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

Todd Williams\textsuperscript{1}, Christopher Corless\textsuperscript{1,2}, Dita Gratzinger\textsuperscript{3}, Michael J. Cascio\textsuperscript{1}, Jennifer Dunlap\textsuperscript{1}, Philipp W. Raess\textsuperscript{1}

\textsuperscript{1} Department of Pathology, Oregon Health & Science University
\textsuperscript{2} Knight Cancer Institute, Oregon Health & Science University
\textsuperscript{3} Department of Pathology, Stanford University

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 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**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2</td>
<td>Positive</td>
<td>74% of nuclei with BCL2 rearrangement</td>
</tr>
<tr>
<td>MYC</td>
<td>Positive</td>
<td>85% of nuclei with MYC rearrangement</td>
</tr>
<tr>
<td>BCL6</td>
<td>Negative for rearrangement</td>
<td></td>
</tr>
</tbody>
</table>
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

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

Potential therapeutic targets
Early event mutations of follicular lymphoma
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

HGBL with MYC & BCL2 &/or BCL6 rearrangements

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

**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

All HGBL with translocations are identified.

Not available at all labs. Expensive.
FISH CASES WITH HIGH GRADE MORPHOLOGY

Morphology

Blastoid

BL

DLBCL/BL

Phenotype & cytogenetics

TdT+

TdT−, cyclin D1−

Diagnosis

B-LBL

HGBL, NOS

BL

HGBL, with MYC and BCL2 and/or BCL6R

DLBCL, NOS

Reduced Cost

Subjective = not reproducible
Will not identify subset of cases with standard DLBCL morphology

PROPOSED SCREENING STRATEGIES TO SUBCLASSIFY HGBL
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
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


High-throughput Sequencing

GCB type
- BCL2, CREBBP, EZH2, B2M, TNFRSF14, MEF2B, KMT2D, MYC

ABC type
- CD79B, PIM1, PRDM1, IRF4, KMT2D, EP300, MYD88

PMBL
- STAT6, GNA13, SOCS1, CIITA, CD58, MFHAS1, ITPKB

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

<table>
<thead>
<tr>
<th>PATHWAY</th>
<th>GENE</th>
<th>COO SUBTYPE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GCB</td>
<td>ABC</td>
<td></td>
</tr>
<tr>
<td>B-cell receptor /</td>
<td>MYD88☆</td>
<td></td>
<td>20-30%</td>
<td></td>
</tr>
<tr>
<td>NFκB pathway</td>
<td>CD79A/B☆</td>
<td></td>
<td>10-20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CARD11</td>
<td>10%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Immunity</td>
<td>TNFRSF14</td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>BCL2☆</td>
<td>30-40%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>PI3K/AKT</td>
<td>GNA13, FOXO1</td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MLL2</td>
<td>20-30%</td>
<td>20-30%</td>
<td></td>
</tr>
<tr>
<td>Epigenetic</td>
<td>EZH2☆</td>
<td>20%</td>
<td></td>
<td>15-20%</td>
</tr>
<tr>
<td>regulation</td>
<td>MEF2B</td>
<td>10-20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CREBBP☆</td>
<td>30-40%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

☆ = 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