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ACUTE LYMPHOCYTIC LEUKEMIA

A broad and integrated diagnostic work-up for a modern management of Acute Lymphoblastic Leukemia (ALL)

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Acute lymphoblastic leukaemia (ALL) represents a biologically and clinically heterogeneous group of diseases characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues. Malignant transformation is a consequence of somatic mutations in a single lymphoid progenitor cell and this mutation might occur at different stages of B- or T-cell development. The diagnosis and classification of ALL is currently a multistep procedure based on morphology, cytochemistry, immunophenotype, cytogenetics, molecular genetics, immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements, multidrug resistance (MDR), genomic profiling and relies on the simultaneous application of multiple techniques [1–4]. Increasing evidence suggests that chromosomal defects and molecular abnormalities are consistently present in patients with ALL, and progress in our understanding of the biologic and genetic characteristics of ALL has not only improved our knowledge of leukemogenesis, but has also allowed the identification of prognostic groups with specific cellular and molecular features [5,6]. Despite the effort to develop risk classification systems that are both reproducible and comparable, a need to refine the ability to distinguish between higher and lower risk patients still remains. In addition to their biologic relevance and their potential prognostic impact, cytogenetic and molecular abnormalities, as well as the immunophenotypic combinations, may offer additional tools for the detection of minimal residual disease (MRD) during the clinical course of the disease. Evidence suggests that a broad and integrated characterization of adult ALL in the context of multicenter protocols is essential for an optimal clinical management [7]. Detailed studies of individual patients need to be conducted at specialized centers, where preservation of viable cells, DNA, RNA, protein lysates, etc. is possible. An integrated biologic approach aimed at identifying prognostic factors implies a coordinated effort through central handling of all patients' samples so that all the necessary investigations can be consistently performed in each individual case and the patients can be enrolled in the same therapeutic protocols. Besides, an integrated approach, using cytogenetic and molecular analysis and leukemia-associated immunophenotypes, can allow to identify suitable markers for monitoring MDR in virtually all childhood and adult ALL cases [8,9]. Recent advances in genome technologies have opened the way for the analysis of gene expression profiles in human leukemias that may lead to innovative genomic-based classifications of hematologic malignancies, as well as to the design of innovative therapeutic strategies.

Morphology and cytochemistry

ALL has been defined by the presence of more than 30% lymphoblasts in the bone marrow (BM) or peripheral blood (PB) according to the French-American-British (FAB) Co-operative group classification system [10]. In the recently proposed World Health Organization (WHO) classification scheme, [11] a blast count above 20% is sufficient for a diagnosis of acute leukemia. The morphologic/cytochemical examination recognizes three morphologic types: L1, L2 and L3 (Table 1). The prognostic significance between the L1 and L2 morphologic subtypes of ALL has never been fully proven; simi-

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Table 1. Blast cell characteristics of ALL subtypes

	Pro-B	Common	Pre-B	B	T
FREQUENCY					
Adults	20–25%	50%		2–5%	20–25%
Children	50–70%	90%	25%	2–5%	15%
MORPHOLOGY	L1/L2	L1/L2	L1/L2	L3	L1/L2
Nucleat TdT	+	+	+	−	+
5'Nucleotidase	+	+	+	−	−
A. phosphatase	−	−	−	−	+
MPO	−	−	−	−	−

larly, immunophenotypic profiles that may be of prognostic significance do not correlate with the L1 or L2 morphology. Only the L3 type of ALL still holds as a distinct entity characterized by its morphology and also by its unique immunophenotypic and genotypic features. Unlike acute myeloid leukemia (AML), no single cytochemical test is specific for ALL; by definition, however, ALL is negative for myeloperoxidase (MPO) in cytochemistry studies and lacks staining with the anti-MPO monoclonal antibody (MoAb). According to the FAB criteria, cases of acute leukemia with more than 3% MPO positive blasts should be classified as AML. More than 95% of cases of L1 and L2 ALL are positive for terminal deoxynucleotidyl transferase (TdT) expression and its detection is useful in distinguishing reactive lymphocytosis from ALL; nevertheless, TdT expression can be found in some cases of AML. Most cases of ALL have a characteristic localized periodic acid-Schiff (PAS) staining pattern, but this finding is not specific for ALL and'block' reactivity can be seen in some AML. Reactivity for nonspecific esterase can be detected in a subset of ALL and is usually weaker than that seen in acute monocytic leukemia. Acid phosphatase and alpha-naphtyl-acetate-esterase (ANAE) reactions give a strong positivity localized to the Golgi region in more than 80% of the blast cells in cases of T-lineage ALL; nowadays, however, they are no longer routinely utilized. Even though no single cytochemical reaction is specific, cytochemistry still represents a relevant component in the integrated diagnostic work-up of acute leukemias that helps to differentiate between ALL and AML.

