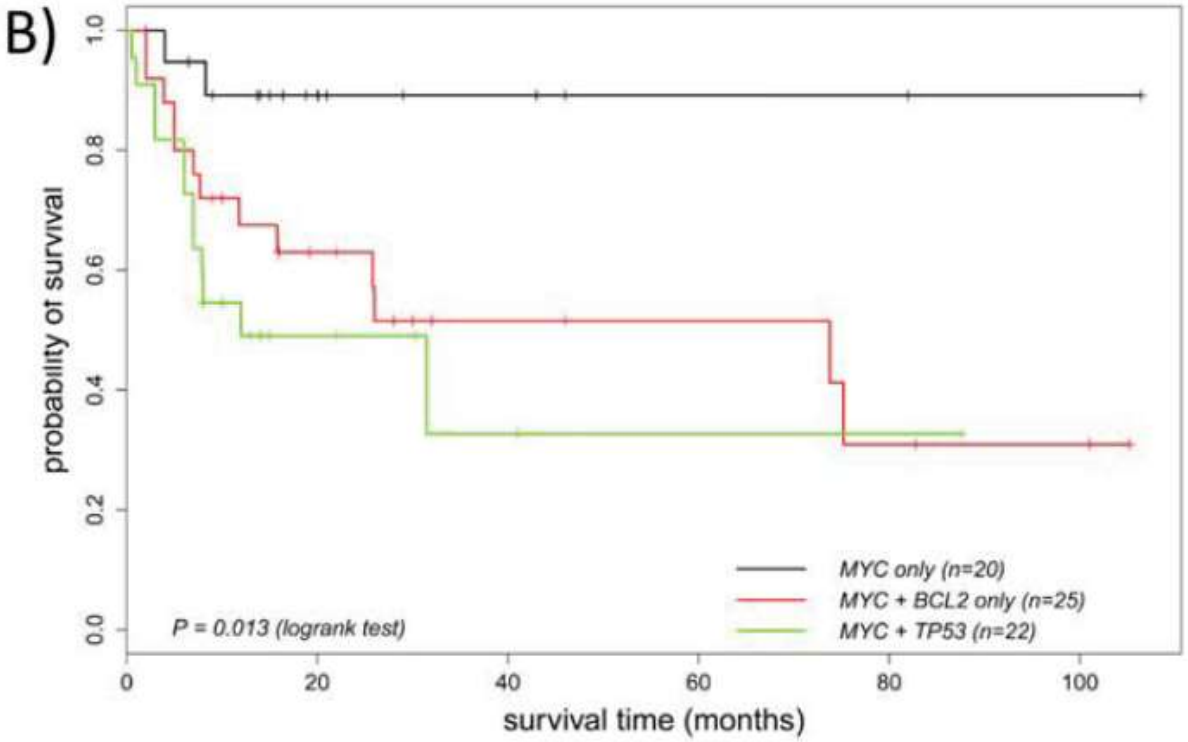


Impact on survival is more pronounced on patients with GCB phenotype.
>50% expression by IHC, best predicts survival effect

Xu-Monette et al. *Blood.* 2012.

The prognosis of *MYC* translocation positive diffuse large B-cell lymphoma depends on the second hit

Impact of *MYC*, *BCL2* and *P53* on survival



MYC/P53 **is as bad/worse than double hit**

BUT…..

