Whole exome sequencing of high grade B cell lymphoma with *BCL2/MYC* rearrangements reveals potentially actionable mutations supportive of transformed follicular lymphoma

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Case SH2017-0160

Clinical History

1995

Diagnosed with follicular lymphoma

Achieves remission with CHOP

1999

Follicular lymphoma recurrence

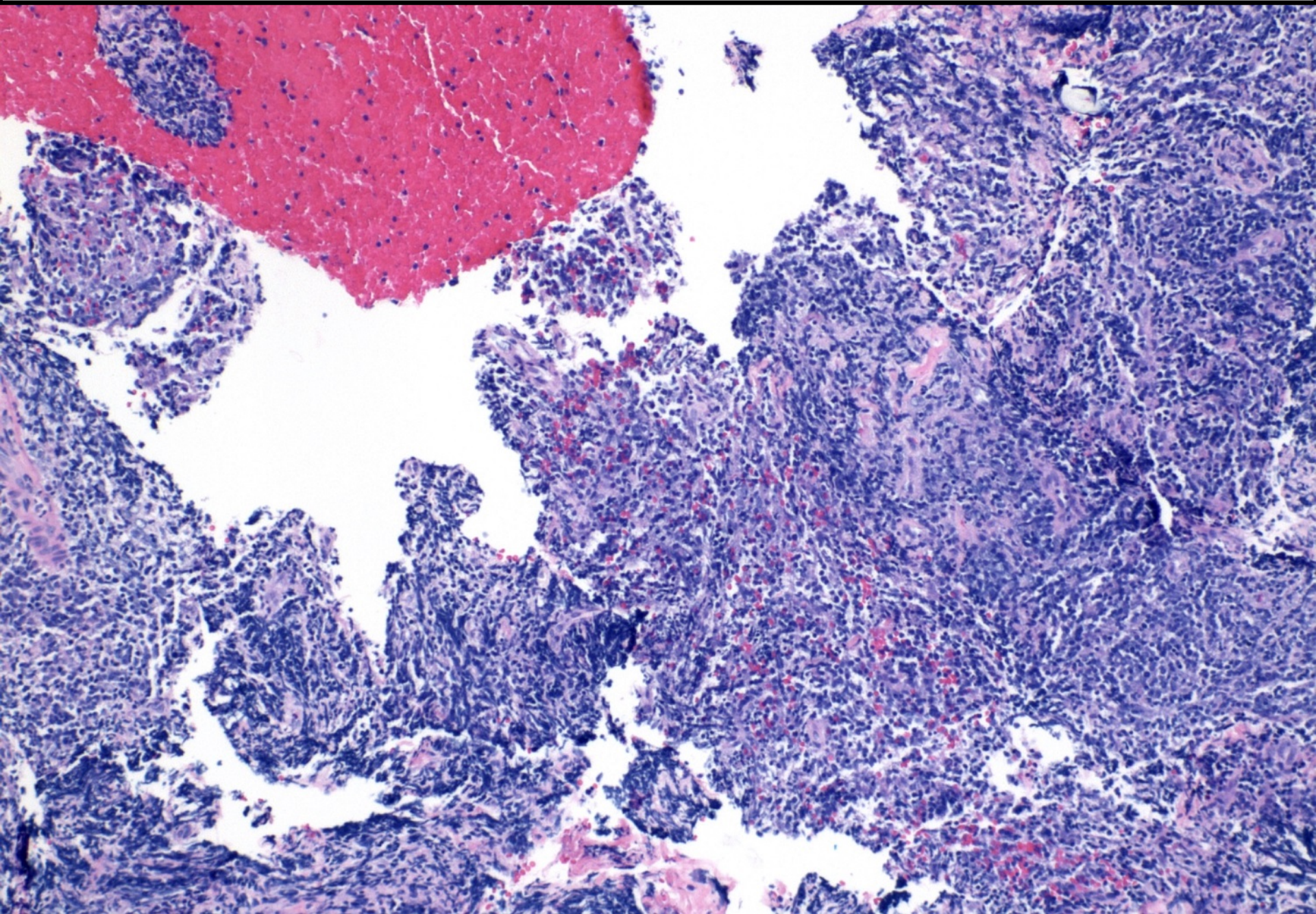
Achieves remission with rituximab

2016

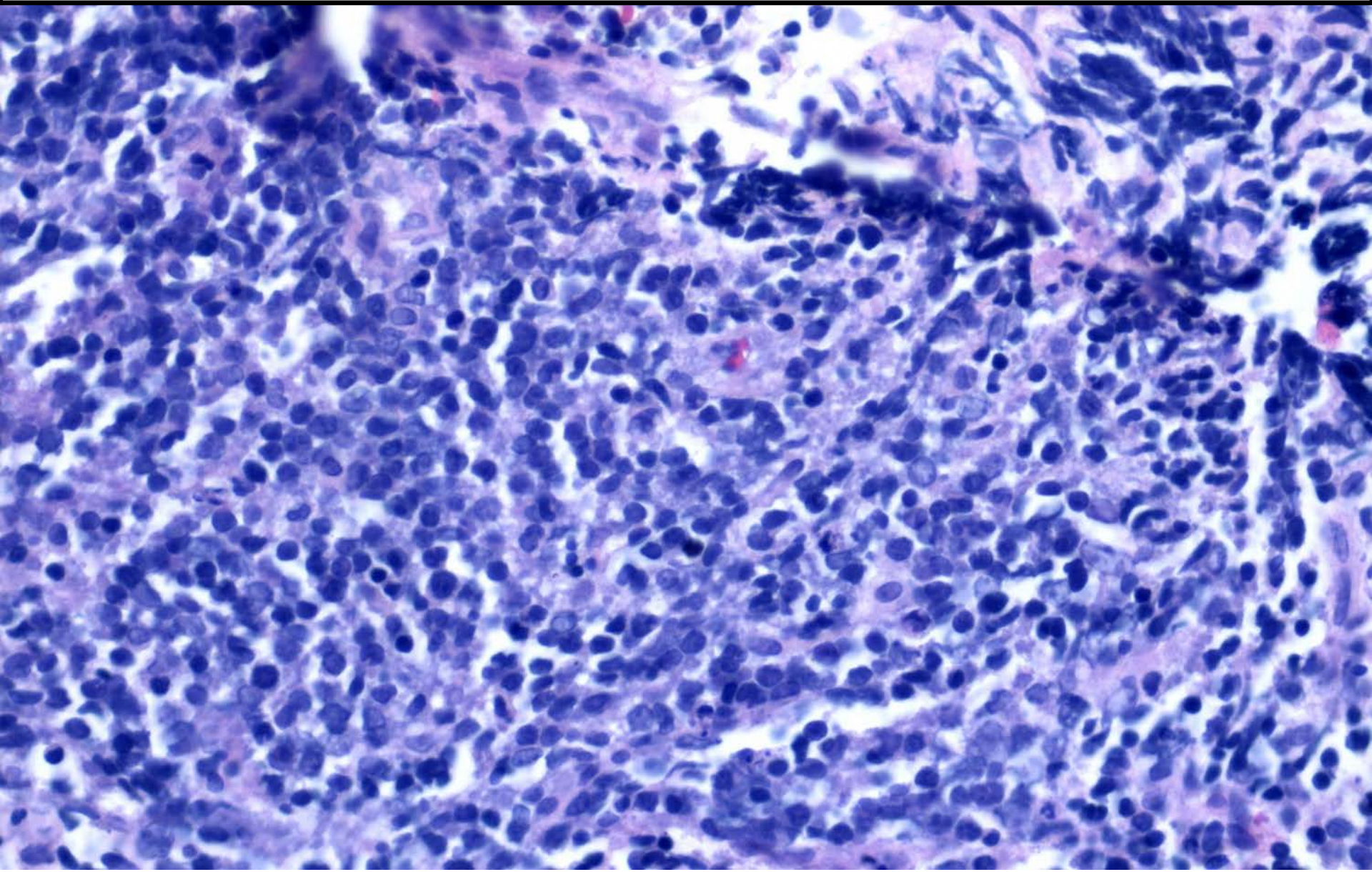
Chest wall mass at site of previous lymphoma

