The history of lymphoma classification and the 2016 WHO revision

A journey from morphology to a multidisciplinary view

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Heterogeneity of Lymphoid Neoplasms

CD20

CD3

14 der(14)  18 der(18)

CLL/SL
MALT
LPL/Waldenström
FL
MCL
DLBCL
Burkitt
ALK+ ALCL
PTCL

Probability of survival

Years
Relevance of a Precise Diagnosis

- Epidemiological characterization
- Distinctive pathogenesis
- Clinical manifestations and evolution of the disease
- Different therapeutic strategies (from wait and see to very aggressive or specific target therapies)
- Neoplasms potentially curable
- Therapeutic regimens with iatrogenic risk
Malignant Lymphomas as Disease Entities
• Non-overlapping (mutually exclusive)
• Stratified according to cell lineage
Why Classify?

- Classification is the “language” of medicine
  - Diseases must be described and defined before they can be diagnosed and treated
- Disease entities should be clearly defined and clinically distinctive
- Consensus on terminology and definitions
  - Essential for both clinical practice and research
Lymphoma Classification: The history

The long & winding road

The early days
(<1975) Morphology

The great divide
(1975-1994) Morphology vs Functional view

Building consensus
(1994-2001)
The REAL Classification
The NHL Project

Courtesy of Dr S Swerdlow
Lymphoma Entities, Basic Discoveries, and Classifications

**Entities & Discoveries**
- HD
- FL
- Burkitt
- MM
  - Sarcoma
  - Sternberg
  - Waldestrom's
  - Macroglobulinemia
- Lymphosarcoma
  - Reticulum Cell Sarcoma

**Classifications**
- American Registry
- Robb-Smith
- Gall & Mallory
- Rappaport
- Dorfman
- BLNI
- Lukes & Collins
- Kiel
- WHO

**Immunology Genetics**
- Lymphocyte Transformation
- B - T cells
- Chromosomal Abnormalities

**Effective Therapy**

Timeline:
- 1900
- 1930
- 1940
- 1955
- 1965
- 1970
- 1975
NIH Meeting in Airlie, VA (1975) of clinicians and Hematopathologists who had proposed classifications.

“No consensus”

Morphological Perspective

National Cancer Institute Sponsored Study of Classifications of Non-Hodgkin’s Lymphomas
Summary and Description of a Working Formulation for Clinical Usage

THE NON-HODGKIN’S LYMPHOMA PATHOLOGIC CLASSIFICATION PROJECT

1982

Functional Perspective

Lukes, USA
Lennert/Kiel

Malignant Lymphomas
Other Than Hodgkin’s Disease
Histology, Immunology, Differential Immunology
K. Lennert
E. K. Müller-Hermelink
H. H. Stein
Springer-Verlag, Berlin Heidelberg New York
WHO Classification: Hematologic Neoplasms

- **“REAL” Classification (ILSG, 1994)**
  - List of Clinicopathologic Entities
  - Cell lineage and Differentiation
  - Integration of Morphological, Immunological, Genetic, Molecular and Clinical Information

- **NHL Classification Project (1999)**
  - University of Nebraska
  - Pathologists and Clinicians of 9 Centers around the world

- **WHO Classification (2001/2008)**
  - The first true international consensus
  - European Association for Haematopathology
  - Society of Hematopathology,
  - Clinical Advisory Committee
  - > 100 Authors
### Agreement Between Referral and Final Diagnosis

<table>
<thead>
<tr>
<th>Referral Diagnosis</th>
<th>Final Pathology Category</th>
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<tbody>
<tr>
<td></td>
<td>Indolent N=304</td>
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<tr>
<td>Indolent*</td>
<td>296 (97%)</td>
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<tr>
<td>Follicular, any grade</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Diffuse large B-cell</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Highly Aggressive</td>
<td>0</td>
</tr>
<tr>
<td>Unspecified B-cell lymphoma</td>
<td>4 (0.2%)</td>
</tr>
<tr>
<td>Other Cancer</td>
<td>0</td>
</tr>
</tbody>
</table>

* CLL/SLL, FL1-2, NMZL

*LaCasce A et al J Clin Oncol 2008*
Lymphomas as Malignant Counterparts of Specific Stages of Lymphocyte Maturation

- Immature B-cell
- Naive B-cell
- Mantle cell
- Centrocye
- Centroblast
- Plasma cell
- Memory B-cells
- B lymphoblastic leukemia/lymphoma
- Mantle cell lymphoma
- Follicular lymphoma
- Burkitt lymphoma
- DLBCL (some)
- Hodgkin lymphoma
- Marginal zone lymphoma
- Lymphoplasmacytic lymphoma
- CLL/SLL
- DLBCL (some)
- Plasma cell neoplasms

