Molecular Hematopathology

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April 2013
Disclosure(s)

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Charles E. Hill, MD, PhD
Outline

• Molecular alterations in hematopathology
• Methods
• Lymphomas
• Leukemias
• Summary
Molecular Alterations in Hematopathology

• Rearrangements
• Physiologic (antigen receptors)
• Pathologic (translocations)
• Mutations
• Gains/Losses
Physiologic Rearrangements

- Normal part of immune system
- Occur as both B and T cells mature
- Determine antigen specificity
- May be used to determine clonality in B and T cells
Pathologic Rearrangements

- Dysregulated normal protein
- Chimeric protein with novel protein function

Enhancer/promoter  Oncogene  Chimeric product
Mutations

• Point mutations (e.g. JAK2 V617F)
• Larger mutations (e.g. FLT3 ITD)
• Activating vs. Inactivating
• Helpful in some situations to assess drug resistance
Gains/Losses

• May affect prognosis
• Gene dosage
• Loss of heterozygosity
Indications for Molecular Testing

- Diagnosis
- Classification
- Prognosis
- Response to therapy/detection of relapse
- Minimal residual disease testing
- Drug selection?
Major Methods

- Southern blot (mostly historical for hematopathology)
- Karyotyping
- FISH
- PCR
  - Various detection methods
  - Q-PCR (Q-RT-PCR)
- Arrays (CGH/SNP, Expression)
- Next Generation Sequencing/Whole Genome Analysis
Southern Blot

• Usually separate nucleic acids by gel electrophoresis
• Immobilization of nucleic acids on nylon
• Target specific probe
• Typically chemiluminescent detection
• Requires large amounts of nucleic acid
Karyotyping

• “Whole Genome Analysis”
• Gross structural abnormalities
• Requires viable cells (metaphase spreads)
• Low sensitivity (~5%)
FISH

- Specific targets
- Very good for balanced translocations
- Copy number variation
- Localizes abnormality to cells of interest
- Moderate sensitivity (~1-2%)
- Can select cells for analysis (e.g. CD138 selection for plasma cells)
Karyotyping

45,X,-X,t(8;21)(q22;q22)
FISH

- Can be performed on multiple specimen types
- Tissue does not need to be viable
- Can detect cryptic translocations
- Large probe size can detect multiple breakpoints
- More sensitive than cytogenetics
- Less sensitive than PCR
- Easier to run single samples
PCR (and variants)

- Very sensitive (1% and much less)
- May be quantitative
- Multiple possible detection methods
- DNA or RNA
- Most commonly used technique for molecular hematopathology
HRM, DGGE, dHPLC, etc.
Arrays

• CGH/SNP arrays
  • “Whole Genome Analysis”
  • Good for copy number variation
  • Not useful for balanced translocation

• Expression arrays
  • Very uncommon for clinical use
  • Can classify some processes into multiple categories
NGS

- Not commonly used clinically
- Very expensive
- True whole genome analysis
- Depth of coverage issues
- Analysis algorithms still have trouble with balanced translocations
## Clonality Testing

<table>
<thead>
<tr>
<th>Target</th>
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<tbody>
<tr>
<td>Immunoglobulin Locus</td>
<td></td>
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<tr>
<td>Heavy Chain</td>
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<tr>
<td>Kappa Light Chain</td>
<td></td>
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<tr>
<td>Lambda Light Chain</td>
<td></td>
</tr>
<tr>
<td>T-cell Receptor Locus</td>
<td></td>
</tr>
<tr>
<td>Gamma</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td></td>
</tr>
<tr>
<td>Alpha</td>
<td></td>
</tr>
</tbody>
</table>
Antigen Receptors

B-cell

Immunoglobulin

T-cell

T-cell receptor

Antigen
IGH@ Rearrangement

1. DJ rearrangement

2. V-DJ rearrangement

3. High power view

Diagram courtesy of Dr. Adam Bagg
IGH@ gene rearrangement and PCR

gel-based PCR product detection

<table>
<thead>
<tr>
<th>Size</th>
<th>Poly</th>
<th>Mono</th>
<th>Mono</th>
<th>Mono</th>
<th>Poly</th>
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</thead>
<tbody>
<tr>
<td>Neg</td>
<td></td>
<td></td>
<td></td>
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</tr>
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</table>
Polyclonal B cells
TRG@ Rearrangement

