2 Classification of Acute Leukemias

Perspective 2

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1. INTRODUCTION

A useful classification of acute leukemia must be reproducible, must impart understanding of leukemogenesis and clinical behavior, and (most importantly) be clinically relevant, which makes such a classification indispensable for designing and comparing clinical trials. Classification systems by their very nature may influence the design of treatment regimens and may even bias investigations of leukemogenesis. The discovery over 30 years ago that the acute lymphoid and acute myeloid leukemias differed in their responses to chemotherapeutic agents set the stage for the development of clinically useful classifications. The first of these was based on the morphologic features of leukemic blasts, with different groups of hematologists establishing their own terminology and diagnostic criteria. This lack of uniform nomenclature and hence comparable classifications posed a major obstacle to rapid progress in the treatment and understanding of leukemia pathobiology.

The first internationally accepted classification of acute leukemia was proposed in 1976 by the French–American–British (FAB) Cooperative Group *(1)*. The initial FAB classification was based solely on morphologic criteria that were subsequently refined in 1981 and 1985 *(2–4)*. Unfortunately, the revisions largely ignored exciting immunologic and cytogenetic discoveries that were contributing to an improved understanding and better treatment designs in acute leukemia. The morphologic, immunologic, and cytogenetic (MIC) classification, introduced in 1988, was the first to recognize the usefulness of cytogenetics for identifying subgroups of acute leukemia *(5,6)*. The MIC system recognized additional subgroups of acute leukemia not discernible in the FAB classification. Modifications of the FAB classification were also recommended by a National Cancer Institute-sponsored workshop in 1990 *(7)*.

Treatment of pediatric leukemia is one of the great successes of modern cancer therapy. Much of the success can be attributed to the recognition of important patient risk factors and the design of effective therapy for patients at high risk of treatment failure *(8)*. Although they are still important, older risk factors based on a patient's physical manifestations or hematologic and biochemical testing have been largely replaced by biologic features of the leukemic cell. Present risk assignments depend heavily on combined morphologic, immunologic, cytogenetic, and (more recently) molecular genetic studies. The recently introduced World Health Organization (WHO) classification takes into account morphologic and immunologic features plus well-studied, common nonrandom chromosomal abnormalities that clearly influence the laboratory and clinical features of

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acute leukemia *(9)*. No doubt current and future gene profiling studies and in-depth studies of gene function, together with a better understanding of host factors and responses to pharmacologic agents, will result in more functionally useful classifications. To paraphrase an old cliché, revised classifications of acute leukemia are almost as certain as new taxes and death. This chapter does not attempt to reiterate the laboratory, biologic, and clinical features of every recognizable type of acute leukemia, as these are available from other sources *(10–12)*, including the preceding chapter in this book, rather, it seeks to highlight the major advances, limitations, and controversies of past and current classifications.

2. DEFINITIONS OF ACUTE LEUKEMIA

Examination of the bone marrow is required by FAB criteria to make a diagnosis of acute leukemia. Thus, acute leukemia is established when at least 30% of the total nonerythroid cells in the marrow are blasts or have features of acute promyelocytic leukemia *(3)*. However, examples of leukemia are encountered in which the blasts comprise >30% of the leukocytes in peripheral blood but less than that in bone marrow. Others recommend that the diagnosis of acute leukemia be accepted when the blast percentage in the peripheral blood of bone marrow is >30% *(13)*. The WHO classification uses >20% blasts in the marrow or peripheral blood as a diagnostic criterion *(9)*.

The definition of a "blast" would appear to be straightforward, but this is often difficult to apply in practice. The FAB group recognized three types of leukemic blasts: lymphoblast, a cell with a high nuclear/cytoplasmic (N/C) ratio, indistinct-to-prominent nucleoli, and the absence of detectable myeloperoxidase by cytochemical staining; type I myeloblast, an agranular cell with a high N/C ratio, uncondensed chromatin, and prominent nucleoli; and type II blast, a cell with type I myeloblast features but containing a few azurophilc granules *(3)*. With the intention of improving interobserver agreement, the 1990 National Cancer Institute Workshop recommended additional leukemic cell types for defining myeloblastic leukemia *(7)*. These included type III myeloblasts, which contained more granules than type II myeloblasts, promyelocytes of acute promyelocytic leukemia, monoblasts and promonocytes of monocytic leukemias, and megakaryoblasts of acute megakaryoblastic leukemia. While these proposals appear to be useful, agreement among observers as to what constitutes a leukemic blast in a given bone marrow specimen often remains an unspoken problem.

The term *acute lymphoblastic leukemia* (ALL) is universally accepted and conveys a clear understanding of the type of leukemia being considered. However, the designation *acute myeloid leukemia* (AML) can be confusing to those outside the hematology–oncology community and sometimes to those within the community. Acute myeloid leukemia, in the strictest sense, refers to malignancies of myelocytic or granulocytic origin. However, in the FAB classification and common usage, this term is applied to leukemias of myeloid, monocytic, erythroid, or megakaryocytic origin. *Acute nonlymphoid leukemia*, a poor substitute for describing all leukemias not of lymphoid lineage, lost favor with hematologists but still creeps into books and journal articles *(7)*.

3. FAB CLASSIFICATION: ACCOMPLISHMENTS AND LIMITATIONS

In 1976, the first FAB Cooperative Group proposal recognized three major hematologic malignancies—acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS)—and proposed subgroups within each of these malignant processes *(1)*. The expectation of the FAB group was that improved uniformity of classification would lead to the identification of clinically relevant subtypes. The lymphoblastic leukemias were divided into three subtypes based on the cytologic features of the leukemic blasts. Initial studies showed that the ALL-L3 subtype was associated with a mature B-phenotype and the t(8;14) chromosomal translocation; however, no similar correlation or reproducible clinical outcomes could be attributed to the L1 and L2 subtypes. Hence, the FAB group revised their classification of ALL in 1981 *(2)*. Subsequent multivariant analysis of clinical studies showed no additional prognostic information beyond that obtained from leukocyte count, age, race, and karyotype ploidy *(14)*. No sooner had the revised FAB classification been proposed than immunologic investigations of ALL showed the importance of differentiating B- from T-lineage ALL and recognizing expression of the common ALL antigen (CALLA) *(14–18)*. The introduction of monoclonal antibody technology led to the development of reagents that opened an era of extensive investigation producing newer and better classifications of ALL based on cell lineage and differentiation rather than morphology alone.

Electron microscopic investigations and rudimentary immunophenotyping studies of the 1970s and early 1980s were not lost on the authors of the FAB classification. By 1985, the FAB group had revised their AML classification and proposed a new AML subtype, acute megakaryoblastic leukemia (AML M7) *(4)*. Morphologic and numerical criteria for AML and MDS subgroups were revised in response to criticisms of the original 1976 proposal, and several years later, criteria were proposed for diagnosing myeloperoxidase-negative myeloid leukemia (AML M0) *(19)*. The FAB group offered no revision of their earlier classifications of lymphoblastic leukemia; indeed, investigators using immunophenotyping were busy proposing new classifications of ALL based on lineage and stage of lymphocyte differentiation. The descriptions of nonrandom cytogenetic abnormalities associated with distinct subgroups of acute leukemia were just making their way to press and thus were not incorporated in the revised FAB classification. The revised classification of AML was not without its critics. Problems identified with the revisions included definitions of what constitutes a leukemic blast, the distinction between acute leukemia and myelodysplastic syndrome based on blast numbers, the use of lysozyme determinations for separating some cases of myeloid from monocytic leukemias, and, importantly, the lack of studies to substantiate that the revisions were clinically useful *(20)*. As discussed later, similar criticisms apply in part to the recently proposed WHO classification.

The FAB classification can be credited with providing the first uniform approach to the classification of acute leukemias, with resultant improvement in separations of ALL, AML, and MDS. More important, this classification facilitated comparisons among treatment protocols by better defining the fre-

Table 1
Morphologic, Immunologic, and Cytogenetic (MIC) Classification of Acute Leukemia[a]

		Immunologic markers						
MIC group	*FAB*	*CD2*	*CD7*	*CD10*	*CD19*	*TdT*	*cIg*	*cIg*	*Karyotype*
Acute lymphoblastic leukemia (ALL)									
Early B-precursor ALL	L1, L2		–	+	+	+	–	–	t(4;11); t(9;22)
Common ALL	L1, L2		–	+	+	+	–	–	6q–; near-haploid; del(12p), or t(9;22)
Pre-B ALL	L1		–	+	+	+	+	–	t(1;19), t(9;22)
B-cell ALL	L3		–	+/–	+	–	–	–	t(8;14); t(2;8); t(8;22)
Early T-precursor ALL	L1, L2	+	+		–	+			t/del(9p)
T-cell ALL	L1, L2	+	+		–	+			6q–

		Immunologic markers						
MIC group	*FAB*	*CD7*	*CD19*	*CD13*	*CD33*	*GPA*	*CD41*	*Karyotype*
Acute myeloid leukemia (AML)								
M2/t(8;21)	M2	–	–	+	+	–	–	t(8;21)(q22;q22)
M3/t(15;17)	M3,M3v	–	–	+	+	–	–	t(15;17)(q22;q12)
M5a/del(11q23)	M5a (M5b,M4)	–	–	+	+	–	–	t/del(11)(q23)
M4Eo/inv(16)	M4Eo	–	–	+	+	–	–	del/inv(16)(q23)
M1/t(9;22)	M1 (M2)	–	–	+	+	–	–	t(9;22)(q34;q11)
M2/t(6;9)	M2 or M4 with basophilia	–	–	+	+	–	–	t(6;9)(p21-22;q34)
M1/inv(3)	M1 (M2, M4, M7) with thrombocytosis	–	–	+	+	–	–	inv(3)(q21q26)
M5b/t(8;16)	M5b with phagocytosis	–	–	+	+	–	–	t(8;16)(p11;p13)
M2 Baso/t(12p)	M2 with basophilia	–	–	+	+	–	–	t/del(12)(p11-13)
M4/+4	M4 (M2)	–	–	+	+	–	–	+4

+, positive; –, negative; no symbol, not specified by MIC workshop.
Abbreviations: FAB, French–American–British Classification; TdT, terminal deoxynucleotidyl transferase; GPA, glycophorin A.
[a] Data from refs. *5* and *6*.

quency and types of leukemias among patients entered on these protocols. Indeed, AML treatment protocols of several cooperative study groups currently incorporate the FAB classification for purposes of patient stratification. Even the findings of current immunologic, cytogenetic, and molecular studies of acute leukemias are frequently put into prospective according to the FAB subtypes studied. However, the limitations of the FAB classification are now obvious. First, the reproducibility of the morphologic separation of the different AML subtypes in various studies ranges from 60 to 90%. Second, immunologic and genetic investigations of the past decade have identified leukemic subtypes not discernible by FAB criteria. Third, FAB criteria for separating MDS from AML are not practical and easily reproducible. Fourth, the FAB classification does not identify patients whose leukemias arise out of a background of MDS and thus may relegate patients to less-than-optimal treatment approaches.