FNA and core biopsy are obtained

Core Biopsy of Left Chest Wall Mass

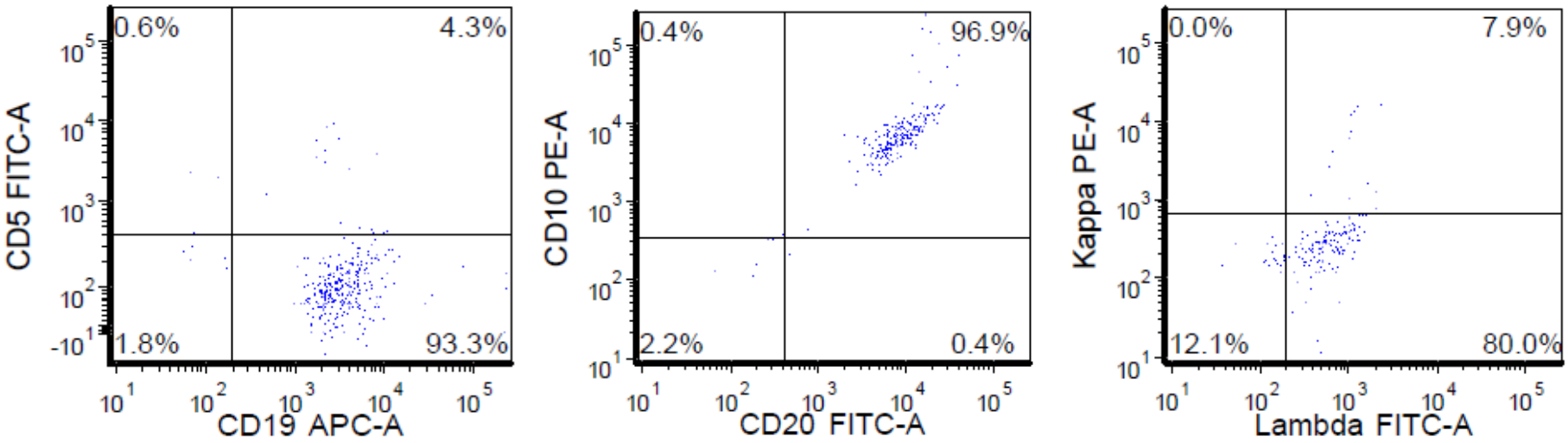


Core Biopsy of Left Chest Wall Mass



IHC: Ki67 ~40%

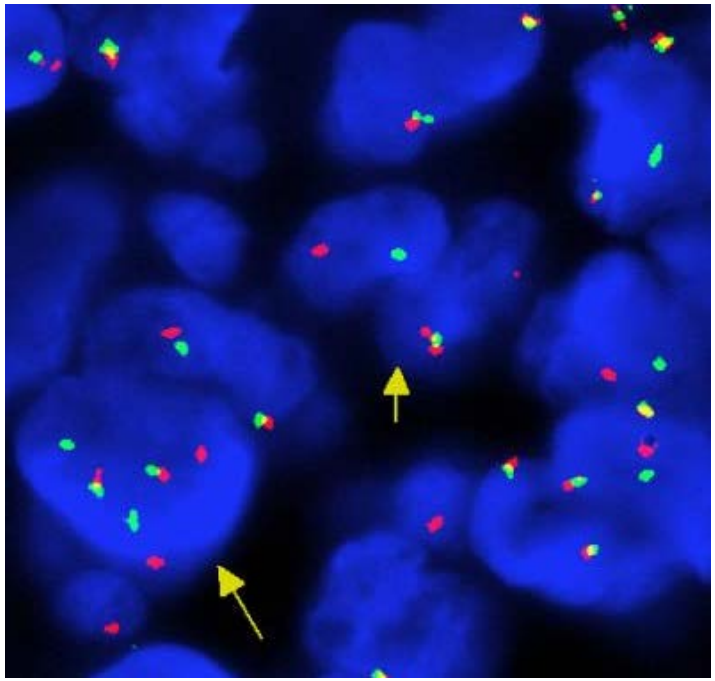
Flow Cytometry



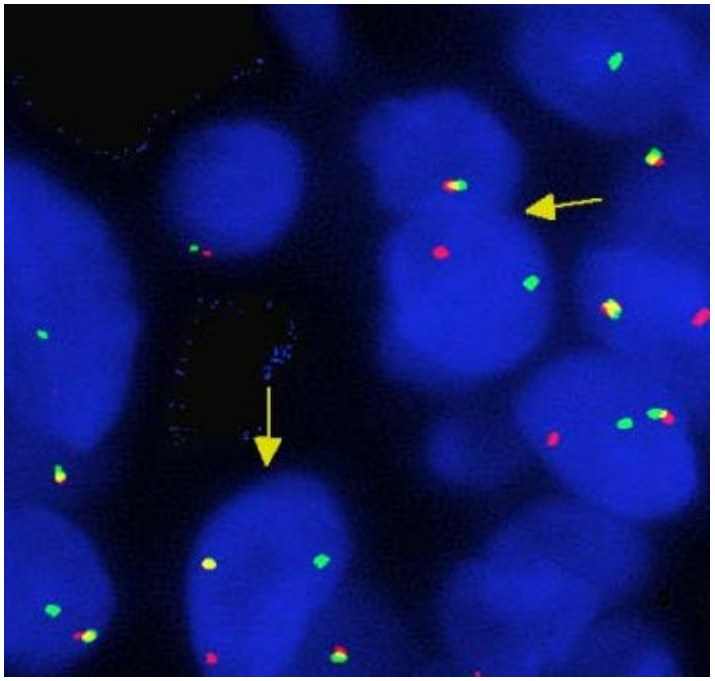
Flow cytometric analysis demonstrated a lambda-restricted mature B cell population expressing CD10, CD19, and CD20, and lacking CD5.

FISH Studies

BCL2 Break-apart



MYC Break-apart



Probe	**Result**	**Comment**
BCL2	Positive	74% of nuclei with *BCL2* rearrangement
MYC	Positive	85% of nuclei with *MYC* rearrangement
BCL6	Negative for rearrangement	

Whole Exome Sequencing Results

- **EZH2** p.Y646F [35% mutant allele frequency (MAF)].
  - Known pathogenic mutation in the SET domain
- **BCL2** p.L86F [35% MAF].
  - Previously confirmed somatic mutation in DLBCL
- BCL2 p.G197S [40% MAF].
  - Variant of unknown significance (VUS), not previously reported
- **CREBBP** splice site mutation in intron 6 [55% MAF].
  - Likely pathogenic due to effects on protein translation. CREBBP mutations are common in DLBCL and follicular lymphoma.
- **TNFRSF14** p.W201* [70% MAF].
  - Likely pathogenic due to effect on protein function, previously reported once in follicular lymphoma
- CARD11 p.C49Y [35% MAF].
  - VUS, previously reported once in DLBCL

