

Acute Lymphoblastic Leukemia

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KEYWORDS

- Acute leukemia • Acute lymphoblastic leukemia
- Lymphoblastic lymphoma • Cytogenetics • Genes

Acute lymphoblastic leukemia (ALL) encompasses a group of lymphoid neoplasms that morphologically and immunophenotypically resemble B-lineage and T-lineage precursor cells. These neoplasms may present predominantly as a leukemic process, with extensive involvement of the bone marrow and peripheral blood or may be limited to tissue infiltration, with absent or only limited (less than 25%) bone marrow involvement. The latter cases are typically designated as lymphoblastic lymphomas (LBLs). ALL and LBLs appear to constitute a biologic continuum, although they may show distinct clinical features. The current World Health Organization Classification of hematopoietic neoplasms designates these disorders as B- or T-lymphoblastic leukemia/lymphoma.¹

EPIDEMIOLOGY

Most ALL cases occur in children, with an incidence of 3 to 4/100,000 in patients 0 to 14 years of age and ~1/100,000 in patients older than 15 years, in the United States.² In children, ALLs represent 75% of all acute leukemias (which in turn represent 34% of all cancers in this age group), with a peak incidence at 2 to 5 years of age.³ This percentage is much lower in adults, in whom acute myeloid leukemias (AMLs) and chronic lymphocytic leukemias are more common.^{3,4} There is a slight male predominance in all age groups and a significant excess incidence among white children.²

ALL presents primarily as de novo disease, with only rare cases occurring as secondary neoplasms.⁵ A variety of genetic and environmental factors have been related to ALL. It occurs with increased frequency in patients with Down syndrome, Bloom syndrome, neurofibromatosis type I, and ataxia-telangiectasia.⁶ In addition, exposure in utero to ionizing radiation, pesticides, and solvents has also been related to an increased risk for childhood leukemia.⁶ Leukemia-specific fusion genes or immunoglobulin (Ig) and clonal Ig gene rearrangements have been identified in neonatal spot (Guthrie) cards of patients who later developed ALL.^{7,8}

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CLINICAL PRESENTATION

The clinical onset of ALL is most often acute, although a small percentage of cases may evolve insidiously over several months.⁹ The presenting symptoms and signs correlate with the leukemic cell burden and the degree of marrow replacement, leading to cytopenias. The most common symptoms include fever (caused by leukemia or a secondary infection secondary to neutropenia), fatigue and lethargy (as a result of anemia), bone and joint pain, and a bleeding diathesis (related to thrombocytopenia). Patients with precursor T-cell ALL/LBL often present with a mediastinal mass with or without associated pleural effusions, which may lead to respiratory distress and other signs of superior vena cava syndrome. Common extramedullary sites of involvement include lymph nodes, liver, spleen, and meninges, whereas less commonly, ALL may infiltrate orbital tissues, testes, tonsils, and adenoids. Rare patients presenting with B-LBL may show skin lesions of lymphadenopathy in the head and neck area or discrete bone lesions.¹⁰ The most common laboratory abnormalities in ALL include anemia, thrombocytopenia, neutropenia, and leucopenia or leukocytosis, with hyperleukocytosis ($>100 \times 10^9/L$) present in approximately 15% of the pediatric patients.⁹ Other common laboratory abnormalities include elevated serum uric acid and lactose dehydrogenase levels, correlating with the tumor burden and degree of tumor lysis.

PATHOLOGIC DIAGNOSIS

Morphology

The classic morphologic features of ALL have been well described in the literature and are best summarized by the categories outlined by the first classification of ALL, the French-American-British (FAB) system, which was based primarily on the microscopic appearance of the leukemic cells, as seen on Wright-Giemsa-stained smears.^{11,12} The FAB classification outlined three morphologic groups of ALL, designated as L1, L2, and L3 (**Fig. 1**). Most commonly, ALL blasts are small to intermediate in size, with scanty cytoplasm, condensed nuclear chromatin, and indistinct or absent nucleoli (FAB L1 subtype). Less commonly, ALL cells may be larger, with moderate amounts of pale basophilic cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli (FAB L2 subtype). Very rarely, ALL may present as the FAB L3 subtype, which consists of large blasts, with abundant deeply basophilic and occasional vacuolated cytoplasm, coarsely clumped nuclear chromatin, and variably prominent nucleoli. Most of the cases presenting with this morphology are Burkitt lymphomas, a subtype of high-grade mature B-cell lymphoma. However, a small subset of precursor B-cell neoplasms, often associated with hypodiploidy, may also present with FAB L3 features (see **Fig. 1**).

When seen in hematoxylin and eosin-stained histologic sections of bone marrow biopsies and tissue infiltrates, the neoplastic lymphoblasts of ALL/LBL may show features that correspond to the FAB categories. The most characteristic appearance is that of a neoplasm with diffuse growth pattern, sometimes with a partial starry-sky appearance, expanding the interfollicular area in subtotally replaced lymphoid organs (such as lymph nodes or tonsils). The blasts are small, with finely granular chromatin and small pinpoint nucleoli. In rare cases, the blasts may have prominent nucleoli (such as in the L2 subtype) or may resemble large-cell lymphoma cells (see **Fig. 1**). Occasional cases of ALL may present with variable degrees of marrow necrosis or associated marrow fibrosis.¹³

Various morphologic variants of ALL have been described, none of which has prognostic significance. The leukemic blasts may contain vacuoles,¹⁴ pale pink or

