Poor Prognosis of Children With Pre-B Acute Lymphoblastic Leukemia Is Associated With the t(1;19)(q23;p13): A Pediatric Oncology Group Study


The prognostic significance of chromosomal translocations, particularly t(1;19) (q23;p13), was evaluated in children with pre-B and early pre-B acute lymphoblastic leukemia (ALL). Patients were treated on a risk-based protocol of the Pediatric Oncology Group (POG) between February 1986 and May 1989. An abnormal clone was detected in 46% (130 of 285) of pre-B cases and 56% (380 of 679) of early pre-B cases. Translocation of any type was associated with a worse treatment outcome than other karyotypic abnormalities: 15 of 66 versus 3 of 64 failed therapy in the pre-B group (P = .001), and 37 of 141 versus 23 of 239 failed in the early pre-B group (P < .001). The t(1;19) (q23;p13) occurred significantly more often in cases of pre-B ALL with a clonal abnormality than in early pre-B ALL cases (29 of 130 versus 5 of 380, P < .001). Among the 285 pre-B cases in which bone marrow was studied cytogenetically, those with t(1;19) had a significantly worse treatment outcome than all others (11 of 29 vs 27 of 256 have failed therapy, P < .001). This difference is significant (P < .001) after adjustment for leukocyte count, age, and other relevant features. Cases with the t(1;19) also had a worse prognosis than pre-B patients with other translocations (4 of 37 have failed, P < .001) or with any other karyotypic abnormality (7 of 101 have failed, P < .001). We conclude that chromosomal translocations confer a worse prognosis for non-T, non-B-cell childhood ALL, and that the t(1;19) is largely responsible for the poor prognosis of the pre-B subgroup.

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MATERIALS AND METHODS

Presenting features and clinical outcome were assessed in 285 children with pre-B ALL and 679 with early pre-B ALL who were entered on the POG 8602 (ALINc 14) between February 1986 and May 1989, and had cytogenetic studies attempted in a central reference laboratory at the University of Alabama at Birmingham (UAB). Ten additional patients with early pre-B and nine with pre-B ALL are excluded from these analyses because of the unavailability of bone marrow for cytogenetic analysis. Children with B-cell or T-cell ALL and infants (less than 1 year of age) are excluded from this protocol and treated on phenotype- or age-directed therapy.

Patients were stratified by risk group (see Table 1) and immunophenotype (early pre-B or pre-B). Informed consent was obtained from the patients, their parents, or both, as deemed appropriate. Those with early pre-B ALL were eligible for randomization after remission induction to receive standard continuation therapy with intermediate-dose methotrexate (IDM) (regimen A, see Fig 1); regimen A

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PRE-B-CELL acute lymphoblastic leukemia (ALL) was first described by Vogler et al1 in 1978. Approximately one fifth of children with non-B, non-T-cell ALL were found to have lymphoblasts expressing cytoplasmic but not surface immunoglobulin (cIg+, sIg-). The early reports did not describe distinctive differences between patients with pre-B ALL and those with early pre-B ALL (cIg+, sIg-, pan-T antigen negative).1-4 A subsequent retrospective study from the Pediatric Oncology Group (POG) showed that the patients with pre-B ALL fared significantly worse, even after statistical adjustment for other known prognostic factors.5 The relative importance of traditional prognostic factors such as age, leukocyte count, and race was shown to depend on immunophenotype.6 These observations have recently been validated in a prospective randomized study with stratification by risk group and immunophenotype (early pre-B vs pre-B, POG study 8036).4

In 1983, Carroll et al5-6 reported that about one fifth or more of children with pre-B ALL had leukemic blasts with a specific chromosomal translocation, t(1;19) (q23;p13), a finding confirmed by other investigators.7 Subsequently, Pui et al10 noted that the presence of translocations in children with pre-B ALL was associated with a significantly increased incidence of traditional high-risk features. However, these reports did not demonstrate associations for the t(1;19) in pre-B ALL and specific negative prognostic factors or poorer outcome compared with pre-B ALL having other translocations. Also, differences in treatment outcome for patients with t(1;19) versus other cases of pre-B or early pre-B ALL have not been assessed.

