Prognostic factors in cytogenetically normal AML

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Case Presentation

• Clinical History: 47 y/o female who presented with WBC count of 60 x 10e3/uL with concern for acute leukemia.

• Laboratory Values:
  • Hgb: 7.3 g/dL, Hct 23%, RBC 2.4 x 10e6/uL, MCV 96 fL, MCH 30.4 pg, MCHC 31.7 g/dL, RDW 18.3%)
  • WBC is significantly increased (WBC: 48.6 x10e3/uL, ANC 1.46 x 10e3/uL, ALC 8.26 x 10e3/uL, AMC 7.29 x 10e3/uL, blasts 26.2 x10e3/uL)
  • Moderate thrombocytopenia (Plt: 69 x10e3/uL, MPV 9.8 fL)

• Morphology: Showed immature monocytes (monoblasts & promonocytes) 87% blasts on manual count

• Flow Cytometry: Immature cells expressed CD4, CD64, CD14 (subset), HLA-DR, CD13, CD33 (moderate), CD11b, & CD11c and negative for CD3 and CD19
Case presentation

• Chromosome Analysis of Bone Marrow showed:
  • NORMAL FEMALE KARYOTYPE, 46,XX[20]
  • INTERPRETATION: Normal

• FISH studies:
  • AML panel and BCR/ABL1 come back normal.
Diagnosis

• The case was signed out as
  • Acute myeloid leukemia, not otherwise specified
  • AML FISH Panel: negative

• Myeloid malignancy panel was pending at time of sign out.
Myeloid malignancy mutation panel result

- Myeloid malignancy mutation panel by next generation sequencing came back as follows
  - NPM1 +
  - FLT3 +
  - DNMT3A +
Outline

• Introduction to AML diagnosis and classification
• Cytogenetically normal AML subtype
  • Genetic mutations in AML
• College of American Pathologists/American society of Hematology (CAP/ASH) guideline for initial diagnostic workup of acute leukemia
• Recent treatment modalities targeting genetic mutations of AML
AML

- Acute myeloid leukemia (AML) is a heterogeneous group of diseases representing clonal proliferations of immature, nonlymphoid, bone marrow–derived cells that most often involve the bone marrow and peripheral blood and may present in extramedullary tissues.
WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed.
Broader Acute leukemia classification
WHO 2017

• AML and related precursor neoplasms
• Acute leukemia of ambiguous lineage
• B-ALL
• T-ALL
• Blastic plasmacytoid dendritic cell neoplasm
Diagnosis of AML

- Clinical features and laboratory values
- Morphology/Microscopy
- Immunophenotype:
  - Flow Cytometry
  - Immunohistochemistry
- Genetic analyses:
  - Conventional cytogenetics
  - Other molecular studies
Microscopy/Morphology

• Equal to or more than 20% blasts in peripheral blood or bone marrow aspirate
• Less than 20% blasts in peripheral blood or bone marrow aspirate
• Blast cell characteristics
• Features of non-blast cells
Microscopy/Morphology

• Equal to or more than 20% blasts in peripheral blood or bone marrow aspirate
  • 200 cell count of peripheral blood and
  • 500 cell count of bone marrow aspirate or core biopsy touch-prep
• Less than 20% blasts in peripheral blood or bone marrow aspirate
• Blast cell characteristics
• Features of non-blast cells
Microscopy/Morphology

- Equal to or more than 20% blasts in peripheral blood or bone marrow aspirate
- Blast cell characteristics
  - Some features are highly suggestive of certain type of specific disease
  - Other features suggestive of AML, NOS subtypes
- Features of non-blast cells
Blast features

Acute promyelocytic leukemia, hypogranular type

Acute megakaryoblastic leukemia
Morphologic features of acute myeloid leukemia with recurrent cytogenetic abnormalities.

(A) Typical, hypergranular acute promyelocytic leukemia (APL) exhibiting numerous well-granulated abnormal promyelocytes and multiple cytoplasmic Auer rods. B and C, Hypogranular variant of APL, exhibiting the characteristic “sliding-plate” (B) and bilobed nuclear morphology (C). Note the absence of significant granulation. (D) Acute myeloid leukemia with t(8;21), exhibiting blasts with prominent perinuclear hofs, occasional salmon-colored granulation, and very fine Auer rods (indistinct, top of photograph). E, Acute myeloid leukemia with inv(16), exhibiting blasts with some suggestion of monocytic features and numerous abnormal eosinophils with basophilic granulation (Wright-Giemsa, original magnification31000 [A through E]).

