Aggressive B-cell lymphomas and gene expression profiling – towards individualized therapy?

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NEW WHO CLASSIFICATION 2008
Diffuse large B-cell lymphoma

1. DLBCL, not otherwise specified (NOS)
2. T-cell/histiocyte-rich large B-cell lymphoma
3. DLBCL of the CNS
4. Cutaneous DLBCL, leg type
5. EBV-positive DLBCL of the elderly
6. Mediastinal large B-cell lymphoma
7. ALK positive large B-cell lymphoma
8. DLBCL associated with chronic inflammation
9. Intravascular large B-cell lymphoma
10. Plasmablastic lymphoma
TOPICS

1. Germinal center B-like DLBCL vs. Activated B-like DLBCL
   - biology, prognosis, diagnostics

2. Genetics
   - prognosis, diagnostics

3. Grey zone between DLBCL and Burkitt lymphoma
   - prognosis, diagnostics

4. EBV-association
   - prognosis, diagnostics
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DLBCL - Gene Expression Profiling
NFκB Activation in ABC DLBCL

Intrinsic biological program or pathogenetic event?
CARD11 activates the NF-κB Pathway
CARD11 mutations in DLBCL

Summary:

- CARD11 mutations are acquired and tumor-specific
- CARD11 mutations occur predominantly in the ABC DLBCL subtype
- CARD11 mutations constitutively activate the NF-κB pathway
- CARD11 mutations are required for survival in ABC DLBCL

Ngo et al., Nature 2006
Lenz et al., Science, 2008
Mutations of multiple genes deregulate NFkB in DLBCL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Percentage of ABC DLBCL</th>
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<tbody>
<tr>
<td>A20 (TNFAIP3)</td>
<td>24%</td>
</tr>
<tr>
<td>CARD11</td>
<td>11%</td>
</tr>
<tr>
<td>RANK</td>
<td>8%</td>
</tr>
<tr>
<td>TRAF5</td>
<td>5%</td>
</tr>
<tr>
<td>TRAF2</td>
<td>3%</td>
</tr>
<tr>
<td>MAP3K7(TAK1)</td>
<td>5%</td>
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</table>

Conclusion: More than 50% of ABC DLBCL carry mutations in positive or negative regulators of NFkB!

Compagno et al., Nature 2009
Mutations and deletions of the A20 gene in ABC-DLBCL

Compagno et al., Nature 2009
Is the distinction of GCB/ABC DLBCL still prognostically relevant in the R-CHOP era?

Investigated samples:
• 176 de novo DLBCL patient samples
• Biopsy obtained prior to treatment
• All patients received R-CHOP-like chemotherapy

Methods:
• Gene expression performed on Affymetrix U133 plus 2.0 arrays
• Classification by gene expression profiling:
  – 78 GCB DLBCL
  – 76 ABC DLBCL
  – 22 unclassified DLBCL

Lenz et al., NEJM, 2008
Overall survival following R-CHOP-like chemotherapy in DLBCL

Lenz et al., NEJM, 2008
The Distinction Between the GCB and ABC Subtypes of DLBCL Retains Prognostic Significance with CHOP-Rituximab Therapy

Lenz et al., NEJM, 2008
Stromal signatures in DLBCL

Lenz et al., NEJM, 2008
The Hans Classifier

- CD10
- bcl-6
- mum-1

GCB (42 cases)

Non-GC (27 cases)

Non-GC (61 cases)

Hans et al., Blood 2003
# The Hans classifier – controversies in the CHOP and R-CHOP era

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Treatment</th>
<th>Survival Association</th>
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<td>Hans et al., Blood 2003</td>
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<td>Haarer et al., Arch Pathol Lab Med 2006</td>
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<td>Muris et al., J Pathol 2006</td>
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<td>Sjo et al., Eur J Haematol 2007</td>
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<td>van Imhoff et al., J Clin Oncol 2007</td>
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<td>Colomo et al., Blood 2003</td>
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<td>De Paepe et al., J Clin Oncol 2005</td>
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<td>Veelken et al., Ann Oncol 2007</td>
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<td>Amen et al., Histopathology 2007</td>
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<td>Wilson et al., JCO 2008 (DE-EPOCH-R)</td>
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<td>RICOVER 60, submitted</td>
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RICOVER 60 - Rituximab treated patients

(Pfreundschuh et al., Lancet Oncology 2008)

n=155

Overall Survival

Non-GCB (n=72)

GCB (n=83)

p=0.718
RICOVER 60 - Rituximab treated patients
centroblastic vs. immunoblastic morphology (n=287)

