Diffuse Large B Cell Lymphoma: Biomarkers for Precision Healthcare

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ACCME/Disclosure

Dr. Rimsza has nothing to Disclose

Educational Objective

• Describe the clinical significance, biology, and methodologies for measuring DLBCL cell-of-origin, oncogene, and immune/microenvironment biomarkers in order to provide precision healthcare for patients.

Outline of presentation

• Precision Medicine
• Surface markers
• DLBCL “Cell of Origin”
• Mutations
• Oncogenes
• Immune markers & microenvironment
**Precision Medicine: The Pathologists' Roles**

- **Diagnosis & Immunophenotype**
- **Microarray & Molecular techniques**
- **Therapeutic Targets**

**Precision Medicine**

- Personalized Medicine/Precision Health/Individualized Medicine
- Customized medical decisions tailored to individual patients.
- Includes diagnostic testing for selecting optimal therapies based on features of the patient or tumor.

- Precision Medicine Initiative announced by President Obama in State of the Union Address Jan 2015
  - Called for $215 million of support in fiscal year 2016
  - $130 million allocated to NIH to build a national, large-scale research participant group
  - $70 million allocated to the National Cancer Institute for cancer genomics

**First Biomarkers of Precision Medicine: B Cell Surface Antigens**

- Targetable with monoclonal antibodies
- Started out with “cold” antibodies, now linked to various drugs and radioactive molecules
- Flow cytometry or Immunohistochemistry

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Method</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>IHC, Flow</td>
<td>1st-3rd generations: rituximab, ofatumumab, obinutuzumab, veltuzumab, ocrelimab, ocaratuzumab</td>
</tr>
<tr>
<td>CD19</td>
<td>Flow</td>
<td>Chimeric antigen receptor T cells (CAR) Bispecific T-cell engager (BITE)-blinatumomab DM4AR3419 linked to maytansinoid derivative</td>
</tr>
<tr>
<td>CD79b</td>
<td>IHC, flow</td>
<td>polatumumab linked to MMAE</td>
</tr>
<tr>
<td>CD22</td>
<td>IHC, flow</td>
<td>pinatumumab linked to MMAE, Combotox linked to ricin</td>
</tr>
<tr>
<td>CD30 (25% of DLBCL)</td>
<td>IHC</td>
<td>SGN-30 conjugated to MMAE-brentuximab vedotin</td>
</tr>
</tbody>
</table>

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References #1-5
Gene Expression Profiling Reveals a New Understanding of DLBCL Biology

Compliments of Lou Staudt

Molecular Cell-of-Origin Model

~15% of DLCBL

A Prognostic Biomarker in R-CHOP Treated Patients

Immunohistochemistry partially reproduces the GEP cell-of-origin classification

83% agreement with GEP, Binary classification

Reference #10
Muris, Choi, “Tally” IHC methods

Muris method: BCL2, CD10, MUM1

Choi method: Added GCET1 & FOXP1 to Hans

Tally method: Choi antibodies, but not algorithmic, additive score, LM02 as tie breaker

<table>
<thead>
<tr>
<th>Stain</th>
<th>ABC Score</th>
<th>GCB Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>MUM1</td>
<td>GCB &gt; ABC</td>
</tr>
<tr>
<td>GCET1</td>
<td>FOXP1</td>
<td>ABC &gt; GCB</td>
</tr>
</tbody>
</table>

If GCB Score = ABC score:
- LM02 > 30% → GCB
- LM02 < 30% → ABC

Numerous Molecular Methods

- RT-PCR for BCL6 and LM02
  - Reference #14
- Gene Expression Profiling
  - Reference #9
  - Reference #15
  - Reference #16
- DNA methylation profiling
  - Reference #17
- MicroRNA profiling
  - Reference #18
- Sequencing
  - Reference #19
- ArrayCGH
  - Reference #20

The Lymph2Cx Assay
Nanostring Technology

Samples from Patients with de novo DLBCL (n = 62)

The IHC algorithms - biopsies with definitive COO

Samples from Patients with de novo DLBCL (n = 62)

- Activated B-cell-like DLBCL/Non-GCB
- Germinal-Center B-cell-like DLBCL
- Unclassified DLBCL

Reference #21
Lymph2Cx: Reproducible results between 2 different cuts at 2 different sites

98% for biopsies with “definitive COO” 95% for all biopsies

Lymph2Cx: Survival Differences

Chosen by Celgene to be FDA-cleared companion diagnostic for Revlimid in ROBUST international Phase III trial of R2-CHOP based on early results showing preferential effect in Non-GCB patients

