Objectives

- Introductory Concepts
- Genetic basis of AML
- Types of mutations and timeline of discovery
- Frequency of genetic aberrations in AML
- Functional mutational categories
- FAB and WHO subtypes
- t-AML
Introductory Concepts

- AML is characterized by an accumulation of granulocyte or monocyte precursors in the bone marrow and blood
- Originates as a consequence of genetic changes in the hematopoietic stem cell which alter growth and differentiation
- Unlike solid tumors, many heme malignancies are associated with single characteristic genetic abnormalities
- Identification of changes can have diagnostic, prognostic, and treatment implications
AML is a genetic disease

• Early pioneering work by Janet Rowley
• Further developed the use of quinacrine fluorescence and Giemsa staining to identify chromosome in the 1970s
• 1972: First to identify translocation (8;21) and postulate that specific translocations caused specific diseases
  • Later discovered t(9;22) and t(15;17)
• Died at age 88 from ovarian CA

Credit
Jewel Samad/Agence France-Presse — Getty Images
Prognostic Implications

**Table 15-3  Cytogenetics and Treatment Outcomes in Acute Myeloid Leukemia**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Incidence (%)</th>
<th>Complete Response Rate (%)</th>
<th>5-Year Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>20</td>
<td>85</td>
<td>60</td>
</tr>
<tr>
<td>inv(16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(8;21), t(15;17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>45</td>
<td>76</td>
<td>38</td>
</tr>
<tr>
<td>Normal, +8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse</td>
<td>30</td>
<td>55</td>
<td>12</td>
</tr>
<tr>
<td>del(5q), -5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(7q), -7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex, 11q23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**At Risk**
- Favorable: 121
- Intermediate: 278
- Unfavorable: 184

**Deaths**
- Favorable: 53
- Intermediate: 168
- Unfavorable: 162

**Estimate (CI) At 5 Years**
- Favorable: 55% (45%, 64%)
- Intermediate: 38% (32%, 44%)
- Unfavorable: 11% (7%, 16%)

Heterogeneity of 3 groups: p < 0.0001
Trisomy 8

- Most frequent cytogenetic aberration; seen in ~10-15% of AML cases
- May be sole karyotypic abnormality (50%) or associated with other aberrations (50%)
- In isolation, associated with older age, lower WBC counts (mean 33.6 vs 51.1), male sex
- Most common genetic aberration in myeloid leukemias of Down’s syndrome
- Constitutional trisomy 8 (cT8) is rare, associated with skeletal abnormalities, urogenital malformations, cognitive deficits

Types of Genetic Alterations

- Karyotypic/chromosomal abnormalities
  - Gain or loss of whole chromosome
  - Translocation
  - Deletion
  - Inversion
- Single gene mutations
  - somatic
  - germline

Kavianpour et al., Tumor Biol, 2016.
Frequency of Genetic Aberrations in AML

- **NPM1 mutant**: 33%
- **TP53 mutant/loss**: 8%
- **biCEBPA mutant**: 4%
- **RUNX1 ~40%**
- **MLL-PTD ~30%**
- **ASXL1 ~30%**
- **SRSF2 ~20%**
- **U2AF1 ~15%**
- **STAG2 ~15%**
- **BCOR ~10%**
- **SF3B1 ~10%**
- **EZH2 ~5%**
- **ZRSR2 ~5%**
- **Complex and Monosomal Karyotype ~50%**
- **GATA2 ~30%**
- **t(15;17)(q22;q21)/PML-RARA**: 13%
- **t(8;21)(q22;q22)/RUNX1-RUNX1T1**: 7%
- **inv(16)(p13q22)/CBFB-MYH11**: 5%
- **11q23/MLL-X**: 4%
- **t(9;22)(q34;q11)/BCR-ABL**: 1%
- **t(6;9)(p23;q34)/DEK-NUP214**: 1%
- **t(5;11)(q35;p15.5)/NUP98-NSD1**: 1%
- **inv(3)(q21q26)/GATA2-EVI1**: 1%
- **Other rare fusions**: 1%

- **DNMT3A ~50%**
- **FLT3-ITD ~40%**
- **Cohesin ~20%**
- **IDH1 ~15%**
- **IDH2-R140 ~15%**
- **PTPN11 ~15%**
- **t(3;5)(q21~25;q31-35)/NPM1-MLF1**
- **t(8;16)(p11;p13)/MYST3-CREBBP**
- **t(16;21)(p11;q22)/FUS-ERG**
- **t(10;11)(p13;q21)/PICALM-MLLT10**
- **t(7;11)(p15;p15)/NUP98-HOXA9**
- **t(3;21)(q26;q22)/RUNX1-MECOM**

