Update on Clinically Relevant Genetic Alterations in AML and Recommendations for Molecular Testing

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Speakers Disclosure

In the past 12 months, we have not had a significant financial interest or other relationship with the manufacturer(s) of the product(s) or provider(s) of the service(s) that will be discussed in my presentation.
Outline

• Introduction and historic background
• Brief review of cytogenetic abnormalities
• Gene mutations
  – 2008 WHO classification
  – 2016 probable updates
  – Comprehensive analysis
  – Recommendations for molecular testing
• Myeloid neoplasms with germline predisposition
Morphological Classification

• FAB-French American British
  – Introduced in 1976
  – Based on the type of cell from which the leukemia developed and its degree of maturity (morphology and cytochemistry)
FAB Classification

- Stem cell
  - Erythroid committed progenitor
  - Myeloid committed progenitor
    - Promonocyte/Monoblast
    - Myeloblast
      - Promyelocyte
        - Neutrophil
  - Megakaryocytic committed progenitor
FAB- Prognosis

A

M3

years from start of therapy

Percent Survival

0 1 2 3 4 5 6 7 8

0 25 50 75 100

AML M0: N = 20 (Censored 8)
AML M1: N = 117 (Censored 43)
AML M2: N = 198 (Censored 87)
AML M3: N = 41 (Censored 32)

B

M4eo

years from start of therapy

Percent Survival

0 1 2 3 4 5 6 7 8

0 25 50 75 100

AML M4: N = 104 (Censored 39)
AML M4 Eo: N = 38 (Censored 30)
AML M5: N = 74 (Censored 30)
AML M6: N = 20 (Censored 10)
Cytogenetic Classification

Chromosomal Abnormalities

• Detected in over half of adult AML
• Most important predictor of outcome
• Define diagnostic categories in WHO classification
  – AML with recurrent cytogenetic abnormalities
  – AML with myelodysplasia related changes

Recurrent Cytogenetic Abnormalities in 2008 WHO Classification

Risk largely determined by recurrent genetic abnormality

Intermediate risk but very heterogeneous

Normal Karyotype 45%

Others

Complex

Standard chemotherapy; without allogeneic stem cell transplant

Up front allogeneic stem cell transplant

High risk

Intermediate risk

Low risk

t(8;21)

inv(16)/t(16;16)

t(6;9)

inv(3)/t(3;3)

t(15;17)

t(16;16)/inv(16)

t(9;11)

t(9;11)

Recurrent Cytogenetic Abnormalities in 2008 WHO Classification

Low risk

High risk

Intermediate risk
Genetic Testing for APL

- Karyotyping
- FISH
  - May miss a small subset of PML-RARA
- RT-PCR
  - More sensitive/Quantifiable
  - Suitable for follow up
2016 Probable Updates on Cytogenetic Subgroups

• Refine APL with PML-RARA
  – Some AMLs with RARA variant translocations (PLZF-RARA and STAT5B-RARA) are associated with poor outcomes and resistance to ATRA

• AML with BCR-ABL1
  – Important to recognize for potential upfront TKI therapy
  – p190 or p210 transcript may be seen
  – NPM1 mutations are frequent
  – Clinical features may be similar to the CML-blast phase
  – Deletion of antigen receptor genes (IGH, TCR), IKZF1 and/or CDKN2A may support a diagnosis of de-novo disease

Konoplev et al., Leuk Lymphoma. 2013 Jan;54(1):138-44.
Gene Mutations in AML

• ~50% of AML have no significant chromosomal abnormality (CN-AML)

• Clinical utility of detecting mutations in AML:
  – Diagnosis (disease defining mutations)
  – Prognosis
  – Targeted therapy
  – Disease monitoring (MRD)
Genomic Landscape of AML

- An average of 13 mutations found in genes
- Of these, an average of 5 are in genes that are recurrently mutated
- There are at least one driver mutation in nearly all AML samples
- A complex interplay of genetic events contributes to AML pathogenesis in individual patients

AMLs have less mutations compared to the other neoplasms.