Immunophenotype

The immunophenotypic characterization of blast cells has several objectives: (a) lineage assignment, (b) evaluation of cell maturation, and (c) assessment of phenotypic aberrations [12–14], Lineage assignment of blast cells by immunophenotype may still represent a major challenge in some acute leukemias; this is mainly due to the cross-lineage antigen expression and it emphasizes the need to use combinations of several lineage-associated markers to establish the lineage affiliation of the blast cells. Flow cytometry is a powerful technique for the characterization of normal and neoplastic hematopoietic cells, the use of highly specific MoAb that recognize distinct epitopes of surface and intracellular antigens has improved the definition of the origin and level of differentiation of acute leukemias. There is no consensus on the best way to report the analyzed data; it is customary to report percentage of blasts expressing each antigen tested and to consider any marker present on more than 20% of blasts as positive: the cut-off level of 20% is arbitrary, however. Another point to consider in leukemia immunophenotyping is the intensity of antigen expression; since differences in fluorescent intensity may be important in distinguishing leukemic cells from normal cells and in discriminating among subtypes of leukemia, quantitative flow cytometry (QFCM) may now be used to measure antigen-binding sites on cells more objectively and this approach may be useful both at diagnosis and during the monitoring of MRD [15]. As shown in Table 2, B-lineage ALL (70–80% of cases) can be classified into four groups according to the expression of B-cell differentiation antigens and cytoplasmic and surface immunoglobulins (Ig); also T-ALL (15–25% of cases) can be classified into four groups based on the level of thymocyte maturation and antigen expression [16]. T-ALL can be further classified according to the subtypes of T-cell receptor (TCR) molecules. Although the affiliation of ALL cases to the B- or T-cell lineage is relatively easy, about 5% of cases remain difficult to classify as ALL or AML; these cases coexpress several lymphoid and myeloid antigens, either on the same cells (biphenotypic leukemia) or on two different populations (hybrid leukemia). There is no consensus regarding diagnostic criteria for such cases. The European Group for the Immunological Characterization of Leukemia (EGIL) [16] has suggested the use of a scoring system based on different combinations of B, T and myeloid antigen expression. According to a strict scoring system, four groups can be identified; the most common group is that in which the blasts coexpress myeloid and B-lymphoid antigens, and less commonly myeloid and T-lymphoid antigens. Cases coexpressing T- and B-lymphoid markers and those with trilineage differentiation are rare. The clinical significance of biphenotypic acute

Table 2. Immunologic classification of All

□ B-lineage ALL:	
■ CD19+ and/or CD79a+ and/or cyCD22+	pro-B ALL (B-I)
■ CD10+ cyIg-	common ALL (B-II)
■ cyIg+ sIg-	pre-B All (B-III)
■ sIg+	mature-B ALL (B-IV)
□ T-lineage ALL:	
● cyCD3+ CD7+	pro-T ALL (T-I)
● CD2+ and/or CD5+ and/or CD8+	pre-T ALL (T-II)
● CD1a+	cortical T ALL (T-III)
● CD1a- mCD3+	mature T ALL (T-IV)
● Anti-TCR α/β+	α/β+ T ALL (group a)
● Anti-TCR γ/δ+	γ/δ+ T ALL (group b)
➢ All with expression of one or two myeloid markers (My+ALL)	

leukemia has not been determined and there has been a lack of uniformity in treatment; for example, there is no agreement as to whether induction therapy should use anti-lymphoid and/or anti-myeloid drugs [17]. To allow reproducible conclusions to be drawn about the optimal treatment of biphenotypic leukemias, increased numbers of patients are required for an objective analysis; due to the rarity of the disease, this will only be possible through multicenter studies.