V\text{\textregistered} segments

1 2 3 4 5 6 7 8 9 10 11

J\text{\textregistered} segments

P1 P P1 P2

C\text{\textregistered}

1 2

J\text{\textregistered} segments

C\text{\textregistered}

VJ rearrangement

High power view

Multiplex PCR

V\text{\textregistered}1-8

V\text{\textregistered}9

V\text{\textregistered}10

V\text{\textregistered}11

Diagram courtesy of Dr. Adam Bagg
Polyclonal T cells
Indications for Clonality Testing

- Atypical lymphoid proliferations
- Equivocal immunophenotype
- Assistance in establishing lineage?
- Monitoring disease (MRD?)
- Multifocal disease
Flow Cytometry

- Sensitive
- Can determine lineage and/or type of lymphoma
- Able to distinguish clonality for B cells (based on immunoglobulin light chain distribution (kappa vs lambda))
- Difficult to distinguish clonality for T cells
- Need intact cells
- Cannot be done on formalin fixed tissue
Limitations of Clonality Analysis

• Tests only determine clonality and must be interpreted in the clinical and pathologic context

• Clonality does not imply malignancy
  – Clonal but not malignant:
    • Lymphomatoid papulosis
    • MGUS
    • Multicentric Castleman Disease
Limitations of Clonality (cont.)

• Not all clones are detectable using PCR based assays
• Sensitivity of 1:1000 cells to 1:100 cells
• Clonal TCR or IgH locus does not define lineage
  – Some lymphomas rearrange both
  – Lineage infidelity in ALL and immunodeficiency states
Interpretation

- Clonality does not equal malignancy
- Lack of detectable clone does not rule out clonality
- Tiered testing - diminishing returns
Better Basis to Behold B- (and T-) cell clones: BIOMED 2

- 8 IG
  - 5 IGH
    - 3 VJ
    - 2 DJ
  - 2 IGK
    - 1 VJ
    - 1 Kde
  - 1 IGL
    - 1 VJ
- 6 TCR
  - 3 TRB
    - 2 VJ
    - 1 DJ
  - 2 TRG
  - 1 TRD

- TRB analysis
  - 38 primers
  - 325 reactions

- hierarchy of IG GR
- 100% IG-lambda+ will be IGK+
- ... but ...
- ~5-10% IG-kappa+ will be IGL+
- IGK and IGL also subjected to SHM, but ? less so
- Kde reaction not affected by SHM

Side courtesy of Dr. Adam Bagg
Lymphoma

- Follicular lymphoma
- Mantle Cell Lymphoma
- MALT lymphoma
- CLL/SLL
- Cutaneous T cell Lymphoma
- Anaplastic Large Cell Lymphoma
- Diffuse Large B Cell Lymphoma
- Others
Histology
Follicular Lymphoma

- 2nd most common lymphoma (~35%)
- Germinal center B cell origin
- Can be nodal or disseminated
- Follicular pattern on morphology
- CD10+, BCL6+, CD43-
- Usually indolent, may transform (~30%)
- Somatic hypermutation can decrease detection of clonal population using IGH@
Follicular Lymphoma

- t(14;18) testing used to aid diagnosis
- Only about 85% positive
- Positive t(14;18) does not establish a diagnosis
- IGH@ positive in about 80%
- IGK@ somewhat better for follicular
- In t(14;18) negative cases, extra copies of chromosome 18/BCL2 or translocation involving BCL6
BCL2 gene rearrangements

BCL2 on 18q21

IGH@ on 14q32

VCR

MBR

ICR

MCR

PCR [~75%]

Southern blot

FISH

Classical cytogenetics

Slide courtesy of Dr. Adam Bagg
IgH Clonality Analysis
IgH – Bcl-2 Translocation
IgH – Bcl-2 Translocation
Bone Marrow Biopsy
Mantle Cell Lymphoma

- Neoplasm of Mantle B cells
- Male predominance
- Nodal, extranodal, disseminated
- Cyclin D1+, CD5+, CD43+
- t(11;14) upregulates Cyclin D1

Image courtesy of Dr. Adam Bagg
Mantle Cell Lymphoma

- t(11;14) most commonly detected by FISH
- IHC for Cyclin D1
- PCR or RT-PCR (limited availability)
Marginal Zone Lymphoma