4. MIC COOPERATIVE GROUP CLASSIFICATION OF ACUTE LEUKEMIA

In 1986, the First MIC Cooperative Study Group published its morphologic, immunologic, and cytogenetic criteria for the classification of ALL *(5)*. Shortly thereafter the second workshop of the MIC group was held to promote similar criteria for the classification of AML *(6)*. The publications of both workshops built on morphologic criteria of the 1985 revised FAB classification. The recommendations of the workshops included retention of the FAB criteria for ALL and AML subgroups L1–L3 and M1–M7, respectively, with no major changes except for recognition of an M2 Baso subgroup. This subtype was applied to M2 leukemias that had evidence of basophilic granules in blast cells and maturing granulocytes. The MIC group also proposed panels of antibodies to B-, T-, erythroid-, megakaryocytic-, and myeloid lineage-associated antigens and immunologic techniques to be used in studying acute leukemias. The second MIC workshop was the first to recommend that bilineage and biphenotypic leukemias be classified as unique categories and stated the importance of recognizing these subtypes in therapeutic trials to establish their laboratory features and clinical significance.

The major emphasis of these workshops was recognition of the increasingly important role played by cytogenetics in the characterization of leukemia. The MIC classification proposed six subtypes of ALL and 10 subtypes of AML that are characterized by unique morphologic, immunologic, and cytogenetic features (Table 1) *(5,6)*. Another 10 karyotypic AML groups (+8, –7, 7q–, 5q–, –Y, +21, 9q–, 17q, 20q–, and +22) without specific morphologic or immunophenotype associations were also proposed. In comparison with the revised FAB classification, the MIC classification was insightful in recognizing the important role that cytogenetics would play in the treatment of acute leukemias. On the downside, the MIC classification was applicable to only 50% of patients with AML. Unfortunately, one-half of patients with AML would not have a karyotypic

Table 2
Immunologic Classification of Acute Leukemias

SJCRH classification (21)		*EGIL classification (90)*	
Immunologic subgroup	*Immunophenotypic profile*	*Immunologic subgroup*	*Immunophenotypic profile*
B-lineage ALL	CD19+/CD22+/cyCD3–/MPO–	B-lineage ALL	CD19+ and/or CD79α+ and/or CD22+
Early pre-B	CD79α±/CD10+/Igμ–	B-I (pro-B)	No B-cell differentiation antigens
		B-II (common B)	CD10+
Pre-B ALL	CD79α+/CD10+/cyIgμ+	B-III (pre-B)	cyIgμ+
Transitional (late) pre-B	CD79α+/CD10+/cyIgμ+/sIgμ+		
Mature B	CD79α+/CD10±/cyIgμ+/sIgμ+/sIgλ+or sIgκ+	B-IV (mature B)	cyIg or sIg κ+ or λ+
T-lineage ALL	CD7+/cyCD3+/CD22–/CD79a±/MPO–	T-lineage ALL	Cytoplasmic/surface CD3+
Pre-T	sCD3–/CD5–/CD1–/CD4–/CD8–/CD10–	T-I (pro-T)	CD7+
Early-T	sCD3–/CD5+/CD1–/CD4–/CD8±/CD10–	T-II (pre-T)	CD2+ and/or CD5+ and/or CD8+
Common-T	$sCD3^{lo}$/CD5+/CD1±/CD4±/CD8±/CD10±	T-III (cortical T)	CD1a+
Late-T	$sCD3^{hi}$/CD5+/CD1–/CD4+ or CD8+/CD10–	T-IV (mature T)	Surface CD3+, CD1a–
		α/β (group a)	TCR α/β+
		γ/δ (group b)	TCR γ/δ+
Early myeloid (AML-M0)	Anti-MPO± but enzymatic MPO-/CD33±/ CD13±/CD15±/CD117±/CD61–/GPA–	Early myeloid (AML-M0)	MPO± but enzymatic MPO–/CD13+/CD33+/ CD65+/and-or CD117+
Myeloid lineage	CD34±/HLA-DR±/MPO±/CD33±/CD13±/ CD15±/CD64- or wk/CD117±/CD61-/GPA-	Myelo/monocytic lineage	MPO+/CD13+/CD33+/CD65+/and-or CD117+
Monocytic lineage	CD34–/HLA-DR+/MPO±/CD33/CD13±/ CD14±/CD15±/CD64+/CD117±/CD61–/GPA–		
Megakaryocytic lineage	CD34±/HLA-DR±/MPO–/CD33±/CD13±/ CD15±/CD64–/CD117±/CD61+/GPA–	Megakaryocytic lineage	CD41+ and/or CD61+ (surface or cytoplasmic)
Erythroid (pure) lineage	CD34±/HLA-DR±/MPO–/CD33±/CD13±/ CD15±/CD64-/CD117±/CD61-/GPA+	Erythroid lineage	Early/immature: unclassified by markers Late/mature: GPA+
Undifferentiated	CD45+/CD34±/CD19±/CD22–/CD79α–/ cyCD3-/CD7±/CD5–/MPO-/CD33±/ CD13-/CD15-/CD117±/CD61–/GPA–	Undifferentiated	Often CD34+/HLA-DR+/CD38+/CD7+

Abbreviations: SJCRH, St. Jude Children's Research Hospital; EGIL, European Group for the Immunological Characterization of Leukemia; MPO, myeloperoxidase; cyIg, cytoplasmic immunoglobulin; sIg, suface immunoglobulin; TCR, T-cell receptor.

Table 3
Correlation of Cytogenetic Abnormalities with Leukocyte Antigen Expression Profiles

Karyotype	*Genes involved*	*Leukemia subtype*	*Leukocyte antigen profile*
t(4:11)(q21;q23)	*AF4*, *MLL*	Early pre-B-ALL	CD45+/CD34+/CD19+/CD24– or wk/CD10– or wk/CD15+
t(12;21)(p12;q22)	*TEL*, *AML1*	Early pre-B- or pre-B-ALL	CD45+/CD34±/CD19+/CD24+/CD10+/CD9– or wk/CD13±/CD33±
t(1;19)(q23;p13)	*PBX1*, *E2A*	Pre-B-ALL	CD45+/CD34–/CD19+/CD24+/CD10+/CD15+/cyIgμ+/sIgμ±
t(9;22)(q34;q11)	*ABL*, *BCR*	Early pre-B- or pre-B-ALL	CD45+/CD34±/CD10+/CD24+/CD9+/CD13±/CD33±
t(8;21)(q22;q22)	*ETO*, *AML1*	AML-M2 (some M1 or M4)	CD45+/CD34+/HLA-DR+/CD19+/CD13 wk+/CD33 wk+/CD56±
t(15;17)(q22;q11)	*PML*, *RARα*	AML-M3 (rare M1 or M2)	CD45+/CD34–/HLA-DR–/CD19–/CD2±/CD13+/CD33+
t(11;17)(q23:q11)	*PLZF*, *RARα*	AML-M3-like	CD45+/CD34–/HLA-DR–/CD19–/CD2±/CD13+/CD33+
inv(16)(p13q22)	*MYH11*, *CBFβ*	AML-M4Eo (some M2)	CD45+/CD34+/HLA-DR+/CD19–/CD2+/CD13+/CD33+/CD14±

Abbreviations: cy, cytoplasmic; s, surface; wk, weak.

change recognized by the MIC classification. The MIC Cooperative Group did not test their classification before its publication. Instead, they recommended that cooperative groups investigate the relationship of specific chromosomal abnormalities to laboratory features and treatment response. It would take another 15 yr before the next morphologic, immunologic, and cytogenetic classification of acute leukemias would be proposed. Despite the insight it provided into the potential clinical significance of chromosomal abnormalities in AML, the MIC classification was not incorporated into cooperative group studies of acute leukemia in the United States.

5. IMMUNOLOGIC INVESTIGATIONS AND CLASSIFICATION OF ACUTE LEUKEMIA

5.1. Lymphoblastic Leukemia

Immunologic studies or immunophenotyping of acute leukemia serve several purposes. Primary among these is to establish or confirm the lineage of a leukemic process. Multiparameter flow cytometric immunophenotyping is also useful for distinguishing acute leukemia from benign proliferations, such as virus-associated lymphoid proliferation or lymphoid regenerative processes following chemotherapy. The immunologic features of a leukemic process may provide prognostic information. As discussed below, expression of CALLA (or CD10) by T-ALL is associated with an improved clinical outcome. Lastly, immunophenotyping is a quick and sensitive technique for detecting small numbers of leukemic blasts in extramedullary sites or in the marrow and blood following treatment (minimal residual disease).

The first immunologic classifications of acute leukemia separated lymphoblastic from myeloblastic lineages and recognized B- and T-lineage ALL subtypes. The first indication that the stage of leukemic cell differentiation might have prognostic significance came from studies of pediatric B-lineage ALL *(14,16–18)*. Subsequent immunologic classifications of ALL followed the development of monoclonal antibodies to cell lineage-associated and differentiation antigens. The production of clinically friendly flow cytometers with multiparameter analysis software complemented the availability of leukocyte monoclonal antibodies. With these new leukocyte reagents and flow cytometers, stages of leukocyte differentiation were delineated in ways not possible with the light microscope. These advances were used to develop new and more useful classifications of leukemias. Indeed, contemporary classifications of ALL correspond to normal stages of B- and T-cell maturation (Table 2) *(21)*.

