Recent advances in the genetics & biology of lymphoma

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NHS Foundation Trust
Lymphoma

- Age standardised incidence: \( \approx 16,500/100,000/y \)
- \( \approx 11,000 \) new cases in UK each year
- Increasing incidence (of diagnosis?)

• DLBCL \( \approx 40\% \) all lymphomas
Diffuse large B cell lymphoma (DLBCL)

WHO 2008

- T cell/histiocyte rich LBCL
- Primary DLBCL of CNS
- Primary cutaneous DLBCL, leg type
- EBV positive DLBCL of the elderly
- Primary mediastinal LBCL
- Intravascular LBCL
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- ALK-positive LBCL
- Plasmablastic lymphoma
- LBCL arising in HHV8-associated MCD
- Primary effusion lymphoma

Diffuse large B cell lymphoma, not otherwise specified

- Median age – 60s
- 40% extranodal
- 50% advanced disease
- Rx: R-CHOP or similar
- 40% cure rate
Germinal centre reaction

- Bcl6
- Blimp1
- Pax5
- XBP1
- GC B cell
- Plasma cell
Germinal centre reaction

Checkpoints (apoptosis arrest)

AID
CSR
DNA breaks
SHM
ATM
p53
p21

Bcl6

Blimp1

Pax5

XBP1

GC B cell

Plasma cell
Germinal centre reaction

Key proteins and processes:
- BCR
- CD40L
- NF-κB
- IRF4
- Bcl6
- Blimp1
- Pax5
- XBP1
- GC B cell
- Plasma cell

Processes:
- Somatic hypermutation
- Clonal expansion
- Selection
- Class switching
- Mutations that increase antigen affinity
- Mutations that reduce antigen affinity
- Differentiation
(One way to define) two types of DLBCL

(Wright et al 2003)

Rx: R-CHOP

(Germinal-center B-cell-like)
(Activated B-cell-like)

(Lenzo et al 2008)

CD10
BCL6
LMO2
A-MYB
JAW1

Ongoing
IGH VDJ mutation

(Loisso et al 2000)

IRF4
Cyclin D2
Flip
CD44
IGHM
FOX1
PRKCB1

IGH VDJ mutated
Distinct genomic abnormalities in ABC vs GCB

### ABC
- **BCL2-R**: 24%
- **BCL6 mut**: 44%
- **TP53 mut**: 24%
- **TP53 del**: 24%

### GCB
- **BCL2-R**: 34%
- **BCL6 mut**: 10%
- **TP53 mut**: 74%
- **TP53 del**: 30%

**CGB**
- Mir-17-92 amp
- REL amp
- PTEN loss
- MDM2 gain/amp
- ING1 loss

**ABC**
- Trisomy 3 / 3p gain
- NFKBIZ amp
- 18q gain/amp: BCL2, MALT1, NFAT2
- CDKN2A/B, INK4A/ARF loss
- SPIB gain/amp

(Lenz et al 2008)

(Iqbal et al 2004; Iqbal et al 2007; Young et al 2007)
**NF-κB dependence of ABC-DLBCL**

A

GCB DLBCL

ABC DLBCL

Cyclin D2
IRF-4
IRF-5
c-FLIP
BCL-2
CCR7
IkB alpha

(Davis et al 2002)

(Davis et al 2002)

Inhibition of MALT1 protease activity is selectively toxic for activated B cell–like diffuse large B cell lymphoma cells

Uta Ferch, Bernhard Kloor, Andreas Gewies, Vera Pfänder, Michael Düwel, Christian Peschel, Daniel Krappmann, and Jürgen Ruland

