Diffuse large B cell lymphoma. Lessons to be learned from molecular analysis
Christian Gisselbrecht MD Paris Saint Louis

11th Educational Course of the EBMT Lymphoma Working Party on "Treatment of Malignant Lymphoma: State-of-the-Art and Role of Stem Cell Transplantation"

Heidelberg (Germany), September 24 - 26, 2015
Heterogeneity in DLBCL

Bio markers: Magic tools?
- Ontogeny
- Evolution of classification
- Prognostic markers
- IHC or not
- Enrichment strategies to guide treatment

- Detection of oncogenic pathways-related biomarkers by immunohistochemistry on FFPE
- Cell of origin - prognostic groups using gene expression in FFPE (NanoString)
- GEP selection of groups for NGS technology
- Recognition of specific oncogenic pathways
- Cell of origin – prognostic groups by immunohistochemistry
- Cell of origin - prognostic groups application of GEP to human lymphomas
- Characterization by immunohistochemistry on FFPE of MUM1 in normal and malignant lymphoid cells
- Cloning of MUM1/IRF4gene
- Characterization by immunohistochemistry on FFPE of BCL6 in normal and malignant lymphoid cells
- Cloning of BCL6 gene
- Modern histologic classifications

Antonino Carbone & Annunziata Gloghini & Yok-Lam Kwong & Anas Younes
Diffuse Large B-Cell Lymphoma: Subgroups and Subtype/Entities

WHO classification 2008

- Diffuse large B-cell lymphoma, not otherwise specified (NOS)
  - Common morphologic variants
    - Centroblastic
    - Immunoblastic
    - Anaplastic
  - Rare morphologic variants
    - Molecular subgroups
      - Germinal centre B-cell-like (GCB)
      - Activated B-cell-like (ABC)
    - Immunohistochemical subgroups
      - CD5-positive DLBCL
      - Germinal centre B-cell-like (GCB)
      - Non germinal centre B-cell-like (non-GCB)
  - Diffuse large B-cell lymphoma subtypes
    - T-cell/histiocyte-rich large B-cell lymphoma
    - Primary DLBCL of the CNS
    - Primary cutaneous DLBCL, leg type
    - EBV positive DLBCL of the elderly
- Other lymphomas of large B cells
  - Primary mediastinal (thymic) large B-cell lymphoma
  - Intravascular large B-cell lymphoma
  - DLBCL associated with chronic inflammation
  - Lymphomatoid granulomatosis
  - ALK-positive LBCL
  - Plasmablastic lymphoma
  - Large B-cell lymphoma arising in HHV8-associated multicentric Castelman disease
  - Primary effusion lymphoma
- Bordeline cases
  - B-cell lymphoma, unclassifiable, intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
  - B-cell lymphoma, unclassifiable, intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

Should we use the same treatment?
Diagnosis and outcome of DLBCL subtypes by GEP. (A) Heat map showing differential expression of genes in GCB, ABC, and PMBL DLBCL subtypes.

Wilson W H Hematology 2013;2013:584-590
Gene-Expression Predictors of Survival among Patients with Diffuse Large-B-Cell Lymphoma Treated with R-CHOP

The Hans Classifier

A surrogate for GEP?

Reproductibility/Concordance: 80%

Hans et al., Blood 2003
Determining Cell-Of-Origin Subtypes In Diffuse Large B-Cell Lymphoma Using Gene Expression Profiling On Formalin-Fixed Paraffin-Embedded Tissue – : NanoString technology

David W. Scott et al (Blood. 2014;123(8):1214-1217)

<table>
<thead>
<tr>
<th>Frozen GEP</th>
<th>NanoString GEP assay – NCI</th>
<th>Hans algorithm</th>
<th>Tally algorithm</th>
<th>Choi algorithm</th>
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<tbody>
<tr>
<td>GCB</td>
<td>28 0 0 21 0 18 3 19 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>7 2 1 5 5 2 8 6 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABC</td>
<td>1 3 25 4 22 0 26 6 20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key Points**


119 DLBCL cases previously subtyped using frozen-GEP, were used to develop a highly accurate and robust NanoString 20 gene assay, applicable to RNA from FFPET that is routinely obtained for diagnosis. This new assay shows excellent performance in archival FFPET, and the rapid turn-around time (<36 hours from FFPET block to result) will allow prospective implementation in future therapeutic trials and, ultimately, clinical practice.
Outcomes in patients with DLBCL after according to cell of origin determined by NanoString technology.
Abstract 84 Accurate Classification Of GCB/ABC and MYC/BCL2 Diffuse Large B-Cell Lymphoma With a 14 Genes Expression Signature and a Simple and Robust RT-MLPA Assay: A CALYM study

