Molecular Pathogenesis of Diffuse Large B cell Lymphoma and advances in Targeted Therapy

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Talking points

✓ Diffuse Large B cell Lymphoma (DLBCL) - pathogenesis
  
  Lymphomagenesis
  Clinical heterogeneity
  Molecular classifications – COO and CCC
  Role of MYC - double-hit and double-expressor lymphomas

✓ DLBCL - treatment

  Improving on R-CHOP with targeted (and not so targeted) therapy

✓ PDE4 inhibitors for the treatment of mature B cell tumors

  Simultaneous targeting the tumor cell and its microenvironment
Learning objectives

✓ Understand the complex molecular basis for the clinical heterogeneity of DLBCL

✓ Be familiar with clinically available biological markers that inform prognosis and may guide treatment

✓ Be able to determine at diagnosis which DLBCL patients will likely experience treatment failure with R-CHOP

✓ Identify investigational treatment options that matches the tumor’s biology
DLBCL is the most frequent NHL

### Non-Hodgkin Lymphoma Subtypes

- Diffuse large B-cell lymphoma (31%)
- Follicular lymphoma (22%)
- Mantle cell lymphoma (6%)
- Composite lymphomas (13%)
- Peripheral T-cell lymphoma (6%)
- Other subtypes with a frequency < 2% (9%)

### Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

<table>
<thead>
<tr>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse large B-cell lymphoma (DLBCL), NOS</td>
</tr>
<tr>
<td>Germinal center B-cell type*</td>
</tr>
<tr>
<td>Activated B-cell type*</td>
</tr>
<tr>
<td>T-cell/histiocyte-rich large B-cell lymphoma</td>
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<tr>
<td>Primary DLBCL of the central nervous system (CNS)</td>
</tr>
<tr>
<td>Primary cutaneous DLBCL, leg type</td>
</tr>
<tr>
<td>EBV+ DLBCL, NOS*</td>
</tr>
<tr>
<td>EBV+ mucocutaneous ulcer*</td>
</tr>
<tr>
<td>DLBCL associated with chronic inflammation</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis</td>
</tr>
<tr>
<td>Primary mediastinal (thymic) large B-cell lymphoma</td>
</tr>
<tr>
<td>Intravascular large B-cell lymphoma</td>
</tr>
<tr>
<td>ALK+ large B-cell lymphoma</td>
</tr>
<tr>
<td>Plasmablastic lymphoma</td>
</tr>
<tr>
<td>Primary effusion lymphoma</td>
</tr>
<tr>
<td>HHV8+ DLBCL, NOS*</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>Burkitt-like lymphoma with 11q aberration*</td>
</tr>
<tr>
<td>High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements*</td>
</tr>
<tr>
<td>High-grade B-cell lymphoma, NOS*</td>
</tr>
<tr>
<td>B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma</td>
</tr>
</tbody>
</table>

WHO recognizes biologic features

B-cell physiology and cancer

V(D)J Recombination

Somatic Hypermutation

Class Switch Recombination

Immature B-cells

Naive B-cells

Ag

Centroblasts

Centrocytes

Dark zone

Light zone

MANTLE ZONE

GERMINAL CENTER

Apoptosis

Memory B-cells

B-CLL

Plasma cells

BONE MARROW

MANTLE ZONE

ALL

BL

FL

DLBCL

MM
DLBCL – clinical heterogeneity

40% of the patients will fail first-line treatment – R-CHOP
International Prognostic Index – IPI
Shipp et al, NEJM 1993

- Age greater than 60 years
- Stage III or IV disease
- Serum LDH elevated
- ECOG performance status ≥ 2, 3, or 4
- Extranodal site > 1

Low risk (0-1 points) - 5yr survival of 73%
L-I risk (2 points) - 5yr survival of 51%
H-I risk (3 points) - 5yr survival of 43%
High risk (4-5 points) - 5yr survival of 26%

- Sub-optimal precision
- Surrogate for biological variables
- Before Rituximab era
Revised IPI and NCCN-IPI

R-CHOP era


- Age > 60 years
- Stage - III or IV disease
- Serum LDH - elevated
- ECOG performance status ≥ 2, 3, or 4
- Extranodal site > 1

Very good (0) - 4-year PFS 94%
Good (1, 2) - 4-year PFS 79%
Poor (3, 4, 5) - 4-year PFS 55%

- Age - 40 to 60 (1), 60 to 75 (2), >75 (3)
- Stage - III or IV disease - 1
- Serum LDH - 1 to 3x (1), >3x (2)
- Performance status ≥ 2 - 1
- Extranodal disease - 1