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**Gene symbol (synonym)**

<table>
<thead>
<tr>
<th>Gene symbol (synonym)</th>
<th>Number of mutated/tested cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABC-DLBCL</td>
</tr>
<tr>
<td><strong>TNFAIP3 (A20)</strong></td>
<td>9/37 (24.3)</td>
</tr>
<tr>
<td><strong>CARD11</strong></td>
<td>4/37 (10.8)</td>
</tr>
<tr>
<td><strong>TNFRSF11A (RANK)</strong></td>
<td>3/37 (8.1)</td>
</tr>
<tr>
<td><strong>TRAF5</strong></td>
<td>2/37 (5.4)</td>
</tr>
<tr>
<td><strong>TRAF2</strong></td>
<td>1/37 (2.7)</td>
</tr>
<tr>
<td><strong>MAP3K7 (TAK1)</strong></td>
<td>2/37 (5.4)</td>
</tr>
<tr>
<td><strong>All genes</strong></td>
<td>19/37 (51.3)</td>
</tr>
</tbody>
</table>

(Compagno et al 2009)
Defective plasma cell differentiation in ABC-DLBCL

Normal GC B cell differentiation

NF-κB → IRF4 → Bcl6 → Blimp1 → Plasma cell

DLBCL with BCL6 mutation / translocation

NF-κB → Proliferation

DDR checkpoint → IRF4

Bcl6 → Blimp1 → Plasma cell

(Saito et al 2007; Wang et al 2002; Pasqualucci et al 2003)
Defective plasma cell differentiation in ABC-DLBCL

Normal GC B cell differentiation

- NF-κB
- IRF4
- Bcl6
- Blimp1
- Plasma cell

DLBCL with PRDM1 (Blimp1) mutation/deletion

- NF-κB
- IRF4
- Bcl6?
- Plasma cell

(Pasqualucci et al 2006; Tam et al 2006; Mandelbaum et al ASH 2009)
• Almost all ABC DLBCL express IgM (ie. no class switch, Ig maintained on surface)

• CSR only on non-productive allele

• Large deletions in switch $\mu$ region on productive allele

• Increased AID-mediated non-VDJ region somatic hypermutation compared to GCB DLBCL

• Increased translocations involving switch regions compared to GCB DLBCL

Aberrant CSR regulation / switch $\mu$ deletions

Repeated futile attempts at CSR

Increased somatic hypermutation / translocations (collateral damage)

Lymphomagenesis
Chronic active BCR signalling in ABC-DLBCL

SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma

Lin teng Chen, 1 Stefano Monti, 2 Przemysław Juszczynski, 1 John Daley, 1 Wen Chen, 1 Thomas E. Witzg, 3 Thomas M. Habermann, 2 Jeffery L. Kuzio, 4 and Margaret A. Shipp 5

1 Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 2 Broad Institute, Cambridge, MA; 3 Mayo Clinic, Rochester, MN; and 4 Department of Pathology, Brigham and Women's Hospital, Boston, MA

(Davis et al 2010)
Genetically-guided therapy for DLBCL

Differential effect of bortezomib + chemo in ABC vs GCB DLBCL

(Dunleavy et al. 2009)
Sporadic Burkitt lymphoma
Children & young adults > older adults
Often extranodal – ileocaecal, kidneys, breasts
70% advanced stage rapidly progressive disease
Treated with intensive chemotherapy
80-90% cure rate

WHO 2000
All cases MYC-R (IGH > IGK > IGL)
All cases Bcl2 negative

A problem:
Diagnostic reproducibility ≈ 60%
Biological and clinical overlap with DLBCL
GEP of aggressive mature B cell lymphomas

- Burkitt lymphoma (BL) N=36
- Molecular BL (mBL) N=44
- Aggressive B-NHL Unclassifiable N=18
- DLBCL N=165
- Non-mBL N=128
- Intermediate N=48