# Recurrent genetic mutations in AML

<table>
<thead>
<tr>
<th>Name</th>
<th>Physiologic Function</th>
<th>Frequency in CN-AML</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutations in current clinical practice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPM1</td>
<td>Nuclear-cytoplasmic shuttling phosphoprotein</td>
<td>30-50%</td>
<td>Favorable</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>Receptor tyrosine kinase</td>
<td>20-30%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>CEBPA</td>
<td>Transcription factor</td>
<td>8-15%</td>
<td>Favorable</td>
</tr>
<tr>
<td>KIT</td>
<td>Receptor tyrosine kinase</td>
<td>30% of CBF-AML</td>
<td>Unfavorable</td>
</tr>
<tr>
<td><strong>Mutations under investigation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLT3-TK</td>
<td>Receptor tyrosine kinase</td>
<td>5-10%</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Transcription factor</td>
<td>10-15%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>IDH1/IDH2</td>
<td>Epigenetic modifier</td>
<td>15-30%</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>Epigenetic modifier</td>
<td>20-30%</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>TET2</td>
<td>Epigenetic modifier</td>
<td>10%</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Chromatin modifier</td>
<td>5-10%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>MLL-PTD</td>
<td>Epigenetic modifier</td>
<td>5-10%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>WT1</td>
<td>Transcription factor</td>
<td>10-15%</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>TP53</td>
<td>Cell cycle regulator</td>
<td>2-5%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>RAS</td>
<td>Membrane associated signaling</td>
<td>10%</td>
<td>Neutral</td>
</tr>
<tr>
<td>PHF6</td>
<td>Chromatin modifier</td>
<td>&lt;5%</td>
<td>Unfavorable</td>
</tr>
</tbody>
</table>

Behdad and Betz. “Molecular Testing in Acute Myeloid Leukemia” in *Diagnostic Molecular Pathology, First Edition. Elsevier*
“Two-hit” pathogenesis paradigm

• Gilliland and Griffin proposed a two step pathogenesis model for AML

• Two types of mutations:
  – Class 1 (promote proliferation):
    • Receptor tyrosine kinase genes (\textit{FLT3}, \textit{KIT}, \textit{RAS})
  – Class 2 (impair differentiation):
    • Chromosomal abnormalities such as t(8;21), inv(16)
    • Gene mutations: \textit{CEBPA}, \textit{RUNX1} and \textit{MLL}, \textit{NPM1}

Gilliland DG, Griffin JD. \textit{Blood} 2002; 100(5): 1532-42
Gene mutations in WHO

• 2008 WHO actionable mutations:
  – Diagnostic mutations (defining a provisional entity): $NPM1$, $CEBPA$
  – Prognostic mutations: $FLT3$, $KIT$

• 2016 possible updates:
  – Possible new entities: $RUNX1$
  – Update on the existing entities: $NPM1$, $CEBPA$
Nucleophosmin 1 (NPM1)

- Nucleolar phosphoprotein, shuttles basic proteins between nuclear and cytoplasmic compartments

- Abnormal cytoplasmic localization of the NPM1 protein (by IHC) led to the discovery of *NPM1* mutations

**NPM1 (5q35)**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Freq (%)</th>
<th>Nucleotide Sequence</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td>GATCTCTG----GCAGT----GGAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLWQWRKSL</td>
</tr>
<tr>
<td>A</td>
<td>80</td>
<td>GATCTCTGTCTGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLCLAVEEVSRLK</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>GATCTCTGCGATGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLCMAVEEVSRLK</td>
</tr>
<tr>
<td>C</td>
<td>&lt; 1</td>
<td>GATCTCTGCATGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLCVAEEVSRLK</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>GATCTCTGCATGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLCLAVEEVSRLK</td>
</tr>
<tr>
<td>E</td>
<td>&lt; 1</td>
<td>GATCTCTG----GCAGTCTCTTGGCCAGGAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLWQSLAQVSLRK</td>
</tr>
<tr>
<td>F</td>
<td>&lt; 1</td>
<td>GATCTCTG----GCAGTCTCTTGGAGAAGGAAGTCTCTTTAAGAAAATAG</td>
<td>DLWQSLAVEKSVSLRK</td>
</tr>
<tr>
<td>~40 more</td>
<td>7</td>
<td>GATCTCTG----GCAGTCTCTTGGAGAAGGAAGTCTCTTTAAGAAAATAG</td>
<td></td>
</tr>
</tbody>
</table>
NPM1 mutations: Clinical Implication

- Most common genetic aberration in AML
- ~30% of adult AML and 5% pediatric AML
- Prognosis: Favorable outcomes in the absence of cytogenetic abnormalities and FLT3-ITD
- Mutations are stable over the course of disease
  - Useful in monitoring of disease and detection of MRD
  - Founder mutations