Functional mutational categories

Functional mutational Spectrum

1. NPM1
2. Signaling Pathway Components
3. Tumor Suppressors
4. Myeloid Transcription Factors
5. Epigenetic Modifiers (DNA methylation/demethylation/chromatin regulation)
6. Spicing Factor Gene Mutations
7. Cohesin Complex Members
NPM1
NPM1 and nucleophosmin

- Nucleophosmin is a protein that shuttles between the nucleus and cytoplasm with multiple functions
  - Molecular chaperone that regulates processing/assembly of ribosomes
  - Involved in control of centrosome duplication during mitosis
  - Implicated in regulation of ARF-p53 tumor suppressor pathway
  - Helps maintain genomic stability
- NPM1 functions both as an oncogene and tumor suppressor gene

Falini et al., NEJM 2005
NPM1 translocations & mutations

- Mutated in ~30% of de novo AML pts
  - Mutated in 50% of pts with nl karyotype AML
  - More than 40 different mutations described
    - Type A, B, and D account for ~90%
- NPM1 gene is a partner in several chromosomal translocations of leukemias and lymphomas that result in fusion proteins
- In isolation, mutation is associated with improved outcomes although mechanism of increased chemosensitivity is unknown

NPM1 and oncogenesis

SIGNALING PATHWAY
FLT3-ITD & FLT3-TKD

• FMS like tyrosine kinase
  • Stimulates cell proliferation upon activation
  • Normally expressed by HSPCs, lost as cells differentiate
• Mutations occur in ~30% of AML
  • ITD (Internal tandem duplication) mutation results in different length duplicates of juxtamembrane domain
  • TKD (tyrosine kinase domain) mutations are point mutations typically involving aspartic acid 835
• FLT3-ITD mutations associated with poor prognosis, higher rate of relapse
  • Exception: if allelic ratio (ratio of mutant to WT) is low
    • Pts with larger ITDs fare worse than those with smaller ITDs
• FLT3-TKD mutations are not thought to be a poor prognostic factor
Mutated FLT3
FLT3 Signaling

Diagram showing the FLT3/ITD signaling pathway, including interactions with Akt, PI-3 kinase, Stat5, transcription factors, and processes like apoptosis, differentiation, and proliferation.
TUMOR SUPPRESSORS
• Tumor suppressors are recessive; loss of function requires:
  • Combination of TP53 mutation and a del of remaining allele
  • TP53 mutation in both alleles
  • Homozygous TP53 mutation due to LOH
  • Certain mutant forms form mixed tetramers with WT P53 protein rendering it incapable of DNA binding (dominant negative effect)
• Mutated in ~7% of AML pts
• Mutations are heterogenous
  • Missense mutation is most frequent type
• Highest incidence (70-80%) in pts with AML evolving from MDS or in AML with complex karyotype
• Mutation associated with lower CR rates and worse survival
TRANSCRIPTION FACTORS
Transcription factors

CBF (Core binding factor) AML

- Includes t(8;21) and inv[16]
- Both result in abnormalities in a transcription factor made up of CBF (Core binding factor) α and β
  - CBF complex regulates expression of genes required for hematopoiesis
- Both are associated with good prognosis
t(8;21)(q22;q22)

• Associated with 5% of AML cases
• t(8;21) results in a RUNX1 (21) RUNX1T1 (8) fusion product
• Commonly associated with loss of a sex chromosome (-X or -Y), or a del(9q)
Normal function of RUNX1/CBF β

- RUNX1 (formerly ETO) gene on chromosome 8 encodes for RUNX1 protein (aka AML1, aka CBF-α2)
  - heterodimerizes with core binding factor β to form a transcription factor
  - mediates transcription factor activation (A) or repression (B) by recruitment of non-DNA binding transcription co-activators
inv[16] [q13q22]

• inversion breakpoint at 16q22 occurs near the end of the coding region of the CBF β gene
• Results in fusion of CBF β on q arm of chromosome 16 with MYH11 on p arm
  • alters fxn of CBF β
• Associated with 8% of AML cases
t(15;17)

- t(15;17) (q22;q11-12)
  - PML gene on chromosome 15
  - RAR-alpha gene on chromosome 17
- Fusion product recruits nuclear corepressors and histone deacetylase, inhibiting transcription of genes required for myeloid differentiation
CEBPA