Early clinical investigations suggested that the stage of leukemic cell differentiation correlated with response to treatment. For example, early studies of childhood B-lineage ALL showed a poorer treatment outcome for pre-B-ALL compared with early pre-B-ALL (22). Subsequent combined immunophenotype and cytogenetic findings showed that this difference in outcome was due to a chromosomal t(1;19)(q23;p13) translocation that is exclusively associated with pre-B-ALL (23,24). More intensive therapy of pre-B-ALL with the t(1;19) translocation now results in treatment outcomes approaching that of early pre-B-ALL. In another example, expression of CALLA (or CD10) was associated with good responses to treatment. However, subsequent cytogenetic findings and improved chemotherapy treatments mitigated the independent prognostic importance of CD10 expression in B-lineage ALL. Clinical studies show that the leukemic cells of most patients with CD10-negative B-lineage ALL have a rearrangement of the *MLL* gene due in some cases to a t(4;11)(p22;q23) translocation (Table 3), a frequent chromosomal abnormality of ALL in patients younger than 12 mo of age. Subsequent studies revealed that chromosome 11q23 translocations, in particular t(4;11), are strong predictors of a poor treatment response that override the predictive importance of CD10 expression (25). Other reports suggest that the intensity of CD45 expression is correlated with a leukemic cell hyperdiploid karyotype (26,27). Associations of leukemic blast expression of other antigens with clinical behavior have not been confirmed by rigorous studies that carefully evaluated the influence of cytogenetic or molecular genetic abnormalities. Immunophenotyping studies have revealed characteristic antigen expression profiles that point to chromosomal abnormalities with prognostic significance but not with the accuracy of cytogenetic or molecular techniques (Table 3). In general, chromosomal abnormalities have largely nullified the usefulness of dividing B-lineage ALL into subgroups based on immunophenotype.

The value of recognizing subtypes of T-ALL by immunophenotyping is more controversial. Similar to B-lineage ALL, T-ALL has been divided into subgroups corresponding to phases of normal T-cell maturation (Table 2) *(21)*. However, attempts to identify immunophenotypic subtypes of T-ALL with prognostic significance have been largely unsuccessful. Previous studies in which T-ALL was classified as early (CD7+,

cytoplasmic CD3+, surface CD3–, CD4–, CD8–, and CD1–), mid or common (CD7+, cytoplasmic CD3+, surface CD3– or weak, CD4+, CD8+, CD1+), or late (surface CD3+, CD1–, CD4+ or CD8+) found that up to 25% of T-ALL cases have antigenic profiles that do not easily fit into a thymic stage of maturation. Furthermore, classifications based on normal T-cell differentiation are largely unsuccessful for predicting response to treatment. Similarly, the prognostic significance of individual antigen expressions by T-ALL blasts, such as CD3, CD2, CD5, and CD34, varies among several large clinical studies *(28–39)*. The disparities may be caused by differences in immunophenotyping methodologies and interpretations or differences in treatment. Multivariant analyses of patients with T-ALL at St. Jude Children's Research Hospital and the Pediatric Oncology Group concur that older age and lack of CD10 expression are independently associated with a poor clinical outcome *(28–30)*. In contrast to B-lineage ALL, characteristic antigen expression profiles in T-ALL are not associated with chromosomal abnormalities *(28)*. As discussed later, gene-expression profiling may point to unique antigenic expressions resulting from genetic abnormalities of leukemic T cells.

5.2. Acute Myeloid Leukemia

Immunophenotyping studies of AML are hampered by the relative lack of monoclonal antibodies to lineage-specific antigens. Additionally, antigen expression profiles of AML only partially correlate with stages of normal marrow myeloid, monocytic, or megakaryocytic differentiation *(40–43)*. The relatively poor correlation is largely owing to asynchronous antigen expression or differences in antigen intensity (intralineage infidelity) with leukemic cell differentiation. Similar to lymphoblastic leukemias, aberrant lymphoid-associated antigen expression (interlineage infidelity) is relatively common and often characteristic of certain cytogenetic abnormalities (Table 3). Older studies based on single-parameter immunophenotyping were inadequate for matching leukemic cell antigen expression with FAB AML subgroups *(44,45)*. However, multiparameter flow cytometric analysis may be more accurate than classic morphologic and cytochemical studies in identifying the lineage(s) involved in a case of AML *(46–52)*. With this approach, leukemic cells can be discriminated from normal hematopoietic cells. Light scatter and CD45 intensity expression can be combined to recognize characteristic patterns that correspond to the FAB AML subtypes. For most practical purposes the primary value of immunophenotyping in AML is to identify megakaryoblastic leukemia and AML subtypes that do not produce enzymatically active myeloperoxidase (AML M0). Although several large studies of adult and pediatric AML do not show any predictive value of the expression of individual leukocyte antigens, this issue continues to be debated *(52–54)*. Expression of CD7, high levels of CD34, or multidrug-resistant antigens, such as p180, may correlate with poor clinical outcomes in adult patients with AML, but such observations have not been used in planning patient treatment *(55–59)*.

5.3. Acute Leukemia with Aberrant Antigen Expression

Current evidence strongly supports the concept that leukemia represents the clonal expansion of a single transformed cell and that most leukemic processes mirror stages of normal leukocyte differentiation. Nonetheless, previous immunologic and molecular studies show that some acute leukemias can display features of one or more hematopoietic lineages (lineage infidelity). Acute leukemias whose blasts simultaneously show characteristics of more than one lineage (e.g., lymphoid plus myeloid) have been termed *acute mixed lineage*, *hybrid*, *chimeric*, or *biphenotypic leukemia (60–65)*. These leukemias should not be confused with the rare cases comprising two or more phenotypic but not necessarily genotypic lineages, variously termed *biclonal*, *bilineal*, or *oligoclonal leukemia*. The leukemias with mixed lineage, hybrid, or biphenotypic features can be defined by morphologic, cytochemical, ultrastructural, and molecular studies, but in most instances they are identified by immunologic studies.

Investigations of the past decade support the concept of two broad categories of acute leukemias with disparate expressions of lineage-associated features. Acute leukemias in the most common category have distinct immunologic, genotypic, and clinical features characteristic of a strong commitment to a single lineage but with one or several aberrant features of another lineage. These include ALL-expressing myeloid-associated antigens (My^+ALL) and AML with lymphoid-associated antigen expression (Ly^+AML). The second category of leukemias displays a mixture of genotypic and antigenic features that make it unclear whether the leukemic blasts are committed to a single lineage of differentiation (i.e., true mixed, hybrid, or biphenotypic leukemias). Recognition of these two categories is clearly a useful advance in leukemia classification, but confusion remains as to their diagnostic criteria, nomenclature, optimal treatment, and prognostic significance. This lack of agreement can be attributed to inconsistencies among studies of these unusual cases, including the patient population studied (pediatric, adult, or a mixture of both), different laboratory methodologies, stringency of the immunologic criteria for defining commitment to lymphoid or myeloid differentiation, and treatment approaches *(64,65)*. Chief among these appears to be the immunologic criteria for defining commitment to the lymphoid or myeloid lineage. For example, definitions vary depending on the immunologic methods employed: single or multiparameter flow cytometry; fluorescence microscopy or immunohistochemistry; the number and type of monoclonal antibodies used; inclusion of antigens that are not lineage-restricted [e.g., CD4, CD11b, CD15, CD10, or terminal deoxynucleotidyl transferase (TdT)]; source and condition of the leukemic samples (e.g., marrow or blood; fresh, old or cryopreserved cells); and the criteria for positive or negative antigen expression.

The criteria used at St. Jude Children's Research Hospital to define My^+ALL, Ly^+AML, and "true mixed" or biphenotypic leukemia are presented in Table 4. The central feature of this classification is the identification of antigens that substantiate lymphoid and myeloid lineage commitment. As shown in Fig. 1, B-lineage ALL is diagnosed when leukemic blasts express CD19 plus CD22 and cytoplasmic CD79α or immunoglobulin, and no cytoplasmic CD3 or myeloperoxidase. The leukemic cells of T-ALL express CD7 plus either surface or cytoplasmic CD3 but do not coexpress surface CD19 and CD22 or cytoplasmic CD79α and myeloperoxi-

Table 4
SJCRH Criteria for My+ ALL, Ly+ AML, and Biphenotypic Leukemia

Ly+ AML[a]

1. Leukemic blasts are MPO+[b] (or ANB+ if AML M5)
2. Leukemic blasts are cyCD3–
3. Leukemic blasts are cyIgμ– and do not coexpress CD22 plus cyCD79α–
4. Leukemic blasts express ≥1 lymphoid-associated antigens: CD2, CD5, CD7, CD19, CD22, CD56, cyCD79α

Biphenotypic acute leukemia

Myeloid/B-lineage biphenotypic acute leukemia:
Leukemic blasts coexpress MPO[b] and cyIgμ, or MPO[b] and cyCD79α plus CD22
Myeloid/T-lineage biphenotypic acute leukemia:
Leukemic blasts coexpress MPO[b] plus cyCD3
Mixed B- and T-lineage acute leukemia:
Leukemic blasts coexpress cyCD3 plus cyIgμ, or cyCD3 and cyCD79α plus CD22

B-lineage My+ALL[a]

1. Leukemic blasts are CD19+ plus CD22+ or cyCD79α+ or cyIg μ+
2. Leukemic blasts are cyCD3–
3. Leukemic blasts are MPO–[b]
4. Leukemic blasts express ≥1 myeloid-associated antigens: CD13, CD14, CD15, CD33, CD36, or CD65

T-lineage My+ ALL[a]

1. Leukemic blasts are CD7+ and cyCD3+
2. Leukemic blasts are CD22–
3. Leukemic blasts are MPO–[b]
4. Leukemic blasts express ≥1 myeloid-associated antigens: CD13, CD14, CD15, CD33, CD36, CD65, CD79α[wk]

Abbreviations: SJCRH, St. Jude Children's Research Hospital; Ly+ AML, acute myeloid leukemia expressing lymphoid (Ly)-associated antigens; My+ ALL, acute lymphoid leukemia expressing myeloid (My)-associated antigens; MPO, myeloperoxidase; ANB, α-naphthyl butyrate esterase; cyCD, cytoplasmic antigen expression; wk, weak.
[a]All four criteria must be fulfilled.
[b]Confirmed by cytochemical, anti-MPO, or ultrastructural study.

dase. AML is diagnosed when leukemic blasts express myeloperoxidase or in its absence, two or more myeloid-associated antigens, including CD13, CD15, CD33, or CD65 but not cytoplasmic CD3, immunoglobulin, or simultaneously CD19, CD22, and cytoplasmic CD79α. A case of My^+ ALL would have the antigenic expression profile defined for B- or T-lineage ALL plus one or more myeloid-associated antigens, such as CD13, CD15, CD33, and CD65 but not myeloperoxidase. A case of Ly^+ AML will display the antigen profile described above for AML plus one or more lymphoid-associated antigens but not cytoplasmic CD3 or coexpression of surface CD19, CD22, and cytoplasmic CD79α.