Chromosomal abnormalities common in \textit{NPM1}-mutated AML

- +8, +4, -Y, \textbf{del (9q)}, +21 common
- Secondary chromosomal abnormalities
- Phenotypic characteristic and expression profile similar to CN- \textit{NPM1} mutated AML
- Prognosis remains good despite these chromosomal abnormalities

NPM1 2016 Updates

- Moved from provisional to permanent entity
- *NPM1*-AML with morphologic evidence of dysplasia
  - In the absence of chromosomal abnormalities and history of MDS; *NPM1* trumps dysplasia
  - In the presence of del(9q), still remains *NPM1*-AML

CCAAT/ enhanced binding protein α CEBPA mutations

• ~5-10% of AML
• Confer favorable outcomes in CN-AML
• Two types of mutations:
  – Biallelic (dmCEBPA)
  – Monoallelic (smCEBPA)
CEBPA Encodes Two Isoforms

- **p42**: Promotes Differentiation
  - TAD1
  - TAD2
  - bZIP
  - Transcription activation

- **p30**: Promotes Proliferation
  - TAD2
  - bZIP
  - DNA Binding and Dimerization
Double Mutation *CEBPA*

Truncating Mutations (nonsense/frameshift) → **p30**

In-frame Mutations (dup/ins/del) → **p42**

**TAD1** | **TAD2** | **bZIP**

Promotes Proliferation

Leukemogenesis

Differentiation

p42 X

p30 X

p2 X
dmCEBPA and NOT smCEBPA Confer favorable outcomes

Fasan A, et al. Leukemia (23 September 2013)
CEBPA
Molecular testing

• Sequencing-based assay is advantageous for CEBPA mutation testing
  – Detection of point mutation
  – Immediate distinction of single and double mutations positive cases
• CEBPA testing is technically challenging
  – Most NGS platforms not optimized
  – Sanger-sequencing assays will maintain an important role
• Missense variants
  – Pathogenic in C-terminal
  – Unknown significance in other regions
• Co-occurring NPM-1 mutations are mainly limited to smCEBPA
CEBPA 2016 Possible Update

- dmCEBPA (not the smCEBPA) upgraded to permanent category
- CEBPA-mutated AML and morphologic evidence of multilineage dysplasia remains in this category

FMS-Like Tyrosine Kinase 3 (FLT3)

- **FLT3L**
- **Juxtamembrane domain**
- **ITD**
- **Point mutation**
- **Disregulated mitogenic activity**
- **Constitutive activity**

Diagram showing the domains of FLT3 and the effects of point mutations and ITD on kinase activity.
FLT3 Mutations

FLT3-ITD
- Found in approximately 20% of AML
- Range 3 - 400 bp (median size 48 bp)
- Always in-frame

- FLT3-TKD mutations found in ~ 5% to 10% of AML
  - Mainly at codons 835 and 836

Frohling S, J Clin Oncol 23:6285-6295, 2005
**FLT3 mutations:**

**Clinical Implications**

- **Prognosis:**
  - *FLT3*-ITD confers worse outcomes
  - Impact of *FLT3*-KD mutations remain controversial

- **Minimal residual disease**
  - Not ideal given variability in relapse

- **Therapy:**
  - Stem cell transplantation
  - Phase II and III clinical trials evaluating FLT3 tyrosine kinase inhibitors underway
**FLT3-ITD:**

Important Considerations

- **FLT3** mutation status may change during relapse
  - May appear or disappear
  - Not founder mutation

- Homozygous mutations (LOH) confer a worse outcome
  - Result of acquired uniparental disomy
  - Mostly seen in relapse
  - Reporting should indicate **FLT3-ITD** high

