ABSTRACT

The cure rate for childhood acute lymphoblastic leukemia (ALL) now exceeds 70%. This success has been achieved in part by improvements in the biologic characterization of newly diagnosed patients. Modern treatment protocols rely on this information to tailor therapy to a patient’s risk of relapse. Patients with favorable genetic features, such as hyperdiploidy or the TEL-AML1 fusion, can be treated with conventional antimetabolite-based therapy to minimize long-term side effects. By contrast, extremely high-risk patients, such as infants with MLL gene rearrangements and cases with BCR-ABL fusion and poor early response, are candidates for allogeneic hematopoietic stem cell transplantation in first remission. Future areas of research include the identification of new genetic subgroups of ALL and the development of novel therapies. The Oncologist 1997;2:374-380

INTRODUCTION

Although acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood, with approximately 2,000 new cases diagnosed in the United States each year, its etiology remains largely unknown [1-3]. Some environmental factors, including exposure to ionizing radiation [4] and parental alcohol and tobacco use [5, 6] are thought to play a causative role in ALL, whereas others, such as residential exposure to magnetic fields, have drawn much attention but have not been proven to increase the risk of this disease [7]. Despite this incomplete understanding of the basis of ALL, improvements in diagnosis and treatment have produced cure rates that now exceed 70% [3, 8-10]. Further refinements in therapy, including the use of risk-adapted treatment protocols, attempt to improve cure rates for high-risk patients while limiting the toxicity of therapy for low-risk patients [9]. This review summarizes the advances in diagnosis and treatment of ALL that have contributed to this success story.

DIAGNOSIS

Immunophenotyping

Unlike most cases of acute myeloid leukemia (AML), which are readily identified by the presence of Auer rods, myeloperoxidase, or esterase, ALL lacks morphologic or cytochemical features to distinguish it from the remaining AML cases. Initial attempts to classify ALL stemmed from the observation that lymphoblasts and normal lymphoid precursors express common antigens. Based on the expression of these antigens, ALL could be broadly classified as either B- or T-lineage [11,12]. Over the past 20 years, classification of leukemias has improved greatly with the availability of large panels of monoclonal antibodies to leukocyte antigens [3]. However, only a few of the 166 cluster of differentiation (CD) groups of antigens are lineage specific and thus clinically useful [13]. These specific markers include immunoglobulin and CD79 for B-lineage ALL and T cell receptor and cytoplasmic CD3 for T-lineage ALL [14]. B-lineage ALLs can be further subclassified as early pre-B, pre-B, transitional pre-B, or B cell [3] (Table 1), and T-lineage ALLs as early-, mid-, or late-thymocyte [15]. The clinical importance of distinguishing these phenotypic subtypes is apparent for B cell ALL; however, distinguishing the subtypes of B-precursor ALL is probably not clinically relevant, and these groups should be treated according to genetic risk factors (discussed below). Among T cell ALL cases, expression of surface CD3 or lack of CD2, CD5, or CD10 expression has been associated with an adverse prognosis [15-19]. None of these findings, however, have been consistently shown to have prognostic impact.

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Approximately 6% of ALL cases express two or more myeloid markers [20]. Once thought to be a poor prognostic subgroup, this type of leukemia is now known to respond well to intensive therapy and should therefore be treated on appropriate risk-based protocols [21, 22].

### Cytogenetic and Molecular Diagnosis

In greater than 90% of ALL cases, some form of specific genetic alteration is present in the leukemic blasts [3, 9]. These alterations include changes in number (ploidy) and structure, with about half of all childhood ALL cases having recurrent translocations (Fig. 1; Table 2). Standard cytogenetic analysis remains an essential tool in diagnosis, providing information regarding the leukemia cell karyotype, including chromosome number and the presence of translocations. However, molecular techniques have greatly improved our ability to correctly detect specific genetic changes [23]. Molecular analysis of diagnostic bone marrow specimens is more sensitive than karyotyping [24, 25], can distinguish lesions that appear identical cytogenetically [26], and can identify translocations that are not seen by routine karyotype analysis [27].