Wang el al, Arch Pathol Lab Med. 2015;139:1215–1223
Microscopy/Morphology

• Equal to or more than 20% blasts in peripheral blood or bone marrow aspirate
• Less than 20% blasts in peripheral blood or bone marrow aspirate
• Blast cell characteristics
• Features of non-blast cells
  • AML with MDS-related dysplasia
  • Increase in number or atypical features of cells like eosinophils, basophils or mast cells
AML with MDS-related changes

• More than or equal to 20% blood or marrow blasts

• Absence of both of following
  • Prior cytotoxic or radiation therapy for an unrelated disease
  • Recurrent cytogenetic abnormality as described in AML with recurrent genetic abnormalities

• Any of the following
  • A previous history of a myelodysplastic syndrome (MDS) or a myelodysplastic/myeloproliferative neoplasm (MDS/MPN)
  • An MDS-related cytogenetic abnormality
  • Multilineage dysplasia greater than 50% in at least two cell lineages
Immunophenotype

Immunophenotyping should be performed in all cases

• Primary method: Flow cytometry
  • Panel must have multiple markers for lineage determination
  • Blast count is usually lower than properly performed manual count

• Additional method: Immunohistochemistry (IHC)
  • Especially if satisfactory marrow aspirate is not obtained for cytometric studies
  • As an additional study to characterize the blast population
Expression of cell-surface and cytoplasmic markers for the diagnosis of AML and Mixed phenotype acute leukemia

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

Genetic analyses

Acute leukemia cytogenetic risk groups are well defined

• Some karyotype abnormalities define specific disease entities

• MDS-related cytogenetic abnormalities are a key feature for diagnosing AML with MDS-related changes

• Normal karyotype requires additional molecular genetic testing
## Tests/procedures for a patient with AML

<table>
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<th>For a patient with AML</th>
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<tbody>
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<td><strong>Tests to establish the diagnosis</strong></td>
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<tr>
<td>Complete blood count and differential count</td>
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<td>Bone marrow aspirate</td>
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<td>Bone marrow trephine biopsy*</td>
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<td>Immunophenotyping</td>
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<td><strong>Genetic analyses</strong></td>
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<tr>
<td>Cytogenetics†</td>
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<tr>
<td>Screening for gene mutations including‡</td>
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<tr>
<td>Screening for gene rearrangements§</td>
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<tr>
<td><strong>Additional tests/procedures at diagnosis (cont’d)</strong></td>
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<td>Analysis of comorbidities</td>
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<td>Biochemistry, coagulation tests, urine analysis**</td>
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<tr>
<td>Serum pregnancy test††</td>
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<tr>
<td>Information on oocyte and sperm cryopreservation‡‡</td>
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<tr>
<td>Eligibility assessment for allogeneic HCT (including HLA typing)a</td>
</tr>
<tr>
<td>Hepatitis A, B, C; HIV-1 testing</td>
</tr>
<tr>
<td>Chest radiograph, 12-lead electrocardiogram, and echocardiography or MUGA (on indication)</td>
</tr>
<tr>
<td>Lumbar punctureb</td>
</tr>
<tr>
<td>Biobankingc</td>
</tr>
<tr>
<td><strong>Sensitiv assessment of response by RT-qPCR or MFCd</strong></td>
</tr>
<tr>
<td>RT-qPCRe,f for NPM1 mutation, CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL1, other fusion genes (if available)d</td>
</tr>
</tbody>
</table>

### Additional tests/procedures at diagnosis

- Demographics and medical historyll
- Detailed family history¶
- Patient bleeding history#
- Performance status (ECOG/WHO score)

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Genetic analyses

†Results from cytogenetics should be obtained preferably within 5 to 7 days. At least 20 bone marrow metaphases are needed to define a normal karyotype, and recommended to describe an abnormal karyotype. Abnormal karyotypes may be diagnosed from blood specimens.

‡Results from NPM1 and FLT3 mutational screening should be available within 48 to 72 hours (at least in patients eligible for intensive chemotherapy), and results from additional molecular genetics within the first treatment cycle. Screening for gene mutations is an evolving field of research; screening for single genes may be replaced by gene panel diagnostics.