Reference #21
Reference #22

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Genes frequently mutated in DLBCL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Effect of Mutation</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARD11</td>
<td>Activating</td>
<td>B-cell receptor signaling</td>
</tr>
<tr>
<td>CD79A/8B</td>
<td>Activating</td>
<td></td>
</tr>
<tr>
<td>TRAF2</td>
<td>Activating</td>
<td></td>
</tr>
<tr>
<td>TRAF5</td>
<td>Activating</td>
<td></td>
</tr>
<tr>
<td>MYD88</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>TNFAIP3</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>CSTM</td>
<td>Inactivating</td>
<td>Antigen presentation</td>
</tr>
<tr>
<td>BCL6</td>
<td>Activating</td>
<td></td>
</tr>
<tr>
<td>ETP1</td>
<td>Activating</td>
<td></td>
</tr>
<tr>
<td>FOXO1</td>
<td>Activating</td>
<td></td>
</tr>
<tr>
<td>GNA13</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>POLR2F</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>Inactivating</td>
<td>Cell cycle/Apoptosis</td>
</tr>
<tr>
<td>BTG1/2</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>CDC20</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>SGK1</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>RCL10</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>FAS</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>RNF5</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>TNFRSF14</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>EZH2</td>
<td>Activating</td>
<td>Chromatin regulation/DNA</td>
</tr>
<tr>
<td>HDAC7</td>
<td>Activating</td>
<td>methylation</td>
</tr>
<tr>
<td>ETP06</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>CREBBP</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>ML12</td>
<td>Inactivating</td>
<td></td>
</tr>
<tr>
<td>MEF18</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>
ABC-DLBCL: mutations in B-cell receptor signaling

**Mutations in:**
- CARD11
- CD79A/B
- MYD88 (gain of function)
- A20

**Lead to activation of:**
- NF-kB (MYD88 or CARD11)
- JAK kinase activation of STAT3 (MYD88)
- PI3K/AKT/mTOR pathway

Reference #23
Reference #24
Reference #25
Reference #26

ABC-DLBCL subtype may respond to agents targeting BCR signaling

**Agents**
- SYK inhibitor
- BTK inhibitor
- PI3K inhibitor
- mTOR inhibitors
- NF-kB pathway inhibitors
  - CARD11
  - Proteosome
  - IkappaB kinase
  - PKC beta

Reference #22
Reference #26

GCB-DLBCL subtype may respond to agents targeting epigenetic-related genes

**Agents**
- Demethylating agents
- Histone deacetylase inhibitors
- EZH2 inhibitors

Reference #27
Reference #28
Reference #29

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MYC abnormalities in DLBCL

- Translocations with Ig or non-Ig genes
  - 10% DLBCL, 30% immunoblastic DLBCL, 30-50% BCLU, IFL
  - As a sole abnormality, unclear prognostic significance
  - Often part of a complex karyotype (compared to BL)
  - More common in GCB

- MYC amplification by FISH
  - 7% of all DLBCL, 22% of GCB
  - Likely poor prognostic implications

- Increased MYC mRNA associated with poor risk
  - Affect NFkappaB and anti-apoptosis (compared to proliferation in BL)

- Altered microRNA

- IHC, until recently, hindered by lack of good antibodies

References #30-37

BCL2 Abnormalities in DLBCL

- Chromosomal abnormalities
  - Translocation (14;18) ~15% overall, mainly GCB
  - Amplification by FISH, ~20% overall, mainly ABC

- Up-regulated gene expression
  - mRNA levels increased in 33%
    - With and without t(14;18) translocations
    - Higher levels on average in ABC subtype

- Up-regulated protein expression
  - IHC (+) in ~40% of cases
    - 62% in ABC-DLBCL
    - 30% in GCB-DLBCL

References #38, #39, #40

Rabbit mAB for MYC IHC

Tonsil: basal epithelial layer(+), lymphatics(-), scattered interfollicular cells (+)

DLBCL: 40-50% of cases are (+); defined as >40% of lymphoma cells are positive

Advantages of Rabbit antibodies:
- More diverse epitope recognition
- Better immune response to small epitope and thus higher specificity
- Higher affinity

References #38, #39

Double Protein/Double Expression MYC(+) and BCL2(+) cases of DLBCL have the worst outcome

Cumulative survival

- MYC^PROT(+)/BCL2(-)
- MYC^PROT(-)
- MYC^PROT(+)/BCL2(+)