Case Summary

History
Recurrent follicular lymphoma

Morphology
Large B-cell lymphoma

Immunophenotype
Lambda restricted
CD10+, CD19+, CD20+, CD5-

FISH
BCL2 rearrangements
MYC rearrangements

Somatic Mutations

- EZH2
- BCL2
- TNFRS14
- CREBBP
- CARD11

Early event mutations of follicular lymphoma

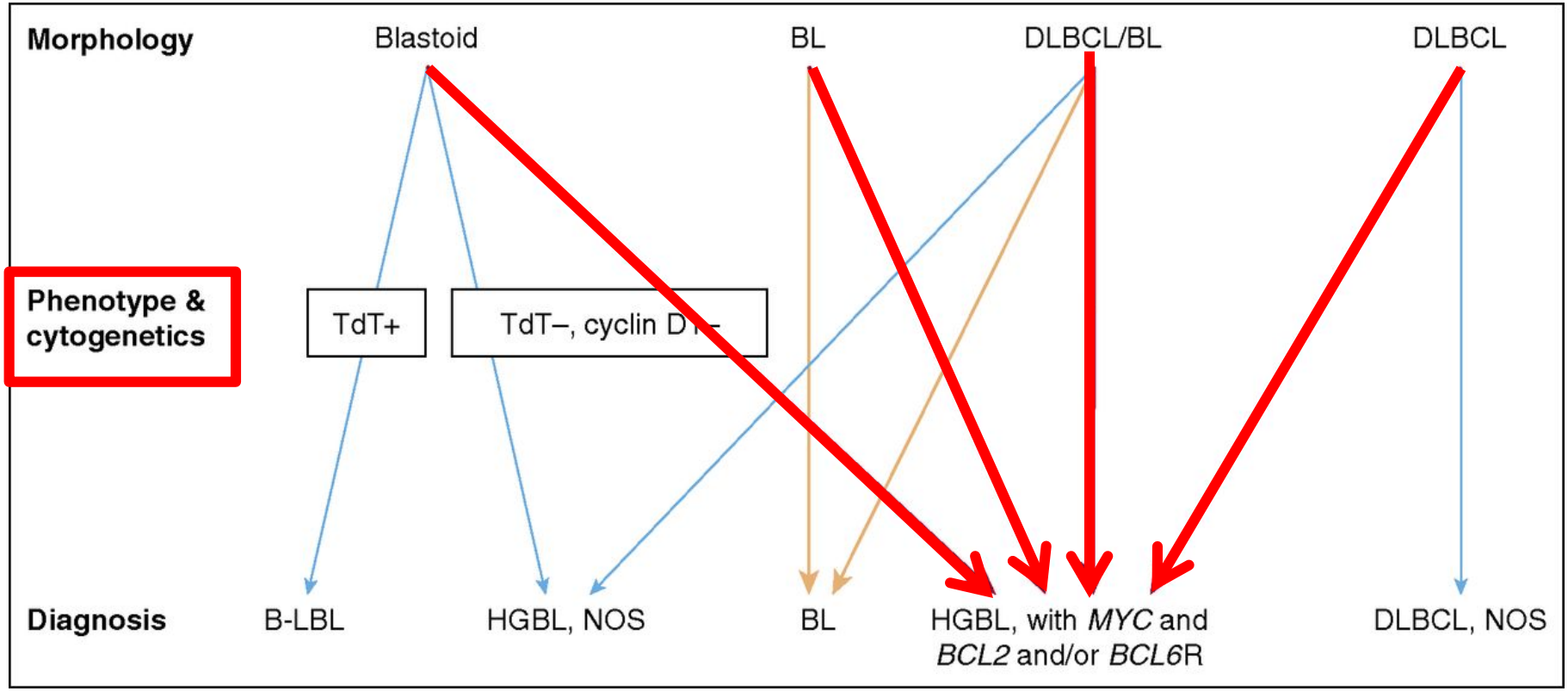
Potential therapeutic targets

Panel Diagnosis
High grade B cell lymphoma with *MYC* and *BCL2* rearrangements

Discussion Objectives

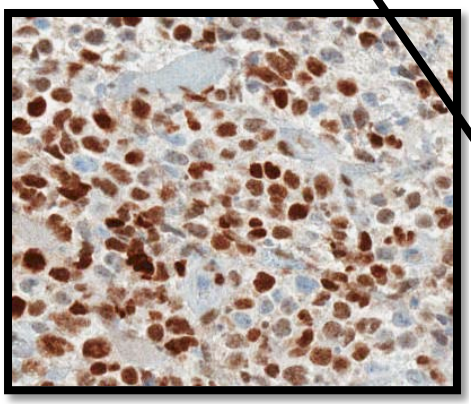
- Molecular diagnosis of high grade B-cell lymphomas
- Proposed screening techniques
- Contribution of NGS to the recognition of these lymphoma subtypes