Other markers are used to identity the maturation level of the blast cells and eventually establish atypical or aberrant phenotypes indicative of specific underlying genetic lesions. A variable proportion of ALL express apparently nonlineage associated markers, e.g., myeloid antigens and CD34. Expression of myeloid associated markers in ALL is well known, but its clinical significance is controversial. The reported incidence of adult ALL showing myeloid antigen expression (My+ ALL) ranges from 15% to 50%, while it varies from 4% to 35% in children [18]. This broad variation may be related to the number of myeloid antigens studied and their degree of lineage specificity, the sensitivity of the MoAb used, the cut-off level and technical factors (e.g., flow cytometric sensitivity and gating strategy). The most frequently expressed myeloid antigens are CD33 ($\sim$25%) and CD13 ($\sim$20%); CD15 and CD14 can be found in $\sim$15% of ALL cases, while CD11c is rarely present on ALL blasts [18,19]. Some studies have shown that the expression of myeloid-associated antigens is a predictor of poor outcome both in childhood and adult ALL, while other studies have not confirmed this observation [20–23]. The presence of myeloid antigens can be useful in the immunologic monitoring of MRD.

CD34 is the most commonly used antigen to define immature hematopoietic progenitor cells. CD34 is present in only 1.5% of BM cells, it is not lineage-restricted and its expression has been documented both in AML and ALL [24]. Overall, about 70% of ALL cases are CD34 positive. The incidence of CD34 expression is more frequent in B-lineage ALL (70–80%) than in T-lineage ALL (20–30%); its expression has also been recorded in a high proportion of Ph chromosome positive ALL. The prognostic significance of CD34 antigen expression in ALL, especially in adult patients, is still not well established, even though its presence does not seem to influence clinical outcome [25,26].

In conclusion, immunophenotype is an essential component of the initial diagnostic evaluation of acute leukemias and is also a valuable tool for monitoring disease after therapy and for the detection of MRD. The quantification of the level of expression of given antigens on the leukemic population may have therapeutic implications; MoAb have, in fact, reached clinical utilization in different lymphoproliferative disorders. This applies, in particular, to antibodies directed against CD20, CD22 and CD52. All three antigens may be expressed by ALL cells. Thus, the percent of positivity and the degree of expression by the leukemic population at diagnosis and at relapse is important when considering the potential clinical utilization of such antibodies for the management of ALL patients.

Cytogenetic and molecular analyses

Cytogenetic and molecular analyses are important in identifying prognostic markers in ALL [5,6,27,28]. The study of cytogenetic abnormalities is the basis for unraveling molecular events that may be involved in the disease, such as the role of fusion transcripts that derive from translocations, tumor suppressor genes from deletions, or the control of cell cycle regulatory genes. There are some limitations associated with cytogenetic studies in ALL: the leukemic cells do not always produce good metaphases and important abnormalities can be missed. Reverse-transcriptase polymerase chain reaction (RT-PCR), DNA flow cytometry, fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH), among other techniques, have made it possible to detect more precisely the molecular and chromosomal defects in common subtypes of ALL. However, detection of chromosomal abnormalities by classic

karyotypic analysis or by molecular techniques has its advantages and inconveniences. Only through a karyotypic analysis can an overall evaluation of the whole genome be carried out and the results obtained can direct further investigations; on the contrary, molecular techniques allow detection of specific abnormalities in situations where karyotyping is difficult (e.g., insufficient metaphases or detection of submicroscopic abnormalities). The chromosomal abnormalities in ALL can be categorized as numerical in nature or structural, with or without numerical abnormalities. Hyperdiploidy is the gain of additional chromosomes so that the total number of chromosomes in a single cell exceeds 46; in ALL, this process seems to be nonrandom and two forms are usually distinguished: ALL with 47–51 chromosomes and ALL with 52 or more chromosomes. Hyperdiploidy is seen in 5–15% of cases of adult ALL and the association with a favorable outcome is less obvious than in childhood ALL. Hypodiploidy (chromosomes <46) is found in 2–8% of cases of ALL and is associated with a poorer outcome. The majority of chromosomal abnormalities found in ALL are structural, usually translocations. More than 30 different nonrandom translocations have been identified in ALL. Since only a relatively limited number of patients have so far been studied and many of these translocations are uncommon, the prognostic implications for most of them have still to be conclusively defined. Most of the more common karyotypic structural rearrangements have been studied at the molecular level. In molecular terms, chromosomal abnormalities or their submicroscopic equivalents are of two general types: those in which the breakpoint occurs within the involved genes, leading to the production of a fusion RNA transcript and a chimeric protein (qualitative change), and those which represent Ig/TCR rearrangement errors (quantitative change). Qualitative abnormalities are found to produce functional fusion genes; one of the most common is the t(9;22)(q34;q11) which forms the *BCR-ABL* fusion gene; another is t(1;19)(q23;p13), where the *E2A* gene fuses with *PBX1*. The rearrangement involving the *MLL* gene on chromosome 11 in the q23 region results in a fusion gene with *AF4* on chromosome 4, band q21. Quantitative abnormalities result from Ig/TCR rearrangement errors which juxtapose the proto-oncogene to regulatory Ig/TCR sequences, leading to deregulated protein expression, for example the SIL-TAL1/tal^{d} deletions on chromosome 1p32 in T-ALL [29]. A list of the main molecular genetic abnormalities identified in ALL and currently used for molecular diagnosis is reported in Table 3, even if this list is not exhaustive and represents a compromise between the current most appropriate molecular method to detect or exclude an abnormality and the most widely used technique. Qualitative fusion transcripts predominate in B-lineage ALL and recombinant errors are rare; in contrast, they are much more frequent in T-ALL, where they represent the majority of molecular abnormalities. Identification of recurring cytogenetic abnormalities and molecular alterations in ALL has had a major impact on risk assessment and a number of structural and chromosomal changes have been incorporated into existing classification systems [5,6]. Within our multicenter GIMEMA ALL 0496 protocol, a central handling of biologic material at presentation is required for all registered cases. This has proven feasible and adequate metaphases could be obtained in over