**FLT3 mutations in APL**

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>FLT3</th>
<th>N</th>
<th>CR (%)</th>
<th>P-value</th>
<th>3-year OS</th>
<th>P-value</th>
<th>3-year DFS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiyoi (1997)</td>
<td>ITD−</td>
<td>59</td>
<td>80%</td>
<td>0.2</td>
<td>60%</td>
<td>0.67</td>
<td>74%</td>
<td>0.34</td>
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<tr>
<td></td>
<td>ITD+</td>
<td>15</td>
<td>93%</td>
<td></td>
<td>53%</td>
<td></td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Shih (2004)a</td>
<td>ITD−</td>
<td>85</td>
<td>95%</td>
<td>1</td>
<td>80%</td>
<td>0.86</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITD+</td>
<td>22</td>
<td>100%</td>
<td></td>
<td>79%</td>
<td></td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Yoo (2006)</td>
<td>ITD−</td>
<td>66</td>
<td>89%</td>
<td>0.54</td>
<td>NR</td>
<td></td>
<td>71%</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>ITD+</td>
<td>9</td>
<td>83%</td>
<td></td>
<td>NR</td>
<td></td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>Kainz (2002)</td>
<td>ITD−</td>
<td>13</td>
<td>53%</td>
<td>&lt;0.05</td>
<td>53%</td>
<td>0.06</td>
<td>81%</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>ITD+</td>
<td>8</td>
<td>100%</td>
<td></td>
<td>85%</td>
<td></td>
<td>46%</td>
<td></td>
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<tr>
<td>Au (2004)</td>
<td>ITD−</td>
<td>65</td>
<td>82%</td>
<td>0.06</td>
<td>73%</td>
<td>0.052</td>
<td>NR</td>
<td></td>
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<tr>
<td></td>
<td>ITD+</td>
<td>17</td>
<td>59%</td>
<td></td>
<td>35%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noguera (2002)</td>
<td>ITD−</td>
<td>57</td>
<td>96%</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td>66%</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>ITD+</td>
<td>33</td>
<td>97%</td>
<td></td>
<td>NR</td>
<td></td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>Callens (2005)</td>
<td>ITD−</td>
<td>72</td>
<td>100%</td>
<td>0.21</td>
<td>89%</td>
<td>0.09</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITD+</td>
<td>45</td>
<td>98%</td>
<td></td>
<td>73%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuchenbauer</td>
<td>Wild</td>
<td>58</td>
<td>NR</td>
<td></td>
<td>88%</td>
<td>0.09</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>-2005</td>
<td>ITD+b</td>
<td>47</td>
<td>NR</td>
<td></td>
<td>78%</td>
<td></td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Chillon (2004)</td>
<td>Wild</td>
<td>44</td>
<td>72%</td>
<td>NS</td>
<td>61%</td>
<td>0.45</td>
<td>80%</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>ITD+c</td>
<td>16</td>
<td>88%</td>
<td></td>
<td>65%</td>
<td></td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>Gale (2005)</td>
<td>Wild</td>
<td>115</td>
<td>88%</td>
<td>0.3</td>
<td>72%</td>
<td>0.5</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITD+d</td>
<td>69</td>
<td>81%</td>
<td></td>
<td>63%</td>
<td></td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

- Patients with ITD had inferior 3-year overall survival compared to patients without ITD
- TKD didn’t reach clinical significance
- Other study: FLT3-ITD(positive) patients had a lower 3-year overall survival rate (62%) compared with FLT3-ITD(negative) patients (82%) (P = 0.006).

c-KIT D816 V mutations

? Role for tyrosine kinase inhibitors

Schnittger S, et al. 2006 Mar 1;107(5):1791-9
AML with *RUNX1* mutation

- Mutations seen in 10-15% of CN-AML
- Can also be seen in MDS and AML-MRC
- Prognosis: poor
- Associated mutations:
  - Generally not seen with *NPM1* and dm-*CEBPA* mutations
  - Frequently seen with *MLL(KMT2A)*-PTD

Epigenetic Modifying Genes: 
*DNMT3a, TET2, IDH1/2*

- Regulate DNA methylation/Contribute to genetic instability
- Found at high frequency in conjunction with other mutations
- Variability in data with regard to prognostic value
- Therapy with hypomethylating agents
- Presence at diagnosis may induce *FLT3*-ITD at relapse

**IDH1 and IDH2 mutations**

- Cumulatively detected in up to 15-30% of AML patients
- All point mutations, affecting codons:
  - R132 of *IDH1*
  - R140 or R172 of *IDH2*
- Same molecular assay used for both gliomas and AML
- Clinical utility:
  - Prognosis: most studies suggest poor prognosis
  - IDH is a target for mutant-selective inhibitors and clinical trials are now beginning ([NCT01915498](https://clinicaltrials.gov/ct2/show/NCT01915498)).

