Update on the WHO Classification of Acute Myeloid Leukemia

Kaaren K. Reichard, MD
Mayo Clinic Rochester
reichard.kaaren@mayo.edu
Conflict of Interest

• Nothing to disclose
Objectives

• Present a practical approach to the diagnosis of AML in 2018

• Review the different diagnostic categories of AML with special focus on newer entities

• Discuss the current state of the impact of molecular testing in AML
Classification of AML

• Not a single disease – complex, heterogenous
• Evolution over the past two decades to incorporate important and/or recently acquired genetic information into classification schemes that are biologically relevant
• Current challenges
Acute Leukemia Diagnosis and Classification

- World Health Organization Classification
- College of American Pathologists/American Society of Hematology (CAP/ASH) Guideline for the Initial Diagnostic Workup of Acute Leukemia
- European LeukemiaNet (ELN) Prognostic Scoring
- National Comprehensive Cancer Network (NCCN) treatment guideline for AML
Practical Approach to AML

• Morphology
  – Blast and non-blast cell morphology
  – Blast count
  – +/- Cytochemistry

• Immunophenotyping
  – Flow cytometry

• Cytogenetics
  – +/- FISH

• Molecular mutations
  – Individual
  – Panel

Complex!
Practical Issues in AML subclassification

• A number of different standard-of-care testing modalities

• Turn-around-time for each modality is different

• Cases need to be amended to integrate subsequently acquired data/as a result need to be revisited several times
  – Challenging: inefficient, lack of time
Systematic Approach to AML

- Clinical history and features (prior myeloid neoplasm, chemotherapy, radiotherapy)
- CBC findings at presentation
  - Hematopoietic failure
- Morphologic review
  - Peripheral blood and bone marrow
  - 200 cell count (PB) and 500 cell count in BM is suggested
  - Blast % based on total bone marrow cells
Systematic Approach to AML

• Morphologic review (cont’d)
  – Blast % based on morphologic differential cell count (not flow cytometry)
  – >20% blood or bone marrow blasts*
  – Blast features
  – Non-blast cell features (and what do we mean by that?)

*several exceptions
Blast enumeration

- Blasts and blast equivalents
  - Myeloblasts
  - Promyelocytes (only in APL)
  - Monoblasts
  - Promonocytes
  - Erythroblasts (only in PEL)
  - Megakaryoblasts
Blast cell features

Acute promyelocytic leukemia with *PML-RARA*

AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*

Some morphologic features suggest specific AML subtypes; others not as specific
Blast cell features
Non-blast cell features

- Presence or absence of multilineage dysplasia
- Granulocytic maturation with salmon-colored granules
- Increases of eosinophils, including abnormal eosinophils
- Increases in basophils or mast cells
Non-blast cell features

AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*

AML with inv(16)(p13.1q22)
  or t(16;16)(p13.1;q22); *CBFB-MYH11*

AML with multilineage dysplasia
Non-blast cell features

Must assess percent dysplastic cells
Cytochemistry

Myeloperoxidase
Highlights myeloid component

Butyrate esterase
Highlights monocytic component
Immunophenotyping

- Standard-of-care on all new acute leukemias
- Flow cytometry is the preferred method
- Immunohistochemistry may be necessary when the bone marrow aspirate is a “dry tap” due to fibrosis or procedural issues
- Immunophenotyping should not replace a manual differential count when adequate smears are available
Immunophenotyping by flow cytometry

• Comprehensive panel covering B, T and myeloid lineage
  – Lack of complete specificity for certain markers for lineage assessment
  – Allows for lineage assignment in the vast majority of cases
  – Some immunophenotypes are highly suggestive of specific AML subtypes

Craig FE, Foon KA. Blood 111:3941, 2008
Cytogenetics in AML

- Standard-of-care in all new diagnoses
- Cytogenetic risk groups are now well-defined

[Graph showing survival rates over years from entry for different cytogenetic abnormalities: t(8;21), t(15;17), inv(16), normal, abn11q23]

AML with adverse cytogenetic abnormalities

% still alive

Years from entry

Cytogenetics in AML

• Some karyotype abnormalities define specific disease entities
• Myelodysplasia-related cytogenetic abnormalities are a key feature for a diagnosis of AML with myelodysplasia-related changes
• Role of FISH studies is not standardized but useful in many situations
Molecular mutation studies in AML

• Relatively new/rapidly expanding
• Baseline survey of testing performed in AML
  – Morphologic assessment 100%
  – Flow cytometry 99.1%
  – Karyotyping 96.2%
  – Molecular 78.2%

## WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

### Acute myeloid leukemia (AML) and related neoplasms

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Description</th>
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<tbody>
<tr>
<td>AML with recurrent genetic abnormalities</td>
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<tr>
<td>AML with (t(8;21)(q22;q22.1);RUNX1-RUNX1T1)</td>
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<td>AML with (PML-RARA)</td>
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<td>AML with (t(6;9)(p23;q34.1);DEK-NUP214)</td>
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<td>AML with (t(3;3)(q21.3;q26.2); GATA2, MECOM)</td>
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<td>AML (megakaryoblastic) with (t(1;22)(p13.3;q13.3);RBMI5-MKL1)</td>
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<tr>
<td>Provisional entity: AML with (BCR-ABL1)</td>
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<tr>
<td>AML with mutated (NPM1)</td>
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<td>AML with biallelic mutations of (CEBPA)</td>
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<td>Provisional entity: AML with mutated (RUNX1)</td>
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<tr>
<td>AML with myelodysplasia-related changes</td>
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<td>Therapy-related myeloid neoplasms</td>
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<td>AML, NOS</td>
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<tr>
<td>AML with minimal differentiation</td>
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<td>AML without maturation</td>
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<td>AML with maturation</td>
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<tr>
<td>Acute myelomonocytic leukemia</td>
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<tr>
<td>Acute monoblastic/monocytic leukemia</td>
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<tr>
<td>Pure erythroid leukemia</td>
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<tr>
<td>Acute megakaryoblastic leukemia</td>
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<tr>
<td>Acute basophilic leukemia</td>
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<tr>
<td>Acute panmyelosis with myelofibrosis</td>
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<tr>
<td>Myeloid sarcoma</td>
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<tr>
<td>Myeloid proliferations related to Down syndrome</td>
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<tr>
<td>Transient abnormal myelopoiesis (TAM)</td>
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<tr>
<td>Myeloid leukemia associated with Down syndrome</td>
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</tbody>
</table>
2016 World Health Organization Classification of AML

- Acute myeloid leukemia (AML) with recurrent genetic abnormalities
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, not otherwise specified
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
Genetic abnormalities in AML

- Molecular pathogenesis of AML is complex
- Moving beyond cytogenetics
- Major gene mutation discoveries through various high throughput sequencing technologies (e.g., NGS)
- Genetic data are now being used to inform both diagnosis and prognosis in AML
Genetic abnormalities in AML

- Recognized and other rare translocations: 30-35%
- \(NPM1\) mutations: 30%
- Chromatin/splicesosome: 13%
- \(TP53\) mutation and chromosomal abn: 10%
- Biallelic CEBPA: 4%

AML updates in 2016 WHO

• AML with biallelic mutations of \textit{CEBPA}
• Provisional entity: AML with mutated \textit{RUNX1}
• Provisional entity: AML with \textit{BCR-ABL1}
• AML with mutated \textit{NPM1}
AML updates in 2016 WHO

• AML with myelodysplasia-related changes (AML-MRC)
  – New criteria
  – Updated MDS-related cytogenetic abnormalities
• New definition for blast percentage in erythroid predominant neoplasms
• Myeloid neoplasms with germline predisposition
AML with biallelic mutations of *CEBPA*

- Biallelic mutations required for this category
- 7-20% of AML cases have a *CEBPA* mutation
  - Approximately half are biallelic
- Should raise the possibility of germline predisposition condition
- No unique morphologic features
- One-quarter of cases may show multilineage dysplasia
AML with biallelic mutations of \textit{CEBPA}

- Immunophenotyping tends to show an increased frequency of CD7 and CD15 expression on the blasts
- Most frequent with a normal or intermediate karyotype
- Although rare, cases with a concurrent myelodysplasia-related cytogenetic abnormality should be diagnosed as AML-MRC for now
- Favorable prognosis
AML with mutated *RUNX1*

- Provisional entity
- Seen in approximately 12% of AML cases
- More frequent in older patients
- Wide morphologic spectrum
- No unique morphologic features
AML with mutated RUNX1

• Diagnosis restricted to *de novo* cases
  – Excluded with history of MDS or therapy
• Cases with myelodysplasia-related cytogenetic abnormalities should be classified as AML with myelodysplasia-related changes
• May be associated with other mutations
  – *KMT2A*-PTD, *IDH1, IDH2* or *ASXL1* mutations
  – Rare *CEBPA* or *NPM1* mutations
AML with mutated *RUNX1*

