Genomics in diffuse large B cell lymphoma (DLBCL)—not as useful as we thought…. OR IS IT?

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April 22, 2017
Duke Controversies in Hematologic Malignancies
Omni Grove Park Inn
Asheville NC
Indeed, one can be nihilistic…

• No improvements made to rituximab-CHOP in 20 years
• Heterogeneity denies unified molecular targets
• 100s of clinical trial failures

or you can fight back.

• Diverse DLBCL subtypes known at molecular level
• Molecular features of DLBCL dictate tumor behavior
• Dozens of "actionable" genomic lesions in DLBCL
• Genomics is key to patient treatment at all points
Refractoriness and relapses: the fundamental issue in DLBCL

- 15% to 25% are refractory to any chemotherapy
- 5% PR patients
- 20% to 30% relapses
- Genomic background is key: Identify and overcome poor outcomes among ALL DLBCL

- 50% to 60% are already cured with previous chemotherapy (R-CHOP)
- We need randomized studies on these selected groups of patients
- We will never improve those cure patients

RCHOP-treated DLBCL patients

% of patients
Guidelines for molecular stratification of DLBCL

**DIAGNOSIS**

**ESSENTIAL:**
- Hematopathology review of all slides with at least one paraffin block representative of the tumor. Rebiopsy if consult material is nondiagnostic.
- An FNA or core needle biopsy alone is not generally suitable for the initial diagnosis of lymphoma. In certain circumstances, when a lymph node is not easily accessible for excisional or incisional biopsy, a combination of core biopsy and FNA biopsies in conjunction with appropriate ancillary techniques for the differential diagnosis (immunohistochemistry, flow cytometry, PCR for IgH and TCR gene rearrangements, and FISH for major translocations) may be sufficient for diagnosis.

- Adequate immunophenotyping to establish diagnosis and GCB versus non-GCB origin
  - IHC panel: CD20, CD3, CD5, CD10, CD45, BCL2, BCL6, Ki-67, IRF4/MUM1, MYC with or without
  - Cell surface marker analysis by flow cytometry: kappa/lambda, CD45, CD3, CD5, CD19, CD10, CD20

**USEFUL UNDER CERTAIN CIRCUMSTANCES:**
- Additional immunohistochemical studies to establish lymphoma subtype
  - IHC panel: Cyclin D1, kappa/lambda, CD30, CD138, Epstein-Barr virus in situ hybridization (EBER-ISH), ALK, HHV8, SOX11
- Karyotype or FISH: MYC, BCL2, BCL6 rearrangements
Fundamental #1: Cell of origin derivation

Lymph2Cx derivation of DLBCL COO and survival

# Fundamental #2: Frequent cytogenetic/mutations in DLBCL

<table>
<thead>
<tr>
<th>Genetic Feature</th>
<th>ABC DLBCL</th>
<th>GCB DLBCL</th>
<th>PMBL</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2 translocation t(14;18)</td>
<td>0%</td>
<td>40%–50%</td>
<td>20%</td>
<td>Alternatively translocated to light chain loci 5–10% of time</td>
</tr>
<tr>
<td>BCL2 amplification</td>
<td>34%</td>
<td>10%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>BCL6 translocation t(3;V) (q27;V)</td>
<td>25%</td>
<td>10%</td>
<td>20%</td>
<td>Variable translocation partners (14q32, 2p11, 22q11, 4p11, 6p21, 11q23)</td>
</tr>
<tr>
<td>3q amplification</td>
<td>25%</td>
<td>0%</td>
<td>&lt;5%</td>
<td></td>
</tr>
<tr>
<td>9q24 amplification</td>
<td>5%</td>
<td>0%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>PRDM1/PRDM1 deletion/mutation</td>
<td>25%</td>
<td>0%</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>REL amplification</td>
<td>0%</td>
<td>15%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Recurrent mutation</td>
<td>CARD11, MYD88, CD79B</td>
<td>EZH2, GNA13, BCL2</td>
<td>PTPN1, SOCS1, STAT6</td>
<td></td>
</tr>
<tr>
<td>MYC rearrangement t(8;14) (q24;q32)</td>
<td>5%–10% of all DLBCLs</td>
<td>25%–30% occur with t(14;18)(q32;q21)</td>
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</tr>
</tbody>
</table>