- Encodes for CCAAT/enhancer binding protein α, a transcription factor essential for myeloid differentiation
  - Also functions to block proliferation
- Mutated in ~10% of AML pts, typically in pts with CN-nl AML
- Biallelic mutation (double mutant) is associated with good prognosis, higher OS
  - Single mutation more often coexist with other mutations (ie FIt3)
CEBPA mutations

Erdogan Taskesen et al. Blood 2011;117:2469-2475
Concurrent mutations in CEBPA single and double mutants

Erdogan Taskesen et al. Blood 2011;117:2469-2475
CEBPA and Flt3 mutations predict OS

Erdogan Taskesen et al. Blood 2011;117:2469-2475
EPIGENETIC MODIFIERS
Epigenetic Modifiers

MLL Fusions

- MLL encodes for histone methyltransferase (KMT2A)
- MLL gene has several translocation partners (121)
  - t(9;11)(p22;q23), t(11;19)(q23;p13.1) and t(6;11)(q27;q23) are most common
- Gives rise to gain-of-function fusion protein (oncogene) associated with poor prognosis
- Present in 7% of adult AML cases; associated with t-AML (topo II exposure)
KMT2a fusion protein

- Fusion proteins induce the aberrant expression of downstream mediators
- Fusion protein down-regulates RNA polymerase II elongation factor allowing for transcriptional elongation free of usual checkpoints

DNMT3A

- DNA Methyltransferase
  - Methylation required to silence expression of TFs related to self-renewal and multipotency (RUNX1, GATA3) allowing differentiation to proceed
- Mutated in ~30% of CN-nl AML
  - Mutations in NPM1 and FLT3-ITD often cosegregate
    - DNMT3A mutated in 50% of NPM1 mutated AML
- Mutations are typically heterozygous with mutant protein interfering with ability of residual WT protein to fxn
  - Results in reduced enzyme activity, focal hypomethylation of specific nucleotides
    - expression of genes that are usually silenced
- Associated with poor prognosis
DNA methylation in early stages of hematopoiesis

Mice double-deficient in DNMT3A and DNMT3B expression show enhanced self-renewal, but no differentiation

Patterns of methylation change during adult hematopoiesis
TET2 & IDH

- TET2 (ten eleven translocation 2) regulates initial step in DNA demethylation
- Mutations occur in 8% of AML pts
  - lead to altered gene expression, impaired differentiation

- Isocitrate Dehydrogenase enzymes (IDH1 & IDH2) catalyze conversion of isocitrate to α-ketoglutarate
- Mutations in IDH reduce α-ketoglutarate production
  - Also cause production of 2-hydroxyglutarate (“oncometabolite”)
    - inhibits fxn of TET2 and other enzymes, leading to altered gene expression
- IDH 1 mutations are found in 7% of AML pts, IDH2 mutations in a further 9%
- Mutations involving different sites are prognostically distinct

Pfeifer et al., Epigenetics Chromatin, 2013;6(10)
MAKING SENSE OF MOLECULAR MARKERS
Putting it all together
MORPHOLOGIC/GENETIC SUBTYPES
Classification Schema

- French-American British (FAB)
  - developed in 1970s
  - based on morphology (cell of origin and degree of immaturity)
- World Health Organization (WHO)
  - developed in 1999
  - incorporates information about cytogenetics
  - determines prognostic subgroups that may help define treatment strategies
### Table 2 | French-American-British (FAB) classification of AML

<table>
<thead>
<tr>
<th>FAB subtype</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Undifferentiated</td>
<td>Myeloperoxidase negative; myeloid markers positive</td>
</tr>
<tr>
<td>M1</td>
<td>Myeloblastic without maturation</td>
<td>Some evidence of granulocytic differentiation</td>
</tr>
<tr>
<td>M2</td>
<td>Myeloblastic with maturation</td>
<td>Maturation at or beyond the promyelocytic stage of differentiation; can be divided into those with t(8;21) AML1–ETO fusion and those without</td>
</tr>
<tr>
<td>M3</td>
<td>Promyelocytic</td>
<td>APL; most cases have t(15;17) PML–RARα or another translocation involving RARα</td>
</tr>
<tr>
<td>M4</td>
<td>Myelomonocytic</td>
<td></td>
</tr>
<tr>
<td>M4&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Myelomonocytic with bone-marrow eosinophilia</td>
<td>Characterized by inversion of chromosome 16 involving CBFβ, which normally forms a heterodimer with AML1</td>
</tr>
<tr>
<td>M5</td>
<td>Monocytic</td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>Erythroleukaemia</td>
<td></td>
</tr>
<tr>
<td>M7</td>
<td>Megakaryoblastic</td>
<td>GATA1 mutations in those associated with Down's syndrome</td>
</tr>
</tbody>
</table>

AML1, acute myeloid leukaemia 1; APL, acute promyelocytic leukaemia; PML, promyelocytic leukaemia; RARα, retinoic-acid receptor-α. Modified from REF.65.

