Precursor T-Cell Acute Lymphoblastic Leukemia/ Lymphoblastic Lymphoma and Acute Biphenotypic Leukemias

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Key Words: Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma; Acute biphenotypic leukemia; Immunophenotype; Karyotype; Molecular findings

Abstract

Session 4 of the 2005 Society of Hematopathology/European Association for Haematopathology Workshop focused on case presentations of precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (pre-T ALL/LBL) and acute biphenotypic leukemia. Pre-T ALL represents approximately 15% of childhood and 25% of adult ALL cases. Pre-T LBL comprises 85% to 90% of LBL and frequently manifests as a mediastinal mass. Gene expression studies have shown distinct subtypes of LYL1+, HOX11+, TAL1+, and MLL+ pre-T ALL/LBL. HOX11 overexpression may correlate with a good prognosis in adult pre-T ALL. ABL gene amplification and NOTCH1 gene mutations in subsets of pre-T ALL/LBL suggest patients may benefit from therapy with tyrosine kinase and γ-secretase inhibitors, respectively. Acute biphenotypic leukemias are characterized by a single population of blasts that express myeloid, T- or B-lineage antigens in various combinations and account for fewer than 4% of all acute leukemias. The blasts have a high incidence of chromosome abnormalities. An accurate diagnosis of pre-T ALL/LBL and acute biphenotypic leukemia requires a multiparametric approach, including examination of morphologic features, immunophenotype, clinical characteristics, and cytogenetic and molecular findings.

Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (pre-T ALL/LBL) is an aggressive neoplasm defined in the French-American-British Cooperative Group classification by the presence of more than 30% lymphoblasts in the bone marrow or peripheral blood.1,2 In the World Health Organization classification of tumors of hematopoietic and lymphoid tissues, a blast count of more than 20% is sufficient for a diagnosis of acute leukemia.3 Until recently, pre-T ALL/LBL had a poorer prognosis than B-lineage ALL/LBL; however, the use of intensive chemotherapy has led to remarkable improvement in rapid treatment outcomes, and recent studies have reported very high remission and overall survival rates. Still, patients with pre-T ALL/LBL remain at high risk for induction failure, early relapse, and isolated central nervous system relapse.4

Acute biphenotypic leukemia is characterized by a population of blasts with coexpression of antigens of more than one cell lineage, usually of myeloid and T or B lineage–specific antigens.5 Less often, these neoplasms express B- and T-lineage antigens.

An accurate diagnosis of pre-T ALL/LBL or acute biphenotypic leukemia requires careful examination of morphologic, immunophenotypic, clinical, cytogenetic, and molecular characteristics. This topic was the focus of Session 4 of the 2005 Society of Hematopathology/European Association for Haematopathology workshop. This article provides an overview of the cases presented and the diagnostic workup for suspected cases of pre-T ALL/LBL and acute biphenotypic leukemia.

Characteristics of Pre–T-Cell ALL/LBL

Figure 1 is a flowchart of the diagnostic workup for pre-T ALL/LBL. Pre-T cell ALL/LBL blasts have L1 to L2
morphologic features or prominent cytoplasmic vacuoles as described in L3 and are positive for cytoplasmic CD3, terminal deoxynucleotidyl transferase (TdT), and, less frequently, CD34. Fewer than 5% of pre-T cell ALL/LBL blasts are negative for TdT. Rarely, the blasts are negative for both TdT and CD34. In such cases, blastic morphologic features, the presence of cytoplasmatic CD3 and other T cell–related markers, and the aberrant expression of surface markers by blasts are important clues for making a correct diagnosis.

Clinical Features

Pre-T ALL/LBL is more common in adolescents than younger children and is more common in boys. It represents approximately 15% of pediatric and 25% of adult ALL cases. The clinical manifestations are variable, and the symptoms may appear insidiously or acutely, depending on the extent of disease. Pre-T LBL represents approximately 85% to 90% of LBL and frequently manifests with a rapidly growing mediastinal mass lesion with pleural effusions and relative sparing of bone marrow hematopoiesis. Arbitrarily, the tumor in the bone marrow, if involved, represents less than 20% of the entire specimen. Pre-T LBL tends to be associated with high peripheral blood blast counts.

Cytomorphologic and Cytochemical Features

In pre-T ALL/LBL, the blasts can exhibit considerable size range—from small lymphoblasts with condensed chromatin and indistinct nucleoli to larger blasts with finely dispersed chromatin and relatively prominent nucleoli.

Unlike the situation in acute myeloid leukemia (AML), no single cytochemical test is specific for ALL/LBL. By definition, ALL/LBL is negative for myeloperoxidase (MPO) as assessed using cytochemical studies; the French-American-British Cooperative Group used the definition of fewer than 3% MPO blasts (Image 1C). MPO is a proteolytic enzyme specific to cells of granulocytic and monocytic lineage. However, faint or block-like MPO cytochemical reactivity can be detected in rare cases of ALL/LBL. Immunophenotypic testing for MPO protein is also helpful, but anti-MPO antibodies are less specific than cytochemical staining because a small subset of ALL/LBL can be positive using this method. TdT expression is not specific to ALL/LBL and can be found in AML. Nevertheless, more than 95% of pre-T ALL/LBL cases are positive for TdT.