• Neoplasm of marginal zone B cells
• Associated with chronic inflammatory processes
  • Infections
  • Autoimmune illness
• MALT lymphoma
• Generally indolent, but can transform
MALT Lymphoma

• Most common in the stomach, but can occur in many places

• Gastric MALT lymphoma associated with *H. pylori* infection

• In early disease, treating with antibiotics can be curative
Extranodal MZLs – MALTomas – site-dependence

- **MALT1**
  - API2
    - stomach
    - lung
  - MALT1
    - 18q21
    - ~50%

- **BCL-10**
  - IGH@
    - parotid
    - ocular adnexa
    - liver
  - BCL-10
    - 1p22
    - ~5%

- **FOXP1**
  - IGH@
    - thyroid
    - ocular adnexa
    - skin
  - FOXP1
    - 3p14
    - ~10%

- t(11;18)
- t(14;18)
- t(1;14)
- t(1;2)
- t(3;14)

Side courtesy of Dr. Adam Bagg
MALT Lymphoma

• Gastric - API2-MALT1 translocation associated with lack of response to antibiotics

• Other translocations probably also associated with independence from inflammatory process

• May test via FISH, PCR, IHC?
Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

• Neoplasm of small B cells
• Male predominance
• CD5+, CD23+, light chain restricted
• Level of maturation associated with prognosis
• Molecular testing not really used for diagnosis
FISH for CLL/SLL

• Used for prognosis
• 17p deletion = poor prognosis (p53)
• 11q deletion = poor prognosis (ATM)
• +12 = Intermediate prognosis (CDK4?)
• Isolated 13q deletion = good prognosis (miRNA’s 15a and 16-1)
CLL/SLL and Somatic Hypermutation

• B cells without SHM are considered less mature than those with SHM
• Normally pre-germinal center B cells have not undergone SHM and memory B cells have
• CLL/SLL with SHM has significantly better prognosis
• Can be tested by molecular methods, but generally use surrogate marker
CLL/SLL and Somatic Hypermutation

- Testing usually by IHC for ZAP70
- Not a great test

Image courtesy of Dr. Adam Bagg
Cutaneous T cell Lymphoma
TRG@ Testing
Mycosis Fungoides

• Not all have demonstrable clonal T cell receptor gene rearrangement
• May use TRG@ testing to determine if clone is in peripheral blood (Sezary Syndrome)
• Indolent, may transform
Sezary Syndrome

• T-cell clone present in skin and peripheral blood
• May detect atypical T-cells not visible on peripheral smear
Anaplastic Large Cell Lymphoma

• Medium-large, atypical cells in background of smaller lymphocytes
• CD30+ (Ki-1), need to distinguish from Hodgkin
• Often CD3 negative
• ALK+ not required for diagnosis

Image courtesy of Dr. Adam Bagg
ALCL

- t(2;5) most common
- Test via FISH, cytogenetics, RT-PCR
- ALK+ yields better prognosis
# ALCL

<table>
<thead>
<tr>
<th>Translocation</th>
<th>ALK Partner</th>
<th>Frequency</th>
<th>ALK Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(2;5)(q23;q25)</td>
<td>NPM1</td>
<td>75%</td>
<td>cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nucleus</td>
</tr>
<tr>
<td>t(1;2)(q21;p23)</td>
<td>TPM3</td>
<td>15%</td>
<td>cytoplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>membrane</td>
</tr>
<tr>
<td>t(2;3)(p23;q21)</td>
<td>TFG</td>
<td>2%</td>
<td>cytoplasm</td>
</tr>
<tr>
<td>inv(2)(p23;q35)</td>
<td>ATIC</td>
<td>2%</td>
<td>cytoplasm</td>
</tr>
<tr>
<td>t(2;17)(p23;q23)</td>
<td>CLTC</td>
<td>2%</td>
<td>cytoplasm</td>
</tr>
<tr>
<td>t(X;2)(q11;p23)</td>
<td>MSN</td>
<td>1%</td>
<td>membrane</td>
</tr>
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</table>
Diffuse Large B cell Lymphoma

• Not a well defined entity, many different categories
• Really multiple types of lymphoma
• Current stratification is based on morphology or clinicopathologic features
DLBCL Morphologic Categories

- Centroblastic
- Immunoblastic
- Anaplastic
- T cell rich
- Plasmablastic
- Lymphomatoid granulomatosis type
DLBCL Clinical Categories