MLL (KMT2A)-PTD

- *MLL-PTDs* are found in 5%-10% of patients with CN-AML
- Result from intragenic duplication of a genomic region between exons 5-11 or 5-12
- Confers an adverse prognosis, irrespective of the presence of *FLT3*-ITD mutation
- Molecular resting: RT-PCR (RNA-based testing)

## Functional classification of mutations in AML

<table>
<thead>
<tr>
<th>Signal Transduction</th>
<th>Differentiation</th>
<th>Epigenetic Regulation</th>
<th>Tumor Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3</td>
<td>RUNX1 (AML1)</td>
<td>TET2</td>
<td>WT1</td>
</tr>
<tr>
<td>KIT</td>
<td>CEBPA</td>
<td>IDH1, IDH2</td>
<td>TP53</td>
</tr>
<tr>
<td>NRAS, KRAS</td>
<td>NPM1</td>
<td>DNMT3A</td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td>PU1</td>
<td>ASXL1</td>
<td></td>
</tr>
<tr>
<td>PTPN11</td>
<td>GATA1, GATA2</td>
<td>EZH2</td>
<td></td>
</tr>
</tbody>
</table>
Is Labeling an AML with a Single Mutation Adequate?

1. The mutations do not occur in isolation
   - Genetic complexity
   - Integrated Genomic profiling

2. AMLs are spatially and temporally heterogeneous
   - Genetic heterogeneity and clonal evolution
Interplay of Genetic Events

<table>
<thead>
<tr>
<th>Cytogenetic Classification</th>
<th>Mutations</th>
<th>Overall Risk Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>Any</td>
<td>Favorable</td>
</tr>
<tr>
<td>Normal karyotype or inter-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mediate-risk cytogenetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Mutant NPM1 and IDH1 or IDH2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Wild-type ASXL1, MLL-PTD, PHF6, and TET2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>FLT3-ITD-negative or posit</td>
<td>Mutant CEBPA</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Intermediate-risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>karyotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Wild-type MLL-PTD, TET2, and DNMT3A and trisomy 8–negative</td>
<td>Intermediate</td>
</tr>
<tr>
<td>FLT3-ITD-negative</td>
<td>Mutant TET2, MLL-PTD, ASXL1, or PHF6</td>
<td>Intermediate</td>
</tr>
<tr>
<td>FLT3-ITD-positive</td>
<td>Mutant TET2, MLL-PTD, DNMT3A, or trisomy 8, without mutant CEBPA</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>Any</td>
<td>Unfavorable</td>
</tr>
</tbody>
</table>

Other genotypes:
- MT3A, or MLL-PTD
- 8q deletion, or without CEBPA mutation
Role of Next Generation Sequencing in Molecular Profiling of AML

• Clinical need for comprehensive molecular profiling
• Cumbersome and expensive using single-gene assays given the growing list of clinically relevant genes
• High throughput sequencing (NGS) is available for clinical testing
  – Potential for “all in one” testing
  – Evaluating for mutant allele frequencies (clonal heterogeneity and evolution)
Northwestern NGS-Myeloid Panel

33 genes (6 full exons and 27 partial)
Algorithmic approach

Morphology and Phenotype = AML

History of Therapy?

Yes

t-AML

No

History of MDS or MDS-MPN

No

Cytogenetic Abnormalities?

No

AML-MRC

Yes

AML with recurrent cytogenetic abnormalities

AML-MRC

CN-AML, AML NOS

Family history of a myeloid neoplasm

Myeloid Neoplasms with Germline Predisposition
AML no history of MDS or therapy

Cytogenetic Abnormalities?

Yes

AML with recurrent cytogenetic abnormalities:
  t(15;17)
  t(8;21)
  inv 16/t(16;16)
  t(6;9)
  inv3/t(3;3)
  BCR-ABL

CN-AML, AML,NOS

No

AML-MRC:
  Complex karyotype
  -7/del(7q)
  -5/del(5q)
  i(17q)/t(17p)
  -13/del(13q)
  del(11q)
  del(12p)/t(12p) – del(9q)**
  idic(X)(q13)
  Balance translocations

Yes

CN-AML, AML,NOS
CN-AML

AML without:
- History of therapy
- History of MDS
- Recurrent or MDS-related Cytogenetic Abnormality

Gene Mutation analysis

AML with NPM1
AML with CEBPA
AML with RUNX1

None

Dysplasia?