Philippe Ruminy, PhD, Herve Tilly, and Fabrice Jardin, MD, PhD et al

Centre Henri Becquerel, Rouen, France

10 genes expression signature incorporated into Reverse Transcriptase Mutiplex Ligation-dependent Probe Amplification assay  RT-MLPA
50 cases: 46 classified as expected ,3 unclassified. Validation on 185 DLBCL.RNA extraction from FFPE

Rapid method: 40 samples in parallel less than 24h
Cheap 5 dollars each can be achieved with FFPE.
## Chemotherapy alternatives to R-CHOP according to subtype?

<table>
<thead>
<tr>
<th>Author</th>
<th>Therapy</th>
<th>Better than R-CHOP-21?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfleischuch, Lancet Oncology 2008</td>
<td>R-CHOP-21 6 cycles</td>
<td>No</td>
</tr>
<tr>
<td>Cunningham, Lancet 2013</td>
<td>R-CHOP-14</td>
<td>No</td>
</tr>
<tr>
<td>Delarue, Lancet Oncology 2013</td>
<td>R-CHOP-14</td>
<td>No</td>
</tr>
<tr>
<td>Recher, Lancet 2011</td>
<td>R-ACVBP</td>
<td>Yes</td>
</tr>
</tbody>
</table>
R-ACVBP Benefit Due to Improvement in ABC Subtype / (aaIPI 1)

229/379 pts available for IHC Hans algorithm

C

PFS

D

OS

GC cases

Non GC cases

Molina, JCO Dec 2014
Optimizing Salvage Therapy for DLBCL Based Upon Cell of Origin: Bio-CORAL Analysis

**R-DHAP salvage**

- **A**
  - Progression-Free Survival (proportion)
  - Time (months)
  - GCB (n = 61)
  - Non-GCB (n = 56)
  - P = .0108

- **C**
  - Overall Survival (proportion)
  - Time (months)
  - GCB (n = 61)
  - Non-GCB (n = 56)
  - P = .0813

**R-ICE salvage**

- **B**
  - Progression-Free Survival (proportion)
  - Time (months)
  - GCB (n = 61)
  - Non-GCB (n = 56)
  - P = .8163

- **D**
  - Overall Survival (proportion)
  - Time (months)
  - GCB (n = 61)
  - Non-GCB (n = 56)
  - P = .3551

*Thieblemont, JCO Nov 2011*
Background

Differential efficacy of treatment within molecular subtypes of DLBCL

First line treatment

R-CHOP

Relapse treatment

DA-EPOCH-B

Overall S.

D. Lenz et al. NEJM 2008

Biomarker

Genetic Mutation
- EZH2
- CREBBP/EP300
- MLL
- β2microglobulin

Cell Surface Protein
- CD20
- CD30
- CD10
- CD37

Intracellular Protein
- Myc
- Bcl2
- pSTAT3
- pAKT
- pERK
## Major recurring genetic alterations in DLBCL

<table>
<thead>
<tr>
<th>Gene defect</th>
<th>Frequency</th>
<th>Location</th>
<th>Mechanism of deregulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL6</td>
<td>35-40%</td>
<td>3q27</td>
<td>t(3;....) and SHM</td>
</tr>
<tr>
<td>BCL2</td>
<td>15% [t(14;18)]</td>
<td>18q21</td>
<td>t(14,18), amplification</td>
</tr>
<tr>
<td></td>
<td>25% (amplification)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cMYC</td>
<td>6-15%</td>
<td>8q24</td>
<td>t(8;14) or variants, SHM</td>
</tr>
<tr>
<td>P53</td>
<td>15-20%</td>
<td>17p</td>
<td>mutation, deletion</td>
</tr>
<tr>
<td>FAS</td>
<td>20%</td>
<td>10q24</td>
<td>mutations (DD), SHM</td>
</tr>
<tr>
<td>SHM</td>
<td>40-50%</td>
<td>IgV, Bcl6, FAS</td>
<td>SHM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bcl6, cMyc, Pax5, RhoH/TTF, PIM1, SHM</td>
<td></td>
</tr>
<tr>
<td>CARD11</td>
<td>&lt;10% (ABC)</td>
<td></td>
<td>mutation</td>
</tr>
</tbody>
</table>

*Adapted from Abramson et al. Blood 2005*
## REPORTED MOLECULAR PROGNOSTIC MARKERS IN DLBCL PATIENTS TREATED WITH R-CHOP