Immunologic Features

Table 1 lists common immunophenotypes for pre-T ALL/LBL. It is strongly recommended, however, that immunologic marker studies be interpreted in conjunction with morphologic studies. Table 2 shows the scoring system proposed by the European Group for the Immunological Classification of Leukaemias. Only CD3 and cytoplasmic MPO are considered highly specific markers for T-cell and myeloid-cell lineages, respectively. Cytoplasmic CD22 and CD79a have been regarded as highly specific markers for B-cell lineages. Nevertheless, the specificity of these markers is not absolute because cytoplasmic CD79a has been found in 10% of T-cell ALLs.

Pre-T ALL/LBL has been classified into subtypes that correspond to stages of normal thymocyte development: early, CD4− and CD8− (pro-thymocyte); CD4+ and CD8+ (early and late cortical thymocyte); and mature, single positive for CD4 or CD8 (mature T cell). In addition to being negative for CD4 and CD8, the early subtype is negative for surface CD3 (but positive for cytoplasmic CD3). The more differentiated subtype is positive for cytoplasmic CD3, in addition to being positive for CD4 and CD8. The mature subtype is positive for surface CD3 (and for cytoplasmic CD3), negative for CD1a, and positive for CD4 or CD8.
Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma. A, Small and large blasts with distinct nucleoli (Wright-Giemsa, ×500). B, Immunofluorescence demonstration of nuclear terminal deoxynucleotidyl transferase staining in blasts (×600). C, Lymphoblasts show no myeloperoxidase cytochemical staining (×1,000). A granulocyte precursor shows positive myeloperoxidase granular staining.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Immunophenotype of Precursor T-Cell ALL/Lymphoblastic Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>TdT</td>
</tr>
<tr>
<td>Pro–T</td>
<td>+</td>
</tr>
<tr>
<td>Pre-T</td>
<td>+</td>
</tr>
<tr>
<td>Cortical T</td>
<td>+</td>
</tr>
<tr>
<td>Mature T</td>
<td>+</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; cCD3, cytoplasmic CD3; sCD3, surface CD3; TdT, terminal deoxynucleotidyl transferase; +, positive; ±, >50% positive; −, negative.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>European Group for the Immunological Classification of Leukaemias Scoring System for Defining Acute Biphenotypic Leukemias*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scoring Points</td>
<td>B Cell</td>
</tr>
<tr>
<td>2</td>
<td>cCD79a (mb-1); cCD22; clgM</td>
</tr>
<tr>
<td>1</td>
<td>CD19; CD20; CD10</td>
</tr>
<tr>
<td>0.5</td>
<td>TdT, CD24</td>
</tr>
</tbody>
</table>

c, cytoplasmic; m, membrane; MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.

* The score depends on specificity for myeloid or lymphoid lineage.

† Demonstrated by cytochemical or flow cytometric analysis.
A significant proportion of pre-T ALL/LBL has been found to have myeloid-associated markers, and in a recent study, myeloid-associated antigens CD13 and CD33 were identified in 15% of 91 cases. CD13 and CD33 are among the most frequently expressed myeloid-associated antigens. CD2 and CD7 can be commonly seen in pre-T ALL/LBL and in up to 20% of AML cases.

Demonstration of myeloid antigen expression is not an adverse prognostic factor for childhood ALL. However, in adult cases, this may not be true. Tsao et al recently reported that in adults with pre-T ALL/LBL, higher levels of CD117 (c-kit) expression were associated with lower complete response rates. Patients with pre-T ALL/LBL with myeloid antigen expression may benefit from AML-type therapy.

Immunohistochemical Analysis

The antigen-retrieval technique allows routinely fixed, paraffin-embedded biopsy sections to be used for antigenic characterization. When fresh cell suspension samples are not available, this method is useful for classifying blastic hematolymphoid malignancies. The following markers are often included in the panel: (1) myeloid-associated markers: MPO, lysozyme, CD117, and CD68; (2) T cell–associated markers: CD1a, CD2, CD3, CD5, CD4, and CD8; (3) B cell–associated markers: CD20, CD22, CD79a, Pax-5/BSAP, and cytoplasmic immunoglobulin κ and λ light-chains. Antibodies against TdT, CD10, and CD34 are also useful in this setting.

Cytogenetic and Molecular Markers

Pre-T ALL/LBL has been associated with a normal karyotype in only 30% to 40% of cases. The most common recurrent cytogenetic abnormality involves chromosome locus 14q11, the site of the TCRα and TCRδ genes, with a variety of partner genes. Translocation of the HOX11 gene to the TCRα locus occurs as a result of t(10;14)(q24;q11) in 5% to 10% of patients with pre-T ALL/LBL. t(5;14)/HOX11L2-positive T-ALL shows higher expression of CD1a+/CD10+ and cytoplasmic CD3+ markers. Other common translocations involving the TCRα locus are t(11;14)(p15;q11) and t(11;14)(p13;q11), and the partner genes include the RBTN1 (LMO1/Ttg1) and RBTN2 (LMO2/Ttg2) genes, respectively. Another common cytogenetic abnormality is the deletion of chromosome 6q, which occurs in 15% to 18% of adult pre-T ALL/LBL cases. Abnormalities of chromosome 9p21 occur in approximately 15% of patients and cause loss of function of the cyclin-dependent kinase inhibitors p16INK4a/p14ARF and p15INK4b. Deletion of p16INK4a can be detected in 80% of children with pre-T ALL/LBL by using the fluorescence in situ hybridization (FISH) methods.

