The Role of Genetic and Molecular Profiling in the Risk Stratification of Acute Myeloid Leukemia

Frederick Racke, M.D., Ph.D.
Medical Director, Hematopathology
Nichols Institute, Quest Diagnostics
San Juan Capistrano, CA

History of Leukemia Classification


2008 WHO classification of acute myeloid leukemia

Acute myeloid leukemia with recurrent genetic abnormalities
- Acute myeloid leukemia with t(8;21)(q22;q22), (RUNX1-RUNX1T1)
- Acute myeloid leukemia with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (CBFB-MYH11)
- Acute promyelocytic leukemia with t(15;17)(q22;q12), (PML-RARA)
- Acute myeloid leukemia with t(6;9)(p23;q34), (DEK-NUP214)
- Acute myeloid leukemia with t(1;22)(p13;q13), (RBM15-MKL1)

Acute myeloid leukemia with myelodysplasia-associated changes

Therapy-related myeloid neoplasms

Provisional entities
- AML with mutated NPM1
- AML with mutated CEBPA
WHo Panel of Monoclonal Antibodies for the Classification of Acute Leukemias

- Hematopoietic precursors: CD34, HLA-DR, TdT, CD45.
- Myeloid: CD13, CD33, CD15, MPO, CD117.
- Megakaryocytic: CD41, CD61.

Primary versus Secondary Aberrations

- Primary
  - necessary for neoplasm
  - simple
  - usually balanced
  - strongly associated with disease type
  - specific gene rearrangement
  - often sole
  - > 200 recognized (recurrent)

- Secondary
  - superimposed on primary
  - multiple
  - may be nonrandom, but non-specific
  - important in tumor progression
  - some may be neutral
  - lead to large-scale genomic imbalances
  - have lineage promiscuity
  - selective advantage regardless of cell type

Ramifications of Primary versus Secondary Aberrations

- Genetic mechanisms underlying tumor initiation and progression totally different
- Elucidation of molecular consequences of secondary aberrations arduous task
- Cytogenetic diagnosis would have to recognize that an unbalanced primary may be secondary to a submicroscopic, truly primary change of major diagnostic and prognostic importance
Leukemia stem cells

- Capable of long term in vitro colony growth and xenogeneic transplantation.
- Unique phenotype: CD34+, CD38-, CD71-, CD90-, CD117-, CD33+, and CD123+.

Cooperating mutations in AML

- **Class 1 mutations**- confer proliferative or survival advantage but do not affect differentiation
  - FLT3-ITD, Ras mutations, C-KIT mutations, BCR-ABL.
- **Class 2 mutations**- impair differentiation and apoptosis
  - PML/RARA, CEBPα, RUNX1/RUNX1T1, CBF/SMMHC, MLL fusions.

AML

- There were about **12,330** new cases of AML in 2010
- About 8,950 deaths from AML in the US in 2010; almost all are adults
- The average age of AML patients is 67 (American Cancer Society)
- Requires at least 20% blasts in the marrow; normal <5%
- WHO (World Health Organization Classification) based on cell lineage, morphology, immunophenotype, genetics (cytogenetics) and clinical presentation
Improved outcome of patients with good risk cytogenetics


Inferior outcome of patients with poor risk cytogenetics


Acute promyelocytic leukemia with t(15;17)

- Arrested maturation at promyelocyte stage.
- Fusion between retinoic acid receptor alpha and PML gene.
- Pharmacologic doses of retinoic acid reverses the differentiation block in APL.

46,XY,t(15;17)(q24;q21)
Acute promyelocytic leukemia, microgranular variant

- Small granules less apparent on Wright stain smears.
- Characteristic blotted morphology.
- Associated with higher white counts at presentation.
- Microgranular variant associated with FLT-3 ITD mutation in APL.

APL with Variant RARA Translocations

<table>
<thead>
<tr>
<th>Chr locus</th>
<th>Gene</th>
<th>Response to ATRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>11q23</td>
<td>ZBTB16</td>
<td>Resistant</td>
</tr>
<tr>
<td>11q13</td>
<td>NUMA1</td>
<td>Probably responsive</td>
</tr>
<tr>
<td>5q35</td>
<td>NPM1</td>
<td>Probably responsive</td>
</tr>
<tr>
<td>17p11.2</td>
<td>STAT5B</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

AML with differentiation and t(8,21)

- Fusion of transcription factor Runx1 with Runx1T1 creating a repressor of Runx1 function.
- Good prognosis AML with cure rates of ~50%.
- C-kit mutations frequent in core-binding factor AMLs and associated with worse outcome.
AML with myelomonocytic differentiation and abnormal eosinophils

- Inv(16) leads to fusion of CBFβ to smooth muscle myosin heavy chain.
- Creates protein that represses normal CBF function.
- Associated with extramedullary involvement including lymphadenopathy.