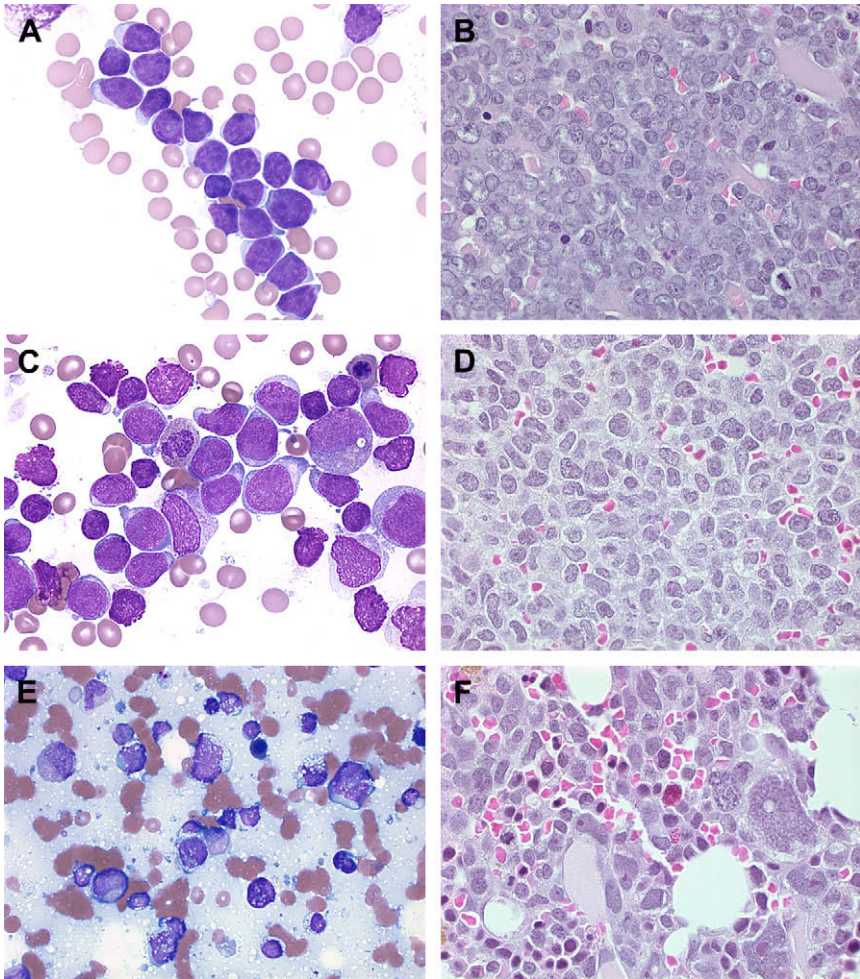


Fig. 1. The morphology of ALL/LBL in smears and paraffin-embedded tissue sections. (A, B) ALL L1. (C–D) ALL L2. (E, F) Morphologic findings in a case of precursor B-ALL with hypodiploidy, resembling a high-grade, mature B-cell lymphoma (A, C, E, Wright-Giemsa stain) (B, D, F, hematoxylin-eosin; original magnification 60x oil immersion).

azurophilic granules (“granular ALL”),^{15–17} or giant inclusions.^{18,19} In some cases, the blasts may show eccentric cytoplasmic uropod-like projections (“hand-mirror cell ALL”).²⁰ Some cases of ALL may show prominent associated eosinophilia (and even hyper eosinophilic syndrome),²¹ which relates to a specific genetic lesion (See later section).

Cytochemistry

Cytochemical staining has been used with decreasing frequency in the diagnosis of ALL due to the availability of immunophenotyping. The leukemic cells of ALL are uniformly negative for myeloperoxidase (MPO), Sudan Black-B, chloroacetate esterase, and nonspecific esterases.²² A notable exception is that of granular ALL,

which frequently shows nonspecific esterase staining localized to the granules and may also show light gray staining with Sudan Black-B.^{1,23} The blasts of ALL are often (75%) positive for PAS and may also be positive for acid phosphatase, more frequently seen in T-cell ALL.²²

Immunophenotype

Immunophenotypic studies are an essential component of the diagnostic workup of ALL/LBL. As opposed to the morphologic features, the lineage of ALL established in this manner subdivides this disease into two broad, clinically and biologically meaningful categories: precursor B-cell ALL (B-ALL) and precursor T-cell ALL (T-ALL).

B-ALL is characterized by the expression of a variety of B-cell-specific antigens, which often include PAX-5 (B-cell-specific activator protein), CD19, CD20, CD22 (surface and cytoplasmic), CD24, and CD79a (cytoplasmic). Notably, CD20, a marker of mature B cells, may be expressed only partially and weakly by the leukemic lymphoblasts or may be entirely negative in B-ALL. A large proportion of B-ALL also express CD10 (common acute lymphocytic leukemia antigen), an antigen consistently expressed by normal B-cell progenitors. Most B-ALL show dim expression of CD45 (leukocyte common antigen), and a subset of these leukemias, most common in children, may be CD45 negative. Other antigens often expressed by the leukemic blasts include progenitor markers often seen in early-stage B-cell precursors, including CD34 and terminal deoxynucleotidyl transferase (Tdt). Depending on their pattern of Ig expression, B-ALL may be classified into early pre-B (or pro-B) ALL, which lacks Ig expression, pre-B-ALL (expression of cytoplasmic μ chains, without Ig light chain expression or restriction), and transitional pre-B-ALL (with cytoplasmic and weak surface expression of μ chains, without Ig light chain expression or restriction).¹ Rare cases of precursor B-ALL may show surface expression of complete IgM with light chain restriction,^{24,25} sometimes associated with other features of mature B cells (such as lack of CD34 and Tdt expression).²⁴ Such cases should be carefully differentiated from mature B-cell neoplasms. Most cases of B-ALL also show expression of one or several myeloid-associated antigens, most often CD13 and CD33 and less often CD11b, CD15, and CD66c. The pattern of immunophenotypic aberrancies correlates to some extent with the underlying genetic lesion in B-ALL (see later section).

