Molecular Markers in Hematologic Malignancy: Ways to locate the needle in the haystack....

Marcie Tomblyn, MD, MS
Associate Member of BMT
H. Lee Moffitt Cancer Center
Objectives

• Review the types of testing for hematologic malignancies

• Understand rationale for molecular testing

• Become familiar with certain disease specific molecular tests
Testing for Heme Malignancies

- **Histology/ Morphology**
  - What the cells look like
- **Immunohistochemistry (IHC)**
  - Staining the cells to identify specific markers
- **Flow cytometry**
  - Looks at individual cells based on staining for specific markers
- **Cytogenetics**
  - Chromosome analysis
- **FISH**
  - Targeting specific chromosomes
- **Molecular studies**
  - Identifying abnormal gene products
Morphology and IHC

ALL with blasts in the peripheral blood (a) and marrow (b).

IHC documents the blasts are positive for TdT (c) and PAX-5 (d).
Flow and Cyto

Clonal population of B-cells expressing CD19 and CD5 and kappa restriction

Conventional cytogenetics showing monosomy 7 and t(8;13)(q24.3;q14)
FISH

**Red signal:** ABL gene on a normal chromosome 9
**Green signal:** BCR on a normal chromosome 22
**Yellow (combined):** BCR/ABL fusion on the Philadelphia chromosome t(9;22)

**Yellow signal:** Trisomy 12 in a patient with CLL
Polymerase Chain Reaction

• Method to rapidly and highly specifically amplify DNA fragments

• Advantages
  – Common, fairly inexpensive
  – Rapid, sensitive and specific

• Disadvantages
  – Requires knowledge of the specific nucleotide sequence
  – Sensitivity may result in false-positive results
Other Techniques

• Gene Expression Profiling
  – Microarray technology to identify a molecular signature of a tumor

• Proteomics
  – Microarray technology to identify protein expression profiles of tissue/cell type
Sensitivity and Specificity

• Sensitivity
  – The ability to detect one malignant cell in many normal cells (the needle in the haystack)

• Specificity
  – The likelihood that the test can discriminate between malignant and normal cells
# Maximum Sensitivity

<table>
<thead>
<tr>
<th>Technique</th>
<th># of blasts required/100,000 cells to detect disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy Standard Expert</td>
<td>5000 blasts</td>
</tr>
<tr>
<td>Microscopy Expert</td>
<td>1000 blasts</td>
</tr>
<tr>
<td>Karyotype analysis</td>
<td>5000 blasts</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>10 blasts</td>
</tr>
<tr>
<td>Polymerase Chain Reaction (PCR)</td>
<td>0.1 blasts</td>
</tr>
</tbody>
</table>
Purpose of Molecular Tests

• Diagnostic accuracy

• Prognostic markers to predict outcomes

• Monitor for minimal residual disease
Prognostication

- Normal karyotype AML with or without Flt3-ITD mutation

AML Model based on molecular mutations

Grossmann V et al. Blood 2012;120:2963-2972
For those still awake.....

It’s time to bury your head!
BCR/Abi

- Fusion protein that results in increased activity of a tyrosine kinase
- Present in CML, ALL (30-35% adult B-cell), and some AML
- Can be followed quantitatively with a Major Molecular Response (MMR) determined as $\leq 0.1\%$ BCR-ABL (ratio of BCR-ABL/BCR)
IgH and T-cell Receptor Gene Rearrangements

- Diverse gene product to allow for wide immunity
- Mutations result in clonal population
- May have false positives due to recovery post-transplant or ongoing infection
CEBP-α

• On chromosome 19q
• Normal function: Transcription factor for maturation of granulocytes
• Mutated in 15 – 20% of patients with AML
• Improved outcomes for patients with this mutation, independent of other mutations
**Flt3**

- Chromosome 13q
- Normal function: tyrosine kinase that is important for proliferation and differentiation of hematopoietic progenitor cells
- Mutated in 30 – 40% of AML patients
  - ITD, D835 point mutation, overexpression without mutation
- Uncontrolled proliferation leads to inferior overall and disease-free survival
NPM1

- On chromosome 5q
- Normal function: controls genomic stability
- Mutation in 50 – 60% AML
  - Either insertion or deletion
  - Increased in women
- Sole mutation present, improved outcomes
  - Outweighed by other negative mutations like FLT3
MLL

- On chromosome 11q
- Normal function: encodes enzyme that regulates homeostasis
- Mutation in 7 – 8% of AML patients as a partial tandem duplication
- Decreases overall survival
**IDH1 and IDH2**

- IDH1 on Chromosome 2q
- IDH2 on Chromosome 15q
- Normal function: critical to the Krebs cycle
- Mutations in 15 – 30% AML patients
- Results in increased expansion of HSCs and impaired differentiation
BCL-1 (CCND1)

- On chromosome 11q
- Normal function: cell cycle regulation
- In Mantle cell lymphoma t(11;14)
  - Moved upstream of IgH gene (chromosome 14)
- Mutation leads to dysregulated cell cycle and proliferation
BCL-2

• On chromosome 18q
• Normal function: inhibit apoptosis and modulates cell cycle progression
• In Burkitt’s lymphoma, moves upstream of IgH t(14;18)
• Overexpression leads to prolonged cell survival
BCL-6

• On chromosome 3q
• Normal function: represses transcription
• Often overexpressed in DLCL
• Mutation leads to increased proliferation
TP53

• On chromosome 17p
• Tumor suppressor that prevents uncontrolled cell growth
• Mutation of 17p found in many cancers
  – CLL, DLCL, solid cancers
CIBMTR Disease Forms

Info on molecular testing now being collected

– AML:  CEBP-α, FLT3-D835 point mutation, FLT3-ITD mutation, IDH1, IDH2, NPM1, MLL
– ALL:  BCR/ABL, TEL-AML/AML1
– MDS:  ASXL1, JAK2, ETV6, EZH2, P53, RUNX1
– Lymphoma:  BCL-1 (CCND1), BCL-2, BCL-6, IgH, TCR
BMT CTN 1202

• Biomarker protocol
• Obtain samples to correlate molecular signatures with clinical outcomes of transplant
  – DNA, RNA, and Protein
• Data collection for post-transplant complications
  – Acute GVHD, chronic GVHD, lung injury, TMA, VOD, serious infections, relapse, death
Summary

• Molecular testing is a powerful tool
  – Guide treatment decisions
  – Can monitor for low levels of disease
• Constantly evolving field with new discoveries
• Impact of various markers requires large populations of patients to determine true importance