§Screening for gene rearrangements should be performed if rapid information is needed for recommendation of suitable therapy, if chromosome morphology is of poor quality, or if there is typical morphology but the suspected cytogenetic abnormality is not present.
## Cytogenetic abnormalities in acute myeloid leukemia

<table>
<thead>
<tr>
<th>Cytogenetic finding</th>
<th>Affected genes</th>
<th>Clinical features</th>
<th>Prognosis</th>
<th>Approximate incidence in de novo AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)</td>
<td>RUNX1/RUNX1T1</td>
<td>Younger adults (average age: 30 years)</td>
<td>Favorable</td>
<td>5 to 7%</td>
</tr>
<tr>
<td>i(15;17)</td>
<td>ALI/RAA4</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(11;17)</td>
<td>ZRS2R/ANRAA</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(11;17)</td>
<td>NUP214/MYH11</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>+8</td>
<td>ALI, NUP214</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(6;9), t(4;11), i(16), or combinations</td>
<td>ALI, NUP214</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(1;12)</td>
<td>LMNA/RUNX1</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(1;12)</td>
<td>ALI, NUP214</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(12;21)</td>
<td>AML1/ETO</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>t(16;16)</td>
<td>ATM/ETO</td>
<td>Older adults (average age: 60 years)</td>
<td>Favorable, high rate with allogeneic HSCT</td>
<td>5 to 6%</td>
</tr>
</tbody>
</table>

**References:**
2016 WHO classification of AML

AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
- AML with PML-RARA
- AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
- 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
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RB15-MKL1
- Provisional entity: AML with BCR-ABL1
- AML with mutated NPM1
- AML with biallelic mutations of CEBPA
- Provisional entity: AML with mutated RUNX1
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, NOS
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monocytic leukemia
- Pure erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis (TAM)
- Myeloid leukemia associated with Down syndrome

From Swerdlow SH, Campo E, Harris NL, et al, eds.
Main categories 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 associated with Down syndrome
Genetic alterations of AML

- Cytogenetic abnormalities are seen in ~50% of patients with AML.
- AML with balanced translocations/inversions
  - These are associated with distinctive clinicopathological features and have prognostic significance
  - AML with these translocation or inversions (first four below) are considered to be acute leukemia without regard to blast cell count, while others are considered controversial, whether those should be categorized as AML when the blast cell count is <20%
  - Most commonly identified balanced abnormalities are
    - t(8;21), inv (16), t(16;16), t(15;17) and t(9;11)
- AML with gene mutations
AML with Recurrent Genetic Abnormalities

- AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- Acute promyelocytic leukemia with *PML-RARA*
- AML with t(9;11)(p22.3;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*
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBMI5-MKL1*
- Provisional entity: AML with *BCR-ABL1*
- **AML with gene mutations**
  - AML with mutated *NPM1*
  - AML with biallelic mutations of *CEBPA*
  - Provisional entity: AML with mutated *RUNX1*
Molecular classes of AML and concurrent gene mutations

Molecular genetic testing

Diagnostic workup should include screening for

• (a) mutations in NPM1, CEBPA, and RUNX1 genes because they define disease categories (provisionally for RUNX1)

• (b) mutations in FLT3 (both for internal tandem duplications [ITDs] together with data on the mutant–to–wild-type allelic ratio, and tyrosine kinase domain mutations at codons D835 and I836);
  • activating mutations of FLT3 are not only prognostic, but may beneficially be affected by tyrosine kinase inhibition

• (c) mutations in TP53 and ASXL1 because they consistently have been associated with poor prognosis

Mutations in AML

• Gene sequencing studies have shown that, on average, de novo AML cases contain more than 10 significant gene mutations, many of which can be broadly grouped into nine categories of genes thought to participate in leukemogenesis,
  • DNA methylation,
  • tumor suppressors,
  • transcription factor fusions,
  • nucleophosmin,
  • signaling molecules,
  • chromatin modification,
  • myeloid transcription factors,
  • the cohesin complex, and
  • the spliceosome complex
Mutations in AML

• The most common genes mutated are,
  • FLT3 (28 percent),
  • NPM1 (27 percent),
  • DNMT3A (26 percent),
  • IDH1 or IDH2 (20 percent),
  • NRAS or KRAS (12 percent),
  • RUNX1 (10 percent),
  • TET2 (8 percent),
  • TP53 (8 percent),
  • CEBPA (6 percent), and
  • WT1 (6 percent)
Mutations in AML

Back to our case

• I will discuss in detail only selected genetic mutations, which were present in the patient.
FLT3

- FLT3 is a class III receptor tyrosine kinase and immunoglobulin receptor superfamily member that is expressed by hematopoietic progenitor cells and downregulated during differentiation.