Rare cases of pre-T ALL/LBL have cytogenetic abnormalities involving 11q23. Reciprocal translocation of t(11;19)(q23;q13.3) involving the MLL gene at 11q23 and ENL at 19q13.3 can be seen in pre-T and pre-B ALL/LBL. MLL gene rearrangements are generally associated with a dismal outcome in ALL/LBL, except in patients with pre-T ALL/LBL who are 1 to 9 years old.

Amplification of the ABL gene was recently reported in 8 (2.9%) of 280 pre-T ALL/LBL cases by Barber et al using a BCR/ABL probe kit and the FISH method. Metaphase FISH analysis revealed that the amplification was extrachromosomal. Others have reported ABL1 amplification or NUP214-ABL1 fusion transcripts in a small subset of T-ALL/LBL cases, and, recently, chromosome 9q34 duplication was reported in approximately one third of cases of pediatric pre-T ALL/LBL.
ALL/LBL. The BCR/ABL1 fusion kinase is frequently associated with chronic myelogenous leukemia and B-cell acute ALL/LBL, but it is rare in T-cell ALL/LBL. The ABL1+ T-ALL/LBL subset, however, is imatinib-sensitive. FISH can promptly identify T-ALL/LBL cases with ABL1 amplification, and ABL1 quantitative real-time polymerase chain reaction (RT-PCR) may be applied to monitor minimal residual disease.

In 1992, Abruzzo et al.41 reported 3 cases of a pre-T ALL/LBL associated with eosinophilia and a high risk of subsequent myeloid malignancy. In some cases, there was an associated t(8;13)(p11;q12) cytogenetic abnormality involving the FGFR1 (RAMP) gene at 8p11 and the FIM/(ZNF198) gene at 13q12.42-45 The fused protein, FIM/FGFR1, contains the FIM putative zinc finger motifs and the catalytic domain of FGFR1. Imatinib mesylate does not inhibit the tyrosine kinase activity of FGFR1. Similar and more selective inhibitors, such as SU4948, have been developed for more specific inhibition,46,47 but it is unknown whether these inhibitors are effective in the treatment of patients with this type of leukemia.

Recently, Weng et al.48 reported that more than 50% of human acute T-cell ALL/LBL has activating mutations that involve the extracellular heterodimerization domain and/or the terminal PEST domain of NOTCH1. The enhanced NOTCH1 pathway is an effective inducer of T-cell ALL/LBL in mice.49 Because the NOTCH1 activation signal can be abrogated by the inhibition of γ-secretase, such as the potent γ-secretase inhibitor used for Alzheimer disease, these findings identify a new potential therapeutic molecular target.

Gene Expression Profiling

Oligonucleotide or complementary DNA microarray technology is being established as an extension of conventional karyotyping and FISH analysis in the diagnosis of leukemia types and as a method of defining previously unrecognized molecular subtypes of ALL/LBL.50 A gene expression profile study of pre-T ALL/LBL showed that molecular signatures link pre-T ALL/LBL oncogenes to specific phases of T-cell differentiation.

Figure 2 illustrates the gene profile of pre-T ALL/LBL. The aberrant activations of LYL1, HOX11, TAL1, and MLL seem to be mutually exclusive in pre-T ALL/LBL.51 LYL1+ pre-T ALL/LBL cases are characterized by expression of early thymocyte genes, including CD34, bcl-2, IL7R, and L-selectin, which reflect the early-arrest double-negative stage of T-cell development. In contrast, HOX11+ and TAL1+ cases are associated with the expression of more mature thymocyte markers. HOX11+ cases express the CD10 and CD1a genes, whereas TAL1+ cases up-regulate LCK, CD3δ, CD3ε, CD6, TCRβ, and TCRα, reflecting differentiation arrest at early and late cortical stages of thymocyte mutation, respectively. The gene expression signature in cases with the MLL-ENL fusion gene shows asynchronous expression of early and more mature thymocyte genes in association with high levels of TCRγ and TCRδ, suggesting that MLL-ENL+ cases are blocked at an early stage of thymocyte differentiation after commitment to the γδ lineage.52 Levels of HOX11 messenger RNA detected by quantitative RT-PCR are elevated in a subset of pre-T ALL/LBL cases, not only in cases with the t(10;14)(q24;q11) but also in cases without this translocation. Overexpression of HOX11 messenger RNA correlates with a good prognosis for patients with pre-T ALL/LBL.53 Less favorable outcomes were seen in subgroups with TAL1+ or LYL1+ gene expression profiles. Therapies targeting newly identified specific molecular abnormalities may be more effective than current therapies.

Prognostic Indicators

Advances in ALL/LBL therapy have changed the risk assignment of some subgroups of patients with pre-T ALL/LBL. Unlike the case in pre-B ALL/LBL, the clinical features and laboratory findings in patients with pre-T ALL/LBL are not predictive of prognosis and, thus, have not been useful for making risk-specific adjustments in therapeutic intensity. Some clinical, laboratory, and biologic characteristics that were previously believed to be prognostically useful now have little value because the treatment of pre-T ALL/LBL has improved dramatically during the past 2 decades.54,55 Pediatric patients with pre-T ALL/LBL are treated as having high-risk disease, and the 5-year event-free survival rate for these children is about 75% with intense therapy, which is similar to that of children with pre-B ALL/LBL. However, patients with pre-T ALL/LBL remain at risk for induction failure, early relapse, and isolated central nervous system relapse.4

