Realistic Pathologic Classification of Acute Myeloid Leukemias

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Key Words: Acute myeloid leukemia; Classification; Cytogenetics; Immunophenotyping; World Health Organization; French-American-British classification

Abstract

Most classification systems of acute myeloid leukemia (AML) rely largely on the criteria proposed by the French-American-British (FAB) Cooperative Group. The recently proposed World Health Organization (WHO) classification of neoplastic diseases of the hematopoietic and lymphoid tissues includes a classification of AMLs. The proposed WHO classification of AMLs includes traditional FAB-type categories of disease, as well as additional disease types that correlate with specific cytogenetic findings and AML associated with myelodysplasia. This system includes a large number of disease categories, many of which are of unknown clinical significance, and there seems to be substantial overlap between disease groups in the WHO proposal. Some disease types in the WHO proposal cannot be diagnosed without detailed clinical information, or they are diagnosed only by the cytogenetic findings. In this report, a realistic pathologic classification for AML is proposed that includes disease types that correlate with specific cytogenetic translocations and can be recognized reliably by morphologic evaluation and immunophenotyping and that incorporates the importance of associated myelodysplastic changes. This system would be supported by cytogenetic or molecular genetic studies and could be expanded as new recognizable clinicopathologic entities are described.

Pathologic classifications must change continuously to reflect advances in our understanding of disease. The pathologic classification of acute myeloid leukemias (AMLs) used by most physicians today, however, does not provide the most information possible for these diseases. The French-American-British (FAB) Cooperative Group provided clear and useful criteria for the pathologic classification of AML, and the classification was modified to incorporate new disease types that required ancillary testing not used in the original descriptions. Some of the FAB categories of AML, such as M3 and M4Eo, correlate with prognostically significant cytogenetic abnormalities; however, the lack of reproducibility and clinical significance of some of the disease groups in the FAB system is now generally acknowledged. In addition, the FAB classification does not accommodate the importance of myelodysplasia-associated changes in adult AML. Alternative classification systems have been proposed that incorporate immunophenotyping, cytogenetic, and myelodysplastic changes, and morphologic and immunophenotypic features are now well described for some AMLs that are highly suggestive of recurring cytogenetic abnormalities.

A list of the provisional World Health Organization (WHO) classification of neoplastic diseases of the hematopoietic and lymphoid tissues has been published that includes a classification for AML. In the WHO system, AMLs are subdivided into 4 major categories: AML with recurrent cytogenetic translocations; AML with multilineage dysplasia; AML and myelodysplastic syndromes, therapy-related; and AML not otherwise categorized. The acceptance of cytogenetic findings and associated myelodysplastic changes in AML, which provide essential prognostic information, are major advances in the classification of these diseases. However, the WHO disease categories are...
seemingly overlapping and include disease types that may not be evident by pathologic review alone. Unlike the classification of lymphoid neoplasms by the WHO, which is based largely on the previously reported Revised European-American Classification of Lymphoid Neoplasms, criteria for the myeloid categories have not been published.

Some categories of the WHO classification, such as “AML with 11q23 (MLL) abnormalities” and the various “AML and myelodysplastic syndromes, therapy-related” types, cannot be classified based on morphologic, cytochemical, or immunophenotypic studies and cannot be offered as timely pathologic diagnoses. In addition, the “AML not otherwise categorized” group includes FAB Cooperative Group–related subtypes that are of questionable clinical relevance and other leukemia types that have not been defined convincingly as clinicopathologic entities.

This article proposes a realistic pathologic classification of AMLs that would contain disease types that can be recognized by a combination of morphologic, cytochemical, and immunophenotyping studies. Major subdivisions of de novo AML and myelodysplasia-associated AML are proposed. Because of the well-recognized prognostic significance of molecular genetic changes, all cases of AML in this system should be studied by routine karyotype analysis and, as needed, molecular genetic analysis to supplement the pathologic diagnosis. This classification is proposed as an alternative to the currently proposed WHO system for AMLs.

### Proposed Classification

Similar to the WHO proposal, a diagnosis of acute leukemia would be based on a bone marrow blast cell count of 20% or more in most cases and should be independent of the marrow blast count for cases with changes of acute promyelocytic leukemia. AML with changes suggestive of t(8;21)(q22;q22), or AML with abnormal eosinophils suggestive of inv(16)(p13q22) or t(16;16)(p13;q11). The clinical similarities between patients with 20% or more blast cells and those with acute leukemia diagnosed with the more traditional 30% cutoff have been demonstrated.