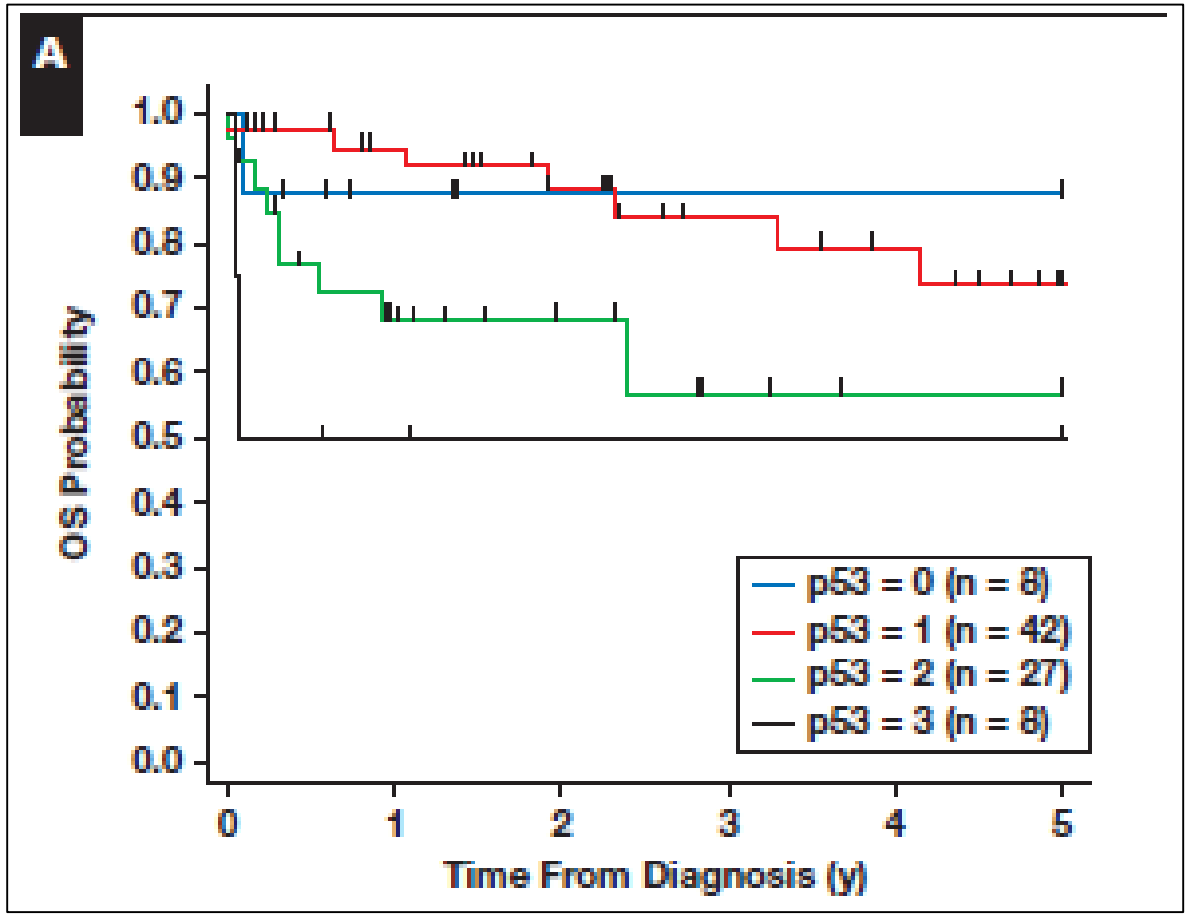
P53 had no independent impact on prognosis (???)

