CHAPTER

18

Acute Myeloid Leukemia

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The successful diagnosis of acute myeloid leukemia (AML) has never been an easy task for the practicing pathologist, and the stakes have always been high. Not only are the morphologic features of a case often challenging, but major, urgent, potentially life-threatening treatment decisions hinge on this diagnosis. In acute promyelocytic leukemia (APL), the challenge is even greater, because any delay, even a few hours, will postpone notification of the clinician that the patient is at high risk for a potentially catastrophic hemorrhage. Consequently, the pathologist is faced with the expectation of providing a “correct” diagnosis as rapidly as possible.

Recent biologic insights in AML have even more dramatically expanded expectations of pathologists, with the advance from traditional morphologic and immunophenotypic evaluations to a biologic assessment of AML. This transition requires that the pathologist understand pathogenetic features of AML, since these mechanistic parameters are directly linked to prognosis, “curability,” many distinctive clinical and morphologic features, optimal therapy, and appropriate minimal residual disease monitoring. The 2008 WHO classification of AML has set a new diagnostic standard in AML diagnosis by utilizing a recurrent genetic abnormality as the defining feature for many types of AML. Thus, the state-of-the-art diagnosis of AML requires an integration of clinical, hematologic, morphologic, immunophenotypic, and genetic features. This process must occur in a systematic fashion and must be logical and cost effective.

**Definition**

Acute myeloid leukemia is a clonal hematopoietic disorder that may be derived from either a hematopoietic stem cell or a lineage-specific progenitor cell. AML is characterized both by a predominance of immature forms (with variable, but incomplete, maturation) and loss of normal hematopoesis. Single or multiple hematopoietic lineages may comprise the leukemic clone. The requisite blast/blast equivalent percentage is 20% in the peripheral blood and bone marrow; a lower percentage is acceptable in cases with AML-defining translocations and in acute erythroid leukemia (see [18.4] “Blasts and Blast Equivalents,” p 380 for discussion of blasts/blast equivalents) [Vardiman 2008b].

The blast percentage is derived from counting all nucleated cells for AML diagnosis with the exception of acute erythroid leukemias in which the blast percentage is based on non-erythroid cells. In extramedullary sites, a diagnosis of myeloid sarcoma is equivalent to AML regardless of blood and bone marrow findings. Finally, in 2 clinical situations (1. prior cytotoxic chemotherapy and/or radiation therapy and 2. Down syndrome), cases with features of myelodysplastic syndrome and those with features of AML are lumped together as “myeloid neoplasms” without regard to blast count, as there are no significant clinical or biologic differences in behavior tied to exceeding the 20% threshold. These basic definitional considerations are summarized in f18.1.
### t18.1 Selected Risk Factors for AML

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inherited Genetic Disorders</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Down syndrome</td>
<td>Acute leukemia 10-20 times that of individuals without trisomy 21</td>
</tr>
<tr>
<td></td>
<td>Elevated risk of acute megakaryoblastic leukemia (~600 times normal)</td>
</tr>
<tr>
<td></td>
<td>Transient abnormal myelopoiesis is a spontaneously remitting “leukemia-equivalent” unique to Down syndrome patients</td>
</tr>
<tr>
<td>Severe congenital neutropenia</td>
<td>Cumulative incidence of MDS and AML after 15 years of G-CSF therapy is 34%; risk is 11% at age 20</td>
</tr>
<tr>
<td></td>
<td>Transformation is particularly associated with the acquisition of secondary mutations to the gene encoding the G-CSF receptor</td>
</tr>
<tr>
<td></td>
<td>Cyclic neutropenia does not appear to share a significant risk of progression to MDS or AML</td>
</tr>
<tr>
<td>Shwachman-Diamond syndrome</td>
<td>Estimated incidence of MDS/AML is 19% at 20 years of age and 36% at 30 years, perhaps due to mitotic spindle destabilization in the absence of SBDS protein and consequent chromosomal instability</td>
</tr>
<tr>
<td>Dyskeratosis congenita</td>
<td>Head and neck squamous cell carcinomas dominate the cancer risk; however, the ratio of observed to expected cases of AML is 195:1 [Alter 2009]</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Ratio of observed to expected incidence of AML is 868:1</td>
</tr>
<tr>
<td></td>
<td>Risk of early transformation is increased in D1 complementation group and in the Ashkenazi Jewish population</td>
</tr>
<tr>
<td><strong>Environmental Exposures</strong></td>
<td></td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>Linked to the risk of secondary AML</td>
</tr>
<tr>
<td>Benzene exposure</td>
<td>Strong dose-response relationship with the development of AML</td>
</tr>
<tr>
<td></td>
<td>Benzene-related cases may be reminiscent of therapy-related AML, a finding that recalls the use of benzene as an early form of chemotherapy</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>Slightly increased risk of AML (average relative risk = 1.6); positive dose-response relationship with the number of cigarettes smoked; cigarette smoke contains benzene, which may be responsible for the increased risk</td>
</tr>
<tr>
<td>Pesticides/herbicides</td>
<td>Slightly increased risk of AML (meta-relative risk: 1.55)</td>
</tr>
<tr>
<td><strong>Chemotherapy</strong></td>
<td></td>
</tr>
<tr>
<td>Topoisomerase II inhibitors</td>
<td>Agents include etoposide and teniposide</td>
</tr>
<tr>
<td></td>
<td>Developing t-AML after such epipodophyllotoxins ~2%-12%, depending on regimen-related factors (see t18.6)</td>
</tr>
<tr>
<td>Alkylating chemotherapy</td>
<td>Agents include: melphalan, cyclophosphamide, nitrogen mustard, chlorambucil, busulfan, carboplatin, cisplatin, dacarbazine, procarbazine, carmustine, mitomycin C, thiota, and lomustine</td>
</tr>
<tr>
<td></td>
<td>Early regimens: relative risks for secondary AML exceed 300</td>
</tr>
<tr>
<td></td>
<td>Less toxic regimens (eg, ABVD): somewhat decreased the risk (see t18.6)</td>
</tr>
<tr>
<td>Anthracyclines and anthracenediones</td>
<td>Agents include daunorubicin, doxorubicin, and mitoxantrone (intercalating agents, but also act as topoisomerase II inhibitors)</td>
</tr>
<tr>
<td></td>
<td>Increased risk of AML may be attributable to the latter mechanism of action</td>
</tr>
<tr>
<td></td>
<td>Risk of secondary AML with these agents is less than with strong topoisomerase II inhibitors such as the epipodophyllotoxins, but incidence still substantial (1 study found relative risk with anthracycline therapy to be on the order of 2.7)</td>
</tr>
<tr>
<td>Anti-tubulin agents</td>
<td>Agents include taxanes, vincristine, and vinblastine (frequently administered with other classes of chemotherapeutic agents)</td>
</tr>
<tr>
<td></td>
<td>Some anecdotal reports suggest an increased risk with taxanes, but other studies show no increase in incidence when taxanes are added to an anthracycline-based regimen</td>
</tr>
<tr>
<td></td>
<td>WHO 2008 classification endorses these drugs as cytotoxic agents implicated in therapy-related hematologic neoplasms</td>
</tr>
</tbody>
</table>

<sup>*</sup>Many other inherited disorders are more weakly associated with increased risk for AML, including Li-Fraumeni syndrome, neurofibromatosis, Noonan syndrome and Klinefelter syndrome; patients with congenital amegakaryocytic thrombocytopenia are also at some increased risk of developing AML ~1/2 reported cancers associated with Diamond-Blackfan anemia are AML; the rarity of these bone marrow failure syndromes somewhat hinders precise risk quantification.

ABVD = adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine; AML = acute myeloid leukemia; G-CSF = granulocyte colony-stimulating factor; MDS = myelodysplastic syndrome

**Epidemiology and Pathogenesis**

The age-adjusted incidence of AML in the United States is 3.4 cases per 100,000 persons [Deschler 2006]. AML can occur in patients of any age, but in general, both the overall incidence and the proportion of total acute leukemias that are myeloid increase with age. Thus, acute lymphoblastic leukemia (ALL) predominates in children, with only one case of AML diagnosed for every 5 cases of ALL [Belson 2007]. For childhood AML, peak incidence occurs in the first year of life, then decreases until age 4, and thereafter remains relatively constant until adulthood [Gurney 1995]. The incidence of AML then increases through adulthood, during which period 70%-80% of acute leukemias are AML, with a marked spike in incidence in the elderly. Much of this increased incidence is attributable to AML with myelodysplasia-related changes, which becomes more common with age, while the incidence of de novo AML remains approximately constant across all adult age groups [Deschler 2006].

One of the central themes in this chapter will be that “AML” may best be considered an umbrella term for a heterogeneous group of myeloid leukemias that differ substantially in cause, age of onset, clinical features, and prognosis. For example, AML with t(1;22)(p13;q13); RBM15-MKL1 is a de novo megakaryoblastic AML arising in infants and very young children, while megakaryoblastic leukemia without the t(1;22) often arises in children in the setting of Down syndrome. Epidemiologic and pathogenetic heterogeneity is the necessary consequence of the increasingly fine distinctions among subtypes of AML. Despite this heterogeneity, we can make some general observations about the disease states and environmental exposures that appear to increase an individual’s risk for developing AML of various subtypes. These are listed and discussed in 18.1. The risk of AML is also substantially increased in patients with other hematopoietic disorders, including myelodysplastic syndrome (MDS), some myeloproliferative neoplasms (MPNs), MDS/MPN overlap syndromes, aplastic anemia, and paroxysmal nocturnal hemoglobinuria, in which case the development of AML may be due to progression of the underlying disease.

In general, AML arises following the accumulation (through inherited genetic mechanisms, environmental influences, sheer random chance, or some combination of these) of specific translocations, mutations, and other genetic alterations. Crucially, leukemogenesis often requires the acquisition of several cooperating genetic lesions. For example, the introduction of the fusion protein RUNX1-RUNX1T1, formed by the t(8;21)(q22;q22), into mice is insufficient to cause leukemia [Mrzek 2008]. Similarly, introduction of an abnormally activated tyrosine kinase, which provides a pro-proliferative signal, results in a myeloproliferative process rather than outright leukemia. However, leukemia supervenes when both abnormalities are present in the mice simultaneously [Grodano 2003]. This and similar observations have given rise to the designation of AML-associated molecular genetic changes as either Class I or Class II, with the former conferring increased proliferation and the latter contributing to arrested hematopoietic differentiation 18.2. This distinction is largely irrelevant for diagnostic purposes, but it does underscore the important idea that cooperating alterations in multiple pathways are usually present in AML.

**Key Steps in the Diagnosis of AML**

An attempt to outline key steps in the diagnosis of AML is made in 18.3 (general approach strategies to all myeloid neoplasms are presented in Chapter 13). Although some of these steps are performed simultaneously or are overlapping, it is important for the diagnostician to understand the importance of basic CBC information in determining the likelihood that a patient has AML. This is especially important for rapid diagnosis of AML and appropriate utilization of specialized tests. Although the WBC is highly variable in AML, it is essential for the diagnostician to assess for hematopoietic failure (ie, neutropenia, anemia, and thrombocytopenia). Although preservation of a single hematopoietic lineage may be observed rarely, hematopoietic failure is an expected finding in AML.

If hematopoiesis is preserved (normal absolute neutrophil count, RBC parameters, and platelet count), a diagnosis of AML is very unlikely, and other diagnostic possibilities should be considered. In our experience, the rare examples of exceptions to this general rule have occurred in circumstances in which the blood and bone marrow examinations were performed for reasons unrelated to hematologic dysfunction, and early/partial involvement by acute leukemia was detected.

### Table 18.2: Class I and Class II Mutations in AML

<table>
<thead>
<tr>
<th>Class I Mutations (Proliferation)</th>
<th>Class II Mutations (Impaired Differentiation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3</td>
<td>PML-RARA</td>
</tr>
<tr>
<td>KIT</td>
<td>RUNX1-RUNX1T1</td>
</tr>
<tr>
<td>RAS</td>
<td>CBFB-MYH11</td>
</tr>
<tr>
<td>PTPN11</td>
<td>MLL fusions</td>
</tr>
<tr>
<td>JAK2</td>
<td>CEBPA</td>
</tr>
</tbody>
</table>

*The mechanism of leukemogenesis for NPM1 mutations remains somewhat unclear.

Reference: [Vardiman 2008b]
Acute Myeloid Leukemia: Diagnostic Steps*

1. Evaluation of an abnormal CBC for possible AML (usually step 1 for the pathologist)
   - Confirm HP failures, assess for blasts/blast equivalents (see step 2), and dysplasia
   - WBC: non-specific; in AML can be low, normal, or high
   - ANC: severe neutropenia characteristic of HP failure; typical in AML, but exceptions occur
   - Circulating blasts: variable number and percent in AML, but key feature to assess in blood
   - RBC features: severe anemia characteristic of HP failure, an expected feature of AML
   - Polychromasia: reduced, since anemia is result of bone marrow production failure
   - Other RBC pathology: non-specific
   - Platelets: severe thrombocytopenia characteristic of HP failure

2. Identify morphologic blasts and blast equivalents in blood (and subsequent bone marrow, if performed)
   - Morphologic assessment of nuclear features key for “blast” designation
   - Distinguish from blast look-alikes and other immature hematopoietic cells
   - Consider APL specifically; alert clinician if suspected based on morphology of immature cells and CBC data
   - Cytochemistry, flow cytometric immunophenotyping, genetics can all be performed on blood as needed

3. Bone marrow examination often performed to address differential diagnoses from blood assessment or for protocol requirements

4. Determine lineage of blasts/blast equivalents (can be performed on blood or bone marrow)
   - Morphology (nucleus and cytoplasm)
   - Cytochemistry
   - Immunophenotype
   - Consider APL specifically and alert clinician if suspected

5. Enumeration of blasts/blast equivalents by morphology and differential cell count
   - Blood (percentage; threshold ≥20%)
   - Bone marrow (percentage; threshold ≥20%): blast count <20%, but Auer rods or other distinctive morphology suggests AML
   - Unique situations compromising blast count:
     - Fibrosis and/or necrosis
     - Predominance (≥50%) of erythroid lineage
     - Marked hypocellularity
     - Technically poor specimen

6. Assess all lineages for dysplasia (core biopsy better for megakaryocyte assessment)

7. Identify biologic subtypes of AML based on:
   - Distinctive morphologic features (eg, multilineage dysplasia)
   - Recurrent cytogenetic subtypes
   - Molecular assessment for NPM1, CEBPA, and FLT3
   - Prior chemotherapy/radiation
   - Antecedent hematologic disorder
   - Constitutional disorder (eg, Down syndrome or other constitutional disorders)

8. For cases lacking diagnostic biologic features, exclude other differential diagnostic possibilities

9. Lineage-based classification for AML, NOS cases lacking identifiable biologic features

*Some diagnostic steps overlapping and/or simultaneous
- ANC = absolute neutrophil count; APL = acute promyelocytic leukemia;
- CBC = complete blood count; HP = hematopoietic; NOS = not otherwise specified; RBC = red blood cell; WBC = white blood cell

Simultaneously with CBC assessment, the blood smear should be reviewed systematically for blasts, blast equivalents, other abnormal cells, and dysplasia (all hematopoietic lineages).

The identification, lineage determination, and enumeration of blasts/blast equivalents is also an integrative process that encompasses routine morphology, cytochemical staining, and flow cytometric immunophenotyping on blood or bone marrow (see [18.4] “Blasts and Blast Equivalents”). Throughout this diagnostic process, the pathologist must remain aware of both the urgency in rapid diagnosis of APL and the numerous diagnostic pitfalls/key problem areas in AML diagnosis (see [18.9] “Diagnostic Pitfalls in AML Diagnosis,” p 419).

