AML: WHO classification, biology and prognosis

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Acute myeloid leukemia

- Clonal expansion of undifferentiated myeloid precursors
- Impaired hematopoiesis and bone marrow failure
- Heterogeneous response to treatment and prognosis

Löwenberg et al, NEJM, 2011
# Acute myeloid leukemia

## Prognosis according age

<table>
<thead>
<tr>
<th>Age</th>
<th>Complete remission</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 - 60 years</td>
<td>80%</td>
<td>40% at 5 years</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>65%</td>
<td>28% at 2 years</td>
</tr>
</tbody>
</table>
FAB classification of AML

**FAB classification of acute myeloblastic leukaemia**

**M0**
- **Morphology:** Acute myeloblastic leukaemia with minimal differentiation.
- **Immunophenotype:** 
  - CD13 +
  - CD33 +
  - CD11b +
  - CD14 +
  - CD15 +

**M1**
- **Morphology:** Acute myeloblastic leukaemia without maturation.
- **Immunophenotype:** 
  - CD13 +
  - CD33 +
  - CD11b +
  - CD14 +
  - CD15 +

**M2**
- **Morphology:** Acute myeloblastic leukaemia with maturation.
- **Immunophenotype:** 
  - CD13 +
  - CD33 +
  - CD11b +
  - CD14 +
  - CD15 +

**M3**
- **Morphology:** Promyelocytic leukaemia.
- **Immunophenotype:** 
  - CD13 +
  - CD11b +
  - CD33 +

**M4**
- **Morphology:** Acute myelomonocytic leukaemia.
- **Immunophenotype:** 
  - CD13 +
  - CD15 +

**M5**
- **Morphology:** Acute monoblastic leukaemia.
- **Immunophenotype:** 
  - CD4 +

**M6**
- **Morphology:** Acute erythroid leukaemia.
- **Immunophenotype:** 
  - CD7 +

**M7**
- **Morphology:** Acute megakaryocytic leukaemia.
- **Immunophenotype:** 
  - CD41 +

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*Photo courtesy of: Acute myeloid leukemia pathophysiology, 2012*

*Bennett et al et al, BJH, 1976*
Modern diagnosis of AML
Based on Grimwade et al, Blood 1998; Grimwade et al, Blood, 2001

Cytogenetic distribution of AML
Impact of specific genetic aberrations on survival in AML

Grimwade et al, Blood 2010
Impact of karyotype complexity on survival for AML patients not belonging to favourable subgroups

Grimwade et al, Blood 2010
Overall survival in AML patients categorized into favourable, intermediate, adverse and very adverse cytogenetic risk groups.


Two or more autosomal monosomy or 1 auto monosomy with structural abn (n=184) = monosomal karyotype*
**Prognostic value of cytogenetics in acute myeloid leukemia**

Cytogenetic analysis of 1975 patients, 18-60 years

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Number of patients (%)</th>
<th>Four-year overall survival, % (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, -X, -Y</td>
<td>1001 (51)</td>
<td>41 (2)</td>
</tr>
<tr>
<td>inv(16)/t(16;16)</td>
<td>120 (6)</td>
<td>70 (4)</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>134 (7)</td>
<td>63 (4)</td>
</tr>
<tr>
<td>Abnormal, no monosomal karyotype</td>
<td>535 (27)</td>
<td>26 (2)</td>
</tr>
<tr>
<td>Monosomal karyotype</td>
<td>184 (9)</td>
<td>4 (1)</td>
</tr>
</tbody>
</table>

Mutational complexity of AML

Organization of mutations into categories of related genes

Comprehensive mutational profiling for risk stratification and clinical management of AML.

Two cooperating classes of mutations in AML

Class I Mutations
- FLT3-ITD
- FLT3-TKD
- RAS
- JAK2
- KIT
- SHP2

Class II Mutations
- PML/RARA
- RUNX1/CBFA2T1
- CBFB/MYH11
- MLL fusions
- CEBPA
- NPM1

proliferative and/or survival advantage

AML

impaired hematopoietic differentiation

molecular therapy, e.g. with FLT3, KIT inhibitors

molecular therapy, e.g. with ATRA

Adapted from Speck & Gilliland, Nat Rev Cancer. 2002
Evolution of mutations in AML