Better survival rates are associated with T-ALL/LBL cases that have a normal karyotype and cases that carry the t(10;14) or overexpress the HOX11 gene.55,56 Markers of drug resistance, such as expression of MDR-1, have been reported to be prognostic factors and also seem to be associated with a lower complete remission rate.57,58 NOTCH1 gene mutations correlate with treatment responses and favorable prognosis in children with pre-T ALL/LBL.59 Finally, assessment of minimal residual disease is important for determining the risk of disease recurrence.57,60

Characteristics of Acute Biphenotypic Leukemia

Figure 1 is a flowchart for the diagnostic workup of acute biphenotypic leukemias (which is identical to that of pre-T ALL/LBL). Acute biphenotypic leukemia is characterized by a single population of blasts that express antigens of more than one lineage, usually myeloid and T or B lineage–specific antigens, or, less commonly, these neoplasms express B- and T-lineage antigens.6 Rarely, the blasts in a case of acute biphenotypic leukemia coexpress markers for all 3 lineages.
The clinical manifestations in patients with acute biphenotypic leukemia are similar to those of patients with other types of acute leukemia. Patients commonly have fatigue, infection, and bleeding. Morphologically, the blasts of acute leukemia of ambiguous lineage may be small with scant cytoplasm and/or large with moderate cytoplasm.

The coexpression of several protein antigens characteristic of myeloid and lymphoid diseases in the same cells makes it difficult to classify these neoplasms. As a result, scoring systems have been used to define these neoplasms, the most popular proposed by the European Group for the Immunological Classification of Leukaemias (Table 2). In this system, various markers are assigned a score of 2, 1, or 0.5, depending on their specificity for myeloid or lymphoid lineage. The cases having a score greater than 2 for the myeloid lineage and a score of 2 for the B- or T-cell lineage are biphenotypic acute leukemia in this system. With this scoring system, only a small number of cases are considered acute biphenotypic leukemia.

A flow cytometric immunophenotypic approach using an optimal number of antibodies and well-defined criteria for the diagnosis of acute biphenotypic leukemia is critical for distinguishing it from ALL/LBL. The hematopoietic progenitor marker CD34 is usually present in cases of acute biphenotypic leukemia, and CD117 is reported in approximately 50% to 60% of cases. Depending on the method used, 30% to 92% of cases of acute biphenotypic leukemia are positive for MPO, and TdT is positive in 78% to 100% of cases.

Acute biphenotypic leukemia cases have a high frequency of structural chromosome abnormalities, including the Philadelphia (Ph) chromosome, rearrangements involving 11q23, and complex cytogenetic abnormalities. The del(6), a common cytogenetic abnormality seen in pre-T ALL/LBL, has been reported in up to 13% of cases. The del(12p) also has been reported by several groups in up to 14% of cases. The prognosis in patients with acute biphenotypic leukemia associated with chromosomal abnormalities seems to be unfavorable, particularly in patients younger than 15 years and in cases with the t(4;11) or Ph chromosome. Expression of cyclin A1 is retained as it is a significant myeloid marker CD34 is usually present in cases of acute biphenotypic leukemia, and CD117 is reported in approximately 50% to 60% of cases. Depending on the method used, 30% to 92% of cases of acute biphenotypic leukemia are positive for MPO, and TdT is positive in 78% to 100% of cases.

Acute Myeloid Leukemia

The leukemic cells in AML, minimally differentiated (AML-M0) and in AML-M7 (acute megakaryocytic leukemia) can manifest as small cells with scant cytoplasm and minimal differentiation and are MPO−. Distinguishing these cases from ALL by morphologic and cytochemical studies alone is extremely difficult, if not impossible. Expression of myeloid markers without the coexpression of a significant number of lymphoid-associated markers is crucial for making a diagnosis of AML-M0. Similarly, the demonstration of megakaryocytic markers such as factor VIII, CD41, CD61, and CD62b is helpful in diagnosing AML-M7. Demonstration by electron microscopy of platelet-peroxidase activity in the endoplasmic reticulum and the perinuclear space of megakaryoblasts supports the diagnosis of AML-M7.

Ph+ Pre-T ALL/LBL vs T-Cell Lymphoblastic Crisis of Chronic Myeloid Leukemia

The Ph chromosome has rarely been reported in pre-T ALL/LBL, and it may be difficult to distinguish those cases from T-cell lymphoblastic crisis of chronic myeloid leukemia (CML), particularly in cases identified initially in blast phase. Approximately 25 cases of Ph+ pre-T ALL/LBL and 44 cases of T-cell lymphoblastic crisis of CML have been reported in the literature. The following features may help to distinguish between these 2 diseases. Male sex and younger age are more common in pre-T ALL/LBL. In most cases of CML in blast phase, there is a history of CML. Bone marrow involvement with numerous lymphoblasts is present in all cases of pre-T ALL/LBL, but is found only in approximately half of cases of CML with T-cell lymphoblastic crisis. The presence of an increased number of residual circulating granulocytic precursors, eosinophils, and basophils favors the diagnosis of Ph+ T-cell

Differential Diagnoses for Pre-T ALL/LBL and Acute Biphenotypic Leukemia

Thymoma and T-Cell ALL/LBL

The clinical findings in patients with thymoma or pre-T ALL/LBL (henceforth referred to as only LBL for this differential diagnosis) are different. Thymoma occurs mostly in adults, and children rarely have this tumor. Pre-T LBL, on the other hand, is a lesion that manifests in adolescents and young adults. However, there can be some overlap, and rarely, patients with thymoma have benign lymphocytosis in peripheral blood. With adequate sampling, the difference between thymoma and pre-T LBL is usually straightforward. However, it is not uncommon to be asked to evaluate small-core biopsy specimens or fine-needle aspiration specimens, and accurate diagnosis established using small specimens can eliminate the need for invasive surgery in patients with pre-T LBL, who can immediately begin chemotherapy.

