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

- Least sensitive
- Most sensitive

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</td>
<td>5000 blasts</td>
</tr>
<tr>
<td>Standard 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

For those still awake.....
It’s time to bury your head!

BCR/Abl

- 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 ≤ 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