Acute Myeloid Leukemia: Importance of Ancillary Studies in Diagnosis and Classification

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Estimated Yearly Incidence of AML
Misconceptions Some Pathologists Have About AML

• Ruling out APL is all that is really important
• Flow cytometry is not helpful in most cases
• The clinician can correlate the pathology findings with cytogenetics, because they will not change the diagnosis
<table>
<thead>
<tr>
<th>Leukemia</th>
<th>Lymphoma</th>
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<tr>
<td>1960’s</td>
<td>Rappaport</td>
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<td>1976 FAB</td>
<td>1982</td>
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<td>-1991</td>
<td>Working</td>
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<td>Formulation</td>
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<td>1994 REAL</td>
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<td>2001 WHO</td>
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What are the Significant Changes of the WHO Classification of AML?
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- Recognized the significance of multilineage dysplasia in AML
- Recognized the significance of prior therapy in AML and MDS
Blast Count in AML

- WHO lower the bone marrow (or blood) blast count for AML to 20% for most cases
- No blast cell minimum for cytogenetic subtypes
RAEBT vs. AML with Multilineage Dysplasia and ≥30% Blasts

Overall Survival

Survival Distribution Function

0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Overall Survival (mo.)

0 1 0 2 0 3 0 4 0 5 0 6 0 7 0 8 0 9 0 1 0 1 1 0 1 2 0 1 3 0

RAEBT (n=24)

AML, MLD or Therapy-related (n=113)

p = 0.5289

City of Hope Data
Cytogenetics in AML
Cytogenetics of Childhood AML


- **normal** 22.8%
- **-7** 1.9%
- **+8 alone** 2.1%
- **+21 alone** 1.5%
- **11q23** 18.4%
- **inv(16)/t(16;16)** 5.9%
- **miscellaneous** 18.6%
  - [one abnormal 7.5%]
  - [two/+ abnormal 11.1%]
- **t(8;21)** 11.7%
- **t(15;17)** 11.5%
- **t(1;22)(p13;q13)** 0.8%
- **t(6;9)(p23;q34)** 1.3%
- **t(10;11)(p13;q21)** 5.6%
- **t(3:5)(q25;q34)** 1.1%
- **t(8;16)(p11;p13)** 0.6%
- **Rare recurrent**
Cytogenetics of Adult AML

- Normal: 40%
- t(15;17): 10%
- inv(16)/t(16;16): 9%
- t(8;21): 8%
- 11q: 7%
- t(15;17): 10%
- Other: 32%

Other include:
- -5 / 5q-
- -7 / 7q-
- +8
- inv(3) / t(3;3)
- Abnormality 13q
- i(17q)
- Abnormality of 17p
- Abnormality of 20q
- Abnormality of 21q
- t(9;22)
- t(6;9)
- del (9q)
- Other trisomy
- -X
- -Y

Complex Karyotypes

SWOG Data
Recurring Cytogenetic Abnormalities in Adult AML

Survival Distribution Function

Overall Survival (mo.)

inv(16) AML (n=30)
t(15;17) AML (n=19)
t(8;21) AML (n=15)
11q23 AML (n=11)

p = 0.0245

## Cytogenetic Risk Groups

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
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<tbody>
<tr>
<td>t(8;21)</td>
<td>Complex (&gt;3) abnormalities</td>
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<tr>
<td>inv(16)/t(16;16)</td>
<td>-7</td>
</tr>
<tr>
<td>t(15;17)</td>
<td>inv(3q)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>del(9q) without t(8;21)</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>11q23, 17p, 20q or 21q</td>
</tr>
<tr>
<td>Single abnormalities</td>
<td>abnormalities</td>
</tr>
<tr>
<td>+8</td>
<td>t(9;22)</td>
</tr>
<tr>
<td>+11</td>
<td>t(6;9)</td>
</tr>
<tr>
<td>-Y</td>
<td>+13</td>
</tr>
<tr>
<td>12p abnormalities</td>
<td>dmin/hsrs</td>
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</tbody>
</table>
Pure Cytogenetic Classification of AML - Overall Survival

Survival Distribution Function

Overall Survival (mo.)