Clinically important molecular alterations in B-precursor ALL include the BCR-ABL, E2A-PBX1, and TEL-AML1 (also termed ETV6-CBFA2) gene fusions, as well as MLL gene rearrangements (Table 2). Despite overall improvements in outcome, patients whose leukemic blasts carry the BCR-ABL fusion [t(9;22)] continue to have a poor prognosis, with event-free survivals (EFS) of only about 30% [25, 28-30]. Our most recent data, however, suggest that BCR-ABL-positive patients who have low presenting leukocyte counts may have outcomes similar to those of patients who lack this alteration [31]. MLL gene rearrangements, which occur in approximately 80% of infant ALL cases and 3% of non-infant cases, are also associated with a dismal outcome despite aggressive chemotherapy [24, 32-37]. By contrast, intensive chemotherapy has overcome the poor prognosis once ascribed to E2A-PBX1 [38-40].

One of the most exciting discoveries in the molecular characterization of childhood ALL is that TEL-AML1 is the

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**Table 1.** Immunologic classification of childhood ALL

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Antigen expression</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pre-B</td>
<td>CD19⁺, CD22⁺, CD79a⁺, CD10±, clg⁺, slgκ-, slgλ⁺</td>
<td>55-60</td>
</tr>
<tr>
<td>Pre-B</td>
<td>CD19⁺, CD22⁺, CD79a⁺, CD10⁺, clg⁺, slgκ⁺, slgλ⁺</td>
<td>20-25</td>
</tr>
<tr>
<td>Transitional pre-B</td>
<td>CD19⁺, CD22⁺, CD79a⁺, CD10⁺, clg⁺, slgκ⁺, slgλ⁻</td>
<td>3-5</td>
</tr>
<tr>
<td>B cell</td>
<td>CD19⁺, CD22⁺, CD79a⁺, CD10⁺, clg⁺, slgκ⁺, slgκ⁺, or slgλ⁻</td>
<td>2-3</td>
</tr>
<tr>
<td>T cell</td>
<td>cCD3⁺ and CD7⁺, plus CD5⁺ or CD2⁺</td>
<td>13-15</td>
</tr>
</tbody>
</table>

Abbreviations: clg = cytoplasmic immunoglobulin; slg = surface immunoglobulin; μ = mu heavy-chain protein; κ = kappa light-chain protein; λ = lambda light-chain protein.

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**Table 2.** Common genetic alterations in childhood ALL

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Molecular alteration</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-precursor ALL</td>
<td>TEL-AML1</td>
<td>25%</td>
</tr>
<tr>
<td>t(12;21)(p13;q22)</td>
<td>E2A-PBX1</td>
<td>6%</td>
</tr>
<tr>
<td>t(1;19)(q23;p13)</td>
<td>BCR-ABL</td>
<td>4%</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>MLL-AF4</td>
<td>4%</td>
</tr>
<tr>
<td>Other 11q23</td>
<td>Other MLL fusions</td>
<td>4%</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>Unknown</td>
<td>20%</td>
</tr>
<tr>
<td>(D&gt;21,16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cell ALL</td>
<td>MYC dysregulation</td>
<td>2%</td>
</tr>
<tr>
<td>t(8;14)(q24;q32)</td>
<td>MYC dysregulation</td>
<td>2%</td>
</tr>
<tr>
<td>t(2;8)(q12;q24)</td>
<td>MYC dysregulation</td>
<td>2%</td>
</tr>
<tr>
<td>t(8;22)(q24;q11)</td>
<td>MYC dysregulation</td>
<td>2%</td>
</tr>
<tr>
<td>T cell ALL</td>
<td>TALI dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(1;14)(p32;q11)</td>
<td>TALI dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(1;7)(p32;q35)</td>
<td>TALI dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(7;9)(q34;q32)</td>
<td>TAL2 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(7;19)(q34;p13)</td>
<td>LYL1 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(10;14)(q24;q11)</td>
<td>HOX11 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(7;10)(q35;q24)</td>
<td>HOX11 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(11;14)(p15;q11)</td>
<td>LMO1 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(7;11)(q35;p13)</td>
<td>LMO2 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(11;14)(p13;q11)</td>
<td>LMO2 dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(1;7)(p34;q34)</td>
<td>LCK dysregulation</td>
<td>8%</td>
</tr>
<tr>
<td>t(7;9)(q34;q34)</td>
<td>TAL1 dysregulation</td>
<td>8%</td>
</tr>
</tbody>
</table>
most common genetic lesion, occurring in about one-fourth of B-precursor cases [27, 41-44]. This fusion, created by a cryptic t(12;21) that is not detectable by cytogenetics, is associated with an increased frequency of expression of myeloid-associated antigens (CD13, CD33, or CDw65) [44, 45] and with a favorable outcome [27, 41, 43, 44]. It thus defines a new group of patients who may be candidates for less intensive therapy [27, 46].