Flow cytometric immunophenotyping of lymphocyte-rich thymoma usually shows an immature T-cell population with an immunophenotypic spectrum of maturation. There is usually variable expression of surface CD3, CD4, and CD8 (smear pattern), as opposed to the uniform distinct population (tight cluster) of T cells seen in pre-T LBL. Immunophenotypic aberrancies are also frequently seen in pre-T LBL cases. TCR gene rearrangements or cytogenetic studies also can help identify pre-T LBL.
lymphoblastic crisis of CML.74 Finally, none of the CML cases in T-lymphoblastic crisis showed bcr-abl involving the minor breakpoint transcript as assessed by RT-PCR.73

Acute Lymphoblastic Transformation of Myelodysplastic Syndrome or Chronic Leukemia

Most cases of myelodysplastic syndrome (MDS) transforming into acute leukemia are of myeloid origin. There are, however, several reports of acute lymphoblastic transformation of refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, chronic myelomonocytic leukemia, and juvenile myelomonocytic leukemia.75-78 Such cases provide clinical evidence that MDS can involve a pluripotent stem cell. In general, ALL transformation of MDS is rare. The median age of patients in the study by Kouides and Bennett76 was 58 years, and the interval between the diagnosis of MDS and ALL was only 1.25 years.

Acute Leukemia of Natural Killer Cells

By convention, high-grade leukemia or lymphomas expressing TdT and lymphoid markers have been classified as pre-B or pre-T ALL or LBL. In rare circumstances, a pathologist may encounter a neoplasm composed of TdT+ blasts with the following immunophenotypic findings: CD56+, CD45+, surface CD3–, CD20–, CD19–, CD13–, CD33–, MPO–, and no evidence of TCRγ gene rearrangements.79,80 In 1 study, the percentage of TdT+ cells detected by immunohistochemical analysis ranged from 5% to 90%.78 These neoplasms were designated as blastic natural killer (NK)-cell lymphoma in the World Health Organization classification, but the histogenesis and name of this tumor has been reconsidered. These neoplasms are now referred to as CD4+, CD56+ hematodermic neoplasm of skin and may be derived from plasmacytoid dendritic cells.81,82 The blasts lack cytoplasmic granules and do not express cytotoxic granule proteins. They are characteristically positive for CD4, CD56, CD123, and TCL1 and usually negative for B and T lineages and myeloid markers.78-80

Aggressive NK-lineage leukemia has a clinical picture characterized by a leukemic phase with concurrent hepatosplenomegaly. The blasts generally show cytoplasmic granules with Wright-Giemsa staining and express cytotoxic proteins such as TIA-1, granzyme B, and/or perforin. The morphologic features and presence of TCRγ and TCRβ gene rearrangements will help distinguish pre-T ALL/LBL from acute leukemia of NK cells. The presence of the restricted subtype of CD158 surface markers supports a diagnosis of acute leukemia of NK cells.83

Difficult-to-Classify Entities

The difficult-to-classify category is an amalgam of several of the previously mentioned categories. It includes cases in which the bone marrow is not completely replaced by leukemic blasts, undifferentiated leukemia, biphenotypic leukemia, t(9;22)+ acute leukemia, and leukemia associated with 11q23 abnormalities, which may show minimal MPO positivity (2%-5%, with only a few faint granules per positive cell). Also included in this category are cases of dedifferentiating relapsed AML that retain myeloid markers but show less MPO activity at relapse than at initial presentation (sometimes <3% activity). Classifying these cases for the purposes of treatment or research protocols often requires a consensus among pathologists and clinicians after consideration of the patient’s history and clinical manifestations, pattern of extramedullary involvement, and the results of cytogenetic, immunophenotypic, and molecular analysis. If the lymphoid determinants overwhelmingly outweigh the myeloid features, a case may be considered lymphoid even in the face of MPO reactivity.

Future Directions

Gene expression profiles using microarray analysis and other techniques such as proteomics or epigenetic modifications will provide more knowledge of the biology of pre-T ALL/LBL and acute leukemia of ambiguous lineage and help identify patients at high risk for treatment failure. Increasing appreciation of the biologic characteristics of pre-T ALL/LBL subsets will lead to more elaborate risk-oriented treatments. The development of new drugs and agents tailored to subset-specific cytogenetic-molecular targets will be vital to the improvement of treatment for pre-T ALL/LBL and acute biphenotypic leukemia.

Summary

The optimal management of patients with T-cell ALL/LBL requires that all cases be assessed by using a multiparametric approach including morphologic, cytochemical, immunologic, and cytogenetic and molecular techniques, further correlated with clinical data. More detailed studies should be conducted at specialized centers in which the preservation of cells, DNA, RNA, and protein for future studies will yield more information about the clinical importance of the expression of various markers, the prevalence and relevance of acute biphenotypic leukemia, and, above all, the mechanisms of leukemogenesis and disease evolution. Such insights will improve the ability of clinicians to treat pre-T ALL/LBL and acute biphenotypic leukemia and prevent refractory disease.

