Molecular Testing of Acute Myeloid Leukemia

No relevant conflict of interest.
Objectives

• To describe the progressive shift toward molecular diagnosis in acute leukemia, especially with regard to Acute Myeloid Leukemia (AML)

• To offer a practical approach for the molecular testing of common AML categories with recurrent genetic abnormalities and other prognostic markers
French-American-British Classification

AML

- M0: AML with Minimal Differentiation
- M1: AML without Maturation
- M2: AML with Granulocytic Maturation
- M3: Acute Promyelocytic Leukemia with t(15;17)
- M4: Acute Myelomonocytic Leukemia (AMML)
- M5: Acute Monoblastic/Monocytic Leukemia
- M6: Acute Erythroid Leukemia
- M7: Acute Megakaryoblastic Leukemia
2001 WHO Classification, 3rd Edition

Context

• 1st and 2nd “Editions” were a series of Journal articles.

• The so called “3rd Edition” of “WHO Classification of Haematopoietic and Lymphoid Tumours” was the first classification scheme to be published as a single volume and gain world-wide acceptance.

• Collaborative Project of:
  – The European Association for Haematopathology.
  – Society for Hematopathology

• “[We] believe that the only thing worse than an imperfect classification is multiple competing classifications.”
2001 WHO Classification, 3rd Edition
Myeloid Neoplasms

- Myelodysplastic Syndromes (MDS)
- Myeloproliferative Disorders (MPD)
- Acute Myeloid Leukemia (AML)
- Myelodysplastic/Myeloproliferative Syndromes (MDS/MPD)
2001 WHO Classification, 3rd Edition

AML Classification

- AML with Recurrent Genetic Abnormalities
  - AML with t(15:17); PML/RARA (FAB M3)
  - AML with t(8:21); RUNX1/RUNX1T1
  - AML with t(16:16) or inv(16); CBFB/MYH11
  - AML with 11q23 rearrangements; MLL
- AML with Multilineage Dysplasia
- AML Associated with Therapy
- AML NOS
  - AML with Minimal Differentiation (FAB M0)
  - AML without Maturation (FAB M1)
  - AML with Granulocytic Maturation (FAB M2)
  - Acute Myelomonocytic Leukemia – AMML (FAB M4)
  - Acute Monoblastic/Monocytic Leukemia (FAB M5)
  - Acute Erythroid Leukemia (FAB M6)
  - Acute Megakaryoblastic Leukemia (FAB M7)
  - Acute Panmyelosis with Myelofibrosis
  - Acute Basophilic Leukemia
  - Myeloid Sarcoma

4 Major Types of AML
17 Subtypes of AML
A few additional sub-subtypes (not listed)
2001 WHO Classification, 3rd Edition

“Blast-phemy”

• A major change with regard to AML definition:
  – FAB classification required \( \geq 30\% \) blasts in the BM in order to diagnose AML.
  – With the 3rd Edition of the WHO the blasts could be \( \geq 20\% \) in the BM OR Blood

• In addition, situations in which a diagnosis of AML could be made even if blasts were \(<20\%\)
  – Myeloid Sarcoma
  – Pure Erythroid Leukemia
  – In the presence of t(15;17), t(8;21) or inv(16)/t(16;16).
Cytogenetically Abnormal Neoplasms Reported in the Literature

2008 WHO Classification, 4th Edition

AML

• AML with Recurrent Genetic Abnormalities (Growing)
  – AML with t(15:17); PML/RARA (FAB M3)
  – AML with t(8:21); RUNX1/RUNX1T1
  – AML with t(16:16) or inv(16); CBFB/MYH11
  – AML with t(9;11); MLLT3/MLL (Narrowed Definition)
  – AML with t(6;9); DEK/NUP214 (New)
  – AML with inv(3) or t(3;3); RPN1/EVI1 (New)
  – AML with t(1;22); RBM15/MKL1 (New)
  – Provisional Entities:
    • AML with mutated NPM1 (New)
    • AML with mutated CEBPA (New)

• AML with Myelodysplasia-Related Changes (Renamed)