Low risk (n=69)
Intermediate risk (n=98)
High risk (n=62)

p < 0.0001

Multilineage Dysplasia in AML

- WHO defines as two or more cell lines with over 50% dysplasia
Overall Survival of Therapy and MDS-associated AML vs. Non-MDS AML

Survival Distribution Function

Non-MDS AML (n=187)
AML, MDS- or Therapy-related (n=113)

p < 0.0001

WHO Classification of Acute Myeloid Leukemia

- Acute myeloid leukemia with recurrent cytogenetic abnormalities
  - AML with t(8;21)(q22;q22), (AML1/ETO)
  - AML with inv(16)(p13q22) or t(16;16)(p13;q22), (CBFβ/MYH11)
  - Acute promyelocytic leukemia (AML with t(15;17)(q22;q12), (PML/RARα) and variants)
  - AML with 11q23 (MLL) abnormalities
Acute Myeloid Leukemia with t(8;21) (RUNX1/RUNX1T1)

- Characteristic blast cell morphology with perinuclear hofs, Auer rods and large salmon-colored granules
Acute Myeloid Leukemia with t(8;21)

- Characteristic blast cell morphology with perinuclear hofs, Auer rods and large salmon-colored granules
Acute Myeloid Leukemia with t(8;21)

CD13 and CD33 positive blast gate
Acute Myeloid Leukemia with t(8;21)

CD19 positive

CD34 positive, CD56 +/-
Acute Myeloid Leukemia with t(8;21)

- Characteristic immunophenotype of CD19+/myeloid antigen +/-CD34+ blast cells in two thirds of cases. A subset are also CD56+
- These morphologic and immunophenotypic features have a high correlation with t(8;21)(q22;q22) or RUNX1/RUNX1T1 fusion
- These cases should be diagnosed as AML without regard to blast cell count (so called “oligoblastic” acute leukemia)
Does CD19+ AML = t(8;21)?
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- Adult AML
  - CD19+ in 10/102 cases (9.8%)

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  – Cytogenetics available on 7 of those 10

Does CD19+ AML = t(8;21)?

- Adult AML
  - CD19+ in 10/102 cases (9.8%)
  - Cytogenetics available on 7 of those 10
  - One (14%) of those 7 cases had t(8;21)

Acute Myeloid Leukemia with t(8;21)

- **Morphology**
  - Perinuclear hofs
  - Large pink cytoplasmic granules
  - Auer rods
  - Associated bone marrow eosinophilia

- **Immunophenotype**
  - Aberrant CD19 expression
  - CD34 expression
  - CD56 expression
CD13, CD33, CD34-positive with partial CD19 and CD56

DX: AML with features of t(8;21)
Acute Myeloid Leukemia with inv(16) or t(16;16) (CBFB/MYH11)

- Blast cell proliferation with or without monocytic differentiation by cytochemistry, with an associated proliferation of abnormal eosinophils
Acute Myeloid Leukemia with inv(16) or t(16;16)

- The eosinophils contain abnormal, basophilic granules
Acute Myeloid Leukemia with inv(16) or t(16;16)
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Acute Myeloid Leukemia with inv(16) or t(16;16)

- The cases express myeloid-associated antigens and may be CD2 positive, but there is no specific immunophenotype for this disease.
- These cases should be diagnosed as AML without regard to blast cell count.
Acute Myeloid Leukemia with inv(16) or t(16;16)

- **Morphology**
  - *Abnormal eosinophils*
  - Myelomonocytic morphology

- **Cytochemistry**
  - Non-specific esterase positive

- **Immunophenotype**
  - Aberrant CD2 expression
DX: AML with abnormal eosinophils, suggestive of inv(16) or t(16;16)
Acute Promyelocytic Leukemia

- Includes microgranular and other variants
- Blasts have folded nuclei with or without cytoplasmic granules and Auer rods
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Acute Promyelocytic Leukemia

- Strong myeloperoxidase positivity
Acute Promyelocytic Leukemia

CD13 and CD33 positive
Acute Promyelocytic Leukemia

HLA-DR weak or negative
Acute Promyelocytic Leukemia

CD34 +/-  CD64 +/-  MPO +++
Acute Promyelocytic Leukemia

• Express myeloid-associated antigens with loss of HLA-DR in the majority of cases and often demonstrates CD2 expression
• These cases should be diagnosed as AML without regard to blast cell count
Does HLA-DR-negative AML = Acute Promyelocytic Leukemia?
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- Adult AML
  - HLA-DR+: 2/7 APLs vs 88/99 other AMLs

Does HLA-DR-negative AML = Acute Promyelocytic Leukemia?