(Hummel et al 2006)
(Dave et al 2006)
### Characteristics of molecular subgroups

<table>
<thead>
<tr>
<th></th>
<th>mBL</th>
<th>Non-mBL</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age &gt;60</strong></td>
<td>9%</td>
<td>69%</td>
<td>55%</td>
</tr>
<tr>
<td><strong>Cell of origin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• GCB</td>
<td>91%</td>
<td>35%</td>
<td>71%</td>
</tr>
<tr>
<td>• ABC</td>
<td>9%</td>
<td>39%</td>
<td>17%</td>
</tr>
<tr>
<td>• Unclassified</td>
<td>9%</td>
<td>26%</td>
<td>13%</td>
</tr>
<tr>
<td><strong>Bcl2 expression</strong></td>
<td>21%</td>
<td>84%</td>
<td>83%</td>
</tr>
<tr>
<td><strong>High proliferation (&gt;95%)</strong></td>
<td>66%</td>
<td>12%</td>
<td>15%</td>
</tr>
<tr>
<td><strong>MYC translocation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>IG-MYC</em></td>
<td>88%</td>
<td>4%</td>
<td>33%</td>
</tr>
<tr>
<td>• non <em>IG-MYC</em></td>
<td>2%</td>
<td>3%</td>
<td>21%</td>
</tr>
<tr>
<td>• no translocation</td>
<td>9%</td>
<td>93%</td>
<td>46%</td>
</tr>
<tr>
<td><strong>IGH-BCL2</strong></td>
<td>2%</td>
<td>11%</td>
<td>21%</td>
</tr>
<tr>
<td><strong>BCL6 translocation</strong></td>
<td></td>
<td>24%</td>
<td>15%</td>
</tr>
<tr>
<td><strong>Genomic complexity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Low</td>
<td>79%</td>
<td>29%</td>
<td>31%</td>
</tr>
<tr>
<td>• High</td>
<td>21%</td>
<td>71%</td>
<td>69%</td>
</tr>
<tr>
<td><strong>Genetic group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>MYC-simple</em></td>
<td>76%</td>
<td></td>
<td>13%</td>
</tr>
<tr>
<td>• <em>MYC-complex</em></td>
<td>13%</td>
<td>7%</td>
<td>40%</td>
</tr>
<tr>
<td>• <em>MYC-negative</em></td>
<td>11%</td>
<td>93%</td>
<td>47%</td>
</tr>
</tbody>
</table>

(Hummel et al 2006)
Genomic complexity in MYC translocated cases

(Boerma et al 2009)
Clinical Implications

mBL: 84% ALL-like Rx
Others: 72% CHOP-like Rx

? Confounding effect of age / lower stage of mBL / MYC-simple disease

(Hummel et al 2006)
Clinical Implications

BL defined by:
- Ki67 approaching 100%
- GCB immunophenotype
- Bcl2 negative
- p53 +ve / p21 –ve
- MYC rearrangement
- No BCL2 / BCL6 rearr

Many had DLBCL morphology

Rx: CODOX-M / IVAC

Survival of patients with a histological diagnosis of DLBCL, reclassified as Burkitt lymphoma following gene expression profiling

(Dave et al 2006)
**MYC – BCL2 “Double hit” lymphomas**


### Presentation
- **Adults** (50% >60y)
- **De novo or transformed follicular lymphoma**
- **Adverse clinical features:** Poor performance status, high IPI, advanced stage, high LDH extranodal disease, BM / PB involvement

### Genetics (Johnson et al 2009)
- **IG-MYC:** 56% (IGH > IGK > IGL) (50% t(8;14) = complex [t(8;14)t(14;18)]
- **Non IG-MYC:** 50% t(8;9)(q24;p13) / (5’ to PAX5); also 1p36, 3p25, 3q27, 4p13, 4p13, 5q13, 12p11, 13q31
  - Almost all have a complex karyotype, incl ≥ 3 translocations
  - Hummel: DH = non-mBL or intermediate

### Histology
- **DLBCL** (35%) or **BCLU / BLL** (65%)
- Most have GCB immunoprofile
- Most have high Ki67 proliferation fraction
- Almost all express Bcl2 strongly

### Outcome
- Very poor survival, median OS <12 months
- If do respond, relapse rapidly

(Niitsu et al 2009)
MYC – BCL2 “Double hit” lymphomas

78 year old man with a rapidly enlarging neck mass
**MYC – BCL2 “Double hit” lymphomas**

**Cytogeneticist: Gavin Cuthbert**

Sequential FISH
(-performed due to low level positivity for IGH/BCL2 and MYC - ~30% abnormal)