AML with 11q23 abnormalities

- Associated with monocytic features.
- Common in young children with AML.
- Also associated with therapy-related AML.

AML with 11q23 abnormalities

<table>
<thead>
<tr>
<th>Chr locus</th>
<th>Gene</th>
<th>Frequency in 11q23 AMLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5p22</td>
<td>MILT3 (AF9)</td>
<td>27-34</td>
</tr>
<tr>
<td>10p12</td>
<td>MILT10 (AF10)</td>
<td>13-18</td>
</tr>
<tr>
<td>19p13.1</td>
<td>ELL</td>
<td>11-18</td>
</tr>
<tr>
<td>6q27</td>
<td>MILT4 (AF6)</td>
<td>10-16</td>
</tr>
<tr>
<td>19p13.3</td>
<td>MILT1 (ENL)</td>
<td>5-8</td>
</tr>
</tbody>
</table>
Summary of 11q23 abnormalities

- **MLL** (HRX/ALL) chimeric oncogenes
  - Can be amplified
  - Can have an internal tandem duplication (ITD)
- Unique age distribution
- >100 partner chromosomes
- Partner contributions have very little similarity
- Different lineages
- *de novo* and secondary
- Poor prognosis

AML with t(6;9) (DEK-NUP214)

- No specific blast morphology
- Frequent erythroid hyperplasia and dysplasia
- Basophilia common
- FLT3 mutations common
- Poor prognosis

AML with inv(3)(q21q26.1) (RPN1-EVI1)

- Mixture of blasts and micromegakaryocytes
- Normal or elevated platelets
- Multilineage dysplasia
- Poor prognosis
AML with t(1;22) (RBM15-MKL1)

- Infantile leukemia
- Megakaryoblastic leukemia
- May present as small round blue cell tumor
- Good prognosis

AML/MDS, therapy related

- 2 major forms
  – Alkylating agent/radiation treatment-related
    - 5-6 year latency period
    - Cytogenetics similar to MDS
  – Topoisomerase II-treatment-related
    - Shorter latency period (2-3 years)
    - Monocytic or myelomonocytic AMLs
    - 11q23 rearrangements.

Therapy-related leukemia with -5/del(5q)/-7/del(7q)

- Long latency (4-6 years)
- Prior exposure to alkylating agents
- Frequently preceding myelodysplastic syndrome (MDS)
- Poor response to therapy
- Short survival
Therapy-related 11q23 [MLL] leukemias

- Short latency period
- Exposure to epipodophyllotoxins
  - DNA topoisomerase II inhibitors
- No prior “pre-leukemia”
- Respond to therapy
- Short survival
- Also contribute nearly all therapy-related ALLs.

46,XY,t(9;11)(p22;q23) MLLT3-MLL
Acute Leukemia of Ambiguous Lineage

- **Primitive acute leukemia**
  - Often express HLA-DR, CD34, CD38, CD45, and CD7.
  - Lack CD79a, cytoCD22, CD3, and mpo.
- **Biphenotypic acute leukemia**
- **Bilineage acute leukemia**
- **Plasmacytoid dendritic cell leukemia/lymphoma**
  - CD4+, CD56+, CD123+ with variable TdT, CD7, CD33, cCD3.
  - Mpo negative but often CD68+

Mixed Phenotype Acute Leukemia (MPAL)

Genetics of MPALs

- **MPAL with t(9;22)(q34;q11.2);BCR-ABL1**
  - Most common genetic abnormality in MPAL
  - Typically B/myeloid but occasionally T/myeloid
  - Often have additional karyotypic abnormalities
- **MPAL with t(v;11q23); MLL rearranged**
  - Often B/myeloid with a pro-B phenotype and monocytic component
  - Various rearrangement partners with AF4 on chr4q21 most common.
Cytogenetic risk stratification in AML

- **Favorable** - t(15;17), t(8;21), inv(16).
- **Intermediate** - NK, t(9;11), -Y, -7q,-9q,-
  11q,+8,+11,+13,+21.
- **Unfavorable** - inv(3)(q21q26), t(6;9), t(6;11), t(11;19), -5q,
  -5, -7, complex karyotype.