Workshop Cases of Pre-T ALL/LBL With Typical Features

Table 3 summarizes workshop cases of pre-T ALL/LBL with typical features. Image 2 illustrates a TdT–case of pre-T ALL/LBL involving the submandibular gland (workshop case 99).
### Table 3

Workshop Cases of Precursor T-Cell Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma With Typical Features

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Site</th>
<th>Immunophenotype</th>
<th>Cytogenetics</th>
<th>FISH/Molecular Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>155/M/34</td>
<td>Primitive acute leukemia, T- vs NK-cell lineage</td>
<td>BM</td>
<td>Positive for TdT, CD2, cCD3, CD7, CD13, CD34, CD117, HLA-DR</td>
<td>46,XY; t(6;22)(q26;q11.2)[20] TCRγPCR, no clonal gene rearrangement</td>
<td></td>
</tr>
<tr>
<td>99/M/29</td>
<td>Pre-T LBL</td>
<td>Submandibular mass</td>
<td>Positive immunohistochemically for CD2, CD3, CD5, CD7, CD34, CD56 (weak), bcl-2</td>
<td>ND</td>
<td>TCRγ and IgH PCR, no clonal rearrangement</td>
</tr>
<tr>
<td>111/M/31</td>
<td>Pre-T LBL</td>
<td>Mediastinal mass</td>
<td>Positive for TdT (weak), CD3, CD4 (equivocal), CD117 (weak)</td>
<td>Normal male karyotype</td>
<td>FISH, negative for MLL gene rearrangement</td>
</tr>
<tr>
<td>112/M/30</td>
<td>Pre-T LBL</td>
<td>BM</td>
<td>Positive for TdT, CD1a, CD2, CD3 (dim), CD4, CD6, CD7, CD8, CD10, CD45</td>
<td>No metaphase</td>
<td>FISH, suggestive of duplication of chromosomes 8, 9, 14, and 22</td>
</tr>
</tbody>
</table>

BM, bone marrow; c, cytoplasmic; FISH, fluorescence in situ hybridization; IgH, immunoglobulin heavy chain; LBL, lymphoblastic lymphoma; ND, not done; NK, natural killer cell; PCR, polymerase chain reaction; Pre-T, precursor T-cell; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

**Image 2** (Case 99) Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma. **A**, Most of the cells are immature (H&E, ×400). **B**, Immunohistochemical studies revealed the blasts involving the submandibular gland are negative for terminal deoxynucleotidyl transferase (×200). **C**, Neoplastic cells were positive for CD34 (×200). **D**, The blasts are strongly positive with CD3 (×200). Contributed by N. Aguilera and colleagues.
Workshop Cases of Pre-T ALL/LBL With Eosinophilia or Unusual Features

Table 4 and Table 5 summarize workshop cases of pre-T ALL/LBL with eosinophilia or unusual features. The cases are as follows: (1) cases 116, 161, and 185, pre-T ALL with eosinophilia; (2) cases with ABL1 amplification by FISH (case 124); (3) cases with MLL gene abnormalities (cases 150 and 182); (4) Ph+ pre-T ALL/LBL (case 218, history of chronic myelomonocytic leukemia) (The mechanisms of this lineage switch in this case are not well understood;74); (5) large cell transformation in pre-T LBL (case 223); and (6) thymoma with γδ pre-T LBL (case 186).

Workshop Cases of Acute Biphenotypic/Bilineage Leukemia/Lymphoma

Table 6 summarizes these workshop cases, including the following: (1) with myeloid/B lineages (case 173); (2) myeloid/T-lineage markers (cases 60, 191, and 221); and (3) cases with NK/myeloid and minimally differentiated

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Site</th>
<th>Immunophenotype</th>
<th>Cytogenetics</th>
<th>FISH/Molecular Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>161/M/32</td>
<td>Pre-T ALL, with eosinophilia</td>
<td>LN, BM</td>
<td>Positive for TdT, CD2, CD3, CD5, CD7, CD13, CD34; blasts positive immunohistochemically for CD3, CD5, CD43, MPO (weak), CD34 (weak)</td>
<td>Complex karyotype</td>
<td>ND</td>
</tr>
<tr>
<td>185/M/11</td>
<td>Pre-T ALL, with eosinophilia</td>
<td>BM</td>
<td>At diagnosis, positive for CD1, CD2, cCD3, CD4, CD5, CD7, TdT; at recurrence, negative for CD1, CD4, CD8, TdT, CD34</td>
<td>At diagnosis, normal karyotype; at recurrence, complex karyotype, including 5q31 abnormality, i(7)(q10)</td>
<td>FISH, 3 signals for 7q31, consistent with i(7)(q10)</td>
</tr>
<tr>
<td>116/M/10</td>
<td>Pre-T LBL, with eosinophilia</td>
<td>Mediastinal mass</td>
<td>Positive for CD10, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD45RO</td>
<td>No metaphase</td>
<td>Pending</td>
</tr>
</tbody>
</table>

All, acute lymphoblastic leukemia; BM, bone marrow; c, cytoplasmic; FISH, fluorescence in situ hybridization; LBL, lymphoblastic lymphoma; LN, lymph node; ND, not done; Pre-T, precursor T-cell; TdT, terminal deoxynucleotidyl transferase.