Rituximab treated patients with centroblastic (cb) vs. immunoblastic (ib) morphology over time. The graph shows a comparison of proportion over months, with a significance level of p=0.005.
RICOVER 60 - Rituximab treated patients
Multivariate analysis of OS
IPI factors and histological subtype (n=287)

<table>
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<th>parameters</th>
<th>rel. risk</th>
<th>95% CI</th>
<th>p-value</th>
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<td>LDH &gt; UNV</td>
<td>2.4</td>
<td>(1.4; 4.0)</td>
<td>0.001</td>
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<tr>
<td>ECOG &gt; 1</td>
<td>2.2</td>
<td>(1.3; 3.8)</td>
<td>0.003</td>
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<tr>
<td>stage III/ IV</td>
<td>1.1</td>
<td>(0.6; 1.9)</td>
<td>0.701</td>
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<tr>
<td>extranodal disease &gt; 1</td>
<td>1.7</td>
<td>(1.0; 3.1)</td>
<td>0.062</td>
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<tr>
<td>ib vs. cb</td>
<td>2.1</td>
<td>(1.1; 3.7)</td>
<td>0.017</td>
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</table>
RICOVER 60 Trial

1. Immunoblastic morphology predicts poor outcome in all patients and in patients treated with R

2. BCL2 expression is predictive in all patients, but not in patients treated with R

3. BCL6 expression is predictive in patients treated with R, but not in all patients

4. BCL2 and BCL6 have only modest impact in Cox models that include the IPI factors

5. The Hans classifier does not predict outcome
GCB versus non-GCB distinction on the basis of CD10, MUM-1 and bcl-6 (25% cut-off levels)

substantial 0.72
moderate/fair 0.42-0.56
moderate 0.42

de Jong, JCO 2007
Improved GCB/ABC immunohistochemical classifiers?

Improved Hans classifier (Choi et al., Clin Cancer Res, 2009)

Muris algorithm: BCL2, CD10, MUM1/IRF4 (Muris et al., J Pathol 2006)

Modified activated B-like algorithm: MUM1/IRF4, FOXP1 (Nyman et al., Mod Pathol 2009)

Combined immunohistochemistry/FISH models
Quantitative RT-PCR: Outcome prediction in DLBCL using a 6 gene model

- 6 gene model predicts in CHOP-treated patients
- 6 gene model predicts in R-CHOP-treated patients
- 6 gene model can be applied in formalin-fixed tissue

Malumbres et al., Blood 2008
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DLBCL – Genetic Alterations

1. Somatic hypermutation of oncogenes (*PIM1*, *MYC* etc.)  50%
2. *BCL6* promoter substitution (translocation)  30-40%
3. *TP53* inactivation  20%
4. *BCL2* translocation - t(14;18)  17%
5. Amplifications of *BCL2/REL/MYC*  20%
6. *MYC* translocation - t (8;14)  6%
Clinical significance of *TP53* mutations in DLBCL

*TP53* mutations in the DNA-binding domain are prognostically important!

Young, K. H. et al. Blood 2008;112:3088-3098
Clinical significance of the MYC-break in DLBCL

Hummel et al., NEJM 2006
Klapper et al., Leukemia 2008
Niitsu et al., Cancer Sci 2008
**Survival of patients with double hit lymphomas**

(double hit = MYC plus BCL2 or BCL6 translocation)

Table 1. Patients' clinical characteristics.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age/Gender</th>
<th>IPI score</th>
<th>Extra-nodal sites</th>
<th>Prior history or concomitant low grade lymphoma</th>
<th>Therapy</th>
<th>Response</th>
<th>SCT</th>
<th>Survival (months)</th>
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<tbody>
<tr>
<td>1</td>
<td>59/M</td>
<td>3</td>
<td>BM, skin, CNS, blood</td>
<td>Yes, FL</td>
<td>CEEP, COPADM</td>
<td>PR</td>
<td>autologous (BEAM)</td>
<td>4</td>
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<tr>
<td>2</td>
<td>65/F</td>
<td>3</td>
<td>pleural effusion, CNS</td>
<td>no</td>
<td>CHOP, IVAM</td>
<td>CR</td>
<td>16</td>
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<tr>
<td>3</td>
<td>45/M</td>
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<td>BM, lung</td>
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<td>COPADM, CYVE</td>
<td>PR</td>
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<td></td>
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<tr>
<td>4</td>
<td>50/F</td>
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<td>BM</td>
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<td>CEEP, DHAP</td>
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<td>10</td>
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<td>13</td>
<td>55/M</td>
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<td>COPADM</td>
<td>Prog</td>
<td>3</td>
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<tr>
<td>14</td>
<td>59/M</td>
<td>3</td>
<td>BM, pleural effusion, peritoneal effusion, CNS, testis</td>
<td>no</td>
<td>R-CHOP</td>
<td>Prog</td>
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<tr>
<td>15</td>
<td>70/F</td>
<td>3</td>
<td>BM, CNS, pleural effusion</td>
<td>Yes, NOS</td>
<td>CHOP</td>
<td>Prog</td>
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<td>16</td>
<td>72/M</td>
<td>3</td>
<td>BM, skin</td>
<td>Yes, NOS</td>
<td>steroids</td>
<td>Prog</td>
<td>1</td>
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</tr>
</tbody>
</table>

