1 Classification of Acute Leukemias

Perspective 1

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1. INTRODUCTION

Research over the last two decades has demonstrated that acute leukemias, originally subdivided by morphologic and cytochemical criteria into myeloid and lymphoblastic varieties, represent highly heterogeneous groups of malignancies that for the most appropriate tailoring of therapy, require an extensive routine diagnostic workup, including immunophenotyping as well as cytogenetic and molecular genetic study. This more detailed characterization of leukemic blasts has provided information complementary to the classical morphology- and cytochemistry-based diagnosis and classification of acute leukemias in terms of our understanding of biologically and clinically relevant subsets; more recently, it has been successfully applied to the detection of minimal residual disease (MRD). Through the use of conventional and molecular cytogenetic analyses, acute leukemias have been recognized as a genetic disease, resulting from a series of acquired or inherited mutations in the structure of certain genes *(1)*. These mutations are passed from the original transformed progenitor cell to its clonal descendants. Most of the genetic aberrations fall into generic classes of functional dysregulation that subvert normal hematopoietic developmental programs by circumvention of cell cycle controls, inhibition of differentiation, and resistance to therapeutic apoptosis in leukemic blasts *(2)*.

The morphologic, cytochemical, and immunophenotypic criteria defined by the French–American–British (FAB) classification *(3–6)* still represent the gold standard for subclassification of acute myeloid leukemia (AML). On the basis of lineage commitment and the degree of blast cell differentiation, these criteria have led to the recognition of eight major AML subgroups (AML M0–M7). Given the distinct clinical behavior and response to treatment of the FAB categories, however, the identification of specific entities, defined according to a combination of morphologic, immunophenotypic and genetic features and clinical syndromes, has become a desirable goal. This objective, originally addressed by the so-called morphologic, immunologic, and cytogenetic (MIC) working classification of AML *(7)*, has been partly reached by the routine application of cytogenetics and molecular genetics to the initial characterization of AML. It has provided valuable insights into

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the pathogenesis of AML as well as treatment strategies targeting the underlying specific molecular abnormalities. Furthermore, a vast array of AML phenotypes and genotypes, which have been identified during the last decade, correlate to various degrees with each other, with the patient's presenting characteristics, and with the clinical behavior of the disease.

Unlike the FAB classification of AML, that of acute lymphoblastic leukemia (ALL) *(3)* has not been shown to have significant immunophenotypic, genetic, and clinical correlates, except for the L3 subtype, and therefore it has been largely replaced by immunophenotypic and genetic schemes. The accurate assignment of leukemic lymphoblasts to specific lineages has been made possible by the use of lineage- and/or maturation-specific monoclonal antibodies (MAbs); hence, the primary diagnosis and subclassification of ALL nowadays rest on immunophenotyping *(8,9)*. The last few years have seen a variety of novel genetic markers that provide crucial information for understanding the biology of both AML and ALL, that can be used as diagnostic and prognostic tools, and that give important clues to rational therapeutic interventions *(1,10)*.

Obviously, a gene-based classification system would be preferable to one relying mainly on indirect measures of blast cell diversity, such as morphology and immunophenotype. There is little doubt that new approaches to acute leukemia classification, such as gene expression profiling with DNA microarrays, will contribute importantly to the identification of acute leukemia subtypes with distinct clinical phenotypes and variable clinical courses *(11–13)*. Future studies should demonstrate whether the molecular classification of acute leukemias on the basis of gene expression profiling will in fact lead to a clinically relevant stratification of AML and ALL into molecularly defined categories and to the development of new therapeutics aimed at the correction of pathologic transcriptional programs.

In the following paragraphs, we start with some general considerations on the techniques currently applied to the classification of acute leukemias. Subsequently, the impact of morphologic and cytochemical analyses, immunophenotyping, and cyto-/molecular genetics for dissecting the cell-biologic heterogeneity of acute leukemias and for defining clinically relevant subsets is covered in greater detail. Finally, controversial issues in the classification of acute leukemias are discussed.

2. MORPHOLOGIC AND CYTOCHEMICAL ANALYSIS

For nearly 100 years, even in the present era of molecular biology, the classification of blood cells and the diagnosis of leukemia have been based on cytomorphologic features after staining. AML therapy depends mainly on the interpretation of the morphologic appearance of blasts in the microscope. Therefore it is necessary to describe the sources, staining techniques, and possible pitfalls. Cytomorphology should also lead to a rational use of the techniques described below, such as immunophenotyping, cytogenetics, fluorescence *in situ* hybridization (FISH), and the polymerase chain reaction (PCR). The morphologic diagnosis of acute leukemias should always be based on the examination of blood *and* bone marrow smears. If the aspiration of bone marrow fails (*punctio sicca*), the examination has to focus on the peripheral blood smears alone, taking into account the histologic results of a trephine biopsy. However, not all staining techniques are useful any more. Absolutely necessary are the May–Grünwald–Giemsa stain, the myeloperoxidase (MPO) reaction, and the nonspecific esterase (NSE) reaction. All other methods seem to be superfluous, especially when other techniques (such as immunophenotyping, cytogenetics, FISH, and molecular genetics) are applied. However, judicious use of the information derived from cytomorphology and cytochemistry allows better, faster, and more efficient use of the techniques described below.

2.1. Staining Techniques for the Diagnosis of Acute Leukemias

Although a detailed discussion of staining and cytochemistry protocols *(14,15)* is beyond the scope of this chapter, some information should be given on how to interpret staining results and avoid mistakes that lead to misdiagnosis. Standard hematologic staining techniques include May–Grünwald–Giemsa (MGG; or Pappenheim, Romanowsky, Wright, Wright-Giemsa). The information obtained by these simple methods leads directly to the diagnosis of AML (e.g., by the detection of Auer rods) or allows the well-directed decision to carry out further investigations. Baselines for the interpretation of a sufficient staining result should be established to permit comparisons with other laboratories. Blood smears and, even more importantly, bone marrow slides with occasional high cellularity and marrow particles should dry for at least 1–3 h for best staining results. Reproducible staining results with the MGG or Pappenheim stain are characterized by three features: (1) they should show erythrocytes in salmon color; (2) the granules in the polymorphonuclear neutrophils (PMNs) should be visible (not fulfilled with the Wright stain for bone marrow); and (3) the platelets should have red–blue colors. If this is not the case, the result of the PMN testing may be misinterpreted as hypogranular, which may lead to the erroneous diagnosis of dysgranulopoiesis.

Selective cytochemical stains improve the accuracy and reproducibility of lineage assessment and traditional AML subclassification. MPO, Sudan black B (SBB), and NSE with α-naphthyl acetate (ANA) or α-naphthyl butyrate (ANB) used as a substrate, are useful in this regard. In most cytochemical determinations of MPO, diaminobenzidine (in some investigations benzidine) is used. Blood films and bone marrow should not be older than 2–5 d for a reliable MPO reaction. Heat or sunlight destroys the cells even more quickly. Promyelocytes, bands, and PMNs show a yellowish to brownish granular stain, which appears strongest in eosinophils; monocytes may show a weak reaction. Lymphoid cells are always negative.

For the interpretation of blasts in AML and to determine peroxidase deficiency, it is always necessary to find some PMNs that are MPO-positive. Otherwise, the reaction may have failed. If this possibility can be excluded, and an MPO defect is detected in more than 50% of the PMNs, one is led to the diagnosis of myelodysplasia with MPO deficiency. In these cases, eosinophils are still positive.

The MPO reaction is still the morphologic gold standard for discriminating between myeloid and lymphoid blasts. Furthermore, Auer rods can be detected up to three times more often

by investigating the MPO reaction than by using MGG stain exclusively *(16)*. For SBB stain, interpretation of the positive reaction is very similar to that of the MPO reaction *(17)*. However, very weak reactivity will be detected better if the peroxidase reaction is used. SBB is reactive in unfixed, air-dried smears much longer than MPO. The detection of esterase is crucial for the definition of monoblasts or monocytes, especially when the cytomorphology in the MGG stain is not typical. For routine purposes, it is helpful to describe the reaction by identifying the diffuse staining of the cytoplasm on a scale from 0 to 4+ (no reaction to very strong reaction). Only when cells have been identified as 3+ or 4+ is it justifiable to describe them as monoblasts or monocytes. Mature monocytes always react less intensively than immature monoblasts. Cells from the monocytic compartment in the bone marrow show a stronger reaction than do monocytes on a peripheral blood smear. The reaction can be inhibited with sodium fluoride. As an internal control, megakaryocytes and macrophages react positively with ANA. Reactivity for ANA on unfixed, unstained smears is stable for weeks.

The NSE reaction is always necessary for AML classification in the FAB system as well as for the diagnosis of chronic myelomonocytic leukemia. Stains such as naphthol ASD-chloroacetate-esterase (specific esterase; CAE), periodic acid-Schiff (PAS), acid phosphatase, dipeptidylaminopeptidase IV (DAP IV), and iron have no further role for the diagnosis of acute leukemia on bone marrow or blood smears.

2.2. Morphologic Classifications of Acute Leukemias

The morphologic classification of acute leukemias requires peripheral blood *and* bone marrow smears (or touch preparations) for all patients. A number of peripheral cells and bone marrow cells (100–200) should be examined. Especially in ALL, the percentage of infiltration sometimes varies widely, and blasts can be found in clusters. The FAB classifications of acute leukemias *(3–6,18,19)* follow an algorithm and are based on several thresholds. In the era of biologic description of disease entities, some of these rules seem to be arbitrary, a limitation considered in the development of new proposals, such as the World Health Organization (WHO) classification discussed below *(20)*. However, the FAB system still forms the basis for the cytomorphologic classification of AML and MDS at diagnosis, but not of ALL. The definition of acute leukemia and the distinction between AML and ALL according to the FAB system are based on two criteria:

1. The percentage of blasts in the bone marrow is >30% of all nucleated cells
2. Three percent or more of blasts show a positive reaction for MPO or SBB in the bone marrow.

The definition of *complete remission* in the acute leukemias has been published by the Cancer and Leukemia Group B (CALGB) and includes the following criteria *(21)*.

1. Bone marrow blasts < 5%.
2. Neutrophils > 1500/ μL.
3. Platelet count > 150,000/μL.

Some other definitions include different thresholds for neutrophils (i.e., whole white blood cell count) and platelets (>100,000/μL). In most study groups, however, the CALGB criteria are accepted and should be standard until other criteria are available.

Measurement of the percentage of blasts in the bone marrow is sometimes difficult after chemotherapy. Immature granulopoietic cells in early regeneration have to be distinguished from remaining myeloid blasts. In critical cases, the cytomorphologic appearance at diagnosis and at remission induction should be analyzed in parallel. In some cases, a control biopsy should be performed after another 7 d of hematopoietic regeneration. For deeper insights into the remission status and MRD, parallel investigations should include cytomorphology, immunophenotyping, cytogenetics (including FISH), and molecular techniques.

3. IMMUNOPHENOTYPING

In the past two decades, the impact of immunophenotyping by flow cytometry in the diagnosis and management of acute leukemia has expanded rapidly. This advance can be attributed mainly to significant progress in laser and computer technologies, the production of several hundred MAbs to a variety of antigens expressed by hematopoietic cells, and the availability of distinct fluorochromes conjugated to MAbs, allowing the simultaneous measurement of at least three or four cellular antigens in combination with two intrinsic parameters (cell size and cytoplasmic complexity) as determined on the basis of the leukemic cells' light-scatter properties [i.e., forward- and side-scatter characteristics (FSC and SSC)] *(9,22–26)*. Given these technical achievements, immunophenotyping by multiparameter flow cytometry has emerged as an optimal method for the immunodiagnosis of hematopoietic maligancies and has largely replaced microscopic analysis with immunocytochemical techniques, because flow cytometry provides an objective, sensitive, and rapid multivariate analysis of high numbers of cells. Accordingly, there is now general consensus that multiparameter flow cytometry is a powerful diagnostic tool for the immunophenotypic characterization of acute leukemias and chronic lymphoproliferative disorders that can be applied to define immunophenotypic subsets, to detect MRD, and (more recently) to develop and monitor antibody-based treatment strategies *(9,22,23,26,27)*.

Most previous studies investigating the diagnostic impact of immunophenotyping and the association between antigen expression and treatment outcome in acute leukemias have used 20% of cells stained with MAbs for surface markers and 10% for more specific markers usually expressed in the cytoplasm (e.g., MPO, CD79α, cytoplasmic CD3) as the general cut points for marker positivity *(28)*. Obviously, these values were chosen arbitrarily and have been criticized *(29)*, since they are not based on physiologic knowledge but rather serve as a convenient means of data collection. Moreover, many clinical studies (see below) describing the immunophenotypic features of acute leukemias and correlating prognosis with immunophenotyping in ALL and AML have been performed with single-color analyses. It is obvious that these studies have not always been adequate to distinguish malignant from normal hematopoietic cells and, more important, have not made use of the information provided by multiparameter flow cytometry *(24)*. Although the ability of three- or four-color immunophenotyping to resolve

unique subsets of malignant cells reliably within a complex population has been convincingly demonstrated, and its application has substantially expanded our understanding of normal and malignant subsets of hematopoietic cells, the clinical relevance of this technique in classification of the acute leukemias has been demonstrated only for flow cytometric detection of MRD (reviewed in ref. *27*). Future studies in the acute leukemias will show whether the analysis of multivariate phenotypic patterns of leukemic blasts, including the density of antigen expression *(30,31)* and its pattern of reactivity (e.g., homogeneous vs heterogeneous), using well-established and hopefully standardized flow cytometric procedures, will provide additional diagnostically and clinically relevant information.

4. GENETIC CHARACTERIZATION

Genetic analysis is an obligatory diagnostic tool in acute leukemia. The results contribute to the confirmation of the diagnosis, but, more important, the karyotype of the leukemic blasts can give important information concerning the prognosis of the disease. Different methods can be used for the genetic characterization of leukemic blasts *(32)*. Chromosome banding analysis provides an overview of all chromosomal abnormalities that can be detected at the level of light microscopy. FISH, Southern blot analysis, or PCR can be used to detect submicroscopic mutations, although for these techniques a preknowledge of the genetic aberrations is mandatory for selecting the right probes.

4.1. Chromosome Banding Analysis

Using improved staining techniques, it had become possible by 1970 to identify individually each of the human chromosomes. This advance allowed the demonstration of recurrent cytogenetic abnormalities associated with specific subtypes of acute leukemia. Such abnormalities are often translocations, a rearrangement of two or more chromosomes that leads to the formation of a new fusion or chimeric chromosome. Also, deletion or amplification of genetic material may occur, consisting of the loss or gain of an entire chromosome or a portion of a chromosome.

Chromosome analysis requires fresh, viable leukemic blasts. Preferentially, bone marrow anticoagulated with heparin is used to study the acute leukemias. Fresh leukemic blasts are prepared directly or grown for 24–72 h in cell culture. Growing cells are then treated with colchicin, which arrests the cells in metaphase, a stage in cell division (mitosis) during which DNA is condensed into readily recognizable chromosomes. The cells are then swollen in a hypotonic solution, fixed, and dropped onto glass slides *(33)*. Different treatments are used to induce a characteristic banding pattern. To obtain a reliable result, a sufficient number of metaphases with good chromosome quality is required. According to an international convention, 20–25 metaphases are completely analyzed.

To interpret cytogenetic data, a nomenclature is needed to describe the karyotype. Currently, the International System for Human Cytogenetic Nomenclature, published in 1995, is used as a standard *(34)*. The normal diploid human cell contains 46 chromosomes, composed of 22 pairs of autosomes (numbered 1–22) and two sex chromosomes. Each chromosome has a constriction called a centromere that divides the chromosome into two arms. The short arm is designated p (for petit), and the long arm q. The arms of each chromosome contain a characteristic pattern of light and dark bands, and each band is identified by a number. The karyotype is reported as a list describing the number of chromosomes, the sex chromosomes present, and any observed abnormalities. Numeric and structural abnormalities can be distinguished. A gain of a complete chromosome is called trisomy and is denoted by a plus sign (e.g., 47,XX,+8), whereas the loss of a chromosome (monosomy) is indicated by a minus sign (e.g., 45,XY,–7). The most common structural abnormalities are translocations (t), deletions (del), inversions (inv), duplications (dup), and isochromosomes (i) *(34,35)*. The general rule in tumor cytogenetics is that only the clonal chromosomal abnormalities found in a tumor should be reported. This means that at least two cells with the same aberration were observed. If the abnormality is a missing chromosome, the same change must be present in at least three cells to be accepted as clonal *(34)*.

Chromosomal aberrations can be subdivided into those that are primary and those that are secondary. Primary aberrations are frequently found as the sole karyotypic abnormality, are often associated with a particular subtype of leukemia, frequently lead to specific gene rearrangements, and are believed to be essential in initiating leukemia. Secondary aberrations, on the other hand, are rarely or never found alone; they develop in cells already carrying a primary abnormality. They usually lead to genomic imbalances and are considered to be important in leukemia progression *(35–37)*.

4.2. Fluorescence In Situ Hybridization

The FISH technique is based on the hybridization of DNA probes that identify specific chromosomal structures. FISH needs suitable DNA probes for defined chromosomal regions known to be involved in karyotypic abnormalities. DNA within metaphase chromosomes or interphase nuclei is denaturated and then hybridized to a probe, which is either directly labeled with a fluorochrome or conjugated with a hapten that can be recognized by specific antibodies bearing a fluorescent tag. The corresponding chromosome structures can be visualized as fluorescent signals *(33)*. The kinds of probes used vary depending on the clinical setting and differential diagnosis. Probes derived from repetitive centromeric sequences, which recognize specific chromosomes, can be used to detect trisomies or monosomies, whereas probes specific for individual genes can be used to detect alterations in gene structure or number. Aberrations detected by such probes include deletions, inversions, translocations, and gene amplifications. Both kinds of probes can be applied on either intact interphase nuclei or metaphase chromosomes. Whole chromosomes can be stained (or "painted") with the collection of probes distributed along the full length of a specific chromosome. The probes can only be applied on metaphase chromosomes and can be used to characterize marker chromosomes of unknown origin. FISH is a helpful tool at diagnosis for the identification of numeric and structural abnormalities and can also be used to monitor effects of therapy. The detection of MRD is hampered by the low sensitivity of this technique.

FISH techniques have provided an increased ability to identify chromosome segments, to analyze and describe complex

rearrangements, and to detect genetic aberrations in interphase nuclei. These advances mandated the development of a FISH nomenclature, which was included for the first time in the International System for Human Cytogenetic Nomenclature in 1995 *(34)*. FISH results can be included in the karyotype description obtained by chromosome banding analysis. If FISH results are obtained on metaphases, the abbreviation *ish* is used. If FISH is carried out on interphase nuclei, the term *nuc ish* is used. The chromosome bands as well as the loci of the probes used are named. The gains and losses of chromosomal material are indicated by + and –; the copy numbers of distinct loci are given by a multiplication sign and the copy number. The juxtaposition of probes is indicated by the abbreviation *con*. For example, the interphase FISH result in cells carrying a *BCR-ABL* rearrangement owing to the translocation t(9;22)(q34;q11) is reported as nuc ish 9q34(ABLx2), 22q11(*BCR*x2)(ABL con *BCR*x1). If probes that are normally juxtaposed are separated owing to an rearrangement, the abbreviation *sep* is used.

