UNDERSTANDING GENETIC AND MOLECULAR MARKERS IN LYMPHOMA

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Data Management Educational Session, Monday 23rd March 2015
Objectives

• To understand the importance of genetic and molecular markers

• To understand the nomenclature used

• To learn specific examples of genetic and molecular markers in lymphoma
Why do we need genetic and molecular markers in lymphoma?

- For diagnostic purposes
- For prognostication
Definition and diagnosis of lymphoma
HETEROGENOUS group of malignant neoplasms arising in the lymphoid system.
Old classifications

60-70′

RAPPAPORT CLASSIFICATION
- Nodular
  - Lymphocytic, poorly differentiated
  - Mixed, lymphocytic and histiocytic
- Histiocytic
- Diffuse
  - Lymphocytic, well differentiated
  - Mixed, lymphocytic and histiocytic
  - Undifferentiated
  - Burkitt's
  - Non-Burkitt's

70-80′

KIEL CLASSIFICATION
- Low grade
  - ML, lymphocytic
  - ML, lymphoplasmacytoid
  - ML, centroblastic, centrocytic
    - Follicular + A-diffuse
    - Diffuse
- High grade
  - ML, centroblastic
  - ML, immunoblastic
  - ML, large cell anaplastic
  - ML, Burkitt's
  - ML, lymphoblastic
  - Rare types

LUKES-COLLINS CLASSIFICATION
- Undefined cell
- T cell
  - Small lymphocyte
  - Cerebriform cell of Sezary's
  - Mycosis fungoides
  - Lymphoplasmacytoid cell
- B cell
  - Small lymphocyte
  - Plasmacytoid lymphocyte
  - Follicular center cell (FCC)
  - Small cleaved
  - Large cleaved
  - Small noncleaved
  - Large noncleaved
  - FCC subtypes: follicular; diffuse; follicular & diffuse
  - Immunoblastic sarcoma
- Histiocytic
- Cell of uncertain classification
- Unclassifiable

80-90′

NEW WORKING FORMULATION for Clinical Use
- Low-Grade
  A. Small lymphocytic (lymphocytic; plasmacytoid)
  B. Follicular, predominantly small cleaved
  C. Follicular, mixed, small cleaved and large cleaved cell
- Intermediate-Grade
  D. Follicular, predominantly large cell, cleaved and/or non-cleaved
  E. Diffuse, small cleaved cell
  F. Diffuse, mixed, large and small cell
  G. Diffuse, large cell, cleaved or non-cleaved
- High-Grade
  H. Large cell, immunoblastic -(B- or T-cell type)
  I. Lymphoblastic
  J. Small noncleaved cell (Burkitt's and non-Burkitt's)
  Miscellaneous
AIM to define specific entities according to:

- Morphology
- Immunophenotype
- Genetic
- Molecular biology
- Clinical presentation and course
Mature B-Cell Neoplasms
- Chronic lymphocytic leukemia / small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- Lymphoplasmacytic lymphoma / Waldenstrom macroglobulinemia
- Heavy chain disease
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extramedullary plasmacytoma
- Extramedullary marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type
- Nodal marginal zone lymphoma
- Follicular lymphoma
- Primarycutaneous follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma, NOS
  (T-cell / histiocyte-rich type; primary CNS type; primary leg skin type & EBV+ elderly type)
- Diffuse large B-cell lymphoma with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK+ large B-cell lymphoma
- Plasmablastic lymphoma
- Large B-cell lymphoma associated with HHV8+ Castleman disease
- Primary effusion lymphoma
- Burkitt lymphoma
- B cell lymphoma, unclassifiable, Burkitt-like
- B cell lymphoma, unclassifiable, Hodgkin lymphoma-like

Mature T-Cell & NK-Cell Neoplasms
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorder of NK-cells
- Aggressive NK-cell leukemia
- Systemic EBV+ T-cell lymphoproliferative disorder of childhood
- Hydroa vacciniforme-like lymphoma
- Adult T-cell lymphoma/leukemia
- Extramedullary T-cell/NK-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Hepato-splenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30+ T-cell lymphoproliferative disorder
- Primary cutaneous gamma-delta T-cell lymphoma
- Peripheral T-cell lymphoma, NOS
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, ALK+ type
- Anaplastic large cell lymphoma, ALK- type

Hodgkin lymphoma (Hodgkin disease)
- Nodular lymphocyte-predominant Hodgkin lymphomas
- Classic Hodgkin lymphomas
- Nodular sclerosis Hodgkin lymphoma
- Lymphocyte-rich classic Hodgkin lymphoma
- Mixed cellularity Hodgkin lymphoma
- Lymphocyte depletion Hodgkin lymphoma