The molecular genetic data essential for the diagnosis of cases of AML with recurrent genetic abnormalities is often delayed compared to rapid morphology, cytochemical, and immunophenotypic assessment. Consequently, a final integrated report may not be generated for several days to several weeks. However, fluorescence in situ hybridization (FISH) testing offers a more rapid turnaround for assessment of specific genetic abnormalities depending upon probe availability. FISH testing is ideal for rapid assessment of AML-defining translocations, including t(15;17) (q22;q21)* in APL, which results in the fusion of PML-RARA.

A lineage-based classification can be used for an initial diagnosis if data supporting a specific biologic subtype is not available. However for the most part, with the exception of APL, lineage-based classification alone is not predictive of genotype. In addition, in the absence of a diagnostic biologic parameter, the diagnostician must exclude many differential diagnostic considerations. This can be especially problematic when specimens are suboptimal, fibrotic, necrotic, or show a blast percentage approximating 20%.

Frequent discussions with the clinical team are essential throughout the diagnostic process. Particular attention must be paid to rapid communication about possible APL, including recommendations for coagulation assessment. This dialogue with the clinical team is also essential to determining factors that can potentially influence the pathologist’s interpretation, such as antecedent hematologic disorders, prior cytotoxic therapy, and recent granulocyte colony-stimulating factor (G-CSF) therapy.

Blasts and Blast Equivalents

Blasts, blast equivalents, and other immature hematopoietic cells must be identified and distinguished from various blast look-alikes [18.4]. For AML diagnosis, blasts include myeloblasts, monoblasts, and megakaryoblasts, while promonocytes are blast equivalents in all types of AML and promyelocytes are blast equivalents exclusively in acute promyelocytic leukemia.

The t(15;17) PML-RARA translocation has been historically denoted as both t(15;17)(q22;q21) and t(15;17)(q22;q12). Currently the most accurate description is actually thought to be t(15;17)(q24;q21). For the sake of simplicity and consistency, we have chosen to use the t(15;17)(q22;q21) designation.
### 18.4 Blasts, Blast Equivalents, Other Immature Cells in Blood and Bone Marrow

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Key Morphologic Features</th>
<th>Cytochemistry</th>
<th>Immunophenotype/Comments*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>Large nucleus with finely dispersed chromatin and variably prominent nucleoli</td>
<td>MPO+</td>
<td>CD34+, CD13+, CD33+, MPO+, HLA-DR+, vCD11c+, wCD45+, CD117 usually +</td>
</tr>
<tr>
<td></td>
<td>Relatively high nuclear/cytoplasmic ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variable number of cytoplasmic granules, may be concentrated in limited portion of cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>Nuclear chromatin slightly condensed; nucleoli variably prominent; nucleus often eccentric, and Golgi zone may be apparent</td>
<td>Strong, uniform MPO+</td>
<td>CD13+, CD33+, MPO+, wCD45+, CD34−, HLA-DR−</td>
</tr>
<tr>
<td></td>
<td>Numerous cytoplasmic granules that may be more dispersed throughout cytoplasm</td>
<td></td>
<td>Loss of HLA-DR and acquisition of strong CD15 and CD11c associated with maturation</td>
</tr>
<tr>
<td></td>
<td>Blast equivalent in APL only</td>
<td></td>
<td>Gradual loss of CD33 also characterizes successive maturation stages</td>
</tr>
<tr>
<td></td>
<td>In APL, intense cytoplasmic granularity usually present</td>
<td></td>
<td>CD34 usually negative in hypergranular variant; often positive in microgranular variant</td>
</tr>
<tr>
<td></td>
<td>Nuclear configuration variable, but nuclear folding and lobulation characteristic of microgranular variant of APL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoblast</td>
<td>Moderate to low nuclear to cytoplasm ratio, nuclear chromatin finely dispersed with variably prominent nucleoli; nuclei round to folded</td>
<td>NSE+</td>
<td>CD34−, HLA-DR+, CD13+, CD33 bright +, CD36/CD64 coexpression, vCD4+, CD11c+, wCD45+</td>
</tr>
<tr>
<td></td>
<td>Abundant, slightly basophilic cytoplasm containing fine granulation and occasional vacuoles</td>
<td></td>
<td>Usually CD34−</td>
</tr>
<tr>
<td></td>
<td>Consistent blast equivalent in AML</td>
<td></td>
<td>Occasional cases moderate CD45+</td>
</tr>
<tr>
<td>Promonocyte</td>
<td>Slightly condensed nuclear chromatin; variably prominent nucleoli</td>
<td>NSE+</td>
<td>CD36/CD64 coexpression, HLA-DR+, CD13+, CD33 bright +, vCD14+, CD4+, CD11c+, CD45+</td>
</tr>
<tr>
<td></td>
<td>Abundant finely granular blue/gray cytoplasm that may be vacuolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very monocytic appearance with nuclear immaturity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Consistent blast equivalent in AML</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythroblast</td>
<td>Relatively high nuclear/cytoplasm ratio</td>
<td>PAS+</td>
<td>Glycophorin A+, hemoglobin A+, CD71+, CD34−, CD45−, MPO−, myeloid antigens negative</td>
</tr>
<tr>
<td></td>
<td>Nucleus round with slightly condensed chromatin; nucleoli variably prominent</td>
<td></td>
<td>CD117 often positive</td>
</tr>
<tr>
<td></td>
<td>Moderate amounts of deeply basophilic cytoplasm that may be vacuolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Included in blast percentage only in acute erythroid leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megakaryoblast</td>
<td>Highly variable morphologic features; often not recognizable without special studies</td>
<td>NA</td>
<td>CD34−, CD41+, CD61+, CD33 bright +, CD13−, HLA-DR− (or dim)</td>
</tr>
<tr>
<td></td>
<td>May be lymphoid-appearing with high nuclear to cytoplasmic ratio</td>
<td></td>
<td>Progressive maturation characterized by loss of CD34 and acquisition of CD42 and von Willebrand factor</td>
</tr>
<tr>
<td></td>
<td>Nuclear chromatin fine to variably condensed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytoplasm may be scant to moderate, is usually agranular or contains a few granules; blebbing or budding of cytoplasm may be evident</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Blasts may form cohesive clumps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastic plasmacytoid dendritic cell§</td>
<td>Variable morphology; not identified in either normal bone marrow or AML</td>
<td>MPO−</td>
<td>CD4+, CD56+, CD123+, CD43+, CD45+, HLA-DR+, vTdT+</td>
</tr>
<tr>
<td></td>
<td>Often not recognizable without immunophenotyping</td>
<td>NSE−</td>
<td>CD123 not specific; seen in subset of AMLs (Discussed in detail in Chapter 19)</td>
</tr>
<tr>
<td></td>
<td>May be lymphoid-appearing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>May show cytoplasmic tadpoles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Key flow cytometric clues to lineage assignment in boldface
†Rare AML lacks surface CD13 and CD33
‡False positive CD41 expression by flow cytometry may be secondary to platelet adherence to blasts
§See Chapter 19

AML = acute myeloid leukemia; APL = acute promyelocytic leukemia; MPO = myeloperoxidase; NSE = non-specific esterase; PAS = periodic acid-Schiff; v = variable antigen expression; w = weak antigen expression

References: [Dohner 2010, Vardiman 2008b]
Erythroblasts are included in the blast percentage only in acute “pure” erythroid leukemia. Nuclear morphologic features are most critical in the decision that a cell is a blast exhibiting finely dispersed rather than condensed nuclear chromatin. Other useful nuclear features of blasts to assess include overall size, nucleoli, and nuclear configuration. Cytoplasmic features are very helpful in lineage determination, i.e., sparse fine granules and Auer rods in myeloblasts, cytoplasmic blebbing in megakaryoblasts, and deeply basophilic, vacuolated cytoplasm in erythroblasts. As their name implies, blastic plasmacytoid dendritic cell neoplasms can be derived from cells with morphologic features of blasts. These cells are not evident in normal bone marrow and are not generally included in the discussion of immature cell populations that need to be identified in cases of possible AML. However, for completeness, blastic plasmacytoid dendritic cells...
are highlighted in 18.4, but the reader is referred to Chapter 19 for comprehensive discussion and illustrations.

Myeloblasts can be morphologically diverse, and the distinction between a granular blast and a promyelocyte with a distinct paranuclear hof (Golgi zone) is key 18.1, 18.2, 18.6. Auer rods are a key cytologic feature of myeloblasts, although Auer rods can be seen in promyelocytes in APL and even rarely in mature cells 18.7. Other unique features of myeloblasts include pseudo Chédiak-Higashi granules, nuclear indentation, deeply basophilic cytoplasm with vacuoles, and, rarely, clumping on aspirate smears mimicking a metastatic process 18.8, 18.9. Associations of some of these unusual morphologic features with immunophenotypic and molecular genetic properties have been reported [Chang 2006, Kussick 2004]. Cytochemical staining for myeloperoxidase is important in establishing the lineage of myeloblasts 18.10. Features of AML on bone

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marrow core biopsy sections are also heterogeneous, but prototypic findings include a predominance of immature cells with dispersed chromatin, variably prominent nucleoli, and moderate amounts of eosinophilic cytoplasm. Monoblasts and promonocytes (blast equivalents) must also be successfully identified based on morphologic and cytochemical features. Flow cytometric immunophenotyping (and to a lesser extent immunohistochemical staining) is a mainstay in assessing immaturity (weak CD45, CD34, CD117) and lineage in AML. Integration of morphology, limited cytochemistry, and immunophenotype is optimal in AML diagnosis. However, the actual blast percentage is best assessed by morphology, and the percentage of CD34+ cells should not substitute for the morphologic percentage. Keep in mind that flow cytometry specimens may show falsely low blast percentages due to hemodilution or falsely elevated blast percentages due to lysis of erythroid cells. Furthermore, many types of blasts (erythroblasts, monoblasts, megakaryoblasts) and blast equivalents (promyelocytes, promonocytes) typically lack CD34 expression.

Based on morphologic differential cell counts, the blast threshold in blood and bone marrow for AML diagnosis is 20% [Vardiman 2008b]. However, as noted earlier, there are exceptions to this general rule, and the blast/blast equivalent percentage must always be assessed in the overall context of a given case. Determining an accurate blast/blast equivalent percentage can be especially challenging in technically suboptimal, necrotic, or fibrotic specimens (see Diagnostic Pitfalls in AML Diagnosis, p 419). In these situations, immunohistochemical techniques may be helpful.
in determining lineage and immature cell populations on tissue sections. Similarly, cases with a marked expansion of the erythroid lineage can be difficult to diagnose (see\textsuperscript{18.9} “Diagnostic Pitfalls in AML Diagnosis,” p 419). Key features to assess include the overall percentage of erythroid lineage cells, the degree of maturation/differentiation of the erythroid lineage, the percentage of myeloid blasts among non-erythroid cells, and the extent of dysplasia.

\textbf{Classification of AML}

In most cases, the integration of traditional clinical, hematologic, morphologic, cytochemical, and immunophenotypic data results in a confident diagnosis of AML. If a patient has Down syndrome, has a known history of antecedent myelodysplasia, or has had prior chemotherapy/radiation therapy, additional biologic information can be provided at the time of traditional assessment. However, the biologic classification of AML will often hinge upon molecular genetic studies. If a positive molecular genetic result is obtained, the AML will be subcategorized among the 9 molecular genetic subtypes in the 2008 WHO classification\textsuperscript{18.5}. If non-contributory molecular genetic and clinical results are obtained, the case will be subclassified based on traditional lineage-based criteria (see\textsuperscript{18.7} “AML, Not Otherwise Specified (NOS),” p 408).

\textbf{Biologic Subtypes of AML}

As illustrated in\textsuperscript{18.2} and\textsuperscript{18.5}, there are essentially 2 ways to categorize specific biologic subtypes of AML: genetic findings and clinical setting. Many details pertaining to these subtypes are listed in\textsuperscript{18.5}, which serves as a quick reference guide for this section. In this section we will describe in some detail those cases of AML categorizable by clinical setting, as well as the genetically defined subtypes of AML.

\textbf{Molecular Genetic Biologic Subtypes of AML}

\textit{Core Binding Factor Acute Myeloid Leukemias}

Core binding factor (CBF) AML includes AML with t(8;21) and AML with inv(16)/t(16;16). These 2 AML cytogenetic groups are often grouped together based on involvement of related CBF machinery and relatively overall favorable prognosis [Marcucci 2005]. The CBF genes are \textit{RUNXI} (21q22, aka \textit{AML1}, \textit{CBFA2}) and \textit{CBFB} (16q22). As part of the CBF heterodimer transcription factor complex, \textit{RUNXI} binds to DNA promoter sequences of genes needed for hematopoiesis, while \textit{CBFB} protects the complex from proteolysis [Okumura 2008, Paschka 2008a, Speck 2002]. The chimeric proteins, produced as a result of the chromosomal rearrangements, contribute to leukemogenesis by dysregulating the normal CBF transcriptional activity [Downing 2003, Helbling 2005].

\textbf{AML with t(8;21)(q22;q22); RUNX1-RUNXI T1}

AML with t(8;21)(q22;q22) is defined by the presence of this translocation, a variant translocation, or molecular \textit{RUNXI-RUNXI T1} fusion, regardless of blast count\textsuperscript{18.3} [Arber 2008a]. Clues and confirmatory tests for this distinct type of AML are listed in\textsuperscript{18.5}.

