Algorithmic Approach to the Classification of Acute Leukemia

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Bullet Points
- The 2008 WHO Classification of Acute Leukemia requires the use of clinical information, morphology, immunophenotyping, cytogenetics and molecular genetics for proper diagnosis and classification
- Morphology remains essential as the starting point for diagnosis and for directing the ordering of appropriate ancillary studies
- Proper diagnosis requires evaluation of all data and may require amendments of initial reports

Key Words: Acute myeloid leukemia, acute lymphoblastic leukemia, mixed phenotype acute leukemia, morphology, immunophenotyping, cytogenetics, gene mutations.

Introduction
The classification of acute leukemia has evolved significantly over the past few decades. The French-American-British (FAB) classification was initially based entirely on the morphologic features of the blast cell population on Romanovsky-stained bone marrow aspirate smears and the results of cytochemical studies. While the FAB classification was modified over time, and eventually included immunophenotyping to distinguish minimally differentiated acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL) and as a means to identify acute megakaryoblastic leukemia, it remained a primarily morphologic classification system. Results of cytogenetic studies were also not part of the FAB classification, however, the FAB M3 and FAB M4Eo subtypes were found to correlate with recurring cytogenetic abnormalities. Other than FAB M3 and M4Eo, however, the morphologic categories of the FAB classification did not represent distinct biologic or clinically significant disease subtypes. The FAB classification also did not address morphologic features of non-blast cells in AML, particularly the presence or absence of co-existing dysplasia.

As immunophenotyping became more available, especially using flow cytometry methods, attempts to classify acute leukemias based on their immunophenotypic features met with variable acceptance. Immunophenotyping of ALL identified more distinct subtypes than the morphologic categories of the FAB, and demonstrated that many cases with FAB L3 morphology were mature B cell neoplasms that represented a leukemic phase of Burkitt lymphoma. The importance of distinguishing ALLs of precursor B cell type from those of precursor T cells is now accepted, but further immunologic subtyping of ALL appears to be of limited clinical or biologic significance. The diagnostic utility of immunophenotyping in AML, other than in the identification of myeloperoxidase- and non-specific esterase-negative AML, has also been questioned. The frequent presence of aberrant antigen expression in acute leukemia has lead to much confusion, including the possible over-diagnosis of acute leukemias of so-called mixed lineage. Despite this, over time it has become clear that some immunophenotypic patterns consistently recur with specific acute leukemia types.
Genetic changes associated with acute leukemia are probably the most important finding in the prognostic stratification of both AML and ALL. With karyotype analysis alone, AML can be subdivided into low, intermediate and high risk groups using current therapeutic regimens, and similar risk groups are defined by karyotype analysis in ALL. These findings led to clinical treatment approaches that emphasized cytogenetic results and gave little, if any, importance to the morphologic or immunophenotypic subtype of a given leukemia. Despite this, the intermediate cytogenetic risk group of AML appears to be a heterogeneous group of disorders, many of which have an apparently normal karyotype. The ongoing discovery of gene mutations in AML, especially normal karyotype AML, provides prognostic information that compliments karyotype data.

Finally, the role of prior therapy in the development or evolution of myelodysplasia and acute leukemia has become clear. These leukemias are now understood to have characteristic behaviors and genetic abnormalities and they were often difficult to classify in prior classification systems.

While a number of classification systems of acute leukemia have been proposed that use some combination of morphology, immunophenotyping and cytogenetic analysis, the 2001 World Health Organization (WHO) Classification of Hematopoietic and Lymphoid Tissues was the first to gain wide acceptance. The 2001 WHO classification most notably recognized the significance of some recurring cytogenetic abnormalities in AML as well as the significance of multilineage dysplasia and prior therapy. In ALL, the 2001 WHO scheme subdivided cases into precursor B and precursor T lineage types and linked these proliferations to their tissue-based lymphoblastic lymphoma equivalents. Although common recurring cytogenetic abnormalities were mentioned, they were not formally incorporated into a subclassification scheme for ALL.