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Site</th>
<th>Immunophenotype</th>
<th>Cytogenetics</th>
<th>FISH/Molecular Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>124/M/49</td>
<td>Pre-T ALL/LBL (ABL1 amplification)</td>
<td>BM and mediastinal mass</td>
<td>Positive for TdT, cCD3, CD4, CD5, CD7, CD8, CD10, CD45, CD79a, CD33 (weak)</td>
<td>Normal karyotype</td>
<td>FISH, ABL signal amplification (~6-20 copies)</td>
</tr>
<tr>
<td>150/F/9</td>
<td>Pre-T ALL with myeloid marker expression</td>
<td>BM</td>
<td>Positive for TdT (weak), cCD3 (weak), CD5, CD7, CD11b, CD33 (weak), CD34, CD38, CD45 (weak), CD123, HLA-DR</td>
<td>Complex karyotype, including t(12;11)(q26.2;q13.3), der(12)(t12;17)(p11.2;q23)</td>
<td>ND</td>
</tr>
<tr>
<td>182/M/9</td>
<td>Pre-T ALL with myeloid marker expression</td>
<td>BM</td>
<td>Positive for TdT, cCD3, CD5, CD7, CD33, CD45</td>
<td>Positive immunohistochemically for TdT (weak)</td>
<td>TCRβ PCR, clonal gene rearrangement</td>
</tr>
<tr>
<td>218/F/53</td>
<td>Pre-T LBL, associated with apparent CMML; clonal relation</td>
<td>LN, neck</td>
<td>Positive for CD2, cCD3, CD5, CD7, CD30 (weak); positive immunohistochemically for TdT (weak)</td>
<td>46,XX,t(12;13)(p13;q12)</td>
<td>ND</td>
</tr>
<tr>
<td>223/F/51</td>
<td>Pre-T LBL, with discrete large cell transformation</td>
<td>LN, axillary; BM (&lt;5%)</td>
<td>Positive for CD2, CD4, CD5, CD7, CD8, CD38, CD45, CD71; positive immunohistochemically for TdT, CD99, CD3, CD4, CD5, CD8, CD1a, CD45</td>
<td>BM, normal karyotype</td>
<td>FISH, negative for Philadelphia chromosome</td>
</tr>
<tr>
<td>186/F/43</td>
<td>Pre-T LBL and thymoma</td>
<td>Mediastinal mass</td>
<td>Positive for TdT (partial), CD2, CD3, CD7, CD45RO, CD13/CD33 (partial/weak), CD117 (partial); positive immunohistochemically for CD3, TdT</td>
<td>No metaphase</td>
<td>TCRβ PCR, clonal gene rearrangement; Southern blot for TCRβ and IgH, negative</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; BM, bone marrow; c, cytoplasmic; CMML, chronic myelomonocytic leukemia; FISH, fluorescence in situ hybridization; IgH, immunoglobulin heavy chain; LBL, lymphoblastic lymphoma; LN, lymph node; ND, not done; PCR, polymerase chain reaction; Pre-T, precursor T-cell; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.
Image 3 (Case 116) Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma with eosinophilia.

A, Biopsy section illustrates lymphoblasts and eosinophils in a 10-year-old boy with a large mediastinal mass (H&E, ×400).

B-E, Immunohistochemical staining of the blasts shows precursor-T lineage (B, CD1a, ×200; C, terminal deoxynucleotidyl transferase, ×200; D, CD3, ×200; E, CD45, ×200). Contributed by K. Rizzo and colleagues.
myeloblastic bilineal acute leukemia (case 197, no TCR gene rearrangement, CD56+, TIA+).

**Workshop Cases of Difficult-to-Classify Entities**

Four cases of difficult-to-classify entities are summarized in Table 7. In these cases, there is not enough data to classify or very primitive blasts.

*From the Division of Pathology and Laboratory Medicine, The University of Texas M.D. Anderson Cancer Center, Houston.*

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**Image 4** (Case 124) Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma with ABL1 amplification. 

**A**, Immunophenotypic studies by flow cytometry identified an aberrant immature T-cell population. **B**, Amplification of the ABL signal was present (~6-20 copies, red) in the majority of nuclei as shown by interphase fluorescence in situ hybridization for BCR/ABL. Contributed by K. Theil and colleagues.
Image 51 (Case 182) Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma with expression of myeloid markers and MLL gene abnormalities. Bone marrow biopsy (A, H&E, ×20) and aspirate smears (B, Wright-Giemsa, ×500) with sheets of blasts and decreased myelopoiesis and erythropoiesis in a 9-year-old boy with bilateral cervical and inguinal adenopathy. Insets (A). Blasts with scant cytoplasm. Megakaryocytes are adequate (H&E, ×500). Inset (B). Blast with distinct nucleolus and basophilic, agranular cytoplasm (Wright-Giemsa, ×1,000). The complex karyotype including t(4;11)(q21;q23) and fluorescence in situ hybridization (FISH) analysis (C). FISH probe LSI MLL 11q23 shows partial deletion and/or rearrangement of MLL in 75% of cells. Flow cytometric immunophenotypic findings are shown (D). Purple, blasts; dark blue, myeloid; light blue, monocytic; green, lymphoid. The blasts are CD45+, CD33+, cCD3+, cMPO–, CD2–, CD4–, and CD8–. Contributed by C. Wilson and colleagues.