Several large studies of childhood My^+ ALL show that myeloid-associated antigen expression does not have independent prognostic significance *(66–71)*. Other studies have failed to consider the impact of genetic abnormalities on clinical outcome in My^+ cases. For example, atypical expression of the myeloid-associated antigen CD15 is common in B-lineage ALL with t(4;11), a translocation that confers a poor outcome in infants and older children independently of immunophenotype *(72)*. By contrast, patients with B-lineage ALL with t(12;21)(p12;q21) have a favorable outcome regardless of the presence or absence of the myeloid-associated antigens CD13 or CD33. The clinical importance of My^+ ALL in adults is still unknown *(75–78)*.

Most studies of pediatric and adult Ly^+ AML find no significant effect of lymphoid antigen expression on clinical outcome except for CD7-positive AML *(65,69,79–82)*. Similar to B-lineage ALL, the aberrant lymphoid antigen expression is largely associated with certain chromosomal abnormalities. For example, favorable cases of AML with t(8;21)(q22;q22) and inv(16)(p13q22) almost always express the lymphoid-associated antigens CD19 and CD2, respectively whereas CD7 is associated with MDS-related and secondary AMLs that frequently display abnormalities of chromosome 7 *(83–89)*.

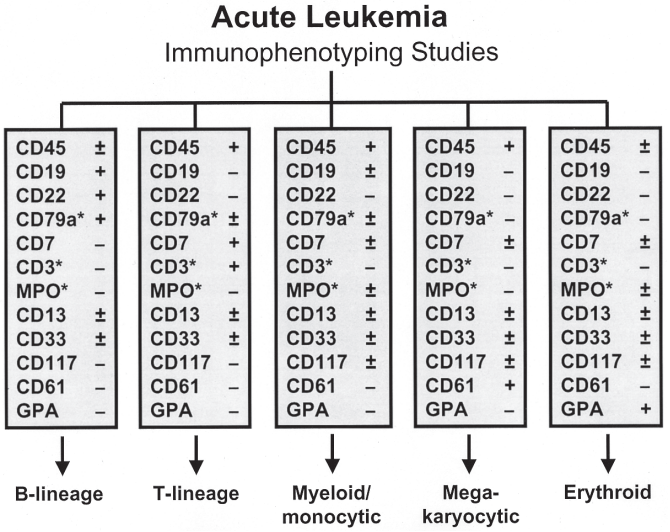

Fig. 1. Basic screening panel for immunophenotyping the major lineages of acute leukemia. The asterisks indicate cytoplasmic antigen expression. Over 98% of B-lineage acute lymphoblastic leukemia (ALL) cases will coexpress CD19, CD22, and CD79α, whereas T-lineage ALL and acute myeloid leukemias (AML) may express CD19 or rarely CD19 plus CD79α, but not CD19 plus CD22 plus CD79α.

An immunophenotypic diagnosis of true mixed or preferably biphenotypic leukemia is considered when the leukemic blasts express MPO plus CD3, MPO plus immunoglobulin, or MPO plus surface CD19, CD22, and cytoplasmic CD79α (Table 4). The European Group for the Immunological Characterization of Leukemia (EGIL) proposed a scoring system for defining biphenotypic leukemias *(90)* in which points are assigned to a lymphoid or myeloid antigen based on its degree of lineage specificity (Table 5). Biphenotypic leukemia is diagnosed when scores exceed 2 for the myeloid lineage plus 2 for

Table 5
EGIL Immunophenotyping Criteria (Scoring System) for Biphenotypic Acute Leukemias

B-lineage	*T-lineage*	*Myeloid*	*Points*[a]
CD79α CyIgμ cy/sCD22	cy/s CD3 TCRα/β TCRγ/δ	MPO	2
CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD13 CD33 CD65s CD117	1
TdT CD24	TdT CD7 CD1a	CD14 CD15 CD64	0.5

Abbreviations: EGIL, European Group for the Immunological Characterization of Leukemia; cy, cytoplasmic; s, surface; TCR, T-cell receptor; MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.

[a]Biphenotypic acute leukemia is defined by >2 points from the myeloid group and >2 points from the B-lineage or T-lineage group.

Data from ref. *90*.

either the B- or T-lineage. The preceding criteria defining biphenotypic leukemia are probably oversimplified, as evidenced by more sophisticated multiparameter flow cytometric analysis. For example, in our studies of such cases, two or more populations of leukemic blasts with discordant immunophenotype profiles may be present in a patient's leukemic specimen. Sometimes, only a minor number of leukemic blasts may have a biphenotypic immunotype, with the greater proportion of blasts demonstrating commitment to a single lineage. The clinical dilemma created by these observations is obvious. Thus, whereas pediatric and adult patients with biphenotypic leukemias appear to have a poor clinical outcome, it will be important to confirm this finding with standardized immunophenotyping methods and criteria for defining biphenotypic leukemia.

6. GENETIC CLASSIFICATION OF ACUTE LEUKEMIA

Studies of pediatric and adult leukemias have conclusively demonstrated the significant impact of genetic abnormalities on clinical response to treatment. Indeed, many specific chromosomal abnormalities have been described that frequently override the importance of morphologic, immunologic, and clinical features. Pediatric patients with ALL and underlying chromosomal hyperdiploidy >50 or t(12;21) have excellent treatment responses, whereas poorer clinical outcomes are associated with chromosomal hypodiploidy <45, or t(1;19), t(9;22), or t(4;11) (Table 6). The t(12;22) and chromosomal hyperdiploidy >50 are uncommon in adults compared with children with ALL (Figs. 2 and 3) *(91,92)*, whereas t(9;22) is more common in adults (Fig. 3) *(92–94)*. In pediatric ALL, more intensive consolidation treatment is given to patients with poor-risk cytogenetic features. This risk-adapted therapeutic approach has been very successful and supports the inclusion of cytogenetics in any classification system for ALL *(8,95)*.

The strikingly different incidences of major cytogenetic abnormalities in adult vs pediatric ALL (Figs. 2 and 3) are not reiterated in AML (Fig. 4). Additionally, the clinical outcomes by cytogenetic group are similar for adult and pediatric patients with AML. Not surprisingly, characteristic morphologic and immunologic features are also associated with many of the nonrandom chromosomal abnormalities in AML (Table 3). Patients whose AML is defined by t(8;21), t(9;11), t(15;17), inv(16), or t(16;16) translocations fare significantly better than those with normal karyotypes, chromosomal 3q translocations or deletions, t(6;9), or monosomy 7 or 7q deletions. Indeed, the leukemic cell karyotype is the strongest prognostic factor in AML. Three cytogenetic risk groups—favorable, intermediate, and adverse—are widely accepted and currently considered in planning treatment *(96–101)*. However, different cooperative groups assign cytogenetic abnormalities to different prognostic subgroups (Table 7).

Despite the association of specific chromosomal abnormalities with clinical outcome, and the intriguing insights afforded by these defects, a clinically useful classification of acute leukemia based solely on cytogenetic studies is not practical for several reasons. The most obvious of these is that a significant number of ALL and AML cases do not have a chromosomal abnormality that defines a leukemic entity or predicts clinical outcome. In AML, most patients are in the intermediate-risk group (Table 7). Furthermore, it is highly probable that within well-defined cytogenetic risk groups, other (unrecognized) genetic lesions influence clinical outcome. For example, despite the relatively good response of myeloblastic leukemias with t(8;21) or inv(16), an unacceptable 40–50% of these patients are not cured with chemotherapy alone, for reasons other than the presence of known high-risk features. This strongly suggests an influence from additional genetic lesions in these leukemias. One possible cooperating genetic abnormality may be the *FLT3* internal tandem duplication (*FLT3* ITD). Recent investigations show that *FLT3* ITD is the most common genetic abnormality in AML, one that adds important prognostic information to all three genetic-risk groups *(102–108)*. The outcomes for patients with AML are significantly worse for those with *FLT3* IDT, but the significance of *FLT3* mutations appears to decline with age *(104–108)*. In one pediatric study, *FLT3* mutations were found in only the favorable and intermediate risk groups *(103)*. Although no study thus far has sufficient numbers of AML patients with favorable cytogenetic features, i.e., t(15;17), t(8;21), or inv(16), to say whether or not *FTL3* mutations are a confounding factor in predicting clinical outcome, it is possible that this or other genetic abnormalities influence treatment response. Thus, the present classification of three major cytogenetic AML risk groups may be an oversimplification and will be inadequate as a clinically useful classification of AML. Predictably, a more fully characterized genetic profile is required to build a useful genetics-based classification of acute leukemias (see the later discussion of gene expression profiling).

7. WHO CLASSIFICATION OF ACUTE LEUKEMIA

Investigations over the last 15 years have demonstrated the importance of immunologic and cytogenetic studies for classi-

Table 6
Cytogenetic Classification of Acute Lymphoblastic Leukemia (ALL)

Karyotype	*Genes involved*	*Leukemia subtype*	*Clinical prognosis*
Hyperdiploid >50[a]		Early pre-B- or pre-B-ALL	Favorable
t(12;21)(p12;q22)	*TEL, AML1*	Early pre-B- or pre-B-ALL	Favorable
t(1;19)(q23;p13)	*PBX1, E2A*	Pre-B-ALL	Good with intensified therapy
t(8;...)(q24;...)[b]	c-*MYC*,—[b]	Mature B-ALL (ALL-L3)	Favorable without central nervous system disease
t(11;19)(q23;p13.3)	*MLL, ENL*	Early pre-B- or T-ALL	Poor in patients <1 yr; favorable in T-ALL
t(4:11)(q21;q23)	*AF4, MLL*	Early pre-B-ALL	Poor in patients <1 or >10 yr of age
t(9;22)(q34;q11)	*ABL, BCR*	Early pre-B- or pre-B-ALL	Poor
Near haploid <30[a]		Early pre-B-ALL	Poor

[a] Chromosomes.
[b] Includes t(8;14)(q24;q32), t(2;8)(p12;q24), and t48;22)(24;q11) where heavy, κ, and λ immunoglobulin genes are involved on chromosomes 14, 2, and 22, respectively.