The 2008 WHO classification (Table 1) expands the genetic disease subtypes of AML, provides broader criteria for the identification of the generally-poor prognosis AMLs with myelodysplasia-related changes and introduces cytogenetic subtypes of ALL. In addition, it provides more restrictive criteria for the diagnosis of mixed phenotype acute leukemia. Morphology, immunophenotyping, cytogenetic analysis, mutation analysis and prior therapy and disease history are all components of the 2008 WHO classification.

**Role of Morphology in Acute Leukemia**

Morphologic examination remains the first step in the evaluation of a specimen for acute leukemia. Review of peripheral blood smears, bone marrow aspirate smears and a bone marrow biopsy is recommended for all suspected cases. The initial morphologic evaluation is critical to the proper ordering of ancillary testing for an individual case. While the diagnosis of acute leukemia can be made with a low marrow blast cell count (<20%) when t(8;21)(q22;q22), inv(16)(p13.1q22), t(16;16)(p13.1;q22) or t(15;17)(q22;q12) are present, the remaining AML types require the identification of 20% or more blasts by morphology. The morphologic evaluation of blasts is significant in several scenarios, but is probably most important in the monocytic proliferations. The diagnosis of acute leukemia versus chronic myelomonocytic leukemia will depend, in part, on the morphologic distinction of bone marrow monoblasts and promonocytes from more mature monocytes. Other blast cell morphologic features of importance include the
bilobed nuclei with or without fine granules and Auer rods to suggest acute promyelocytic leukemia, which should result in a clinical work-up for disseminated intravascular coagulopathy as well as rapid molecular confirmation of the t(15;17)(q22;q12). The morphologic identification of blasts characteristic of t(8;21)(q22;q22) with perinuclear hofs and chunky pink cytoplasmic granules is important as these cells may be misinterpreted as more mature granulocytes leading to a delay in the diagnosis of acute leukemia. Non-blast morphologic features of importance are the abnormal eosinophils with large, basophilic granules of inv(16)(p13.1q22) and t(16;16)(p13.1;q22), and the presence of multilineage dysplasia for a diagnosis of AML with myelodysplasia-related changes (Table 2). These, as well as less specific morphologic features of some disease types, add valuable information to the diagnosis and classification of acute leukemias, especially acute myeloid leukemia.

In summary, the key roles of morphology in acute leukemia are:

1. Identification of a blast cell proliferation of 20% or more
2. Differentiating blasts and promonocytes from mature monocytes
3. Identification of blast cell features suggestive or recurring cytogenetic abnormalities
   a. Hypergranular and hypogranular blasts of acute promyelocytic leukemia
   b. Blasts with features suggestive of t(8;21)(q22;q22)
4. Identification of non-blast cell features that are suggestive or diagnostic of specific disease types
   a. Abnormal eosinophils of inv(16)(p13.1q22) or t(16;16)(p13.1;q22)
   b. Presence of multilineage dysplasia

Role of Cytochemistry in Acute Leukemia
Cytochemical studies are no longer necessary for the diagnosis of most cases of acute leukemia. With the exception of iron stains, many laboratories no longer perform these studies on a routine bases. Cytochemical studies are most useful in defining the subtypes of AML, not otherwise specified, although the clinical utility of the cytochemical subtypes of AML is questionable.