Changes in chromosome number, which often occur in the absence of a specific translocation, also significantly influence the prognosis of childhood ALL [47]. Hyperdiploidy, defined as a DNA index (DI) ≥ 1.16, occurs in about 20% of B-precursor patients and was a favorable prognostic factor on several different protocols, including those based on antimetabolite therapy [48, 49]. The good outcome of hyperdiploid patients is likely due to a combination of factors, including increases in leukemic blast accumulation of methotrexate polyglutamates [50, 51], sensitivity to antimetabolites [52], and propensity for apoptosis [53]. Heterogeneity within the hyperdiploid group is demonstrated by the superior outcome of patients with DI ≥ 1.16 and trisomies of chromosomes 4 and 10 compared to those without both trisomies [54]. Patients with hypodiploidy (<45 chromosomes) have a poor outcome and may be candidates for alternative therapy [55].

**RISK CLASSIFICATION**

With the recognition of distinct prognostic subgroups, contemporary protocols stratify children with B-precursor ALL into groups designated “high-risk” or “standard-risk.” Risk classification is based, in part, on clinical features, the most important of which have been presenting age and leukocyte count [56]. Participants of a recent workshop sponsored by the National Cancer Institute defined standard-risk ALL as B-precursor cases with age between one and ten years and an initial leukocyte count of less than 50 × 10^9/L. All other patients are considered high-risk [57]. Using these criteria, four-year EFS estimates for the two groups are 80% and 65%, respectively [57]. However, the EFS estimates for hyperdiploid patients within both risk groups are approximately 89%, suggesting that genetic factors may more accurately predict outcome than do age and leukocyte count.

Further evidence that genetic features of leukemic blasts may be the best factors on which to base risk classification schemes comes from patients with TEL-AML1 expression, who have an excellent outcome (EFS approximately 90%) regardless of age or leukocyte count [27]. Similarly, infants less than one year of age were once considered a very high-risk group, whereas now only those infants with MLL gene rearrangements fall into this classification. The 20% of infants without this genetic feature may have similar outcomes to non-infants with ALL [32-34, 58]. We therefore propose a risk classification scheme based largely on genetic features of leukemic blasts (Table 3) [9, 37]. According to this scheme, patients with hyperdiploidy or TEL-AML1 comprise the good-risk group, while patients with MLL gene rearrangements or expression of BCR-ABL are considered high-risk. All other patients would be intermediate risk. We should stress, however, that even specific genetic features are not perfect predictors of outcome. Additional clinical and biologic information, including rate of cytoreduction [59-61], in vitro drug sensitivity [62], and growth of leukemic blasts in model systems (e.g., mice with severe combined immunodeficiency or stromal-supported cell cultures) [53, 63] help refine this classification system and improve our ability to direct treatment.

**TREATMENT**

With the exception of B cell ALL, which is treated with specific therapy that emphasizes high-dose cyclophosphamide, cytarabine, and methotrexate, as well as intensive intrathecal therapy [64-66], the treatment of childhood ALL includes four components: remission induction, consolidation, continuation, and treatment of subclinical central nervous system (CNS) leukemia [56]. Induction therapy generally consists of three or four drugs, including a glucocorticoid, vincristine, and asparaginase for standard-risk patients, with an anthracycline added for high-risk patients. These regimens have achieved complete remission rates of 97% to 99%. Yet, the finding that long-term outcome improves if the leukemic cell burden is rapidly reduced [59-61] has led to intensification of induction therapy with four to six drugs. Improvements in outcome for high-risk patients may therefore be due, at least in part, to more intense induction [40, 67-69]. Recently, we asked whether G-CSF reduced the morbidity associated with such intense induction regimens [70]. Although minor clinical effects

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**Table 3. Genetic risk classification**

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Genetic features</th>
<th>Patients affected (%)</th>
<th>Recommended therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>DNA index ≥ 1.16 or TEL-AML1 fusion</td>
<td>40-50</td>
<td>Anti-metabolite-based</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>E2A-PBX1 fusion and all others not in low- or high-risk groups</td>
<td>40-50</td>
<td>Intensive chemotherapy</td>
</tr>
<tr>
<td>High risk</td>
<td>BCR-ABL fusion or MLL rearrangement</td>
<td>10</td>
<td>Allogeneic bone marrow transplantation in first remission</td>
</tr>
</tbody>
</table>
were achieved, G-CSF did not reduce the hospitalization rate or improve long-term survival.