T-ALL is characterized by expression of T-lineage-associated antigens (CD2, CD3, CD4, CD5, CD7, CD8) as well as CD1a, CD10, CD34, CD99, HLA-DR, and Tdt. The pattern of antigen expression may be used to subclassify T-ALLs according to the stages of normal thymocyte development that they resemble^{1,26} (**Table 1**). A subset of T-ALL, typical of the cortical subtype, may show coexpression of weak CD79a.²⁷ Notably, some T-ALL/LBL may be negative for Tdt,²⁸ which may raise the differential diagnosis with mature T-cell lymphomas, particularly difficult in cases that are also

Table 1									
Subclassification of T-ALL according to stages of normal thymocyte maturation									
T-ALL Subtype	CD1a	CD2	cCD3	sCD3	CD4	CD5	CD7	CD8	CD34
Pro-T	–	–	+	–	–	–	+	–	+
Pre-T	–	+	+	–	–	±	–	–	±
Cortical T	+	+	+	–	+	±	+	+	–
Medullary T	–	+	+	+	± ^a	±	+	±	–

^a Medullary stage T-ALL shows either CD4 or CD8 expression.

negative for HLA-DR and CD34. T-ALL may frequently express myeloid antigens, including CD11b, CD13, CD15, and CD33. Rare cases express CD117 (c-kit). This latter feature is seen most often in association with a recently described subset of T-ALL that appear to resemble early T-cell precursors recently migrated from the bone marrow to the thymus.²⁹ These genetically heterogeneous cases share a CD1a-, CD8-, CD5 weakly+, CD117+, CD34+, HLA-DR+, CD13+, CD33+, CD11b+, or CD65+ immunophenotype and appear to invariably correlate with a poor response to therapy, including remission induction failure and/or hematologic relapse. Therefore, an aggressive therapeutic approach, including hematopoietic stem cell transplantation, is warranted in these patients.

GENETICS OF ACUTE LYMPHOBLASTIC LEUKEMIA

Although relatively homogeneous at the morphologic and immunophenotypic level, ALL/LBLs show significant heterogeneity at the genetic level. Their genetic lesions define disease subsets with distinct biology and response to therapy and are used in the risk stratification schemas for most current treatment protocols.

Genetics of Precursor B-cell Acute Lymphoblastic Leukemia

Genetic Subgroups

B-ALLs include several cytogenetic subgroups with distinct biologic and pharmacologic features¹ that are very important in the current risk stratification of these patients. These subgroups account for ~60% to 80% of cases in children and adults and can be identified by conventional cytogenetics, molecular diagnostics (reverse transcriptase polymerase-chain reaction), flow cytometry (cell cycle analysis/DNA index),^{9,30,31} and, currently in preclinical trials, gene expression profiling using oligonucleotide arrays. The remaining ALL cases are still characterized only on the basis of the morphologic and immunophenotypic features. Gene expression profiling studies have shown that these cytogenetic subgroups, although extensively overlapping in morphology and immunophenotype, have distinct gene expression signatures.^{32,33} and in vitro sensitivities to drugs,^{34–36} which correlate with different patterns of drug-metabolizing enzyme gene expression.³⁷ Genome-wide genetic analyses, using single nucleotide polymorphism arrays and genomic DNA sequencing, have found that these cytogenetic subgroups also contain distinct alterations in genes encoding principal regulators of B-lymphocyte development and differentiation.³⁸ These high-throughput methodologies have provided a starting point for a much more detailed molecular characterization of these genetic subgroups.^{39–42} The clinicopathologic and molecular features of the main genetic subtypes of B-ALL are summarized in **Table 2**.

Genetics of T-cell Acute Lymphoblastic Leukemia

Although extensive molecular studies combined with gene expression profiling have uncovered a wealth of information regarding the molecular biology of T-ALL, none of this data is currently used in therapy decisions in this disease, likely due to the smaller numbers of cases available for evaluation in this less common form of ALL. However, since some of these genetic alterations appear to correlate with outcome, it is likely that at least some of them will enter the clinical realm in the near future.