- Once physiologically activated through FLT3 ligand binding, phosphorylation of regions in the juxtamembranous domain leads to growth induction and apoptosis inhibition through STAT5 and MAPK signaling.
Chromosomal location

Cytogenetic Location: 13q12.2, which is the long (q) arm of chromosome 13 at position 12.2

Molecular Location: base pairs 28,003,274 to 28,100,592 on chromosome 13 (Homo sapiens Annotation Release 109, GRCh38.p12) (NCBI)
FLT3

- FLT3 mutations occur in approximately 30% of acute myeloid leukemia (AML) patients

- Two major types of genetic abnormalities of FLT3 have been described:
  - ITD (internal tandem duplication) of the juxtamembranous domain
  - Missense mutation resulting in the amino acid change at D835.

- ITD is more common, occurring in approximately 23%, with the point mutation seen in about 7% of cytogenetically normal AMLs.

- Functionally, these lesions result in the constitutive activation of the tyrosine kinase domains through autophosphorylation, leading to a persistent “on” signal in the transformed leukemic cell.

- Clinically only the ITDs have prognostic relevance.
Diagram of FLT3 structure. Shown in schematic fashion are the 5 immunoglobulin-like folds that make up the ligand-binding extracellular domain, single transmembrane domain, and cytoplasmic domain made up of a kinase domain interrupted by a kinase insert. The juxtamembrane domain where internal tandem duplications (ITDs) occur and aspartic acid 835 where most kinase domain mutations occur are indicated by arrows (Small D. FLT3 Mutations: Biology and Treatment. Hematology Am Soc Hematol Educ Program. 2006).
FLT3

• FLT3 has been shown to be one of the single most pertinent prognosticators for overall survival in AML patients, and this correlation with poor prognosis is independent of the powerfully prognostic karyotypic groups.

• A number of variables appear to affect the prognostic associations of FLT3 ITDs.
  • Only when the mutant allele burden is greater than the wild-type allele, >50%, does its prognostic pertinence emerge.
  • However, even very small FLT3 ITD mutations (clone size of 0.2% to 2%) are important to detect because they may survive chemotherapy and expand over time.
FLT3

• FLT3 inhibitors have been used in the therapy of patients with such mutations.

• Interestingly resistance develops because of the expansion of mutant clones, in particular those occurring in and around D835 as well as those with gatekeeper mutations affecting F691
The *NPM1* gene provides instructions for making a protein called nucleophosmin. Nucleophosmin shuttles back and forth between the nucleolus, nucleoplasm and the cytoplasm. It is thought to play a part in many cellular functions, including protein formation, DNA replication, and cell replication.

In the nucleolus, nucleophosmin attaches to another protein called ARF, keeping it in the proper location and protecting it from being broken down. The ARF protein is considered a tumor suppressor because it is involved in pathways that prevent cells from growing and dividing in an uncontrolled way.
Chromosomal location

Cytogenetic Location: 5q35.1, which is the long (q) arm of chromosome 5 at position 35.1

Molecular Location: base pairs 171,387,116 to 171,410,884 on chromosome 5 (Homo sapiens Annotation Release 109, GRCh38.p12) (NCBI)
NPM1

• NPM1 mutations occur in approximately 20-25% of acute myeloid leukemia patients and approximately 50% to 60% of cytogenetically normal AMLs.

• The gene encoding nucleophosmin (NPM1) is, according to most but not all studies, the most frequently mutated gene in AML.

• Mutations in this gene, which are typically small insertions (usually of 4 bp, sometimes up to 11 bp) in the coding region of the terminal exon (exon 12).

• Mutations alter tryptophan residues required for proper nucleolar localization and create a putative nuclear export signal at the C terminus of the protein.