In 1996, two methods were published that are able to identify each of the 24 different human chromosomes (22 autosomes and the two sex chromosomes, X and Y) with uniquely distinctive colors *(38–40)*. The new techniques complement standard cytogenetics and are especially helpful in deciphering complex or hidden chromosomal rearrangements *(38,41)*.

4.3. Comparative Genomic Hybridization

If no viable cells are available or cells do not proliferate in vitro, chromosome analysis cannot be performed. Recently, an alternative approach was introduced that does not rely on tumor metaphases. This technique, called comparative genomic hybridization (CGH), provides an overview of DNA sequences and copy number changes (losses, deletions, gains, amplifications) in a tumor specimen and maps theses changes on normal chromosomes *(42,43)*. CGH is based on the *in situ* hybridization of differentially labeled total genomic tumor DNA and normal reference DNA to normal human metaphase chromosomes. Copy number variations among the different sequences in the tumor DNA are detected by measuring the tumor/normal fluorescence intensity ratio for each locus in the normal metaphase chromosomes. CGH only detects changes that are present in a substantial proportion of tumor cells (>50%). It does not reveal translocations, inversions, and other aberrations that do not change copy number. In leukemia, this method is only helpful in cases in which no metaphases of the leukemic cell clone can be obtained or if it is suspected that only the normal hematopoietic cells, not the leukemic blasts, divide in vitro. In ALL, this technique seems to add more information to chromosome banding analysis than it does in AML *(44–48)*.

4.4. Polymerase Chain Reaction

The PCR technique permits in vitro production of many copies of a defined DNA sequence up to several kilobases long, provided DNA sequences flanking this region on both sides are known *(49)*. The amplified DNA fragment can be detected as a band after gel electrophoresis. PCR can detect translocations, inversions, insertions, deletions, duplications, point mutations, and amplifications. Its advantage and disadvantage are one and the same: its extreme sensitivity. The technique can detect as few as 1 leukemic cell in 10^4–10^6 normal cells and therefore is very useful for the detection of MRD after cytoreductive therapy. A problem is the risk of false-positive results owing to contamination of reagents. New opportunities are emerging with the development of quantitative PCR. With techniques called real-time PCR, quantification of PCR products is more easily and more accurately reached than before, allowing one to determine the kinetics of leukemic cells during and after chemotherapy.

4.5. DNA Microarray Technology

DNA microarray technology allows one to assay thousands of unique nucleic acid samples simultaneously. This technology consists of the immobilization or synthesis of nucleic acids at high density on a solid support. The array is used as a detector for the hybridization of a complementary, fluorescently labeled nucleic acid probe *(11,12)*. Gene expression profiles can be determined, and sequence analysis of polymorphisms and detection of mutations can be performed with this new technique. In contrast to conventional methods that are limited to the analysis of expression of a few genes, the DNA array technology is capable of analyzing the profile of all genes expressed in a cell or organ. The first experiments on cell lines established from leukemia have proved that this new technique is reproducible and allows the analysis of downstream genes of leukemia-specific fusion transcripts. Golub et al. analyzed 27 patients with ALL and 11 with AML and were able to define 50 of 1100 genes that allow ALL to be distinguished from AML *(13)*. Gene expression profiling with the help of DNA microarrays might add useful information for the classification of acute leukemias and, more important, will lend insight into the pathogenetic mechanisms of leukemia in the future.

5. FAB-CLASSIFICATION OF AML

The FAB classification of AML follows an algorithm and distinguishes 11 different subtypes *(3–6,18,19)*. These strict definitions of AML do not take into account underlying biologic characteristics *per se*. Without the FAB categories, however, chromosomally defined entities and most correlations between morphology and cytogenetics would have been missed in the last 25 years. Moreover, correlations between diagnostic and therapeutic results in AML were first recognized by use of the FAB system. This is still the case today.

5.1. Correlations of Morphologic and Cytochemical Features with Immunophenotyping, Cytogenetics, and Molecular Genetics

Since publication of the original FAB classification for acute leukemias in 1976, several revisions have appeared. Although the concordance rate among observers differed from 65% to 80% *(3,50–52)*, it was possible to correlate specific and recurrent morphologic features with cytogenetic and immunophenotypic results. In AML, use of the FAB system led to the description of more specific morphologic details and correlations with cytogenetic results (Table 1):

1. AML M2 (or-M1), showing dysgranulopoiesis, increase of normal eosinophils, very mature blasts (type II and type III), and long, needle-like Auer rods were found to be associated cytogenetically with t(8;21) *(53,54,55,56)*.

Table 1
FAB Classification of AML

Subtype	*Definition*
AML-M0	No maturation, MPO <3%, *but* myeloid markers by immunophenotyping present
AML-M1	Blasts ≥ 90% of nonerythroid cells, MPO ≥ 3%
AML-M2	>10% of myeloid cells show maturation from promyelocytes onwards, monocytes <20%
AML-M3	Predominant cells are highly abnormal promyelocytes
AML-M3v	Predominant cells are bilobulated blasts with strong MPO reactivity
AML-M4	Myelomonocytic blast cells, with monocytic component >20% but <80%
AML-M4eo	Like M4, with abnormal eosinophils (usually >5%)
AML-M5a	Monoblasts ≥ 80% in bone marrow
AML-M5b	Monoblasts and monocytes ≥ 80% in bone marrow
AML-M6	Erythroblasts ≥ 50% of total nucleated cells, *and* ≥ 30% of nonerythroid cells are blasts
AML-M7	Blasts demonstrated to be megakaryoblasts by immunophenotyping (CD41+, CD61+)

Abbreviations: FAB, French–American–British; MPO, myeloperoxidase.

2. AML M2 baso showed an increase of basophils besides the typical M2 morphology and was in some cases correlated with t(6;9) *(57)*.
3. AML M2, with increased megakaryocytes, mostly with two little nuclei, and in many cases normal or even elevated platelet counts, was seen in cases with inv(3)(q21q26) *(58,59,60)*.
4. AML M3 *(61)* and its variant (AML M3v) *(19)* were found to bear the same cytogenetic abnormality, namely, t(15;17).
5. In AML M4, with abnormal eosinophils (M4eo), inv(16) or t(16;16) was detected *(62–65)*. In contrast to normal eosinophils, these abnormal eosinophils show large basophilic granules and abnormal granular positivity with CAE.
6. In cases of AML M4 or AML M5 with erythrophagocytosis, t(8;16) was found.
7. Another correlation was observed in cases with dysplastic granulopoiesis and pseudo-Pelger-Huët anomalies, sometimes showing alterations in chromosome 17p involving the *p53* gene *(66)*.
8. In cases of monocytic AML (M4, M5), the 11q23 chromosome region was frequently involved.

The strongest correlations between cytomorphology and cytogenetics were clearly found in AML M3(v) and AML M4eo; all other associations are much weaker. It should be noted, however, that only cytogenetic and/or molecular genetic analyses can lead to the definition of a biologic entity. For example, the threshold of 5% abnormal eosinophils for the diagnosis of AML M4eo is arbitrary, because some cases show < 1% clearly abnormal eosinophils.

5.2. Clinical Impact of Specific Morphologic Features on Diagnosis

In AML, but not ALL, more detailed morphologic approaches have led to deeper insights into the biology of acute leukemias and allow one to predict the prognosis more precisely. The presence of dysplasia, for example, is now included in the new WHO proposal *(20)*. It therefore seems appropriate to present some data from the literature as well as our own data [from the AML Cooperative Group (AMLCG)].

5.2.1. Auer Rods

The detection of Auer rods can lead to the diagnosis of AML. However, the AMLCG study detected Auer rods in only 45.5% of 601 cases of AML *(67)*. It is well known that Auer rods may give important information in AML, as follows *(4,16,68,69)*:

1. In cases with MPO < 3% (normally classified as AML M0), the detection of Auer rods alone leads to the diagnosis of AML M1, by definition.
2. In AML M1 and-M2 carrying t(8;21), very often long, thin, so-called needle-like Auer rods can be seen, and one may even predict this aberration before the cytogenetic result is available.
3. In most cases of AML M3 (and more or less in M3v), bundles of Auer rods can be seen in the cytoplasm. These cells are called faggot cells. However, only the detection of t(15;17) or the *PML/RARα* fusion gene will make the diagnosis absolutely safe.

In pediatric AML studies, the detection of Auer rods was correlated with a better prognosis *(70,71)*. In our study of 601 adult patients with AML, the detection of Auer rods was correlated with a better prognosis with respect to overall survival ($p = 0.0001$), relapse-free survival ($p = 0.01$), and event-free survival ($p = 0.0003$). This was also true when patients with t(8;21) or t(15;17) were excluded from the cohort of Auer rod-positive patients *(67)*.

5.2.2. Dysplastic Features at Diagnosis

The detection of dysplasia was described as essential in myelodysplastic syndromes but was also investigated in *de novo* AML at diagnosis *(67,72–83)* and during remission *(74)*. However, studies in AML were mostly retrospective, and criteria for the definition of dysplasia were not generally accepted. This makes it very difficult to compare results. The WHO classification referred to this morphologic feature and defined an AML subgroup with multilineage dysplasia as the presence of dysplastic features in two or more cell lines *(20)*. In our investigations, we followed the definitions of Goasguen and Bennett *(76)*:

1. *Dysgranulopoiesis (DysG)* was defined as agranular or hypogranular features, or hyposegmented nuclei (pseudo Pelger-Huët anomaly), in >50% of at least 10 PMNs. At least 25 cells were observed, but usually 100 cells were counted. MPO deficiency in the PMNs was defined as >50% MPO-negative cells in at least 10 PMNs after strong positivity of eosinophils or other PMNs was confirmed.
2. *Dyserythropoiesis (DysE)* was defined as dysplastic features in >50% of at least 25 erythroid precursors, including megaloblastoid aspects, karyorrhexis, nuclear particles, or multinuclearity. A minimum of 25 cells have to be counted.

Table 2
Relationships Among Morphology, Immunophenotypic Features, and Genetic Aberrations in AML[a]

Antigen	*M0*	*M2t(8;21)*	*M3t(15;17)*	*M4Eoinv(16)*	*M5*	*M5t(9;11)*	*M7*
MPO	+/–	+	+	+	–/+	–	–
CD2	–			+/–			
CD7	–/+	–	–	–	–/+	–	–/+
CD13	+/–	+	+	+	+/–	–/+	+/–
CD14	–	–	–	+/–	+/–	–/+	–
CD15	–	+/–	–/+	+/–		+	–
CD19	–	+/–					
CD33	+/–	+/–	+	+	+	+	+/–
CD34	+/–	+/–	–	–/+			
CD56		+/–				+/–	
CD41/CD61	–	–	–	–	–	–	+
CD64	–	–	+/–	+	+	+/–	
CD65	–/+	+/–	–/+	+	+/–	+	+/–
CD117	+/–	+/–	–/+	+/–	–/+		
HLA-DR	+/–	+	–	+	+	+	+/–

[a] –, antigen not expressed; –/+, antigen expressed in <50% of patients; +/–, antigen expressed in the majority of patients; +, antigen expressed; open fields represent partial expression without specificity for diagnosis or lack of reliable data.

3. Dysmegakaryopoiesis (DysM) was diagnosed when at least three megakaryocytes or >50% of at least six cells showed dysplastic features, such as microkaryocytes or multiple separated nuclei or very large single nuclei.

Trilineage dysplasia (TLD) was diagnosed when DysG, DysE, and DysM were detectable. In the AMLCG series of 601 prospectively analyzed patients with *de novo* AML, no dysplasia was seen in 45.1%, DysG was seen in 16.7%, DysE in 10.2%, DysM in 22.3%, and trilineage dysplasia in 14.3%. Although there was a trend toward better overall survival in patients without any dysplasia in comparison with multilineage dysplasia (i.e., two cell lines) or TLD, none of the differences tested were statistically significant in this cohort *(67)*.

The analysis was also not able to demonstrate significant differences between patients with no dysplasia and one-lineage dysplasia (combined group) vs patients with multilineage dysplasia, after we excluded all patients with specific cytogenetic abnormalities according to the definition of the WHO proposal *(20)*.

Another cohort was defined for subgroup analysis: we investigated only patients with normal karyotype with respect to dysplastic features. Patients with no dysplasia or single-lineage dysplasia showed no significant difference from patients with multilineage dysplasia in relapse-free ($p = 0.4$) or event-free survival ($p = 0.1$). These results stress the need for further investigations. Subgroup analysis has to be performed before multilineage dysplasia in AML can be proved to define an independent biologic entity.

5.2.3. Dysplasia and Its Correlation with Secondary AML

In many studies, the detection of dysplasia in one to three cell lineages has been used to define a case as *secondary* AML after an antecedent myelodysplasia. There is no clinical evidence that this conclusion is true *(79)*. The discrimination between *de novo* AML and AML after a myelodysplastic syndrome (MDS) can be made only from the history of the patient (i.e., abnormal white blood cells and unexplained anemia or thrombocytopenia in the past). If this is not possible, and the patient's history with respect to fatigue or other adverse features lasts only some weeks or up to 2–3 mo, one cannot predict that any AML with specific dysplastic features evolved from a preceding MDS. Drawing conclusions like this does not improve understanding of AML pathogenesis and may even lead to wrong treatment decisions.

6. IMMUNOPHENOTYPING OF AML

Immunophenotyping by flow cytometry has been instrumental in recognizing minimally differentiated AML (AML M0), acute megakaryoblastic leukemia (AML M7), and AML coexpressing lymphoid-associated antigens *(5,6,25,28)*. It has been especially helpful in distinguishing AML with monocytic differentiation from AML M0/M1 or AML subtypes with granulocytic differentiation (i.e., AML M2/M3) *(84)*. The diagnostic sensitivity of a comprehensive panel of MAbs to myeloid and lymphoid lineage as well as progenitor cell-associated antigens has been demonstrated in both childhood and adult AML *(25,85–88)*. Although none of the antimyeloid MAbs used in these studies recognized the blast cells of all AML patients, nearly all AML cases can nowadays be detected by using a combination of two or three panmyeloid reagents (i.e., CD13, CD33, CD65) with MAbs to MPO that detect both the proenzymatic and the enzymatic forms of MPO. Except for MPO and megakaryocyte-associated antigens (e.g., CD41a, CD61), however, expression of myeloid-associated markers is not restricted to AML. Attempts to correlate immunophenotypic features with the various AML subtypes (AML M1 through AML M6) according to the FAB classification have been largely unsuccessful (22,25,89). Although some AML subtypes (e.g., AML M3; see below) show a characteristic immunophenotypic profile, there are few entirely consistent relationships between morphology and immunophenotype (Table 2). Thus, cases with identical antigen expression may belong to different FAB subtypes, and different immunophenotypic features are found in the same FAB subtypes.

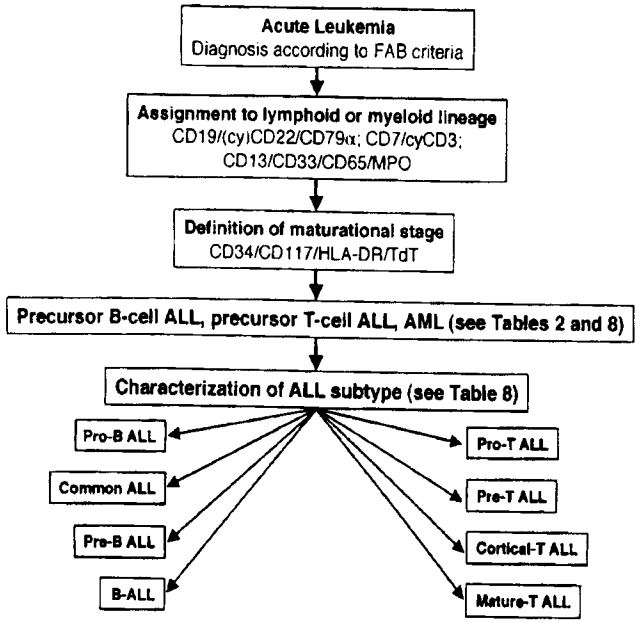

Fig. 1. Flow chart indicating the antigens essential for lineage assignment and definition of maturational stage in AML and ALL.

In AML, interpretation of immunophenotyping studies may be confusing, because leukemic blasts in bone marrow and peripheral blood specimens are frequently admixed with normal hematopoietic cells, and the blast cell population can also be heterogeneous. Therefore, various multiparameter flow cytometry techniques have been proposed that facilitate the identification of leukemic cells. Among these techniques, the leukocyte common antigen (CD45)/side-scatter (SSC) gating procedure allows an efficient discrimination between the blast cell population and the normal cells and facilitates the analysis of leukemic blasts present in low proportions (90,91). The use of CD45/SSC gating, primarily gated on blast cells identified by virtue of low/intermediate CD45 density, correlates with bone marrow differential and provides characteristic flow-cytometric profiles for most subtypes of AML *(25,84,91)*. Moreover, this gating strategy has been demonstrated to give similar results in leukemic specimens enriched for leukemic blasts by density-gradient separation techniques and in lysed whole bone marrow or peripheral blood samples *(91)*. Based on these observations, several authors suggested that CD45/SSC gating should replace forward scatter (FSC)/SSC gating, and that this method could contribute to reduced costs without affecting diagnostic quality *(91–93)*.

By applying multiparameter flow cytometry to immunophenotyping studies, it has recently been shown that the antigenic profiles of AML differ significantly from the antigen expression pattern on normal bone marrow elements. These phenotypic aberrations of leukemic blasts, often referred to as asychronous or aberrant antigen expression, are probably related to the underlying genetic alterations as well as to a disturbed regulatory control of particular proteins *(94–96)* and may be useful in both screening for genetic abnormalities and monitoring of MRD.

Several antigen-screening panels for immunophenotyping of AML have been recommended *(25,28,97,98)*, mainly including MAbs directed toward antigens expressed by early hematopoietic progenitors and relatively lineage-restricted antigens. The screening panel currently applied to immunophenotypic characterization of acute leukemias in our institution is presented in Fig. 1.

In the following sections, we describe the immunophenotypic features of AML M0 and AML M7 and summarize the antigenic profiles that may be associated with clinically relevant entities such as AML with t(8;21), acute promyelocytic leukemia with t(15;17), AML with abnormal bone marrow eosinophilia and inv(16) or t(16;16), and AML with 11q23 abnormalities (Table 2). Although the relevance of immunophenotyping for the identification of AML subtypes carrying a specific genetic abnormality has been questioned, recent studies based on multivariate phenotypic pattern and light scatter characteristics instead of individual antigen expression have demonstrated markedly improved sensitivity and specificity of immunophenotyping (94,99).

6.1. AML Minimally Differentiated (*AML M0*)

Leukemias of the M0 subtype, which cannot be recognized on morphologic grounds alone, comprise 3–6% of pediatric and up to 10% of adult AML cases. In 1991, the FAB Cooperative Group listed morphologic, cytochemical, and immunophenotypic diagnostic criteria and proposed the designation M0 for these leukemias *(6)*. These criteria included negative cytochemical reactions to MPO and SBB stains, no evidence of lymphoid differentiation by immunophenotyping, and expression of myeloid antigens (e.g., CD13 or CD33) or the demonstration of the enzyme MPO by immunophenotyping and/or electron microscopy.