FAB classification of acute myeloblastic leukaemia

M0  Acute myeloblastic leukaemia with minimal differentiation

Morphology:
Can resemble LLA-L2 blasts. Medium-sized blasts, rounded nucleus, fine chromatin, basophilic non-granular cytoplasm, prominent nucleoli.

Immunophenotype
- CD13 +
- CD33 +
- CD11b +
- CD11c +
- CD14 +
- CD15 +

M1  Acute myeloblastic leukaemia without maturation

Morphology:
Medium-sized blasts with high nucleo-cytoplasm (n/c) ratio, rounded nuclei with immature, dispersed chromatin with one or more prominent nucleoli. Blasts can show fine azurophilic granulation or isolated Auer rods in the cytoplasm in 5% to 10% of cases.

Immunophenotype
- MPO +
- CD13 +
- CD33 +
- CD117 +
- CD34 +/-

M2  Acute myeloblastic leukaemia with maturation

Morphology:
Small to medium-sized blasts with high nucleo-cytoplasm (n/c) ratio and rounded nuclei sometimes located in a corner of the cytoplasm. The nucleus shows dispersed, immature chromatin with one or more nucleoli. The cytoplasm is basophilic and can contain traces of primary azurophilic granulation or isolated Auer rods.

Immunophenotype
- MPO +
- CD34 +/-
- CD13 +
- CD15 +
- HLA-DR +
- Sudan black +
- CD117 +/

M3  Promyelocytic leukaemia

Morphology:
Abundant, intensely azurophilic granulation. The nucleus is usually monoytic in appearance (reniform) and is either irregular or bilobed with a deep cleft. Scarcely basophilic cytoplasm due to the proliferation of azurophilic granulation. Some atypical promyelocytes also contain elongated or splinter-shaped crystalline cytoplasmic inclusions specific to this type of leukaemia. These usually form clumps, but differ from Auer rods in that they show a tubular substructure on electronic microscopy.

Immunophenotype
- CD13 +
- CD33 +
- HLA-DR -
- CD34 -

Photo courtesy of: Acute myeloid leukemia pathophysiology, 2012
<table>
<thead>
<tr>
<th>M4</th>
<th>Acute myelomonocytic leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology:</strong></td>
<td>Large blasts, moderate nucleo-cytoplasm ratio and variable basophilia. The nucleus may be rounded, kidney-shaped or irregular. Nucleoli are usually prominent.</td>
</tr>
<tr>
<td><strong>Immunophenotype:</strong></td>
<td>-CD13 +</td>
</tr>
<tr>
<td></td>
<td>-CD15 +</td>
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<tr>
<td></td>
<td>-CD33 +</td>
</tr>
<tr>
<td></td>
<td>-CD11b +</td>
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<tr>
<td></td>
<td>-CD11c +</td>
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<tr>
<td></td>
<td>-CD14 +</td>
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<td></td>
<td>-CD64 +</td>
</tr>
<tr>
<td></td>
<td>-CD4 +</td>
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<table>
<thead>
<tr>
<th>M5</th>
<th>Acute monocytic leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M5a acute monoblastic leukaemia:</strong></td>
<td>Large blasts with rounded nucleus and dispersed, immature chromatin (1-3 nucleoli) and moderately large and intensely basophilic cytoplasm. The cytoplasm may show some Auer rods and/or prolongations and granulations.</td>
</tr>
<tr>
<td><strong>M5b acute monocytic leukaemia:</strong></td>
<td>Promonocytes have a rounded or kidney-shaped nucleus with a less basophilic cytoplasm that is more highly granulated than monoblasts and contains some vacuoles. A finding of erythrophagocytosis together with monoblastic blasts suggests a t(8;16) translocation.</td>
</tr>
<tr>
<td><strong>Immunophenotype:</strong></td>
<td>-CD14 +</td>
</tr>
<tr>
<td></td>
<td>-CD68 +</td>
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<tr>
<td></td>
<td>-CD4 +</td>
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<tr>
<td></td>
<td>-CD11c +</td>
</tr>
<tr>
<td></td>
<td>-HLA-DR +</td>
</tr>
<tr>
<td></td>
<td>-CD64 +</td>
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</table>