Role of Immunphenotyping in Acute Leukemia
Immunphenotyping is advised in the work-up of all potential acute leukemias and is required to properly diagnose ALL or mixed phenotype acute leukemia. Immunphenotyping of acute leukemia is usually performed by flow cytometry and, because of the relatively high frequency of aberrant antigen expression in acute leukemia, should include a large enough battery of antibodies to clearly define the lineage of the blast cell population and to detect aberrant antigenic expression patterns that may be used to detect residual disease following therapy. While immunphenotyping is required to identify precursor B, precursor T and myeloid blast populations, it is also useful in identifying aberrant antigen patterns that correspond to specific disease groups. In AML, the combination of distinctive morphologic and immunphenotypic features is most helpful. Relatively specific antigen expression patterns are also seen in some types of precursor B lymphoblastic leukemias. Table 3 summarizes the more common antigenic patterns in acute leukemia.
Immunophenotyping should not be used as a substitute for a visual bone marrow aspirate blast cell count, as the diagnosis of acute leukemia, in most settings, is based on a morphologically derived blast cell count of 20% or more. However, in cases in which a cellular bone marrow aspirate cannot be obtained due to marrow fibrosis, a CD34 stain of the bone marrow biopsy may provide valuable information for establishing the diagnosis of acute leukemia if the blasts express CD34. It should be kept in mind, however, that in approximately 25% of all cases of AML the blasts do not express CD34.

In summary, the key roles of immunophenotyping in acute leukemia are:
1. Establish precursor T, precursor B or myeloid lineage of the blast cell population
2. Identify aberrant antigen expression patterns that are suggestive of specific disease categories

Role of Cytogenetics in Acute Leukemia
Cytogenetic studies (karyotype analysis) should be performed on all new acute leukemias or suspected leukemias. The diagnostic pathologist should review the final cytogenetic results on all cases and amend cases in which a recurring AML or ALL cytogenetic abnormality included in the 2008 WHO classification (Table 1) is identified or if an abnormality sufficient to diagnose AML with myelodysplasia-related changes is detected (see Table 4). Some of the recurrent cytogenetic abnormalities are difficult or impossible to detect on routine karyotype and other molecular methods should be employed to investigate them. Specifically, all pediatric B-ALL cases should be studied by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) for evidence of t(12;21)(p13;q22)/ETV6-RUNX1 because this is characteristically a cryptic abnormality. Case of AML with morphologic evidence of abnormal eosinophils should be further investigated for inv(16)(p13.1q22), t(16;16)(p13.1;q22) if these abnormalities are not identified by karyotype analysis, as this abnormality may also be subtle.

In summary, the key roles of cytogenetics in acute leukemia are:
1. Detection of recurring cytogenetic abnormalities
2. Detection of myelodysplasia-related abnormalities

Role of Mutations Analysis in Acute Leukemia
Currently, the major role of mutation analysis is in AML. Mutations in AML are grouped into two classes. Class I mutations provide a proliferative and/or survival advantage and do not affect differentiation. Class II mutations impair hematopoietic differentiation and subsequent apoptosis. While a large number of genes undergo mutations in AML, the clinical relevance of many is still being investigated and only a small subset have been included in the 2008 WHO classification. Class I mutations, which include mutations of FLT3-ITD, FLT3-TKD and KIT, appear to occur late in AML and some may appear at relapse. These tend to occur in more than one subtype of AML and are considered as prognostic factors in AML. Class II mutations, which include mutations of CEBPA and probably NPM1 appear to occur early in the development of AML, similar to some of the recurrent cytogenetic abnormalities in AML. CEBPA and NPM1 mutations also do not appear to commonly occur in combination with the recurrent cytogenetic abnormalities in AML, suggesting that they may represent
biologically distinct entities. For this reason, AML with mutations of CEBPA and NPM1 are included as provisional entities in the 2008 WHO classification.

Evaluation of NPM1 and CEBPA appears warranted in most cases of AML, other than those with the other recurring genetic abnormalities, especially in cases of normal karyotype or intermediate-risk karyotype AML, as the detection of these mutations in the absence of a FLT3 mutation appear to confer a good prognosis. Detection of KIT mutations appears to be most relevant to AMLs with t(8;21)(q22;q22), inv(16)(p13.1q22), or t(16;16)(p13.1;q22), and routine study of this gene in other AML types is probably not warranted. FLT3-ITD mutations occur across a broader spectrum of disease types, including AML with normal karyotype, AML with t(15;17)(q22;q12), AML with t(6;9)(p23;q34) and the provisional entity of AML with mutated NPM1, generally conferring a poorer prognosis than similar cases without FLT3 mutations. Because of their associations with other recurring genetic abnormalities, FLT3 and KIT mutations are viewed more as important prognostic indicators rather than as defining possible disease entities.