<table>
<thead>
<tr>
<th>Favorable</th>
<th>Unfavorable</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMO2</td>
<td>MYC</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>CD5</td>
</tr>
<tr>
<td>P21</td>
<td>BCL2 (?)</td>
</tr>
<tr>
<td>CD30?</td>
<td>Mutated p53</td>
</tr>
<tr>
<td>CD40</td>
<td></td>
</tr>
<tr>
<td>Caspase 3a</td>
<td>Ki-67</td>
</tr>
<tr>
<td>Beclin 1</td>
<td>VEGFR2</td>
</tr>
<tr>
<td>SOCS1 major</td>
<td>High microvessel density</td>
</tr>
<tr>
<td></td>
<td>Indoleamine 2,3-dioxygenase</td>
</tr>
<tr>
<td></td>
<td>RCOR1 del</td>
</tr>
<tr>
<td></td>
<td>CDKN2A/2B del</td>
</tr>
<tr>
<td></td>
<td>SOCS1 minor</td>
</tr>
</tbody>
</table>
LIMITATIONS OF BIOMARKER STUDIES

- Discrepant results
- Small retrospective patient cohorts
- Patient selection and treatment variability
- Lack of uniformity in methodology
- Failure to control for other biologic processes that may confound outcome
- Have not been validated in independent population treated with standard of care

Clinical IPI remains an independent factor when challenges with most biomarkers.
Impact of MYC and BCL2 Protein Expression in DLBCL treated with R-CHOP

- Recently available MYC antibody measures MYC protein expression by IHC
- MYC translocation seen in 11%, but MYC overexpression in 33%
- MYC translocation and MYC expression only prognostic if BCL2 overexpressed
Overall Survival of Patients with DLBCL According to MYC and BCL2 Translocation or MYC and BCL2 Protein Expression

- Other (n = 236)
- MYC+/BCL2+ (n = 55)
- DHIT (n = 14)

5% double hit

P < .001
*P = .014 (MYC+/BCL2+ v other)

20% “dual expressors”
OS and PFS after RCHOP in patients with (DLBCL) harboring gene breaks in MYC, BCL2, or both.

Tina Marie Green et al. JCO 2012;30:3460-3467
TTP for patients with DLBCL after treatment with RCHOP according to cell of origin and immunohistochemistry for MYC and BCL2.

What about dual expressors?

NanoString technology

David W. Scott et al. JCO 2015;33:2848-2856

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GCB / non GCB

MYC / BCL2

GCB

MYC > 40%
FLt
Immunoblasts
BCLU

FISH MYC, (BCL2-BCL6), IG

MYC-IG
SH/DoubleHit

MYC BCL2 Double Expressors

non GCB

MYC > 40%
BCL2 > 50-70%

Algorithm for clinical use

Adapted treatment
Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis

A

B

C

D

E

Treatment strategies for aggressive lymphomas: what works?

Comparison of long-term, progression-free, and overall survival.

Overall survival by SCT versus observation in first complete remission.

**Key Points**

- A subset of DHL patients may be cured, and some patients may benefit from intensive induction.
- Further investigations into the roles of SCT and novel agents are needed.

Headlines news!
How to make a choice or to combine new treatments?
Are we moving towards cell-of origin as a guide to therapy?

<table>
<thead>
<tr>
<th>Non GC-B type</th>
<th>GC type</th>
</tr>
</thead>
<tbody>
<tr>
<td>• NF-kB activated</td>
<td>• Epigenetic regulation mutant/lost</td>
</tr>
<tr>
<td>• BCR signalling</td>
<td>• EZH2, CREBBP</td>
</tr>
<tr>
<td>• TLR/IRAK signalling</td>
<td>• ? EPOCH, DHAP</td>
</tr>
<tr>
<td>• Bortezomid</td>
<td>• GSK 126</td>
</tr>
<tr>
<td>• Ibrutinib</td>
<td>• EPZ6438</td>
</tr>
<tr>
<td>• Fostamatinib</td>
<td></td>
</tr>
<tr>
<td>• Lenalidomide</td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>Example Agent</td>
</tr>
<tr>
<td>-------------------------</td>
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</tr>
<tr>
<td>NF-κB</td>
<td>Bortezomib</td>
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<tr>
<td>PI3 Kinase</td>
<td>CAL-101</td>
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<tr>
<td>PKCβ</td>
<td>Enzastaurin</td>
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<tr>
<td>BTK</td>
<td>Ibrutinib</td>
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<tr>
<td>Syk</td>
<td>Fostamatinib</td>
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<td>Multi-target/</td>
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<tr>
<td>Angiogenesis</td>
<td>Lenalidomide</td>
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<tr>
<td>EZH2</td>
<td>EZH2 Inhibitor</td>
</tr>
<tr>
<td>Bcl2 family</td>
<td>ABT-263</td>
</tr>
</tbody>
</table>
NFkB signalling pathway is constitutively activated in ABC DLBCL