References


<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
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<th>Site</th>
<th>Immunophenotype</th>
<th>Cytogenetics</th>
<th>FISH/Molecular Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>173/M/3</td>
<td>Acute leukemia/lymphoma, mixed lineage</td>
<td>BM; cervical LN; mediastinal mass</td>
<td>BM positive for MPO, cCD3, sCD3 (partial), CD7, CD24, CD13 (dim), CD33, CD45, CD117, LN positive for CD3, CD5, CD43, CD45, EMA</td>
<td>Complex karyotype</td>
<td>ND</td>
</tr>
<tr>
<td>191/F/65</td>
<td>Biphenotypic acute leukemia</td>
<td>BM; cervical LN</td>
<td>BM positive for CD34, CD33, CD117 (subset), CD15, CD11c, CD38, cCD3, CD2, CD5, CD7, CD4 (dim), CD22 (dim), CD66, CD71, MPO, and TdT (equivocal); LN immunohistochemically positive for CD34 (diffuse) and variable for CD2, CD7, CD15, MPO</td>
<td>Normal karyotype</td>
<td>TCR/PCR, no clonal gene rearrangement</td>
</tr>
<tr>
<td>221/M/61</td>
<td>Biphenotypic acute leukemia</td>
<td>BM</td>
<td>Positive for TdT (partial), CD2, cCD3 (dim), CD7, CD13, CD15, CD33 (partial), CD34, CD45 (dim), CD117</td>
<td>Normal karyotype</td>
<td>ND</td>
</tr>
<tr>
<td>197/M/65</td>
<td>Acute leukemia, bilineal/primitive immunophenotype</td>
<td>BM</td>
<td>Population 1 positive for cCD3 (dim to moderate), CD7 br+, CD56 br+, CD34, CD45 (negative to dim), CD117 br+; population 2 positive for CD45 (dim to moderate), CD13 br+, CD33 moderate +, MPO subset + LN immunohistochemically positive for cCD3, CD7, CD10, TdT, MPO</td>
<td>46;XY,t(4;12)(q12;p13)</td>
<td>TCR/PCR, no clonal gene rearrangement, Southern blot for TCRβ rearrangement, negative</td>
</tr>
<tr>
<td>60/M/34</td>
<td>Pre-T LBL and AML (bilineal vs independent tumors)</td>
<td>Neck LN; BM</td>
<td>LN immunohistochemically positive for cCD3, CD7, CD10, TdT, MPO; BM positive for CD7, CD13, CD33, CD34, CD117, MPO</td>
<td>46;XY,del(5)(q33);46,del(6)(q21q23)</td>
<td>TCR/PCR, pending</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; BM, bone marrow; br, bright; cCD3, cytoplasmic CD3; EMA, epithelial membrane antigen; FISH, fluorescence in situ hybridization; LN, lymph node; MPO, myeloperoxidase; ND, not done; PCR, polymerase chain reaction; s, surface; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase; +, positive.


Image 6I (Case 60) Biphenotypic acute leukemia/lymphoma.

A, Bone marrow biopsy specimen of acute myeloid leukemia (H&E, ×400).

B, The blasts show positive myeloperoxidase (MPO) staining (×400).

C, Lymph node with precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (H&E, ×400).

D, The blasts show positive immunohistochemical staining for CD3 (×500).

E, The majority of blasts are negative for MPO (×500). Contributed by Y. Ko.
Table 7
Workshop Cases of Difficult-to-Classify Entities

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Site</th>
<th>Immunophenotype</th>
<th>Cytogenetics</th>
<th>FISH/Molecular Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>111/M/31</td>
<td>Pre-T LBL</td>
<td>Mediastinal mass</td>
<td>Immunohistochemically positive for TdT and CD117 (focal and weak), CD3, CD4 (equivocal); flow cytometry, no aberrant antigen loss and CD4/CD8 ratio, 3:1</td>
<td>Normal karyotype</td>
<td>FISH, negative for MLL gene rearrangement</td>
</tr>
<tr>
<td>160/F/61</td>
<td>Pre-T ALL/LBL</td>
<td>BM</td>
<td>Positive for cCD3, sCD3, CD5, CD7, CD19 (partial), CD38, CD58, CD79a (partial), CD117 (partial)</td>
<td>Complex karyotype</td>
<td>TCRγ-PCR; clonal gene rearrangement; IgH PCR, no monoclonal rearrangement; TCRβ Southern blot, no rearrangement</td>
</tr>
<tr>
<td>162/M/59</td>
<td>Blastic T-cell leukemia/lymphoma</td>
<td>Mediastinal mass; PB; BM</td>
<td>Positive for CD2, CD5, CD7, CD45 (bright), TCRβ; immunohistochemically positive for CD3, CD5</td>
<td>NA</td>
<td>TCRγ PCR, clonal gene rearrangement, (pending)?</td>
</tr>
<tr>
<td>241/F/63</td>
<td>Acute leukemia consistent with NK lineage; γδ myeloid markers</td>
<td>Inguinal LN</td>
<td>Positive for CD5, CD7, CD33, CD34, CD56, immunohistochemically positive for TdT (occasional)</td>
<td>add(12)(11)</td>
<td>Southern blot for immunoglobulin Jγ and TCRβ, germline</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; BM, bone marrow; c, cytoplasmic; FISH, fluorescence in situ hybridization; IgH, immunoglobulin heavy chain; LBL, lymphoblastic lymphoma; LN, lymph node; NA, not available; NK, natural killer cell; PB, peripheral blood; PCR, polymerase chain reaction; Pre-T, precursor T-cell; s, surface; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.


57. Tafuri A. Multidrug resistance proteins MDRI/P-gp, MRPI, and LRP in adult ALL patients uniformly treated according to the GIMEMA 0496 protocol: poor prognostic impact of MDRI/P-gp [abstract]. Blood. 1999;94:1265a.