FLT3-ITD and NPM1 mutations studies are performed using fairly straightforward PCR based methods and are offered in many laboratories. Unfortunately, reliable detection of CEBPA mutations requires extensive sequencing. Until more rapid and reliable methods are developed for CEBPA mutation detection, this test will probably not be widely available for some time.

Key points related to gene mutations in AML are:

1. FLT3-ITD mutations provide prognostic information across a wide array of AML types
2. KIT mutations provide prognostic information for AMLs with t(8;21)(q22;q22), inv(16)(p13.1q22), or t(16;16)(p13.1;q22)
3. NPM1 mutations should always be interpreted with results of FLT3-ITD

Role of Clinical Information in the Diagnosis of Acute Leukemia

Vital clinical information for the accurate classification of acute leukemia would include the presence of Down Syndrome, a history of myelodysplasia and a history of cytotoxic therapy for a prior neoplastic or non-neoplastic disorder. The myeloid proliferations of Down Syndrome are distinct and are now classified separately. A history of prior myelodysplasia in a newly diagnosed case of AML would automatically place a case into the category of AML with myelodysplasia-related changes. While many of these patients will show the presence of multilineage dysplasia and/or have a myelodysplasia-associated cytogenetic abnormality, rare cases will not show those features and will be missed without an accurate clinical history.

Similarly, a history of prior cytotoxic therapy in a patient with newly diagnosed AML would place a case into the category of therapy-related myeloid neoplasms. Many of these patients may otherwise show features of AML with myelodysplasia-related changes, but they are best classified as therapy-related. Others may have a genetic abnormality included among those listed as identifying a distinct genetic entity. In all such cases of therapy-related AML, it is recommended that the genetic abnormality be appended to the final diagnosis.
While ALL arising from myelodysplasia and therapy-related ALL are rare, they may occur. While specific categories for each are not included in the 2008 WHO classification, recognition of such associations is warranted to better understand these rare types of ALL.

**Priorities for Acute Leukemia Classification**

1. Recurrent cytogenetic abnormalities (other than provisional entities) and therapy-related myeloid neoplasm categories trump all others
   a. Rare cases of therapy-related disease with a recurrent genetic abnormality should be diagnosed as therapy-related disease with clear identification of the genetic abnormality (example: Therapy-related AML with t(8;21)(q22;q22))
   b. Cases with of AML with complex karyotypes that include an abnormality in the recurrent cytogenetic abnormality group should be diagnosed as one of the AMLs with recurrent genetic abnormalities

2. AML with myelodysplasia-related features trumps AML, not otherwise specified and provisional entities of AML with mutated *NPM1* and AML with mutated *CEBPA*
   - Cases of AML with myelodysplasia-related changes, no history of myelodysplasia, and intermediate risk cytogenetics may also have *NPM1* mutations (with or without *FLT3* mutations). Until the significance of this combination is clarified, cases of this type should be classified as “AML with myelodysplasia-related changes (multilineage dysplasia) and mutated *NPM1*.”
Table 1. 2008 WHO Classification of Acute Leukemia

**Acute myeloid leukemia with recurrent genetic abnormalities**
- AML with t(8;21)(q22;q22); (*RUNX1*RUNX1T1*)
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); (*CBFB-MYH11*)
- APL with t(15;17)(q22;q12); (*PML-RARA*)
- AML with t(9;11)(p22;q23); (*MLLT3-MLL*)
- AML with t(6;9)(p23;q34); (*DEK-NUP214*)
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); (*RPN1-EVI1*)
- AML (megakaryoblastic) with t(1;22)(p13;q13); (*RBM15-MKL1*)