The peripheral blood findings are variable, but circulating blasts accompanied by cytopenias are common\textsuperscript{18.15}. Circulating mature granulocytes including neutrophils are a
### AML Subtype

#### Clinical Features

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Young adults, myeloid sarcomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%-15% of pediatric AML</td>
</tr>
<tr>
<td></td>
<td>7% of adult AML</td>
</tr>
</tbody>
</table>

#### CBC/Blood Morphology

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Cytopenias, circulating blasts with evidence of neutrophilic maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blasts may be &lt;20%</td>
</tr>
<tr>
<td></td>
<td>Long tapered Auer rods</td>
</tr>
<tr>
<td></td>
<td>Salmon-colored cytoplasm, rim of basophilia</td>
</tr>
<tr>
<td></td>
<td>Manifests as AML with maturation</td>
</tr>
</tbody>
</table>

#### BM Aspirate

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Blasts may be &lt;20%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal eosinophils with admixed eosinophil/ basophil granules</td>
</tr>
<tr>
<td></td>
<td>Variable monocytic component</td>
</tr>
</tbody>
</table>

### AML Subtype

#### Clinical Features

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Extramedullary disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10% - 15% of pediatric AML</td>
</tr>
<tr>
<td></td>
<td>8% adult AML</td>
</tr>
</tbody>
</table>

#### CBC/Blood Morphology

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Although variable, higher WBC and blast counts vs t(8;21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monocytic cells, myeloid blasts predominate</td>
</tr>
<tr>
<td></td>
<td>Cytopenias</td>
</tr>
</tbody>
</table>

#### BM Aspirate

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Abnormal promyelocytes predominate (blasts may be &lt;20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypergranular variant (majority of cases): packed with granules, numerous Auer rods in single cell</td>
</tr>
<tr>
<td></td>
<td>Microgranular variant (20% of cases): reniform, bilobed nuclei with scant to inconspicuous granules</td>
</tr>
</tbody>
</table>

### AML Subtype

#### Clinical Features

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Restricted to infants or young children (&lt;3 years of age) without Down syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marked organomegaly</td>
</tr>
</tbody>
</table>

#### BM Aspirate

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Megakaryoblasts with basophilic cytoplasm and cytoplasmic blebbing; smaller blasts may show a more lymphoblastic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micromegakaryocytes are present Granulocytic and erythroid dysplasia not prominent</td>
</tr>
</tbody>
</table>

### AML Subtype

#### Clinical Features

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Typically, de novo acute leukemia in older adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC may be relatively high</td>
</tr>
</tbody>
</table>

#### BM Aspirate

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Many, but not all cases show monocytic differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80%-90% of acute monocytic leukemias have mutated NPM1</td>
</tr>
<tr>
<td></td>
<td>High blast percentage</td>
</tr>
</tbody>
</table>

### AML Subtype

#### Clinical Features

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Occurs in 6%-15% of de novo AML; no age or sex predilection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relatively high hemoglobin and blood blast count</td>
</tr>
<tr>
<td></td>
<td>Relatively low platelet count</td>
</tr>
</tbody>
</table>

#### BM Aspirate

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Most cases show features of AML with or without maturation; no specific morphologic features</th>
</tr>
</thead>
</table>

---

*The t(15;17) PML-RARA translocation has been historically denoted as both t(15;17)(q22;q21) and t(15;17)(q22;q12); currently the most accurate description is actually thought to be t(15;17)(q24;q21); for the sake of simplicity and consistency, we have chosen to use the t(15;17)(q22;q21) designation.*

**AML** = acute myeloid leukemia; **APL** = acute promyelocytic leukemia; **ATRA** = all trans retinoic acid; **BM** = bone marrow; **CBC** = complete blood count; **DIC** = disseminated intravascular coagulation; **HP** = hematopoiesis; **IHC** = immunohistochemistry; **MDS** = myelodysplastic syndrome; **MPN** = myeloproliferative neoplasm; **MPO** = myeloperoxidase; **MRD** = minimal residual disease; **PML** = promyelocytic leukemia; **WBC** = white blood cell count.
<table>
<thead>
<tr>
<th>BM Biopsy</th>
<th>Flow Cytometry</th>
<th>Confirmatory Tests/Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercellular</td>
<td>Blasts: CD34+, CD117+, CD13+, CD33+, CD19+, CD79a+, TdT+ or CD56+ Frequent</td>
<td>Favorable subtype</td>
</tr>
<tr>
<td>increased blasts with evidence of granulocytic maturation</td>
<td>subpopulations reflecting granulocytic maturation in background</td>
<td>Cytogenetics</td>
</tr>
<tr>
<td>Megakaryopoiesis and erythropoiesis decreased</td>
<td>IHC: PAX5+, CD10+</td>
<td><strong>KIT</strong> activating mutations (12%-47%) may confer worse overall survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Molecular monitoring of <strong>RUNX1/RUNX1T1</strong> for MRD</td>
</tr>
<tr>
<td>Hypercellular; admixture of immature and mature monocytic and</td>
<td>2 distinct populations:</td>
<td>Favorable subtype</td>
</tr>
<tr>
<td>granulocytic components</td>
<td>blasts (CD34+, CD117+, weak CD45+, myeloid antigens +) monocyte cells (CD45+,</td>
<td>Cytogenetics</td>
</tr>
<tr>
<td></td>
<td>bright CD36/CD64 coexpression, CD33 bright+, CD34–, CD117–, HLA-DR+)</td>
<td>Molecular or FISH for <strong>CBFB-MYH11</strong> if suspect cryptic fusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trisomy 22 often also seen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If +22 identified, may need FISH/molecular if karyotype otherwise normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>KIT</strong> mutations (25%); significance unclear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Molecular negativity may be associated with long-term remission</td>
</tr>
<tr>
<td>Hypercellular, dominated by cells with obvious granules in hypergranular</td>
<td>Classically CD34–, HLA-DR–, CD33 bright+ CD2 and CD34 expression common in</td>
<td>Intermediate risk</td>
</tr>
<tr>
<td>variant</td>
<td>microgranular variant CD56 in 20% of cases; associated with worse outcome</td>
<td>Cytogenetics</td>
</tr>
<tr>
<td>Antibodies against PML show a nuclear granular pattern vs a speckled</td>
<td></td>
<td>**FISH for <strong>PML-RARA</strong> needed in rare cases with negative cytogenetics and FISH</td>
</tr>
<tr>
<td>pattern in non-APL promyelocytes</td>
<td></td>
<td>MRD monitoring (see Chapter 36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATRA overcomes maturation block; used in combination with chemotherapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arsenic trioxide also used in therapy of APL</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>FLT3</strong> mutations (~30%-40%); likely adverse effect</td>
</tr>
<tr>
<td>Hypercellular, monoblasts and promonocytes IHC: CD68+, lysozyme+, CD163+</td>
<td>Coexpression of CD36/CD64, HLA-DR+, CD33 bright+, CD4 weak+, CD56+, CD45+</td>
<td>Poor prognosis</td>
</tr>
<tr>
<td></td>
<td>CD34–, CD117–, CD14 variable, often negative/dim</td>
<td>Cytogenetics</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>FISH for MLL</strong></td>
</tr>
<tr>
<td>Background myelodysplasia (&gt;60% of cases)</td>
<td>Typical myeloid phenotype:</td>
<td>Poor risk</td>
</tr>
<tr>
<td></td>
<td>CD45 weak+, CD34+, CD117+, CD13+, CD33+</td>
<td>Cytogenetics</td>
</tr>
<tr>
<td>Small, hypolobated megakaryocytes Cellularity and fibrosis variable</td>
<td>Blasts typically express CD34, CD13, CD33; may express CD7, CD41 and CD61</td>
<td>Poor risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytogenetics</td>
</tr>
<tr>
<td>Micromegakaryocytes are present</td>
<td>Blasts typically express CD41 and CD61; CD42b more variable. CD36 may be</td>
<td>Patients may respond to intensive chemotherapy</td>
</tr>
<tr>
<td>There is usually collagen and/or reticular fibrosis</td>
<td>positive. CD34, MPO, CD45 frequently negative, somewhat complicating lineage</td>
<td></td>
</tr>
<tr>
<td>Overall appearance may suggest metastatic tumor</td>
<td>expression (CD36/CD64)</td>
<td></td>
</tr>
<tr>
<td>Usually markedly hypercellular</td>
<td>Blasts typically CD34 negative, with expression of CD14, CD11b and CD68</td>
<td>Molecular (typically sizing via capillary electrophoresis for exon 12 mutations; “type A” mutation, a 4 base pair TCTG insertion most common); favorable outcome</td>
</tr>
<tr>
<td></td>
<td>Monocytic antigens typically expressed (CD36/CD64)</td>
<td>IHC for abnormal cytoplasmic NPM1 protein endorsed by WHO; some studies suggest molecular analysis may be preferred</td>
</tr>
<tr>
<td>Most cases show features of AML with or without maturation; no specific</td>
<td>Blasts express typical myeloid antigens: usually CD34+, express CD7 in the</td>
<td>Molecular (mutations described throughout gene, with 2 “hot-spots” at N-terminal and C-terminal)</td>
</tr>
<tr>
<td>morphologic features assist in identifying <strong>CEBPA</strong> mutated cases</td>
<td>majority of cases</td>
<td>Favorable outcome</td>
</tr>
</tbody>
</table>

**AML** = acute myeloid leukemia; **APL** = acute promyelocytic leukemia; **ATRA** = all trans retinoic acid; **BM** = bone marrow; **CBC** = complete blood count; **HP** = hematopoiesis; **IHC** = immunohistochemistry; **DIC** = disseminated intravascular coagulation; **MDS** = myelodysplastic syndrome; **MPN** = myeloproliferative neoplasm; **MPO** = myeloperoxidase; **MRD** = minimal residual disease; **PML** = promyelocytic leukemia; **WBC** = white blood cell count
### Clues and Confirmatory Tests for Biologic Types of AML (continued from previous spread)

<table>
<thead>
<tr>
<th>AML Subtype</th>
<th>Clinical Features</th>
<th>CBC/Blood Morphology</th>
<th>BM Aspirate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Other Biologic Subtypes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML with MDS-related changes (3 criteria; only 1 required)</td>
<td>Elderly; patient may have antecedent MDS, MDS/MPN (#1 criterion)</td>
<td>Cytopenias</td>
<td>Dysplasia of ≥50% of erythroid and/or granulocytic cells (#2 criterion)</td>
</tr>
<tr>
<td>Therapy-related myeloid neoplasm</td>
<td>History of chemotherapy and/or radiation therapy</td>
<td>Cytopenias</td>
<td>Cases may meet criteria for AML, MDS, or MDS/MPN (see t18.8)</td>
</tr>
<tr>
<td>Down syndrome (DS)-related myeloid proliferation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient abnormal myelopoiesis (TAM)</td>
<td>Diagnosis of DS (including mosaic cases)</td>
<td>Blasts are morphologically indistinguishable from those in myeloid leukemia associated with DS</td>
<td>Blasts are morphologically indistinguishable from those in myeloid leukemia associated with DS</td>
</tr>
<tr>
<td></td>
<td>Neonate/fetus</td>
<td>Basophilia may be present</td>
<td>Erythroid and megakaryocytic dysplasia typically present</td>
</tr>
<tr>
<td>Myeloid leukemia associated with DS</td>
<td>Diagnosis of DS (including mosaic cases)</td>
<td>Blasts have megakaryoblastic features, with basophilic cytoplasm, coarse granules, and cytoplasmic blebbing</td>
<td>Myeloid neoplasms falling into this category may show &lt;20% blasts, as MDS and AML in this clinical setting are not clinically or biologically distinct</td>
</tr>
<tr>
<td></td>
<td>Infant/young child (usually &lt;4-5 yrs of age)</td>
<td>Erythroid precursors, marked anisopoikilocytosis, and giant platelets may be seen</td>
<td>Dyserythropoiesis and dysgranulopoiesis</td>
</tr>
</tbody>
</table>

AML = acute myeloid leukemia; APL = acute promyelocytic leukemia; ATRA = all trans retinoic acid; BM = bone marrow; CBC = complete blood count; DIC = disseminated intravascular coagulation; HP = hematopoiesis; IHC = immunohistochemistry; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasm; MPO = myeloperoxidase; MRD = minimal residual disease; PML = promyelocytic leukemia; WBC = white blood cell count

#### References by subtype

- **8;21**

- **inv(16)**

- **15;17**

- **9;11**

- **6;9**

---

**f18.3** Partial karyotype showing chromosomes 8 and 21 illustrates t(8;21) (q22;q22). The abnormal chromosome of each pair is each on the right. (Giemsa trypsin Wright)

**f18.15** Peripheral blood smear from a patient with acute myeloid leukemia and t(8;21) shows prominent myeloid and monocytic maturation. Blasts are present, but maturation is prominent. Note profound anemia and thrombocytopenia. (Wright)
A CUTE MYELOID LEUKEMIA

usual finding; monocytes may also be present. In the bone marrow, the blast count is variable and in some cases is less than the otherwise required 20% (Chan 1997, Wong 2009b, Xue 1994). Blasts with distinctive, long, thin Auer rods with tapered ends are characteristic and may even be seen in mature neutrophils and eosinophils. Additional distinctive findings include salmon-colored cytoplasm with a rim of basophilia in maturing granulocytes, and dysplastic features of the mature granulocytes, including pseudo Pelger-Huët nuclei and nuclear/cytoplasmic dysynchrony (Nakamura 1997). The bone marrow core biopsy is hypercellular with evidence of granulocytic maturation (Nakamura 1997).

AML with t(8;21) may be associated with systemic mastocytosis. In some cases the mastocytosis may be occult at diagnosis, becoming more apparent and predominant after successful therapeutic reduction of the acute leukemia.

**BM Biopsy**

| Abnormal megakaryocytes | Aberrant antigen maturation profile variable; myeloid blasts (CD34+) | MDS-associated cytogenetic abnormalities (#3 criteria), not all cases (see t18.7) |

| Cases may meet criteria for AML, MDS, or MDS/MPN (see t18.9) | Variable; often aberrant | Two common subtypes: chromosomal losses/gains or 11q23 (MLL) translocation |

| Blast % may be lower than blood blast count | Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b. | GATA1 mutation |

| Variable; often aberrant | Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b. | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

| Very dense fibrosis may be present | In contrast to TAM, blasts are CD34- in ~50% of cases. CD56 and CD41 also more likely to be negative | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

| Highly atypical megakaryocytes | In contrast to TAM, blasts are CD34- in ~50% of cases. CD56 and CD41 also more likely to be negative | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

**Flow Cytometry**

| MDS-associated cytogenetic abnormalities (#3 criteria), not all cases (see t18.7) | MDS-associated cytogenetic abnormalities (#3 criteria), not all cases (see t18.7) | MDS-associated cytogenetic abnormalities (#3 criteria), not all cases (see t18.7) |

| Cases may meet criteria for AML, MDS, or MDS/MPN (see t18.9) | Variable; often aberrant | Two common subtypes: chromosomal losses/gains or 11q23 (MLL) translocation |

| Blast % may be lower than blood blast count | Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b. | GATA1 mutation |

| Variable; often aberrant | Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b. | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

| Very dense fibrosis may be present | In contrast to TAM, blasts are CD34- in ~50% of cases. CD56 and CD41 also more likely to be negative | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

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**Confirmatory Tests/Other**

| Abnormal megakaryocytes | Aberrant antigen maturation profile variable; myeloid blasts (CD34+) | MDS-associated cytogenetic abnormalities (#3 criteria), not all cases (see t18.7) |

| Cases may meet criteria for AML, MDS, or MDS/MPN (see t18.9) | Variable; often aberrant | Two common subtypes: chromosomal losses/gains or 11q23 (MLL) translocation |

| Blast % may be lower than blood blast count | Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b. | GATA1 mutation |

| Variable; often aberrant | Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b. | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

| Very dense fibrosis may be present | In contrast to TAM, blasts are CD34- in ~50% of cases. CD56 and CD41 also more likely to be negative | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

| Highly atypical megakaryocytes | In contrast to TAM, blasts are CD34- in ~50% of cases. CD56 and CD41 also more likely to be negative | GATA1 mutation; cases of MDS or AML occurring in patients with DS who are older than age 5 and lack GATA1 mutation are considered “conventional” MDS or AML |

**BM Biopsy**

- Abnormal megakaryocytes
- Cases may meet criteria for AML, MDS, or MDS/MPN (see t18.9)
- Blast % may be lower than blood blast count
- Very dense fibrosis may be present
- Highly atypical megakaryocytes

**Flow Cytometry**

- Aberrant antigen maturation profile variable; myeloid blasts (CD34+)
- Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b.
- Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b.

**Confirmatory Tests/Other**

- MDS-associated cytogenetic abnormalities (#3 criteria), not all cases (see t18.7)
- Variable; often aberrant
- Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b.
- Positive for CD34, CD56, CD13, CD33, CD7, CD4, CD41, CD61, CD42b.