- Primary CNS lymphoma
- Primary effusion lymphoma
- Cutaneous large B cell lymphoma
- Intravascular large cell lymphoma
- Thymic large B cell lymphoma
Gene Expression and DLBCL

• Study done by Alizadeh, et al. at Stanford (Nature. 2000; 403 (6769): 503-11)
• Showed that DLBCL has two distinct gene expression groups
• Germinal center-like B cell lymphoma (good prognosis)
• Activated B cell like lymphoma (poor prognosis)
Gene Expression and DLBCL

- Not routinely performed clinically
- Difficult to interpret
- IHC used most commonly to categorize (CD10, BCL6, MUM1, etc.?)
Other B Lymphomas

• Burkitt – MYC translocations (t(8;14), etc.)

• Lymphoplasmacytic lymphoma - deletions of 6q23 (50%) and 13q14, gains of 3q13-q28, 6p and 18q

• Splenic marginal zone lymphoma – deletion 7q21-32 (40%) or translocation involving CDK6
Other T Lymphomas

• T PLL – inv 14(q11;q32) (80%) or t(14;14)(q11;q32) (10%)
• Hepatosplenic T cell lymphoma – iso(7q)
• Enteropathy associated T cell lymphoma – 70% homozygous for DQB1*02
Myeloma

• Typically FISH panel and conventional cytogenetics
• FISH panels vary (especially for hyperdiploidy)
• May perform arrays (CNV or expression)
## Myeloma

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Prognosis</th>
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<tbody>
<tr>
<td>del 13q</td>
<td>Poor</td>
</tr>
<tr>
<td>Trisomy (odd #’s except 13)</td>
<td>Good</td>
</tr>
<tr>
<td>1q+</td>
<td>Poor</td>
</tr>
<tr>
<td>Del 17p (TP53)</td>
<td>Poor</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>Good?</td>
</tr>
<tr>
<td>t(4;14), t(14;16)</td>
<td>Poor</td>
</tr>
</tbody>
</table>
Leukemia

• MPN’s
• CML
• PV/ET/PMF
• AML
• ALL
Chronic Myeloproliferative Neoplasms

- Chronic myelogenous leukemia
- Polycythemia vera
- Essential Thrombocythemia
- Primary myelofibrosis
- Myelodysplastic syndromes
CML

- 1-2 per 100,000 population
- 1.75:1 male predominance
- t(9;22) – BCR-ABL1
- Nowell and Hungerford – “Philadelphia Chromosome” (der 22)
- Janet Rowley
- Molecular monitoring standard of care
CML

- BCR – Breakpoint Cluster Region
- ABL1 - V-abl Abelson murine leukemia viral oncogene homolog 1
- Translocation produces fusion protein with novel function
- RT-PCR
BCR-ABL

- e1a2: ALL p190
- e13a2
- e13a3
- e14a2
- e14a3
- e19a2: CML p210
- CML p230
Why test?

- BCR-ABL1 targeted by small molecules
- Prognosis
- Monitoring response
- Prediction of relapse
Testing for BCR-ABL1

- Karyotype
- FISH
- PCR
- RT-PCR
  - Competitive RT-PCR
  - Nested RT-PCR
  - qRT-PCR
NCCN Guidelines

From NCCN 2.2013, qPCR should be performed:

At diagnosis

Every 3 months when responding to therapy. After CCyR, every 3 months for 3 years, then every 3-6 months

For rising transcript levels (1 log) with MMR, repeat in 1-3 months
QRT-PCR
Harmonizing Results

- Median measurement of 30 shared baseline samples established as 100%
- 0.1% is Major Molecular Response
- For example, if median ratio BCR-ABL/control is 0.75, MMR = 0.00075
- International Scale defined by these two points
What does an IS do?