Low (0-1) - 5-year PFS 96%
L-I (2-3) - 5-year PFS 77%
H-I (4-5) - 5-year PFS 56%
High (≥6) - 5-year PFS 38%

Surrogate for biological variables
Identify biologically discrete subsets of DLBCL – tailored treatment
DLBCL – molecular classification
gene expression profile / cell-of-origin

Hundreds of genes define the signatures limiting clinical implementation
- Lymph2Cx cell-of-origin assay: Nanostring technology FFPE
DLBCL – molecular classification

Clinically useful IHC-based classifier

![Diagram showing molecular classification of DLBCL types with CD10, bcl6, MUM1, and GCB/Non-GCB markers.](image)

Hans et al. Blood 2004

![Survival analysis graph showing survival rates for GCB-DLBCL and Non-GC-DLBCL with a p-value of 0.007.](image)
DLBCL – molecular classification

gene expression profile / consensus clustering

Nature Medicine 2001
Blood 2003
Blood 2005

SYK inhibition

Fatty acid oxidation (FAO) inhibition

BCR and Oxphos highly translational
Primary Mediastinal BCL
distinct pathogenesis

Similarity to Hodgkin Lymphoma

9p24 amplification – opportunity for targeted interventions
Chromosomal translocations

Typically involves the *IGH* locus

The net result is gain of function of the partner gene

It has prognostic value and influence treatment decisions
Double (or triple) hit DLBCL

MYC and BCL2 (or BCL6) translocation

~ 5% of DLBCL

Very poor prognosis – OS 8months R-CHOP

DHL are GCB - DLBCL
MYC/BCL2 protein co-expression in DLBCL


30% of DLBCL are MYC/BCL2 +

Significant adverse impact: 30% vs. 75% overall survival

Supersedes GCB/ABC classifier as predictor

Associated with ABC (66% MYC/BCL2+ are ABC-DLBLC)

Reproducibility still a problem – examiner and reagents
Double-expressor lymphoma (DEL) a discrete entity?

Diffuse large B-cell lymphoma, NOS

- Distinction of GCB vs ABC/non-GC type required with use of immunohistochemical algorithm acceptable, may affect therapy.
- Coexpression of MYC and BCL2 considered new prognostic marker (double-expressor lymphoma).
- Mutational landscape better understood but clinical impact remains to be determined.

Minimal Recommendations

- Test for chromosomal translocations - MYC, BCL2, BCL6
- Test for MYC and BCL2 expression using IHC
- Test for CD10, BCL6, MUM1 expression using IHC – Lymph2Cx
- Construct a IPI score for all DLBCL patients
- Use these data to make an informed decision on treatment
- Bank the tumor and non-tumoral tissue
**Talking points**

- **Diffuse Large B cell Lymphoma (DLBCL) - pathogenesis**
  - Lymphomagenesis
  - Clinical heterogeneity
  - Molecular classifications – COO and CCC
  - Role of MYC - double-hit and double-expressor lymphomas
  - Mutational landscape

- **DLBCL - treatment**
  - Improving on R-CHOP with targeted (and not so targeted) therapy

- **PDE4 inhibitors for the treatment of mature B cell tumors**
  - Co-targeting the tumor cell and its microenvironment
Diffuse Large B-Cell Lymphoma Treatment

Front line treatment: R-CHOP

R = Rituximab
C = Cyclophosphamide
H = Doxorubicin Hydrochloride (Hydroxydaunomycin)
O = Vincristine Sulfate (Oncovin)
P = Prednisone

Outside a clinical trial nearly all patients should receive R-CHOP

~ 60% of patients respond
Three seemingly indistinguishable DLBCL cases

Case 1 - Female, 65 years of age, enlarged cervical lymph node.
Excisional biopsy confirms DLBCL.
Stage – III
LDH = 200U/L, ECOG = 2, no extranodal site
IPI – high intermediate (IPI - 43% 5-year OS; R-IPI 55%; NCCN-IPI 56%)

Case 2 - Male, 59 years of age, enlarged axillary lymph node
Excisional biopsy confirms DLBCL.
Stage – III
LDH = 500U/L, ECOG = 3, no extranodal site
IPI – high intermediate (43% 5-year OS; R-IPI 55%; NCCN-IPI 56%)