Clipson et al. *J Path: Clin Res.* 2015

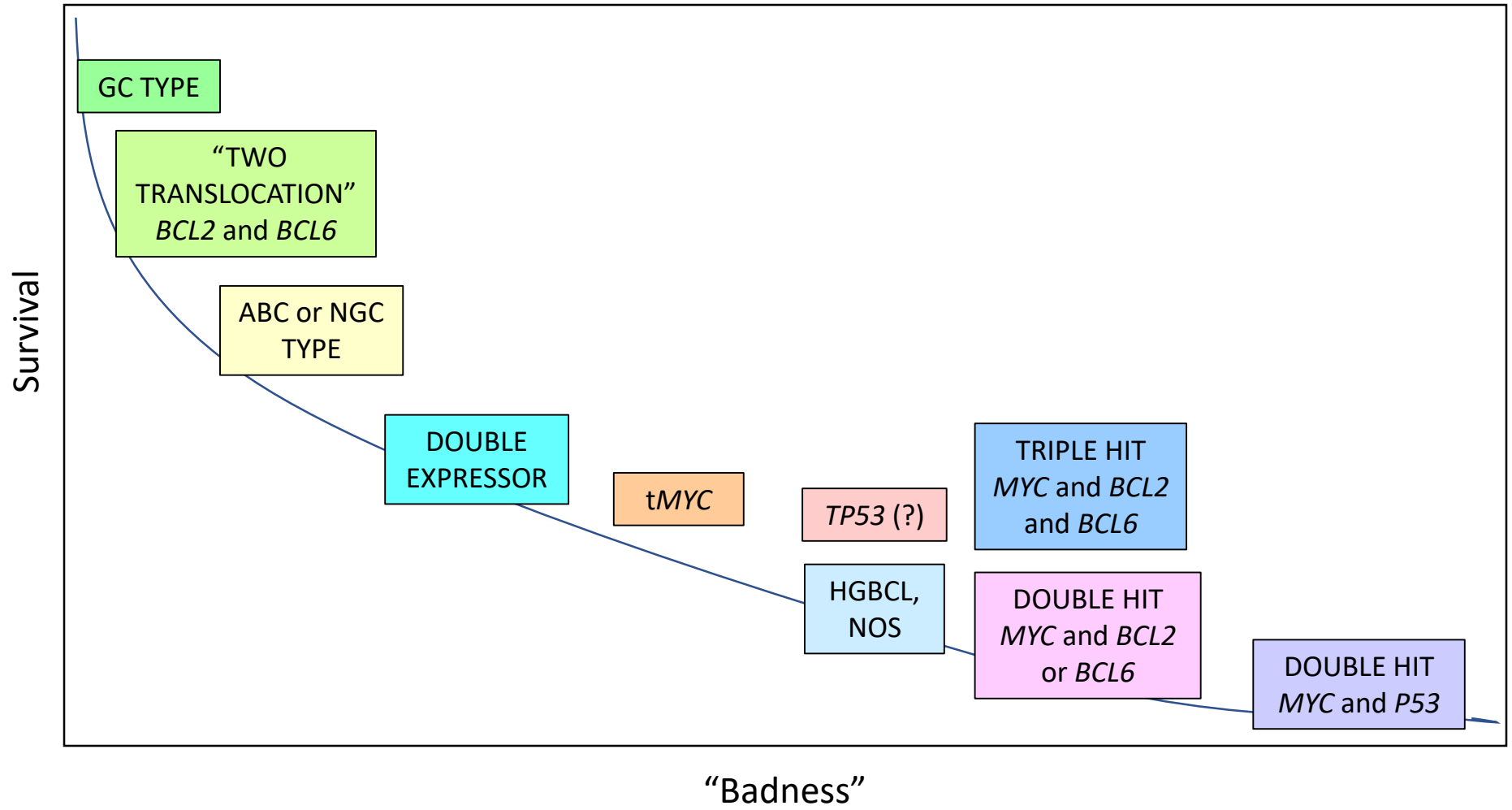
p53 Expression Is a Strong Marker of Inferior Survival in De Novo Diffuse Large B-Cell Lymphoma and May Have Enhanced Negative Effect With MYC Coexpression

A Single Institutional Clinicopathologic Study

Yi Xie, MD PhD,[1] Mohmad Ajaz Bulbul, MD,[2] Lingyun Ji, MS,[3] Casey M. Inoueye, MD,[4] Susan G. Groshen, PhD,[3] Anil Tulpule, MD,[5] Dennis P. O'Malley, MD,[6] Endi Wang, MD, PhD,[7] and Imran N. Siddiqi, MD, PhD[1]