• So the mutant nucleophosmin protein is predominantly localized to the cytoplasm and through dimerization causes the mislocalization of the wild-type protein as well.
  • This leads to the mislocalization and destabilization of p14ARF and to the inhibition of TP53 activity.
NPM1

• *NPM1* mutations has good prognosis in patients with AML.
• This benefit, however, is affected by the *FLT3* status.
• *FLT3* ITDs are enriched in AMLs with *NPM1* mutations and their presence abolishes the good prognostic effect of *NPM1* mutations.
• It has also been suggested that *NPM1* mutations are only good when accompanied by *IDH2* mutations.
• Combining the status of these two of *NPM1* and *FLT3* allows stratification into three prognostic groups.
  • good (*FLT3*-ITD^−/*NPM1^+),
  • intermediate (*FLT3*-ITD^−/*NPM1^− or *FLT3*-ITD^+/*NPM1^+),
  • poor (*FLT3*-ITD^+/*NPM1^−)
**DNMT3A**

- *DNMT3A* encodes an epigenetic regulator that mediates de novo methylation of CpG dinucleotides.
- It is one of the frequently mutated genes in AML (~20%), often together with *NPM1* and *FLT3* mutations.
- It is usually associated with a poor prognosis.
  - Missense mutations (typically affecting R882) are associated with a poor prognosis,
  - whereas truncating mutations seem to be neutral.
- The adverse effect of *DNMT3A* mutations can be overcome by escalating the dose of anthracyclines in induction chemotherapy.
Chromosomal location

Cytogenetic Location: 2p23.3, which is the short (p) arm of chromosome 2 at position 23.3
Molecular Location: base pairs 25,232,961 to 25,342,590 on chromosome 2 (Homo sapiens Annotation Release 109, GRCh38.p12) (NCBI)
CEBPA

- CEBPA (CCAAT enhancer binding protein alpha) encodes a key transcription factor that regulates myeloid cell differentiation and proliferation.
- Mutations are seen in approximately 10% of all AMLs, typically with relatively well preserved platelet counts, and are associated with a favorable prognosis.
RUNX1

• This gene, which is involved in both the t(8;21) translocation in AML and the t(12;21) translocation of ALL and is also the target of point mutations.
• Thus, RUNX1 mutations occur in approximately 25% of these AMLs and are associated with TdT positivity.
• They are associated with a poor prognosis.
• These mutations lead to a platelet disorder as well and a risk of leukemia of 35%.
KIT

- *KIT* mutations are particularly common in CBF AMLs, with a t(8;21) or inv(16),
- It occurs in approximately 20% of cases.
- They tend to be associated with a poor prognosis in these typically good-prognosis AMLs.
- The presence of these mutations may also have therapeutic relevance.
TP53

• The gene encoding this prototypic tumor suppressor is mutated in approximately 7% of all AMLs.

• Mutations are also enriched in therapy-related AML. It has been suggested that TP53 mutations are not directly induced by cytotoxic chemotherapy.

• Rather, they are likely to reflect rare age-related mutations that are resistant to chemotherapy and that expand preferentially after treatment.
IDH1 and IDH2

- IDH1 and IDH2 are NADP-dependent enzymes that convert isocitrate to alpha-ketoglutarate in the Krebs cycle.
- In some studies, IDH2 mutations associate with improved overall survival.
- In addition, IDH-mutant cells depend on BCL2, and thus therapeutic inhibition of BCL2 may have a role.
TET2

• TET2 is involved in epigenetic regulation facilitating the conversion of 5-methylcytosine into 5-hydroxymethylcytosine.

• Mutations of TET2 that occur in approximately 10% to 15% of AMLs lead to loss of function and are likely to occur early in leukemogenesis.

• They are usually mutually exclusive of IDH1/IDH2 mutations.
KMT2A

• There is usually a cytogenetic pointer to the presence of a KMT2A partial tandem duplication in that approximately 90% of cases with trisomy 11 are associated with this abnormality.

• It is also present in approximately 10% of AMLs with normal cytogenetics.

• It is prognostically important, associated with an unfavorable outcome.
Overall survival in acute myeloid leukemia

This figure illustrates overall survival in adult subjects with acute myeloid leukemia (AML, age range 15 to 66 years, median 52 years), according to the following cytogenetic risk categories: Favorable risk (median survival 7.6 years): t(8;21); inv(16) or t (16;16); del(9q). Intermediate risk (median survival 1.3 years): normal karyotype; -Y; del(5q); loss of 7q; t(9;11); +11; del(11q); abn(12p); +13; del(20q); +21. Adverse risk (median survival 0.5 years): complex karyotype (≥3 abnormalities); inv(3) or t(3;3); t (6;9); t(6;11); −7; +8 (sole abnormality); +8 with one other abnormality other than t(8;21), t(9;11), inv(16), or t(16;16); t (11;19)(q23;p13.1).