Table 3. Main chromosomal abnormalities characterized at the molecular level in ALL

Disease	Gene Involved		Abnormality	Incidence	Molecular detection §
B-ALL	*BCR*	*ABL*	†(9;22)(q34;q11)	Adults: 30% Childern: 3%	RT-PCR
	c-MYC	*IgH*	†(8;14)(q24;q32)	1%	FISH
	E2A	*PBX1*	†(1;19)(q23;p13)	5%	RT-PCR
	E2A	*HLF*	†17;19)(q22;p13)	$<1\%$	RT-PCR
	IL3	*IgH*	†(5;14)(q31;q32)	$<1\%$	DNA-PCR
	MLL	*AF1P*	†(1;11)(p32;q23)	$<1\%$	RT-PCR
	MLL	*AF4*	†(4;11)(q21;q23)	Adults: 5% Infants: 60%	RT-PCR
	MLL	*AF9*	†(9;11)(q21;q23)	$<1\%$	RT-PCR
	MLL	*ENL*	†(11;19)(q23;p13)	$<1\%$	RT-PCR
	TEL	*AML1*	†(12;21)(p13;q22)	Adults: $<1\%$ Children: 20%	RT-PCR
T-ALL	*c-MYC*	*TCRα/δ*	†(8;14)(q24;q11)	2%	FISH
	HOX11	*TCRα/δ*	†(10;14)(q24;q11)	5–10%	Southern
	LMO1	*TCRα/δ*	†(11;14)(p15;q11)	1%	Southern
	LMO2	*TCRα/δ*	†(11;14)(p13;q11)	5–10%	Southern
	SIL	*TAL1*	Normal 1p32	Adults: 10% Children: 20%	RT-PCR
	TAL1	*TCRα/δ*	†(1;14)(p32;q11)	1–3%	Southern
	TCL1	*TCRα/δ*	inv(14)(q11;q32)	$<1\%$	FISH

70% of enrolled cases. The opportunity offered by this protocol to combine molecular and cytogenetic data in the framework of a therapeutic trial, has allowed an integrated molecular-cytogenetic classification to be proposed [30] which categorizes adult ALL cases into subgroups which are as homogeneous as possible based on defined genetic alterations and has also enabled a group of patients to be identified without known cytogenetic or molecular changes. The results obtained by this integrated classification have enhanced the importance of a broad genetic characterization of patients with ALL and offer a further biologic basis for stratified treatment approaches.