More recently, stricter guidelines for excluding lymphoblastic and megakaryoblastic leukemias have been proposed. They are based on the availability of more specific lineage-restricted MAbs, the use of multicolor flow cytometry, and the cytoplasmic detection of myeloid antigens in fixed cells (e.g., CD13, MPO) *(25,28,100)*. According to these criteria, acute leukemias devoid of detectable MPO can only be classified as AML M0 in the absence of lineage-restricted lymphoid (e.g., CD3, CD22, CD79α, TCRβ) and megakaryocytic antigens (e.g., CD41, CD61). Most M0 cases express CD13, CD33, and CD65 as well as progenitor cell-associated antigens, such as HLA-DR, CD7, CD34, and CD117 *(101–104)*, whereas other myeloid lineage-associated antigens (e.g., CD14, CD15) are rarely found. The reliability of anti-MPO antibodies for detecting minimal myeloid differentiation in cases with CD13 and CD33 negativity has been demonstrated *(102,105,106)*. Up to 80% of M0 cases may have a complex composite immunophenotype with expression of myeloid as well as non-lineage-restricted lymphoid markers, including CD2, CD7, CD4, CD19, and terminal deoxynucleotidyl transferase, which makes them difficult to classify as AML, ALL, or biphenotypic acute leukemia (see below) *(100)*. Cytogenetic studies reveal a variety of clonal abnormalities (such as complex karyotypes, anomalies of chromosome 5 and/or 7, trisomy 8, and trisomy 13, reflecting the heterogeneity of minimally differentiated AML) and indicate that AML M0, very similar to AML M1, is not a unique leukemia subtype but probably includes distinct malig-

nant myeloid processes with different underlying cytogenetic and/or molecular genetic defects *(101,102)*. The cytogenetic abnormalities and the higher level of P-glycoprotein expression described in most but not all studies may contribute to the poor treatment outcome that has been observed in adults with AML M0 *(102,103,107)*.

6.2. AML M2 Harboring t(8;21)

Several studies have described the distinctive immunophenotypic features of AML M2 cases harboring the translocation t(8;21), which complement the characteristic morphologic findings of this AML subtype *(96,108–111)*. These features include expression of CD13, CD15, CD33, CD34, CD65, and HLA-DR with frequent coexpression of the B-cell-associated antigen CD19 and the neural cell adhesion molecule CD56. Notably, however, recent case reports of adult patients with a so-called myeloid surface antigen-negative phenotype have been published *(112–114)*, indicating that low levels or absence of the panmyeloid antigens CD13 and CD33 may occur in parallel with expression of MPO as detected by cytochemical staining or flow cytometry. Controversial findings have been described regarding the frequency and intensity of CD19 and/or CD56 expression in AML M2 with t(8;21) *(87,115)* and have led to questions as to whether these aberrant phenotypic features occur frequently enough to allow selection of cases for molecular screening on the basis of immunophenotyping *(110)*. This discrepancy may be caused by the usually weak and variable expression of CD19 and CD56 on AML cells, thus requiring special gating strategies to separate blasts from whole mononuclear cell fractions in flow-cytometric analysis and by other methodologic aspects, including the use of different CD19 MAbs and the application of different staining techniques. Recent studies have shown overexpression of CD34 in an asynchronous combination with cytoplasmic MPO and have suggested that quantification of CD34 expression might be useful for both rapid diagnosis and remission assessment in AML with t(8;21) *(96)*.

6.3. Acute Promyelocytic Leukemia with t(15;17)

The characteristic but not unique immunophenotypic features of acute promyelocytic leukemia APL with the t(15;17) include (1) absence or low expression of HLA-DR, CD7, CD14, CD15 (as detected with the MAbs VIM-D5 or VIM-C6), and CD34; (2) variable expression of CD11b, CD65, and CD117; and (3) weak expression of CD64 and strong expression of CD9, CD13, CD33, CD68, and MPO *(84,87,116–118)*. More recently, the combined use of three phenotypic criteria [i.e., presence of a single blast cell population, heterogeneous reactivity for CD13 and the pattern of expression of CD34/CD15 as detected by the Leu-M1 MAb] *(94)*, and the availability of antibody reagents directed against the promyelocytic leukemia (PML) protein *(119)* have improved the sensitivity and specificity of immunophenotyping studies for rapid screening of APL, especially in cases with M3v morphology or rare cases of t(15;17)-positive leukemias resembling AML M1 and AML M2 *(120,121)*. In addition, immunophenotyping has been helpful in differentiating acute myelomonocytic or monocytic leukemias from the microgranular variant, which usually, unlike AML M4 or-M5, does not express CD4, CD14, CD36, and HLA-DR and exhibits distinct light-scatter characteristics owing to its high MPO content. Recent studies suggested a correlation between immunophenotypic characteristics and morphologic, molecular-genetic, and clinical features of childhood and adult APL that may be useful for a better determination of the biologic and clinical heterogeneity of this subtype. A strong association of the S (short) transcript resulting from a break at *bcr3* of the *PML* gene and M3v morphology with CD2 positivity has been described *(118,122,123)*, as well as a more frequent expression of CD56 in APL with the S-isoform subtype *(124)*. Interestingly, both aberrant immunophenotypic features were of prognostic significance, with CD2 positivity predicting a better complete response (CR) rate and event-free survival in APL *(118)*, whereas CD56 was associated with a poor treatment outcome in a small series of adult patients treated with various treatment protocols *(124)*.

Immunophenotypic features of rare AML cases, which morphologically resemble AML M3 and do not express the *PML-RARα* fusion gene but show rearrangement of the *RARα* locus with genes other than *PML* on chromosome 15, such as t(11;17) and t(5;17), are similar to the pattern seen in typical AML M3. Interestingly, expression of CD56 in parallel with functional natural killer (NK) cell-mediated cytotoxicity was observed in four cases with t(11;17) *(125,126)*.

6.4. *AML M4Eo*

The diagnostic value of multiparameter flow cytometric analysis for the detection of small subpopulations with aberrant immunophenotypic features has been nicely illustrated in AML M4Eo by demonstrating expression of CD2 on leukemic blasts with inv(16) or t(16;16) *(127,128)*. By using multiparameter flow cytometry, two major leukemic cell populations are evident in AML M4Eo with expression of panmyeloid and granulocytic or monocytic antigens, including CD4, CD13, CD14, CD15, CD33, and CD65. Similar to AML M2 with t(8;21), other characteristic features of AML M4Eo include frequent positivity of CD34 and the absence of CD7 *(87)*. The availability of MAbs to the chimeric CBFβ-MYH11 protein may be used in flow cytometric analyses to screen for the inv(16) abnormality *(129)*.

6.5. *AML M5* with 11q23 Aberrations

Previous studies in patients with acute myelomonocytic leukemias associated with 11q23 aberrations failed to show a specific immunophenotypic pattern on leukemic blasts that might be used to distinguish acute myelomonocytic leukemias with 11q23 translocations from FAB M4 or M5 cases without 11q23 involvement *(130,131)*. However, results in children and adults with *MLL* rearrangements, usually owing to t(9;11), disclosed characteristic features such as strong expression of HLA-DR, CD33, CD65, and CD4, whereas other myeloid lineage-associated antigens (e.g., CD13, CD14) and CD34 were detected in <30% of cases *(87,132,133)*. Furthermore, we *(133)* and others *(134)* have observed a frequent coexpression of CD56 in AML with monocytic differentiation and rearrangement of the *MLL* gene.

Interestingly, previous studies testing the reactivity of a MAb, 7.1, which detects the human homolog of the rat NG2 chondroitin sulfate proteoglycan molecule, have noted strong associations among blast cell expression of the NG2 molecule, FAB M4/M5 morphology, and 11q23 abnormalities in child-

hood AML *(135,136)*. In agreement with these results, we recently demonstrated in a large series of patients, including children and adults with AML, that the MAb 7.1 is a sensitive but not entirely specific marker for the identification of 11q23-associated AML *(133)*. Moreover, we observed frequent coexpression of NG2 and CD56 in AML with monocytic differentiation, raising the question of whether these molecules, both probably involved in cell adhesion and migration mechanisms, have any pathophysiologic impact on the clinical behavior of this AML subset.

Recent studies suggested that addition of CD64 and CD45 intensity vs logarithmic side-scatter analysis to CD14, a highly specific but relatively insensitive monocytic marker, may greatly improve flow cytometric detection of AML with monocytic differentiation *(84)*. In addition, data from a large series of Japanese adult patients suggested that AML with myelomonocytic differentiation, often associated with 11q23 abnormalities or with inv(16), exhibited a typical surface antigen expression pattern (i.e., $CD34^{low}$, $CD33^{high}$, $CD11b^{high}$, GM-CSF-R^{high}, and $CD4^{positive}$) *(137)*.

In future studies, it will be important to determine whether the analysis of these phenotypic features by multiparameter flow cytometry will contribute to a more relevant subdivision of AML with monocytic differentiation and whether such studies have prognostic implications.

6.6. Acute Megakaryocytic Leukemia (AML M7)

The differentiation of AML M7 from ALL, AML M0, and sometimes small tumors in children is usually not possible by morphologic and cytochemical studies. Therefore, the diagnosis of AML M7 must be confirmed by immunophenotypic detection of different platelet glycoproteins indicating megakaryocytic differentiation (e.g., CD41a, CD61) or by ultrastructural demonstration of platelet peroxidase *(5,18)*. Immunophenotyping studies, however, are more readily performed than ultrastructural studies and have largely replaced the latter *(138,139)*. Leukemic blasts in AML M7 express CD61, CD41a, and (less frequently) CD42b. In addition, most cases express CD4, CD33, CD34, CD36, HLA-DR, and (less frequently) CD13. Coexpression of lymphoid antigens, especially CD7 or CD2, has been described. Cytoplasmic expression of platelet glycoproteins may precede the cell surface expression of these markers and should be tested in cases with undifferentiated morphology and negative or inconclusive cytochemistry to differentiate AML M7 from AML M0 and ALL *(140)*. Caution must be exercised, as platelet adherence to leukemic blasts as well as nonspecific binding of glycoproteins IIb/IIIa to AML M5 may result in false-positive CD41a and CD61 staining results. Therefore, confirmation of flow cytometric results by cytospin immunofluorescence should be performed in all cases with equivocal immunophenotyping and morphologic findings.

Recently, characteristic immunophenotypic features have been described within the CD34+ stem-cell compartment in patients with AML M7 *(141)*. In comparison with CD34+ cells in AML M0 through AML M6 subtypes, the CD34+ megakaryoblasts expressed CD61 and glycophorin A but were CD38–. These results are in line with the hypothesis of a common immature progenitor cell for the megakaryocytic and erythroid cell lineages *(142)* and the expression of megakaryocytic antigens occasionally observed in acute erythroleukemia as well as the positivity of glycophorin A in some cases of AML M7.

The chromosomal abnormalities associated with AML M7 include t(1;22)(p13;q13) *(143)*, constitutional or acquired trisomy of chromosome 21 *(144–147)*, and occasionally rearrangements of 3q21 and 3q26. In contrast to t(1;22), which is exclusively observed in infants and is not associated with any dysplastic features, rearrangements of 3q21 and 3q26 are not specific for AML M7, have been demonstrated in all subtypes of AML except AML M3, and occur mainly in older patients whose leukemic blasts may display dysplastic features *(60)*.

It should be noted that infants with Down's syndrome often present with a transient myeloproliferative disorder, which, by immunophenotyping studies, commonly shows evidence of megakaryocytic as well as erythroid differentiation and by light microscopy or immunophenotyping is indistinguishable from AML *(146,147)*. Leukemic blasts in children with Down's syndrome and AML M7 often show evidence of erythroid differentiation as well and coexpress CD7 *(146,147)*.

6.7. Prognostic Implications of Immunophenotyping in AML

The prognostic significance of surface antigen expression in AML is still a matter of controversy. Although some investigators, especially in childhood AML, could not show any correlation between the expression of individual progenitor-, myeloid- or lymphoid-associated antigens and treatment outcome *(87,148,149)*, others suggested a significant influence of specific antigens or combined phenotypic features on the CR rate and/or CR duration and survival. Among the antigens implicated as having an adverse prognostic effect are CD7, CD9, CD11b, CD13, CD14, HLA-DR, CD34, and TdT *(106,150–153)*. On the other hand, the presence of CD15, CD65, and CD2 has been associated with a better treatment outcome *(154,155)*. Other authors could not confirm these findings (e.g., CD2, CD7, CD34, TdT) *(87,148,149,155–157)*. The comparability of most of these results, however, is hampered by methodologic differences such as the choice of MAbs and techniques applied to the detection of antigen expression, inconsistencies in criteria for defining antigen positivity, and variation in the patient populations studied (i.e., children and/or adults) or the treatment administered.

Moreover, the prognostic value of correlating clinical outcome with specific antigens rather than evaluating the composite immunophenotype must be questioned in view of recent findings demonstrating that expression of particular antigens can be associated with favorable as well as poor prognostic genetic aberrations. For example, t(8;21), inv(16), chromosome 5 and 7 aberrations, and complex karyotypes were more frequently observed in CD34+ AML *(86,158)*, and CD19 coexpression may occur in AML with either t(8;21) or t(9;22) *(87,159,160)*. These results suggest that CD34+ and/or CD19+ AML comprise a heterogeneous group of patients with good as well as poor risk factors. Recent data suggesting a prognostic role of CD56 expression in AML with t(8;21) *(111)*

and APL *(124)* but not in AML with 11q23 translocations *(130)* are in line with this statement. Moreover, in future studies, expression of surface antigens should also be interpreted in the context of other cell biologic features, including differentiation stage and functional characteristics reflecting cellular resistance mechanisms to cytotoxic drugs (e.g., multidrug-resistance phenotype, expression of apoptosis-regulating proteins) *(157,158,161–163)*. Our own results in a large series of untreated children and adults with *de novo* AML enrolled in the German AML-Berlin-Frankfurt-Münster (BFM) and AMLCG studies do not show any influence of the expression of individual myeloid-, lymphoid-, and progenitor-cell-associated antigens on prognosis *(87,156,164,165)* and thus do not indicate that immunophenotyping alone can be applied in risk stratification in AML at diagnosis. These findings are in line with other recent studies in children *(148,149)* and adults *(85)* with AML.

7. GENETIC ABNORMALITIES IN AML

Cytogenetic analysis is the most important diagnostic tool for determining prognosis in AML *(166–169)*. Cytogenetic studies have revealed that acquired clonal chromosome aberrations can be observed in most patients with AML *(35)*. Numerous recurrent karyotypic abnormalities have been discovered in AML (Table 3) *(37,170)*. Chromosome analysis has paved the way for molecular studies that have identified genes involved in the process of leukemogenesis *(171)*. Furthermore, the identification of specific chromosomal abnormalities and their correlation with cytomorphologic features, immunophenotype, and clinical outcome have led to a new understanding of AML as a heterogeneous group of distinct biologic entities. The importance of cytogenetic findings in AML for classification and for the understanding of pathogenetic mechanisms is increasingly appreciated in a clinical context and also in the new WHO classification, which uses cytogenetic abnormalities as a major criterion *(20)*.

The incidence of abnormal karyotypes in AML has been reported to be 55–78% in adults and 77–85% in children *(35,172–178)*. However, a substantial proportion of patients with AML have no chromosome abnormalities. Although it is possible that normal karyotypes may be attributed in some cases to the existence of nonmalignant cells dividing preferentially in vitro, the fact that in many patients the normal karyotype observed at diagnosis remains normal at relapse suggests that the absence of cytogenetic aberrations is a real phenomenon rather than a failure to detect aberrations *(179,180)*. Recent data indicate that a proportion of cytogenetically normal patients displays submicroscopic gene alterations that can only be detected by molecular methods. For instance, approx 6% of adult AML patients with a normal karyotype display a partial tandem duplication within the *MLL* gene *(181,182)*.

Attempts to classify cytogenetic data in AML have led to recognition of two distinct karyotypic patterns. One is characterized by balanced rearrangements leading to specific gene rearrangements, whereas in the other, unbalanced aberrations result in large-scale genomic imbalances. According to the hypothesis of Johansson et al. *(36)*, there are no unbalanced primary aberrations. An unbalanced "primary" abnormality is secondary to a submicroscopic, truly primary change. Therefore, in cases without a balanced primary abnormality, molecular analysis might reveal the underlying primary defect. Especially in patients with complex aberrant karyotypes, who show a variety of different unbalanced aberrations, submicroscopic abnormalities (such as mutations in DNA repair genes) leading to genetic instability must be suspected.

Table 3
Chromosomal Abnormalities in AML

Cytogenetic change	*Fusion gene*	*FAB subtype*	*Frequency*	
			Children (%)	*Adult (%)*
t(8;21)(q22;q22)	*AML1-ETO*	M2/M1	10–15	8–12
inv(16)(p13q22)	*CBFβ-MYH11*	M4eo	6–12	8–12
t(15;17)(q22;q12)	*PML-RARα*	M3/M3v	8–15	8–10
t(9;11)(p22;q23)	*MLL-AF9*	M5a	8–10	1–2
t(3;21)(q26;q22)	*AML1-EAP/EVI1*	—	1	<1
t(6;9)(p23;q34)	*DEK-CAN*	M1/M2	1–2	Rare
inv(3)(q21q26)	*EVI-?*[a]	—	<1	1–2
t(1;22)(p13;q13)		M7	2	–
+8 sole		—	1–4	3–5
+11 sole		M1/M2	–	<1
Complex		—	6	10–20

[a]?, unknown.

7.1. Primary Chromosome Abnormalities and Their Molecular Correlates

Primary chromosomal aberrations are frequently found as the sole karyotypic abnormality and are often specifically associated with a particular AML subtype. On average, 55% of AML patients with karyotypic abnormalities have only one rearrangement (15–20% have gain or loss of a single chromosome) *(35)*.

7.1.1. t(8;21)(q22;q22)/*AML1-ETO*

A t(8;21)(q22;q22) was first identified in 1973 *(183)*. This is the most frequent abnormality in AML in children (incidence, 10–15%) and occurs in approx 7–10% of patients with AML in Europe and the United States *(35,184)*. However, its frequency varies, and it is reported to be particularly common in Japan (37.5%) and in South Africa (62.5%) *(35)*. The 8;21-translocation is more frequent in the young and is rare beyond the age of 50. In >90% of patients, it is associated with a FAB-M2 subtype (around 10% show a M1-subtype) *(55,185)*. The translocation breakpoints have recently been cloned *(186,187)*. The breakpoints in 21q22 cluster to a limited region of the *AML1* gene, which is an important transcription factor in hematopoietic cells *(188,189)*. The 8q22 breaks cluster to the locus of a gene with putative zinc finger DNA binding motifs called *ETO* or *MTG8*. The translocation leads to a consistent hybrid gene encoding a novel message that can be consistently detected by reverse transcriptase RT-PCR *(190)*. Data on the detection of MRD with RT-PCR demonstrate that even in patients in long-term clinical remission, *AML1-ETO* fusion transcripts are still detectable *(191)*. New methods allowing the quantification of transcripts may be more helpful for treatment decisions because the kinetics of the amount of transcripts may be more important than the observation that *AML1-ETO* transcripts are still detectable *(192)*.

To elucidate the role of *AML1-ETO* in leukemogenesis, mice with a knocked-in *AML1-ETO* gene were generated *(193)*. These studies suggest that *AML1-ETO* not only neutralizes the normal biologic activity of *AML1* but also directly induces aberrant hematopoietic cell proliferation *(194)*.