ABC, Activated B cell-like; DLCBL, diffuse large B-cell lymphoma; GCB, germinal center B cell-like; PMBL, primary mediastinal B-cell lymphoma

Gene mutations in DLBCL: the “long tail”

- Hundreds of recurrent genomic lesions known now in DLBCL
- Survival and “actionability” now can be paired to mutations

Dave et. al., Hematologic Malignancies Research Consortium, ASH 2016
How do we use this information to:
1) stratify prognosis and 2) improve outcomes for patients?
Importance of MYC in DLBCL

- MYC has many known oncogenic functions
- Frequently expressed/rearranged in DLBCL
- Collaborates with other genes (BCL2)

<table>
<thead>
<tr>
<th>Genetic feature</th>
<th>DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC translocation</td>
<td>5%–15%</td>
</tr>
<tr>
<td>MYC-IG translocations</td>
<td>IG and non-IG</td>
</tr>
<tr>
<td>MYC overexpression</td>
<td>Variable</td>
</tr>
<tr>
<td>BCL2/BCL6 translocation in addition to MYC</td>
<td>50%–70%</td>
</tr>
<tr>
<td>Complex karyotype</td>
<td>Yes</td>
</tr>
<tr>
<td>BCL2 protein expression</td>
<td>Variable</td>
</tr>
<tr>
<td>ID3/TCF3 mutations</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Nature Reviews Cancer 2008, 8, 976-990.
MYC, BCL2 and BCL6 in DLBCL

• **Double/triple hit** lymphomas:
  • 10-15% of DLBCLs have MYC/Ig rearrangements
  • Majority of MYC/IgH rearrangements occur with rearrangement of BCL2 and/or BCL6
  • CCND1, BCL3, other genes may be involved
  • Frequently germinal center (GCB) phenotype/CD10+ (~90% of cases)

• **Double/triple expressor** lymphomas:
  • Homogenetic rearrangement (at least 30% of cases)
  • Have evidence of protein expression (IHC) of MYC/BCL2/BCL6 in tumor cells
  • Activated B cell (ABC) DLBCL phenotype more common

Figure from *Diagnostic Pathology* 2017; **12**:3.
MYC rearrangement is associated with a poor outcome with RCHOP treated DLBCL

- UK series, 300 newly diagnosed DLBCLs
- Treated with rituximab-CHOP, observed
- Integrated IHC/FISH data analyzed
- Additional reports from other centers similar

Improving double hit lymphoma induction and consolidation strategies

- Multicenter series 311 DHLs
- Retrospective outcomes
- SCT in first CR:
  - Total = 39
  - HDT/autoHSCT = 28
  - Allo HSCT = 11

* p<0.05 for CR rate by Fisher's exact test, two-tailed.

Double/triple protein expressors may benefit from intense consolidation strategies

S9704 consort diagram

MYC+ IHC

Auto HSCT

No transplant

Point #1: Evaluate/act on MYC status in all DLBCL up front

- MYC rearrangements associated with unacceptable survival in RCHOP
- Various algorithms used in laboratory to evaluate expression/rearrangement
- Potential algorithms (including clinical trial):
  - DHL: REPOCH/R-HyperCVAD with consideration of allogeneic HSCT
  - Double/triple expressor: RCHOP, consider CNS prophylaxis and autologous HSCT
Novel agents for ABC DLBCL: lenalidomide and ibrutinib
Lenalidomide in relapsed/refractory DLBCL