• Adult AML
  – HLA-DR+: 2/7 APLs vs 88/99 other AMLs
  – p <0.0001

Does HLA-DR-negative AML = Acute Promyelocytic Leukemia?

- Adult AML
  - HLA-DR negative in 16/106 (15.1%)

Does HLA-DR-negative AML = Acute Promyelocytic Leukemia?

- Adult AML
  - HLA-DR negative in 16/106 (15.1%)
  - t(15;17) was detected in only 5 of those 16 (31%)

Does HLA-DR-negative AML = Acute Promyelocytic Leukemia?

- Adult APL vs. other AML
  HLA-DR negative \( p < 0.0001 \)

Does HLA-DR-negative AML = Acute Promyelocytic Leukemia?

- Adult APL vs. other AML
  - HLA-DR negative \( p < 0.0001 \)
  - CD4 negative \( p = 0.0084 \)
  - CD11c negative \( p < 0.0001 \)
  - CD36 negative \( p = 0.0297 \)
  - CD117 negative \( p = 0.0422 \)
  - CD2 positive \( p = 0.0293 \)

Acute Promyelocytic Leukemia

- Morphology
  - *Bilobated nuclei*
  - *Abundant cytoplasmic granules*
  - Cells with numerous Auer rods

- Cytochemistry
  - *Strong MPO in every cell*

- Immunophenotype
  - *Lack of HLA-DR*
  - *Strong cMPO*
  - Aberrant CD2 expression
CD13, CD33, MPO (strong) positive, HLA-DR negative.
Acute Myeloid Leukemia with 11q23 \((MLL)\) Abnormalities

- Blast cell proliferation, usually with monocytic or myelomonocytic features
Acute Myeloid Leukemia with 11q23 (MLL) Abnormalities

- Blast cell proliferation, usually with monocytic or myelomonocytic features
- More common in children
- Frequently therapy-related when occurring in adults, although associated multilineage dysplasia is often no apparent
- No specific morphologic or immunophenotypic features
Acute Myeloid Leukemia with 11q23 (MLL) Abnormalities

CD34 negative  CD56 +/-  CD64 positive
Acute Myeloid Leukemia with 11q23 (MLL) Abnormalities

MPO negative

CD11c
Acute Myeloid Leukemia with 11q23 (MLL) Abnormalities

• Diagnostic criteria
Acute Myeloid Leukemia with 11q23 (MLL) Abnormalities

- Diagnostic criteria
WHO Classification of Acute Myeloid Leukemia

- Acute myeloid leukemia with multilineage dysplasia
- Acute myeloid leukemia and myelodysplastic syndrome, therapy related
  - Alkylating agent related
  - Topoisomerase II inhibitor-related

WHO Classification of Tumours, 2001
AML with Multilineage Dysplasia
AML with Multilineage Dysplasia
Therapy-Related AML/MDS

Alkylating agent-related

Topo-II inhibitor-related
WHO Classification of Acute Myeloid Leukemia

• Acute myeloid leukemia not otherwise categorized
  • AML, minimally differentiated
  • AML, without maturation
  • AML, with maturation
  • Acute myelomonocytic leukemia
  • Acute monoblastic and monocytic leukemia
  • Acute erythroid leukemia
  • Acute megakaryoblastic leukemia
  • Acute basophilic leukemia
  • Acute panmyelosis with myelofibrosis
  • Myeloid sarcoma

• Acute leukemias of ambiguous lineage
What else is there to know about AML?
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- What is erythroleukemia?
Acute Erythroid Leukemia

- FAB (M6) requires >50% bone marrow erythroid precursors and 30% or greater blasts (myeloblasts) in the non-erythroid cells
- WHO includes two types
  - M6-like except 20% or greater blasts
  - Pure erythroid leukemia; over 80% immature erythroid cells
- Other groups have described “M6C”
  - Over 30% erythroid cells in marrow and over 30% myeloblasts
Acute Erythroid Leukemia

- Almost always associated with dysplastic changes of other cell lines
- Complex cytogenetic abnormalities are usually present, similar to the myelodysplasias and the other MDS-associated acute myeloid leukemias
- Why aren’t these just cases of MDS or AML with multilineage dysplasia?
What else is there to know about AML?

- What is acute megakaryoblastic leukemia?
What else is there to know about AML?

- What is acute megakaryoblastic leukemia?