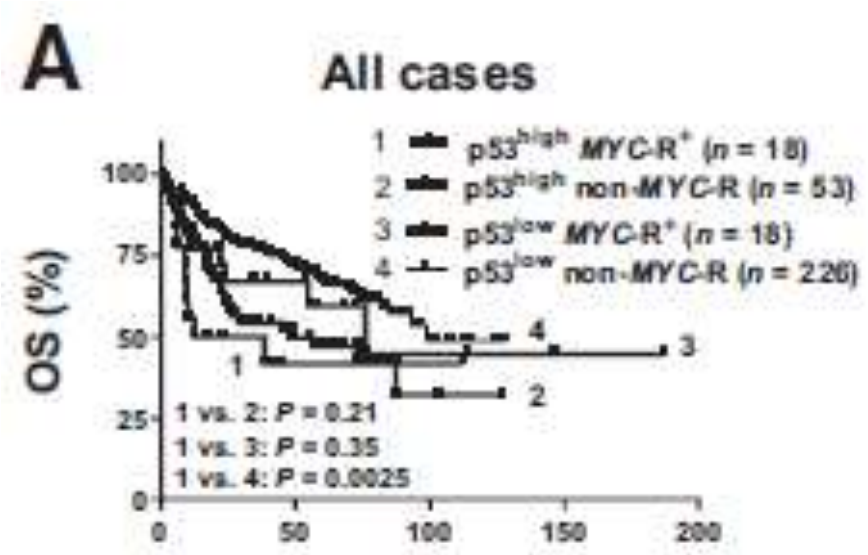
Survival in DLBCL and HGBCL types

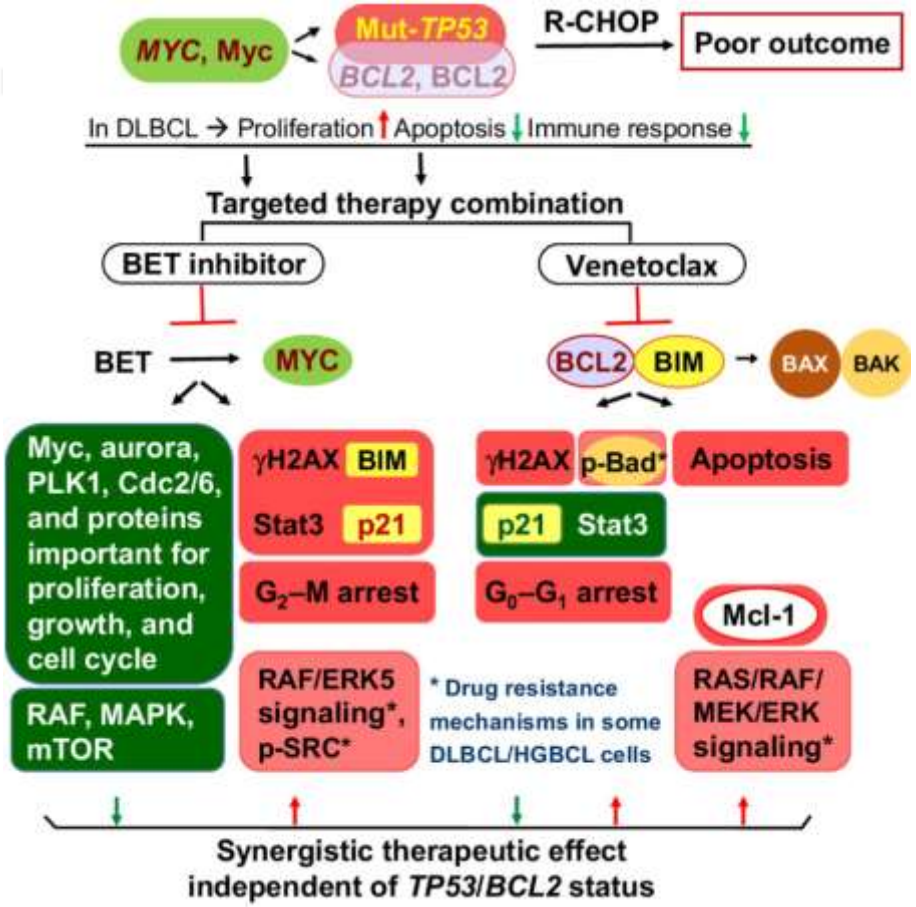


Mol Cancer Res. 2021 Feb;19(2):249-260. doi: 10.1158/1541-7786.MCR-20-0466. Epub 2020 Nov 5.

Aggressive B-cell Lymphoma with MYC/TP53 Dual Alterations Displays Distinct Clinicopathobiological Features and Response to Novel Targeted Agents

Manman Deng [# 1 2], Zijun Y Xu-Monette [# 1], Lan V Pham [# 3], Xudong Wang [# 1], Alexandar Tzankov [4], Xiaosheng Fang [1], Feng Zhu [1], Carlo Visco [5], Govind Bhagat [6], Karen Dybkaer [7], April Chiu [8], Wayne Tam [9], Youli Zu [10], Eric D Hsi [11], Hua You [12], Jooryung Huh [13], Maurilio Ponzoni [14], Andrés J M Ferreri [14], Michael B Møller [15], Benjamin M Parsons [16], Fredrick Hagemeister [17], J Han van Krieken [18], Miguel A Piris [19], Jane N Winter [20], Yong Li [21], Bing Xu [22], Phillip Liu [23], Ken H Young [24 25]