BM; bone marrow; CNS, central nervous system; PR, partial response; CR, complete response; SCT, stem cell transplantation; TBI, total body irradiation; Cy, cyclophosphamide; Bu, busulfan.

Le Gouill et al., Haematologica 2007
Survival curves of patients with BCL2+/MYC+ lymphomas according to the timing of MYC+ rearrangement and treatment regimen

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The new WHO classification:

BCLUWFIBDLBCLABL
Gene Expression Differentiates Burkitt Lymphoma from all Subgroups of Diffuse Large B Cell Lymphoma

<table>
<thead>
<tr>
<th>Burkitt Lymphoma</th>
<th>Diffuse Large B-cell Lymphoma (DLBCL)</th>
</tr>
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<tbody>
<tr>
<td>Classic</td>
<td>ABC</td>
</tr>
<tr>
<td>Atypical</td>
<td>GCB</td>
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<tr>
<td></td>
<td>PMBL</td>
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<tr>
<td></td>
<td>Unclass</td>
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**GENE**
- Stage 1 Predictor (MYC Targets)
  - MYC
  - TERT
  - NS
  - NUP
  - MAK
  - RFC3
  - BYSL
  - IL6
  - CDC7
  - FCL1A
  - LAUTS2
  - MYBL1
  - BMP7
  - ITPR3
  - CDC2
  - BACH2
  - TTK
  - MME
  - ALOX5
  - TOP1
  - JAK2
  - HLA-F
  - PIM1
  - CASP8
  - HLA-E
  - CCL17
  - VMP1
  - TRAF1
  - HCK
  - HLA-G
  - JAK3
  - BAFF
  - NFKBIA
  - LMO2
  - CD44
  - CD40L
  - BIC
  - STAT3
  - BCL2A1

**Stage 2 Predictor**

Dave et al., NEJM 2006
A molecular grey zone between DLBCL and BL

Training set

Test set

Hummel et al., NEJM 2006
B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL and BL (BCLUWFIBDLBCLABL)

- Highly proliferative lymphomas with morphological and phenotypic features between Burkitt and DLBCL; occasionally leukemic
- A proportion of these cases carry double hit oncogenic events involving c-myc
- Clinically aggressive
- These cases should be recognized as a “practical category” but it is not clear they represent a specific disease entity
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7. ALK positive large B-cell lymphoma
8. DLBCL associated with chronic inflammation
9. Intravascular large B-cell lymphoma
10. Plasmablastic lymphoma
DLBCL and EBV

- **Secondary to other defined disorders**
  - Primary immune disorders, HIV
  - Post-transplant lymphoproliferative disorder
  - Associated with chronic inflammation
  - Lymphomatoid granulomatosis

- **Primary (DLBCL of the elderly)**
  - Without any known immunodeficiency
  - age > 50 years
  - More advanced stage
  - More than one extranodal involvement
  - Higher IPI risk group
  - B symptoms
  - Poorer outcome to initial treatment

*Park et al., Blood 2007*
Summary / Role of Pathology

1. Fundamental biological differences between GCB/ABC DLBCL
   
   Mutations of multiple genes deregulate NFκB in ABC DLBCL – relevant therapeutic target!

2. Prognostic gene expression signatures from the CHOP era remain relevant in the R-CHOP era

3. Need to develop a diagnostically applicable test to distinguish GCB/ABC DLBCL for future clinical trials
   
   Immunohistochemical algorithms for DLBCL subtype distinction (e.g. Hans) remain controversial
Summary / Role of Pathology

4. Negative prognostic impact of MYC translocations
   More FISH in the diagnostic setting

5. Practical category in the new WHO: BCLUWFIBDLBCLABL
   ‘B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL’
   frequent (ca. 50%): ‘double hit’ lymphomas (MYC/BCL2)

6. EBV-associated DLBCL of the elderly
   age >50 years
   likely to be prognostically unfavorable
   implies increased EBER testing in diagnostic setting