Recurrent chromosomal abnormalities in T-ALL often include reciprocal translocations that disrupt developmentally important transcription factor genes, as a result of rearrangements to loci for the T-cell receptor (*TCR*) genes, most commonly *TCR α* (14q11.2) and *TCR β* (7q35). Common examples include the t(1;14) (p32;q11) (3% of

Table 2
Cytogenetic subtypes of precursor B-ALL and their clinicopathologic features

Cytogenetic Subgroup	Frequency (%)	Cytogenetic Abnormality	Fusion Gene	Unique Morphologic Features	Unique Immunophenotypic Features	Additional Molecular Abnormalities	Pharmacologic Features	Prognostic Category
Hyperdiploid ALL	27–29 (P) 6–8 (A)	51–65 chromosomes (+4, +14, +21, +X)	NA	NA	NA	Uncommon BCDG mutations (13%), FLT3 mutations (21%–25%)	Higher sensitivity to MTX, MP	Favorable/low risk (P) Unfavorable (A)
ALL with t(12;21)	22–25 (P) 1–2 (A)	t(12;21) (p13;q22)	<i>TEL/AML1 (ETV6/RUNX1)</i>	NA	Early pre-B-ALL, My+	Monoallelic <i>PAX5</i> deletions (28%)	Higher sensitivity to asparaginase	Favorable/low risk (P)
ALL with t(1;19)	3–6 (P) 5–7 (A)	t(1;19) (q23;p13)	<i>E2A (TCF3)/PBX1</i>	NA	Pre-B-ALL, CD34-/dim+, CD20-, CD9++	NA	NA	Standard risk (P) Unfavorable (A)
Philadelphia+ ALL	2–3 (P) 20–30 (A)	t(9;22) (q34;q11.2)	<i>BCR/ABL (P190, P210)</i>	ALL L1, sometimes granular blasts	NA	IKZF1 (Ikaros) deletions, BCDG mutations in 66%	NA	Unfavorable/high risk (P,A)

ALL with t(v;11q23); <i>MLL</i> rearranged	2–3 (P) 5–7 (A)	t(4;11) (q21;q23) t(19;11) (p13;q23)	<i>AF4/MLL</i> <i>ENL/MLL</i>	NA	Early pre-B, CD10-, CD15+, sCD22-, CD65+, NG2+	FLT3 mutations (18%) Increased expression of <i>HOX</i> genes	Higher sensitivity to cytarabine	Unfavorable/ high risk (P,A)
Hypodiploid ALL	5%–6% (P) NA (A)	<46 chromosomes (typically near haploid or low hypodiploid)	NA	NA	NA	BCDG mutations in 100%	NA	Unfavorable/ High risk
ALL with eosinophilia	<1	t(5;14 q31;q32)	<i>IL3/IGH</i>	Increased dysplastic eosinophils	NA	NA	NA	NA

Abbreviations: A, % in adults; BCDG, B-cell development genes (eg, PAX5, EBF1, IKZF1, LEF1, TCF3, BLNK); FLT3, fms-related tyrosine kinase 3; MP, mercaptopurines; MTX, methotrexate; My, myeloid antigens; NA, not applicable, not known; P, % in pediatric patients.

cases), involving the *TAL/SCL1* gene (1p32), the t(10;14) (q24;q11.2) disrupting the *HOX11* transcription factor gene (10q24),^{43,44} the translocation t(5;14) (q35;q32), with rearrangements of the *HOX11L2* gene,^{45,46} and the t(11;14) and t(7;11) involving the *LMO1*(11p15) and *LMO2*(11p13) oncogenes, respectively.⁴⁷ Less common translocations that do not involve the *TCR* genes include the t(9;17) and the t(10;11) (p12;q14), leading to the *AF10-CALM* fusion gene.^{48,49} The latter is associated with a mature or immature TCR $\gamma\delta$ phenotype and in limited studies, is associated with a poor prognosis for the subset of patients with immature TCR $\gamma\delta$ leukemia.^{50,51}

T-ALL may also harbor molecular lesions present without detectable cytogenetic abnormalities. Up to 50% of T-ALL contains activating mutations of *TAL1/SCL*, independent of detectable translocations. These mutations correlate with leukemias arrested at the late cortical stage of thymocyte maturation, which express TCR $\alpha\beta$, and appear to have an inferior prognosis.^{52,53} Approximately 30% of T-ALL contain *HOX11(TLX1)*, more common in adults than in children. These cases correspond to an early cortical thymocyte phenotype expressing TCR $\alpha\beta$ and appear to have a superior survival.^{52–54} *LYL1* mutations are present in up to 22% of pediatric T-ALL, and they correlate with the double-negative early thymocyte stage of differentiation⁵² and with an inferior survival. *MLL* gene mutations are present in 4% to 8% of T-ALLs, are associated with maturation arrest at early thymocyte stages and expression of TCR $\gamma\delta$, and have no impact on prognosis.⁵³ Activating mutations of the *NOTCH1* gene are present in more than 50% of T-ALL.⁵⁵

Limited studies have suggested a correlation between these genetic abnormalities present in T-ALL blasts and the patient age, possibly corresponding to different stages of thymic development and involution.⁵⁶

Immunoglobulin and T-Cell Receptor Gene Rearrangements in Acute Lymphoblastic Leukemia

Rearrangements of *Ig* and/or *TCR* genes are present in most ALL/LBL. These rearrangements are not always lineage specific, as B-ALL may contain *TCR* rearrangements,^{57,58} and T-ALL may contain *Ig* rearrangements,⁵⁹ possibly due to continuous recombinase activity in the malignant hematopoietic cells.⁶⁰