**IGH/BCL2** (dual fusion)

**MYC** (break apart)

Further interphase FISH
**IGH-MYC** fusion negative
**IGK** split
Abnormal **BCL6** signal (FFR)
MYC – BCL2 “Double hit” lymphomas

73 year old woman with lymphadenopathy, PB lymphocytosis & bone marrow infiltration

G-banded karyotype from bone marrow preparations showing 47,XX,add(8)(q24),?t(14;18)(q32;q21),-16,+mar1,+mar2

C.P. - dob. 1933
MYC – BCL2 “Double hit” lymphomas

Cytogeneticist: Gavin Cuthbert

IGH/BCL2 dual fusion
- Fusion: der(14)
- Fusion: der(18)

MYC/BCL6 dual fusion
- Fusions: marker
- Fusion: der(3)
- Fusion: der(8)

C.P. - dob. 1933
MYC – BCL2 “Double hit” lymphomas

Whole chromosome painting

- marker
- wcp3
- wcp8
- der(3)
- der(8)

Composite image of key abnormal chromosomes

- Partial G-banded karyotype featuring images from 2 metaphases
- MYC/BCL6 dual colour FISH
- wcp3 and wcp8

revised karyotype: 47,XX,t(3;8)(q27;q24.1),t(14;18)(q32;q21),+rea(8)t(3;8),?del(16)(q1?)

C.P. - dob.1933
## WHO 2008: BCLU-IDB

<table>
<thead>
<tr>
<th></th>
<th>Burkitt lymphoma</th>
<th>BCLU-IDB</th>
<th>DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Small / medium-size cells</td>
<td>Small / medium or mixture</td>
<td>Large cells</td>
</tr>
<tr>
<td><strong>Immunophenotype</strong></td>
<td>GCB CD10+ Bcl6+ Bcl2-/weak</td>
<td>Mostly as BL Bcl2 strong in DH ?non-GCB if DH</td>
<td>GCB Non-GCB</td>
</tr>
<tr>
<td><strong>Proliferation fraction</strong></td>
<td>&gt;90% homogeneous</td>
<td>Often as BL May be as DLBCL</td>
<td>Commonly &lt;90% heterogeneous</td>
</tr>
<tr>
<td><strong>MYC rearrangement</strong></td>
<td>Yes (5% lack MYC-R)</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>MYC translocation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• IG-MYC</td>
<td>Yes</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
<tr>
<td>• non IG-MYC</td>
<td>No</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>BCL2 or BCL6 rearranged but not MYC</strong></td>
<td>No</td>
<td>Rare</td>
<td>Sometimes</td>
</tr>
<tr>
<td><strong>Genetic group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• MYC-simple</td>
<td>Yes</td>
<td>Rare</td>
<td>Rare (?never)</td>
</tr>
<tr>
<td>• MYC-complex</td>
<td>Rare</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Double hit</strong></td>
<td>No</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
</tbody>
</table>
DLBCL with MYC translocations

- 5-10% DLBCL have MYC rearrangement in absence of BCL2 / BCL6 rearrangements
- No defining high risk clinical features
- No consistent histological features
  - 50-75% GCB GEP profile / immunophenotype
  - Only a trend to high proliferation fraction
- Independent prognostic factor for PFS & OS in CHOP / R-CHOP treated DLBCL

(Klapper et al 2008)
(Savage et al 2009)
A personal view & questions

1. Burkitt lymphoma
2. Non-\textit{MYC} DLBCL
3. \textit{MYC} complex – intermediate
4. \textit{MYC} complex – DLBCL
5. Non-\textit{MYC} intermediate

- How should 3-5 be treated?
- How should we capture genomic complexity, not just \textit{BCL2/6} translocations?
- How should we identify 5?
- Should all aggressive B cell lymphomas have \textit{MYC} +/- other FISH or karyotyping?
- How must we design diagnostic systems to capture the clinically relevant genetic information that is headying our way?
Acknowledgements

Not my work!

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