WHO Classification - 2016 update

- Diagnostic criteria
  - Morphology
  - Phenotype
  - Clinical Criteria
  - Molecular
  - Infectious agents
- Early steps in lymphoid neoplasms
- Categories with overlapping features between entities
- Introducing Personalized Medicine
Follicular lymphoma

CD10

Bcl-2

14 der(14)

18 der(18)
### Expert Pathologist Agreement With the Consensus Diagnosis

<table>
<thead>
<tr>
<th>Consensus Diagnosis</th>
<th>Dx 1 (%)</th>
<th>Dx 2-1 (%)</th>
<th>Dx2 (%) + Phenotype</th>
<th>Dx 3-2 (%)</th>
<th>Dx 3 (%)</th>
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</thead>
<tbody>
<tr>
<td>Marginal zone B-cell, MALT</td>
<td>84</td>
<td>2</td>
<td>86</td>
<td>0</td>
<td>86</td>
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<tr>
<td>Small lymphocytic (CLL)</td>
<td>84</td>
<td>3</td>
<td>87</td>
<td>0</td>
<td>87</td>
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<tr>
<td>Lymphoplasmacytoid</td>
<td>53</td>
<td>3</td>
<td>56</td>
<td>0</td>
<td>56</td>
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<tr>
<td>High grade B-cell, Burkitt-like</td>
<td>47</td>
<td>6</td>
<td>53</td>
<td>0</td>
<td>53</td>
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<tr>
<td>Marginal zone B-cell, nodal</td>
<td>55</td>
<td>8</td>
<td>63</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>77</td>
<td>10</td>
<td>87</td>
<td>0</td>
<td>87</td>
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<tr>
<td>Diffuse large B-cell</td>
<td>73</td>
<td>14</td>
<td>87</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>Precursor T-lymphoblastic</td>
<td>52</td>
<td>35</td>
<td>87</td>
<td>2</td>
<td>89</td>
</tr>
<tr>
<td>Anaplastic large T/null-cell</td>
<td>46</td>
<td>39</td>
<td>85</td>
<td>0</td>
<td>85</td>
</tr>
</tbody>
</table>

*NHL project, Blood 1997; 89: 3909-3918*
“Phenotype an numbers as diagnostic criteria”

Chronic Lymphocytic Leukemia

- Current definitions (> $5 \times 10^9$/L monoclonal lymphocytes with the CLL phenotype)
- SLL is the same disease but restricted to tissues of non-leukemic (< $5 \times 10^9$/L) patients without cytopenias

WHO 2008
Nodal Peripheral T-cell Lymphomas of TFH Origin

- Gene expression profiling and mutation analysis has helped to clarify the interrelationship among nodal T-cell lymphomas of TFH origin.

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- CD10
- CXCL13
- PD1
- ICOS
- BCL6
Gene expression profiling allowed reclassification of 14% of PTCL, NOS as AITL

Gene expression signatures of PTCL; Iqbal et al. *Blood* 2014
Clinical criteria in diagnosis

**DLBCL Topographic site**

- Primary mediastinal
- Intravascular
- Primary CNS
- Primary cutaneous DLBCL, leg type
Pediatric lymphomas (come of age)

Follicular Lymphoma Pediatric Type

- Children and young adults
- Striking male predominance
- Nodal presentation, head and neck
- Grade 3, blastic
- No diffuse areas
- High proliferation rate
- Lack of t(14;18)
- Excellent prognosis
- Local therapy / Watch & wait recommended

Louissaint A Jr et al Blood. 2012, 120:2395-404
### Genetic alterations in Pediatric Type Follicular Lymphoma

<table>
<thead>
<tr>
<th>Genes</th>
<th>PTFL (n=42) (%)</th>
<th>t(14;18)-neg FL (%)</th>
<th>tt(14;18)-pos FL* (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFRSF14</td>
<td>51</td>
<td>36</td>
<td>18-46</td>
<td>Ns</td>
</tr>
<tr>
<td>KMT2D</td>
<td>16</td>
<td>36</td>
<td>67-82</td>
<td>Ns</td>
</tr>
<tr>
<td>CREBBP</td>
<td>3</td>
<td>45</td>
<td>33-64</td>
<td>0.001</td>
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<tr>
<td>FOXO1</td>
<td>5</td>
<td>27</td>
<td>-</td>
<td>Ns</td>
</tr>
<tr>
<td>GNA13</td>
<td>11</td>
<td>0</td>
<td>-</td>
<td>Ns</td>
</tr>
<tr>
<td>EZH2</td>
<td>0</td>
<td>18</td>
<td>7-20</td>
<td>0.0049</td>
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</tbody>
</table>

Schmidt J et al Blood 2016

<table>
<thead>
<tr>
<th>Genes</th>
<th>PTFL (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP2K1</td>
<td>9 (38%)</td>
</tr>
<tr>
<td>MAPK1</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>RRAS</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