Diagnostic Approach to High Grade B Cell Lymphomas



Swerdlow *et al*. Blood 2016;127:2375-2390

Distribution of HGBL by COO, cytogenetic status, and IHC



IHC expression of MYC & BCL2

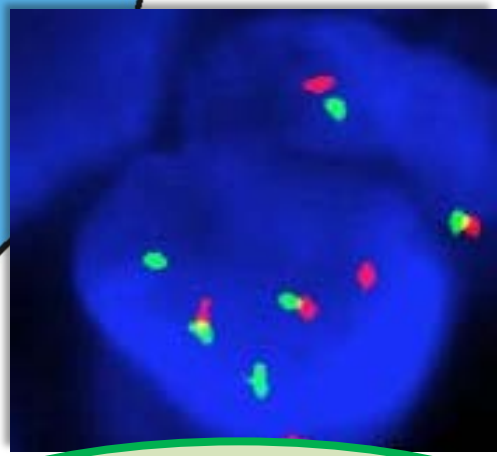
Unclassified subtype

ABC subtype

GCB subtype

Double expression

Double hit

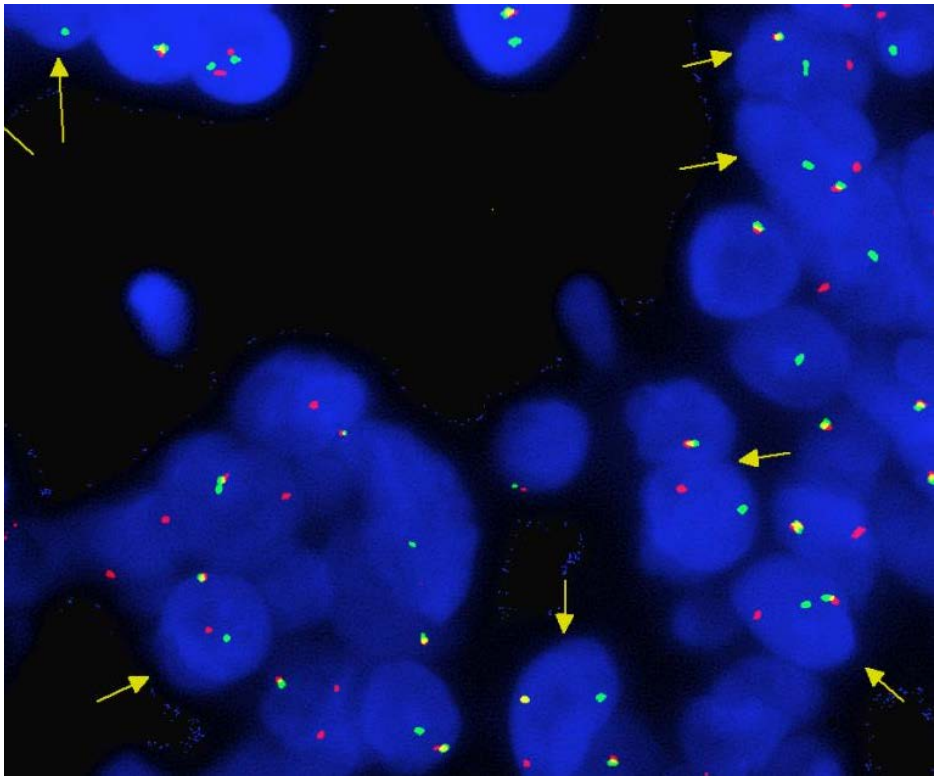


HGBL with MYC & BCL2 &/or BCL6 rearrangements

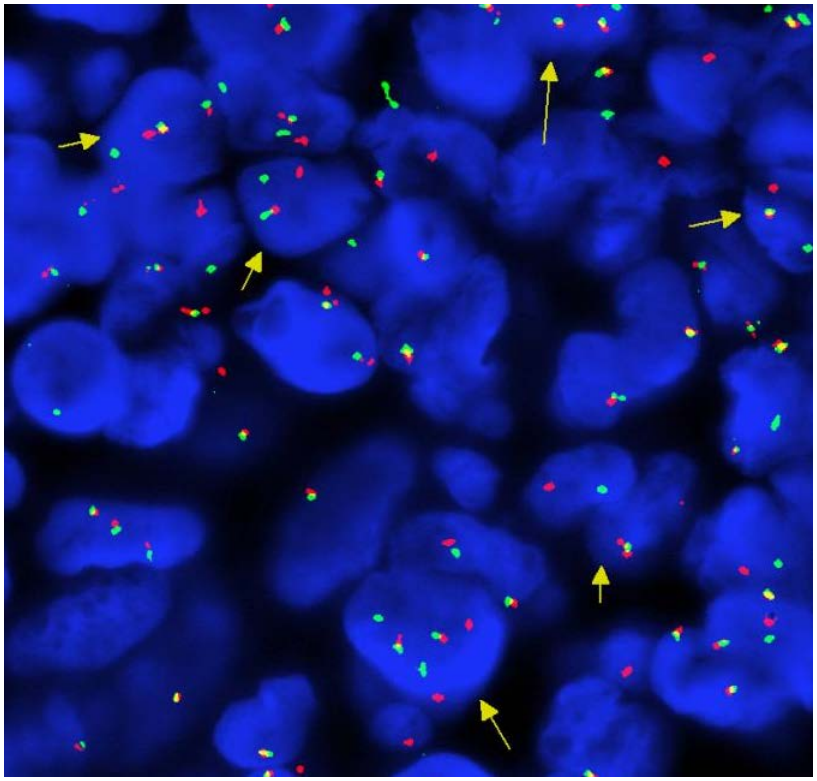
Friedberg JW. Blood. 2017 Aug 3;130(5):590-596.

Diagnosis based on molecular studies that identify rearrangements of MYC and BCL2 genes

MYC break-apart probes



BCL2 break-apart probes



What cases should be FISH'd?