  An acute leukemia with blasts showing megakaryocytic features by immunophenotyping or platelet peroxidase electron microscopy
Acute Megakaryoblastic Leukemia

- Cytoplasmic blebs are common, but are not specific for this type of leukemia
- Commonly associated with marrow fibrosis
- Blasts are myeloperoxidase negative by cytochemistry, but may express myeloid-associated markers and should be positive for two megakaryocyte-associated markers (CD41, CD42, CD61, vWF, Ulex)
- Demonstration of platelet peroxidase by electron microscopy may also be helpful
Acute Megakaryoblastic Leukemia

Megakaryoblast

Non-Megakaryoblasts
Acute Megakaryoblastic Leukemia

Appear to be at least three types

- Adult type
  - Associated with multilineage dysplasia and MDS-like cytogenetic abnormalities
  - May be related to acute panmyelosis with myelofibrosis

- Childhood type
  - Associated with trisomy 21
  - Occurs at an older age (>2 yrs) than transient myeloproliferative disorder of Down Syndrome

- Infant type
  - Associated with t(1;22)
Acute Megakaryoblastic Leukemia with t(1;22)(p13;q13)

- Infant leukemia
- Nonspecific cytoplasmic blebs
- MPO negative; CD41, CD61 positive
- \textit{RBM15/MKL1 (OTT/MAL)} fusion
What else is there to know about AML?

- Other translocations
- Mutations
Recurrent Cytogenetic Abnormalities in AML with Multilineage Dysplasia

- Chromosome 5 and 7 abnormalities
- Complex karyotypes
- Balanced abnormalities
  - inv(3)(q21q26)/t(3;3)(q21;q26), *EVI1*
  - t(6;9)(23;q34), *DEK-CAN*
  - t(3;5)(q25;q31), *NPM-MLF1*
Mutations in AML

- *NPM1*
- *FLT3*
- *CEPBA*
- *MLL*
- *CKIT*

Mutations in AML

- **FLT3**
  - Mutations occur in 10-15% of childhood AMLs and 20-28% of adult AMLs
  - More frequent in APL, normal karyotype AML or t(6;9) AML
  - Mutation associated with decreased disease free survival in adults
  - Clinical trials with FLT3 inhibitors are underway

FLT3 Mutations

Internal Tandem Duplications (ITD)

Point Mutations (D835)
Significance of \textit{FLT3} Mutations in Adult AML with Normal Karyotypes
Mutations in AML

• *NPM1*
  – 25-30% of all AMLs
  – Up to half of AMLs with normal karyotypes
  – Women, high WBC and Plt counts
  – Mutated *NPM1* associated with a good prognosis unless associated with a *FLT3* mutation

NPM1 Mutations
NPM1 Mutations

Mutations in AML

• *CEPBA*
  – Mutations occur in 7-11% of AMLs
  – Mutations associated with a favorable prognosis, unless accompanied by a *FLT3* mutation

Preudhomme et al. *Blood* 100:2717, 2002
Mutations in AML

- **KIT**
  - Mutations occur in 22-30% of AMLs with t(8;21) and inv(16)
  - Involve exon 17 (usually D816V) or exon 8
  - Mutations in these disease groups associated with a worse prognosis

AML Classification

- AML with Recurring Genetic Abnormalities
- AML, Myelodysplasia-Related
- AML, Therapy-Related
- AML, NOS
AML Classification

• AML with Recurring Genetic Abnormalities
  – AML with t(8;21)
  – AML with inv(16)
  – AML with t(15;17)
  – AML with t(9;11)
  – AML with t(1;22)
  – AML with t(9;22)
  – AML with NPM1 mutations
  – AML with CEPBA mutations

• AML, Myelodysplasia-Related
• AML, Therapy-Related
• AML, NOS
AML Classification

• AML with Recurring Genetic Abnormalities
• AML, Myelodysplasia-Related
  – AML following MDS
  – AML with multilineage dysplasia
  – AML with MDS-related cytogenetics
• AML, Therapy-Related
• AML, NOS
AML Classification

- AML with Recurring Genetic Abnormalities
- AML, Myelodysplasia-Related
- AML, Therapy-Related
- AML, NOS
Summary

- A combined morphologic, immunophenotypic and genetic approach is necessary for the accurate classification of AML and allows for identification of prognostically significant diseases types.
- The ongoing discovery of genetic subtypes of AML will impact future classification and therapy.