Case-Control Study Suggests a Favorable Impact of TEL Rearrangement in Patients With B-Lineage Acute Lymphoblastic Leukemia Treated With Antimetabolite-Based Therapy: A Pediatric Oncology Group Study

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TEL gene rearrangement is the most common genetic lesion in pediatric acute lymphoblastic leukemia (ALL), occurring in about 25% of B-lineage cases. We previously showed that, among patients treated on St Jude protocols, TEL rearrangement independently conferred an excellent prognosis. To extend these results to patients treated with antimetabolite-based therapy, we performed Southern blot analysis to determine the TEL gene status of 104 cases of B-lineage ALL treated on Pediatric Oncology Group 8602, matched on age, gender, and leukocyte count. There were 52 failures among the 27 patients with germline TEL, compared with only 8 failures among 27 patients in the rearranged group. Based on a two-sided logistic regression analysis, stratified for age (subdivided at 10 years), leukocyte count (subdivided at 50,000/B lineage), and gender, the estimated odds of failing by 4 years in the germline TEL group is 5.4 times that of the rearranged TEL group, with 95% confidence from 1.9 to 15.6, two-sided

P = .0009. Thus, the presence of a rearranged TEL gene is also associated with an improved survival among patients treated with antimetabolite-based therapy. Our results indicate that all newly diagnosed ALL patients should be screened for TEL gene rearrangements and suggest that these patients are candidates for less intensive therapy.

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were subdivided into eight strata, as shown in Table 1. A stratified random sample of 80 cases (failures) were selected according to its natural distribution, along with a stratified random sample of 80 controls in the identical number per stratum. Failures were intentionally overrepresented as 50%, rather than the natural distribution of 29%, to increase statistical power to answer the question study. Under the assumptions that the frequency of rearranged TEL would be approximately 25% and the difference in 4-year event-free survival would be 25% for germline versus rearranged TEL, ie, 55% to 65% versus 80% to 90%, a sample size of 104 patients would be needed to have 80% power to detect the difference (P < .05, two-sided). The exact conditional Mantel-Haenszel test was used to compare the difference.

The list of 160 patients, which was prioritized in random order, was sent blinded as to outcome to the lead investigator, who performed Southern blot analysis for TEL rearrangement until 104 successful results were obtained. This led to 140 attempted cell bank searches, of which 115 had frozen cells available and 104 had successful assay results.

It is important to note that one cannot use data from a retrospective study that relies on the use of cryopreserved materials to obtain valid Kaplan-Meier estimates, irrespective of whether one distorted the natural distribution or not, if a substantial number of patients samples are missing. Even in this study, a potential bias might have occurred if past studies had disproportionately removed cryopreserved samples from patients in long-term remission with germline TEL or samples from rearranged cases that had an adverse event.

**Molecular analysis.** Genomic DNA obtained from Ficoll-Hypaque–enriched leukemic blasts was analyzed for TEL gene rearrangements as previously described. Briefly, 10 μg of high molecular weight DNA was digested with BamHI, separated electrophoretically in 0.8% agarose gels, and transferred to nylon membranes. Membranes were then hybridized with an [α-32P]-labeled 466-bp Sac I/BamHI TEL cDNA fragment (probe pSB). After high stringency washes, membranes were analyzed by autoradiography.

**RESULTS**

Hyperdiploid DNA content is associated with an excellent prognosis in pediatric ALL. To determine if TEL rearrangement can identify another subgroup of patients with a good prognosis, we performed Southern blot analysis on 104 non-hyperdiploid B-lineage ALL cases selected as described above. All blots were hybridized with probe pSB (Fig 1), which detects the majority of TEL rearrangements. A representative blot is shown in Fig 2. Overall, 27 cases showed rearrangements of TEL. Because our previous study showed that nearly all cases with TEL gene rearrangements also express the TEL-AML1 chimeric transcript, the present 27 cases were not analyzed for TEL-AML1 expression.

Table 1 describes the results of the study according to stratum and TEL results, including those cases with no cells available or unsatisfactory results. There were 52 failures among the 77 patients with germline TEL, compared with only 8 failures among the 27 patients in the rearranged group. Based on a two-sided Mantel-Haenszel stratified analysis, the estimated odds ratio (ie, the ratio of failure in germline TEL cases to that for rearranged TEL cases) is 5.4 (95% confidence interval, 1.9 to 15.6; P = .0009, two-sided). Under a worst case scenario to check for bias, we also grouped the “No Cells” group with the germline group and the unsatisfactory group with the rearranged group. The estimated odds ratio would then be 2.3 (95% confidence interval, 0.98 to 5.3; P = .055, two-sided).