Case 3 - Male, 55 years of age, enlarged cervical lymph node
Excisional biopsy confirms DLBCL.
Stage – III
LDH = 800U/L, ECOG = 4, BM +
IPI – high intermediate (43% 5-year OS; R-IPI 55%; NCCN-IPI 38%)

Ordered IHC for CD10, BCL6, MUM1, MYC and BCL2; karyotyping/FISH
Three seemingly indistinguishable DLBCL cases

Case 1

- CD10+, BCL6/MUM1- (GCB-DLBCL), BCL2/MYC negative IHC, no translocations
- R-CHOP (community or academic setting)
- Likely to be cured
Three seemingly indistinguishable DLBCL cases

Case 2

- CD10-, BCL6/MUM1+ (non-GCB), BCL2/MYC positive IHC, no translocation

- ABC, double-expressor lymphoma

There are currently no prospective data to guide the management of DEL

Enrolled in a clinical trial designed for ABC-DLBCL that uses biological agents

* R-CHOP vs DA-EPOCH-R negative (higher toxicity) - CALGB/Alliance Phase 3, ASH 2016
Case 2 – DLBCL – ABC, MYC/BCL2+

ROBUST trial: Phase 3, Randomized, Double-Blind, Multicenter Study

Revlimid + R-CHOP (R2-CHOP) vs. Placebo + R-CHOP
Untreated ABC DLBCL (Lymph2Cx - Nanostring technology).

R-CHOP + Ibrutinib: Phase 3, Randomized, Double-Blind, Multicenter Study

Ibrutinib + R-CHOP vs. Placebo + R-CHOP
Untreated Non-GCB DLBCL (IHC).

- Phase Ib – 33 treatment naïve pts, safe
  
  Lancet Oncol 2014; 15: 1019–26

- Phase I/II – 80 relapse/refractory pts
  
  Nature Medicine 2015
Three seemingly indistinguishable DLBCL cases

Case 3

- CD10+, BCL6/MUM1- (GCB), BCL2/MYC + IHC, IGH/MYC & IGH/BCL2 + (FISH)
- Double-hit lymphoma
- Unlikely to survive with R-CHOP

- Dose-intensive regimens - more advanced concept than rational targeting

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>Age &lt; 60</th>
<th>DH/TH at Transformation of low grade</th>
<th>DLBCL Morphology</th>
<th>ASCT in CR1</th>
<th>Achieved EFS12</th>
<th>Median OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-CHOP</td>
<td>33</td>
<td>36%</td>
<td>9%</td>
<td>59%</td>
<td>12%</td>
<td>31%</td>
<td>17.7</td>
</tr>
<tr>
<td>R-EPOCH</td>
<td>17</td>
<td>35%</td>
<td>29%</td>
<td>36%</td>
<td>17%</td>
<td>47%</td>
<td>13.5</td>
</tr>
<tr>
<td>R-CODOX-M/IVAC</td>
<td>15</td>
<td>93%</td>
<td>7%</td>
<td>22%</td>
<td>33%</td>
<td>69%</td>
<td>Not reached</td>
</tr>
<tr>
<td>R-HyperCVAD</td>
<td>6</td>
<td>50%</td>
<td>33%</td>
<td>0%</td>
<td>0%</td>
<td>33%</td>
<td>12.3</td>
</tr>
</tbody>
</table>

- Targeted: Phase 1/2 – BET inhibitor (INCB057643)
Primary Mediastinal BCL
distinct pathogenesis - targeted treatment

Role of checkpoint inhibitors

KEYNOTE-013 - Phase Ib trial of Pembrolizumab in R/R PMBCL

Interim report - 9 patients, 44% objective response
Primary Testicular Lymphoma
distinct pathogenesis – targeted treatment
Role of checkpoint inhibitors

Pilot, off-label - four patients R/R PTL and one PCNSL – 100% objective response (80% complete)

Multi-institutional phase 2 open-label, single-arm trial of nivolumab in R/R PCNSL and PTL (CA209-647)

Blood, 2016
Diffuse Large B-Cell Lymphoma
other actionable targets

• EZH2 inhibitor (tazemetostat) - Phase 1/2 - R/R DLBCL
  *fast track designation for DLBCL with mutant EZH2

• CAR-T-cells - CD19, κ or λ, and CD30
  Several trials open for R/R B cell lymphomas
  Combination with checkpoint inhibitors
Talking points

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  Lymphomagenesis
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  Role of MYC - double-hit and double-expressor lymphomas
  Mutational landscape

