Diffuse Large B Cell Lymphoma: Biomarkers for Precision Healthcare

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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.
Precision Medicine: The Pathologists’ Roles

- Diagnosis & Immunophenotype
- Microarray & Molecular techniques
- Therapeutic Targets
Outline of presentation

- Precision Medicine
- Surface markers
- DLBCL “Cell of Origin”
- Mutations
- Oncogenes
- Immune markers & microenvironment
Precision Medicine

- Personalized Medicine/Precision Health/Individualized Medicine
- Customized medical decisions tailored to individual patients.
- Includes diagnostic testing for selecting appropriate/optimal therapies based on the context of a patient’s, or tumor’s, genetics.

- Precision Medicine Initiative announced by President Obama in State of the Union Address Jan 20, 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 to lead efforts in 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>
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<tbody>
<tr>
<td>CD20</td>
<td>IHC, Flow</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; - 3&lt;sup&gt;rd&lt;/sup&gt; generations: rituximab, ofatumomab, obinutuzumab, veltuzumab, ocrelizumab, ocaratuzumab</td>
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<tr>
<td>CD19</td>
<td>Flow</td>
<td>Chimeric antigen receptor T cells (CAR) Bispecific T-cell engager (BiTE)-blinatumomab DM4SAR3419 linked to maytansinoid derivative</td>
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<td>CD79b</td>
<td>IHC, flow</td>
<td>polatuzumab linked to MMAE</td>
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<td>CD22</td>
<td>IHC, flow</td>
<td>pinatuzumab linked to MMAE</td>
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<tr>
<td>CD30</td>
<td>IHC</td>
<td>SGN-30 conjugated to MMAE-brentuximab vedotin</td>
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References #1-5
Outline of presentation

- Precision Medicine
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Molecular Cell-of-Origin Model: Gene expression signatures predict survival in patients treated with R-CHOP
Molecular Subtypes Correspond to Stage of Differentiation

GCB-DLBCL  ABC-DLBCL  PBL

↑NFkB & IRF4

Germinal Center  Activated B Cell

Plasma Cell

B cell differentiation: Loss of B-cell markers; acquisition of plasma cell markers
Molecular Types are 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>Tally core: 1 point for each (+) stain:</th>
<th></th>
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<tbody>
<tr>
<td>GCB</td>
<td>ABC</td>
</tr>
<tr>
<td>CD10</td>
<td>MUM1</td>
</tr>
<tr>
<td>GCET1</td>
<td>FOXP1</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
Nanosting Technology

Samples from Patients with *de novo* DLBCL (n = 67)

Activated B-cell-like DLBCL/Non-GCB
Germinal-Center B-cell-like DLBCL
Unclassified DLBCL
The IHC algorithms - biopsies with definitive COO

Samples from Patients with de novo DLBCL (n = 67)

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

Reference #21
Lymph2Cx: Reproducible Results Between 2 Sites

- 98% for biopsies with "definitive COO"
- 95% for all biopsies
Lymph2Cx: Survival Differences Retained

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
Outline of presentation

• Precision Medicine
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<table>
<thead>
<tr>
<th>Gene</th>
<th>Effect of Mutation</th>
<th>Pathway</th>
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</thead>
<tbody>
<tr>
<td>CARD11</td>
<td>Activating</td>
<td>B-cell receptor signaling</td>
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<td>CD79A/B</td>
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<td>TRAF2</td>
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<td>TRAF5</td>
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<td>MYD88</td>
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<td>CD58</td>
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<tr>
<td>B2M</td>
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<td>BCL6</td>
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<td>B-cell differentiation</td>
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<tr>
<td>ETS1</td>
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<tr>
<td>FOXO1</td>
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<td>IRF4</td>
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<td>GNA13</td>
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<td>TP53</td>
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<td>Cell cycle/Apoptosis</td>
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<tr>
<td>BTG1/2</td>
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<tr>
<td>CCND3</td>
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<td>SGK1</td>
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<td>FAS</td>
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<td>TNFRSF14</td>
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<td>EZH2</td>
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<td>Chromatin regulation/DNA methylation</td>
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<td>EP300</td>
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<td>CREBBP</td>
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<td>MLL2</td>
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<tr>
<td>MEF2B</td>
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</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

- **Tonic BCR signaling**
  - SYK inhibitor
  - BTK inhibitor
  - PI3K inhibitor
  - mTOR inhibitors
  - NF-κB pathway inhibitors
    - CARD11
    - Proteosome
    - IkappaB kinase
    - PKC beta

- **Agents**
  - fostamatinib
  - Ibrutinib
  - CA-101
  - rapalogs, small molecule
  - lenalidomide*
    - fostamatinib
    - bortezomib, carfilzomib
    - PS1145
    - enzastaurin

Reference #22
Reference #26
GCB-DLBCL subtype may respond to agents targeting epigenetic-related genes

- **Histone methyltransferase mutations**
  - MLL2 inactivated
    - disrupts H3K4 methylation that “marks” activated genes
  - MEF2b inactivated
    - cooperates with CREBBP and EP300 in acetylating histones
  - EZH2 activated
    - methylates H3K27 to interfere with histone acetylation

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

References:
- Reference #27
- Reference #28
- Reference #29
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MYC abnormalities in DLBCL

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

- MYC amplification by FISH
  - 7% of all DLBCL, 22% of GCB subtype of DLBCL
  - 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
Rabbit mAB: MYC IHC for detection of Double Protein/Double Expression

Tonsil: basal epithelial layer (+), lymphatics (-), scattered inter-follicular 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

Reference #38
Reference #39
Reference #40
BCL2 Abnormalities in DLBCL

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

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

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

Reference #41
Reference #38
DP/DE MYC(+) and BCL2(+) cases of DLBCL have the worst outcome
False-negative BCL2 staining can occur with mouse vs. rabbit mAB—possibly related to mutations or phosphorylation of BCL2

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

Reference #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 DH, likely aggressive (conflicting data)
  - more frequently extranodal
  - more frequently GCB
  - usually DLBCL, BL, or HGLUC

References:
- #43
- #44
- #45
Double and Triple “Hit” (DH/TH) vs. Double Protein or Double Expressing (DP or DE)

• MYC-BCL2 > TH> MYC-BLC6

• Not just translocations, amplifications and other abnormalities are also important

• DP more common than DH

• Cannot use Ki67 to screen for MYC abnormalities
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 precise 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>
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<tbody>
<tr>
<td>PD1/CD279</td>
<td>IHC for NSCLC</td>
<td>nivolumab and pembrolizumab</td>
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<td>CTLA-4</td>
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<td>ipilimumumab</td>
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<td>CD137, TNFRSF9</td>
<td>IHC</td>
<td>Agonistic antibodies in mice</td>
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<tr>
<td>CD47</td>
<td>IHC</td>
<td>Interrupts SIRP1 inhibition of phagocytosis</td>
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<td>Major Histocompatibility Class II, HLA-DR</td>
<td>IHC, flow</td>
<td>Histone deacetylase inhibitors</td>
</tr>
</tbody>
</table>

References #50-54
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

Reference #55
Reference #56
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


27. Thieblemont C, Briere J, Mounier N, et al. The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in