Multidrug resistance

Some investigators have studied MDR-1 gene expression in leukemic cells from ALL patients in an attempt to demonstrate a correlation with treatment response and/or patient follow-up. All studies reached the conclusion that the overexpression of the gene is probably partially implicated in the chemoresistance phenomenon; this resistance can be expressed at diagnosis and can also be acquired after treatment. Expression of MDR-1 at diagnosis has no effect on the probability of entering CR in pediatric ALL patients, but the CR rate in adult ALL appears significantly lower in MDR positive cases compared with MDR negative cases [31]. The MDR-1 gene encodes for a membrane P-glycoprotein p170 (P-gp) that acts as an adenosine triphosphate (ATP) dependent efflux pump. The expression of this gene confers resistance to some chemotherapeutic agents such as vinca alkaloids, anthracyclines, etc. However, the exact prognostic significance of this resistance mechanism is still unclear [32]. Several studies have found a correlation between high P-gp expression levels and/or P-gp function, and poor response to chemotherapy in AML [33] and, to a lesser extent, in ALL [31,34]. One of the main reasons for these contradictory results may be methodologic problems in P-gp detection. The methods currently used are: (a) measurement of P-gp function by efflux studies, (b) P-gp expression levels by MoAb, and (c) MDR-1 gene expression encoding for (P-gp) by RT-PCR. However, each of these methods has disadvantages. MDR-1 detection could thus represent a valuable biologic parameter in the diagnostic screening of ALL patients. The inclusion of this parameter may result in the design of biologically based risk adapted therapeutic strategies for the management of adult ALL. The adoption of protocols based on drugs that are not P-gp substrates may offer therapeutic advantages for CR achievement for ALL patients expressing the MDR-1 protein.

Minimal residual disease

One of the most important challenges in leukemia treatment is to accurately distinguish patients who require more intensive (and potentially more toxic) therapy from those in whom high cure rates can be achieved with less intensive therapy. MRD studies can provide a direct measurement of leukemic cell responses to chemotherapy. This information can be used to improve strategies of risk assessment and treatment selection in the management of ALL patients. Nevertheless, before using MRD data to guide therapy, further analysis is required to conclusively establish the predictive value of MRD findings. Leukemia cells can be potentially distinguished from normal hematopoietic progenitors on the basis of morphologic and cytochemical properties, immunophenotype, karyotypic or genetic abnormalities, and Ig/TCR gene rearrangements. These different characteristics have been exploited in an attempt to detect small numbers of blasts among normal cells and a variety of techniques have been developed for the detection of residual disease. The conventional criteria for remission in patients with acute leukemia are based on the morphologic examination of BM samples and patients are considered to be in CR when BM aspirates contain less than 5% blasts. At the time of morphologic CR, however, the extent of MRD varies considerably. The methods for MDR analysis include cytogenetics, FISH, Southern blotting, immunophenotype and PCR techniques. The applicability of these techniques for MRD detection depends on three parameters: (a) specificity to discriminate between malignant and normal cells without false positive results), (b) sensitivity limit of at least 10^{-3}, and (c) reproducibility and applicability (easy standardization and rapid collection of results for clinic application) [8,9,35,36]. Only a proportion of leukemias have specific markers such as chromosomal translocations, e.g., t(9;22), t(4;11) or t(1;19), and conventional karyotypic analysis may be used to monitor MRD if an abnormal karyotype is present at diagnosis; however, its low specificity and the risk of analyzing metaphases from normal cells represent major obstacles in its routine use. The main advantage of FISH is that it provides interpretable information based on interphase cells with a low proliferative rate. Nonetheless, the sensitivity of FISH analysis for MRD monitoring is limited. Immunophenotyping techniques using multicolor-gated flow cytometry are based on the aberrant expression of antigens by the leukemic cell population and on the identification of markers that may be found on malignant cells in combinations that are normally not observed in normal BM and PB cells. For a productive detection of MRD in ALL, it is necessary to distinguish leukemic lymphoblasts from their normal counterparts and the intensity of expression may also help in distinguishing leukemic cells

from normal progenitors [15,37]. Some immunophenotypic combinations present on leukemic cells are confined to certain normal tissues and are not found in normal BM or PB cells. Overall, flow cytometry can be utilized to monitor MRD in about 85–90% of cases.