<table>
<thead>
<tr>
<th>M6</th>
<th>Acute erythroid leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M6a erythroid leukaemia with proliferation of mixed blasts:</strong></td>
<td>Morphology of erythroblasts in peripheral blood is greatly changed, with schistocytes, “pincered” or mushroom-shaped cells, and spiculated echinocyte and acanthocyte cells.</td>
</tr>
<tr>
<td><strong>M6b pure erythroid leukaemia:</strong></td>
<td>Erythroblasts make up 80% of bone marrow cells, with less than 3% myeloid cells. Erythroblasts in peripheral blood consist of macrocytes, basophilic stippling, Howell-Jolly bodies or Cabot rings.</td>
</tr>
<tr>
<td><strong>Immunophenotype:</strong></td>
<td>-CD13 +</td>
</tr>
<tr>
<td></td>
<td>-CD68 +</td>
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<tr>
<td></td>
<td>-CD33 +</td>
</tr>
<tr>
<td></td>
<td>-CD15 +</td>
</tr>
<tr>
<td></td>
<td>-Glycoporphin A +</td>
</tr>
<tr>
<td></td>
<td>-Glycoporphin C +</td>
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</table>

<table>
<thead>
<tr>
<th>M7</th>
<th>Acute megakaryocytic leukaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology:</strong></td>
<td>Highly immature, polymorphic blasts. The nucleus is eccentric with dispersed, reticulated chromatin and 1-3 prominent nucleoli. The cytoplasm is non-granular, basophilic, and very similar in appearance to platelets, with pseudopods or granulations. Micromegakaryocytes and fragments of megakaryoblasts are seen in peripheral blood (giant platelets, some highly degranulated).</td>
</tr>
<tr>
<td><strong>Immunophenotype:</strong></td>
<td>-CD41 +</td>
</tr>
<tr>
<td></td>
<td>-CD61 +</td>
</tr>
<tr>
<td></td>
<td>-CD42 +</td>
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<td>-CD13 +</td>
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<td>-CD33 +</td>
</tr>
<tr>
<td></td>
<td>-CD34 +</td>
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</table>
### WHO classification schema

- Takes into account etiologic, immunophenotypic, cytologic and morphologic features
- Accounts for genetic and clinical diversity

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>AML with recurrent genetic abnormalities</td>
<td>t(8;21), inv(26) or t(16;6), t(15;17), 11q23 (MLL) abnormalities, others</td>
</tr>
<tr>
<td>AML with MDS-related changes</td>
<td>Multilineage dysplasia without NPM1 or CEBPA mutation; h/o MDS; MDS-related cytogenetic abnormality</td>
</tr>
<tr>
<td>Therapy related AML (t-AML)</td>
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</tr>
<tr>
<td>AML, NOS</td>
<td>Not otherwise fitting into other categories; Acute Erythroid Leukemia, Pure Erythroid Leukemia</td>
</tr>
<tr>
<td>Myeloid Sarcoma</td>
<td></td>
</tr>
<tr>
<td>Myeloid proliferations of Down Syndrome</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated and Biphenotypic Acute Leukemias</td>
<td>Both lymphocytic and myeloid features</td>
</tr>
</tbody>
</table>

Yardman et al., Blood 2002 100:2292-2302.
Arber et al., Blood 2016 127:2391-2405
## t-AML

<table>
<thead>
<tr>
<th>Type of Disease</th>
<th>Unbalanced 5q-/-5 7q-/-7 +8, %</th>
<th>Balanced 11q23 21q22 17q21 16q22, %</th>
<th>Normal Karyotype, %</th>
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</thead>
<tbody>
<tr>
<td>De novo AML</td>
<td>15-25%</td>
<td>15-20%</td>
<td>40-50%</td>
</tr>
<tr>
<td>t-AML</td>
<td><strong>40-50%</strong></td>
<td>15-20%</td>
<td>10-15%</td>
</tr>
</tbody>
</table>

- Exposure to alkylating agents closely associated with 5q-/-5 and 7q-/-7
  - Long latency (4-7yrs)
  - Often associated with preceding MDS
- Topo II inhibitors associated with development of balanced translocations (including 11q23)
  - Short latency (6mo-5yrs)

Yardman et al., Blood 2002 100:2292-2302.
Questions?