**References**

- Chan 1997
- Wong 2009
- Xue 1994
- Martinelli 2003
- Nazifova 1997
- Raza 2004
- Testoni 1999
- Duchayne 2003
- Mercher 2001
- Nuñez 2009
- Paredes-Aguirre 2003
- Friebe 1993
- Bedar 2010
- Falini 2005
- Konoplev 2009
- Schnittger 2005
- Tsoa 2006
- Yu 2008
- Yan 2003
- Baldus 2007
- Biener 2005
- Dufour 2010
- Finishing 2004
- Konshneider 2009
- Pabst 2009
- Schlenk 2008
- Wouters 2009
- Arber 2008a
- Warrt 2008
- Cadek 2009
- Knight 2009
- Pedersen-Bjergaard 2008
- Rund 2005
- Seedhouse 2007
- Vardiman 2008
- Massey 2005
- Xavier 2009
- Zupanzyk 2003

**Abbreviations**

- AML = acute myeloid leukemia
- APL = acute promyelocytic leukemia
- ATRA = all trans retinoic acid
- BM = bone marrow
- CBC = complete blood count
- HP = hematopoiesis
- IHC = immunohistochemistry
- DIC = disseminated intravascular coagulation
- MDS = myelodysplastic syndrome
- MPN = myeloproliferative neoplasm
- MPO = myeloperoxidase
- MRD = minimal residual disease
- PML = promyelocytic leukemia
- WBC = white blood cell count

**Cytogenetic Abnormalities**

- MDS-related changes (Arber 2008b, Warrt 2008)

18: ACUTE MYELOID LEUKEMIA
Long, tapered Auer rods and abnormal cytoplasmic granulation are evident in this bone marrow aspirate smear from a patient with acute myeloid leukemia and t(8;21). (Wright)

This neutrophil from a case of acute myeloid leukemia with t(8;21) shows Auer rods within the cytoplasm. (Wright)

Systemic mastocytosis was evident in conjunction with acute myeloid leukemia associated with t(8;21) in this bone marrow aspirate smear. Note features of acute myeloid leukemia with t(8;21) as well as numerous mast cells. (Wright)

Substantial maturation is evident in this bone marrow core biopsy section from a patient with acute myeloid leukemia and t(8;21). (H&E)

Systemic mastocytosis was evident in conjunction with acute myeloid leukemia and t(8;21) in this bone marrow aspirate smear. Note features of acute myeloid leukemia with t(8;21) as well as numerous mast cells. (Wright)

This bone marrow core biopsy section from a patient with concurrent acute myeloid leukemia with t(8;21) and systemic mastocytosis shows numerous admixed mast cells. (immunoperoxidase for tryptase)
also occur after previous chemotherapy (therapy-related) or in blast phase of chronic myelogenous leukemia (CML) [Arber 2002, Gustafson 2009, Yin 2004]. Frequent secondary cytogenetic aberrations include loss of a sex chromosome (–Y in men and –X in women) and deletion 9q [Kuchenbauer 2006, Mrozek 2008a]. KIT activating mutations have been identified in t(8;21) and confer an inferior overall survival [Boissel 2006, Cairoli 2006, Paschka 2006, Schnittger 2006]. FLT3 mutations are seen in 4%-12% of AML with t(8;21), although only a minority are of the internal tandem duplication type tied most definitively to prognosis in cytogenetically normal cases (see [T.8.6 “Prognostic Factors in AML,” p 417]) [Boissel 2006, Care 2003, Shimada 2006, Srirana 2008]. Nevertheless, some reports suggest that FLT3 mutation is an adverse prognostic finding in AML with t(8;21) [Boissel 2006].
The peripheral blood abnormalities are variable, but leukocytosis, anemia, and thrombocytopenia are common. Monocytic cells are abundant with variable numbers of monoblasts, myeloblasts, and promonocytes. The abnormal eosinophils (described below) are generally inconspicuous or absent in the blood.

The bone marrow is hypercellular with a variable blast count. The morphologic features show a spectrum of mature and immature monocytic cells and myeloblasts. The monocytic and granulocytic components are confirmed by positive nonspecific esterase and myeloperoxidase cytochemical stains, respectively. In addition, the bone marrow contains abnormal eosinophils with mixed eosinophilic and basophilic granules. The abnormal basophil granules within these eosinophils lack both myeloperoxidase and toluidine blue reactivity, distinguishing them from basophil granules. Significant maturation may be evident with relatively low numbers of blasts. Both maturation and significant eosinophilia are evident on bone marrow biopsy sections.

Immunophenotypically, AML with inv(16)/t(16;16) reveals distinct populations corresponding to the admixture of blasts, granulocytes, and monocytic cells. An immunohistochemical antibody stain (AH107) against the CBFB-MYH11 fusion protein may aid in the diagnosis.

Patients with AML with inv(16)/t(16;16) have a favorable prognosis similar to t(8;21). The inv(16) may occur rarely after previous chemotherapy or in blast phase of CML. KIT mutations are present in ~30% of patients, the significance of which is unclear.
Activating mutations in NRAS, KRAS, KIT, or FLT3 are seen in ~70% of cases [Boissel 2006, Valk 2004].

**APL with t(15;17)(q22;q21); PML-RARA**

AML with t(15;17)(q22;q21)/PML-RARA (aka APL) is a distinct clinicopathologic entity defined by the presence of the PML-RARA fusion, regardless of blast count [Arber 2008a]. Because of the propensity for life-threatening coagulopathy, it is imperative that cases of APL be rapidly identified [Lock 2004, Stein 2009]. This can be accomplished with a myeloperoxidase cytochemical stain, antibody against the PML protein, or FISH.

Two typical morphologic variants of APL are recognized (hypergranular and microgranular), plus a third variant that is quite rare (hyperbasophilic). Hypergranular APL often presents with peripheral leukopenia with rare, if any, circulating promyelocytes, while the bone marrow is typically packed with abnormal, hypergranular promyelocytes [Bennett 2000]. Occasional/rare cells packed with Auer rods are noted [Arber 2008a]. There is little to no maturation beyond the promyelocyte stage. These hypergranular promyelocytes are intensely myeloperoxidase or Sudan black B positive, a very useful feature in the rapid diagnosis of APL [Bennett 2000]. On bone marrow core biopsy sections, APL cells show moderate amounts of eosinophilic cytoplasm. Because of the degree of maturation, CD34 is characteristically negative.

Microgranular APL is morphologically distinct from the hypergranular variant in both nuclear and cytoplasmic features. Microgranular promyelocytes exhibit marked nuclear irregularities including reniform, lobulated, and monocyte-like nuclei, while the cytoplasm shows subtle often inconspicuous granulation. The rare hyperbasophilic variant is characterized by nuclear regularity and basophilic cytoplasm; features overlap with microgranular APL. It is essential to distinguish microgranular APL from a true monocytic leukemia.
Cytochemically, both the hypergranular and microgranular APL cases show intense myeloperoxidase and Sudan black B positivity in the leukemic cells. This marked degree of staining is quite compelling for a diagnosis of APL and is very useful in distinguishing APL from morphologically similar disorders.

Immunophenotypically, hypergranular and microgranular APL show some similarities but also key differences. The hypergranular variant is typically CD34 negative, HLA-DR negative, with bright CD33, whereas the microgranular variant is often CD34 positive with aberrant CD2 expression (90%) [Edwards 1999, Kaleem 2003, Lin 2004, Paietta 2003]. The lack of CD34 and HLA-DR is not specific for APL, thus complete reliance on flow cytometric immunophenotyping to diagnose APL is to be avoided [Moon 2007, Ochsleghl 2009].
The distinctly folded, sliding plate appearance of nuclei in microgranular acute promyelocytic leukemia is evident in this peripheral blood smear. Note marked leukocytosis. (Wright)

The distinctive nuclear features of microgranular acute promyelocytic leukemia are evident on high magnification of this peripheral blood smear. Note marked leukocytosis. (Wright)

Rare hypergranular promyelocytes are present in microgranular acute promyelocytic leukemia in blood. (Wright)

This composite compares microgranular acute promyelocytic leukemia with acute monocytic leukemia. Note the differences in nuclear configuration and the greater amounts of cytoplasm in acute monocytic leukemia. (Wright)

Intense myeloperoxidase positivity, characteristic of all types of acute promyelocytic leukemia, can be identified by either cytochemical stain or immunoperoxidase stain. (myeloperoxidase cytochemical and immunoperoxidase stains)
The differential diagnostic considerations vary between hypergranular and microgranular APL largely due to their different cytologic appearances. A wave of granulocytic recovery after transient agranulocytosis, G-CSF therapy, or any other prominent promyelocyte population can mimic hypergranular APL. Key cytologic factors in granulocytic recovery include prominent paranuclear hofs (Golgi regions), round nuclei, and absence of Auer rods [Harris 1994, Innes 1987]. In addition, CD117 and CD11b expression may aid in distinguishing APL from a benign process [Rizzatti 2002]. As noted above, the microgranular variant of APL may be mistaken for acute monocytic leukemia (AMoL); however, the typical bilobed (sliding plate) appearance of microgranular APL is not seen in AMoL (see 18.41). Striking uniform myeloperoxidase cytochemical activity in the leukemic cells also indicates APL, while greater amounts of cytoplasm and nonspecific esterase positivity characterize monocytic leukemias (see 18.14).

Patients with de novo t(15;17) have a favorable prognosis. t(15;17) AML may also arise after previous chemotherapy or in blast phase of CML [Beaumont 2003, Chung 2008, Hasen 2008, Pulsoni 2002]. Occasionally the molecular fusion is present but is cyogenetically and/or FISH cryptic requiring PCR-based confirmation [Wang 2009a, Wang 2009b]. FLT3 mutations are detected in 30%-40% of APL cases and may be associated with shorter survival times [Callens 2005].

Acute Myeloid Leukemias with Variant RARA Translocations

Occasionally, a case will exhibit many features suggestive of APL but lack the t(15;17) translocation; alternative translocation partners for RARA on chromosome 17 have been noted in these cases 18.6. Morphologic differences from classic APL include less prominent nuclear bilitation, absence of Auer rods, and pelgeroid neutrophils. The responsiveness of these variants with ATRA varies 18.6.

AML with t(9;11)(p22;q23); MLLT3-MLL

In the WHO 2008 classification, AML with t(9;11) is the only translocation involving the MLL gene included as a distinct biologic subtype 18.7 [Arber 2008a]. Clues and confirmatory tests in AML with t(9;11) are detailed in 18.5.

In the peripheral blood and bone marrow, AML with t(9;11) manifests as a proliferation of monoblasts and/or promonocytes (blasts/blast equivalents ≥20%) i18.4. Typical APL: hypergranular, morphology, and immunophenotype

<table>
<thead>
<tr>
<th>Variant Partners</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZBTB16(11q23)</td>
<td>Morphologic differences from classic APL: blasts with regular nuclei, increased Pelger-Huët-like neutrophils; ATRA resistant</td>
</tr>
<tr>
<td>NUMA1(11q13)</td>
<td>ATRA-sensitive</td>
</tr>
<tr>
<td>NPM1(5q35)</td>
<td>Morphologic differences: no Auer rods</td>
</tr>
<tr>
<td></td>
<td>Predominantly hypergranular</td>
</tr>
<tr>
<td></td>
<td>ATRA-sensitive</td>
</tr>
<tr>
<td>STAT5B(17q11.2)</td>
<td>ATRA-resistant</td>
</tr>
<tr>
<td>PRKARIA(17q23-24)</td>
<td>Single case report</td>
</tr>
<tr>
<td></td>
<td>Cryptic fusion</td>
</tr>
<tr>
<td></td>
<td>Typical APL: hypergranular, morphology, and immunophenotype</td>
</tr>
</tbody>
</table>

AML = acute myeloid leukemia; APL = acute promyelocytic leukemia; ATRA = all trans retinoic acid


18: Acute Myeloid Leukemia
AML with t(9;11) is associated with an intermediate prognosis and reportedly fares better than AMLs with other MLL translocation partners [Balgobind 2009, Mrozek 1997]. AMLs with MLL rearrangements other than t(9;11) are diagnosed as AML, not otherwise specified, or as therapy-related leukemia with t(v;11q23) in the setting of prior cytotoxic treatment. If the cytogenetics reveal t(2;11)(p21;q23) or t(11;16)(q23;p13), these should be diagnosed as AML with myelodysplasia-related changes [Arber 2008a].

AML with Variant MLL Translocations

Although >60 fusion partner genes are known for MLL, the more common translocations in AML include 6q27 (MLLT4), 10p12 (MLLT10), 19p13.1 (ELL), and 19p13.3 (MLLT1), all resulting in fusion genes [Martineau 1998, Meyer 2009, Moorman 1998]. AML with MLL abnormalities accounts for ~3% of all AML [Schoch 2003]. In neonates, acute monoblastic leukemias frequently harbor MLL translocations, and overall survival may depend on the translocation partner [Balgobind 2009]. Given that the MLL gene is not invariably involved when abnormalities of 11q23 are detected by conventional cytogenetics and that MLL rearrangements (including those resulting from MLL partial tandem duplication) may be missed by standard cytogenetics, molecular or FISH techniques may be necessary to identify and verify an MLL abnormality [Abdou 2002, Caligiuri 1998, Mathew 1999, Watanabe 2003]. Since 11q23 is a topoisomerase II cleavage site, it has been experimentally demonstrated that topoisomerase II inhibitors (eg, etoposide) increase chromosomal recombination events involving this locus (see “Therapy-Related AML,” p 403) [Buono 2009, Chatterjee 1999, Le 2009, Libura 2005]. MLL amplification in AML is associated with poor outcome. MLL-rearranged leukemias often cause high-level expression of Homeobox genes such as HOXA9 [Faber 2009, Roth 2009].

AML with t(6;9)(p23;q34); DEK-NUP214

AML with t(6;9)(p23;q34) is rare and estimated to comprise approximately 1% of all adult and pediatric AML cases [Byrd 2002, Slovak 2000]. Detailed clues and confirmatory tests in this type of AML are shown in t18.5 [Alabekh 1997, Slovak 2006]. The CBC exhibits findings of bone marrow failure (ie, thrombocytopenia, neutropenia, anemia), circulating blasts, and often basophilia. The blasts may contain Auer rods and be cytochemically positive for myeloperoxidase and/or nonspecific esterase, thus exhibiting hybrid myeloid and monocytic features [Arber 2008a]. The bone marrow is hypercellular with ≥20% blasts. Background trilineage
dysplasia is common as is basophilia. Increased ring sideroblasts may be seen. CD34 and CD117 are generally positive on the blasts with a subset expressing TdT.

The t(6;9) tends to be the sole cytogenetically evident, although easily overlooked, abnormality, which results in the formation of a chimeric fusion gene DEK-NUP214 (NUP214 once known as CAN) [Seekam 1992, von Lindern 1992]. This resultant oncoprotein is likely to work together with other cryptic genetic aberrations to ultimately contribute to the development of AML [Deguchi 2002].

Up to 80% of t(6;9) AML cases may have an internal tandem duplication mutation of the tyrosine kinase FLT3, which may play a key role in leuкоemogenesis by impeding normal cellular proliferation [Oyarzo 2004, Parcells 2006, Thiede 2002]. Increased global protein synthesis specifically in t(6;9) myeloid cells as a result of increased translation may be a mechanism underlying leukemogenesis [Ageberg 2008].

Patients with t(6;9) AML have an overall poor prognosis (5-year survival estimate in adults is 9%) [Slovak 2006]. Higher WBC counts predict shorter overall survival, while higher bone marrow blast counts predict shorter disease-free survival [Slovak 2006]. Allogeneic stem cell transplant may improve overall survival [Slovak 2006].

**AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1**

Rarely, a case of AML will bear either the inv(3) (q21;q26.2) or the t(3;3)(q21;q26.2), both of which generate fusion of RPN1 and EVII [Martinelli 2003, Nucifora 1997]. The morphologic hallmark of these cases is the presence of conspicuously atypical megakaryocytes (usually small in size with monolobated or bilobated nuclei) in a background of multilineage dysplasia. A similar picture is present in the peripheral blood, with notably prominent platelet-associated abnormalities (including giant, hypogranular forms and circulating megakaryocytic nuclei) in a background of granulocytic dysplasia [Iottierzio Bellomo 1992]. EVII also serves as a translocation partner with genes other than RPN1, in which case the process is not considered to fall within this specific biologic subtype. AML with RPN1-EVI1 is a highly aggressive disease with a median survival of only 5.5 months, though response to alternate treatment regimens may be better. It is to be hoped that wider recognition of the entity, facilitated by its specific
inclusion in the WHO 2008 system, may foster more effective therapies.