Reference #38
False-negative BCL2 staining can occur with mouse vs. rabbit mAB—possibly related to mutations or phosphorylation of BCL2

![Image of staining results]

124 disagreement rates:
28% of cases (26/94) with E17
46% of cases (43/94) with SP66

References #42

BCL6 abnormalities in DLBCL

- 21% rearrangements at 3q27
  - Translocations with Ig and >20 non-Ig genes
  - Intra-chromosomal abnormalities including interstitial deletions and inversions
- If Double Hit, likely aggressive (conflicting data)
  - more frequently extranodal
  - more frequently GCB
  - usually DLBCL, BL, or HGLUC

References #43 #44

Double and Triple “Hit” vs. Double Protein/Double Expression

- MYC-BCL2 > Triple Hit > MYC-BCL6
- Not just translocations, amplifications and other abnormalities are likely also important
- “Double Protein/Expression” more common than “Double Hit”
- Cannot reliably use Ki67 to screen for MYC abnormalities since it will be <90% in some cases

References #34

“A Clinician’s Perspective 2015”

- All patients with DLBCL should be tested for MYC and BCL2 by IHC, as their presence defines protein co-expressing lymphoma.
- Ideally, all patients with DLBCL would have FISH testing for MYC rearrangements.
- A compromise would be to use MYC-IHC to screen patients for further testing with FISH. Although the cut-off is uncertain
- Patients found to have MYC rearrangements should have subsequent FISH for BCL2 and BCL6 rearrangements.
- All patients with BCLU, immunoblastic DLBCL and transformed indolent lymphomas should have FISH for MYC rearrangements.
Both COO and MYC/BCL2 are prognostically important

- “Diffuse large B-cell lymphoma Cell-of-origin Classification using the Lymph2CX Assay in the Context of BCL2 and MYC Expression Status”

- “Prognostic Significance of Diffuse Large B Cell Lymphoma Cell of Origin Determined by Digital Gene Expression in Formalin Fixed Paraffin-Embedded Tissue Biopsies”

MYC protein, BCL2 protein, Activated B cell subtype are more frequent in older patients

Where to draw the treatment line?

New treatments for DH and DP, possibly DA-EPOCH-R

Therapeutic Targeting of MYC and BCL2

- **MYC**
  - Disruption of Myc/Max dimers
  - BET Bromodomain Inhibition, JQ1
  - G-quadruplex inhibitors of DNA secondary structure

- **BCL2**
  - BH3 mimetics
    - ABT199, Obatodax, AT101
  - Antisense
    - Oblimersen
  - G-quadruplex inhibitors of DNA secondary structure
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B Lymphomas Interact with Microenvironment by Exploiting Normal B-T cell Communication

- Recruitment (Hodgkin Lymphoma)
- Re-education (follicular lymphoma)
- Effacement (Burkitt lymphoma)

Immune & Microenvironment

<table>
<thead>
<tr>
<th>Marker</th>
<th>Method</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1/CD279</td>
<td>IHC for NSCLC</td>
<td>nivolumab and pembrolizumab</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>ipilimumab</td>
<td></td>
</tr>
<tr>
<td>CD137, TNFRSF9</td>
<td>IHC</td>
<td>Agonistic antibodies in mice</td>
</tr>
<tr>
<td>CD47</td>
<td>IHC</td>
<td>Interrupts SIRP1 inhibition of phagocytosis</td>
</tr>
<tr>
<td>Major Histocompatibility</td>
<td>IHC, flow</td>
<td>Histone deacetylase inhibitors</td>
</tr>
<tr>
<td>Class II, HLA-DR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Minimal Residual Disease: Precision Health Decisions Following Treatment

- Clonotypic IgH by high throughput sequencing
  - Pre-Tx: 82% in cell-free plasma vs 71% in leukocytes
  - Pre-relapse: 100% in plasma vs 30% in cells
- Plasma IgH vs. PET/CT
  - Specificity: 100% vs 56% (p<0.0001)
  - Sensitivity: 31% vs 55% (p=0.4)
  - Plasma positivity often preceded PET/CT detection

- Tumor specific mutations
Practical Suggestions for DLBCL Biomarkers

- Immunophenotype, including pan-B cell markers CD20
- Cell of Origin
  - IHC: CD10, BCL6, MUM1, other
  - Molecular, Lymph2Cx or other
- MYC, BCL2, BCL6; IHC and FISH
- Other: per local practice, clinical trials

Traditional Chemotherapy vs. Precision Medicine

CHALLENGE TO PATHOLOGY: Reproducible techniques and standardized reporting.

Thank you & Questions