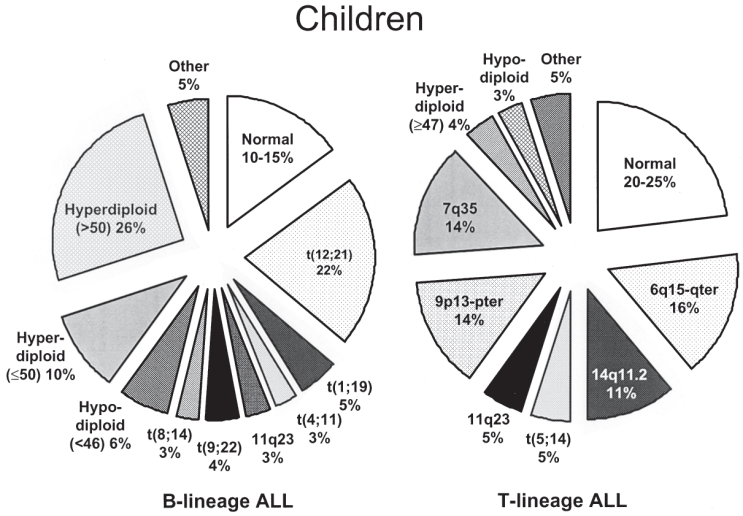

Fig. 2. Recurring chromosomal abnormalities in pediatric acute lymphoblastic leukemia (ALL) as detected by classic cytogenetics and fluorescense in situ hybridization.

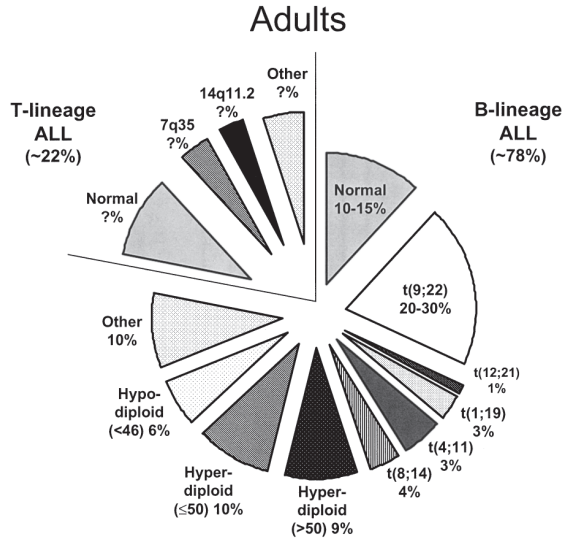

Fig. 3. Recurring chromosome abnormalities in adult acute lymphoblastic leukemia (ALL) as detected by classic cytogenetics and fluorescense in situ hybridization. The pie chart is divided into B- and T-lineage ALL with further subdivision into chromosomal abnormalities. Chromosome 7q abnormalities include translocations t(7;11)(q35;p13), t(7;10)(q35;q24), and others involving the *TCR*β gene. Chromosome 14q abnormalities include translocations t(11;14), t(10;14), t(8;14), and others involving the *TCR*α/*TCR*δ gene complex. The t(11;19) translocation involving *MLL* is the most common T-ALL abnormality of chromosome 11q23. Relative incidences in chromosome abnormalities for adult T-ALL all not available.

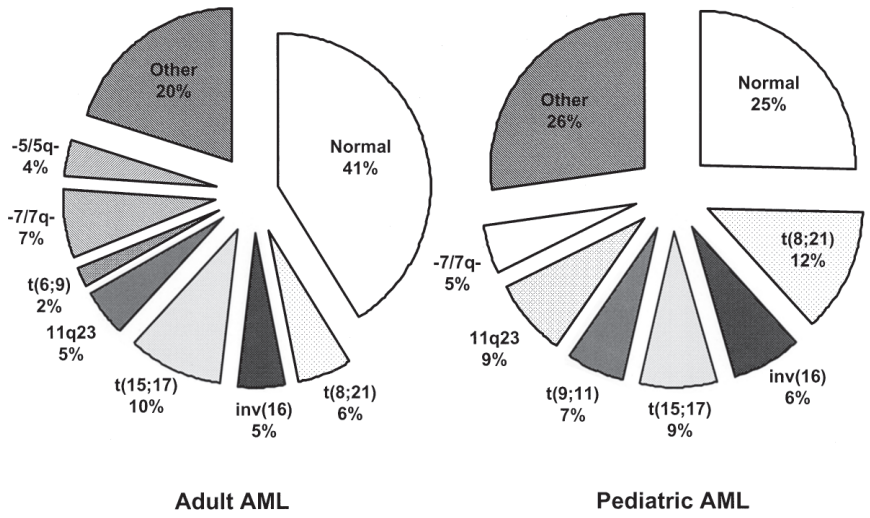

Fig. 4. Recurring chromosomal abnormalities in pediatric and adult acute myeloid leukemia (AML) as detected by classic cytogenetics and fluorescense *in situ* hybridization.

Table 7
Cytogenetic Classifications of Acute Myeloid Leukemia

Group	*CALGB (96)*	*MRC (97,98,100)*	*GAMLCG (101)*	*SWOG (99)*
Favorable	t(15;17)	t(15;17) with any abnormality	t(15;17)	t(15;17) with any abnormality
	inv(16)/t(16;16)/del(16)	inv(16)/t(16;16)/del(16q) with any other abnormality	inv(16)/t(16;16)	inv(16)/t(16;16)/del(16q) with any other abnormality
	t(8;21)	t(8;21) with any other abnormality	t(8;21)	t(8;21) without del(9q) or complex karyotype
Intermediate	Normal karyotype	Normal karyotype	Normal karyotype	Normal karyotype
		+8, –Y, +6, der(12p)	Other abnormalities	+8, –Y, +6, der(12p)
		11q23 abnormality		
		del(9q) or del(7q) without other abnormality		
		Complex karyotypes (≥3 but <5 abnormalities)		
		All abnormalities of unknown prognostic significance		
Unfavorable	Other abnormalities	–5/del(5q) –7	–5/del(5q). –7/del(7q)	–5/del(5q). –7/del(7q)
		inv(3q), del(9q), 17p abnormality	inv(3), 17p	inv(3), 17p abn, 20q, +13,
		t(6;9)	12p	t(6;9)
		t(9;22)		t(9;22)
			11q23	11q23 abnormality
				(8;21) with del(9q) or complex karyotype
		Complex karyotypes with ≥5 abnormalities	Complex karyotype	Complex karyotypes with ≥3 abnormalities
Unknown	—	—	—	All other clonal karyotypes with <3 chromosomal abnormalities

Abbreviations: CALGB, Cancer and Leukemia Group B; MRC, Medical research Council; SWOG, Southwestern Oncology Group; GAMLCG, German AML Cooperative Group.

Table 8
World Health Organization (WHO) Classification of Acute Leukemia with Corresponding FAB Classification Subtypes

WHO classification[a]	*Corresponding FAB subtypes*[b]
Precursor lymphoblastic leukemia/lymphoblastic lymphoma	
Precursor B-cell acute lymphoblastic leukemia/lymphoma	L1, L2
Precursor T-lymphoblastic leukemia/lymphoblastic lymphoma	L1, L2
Burkitt's lymphoma/leukemia	
Endemic Burkitt's lymphoma/leukemia	L3
Sporadic Burkitt's lymphoma/leukemia	L3
Immunodeficiency-associated Burkitt's lymphoma/leukemia	L3
AML with recurrent genetic abnormalities	
AML with t(8;21)(q22;q22); *AML1-ETO*	M2>M1>M4>M0
AML wth abnormal marrow eosinophilia and inv(16)(p13q22) or t(16;16)(p13;q22): *CBFβ-MYH11*	M4Eo>M4>M2>M1
Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARα*	M3>M2>M1
AML with 11q23 abnormalities; *MLL* rearrangements	M5>M4>M2>M1>M0
AML with multilineage dysplasia	
Following a myelodysplastic syndrome or myeloproliferative disorder or without antecedent myelodysplastic syndrome	M2>M4>M6
AML and myelodysplastic syndrome, therapy-related	
Alkylating agent-related	M2>M4>M6
Topoisomerase type II inhibitor-related	M5>M4>M2>M1
Other types	
AML not otherwise categorized	
Acute myeloid leukemia minimally differentiated	M0
Acute myeloid leukemia without maturation	M1
Acute myeloid leukemia with maturation	M2
Acute myelomonocytic leukemia	M3
Acute monoblastic leukemia	M4
Acute erythroid leukemia	M5
Acute megakaryoblastic leukemia	M7
Acute basophilic leukemia	—
Acute panmyelosis with myelofibrosis	M7; ? M1; ? MDS
Myeloid sarcoma	—

Abbreviations: FAB, French–American–British;MDS, myelodysplastic syndrome.
[a]For details, see ref *6*.
[b]For details, see refs. *3* and *4*.

fication and treatment of pediatric lymphoblastic malignancies. The value of these studies for the classification of adult and pediatric acute myeloid malignancies has come to light more recently. The indispensability of these studies has not been lost on the framers of the recently introduced WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue *(6)*. Whereas the FAB classification attempted and partly achieved a standardized morphologic classification of hematopoietic malignancies, and the MIC classification recognized the importance of several major nonrandom cytogenetic abnormalities, the newer WHO classification purports to go beyond these classifications by continuously recognizing new clinically relevant molecular genetic lesions. Interestingly, the newest WHO classification system continues to rely heavily on classic morphology for identification of a substantial proportion of AML cases, since understanding of the genetic basis of leukemogenesis is largely incomplete. Indeed, the morphologic features of leukemic blasts are but one manifestation of their underlying genetic abnormalities.

The WHO classification stratifies hematopoietic neoplasias by major lineage: lymphoid, myeloid, histiocytic/dendritic, and mast cell. Within each major group, distinct disease entities are defined by a combination of clinical syndrome and morphologic, immunophenotypic, and genetic features (Table 8) *(9)*. A cell of origin is suggested for each of the acute leukemias. As admitted by the authors of the WHO classification, this cell often represents the stage of differentiation of the malignant cells rather than the cell in which the initial transforming event occurs. In some myeloid leukemias, the cell of origin is known to be a multipotential stem cell, even though most of leukemic cells may be committed to a particular lineage or a late stage of myeloid differentiation (e.g., AMLs arising from MDS).