The detection of leukemia-associated clonal genetic changes at the karyotypic and genetic levels has been extensively tested by molecular biology techniques, based on PCR analysis. Two types of PCR targets can be used to detect MRD in ALL patients: leukemia-specific breakpoint fusion regions of chromosome rearrangements (translocations, deletions or inversions) or junctional regions of leukemia clone-specific rearranged Ig/TCR genes. The presence, at diagnosis, of one of these transcripts allows the monitoring of MRD during the clinical follow-up (e.g., *BCR-ABL*, *ALL-AF4*, *E2A-PBX1*). The Ig heavy chain genes IgH undergo rearrangements in 90–95% of B-lineage ALL cases. TCR gene rearrangements occur in 95% of T-lineage ALL and in 50–70% of B-lineage ALL [29]. Because such rearrangements are clonal, analysis of Ig and TCR gene configurations can be used to track the persistence of malignant clones whose rearrangements have been determined at diagnosis. One of the aims of MRD investigations is to estimate the amount of residual tumor rather than to establish its presence and current PCR methods do not allow an easy and accurate MRD quantification. More recently, real-time quantitative PCR (RQ-PCR) has been used for MRD detection in ALL [38]. This method exploits the 5'-3' nuclease activity associated with Taq polymerase and uses a fluorogenically labeled target-specific DNA probe; this probe is designed to anneal between the forward and reverse oligonucleotide primers used for PCR amplification. The RQ-PCR technique appears to be a sensitive, reproducible and quick method for quantifying MRD. The greatest obstacle to the routine use of MRD studies in ALL therapy protocols is that none of the techniques currently available for MRD detection can be applied to all patients. Because PCR may detect residual leukemic cells in cases not amenable to flow cytometric investigation, and vice-versa, it is possible to apply the two techniques in tandem. Correlative studies have demonstrated that detection of MRD by flow cytometry or by PCR analysis of leukemia-specific markers is strongly associated with subsequent relapse. It should again be stressed that all the above can be attempted only through a broad, integrated and uniform characterization of all cases enrolled in multicenter studies. Only through such a broad effort it will be possible to conclusively define the role and impact of biologically based MRD monitoring for the management of ALL patients.

Gene expression profiling

Genomic profiling is becoming a reality that may profoundly modify the management of ALL patients. Several studies have identified unique gene expression signatures characteristic of various hematologic and non-hematologic cancers [39,40]. The potential exploitation of microchip analysis is manifold: it can define the genetic signature of given neoplastic populations, it can help define the lineage affiliation of the tumor, it can identify sets of genes that characterize subsets of patients with distinct responses to treatment and, ultimately, have a prognostic impact, it may identify new targets for future therapies based on the under- or overexpression of given genes, it may allow definition of drug susceptibility or resistance, etc. These innovative technologies have been recently utilized in both childhood and adult ALL [41–44]. In pediatric ALL, it has been shown that distinct gene expression profiles could be found in each of the prognostically important leukemia subtypes, based on immunophenotypic and cytogenetic/ molecular features [41]. In a study dedicated specifically to childhood T-ALL, microchip analysis could identify previously unrecognized molecular subtypes of T-ALL and associate the activation of particular oncogenes to defined stages of normal thymocyte development [42]. Hierarchical clustering of all adult ALL samples based on gene expression profile identified two well-defined groups which correlated precisely with the T- or B-cell immunophenotype of the leukemic cells. Further analysis identified gene expression profiles associated with the presence of either ALL1-AF4, BCR-ABL or E2A-PBX1 gene rearrangements. Furthermore, an integrated analysis of childhood and adult ALL highlights a strong similarity between cases which harbor specific rearrangement regardless of patient's age [44]. With the use of these technologies, it has been shown that genetically defined subgroups express different sets of genes. In individual cases, the genetic lesion could be classified by microarray analysis, while being negative by RT-PCR [41]. Evidence has also been provided that the lineage affiliation of rare cases with unique phenotypic features may be clarified on the basis of the genomic profile [45]. Times are mature to verify whether these innovative technologies will change our approach to the characterization of leukemias. Should this be the case, it is foreseeable that in the near future all new cases will undergo a rapid gene chip analysis that may possibly substitute many of the analyses routinely carried out nowadays through the efforts of numerous laboratories. As our knowledge increases, it is also likely that ad hoc chips will be designed that contain a number of genes sufficient for an adequate classification of given diseases, rather than using the broad chips presently available. It appears realist to hypothesize that we are

in the verge of a time when subgroups of patients will be classified on the basis of the genomic profile and that the latter will direct the therapeutic strategy in terms of both drug decisions and treatment aggressiveness. Finally, in the very near future, great attention will be paid to the identification of new therapies aimed at targeting the specific regulatory pathways operational in the different leukemia subtypes.

Conclusions

ALL are classified on the basis of the presumed cell of origin and do not represent a single disease but rather a heterogeneous collection of diseases with different genetic profiles and differences in clinical progression, treatment and outcome. Following a diagnostic work-up, prognostic data are routinely achieved through physical examination, serum biochemical profiles, peripheral blood count and bone marrow morphology. Over the years, information obtained through karyotype, molecular genetics, extensive immunophenotype, multidrug resistance and, more recently, genomic profiling is progressively contributing to a better understanding of the biology of this complex disease, to the identification of subgroups of patients with a different clinical outcome, to the more precise monitoring of MRD, to the use of different therapeutic protocols based on prognostic indicators and, recently, also to the design of innovative and specific treatment strategies.

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