7.1.2. t(15;17)(q22;q12)/*PML-RARα*

The t(15;17) is specifically associated with APL and is found in virtually all cases with an M3 or an M3v FAB subtype if optimal chromosome analysis is performed *(61)*. The incidence of t(15;17) in different APL series has varied between 41% in the early days of cytogenetics and 100% in recent series *(35)*. This is because t(15;17) can only be detected when chromosomes are of good quality. Furthermore, it is important that APL cells be cultivated for 24 and 48 h before chromosome preparation, as it has been shown that direct preparation yields mostly only normal metaphases, whereas abnormal metaphases increase after culturing for 24 and 48 h *(195)*.

In 1976, Golomb et al. *(196)* described for the first time an abnormal chromosome 17 in two APL patients. Rowley et al. *(61)* in 1977 found that the rearranged chromosome arose through a reciprocal translocation between the long arms of chromosomes 15 and 17. When the translocation breakpoints were cloned, it turned out that the retinoic receptor α gene (*RARα*) on chromosome 17 and the *PML* gene on chromosome 15 are involved *(197)*. As a result of the translocation, the truncated *RARα* gene is moved to chromosome 15, where it is fused to PML and gives rise to a *PML-RARα* hybrid gene that can be consistently detected by RT-PCR *(198,199)*. Experiments with transgeneic mice expressing *PML-RARα* suggest that this fusion product is not sufficient to cause APL directly, but its expression alters myeloid development, resulting in an accumulation of myeloid precursors that may be susceptible to cooperative transforming events *(200)*.

Remarkably, APL with the *PML-RARα* rearrangement responds—albeit temporarily—to treatment with all-*trans*-retinoic acid (ATRA). After an initial transient proliferation, the APL cells differentiate, senesce, and die. The introduction of ATRA in chemotherapy protocols has improved outcome dramatically *(201,202)*. As ATRA treatment has to start immediately in all cases with clinical suspicion or cytomorphologic evidence of AML M3 or AML M3v, t(15;17)/*PML-RARα* has to be confirmed or excluded. In our hands the FISH technique is the quickest method, as a result can be obtained within 4 h. Chromosome banding analysis has to be performed as well to detect variant translocations or additional abnormalities. PCR also has be performed at diagnosis to allow the monitoring of MRD during and after treatment. In most patients, the *PML-RARα* transcript cannot be detected by PCR after treatment. Several clinical trials were able to show that if PCR becomes positive again, patients will relapse *(203,204)*. Data obtained using the new real-time PCR technique, allowing quantitation of MRD, show a decrease of *PML-RARα* copy numbers during therapy and an increase at the time of relapse *(205)*.

7.1.3. inv(16)(p13q22)/t(16;16)(p13;q22)/*CBFβ-MYH11*

There is a close association between inv(16) and its variant t(16;16) in AML M4 with abnormal eosinophils *(62)*. Although different FAB subtypes have been reported in patients with inv(16), there is compelling evidence that AMLM4eo/inv(16) is a biologic entity. On the molecular level, the smooth muscle myosin heavy-chain gene (*MYH11*) on 16p13 is fused to the *CBFβ* gene on 16q22 *(206,207)*. The fusion transcript is detectable in all patients with AML M4eo by RT-PCR *(208)*. Data on whether RT-PCR can detect more patients with t(8;21)/*AML1-ETO* or inv(16)/t(16;16)/*CBFβ-MYH11* than chromosome banding analyses are conflicting. Results from our own group, obtained by screening 250 AML patients for *AML1-ETO* and *CBFβ-MYH11*, showed no discrepancy between results of cytogenetics and RT-PCR (unpublished observations). These results correspond to data from the CALGB but are in contrast to results published by Langabeer et al. and Ritter et al. *(209–211)*. This discrepancy may reflect several factors, including number and quality of metaphases obtained for cytogenetic analysis.

The *CBFβ-MYH11* fusion gene seems to have a dominant-negative function, as knocked-in mice heterozygous for *CBFβ-MYH11* have a phenotype very similiar to that resulting from homozygous deletions of *CBFβ (212)*. The prognostic impact of inv(16) is still in question. Some data from clinical studies show a favorable outcome, whereas in other studies only an intermediate prognosis was observed *(168,175–177,213)*.

7.1.4. 11q23 Abnormalities/*MLL* Rearrangements

The *MLL* gene is involved in more than 30 different translocations identified at the present time *(214)*. Nineteen of these translocations have already been cloned, and all lead to in-frame fusions. The partner genes do not appear to have any unifying characteristics that would clarify their role in the leukemogenic process, although a few share similiar domains. In addition to translocations, *MLL* is involved in a partial tandem duplication *(181,182)*. The function of the intact *MLL* protein in mammalian cells is only poorly understood, and how *MLL* is involved in the leukemic process remains unknown *(1)*.

The most common translocations affecting the *MLL* gene in AML are t(9;11)(p21;q23) *(215)*, t(6;11)(q27;q23) *(216)*, and t(11;19)(q23;p13) *(217)*. They are usually observed in the M5a, M5b, or M4 FAB subtypes. In therapy-related AML, 11q23 abnormalities are frequently observed, especially after treatment with one of the epipodophyllotoxins *(218)*. Patients with 11q23 abnormalities seem to have an intermediate to poor prognosis, with generally high remission rates but short survivals. Recent data from Mrózek et al. *(219)* show that significant differences in clinical outcome may exist within this cytogenetic category, in that patients with t(9;11)(p22;q23) had a significantly better outcome than cases with other translocations involving 11q23 *(219)*. Also, in children, t(9;11) seems to be associated with a better prognosis than other 11q23 rearrangements *(184)*.

7.1.5. inv(3)(q21q26)/t(3;3)(q21;q26)

Abnormalities involving breakpoints at both 3q21 and 3q26 have been described in AML in all FAB subtypes, except for AML M3/M3v and AML M4eo. Although no association with a distinct FAB subtype exists, these patients often show a typical bone marrow morphology, with an increased number of megakaryocytes and numerous micromegakaryocytes. In the

peripheral blood, normal or even elevated platelet counts are often observed. Clinical outcome in these patients is very poor *(60,220)*.

7.1.6. t(6;9)(p23;q34)/*DEK-CAN*

t(6;9) has been reported in more than 50 AML patients, mostly as the sole anomaly. It is not associated with a distinct FAB subtype, although many cases have been classified as M2 or M4 *(221)*. An increase of basophils in the bone marrow was reported in about 50% of patients. On the molecular level, the *DEK* gene on 6p23 is fused to the *CAN* gene on 9q34. The function of the fusion gene is unknown, but it seems to have a nuclear localization. t(6;9) seems to be associated with an early age of onset (mean age, 38 yr) and a poor prognosis, although few data on clinical outcome are available *(222)*.

7.1.7. t(8;16)(p11;p13)

This translocation has been reported in more than 30 cases, the majority with myelomonocytic or monoblastic leukemia, especially M5b. Erythrophagocytosis was often demonstrated. Most patients are young, sometimes infants. Usually t(8;16) is the only change. Response to chemotherapy was reported to be poor *(223,224)*. The translocation t(8;16)(p11;p13) consistently disrupts two genes: *CBP* on chromosome 16p13.3 and *MOZ* on chromosome 8p11 *(225)*.

There are several other karyotypic abnormalities that have been described as primary chromosomal aberrations in AML. These include some rare balanced translocations, such as t(1;3)(p36;q21), t(1;22)(p13;q13), t(3;21)(q26;q22), t(7;11)(p15;p15), t(11;17)(q23;q25), and t(16;21)(p11;q22), which have been identified as recurrent translocations. However, only a few patients with these alterations have yet been described, so that no reliable information on prognosis is available. On the other hand, several unbalanced abnormalities including gains and losses of whole chromosomes or deletions are thought to be primary abnormalities, but in none of these have the pathogenetic mechanisms been resolved.

7.1.8. Monosomy 5/Deletion 5q

Loss of part of the long arm of chromosome 5 or total monosomy 5 show no distinctive FAB subtype preference and are usually accompanied by secondary aberrations, most often in complex aberrant karyotypes *(35)*. The incidence of *de novo* AML is around 7% of aberrant cases compared with 17% in secondary AML *(226)*. Abnormalities of chromosome 5 are associated with a poor outcome *(175–177)*. Whether this is mainly owing to the complex aberrant karyotypes associated with these changes is unclear, because they are only rarely observed as the sole abnormality.

7.1.9. Monosomy 7/Deletion 7q

After trisomy 8, monosomy 7 is the second most frequent solitary numeric abnormality in AML *(35)*. As the sole anomaly, it is found in 3% of cytogenetically abnormal cases and is observed as one of several changes—usually in complex aberrant karyotypes—in 12%. There is no preference for a particular FAB subtype. Both monosomy 7 and deletions of the long arm seem to be particularly frequent in therapy-associated AML, especially after treatment with alkylating agents *(227,228)*. Abnormalities of chromosome 7 are associated with a poor prognosis *(175–177)*. In one study, deletion of the long arm of chromosome 7 was not an unfavorable prognostic factor *(178)*.

7.1.10. Trisomy 8

A gain of chromosome 8 is the most frequent numeric abnormality in AML, occurring as a solitary change in 5% of all cytogenetically abnormal AML cases. If cases with multiple aberrations are also considered, the frequency of +8 triples *(35)*. Trisomy 8 is a frequent additional aberration in patients with t(8;21), inv(16), t(15;17), or t(9;11) and is often observed as an abnormality in complex aberrant karyotypes. The accompanying abnormalities seem to determine the prognosis. In a study of 51 patients with trisomy 8, patients with +8 as the sole cytogenetic anomaly had an intermediate prognosis and patients with +8 in addition to favorable chromosomal aberrations maintained a good clinical outcome, whereas +8 in combination with other abnormalities determined the worst prognosis (event-free survival, 37.5% vs 55.0% vs 9.0% respectively) *(229)*.

7.1.11. Deletion 9q

Deletions of the long arm of chromosome 9 can be found as the sole abnormality without a FAB subtype preference, but they are also observed as secondary changes, in particular in addition to t(8;21) and less often in addition to t(15;17) or inv(16) *(230,231)*.

7.1.12. Trisomy 11

Like other trisomies, trisomy 11 can occur as a single abnormality but is more frequently seen together with other numeric or structural cytogenetic changes. Although it is the third most common trisomy in AML, few clinical data are available on this abnormality. Isolated trisomy 11 is predominantly associated with older age, M2 and M1 FAB subtypes, and a poor response to standard chemotherapy with an overall unfavorable prognosis *(232)*. Recently, a partial tandem duplication of the *MLL* gene was reported as a recurrent molecular defect in 37.5–91% of AML cases with trisomy 11 *(182,233)*. The mechanism by which the partial tandem duplication contributes to leukemogenesis is currently unknown.

7.1.13. Trisomy 13

About 25% of patients with trisomy 13 show no other abnormality. In a review of 29 patients, all FAB subtypes except M6 and M7 were observed in patients with trisomy 13 as the sole cytogenetic change. The median age was 60 years. Patients responded well to induction therapy, but relapse occurred quickly and survival was short *(234,235)*.

7.1.14. Trisomy 21

Trisomy 21 is present in 5% of all AML patients and in less than 1% as the sole anomaly. There seems to be no FAB type specificity *(236)*.

Other rare trisomies observed as the sole abnormality in AML involve chromosomes 4, 9, or 22 *(35,37,170)*.

7.1.15. Complex Aberrant Karyotype

A group of 10–20% of patients show so-called complex aberrant karyotypes, which are associated with a very poor prognosis *(175–178,238)*. The definition of a complex aberrant karyotype varies among different study groups. Most commonly, it is defined as at least three cytogenetic abnormalities. This definition seems to be insufficient, as patients with balanced translocations such as t(8;21) or inv(16) and additional

Table 4
Cytogenetic Classification Systems of Different Clinical Study Groups for AML

Author and reference	*Favorable*	*Intermediate*	*Unfavorable*
AMLCG, 1999 *(169)*	t(8;21), t(15;17), inv(16), t(16;16)	Normal, other abnormalities	–5/5q–, –7/7q–, inv(3), 11q23, 12p,17p, complex
CALGB, 1998 *(246)*	t(8;21),inv(16), t(16;16),del(16)	Normal	Other abnormalities
Döhner, 1998[a]	t(8;21), t(15;17)	inv(16), 11q23, all other abnormalities	–5/5q–, –7/7q–, inv(3), 12p,17p
Gale et al., 1995 *(247)*	t(8;21),inv(16), t(16;16), del(16), t(15;17)	+8, +21, t(6;9), other abnormalities	t(9;22),–5,–7, del(11)
SWOG, 1997 *(248)*	t(8;21),inv(16), t(16;16),+14	Normal, other abnormalities	–5/5q–, –7/7q–, +13, inv(3), 11q23, 17p, 20q–,+13, dm, HSR, complex
EORTC[a]	t(8;21), inv(16)		–5/5q–, –7/7q–, 11q23, complex
MRC, 1998 *(168)*	t(8;21), t(15;17), inv(16)	Normal,+8,+21,+22,del(7q), del(9q), abn(11q23), all other numeric or structural abnormalities	–5/5q–, –7, abn 3q, complex

[a]Personal communication or protocol of the study group.

aberrations fulfill this criterion as well but belong to completely different biologic entities. The "real" complex aberrant karyotype shows unbalanced karyotypic abnormalities. The incidence of complex aberrant karyotypes is age-dependent. The incidence in patients younger than 60 yr is <10%, while complex aberrant karyotypes are found in up to 20% of patients older than 60 years. Prognosis is equally poor in all age groups, with less than 10% of patients surviving longer than 1 yr *(238)*.

7.2. Secondary Chromosomal Abnormalities

Secondary chromosomal aberrations are rarely or never found alone, rather, they develop in cells already carrying a primary abnormality. Although less specific than the primary changes, secondary aberrations nevertheless demonstrate nonrandom features with distribution patterns that appear to depend on the primary abnormality and to a lesser degree on the type of leukemia (AML or ALL) *(239)*. In contrast to primary aberrations, which are often balanced rearrangements, such as translocations or inversions, common secondary aberrations almost exclusively lead to genomic imbalances (gains and losses of whole chromosomes, deletions, or unbalanced translocations).

The biologic and clinical significance of particular secondary aberrations associated with specific primary changes in AML is largely unexplored. Published data on the prognostic impact of secondary aberrations are conflicting. Although no influence on prognosis of secondary abnormalities in patients with t(8;21)(q22), inv(16)(p13q22), or t(15;17)(q22;q12) was reported in the large Medical Research Council 10 (MRC10) trial and some smaller studies *(229,240–244)*, a negative prognostic impact of additional abnormalities was noted for patients with t(8;21) and t(15;17) in one study each *(241,245)*. The European 11q23 Workshop analyzed 125 patients with t(9;11)(p22;q23). Additional chromosomal abnormalities did not impair prognosis *(215)*. More studies are needed to assess the clinical consequences of secondary aberrations in AML. Since the number of patients representing one distinct entity is usually too small for meaningful clinical comparisons, intergroup analysis affords the only available means by which to answer these important questions.

7.3. Implications of Chromosomal Abnormalities for Prognosis

The karyotype of the leukemic blast cell is the most important independent prognostic factor in AML. For clinical purposes, karyotype analysis allows one to discriminate among three major prognostic groups. A favorable outcome under currently used treatment regimens was observed in several studies in patients with t(8;21)(q22;q22), inv(16)(p13q22), or t(15;17)(q22;q11-12). Chromosomal aberrations with an unfavorable clinical course include –5/del(5q), –7/del(7q), inv(3)/t(3;3), and a complex aberrant karyotype. The remainder are assigned to an intermediate prognostic group. This group is highly heterogeneous because it includes patients with a normal karyotype and rare chromosome aberrations with a yet unknown prognostic impact. This group will need further subdivision in the future.

There is as yet no consensus concerning the final details among large clinical study groups on how to classify AML patients according to karyotype and prognosis. Different groups assign cytogenetic categories to different prognostic subgroups according to their experience (Table 4) *(168,169,246–248)*. It has to be kept in mind that treatment itself influences the impact of prognostic parameters. The major objective for the future is to find the best therapy for each biologic entity. To reach this objective, the biologic entities have to be clearly defined, and large well-designed prospective trials are needed to allow a randomized comparison of different treatment strategies even in small subgroups. For APL, this goal has already been achieved. There is worldwide agreement to treat this subgroup of patients within separate trials, implementing ATRA.

Data from Bloomfield et al. *(246)* suggest that patients with t(8;21) or inv(16) benefit from treatment with high-dose cytarabine. Compared with other cytogenetic risk groups, patients with t(8;21) or inv(16) had the best outcome overall and demonstrated the greatest benefit from increasing doses of cytarabine *(246)*. These data also stress that different treatment strategies can influence the prognosis of distinct cytogenetic subgroups. One important finding concerning cytogenetic

abnormalities and prognosis was that the incidence of distinct chromosome abnormalities varies with age, whereas the prognosis of defined cytogenetic aberrations is independent of age *(249)*.

8. FAB CLASSIFICATION OF ALL

The lineage assignment, subclassification of precursor B- or T-cell leukemia, and stratification of treatment according to cell-biologic risk groups in ALL are in the domain of immunophenotyping and cyto-/molecular genetic analyses. The morphologic categories L1–L3, originally proposed by the FAB group, no longer have clinical importance, with the exception of the L3 subtype *(3,14,250)*. Even in cases that display the L3 morphology (relatively uniform blasts with intensively basophilic cytoplasm and sharply defined, fat-containing vacuoles) the results of cytogenetic analysis and immunophenotyping should be considered before a definite diagnosis of L3-type Burkitt cell leukemia is made. The morphologic appearance of L3 can be imitated by the AML subtypes M0, M1, or M5 or even by several undifferentiated solid tumors. Also, in rare cases of L1- and L2-ALL, vacuolation can be seen *(14)*.

In some cases of ALL, >40% of the lymphoblasts have a hand-mirror shape, but this feature has also been described in rare instances of AML. At present, this morphologic finding merely seems to identify morphologic variants without distinguishing clinical correlations *(14)*.

It has been suggested that a number of *BCR-ABL*-positive ALL cases show a unique morphologic appearance: in addition to the dominant lymphoid blast cell population, there are larger blasts with myeloid characteristics, some of the latter even showing a positive MPO reaction (mostly <3%). This may be confused in some cases with the diagnosis of AML M0 or even AML M1 with the Philadelphia translocation *(14)*. Conversely, some AML are Philadelphia chromosome-positive *(251)*.

9. IMMUNOPHENOTYPING OF ALL

Since the demonstration by Borella and Sen *(252)* that in some children with ALL, leukemic lymphoblasts are of thymic origin, immunophenotyping has become essential in the diagnosis of ALL and has substantially contributed to a more precise and biologically oriented classification of the disease (reviewed in refs. *8, 22, 23, 26,* and *253–255*). During the last two decades, immunophenotyping in ALL, initially performed with polyclonal antisera and subsequently with a rapidly expanding panel of MAbs, has mainly been applied to distinguishing ALL from AML, lineage assignment of leukemic blasts, phenotypic characterization of pathologic cell subsets, and examining the role of membrane antigen expression in predicting treatment response (reviewed in refs. *8, 9, 23, 25, 254*). Additionally, based on observations that leukemic blasts frequently show aberrant or asynchronous antigen expression compared with normal hematopoietic cell differentiation, leukemia-associated phenotypic features have been routinely used to detect MRD in ALL (reviewed in ref. *27*). More recently, immunophenotyping in conjunction with cytogenetic and molecular genetic studies has identified biologically and clinically distinct subsets within the major diagnostic subgroups of precursor B- and T-cell ALL and has become decisive in monitoring risk groups in therapeutic studies (reviewed in refs. *8* and *254–256*).