The WHO classification departs from the FAB and MIC classifications by combining ALL with lymphoblastic lymphomas. The authors conclude that laboratory evidence justifies the concept that B-precursor and T-lineage ALL and B- and T-lymphoblastic lymphomas, respectively, as well as ALL-L3 and Burkitt's lymphoma, are different clinical manifestations

of the same neoplasm *(9)*. Surprisingly, this line of reasoning is not applied to myeloid sarcomas, which are recognized as an entity related to but separate from other AMLs. Major differences between the WHO and FAB classifications of acute leukemias and myelodysplastic syndromes include:

- Replacing the morphologic terms of L1 and L2 ALL with an immunologic classification consisting of precursor-B and precursor-T lymphoblastic leukemias that are further subgrouped by cytogenetic abnormalities
- Grouping L3 ALL with Burkitt's lymphoma
- Lowering the blast count from 30 to 20% for the diagnosis of AML, with elimination of the myelodysplastic subgroup of refractory anemia with excess blasts in transformation (RAEB-IT)
- Revision of the MDS subdivision based on number of dysplastic lineages, presence of ringed sideroblasts, and blast percentage
- Recognition of distinct cytogenetic AML subtypes
- New category of AML with multilineage dysplasia with or without an antecedent MDS
- New category of Therapy-Related AML
- New category of Acute Leukemia of Ambiguous Lineage
- Inclusion of a pure erythroid leukemia (M6b) in the AML Not Otherwise Categorized subgroup
- Recognition of the rare acute basophilic leukemia also in the AML Not Otherwise Categorized subgroup.

The authors of the WHO classification invested considerable time in its development, cautiously incorporating current biologic insights and discarding irrelevant or outdated information. Although the proposed WHO classification is an improvement over previous classifications, critical questions remain as to its laboratory application and clinical usefulness. Potential problems revolve around the standardization of morphologic criteria. Lowering the blast count from 30 to 20% for the diagnosis of AML will not solve the dilemma of distinguishing *de novo* AML from MDS or the difficulty that morphologists often experience in differentiating leukemic blasts from slightly more mature cells (e.g., myeloblasts from early promyelocytes). Elaborate previous proposals for distinguishing among type I, II, and even III blasts have not been useful *(7)*. Thus, the problem that existed with the FAB requirement of 30% blasts for defining AML will persist. A similar problem exists in defining the morphologic criteria for dysplasia. Not infrequently, dysplastic changes may be subtle or present in only a small percentage of cells, undoubtedly leading to problems in differentiating AML with Multilineage Dysplasia from AML Not Otherwise Categorized. The WHO classification attempts to clarify the difference between these two categories by requiring that the latter show dysplasia in at least 50% of cells. However, a case with <50% dysplastic cells, 40% for example, will be excluded from the AML with Dysplasia Category. Supporting data for such separation do not exist. If multilineage dysplasia in AML is truly a unique feature, why artificially separate AML with less or more than 50% dysplastic cells? Furthermore, it should be remembered that investigators disagree over the clinical significance of AML presenting with multilineage dysplasia.

Another problem facing investigators who plan to use the WHO classification will be the standardization of immunologic and genetic testing and the criteria for interpreting these tests. How will new discoveries of genetic abnormalities be incorporated into the WHO classification in a timely fashion? At this writing there is already evidence that point mutations (e.g., *PT53*, *FLT3*, and *P16*), predict a poorer therapeutic response. Quite likely, some cooperative groups but not others will base their treatment programs on such discoveries, making intergroup comparisons difficult. Finally, the WHO classification was not subjected to clinical testing before being introduced to the international community of hematologists and oncologists. Hence, its reproducibility and the methods best suited to acquiring informative results will not be clear for several more years. Even with these caveats, the WHO classification of acute leukemias should improve comparisons among different study groups.

8. GENE EXPRESSION PROFILING IN THE CLASSIFICATION OF ACUTE LEUKEMIA

The phenotypic and cytogenetic diversity of acute leukemia is accompanied by a corresponding diversity in gene expression patterns. Gene expression profiling using cDNA microarrays permits simultaneous analysis of multiple gene markers and has been used successfully to categorize a variety of malignancies *(109–115)*. Advances in bioinformatics are not only making it possible to categorize leukemias into recognizable morphologic and cytogenetic subtypes but also show strong promise of being able to recognize additional types that may aid in predicting disease course *(110,116)*. The ability of gene profiling to identify currently recognized subtypes of leukemia is not unexpected, since gene expressions dictate morphologic, immunophenotype, and other leukemic cell manifestations of ALL and AML. Ultimately, this approach to leukemia classification may allow disease aggressiveness and treatment responsiveness to be reliably predicted for individual cases.

The first report of gene profiling by DNA microarray analysis, specifically applied to human leukemia, demonstrates the ability of this technology to distinguish AML accurately from ALL, as well as B- from T-lineage ALL *(110)*. In this study, investigators were able to identify 50 genes that would serve as a class predictor of AML or ALL in the vast majority of cases, with 100% accuracy. In a more recent study of a large number of ALL samples, investigators accurately distinguished B-lineage from T-lineage ALL, while identifying several important prognostic cytogenetic subgroups of B-lineage ALL—hyperdiploid > 50, t(12;21), t(1;19), and *MLL* rearranged—with 95–100% accuracy *(116)*. A novel group of B-lineage ALL cases with a unique gene profile were also identified. Surprisingly, initial analysis of the study data strongly suggested that gene profiling is capable of predicting those patients with ALL who will fail contemporary multiagent chemotherapy. Gene profiling also appears to be a promising technique for predicting resistance to the tryosine kinase-inhibiting agent ST1571. In a study of ALL with t(9;22) translocations, the gene expression profiles discriminated all patients who were sensitive to ST1571 from those resistant to this kinase inhibitor *(117)*. In another study of pediatric T-ALL patients, gene expression signatures delineated novel molecular pathways that may drive the malignant transformation of developing T cells *(118)*. Using oligo-

nucleotide microarrays, these investigators identified several gene expression signatures indicative of leukemic cell arrest that corresponded to specific stages of normal thymocyte development: *LYL1*$^+$, *HOX11*$^+$, and *TAL1*$^+$ signatures, corresponding to pro-T, early cortical, and late cortical thymocyte stages, respectively. Activation of *HOX11L2* was further identified as a novel event in T-ALL leukemogenesis. *HOX11* expression was associated with a favorable prognosis, whereas activation of *TAL1*, *LYL1*, and *HOX11L2* was found to predict a poorer treatment outcome.

In yet another study, gene expression profiles showed that ALL cases possessing a rearranged *MLL* gene have a highly uniform and distinct gene expression pattern that distinguishes them from conventional ALL or AML *(119)*. The leukemias with rearranged *MLL* genes expressed some lymphoid- and myeloid-specific genes, but at lower levels than other cases of ALL and AML. These leukemias also expressed genes characteristic of progenitor cells. The investigators contend that their observations support the derivation of *MLL*$^+$ leukemia from a very early B-cell progenitor that has the potential to differentiate in either the lymphoid or myeloid/monocytic pathway. This study also supports a model of leukemogenesis in which a specific chromosomal translocation results in a distinct type of leukemia, rather than a model in which all cells bearing translocations converge on a common pathway of leukemogenesis.

Gene-expression profiling will no doubt lead to other remarkable discoveries in acute leukemia. For example, this molecular genetic strategy will make it possible to examine the full spectrum of deletions and additions of genetic loci, mutations, and rearrangements in tyrosine kinases, hematopoietic transcription factors, and even single nucleotide polymorphisms—all of which can influence response to treatment. Thus, with gene profiling, one can produce a fingerprint for each leukemia patient that will direct optimal therapy and predict clinical outcome. Leukemia gene-expression fingerprints may in fact replace classifications of acute leukemia as we now know them. The present limitations of microarray technology include its cost and availability. Most reports of gene profiling in acute leukemia are retrospective, with unblinded analyses, and focus on samples with a high percentage of leukemic blasts. Whether the spectacular results of these initial reports can be reproduced prospectively and performed on the entire spectrum of leukemic samples, including those with low blast cell percentages, remains to be seen.

9. SUMMARY AND RECOMMENDATIONS

A classification of acute leukemia should be reproducible, should impart an understanding of leukemogenesis and clinical behavior, and should be clinically relevant. Each of the classifications presented above fails to satisfy all three of these requirements fully. The WHO classification is a theoretical improvement over all the others, but its reproducibility and clinical relevance have not been tested. It is not even clear that any single classification would satisfy all users. The WHO classification attempts to categorize acute leukemias by combining clinical and biologic features. As a result, its biologic criteria are oversimplified and may not be relevant as new therapies are developed. It may be more useful to devise separate clinical and biologic classifications. For example, the laboratory investigator would be most interested in a detailed biologic classification, whereas the physician would favor a more clinically relevant categorization. Indeed, with some recent exceptions, acute leukemia treatments are not so refined as to require a classification that would accommodate every conceivable subtype of ALL or AML.

Presently, the WHO classification offers the best system for comparing clinical trials. However, to be more relevant, it must be modified to include additional chromosomal or molecular genetic abnormalities that are clinically relevant [e.g., t(11;17) and t(8;16) in AML]. The Multilineage Dysplasia category of AML will be difficult to reproduce among different investigators and needs further refinement. The AML Not Otherwise Categorized subgroup is a waste bin of different leukemias and will no doubt vary in size and complexity depending on the skill of the morphologist and the availability of sophisticated molecular assays.

It may well be that the explosion of new information coming from gene expression profiling studies will render the WHO classification obsolete before it can be fully tested in clinical trials. This new technology will undoubtedly provide a more exact model of leukemogenesis, which in turn may suggest new modes of treatment requiring revised classifications of the lymphoid and myeloid leukemias. We can look forward to the day when each patient's leukemia will be classified by its gene expression profile. Treatment will be based not only on this profile, but also on the patient's intrinsic genetic profile, which largely determines how he or she will respond to therapy.

REFERENCES

1. Bennett JM, Catovsky D, Daniel M-T, et al. Proposals for the classification of acute leukemias. French-American-British (FAB) co-operative group. Br J Haematol 1976;33:451–458.
2. Bennett JM, Catovsky D, Daniel M-T, et al. The morphologic classification of acute lymphoblastic leukemia: concordance among observers and clinical correlations. Br J Haematol 1981;41:553.
3. Bennett JM, Catovsky D, Daniel M-T, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985;103:620–625.
4. Bennett JM, Catovsky D, Daniel M-T, et al: Criteria for the diagnosis of acute leukemia of megakaryocytic lineage (M7). A report of the French-American-British Cooperative Group. Ann Intern Med 1985;103:460–462.
5. First MIC Cooperative Study Group. Morphologic, immunologic, and cytogenetic (MIC) working classification of acute lymphoblastic leukemia. Cancer Genet Cytogenet 1986;23:189–197.
6. Second MIC Cooperative Study Group. Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukaemias. Br J Haematol 1988;68:487–494.
7. Cheson BD, Cassileth PA, Head DR, et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. J Clin Oncol 1990;8: 813–819.
8. Pui C-H, Boyett JM, Rivera GK, et al. Long-term results of Total Therapy studies 11, 12, 13A for childhood acute lymphoblastic leukemia at St Jude Children's Research Hospital. Leukemia 2000;14:2286–2294.
9. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC, 2001.