Louissaint A et al Blood 2016
“Large B-cell lymphoma with IRF4 rearrangement”

- New provisional entity segregated from other pediatric FL
- Waldeyer’s ring, head and neck nodal, bowel presentation
- Most commonly in children/young adults
- Follicular and diffuse areas with grade 3
- Germinal center phenotype (CD10/BCL6)
- BCL2 expression but no t(14;18)
- Strong IRF4 expression and IRF4 translocation
- Cases without the genetic alteration may be detected
- Treatment is often required

Molecular Definition of entities
ALK + and ALK – ALCL are Different Entities

Molecular Subtypes of DLBCL

- Acceptance (reluctantly) that, even if imperfect, IHC methods can be used for the diagnosis (Hans algorithm remains the most popular).
- Molecular methods for FFPE tissues on the horizon.
Lymphoma entities related to Infectious agents

<table>
<thead>
<tr>
<th>EBV+ Lymphoid neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>- EBV + LBCL of the elderly</td>
</tr>
<tr>
<td>- Extranodal NK/T-cell lymphoma, nasal type</td>
</tr>
<tr>
<td>- Epstein-Barr virus (EBV) positive T-cell lymphoproliferative diseases of childhood</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HHV8+ associated lymphoid neoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Primary effusion lymphoma</td>
</tr>
<tr>
<td>- HHV8 positive DLBCL, NOS</td>
</tr>
</tbody>
</table>

Early steps in Follicular and Mantle cell Lymphoma
“In Situ” and early involvement lesions

• “in situ” follicular “neoplasia”
  – Incidental finding
  – Low incidence of progression (<5%)
  – Need to exclude systemic lymphoma

• Partial involvement by FL
  – Stages I and II
  – 50% progress to overt FL

• “in situ” mantle cell “neoplasia”
• Mantle zone MCL
  – progress to overt FL

Jegalian AG et al Blood 2011
Mamessier E et al Haematologica 2014; 99: 802–810

Cyclin D1
Indolent T-cell Lymphoproliferative diseases of low malignant potential

Multiple mucosal polyps
Can affect entire GI Tract

Most common in:
small intestine
colon

Less often:
stomach
oral mucosa

Perry et al., Blood 2013, Indolent T-LPD of the GI Tract
Gray Zone Lymphomas in the WHO Classification

- Recognition of biological and pathological continuum in certain entities
- Not a single criteria recognizes these categories
- Not specific entities, but working categories that need further studies
- Keep purity of well defined entities
- Challenging for clinical management. BL, HL and DLBCL protocols differ substantially
### Diagnostic value of somatic mutations in mature small B-cell lymphoid neoplasms

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mutations/Markers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairy Cell Leukemia (v)</td>
<td><em>MAP2K1</em></td>
<td></td>
</tr>
<tr>
<td>Hairy Cell Leukemia (c)</td>
<td><em>MYD88 L265P</em></td>
<td></td>
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<tr>
<td>Waldenstrom M/LPL</td>
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<tr>
<td>79-100% HCL</td>
<td></td>
<td></td>
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<tr>
<td>4% Plasma cell myeloma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NHL (Other BRAF mut)</td>
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<td></td>
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<tr>
<td>50% HCLv</td>
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<tr>
<td>50% HCLc IGHV4-34</td>
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<td></td>
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<tr>
<td>0% HCL BRAFmut</td>
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<td></td>
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<tr>
<td>90% WM</td>
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</tr>
<tr>
<td>29% DLBCL-ABC</td>
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<td></td>
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<tr>
<td>6% MZL</td>
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<tr>
<td>3% CLL</td>
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</tbody>
</table>
Somatic Mutations and CNA in CLL and MBL
(Whole genome/exome sequencing)

Puente X et al Nature 2015
## Recurrently Mutated Pathways in Lymphoid Neoplasms

<table>
<thead>
<tr>
<th>Pathway</th>
<th>U-CLL</th>
<th>M-CLL</th>
<th>MCL</th>
<th>FL GCB</th>
<th>ABC</th>
<th>BL</th>
<th>SMZL</th>
<th>HCL</th>
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<tr>
<td>BCR-signaling</td>
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<td>NFkB</td>
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<td></td>
<td>+</td>
<td>+/-</td>
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<td>Chromatin Remodeling</td>
<td>+/-</td>
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<td>+</td>
<td>+/-</td>
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<td>TLR/MYD88</td>
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<td>DNA-damage</td>
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</tr>
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</table>
Clinical Relevance of Mutational Profiles in Lymphoid Neoplasms

- Diagnostic criteria to refine entities
- Identification of subsets of patients
- Prognostic and predictive significance
- Monitoring disease evolution: Dynamic evolution of mutational landscape
- Targets for therapy: Actionable mutations