Provisional entity: AML with mutated NPM1
Provisional entity: AML with mutated CEBPA

**Acute myeloid leukemia with myelodysplasia-related changes**

**Therapy-related myeloid neoplasms**

**Acute myeloid leukemia, not otherwise specified**
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Acute erythroid leukemias
  - Pure erythroid leukemia
  - Erythroleukemia, erythroid/myeloid
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

**Myeloid Proliferations related to Down Syndrome**
- Transient abnormal myelopoiesis
- Acute myeloid leukemia associated with Down syndrome

**Acute leukemias of ambiguous lineage**
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
- Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
- Mixed phenotype acute leukemia, B/myeloid, NOS
- Mixed phenotype acute leukemia, T/myeloid, NOS
- Mixed phenotype acute leukemia, NOS – rare types
- Natural killer (NK)-cell lymphoblastic leukemia/lymphoma

**B lymphoblastic leukemia/lymphoma, NOS**

**B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities**
- B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
- B lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
- B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); *TEL-AML1 (ETV6-RUNXI)*
- B lymphoblastic leukemia/lymphoma with hyperdiploidy
- B lymphoblastic leukemia/lymphoma with hypodiploidy (Hypodiploid ALL)
- B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
- B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *E2A-PBX1 (TCF3-PBX1)*

**T lymphoblastic leukemia/lymphoma**
Table 2. Criteria for the diagnosis of AML with myelodysplasia-related features.

- \[ \geq 20\% \text{ blood or marrow blasts} \]

AND

- Any one of the following:
  - Previous history of myelodysplastic syndrome
  - Myelodysplastic syndrome-related cytogenetic abnormality
  - Multilineage dysplasia
  
  AND

- Absence of both:
  - Prior cytotoxic therapy for an unrelated disease
  - Recurring cytogenetic abnormality as described in AML with recurrent genetic abnormalities

Table 3. Relatively-Unique Immunophenotypic Patterns in Acute Leukemia

<table>
<thead>
<tr>
<th>Acute Leukemia Type</th>
<th>Characteristic Antigen Pattern*</th>
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<tbody>
<tr>
<td>AML with t(8;21)(q22;q22); RUNX1-RUNX1T1</td>
<td>MPO(^+)/CD13(^+)/CD33(^\text{weak})/HLA-DR(^-)/CD34(^+)/CD19(^+)</td>
</tr>
<tr>
<td>AML with t(15;17)(q22;q12); PML-RARA</td>
<td>MPO(^++)/CD33(^+)/CD13(^\text{hetero})/HLA-DR(^-)/CD34(^++)/CD2(^++)</td>
</tr>
<tr>
<td>B-ALL with t(9;22)(q34;q11.2); BCR-ABL1</td>
<td>CD19(^+)/CD10(^+)/TdT(^+)/CD13(^+)/CD33(^+)/CD25(^+)</td>
</tr>
<tr>
<td>B-ALL with t(v;11q23); MLL rearranged</td>
<td>CD19(^+)/CD10(^+)/TdT(^+)/CD24(^+)/CD15(^+)</td>
</tr>
<tr>
<td>B-ALL with t(12;21)(p13;q22); ETV6-RUNX1</td>
<td>CD19(^+)/CD10(^++)/CD9(^-)/TdT(^+)/CD13(^-)/CD34(^+)</td>
</tr>
<tr>
<td>B-ALL with t(1;19)(q23;p13.3); TCF3-PBX1</td>
<td>CD19(^+)/CD10(^+)/CD9(^++)/TdT(^+)/c(^+)/CD34(^++)</td>
</tr>
</tbody>
</table>

+, positive; ++, bright positive; -, negative; -/+ , positive in a minority of cases; weak, weak expression; hetero, heterogeneous expression.