Double-hit Signature with *TP53* Abnormalities Predicts Poor Survival in Patients with Germinal Center Type Diffuse Large B-cell Lymphoma Treated with R-CHOP

Joo Y. Song[1,2], Anamarija M. Perry[3], Alex F. Herrera[2,4], Lu Chen[2,5], Pamela Skrabek[6], Michel R. Nasr[7], Rebecca A. Ottesen[8], Janet Nikowitz[8], Victoria Bedell[1,2], Joyce Murata-Collins[1], Yuping Li[1], Christine McCarthy[8], Raju Pillai[1,2], Jinhui Wang[9], Xiwei Wu[9], Jasmine Zain[2,4], Leslie Popplewell[2,4], Larry W. Kwak[2,4], Auayporn P. Nademanee[2,4], Joyce C. Niland[8], David W. Scott[10], Qiang Gong[1], Wing C. Chan[1,2], and Dennis D. Weisenburger[1,2]

- Cell-of-origin
- MYC, BCL2, BCL6 (DHL, THL)
- GEP (DHITsig) double hit signature without MYC
- Double expressor

GROUPS

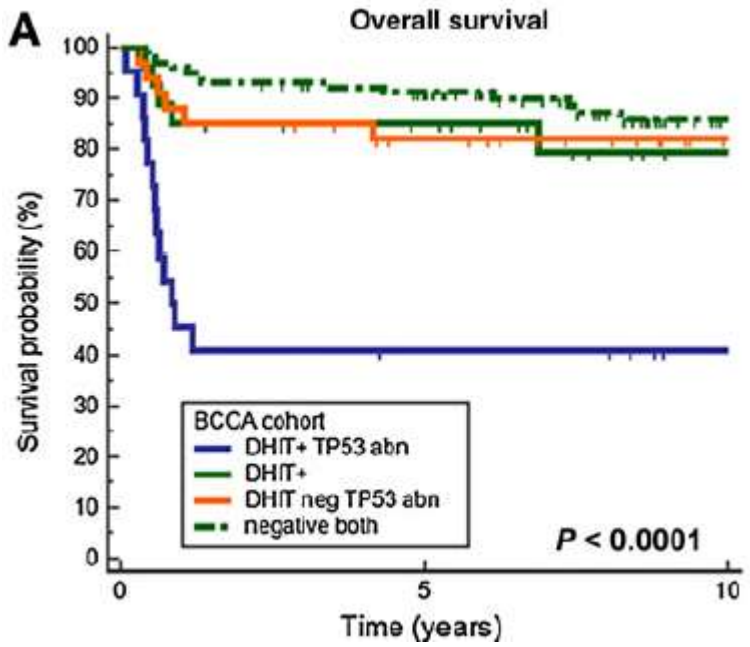
- GCB1: *TP53* with DHITsig
- GCB2: DHITsig, TP53 negative
- GCB3: *EZH2* mutation and/or *BCL2* translocation positive (AKA EZB)
- GCB4: no DHITsig, lack *EZH2*, lack *BCL2*, lack *TP53*

	% of cases	OS/PFS		
GCB1 (DHsig+TP53)	8%	9.2/6.1	Very poor prognosis	Clinical trial
GCB2 (Dsig, no TP53)	9%		Good prognosis	R-CHOP +/- anti-BCL2 or anti-EZH2
GCB3	32%		Intermediate prognosis	Clinical trial using R-CHOP and anti-BCL2 and/or anti-EZH2
GCB4	21%		Good prognosis	R-CHOP

"Our cases that were DHITsig positive with *TP53* abnormalities had a dismal prognosis,

which confirms that the *TP53* abnormality is key to the aggressiveness of these lymphomas.

This is also consistent with recent findings that not all double- or triple-hit lymphomas have a poor prognosis"



SUMMARY

- Can’t find what you don’t look for!

- Aggressive B cell lymphoma
  - Screen with MYC & BCL2 IHC (double expressor)
  - Perform FISH in all candidate cases (*MYC, with reflex to BCL2, BCL6)*
  - Screen for P53 (IHC) (mutant high or null => *TP53* mutation status)
  - Can have very aggressive behavior and different therapy

- It is unclear if the WHO22 and ICC (ICK!) will address HGBCL in a meaningful way, but changes will undoubtedly be coming

Questions?

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