Survival curves for three mutations
FLT3, NPM1 and DNMT3A

Papaemmanuil, et al. NEJM 374;23, 2016
Survival curves comparing MLL and FLT3 and DNMT3A vs IDH2

Papaemmanuil, et al. NEJM 374;23, 2016
Initial Diagnostic Workup of Acute Leukemia

Guideline From the College of American Pathologists and the American Society of Hematology

Daniel A. Arber, MD; Michael J. Borowitz, MD, PhD; Melissa Cessna, MD; Joan Ezzell, MD; Kathryn Foucar, MD; Robert P. Hasserjian, MD; J. Douglas Rizzo, MD; Karl Theil, MD; Sa A. Wang, MD; Anthony T. Smith, MLS; R. Bryan Rumble, MSc; Nicole E. Thomas, MPH, CT(ASCP)™; James W. Vardiman, MD

- Context.—A complete diagnosis of acute leukemia requires knowledge of clinical information combined with morphologic evaluation, immunophenotyping and karyotype analysis, and often, molecular genetic testing. Although many aspects of the workup for acute leukemia are well accepted, few guidelines have addressed the different aspects of the diagnostic evaluation of samples from patients suspected to have acute leukemia.

- Objective.—To develop a guideline for treating physicians and pathologists involved in the diagnostic and prognostic evaluation of new acute leukemia samples, including acute lymphoblastic leukemia, acute myeloid leukemia, and acute leukemias of ambiguous lineage.

- Design.—The College of American Pathologists and the American Society of Hematology convened a panel of experts in hematology and hematopathology to develop recommendations. A systematic evidence review was conducted to address 6 key questions. Recommendations were derived from strength of evidence, feedback received during the public comment period, and expert panel consensus.

- Results.—Twenty-seven guideline statements were established, which ranged from recommendations on what clinical and laboratory information should be available as part of the diagnostic and prognostic evaluation of acute leukemia samples to what types of testing should be performed routinely, with recommendations on where such testing should be performed and how the results should be reported.

- Conclusions.—The guideline provides a framework for the multiple steps, including laboratory testing, in the evaluation of acute leukemia samples. Some aspects of the guideline, especially molecular genetic testing in acute leukemia, are rapidly changing with new supportive literature, which will require on-going updates for the guideline to remain relevant.

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Supplemental digital content is available for this article at www.archivesofpathology.org in the October 2017 table of contents.
From the Department of Pathology, University of Chicago,
CAP/ASH AML Genetic Testing Recommendations

• Karyotype

• Molecular testing
  • For all or most cases
    • FLT3-ITD, NPM1, CEBPA, RUNX1
    • Others: IDH1, IDH2, TET2, WT1, DNMT3A, TP53
  • For select cases
    • KIT for core binding factor leukemias
    • PML-RARA if APL suspected

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Novel treatments

• The Food and Drug Administration (FDA) recently approved two new treatments for some adult patients with acute myeloid leukemia (AML)
  • enasidenib (Idhifa), a drug that targets aberrant forms of the IDH2 protein, and
  • Novartis’s midostaurin (Rydapt®) was approved in combination with chemotherapy for a subset of patients with a mutation FLT3
• Specific treatment for FLT3 needs to be started within 8 days
• Significance: If we order NGS panel for the myeloid malignancy mutation panel, the results are delayed (10-16 days) so it is better to order the individual mutation study analysis so that clinical team can start treatment.
Take home message

• Results from NPM1 and FLT3 mutational screening should be available within 48 to 72 hours (at least in patients eligible for intensive chemotherapy), and results from additional molecular genetics within the first treatment cycle.

• Screening for gene mutations is an evolving field of research; screening for single genes may be replaced by gene panel diagnostics.
Questions
Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

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The first edition of the European LeukemiaNet (ELN) recommendations for diagnosis and management of acute myeloid leukemia (AML) in adults, published in 2010, has found broad acceptance by physicians and investigators caring for patients with AML. Recent advances, for example, in the discovery of the genomic landscape of the disease, in the development of assays for genetic testing and for detecting minimal residual disease (MRD), as well as in the development of novel antileukemic agents, prompted an international panel to provide updated evidence- and expert opinion-based recommendations. The recommendations include a revised version of the ELN genetic categories, a proposal for a response category based on MRD status, and criteria for progressive disease. (Blood. 2017; 129(4):424-447)