Current procedures for the diagnosis, lineage affiliation, and characterization of maturational stages of ALL are outlined in Fig. 1. It should be emphasized that both the lineage affiliation and the definition of maturational stage in ALL are based on patterns of antigen expression demonstrated by an appropriate selection of CD MAbs rather than on the presence or absence of a single antigen. In addition, it is noteworthy that the dominant phenotype of a leukemic cell population reflects the degree of maturation achieved by a leukemic clone and may not correspond to the initial target cell of the disease, mostly a more immature progenitor cell.

The following sections briefly discuss significant associations between immunophenotypic features and numeric and/or structural chromosomal abnormalities that have recently contributed to a refined ALL classification, especially in precursor B-cell ALL. It should be noted that accurate phenotypic predictions of specific translocations in precursor B-cell ALL could not be obtained by simply classifying antigen expression as either positive or negative but that they require more complex descriptions of patterns of expression or combinations of antigens.

9.1. B-Cell Precursor ALL

9.1.1. t(4;11)(q21;q23)

The t(4;11)(q21;q23) chromosomal abnormality occurs in about 2–6% of both children and adults with ALL and has been associated with characteristic immunophenotypic and clinical features [e.g., high leukocyte counts, predominance of females in infants, frequent organ enlargement, and increased incidence of central nervous system (CNS) leukemia at diagnosis); reviewed in refs. *257* and *258*]. Previous reports, mainly in infant ALL, have suggested that t(4;11)-associated acute leukemias mostly originate in multipotent or very early CD10-negative B-progenitor cells with a high frequency of myeloid-antigen positivity *(259,260)*. Recent studies have analyzed the immunophenotypic and genotypic features of this subgroup in greater detail. In the vast majority of ALL cases with t(4;11), leukemic blasts show a typical antigenic profile (e.g., CD19+, CD10–, CD24– or weakly +, cyIgM – or +, CD15 and/or CD65s+) indicative of an immature pro-B phenotype with frequent coexpression of particular myeloid antigens (i.e., CD15, CD65s). This clear-cut association of immunophenotypic features with t(4;11), initially described in infant ALL *(261–263)*, has also been recently found in adult patients *(264–270)*. Southern blot analysis revealed Ig heavy-chain gene rearrangements in virtually all cases as well as oligoclonal disease in some of them *(261)*, thus underlining the early B-cell commitment of blast cells with this cytogenetic abnormality. Based on our experience in a large series of childhood and adult ALL patients with 11q23 rearrangements *(261,268,271,272)*, these features, especially the missing or weak expression of CD24 compared with CD19, and the coexpression of CD65s, usually associated with negativity of other panmyeloid antigens (e.g., CD13, CD33), are highly predictive for the cytogenetic and/or molecular demonstration of *MLL* rearrangements, mostly owing to a t(4;11), or more rarely, other 11q23 aberrations. More recently, the 7.1 MAb, which recognizes a specific an-

tigen of the chondroitin sulfate proteoglycan family, has been demonstrated to detect with high sensitivity childhood ALL with *MLL* rearrangements, but it does not distinguish between the different translocation partners involved in *MLL* rearrangements *(133,273)*, thus not obviating the need for molecular genetic analyses. Our own results in a large number of childhood and adult ALL patients suggest that carefully constructed antibody panels, including 7.1, may be helpful for the identification of ALL carrying *MLL* rearrangements and for detection of MRD by flow cytometry *(133)*.

9.1.2. Philadelphia Translocation

The Philadelphia (Ph) translocation, or t(9;22)(q34;q11), occurring in 15–30% of adults and 3–5% of children with ALL, is usually associated with a common or pre-B ALL phenotype *(264,274–283)*. Some studies have identified a low proportion of immature CD10-precursor B-cell, B- or pure T-lineage features in Ph+ ALL *(274,284)*. Recent data have indicated that, similar to chronic myelogenous leukemia, a primitive hematopoietic cell is the target for the leukemic transformation in Ph+ ALL *(285)*. Although several immunophenotypic features, such as increased myeloid antigen expression *(267,282)*, positivity of the KOR-SA3544 antigen, *(286)*, coexpression of CD34 and CD10 *(270)*, and expression of CD25 *(280)* have been suggested to be associated with Ph+ ALL, there does not appear to be a definitive correlation between this translocation and immunophenotype. Our data in childhood and adult ALL *(279,287)*, as well as the findings of others *(288,289)*, suggest that myeloid antigens are not coexpressed more frequently in Ph+ ALL than in Ph– ALL cases. No extensive analyses have yet been performed to investigate the correlation between surface antigen expression and the two different breakpoint cluster regions (major or minor bcr) detected on chromosome 22 in Ph+ ALL. Preliminary results in adult ALL suggested that rearrangements in the major bcr are more common in My+ than in My– ALL *(279)*.

The monoclonal antibody KOR-SA3544 was previously reported to recognize Ph+ ALL with high sensitivity *(286)*. More recent studies, however, have shown that this antibody specifically recognizes the nonspecific crossreacting antigen (NCA)-50/90 (CD66c), one of the carcinoembryonic antigen (CEA)-related glycoproteins *(290)*, and reacts with distinct subsets of precursor B-cell ALL, including Ph+ ALL, *ETV6-AML1*-negative ALL, and hyperdiploid ALL *(291,292)*.

9.1.3. *ETV6-AML1*

The *ETV6-AML1* fusion gene, created by t(12;21), is the most common translocation in childhood ALL, occurring in about 20–25% of cases, and is present in <3% of adult ALL cases. The *ETV6-AML1* rearrangement is restricted to patients with nonhyperdiploid precursor B-cell ALL, and most cases display a common ALL or, less frequently, a pre-B ALL phenotype. The high frequency of myeloid antigen expression (i.e., CD13 and/or CD33) originally reported in a large series of children with *ETV6-AML1* rearrangement enrolled in the German and Italian multicenter trials *(293)* was confirmed by other reports *(294–296)*. Interestingly, other characteristic immunophenotypic features have recently been described that are highly predictive of *ETV6-AML1* rearrangement and may be used as a screening test for this genetic abnormality *(296)*. These include negativity of CD66c *(292)* and complete or partial lack of both CD9 and CD20 expression *(296,297)*.

9.1.4. t(1;19)(q23;p13)

Previous studies have indicated that t(1;19)(q23;p13), found in 5–6% of childhood and <5% of adult ALL cases, is strongly associated with cytoplasmic μ+ pre-B ALL *(298)*. Although subsequent studies confirmed the close association between t(1;19) and pre-B phenotype in both childhood and adult ALL *(299)*, this abnormality was also detected in some cytoplasmic μ– common ALL *(274,299,300)*. More recently, some authors described a pattern of surface antigen expression (i.e., CD9+, CD19+, CD22+, CD20±, CD34–, $CD45^{high}$) that is characteristic of ALL with t(1;19) but lacks specificity *(301,302)*. Polyclonal and monoclonal antibodies are now available for the flow-cytometric detection of the E2A-PBX1 protein in the nucleus of t(1;19)+ leukemic blasts *(303)*.

9.2. B-ALL

While in precursor B- and T-cell ALL, specific cytogenetic abnormalities do not show a close relationship to the FAB subtype, leukemic blasts in mature surface immunoglobulin (sIg)+ B-ALL are usually characterized by FAB L3 morphology and invariably exhibit t(8;14)(q24;q32) (in 75–85% of the patients) or one of the variant translocations t(2;8)(p11-12;q24) and t(8;22)(q24;q11). The high predictive value of these B-ALL-associated chromosomal anomalies for L3 morphology has recently been demonstrated *(37)*. It should be noted, however, that exceptions to these associations have been described. These include pediatric ALL patients with the 8;14 translocation, FAB L3 morphology, and clinical and laboratory features consistent with B-cell ALL, whose leukemic blasts displayed a less differentiated B-precursor immunophenotype *(304)* or lacked surface and cytoplasmic Ig *(305)*, and adults with sIg light-chain-positive ALL of L1 or L2 morphology without t(8;14) or its variants *(306)*. These unusual findings demonstrate the importance of evaluating patients with a combination of diagnostic tools in order to identify those who do not fit the recognized subgroups for a given disease. Furthermore, clinical behavior and outcome data in these patients suggest a hierarchy of clinical relevance of laboratory tests, with cytogenetic evidence of t(8;14) or one of the variant translocations being of greatest importance for assigning patients to B-cell ALL-specific protocols *(304)*; treatment outcome of other subtypes without one of these three translocations appears to be similar to those for precursor B-cell ALL.

9.3. T-Lineage ALL

Similar to precursor B-cell ALL, precursor T-cell ALL is manifested by a strong interpatient biologic heterogeneity, and leukemic transformation may occur at distinct stages of T-cell ontogeny, which results in lymphoblasts with immunophenotypic features corresponding to immature or more mature T-cell progenitors. In marked contrast to precursor B- or mature B-cell ALL, however, clear-cut relationships have not yet been established between specific chromosomal changes, occurring in about 44–61% of pediatric T-lineage ALL patients *(307,308)* and the maturational stage of T-ALL blasts or a particular pattern of surface antigen expression *(281,307–310)*.

Moreover, it has not been possible to identify characteristic phenotypic features within groups of frequent chromosomal abnormalities in T-ALL *(307,309)*, except for children with t(11;14), whose leukemic blasts expressed a profile of membrane surface antigens (i.e., CD4+, CD8+, CD3±) that has been associated with more mature thymocytes *(309,311)*. Most of these data were derived from cytogenetic analyses in childhood ALL, and detailed results are not yet available as to the correlation of immunophenotype and karyotype in adult patients with precursor T-cell ALL.

Alterations of the *TAL1* protooncogene on chromosome 1p32, either by translocation or other rearrangements, have recently been shown to represent the most common nonrandom genetic defect associated with precursor T-cell ALL, occurring in about 10–25% of patients *(312–314)*. Several reports unanimously demonstrated that *TAL1* alteration in T-ALL exclusively occurred in CD3– or CD3+ T-ALL of the αβ lineage, whereas no clear association of *TAL1* gene rearrangements with a distinct stage of thymocyte maturation could be detected *(312–315)*.

10. PROGNOSTIC IMPACT OF IMMUNOPHENOTYPING IN ALL

The lack of standardized criteria in the past for the classification of immunophenotypic subgroups, the paucity of controlled prospective studies on the treatment outcome of precursor B- and T-cell ALL subsets, and the different treatment strategies administered complicate the assessment of the prognostic impact of immunophenotyping studies in ALL. In addition, the strong correlation between certain immunophenotypic subgroups and cytogenetic or clinical features (see above) has called into question the value of immunophenotyping as an independent predictor of treatment outcome. Finally, several studies have shown that the prognostic impact of immunophenotypic subgroups as well as chromosomal abnormalities is diminished by the improved efficacy of chemotherapy; hence, prognostic factors must be evaluated in the context of the therapy delivered *(270,299,316–319)*.

In precursor B-cell ALL, no substantial differences in remission rates were recorded for immunophenotypic subgroups, but several studies revealed an association between the maturational stage of B-lymphoblasts and the duration of remission. Most studies in both childhood and adult ALL have reported a worse prognosis for patients whose leukemic blasts express an immature CD10-negative pro-B phenotype, also referred to as early pre-B or null-ALL *(254,269,270,320–323)*, which, however, was frequently associated with adverse biologic (e.g., 11q23 rearrangements) and clinical features (e.g., high tumor burden, age < 1 yr).

Cytogenetic and molecular genetic studies have provided conclusive evidence that children and adults with common and pre-B ALL differ significantly with respect to the incidence of the known favorable or unfavorable chromosomal translocations. For instance, t(9;22) accounts for up to 55% of adult and <5% of children with CD10+ precursor B-cell ALL, whereas the reported frequency for t(12;21), associated with a good prognosis in most recent studies, ranges between 12 and 36% in childhood common or pre-B ALL and rarely occurs in adult patients *(264,275,289)* (reviewed in ref. *324*). These findings may partially explain the striking differences observed in treatment outcome between children and adults with common or pre-B ALL.

Confirmation of the prognostic importance of the pre-B ALL immunophenotype has been limited to sequential studies of the Pediatric Oncology Group (POG), since, until recently, this was the only group performing cytoplasmic μ testing in the context of large prospective clinical trials. Previous studies of the POG suggested that the pre-B phenotype might be an independent prognostic marker for reduced event-free survival *(298)*. However, more recent data show that only the subgroup of children with pre-B ALL and t(1;19) has a worse treatment outcome *(325)*. By contrast, the German ALL-BFM trials, the analysis of the MRC UKALL Trial XI, and a single-center study did not reveal any significant differences in remission duration between common and pre-B ALL *(320,326)*.

In children with precursor B-cell ALL, prognosis has been linked to other immunophenotypic features, such as CD20, CD34, and CD45 expression, and it has been suggested that the lack of CD20 and CD45 antigens or the presence of CD34 on leukemic blasts may be associated with a longer event-free survival *(327–330)*. However, in view of the relationship of these immunophenotypic features (e.g., absent CD45) to other biologically favorable characteristics *(297,329,331)*, their prognostic significance has to be evaluated in further studies by adjusting results for the presence of other risk factors.

Several studies in childhood and adult ALL have shown that a remarkable prognostic improvement of B-ALL is achieved by the development of intensive treatment strategies, especially adapted to the biologic and clinical features of this disease *(318,319)*. These data impressively illustrate that more effective treatment can offset the negative prognostic impact of biologic characteristics, such as the immunophenotype or chromosomal translocations.

In T-lineage ALL, various immunophenotypic features seem to be associated with an increased risk of treatment failure, including an immature pro-/pre-T-ALL phenotype, membrane expression of CD3 or MHC class II antigen, and negativity of CD2, CD5, THY antigen (similar to CD1), or CD10 *(254, 320,327,332–336)*. The prognostic impact of these factors, however, has differed according to the treatment strategies used, and immunophenotyping still represents a controversial prognostic factor that has not been routinely used for risk classification or assignment to novel treatment strategies in high-risk precursor T-cell ALL patients.

In our experience and in other studies, children and adults with pre-T ALL differ markedly with respect to their phenotypic and genotypic features, suggesting an arrest of adult pro-/pre-T ALL at a less mature differentiation stage than in childhood, which may be closely related to the worse treatment outcome observed in these patients *(327,332,334,337,338)*. Similar results have previously been published in adolescent and adult patients, whose leukemic blasts showed CD7 antigen expression with an absence of myeloid, B-, or more mature T-cell differentiation antigens *(339)*. Most interestingly, these leukemic blasts were capable of multilineage differentiation in vitro both spontaneously and after stimulation with appropriate cytokines, sug-

Table 5
Frequency of Ploidy Groups: Adult versus Childhood ALL

Ploidy group	Frequency (%)	
	Adult ALL	Childhood ALL
Normal karyotype	26–34	8–56
Hypodiploidy < 46	2–8	5–7
Pseudodiploidy	7–59	3–42
Hyperdiploidy 47–50	7–17	8–16
Hyperdiploidy > 50	4–9	14–28
Near-triploidy	3	<1
Near-tetraploidy	2	1

Table 6
Chromosomal Abnormalities in ALL

Abnormality	Disease subtype	Fusion gene	Frequency	
			Childhood (%)	Adult (%)
t(1;19)(q23;p13)	Pre-B-ALL	*E2A-PBX1*	5–6	3
t(4;11)(q21;q23)	Pro-B ALL	*MLL-AF4*	2	6
t(9;22)(q34;q11)	c-ALL	*BCR-ABL*	2–5	25–30
t(8;14)(q24;q32)	B-ALL	*MYC-IGH*	3	5
t(10;14)(q24;q11)	T-ALL	*HOX11-TCR*	1	3
t(12;21)(p13;q22)	Pre-B-ALL	*ETV6-AML1*	10–20	<1
9p	T-, pre-B-ALL	$p16^{INK4A}$	7–12	15
6q	c-, pre-B-,T-ALL	?	4–13	6
14q11	T-ALL	*TCR*	1	6

gesting that acute leukemia in these patients evolved from in vivo transformation of immature pluripotent hematopoietic cells that respond poorly to conventional chemotherapy. Consistent with this hypothesis, patients with immature precursor T-cell ALL showed more frequent coexpression of CD34, CD117, and/or myeloid antigens *(327,338,340)*.

Further attempts to identify additional prognostically relevant subgroups of precursor T-cell ALL have been largely unsuccessful in both childhood and adult ALL *(332,341)*. However, at least three multicenter trials in childhood ALL, using similar maturational staging systems, have recently lent strong support to evidence that children with cortical (CD1a+) precursor T-cell ALL have a better early response to treatment, as illustrated, for instance, by the in vivo response to corticosteroids, and a significantly longer duration of event-free survival than in those with an immature or mature precursor T-cell phenotype *(310,320,342)*. Similar data, showing a significant improvement in survival of adult patients with CD1, CD2, CD4, and CD5 expression compared with patients not expressing these antigens, have been recently published by the Cancer and Leukemia Group *(270)*. Although, it is unclear at present why patients with a cortical immunophenotype respond better to treatment, recent investigations of apoptosis-related parameters, including spontaneous apoptosis in vitro and modulation of apoptosis by interleukin-7, suggested that maturational stages of precursor T-cell ALL may differ as to their accessibility to apoptotic programs, with lymphoblasts expressing CD1a or exhibiting a selection-related phenotype being more susceptible to apoptosis than leukemic lymphoblasts with an immature phenotype *(342,343)*.

Several studies have suggested that a subclassification of membrane CD3+ precursor T-cell ALL according to T-cell antigen receptor (TCR) αβ or γδ expression provides valuable clinical information, because TCR-γδ+ cases represent an important, albeit rare, subgroup of precursor T-cell ALL with distinctive clinicopathologic features and prognosis *(344–346)*. Further prospective studies are needed to characterize more thoroughly the cell-biologic features of TCR-γδ+ lymphoblasts and to confirm the better prognosis of this subgroup compared with TCR-αβ+ precursor T-cell ALL *(346)*.

11. GENETIC ABNORMALITIES IN ALL

Clonal karyotypic abnormalities are found in 60–85% of ALL cases *(172,267,281,347)*. The Third International Workshop on Chromosomes in Leukemia found the majority of cytogenetic changes in cases with B-cell precursor ALL, with only 39% occurring in T-cell ALL. In children, an abnormal karyotype was observed in 61% of T-ALL cases *(308)*.

Gross numeric disturbances, in particular, massive hyperdiploidy, are more frequent in ALL than in AML. More than 30% of cases are hyperdiploid and around 10% hypodiploid. More than 30 different recurring chromosome aberrations are known in ALL. Taken together, these aberrations are present in two-thirds of all cytogenetically abnormal ALL cases. Some of them are of particular clinical importance or are specifically associated with distinctive immunophenotypes.