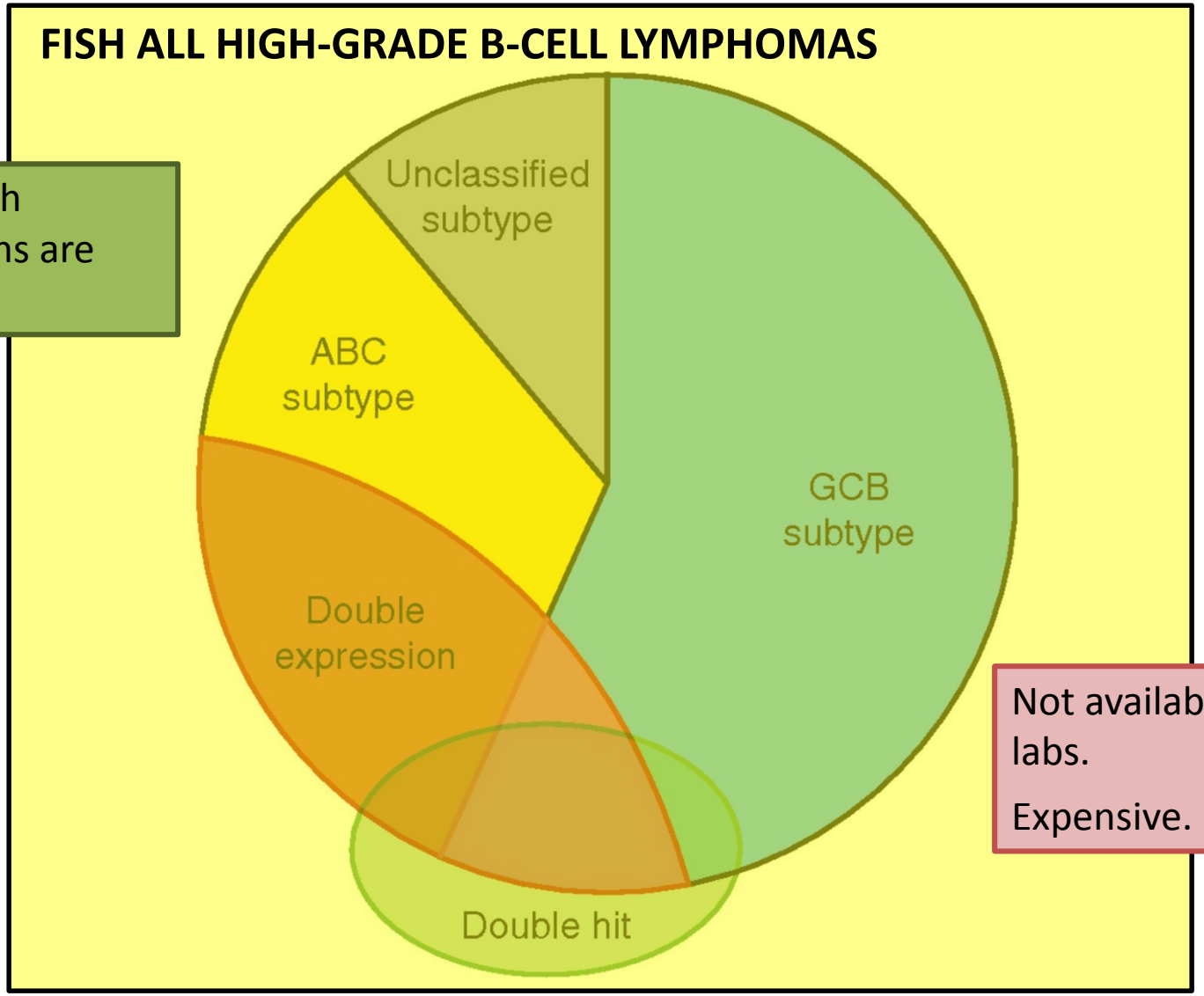
Currently, no consensus guidelines

a) FISH all three genes for all cases

b) FISH cases that exhibit high grade morphology

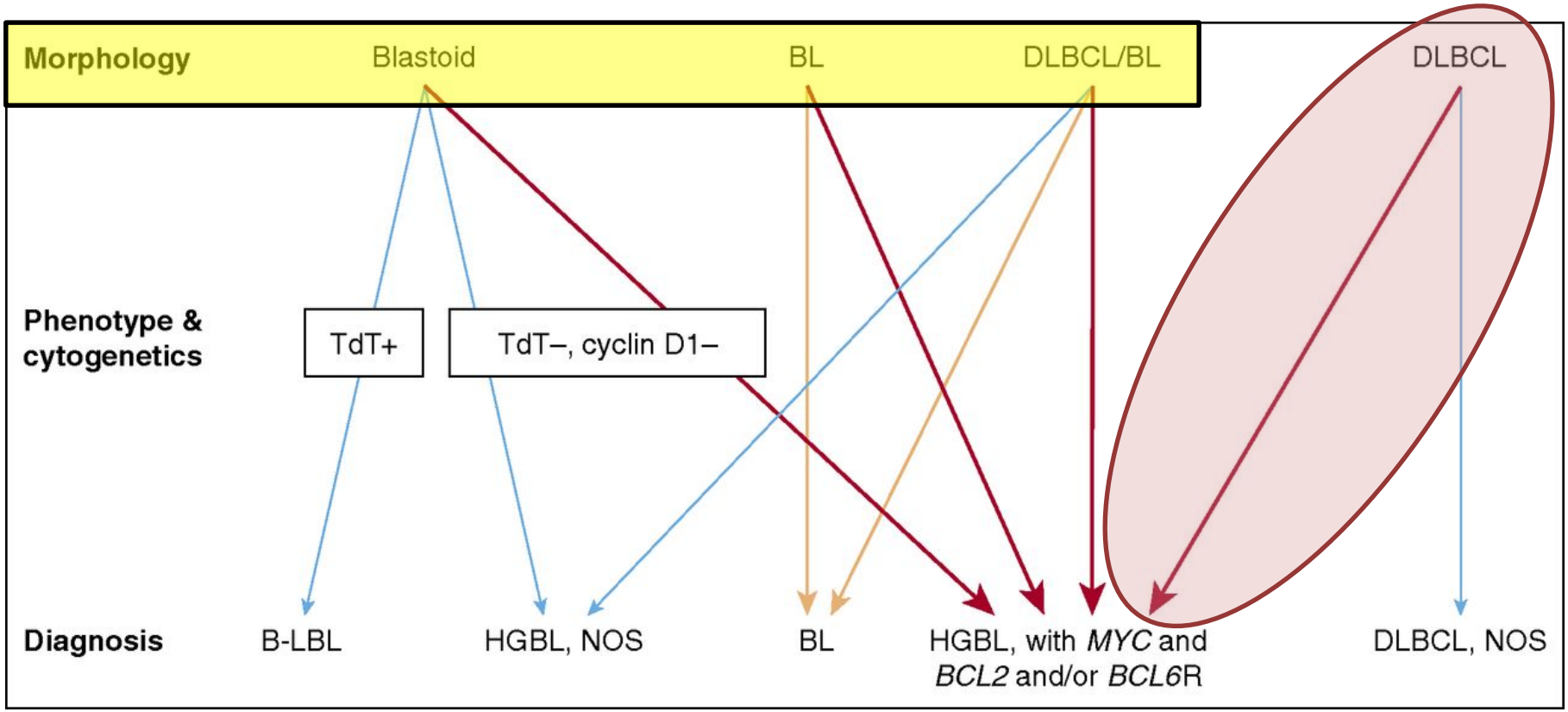
c) FISH cases with GCB immunophenotype

d) FISH cases with MYC immunohistochemical expression >40%



PROPOSED SCREENING STRATEGIES TO SUBCLASSIFY HGBL

FISH CASES WITH HIGH GRADE MORPHOLOGY



Reduced Cost

Subjective = not reproducible

Will not identify subset of cases with standard DLBCL morphology

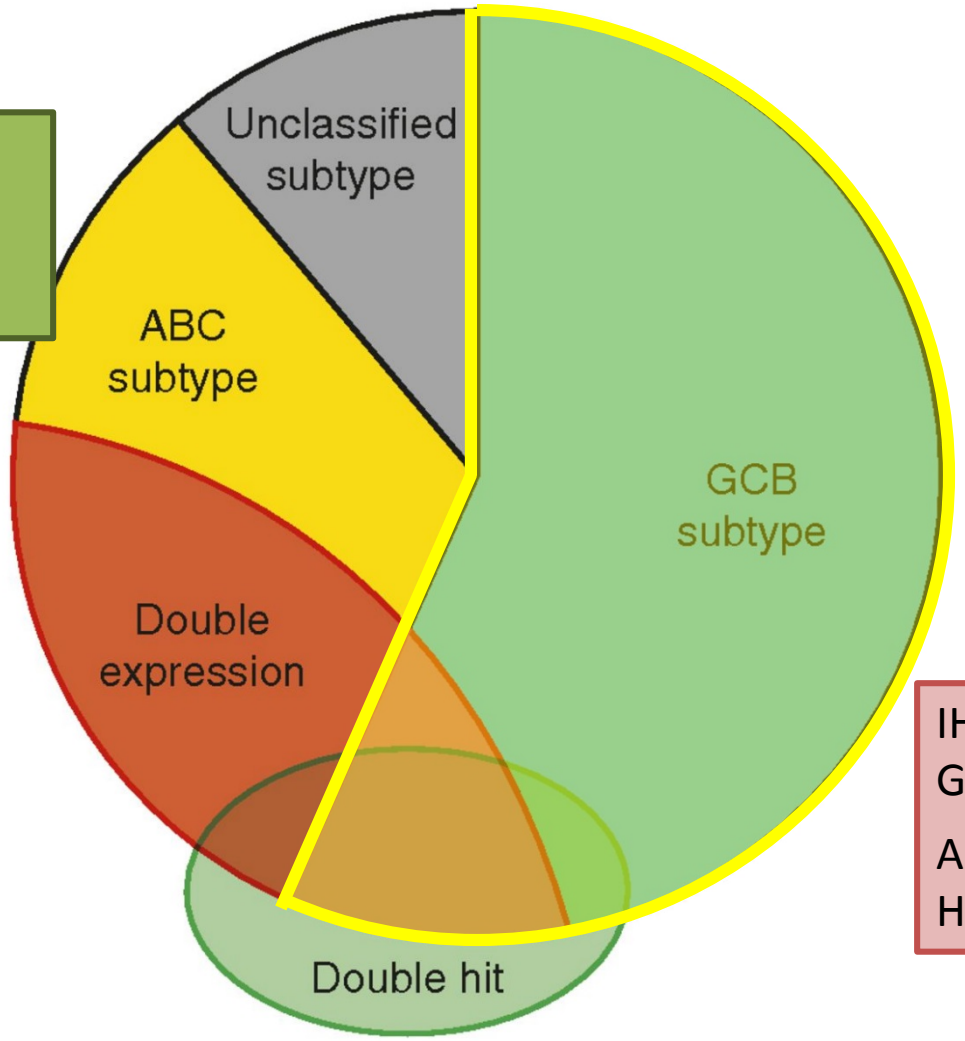
PROPOSED SCREENING STRATEGIES TO SUBCLASSIFY HGBL

FISH CASES WITH GCB IMMUNOPHENOTYPE

90-95% of DH-HGBL

Reduces FISH by ~50%

Reduced cost