Welch et al, Cell, 2012
Patterns of relapse in AML

WHO classification

Contents

Chapter 7: Myeloid neoplasma with germline predisposition

Chapter 8: Acute myeloid leukemia and related precursor neoplasms

Chapter 9: Blastic plasmacytoid dendritic neoplasm

Chapter 10: Acute leukemias of ambiguous lineage
  Mixed phenotype acute leukemia (MPAL)
Principles WHO classification

- Integration of all available information
  - Definition, ICD-O Code, Synonyms
  - Epidemiology
  - Clinical features
  - Microscopy
  - Immunophenotype
  - Genetic profile
  - Prognosis and predictive factors
# Tests/ procedures

**For a patient with AML**

<table>
<thead>
<tr>
<th>Tests to establish the diagnosis</th>
<th>Additional tests/procedures at diagnosis (cont'd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count and differential count</td>
<td>Analysis of comorbidities</td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
<td>Biochemistry, coagulation tests, urine analysis**</td>
</tr>
<tr>
<td>Bone marrow trephine biopsy*</td>
<td>Serum pregnancy test‡‡</td>
</tr>
<tr>
<td>Immunophenotyping</td>
<td>Information on oocyte and sperm cryopreservation‡‡</td>
</tr>
</tbody>
</table>

**Genetic analyses**

<table>
<thead>
<tr>
<th>Cytogenetics†</th>
<th>Eligibility assessment for allogeneic HCT (including HLA typing)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening for gene mutations including‡</td>
<td>Hepatitis A, B, C; HIV-1 testing</td>
</tr>
<tr>
<td><em>NPM1, CEBPA, RUNX1, FLT3, TP53, ASXL1</em></td>
<td>Lumbar puncture§</td>
</tr>
<tr>
<td>Screening for gene rearrangements§</td>
<td>Biobanking¢</td>
</tr>
<tr>
<td><em>PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL1,</em> other fusion genes (if available)</td>
<td>Sensitive assessment of response by RT-qPCR or MFCd</td>
</tr>
</tbody>
</table>

**Additional tests/procedures at diagnosis**

<table>
<thead>
<tr>
<th>Demographics and medical history¹¹</th>
<th>RT-qPCR² for <em>NPM1</em> mutation, <em>CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL1,</em> other fusion genes (if available)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detailed family history¶</td>
<td>MFCf</td>
</tr>
<tr>
<td>Patient bleeding history#</td>
<td></td>
</tr>
<tr>
<td>Performance status (ECOG/WHO score)</td>
<td></td>
</tr>
</tbody>
</table>
## Markers for the diagnosis of AML and MPAL

<table>
<thead>
<tr>
<th>Expression of cell-surface and cytoplasmic markers</th>
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<tbody>
<tr>
<td><strong>Diagnosis of AML</strong>†</td>
</tr>
<tr>
<td><strong>Precursors</strong>‡</td>
</tr>
<tr>
<td><strong>Granulocytic markers</strong>‡</td>
</tr>
<tr>
<td><strong>Monocytic markers</strong>§</td>
</tr>
<tr>
<td><strong>Megakaryocytic markers</strong>‖</td>
</tr>
<tr>
<td><strong>Erythroid markers</strong></td>
</tr>
<tr>
<td><strong>Diagnosis of MPAL</strong>¶</td>
</tr>
<tr>
<td><strong>Myeloid lineage</strong></td>
</tr>
<tr>
<td><strong>T-lineage</strong></td>
</tr>
<tr>
<td><strong>B-lineage</strong>***</td>
</tr>
</tbody>
</table>

Blood, 2017, Döhner et al.
8: Acute myeloid leukemia and related precursor neoplasms

- AML with recurrent genetic abnormalities
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML not otherwise specified
- Myeloid sarcoma
- Myeloid proliferations associated with Down syndrome
AML with recurrent genetic abnormalities

- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1;1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- Acut promyelocytic leukemia with PML-RARA FAB M3
- AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3
- AML with t(6;9)(p23;q34.1); DEK-NUP214
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM (=EVI1)
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); RBM15-MKL1
- AML with BCR-ABL1
- AML with with gene mutations
  - AML with mutated NPM1
  - AML with biallelic mutation of CEBPA
  - AML with mutated RUNX1
AML with recurrent genetic abnormalities favorable prognosis

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- AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); RBM15-MKL1
- AML with BCR-ABL1
- AML with with gene mutations
  - AML with mutated NPM1
  - AML with biallelic mutation of CEBPA
  - AML with mutated RUNX1
## 2017 ELN risk genetic stratification