The proposed system is summarized in Table 2.

### AML, De Novo

**AML, Not Otherwise Specified**

De novo cases without changes suggestive of cytogenetic abnormalities or without associated myelodysplastic changes would be placed in a subcategory of AML, not otherwise specified (NOS) but may be further subdivided into those with and without monocytic differentiation, if desired. However, the poor reproducibility of subclassifying these types of cases, particularly FAB M2 and M4 AML, is well documented. This subcategory would include the non–myelodysplasia-associated minimally differentiated AMLs (FAB type M0) and some cases of AML with 11q23 abnormalities that cannot be predicted by morphologic or cytochemical examination or immunophenotyping. The morphologic features of cases in this category are variable. Blast cells may range from having oval nuclei with scant, agranular cytoplasm to cells with folded nuclei and abundant, granular cytoplasm with Auer rods and would include most cases of the de novo M0, M1, M2, M4, and M5 leukemias of the FAB classification. By definition, multilineage dysplastic changes of the non–blast cell populations are not present. Cytochemical analysis reveals myeloperoxidase or Sudan black B positivity (>3%) in most cases, but cases

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<th>Proposed World Health Organization Classification of Acute Myeloid Leukemias (AMLs)</th>
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<tr>
<td>AMLs with recurrent cytogenetic translocations</td>
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<td>AML with t(8;21)(q22;q22), AML1(ETO)/CBF</td>
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<td>Acute promyelocytic leukemia [AML with t(15;17)(q22;q11-12)] and variants, PML/RARA</td>
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<td>AML with abnormal bone marrow eosinophils [inv(16)(p13q22) or t(16;16)(p13;q11)], CBFB/MYH11</td>
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<td>AML with 11q23 (MLL) abnormalities</td>
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<td>AML with multilineage dysplasia</td>
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<td>With prior myelodysplastic syndrome</td>
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<td>Without prior myelodysplastic syndrome</td>
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<td>AML and myelodysplastic syndrome, therapy-related</td>
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<td>Alkylating agent-related</td>
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<td>Epipodophyllotoxin-related</td>
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<tr>
<td>Other types</td>
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<td>AML not otherwise categorized</td>
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<td>AML, minimally differentiated</td>
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<td>AML, without maturation</td>
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<td>AML, with maturation</td>
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<td>Acute myelomonocytic leukemia</td>
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<td>Acute monocytic leukemia</td>
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<td>Acute erythroid leukemia</td>
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<td>Acute megakaryocytic leukemia</td>
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<td>Acute basophilic leukemia</td>
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<td>Acute panmyelosis with myelofibrosis</td>
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<tr>
<th>Table 2</th>
<th>Realistic Pathologic Classification of Acute Myeloid Leukemia</th>
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<td>Acute myeloid leukemia, de novo</td>
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<td>Acute myeloid leukemia (with or without monocytic features), not otherwise specified</td>
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<td>Acute myeloid leukemia with changes suggestive of t(8;21)(q22;q22) CD56+</td>
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<td>CD56–</td>
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<td>Acute promyelocytic leukemia</td>
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<td>Variant: acute promyelocytic leukemia with features suggestive of t(11;17)(q23;q21)</td>
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<td>Acute myeloid leukemia with abnormal eosinophils suggestive of inv(16)(p13q22) or t(16;16)(p13;q11)</td>
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<td>Acute megakaryoblastic leukemia</td>
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<td>Acute myeloid leukemia, myelodysplasia-associated</td>
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<td>Acute myeloid leukemia, treatment-related</td>
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<td>Acute myeloid leukemia arising from myelodysplasia</td>
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<td>Acute myeloid leukemia with associated myelodysplasia</td>
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Acute myeloid leukemia, not otherwise specified. The blast cells do not show morphologic features that would suggest any of the specific de novo acute myeloid leukemia (AML) types and would be considered AML-M1 in the French-American-British classification.

AML with changes suggestive of t(8;21)(q22;q22). The blast cells, including those with arrows, have cytoplasmic granules that might be mistaken for promyelocytes. Coalescing granules that are salmon-pink are present (arrows) but are best seen by focusing up and down on the actual slide. Associated eosinophils are present, but they do not show abnormalities of granulation. The blast cells expressed myeloid-associated antigens with CD34 and CD19 expression. Cytogenetic studies confirmed the presence of t(8;21).

Acute promyelocytic leukemia. The blasts have fine cytoplasmic granules and characteristic folded or bilobed nuclei. The blasts showed strong myeloperoxidase positivity by cytochemistry and were myeloid antigen positive with loss of HLA-DR. Cytogenetic studies confirmed the t(15;17).