We report here the prognostic significance of chromosomal translocations, focusing on the t(1;19) (q23;p13), in relation to other risk factors among patients treated on the POG 8602 treatment study (ALINc 14), which was designed for patients with pre-B or early pre-B ALL. The unfavorable prognosis associated with the pre-B immunophenotype was found to be primarily attributable to the presence of translocations, especially t(1;19). These findings can be used to refine stratification and risk-directed therapy.
plus intensive L-asparaginase (regimen B); regimen A plus high-dose cytarabine (regimen C); or with IDM and cytarabine spread out over the first 18 months of therapy (regimen D). All patients received "backbone" therapy with oral 6-mercaptopurine and intra-muscular methotrexate. Central nervous system (CNS) preventive therapy consisted of triple intrathecal therapy (hydrocortisone, methotrexate, cytarabine).

Randomization of patients with pre-B ALL was restricted to regimen B or C because of the relatively small numbers of cases. Of the 294 children with pre-B ALL, 89 are receiving regimen B and 198 regimen C; 6 are awaiting treatment, and 1 received regimen D in error. Of 10 patients with pre-B ALL and t(1;19), 10 were treated according to regimen B and 19 according to regimen C. Patients with pre-B ALL were restricted to regimen C initially until regimen B opened (14 months after the study opened); patients were then randomized to regimen B or C because of the relatively small numbers of cases. Of 10 patients with early pre-B ALL and the nine with pre-B ALL who did not have a sample of bone marrow sent for cytogenetic analysis are included in this summary of treatment assignments, but not in any of the analyses in this report. They remain on study but are not included in this report since we wish to focus on those children who had centrally performed cytogenetic studies.

**Cytochemistry and immunologic cell markers.** Leukemic cells were examined morphologically and classified according to French-American-British (FAB) criteria. In addition, cells were examined for cytochemical reactivity to Sudan-black B, myeloperoxidase, and nonspecific esterase by standard techniques. Bone marrow cells were sent to St Jude Children's Research Hospital (SJCRH, Memphis, TN) Cell Marker Reference Laboratory for clg and sIg testing as previously described. Only cytoplasmic μ (not other heavy chain classes) was assessed. HLA-DR and common ALL (CALLA) antigens were identified at Duke University's Immunology Reference Laboratory (Durham, NC) by a standard immunofluorescence method using MoAbs (CD3, CD5, CD7) with flow cytometry.

### Table 1. Criteria for Risk Classification

<table>
<thead>
<tr>
<th>Feature</th>
<th>Age (y)</th>
<th>&lt;1</th>
<th>1-2</th>
<th>3-6</th>
<th>6-10</th>
<th>&gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC level (× 10^9/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>10-99</td>
<td></td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>&gt;100</td>
<td></td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Liver or spleen below umbilicus</td>
<td></td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>umbilical; extramedullary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>leukemia</td>
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</table>

Abbreviations: A, good-risk group; B, poor-risk group.
Immunologic classification. All newly diagnosed cases of ALL were classified as pre-B, B, T, or early pre-B ALL according to the following criteria: pre-B, $\geq 10\%$ of marrow lymphoblasts $\text{clg}^+$; B, $\geq 10\%$ of marrow lymphoblasts $\text{clg}^+$; and T, CD5, or CD7 without HLA-DR expressed on $\geq 20\%$ of the leukemic blasts. The early pre-B group included all patients with ALL who had complete immunophenotyping and were not classified by the above criteria, regardless of the expression of CALLA or HLA-DR.

Cytogenetics. Cytogenetic studies were performed at a central reference laboratory (UAB). Samples (usually bone marrow) were placed in sterile tubes containing RPMI 1640 supplemented with 15% fetal calf serum and shipped overnight to the cytogenetic laboratory. On arrival, cells were placed in fresh medium and subjected to short-term (24-hour) culture at 37°C. The cells were then exposed to colcemid (0.05 pg/mL) for 2.5 hours at 4°C. Routine methods were used for culture harvest, slide preparation, and GTG-banding. In every case, an attempt was made to completely analyze a minimum of 20 metaphase cells. A clone was defined as: (1) two or more metaphases with an identical structural abnormality; (2) two or more metaphases with an identical extra chromosome; or (3) three or more metaphases with an identical missing chromosome. Cytogenetic results were subgrouped for the present analyses as follows: normal (satisfactory banding, with no clonal abnormality detected); t(1;19) present; other translocation present with t(1;19) excluded; other clonal abnormality present (all translocations excluded); and unassignable (no karyotype determined).

Statistical analysis. The design of the ALinC 14 study was based in part on the results of our previous ALinC 13 study, in which children with pre-B ALL had a worse prognosis than those with early pre-B ALL.4 Therefore, we planned to compare the event-free survival (EFS) for children with early pre-B or pre-B ALL by risk group and immunophenotype (early pre-B or pre-B) according to treatment designated groups (A to D). Infants less than 1 year of age did not enter this study.