**AML with t(1;22)(p13;q13); RBM15-MKL1**

AML with t(1;22)(p13;q13) is an exceedingly rare diagnosis (<1% of cases) that should be considered only in children <3 years of age; most commonly, presentation is in infancy. The t(1;22) involves RBM15 and MKL1. The latter gene is named for its association with megakaryoblastic leukemia and encodes a protein involved in chromatin organization, while RBM15 regulates c-myc; the fusion event may disturb both of these activities [Mercher 2001, Niu 2009]. As befits an AML involving MKL1, this is a megakaryoblastic leukemia, and these blasts may show distinct clumping on aspirate smears and are often mixed with micromegakaryocytes [Duchayne 2003]. Other clues to the possible presence of this subtype are organomegaly, the lack of significant erythroid or granulocytic dysplasia, and the presence of bone marrow fibrosis [Paredes-Aguilera 2003, Ribeiro 1993]. The fibrosis is often so extensive as to suggest a differential diagnosis of metastatic disease, an impression heightened by the relatively cohesive nature of the megakaryoblasts. Given a megakaryoblastic proliferation in an infant, it is also prudent to completely exclude a diagnosis of Down syndrome and its associated myeloid proliferations [Hama 2008]. The importance of recognizing the t(1;22) subtype as a distinct diagnostic entity is underscored by studies showing a significant survival advantage with intensive chemotherapy [Duchayne 2003].

**AML with Mutated NPM1 and CEBPA**

Several recurrent gene mutations now share the stage with the well-established translocations described above. The WHO 2008 scheme endorses mutations in 3 genes (NPM1, CEBPA, and FLT3) as especially diagnostically or prognostically significant. However, the inclusion of these gene mutations has occasioned a good deal of strenuous debate, centering on a fundamental (almost philosophical) question: at what point does a genetic aberration tied to a given biologic behavior cease to be simply a prognostic factor and instead become definitional of a specific subtype of AML? FLT3, for example, is associated with a relatively poor prognosis but is found in a wide array of otherwise well-defined AML subtypes, such as APL. For this reason, the WHO relegates FLT3 mutation status to a prognostic factor rather than a diagnostic subgroup. In contrast, NPM1 and CEBPA mutations define specific subtypes of AML, though these entities are only provisional categories. We will adhere to this scheme and discuss NPM1 and CEBPA here as biologic subtypes of AML, while reserving consideration of FLT3 for the subsequent section on prognostic factors.
AML with Mutations of NPM1

NPM1 mutations are frequent in karyotypically normal AML, occurring in between 50% and 60% of such cases [Falini 2005]. NPM1 encodes the protein nucleophosmin, which appears to function primarily as a shuttle for other proteins between the nucleus and the cytoplasm. It may also play a role in cell cycle control and in the regulation of centromere duplication to facilitate orderly mitosis [Falini 2007, Tsiou 2006, Yu 2006, Yun 2003]. NPM1 mutations are typically insertions of variable length that produce a frameshift sufficient to alter the nuclear localization sequence, leading to aberrant accumulation of the protein in the cytoplasm i18.5. Though immunohistochemical techniques may be applied to detect this abnormal cytoplasmic nucleophosmin, direct PCR amplification of the affected region with sizing by capillary electrophoresis is widely available in molecular diagnostic laboratories [Konoplev 2009, Luo 2010]. NPM1 mutations have been especially associated with AML showing monocytic differentiation and absence of CD34 expression. Although neither feature is completely sensitive or specific for predicting an NPM1 mutation, the fact that up to 90% of acute monocytic leukemias have mutated NPM1 provides a powerful diagnostic clue [Schnittger 2005]. Mutated NPM1 is associated with a good prognosis with one exceptionally important caveat: NPM1 mutations are rather frequently (ie, 40%) present in tandem with FLT3 mutations, in which case the adverse prognostic effect of the FLT3 mutation essentially negates the otherwise beneficial behavior associated with the NPM1 mutation [Baldus 2007, Schlenk 2008]. Thus, testing for NPM1 should always be performed together with FLT3 analysis.

AML with CEBPA Mutations

Mutations of the CCAAT/enhancer binding protein α (CEBPA) gene define the second provisional subtype of AML with gene mutations. CEBPA encodes a transcription factor essential for granulocytic maturation [Korschmiede 2009]. Mutations occurring in AML cluster in the N-terminal and C-terminal regions, inducing frameshift and missense mutations, respectively, that presumably contribute to leukemogenesis by interfering with the protein’s contribution to terminal differentiation. CEBPA mutations are somewhat less common than NPM1 mutations, and are present in between 15% and 20% of cytogenetically normal cases—most commonly, but not exclusively, in cases with the morphologic features of AML with or without maturation [see i18.7 “AML, Not Otherwise Specified (NOS),” p 408] [Baldus 2007]. Mutated CEBPA in isolation confers a relatively good prognosis, though recent data suggest that this effect is limited to only those cases that carry biallelic CEBPA mutations [Bierne 2005, Frohling 2004, Hu 2009, Pubot 2009, Schlenk 2008, Wouters 2009].

Diagnostic Strategies for Molecular Assessment in AML

The inclusion of NPM1 and CEBPA mutation status in the AML classification scheme presents a challenge to the diagnostician. In contrast to conventional cytogenetics and flow cytometric immunophenotyping, which are routinely performed in newly diagnosed cases of AML, molecular analysis for gene mutations represents a new frontier. When should this specialized testing be ordered? The best answer, as of this writing, is, in cytogenetically normal cases and/or when, after consultation with the clinical team, it is determined that gene mutation status will provide information useful in patient management. The prognostic significance of NPM1, CEBPA (and FLT3) mutations is clearly established in cytogenetically normal cases [Mrozek 2007]. In other situations, including AML with myelodysplasia-related changes and cytogenetically abnormal cases that do not fulfill criteria for AML with myelodysplasia-related changes, the significance of these gene mutations remains less certain. However, our understanding of gene mutations is constantly evolving, with recent data demonstrating that NPM1 mutation status may carry similar prognostic connotations even in karyotypically abnormal AML [Haefer 2009a]. While, in general, strict adherence to the WHO classification scheme is desirable, communication with clinicians on a case-by-case basis is essential to its rational application in this area. Thus, at large research institutions with multiple treatment protocols and studies, maximum effort in all cases (even those carrying cytogenetic abnormalities or myelodysplasia-related changes) to identify these and other mutations might indeed be indicated. In contrast, such efforts might be excessive in an elderly or infirm patient ineligible for aggressive treatment options. It is likely that diagnostic strategies employing these gene mutations will continue to evolve as further studies more clearly define their impact in the full spectrum of morphologic subtypes of AML.

18.62 Clinical Biologic Subtypes of AML

AML with Myelodysplasia-Related Changes

AML with myelodysplasia-related changes is a broad category that may be invoked by clinical setting, genetic abnormality, or morphologic findings [Arber 2008b]. Specifically, these cases of AML satisfy one or more of the following criteria:

1. the patient has a history of previously diagnosed MDS or MDS/MPN overlap syndrome
2. cytogenetic abnormalities associated with MDS are present i18.7, i18.9
3. there is multilineage dysplasia, defined for this purpose as dysplasia involving at least half of the cells in 2 separate bone marrow lineages i18.51, i18.52, i18.53, i18.54
**t18.7 Cytogenetic Abnormalities Sufficient to Diagnose AML with Myelodysplasia-Related Changes***

Complex karyotype (≥3 unrelated abnormalities, none of which is included in the AML with recurrent cytogenetic abnormalities subgroup); such cases should be classified in the appropriate cytogenetic group:

-7/del(7q)
-5/del(5q)
i(17q)/t(17p)
-13/del(13q)
del(11q)
del(12p)/t(12p)
del(9q)
idic(X)(q13)
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;p21)
t(3;5)(q25;q34)

*Patients with a diagnosis of AML with myelodysplasia-related changes should not have a history of prior cytotoxic therapy; in such a case, the “therapy-related myeloid neoplasm” category takes precedence over any MDS-related changes, and the disease should be classified as such; t(11;16)(q23;p13.3) and t(3;21)(q26.2;q22.1) occur in therapy-related cases with particular frequency.

Reference: [Arber 2008a]

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**i18.9 This karyogram shows a complex karyotype including monosomy of many chromosomes as well as multiple marker chromosomes. The abnormal chromosomes are on the right. (Giemsa trypsin Wright)**

**i18.51 This peripheral blood smear shows profound pancytopenia, a circulating blast, and dysplastic neutrophils. (Wright)**

**i18.52 This bone marrow aspirate smear in acute myeloid leukemia with myelodysplasia-related changes shows highly abnormal multinucleated erythroid precursors, increased myeloblasts, and dysplastic maturing granulocytic cells. (Wright)**

**i18.53 This bone marrow core biopsy in acute myeloid leukemia with myelodysplasia-related changes shows highly atypical multinucleated erythroid cells in the approximate size range of megakaryocytes. Note increased blasts and dysplastic megakaryocytes. (H&E)**
Morphologic and immunohistochemical assessment of bone marrow core biopsies can be used to enumerate immature cells and assess for multilineage dysplasia **i18.55, i18.56, i18.57, i18.58** [Ngo 2008].

In addition, these cases by definition lack the clinical history (eg, prior cytotoxic therapy) and genetic abnormalities [eg, t(15;17)] that would identify them as belonging to other specific biologic subtypes of AML. However, there is a minor exception to this rule: some cases of AML with myelodysplasia-related changes will also carry mutated NPM1 or CEBPA, which, as described above, could theoretically place them into the provisional categories reserved for cases with such mutations. Because the prognostic significance of NPM1 and CEBPA mutations is unclear in the setting of myelodysplasia-related changes, the WHO 2008 classification recommends that these rare cases of AML with myelodysplasia-related changes be classified as such and not as AML with mutated NPM1 or CEBPA.
cases be assigned to the MDS-related category, with a note appended to the diagnostic line identifying the coexisting mutation. Cases of AML with myelodysplasia-related changes have a generally poor prognosis, though this may be primarily attributable to the frequent presence of high-risk cytogenetic abnormalities rather than history or morphology per se, as suggested by recent authors [Wandt 2008]. However, in comparison to AML, NOS, cases of AML with MDS-related changes have a significantly worse overall survival [Weinberg 2009].

**Therapy-Related AML**

One clinical setting that automatically triggers a specific subclassification of AML is a history of prior cytotoxic chemotherapy and/or radiation treatment [Vardiman 2008a]. Agents that have been implicated are listed in [18.1](#). There are 2 broad categories of therapy-related myeloid neoplasms: those that follow treatment with alkylating agents and those that follow agents directed against the enzyme topoisomerase II. Although in both cases, AML arises following direct DNA damage induced by the therapeutic agent, the genetic and clinical features of the resulting processes are quite different, as summarized in [18.8](#). Because chemotherapeutic regimens often include both alkylating agents and topoisomerase II inhibitors, the WHO 2008 recommendation is to group all therapy-related myeloid neoplasms (t-AMLs) together rather than assigning them to specific categories based on the specific therapeutic agent used. This is in contrast to the 2008 WHO classification, which distinguished between alkylating agent-related and topoisomerase II inhibitor-related AMLs.

### Table: Comparison of Clinicopathologic Features of 2 Types of Therapy-Related Myeloid Neoplasms

<table>
<thead>
<tr>
<th>Feature</th>
<th>Secondary to Alkylating Agent Therapy</th>
<th>Secondary to Topoisomerase II Inhibitor Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy</td>
<td>Alkylating agents or radiotherapy induce permanent genetic abnormalities</td>
<td>Epipodophyllotoxins and related agents that inhibit DNA-topoisomerase II</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>Linked to polymorphisms in genes involved in detoxification or major DNA repair pathways</td>
<td>11q23 is cleavage site for topoisomerase II; double-stranded DNA cleavage; repair blocked by inhibitor</td>
</tr>
<tr>
<td>Latent period</td>
<td>2-11 years; shorter for patients with higher cumulative alkylating agent dose</td>
<td>Brief, &lt;1-3 years</td>
</tr>
<tr>
<td>Blood</td>
<td>Myelodysplastic phase characterized by severe cytopenias, trilineage dysplasia, and basophilia</td>
<td>Abrupt onset of cytopenias in most cases; some cases have short cytopenic “prodrome” phase</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Usually hypercellular, but may be hypocellular</td>
<td>Hypercellular</td>
</tr>
<tr>
<td>Cytogenetics/molecular</td>
<td>–5/del(5q), –7/del(7q) (multistep leukemogenesis)</td>
<td>Balanced 11q23 translocations in most cases</td>
</tr>
<tr>
<td></td>
<td>Complex karyotypic abnormalities frequent</td>
<td>Balanced 21q22 translocation, t(15;17), and inv(16) in occasional cases</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>Poor response to conventional antileukemic therapy</td>
<td>Good achievement of complete remission; relapse frequent especially in 11q23-associated cases</td>
</tr>
<tr>
<td>Survival</td>
<td>Poor</td>
<td>Variable; poor in 11q23 group</td>
</tr>
</tbody>
</table>

than specifying subtypes [Vardiman 2008a]. Furthermore, therapy-related cases with the morphologic and clinical features of myelodysplastic syndrome or MDS/MPN overlap syndromes share the poor prognosis of therapy-related cases with features of acute leukemia. For this reason, all such processes are grouped together as “therapy-related myeloid neoplasms,” regardless of blast count [Czader 2009, Vardiman 2008a]. Multilineage dysplasia is frequently noted in cases of t-AML [i18.59, i18.60, i18.61, i18.62, i18.63, i18.64, i18.65, i18.66, i18.67, i18.68, i18.69, i18.70]. Fibrosis is common in t-myeloid neoplasms [i18.71, i18.72] [Czader 2009, Vardiman 2008a].
This bone marrow aspirate smear composite shows prominent monoblastic features in this case of therapy-related acute myeloid leukemia linked to topoisomerase II inhibitor therapy. (Wright)

This bone marrow core biopsy section shows side-by-side therapy-related acute myeloid leukemia with residual chronic lymphocytic leukemia. (H&E)

Composite of therapy related-acute myeloid leukemia shows increased myeloblasts, disrupted erythroid colonies, abnormal localization of immature granulocytic cells, and increased and dysplastic megakaryocytes. (immunoperoxidase for CD34, hemoglobin A, myeloperoxidase, and CD42b)

Residual myeloma is readily apparent by immunohistochemical staining for CD138, with persistence of highly atypical plasma cells on bone marrow aspirate smear (inset). Areas not effaced by residual myeloma were effaced by therapy-related acute myeloid leukemia. (immunoperoxidase for CD138 and Wright)
Down Syndrome-Associated AML and Transient Abnormal Myelopoiesis