Ig and TCR rearrangements in B-cell acute lymphoblastic leukemia

Most B-ALLs (97%) contain *Ig* gene rearrangements, involving the heavy chain gene (*IgH*) (>95%), kappa light chain gene (*IgK*) (30%), or lambda light chain gene (*IgL*) (20%).⁵⁹ These rearrangements are often oligoclonal (multiple *IgH* rearrangements present in 30%–40%, multiple *IgK* rearrangements in 5%–10%).⁶¹ B-ALL may also contain *TCR* rearrangements and/or deletions, involving the *TCR* β , γ , and/or δ genes (in 35%, 55%, and 90% of the cases, respectively).⁵⁸ These may be biclonal or oligoclonal (*TCR* β in 3%, *TCR* γ in 10%).⁵⁹ The oligoclonality seen in these neoplasms may be due to continuing rearrangements⁶⁰ and secondary rearrangements, which may account for changes in patterns of *Ig* and *TCR* gene rearrangements often seen in B-ALL at relapse.⁵⁸

Ig and TCR rearrangements in T-cell acute lymphoblastic leukemia

T-ALL/LBLs typically contain *TCR* gene rearrangements (95%–100%). A lower frequency is observed in rare pro-T ALLs, where *TCR* genes are in germline configuration in about 10% of the cases.⁵⁹ TCR $\alpha\beta$ + T-ALLs contain *TCR* β (100%) and *TCR* γ (100%) gene rearrangements and have at least one deleted *TCR* δ allele, whereas deletion of the second allele is seen in 65% of the cases. TCR $\gamma\delta$ + T-ALLs have *TCR* δ and *TCR* γ rearrangements (100% of the cases for each) and typically also contain *TCR* β rearrangements (95%). In contrast to B-ALL, lineage-inappropriate *Ig* gene

rearrangements are encountered only in ~20% of T-ALL, typically as incomplete *IgH* (D_H - J_H) rearrangements. In addition, oligoclonality is seen only rarely in diagnostic T-ALL samples.⁶² Relapsed T-ALL may show secondary *TCR γ* and *TCR β* rearrangements in 15% to 20% of the cases.⁶²

Differential Diagnosis

ALL/LBL may overlap morphologically and immunophenotypically with a variety of benign and malignant entities.

BENIGN PROLIFERATIONS

Expansions of benign B-cell precursors (“hematogones”) and normal thymocytes may enter the differential diagnosis of B-ALL/LBL and T-ALL/LBL, respectively, due to their close morphologic and immunophenotypic resemblance to their neoplastic counterparts.

Benign precursor B cells (hematogones) are normal precursor B cells found primarily in the bone marrow but also in small numbers in extramedullary sites, such as peripheral blood, lymph nodes, and tonsils.^{63–66} They may increase in numbers (sometimes to a significant proportion of the normal cells) in bone marrows from patients of any age but most often in children and young adults,⁶⁷ in a variety of reactive or regenerative conditions. These include viral infections and marrow recovery after infection, chemotherapy, and bone marrow transplantation.⁶⁷ Morphologically, hematogones include cells that resemble ALL L1-type lymphoblasts and immature and mature small lymphocytes. There is also an immunophenotypic range of maturation, which can be summarized in several distinct stages.^{68,69} Typically, Stage I hematogones (CD34+, Tdt+ cells) represent a very minor component of the reactive hematogone expansions. However, in certain settings, especially recovery postchemotherapy, this subset may become more prominent (or even predominant), raising the differential diagnosis with recurrent leukemia.⁷⁰ Flow cytometric analysis is often essential for this distinction, as in most cases B-ALL will show immunophenotypic aberrancies not expected in benign hematogone expansions.⁶⁸

Normal cortical thymocytes are important in the differential diagnosis of precursor T-ALL/LBL, in samples obtained from mediastinal lesions, which may contain normal or hyperplastic thymic cortex, in addition to neoplastic tissue, and from cervical lesions, which may contain ectopic thymic tissue or ectopic thymoma. The morphologic distinction is typically clear, primarily based on the presence of the characteristic thymic architecture and the more mature-appearing thymocyte features. However, significant overlap may occur at the immunophenotypic level. Therefore, findings of Tdt+, CD34+, and CD1a+ precursor T-cells on flow cytometry of mediastinal or cervical masses (especially in children and young adults) should always be correlated with the morphologic findings. Similar to hematogones, when examined immunophenotypically, normal thymocytes show a characteristic sequence of maturation, whereas T-ALL/LBLs show immunophenotypic aberrancies in all cases, which should be useful in the differential diagnosis.⁶⁸

Malignant Neoplasms

A variety of malignant neoplasms with overlapping morphologic and immunophenotypic features may enter the differential diagnosis of ALL/LBL.

Other Blastic Hematopoietic Neoplasms may include AML, mixed-lineage leukemias, plasmacytoid dendritic cell (DC2) leukemias, and, especially in adults, blastic mantle cell lymphoma. AML may present as a tissue infiltrate (termed myeloid or