Because data regarding the presence or absence of a chromosomal translocation, presence of a clonal abnormality, and presence of the t(1;19) were known for over one half of these patients, we performed a retrospective analysis of the potential effect of these variables on treatment outcome within each phenotype.

Comparison of EFS (the time from registration to induction failure, relapse, death, or last contact) was done by the logrank method.15 The t(1;19) comparisons within pre-B ALL groups were also stratified individually for risk group (Table 1), leukocyte count (subdivided at $10 \times 10^9/L$), sex, race (black vs nonblack), age (subdivided at 10.0 years), and DNA index (subdivided at 1.16). EFS is displayed by the Kaplan-Meier method16 with SEs of Peto et al.15

Among patients with pre-B ALL, those with the t(1;19) were compared with all others with respect to white blood cell (WBC) levels by the Wilcoxon test17 and with respect to racial distribution (proportion of black patients) by the exact unconditional Z-test.18

RESULTS

Clinical and biologic characteristics. The distribution of cytogenotypic findings is shown in Table 2. Successful cytotypes were obtained for 214 of 285 (75%) children with pre-B and 526 of 679 (77%) with early pre-B ALL. Clonal abnormalities were noted in 130 patients (46%) with pre-B and 380 (56%) with early pre-B ALL. Among the 285 children with pre-B ALL who had a sample sent to the reference laboratory for cytogenetic evaluation, 29 had a t(1;19), 37 had a translocation other than t(1;19), 64 had some other clonal abnormality, 84 had a normal karyotype, and in 71 karyotyping was unsuccessful. Among the patients with early pre-B ALL, 5 had a t(1;19), 136 had a translocation other than t(1;19), 239 had some other clonal abnormality, and the remaining patients had a normal karyotype (n = 146) or no karyotype was obtained (n = 153). Among cases with a demonstrated clonal abnormality, the t(1;19) (q23;p13) occurred significantly more often in pre-B than in early pre-B ALL (22% vs 1%, P < .001).

Table 2. Distribution of Cytogenotypic Findings

<table>
<thead>
<tr>
<th></th>
<th>Pre-B</th>
<th>Early Pre-B</th>
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<tbody>
<tr>
<td></td>
<td>(n = 285)</td>
<td>(n = 679)</td>
</tr>
<tr>
<td>Successfully karyotyped</td>
<td>214</td>
<td>526</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>84</td>
<td>146</td>
</tr>
<tr>
<td>Clonal abnormality</td>
<td>130</td>
<td>380</td>
</tr>
<tr>
<td>Translocation other than t(1;19)</td>
<td>37 (29%)</td>
<td>136 (36%)</td>
</tr>
<tr>
<td>t(1;19)</td>
<td>29 (22%)</td>
<td>5 (1%)</td>
</tr>
<tr>
<td>Other abnormality</td>
<td>64 (49%)</td>
<td>239 (63%)</td>
</tr>
</tbody>
</table>

All patients with pre-B or early pre-B ALL who had bone marrow samples sent to UAB cytogenetic reference laboratory. Nine additional children with pre-B ALL and 10 with early pre-B ALL were entered on study but are excluded from these analyses because no cytogenetic samples were sent to the cytogenetic reference laboratory for analyses.

In general, the presence of a translocation was associated with a worse treatment outcome than were other cytotypic abnormalities: 15 of 66 versus 3 of 64 failed therapy within the pre-B group (P = .001), and 37 of 141 versus 23 of 239 within early pre-B ALL (P < .001; see Fig 2). However, among cases of pre-B ALL, those with the t(1;19) had a worse EFS than those with other translocations (11 of 29 vs 4 of 37 have failed therapy, P = .01; see Fig 3). In fact, if children with pre-B ALL and t(1;19) are removed from the pre-B ALL cohort, the remaining children with translocations fare no worse than do those with a normal karyotype or those with any clonal abnormality except a t(1;19). None of the five cases with the t(1;19) and an early pre-B cell phenotype have failed as of yet.