Previously termed M0 leukemia and some cases with monocytic features are negative. Those with monocytic differentiation are positive for nonspecific esterase (alpha-naphthyl butyrate esterase or alpha-naphthyl acetate esterase with sodium fluoride inhibition) in at least 20% of blast cells. Immunophenotyping studies confirm the myeloid lineage of the cells, with expression of CD13 and CD33 being most common, but myeloperoxidase and other myeloid-associated antigens frequently are expressed. No specific recurring cytogenetic abnormality is seen in this group, but cytogenetic studies are essential for providing supplemental prognostic information.

AML With Changes Suggestive of t(8;21)(q22;q22)

AMLs with t(8;21)(q22;q22), producing an AML1/ETO fusion, have morphologic and immunophenotypic characteristics that can be useful in suggesting the presence of this abnormality. This leukemia type represents approximately 10% of AMLs. This type of AML often is associated with extramedullary myeloid tumors but has a high rate of remission and prolonged disease-free survival with current therapeutic approaches for AML. These cases represent a subgroup of FAB-M2 AMLs and should be considered acute leukemias without regard to blast cell count, because many of the neoplastic cells in this disease have abundant granules that may be considered promyelocytes by some. The neoplastic cells typically have large salmon-colored granules and Auer rods and often demonstrate perinuclear clearing suggestive of a hof. An associated increase in normal-appearing bone marrow eosinophils is common. The blasts are positive for myeloperoxidase or Sudan black B by cytochemical analysis. They usually express myeloid-associated antigens, but rare cases are CD13− and CD33−. The neoplastic cells usually are
CD34+, and up to two thirds of cases express CD19. CD56 expression is found on approximately half of the cases, and reports of decreased survival and shortened remission time in the CD56 group may warrant subclassifying these cases into CD56+ and CD56− types. Detection of the morphologic and immunophenotypic features typical of this category should result in further cytogenetic or molecular genetic testing to confirm the presence of this karyotypic abnormality, as well as other abnormalities. Additional karyotype abnormalities, other than loss of sex chromosomes, are associated with worse outcome.

**Acute Promyelocytic Leukemia**

Acute promyelocytic leukemia includes cases of FAB-M3 leukemia with characteristic morphologic features that are associated with abnormalities of the RARα gene on chromosome 17. Patients have a high occurrence of disseminated intravascular coagulopathy and generally respond favorably to therapeutic regimens that include all-trans-retinoic acid.

In this disorder, abnormal promyelocytes are the neoplastic cell type, and the diagnosis is made independent of the blast cell count. The abnormal cells have oval to folded nuclei that may appear monocytoid. Immature cells with folded, monocytoid nuclei are present admixed with abnormal eosinophils (arrow). The eosinophils contain characteristic large basophilic granules admixed with more normal eosinophil granules. Cytogenetic studies confirmed the presence of inv(16). Acute megakaryoblastic leukemia. The blast cells are large with abundant, finely granular, lightly basophilic cytoplasm. The cells are myeloperoxidase negative by cytochemistry but express megakaryocyte-associated markers CD41 and CD61. Myelodysplasia-associated acute myeloid leukemia. Abnormal hypogranular neutrophils with hypolobated nuclei are present, as well as megaloblastoid erythroid precursor, admixed with the blast cells. Monolobated megakaryocytes with hyperchromatic nuclei also were present in the specimen. (Wright-Giemsa, ×1,000)
The neoplastic cells are characteristically strongly positive for myeloperoxidase or Sudan black B by cytochemical analysis, and some cases may show a subset of neoplastic cells to be positive for nonspecific esterase. The cells express most of the commonly studied myeloid-associated antigens, often (25%-30%) aberrantly express CD2, and are usually HLA-DR negative.27 CD2 expression seems to be more common in the microgranular variants of the disease.28 A pathologic diagnosis of acute promyelocytic leukemia should result in cytogentic or molecular genetic studies.

The t(15;17)(q22;q21), resulting in a PML/RARα fusion, is the most commonly identified abnormality, but other translocations of RARα on chromosome 17 with PLZF at 11q23, NuMA at 11q13 and NPM at 5q35 may occur. Cases that lack an RARα translocation or have a translocation involving PLZF do not respond to all-trans-retinoic acid and require a different therapeutic approach.29,30 The morphologic and immunophenotypic features of these PLZF/RARα cases have been reported recently.31 Cases with this abnormality are described as having more round nuclei, variable coarse or fine cytoplasmic granules, and frequent cells with condensed nuclear chromatin, termed “Pelger-like,” cells, compared with PML/RARα cases. Such distinctive morphologic features in a clinically significant subtype of acute promyelocytic leukemia warrant inclusion as a subtype of this disease in the classification.