monoblastic sarcoma) with or without an associated leukemic process and is often considered in the differential diagnosis of ALL/LBL, especially in cases with larger blasts and prominent nucleoli. In some cases, myeloid sarcoma may be associated with immature eosinophilic elements. Extensive immunophenotyping by flow cytometry typically allows for an easy distinction. However, in paraffin-embedded tissue samples, only a more limited panel of immunohistochemical markers can be applied, rendering this differential diagnosis more difficult. A panel of markers including those for MPO, lysozyme, Tdt, PAX5, and CD3 typically suffices. Notably, AML with t(8;21) may be occasionally challenging in this context, as it may express CD19, CD79a, and PAX5, in addition to MPO.⁷¹ Tdt expression may also be encountered in other types of AML.⁷² *Acute mixed-lineage leukemias* (particularly acute biphenotypic leukemias) may be considered in the differential diagnosis of ALL in cases expressing several myeloid antigens. Scoring systems devised for this purpose should be applied in such cases. *DC2 leukemia* is a rare type of leukemia (<1% of all leukemias)⁷³ thought to derive from a subtype of antigen-presenting cells, the plasmacytoid DC2 cells. Morphologically they may resemble ALL L2 or may show ample cytoplasm, with pseudopodia and cytoplasmic vacuoles lined along the cell outlines like a “string or pearls.” The leukemic cells express CD4, CD56, HLA-DR, as well as the DC2-specific antigens CD123, BDCA-2, and BDCA-4. Some cases may express lymphoid and myeloid antigens of low specificity (such as CD2, CD22, Tdt, and CD33), but they are typically negative for other myeloid antigens, CD3, TCR, CD79a, and CD34.^{73,74} At present, these leukemias are treated similar to ALL. *Blastic mantle cell lymphoma* may rarely enter the differential diagnosis of ALL/LBL. The characteristic immunophenotype of this lymphoma, CD5+, CD10-, CD20+, Tdt-, and cyclin D1+, should allow for easy differentiation.⁷⁵

Burkitt lymphoma/leukemia

Typically, the distinction relies on the distinctive morphologic appearance of Burkitt lymphoma, combined with a mature B-cell immunophenotype, CD10+, CD20+, surface Ig positive with light chain restriction, CD34-, Tdt-, and negative for myeloid antigens. This distinction may be difficult in the setting of so-called “precursor B or atypical Burkitt lymphoma,” which contains the classic Burkitt-associated chromosomal translocations but has immunophenotypic features closer to precursor B-cell neoplasms.^{76,77} The immunophenotype includes features of precursor B cells (eg, expression of Tdt and CD34, dim CD20) and of mature B cells (surface IgM with light chain restriction). In the setting of such combinations of features, ALL should be diagnosed with caution, and findings should be correlated with cytogenetics, because in the presence of the t(8;14) or its variants, the patients are treated according to Burkitt lymphoma protocols.⁷⁷

Peripheral T-Cell lymphoma

A variety of mature (or peripheral) T-cell lymphomas may present with leukemic involvement of the bone marrow and/or peripheral blood, which may morphologically mimic T-ALL. These include T-cell prolymphocytic leukemia, adult T-cell leukemia/lymphoma, Sézary syndrome, and rarely anaplastic large-cell lymphoma (ALCL).⁷⁸ The differential diagnosis depends on a combination of morphologic features, clinical presentation, and the finding of a mature T-cell immunophenotype in the latter lymphomas. Certain immunophenotypic features, such as expression of CD30 and the ALK protein, can also be used to exclude T-ALL. In this context, one should also remember that some mature T-cell lymphomas, especially ALCL, may express

myeloid antigens, such as CD33, a feature typically associated with T-ALL.⁷⁹ Last, the presence of genetic lesions such as the t(2;5) for ALCL would further exclude ALL.

Non-hematopoietic neoplasms

A variety of blastic small blue-cell tumors of childhood may enter the differential diagnosis of ALL/LBL in the pediatric age group. *Ewing sarcoma* is the childhood nonhematopoietic neoplasm most likely to be considered in the differential diagnosis of LBL, especially in small samples. It is typically composed of small blastic cells without prominent nucleoli, which are CD45- and CD99+, similar to a subset of precursor B-cell ALL.⁸⁰ On further evaluation, Ewing sarcoma is always negative for B-lineage markers and Tdt, which should be included in diagnostic panels for small blue-cell tumors of children. *Merkel cell carcinoma*, most often seen in adults, may show immunophenotypic overlap with B-ALL/LBL, as both PAX-5 and Tdt expression have been reported in this tumor.^{81,82} Notably, PAX-5 expression, although largely specific for B-lineage in hematopoietic neoplasms, may be seen in a variety of other nonhematopoietic tumors, such as neuroendocrine carcinomas, and a variety of other subtypes of carcinomas.^{82–85} In all of these entities, correlation with clinical presentation and morphology and applying the appropriate panels of immunohistochemical stains allow for accurate distinction from ALL/LBL.

PROGNOSIS AND TREATMENT

Prognosis

The prognosis of ALL has improved dramatically over the past several decades as a result of adapting therapy to the level of risk for relapse, improvements in supportive care, and optimization of the existing chemotherapy drugs. The outcome of pediatric ALL has evolved from an overall survival of less than 10% in the 1960s to approximately 75% to 80% at present.⁹ However, adult patients have a less optimistic outlook. The remission rates have reached 85% to 90%, with overall survival rates of only 40% to 50%.⁸⁶ About 75% of the patients present with poor risk features and have a disease-free survival of 25%, and only 25% present with standard risk features that confer disease-free survival greater than 50%.³⁰ ALL patients are stratified and treated according to algorithms that integrate the presenting features (patient age, leukocyte counts, the presence or absence of central nervous system [CNS], or testicular involvement), leukemia features (lineage, genetic subgroup), and of early therapy response (measuring the dynamic of disease clearance in the first 1–2 weeks of therapy).^{9,30} Patients at low risk for relapse are treated primarily with antimetabolite therapy. Pediatric patients presenting with high-risk features or showing induction failure or persistent minimal residual disease (MRD) after the first 2 weeks of induction receive more aggressive therapy and are considered for allogeneic hematopoietic stem-cell transplantation. All the remaining cases are classified as standard risk for relapse and are treated with intensive multiagent chemotherapy regimens.