- An International Scale helps standardize quantification
- An IS does not improve pre-analytic issues
- An IS does not reduce inherent assay variability
- An IS does improve reporting
Control Genes and Quantification

- ABL1 is the most commonly used control gene
- BCR was used as control gene for IRIS trial labs
- GUSB used by some
- EAC found ABL1, GUSB, and B2M suitable (Beillard, Leukemia, 2003, 17:2474)
- BCR was not reported in study by EAC
Kinase domain mutations

P = P loop
- ATP-binding site
- ? worst mutations

B = Binding domain
- where imatinib binds

C = Catalytic domain

A = Activation loop
- conformation altered
- affects imatinib binding
- closed: inactive
- open: active

> 100 different mutations

2-10% of patients

>10% of patients

green

red

Slide courtesy of Dr. Ada, Bagg
MPN

- BCR-ABL1
- V617F of JAK2 (exon 14)
- Exon 12 of JAK2
- MPL (5-10% of ET)
Signalling Through the Erythropoietin Receptor

Diagram: EPO-R (Erythropoietin Receptor) activates JAK2, which in turn activates STAT5.
V617F JAK2

• Disrupts “autoinhibitory loop”
• Activating mutation
• Appears as though EpoR is occupied all the time
• Somatic in erythro-myeloid precursors
V617F JAK2

- Single base change eliminates a BsaX restriction enzyme site
- PCR followed by digestion and electrophoresis
- PCR
- Q-PCR
JAK2 Testing

• Aid in confirming diagnosis

• Possible future targeted therapies

• Reclassification of myeloproliferative disorders
JAK2 Assay

Controls
V617F JAK2
AML with recurrent cytogenetic abnormalities

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Prior Class</th>
<th>Genes</th>
<th>Prognosis</th>
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<tbody>
<tr>
<td>t(8;21)</td>
<td>M2</td>
<td>RUNX1-RUNXT1</td>
<td>Good</td>
</tr>
<tr>
<td>t(15;17)</td>
<td>M3</td>
<td>PML-RARA</td>
<td>Good</td>
</tr>
<tr>
<td>inv 16</td>
<td>M4Eo</td>
<td>CBFB-MYH11</td>
<td>Good</td>
</tr>
<tr>
<td>t(11q23)</td>
<td>M4/M5</td>
<td>MLL-?</td>
<td>Poor</td>
</tr>
<tr>
<td>t(1;22), t(6;19), t(8;16), inv 3</td>
<td></td>
<td></td>
<td>all poor prognosis</td>
</tr>
</tbody>
</table>
Acute promyelocytic leukemia

- Distinguished by Auer rods in cytoplasm of blasts
- DIC in 85%
- Anemia, thrombocytopenia due to blast proliferation
• t(15;17) – PML-RARA
• ATRA responsive
• Multiple breakpoints in PML
  • Short, Long, Variant
• PCR, RT-PCR, QRT-PCR, FISH
Variant APL

• t(11;17) – PLZF-RARA
• Not ATRA responsive
• t(5;17) – NPM1-RARA
• ATRA responsive?
AML with Normal Cytogenetics

• Not genetically stable, no gross chromosomal abnormalities
• Mutations common
• Many associated with prognosis
FLT3

- FLT3 = FMS-like Tyrosine Kinase 3
- Over-expressed on AML blasts
  - ~20-30% of AML cases
  - Also on t(15;17) blasts
- Typical mutation is Internal Tandem Duplication (and D835)
- Reduced overall survival
  - Controversial in older patients
  - Correlates with poor prognosis in younger patients with normal cytogenetics
• Nucleophosmin
• Most common mutation in AML with normal cytogenetics (~60%)
• Good prognosis
• No effect if FLT3 mutated
• Common insertion
## ALL vs Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Translocation</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>t(4;11)</td>
<td>AFF1-MLL</td>
</tr>
<tr>
<td>Children</td>
<td>t(12;21)</td>
<td>ETV6-RUNX1</td>
</tr>
<tr>
<td>Adults</td>
<td>t(9;22)</td>
<td>BCR-ABL1</td>
</tr>
</tbody>
</table>
t(4;11) ALL

- Most common leukemia of infants
- May be present/diagnosed at birth
- Poor prognosis
t(12;21) ALL

- Most common translocation in ALL of children (~25%)
- Good prognosis
- Cryptic
  - Need FISH or molecular studies
t(9;22) ALL

- Poor prognosis
- Different breakpoints (e1a2 vs other)
- p190 vs p210
- Most common translocation in adult ALL
- Quantitative testing?
Other Changes

• C-KIT mutations in mastocytosis
• IDH1/2
• TET2
• Gene amplifications
• Pharmacogenetics
• Etc, etc, etc … … …
Summary

• Molecular testing integral for hematopathology

• Hematologic neoplasms often defined by molecular abnormalities

• Diagnosis, response, MRD, relapse