• AML Associated with Therapy

• AML NOS (Diminishing – now 25-30% of AML)
  – AML with Minimal Differentiation (FAB M0)
  – AML without Maturation (FAB M1)
  – AML with Granulocytic Maturation (FAB M2)
  – Acute Myelomonocytic Leukemia – AMML (FAB M4)
  – Acute Monoblastic/Monocytic Leukemia (FAB M5)
  – Acute Erythroid Leukemia (FAB M6)
  – Acute Megakaryoblastic Leukemia (FAB M7)
  – Acute Panmyelosis with Myelofibrosis
  – Acute Basophilic Leukemia

• Myeloid Sarcoma (Promoted)

• Myeloid Proliferations Related to Down Syndrome (New-ish)
  – Transient Abnormal Myelopoiesis
  – Myeloid Leukemia associated with Down Syndrome

• Blastic Plasmacytoid Dendritic Cell Neoplasms (Un-misclassified)

7 Major Types of AML

24 Subtypes of AML

A few additional sub-subtypes (not listed)
2008 WHO Classification, 4th Edition
AML

• AML with Recurrent Genetic Abnormalities (Growing)
  – AML with t(15:17); PML/RARA (FAB M3)
  – AML with t(8:21); RUNX1/RUNX1T1
  – AML with t(16:16) or inv(16); CBFB/MYH11
  – AML with t(9;11); MLLT3/MLL (Narrowed Definition)
  – AML with t(6;9); DEK/NUP214 (New)
  – AML with inv(3) or t(3;3); RPN1/EVI1 (New)
  – AML with t(1;22); RBM15/MKL1 (New)
  – Provisional Entities:
    • AML with mutated NPM1 (New)
    • AML with mutated CEBPA (New)
So how do you incorporate all these changes in your offering of molecular testing in AML?
New Molecular Markers of AML Prognosis

**FLT3, NPM1, CEBPA, KIT, MLL, IDH1/2 and WT1**

- These mutations are known to have substantial impact on outcomes.
- The problem is:
  - These mutations can occur individually or together in various combinations.
  - Can occur in association with genetically defined types of AML.
  - Predominantly lack specific morphologic features.
  - Can improve prognosis in some circumstances and worsen prognosis in others.
2008 WHO AML Classification, 4th Edition
AML with Mutated NPM1 or CEBPA

• These two were chosen as **Provisional** categories:
  – Occur with among the greatest frequency.
  – Don’t generally occur in AML with Recurrent Cytogenetic Abnormalities.
  – Usually don’t occur together.
  – May have some identifying morphologic features.
  – Occur mainly in *de novo* AML.
  – Improve prognosis; comparable to AML with t(8;21) or AML with inv(16)/t(16;16) – thus, may affect management
AML with Mutated NPM1 or CEBPA

![Graph showing relapse-free survival over time for different genotypes](image)

- Mutant NPM1 without FLT3-ITD
- Mutant CEBPA
- Other genotypes

Relapse-Free Survival (%)

Time (years)

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Other genotypes</th>
<th>Mutant NPM1 without FLT3-ITD</th>
<th>Mutant CEBPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>212 90 60 38 27 14 9 5 4 2 0</td>
<td>136 102 83 64 50 34 24 13 8 3 1</td>
<td>62 40 32 26 17 11 6 4 4 3 0</td>
</tr>
</tbody>
</table>

P < .001

Marcucci G. JCO. 2011; epub
AML with Mutated *NPM1* or *CEBPA*
# New Molecular Markers of AML Prognosis

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Established</td>
</tr>
<tr>
<td>Favorable</td>
<td>NPM1</td>
</tr>
<tr>
<td></td>
<td>CEBPA</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>FLT3-ITD</td>
</tr>
<tr>
<td></td>
<td>KIT</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfavorable Over expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical Testing</td>
<td>Recommended</td>
</tr>
</tbody>
</table>

Old Molecular Markers of AML Prognosis
## Recommended Molecular Testing
### AML Classification & Risk Stratification