- ABC DLBCL are **less curable**
- More than 50% ABC DLBCL carry mutations in positive or negative regulators of NFkB (Compagno et al. Nature 2009)
- anti-apoptotic effect and can inhibit chemotherapy

- A20 (TNFAIP3): 24%
- CARD11: 11%
- RANK: 8%
- TRAF5: 5%
- TRAF2: 3%
- MAP3K7 (TAK1): 5%

Adapted from Küppers et al. 2009
Survival curves in diffuse large B-cell lymphoma. (A) PFS curve and (C) PFS stratification based on IPI; and COO (E). (B) Overall survival (OS) curve and OS stratification based on IPI (D) and subtype (F).

Bortezomib Plus CHOP-Rituximab for Previously Untreated Diffuse Large B-Cell Lymphoma and Mantle Cell Lymphoma

DLBCL: 35 pts
ORR 100%

Randomized studies
On going

Jia Ruan  J Clin Oncol 29:690-697. © 2010
Microenvironment & DLBCL: expression of lymph node signature predicts outcome in R-CHOP DLBCL patients

Extracellular matrix, histiocytes

Angiogenesis

Vessel density

Antiangiogenic agents?

Lenz et al, NEJM 2008
Kaplan-Meier analysis of (A) PFS and (B) OS by treatment arm.
MAIN study: avastin in combination with R-CHOP

787 pts enrolled
R-CHOP = RA-CHOP
But RA-CHOP more toxic: increased cardiac events

No efficacy
Targeting Microenvironment – IMiDs: Lenalidomide

T-Cell Effects
- ↑ Immune synapse formation
- ↑ T cell activation and proliferation
- ↑ CD8+ T effector cell activity

NK-Cell Effects
- ↑ Immune synapse formation
- ↑ ADCC
- ↑ Direct NK-mediated killing

Microenvironmental Effects
- ↓ FGF2
- Altered cytokine levels
- ↑ IgG production

B-CLL Cell Effects
- ↑ APC function
- ↓ CXCR4 expression

NHL Cell Effects
- NHL tumor cell death

Expansion of T cells
- IL2
- Actin
- IFNγ
- CD80
- CD86
- CXCR4
- ICAM-1
- CD40
- SDF1α
- FGF2
- IgG
- NLC
- Stromal cell

ADCC
- Granzyme B
- Granzyme A
- CD20
- RTX

Direct NK killing

T Cell

NHL
Lenalidomide Combined With R-CHOP Overcomes Negative Prognostic Impact of Non-Germinal Center B-Cell

R2-CHOP (Len days 1-10) / 64 pts / historical controls / GC/non GC Hans*
Other Combinations Len-R-CHOP in DLBCL

(REAL07) 49 pts / R-CHOP + Len 15mg d 1-14 / med age 69 (64-71) / 61% high-interm or high risk IPI

CR rate 81% in GC and 88% in non-GC (NS)

PFS based on COO / Hans

Phase III / LRCHOP21 vs RCHOP21 in untreated non-GCB DLBCL

6-8 cycles R-CHOP / high-intermediate or high-risk IPI
CR → rand Len or R2

<table>
<thead>
<tr>
<th>Response</th>
<th>Len (n = 22)</th>
<th>R2 (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2y DFS</td>
<td>90%</td>
<td>82%</td>
</tr>
<tr>
<td>2y OS</td>
<td>96%</td>
<td>72%</td>
</tr>
</tbody>
</table>

REMARC trial → Len Maintenance vs placebo post R-CHOP

Chiappella et al. ASH 2013, Abstract 850
Vitolo, Lancet Oncol, May 2014

Reddy et al. ASH 2013, Abstract 3661
Senior

**DLBCL**
- ≥ 80 years old
- ECOG : 0-2
- LVEF > 50%
- Creatinin clearance (MDRD) ≥ 40 ml/min

**DLBCL Evaluation - Randomization**
- Stratification on
  - CD10 expression: Positive/Negative
  - age: 80 to <80; ≥80 years old

**Follow-up phase:**
- every 3 months the first two years then every 6 months

**6-R miniCHOP (3 weeks cycles)**
- Pre-phase
  - D-7 to D-4
- R miniCHOP (IV)
  - D1 to D14
- R miniCHOP (SC)
  - D1 to D14
- Evaluation → Follow-up phase