IHC misses 10-15% of GCB

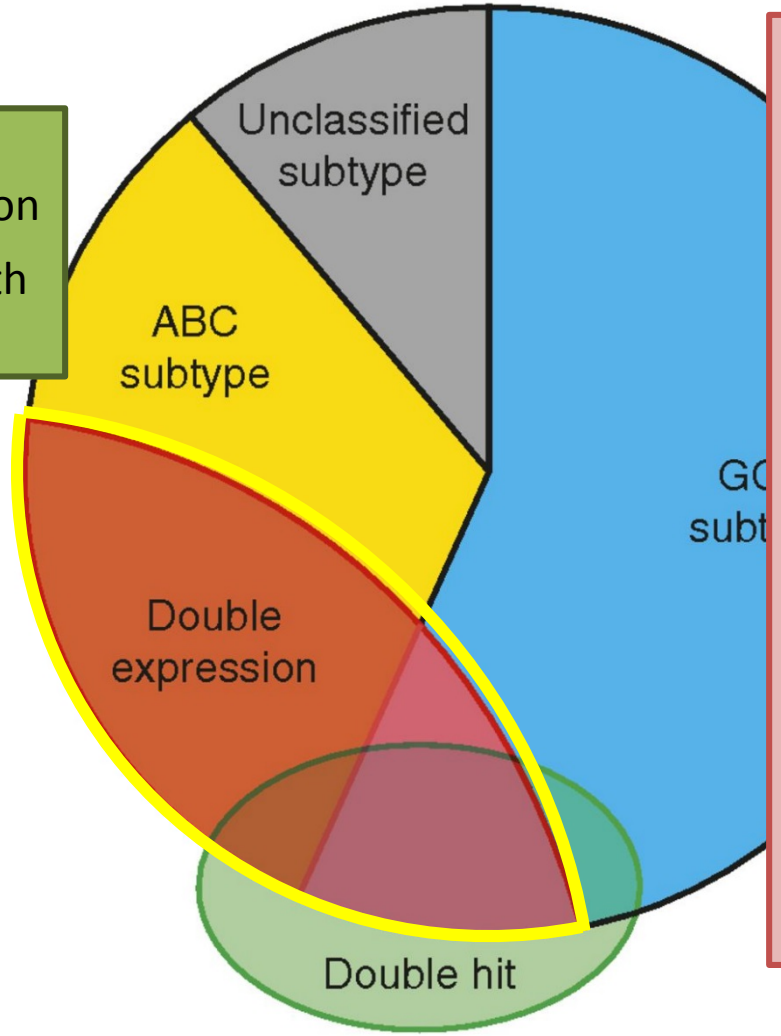
A subset (~10%) of DH-HGBL still missed

PROPOSED SCREENING STRATEGIES TO SUBCLASSIFY HGBL

FISH CASES THAT DEMONSTRATION MYC IHC EXPRESSION

MYC translocations increase MYC expression

MYC IHC correlates with MYC RA



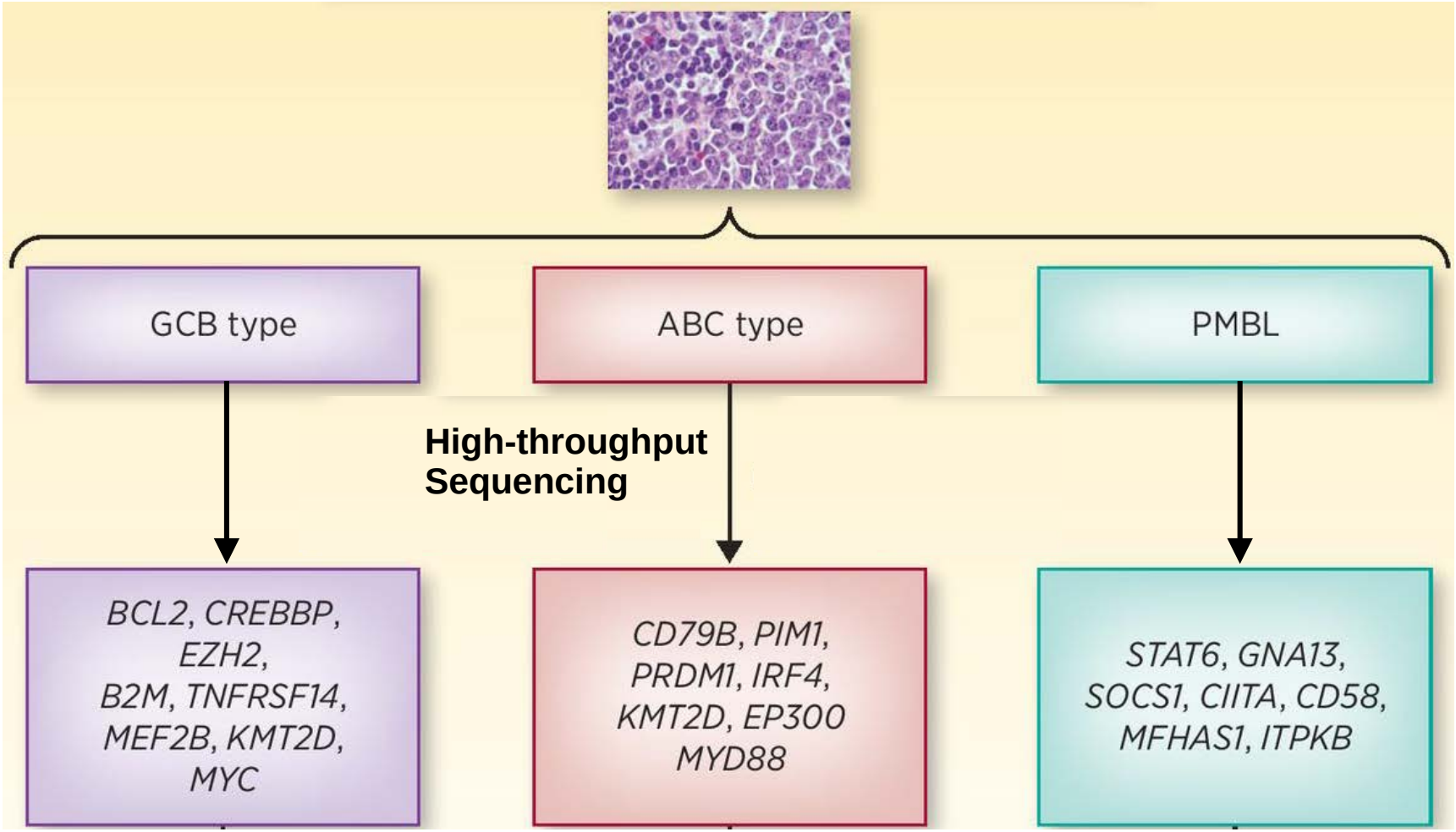
MYC IHC is negative in 10-26% of cases with *MYC* rearrangement

- 26% (9/34) of DLBCL. Johnson *et al*. JCO 2012
- 19% (6/32) of DLBCL. Wang *et al*. AJSP 2015
- 12% (5/41) of HGBL [*50% MYC IHC cutoff*] Kluk *et al*. AJCP 2016
- 22% (9/38) of double/ triple hit lymphoma. Moore *et al*. AJSP 2017
- 10% (6/58) of HGBL. Raess *et al*. Leuk Lymphoma 2017