Therapy of acute lymphoblastic leukemia

In most centers, the treatment of ALL involves short-term intensive chemotherapy (with high-dose methotrexate, cytarabine, cyclophosphamide, dexamethasone or prednisone, vincristine, L-asparaginase, and/or an anthracycline).^{9,30} This is followed by intensification or consolidation therapy to eliminate residual leukemia, prevent or eradicate CNS leukemia, and ensure continuation of remission. Radiation may be used for patients showing evidence of CNS or testicular leukemia, although this approach is controversial at the current time, especially in children.⁹ In adult patients, the use of growth factors such as granulocyte colony-stimulating factor that accelerate hematopoietic recovery has greatly improved the success rate of ALL therapy.⁸⁷

Pharmacogenetics of acute lymphoblastic leukemia

Increasing numbers of pharmacogenetic studies have shown that germline polymorphisms and mutations present in ALL patients may affect the levels of expression and functionality of drug-metabolizing genes. They may lead to an increase in the likelihood of leukemia in their carriers, may influence the response of leukemic blasts to specific chemotherapy agents, and may also increase the probability of developing secondary (treatment-related) malignancies.^{88,89} Such genes are those encoding for thiopurine methyltransferase, glutathione S-transferase, cytochrome P450 3A4, and methylene-tetrahydrofolate reductase. Furthermore, leukemic blasts with various chromosomal abnormalities (such as hyperdiploid ALL) and therefore additional copies of the wild-type genes for drug-metabolizing enzymes may differ from the somatic cells with respect to their production of these enzymes, leading to altered resistance to the related drugs.⁹⁰ Thus, chemotherapy regimens need to be tailored not only to the specific features of each acute leukemia but also to the patients' individual genetic background.

MINIMAL RESIDUAL DISEASE STUDIES IN ACUTE LYMPHOBLASTIC LEUKEMIA

The utility of MRD studies has been well documented in the management of children with ALL⁹¹ and in further stratification of adults with standard-risk ALL.⁹² They include several types of techniques that can detect amounts of residual leukemia that cannot be identified reliably using morphologic examination or conventional flow cytometry ("submicroscopic disease"). The technical approaches currently used for the detection and quantification of MRD include flow cytometry and molecular analysis (polymerase-chain reaction [PCR]) for leukemia-specific *Ig* and *TCR* gene rearrangements or fusion transcripts. Although generally there is good correlation between these methodologies in most cases, the use of both techniques is the ideal approach for adequate MRD monitoring in all patients.

Flow cytometric detection of MRD (FC-MRD) is based on the presence of an aberrant leukemia-associated immunophenotype, distinct from that of normal (benign) lymphoid precursors present in the bone marrow and peripheral blood. For T-ALL, the detection of cells coexpressing T-lineage antigens and Tdt or CD34 in peripheral blood or marrow is sufficient to support the presence of MRD, since normal T-cell precursors are not normally encountered outside the thymus. For B-ALL, the distinction is much more difficult, because, as previously discussed, normal precursor B cells (hematogones) can be encountered at all anatomic sites, and more refined analyses are required to differentiate these cells from leukemic B lymphoblasts. Additionally, benign progenitor cells occurring in the postchemotherapy or post-transplantation settings may have immunophenotypic features distinct from those encountered in reactive conditions. In addition, leukemic cells may show immunophenotypic shifts during therapy and between initial diagnosis and relapse.⁹³ These factors have to be considered when constructing panels of markers adequate for MRD monitoring and analyzing the data in this setting. Aberrant leukemia-associated immunophenotypes can be identified in 95% of pediatric ALL.^{94,95} Markers typically included in FC-MRD panels include myeloid antigens commonly expressed in B-cell leukemia (e.g. CD13, CD15, CD33, CD65, CD66c) as well as markers often expressed inappropriately for stage of maturation in ALL (CD21, normally only coexpressed on mature B cells; CD38, lower than normal cells; CD58, higher than normal cells). The sensitivity of FC-MRD in routine clinical samples is typically 1 in 10⁴ cells.⁹⁶ Under ideal experimental conditions and when leukemic cells have a very distinct immunophenotype and at least 1 x 10⁷ cells are available, the sensitivity may be as high as 1 in 10⁵ cells.⁹⁶

Advantages of FC-MRD detection (as opposed to PCR) include the possibility of direct quantitation and that of excluding dying cells and cellular debris from analysis. Disadvantages include a lower sensitivity and the difficulties resulting from immunophenotypic shifts in the leukemic cells, with possible disappearance of some of the aberrancies used to identify MRD in a specific case.⁹⁶ FC-MRD detection can be applied in a different manner to peripheral blood and bone marrow samples, depending on the lineage of ALL.⁹⁷ In T-ALL, monitoring MRD levels using peripheral blood is an acceptable substitute for bone marrow samples, as the MRD levels at the two locations always match. For B-ALL, bone marrow residual disease may be found without peripheral blood involvement, and often the levels of MRD found in the bone marrow significantly exceed those found in the blood. The prognostic value of FC-MRD has been demonstrated in retrospective and prospective studies.⁹⁵ At the current time, it is hoped that high-throughput methodologies for genetic analysis in ALL will lead to the discovery of new leukemia-associated markers that can be used in FC-MRD studies.⁹⁵

PCR for Ig or TCR gene rearrangements. If using PCR with consensus primers (including heteroduplex and GeneScan strategies), the detection limit of MRD is 1% to 5% depending on the number of polyclonal B- or T-lymphocytes present in the sample.⁵⁹ Since this sensitivity is comparable to the use of morphologic and immunophenotypic evaluation, alternative approaches have been employed. The sensitivity attained by using patient-specific, junctional region-specific oligonucleotide probes is more suitable for MRD detection. These probes are generated through sequencing of the junctional regions of the rearranged *Ig* and/or *TCR* genes found in the leukemic cells of each patient at the time of initial diagnosis.^{98,99} They are then used in real-time quantitative PCR (RQ-PCR) assays in follow-up samples. Through this approach, the sensitivity of MRD detection has increased to 10^{-4} to 10^{-6} (ie, 1–100 leukemic cells among 10^6 normal cells).