**Evaluation → Follow-up phase**
- D-7 to D-4
- R miniCHOP (IV)
- R miniCHOP (SC)
- Follow-up phase
- 6-R miniCHOP (3 weeks cycles)

**Aspirin 100 mg**
- or LWMH

**Graph**
- ABC: 65% vs. non-ABC: 21%
REMARC study design

**Induction**
- **At diagnosis**
- **R-CHOP x 8/6**
- SD, PD study withdrawal

**CR/PR**
- Until 3 months after D1 of last R-CHOP

**Maintenance**
- **ARM 1**
  - Lenalidomide 25mg/day (cl creat 30-60=10)
  - 3 weeks every 4 weeks over 24 months

- **ARM 2**
  - Placebo
  - Daily for 3 weeks every 4 weeks over 24 months

**CR/PR**

**REGISTRATION**
Targeting “Drivers” or Key Kinases in DLBCL

Key Pathway Related Mutations Contribute to BCR / Signaling in ABC DLBCL

Rochewski, Nat Rev Clin Oncol, Jan 2014
PKCβ as a rational therapeutic target for DLBCL patients

- Feasible (one grade 4 toxicity; 7 grade 3)
- 12/55 patients experienced FFP for > two cycles
- 8 pts remained FFP for > four cycles

- Phase II study
- 55 pts with relapsed or refractory DLBCL
- PKCβ inhibitor (enzastaurin)
- orally
- Limited activity!

The level of expression of the target gene product can be determined by IHC in FFPE tissues

Robertson MT et al. J Clin Oncol 2007
PKCβ as a rational therapeutic target for DLBCL patients

Maintenance treatment: PRELUDE
Michael Crump, et al ASH 2013 Abst.371

Primary Endpoint: Disease-free survival
Secondary Endpoints: Overall survival, Safety, pharmacokinetic & Biomarker assessment

• DFS at 24 and 48 months were 79% and 70% for the enzastaurin arm, and 75% and 71% for placebo, respectively.
Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomized, phase 1b study.

**Ongoing combinations:**
- R-CHOP+/- ibrutinib (PHOENIX) in non-GC subtype DLBCL
- R2-I in r/r DLBCL

**Ph I: well tolerated, dose ph II 560 mg qd**

**Best Response DLBCL**  
N = 23

- **ORR** 100%
- **CR** 91%
- **PR** 9%

Younes, Lancet Oncology, Aug 2014
CD30 expression per IHC assessed by visual and computer-assisted methods.

In DLBCL

A. CR in 83-Year-Old Male with Relapsed DLBCL
   - 0% CD30 expression on neoplastic cells

B. 1.4% CD30 expression on all cells

C. CR in 76-Year-Old Male with Refractory DLBCL
   - 1% CD30 expression on neoplastic cells

D. 34% CD30 expression on all cells

Brentuximab vedotin demonstrates objective responses in a phase 2 study of relapsed/refractory DLBCL with variable CD30 expression

Key Points
- Brentuximab vedotin was active in DLBCL across a range of CD30 expression levels, and objective responses occurred in 44% of patients.

Jacobsen et al
Brentuximab vedotin demonstrates objective responses in a phase 2 study of relapsed/refractory DLBCL with variable CD30 expression

Key Points
- Brentuximab vedotin was active in DLBCL across a range of CD30 expression levels, and objective responses occurred in 44% of patients.

Randomized study
On going

Jacobsen et al

*Blood. 2015;125(9):1394-1402*
Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study.


Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study.


Abstract 704 Genomic Profiling Combining DNA and RNA Analysis of 112 Formalin-Fixed Paraffin-Embedded Diffuse Large B Cell Lymphoma Specimens Identifies a High Frequency of Clinically Relevant Genomic Alterations
Andrew M. Intlekofer, MD, PhD1 et al
Conclusion
Take Home Messages

• In Diffuse Large B cell Lymphoma biomarkers will help for diagnosis, characterization of subtypes and guide treatment.
• Several studies are on going to validate the role of biomarkers and treatment

• **AT DIAGNOSIS:** young patient who can fit intensive treatment
  • GC non GCB, at least by IHC
  • Myc, Bcl2 first IHC
  • completed by FISH if necessary
• **AT RELAPSE:**
  • GC or non-GCB if not done before,
  • add CD30
  • or any marker which can be associated with new drugs.
• All investigators:
  • Corinne Haioun
  • Hervé Tilly
  • Thierry Lamy
  • Gilles Salles
• All pathologists:
  • LYSA-P Thierry Molina
  • Philippe Gaulard
  • Josette Briere

and the patients