The distribution of chromosomal abnormalities differs between childhood and adult ALL *(348)*. The two most striking examples are the Philadelphia translocation t(9;22)(q34;q11), which occurs in about 3–6% of cases in childhood but in 15–30% of adult cases. On the other hand, the translocation t(12;21)(p13;q22) occurs in about 10–15% of childhood ALL but is found in <3% of adults with this disease. These differences in ALL genetic features between age groups contribute to the differences in response to therapy *(349)*. However, the prognostic impact of distinct karyotypic abnormalities seems to be comparable between age groups: for example, the Philadelphia translocation is associated with the poorest outcome in childhood as well as in adult ALL. However, recent data suggest that within the subgroup with Ph+ childhood ALL, some patients, who can be identified by young age and leukocyte counts of <50.000/μL, benefit from intensive chemotherapy *(350)*.

11.1. Chromosomal Abnormalities and Their Molecular Correlates

In ALL, it is common to classify karyotypes into ploidy groups, on the one hand, and groups with specific rearrangements, on the other hand. Commonly recognized ploidy groups are low hyperdiploidy (modal number 47–50), high hyperdiploidy (>50), near-triploidy, near-tetraploidy, near-haploidy, hypoploidy (45 and lower), pseudodiploidy (normal number of chromosomes with structural changes) and normal karyotype (Table 5) *(351)*. An array of nonrandom structural rearrangements has been described (Table 6).

11.1.1. Hypoploidy <46 Chromosomes

Modal chromosome numbers of 45 and less are rare, especially the near-haploid numbers of 24–36. Commonly lost are chromosomes 1, 5, 6, 10, 11, 18, 19, 21, and 22. Near-haploid

cases almost always have only numeric changes. Many cases also have a hyperdiploid population twice the near-haploid number. Hypoploidy confers a rather poor prognosis in adults as well as children with ALL *(281,352)*.

11.1.2. Pseudodiploidy

A normal chromosome number with structural changes is the most frequently found abnormal karyotype in ALL. Nearly two-thirds of cases have recurrent translocations. Most cases have structural changes with only a few combining structural and numeric aberrations. The prognosis depends on the specific chromosome rearrangements rather than on chromosome number in this subgroup. More accurate identification of underlying specific structural aberrations will make the pseudodiploid group superfluous in a prognostically useful classification.

11.1.3. Hyperdiploidy 47–50 Chromosomes

Somewhat less than one-fifth of all ALL cases belong to this category. Most cases have 47 or 48 chromosomes. About one-fourth of cases show only numeric changes, whereas in the others, the pattern of structural abnormalities is similar to that of ALL in general. The chromosomes mainly gained in this group are X, 5, 8, 10, and 21 *(347)*. In childhood, numbers between 47 and 50 are associated with an intermediate prognosis.

11.1.4. Hyperdiploidy >50 Chromosomes

Chromosome numbers cluster around 51–55. There seems to be a certain pattern of gained chromosomes. Frequently gained chromosomes are 4, 6, 8, 10, 14, 17, and 21. About half of all childhood and adult ALL cases with hyperdiploidy >50 show additional structural chromosomal rearrangements, with t(9;22)(q34;q11) being the most common in adults *(353)*. In children with ALL, a modal number between 51 and 55 is associated with a favorable prognosis. The combination of trisomies of both chromosomes 4 and 10 with hyperdiploid ALL identifies a subgroup of patients with an extremely favorable outcome and a strong likelihood of cure with antimetabolite-based chemotherapy *(353)*. In adult ALL, the impact of hyperdiploidy on prognosis appears to be less significant. This is mainly because hyperdiploidy in adulthood is often accompanied by a t(9;22)(q34;q11) *(267,354)*. Poor-risk structural rearrangements override the prognostic impact of otherwise favorable numeric changes. If adults who show only numeric changes and a modal number >50 are taken into account, the prognosis is favorable.

11.1.5. Near-Triploidy and Near-Tetraploidy

Near-triploidy and near-tetraploidy are more frequent in adult ALL than in childhood ALL. Although near-triploidy seems to be associated with a poor outcome, the prognosis associated with near-tetraploidy is in the range of that conferred by a normal karyotype. These data have to be interpreted cautiously because the number of reported cases is still small.

11.1.6. Single Chromosome Gains and Losses

The incidence of a trisomy or monosomy as the only karyotypic anomaly in ALL is low. In childhood ALL, the most common changes are trisomy 8, monosomy 20, and trisomy 21. In adults, the data are scanty, so that the significance of monosomies or trisomies as isolated karyotypic changes is unclear.

11.1.7. t(12;21)(p13;q22)/*ETV6-AML1*

The translocation t(12;21) was long considered a rarity, since it was detected in <0.05% of patients analyzed by chromosome banding *(347)*. However, this rearrangement is difficult to detect, because only small segments are translocated. After the *ETV6* (or *TEL*) and *AML1* (also rearranged in AML) genes were cloned, molecular tools for the detection of *ETV6-AML1* rearrangement became available *(355)*. With molecular techniques such as FISH, Southern blot, and RT-PCR, *ETV6-AML1* fusion transcripts were detected in up to 27% of children, making it the most common genetic abnormality in pediatric ALL *(356,357)*. Interestingly, the nonrearranged *ETV6* allele seems to be deleted in almost all t(12;21) cases. The *ETV6-AML1* rearrangement is associated with a B-cell precursor immunophenotype and confers an excellent prognosis *(293)*. In adult ALL, the frequency of *ETV6-AML1* rearrangements is low (3–4%) and little is known about its prognostic significance *(358,359)*.

11.1.8. t(9;22)(q34;q11)/*BCR-ABL*

The Philadelphia translocation is the most frequent rearrangement in adult ALL. Its incidence is age-dependent. In childhood ALL, it occurs in about 3–6% of cases, whereas in adults, the frequency is at least 15–30% *(267,360)*. Owing to the reciprocal translocation between the long arms of chromosome 9 and 22, the large 3' segment of the *ABL* gene from chromosome 9 is translocated to the 5' part of the *BCR* gene on chromosome 22, creating a hybrid *BCR-ABL* gene that is transcribed into a chimeric *BCR-ABL* mRNA, which generates a protein with an increased tyrosine phosphokinase activity compared with normal human c-*ABL*. Depending on the breakpoint within the *BCR* gene, two different fusion proteins can occur. If the break takes place in the major breakpoint cluster region (M-bcr), a fusion protein of 210 kDa, called $p210^{BCR\text{-}ABL}$, is created. This fusion protein is observed in about 50% of adult Ph+ ALL and 20% of childhood Ph+ ALL cases. The remaining patients show a break within the minor breakpoint cluster region (m-bcr), translating into a smaller *BCR-ABL* fusion protein called $p190^{BCR\text{-}ABL}$ *(361)*. In vitro studies show that $p190^{BCR\text{-}ABL}$ is a more active tyrosine kinase than $p210^{BCR\text{-}ABL}$. An association between breakpoint location and clinical features and prognosis has not been found so far *(264,362)*. Only one study analyzing 36 patients with Ph+ ALL after bone marrow transplantation reports that the expression of $p190^{BCR\text{-}ABL}$ was associated with a higher risk of relapse than was the expression of $p210^{BCR\text{-}ABL}$ *(363)*. Some rare breakpoints also exist within the *BCR* gene, which lead to proteins of different sizes.

Chromosome banding analysis seems to underestimate the incidence of the *BCR-ABL* fusion gene, and Ph-negative cytogenetics with positive tests for the *BCR-ABL* fusion gene have been documented, although in rare instances *(364–366)*. Molecular tools for the detection of the *BCR-ABL* rearrangement include FISH and PCR, which are complementary to cytogenetics. The necessity for molecular screening for the *BCR-ABL* rearrangement in B-lineage ALL is debatable and largely depends on the availability and quality of chromosome banding analysis. Considering the high prognostic impact of the *BCR-ABL* rearrangement, screening with either FISH or PCR is justified, especially if only suboptimal chromosome banding analysis is available or a normal karyotype was observed.

Table 5
Frequency of Ploidy Groups: Adult versus Childhood ALL

Ploidy group	Frequency (%)	
	Adult ALL	Childhood ALL
Normal karyotype	26–34	8–56
Hypodiploidy < 46	2–8	5–7
Pseudodiploidy	7–59	3–42
Hyperdiploidy 47–50	7–17	8–16
Hyperdiploidy > 50	4–9	14–28
Near-triploidy	3	<1
Near-tetraploidy	2	1

Table 6
Chromosomal Abnormalities in ALL

Abnormality	Disease subtype	Fusion gene	Frequency	
			Childhood (%)	Adult (%)
t(1;19)(q23;p13)	Pre-B-ALL	*E2A-PBX1*	5–6	3
t(4;11)(q21;q23)	Pro-B ALL	*MLL-AF4*	2	6
t(9;22)(q34;q11)	c-ALL	*BCR-ABL*	2–5	25–30
t(8;14)(q24;q32)	B-ALL	*MYC-IGH*	3	5
t(10;14)(q24;q11)	T-ALL	*HOX11-TCR*	1	3
t(12;21)(p13;q22)	Pre-B-ALL	*ETV6-AML1*	10–20	<1
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Further attempts to identify additional prognostically relevant subgroups of precursor T-cell ALL have been largely unsuccessful in both childhood and adult ALL *(332,341)*. However, at least three multicenter trials in childhood ALL, using similar maturational staging systems, have recently lent strong support to evidence that children with cortical (CD1a+) precursor T-cell ALL have a better early response to treatment, as illustrated, for instance, by the in vivo response to corticosteroids, and a significantly longer duration of event-free survival than in those with an immature or mature precursor T-cell phenotype *(310,320,342)*. Similar data, showing a significant improvement in survival of adult patients with CD1, CD2, CD4, and CD5 expression compared with patients not expressing these antigens, have been recently published by the Cancer and Leukemia Group *(270)*. Although, it is unclear at present why patients with a cortical immunophenotype respond better to treatment, recent investigations of apoptosis-related parameters, including spontaneous apoptosis in vitro and modulation of apoptosis by interleukin-7, suggested that maturational stages of precursor T-cell ALL may differ as to their accessibility to apoptotic programs, with lymphoblasts expressing CD1a or exhibiting a selection-related phenotype being more susceptible to apoptosis than leukemic lymphoblasts with an immature phenotype *(342,343)*.

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The distribution of chromosomal abnormalities differs between childhood and adult ALL *(348)*. The two most striking examples are the Philadelphia translocation t(9;22)(q34;q11), which occurs in about 3–6% of cases in childhood but in 15–30% of adult cases. On the other hand, the translocation t(12;21)(p13;q22) occurs in about 10–15% of childhood ALL but is found in <3% of adults with this disease. These differences in ALL genetic features between age groups contribute to the differences in response to therapy *(349)*. However, the prognostic impact of distinct karyotypic abnormalities seems to be comparable between age groups: for example, the Philadelphia translocation is associated with the poorest outcome in childhood as well as in adult ALL. However, recent data suggest that within the subgroup with Ph+ childhood ALL, some patients, who can be identified by young age and leukocyte counts of <50.000/μL, benefit from intensive chemotherapy *(350)*.

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Modal chromosome numbers of 45 and less are rare, especially the near-haploid numbers of 24–36. Commonly lost are chromosomes 1, 5, 6, 10, 11, 18, 19, 21, and 22. Near-haploid

The prognosis of Ph+ ALL is poor in childhood *(367)* as well as in adults *(267,281,360)*. Recent studies show that in childhood ALL a subgroup of patients seems to benefit from intensive chemotherapy *(283,350)*. A study in adult ALL was able to show that karyotypic abnormalities in addition to the Ph translocation have prognostic impact *(279)*.

11.1.9. 11q23/*MLL* Abnormalities

A common molecular event is the disruption of the *MLL* gene (mixed-lineage leukemia or myeloid-lymphoid leukemia) located on chromosome 11, band 11q23. *MLL* rearrangements occur in ALL as well as in AML. More than 30 chromosomal loci are known to participate in *MLL* rearrangements *(214)*. Although some patterns and associations between the partner genes are beginning to emerge, it is not yet possible to frame a single hypothesis for 11q23 leukemic transformation. In ALL, the most commonly involved partner genes of *MLL* are *AF4* (4q21) and *ENL* (19p13).

The translocation t(4;11)(q21;q23) was first described by Oshimura et al. in 1977. It is observed in >60% of infants with ALL, 2% of children, and 3–6% of adults *(262,281)*. It is associated with young age (generally under 2 yr), female sex, and high white blood cell counts, and is frequently associated with organomegaly and CNS involvement.

The clinical outcome for both adults and children with t(4;11)(q21;q23) has been poor regardless of age *(262, 267,281,368)*. However, intensifying treatment strategies seems to improve prognosis substantially. Although data in adult ALL with t(4;11) are still scarce, risk-adapted therapy might benefit this group, as it did in children *(268)*.

11.1.10. t(1;19)(q23;p13)/*E2A-PBX1*

This translocation was first described in 1984 by Carroll et al *(369)*. Subsequent studies confirmed it as one of the most common translocations in childhood ALL, with an incidence of 5–6% overall and 25% of pre-B cell-ALL cases *(299)*. In adult ALL, it is present in <5% of cases *(267,370,371)*. Clinical characteristics include a high white blood cell count, high lactate dehydrogenase levels, and a pseudodiploid karyotype. At the molecular level, the *E2A* gene on chromosome 19 is fused to *PBX1* on chromosome 1. *E2A* encodes two transcription factors, E12 and E47, via alternative splicing, which are considered essential for normal lymphopoiesis and regulation of B-cell development. A study in pediatric ALL compared the value of chromosome banding analysis and the PCR technique for detection of the t(1;19) and found that more cases were detected by PCR. In half of these additional cases, no metaphases were obtained, whereas in the others, only normal metaphases were observed *(372)*. Improving culturing techniques can reduce these discrepancies. Pediatric studies have shown that children with t(1;19) fare poorly on standard treatment protocols but that a favorable outcome can be achieved with intensified treatment *(325,373)*. As incidence of this rearrangement is low in adults, no data on its prognostic impact are available.

11.1.11. t(8;14)(q24;q32) and Variants t(2;8)(p12;q24), and t(8;22)(q24;q11)

t(8;14) and its variants are associated with mature B-cell neoplasia with remarkable specificity. They are found in both leukemia and lymphoma of the Burkitt type. The crucial event in all three translocations is the juxtaposition of the oncogene *MYC*, located on 8q24, with either the immunglobulin heavy-chain locus (14q32), the immunglobulin light-chain locus κ (2p12), or the immunglobulin light-chain locus l (22q11). Under the influence of transcription-stimulating sequences in the constitutively active immunglobulin locus, the transcription of *MYC* is increased, ultimately leading to neoplastic growth. t(8;14) is the most common (85%) of these rearrangements, whereas t(2;8) and t(8;22) are found in around 5 and 10% of patients, respectively *(35)*.

Mature B-cell ALL was long believed to be associated with poor prognosis. The introduction of short-term dose-intensive regimens such as hyperfractionated cyclophosphamide, high-dose methotrexate, and cytarabine has significantly improved clinical outcomes in children as well as adults *(318,374, 375)*.

11.1.12. 6q Deletions

Deletions of the long arm of chromosome 6 are common in ALL, occurring in 5–10% of cases with clonal abnormalities, being the only change in about two-thirds *(35)*. PCR-based techniques to detect the loss of heterozygosity have yielded evidence that deletions of 6q seem to be more frequent when identified by this method compared with chromosome banding techniques. With molecular techniques loss of heterozygosity on the long arm of chromosome 6 occurred in up to 32% of cases *(376)*. Such deletions are not specific for ALL but rather seem to be characteristic of lymphoid malignancies. Molecular techniques have defined a minimal deleted region, but so far the putative tumor suppressor gene on the long arm of chromosome 6 has not been cloned *(376)*. Clinical studies have either confirmed no difference in prognosis or reported improved outcomes for 6q deletions *(267,354)*.

11.1.13. 9p Abnormalities

Loss of 9p material can occur owing to deletions or unbalanced translocations. The commonly deleted region was found to be 9p21-p22. From 7 to 12% of childhood and 12 to 15% of adult ALL cases show 9p abnormalities *(267,347,360)*. They occur in B-lineage as well as in T-lineage ALL. An association with high-risk clinical features such as older age, high leukocyte counts, bulky disease, and an increased incidence of CNS disease was reported *(281)*. Candidates for the putative tumor suppressor gene at 9p21 are $p16^{INK4A}$ and $p15^{INK4B}$. They both encode proteins that inhibit the cyclin-dependent kinases CDK4 and CDK6 and play a crucial role in cell cycle progression. The main mechanism for $p16^{INK4A}$ inactivation is biallelic deletions with $p15^{INK4B}$ gene codeletion in most but not all cases. Point mutations within $p16^{INK4A}$ are rare *(281)*.

11.1.14. t/dic(9;12)(p11-12;p11-13)

This rare group of chromosomal abnormalities leads to loss of parts of the short arms of chromosomes 9 and 12. The most common rearrangement in this group is dic(9;12), which is associated with young age, no CNS involvement, B-progenitor phenotype, and an excellent prognosis *(377)*.

11.1.15. 12p Deletions

Partial monosomy 12p owing to deletion or unbalanced translocations is observed in about 5% of ALL cases *(267)*. It usually occurs within complex aberrant karyotypes and only

rarely as the sole abnormality. The presence of 12p deletions seems to have no impact on prognosis *(267)*.

11.1.16. Abnormalities Involving the *T-Cell Receptor* Genes

Translocations involving the *TCR* genes are among the most common aberrations in T-cell ALL *(281)*. Most patients with T-cell ALL show a normal karyotype by cytogenetics, but those who show an abnormality have about 50% of breakpoints that map to loci of one of the *TCR* genes. These include chromosome 14 band q11 (*TCR*-α and *TCR*-δ), 7q32-36 (*TCR*-β), and 7p15 (*TCR*-γ). A number of distinct chromosomal translocations have been identified. A common theme is the juxtaposition of *TCR* promotor/enhancer elements to a variety of putative or proven transcription factors located at breakpoints on the partner chromosomes. The most common translocation in T-cell ALL is t(10;14)(q24;q11), occurring in 7–14% of ALL of T-lineage origin. The *HOX11* gene, a homeobox gene, located on 10q24, becomes transcriptionally activated. The prognosis of t(10;14) seems to be favorable in adults *(267,360,378)*. Other recurrent translocations in T-cell ALL involving TCR genes are t(8;14)(q24;q11) *(379)*, t(11;14)(p15;q11) *(311)*, t(11;14)(p13;q11) *(311)*, and t(7;9)(q34;q34) *(308,380)*.

11.1.17. *TAL1* Gene Rearrangements

Approximately 20–30% of children and 10–30% of adults with T-cell ALL have rearrangements within a gene called *TAL1* (or *TCL5* or *SCL*) on chromosome 1, band p33. Two mechanisms of rearrangements exist. About 3% occur by a balanced translocation t(1;14)(p33;q11) that results in a rearrangement between the *TAL1* gene and the *TCRα/δ* locus *(381)*. In 25–30% of pediatric patients with *TAL1* alterations, the changes are not detectable by chromosome banding analysis *(35)*. In these patients, submicroscopic deletions within chromosome band 1p33 fuse the coding exons of *TAL* to the first noncoding exon of *SIL* (SCL-interrupting locus) *(314)*. This rearrangement can be detected either by Southern blot or PCR analysis. Both mechanisms lead to a transcriptional activation of *TAL1*. Outcome seems not to differ significantly between patients with or without *TAL1* rearrangement *(281)*.