<table>
<thead>
<tr>
<th>Risk category*</th>
<th>Genetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Favorable</strong></td>
<td>t(8;21)(q22;q22.1); <em>RUNX1-RUNX1T1</em></td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <em>CBFB-MYH11</em></td>
</tr>
<tr>
<td></td>
<td>Mutated <em>NPM1</em> without <em>FLT3-ITD</em> or with <em>FLT3-ITDlow†</em></td>
</tr>
<tr>
<td></td>
<td>Biallelic mutated <em>CEBPA</em></td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td>Mutated <em>NPM1</em> and <em>FLT3-ITDhigh†</em></td>
</tr>
<tr>
<td></td>
<td>Wild-type <em>NPM1</em> without <em>FLT3-ITD</em> or with <em>FLT3-ITDlow†</em> (without adverse-risk</td>
</tr>
<tr>
<td></td>
<td>genetic lesions)</td>
</tr>
<tr>
<td></td>
<td>t(9;11)(p21.3;q23.3); <em>MLLT3-KMT2A</em></td>
</tr>
<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>
</tr>
<tr>
<td></td>
<td>inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); <em>GATA2,MECOM(EVI1)</em></td>
</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>
<tr>
<td></td>
<td>Wild-type <em>NPM1</em> and <em>FLT3-ITDhigh†</em></td>
</tr>
<tr>
<td></td>
<td>Mutated <em>RUNX1¶</em></td>
</tr>
<tr>
<td></td>
<td>Mutated <em>ASXL1¶</em></td>
</tr>
<tr>
<td></td>
<td>Mutated <em>TP53#</em></td>
</tr>
</tbody>
</table>

Blood, 2017, Döhner et al.
AML with myelodysplasia-related changes

- ≥ 20% blasts in PB or BM
- AND one of the following:
  - History of MDS or MDS/MPN
  - Myelodysplasia-related cytogenetic abnormality
    - Complex karyotype: 3 or more chromosomal abnormalities
    - Unbalanced abnormalities: -7, del(7q), -5, del(5q), i(17q), t(17q), -13, del(13q), del(11q), del(12p), t(12p) or idic(X)(q13)
    - Balanced abnormalities: t(11;16)(q23.3;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21.2), 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;q21.2) or t(3;5)(q25.3;q35.1)
  - Multilineage dysplasia: dysplasia in ≥50% of cells in ≥2 myeloid lineages
- AND absence of both prior cytotoxic therapy for unrelated disease and aforementioned recurring genetic abnormalities
Therapy-related myeloid neoplasms

- t-AML, t-MDS or t-MDS/MPN
- Excluded: progression from MPN or evolution of primary MDS or MDS/MPN to AML (secondary AML)
- Cytotoxic agents implicated in therapy-related myeloid neoplasms
  - Alkylating agents
  - Ionizing radiation therapy
  - Topoisomerase II inhibitors
  - Others
AML not other specified

- AML with minimal differentiation  FAB M0
  - MPO negative, CD13+, CD117+, CD33+ (60%)
- AML without maturation  FAB M1
  - >90% blasts of NEC
- AML with maturation  FAB M2
- Acute myelomonocytic leukemia  FAB M4
- Acute monoblastic/monocytic leukemia  FAB M5a/b
- Acute erythroid leukemia  FAB M6
- Acute megakaryoblastic leukemia  FAB M7
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis
Myeloid sarcoma

- Tumor mass consisting of myeloid blasts with or without maturation
- Occurring in other anatomical site than bone marrow
- Not: Infiltration of any site of the body by myeloid blasts in a patient with AML
- Localization, any site, most frequent:
  - Skin, lymph nodes, GI tract, bone, soft tissue, testes
Molecular classes of AML and concurrent gene mutations in adult patients ≤65 years
Genomic classification and prognosis in AML

11 discrete genetic subsets of AML on the basis of the expression and coexpression of particular mutations

Molecular subclassification and overall survival

- 11 discrete genetic subsets of AML on the basis of the expression and coexpression of particular mutations.