The 29 pre-B cases with an identified t(1;19) had a significantly worse treatment outcome than all other pre-B cases (11 of 29 vs 27 of 256 have failed therapy, respectively, P < .001, see Fig 3). Also, patients with the t(1;19) had a worse prognosis than pre-B cases with any other detectable cytogenetic abnormality (11 of 29 vs 7 of 101 have failed, P < .001; see Fig 3). Their worse treatment outcome remains significant even after stratifying on an individual basis by risk group, leukocyte count, age, and DNA index 1.16; all analyses achieved significance at the P < .001 level.
except DNA index ($P = .015$) by logrank analysis. Finally, after adjustment for treatment was made for patients with pre-B ALL, EFS remained significantly worse for those with a t(1;19) compared to all other pre-B cases with a demonstrated clonal abnormality and to all other pre-B patients, regardless of cytogenetic findings, $P < .001$ for both comparisons. These data show the independent prognostic importance of t(1;19).

If cases with the t(1;19) are excluded from patients in both immunophenotypic groups with clonal abnormalities detected, the prognoses of children with pre-B ALL (n = 101) are significantly better than those of children with early pre-B ALL (n = 375, $P = .02$).

Within the group of children with pre-B ALL, those with a t(1;19) were significantly more likely to be black (12 of 29 v 28 of 256, $P < .001$) and to have higher leukocyte counts (median, 25 x 10^9/L v 14 x 10^9/L, $P = .026$ by two-sided Wilcoxon test).

**DISCUSSION**

Results of this study indicate that the poor prognosis that has been ascribed to the pre-B immunophenotype in childhood ALL appears to be restricted to children with translocations, particularly the t(1;19) (q23;p13). Randomized trials often rely on some form of stratification to ensure comparability among patient groups; these preliminary results suggest that knowledge of chromosomal translocations is important for study design. Children who have pre-B ALL without a translocation have a treatment outcome at least as good as that of children who have early pre-B ALL without a translocation in the context of this therapy. Our findings that chromosomal translocations are associated with a poor treatment outcome in early pre-B and pre-B ALL confirm previous observations.

The finding showing that children with pre-B ALL and a t(1;19) have an even worse estimated EFS than those with other translocations is not surprising. Other specific chromosomal translocations, such as t(9;22) (q34;q11), have previously been shown to confer an especially poor prognosis. Approximately 25% of children with ALL have a pre-B immunophenotype, and a similar proportion of children with pre-B ALL have a t(1;19). Because of the frequency of the t(1;19) and its strong association with a poor prognosis, this chromosomal translocation assumes special importance for therapy planning.

Two consecutive trials of the POG showed that children with pre-B ALL had poorer survival than those with early pre-B ALL. The greater proportions of black patients and of patients with higher leukocyte counts within the pre-B patient subgroup partially explain the less favorable treatment response. Here we show that both black race and higher leukocyte counts are strongly associated with t(1;19) within the pre-B phenotype. However, both of our previous studies (ALinC 12 and 13) indicated independent prognostic importance for the pre-B immunophenotype. We have not yet demonstrated a worse overall EFS for children with pre-B ALL as compared to those with early pre-B ALL in this ALinC 14 study, in contrast to our previous findings.
reason for this discrepancy is unclear, but it may be due to significant improvement in early EFS in this study compared with our other trials or, less likely, to the short follow-up of patients treated in this study.

Previous studies have been unable to define specific subgroups of pre-B cases with a worse prognosis. Recently, Pui et al. showed that, within the pre-B phenotype, patients with chromosomal translocations had a significantly higher incidence of traditional high-risk factors, including black race, higher WBC level, DNA index ≥1.16, and higher lactate dehydrogenase levels. Our data extend our previous findings and those of Pui et al by showing the independent prognostic importance of the t(1;19) in children with pre-B ALL.

Mellenten et al. recently found that the E2A gene, which encodes proteins with properties of the Ig enhancer-binding factors E12 and E47, maps to chromosome region 19p13.2-3.3. The breakpoint region noted on chromosome 19 in the t(1;19) All 10 pre-B leukemias or cell lines carrying the t(1;19) contained rearrangements of the E2A gene. These rearrangements altered the E2A transcriptional unit, resulting in the synthesis of a transcript larger than the normalized E2A messenger RNAs (mRNAs) in one cell line studied. Subsequently, Nourse et al. and Kamps et al. identified cDNAs encoding an 85-Kd fusion protein that has the features of a chimeric transcription factor in which the DNA binding domain of E2A is replaced by the putative DNA binding domain of a homeoprotein from chromosome 1 which they named Prl. They demonstrated further that an identical E2A-Prl mRNA junction was created in several t(1;19) cell lines, indicating that the fusion transcripts and predicted chimeric protein are consistently observed in this translocation. Based on this information it may be concluded that this fusion protein is likely to contribute to the ALL phenotype by directly altering the expression of genes normally responsive to the Prl homeoprotein.

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