Perhaps the paradigmatic example of AML that is defined by the clinical setting in which it arises is myeloid leukemia associated with Down syndrome, which is very often the sequela to an unusual phenomenon, specific to neonates with Down syndrome, called “transient abnormal myelopoiesis” (TAM, aka transient myeloproliferative disorder) [Baumann 2008]. Immunophenotypically, morphologically, and clinically, TAM is essentially indistinguishable from acute myeloid leukemia, but it shows the unique and mysterious property of spontaneous regression [i18.73, i18.74, i18.75, i18.76]. The incidence of TAM is quite high in Down syndrome, occurring in at least 1 in 10 neonates. The true incidence could be even higher, since differential counts on the CBCs of apparently healthy newborns with Down syndrome may not be routinely obtained. In addition, TAM can occur in utero, in which case it may cause fetal hydrops and spontaneous abortion. If series of both in utero and neonatal cases are combined, it is estimated that perhaps 20% of conceptions with trisomy 21 that reach at least late-term gestation experience TAM [Zipursky 2003]. The natural history of TAM is spontaneous hematologic remission, though approximately 20% of patients succumb before this occurs to associated morbidities, including cardiopulmonary failure in the setting of ascites, and fatal progressive hepatic fibrosis secondary to the leukemic infiltration and cytokine secretion [Massey 2006, Zipursky 2003]. Some 20% of patients with TAM later develop a non-remitting myeloid leukemia associated with Down syndrome (MLADS), which is usually megakaryoblastic in nature and occurs before the age of 4 [i18.77, i18.78, i18.79].
Both megakaryoblasts and differentiating megakaryocytes are evident in this peripheral blood smear from a Down syndrome neonate with transient abnormal myelopoiesis. Note large megakaryocyte fragments. (Wright)

This bone marrow core biopsy is from a 4-week-old Down syndrome baby with transient abnormal myelopoiesis. Note increased immature cells and markedly increased small abnormal megakaryocytes. (H&E)

This bone marrow core biopsy section is from a 4-week-old Down syndrome infant with transient abnormal myelopoiesis. At high magnification, a marked increase in immature blastic cells is evident in conjunction with abnormal megakaryocytes. (H&E)

This peripheral blood smear is from a 22-month-old Down syndrome baby with acute megakaryoblastic leukemia. Note profound pancytopenia, circulating megakaryoblasts, and dysplastic platelet. (Wright)

This bone marrow aspirate smear shows Down syndrome-associated acute megakaryoblastic leukemia. (Wright)

This composite of Down syndrome-associated acute megakaryoblastic leukemia shows a comparison of the morphologic features in conjunction with the markedly increased megakaryocytic component highlighted by CD31. (H&E and immunoperoxidase for CD31)
AML, Not Otherwise Specified (NOS)

Despite attempts to utilize a biologic-based classification of AML to the greatest extent possible, the 2008 WHO classification has included 11 subtypes of AML, not otherwise specified (NOS) [Arber 2008c, Vardiman 2009]. A lineage-based system is used to subclassify those cases of AML that lack any specific AML-defining biologic characteristic. Consequently, the AML, NOS category is reserved for cases that fulfill general criteria for AML but lack: a) an AML recurrent cytogenetic or molecular abnormality, b) a link to prior chemotherapy, c) multilineage dysplasia involving the majority of cells, d) MDS-type cytogenetic abnormalities, e) an association with Down syndrome, or f) a history of MDS or MDS/MPN.

### 18.9 Acute Myeloid Leukemia, Not Otherwise Specified (AML, NOS)

<table>
<thead>
<tr>
<th>Type</th>
<th>Typical Features</th>
<th>Other/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with minimal differentiation</td>
<td>Predominance of agranular blasts</td>
<td>Myeloid lineage confirmed by immunophenotyping; aberrant features common</td>
</tr>
<tr>
<td></td>
<td>Lineage of blasts <em>not</em> apparent by morphology or cytochemistry</td>
<td>Cytogenetic abnormalities common; if MDS-related cytogenetic abnormality,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>classify case as AML with MDS-related change</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No recurrent cytogenetic abnormalities; poor prognosis</td>
</tr>
<tr>
<td>AML without maturation</td>
<td>Blasts ≥90%; blasts exhibit myeloid features by morphology (granules, Auer rods) and/or cytochemistry (MPO+)</td>
<td>Myeloid lineage confirmed by flow cytometric immunophenotyping, aberrant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>features common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No recurrent cytogenetic abnormalities</td>
</tr>
<tr>
<td>AML with maturation</td>
<td>≥20% blasts in blood or bone marrow</td>
<td>Myeloid antigens expressed; occasional aberrant features</td>
</tr>
<tr>
<td></td>
<td>≥10% maturing granulocytic lineage cells</td>
<td>Key differential diagnostic considerations are RAEB-2 and AMML</td>
</tr>
<tr>
<td></td>
<td>&lt;20% monocytes</td>
<td>No recurrent cytogenetic abnormalities</td>
</tr>
<tr>
<td>AMML</td>
<td>Blasts and blast equivalents (promonocytes) ≥20% in blood or bone marrow</td>
<td>Myelomonocytic lineage confirmed by flow cytometry; aberrant features common</td>
</tr>
<tr>
<td></td>
<td>≥20% maturing granulocytic lineage cells</td>
<td>No recurrent cytogenetic abnormalities</td>
</tr>
<tr>
<td></td>
<td>≥20% monocytes</td>
<td>Monocytosis common</td>
</tr>
<tr>
<td></td>
<td>Cytochemically stains MPO+ and NSE+</td>
<td>Blood picture may closely resemble CMML</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Key differential diagnoses include CMML, AMoL and AML with maturation, and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>microgranular APL</td>
</tr>
<tr>
<td>AMML t(8;16) (p11;p13)</td>
<td>Fulfills AMML criteria</td>
<td>Disruption and fusion of MYST3 (8p11) and CREBBP (16p13)</td>
</tr>
<tr>
<td></td>
<td>Ingestion of erythrocytes by myelomonocytic blasts</td>
<td>Rare AML subtype, &lt;0.5% de novo AML (see text) and &lt;2% t-AML</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DIC, extramedullary disease including CNS infiltration common</td>
</tr>
<tr>
<td>Acute monocytic leukemia, 2 types</td>
<td>≥80% monocyte lineage cells ≤20% neutrophilic lineage cells</td>
<td>Monocytic lineage confirmed by immunophenotyping; aberrant features common</td>
</tr>
<tr>
<td></td>
<td>If monoblasts predominate, termed <em>acute monocytic leukemia</em>; if promonocytes predominate, termed <em>acute monocytic leukemia</em></td>
<td>Extramedullary lesions common (CNS, skin, gingiva)</td>
</tr>
<tr>
<td></td>
<td>NSE+ in majority of cells</td>
<td>Key differential diagnosis includes CMML, microgranular APL, AMML</td>
</tr>
<tr>
<td>Acute erythroid leukemia, 2 types</td>
<td>Erythroleukemia consists of ≥50 erythroid cells and ≥20% myeloblasts (percent based on non-erythroid cells)</td>
<td>Dysplasia often prominent; if ≥50% in 2 lineages, designation of AML with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDS-related changes should be used; if MDS-related cytogenetic features</td>
</tr>
<tr>
<td></td>
<td></td>
<td>present, should be called AML with MDS-related changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Must also distinguish from RAEB and non-neoplastic disorders</td>
</tr>
<tr>
<td>Acute megakaryoblastic leukemia</td>
<td>≥20% blasts in blood or bone marrow</td>
<td>Maturation of megakaryocytic lineage may be present</td>
</tr>
<tr>
<td></td>
<td>&gt;60% of blasts are megakaryoblasts</td>
<td>Megakaryocytic lineage confirmed by immunophenotyping (CD61+, CD41+,</td>
</tr>
<tr>
<td></td>
<td>Clumps (pseudometastasis)</td>
<td>CD42b+); may also be CD36+</td>
</tr>
<tr>
<td></td>
<td>Variable cytological features</td>
<td>Down syndrome associated cases excluded</td>
</tr>
<tr>
<td></td>
<td>Pure megakaryoblastic vs multilineage</td>
<td>Differential diagnosis includes Burkitt leukemia, megaloblastic anemia, and</td>
</tr>
<tr>
<td></td>
<td>Fibrosis common</td>
<td>other acute leukemias</td>
</tr>
<tr>
<td></td>
<td>Micromegakaryocytes not included in blast percentage</td>
<td>Differential diagnoses include APMF, metastastic lesions, MPN</td>
</tr>
<tr>
<td>Acute basophilic leukemia</td>
<td>AML with primary basophilic differentiation</td>
<td>Immunophenotyping to confirm basophilic differentiation (CD123+, CD203c+, CD9+)</td>
</tr>
<tr>
<td></td>
<td>Coarse basophilic granules (toluidine blue, Alikan blue positive)</td>
<td>Must exclude BCR-ABL1 cases and t(6;9) cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Key differential diagnosis is mast cell leukemia</td>
</tr>
<tr>
<td>Acute panmyelosis with myelofibrosis</td>
<td>≥20% myeloid blasts</td>
<td>Immunohistochemical staining to confirm increased myeloblasts and other</td>
</tr>
<tr>
<td></td>
<td>Proliferation of all hematopoietic lineages</td>
<td>lineage involvement</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td>If MDS-related cytogenetic features detected, should be classified as AML</td>
</tr>
<tr>
<td></td>
<td>Absent or minimal splenomegaly</td>
<td>for MDS-related changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differential diagnoses include acute megakaryoblastic leukemia, primary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>myelofibrosis, post polycythemic PV, metastastic lesions</td>
</tr>
</tbody>
</table>

*These AML cases lack any distinctive clinical (ie, not therapy-related), morphologic (ie, multilineage dysplasia), or molecular/genetic features to allow inclusion in biologic categories

AEL = acute erythroblastic leukemia; AML = acute myeloid leukemia; AMML = acute myelomonocytic leukemia; AMoL = acute monocytic leukemia; APL = acute promyelocytic leukemia; APMF = acute panmyelosis with myelofibrosis; CMML = chronic myelomonocytic leukemia; CNS = central nervous system; DIC = disseminated intravascular coagulation; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasm; MPO = myeloperoxidase; NSE = nonspecific esterase; RAEB = refractory anemia with excess blasts

AML with Minimal Differentiation

Agranular, lineage-indeterminate blasts predominate in this AML, NOS subtype, and flow cytometric immunophenotyping is required for delineation of myeloid lineage [Arber 2008c, Barbaric 2007, Bene 2001, Kaleem 2001]. There are no recurrent cytogenetic abnormalities, and prognosis is poor.

AML without Maturation

Myeloid lineage blasts, based on morphologic and cytochemical properties, predominate in this AML, NOS subtype [Arber 2008c, Barbaric 2007, Bene 2001, Kaleem 2001]. The blast percentage exceeds 90%, and there is no significant maturation of these leukemia cells.

AML with Maturation

The designation “with maturation” is applied to AML cases (≥20% blast threshold met) in which at least 10% of cells are promyelocytes, myelocytes, metamyelocytes, or neutrophils [Arber 2008c, Barbaric 2007, Bene 2001, Kaleem 2001]. However, a significant monocytic component is not present. Although dysplasia is common in AML with maturation, both the extent of dysplasia and the cytogenetic findings cannot fulfill AML with MDS-related changes criteria (see “AML with Myelodysplasia-Related Changes,” p 400).

Acute Myelomonocytic Leukemia (AMML)

Blasts and promonocytes must be at least 20%, and substantial granulocytic and monocytic lineage involvement must be present to justify a diagnosis of acute myelomonocytic leukemia (AMML) [Arber 2008c, Barbaric 2007, Bene 2001, Kaleem 2001]. Both
cytochemical staining and flow cytometric immunophenotyping are useful in confirming the dual lineage involvement in AMML. Careful attention to promonocyte enumeration is key to distinguishing AMML from CMML. Similarly, both strong uniform myeloperoxidase positivity and confirmation of PML-RARA fusion are critical in distinguishing AMML from microgranular APL.

**AMML with t(8;16)(p11;p13); MYST3-CREBBP**

A very rare subtype of AMML has distinctive genetic and morphologic features. Cases of AML with t(8;16)(p11;p13) show prominent erythrophagocytosis by blasts, prominent cytoplasmic granulation, and frequent extramedullary disease. Cytochemical nonspecific esterase and myeloperoxidase positivity are present, supporting a granulocytic and monocytic lineage despite the...
A CUTE MYELOID LEUKEMIA

The incidence of t(8;16) is low (0.2%-0.4% of de novo AML) [Haferlach 2009b, Mitelman 1992]. These patients have a poor prognosis [Becher 1988, Bernasconi 2000, Haferlach 2009b, Heim 1987, Velloso 1996].

Acute Monocytic Leukemias

Immature monocytic cells predominate in this AML, NOS type; 2 subtypes are designated based on whether monoblasts predominate (ie, acute monoblastic leukemia) or minimally more differentiated blast equivalent promonocytes predominate (ie, acute monocytic leukemia) t18.9, i18.91, i18.92, i18.93, i18.94. Very rare acute monocytic leukemias can exhibit histiocytic differentiation [Boeckx 2007, Esteve 1995]. Both cytochemical nonspecific esterase staining and flow cytometric immunophenotyping can be used to document monocytic differentiation, but nonspecific

Ingestion of multiple red blood cells by a leukemic blast is evident on the cytospin smear from a patient with acute myelomonocytic leukemia and t(8;16).

This peripheral blood smear illustrates the typical cytologic features of acute monoblastic leukemia. Note that virtually all nuclei are oval to round in shape and there is little evidence of monocytic maturation. (Wright)

This composite illustrates the cytologic and cytochemical features of acute monoblastic leukemia. Note strong nonspecific esterase positivity. (Wright and nonspecific esterase cytochemical stain)

Acute monocytic leukemia with maturation including atypical monocytes is evident in this peripheral blood smear from a patient with marked leukocytosis and profound anemia and thrombocytopenia. (Wright) (courtesy T Keith, MD)

This composite illustrates the features of acute monocytic leukemia on bone marrow core biopsy sections. Note widely spaced nuclei due to abundant amounts of cytoplasm, homogeneous uniform cell infiltrates, and increased tingible body macrophages. (H&E)
Esterase stains may be negative or weak in up to 20% of cases [Arber 2008c, Dunphy 2004]. Extramedullary disease is common in acute monoblastic/monocytic leukemias. Key differential diagnostic considerations include CMML, microgranular APL, and AMML.

Acute Erythroid Leukemia

The acute erythroid leukemias are further divided into 2 subtypes. In cases designated as “erythroleukemia,” both a myeloblast component (≥20% of non-erythroid cells) and an erythroid component (≥50% of all cells) are present, while the term “pure erythroid leukemia” is reserved for cases with ≥80% erythroblasts with little, if any, granulocytic lineage involvement [Arber 2008c]. Dysplasia is common in erythroleukemia, but it must be ≤50% of the lineage cells. If ≥50% of...
A CUTE MYELOID LEUKEMIA

18.100 This composite of immunohistochemical stains from a case of acute erythroid leukemia shows increased myeloblasts by CD34 staining a and numerous erythroblasts by CD117 staining b. (immunoperoxidase for CD34 and CD117)

18.101 This bone marrow aspirate smear in acute pure erythroid leukemia shows an overwhelming predominance of erythroblasts. (Wright)

18.102 Both abnormal erythroblasts, marked anisopikilocytosis, and dysplastic neutrophils are evident in the peripheral blood in a patient with acute erythroid leukemia. (Wright)

18.103 Markedly increased erythroblasts with dispersed chromatin and oblong nuclei efface this bone marrow core biopsy section in acute pure erythroid leukemia. (H&E)

18.104 A marked predominance of uniform megakaryoblasts with striking cytoplasmic blebbing is evident on this bone marrow aspirate smear from a 15-month-old male with acute megakaryoblastic leukemia not related to Down syndrome. (Wright)

cells in at least 2 lineages are dysplastic, a diagnosis of AML with MDS-related changes is warranted. The diagnosis of pure erythroid leukemia is challenging because erythroblasts can closely resemble Burkitt leukemia, acute megakaryoblastic leukemia, and other neoplasms. Immunophenotyping is essential in lineage delineation i18.101.