Despite the proven diagnostic and prognostic value of cytogenetic analyses in ALL, there are compelling reasons to add molecular techniques. In some cases, clinically important genetic changes may be missed by cytogenetic evaluation, even in sophisticated laboratories with a high rate of successful analysis. Some important genetic alterations are not identifiable by routine karyotyping. Examples are the *ETV6-AML1* fusion gene in B-lineage ALL and the *TAL1* rearrangements in T-cell ALL. Furthermore, molecular techniques are able to identify patients carrying a *BCR-ABL* rearrangement who do not show a Ph translocation in chromosome banding. Therefore, especially for those aberrations with a high prognostic impact, molecular screening should be considered if chromosome banding analysis fails or shows a normal karyotype *(382)*. Complementary techniques such as FISH and CGH must be used in selected cases if chromosome banding studies are not able to clarify the karyotype completely.

11.2. Implications of Chromosome Abnormalities on Prognosis

The implication of every prognostic factor on clinical outcome has to be analyzed in the background of the treatment strategies applied, because therapy has a major impact on the relevance of prognostic parameters. In terms of currently used standard protocols, t(12;21), dic(9;12), hyperdiploidy with a chromosome number between 51 and 55, and the t(10;14) in T-lineage ALL are associated with a favorable outcome. Patients with a normal karyotype, a deletion of the long arm of chromosome 6, or a 9p or 12p deletion show an intermediate prognosis. For patients with t(9;22), outcome is dismal even with intensive treatment strategies including allogeneic bone marrow transplantation. For patients with t(1;19), t(4;11), or t(8;14) and variants, prognosis could be improved with intensified therapy and risk-adapted treatment strategies *(268,318,373–375)*.

As therapy improves, the importance of various cytogenetic abnormalities in acute leukemia may change or diminish. There is evidence in childhood ALL that with more intensive chemotherapy, all patients fall into a single good-prognosis group, regardless of ploidy or specific karyotypic abnormalities. Even in the poor-prognostic subgroup of Ph-positive patients changes in treatment strategies may improve outcome. Recent studies in Ph-positive childhood ALL show that intensive chemotherapy improved outcome in a subgoup of patients *(283,350)*.

12. CONTROVERSIAL ISSUES

12.1. Which Standard Staining Techniques Should Be Applied to the Morphologic and Cytochemical Diagnosis of AML?

The diagnosis of AML is based first of all on morphologic features. This is because staining is quick and leads to a reliable diagnosis in most cases, with the exception of the subgroups AML M0 and AML M7. For all other catogeries, staining with MGG, MPO, and NSE (ANA or ANB) has to be performed on bone marrow aspirates and on blood films in parallel. Thus, cytomorphology will probably still be the backbone for diagnosis of AML in the next decade. Its value for the diagnosis of ALL may be restricted mainly to confirm the L3-type Burkitt cell leukemia in combination with immunophenotyping and cytogenetics. The role of cytomorphology for the definition of remission status has to be newly defined in comparison with other methods. Cytomorphology gives limited prognostic information.

12.2. Is the Updated AML Classification as Proposed by the WHO Useful?

This new proposal for the classification of AML follows a hierarchy beginning with cytogenetic entities and ending with cytomorphologic descriptions, as has been used in the FAB system. In addition, dysplastic features and the history of the AML with preceding MDS or chemotherapy is considered for classification subgroups.

The proposal has taken a major step toward classification based on biology. The new threshold for AML (>20% blasts) follows the same philosophy, because the biology of RAEB-T and AML were found to be the same. Also, the inclusion of AML after MDS and after preceding chemotherapy will help to clarify terminology and classification. With respect to the sub-

category of multilineage dysplasia, further prospective evaluations of its biologic relevance are urgently needed. For further studies, the FAB categories and the WHO classification should be investigated in parallel for clinical and biologic relevance.

In the next few years, the classification of acute leukemias will move further from morphology-based categories to subtypes that rely on immunophenotypic and mainly genetic characteristics. Standard cytogenetics will be replaced more and more by molecular methods, including microarray expression profiling and protein analysis. The main objective for the cytomorphologic and cytochemical approach will be to regulate and place all other techniques within an algorithm to make diagnosis quick, thus pointing the way to recognition of the leukemia as a biologic entity.

12.3. Which Cellular Antigens Have Proved Particularly Helpful in Lineage Assignment and Subclassification of Acute Leukemias?

Immunophenotyping studies have used different approaches to diagnose and subdivide the acute leukemias *(28,97,98)*. These include, first, the application of a general and comprehensive panel of MAb combinations; second, the use of a "minimal" screening panel for lineage assignment of the predominant blast population followed by a more thorough, secondary set of MAbs chosen according to the results obtained with the screening panel; and finally, the selection of a "targeted" approach based on morphologic, cytochemical, and clinical information. Obviously, the sequential or "targeted" approach is associated with some savings in reagent costs, but it requires more time and planning and depends on the availability of supportive additional information. Irrespective of the approach used, the MAb panel(s) should be sufficiently broad to allow lineage assignment, characterization of the definitive phenotype and maturational stage of the blast population, detection of aberrant antigen expression, and identification of normal cell populations possibly present in the specimen (e.g., normal mature T-cells or precursor B-cells). Over the past decade, the increasing availability of MAbs with specificity for hematopoietic precursors and differentiation antigens has improved our ability not only to recognize various subtypes of precursor B- and T-cell ALL, as well as AML, but also to characterize leukemic stem cells and to extend our knowledge about the hematopoietic stem-cell hierarchy that is susceptible to leukemic transformation *(2,383)*.

A flow chart representing the currently relevant antigenic targets in the immunophenotypic analysis of acute leukemias is shown in Fig. 1, and a brief description of these antigens and their cellular distribution is given in Table 7. These antigenic targets include lineage-specific markers for the lymphoid or myeloid differentiation, antigens mainly expressed by hematopoietic precursors, and antigens more closely associated with distinct maturational stages of myeloid and B- or T-cells. In this context, it is noteworthy that MAbs within CD groups recognize the same cellular antigen, but not necessarily an identical epitope, and thus may differ in their reactivity to leukemic blasts. Additionally, the directly fluorochrome-conjugated MAbs in dual- or multicolor immunofluorescence assays for the analysis of leukemia-associated antigens are associated with different thresholds for recognition of antigen expression. Therefore, published results in acute leukemias from different laboratories, especially regarding weakly expressed antigens, may not be readily comparable.

It should be noted that the most specific markers for the lymphoid or myeloid lineages are expressed in the cytoplasm at the immature stages of cell differentiation *(384–386)*, and their flow-cytometric detection requires appropriate permeabilization and fixation procedures *(387)*. However, in view of the coexpression of lineage-specific molecules such as CD79α in T-lymphoblastic leukemia/lymphoma *(388)* or acute myeloid leukemias *(389)* and MPO in ALL *(390,391)*, the importance of these markers for lineage assignment of leukemic blasts has to be confirmed in further clinical studies. The same applies to MAbs recognizing distinct epitopes of the human pre-B-cell receptor that may improve the precision of precursor B-cell ALL subclassification *(392)*.

Terminal deoxynucleotidyl transferase (TdT), initially considered as a specific marker of immature lymphoid cells, can be detected in AML with varying incidence, probably because of the application of techniques with different sensitivities (reviewed in ref. *393*). Hence, TdT may be used as a marker of hematopoietic immaturity or to corroborate the diagnosis of ALL in the presence of other lymphoid-specific antigens *(21)* but not as a lymphoid-specific antigen. In our experience, this quite comprehensive panel of MAbs, especially when used in combination with a multiparameter (three-color or more) flow-cytometric procedure, is suitable for answering the most relevant questions (see above) in diagnostic immunophenotyping and monitoring of acute leukemias. On the basis of these immunophenotypic analyses, a firm diagnosis of AML and ALL as well as a subclassification of precursor B- and T-cell ALL can be made in >98% of cases.

Although a detailed discussion of multiparameter flow cytometry is beyond the scope of this chapter, it should be mentioned that in view of its potential clinical utility, many attempts have been undertaken to standardize and validate laboratory procedures used in the immunophenotypic analysis, to select appropriate combinations of MAbs, and to standardize data analysis and interpretation, as well as data reporting *(24,28,30,97,98,394,395)*. The broad range of topics to be considered in this process illustrates the scope of the problem of achieving consensus. Thus, a major challenge for the future will be to improve the intra- and interlaboratory reproducibility of flow-cytometric immunophenotyping by performing stable, calibrated, and standardized measurements in such a way that identical neoplastic cells provide identical phenotypic patterns, whenever they are analyzed at different times and in different laboratories *(24)*. Hopefully, the recent consensus recommendations will contribute to achieving this goal by successfully implementing adequate flow-cytometric practices in hematopoietic malignancies.

Advances in immunophenotyping by using state-of-the-art flow cytometry and three or four fluorochromes conjugated with MAbs facilitate a multiparametric measurement of intrinsic and extrinsic cellular properties of leukemic cells *(23)*. From these studies, much more information as to the immunophenotypic characteristics of leukemic lymphoblasts

Table 7
Clusters of Differentiation (CD) Antigens Useful in the Diagnosis and Classification of AML and ALL

CD group	*Molecular and functional characteristics*	*Cellular reactivity within the lymphohematopoietic system*	*Comments on diagnostic value in leukemia diagnosis*
Myeloid lineage			
CD13	150-kDa type II membrane glycoprotein, homodimer, aminopeptidase N	Early committed progenitors of granulocytes and monocytes (CFU-GM) and maturing cells of these lineages	Expressed in most AML, coexpressed in 20–35% of ALL
CD14	55-kDa glycosylphosphatidylinositol-linked glycoprotein; LPS receptor	Mature monocytes (strong), macrophages, granulocytes (weak)	Expressed predominantly in mature myelomonocytic leukemias (AML-M4, M5b)
CD15	Carbohydrate, 3-FAL, X-hapten, Lewis-X (Le^X); adhesion molecule, ligand for E-, P-, L-selectin (CD15s)	Mature granulocytes and monocytes, myeloid and monocytic cells, Langerhans cells	Expressed in 50% of AML, aberrantly expressed in 5–10% of ALL, predominantly in pre-B-ALL with t(4;11)
CD33	67-kDa transmembrane protein, sialoadhesin	Myeloid and monocytic cells, early erythroblasts, megakaryoblasts	Expressed in most AML, coexpressed in 20–35% of ALL
CD36	88-kDa glycoprotein, platelet gpIIIb, gpIV	Megakaryocytes, platelets, mature monocytes and macrophages, erythroid precursors	Expressed predominantly in AML-M5, M6, M7
CD41	Platelet glycoprotein IIb, αIIb integrin chain, forms complex with CD61/β3 integrin chain	Megakaryocytes and platelets	Expressed in AML-M7
CD42b	Platelet glycoprotein Ibα, forms complex with CD41c (disulfide bond) and CD41a, CD41d; CD41a–d: receptor for vWf (von Willebrand factor)	Megakaryoctes and platelets, absence of CD42 complex leads to Bernard-Soulier syndrome	Expressed in AML-M7
CD61	Platelet glycoprotein IIIa, β3 integrin chain, forms complex with CD41/αIIβ integrin chain	Megakaryocytes and platelets	Expressed in AML M7
CD64	72-kDa glycoprotein, high-affinity IgG Fc receptor (FcγRI), receptor-mediated endocytosis of IgG-antigen complexes, antibody-dependent cellular cytotoxicity	Monocytes and macrophages, immature granulomonocytic progenitors, subset of dendritic cells, early myeloid lineages	Expressed in monoblastic/monocytic leukemia and in subsets of immature AML
CD65/CD65s	Carbohydrate, ceramide-dodecasaccharide/sialylated-CD65	Mature granulocytes, myeloid cells, monocytes	Expressed in most AML, aberrantly expressed in 5–10% of ALL, predominantly in pro-B-ALL with t(4;11)
CD66c	90-kDa GPI-linked glycoprotein, member of the carcinoembryonicantigen family	Mature granulocytes, myeloid cells, monocytes	Expressed by distinct subsets of B-cell precursor ALL (e.g., Ph^+, *ETV6-AML1*$^-$, and hyperdiploid cases)
T-lineage			
CD1a	49-kDa type I transmembrane glycoprotein, MHC I-like; binds to β_2-microglobulin; non-peptide antigen-presenting molecule	Cortical thymocytes, Langerhans cells	Defines cortical precursor T-cell ALL
CD2	50-kDa type I transmembrane glycoprotein, LFA-1; receptor for CD58 (LFA-3); adhesion and signal transducing molecule	Thymic and mature T-cells, most NK cells	Expressed in 70–85% of precursor T-cell ALL and approx. 10% of AML (especially M3 and M4Eo subtypes)
CD3	Complex of six polypeptide chains, component of the TCR (associated with TCRαβ or TCRγδ)	Thymic and mature T cells	Cytoplasmic expression defines precursor T-cell ALL, membrane expression in 25% of T-lineage ALL defines mature precursor T-cell ALL

Continued on next page

Table 7 (Continued)
Clusters of Differentiation (CD) Antigens Useful in the Diagnosis and Classification of AML and ALL

CD group	*Molecular and functional characteristics*	*Cellular reactivity within the lymphohematopoietic system*	*Comments on diagnostic value in leukemia diagnosis*
T-lineage (Cont.)			
CD4	55-kDa transmembrane glycoprotein, receptor for MHC class II molecules, receptor for HIV envelope glycoprotein (gp120)	Subset of thymocytes and mature T-cells (helper/inducer), monocytes, macrophages	Variably expressed by preT-, cortical, or mature precursor T-cell ALL, and AML (especially of monocytic origin)
CD5	67-kDa glycoprotein, scavenger receptor cysteine-rich (SRCR) family, costimulatory molecule and receptor for CD72	Thymic (weak expression) and mature (strong expression) T-cells,subset of mature B-cells	Expressed by 90–95% of precursor T-cell ALL
CD7	40-kDa glycoprotein,	T cells, NK cells, hematopoietic stem cells	Expressed in virtually all precursor T-cell ALL and approx. 15% of AML
CD8	32-kDa, αα homodimer or αβ heterodimer, coreceptor with TCR for MHC	Subset of thymocytes and mature T-cells (suppressor/cytotoxic), NK cells	Variably expressed by precursor T-cell ALL
CD group	Molecular and functional characteristics	Cellular reactivity within the lymphohematopoietic system	Comments on diagnostic value in leukemia diagnosis
B lineage			
CD19	95-kDa glycoprotein, associates with CD21; signal transduction	Expression from the earliest recognizable B-lineage cells to mature B-cells, follicular dendritic cells	Expressed in virtually all B-cell precursor ALL and a subset of AML [especially AML-M2 with t(8;21)]
CD20	33–37-kDa phosphoprotein, B-cell activation	B-cells	Expressed in 40% of B-cell precursor ALL
CD22	135-kDa type I glycoprotein, adhesion and signaling	Precursor and mature B-cells	Cytoplasmic expression in virtually all B-cell precursor ALL,membrane expression in B-cell precursor ALL and B-ALL
CD24	35–45-kDa glycosylphosphatidylinositol-linked glycoprotein	Precursor and mature B-cells, neutrophil granulocytes	Expressed in >90% of B-cell precursor ALL and some AML
CD79α	40–65-kDa glycoprotein, associated with CD79α; component of B-cell antigen receptor	Precursor and mature B-cells, plasma cells	Expressed in virtually all B-cell precursor ALL and aberrantly in some AML
NK			
CD56	175–220-kDa glycoprotein, neural cell adhesion molecule (NCAM), homotypic and heterotypic cell adhesion (in neural development)	NK cells, subset of T-cells	Expressed in some AML with t(8;21), t(15;17), acute monocytic leukemia and NK cell neoplasms
Non-lineage-associated			
CD10	100-kDa glycoprotein, zinc metalloprotease, neutral endopeptidase, common ALL antigen (CALLA)	Lymphoid precursors, germinal center B-cells, mature neutrophil granulocytes	Defines common ALL, expressed in approx. 40% of T-cell precursor ALL
CD34	105–120-kDa type I transmembrane glycoprotein, cell adhesion	Early lymphohematopoietic stem and progenitor cells	Expressed in 60–70% of B-cell precursor ALL, <10% of T-cell precursor ALL, and 40–50% of AML
CD45	180–220-kDa glycoprotein, leukocyte common antigen (LCA), tyrosine phosphatase, T- and B-cell antigen receptor-mediated activation	Expressed, typically at high levels, on all hematopoietic cells	Expressed in 90% of all B-cell precursor ALL, nearly all T-cell precursor ALL, and nearly all AML
CD117	145-kDa glycoprotein, tyrosine kinase receptor type 3, *c-kit*, stem cell factor (SCF) receptor	Hemopoietic stem and progenitor cells	Expressed in approx. 85–90% of B-cell precursor ALL and virtually all other subtypes of acute leukemia

Abbreviations: LFA, leukocyte factor-associated antigen; LPS, lipopolysaccharide; NK, natural killer; TCR, T-cell receptor.

and their functional features has emerged, which could be helpful in answering specific biologic as well as clinical questions; these data have not yet been applied on a large scale to routine immunophenotyping of ALL within clinical trials.

The enormous progress achieved over the last two decades in the identification of leukocyte surface antigens *(396)* has led to a deeper insight into the functional significance of various molecules that have proved useful in immunophenotyping of acute leukemias, as well as of novel molecules, more recently emerging as key regulators of cell proliferation, differentiation, adhesion, and induction of apoptosis. These novel markers, including costimulatory molecules *(397)* hematopoietic growth factor receptors *(398)*, adhesion molecules *(399)*, and chemokine receptors *(400)*, may hopefully complement the phenotypic classification in the future by providing a more clinically relevant functional subdivision of AML and ALL. In addition, considerable interest has focused on the expression of molecules mediating the multidrug resistance phenotype *(157)* and of proteins that play an important role in the regulation of drug-induced apoptosis in acute leukemias *(401)*. Preliminary results in both childhood and adult acute leukemias suggest that the expression of at least some of these molecules can be easily detected by flow cytometry and may provide clinically relevant information (e.g., Bcl-2-related proteins, death receptors for FasL and TRAIL, and caspases) *(158,161,402,403)*. However, further prospective investigations, preferably in the context of controlled clinical trials, are needed to clarify the importance of these molecules in the development of cytotoxic drug resistance in acute leukemia and to determine their prognostic significance compared with other parameters (e.g., in vitro drug sensitivity testing, early in vivo treatment response). These studies will hopefully also contribute to the elucidation of the precise mechanisms involved in the apoptotic killing of acute leukemia cells and of the strategies by which malignant cells escape killing by cytotoxic drugs *(401)*.

12.4. Should We Continue to Subdivide Precursor B- and T-Cell ALLs into Distinct Immunophenotypic Subgroups, and Which Subgroups Should be Identified?