PCR for leukemia-specific fusion transcripts. This methodology detects and quantifies fusion transcripts that correspond to the leukemia-associated translocations such as t(9;22), t(4;11), t(1;19), or t(12;21) (See **Table 2**), typically via reverse transcriptase RQ-PCR assays.⁹¹ It can only be applied to less than 50% of ALLs, which contain such fusion genes. The sensitivity of this methodology is as high as 10^{-4} , depending of the amount and quality of the RNA available in the sample.^{91,100} Due to its high sensitivity and lack of patient specificity, a major pitfall is related to false-positive results due to cross-contamination.⁹¹ Although this modality appears to correlate well with the other two methodologies in most patients,¹⁰⁰ its use in the prediction of outcome in ALL remains to be established.⁵⁹

RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA

The overall frequency of relapse in ALL is approximately 25% in children and 50% in adults, with a rate that is highly dependent on the immunophenotypic and genetic subtype or otherwise-defined risk category of ALL.^{9,30,87} Recent insights have identified additional genetic predictors of relapse, such as deletions of the *IKZF1* (*Ikaros*) gene.¹⁰¹ The genetic subgroup also determines the characteristics of the relapse and the prognosis of these patients. For instance, relapse in patients with Philadelphia-positive ALL, representing approximately 10% of the relapsed ALL (rALL) in some studies, typically occurs following a short complete remission (CR) and correlates with an extremely poor prognosis, as a second CR cannot be induced in many of these patients. ALLs with *TEL/AML1* most often relapse following a long first CR, and a second CR is relatively easy to induce and maintain, often for long periods of

time. Most ALLs relapse in the first 3 to 5 years from diagnosis. Only a very small percentage relapse more than 5 years from diagnosis, and relapses may occur 10 to 20 years later in a minority of patients.¹⁰² rALL may involve the bone marrow or extramedullary tissues (most often at “sanctuary sites,” such as CNS, testis, ovary) or both. The isolated bone marrow relapses appear to correlate with a less favorable prognosis than the isolated extramedullary or combined relapses.⁹

The morphologic and immunophenotypic features of rALL are often largely similar to those seen at the time of initial diagnosis. However, variable immunophenotypic shifts may also be observed, whereby some antigens (most often Tdt, CD10, HLA-DR, myeloid antigens) may increase or decrease in intensity or even be lost at relapse.^{93,103} Such variations may be found in 34% to 73% of pediatric B-ALL and 15% of pediatric T-ALL.^{93,103} The most extreme variations consist of lineage switch, from B-ALL to T-ALL, or from ALL to AML or biphenotypic leukemia. Conventional cytogenetics and molecular analysis typically identify the translocations and fusion transcripts present at the time of initial diagnosis. In addition, cytogenetic analysis often (75%) identifies newly acquired abnormalities.²² Very rarely, an entirely different karyotype may be identified, suggesting the possibility of a second *de novo* ALL. Molecular studies document shifts in the pattern of *Ig* and *TCR* gene rearrangements and even acquisition of new fusion genes.¹⁰⁴ In rare cases (0.5%–1.5%), detailed molecular studies may support a new (second) ALL, distinct from the previous disease.¹⁰⁴ It appears that a combination of immunophenotypic and molecular studies may distinguish three categories of ALL relapse: rALL similar to the leukemia present at initial diagnosis, rALL clonally derived from the initial leukemia, and, rarely, a second *de novo* ALL.¹⁰⁴ Recent high-throughput genomic studies using SNP arrays and comparing diagnostic and relapse ALL samples correlate with these findings.¹⁰⁵ In these latter studies, a minority (8%) of rALL were similar to the initial diagnostic samples, a third (34%) were consistent with clonal evolution of the cells predominant at diagnosis, and half (52%) were shown to have derived by clonal evolution from minor clones present at the time of diagnosis that appeared to precede the dominant clones in the sequence of genetic lesions (“ancestral clones”). About 6% of rALL were completely genetically distinct from the diagnostic samples, suggesting *de novo* disease.

SUMMARY

Although relatively homogeneous at the morphologic and immunophenotypic level, ALL/LBLs encompass a family of extremely heterogeneous disorders when examined at the genetic level. This heterogeneity is reflected in the outcome of pediatric and adult patients in the context of contemporary therapies. High-throughput analysis methodologies have begun to characterize this heterogeneity and, although only in their early stages, have begun to uncover new clinically significant disease subsets, previously unidentified markers useful for MRD monitoring, mechanisms and predictors of disease relapse, germline polymorphisms important in individualized therapy, and new attractive therapeutic targets. These insights are likely to further improve the treatment outcome of patients with ALL.

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