AML With Abnormal Eosinophils Suggestive of inv(16)(p13q22) or t(16;16)(p13;q11)

AMLs containing abnormal eosinophils with basophilic granules are associated with a characteristic cytogenetic abnormality and an improved survival.10,32,33 The molecular abnormality in these leukemias results in the disruption of the normal hematopoiesis-related core binding factor that also is disturbed in the t(8;21) AMLs. This leukemia type also has an improved prognosis compared with other AMLs, similar to the t(8;21) AMLs.10,12,17 This disease group includes cases of FAB-M4Eo, but also includes some cases that fulfill other FAB categories, particularly M2. The blast cells are often myelomonocytic in appearance with abundant cytoplasm and folded nuclei but may have a less differentiated appearance with cytoplasmic granules and more round nuclei. The blast cell proliferation is accompanied by an abnormal eosinophil population containing basophilic granules admixed with the more normal-appearing eosinophilic granules. Image 1D. With these morphologic features, the diagnosis of this disease category should be made without regard to the blast cell count. The presence of increased eosinophils without abnormal basophilic granules is a nonspecific finding and is not sufficient for a diagnosis of this type of acute leukemia on morphologic grounds alone. The blast cells usually are positive for myeloperoxidase and Sudan black B, and a subpopulation may be positive for nonspecific esterase. The abnormal eosinophils are weakly positive for chloracetate esterase. The blast cells express most of the myeloid-associated antigens, and some cases aberrantly express CD2.34,35 This pathologic diagnosis should result in cytogenetic or molecular genetic confirmation of t(16;16)(p13;q22) or inv(16)(p13q22), both of which result in a fusion of the CBFβ/MYH11 (also known as SMMHC) genes.

Acute Megakaryoblastic Leukemia

Acute megakaryoblastic leukemia includes de novo AML cases designated M7 in the FAB classification and has characteristic ultrastructural and immunophenotypic features. FAB-M7 leukemias occur in 2 age groups: young children and adults.3,36,37 Acute megakaryoblastic leukemia often is associated with organomegaly and a poor outcome.38 Many of the adult-type cases have associated dysplastic changes and would be considered myelodysplasia-associated AMLs rather than de novo acute megakaryoblastic leukemias in the proposed system.

The blast cells are variable in appearance, ranging from undifferentiated small cells with fine nuclear chromatin and varying amounts of agranular basophilic cytoplasm that may form blebs or pseudopods to larger cells with azurophilic granules that may have the appearance of L-2 lymphoblasts. The morphologic features of the blast cells, however, are not sufficiently specific for diagnosis, and ancillary testing is necessary. Marrow fibrosis is more common with this type of AML and may result in an inability to aspirate suitable marrow material for smear evaluation or some ancillary studies.

Cytochemical studies for myeloperoxidase, Sudan black B, and alpha-naphthyl butyrate esterase are characteristically negative, but the blast cells are positive for acid phosphatase and alpha-naphthyl acetate esterase (sodium-fluoride sensitive). Diagnosis requires detection of platelet peroxidase by electron microscopy or platelet- or megakaryocyte-associated antigen expression by immunophenotyping. Because nonspecific megakaryocyte antigen expression may occur in other types of AML,19 expression of at least 2 megakaryocyte-associated antigens (including CD41, CD42, CD61, von Willebrand factor, and Ulex europaeus) should be identified for diagnosis of this type of leukemia.

At the genetic level, there seem to be 3 types of acute megakaryocytic leukemia: Down syndrome–associated, t(1;22)(p13;q13)-associated, and those with other abnormalities.38 This disorder is distinct from the neonatal transient myeloproliferative disorder of Down syndrome, but it may occur within 1 to 2 years of resolution of that neonatal proliferation.38 The t(1;22)(p13;q13) is the other common recurring cytogenetic abnormality of the childhood disease.39,40 The t(1;22)-positive cases usually occur in the neonatal
Discussion

The proposed classification offers a realistic pathologic approach to AML. It requires appropriate clinical information and respects the essential contributions of cytogenetic analysis in AML. Morphologic, cytochemical, and immunophenotypic evaluation, however, cannot detect all relevant cytogenetic and molecular genetic abnormalities. For example, AMLs with MLL rearrangements do not offer sufficiently distinct features to be a separate category in this classification. To include them as a category would incorrectly imply a consistent ability to detect these cases. The prognostic significance of the various 11q23 translocations involving the MLL gene are well described, however,12,48 and reports may need to be amended when abnormalities of MLL are identified after the initial pathologic evaluation. Follow-up cytogenetic and molecular genetic studies are essential to this system to confirm the suggested abnormalities or to identify abnormalities that cannot be suggested reliably on morphologic, cytochemical, or immunophenotypic grounds alone.