Research over the past two decades, by applying immunophenotypic, cytogenetic, and molecular genetic techniques, has provided valuable information as to the diagnosis and classification of ALL. It has been demonstrated that precursor B- and T-cell ALL represents highly heterogeneous groups of malignancies that for the most appropriate tailoring of treatment strategies require a complex classification system, taking into account genotypic as well as immunophenotypic and clinical features (reviewed in ref. *348*). To be clinically useful, this classification system has to be easy, applicable to most patients in clinical trials, rapid to assess, reproducible, cost-effective, and capable of identifying biologically and clinically relevant entities.

In the past, criteria applied to define subgroups of precursor B- and T-cell ALL patients have differed markedly, and various immunophenotypic classifications of ALL have evolved, with most of them reflecting normal B- and T-cell ontogeny. The terms used to designate ALL immunophenotypic subgroups have been mainly based either on the presumptive B- and T-cell differentiation stages of normal lymphopoiesis, which define four to six subgroups of precursor B-cell ALL *(404,405)* and three to four subgroups of precursor T-cell ALL *(332,334,404,406)*, or on the expression of CD10, cytoplasmic or sIg, and different T-cell antigens, thus distinguishing broader categories of precursor B- and T-cell ALL *(341,384,407)*. More recently, the European Group for the Immunological Characterization of Leukemias (EGIL) has proposed guidelines for the immunophenotypic subclassification of B- and T-lineage ALL into different categories according to the degree of B- or T-lymphoid differentiation of the blast cells *(28)* (Table 8). Although conceptually, most of these models are useful in classifying ALL, several studies have shown that the immunophenotypic features of precursor B- and T-cell ALL and the normal stages of B- and T-lymphocyte development differ remarkably and that asynchronous or aberrant combinations of B- or T-cell-associated antigens, which do not reflect normal lymphoid ontogeny, have been demonstrated in the vast majority of precursor B- and T-cell ALL cases *(384,408–411)*. Based on these observations, it was suggested that leukemic blasts in precursor B- and T-cell ALL may not originate from phenotypically identical normal cellular counterparts or may result from the malignant proliferation of rare normal lymphoid cells not detectable with presently available methods *(408)*. Alternatively, asynchronous or aberrant expression of antigens in ALL has been postulated to reflect genetic alterations that occur during early stages of lymphoid differentiation (reviewed in ref. *412*).

In view of these data and only weak evidence in favor of basing treatment strategies on immunophenotyping studies, the clinical importance of subclassification into distinct precursor B- and T-cell subsets has been questioned *(348)*, and alternative criteria not representing a classification of ALL derived from an

Table 8
Classification of ALL

Category	*Definition*
B-lineage ALL[a]	**CD19+ and/or CD79α+ and/or CD22+**
Pro-B (B-I)	No expression of other B-cell differentiation antigens
Common (B-II)	CD10+
Pro-B (B-III)	Cytoplasmic IgM+
Mature B (B-IV)	Cytoplasmic or surface κ or λ+
T-lineage ALL[b]	**Cytoplasmic/membrane CD3+**
Pro-T (T-I)	CD7+
Pre-T (T-II)	CD2+ and/or CD5+ and/or CD8+
Cortical T (T-III)	CD1a+
Mature T (T-IV)	Membrane CD3+, CD1a-
α/β + (group a)	Anti-TCR α/β+
γ/δ + T-ALL (group b)	Anti-TCR γ/δ+
ALL with myeloid antigen expression (My + ALL)	

[a] Positive for at least two of the three markers. Most cases are terminal deoxynucleotidyl transferase (TdT)+, HLA-DR+ except for B-IV, which often is TdT–.

[b] Most cases are TdT+ HLA-DR-CD34–, but these markers are not considered for diagnosis or disease classification.

Table 9
Scoring System for Biphenotypic Acute Leukemias (BALs)[a]

Points	*B-lineage*	*T-lineage*	*Myeloid lineage*
2	CD79α cy IgM (cy) CD22	CD3 (cy/m) anti-TCR α/β anti-TCR γ/δ	MPO
1	CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD13 CD33 CD65s CD117
0.5	TdT CD24	TdT CD7 CD1a	CD14 CD15 CD64

Abbreviations: cy, cytoplasmic; m, membrane; TCR, T-cell receptor; MPO, myeloperoxidase; Tdt, terminal deoxynucleotidyl transferase.

[a]Total scores must exceed 2 for the myeloid lineage and 1 for the lymphoid lineages. The value of each marker is given in the far left column.

ontologic model have been proposed *(413)*. However, there is now a great deal of evidence that several immunophenotypic subgroups of precursor B- and T-cell ALL have innate cell biologic and clinical features that should be taken into consideration when interpreting treatment outcome. Moreover, intrinsic pharmacologic differences in responsiveness to cytotoxic drugs, recently described in immunophenotypic subgroups, may contribute to devising truly lineage- and/or subgroup-specific therapeutic interventions for patients with ALL *(414,415)*.

In precursor B-cell ALL, the following subclassification may provide the most useful information to guide treatment selection: (1) pro-B ALL, frequently associated with 11q23 rearrangements in both childhood and adult ALL; (2) common and pre-B ALL, with both subsets harboring a high degree of genotypic diversity [e.g., Ph translocation, t(12;21), t(1;19), hyperdiploidy]; and probably (3) transitional pre-B ALL or late pre-B ALL, characterized by leukemic blasts that express both cytoplasmic μ and sIg μ heavy chains without κ or λ light chains, and show an excellent response to precursor B-cell-directed treatment *(416)*. Distinctions such as that between pro-B and common/pre-B ALL are especially helpful in patients in whom karyotyping has not been possible, since it may direct molecular genetic studies for gene rearrangements (e.g., *MLL* or *BCR-ABL*).

In precursor T-cell ALL, no clear-cut correlations of immunophenotypic subgroups with genetic abnormalities have yet been established. However, recent studies suggest that immature subgroups with a pro-T and pre-T phenotype differ markedly with respect to their cell biologic features and treatment response from the predominant subgroup, which has a CD1a-positive phenotype *(310,320,342)*, thus corroborating the value of immunophenotypic subclassification of precursor T-cell ALL. The relationships of other precursor T-cell ALL subgroups (i.e., pro-T vs pre-T, αβ- vs γδ-positive ALL) with cell biologic features and treatment outcome are still unclear and have to be prospectively evaluated within controlled clinical trials in both childhood and adult ALL.

12.5. Which Criteria Should Be Used to Establish Aberrant Antigen Expression and to Classify Biphenotypic Acute Leukemia?

The widespread application of flow-cytometric immunophenotyping with a large panel of MAbs to myeloid- and lymphoid-associated differentiation antigens has led to the recognition of acute leukemias with blasts coexpressing antigens associated with different lineages. Different hypotheses have been postulated to explain the origin of these acute leukemias, which often display heterogeneous cell biologic features, leading to the concept of "lineage infidelity" *(95)*, which implies aberrant phenotypic features frequently resulting from specific genetic alterations, and the theory of "lineage promiscuity" *(417)*, whereby the malignant transformation occurs in a progenitor cell with the capacity for both myeloid and lymphoid differentiation. Unfortunately, much controversy has surrounded the criteria for identifying such leukemias, and a variety of terms have been used to refer to these acute leukemias, such as hybrid, biphenotypic, mixed-lineage, myeloid antigen-positive ALL (My+ ALL), and lymphoid antigen-positive AML (Ly+ AML), thus causing considerable confusion and complicating the assessment of the clinical importance of these observations.

More recently, strict and well-defined criteria have been proposed that were aimed at distinguishing biphenotypic acute leukemia (BAL), also referred to as "true" mixed-lineage leukemia, from those cases with aberrant expression of one or more markers from another lineage (e.g., My+ ALL and Ly+ AML) *(25,28,340,418)*. Criteria and scoring systems applied to the diagnosis of BAL are based on the number and degree of specificity of the markers (lymphoid and myeloid) expressed by the leukemic blasts and have been described in detail elsewhere *(25,28,340)*. The diagnosis of both BAL (Table 9) and My+ ALL or Ly+ AML requires multiparametric flow cytometry with at least two fluorochromes conjugated to different MAbs to demonstrate coexpression of lineage-specific (e.g., MPO, CD22, CD79α, CD3) and/or lineage-associated antigens. Other markers with a high degree of specificity (e.g., MAbs recognizing surrogate light-chain components) *(392)* will be incorporated into these scoring systems in the near future and hopefully improve its reliability. Although extensive data on the cell biologic features and response to treatment of BAL or mixed-lineage leukemia are not yet available, preliminary results suggest that they represent an uncommon subtype with distinct genetic (e.g., Ph translocation, 11q23 rearrangements, complex cytogenetic abnormalities) and clinical features as well as a poor prognosis *(418–422)*. Based on immunophenotyping, cytogenetic and molecular genetic findings, and the documented phenomenon of in vivo as well as in vitro phenotypic switches in some cases of BAL, it has been suggested that these leukemias arise in a multipotent progenitor cell with the capability of differentiating along both myeloid and lymphoid lineages *(421)*.

In contrast to BAL, My+ ALL and Ly+ AML occur frequently. Their incidence has varied considerably among independent studies, both overall and with regard to individual antigens (reviewed in refs. *88* and *412*), ranging from 5 to >50% for My+ ALL and from 10 to 30% for Ly+ AML. This wide variability has been attributed to a number of causes, including

the lack of consistent criteria for the diagnosis of My+ ALL or Ly+ AML and for defining positive results, the utilization of various panels of MAbs, the lack of lineage specificity of most of the MAbs used, and several technical factors (e.g., distinct sensitivities of fluorochromes and flow cytometers, inconsistent gating strategies) *(88,282,412,419)*. In view of previous studies pointing to myeloid-antigen expression as a predictor of poor prognosis in both childhood and adult ALL *(423,424)*, considerable interest has focused on the cell biologic features and clinical significance of this subgroup of acute leukemias. Several recent studies, including more than 4000 pediatric patients with ALL *(425–427)* and our own data in almost 5000 children treated within the ALL-BFM 86, 90, and 95 trials *(320,327,428)*, have failed to demonstrate an association of My+ ALL with poor outcome. In some of these studies, myeloid-associated antigen expression was clearly associated with certain genetic features of leukemic cells, particularly *MLL* and *ETV6-AML1* rearrangements *(293,427–429)*. In contrast to childhood My+ ALL, the clinical importance of myeloid-associated antigen expression in adult ALL is still unknown. The presence of myeloid-associated antigens has been associated with a poor outcome in some *(423,430)* but not all studies *(270,431,432)*. Most of these studies, however, included only a relatively small number of patients, have not always carefully excluded minimally differentiated AML (AML M0) *(423)*, and, most important, have not adequately taken into account the prognostic importance of specific genetic abnormalities frequently found in adult patients with My+ ALL, such as Ph positivity or 11q23 rearrangements *(268,270,282,433)*. Further prospective studies, consistently based on well-defined diagnostic criteria, are urgently needed to elucidate more accurately the biologic heterogeneity of My+ ALL and to establish its clinical relevance in adult patients.

A critical review of data published in the literature revealed that most retrospective and prospective studies failed to demonstrate any prognostic significance for Ly+ AML, except for CD7+ AML *(88)*. The latter subgroup has been associated with more frequent expression of progenitor-associated markers (e.g., CD34, CD117, HLA-DR, TdT), concomitant rearrangements of *Ig* and/or *TCR* gene rearrangements, and poor prognosis in most (but not all) studies in both childhood and adult AML *(86,87,162,434–437)*. It should be noted that immature CD7+ AML and pro-/pre-T ALL occasionally show biologic similarities, such as reactivity with MAbs recognizing antigens expressed on both immature T-cell ALL and AML *(438)*, responsiveness to several growth factors *(437)*, expression of *c-kit* at the mRNA and protein levels *(164,165,340,439)*, expression of the multidrug resistance phenotype *(440)*, and similar *TCRδ* gene rearrangements *(441)*, suggesting that in at least some CD7+ acute leukemias, malignant transformation has arisen in a pluripotent progenitor cell with variable differentiation potential along both myeloid and T-lymphoid lineages *(429)*.

Given the significant associations between expression of several lymphoid-associated antigens by AML and specific genetic abnormalities, such as CD19 in AML with t(8;21) and CD2 in AML with t(15;17), as well as AML with inv(16) or t(16;16), cytogenetic and molecular data have to be incorporated into the classification of Ly+ AML, and future studies evaluating the prognostic significance of Ly+ AML have to take into consideration its genetic background.

12.6. Which Conventional and Molecular Cytogenetic Techniques are Necessary to Identify Cytogenetic Alterations and to Provide Cytogenetic Information that Has Clinical Relevance?

In general, classical cytogenetics using banding techniques is still the gold standard for the genetic classification of acute leukemias. These techniques should be performed in each patient with acute leukemia at diagnosis as well as at relapse. New techniques such as FISH, Southern blot, and PCR analyses have added important information to the more sophisticated subgrouping of acute leukemia. These techniques should be used on demand in cases in which these investigations can give information that cannot be obtained by banding analysis. Screening with these techniques for each detectable genetic aberration is very expensive and labor-intensive and thus not cost-effective. In childhood B-cell precursor ALL, screening with RT-PCR or FISH for the detection *ETV6-AML1* rearrangement seems mandatory, because t(12;21) is mostly not detectable with conventional cytogenetics. Furthermore, in cases of T-cell precursor ALL, checking for *TAL1* rearrangements with Southern blotting or RT-PCR should be performed. If no banding analysis is available, screening for the most important abnormalities regarding prognosis is recommended: in B-cell precursor ALL: *BCR-ABL* rearrangement, *E2A-PBX1* rearrangment, *MLL* rearrangements, hyperdiploidy, 9p deletions; in mature B-ALL: translocations involving *MYC*; in precursor T-cell ALL: *TAL1* rearrangement and in AML: rearrangements of *AML1-ETO*, *PML-RARα*, *CBFβ-MYH11*, *MLL* rearrangements, monosomies 5/7, and deletions 5q/7q and 17p.

12.7. Do We Need an International Cytogenetic Classification for Acute Leukemias and Which Aspects are Important?

An international cytogenetic classification is urgently needed to allow a comparison among different studies. On the one hand, a hierarchical classification according to primary chromosome aberrations is needed; on the other hand, for clinical use a prognostic grouping for distinct cytogenetic abnormalities is required. This is problematic because the prognosis of cytogenetic subgroups is influenced by therapy. Therefore, a biologically orientated classification is necessary that will allow analysis of the impact of certain treatments on a cytogentically defined subgroup of patients.

In AML, the favorable cytogenetic subgroup is well defined, and nearly all study groups agree that patients with t(15;17), t(8;21), or inv(16)/t(16;16) belong to this subgroup. The impact of additional abnormalities on favorable aberrations has to be determined in metaanalyses. For the intermediate and unfavorable subgroups, discrepancies occur. The intermediate subgroup is a mixture of patients with normal karyotypes, karyotypic abnormalities with proven intermediate prognosis, and karyotypic aberration of unknown prognostic significance owing to low frequency of these aberrations. For future analysis, more informative results can be obtained if the intermediate group is analyzed in three subgroups, as mentioned above. In patients

with a normal karyotype, molecular studies will help to define distinct entities within this probably heterogeneous group. Metaanalyses will help to clarify the prognostic impact of rare abnormalities of as yet unknown prognostic impact. Concerning karyotypic abnormalities assigned to the unfavorable group, the definition of complex aberrant karyotypes in particular has to be standardized and based on biologic features, rather than on the number of abnormalities. Further molecular studies in this subgroup may clarify the underlying biologic mechanism leading to genomic instability and a poor prognosis.

Cytogenetic classifications for clinical use based on prognostic impact will change with the implementation of new treatment strategies. Therefore, reports on the prognostic impact of distinct cytogenetic abnormalities must always be evaluated in the background of the applied treatment.

12.8. Do We Need Cytogenetic Analyses in Relapsed AML?

Only a few studies on the cytogenetics of relapsed AML have been published. These studies and our own data, however, show that karyotype is a prognostic factor in AML at relapse. From 50 to 60% of patients with a favorable or intermediate karyotype in relapse achieve a complete remission, contrasted with only 20% of cases with unfavorable cytogenetics *(442)*. Relapse occurs later in patients with a favorable or intermediate karyotype than in patients with unfavorable cytogenetics *(443)*. Compared with cytogenetics at diagnosis, the same karyotype is found in relapse in 40–50% (20–30% show normal karyotypes and about 20% the same aberrant karyotype at diagnosis and in relapse). From 15 to 20% of patients show a normal karyotype at diagnosis and an aberrant one at relapse, whereas 25% of those who had an aberrant karyotype at diagnosis gain additional chromosomal aberrations at relapse. In 2–5%, a new clone is observed at relapse that is unrelated to the clone observed at diagnosis *(179,180)*. In these patients, secondary AML rather than a relapse of the primary AML should be suspected.

12.9. What Is the Impact of Cytogenetics on Therapy-Related AML?

Leukemia secondary to chemotherapy accounts for 10–15% of all AML cases. The therapy-related (t-)AMLs are usually divided into two subgroups, depending on whether the patient has received alkylating agents or drugs targeting topoisomerase II. t-AMLs related to alkylating agents are frequently characterized by a preceding myelodysplastic phase, a long interval between cytotoxic treatment and the appearance of t-AML (36–72 mo), cytogenetic abnormalities involving chromosomes 5 and 7, and often complex aberrant karyotypes and a poor response to therapy. t-AML related to therapy with topoisomerase II inhibitors usually presents as overt leukemia without a myelodysplastic phase with either an M4 or an M5 subtype according to FAB classification, has a short latency period (12–36 mo), and shows balanced chromosome aberrations, primarily translocations involving chromosome bands 11q23 and 21q22 and a more favorable response to chemotherapy *(218,444–448)*. Translocations involving 11q23 predominate following therapy with epipodophyllotoxins, whereas patients with translocations to 21q22, inv(16), and t(15;17) most often have received anthracyclines. However, a multivariate analysis in a large series of patients with t-AML and balanced translocations showed that younger age and not a specific type of DNA topoisomerase II inhibitor seems to predispose to the development of t-AML with 11q23 translocations *(449)*. In accord with these data, our own findings indicate that patients with balanced chromosomal aberrations such as t(8;21), inv(16), t(15;17), or t(11q23) were significantly younger than those with other abnormalities (median age 45 vs 60 yr) and showed a shorter latency period between the primary tumor and t-AML (30 vs. 81 mo) *(450)*.

Compared with *de novo* AML, t-AML has a higher incidence of clonal chromosome abnormalities. In 75–96% of cases, karyotypic aberrations are detected *(226,450–452)*. The spectrum of such changes is comparable, but the distribution varies, as 11q23 abnormalities and complex aberrant karyotypes occur more often in patients with t-AML than in *de novo* AML *(447,450,453,454)*.

Overall, t-AML responds less well to treatment than does its *de novo* counterparts. Recent data show that, as in *de novo* AML, cytogenetics are an important prognostic factor in t-AML; moreover, if corresponding cytogenetic subgroups are compared according to response, outcome does not differ much *(450,451,454–456)*.

Work is in progress to identify parameters that predispose patients to the development of t-AML. Defects in DNA repair mechanisms as well as polymorphisms in enzymes involved in the metabolism of antileukemia drugs are under investigation *(457,458)*. However, as t-AML is a secondary and in some cases a tertiary neoplasm, and in a quite high proportion of patients surgery was the only treatment for the primary tumor, a predisposition of cancer patients to leukemia, independent of previous chemo- or radiotherapy, cannot be excluded *(444)*.

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