[18.7.7] Acute Megakaryoblastic Leukemia

The diagnosis of acute megakaryoblastic leukemia can be uniquely challenging because of the frequent association with fibrosis, which can preclude aspiration for morphology and flow cytometric immunophenotyping i18.9. Consequently, immunohistochemical stains are often required to help confirm both the minimal 20% blast percentage necessary for a diagnosis of AML and to confirm that at least 50% of the blasts are megakaryo-cytic i18.104, i18.105, i18.106, i18.107, i18.108, i18.109, i18.110, i18.111.
18.105 This bone marrow clot section is effaced by megakaryoblasts in this 15-month-old male with acute megakaryoblastic leukemia not related to Down syndrome. (H&E)

18.106 This circulating megakaryoblast is nondescript and megakaryoblastic lineage is not apparent. (Wright)

18.107 Markedly increased immature megakaryocytic lineage cells are evident in this bone marrow core biopsy section from an adult with acute megakaryoblastic leukemia. (H&E)

18.108 Striking bone marrow fibrosis is evident in this bone marrow core biopsy section of acute megakaryoblastic leukemia. (reticulin)

18.109 Immunoperoxidase staining for CD61 highlights tremendous numbers of megakaryocytic cells on this bone marrow core biopsy section of acute megakaryoblastic leukemia. (immunoperoxidase for CD61)

18.110 Admixed immature granulocytic cells are highlighted by myeloperoxidase staining in this bone marrow core biopsy section in acute megakaryoblastic leukemia (see 18.109). (myeloperoxidase cytochemical stain)
Both circulating megakaryoblasts and more differentiated megakaryocytic lineage cells are evident in this peripheral blood smear from a patient with a high-grade megakaryocytic neoplasm. Note tremendously enlarged and agranular circulating megakaryocyte fragments. (Wright)

As emphasized, early differentiating megakaryocytic lineage cells and dysplastic megakaryocytes such as micromegakaryocytes are not included in the blast percentage. Similarly, cases of acute megakaryoblastic leukemia linked to biologic genetic features such as Down syndrome or t(1;22) are not included in this AML, NOS category.

Acute megakaryoblastic leukemia is more common in children than adults. Pediatric cases of this leukemia show a similar favorable outcome regardless of whether or not they are linked to Down syndrome. Outcome in adults is unfavorable.

The differential diagnosis of acute megakaryoblastic leukemia includes other acute leukemias as well as diverse disorders associated with bone marrow fibrosis, including chronic myeloproliferative neoplasms and metastatic lesions.

Acute Basophilic Leukemia

De novo acute leukemias that exhibit basophilic differentiation are very uncommon and must be distinguished from BCR-ABL1-related disorders such as basophilic blast phase of chronic myelogenous leukemia. Genetic testing is essential in making this distinction as well as in identifying cases of AML with t(6;9), which are excluded from this AML, NOS group. Basophilic differentiation is best determined by either metachromatic staining (toluidine blue and alcin blue positivity) or by flow cytometric immunophenotyping to exclude mast cell leukemia and other myeloid lineage leukemias, as well as to provide support for basophilic lineage differentiation (CD123+, CD203c+, CD9+) [Arber 2008c]. Electron microscopy can also be used to identify theta granules [Peterson 1991, Siviezel 2003].

Acute Panmyelosis with Myelofibrosis

The diagnosis of this rare subtype of AML, NOS, is problematic both because of the obligatory myelofibrosis and because diagnostic requirements include confirmation that blasts exceed 20% and documentation of expanded myeloid, erythroid, and megakaryocytic populations in a bone marrow that is typically in aspirable. There is a heavy reliance on immunohistochemical staining to establish this diagnosis.

The potential overlap with myeloproliferative neoplasms is substantial. Therefore, cases of acute panmyelosis with myelofibrosis (APMF) must exhibit an acute onset, lack significant splenomegaly, and lack teardrop erythrocytes. Confirmation of an AML-defining blast threshold by immunohistochemical staining for CD34 is critical in distinguishing APMF from myelodysplasia with fibrosis.
A CUTE MYELOID LEUKEMIA (see t16.12). Estimation of the extent of lineage dysplasia is important to distinguish APMF from AML with MDS-related changes. Finally, a metastatic lesion must be excluded by morphologic and immunohistochemical assessment.

Prognostic Factors in AML

Cytogenetics

Pretreatment conventional cytogenetic studies identify an acquired clonal abnormality in approximately 50%-60% of patients with de novo AML, of which 10%-20% are complex (>3 chromosomal aberrations). In approximately 40%-50% of cases, no karyotypic abnormality is detected using typical banding techniques, yielding an AML with normal karyotype [Byrd 2002, Cheng 2009, Grimwade 1999, 2001, Mrozek 2004, Slovak 2000]. Molecular (submicroscopic) characterization of the karyotypically normal AMLs is an area of active investigation (see [18.8.2] “Molecular Genetics”).

Based on several large studies, a cytogenetic risk stratification system has been proposed for AML that categorizes specific karyotypic abnormalities as favorable, intermediate, or unfavorable t18.10. The majority of large studies agree that the AML patients with t(15;17), inv(16)/t(16;16), or t(8;21) have a favorable prognosis compared with those that have a complex karyotype and monosomy 7 [Mrozek 2008a]. Of note, while additional chromosomal changes with t(8;21), inv(16)/t(16;16), or t(15;17) may be seen, these have not been generally shown to affect prognosis [Byrd 2002, Kelly 2009, Schlenk 2004]. FLT3 mutations may occur in t(8;21) and inv(16), and although not well established, may suggest an adverse prognosis [Boissel 2006]. A monosomal karyotype in AML may portend a particularly unfavorable prognosis [Breems 2008, Weinberg 2010].

In patients ≥60 years with de novo AML, the significance of some of cytogenetic risk groups may differ from younger patients. A very complex karyotype, defined as ≥5 chromosomal abnormalities, is associated with an unfavorable risk in the older age group, are non-complex karyotypes showing a rare aberration such as trisomy 4, abnormalities of 3q, t(6;9)(p23;q34), and double minutes [Farag 2006, Grimwade 2001].

Prognostic Risk of Cytogenetic Abnormalities in Patients <60 Years with De Novo AML

<table>
<thead>
<tr>
<th>Prognostic Risk Group</th>
<th>Cytogenetic Finding</th>
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<tbody>
<tr>
<td>Favorable</td>
<td>t(15;17)(q22;q21)</td>
</tr>
<tr>
<td></td>
<td>t(8;21)(q22;q22)</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13;q22)/t(16;16)(p13;q22)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal karyotype</td>
</tr>
<tr>
<td></td>
<td>t(9;11)(p22;q23)</td>
</tr>
<tr>
<td></td>
<td>del(7q), del(9q)*</td>
</tr>
<tr>
<td></td>
<td>–Y, +11, +13, +21</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>Complex karyotype†</td>
</tr>
<tr>
<td></td>
<td>inv(3)(q21;q26)/t(3;3)</td>
</tr>
<tr>
<td></td>
<td>t(6;9)(p23;q34)†</td>
</tr>
<tr>
<td></td>
<td>–5, –7</td>
</tr>
</tbody>
</table>

*Some may classify as unfavorable
†≥3 chromosomal abnormalities
‡In intermediate risk group in [Byrd 2002]

AML = acute myeloid leukemia

Molecular Genetics

In addition to the gene mutations (NPM1 and CEBPA) that currently define provisional biologic subtypes of AML (see [18.6] “Biologic Subtypes of AML,” p 385), there is an ever-expanding catalogue of additional genetic alterations that occur in significant numbers in AML of various subtypes. These include mutations (e.g., TET2, MLL, KRAS, NRAS, WT1) and alterations in gene expression levels (e.g., BAALC, ERG, MNI, EVII, MNI, PRAME, MLL, WT1, RHAMM) [Abdel-Wahab 2009, Baldus 2007, Gadzik 2008, Paschka 2008b, Santamaria 2009, Schlenk 2008]. As mentioned in [18.6] “Biologic Subtypes of AML,” KIT mutations are particularly associated with a relatively adverse prognosis in the core binding factor AMLs [t(8;21) and inv(16)/t(16;16)] [Caironi 2006, Care 2003, Paschka 2006]. In general, these alterations contribute to leukemogenesis and carry prognostic significance, but, in contrast to NPM1 and CEBPA mutations, they do not presently define distinct biologic or clinical entities. Scoring systems incorporating many of these alterations have been developed and appear to be useful in predicting prognosis [Santamaria 2009]. A major challenge in the coming years will be the rational and cost-effective implementation of such “multivariate” molecular assays.

Standing out from this “alphabet soup” is FLT3, which encodes a membrane-bound receptor tyrosine kinase. Activating FLT3 mutations occur in 2 forms, only 1 of which is at present incontrovertibly significant for AML prognosis. So-called internal tandem duplications (ITD) affecting the juxtamembrane portion of the protein correlate with poor prognosis, an association that has been particularly documented in karyotypically normal cases [Kottaridis 2001, Schlenk 2008, Stirewalt 2003, Zwaan 2003]. In contrast, the significance of mutations affecting the tyrosine kinase domain (TKD) of the protein is controversial, with recent data suggesting that FLT3-TKD mutations may impact prognosis, though perhaps differently, in distinct subtypes of AML [Bacher 2008, Frohling 2002, Mead 2007, Whitman 2008, Yanada 2005]. Clinical trials are currently evaluating the pharmacologic effectiveness of specific FLT3 inhibitors [Sanz 2009a].

As of this writing, a few general observations may be made about the clinical situations in which FLT3 analysis is indicated. First, FLT3 must be examined in all cases submitted for NPM1 mutation analysis, since an FLT3 mutation in an NPM1-mutated case drastically reduces the beneficial prognosis otherwise associated with the latter finding. Second, because FLT3-ITD status is among the strongest independent prognostic factors in cytogenetically normal AML, molecular analysis for alterations at this location is eminently defensible. Third, given the burgeoning interest in targeted anti-FLT3 therapy, the practicing pathologist may find him- or herself fielding requests for FLT3 mutation analysis for a wide array of AML subtypes. In such a situation, the diagnostician should remain cognizant of the fact that the exact prognostic significance of FLT3 mutations may be variable or undocumented in the context of these distinct AML subtypes.

Flow Cytometry

Aside from the fact that certain immunophenotypic profiles tend to “track” with specific AML categories, certain aberrant immunophenotypic findings have prognostic significance. These distinctive immunophenotypic features are also useful in the assessment for minimal residual disease. Potential predictive markers in AML diagnosis are listed in [18.11] [Al-Mawali 2009b, Casasnovas 1998, Del Poeta 1995, Lacombe 2009, Mason 2006, Smith 1992, Terwijn 2009].

The prognostic value of immunophenotyping in AML is controversial [Bradstock 1993, Rull 1991]. Many of the studies have been small, single-institution studies, with few results being confirmed in large clinical trials. The different results likely stem from a variety of technical, analytic, and population-based factors. In particular, how the blast population is gated and the cut-off point (or percentage) for selection of antigen positivity is problematic.

Other Prognostic Factors

Additional prognostic factors delineated in various AML outcome studies in adults include age, WBC, de novo vs secondary AML, performance status, and rapidity in the

<table>
<thead>
<tr>
<th>Marker</th>
<th>Prognostic Significance</th>
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<tbody>
<tr>
<td>CD25</td>
<td>Adverse overall and relapse-free survival</td>
</tr>
<tr>
<td>CD15</td>
<td>Favorable prognosis</td>
</tr>
<tr>
<td>CD7</td>
<td>Controversial; lower complete remission rate; adverse prognosis in several studies</td>
</tr>
<tr>
<td>CD11b</td>
<td>Adverse prognosis</td>
</tr>
<tr>
<td>CD56</td>
<td>Lower complete remission rate</td>
</tr>
<tr>
<td>Expression of lymphoid antigens</td>
<td>Lower complete remission rate (adults)</td>
</tr>
<tr>
<td>Leukemia-associated phenotype (LAP) (aberrant/asyncronous antigen profile patterns)</td>
<td>No prognostic significance (majority of pediatric patients)</td>
</tr>
<tr>
<td>Early clearance of peripheral blasts</td>
<td>Lower complete remission rate; adverse prognosis</td>
</tr>
</tbody>
</table>

clearance of blasts from either blood or bone marrow [Derolf 2009, Dohner 2010, Hussein 2008, Lacombe 2009, Wheatley 2009]. Although overall survival in adults with AML has improved over time, the survival time for advanced elderly patients (≥80 years) with AML has not improved [Derolf 2009]. Improved survival times for children with AML have also been noted, although factors predictive of inferior survival include age >16 years, non-white ethnicity, absence of a related donor, WBC ≥100 × 10⁹/L, and adverse karyotype [Lange 2008].

### Diagnostic Pitfalls in AML Diagnosis

#### Low Blast Count AML

AMLs with t(8;21), inv(16)/t(16;16), or t(15;17) as described earlier and in t18.5 may be diagnosed when the blast count is <20% i18.15, i18.28. Clues to identifying these low blast count AML cases include severe peripheral cytopenias with variable numbers of blasts, Auer rods, abnormal salmon-colored granules and/or abnormal eosinophil precursors, or distinctive flow cytometric studies. All of these features are associated with the more overt AMLs with these same cytogenetic abnormalities t18.5. Cytogenetic studies are essential in confirming the AML-defining translocations and excluding other differential diagnostic considerations.

#### Hypocellular AML

Rare cases of AML present in a markedly hypocellular bone marrow i18.116, i18.117. Causes of this uniquely reduced cellularity are unknown. The key challenge in these cases is to document that blasts exceed the 20% threshold. Further subclassification may or may not be feasible. Distinction from hypocellular MDS, hypocellular hairy cell leukemia, and aplastic anemia can usually be achieved by the integration of morphology and immunohistochemical stains i18.117.

#### Hypocellular acute myeloid leukemia is evident in this composite illustrating morphologic features a and markedly increased immunophenotypic blasts b. (H&E and immunoperoxidase for CD34)

#### AML with Necrosis

The delineation of lineage and stage of maturation is uniquely challenging in extensively necrotic specimens. Bone marrow aspirate smears may contain insufficient intact cells for adequate assessment. Similarly, both flow cytometric immunophenotyping and conventional karyotyping are frequently unsuccessful due to low viability and paucicellularity. The successful diagnosis of extensively necrotic AML is most often achieved by serial sectioning of generous core biopsy sections and extensive immunohistochemical assessment i18.118, i18.119, i18.120. Distinction from other necrotic bone marrow infiltrates rests largely with comprehensive immunohistochemical staining. However, blood smears should be reviewed for evidence of circulating viable neoplastic cells that could be assessed for lineage and stage of maturation by flow cytometric techniques.

#### AML with Fibrosis

Fibrosis is a defining feature of APMF and is highly characteristic of acute megakaryoblastic leukemia and therapy-related myeloid neoplasms. Beyond these specific AML subtypes, mild to moderate reticulin fibrosis can be encountered in almost any other AML subtype. In light of the inaspirability of these fibrotic bone marrows, definitive diagnosis of AML often relies upon immunohistochemical assessment of good quality bone marrow core biopsy sections to confirm by estimate that CD34+ blasts are ≥20% and to confirm myeloid lineage by CD33, MPO, and other myeloid antigen expression i18.121, i18.122. CD 117 staining is also useful in delineating immature myeloid and some monocytic cells. Distinction from other fibrotic neoplastic and
non-neoplastic disorders is achieved largely through immuno-
histochemical studies (see t16.12).