No
AML, NOS

Yes
AML-MRC
Which Molecular Assays are Essential for Newly diagnosed AML

• In CN-AML:
  – NPM1: Can be Single Gene Assay (SGA) or part of NGS-myeloid
  – FLT3-ITD: Has to be evaluated by SGA even if part of NGS
  – CEBPA: Can be SGA or part of NGS-myeloid
  – RUNX1: Is usually part of NGS-Myeloid

• In inv (16) and t(8;21) AML:
  – KIT: Can be SGA or part of NGS-myeloid
Which molecular assays are currently optional but recommended in newly diagnosed AML

- IDH1/2: SGA or part of NGS-myeloid
- TET2, DNMT3A, WT1: Usually part of NGS-Myeloid
- MLL-PTD: SGA (RNA based)
- In t(15;17):  
  - FLT3-ITD
- In AML-MRC with Complex karyotype:  
  - TP53: Usually part of NGS-Myeloid
Myeloid Neoplasms with Germline Predisposition

*Familial AML and MDS*
MDS/AML with Germline Predisposition

• Traditionally considered rare
• Most well described are those associated with syndromic bone marrow failure disorders, e.g. Fanconi anemia – usually present in childhood
• Also occur in Down Syndrome and Neurofibromatosis 1
• Increasing recognition of cases with autosomal dominant inheritance and MDS/AML as a main clinical feature - can present at any age
# Myeloid Neoplasms with Germline Predisposition

**WHO Provisional 2016**

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Incidence of Known Familial MDS/AML

• Not well characterized
• NGS study of sporadic acute leukemia in childhood showed pathogenic or probable pathogenic mutations in 26 of 588 (4%)*
• Challenges
  – Many families with MDS/AML clustering are negative for known mutations
  – Many familial genes also found as somatic mutations

### Myeloid Neoplasms with Germline Predisposition

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AML with Germline $CEBPA$ mutation

- No prior blood abnormalities or physical findings - nearly all develop AML as *children or young adults*
- Biallelic $CEBPA$ mutations – 1 germline and a second acquired at time of AML progression
- Biallelic $CEBPA$ mutations new entity in 2016 WHO update – subset could be familial - current data indicates prevalence could be up to 1% of AML
- AML morphology FAB M1-M2 with frequent Auer rods, aberrant CD7 expression, normal karyotype
- Multiple relapses common but overall favorable prognosis
AML with Germline *DDX41* mutation

- Only a limited number of pedigrees reported
- Subset have biallelic mutations – germline and another acquired somatic *DDX41* mutation
- **Long latency** – average age of onset 61 years
- High grade myeloid neoplasms with normal karyotype and poor prognosis – MDS with multilineage dysplasia, MDS with excess blasts, MDS with 5q-, AML
- Lymphoid neoplasms also reported
### Myeloid Neoplasms with Germline Predisposition

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Myeloid neoplasms with germline predisp. and pre-existing platelet disorder

• Germline mutations associated with autosomal disorders with variable thrombocytopenia/bleeding tendencies
  – \textit{RUNX1} - Familial platelet disorder with propensity to myeloid malignancy
    • \textit{RUNX1} mutations also occur in sporadic myeloid neoplasms
  – \textit{ANKRD26} - Thrombocytopenia 2
  – \textit{ETV6} - Thrombocytopenia 5
• Increased risk of MDS/AML often at young age (other malignancies reported)
• Challenge for pathologists – dysmegakaryopoiesis often part of the disorder - may not indicate a neoplasm
Germline ANKRD26 Mutation
## Myeloid Neoplasms with Germline Predisposition

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50 y/o F - Presented with circulating blasts

**CBC**
- WBC 8.7 K/uL
- Hb 9.5 g/dL
- Hct 30.6%
- MCV 80 fl
- RDW 23.8%
- PLT 55 K/uL

**Differential**
- Neutrophils 56%
- Lymphocytes 13%
- Monocytes 3%
- Eosinophils 3%
- Basophils 3%
- Blasts 24%
- NRBC’s 1/100
PMH and Family History

- Patient – 25 yr history of thrombocytopenia
- Family history of thrombocytopenia - mother, maternal aunt, siblings and their children and her children – mother, maternal aunt died of MDS
Touch Preparation (No aspirate)

FC - CD34+, CD117+, CD13+, CD33+, MPO+, HLADR+, partial CD7+, neg for other lymphoid and neg for monocytic markers
Karyotype