✓ DLBCL - treatment

  Improving on R-CHOP with targeted (and not so targeted) therapy

✓ PDE4 inhibitors for the treatment of mature B cell tumors

  Simultaneous targeting the tumor cell and its microenvironment
PDE4 inhibitors to treat DLBCL

PDE4B expressed in fatal DLBCL

PDE4 inhibitors are FDA-approved for inflammatory/auto-immune disorders
The cyclic-AMP/PDE4 signaling axis
PDE4 inhibition suppresses BCR-related kinases

**PI3K**
- Vehicle
- Roflumilast
- Idelalisib
- Combination

**SYK**
- Rational combined targeting of phosphodiesterase 4B and SYK in DLBCL

**AKT**
- **ABC-DLBCL**

**BTK**
- -
- +

**pBTK**
- -
- +

**BTK**
- -
- +

**PI(3,4,5)P3**
- 0
- 1
- 2
- 3
- 4

**Tumor Volume**
- Day 0
- Day 7
- Day 10
- Day 14

**PI3K Activity**
- V
- R
- Id
- R+Id

**Gene Set Enrichment Analysis Unveils the Mechanism for the Phosphodiesterase 4B Control of Glucocorticoid Response in B-cell Lymphoma**
- Sang-Woo Kim, Deepak Rai, Morgan R. McKellar, and Ricardo C.T. Aguilar

**Clinical Cancer Research**
- Cancer Therapy: Preclinical

**Blood, 2005**
- The phosphodiesterase PDE4B limits cAMP-associated PI3K/AKT-dependent apoptosis in diffuse large B-cell lymphoma
- Peter G. Smith, Fengshi Wang, Kathleen N. Wilkinson, Kerry J. Savage, Ulf Klein, Donna S. Neuberg, Gideon Sollag, Margaret A. Shipp, and Ricardo C. T. Aguilar

**Blood, 2009**
- Rational combined targeting of phosphodiesterase 4B and SYK in DLBCL
- Sang-Woo Kim, Deepak Rai, Morgan R. McKellar, and Ricardo C.T. Aguilar

**CCR, 2011**
- Gene Set Enrichment Analysis Unveils the Mechanism for the Phosphodiesterase 4B Control of Glucocorticoid Response in B-cell Lymphoma
Clinical initiatives

Safety and Pharmacodynamics of the PDE4 Inhibitor Roflumilast in Advanced B-cell Malignancies

Kevin Kelly\textsuperscript{1,2}, Alex Mejia\textsuperscript{1,2}, Avvari N. Suhasini\textsuperscript{1}, An-Ping Lin\textsuperscript{1}, John Kuhn\textsuperscript{3}, Anand B. Karna\textsuperscript{1,2}, Steven Weitman\textsuperscript{2,4}, and Ricardo C.T. Aguilar\textsuperscript{1,2,5,6}

\textit{CCR}, 2016
PDE4 and the DLBCL microenvironment

PDE4 inhibition suppresses vascular remodeling in non-neoplastic models

Angiogenesis is a risk factor in DLBCL

VEGF levels

Microvessel density

Phase 3 trial of R-CHOP + Avastin was negative
PDE4 inhibition suppresses lymphoma angiogenesis in vivo

Pde4b+/+ x Pde4b-/-

Eμ-Myc Tg /+

Eμ-Myc lymphoma cells

Clinical signs of lymphoma (~10 days)

Vehicle

Roflumilast – 5mg/kg/day (gavage)

Cohort 1

Cohort 2

Cohort 3

p=0.03

p=0.01

p=0.04

Microvessel density
mean vessel number - three hotspots

Microvessel density
mean vessel number - three hotspots

Anti-CD34

Pde4b+/+

Pde4b-/-

Eμ-Myc Tg /+;Pde4b-/-

Vehicle

Roflumilast

Anti-CD34
Phosphodiesterase 4 inhibitors have wide-ranging activity in B-cell malignancies

Phase I/II – PDE4i + R-CHOP in untreated DLBCL
Conclusions

- DLBCL is clinically and biologically heterogeneous – IPI should be interpreted with caution
- GCB vs. ABC (IHC), MYC and BCL2 expression (IHC), and MYC, BCL2, BCL6 rearrangement should be standard of care
- Double expressor lymphomas have poor outcome – candidates for trial; DHL must be treated with dose intensive regimen
- Immunomodulation – niche application (PMBCL, PTL, PCNSL)
- Solid rationale to further test PDE4 inhibitors in DLBCL
- Multiple targeted approaches in the pipeline – power of genetics far from saturated. Inhibiting MYC is likely the “holly grail”
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