10. Pui C-H, ed. Childhood Leukemias. Cambridge: Cambridge University Press, 1999.
11. Brunning RD, McKenna RW. Atlas of Tumor Pathology. Tumors of the Bone Marrow. Fascicle 9. Washington, DC: AFIP, 1994.
12. Foucar, K. Bone Marrow Pathology, 2nd ed. Chicago: ASCP, 2001.
13. Cason JD, Trujillo JM, Estey EH, et al. Peripheral acute leukemia: high peripheral but low-marrow blast count. Blood 1989;74: 1758–1761.
14. Kalwinsky DK, Roberson P, Dahl G, et al. Clinical relevance of lymphoblastic biological features in children with acute lymphoblastic leukemia. J Clin Oncol 1985;3:477–484.
15. Sen L, Borella L. Clinical importance of lymphoblasts with T markers in childhood acute leukemia. N Eng J Med 1975;92:828–832.
16. Greaves MF, Janossy G, Peto J, et al. Immunologically defined subclasses of acute lymphoblastic leukaemia in children: their relationship to presentation features and prognosis. Br J Haematol 1981;48:79–97.
17. Morgan E, Hsu CCs,. Prognostic significance of the acute lymphoblastic leukemia (ALL) cell-associated antigens in children with null-cell ALL. Am J Pediatr Hematol Oncol 1980;2:99–102.
18. Sallan SE, Ritz J, Pesando J, et al. Cell surface antigens: prognostic implications in childhood acute lymphoblastic leukemia. Blood 1980;55:395–402.
19. Bennett JM, Catovsky D, Daniel M-T, et al: Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML M0). Br J Haematol 1991;78:325–329.
20. Bloomfield CD, Brunning RD. The revised French-American-British classification of acute myeloid leukemia: is new better? Ann Intern Med 1985;103:614–616.
21. Behm FB, Campana D. Immunophenotyping In: Childhood Leukemias. Pui CH, ed. Cambridge: Cambridge Univiversity Press, 1999. p. 111.
22. Crist W, Boyett J, Roper M, et al. Pre-B cell leukemia responds poorly to treatment: a Pediatric Oncology Group study. Blood 1984;63:407–414.
23. Crist WM, Carroll AJ, Shuster JJ, et al. Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): a Pediatric Oncology Group study. Blood 1990;76:117–122.
24. Raimondi SC, Behm FG, Roberson PK, et al. Cytogenetics in pre-B acute lymphoblastic leukemia with emphasis on prognostic implications of the t(1;19). J Clin Oncol 1990;8:1380–1388.
25. Pui CH, Frankel LS, Carroll AJ, et al. Clinical characteristics and treatment outcome of childhood acute lymphoblastic leukemia with the t(4;11)(q21;q23): a collaborative study of 40 cases. Blood 1991;77:440–447.
26. Behm FG, Raimondi SC, Schell MJ, et al. Lack of CD45 antigen on blast cells in childhood acute lymphoblastic leukemia is associated with chromosome hyperdiploidy and other favorable prognostic factors. Blood 1992;79:1011–1016.
27. Borowitz MJ, Shuster JJ, Carroll AJ, et al. Prognostic significance of fluorescence intensity of surface marker expression in childhood B-precursor ALL. A Pediatric Oncology Group study. Blood 1997;89:3960–3966.
28. Pui C-H, Behm FG, Singh B, et al. Heterogeneity of presenting prognostic features and their relation to treatment outcome in 120 children with T-acute lymphoblastic leukemia. Blood 1990;75: 174–179.
29. Ludwig WD, Harbott J, Bartram CR, et al. Incidence and prognostic significance of immunophenotypic subgroups in childhood acute lymphoblastic leukemia: experience of the BFM study 86. Recent Results Cancer Res 1993;131:269–282.
30. Shuster JJ, Falletta JM, Pullen DJ, et al. Prognostic factors in childhood T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood 1990;75:166–173.
31. Crist WM, Shuster JJ, Falletta J, et al. Clinical features and outcome in childhood T-cell leukemia-lymphoma according to stage of thymocyte differentiation: a Pediatric Oncology Group study. Blood 1998;72:1891–1897.
32. Gerand R, Voisin S, Papin S, et al. Characteristics of pro-T ALL subgroups: comparison with late T-ALL. The Groupe d'Etude Immunologique des Leucemies. Leukemia 1993;7:161–167.
33. Uckun FM, Sensel MG, Sun L, et al. Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. Blood 1998; 91:735–746.
34. Garand R, Vannier JP, Bene MC, et al. Comparison of outcome, clinical, laboratory, and immunological features in 164 children and adults with T-ALL. The Groupe d'Etude Immunolgique des Leucemies. Leukemia 1990;4:739–744.
35. Cascavilla N, Musto P, D'Arena G, et al. Are "early" and "late" T-acute lymphoblastic leukemia different diseases? A single center study of 34 patients. Leuk Lymphoma 1996;21:437–442.
36. Niehues T, Kapaun P, Harms DO, et al. A classification based on T cell selection-related phenotypes identifies a subgroup of childhood T-ALL with favorable outcome in the COALL studies. Leukemia 1999;13:614–617.
37. Czuczman MS, Dodge RK, Stewart CC, et al. Value of immunophenotype in intensively treated adult acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8364. Blood 1999;93:3931–3939.
38. Uckun FM, Steinherz PG, Sather H, et al. CD2 expression on leukemic cells as a predictor of event-free survival after chemotherapy for T-lineage acute lymphoblastic leukemia: a Children's Cancer Group study. Blood 1996;88:4288–4295.
39. Pui CH, Behm FG, Crist WM. Clinical and biological immunologic marker studies in childhood acute lymphoblastic leukemia. Blood 1993;82:343–362.
40. Terstappen LWMM, Loken MR. Myeloid differentiation in normal bone marrow and acute myeloid leukemia assessed by multidimensional flow cytometry. Anal Cell Pathol 1990;2:229–340.
41. Terstappen LWMM, Safford M, Konemann S, et al. Flow cytometric characterization of acute myeloid leukemia. Part II. Phenotypic heterogeneity at diagnosis. Leukemia 1991;5:757–767.
42. Orfao A, Chillon MC, Bortoluci AM, et al. The flow cytometric pattern of CD34, CD15, and CC13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML-RARα gene rearrangements. Haematologica 1999;84:405–412.
43. Porwit MacDonald A, Janossy G, Ivory K, et al. Leukemia-associated changes identified by quantitative flow cytometry. IV. CD34 overexpression in acute myelogenous leukemia M2 with t(8;21). Blood 1996;87:162–169.
44. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood 1997;90:169–186.
45. Neame PB, Soamboonsrup P, Browman GP, et al. Classifying acute leukemia by flow cytometric analysis: a combined FAB-immunologic classification of AML. Blood 1986;68:1355–1362.
46. Behm FG. Diagnosis of childhood acute myeloid leukemia. Clin Lab Med 1999;19:187–237.
47. Krasinskas AM, Wasik MA, Kamoun M, et al. the usefulness of CD64, other monocytic-associated antigens, and CD45 gating in the subclassification of acute myeloid leukemias with monocytic differentiation. Am J Clin Pathol 1998;110:797–805.
48. Borowitz MJ, Guenther KL, Shults KE, Stelzer GT. Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. Am J Clin Pathol 1993;100:534–540.
49. Lacombe F, Durieu F, Briais A, et al. Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia. Leukemia 1997;11:1878–1886.
50. Rainer RO. Hodges L. Steltzer GT. Cd45 gating correlates with bone marrow differential. cytometry 1995;22:139–145.
51. Sun T, Sangaline R, Ryder J, et al. Gating strategy for immunophenotyping of leukemias and lymphoma. Am J Clin Pathol 1997;108:152–157.
52. Orfao A, Vidriales B, Gonzalez M, et al. Diagnostic and prognostic importance of immunphenotyping in adults with acute myeloid leukemia. Recent Results Cancer Res 1993:369–379.