- 102 relapsed/refractory DLBCLs
- Lenalidomide versus IC
- Gene expression and correlatives
Optimizing frontline treatment of ABC DLBCL with lenalidomide

Phase II trial lenalidomide/rituximab-CHOP (R²-CHOP)

<table>
<thead>
<tr>
<th>Subtype, treatment</th>
<th>PFS, % (95% CI)</th>
<th>OS, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 months</td>
<td>24 months</td>
</tr>
<tr>
<td>GCB:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– R-CHOP</td>
<td>73 (62–85)</td>
<td>64 (53–78)</td>
</tr>
<tr>
<td>– R²-CHOP</td>
<td>64 (49–84)</td>
<td>59 (44–80)</td>
</tr>
<tr>
<td>Non-GCB:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– R²-CHOP</td>
<td>72 (55–94)</td>
<td>60 (41–87)</td>
</tr>
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- 2 Phase II studies have evaluated lenalidomide with RCHOP induction
- Addition of lenalidomide to frontline RCHOP tolerated (15 mg d1-14)
- Poor PFS/OS in ABC DLBCL phenotype appear negated

ROBUST Phase III study design (NCT02285062)
Point #2: Assay and address cell of origin at diagnosis/relapse

- 2 Multi-center RCTs currently evaluating lenalidomide in combination with RCHOP induction in ABC DLBCL
- Lenalidomide no better than other therapies in GCB DLBCL
- COO may be helpful in picking salvage therapy (R-DHAP in GCB DLBCL)
Multiplexed next generation sequencing panels for precision cancer therapy

- Information on status of hundreds of mutations/copy number variation/rearrangements
- Can provide additional information in DLBCL beyond MYC/COO
- “Basket trials” for alterations is potential benefit
- Consider value of NGS to tell you what NOT to do…
Targeting B cell receptor signaling in DLBCL

- Phase II multicenter evaluated ibrutinib in DLBCL
- 70 patients with R/R DLBCL
- Ibrutinib 560 mg daily
- Targeted gene profiling
  - CARD11, MYD88, CD79A/B, TNFAIP3,
Molecular subgroups and response to BCR inhibition in DLBCL

Presumed resistance mutations

Point 3: DLBCL precision medicine will require genomics to improve outcomes

- Clinical high throughput genomics use will become more prevalent in hematologic malignancies (m7FLIPI, etc.)
- Vast genomic heterogeneity dictates we go beyond current assessment to provide best care in relapsed/refractory disease
- Off label “basket” targeting of alterations possible in DLBCL