Consolidation, or intensification, therapy is given soon after remission is achieved in an attempt to further reduce the leukemic cell burden before the emergence of drug resistance [56]. In this phase of therapy, the drugs are used at higher doses than they are in induction [67], or different drugs are used, such as high-dose methotrexate and 6-mercaptopurine [40, 59, 71], epipodophyllotoxins with cytarabine [40, 72], or multiagent combination therapy [69]. Consolidation therapy, first used successfully in the treatment of high-risk patients, also appears to improve the long-term survival of standard-risk patients [59, 72]. Similarly, the addition of intensive reinduction therapy administered soon after remission is achieved is beneficial for both high- and standard-risk ALL patients [59, 73, 74].

Whereas B cell ALL is treated with a two- to eight-month course of intensive therapy [64-66], B-precursor and T cell ALL require approximately two to two and one-half years of continuation therapy to achieve acceptable cure rates. Attempts to reduce this timeframe have resulted in high relapse rates after therapy was stopped [75]. Most contemporary protocols include a continuation phase based on weekly parenteral methotrexate given with daily oral 6-mercaptopurine, interrupted by monthly pulses of vincristine and a glucocorticoid. Although these pulses have improved outcome [73,76], they are associated with avascular necrosis of the bone [77]. High-risk ALL patients may also benefit from intensified continuation therapy that includes the rotational use of drug pairs [40, 78]. The improvements in relapse-free survival gained by intensification with anthracyclines or epipodophyllotoxins must be weighed against the late sequelae of these agents, which include cardiotoxicity [79-81] and treatment-related acute myeloid leukemia [82, 83].

Treatment of subclinical CNS leukemia, pioneered at St. Jude Children’s Research Hospital, is also an essential component of ALL therapy. Although cranial irradiation effectively prevents overt CNS relapse, concern over subsequent neurotoxicity and brain tumors has led many investigators to replace irradiation with intensive intrathecal and systemic chemotherapy for most patients. This strategy has produced excellent results, with CNS relapse rates less than 2% in some studies [84, 85]. Whether cranial irradiation is necessary for patients with very high-risk ALL is uncertain. The Children’s Cancer Group recently reported that craniospinal irradiation combined with intensive systemic and intrathecal therapy eliminates the poor prognostic impact generally ascribed to the presence of CNS leukemia at diagnosis [86]. Another recent study suggests that among children with T cell ALL whose initial leukocyte counts are greater than 100 x 10^9/l, those treated with intensive triple intrathecal therapy alone have an inferior outcome compared to those who receive 12 Gy cranial irradiation [87]. This trial was not randomized, and the comparison groups did not receive identical systemic chemotherapy. Notwithstanding this finding, 12 Gy cranial irradiation appears adequate for prophylactic treatment of subclinical CNS leukemia, even in patients with T cell ALL and high leukocyte counts [87].

We have yet to find the optimal treatment of very high-risk ALL patients (those with **BCR-ABL** or **MLL** gene rearrangements). Children with the **BCR-ABL** fusion and initial leukocyte counts less than 25 x 10^9/l may be cured with intensive chemotherapy [31], yet patients with **BCR-ABL** and high leukocyte counts as well as those with **MLL** rearrangements have long-term event-free survival of less than 20%. Many institutions therefore treat these patients with allogeneic bone marrow transplantation in first remission [88-91]. For patients without a matched family donor, unrelated donor transplantation is a reasonable treatment option [92]. Results of stem cell transplantation, often reported from single institutions, have been inconsistent and sometimes disappointing. Large, multi-institutional, controlled trials are clearly needed to determine the effectiveness of this therapeutic modality for this patient population.

**CONCLUSIONS**

More than two-thirds of children with ALL can now be cured. Because of the diverse nature of the disease, we favor risk-directed therapy for all patients based on the molecular characterization of their leukemic cells at diagnosis. Our future goals include the identification of new genetic subgroups of ALL and the development of new therapies to directly target the oncogenic products of ALL translocations.

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