- Cases with both *RUNX1* and *NPM1* should be classified as AML with mutated *NPM1*
- Cases with *RUNX1* and biallelic *CEBPA* mutations should be classified as AML with biallelic mutation of *CEBPA*
- Poor response to therapy with shortened survival
- Germline mutations should be evaluated
AML with $BCR-ABL1$

- *De novo* AML in which patients show no evidence (either before or after therapy) of chronic myeloid leukemia (can be quite challenging)
- Cases of mixed phenotype acute leukemia, therapy-related are excluded
- Provisional entity
AML with *BCR-ABL1*

- Rare (<1% of AMLs)
- Compared with CML, less frequent splenomegaly, lower PB basophilia, fewer dwarf megakaryocytes
- If another recurring genetic abnormality is present, that abnormality drives the final classification
- Patients may benefit from tyrosine kinase inhibitor therapy
AML with mutated \textit{NPM1}

- Occurs in $\sim50\%$ of adult cases with normal karyotype
- Strong association with monocytic differentiation
- Mutation may be detected by immunohistochemical and/or molecular techniques
AML with mutated *NPM1*

- Multilineage dysplasia allowed if no history of MDS, MDS/MPN or MDS-related cytogenetic abnormality
- Usually associated with normal karyotype
- Secondary mutations include *FLT3* and *DNMT3A*
- Favorable prognosis with normal karyotype and absence of *FLT3*-ITD
AML with myelodysplasia-related changes

• Revised AML-MRC criteria when only multilineage dysplasia (MLD) present
  – If *NPM1* mutation identified, diagnose as AML with *NPM1* mutation (despite MLD)
  – If biallelic *CEBPA* mutation identified, diagnose as AML with *CEBPA* mutation (despite MLD)
  – If AML-MRC diagnoses is based on history of MDS or on MDS-related cytogenetics, then retain AML-MDS even if *NPM1*, biallelic *CEBPA* mutations identified
  – Slight modifications to the cytogenetic abnormalities
MDS-related cytogenetic abnormalities

- Complex karyotype*
- Unbalanced abnormalities
  - -7/del(7q)
  - -del(5q)/t(5q)
  - i(17q)/t(17p)
  - -13/del(13q)
  - del(11q)
  - del(12p)/t(12p)
  - del(q)
  - idic(X)(q13)

- Balanced abnormalities
  - t(11;16)(q23.3;p13.3)
  - t(3;21)(q26.2;q22.1)
  - t(1;3)(p36.3;q21.1.2)
  - t(2;11)(p21;q23.3)
  - t(5;12)(q32;p13.2)
  - t(5;7)(q32;q11.2)
  - t(5;17)(q32;p13.2)
  - t(5;10)(q32;q21)
  - t(3;5)(q25.3;q35.1)

* >3 abnormalities
**NPM1 and CEBPA mutations and multilineage dysplasia**

- **NPM1**
  - No significance of multilineage dysplasia in the presence of *NPM1* mutation, a normal karyotype and no history of MDS
  - Multilineage dysplasia found in 74/318 (23%) de novo *NPM1* mutated AML

- **CEBPA mutations**
  - Multilineage dysplasia found in 28/108 (26%) of *CEBPA* mutated AML patients
  - No significant survival difference with multilineage dysplasia

Updated blast percent based on all BM cells even in erythroid predominant cases

• Erythroblasts counted as blasts in pure erythroid leukemia and must exceed 80% of total cells

• Note that cases previously fulfilling criteria for acute erythroid leukemia, myeloid/erythroid may now fall into MDS
Myeloid neoplasms with germline predisposition

- New category in updated WHO
- Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction
  - AML with germline CEBPA mutation
  - Myeloid neoplasms with germline DDX41 mutation*
- Myeloid neoplasms with germline predisposition and pre-existing platelet disorders
  - Myeloid neoplasms with germline RUNX1 mutation*
  - Myeloid neoplasms with germline ANKRD26 mutation*
  - Myeloid neoplasms with germline ETV6 mutation*
- Myeloid neoplasms with germline predisposition and other organ dysfunction
  - Myeloid neoplasms with germline GATA2 mutation
  - Myeloid neoplasms associated with bone marrow failure syndromes
  - Myeloid neoplasms associated with telomere biology disorders
  - Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders
  - Myeloid neoplasms associated with Down syndrome*
CAP/ASH AML Genetic Testing Guidelines