PROPOSED SCREENING STRATEGIES TO SUBCLASSIFY HGBL

WHOLE EXOME SEQUENCING FOR LYMPHOMA?

Contribution of High-Throughput Sequencing Technologies to Classification of Large B Cell Lymphomas



Adapted from Lim *et al*. Clin Cancer Res. 2016 Jun 15;22(12):2829-31.

Contribution of High-Throughput Sequencing Technologies to Classification of Large B Cell Lymphomas

PATHWAY	GENE	COO SUBTYPE	
		GCB	ABC
B-cell receptor / NFκB pathway	*MYD88* ★		20-30%
	CD79A/B ★		10-20%
	CARD11	10%	10%
Immunity	*TNFRSF14*	10%	
Apoptosis	*BCL2* ★	30-40%	10%
PI3K/AKT	*GNA13, FOXO1*	10%	
Epigenetic regulation	*MLL2*	20-30%	20-30%
	EZH2 ★	20%	
	MEF2B	10-20%	
	CREBBP ★	30-40%	15-20%

★ = actionable target

Adapted from Bohers *et al*. Leuk Lymph 2015 May;56(5):1213-22.

Summary

- The diagnostic approach to high grade B cell lymphomas requires integration of morphologic, phenotypic, and cytogenetic/FISH findings

- Performing FISH on all cases of large B cell lymphoma is the only strategy which will identify all high grade B cell lymphomas with MYC and BCL2 and/or BCL6 rearrangements
  - Alternate strategies miss cases which may warrant different treatment

- High-throughput sequencing technologies have identified characteristic and potentially actionable mutations in GCB, ABC, and PMBL subtypes

Thank you!

Panel Diagnosis

High grade B cell lymphoma with *MYC* and *BCL2* rearrangements