* none of these patterns is entirely specific and must be correlated with cytogenetic studies for confirmation
Table 4. Cytogenetic abnormalities sufficient to diagnose AML with myelodysplasia-related features when ≥20% blood or marrow blasts are present

<table>
<thead>
<tr>
<th>Complex karyotype*</th>
<th>Balanced abnormalities</th>
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<tbody>
<tr>
<td>Unbalanced abnormalities</td>
<td>t(11;16)(q23;p13.3)**</td>
</tr>
<tr>
<td>-7/del(7q)</td>
<td>t(3;21)(q26.2;q22.1)**</td>
</tr>
<tr>
<td>-5/del(5q)</td>
<td>t(1;3)(p26.3;q21.1)</td>
</tr>
<tr>
<td>i(17q)/t(17p)</td>
<td>t(2;11)(p21;q23)</td>
</tr>
<tr>
<td>-13/del(13q)</td>
<td>t(5;12)(q33;p12)</td>
</tr>
<tr>
<td>del(11q)</td>
<td>t(5;7)(q33;q11.2)</td>
</tr>
<tr>
<td>del(12p)/t(12p)</td>
<td>t(5;17)(q33;p13)</td>
</tr>
<tr>
<td>del(9q)</td>
<td>t(5;10)(q33;q21)</td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td>t(3;5)(q25;q34)</td>
</tr>
</tbody>
</table>

* ≥ 3 unrelated abnormalities, none of which are included in the AML with recurrent genetic abnormalities subgroup (such cases should be classified in the appropriate cytogenetic group); ** these abnormalities most commonly occur in therapy-related disease and therapy-related AML should be excluded before using these abnormalities as evidence for a diagnosis of AML with myelodysplasia-related features.

Selected References

- Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid


Algorithmic Approach to the Classification of Acute Leukemia, other than Mixed Phenotype Acute Leukemia

Morphologic Review

≥ 20% Blood or Marrow Blasts
< 20% Blood or Marrow Blasts

Immunophenotype

Cytogenetics

Myeloid

Precursor T

Precursor B

History and Cytogenetics

Myeloid proliferation of Down Syndrome

T-ALL

Morphology for multilineage dysplasia

Therapy-related AML

AML with recurrent genetic abnormalities

AML with myelodysplasia-related changes

B-ALL, NOS

AML, NOS

Algorithmic Approach to the Classification of Acute Leukemia, other than Mixed Phenotype Acute Leukemia

Not acute leukemia

B-ALL with recurrent genetic abnormalities

AML with recurrent genetic abnormalities

Normal or others

Recurrent genetic abnormality

None

Present Absent

Recurrent genetic abnormality

t(8;21), inv(16), t(16;16) or t(15;17)

Normal or others

Recurrent genetic abnormality

Not acute leukemia
Algorithmic Approach to the Classification of Acute Leukemia

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COMPONENTS OF A CLASSIFICATION SYSTEM

- Clinical Features
- Morphology
- Cytochemistry
- Immunophenotype
- Cytogenetic/ Molecular Genetic
Clinical Features and Acute Leukemias
### Clinical Features and Acute Leukemias

<table>
<thead>
<tr>
<th>Age</th>
<th>History</th>
<th>Therapy Response</th>
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<tbody>
<tr>
<td>Elderly</td>
<td>de novo</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Adult</td>
<td>Prior therapy</td>
<td>HSC transplantation</td>
</tr>
<tr>
<td>Pediatric</td>
<td>Toxin exposure</td>
<td></td>
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<tr>
<td>Infant</td>
<td>Prior MDS</td>
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</table>
2008 WHO Classification

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

- Limited role in ALL
- In the absence of Auer rods, must be coordinated with immunophenotyping
- Specific AML blast cell characteristics still helpful
  - AML with t(8;21)(q22;q22); (RUNX1;RUNX1T1)
  - AML with inv(16)(p13.1q22) or t((16;16)(p13.1;q22); (CBFB-MYH11)
  - APL with t(15;17)(q22;q12); (PML-RARA)
- Blast counts still necessary
- Identification of multilineage dysplasia
Role of Morphology in Acute Leukemia

- AML with t(8;21)
- AML with inv(16)
- Acute Promyelocytic Leukemia
Role of Morphology in Acute Leukemia

- Diagnosis is still made, in most cases, based on a morphologic blast cell count.