Post-Transplant Lymphoproliferative Disorders (PTLD)
- Plasmacytic hyperplasia
- Infectious mononucleosis like PTLD
- Polymorphic PTLD
- Monomorphic PTLD (B & T/NK cell types)
- Classic HD type PTLD

Histioctytic and Dendritic Cell Neoplasms
- Histioctytic sarcoma
- Langerhans cell histiocytosis
- Langerhans cell sarcoma
- Interdigitating dendritic cell sarcoma
- Follicular dendritic cell sarcoma
- Fibroblastic reticular cell tumor
- Indeterminate dendritic cell sarcoma
- Disseminated juvenile xanthogranuloma
Morphology

- cHL Mixed cellularity
- cHL Nodular sclerosis
- Follicular lymphoma
- Diffuse large B-cell lymphoma
Immunophenotype

- Markers called ‘CD...’ (i.e. CD20)
- In lymph nodes assessed by immunohistochemistry (IHC)
Genetic markers

• Chromosomal abnormalities
  – Translocations
  – Deletions
  – Additions

• Detected by chromosome analysis (=cytogenetics=G-band) or FISH (=fluorescent in situ hybridization)
Molecular markers

- Result of chromosomal/genetic abnormalities: abnormal fusion DNA/RNA
- Detected by PCR
Genetic markers in lymphoma: Burkitt lymphoma

- Translocation of chromosome 8 (C-MYC gene) with
  - Chromosome 14 (IgH: heavy chain of immunoglobulins)
  - Chromosome 2 (Igκ: kappa light chain)
  - Chromosome 22 (Igλ: lambda light chain)
Genetic markers in lymphoma: t(8;14) by cytogenetics
Genetic markers in lymphoma: t(8;14) by FISH
Molecular markers: *BCL-2/IgH* rearrangement by PCR
Other examples of genetic markers in lymphoma

<table>
<thead>
<tr>
<th>Chromosomal translocation</th>
<th>Effect</th>
<th>Lymphomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(14;18)(q32;q21)</td>
<td>$BCL2$ deregulation</td>
<td>FL, DLBCL</td>
</tr>
<tr>
<td>3q27 rearrangements</td>
<td>$BCL6$ deregulation</td>
<td>DLBCL, PMBCL, FL</td>
</tr>
<tr>
<td>8q24 rearrangements</td>
<td>$MYC$ deregulation</td>
<td>BL, DLBCL, FL, MCL</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>$CCND1$ deregulation</td>
<td>MCL, MM</td>
</tr>
<tr>
<td>16p13 rearrangements</td>
<td>$CIITA$ and $SOCS1$ disruption</td>
<td>PMBCL, HL</td>
</tr>
<tr>
<td>t(11;18)(q21;q21)</td>
<td>$BIRC3$-$MALT1$ fusion gene</td>
<td>MALT</td>
</tr>
<tr>
<td>t(14;18)(q32;q21)</td>
<td>$MALT1$ deregulation</td>
<td>MALT</td>
</tr>
<tr>
<td>2p23 rearrangements, usually t(2;5)(p23;q35)</td>
<td>Fusion genes with $ALK$ (usually $NPM$-$ALK$)</td>
<td>ALK+ALCL</td>
</tr>
</tbody>
</table>
The same abnormality can be detected by different techniques

- t(14;18): cytogenetics, FISH
- **BCL-2/IgH** rearrangement: PCR
- bcl-2 over-expression: IHC
How genetic/molecular markers can define a specific type of lymphoma: anaplastic large cell lymphoma

ALCL ALK+ or ALCL ALK-
How genetic/molecular markers can define a specific type of lymphoma: ALK+ anaplastic large cell lymphoma
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How molecular markers can help in the diagnosis of a specific type of lymphoma: MYD88 and LPL/WM

MYD88 L265P Somatic Mutation in Waldenström’s Macroglobulinemia

<table>
<thead>
<tr>
<th>Submitted Diagnosis</th>
<th>No. Positive for MYD88 L265P/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL</td>
<td>13/13a</td>
</tr>
<tr>
<td>SMZL</td>
<td>0/6b</td>
</tr>
<tr>
<td>CLL/SLL</td>
<td>0/9</td>
</tr>
<tr>
<td>PCM</td>
<td>0/8c</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>0/7</td>
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<tr>
<td>Hairy cell leukemia</td>
<td>1/13</td>
</tr>
<tr>
<td>Hairy cell leukemia variant</td>
<td>0/2</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>0/6</td>
</tr>
</tbody>
</table>