46,XX,del(7)(q22q36)[20]
Molecular Results

• No mutations
  – FLT3 ITD, FLT3 D835, NPM1 or cKIT
  – RUNX1 or CEBPA

• GATA2 mutations in region of 2nd Zinc finger:

![Diagram showing mutations in GATA2 region with Thr358Asn and Leu359Val highlighted]
Diagnosis

- AML with germline \textit{GATA2} mutation
GATA2

- Encodes zinc finger transcription factors essential for hematopoietic differentiation and lymphatic formation
- Critical for genesis and function of hematopoietic stem cells and preferential differentiation to erythroid/megakaryocyte lineages
- Mutations cause loss of mutated allele → haploinsufficiency, transmitted with autosomal dominant inheritance
- Mutations heterogeneous – not common in sporadic AML

Clinical syndromes associated with germ line GATA2 mutations

- Familial MDS/AML
- Emberger: lymphedema with warts and predisposition to MDS/AML
- MonoMAC: monocytopenia and susceptibility to Mycobacterium avium complex infections, predisposition to MDS/AML
- Dendritic cell, monocyte, NK and B lymphocyte deficiency with susceptibility to viral infections
- Chronic neutropenia
Clinical features of GATA2 deficiency by organ system

Head & neck:
- Embolic stroke
- Sensorineural hearing loss
- Hypothyroidism
- HSV ulcers

Thorax:
- Pulmonary alveolar proteinosis
- Ventilatory and diffusion defects
- Culture-negative endocarditis
- Solid organ tumors

Bone marrow:
- Cytopenias
- MDS/AML
- Disseminated NTM infection
- Severe C. difficile infection

Abdomen:
- Disseminated NTM infection
- Severe C. difficile infection

Upper extremities:
- Chronic arthralgias
- Clubbing
- Extragenital warts

Pelvis:
- Miscarriage
- Genital dysplasia
- Genital warts

Lower extremities:
- Panniculitis/Erythema nodosum
- Bony infarcts
- Lymphedema
- Deep venous thrombosis

Horwitz M S Blood 2014;123:799-800

Spinner M A et al. Blood 2014;123:809-821
Hematologic Manifestations of GATA2 deficiency

- Cytopenias common (hematologic parameters prior to MDS/AML normal in <10%)
  - Decreased B cells (86%), NK cells (82%), monocytes (78%), CD4+ T cells (51%), neutrophils (47%)
- MDS/AML develops in ~70% at med. age 29 yrs (4-78 yrs)

Hypocellular, atypical megs, ↑ reticulin fibrosis

Spinner M A et al. Blood 2014;123:809
Micol JB et al. Haematologica 2014;99:201
AML/MDS with Germline GATA2 mutation

• Frequent del(7) or monosomy 7
• Acquisition of additional mutations likely important in leukemogenesis – somatic mutations of ASXL1
• Poor outcome unless transplanted – severe infection frequent cause of death

Patient Follow Up

• 7+3 induction therapy with residual disease
• Recurrent Legionella pneumonia, respiratory failure requiring intubation
• Received double cord allo-HSCT - 5 months s/p tx no residual disease, normal karyotype, neg FISH for del(7)
• Continued respiratory failure, pneumonia and septic shock – expired 6 mo post transplant
• Family members declined testing for GATA2
Why is it important to recognize MDS/AML with germline predisposition?

• Prognostic and therapeutic implications
• Diagnostic implications
• Identification and follow up of mutation carriers – i.e. consider baseline bone marrow biopsy for mutation carriers
• Unrelated donor or mutation negative donors for stem cell transplant
• Aid in understanding biology of *de novo* AML
How to identify familial MDS/AML

• Familiarity with predisposition syndromes (clinical findings, biallelic CEBPA, cytopenias, etc)
• Careful family history (i.e. any close relatives with cytopenias, aplastic anemia, MDS, acute leukemia?)
• Young age of onset of MDS/AML
Lab Testing

• Test for germline mutation in specimen containing only non-leukemic cells
  – Skin fibroblasts (recommended)
Take home message....

• AML/MDS with germline predisposition rare but likely more common than currently appreciated – more entities continue to be recognized
• Family history, early disease onset, disease in multiple close relatives, etc. raise suspicion
• Genetic testing is available
• Clinical implications for diagnosis, management and therapy of patients and their families
• Will be included in WHO update 2016 as provisional entity