53. Smith FO, Lampkin BC, Versteeg C, et al. Expression of lymphoid-associated cell surface antigens by childhood acute myeloid leukemia cells lacks prognostic significance. Blood 1992;79:2415–2422.
54. Kuerbitz SJ, Civin CI, Krischer JP, at al. Expression of myeloid-associated and lymphoid-associated cell-surface antigens in acute myeloid leukemia: a Pediatric Oncology Group study. J Clin Oncol 1992;10:1419–1429
55. Selleri C, Notaro R, Catalando L, et al. Prognostic irrelevance of CD34 in acute myeloid leukemia. Br J Haematol 1992;82:479–482.
56. Lee EJ, Yang J, Leavitt RD, et al. The significance of CD34 and TdT determination in patients with untreated *de novo* acute myeloid leukemia. Leukemia 1992;6:1203–1209.
57. Myint H, Lucie NP. The prognostic significance of the CD34 antigen in acute myeloid leukemia. Leuk Lymphoma 1992;7:425–429.
58. Solary E, Casanovas R-O, Campos L, et al. Surface markers in adult acute myeloblastic leukemia: correlation of CD19+, CD34+ and CD14+/DR– phenotypes with shorter survival. Leukemia 1992;6:393–399.
59. Geller RB, Zahurak M, Hurwitz CA, et al. Prognostic importance of immunophenotyping in adults with acute myelocytic leukaemia: the significance of the stem-cell glycoprotein CD34 (My10). Br J Haematol 1990;76:340–347.
60. Kuerbitz SJ, Civin CI, Krischer JP, et al. Expression of myeloid-associated and lymphoid-associated cell-surface antigens in acute myeloid leukemia of childhood: a Pediatric Oncology Group Study. J Clin Oncol 1992;9:1419–1429.
61. Ben-Bassat I, Gale RP, Hybrid acute leukemia. Leuk Res 1986;8:929–936.
62. Das Gupta A, Advani SH, Nair CN, et al. Acute leukemia and coexpression of lymphoid and myeloid phenotypes. Hematol Oncol 1987;5:189–196.
63. Mirro J, Zipf TF, Pui C-H, et al. Acute mixed lineage leukemia: clinicopathologic correlations and prognostic significance. Blood 1985;66:1115–1123.
64. Drexler HG, Theil E, Ludwig W-D. Review of the incidence and clinical relevance of myeloid antigen-positive acute lymphoblastic leukemia. Leukemia 1991;5:637–645.
65. Drexler HG, Theil E, Ludwig W-D. Acute myeloid leukemia expressing lymphoid-associated antigens: diagnostic incidence and prognostic significance. Leukemia 1993;7:489–498.
66. Borowitz MJ, Shuster JJ, Land VJ, et al. Myeloid antigen expression in childhood acute lymphoblastic leukemia. N Engl J Med 1991;325:1379–1380.
67. Ludwig W-D, Harbott J, Bartram CD, et al. Incidence and prognostic significance of immunophenotypic subgroups in childhood acute lymphoblastic leukemia: experience of BFM Study 86. In: Recent Advances in Cell Biology of Acute Leukemia. Ludwig W-D, Thiel E, eds. New York: Springer-Verlag, 1993. pp. 269–282.
68. Pui C-H, Behm FG, Singh B, et al. Myeloid-associated antigen expression lacks prognostic value in childhood acute lymphoblastic leukemia treated with intensive multiagent chemotherapy. Blood 1990;75:198–202.
69. Pui C-H, Raimondi SC, Head DR, et al. Characterization of childhood acute leukemia with multiple myeloid and lymphoid markers at diagnosis and at relapse. Blood 1991;78:1327–1337.
70. Pui C-H, Schell MJ, Raimondi SC, et al. Myeloid-antigen expression in childhood acute lymphoblastic leukemia. N Engl J Med 1991;325:1378–1379.
71. Uckun FM, Sather HN, Gaynon PS, et al. Clinical features and treatment outcomes of children with myeloid antigen positive acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood 1997;90:28–35.
72. Behm FG, Raimondi SC, Frestedt JL, et al. Rearrangement of the *MLL* gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood 1996;84: 2870–2877.
73. Raynaud S, Mauvieux L, Cayuela JM, et al. TEL/AML1 fusion gene is a rare event in adult acute lymphoblastic leukemia. Leukemia 1996;10:1529–1530.
74. Aguiar RC, Sohal J, van Rhee F, et al. TEL-AML1 fusion in acute lymphoblastic leukemia of adults. Br J Haematol 1996;95: 673–677.
75. Sobol RE, Mick R, Royson I, et al. Clinical importance of myeloid antigen expression in adult lymphoblastic leukemia. N Engl J Med 1987;316:1111–1117
76. Boldt DH, Kopecky KJ, Head D, et al. Expression of myeloid antigens by blast cells in acute lymphoblastic leukemia of adults. The Southwest Oncology Group experience. Leukemia 1994; 8:2118–2126.
77. Lauria F, Raspadori D, Martinelli G, et al. Increased expression of myeloid antigen markers in adult acute lymphoblastic leukaemia patients: diagnostic and prognostic implications. Br J Haematol 1994;87:286–292.
78. Larson RA, Dodge RK, Burns CP, et al. A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. Blood 1995;85:2025–2037.
79. Creutzig U, Harbott J, Sperling C et al. Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87. Blood 1995;86:3097–3108
80. Saxena A, Sherdan DP, Card RT, et al. Biologic and clinical significance of CD7 expression in acute myeloid leukemia. Am J Hematol 1998;58:278–284.
81. Jensen AW, Hokland M, Jorgensen H, et al. Solitary expression of CD7 among T-cell antigens in acute myeloid leukemia: identification of a group of patients with similar T-cell receptor β and δ rearrangements and course of disease suggestive of poor prognosis. Blood 1991;78:1292–1300.
82. Kita K, Miwa H, Nakase K, et al. Clinical importance of CD7 expression in acute myelocytic leukemia. The Japan Cooperative Group of Leukemia/Lymphoma. Blood 1993;81:2399–2405.
83. Kita K, Nakase K, Miwa H, et al. Phenotypical characteristics of acute myelocytic leukemia associated with the t(8;21)(q22;q22) chromosomal abnormality: frequent expression of immature B-cell antigen CD19 together with stem cell antigen CD34. Blood 1992;80:470–477.
84. Tsuchiya H, ElSonbaty SS, Nagano K, et al. Acute myeloblastic leukemia (ANLL-M2) with t(8;21)(q22;q22) variant expressing lymphoid but not myeloid surface antigens with a high number of G-CSF receptors. Leuk Res 1993;17:375–377.
85. Khalil SH, Jackson JM, Quri MH, Pyle H. Acute myeloblastic leukemia (AML M2) expressing CD19 B-cell lymphoid antigen without myeloid surface antigens. Leuk Res 1994;18:145.
86. Hurwitz CA, Raimondi SC, Head D, et al. Distinctive immunophenotypic features of t(8;21)(q22;q22) acute myeloblastic leukemia in children. Blood 1992;80:3182–3188.
87. Seymour SA, Pierce HM, Kantarijian MJ, Keating MJ, Estey EH. Investigation of karyotypic, morphologic and clinical features in patients with acute myeloid leukemia blast cells expressing neural cell adhesion molecule (CD56). Leukemia 1994;8:623–626.
88. Adriaansen HJ, te Boekhorst PAW, Hagemeijer AM, et al. Acute myeloid leukemia M4 with bone marow eosinophilia (M4Eo) and inv(16)(p13q22) exhibits a specific immunophenotype with CD2 expression. Blood 1993;81:3043–3051.
89. Paietta E, Wiernik PH, Andersen J, Bennett J, Yunis J. Acute myeloid leukemia M4 with inv(16)(p13q22) exhibits a specific immunophenotype with CD2 expression. Blood 1993;82:2595.
90. Béné MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. Leukemia 1995;9: 1783–1785.
91. Raynaud S, Mauvieux L, Cayuela JM, et al. TEL/AML1 fusion gene is a rare event in adult acute lymphoblastic leukemia. Leukemia 1996;10:1529–1530.
92. Aguiar RC, Dodge RK, Mrozek K, et al. Prospective karyotype analysis in adult acute lymphoblastic leukemia. The Cancer and Leukemia Group B experience. Blood 1999;93:3983–3993.
93. Secker-Walker LM, Craig JM, Hawkins JM, Hoffbrand AV. Philadelphia positive acute lymphoblastic leukemia in adults: age distri-

bution, BCR breakpoint and prognostic significance. Leukemia 1991;5:196–199.
94. Westbrook CA, Hooberman AL, Spino C, et al. Clinical significance of the BCR-ABL fusion gene in adult acute lymphoblastic leukemia: a Cancer and Leukemia Group B study (8762). Blood 1992;80:2983–2990.
95. Reiter A, Schrappe M, Ludwig WD, et al. Chemotherpay in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 1994;84:3122–3133.
96. Bloomfield CD, Shuma C, Regal L, et al. Long-term survival of patients with acute myeloid leukemia: a third follow-up of the Fourth International Workshop on Chromosomes in Leukemia. Cancer 1997;80:2191–2198.
97. Grimwald D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 1998; 92:2322–2333.
98. Wheatley K, Burnett Ak, Goldstone AH, et al. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council Adult and Children's Leukaemia Working Parties. Br J Haematol 1999;107:69–79.
99. Leith CP, Kopecky KJ, Godwin J, et al. Acute myeloid leukemia in the elderly: assessment of multi-drug resistance (MDD1) and cytogenetics distinguish biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group Study. Blood 1997;89:3323–3329.
100. Grimwald D, Walker H, Harrison G, et al. The predictive value of hierachial cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML 11 trial. Blood 2001:98:1312–1320.
101. Büchner T, Hiddemann W, Wörmann B, et al. Double induction strategy for acute myeloid leukemia: the effect of high-dose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine: a random trial by the German AML Cooperative Group. Blood 1999;93:4116–4124.
102. Iwai T, Yokota S, Nakao M, et al. Internal tandem duplication of the *FLT3* gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group. Japan. Leukemia 1999;13:38–43.
103. Kondo M, Horibe K, Takahashi Y, et al. Prognostic value of internal tandem duplication of the *FLT3* gene in childhood acute myelogenous leukemia. Med Pediatr Oncol 1999;33:525–529.
104. Meshinchi S, Woods WG, Stirewalt DL, et al. Prevalence and prognostic significance of *FLT3* internal tandem duplication in pediatric acute myeloid leukemia. Blood 2001;97:89–94
105. Rombouts WJ, Blokland I, Lowenberg B, Ploemacher RE. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tanden duplications in the FLT3 gene Leukemia 2000;14:675–683.
106. Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood 1999; 93:3074–3080.
107. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a *FLT3* internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy. Analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001;98:1752–1759.
108. Stirewalt DL, Kopecky KJ, Meshinchi S, et al. *FLT3*, *RAS*, and *TP53* mutations in elderly patients with acute myeloid leukemia. Blood 2001;97:3589–3595.
109. Khan J, Wei JS, Saal LH, et al. Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nat Med 2001;7:673–679.
110. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531–537.
111. Dhanasekaran SM, Barrette T, Ghosh D, et al. Delineation of prognostic biomarkers in prostate cancer. Nature 200;412:822–826.
112. Ramaswamy S, Tamayo P, Rifkin R, et al. Multiclass cancer diagnosis using tumor gene expression signatures. Proc Natl Acad Sci USA 2001;98:15,149–15,154.
113. Shipp MA, Ross KN, Tamayo P, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nat Med 2002:8;68–74.
114. Alizadeh AA, Elsen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–511.
115. Khan J, Wei JS, Ringnér M, et al. Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nat Med 2001;7:673–679.
116. Yeoh E-J, Williams K, Patel D, et al. Expression profiling of pediatric acute lymphoblastic leukemia (ALL) blasts at diagnosis accurately predicts both risk of relapse and of developing therapy-induced acute myeloid leuekmia (AML). Blood Suppl. 1 2001.
117. Hofmann W-K, de Vos S, Elashoff D, et al. Resistance of Ph+ acute lymphoblastic leukemia to the tyrosine kinase inhibitor ST1571 (Glivee) is associated with distinct gene expression profiles. Blood (suppl 1) 2001.
118. Ferrando AA, Neuberg DS, Stuanton J, et al. Gene expression signatures define novel oncogenic pathways in T-cell acute lymphoblastic leukemia. Cancer Cell 2002;1:75–87.
119. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002;30:41–47

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