How genetic/molecular markers can help in the diagnosis of a specific type of lymphoma

AIM to define specific entities according to:

• Clinical presentation and course
• Morphology
• Immunophenotype
• Genetic
• Molecular biology
How genetic/molecular markers can help in the diagnosis of a specific type of lymphoma

- **Clinical presentation and course:** male, 65 yrs, good performance status, asymptomatic, 12 months 2cm cervical, axillary lymph nodes, splenomegaly
  - ?indolent lymphoma

- **Morphology:** LN biopsy shows diffuse infiltration by small/medium lymphocytes
  - ?MCL, ?SLL

- **Immunophenotype:** B-cell markers, CD5+
  - ?MCL, ?SLL

- **Genetic**
  - t(11;14): MCL
  - 13q-, 11q-, 12+: CLL

- **Molecular biology**
  - Cyclin d1 over-expression: MCL
Is it really so important to distinguish different types of lymphoma?

5-yr OS >70%

5-yr OS <30%
Prognosis and impact on treatment
Impact on prognosis of genetic/molecular markers: 17p del in CLL
Impact on treatment of genetic/molecular markers: 17p del in CLL

Treated with fluda/alkylating

Gónzalez et al, J Clin Oncol, 2011

Treated with alemtuzumab

Stilgenbauer et al, J Clin Oncol, 2009
Impact on treatment of genetic/molecular markers: 17p del in CLL

The poor prognosis of 17p- can be abrogated by allogeneic transplant

Dreger et al, Blood, 2010
Prognostic value of genetic/molecular markers: double/triple hit lymphomas

- Presence of BCL-2/C-MYC/BCL-6 translocations

Green et al, J Clin Oncol, 2012

Can SCT eradicate the poor prognosis associated with DHL?
How to record genetic markers

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>Abnormality</th>
<th>Absent</th>
<th>Present</th>
<th>FISH used</th>
<th>Not evaluated</th>
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</thead>
<tbody>
<tr>
<td>Mantle cell lymphoma or Waldenstrom macroglobulinaemia</td>
<td>del 17p</td>
<td>☐</td>
<td>☐</td>
<td>☐ No</td>
<td>☐</td>
</tr>
<tr>
<td>BL or Intermediate BL/DLCBL</td>
<td>t(2;8)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td></td>
<td>t(8;14)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td></td>
<td>t(8;22)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td></td>
<td>t(14;18)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td></td>
<td>myc rearrangement</td>
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<td>✔</td>
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<td></td>
<td>BCL-2 rearrangement</td>
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<td>✔</td>
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<td></td>
<td>BCL-6 rearrangement</td>
<td></td>
<td></td>
<td>✔</td>
<td></td>
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<tr>
<td>Peripheral T-cell lymphoma</td>
<td>t(2;5)</td>
<td>☐</td>
<td>☐</td>
<td>☐ No</td>
<td>☐</td>
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</table>
How to record IHC

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>Phenotype</th>
<th>Positive</th>
<th>Negative</th>
<th>Not evaluated</th>
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<tbody>
<tr>
<td>Mantle cell lymphoma</td>
<td>Cyclin D1</td>
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<tr>
<td></td>
<td>SOX11</td>
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<tr>
<td>BL or Intermediate BL/DLCBL</td>
<td>MYC</td>
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<tr>
<td>Intermediate BL/DLCBL</td>
<td>BCL-2/IgH</td>
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<td></td>
<td>BCL-6</td>
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<tr>
<td>Peripheral T-cell lymphoma</td>
<td>ALK</td>
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How to record molecular markers

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>Marker</th>
<th>Present</th>
<th>Absent</th>
<th>Not evaluated</th>
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<tbody>
<tr>
<td>Mantle cell lymphoma</td>
<td>TP53 mutation</td>
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<tr>
<td>BL or Intermediate BL/DLBCL</td>
<td>myc rearrangement</td>
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<tr>
<td>Intermediate BL/DLBCL</td>
<td>BCL-2 rearrangement</td>
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<tr>
<td>Intermediate BL/DLBCL</td>
<td>BCL-6 rearrangement</td>
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<tr>
<td>Waldenstrom macroglobulinaemia</td>
<td>MYD88 mutation</td>
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<td></td>
<td>TP53 mutation</td>
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<td>Peripheral T-cell lymphoma</td>
<td>ALK rearrangement</td>
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</table>
Conclusions

• Genetic/molecular abnormalities define specific entities

• Genetic/molecular abnormalities help in the differential diagnosis of lymphoma

• Genetic/molecular abnormalities define the prognosis and

• Genetic/molecular abnormalities guide the treatment
Thank you!!