- In cases without adequate aspirate material, including cases with marrow fibrosis, estimates from CD34 stained slides may be useful.

- Flow cytometry should not replace the blast cell count.
Role of Morphology in Acute Leukemia

Detection of multilineage dysplasia

- Surrogate for myelodysplasia-related cytogenetic abnormalities or prior MDS
- Significance of multilineage dysplasia in the absence of prior MDS and with a normal karyotype more controversial
Myelodysplasia-related Changes (MDS) in AML

Survival Distribution Function

Overall Survival (mo.)

Non-MDS AML (n=187)

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

$p < 0.0001$

Acute myeloid leukaemia with myelodysplasia-related changes

- Multilineage dysplasia, or
- History of myelodysplasia, or myelodysplastic/myeloproliferative neoplasm, or
- MDS-related cytogenetic abnormalities, and
- Absence of the specific genetic abnormalities of AML with recurrent genetic abnormalities
Product-Limit Survival Function Estimates

Logrank p<.0001

From Weinberg et al., Blood 2009
Role of Cytochemistry in Acute Leukemia

- Useful in subclassifying AML, not otherwise specified cases
- Cytochemical subtypes of no clinical significance
Immunophenotyping in Acute Leukemia

- Essential for precursor B and T ALL, minimally differentiated AML, and mixed phenotype acute leukemia

- Characteristic immunophenotypes for AML types or for MRD
  - AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
    - MPO+/CD13+/CD33<sub>weak</sub>/HLA-DR+/CD34+/CD19+
  - B-ALL with t(v;11q23); MLL rearranged
    - CD19+/CD10-/TdT+/CD24-/CD15+

- Epidemic of “mixed-lineage” leukemias
## EGIL Scoring System For Biphenotypic Acute Leukemias

<table>
<thead>
<tr>
<th>Points</th>
<th>B-lineage</th>
<th>T-lineage</th>
<th>Myeloid lineage</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>CD79a</td>
<td>CD3(m/cyt)</td>
<td>anti-MPO</td>
</tr>
<tr>
<td></td>
<td>cyt IgM</td>
<td>anti-TCR α/β</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cytCD22</td>
<td>anti-TCR γ/δ</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>CD19</td>
<td>CD2</td>
<td>CD117 (c-kit)</td>
</tr>
<tr>
<td></td>
<td>CD10</td>
<td>CD5</td>
<td>CD13</td>
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<tr>
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<td>0.5</td>
<td>TdT</td>
<td>TdT</td>
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<td>CD24</td>
<td>CD7</td>
<td>CD15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD1a</td>
<td>CD64</td>
</tr>
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Leukemia 1995;9:1783  
Leukemia 1998;12:2038
Myeloid
- Myeloperoxidase, or
- Monocytic differentiation (2 or more: NSE, CD11c, CD14, CD64, lysozyme)

T lineage
- Cytoplasmic or surface CD3

B lineage
- Strong CD19 plus strong expression of at least 1 of CD79a, cCD22, CD10, or
- Weak CD19 plus strong expression of at least 2 of CD79a, cCD22, CD10
Mixed Phenotype Acute Leukemia

- With $t(9;22)(q34;q11.2); BCR-ABL1$
- With $t(v;11q23); MLL$ rearranged
- B/myeloid, NOS
- T/myeloid, NOS
- NOS, rare types
  - T/B
  - T or B/megakaryocyte
  - T or B/erythroid
Cytogenetics is now recognized as one of the most important prognostic features in AML, ALL and MPAL.

Molecular genetic studies essential for some cases (i.e. detection of t(12;21) in pediatric ALL).