# Proposed genomic classification of AML

**Table 1. Proposed Genomic Classification of Acute Myeloid Leukemia (AML).**

<table>
<thead>
<tr>
<th>Genomic Subgroup</th>
<th>Frequency in the Study Cohort (N = 1540)</th>
<th>Most Frequently Mutated Genes&lt;sup&gt;a&lt;/sup&gt; gene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with NPM1 mutation</td>
<td>418 (27)</td>
<td>NPM1 (100), DNMT3A (54), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (39), NRAS (19), TET2 (16), PTPN11 (15)</td>
</tr>
<tr>
<td>AML with mutated chromatin, RNA-splicing genes, or both&lt;sup&gt;†&lt;/sup&gt;</td>
<td>275 (18)</td>
<td>RUNX1 (39), MLL&lt;sup&gt;ITD&lt;/sup&gt; (25), SRSF2 (22), DNMT3A (20), ASXL1 (17), STAG2 (16), NRAS (16), TET2 (15), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (15)</td>
</tr>
<tr>
<td>AML with TP53 mutations, chromosomal aneuploidy, or both&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>199 (13)</td>
<td>Complex karyotype (68), -5/5q (47), -7/7q (44), TP53 (44), -17/17p (31), -12/12p (17), +8/8q (16)</td>
</tr>
<tr>
<td>AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFβ–MYH11</td>
<td>81 (5)</td>
<td>inv(16) (100), NRAS (53), +8/8q (16), +22 (16), KIT (15), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (15)</td>
</tr>
<tr>
<td>AML with biallelic CEBPA mutations</td>
<td>66 (4)</td>
<td>CEBPA&lt;sup&gt;biallelic&lt;/sup&gt; (100), NRAS (30), WT1 (21), GATA2 (20)</td>
</tr>
<tr>
<td>AML with t(15;17)(q22;q12); PML–RARA</td>
<td>60 (4)</td>
<td>t(15;17) (100), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (35), WT1 (17)</td>
</tr>
<tr>
<td>AML with t(8;21)(q22;q22); RUNX1–RUNX1T1</td>
<td>60 (4)</td>
<td>t(8;21) (100), KIT (38), −Y (33), −9q (18)</td>
</tr>
<tr>
<td>AML with MLL fusion genes; t(x;11)(x;q23)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>44 (3)</td>
<td>t(x;11q23) (100), NRAS (23)</td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); GATA2, MECOM(EVI1)</td>
<td>20 (1)</td>
<td>inv(3) (100), −7 (85), KRAS (30), NRAS (30), PTPN11 (30), ETV6 (15), PHF6 (15), SF3B1 (15)</td>
</tr>
<tr>
<td>AML with IDH2&lt;sup&gt;R172&lt;/sup&gt; mutations and no other class-defining lesions</td>
<td>18 (1)</td>
<td>IDH2&lt;sup&gt;R172&lt;/sup&gt; (100), DNMT3A (67), +8/8q (17)</td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34); DEK–NUP214</td>
<td>15 (1)</td>
<td>t(6;9) (100), FLT3&lt;sup&gt;ITD&lt;/sup&gt; (80), KRAS (20)</td>
</tr>
<tr>
<td>AML with driver mutations but no detected class-defining lesions</td>
<td>166 (11)</td>
<td>FLT3&lt;sup&gt;ITD&lt;/sup&gt; (39), DNMT3A (16)</td>
</tr>
<tr>
<td>AML with no detected driver mutations</td>
<td>62 (4)</td>
<td></td>
</tr>
<tr>
<td>AML meeting criteria for ≥2 genomic subgroups</td>
<td>56 (4)</td>
<td></td>
</tr>
</tbody>
</table>

Genomic classification and prognosis in AML

- The driver landscape in AML reveals distinct molecular subgroups that reflect discrete paths in the evolution of AML, informing disease classification and prognostic stratification.

- Prospective studies may elucidate distinct approaches to their management.
Prognostic value of minimal residual disease detection in AML with flow cytometry

- 517 AML patients, 18-60 years
- 85% of all AMLs:
  - Leukemia-associated phenotype by immunoflow cytometry is determined at diagnosis
  - Minimal residual disease assessment in complete remission:
    - After chemotherapy induction cycle 1
    - After chemotherapy cycle 2
    - After consolidation treatment

Terwijn et al. J Clin Oncol 2013
Relapse incidence by minimal residual disease

A: After chemotherapy induction cycle 1
B: After chemotherapy cycle 2
C: After consolidation treatment
Relapse incidence by minimal residual disease

After chemotherapy cycle 2
D: Good risk
C: Intermediate risk
F: Poor risk
Literature AML