Blasts In The Bone Marrow: Information From Aspirate Smears, Biopsy Sections and Flow Cytometry
Importance of Accurate and Precise Identification of Bone Marrow Blasts

- Diagnosis
- Classification
- Prognosis
- Treatment decisions
Confounding Issues for Accurate and Precise Assessment of Blasts

• Technical
  – Specimen collection and quality of specimen
  – Quality of the preparation

• Other factors
  – Drug induced changes
    • G-CSF, chemotherapy, etc

• Limitations of method
  – Aspirate smears, biopsy sections, flow cytometry
Modalities for Assessing Blasts in the Bone Marrow

1. Bone Marrow Aspirate Smears
Myeloblasts Types 1 and 2 and a Promyelocyte
# Aspirate Smears For Assessment Of Blasts In Bone Marrow

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Historical standard</td>
<td>• Hemodilution—myelofibrosis</td>
</tr>
<tr>
<td>• Rapid and ease</td>
<td>• Technical quality of smear</td>
</tr>
<tr>
<td>• Allows for identification of abnormal features</td>
<td>• Limited cell count subject to statistical error</td>
</tr>
<tr>
<td>• Lineage identification</td>
<td>• Variation in morphologic criteria for a blast (subjectivity)</td>
</tr>
<tr>
<td>• Assessment of background features--myelodysplasia</td>
<td>– Distinction of Type 2 &amp; 3 blasts from promyelocytes</td>
</tr>
<tr>
<td>• Cytochemical evaluation</td>
<td>– Normal from neoplastic</td>
</tr>
<tr>
<td>– MPO, NSE</td>
<td>– Myeloblast and lymphoblast</td>
</tr>
</tbody>
</table>
Modalities for Assessing Blasts in the Bone Marrow

2. Bone Marrow Trephine Biopsy Sections
## Biopsy Sections For Assessment Of Blasts In Bone Marrow

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Truest visualization of intact marrow architecture</td>
<td>• Immunohistochemistry is required to distinguish blasts from other immature cells and to identify blast lineage</td>
</tr>
<tr>
<td>• Recognition of abnormal relationships---ALIP</td>
<td>• Distinction of normal from abnormal blasts may be problematic</td>
</tr>
<tr>
<td>• Blast counts not altered by myelofibrosis</td>
<td>• Difficult to identify small numbers of neoplastic blasts and</td>
</tr>
<tr>
<td>• Applicability of immunohistochemistry</td>
<td>• CD34 negative blasts</td>
</tr>
<tr>
<td>• Identification of lineage</td>
<td></td>
</tr>
</tbody>
</table>
Modalities for Assessing Blasts in the Bone Marrow

3. Flow Cytometry
## Enumeration of Blasts in Multiple Aspirate Specimens

<table>
<thead>
<tr>
<th>Aspirate Pull</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HLA-DR+/CD11b-CD34+/HLA-DR+</td>
<td>3.2</td>
<td>2.0</td>
<td>1.8</td>
</tr>
<tr>
<td>2. HLA-DR+/CD11b-CD34+/HLA-DR+</td>
<td>2.9</td>
<td>1.9</td>
<td>1.4</td>
</tr>
<tr>
<td>3. HLA-DR+/CD11b-CD34+/HLA-DR+</td>
<td>1.3</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>4. HLA-DR+/CD11b-CD34+/HLA-DR+</td>
<td>1.1</td>
<td>0.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Criteria to Define a Phenotypic Myeloblast

- Combining CD45 and side scatter with different reagent combinations:
  - CD34 and HLA-Dr/CD11b or CD117
  - Correct for lymphoblasts
- Redundancy covers for aberrant loss of any one antigen on the myeloblast population
- Expressing the proportion of myeloblasts / non-erythroid cells
  - Circumvents the problem of variable erythroid cell lysis in the preparation
Normal blasts
Hematogones

- Bone marrow B-cell precursors

- Size and morphology bridge mature lymphocytes and neoplastic lymphoblasts

- Large percentages seen in healthy infants and young children

- Increased in
  - Regenerating marrows
  - Autoimmune or congenital cytopenias
  - Lymphoma, neuroblastoma
  - AIDS

Mckenna Leuk and Lymph 2004
Clinical Necessity Of Identifying Neoplastic Blasts In low percentage

• Distinction of normal from low level neoplastic blasts at diagnosis of some myeloid neoplasms (MDS) and in post-therapy marrow

• Minimal residual disease (MRD) assessment
Sensitivity of Methods for Detection of Neoplastic Blasts in Bone Marrow

- Sensitivity
  - Bone marrow smears ---- 1% to 5%
  - Bone marrow biopsies --- ?
  - Cytogenetic banding ----- 1% to 5%
  - FISH ---------------------- 0.1% to 5%
  - Flow Cytometry -------- 0.01% to 0.005%
  - Molecular (PCR) --------- 0.01% to 0.0001%
Prognostic Significance of End of Consolidation MRD in ALL

Blood; 111: 5477-5485
Minimal Residual Disease Detection
# Flow Cytometry Assessment For Bone Marrow Blasts

## Advantages

- Best sensitivity
  - MRD assessment
- Large number of cells counted
  - Dramatically reduces counting statistical error
- Objective criteria can be used to define a blast
- Lineage identification

## Issues

- Specimen hemodilution
  - Aspirate procedure
  - Preparation
- Frequent lack of correlation with blast counts on aspirates and sections
- Lack of consensus criteria to define a phenotypic myeloblast
- No visualization of cells
Summary--Generalizations

• Aspirate smears typically provide the most accurate quantification of blasts in the marrow and frequently identify lineage

• Trephine biopsies are superior for quantification of blasts in fibrotic bone marrow and provide material for immunophenotyping

• Flow cytometry is most sensitive for detection of low numbers of blasts and MRD and best for lineage characterization
Summary

• Bone marrow smears, biopsy sections and flow cytometry are all effective methods for assessment of blasts in the bone marrow
• Each may independently contribute diagnostic information
Summary

• One of the three methods may prove most suitable in a given clinical setting
• Often it is the integration of data from these different modalities that provides diagnostic information
• For optimal practice of bone marrow hematopathology all 3 methodologies should be used