- Tools are at our fingertips!
The future: pathways and novel targets in DLBCL

<table>
<thead>
<tr>
<th>Affected gene</th>
<th>Pathway</th>
<th>Molecular subtype</th>
<th>Therapeutic agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYK</td>
<td>BCR/NFκB</td>
<td>ABC</td>
<td>SYK inhibitors, e.g. fostamatinib</td>
</tr>
<tr>
<td>BTK</td>
<td>BCR/NFκB</td>
<td>ABC</td>
<td>BTK inhibitors, e.g. dirafitinib, ACP-196, ONO-4655, CC-292</td>
</tr>
<tr>
<td>PKCβ</td>
<td>BCR/NFκB</td>
<td>ABC</td>
<td>PKC inhibitors, e.g. enastaurin, somistrain</td>
</tr>
<tr>
<td>IKK</td>
<td>BCR/NFκB</td>
<td>ABC</td>
<td>IKK inhibitors, e.g. PS-1145, MLX015</td>
</tr>
<tr>
<td>NFκB</td>
<td>BCR/NFκB</td>
<td>ABC</td>
<td>proteasome inhibitors, e.g. bortezomib, carfilzomib</td>
</tr>
<tr>
<td>SP1β</td>
<td>Microenvironment/NF</td>
<td>ABC</td>
<td>Immune modulators, e.g. lenalidomide</td>
</tr>
<tr>
<td>IRF4</td>
<td>Microenvironment/NF</td>
<td>ABC</td>
<td>Immune modulators, e.g. lenalidomide</td>
</tr>
<tr>
<td>MYD88</td>
<td>Toll-like receptor</td>
<td>ABC</td>
<td>IRAK inhibitors</td>
</tr>
<tr>
<td>IRAK</td>
<td>Toll-like receptor</td>
<td>ABC</td>
<td>IRAK inhibitors</td>
</tr>
<tr>
<td>MALT1</td>
<td>Toll-like receptor/CM</td>
<td>ABC</td>
<td>MALT1 inhibitors, e.g. MI-2</td>
</tr>
<tr>
<td>JAK2/3</td>
<td>JAK/STAT</td>
<td>ABC</td>
<td>JAK inhibitors, e.g. pacritinib, ruxolitinib, lestaurtinib</td>
</tr>
<tr>
<td>STAT2/6</td>
<td>JAK/STAT</td>
<td>ABC</td>
<td>STAT inhibitors</td>
</tr>
<tr>
<td>PI3K</td>
<td>PIK3AKT/mTOR</td>
<td>GCB</td>
<td>PIK3 inhibitors, e.g. idelalisib, buparlisib, rigosertib</td>
</tr>
<tr>
<td>AKT</td>
<td>PIK3AKT/mTOR/DLBCL**</td>
<td>AKT inhibitors, e.g. MK2206, perifosine</td>
<td></td>
</tr>
<tr>
<td>mTOR</td>
<td>PIK3AKT/mTOR/DLBCL**</td>
<td>mTOR inhibitors, e.g. temsirolimus, everolimus, ridaforolimus, sirolimus</td>
<td></td>
</tr>
<tr>
<td>ERK</td>
<td>PIK3AKT/mTOR/DLBCL**</td>
<td>ERK inhibitors, e.g. sorafenib</td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td>Apoptotic signaling</td>
<td>GCB, ABC</td>
<td>BET-bromodomain inhibitors</td>
</tr>
<tr>
<td>BCL2</td>
<td>Apoptotic signaling</td>
<td>GCB, ABC</td>
<td>BCL2 inhibitors, e.g. GDC-199 (venetoclax)</td>
</tr>
<tr>
<td>BCL6</td>
<td>Apoptotic signaling</td>
<td>GCB, ABC</td>
<td>BCL6 inhibitors, e.g. 79-6</td>
</tr>
<tr>
<td>CREBBP</td>
<td>Histone modification</td>
<td>GCB, ABC</td>
<td>HDAC inhibitors, e.g. vorinostat, panobinostat, entinostat, mocetinostat</td>
</tr>
<tr>
<td>EP300</td>
<td>Histone modification</td>
<td>GCB, ABC</td>
<td>HDAC inhibitors, e.g. vorinostat, panobinostat, entinostat, mocetinostat</td>
</tr>
<tr>
<td>MLL2</td>
<td>Histone modification</td>
<td>GCB, ABC</td>
<td>HDAC inhibitors, e.g. vorinostat, panobinostat, entinostat, mocetinostat</td>
</tr>
<tr>
<td>EZH2</td>
<td>Histone modification</td>
<td>GCB, ABC</td>
<td>EZH2 inhibitors, e.g. non-selective EZH2 inhibitor (EZH-Nep), GSK126, GSK148</td>
</tr>
</tbody>
</table>

Rational approach to genomics in DLBCL

- Assess IHC COO algorithm and MYC/BCL2/BCL6 at diagnosis
- Strongly consider MYC/BCL2/BCL6 FISH in MYC IHC+ cases
- Screen up front for ABC DLBCL patients for up front studies
- Discuss transplant options with DHL and high risk MYC protein expressors
- Consider next generation sequencing for multiple relapsed/refractory DLBCL after multiple rounds of therapy
Thoughts? Questions? Complaints?