Myeloid Proliferations with Abundant Erythroid Cells

Myeloid proliferations with abundant erythroid cells can generate a challenging set of differential diagnostic possibilities, including both benign and malignant processes t18.12 [Arber 2008c, Morice 2005, Tso 2009, Vardiman 2008b]. Arriving at the correct diagnosis most often requires the assimilation of a substantial amount of data, including hematologic indices, morphology, additional laboratory studies (eg, vitamin B₁₂, folate, and zinc levels), and genetics. In cases that are clearly malignant, adherence to the diagnostic criteria for each myeloid neoplasm category will lead to the correct WHO 2008 subclassification [Vardiman 2008b]. By convention, erythroblasts
### Differential Diagnostic Possibilities for Myeloid Proliferations with Abundant Erythroid Cells

<table>
<thead>
<tr>
<th>Diagnostic Possibility</th>
<th>Comments/Bone Marrow Features</th>
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</thead>
</table>
| Acute erythroid leukemia:  
  pure erythroid type  
  erythroleukemia (erythroid/myeloid) |  
  ≥50% erythroid cells  
  ≥80% erythroblasts  
  Myeloblasts ≤20% of non-erythroid cells |
| Myelodysplastic syndrome | <20% myeloblasts; variable erythroid percentage (see Chapter 16) |
| AML with myelodysplasia-related changes | ≥20% blasts; ≥50% of dysplastic cells in at least 2 lineages |
| AML, NOS, with increased erythroid precursors | ≤50% erythroid lineage cells |
| Polycythemia vera | Erythroid predominance with complete maturation; minimal/no dysplasia  
  Blasts not increased  
  Peripheral erythrocytosis  
  Low erythropoietin level  
  Increased megakaryocytes, usually hyperlobated  
  Bone marrow hypercellularity with megakaryocyte abnormalities  
  JAK2 V617F mutation positive in 95% of cases |
| Non-neoplastic erythroid proliferations:  
  megaloblastic anemia | Erythroid hyperplasia with left shift, sieve-like chromatin, giant metamyelocytes (see Chapter 6) |
| florid hemolytic anemia | Erythroid hyperplasia with intact maturation; nuclear budding, multinucleation (see Chapter 6) |
| florid erythroid regeneration post chemotherapy  
  recombinant erythropoietin administration  
  congenital dyserythropoietic anemia | Intact maturation, minimal dysplastic change (mainly seen in most mature cells)  
  Erythroid hyperplasia with left shift (see Chapter 35)  
  Ineffective erythropoiesis, marked dyserythropoiesis; multinucleation (see Chapter 6) |
| Tumors mimicking erythroblasts:  
  Burkitt/ other lymphoma types  
  metastatic tumors  
  other acute leukemias  
  myeloma | Immunophenotypic studies reveal lineage |

### References
- [Arber 2008c, Morice 2005, Tso 2009, Vardiman 2008b]

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should predominate in acute erythroid leukemia, while myelodysplasia tends to show an admixture of all stages of erythroid maturation \[18.96, 18.101\] (see Chapter 16). Distinguishing high-grade myelodysplasia with abundant erythroid cells (≥50%) from AML with MDS-related changes or acute erythroid leukemia hinges on the percent of non-erythroid cells that are myeloblasts. Clinical heterogeneity is well-described in acute erythroleukemia; many patients may not require the urgent therapy needed in other settings of acute leukemia (ie, clinically more akin to MDS). In such scenarios it is prudent to communicate with the submitting physician regarding the diagnostic criteria utilized and the clinical heterogeneity of cases with ≥50% erythroid lineage cells that fulfill AML criteria.

Megaloblastoid change is a frequent dysplastic morphologic feature in myeloid malignancies. Without other types of overt dysplasia, an increase in blasts, or Auer rods, megaloblastic anemia must remain in the differential diagnosis. Genetic studies are often helpful in addition to serum vitamin B₁₂, folate, and methylmalonic acid levels (see Chapter 6).

**G-CSF Therapy**

Therapeutic doses of recombinant G-CSF or granulocyte macrophage colony-stimulating factor (GM-CSF) act similarly and may induce a variety of cellular changes (eg, transient increases in peripheral blood or bone marrow blasts and/or neutrophil dysplasia) that may mimic a myeloid neoplasm (see \[t10.6\] [Meyerson 1998]). Consequently, it is essential that the diagnostician have information about cytokine therapy as part of routine bone marrow examination. In de novo presentations of bone marrow left-shifted myeloid hyperplasia and increased blasts, but lacking definitive dysplasia, Auer rods, or abnormal karyotype, one should
exercise extreme caution prior to diagnosing a myeloid malignancy. A reactive process such as CSF effect, infection, or paraneoplastic phenomenon secondary to an underlying solid tumor must be ruled out.

### Minimal Residual Disease, Post-Therapy Changes, and Relapse

Minimal residual disease monitoring in AML by either flow cytometric immunophenotyping or molecular techniques can potentially improve assessment of ongoing treatment response and ultimately improve outcomes [Al-Mawali 2009a]. Established minimum residual disease (MRD) assays include PCR amplification of known genetic aberrations or flow cytometric detection of aberrant immunophenotypes [Kern 2008, Shook 2009]. Quantitative PCR assessment of APL is addressed in Chapter 36.

Flow cytometric immunophenotyping may attain a sensitivity of aberrant cell detection of $10^{-4}$ compared with the traditional techniques of morphology (1%-5%) and cytogenetics (1%-5%) (see Chapter 36). The sensitivity of detection of MRD by molecular techniques is in the range of $10^{-5}$.

Several published reports indicate that the detection of MRD is a valuable tool in predicting relapse [Feller 2004, San Miguel 1997, 2001, Venditti 2002]. The lack of flow cytometric detection of leukemic cells after induction chemotherapy may argue against early AML relapse, and a threshold of ≤0.15% residual leukemic cells post induction and post consolidation therapy predicts for better overall survival (see Chapter 36 for more details) [Al-Mawali 2009a, Drach 1992].

The morphologic spectrum of bone marrow features during induction chemotherapy is detailed in Chapter 35. Distinctive post-therapy findings in AML include striking non-neoplastic dysplastic megakaryocytic proliferations and ATRA-induced morphologically abnormal maturing promyelocytes in APL [Rosenthal 1991, Tohyama 2003]. Relapse of AML is generally characterized by a marked decline in cell counts in blood, variable numbers of circulating blasts, and variable extent of bone marrow effacement [18.124, 18.125]. These relapsed AMLs generally exhibit similar morphologic, immunophenotypic, and genetic features to the original leukemia, although clonal evolution may occur. Rare AML patients develop therapy-related secondary AML, creating a unique diagnostic challenge. In general, the characteristic morphologic and cytogenetic features of t-AML will be distinct from those of the original leukemia.

### Differential Diagnosis of AML

Depending upon the lineages involved and the extent of maturation, the differential diagnosis of AML is diverse.
The primary differential diagnostic considerations for specific AML subtypes have been included earlier in the discussion of specific AML subtypes. Similarly, the differential diagnosis of hypoplastic, fibrotic, and necrotic AML is included in [18.9] “Diagnostic Pitfalls in AML Diagnosis,” p 419. Because myeloid neoplasms with abundant erythroid lineage cells are particularly problematic, an earlier section focuses on the differential diagnosis of these erythroid predominant lesions. For AML, definitive diagnosis hinges on accurate blast enumeration to allow distinction from high-grade MDS and MDS/MPN. The diagnostician must be aware that a diagnosis of AML is warranted in cases with ≤20% blasts if an AML-defining translocation is identified (see “Low Blast Count AML,” p 419). Transformations into AML by other myeloid neoplasms must also be recognized. This is best achieved when comprehensive clinical information is readily available. Previous bone marrow specimens should be compared systematically to current specimens to clarify issues of transformation of an underlying hematologic disorder such as MDS, MPN, and MDS/MPN. Auer rods are for the most part indicative of AML, but they may be present in rare patients with MDS (see Chapter 16). However, Auer rod-like inclusions have also been noted in lymphomas and myeloma (see i23.20).

Morphologic look-alikes of myeloid blasts include granular ALL, blastic plasmacytoid dendritic cell neoplasm, aggressive NK cell leukemia, myeloma, and lymphomas [Groom 1991, Pitman 2007]. Although bone marrow core biopsy section morphology generally allows distinction, the bone marrow aspirate smear appearance of rhabdomyosarcoma, neuroblastoma, medulloblastoma, and other metastatic lesions can closely mimic acute leukemia (see Chapter 29) [Chen 2004, Etzell 2006]. Even immunophenotypic overlap between metastatic lesions and AML has been noted [Ettell 2006].

A systematic evaluation including cytochemical stains and immunophenotyping can aid in these distinctions. Benign disorders that can mimic selected AML subtypes include megaloblastic anemia, G-CSF therapy, arsenic toxicity, and other toxic bone marrow insults.

### Components of the Diagnostic Interpretation

The components of the diagnostic interpretation of AML must include information regarding blast/blast equivalent enumeration, lineages involved, extent of maturation, and extent of dysplastic features of all lineages. The integration of molecular genetic information is also essential. t18.13 provides tips and strategies for this process.

#### t18.13 Diagnosis of AML: Key Tips/Strategies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WHO Criteria</th>
<th>Comments/Tips/Caveats</th>
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<tbody>
<tr>
<td>Percent blasts/blast equivalents</td>
<td>≥20% for AML (blast threshold based on percentage of non-erythroid cells for AML subtypes with ≥50% erythroid cells in BM)</td>
<td>20% threshold may be met in blood or BM&lt;br&gt;Difficult to count blasts on inaspirable specimens&lt;br&gt;Promelocytes are blast equivalents only for APL&lt;br&gt;Promonocytes are always blast equivalents&lt;br&gt;Erythroblasts are blast equivalents only in acute pure erythroid leukemia&lt;br&gt;Distinction from MDS and MDS/MPN based largely on blast percentage and genotype&lt;br&gt;Flow cytometry is not a substitute for morphology in determining blast percent; not all blasts are CD34+</td>
</tr>
<tr>
<td>Lineage of blasts</td>
<td>Must confirm lineage of immature cells by morphology, cytochemistry, and/or IP for acute myelomonocytic, monocytic, megakaryoblastic leukemias</td>
<td>Morphology, cytochemical stains, and multicolor flow cytometric IP integrated into myeloid lineage determination and exclusion of ALL&lt;br&gt;May have to rely on IHC staining on core biopsy for inaspirable specimens</td>
</tr>
<tr>
<td>Cytogenetics/FISH/molecular</td>
<td>Biologic parameters are integral part of WHO classification of AML &lt;br&gt;A specific cytogenetic or molecular finding defines many AML subtypes</td>
<td>Recurrent cytogenetic abnormalities that define AML subtypes include t(8;21), t(15;17), inv(16), t(9;11), t(6;9), inv(3), t(1;22)&lt;br&gt;Molecular abnormalities that define AML subtypes include NPM1 and CEBPA mutations</td>
</tr>
</tbody>
</table>

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; AML, NOS = AML, not otherwise specified; APL = acute promyelocytic leukemia; BM = bone marrow; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; IP = immunophenotype; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasm
**[18.13] Clues and Caveats**

1. Clinical, morphologic, cytochemical, immunophenotypic, and molecular genetic features should all be integrated into AML diagnosis.
2. Hematopoietic failure (anemia, neutropenia, and thrombocytopenia) is an expected feature of all types of acute myeloid leukemia; the WBC count is highly variable.
3. Exceptions to the 20% threshold for AML diagnosis include cases with AML-defining cytogenetic abnormalities [(eg, t(8;21), t(15;17), and inv(16)] and acute erythroid leukemia.
4. Promyelocytes are blast equivalents only in APL.
5. Promonocytes (blast equivalents) have slightly folded nuclei, dispersed chromatin, nucleoli, and moderate amounts of basophilic cytoplasm.
6. Promonocyte enumeration is critical in the distinction between acute and chronic monocytic leukemias.
7. Erythroblasts are included in the blast percentage only in cases of acute “pure” erythroid leukemia.
8. Maturing megakaryocytic lineage cells and micromegakaryocytes are not blast equivalents.
9. Rapid diagnosis of APL is imperative due to risk of hemorrhage.
10. Classic hypergranular APL is usually associated with a very low WBC, while marked leukocytosis is typical in microgranular APL.
11. Flow cytometric immunophenotyping is not the optimal modality to diagnose APL; morphology and cytochemistry are superior for diagnosis, which should be confirmed by genetic testing.
12. The biologic classification of AML includes cases defined by specific cytogenetic or molecular features, as well as cases defined by clinical parameters: underlying Down syndrome, prior chemotherapy, or antecedent myeloid neoplasm (eg, myelodysplasia).
13. Therapy-related disorders include cases of AML, myelodysplasia, and hybrid myelodysplastic/myeloproliferative diseases, but, due to similarities in outcome, these subtypes are merged into the single category of therapy-related myeloid neoplasms.
14. Down syndrome-associated neoplasms include transient abnormal myelopoiesis (spontaneously regressing neonatal disorder) and myeloid leukemia associated with Down syndrome (typically acute megakaryoblastic leukemia in ≤4-year-old child).
15. Transient abnormal myelopoiesis and Down syndrome-associated acute megakaryoblastic leukemia can be morphologically indistinguishable.
16. Distinctive morphologic clues to AML with t(8;21) include low blast count, long, tapered Auer rods, and salmon-colored cytoplasmic granulation with a rim of basophilia.
17. Distinctive morphologic clues to AML with inv(16) include increased bone marrow eosinophils with mixed eosinophil/basophil granules.
18. Distinctive clues to APL include marked leukopenia, profound thrombocytopenia, hypergranular promyelocytes, stacks of Auer rods, maturation block at promyelocyte stage, intense myeloperoxidase positivity, and coagulopathy.
19. Distinctive morphologic and CBC features of microgranular APL include leukocytosis, folded (sliding plate) nuclei, rare hypergranular promyelocytes, maturation block at promyelocyte stage, intense myeloperoxidase positivity, and coagulopathy.
20. There is substantial morphologic overlap between microgranular APL and acute monocytic/myelomonocytic leukemias.
21. Three different scenarios are used to classify a case as AML with MDS-related changes:
   a. History of MDS or MDS/MPN (but not linked to antecedent therapy)
   b. MDS-associated cytogenetic abnormality
   c. Multilineage (at least 2 lineages) dysplasia involving at least 50% of cells in each affected lineage.
22. The lack of nonspecific esterase positivity does not exclude a diagnosis of acute monocytic/myelomonocytic leukemia if morphology and flow cytometric immunophenotyping support the diagnosis.
23. Recurrent AML-defining cytogenetic abnormalities prevail over other findings in AML classification, although therapy-related myeloid neoplasm with an AML-defining cytogenetic abnormality should be classified as therapy-related AML with t(15;17), etc.
24. If a case of AML has complex cytogenetic abnormalities that include an AML-defining cytogenetic abnormality [eg, t(8;21), t(15;17), etc.], it should be classified within the AML-defining cytogenetic abnormality group.

**[18.14] References**


18: A C U T E M Y E L O I D L E U K E M I A


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Spencer DV, Cavalier M, Kalpathti R, Quigley DJ [2007] Inverted and deleted chromosome 16 with deletion of 3′CBFB identified by fluorescence in situ hybridization. Cancer Genet Cytogenet 179:82-84.


Weinberg O, Seetharam M, Ren L, et al [2010] Acute myeloid leukemia (AML) with monosomal karyotype is characterized by absence of NPM1 and FLT3 mutations, worse clinical outcome and usually falls within AML with myelodysplasia-related changes (MRC). Mod Pathol 23:328A.