Genetic/Molecular alterations affecting outcome in NK AML

- **Favorable**
  - NPM1 mutations
  - CEBPα mutations
- **Unfavorable**
  - FLT3-ITD
  - MLL-PTD
  - BAALC overexpression
  - ERG overexpression

FLT3 Mutations

- Occur in about 25% of AMLs
- Most are activating internal tandem duplications but some activating TKD mutations also occur
- Occur frequently in
  - AML with NPM1 mutations (40%)
  - APL (40-50%)
  - t(6;9)(p23;q34)/DEK-NUP214 (75%)
- Confers poor prognosis, in particular when there is a high mutant allelic burden
CCAAT/Enhancer-Binding Protein α (CEBPA)

• Mutations can be seen throughout N-terminus and/or C-terminus
• Present in approx. 10% of AML cases, usually in NK AML
• Germline mutations have been reported
• Improved outcome with biallelic mutations and in the absence of FLT3-ITD

Nucleophosmin-1 mutations

• NPMc+ AML
  – 50% of NK AML
  – Good prognosis if without FLT3m
  – Heterozygous mutations located in exon 12 involving c-terminus
  – Although associated with a good prognosis, detectable NPM1 mutations post-therapy have strong prognostic impact.

Erg staining in AML TMA
Patterns of ERG expression

Molecular risk factors: normal karyotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>Increased survival</td>
</tr>
<tr>
<td>CEBPα</td>
<td>Increased remission duration, OS, and DFS</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>Decreased remission duration and OS</td>
</tr>
<tr>
<td>BAALC</td>
<td>Increased induction failure and decreased OS</td>
</tr>
<tr>
<td>ERG</td>
<td>Decreased OS</td>
</tr>
<tr>
<td>MLL-PTD</td>
<td>Decreased remission duration</td>
</tr>
</tbody>
</table>

Novel molecular genetic markers in AML

- TET2-mutated in 10%
- ASXL1- mutated in 3-5% of young AMLs and 16% of elderly
- IDH1/2-mutated 15-30%, increasing in elderly. Mutually exclusive with TET2m
- PHF6-mutated in 3%
- DMNT3A-mutated in 20% of adult AML, occurring throughout reading frame. 2nd most common mutation to FLT3m
Novel molecular genetic markers in AML

- ASXL1, TET2 and PHF6 mutations associated with very adverse overall survival for FLT3neg NK AML.
- IDH stratifies NPM1 prognosis
  - FLT3neg NPM1/IDH mut have survival better than core-binding factor AMLs
  - FLT3neg NPM1mut/IDHneg relatively poor outcome
- TP53 mutations associated with aneuploidy but also impacts prognosis independently

**Revised Risk Stratification**

<table>
<thead>
<tr>
<th>Cytogenetic Class</th>
<th>Mutations</th>
<th>Overall Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>Any</td>
<td>Favorable</td>
</tr>
<tr>
<td>Normal Karyotype</td>
<td>FLT3-ITDneg</td>
<td>Mut NPM1/IDH1/2</td>
</tr>
<tr>
<td></td>
<td>FLT3-ITDneg</td>
<td>Wild type ASXL1, MLL-PTD PHF6 and TET2</td>
</tr>
<tr>
<td></td>
<td>FLT3-ITD+/−</td>
<td>Mut CEBPa</td>
</tr>
<tr>
<td></td>
<td>FLT3-ITD+</td>
<td>Wild type MLL-PTD, DNMT3A, TET2, and Tri8neg</td>
</tr>
<tr>
<td></td>
<td>FLT3-ITDneg</td>
<td>Mutant ASXL1, MLL-PTD PHF6, and TET2</td>
</tr>
<tr>
<td></td>
<td>FLT3-ITD+</td>
<td>Mutant DNMT3A, MLL-PTD PHF6, TET2, or Tri8 w/o CEBPa</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>Any</td>
<td>Unfavorable</td>
</tr>
</tbody>
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**Risk stratification for Molecular and Cytogenetic data: ELN recommendations**

<table>
<thead>
<tr>
<th>Genetic Group</th>
<th>Subsets</th>
</tr>
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</table>
| Favorable    | t(8;21); RUNX1-RUNX1T1  
|              | inv(16);CBFB-MYH11  
|              | NPM1 w/o FLT3-ITD CEBPa |
| Intermediate-1| NPM1mut w FLT3-ITD  
|               | NPM1wt w FLT3-ITD |
| Intermediate-2| t(9;11);MLLT3-MLL |
|               | Cyto genetic not classified as favorable or adverse |
| Adverse      | inv(3);RP11-EVI1,  
|              | 11q23;MLL rearranged, -5 or del(5q),  
|              | -7, 8q24.1 and complex karyotype |

Summary

• Cytogenetics remains an important determinant in the risk stratification of AML.

• There is an expanding number of mutations that are being integrated with cytogenetic results to create a more layered risk stratification profile.

• Many of the mutations are being identified in “actionable” targets that will lead to not only prognostic implications but therapeutic ones as well.

Questions?