• Karyotype

• Molecular testing
  – For all or most cases
    • FLT3-ITD, NPM1, CEBPA, RUNX1
    • Others: IDH1, IDH2, TET2, WT1, DNMT3A, TP53, others
  – For select cases
    • KIT for core binding factor leukemias
    • PML-RARA if APL suspected
## 2017 ELN AML genetic risk stratification

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Genetic abnormality</th>
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<tbody>
<tr>
<td><strong>Favorable</strong></td>
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<tr>
<td></td>
<td>Mutated <em>NPM1</em> without <em>FLT3-ITD</em> or with <em>FLT3-ITD</em>&lt;sub&gt;low&lt;/sub&gt;</td>
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<tr>
<td></td>
<td>Biallelic mutated <em>CEBPA</em></td>
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<tr>
<td><strong>Intermediate</strong></td>
<td>Mutated <em>NPM1</em> and <em>FLT3-ITD</em>&lt;sub&gt;high&lt;/sub&gt;†</td>
</tr>
<tr>
<td></td>
<td>Wild-type <em>NPM1</em> without <em>FLT3-ITD</em> or with <em>FLT3-ITD</em>&lt;sub&gt;low&lt;/sub&gt; (without adverse-risk genetic lesions)</td>
</tr>
<tr>
<td></td>
<td>t(9;11)(p21.3;q23.3); <em>MLLT3-KMT2A</em></td>
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<tr>
<td></td>
<td>Cytogenetic abnormalities not classified as favorable or adverse</td>
</tr>
<tr>
<td><strong>Adverse</strong></td>
<td>t(6;9)(p23;q34.1); <em>DEK-NUP214</em></td>
</tr>
<tr>
<td></td>
<td>t(v;11q23.3); <em>KMT2A</em> rearranged</td>
</tr>
<tr>
<td></td>
<td>t(9;22)(q34.1;q11.2); <em>BCR-ABL1</em></td>
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</tr>
<tr>
<td></td>
<td>−5 or del(5q); −7; −17/abn(17p)</td>
</tr>
<tr>
<td></td>
<td>Complex karyotype, monosomal karyotype</td>
</tr>
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<td></td>
<td>Wild-type <em>NPM1</em> and <em>FLT3-ITD</em>&lt;sub&gt;high&lt;/sub&gt;</td>
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<td>Mutated <em>ASXL1</em></td>
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<tr>
<td></td>
<td>Mutated <em>TP53</em></td>
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</table>
Mutation Testing in AML

• Diagnosis:
  – WHO, ELN, CAP/ASH: NPM1, CEBPA, RUNX1

• Prognosis:
  – WHO: FLT3-ITD, KIT, others
  – CAP/ASH: FLT3-ITD, DNMT3A, IDH1, IDH2, KIT, TET2, TP53, WT1, others
  – ELN: FLT3-ITD, ASXL1, TP53

• Targets: FLT3, IDH1, IDH2
ASXL1 in AML

- Early event in leukemogenesis
- 3-19% mutation frequency
- Frequency increases with age (< 60 years: 5-10%; > 60 years: 10-18%)
- Mutations appear to be more frequently associated with AML with myelodysplasia-related changes and a worse overall survival
- ASXL1/RUNX1 and ASXL1/SRSF2 mutations particularly poor outcome
TP53 in AML

- Frequency: 5-15%
  - Frequency increases with age (< 60 years: ~5-10%; > 60 years: ~10-20%)
- Associated with complex karyotype, monosomomal karyotype, −5/5q−, −7/7q−, abn(17p)
- Associated with very poor outcome
- TP53 mutation and complex karyotype provide independent prognostic information, with the combination of both lesions having dismal outcome
**AML required/key information for reporting**

- **Clinical:** History of chemo/MDS
- **Morphology:** Blast %, Dysplastic %
- **Flow Cytometry:** Confirm myeloid (all cases) (CD33, CD13, MPO)
- **Cytogenetics:** AML-defining vs other (all cases)
- **Molecular:** FLT3, NPM1, CEBPA, RUNX1, BCR-ABL1, KIT, others, (selected genes)
AML Diagnosis in 2018

• Maintain morphologic, immunophenotypic and cytogenetic knowledge

• Integrated diagnosis: incorporate clinical history and pertinent molecular data

• Identify early disease, assess for minimal residual disease, recognize specific entities amenable to specific/targeted therapy
Summary

• Comprehensive approach to the initial diagnostic workup of AML includes utilizing multiple specialized testing.

• Molecular genetic testing is complex and refining our approach to AML.

• Comprehensive and integrated reporting is critical for the most accurate diagnostic and prognostic determination in AML.
Questions?