In general, the myelodysplasia-associated AMLs in the proposed system would not be categorized further into specific disease groups. However, rare cases of acute promyelocytic leukemia, t(8;21) AML, and inv(16) AML are reported to arise as therapy- or toxin-related acute leukemia,49-52 and diagnoses such as myelodysplasia-associated AML, acute promyelocytic type, or myelodysplasia-associated AML with changes suggestive of t(8;21)(q22;q22) may be appropriate in these instances. Some overlap between the AML, NOS group and the myelodysplasia-associated AML group may occur, especially in elderly patients who have insufficient non–blast cell elements for evaluation of dysplasia and no history of myelodysplasia.

Other types of acute leukemia are included in the FAB classification and the provisional WHO classification that are not included in the currently proposed system. Various types of erythroleukemia are described in the literature, including the FAB-M6 type and pure erythroid leukemia.25,34 These cases have a high association with myelodysplasia, and most could be diagnosed as myelodysplastic syndromes or as AML, myelodysplasia-associated. Acute basophilic leukemia, proposed by the WHO, does not seem to be a distinct disease type.55-57 Many cases that might be diagnosed as acute basophilic leukemia have t(9;22)(q34;q11) or t(6;9)(p23;q34), but no other specific recurring cytogenetic abnormalities are reported that would suggest a specific entity. The t(9;22) and t(6;9) might be suggested when increased basophils are present, but previously reported cases have shown morphologic variation, and no features are specific for these translocations.58,59 The t(6;9) AMLs, however, often have evidence of dyserythropoiesis and might be classified as myelodysplasia-associated AMLs in this system. The category of acute panmyelosis with myelofibrosis in the provisional WHO classification also seems to represent a heterogeneous group of diseases. The earlier criteria for acute myelofibrosis included relatively nonspecific pathologic criteria of marrow fibrosis with a proliferation of immature cells.60 Many of the cases reported in the literature have features of acute megakaryoblastic leukemia with extensive marrow fibrosis61,62 while others have lower numbers of pluripotent blast cells that may represent myelodysplastic syndromes or transformation of chronic myeloproliferative disorders with associated fibrosis.63,64
Until more specific criteria for the diagnosis of acute basophilic leukemia or acute panmyelosis with myelofibrosis are presented that demonstrate them as distinct from the aforementioned diseases, it would seem premature to adopt them as specific disease types.

Finally, this classification does not directly address cases of biphenotypic acute leukemia. Lymphoid antigen expression is relatively common in AML. Some lymphoid antigens, such as CD19 and CD2, are expressed commonly in specific subtypes of AML in this system, and such antigen expression should not be regarded as evidence of biphenotypic acute leukemia. Although the definition and clinical significance of biphenotypic acute leukemia are controversial, the criteria and modified weighted point system for biphenotypic acute leukemia proposed by the European Group for the Immunological Classification of Leukemias (EGIL) greatly reduce the number of cases that fall in that category. The EGIL proposal offers useful guidelines for differentiating AML with aberrant lymphoid antigen expression from the rare biphenotypic leukemias.

The proposed classification of AML attempts to incorporate current knowledge of disease types into a practical and useful system that can result in a relatively rapid and useful pathologic diagnosis. It recognizes the importance of cytogenetic studies, and diagnoses could be modified once cytogenetic results become available to incorporate this additional information. Additional morphologic, cytochemical, and immunophenotypic features of clinically significant AML types will become available in the future, and this system could be modified easily to incorporate those future advances. The proposed classification includes the type of information that usually is available during the time that initial treatment decisions need to be made. Hopefully, future advances in cytogenetic and molecular genetic testing will result in very rapid and economic testing so that the results of these important tests will be available at all centers before therapy. In addition, evaluation of the reproducibility of this system among pathologists would be needed, and clinical studies are warranted to validate any proposed classification system. Until any classification system has been evaluated in this manner, however, the proposed system offers one approach to these diseases.

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