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Perform Test</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Favorable</strong></td>
<td>t(15;17)</td>
<td><em>FLT3?</em></td>
</tr>
<tr>
<td></td>
<td>t(8;21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inv(16)/t(16;16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>KIT</em></td>
<td>Prognosis/Therapy</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td>Normal karyotype</td>
<td>Prognosis/Therapy</td>
</tr>
<tr>
<td></td>
<td>Nonrecurrent Abnormalities</td>
<td>Prognosis/Classification</td>
</tr>
<tr>
<td></td>
<td><em>FLT3-ITD</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>NPM1</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>CEBPA</em></td>
<td></td>
</tr>
<tr>
<td><strong>Unfavorable</strong></td>
<td>Inv(3)/t(3;?)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(6;9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(6;11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complex karyotype</td>
<td></td>
</tr>
</tbody>
</table>
AML

CEBPA

• Name: CCAAT/enhancer binding protein (C/EBP) α
• Gene: 19q13.1
• Role: Transcription factor
• Consequence: decreased transcription
• Prognostic Significance : Good
• Hematopathologic association: Coexpression of T cell antigens
• Frequency in AML: 10%
AML

CEBPA Mutations

• Two major categories:
  – N-terminal insertions and deletions result in truncated protein that lacks an activation domain.
  – C-terminal mutations alter the dimerization and DNA-binding properties of CEBPA, leading to a decrease in protein activity.

• CEBPA can also be silenced through promoter hypermethylation, correlates with prognosis.

• Mutation detection: Slow adoption of CEBPA testing in clinical laboratories.
AML

**NPM1**

- **Name**: Nucleophosphosphomin
- **Gene**: 19q13.1
- **Role**: Shuttle protein, promotes cell growth
- **Consequence**: Cytoplasmic mislocalization; dysregulated p53
- **Prognostic Significance**: Good
- **Hematopathologic association**: Cup like nuclear invaginations, CD34-
- **Frequency in AML**: 25-30%
AML

NPM1 Mutations

- Most are small insertions (4–11 bp) in exon 12
- Mutation detection: Mislocalized protein can be detected by IHC, flow cytometry, PCR-CE, Sequencing
**AML**

**FLT3**

- **Name**: FMS-related tyrosine kinase 3
- **Gene**: 13q12
- **Role**: Receptor tyrosine kinase
- **Consequence**: Increased signal transduction
- **Prognostic Significance**: Poor
- **Hematopathologic association**: Cup like nuclear invaginations
- **Frequency in AML**: 12%
AML

**FLT3 Mutations**

- *FLT3* internal tandem duplications (ITD) are significant
- *FLT3* (TKD) mutations are not significant
- Location of ITD: mutations that integrate into the β-2 sheet of KD1 are associated with a particularly adverse prognosis
- Variability of length of ITD: Not sure
- Mutation detection: PCR-CE
AML

KIT

• Name: v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
• Gene: 4q11-q12
• Role: Receptor tyrosine kinase
• Consequence: Increased signal transduction
• Prognostic Significance: Poor in adults
• Hematopathologic association: CBF-AML (16-46%)
• Frequency in AML: 2-8%
AML

*KIT* Mutations

Mutations tend to occur in:
- exon 8 (dimerization domain)
- exon 11 (JM domain, ITD)
- exon 17 (AL, mostly D816V)

Detection:
- DHPLC
- Sequencing
- AS-PCR
Recommended Molecular Testing
AML Classification & Risk Stratification - Summary

PROGNOSIS

- **Good**
  - t(15;17)
  - t(8;21)/inv(16)/t(16;16)
- **Intermediate**
  - t(9;11)
  - t(6;9)
  - t(6;11)
- **Poor**
  - Complex karyotype

- **NPM1+/FLT3-**
  - CEBPA+
- **NPM1-/FLT3+**
  - Normal karyotype
  - Nonrecurrent Abnormalities

* NPM1+/FLT3+, NPM1-/FLT3-, and CEBPA- cases.
Land Ahoy!
Molecular Testing of Acute Myeloid Leukemia Demystified