Mutation analysis provides additional information for many AML types.
Cytogenetics 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

Genetics in the 2008 WHO Classification

- Expansion of AML with recurrent genetic abnormalities category
  - Includes provision entities of AML with mutated NPM1 and AML with mutated CEPBA
  - Recognizes prognostic role of FLT3 and KIT mutations in specific AML types

- ALL with recurrent genetic abnormalities
  - Including hyperdiploid and hypodiploid ALL

- Genetic criteria for inclusion into AML with myelodysplasia-related changes
AML with recurrent genetic abnormalities - 1

- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*
- AML with t(9;11)(p22;q23); *MLLT3-MLL*
AML with recurrent genetic abnormalities - 2

- AML with t(6;9)(p23;q24); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1

Provisional entities
- AML with mutated NPM1
- AML with mutated CEBPA
B Lymphoblastic Leukemia/Lymphoma with Recurrent Genetic Abnormalities

- With t(9;22)(q34;q11.2); BCR-ABL1
- With t(v;11q23); MLL rearrangement
- With t(12;21)(p13;q22); TEL-AML1 (ETV6-RUNX1)
- With hyperdiploidy
- With hypodiploidy
- With t(5;14)(q31;q32); IL3-IGH@
- With t(1;19)(p23;p13.3); E2A-PBX1 (TCF3-PBX1)
MDS-related cytogenetic abnormalities

- **Complex karyotype***
  - **Unbalanced abnormalities**
    - -7/del(7q)
    - -5/del(5q)
    - i(17q)/t(17p)
    - -13/del(13q)
    - del(11q)
    - del(12p)/t(12p)
    - del(9q)
    - idic(X)(q13)

- **Balanced abnormalities**
  - t(11;16)(q23;p13.3)**
  - t(3;21)(q26.2;q22.1)**
  - t(1;3)(p36.3;q21.1)
  - t(2;11)(p21;q23)**
  - t(5;12)(q33;p12)
  - t(5;7)(q33;q11.2)
  - t(5;17)(q33;p13)
  - t(5;10)(q33;q21)
  - t(3;5)(q25;q34)

***>3 abnormalities

** must exclude therapy-related disease
Algorithmic Approach

Morphologic Review

- >20% Blood or Marrow Blasts
- <20% Blood or Marrow Blasts
Algorithmic Approach

If >20% blood or marrow blasts are present, then:

- Mixed?
- Myeloid
- Precursor B
- Precursor T

If Immunophenotype is Mixed, then:

T-ALL
Algorithmic Approach

Precursor B

Cytogenetics

- t(9;22), t(v;11q23), t(12;21), t(1;19), 1(5;14), hyperdiploid, or hypodiploid
- No recurrent genetic abnormality

B-ALL with recurrent genetic abnormality

B-ALL, not otherwise specified
Algorithmic Approach

Myeloid

History and Genetics

- Therapy-related AML
  - Hx of cytotoxic therapy
  - Down syndrome

- AML with myelodysplasia-related changes
  - Prior MDS or MDS-related cytogenetics
  - Recurrent genetic abnormality

- AML with recurrent genetic abnormality
  - Present

- AML, not otherwise specified
  - Absent

- Myeloid proliferation of Down Syndrome
  - None
Algorithmic Approach

- <20% Blood or Marrow Blasts
  - Cytogenetics
    - t(8;21, inv(16), t(16;16) or t(15;17)
    - t(5;14)
    - Normal or other abnormalities
  - AML with recurrent genetic abnormality
  - ALL with t(5;14)
  - Not acute leukemia
The 2008 WHO Classification of Acute Leukemia requires the use of clinical information, morphology, immunophenotyping, cytogenetics and molecular genetics for proper diagnosis and classification.

Morphology remains essential as the starting point for diagnosis and for directing the ordering of appropriate ancillary studies.

Proper diagnosis requires evaluation of all data and may require amendments of initial reports.
Questions?