**DISCUSSION**

Risk-based treatment protocols for childhood ALL tailor the intensity of therapy to the risk of relapse. Patients pre-

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### Table 1. TEL Results by Stratum

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Leukocyte Count (× 10^9/L)</th>
<th>Sex</th>
<th>Germline</th>
<th>Rearranged</th>
<th>No Cells</th>
<th>Unsatisfactory</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;9.99</td>
<td>≥50</td>
<td>M</td>
<td>15/19*</td>
<td>3</td>
<td>1/10</td>
<td>2/4</td>
<td>21/42</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>≥50</td>
<td>M</td>
<td>9/14</td>
<td>0/3</td>
<td>2/4</td>
<td>1/1</td>
<td>12/22</td>
</tr>
<tr>
<td>&lt;9.99</td>
<td>&gt;50</td>
<td>M</td>
<td>5/10</td>
<td>1/4</td>
<td>3/4</td>
<td>1/2</td>
<td>10/20</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>&gt;50</td>
<td>M</td>
<td>5/7</td>
<td>0/0</td>
<td>0/1</td>
<td>0/1</td>
<td>5/9</td>
</tr>
<tr>
<td>&lt;9.99</td>
<td>≤50</td>
<td>F</td>
<td>5/7</td>
<td>2/7</td>
<td>1/3</td>
<td>1/1</td>
<td>9/18</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>≤50</td>
<td>F</td>
<td>2/3</td>
<td>0/0</td>
<td>0/1</td>
<td>1/2</td>
<td>3/6</td>
</tr>
<tr>
<td>&lt;9.99</td>
<td>&gt;50</td>
<td>F</td>
<td>8/12</td>
<td>2/4</td>
<td>0/2</td>
<td>0/0</td>
<td>10/18</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>&gt;50</td>
<td>F</td>
<td>3/5</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>3/5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>52/77</td>
<td>8/27</td>
<td>7/25</td>
<td>6/11</td>
<td>73/140</td>
</tr>
</tbody>
</table>

* Number of failures/cases studied.
dicted to be at lower risk of relapse are generally treated with antimitabolite-based therapies, which are well-tolerated and associated with few long-term side effects. Higher-risk patients are treated more intensively, often with chemotherapy regimens that emphasize epipodophyllotoxins, anthracyclines, or alkylating agents. Although these intensive treatment regimens are more efficacious, patients are at increased risk of developing secondary acute myeloid leukemia or cardiomyopathy.\textsuperscript{26-28}

The National Cancer Institute recently sponsored a workshop that adapted uniform age and leukocyte criteria to define clinical risk groups for children with B-lineage ALL.\textsuperscript{29} By consensus, the participants recommended an age of 1.00 to 9.99 years and a presenting leukocyte count of less than $50 \times 10^9/L$ as standard-risk criteria, with other combinations of these two features considered higher-risk. Four-year event-free survival estimates for these two categories were 80% and 65%, respectively. We recently showed that \textit{TEL} rearrangement is associated with an exceptionally good outcome and provides independent prognostic information even among the workshop consensus risk groups.\textsuperscript{18} Among patients with a favorable age and leukocyte count by the workshop criteria, \textit{TEL} status distinguished those with an excellent 5-year event-free survival from those with an intermediate outcome.\textsuperscript{18} It also identified long-term event-free survivors among patients with an unfavorable age or leukocyte count; only 1 of 11 patients with rearranged \textit{TEL} in this group failed therapy.\textsuperscript{18} Likewise, in the present study, the prognostic impact of \textit{TEL} rearrangement was not affected by age or leukocyte count (Table 1).

Because our previous studies included only patients treated on St Jude protocols, it was necessary to determine the impact of \textit{TEL} status among patients treated less intensively, because treatment effects may dramatically alter the clinical significance of prognostic factors. For example, the t(1;19) is associated with a poor outcome among patients treated with conventional antimitabolite-based therapies.\textsuperscript{2} However, when these patients are treated more intensively, their prognosis is similar to that of patients who lack this translocation.

In the present study, we have studied the prognostic significance of \textit{TEL} gene rearrangements among patients treated with conventional antimitabolite-based therapy. We analyzed only nonhyperdiploid cases that lacked the t(4;11) and t(9;22) so that we could determine the impact of \textit{TEL} status among cases that did not have other prognostically important genetic features. Furthermore, our previous results indicate that \textit{TEL} rearrangements generally occur only in nonhyperdiploid cases that lack other detectable translocations.\textsuperscript{18} We have now shown that, among 104 B-lineage ALL cases treated on POG 8602, \textit{TEL} gene rearrangement conferred a significantly improved prognosis. Germline \textit{TEL} cases had an estimated 5.4-fold increased risk of relapse compared with the rearranged \textit{TEL} cases. These results, together with our previous observations,\textsuperscript{18} confirm the excellent prognosis of patients whose leukemic blasts carry \textit{TEL} gene rearrangements.

\textit{TEL} status should thus be used as one of the key prognostic factors for treatment assignment in ALL, but must be confirmed in prospective studies, because retrospective studies may be biased by the availability of diagnostic bone marrow specimens. In this regard, we are now conducting a prospective study of the impact of \textit{TEL} gene status on all newly diagnosed B-lineage ALL patients entered on POG protocols. Studies are also underway to determine the basis of the chemoresponsiveness of blasts carrying rearranged \textit{TEL}. In addition, several laboratories are studying the biologic properties of the \textit{TEL-AML1} chimeric protein. For example, Hiebert et al\textsuperscript{30} have shown that \textit{TEL} converts \textit{AML1} from an activator to a repressor of transcription. Future studies should further elucidate the mechanisms by which \textit{TEL-AML1} leads to oncogenic transformation.

In summary, our results indicate that patients with rearranged \textit{TEL} are candidates for less intensive therapy. In addition, these studies should lead to an improved genetically based risk classification scheme for pediatric ALL. Ulti-
mately, all patients should be classified by genetic features, leading to further improvements in outcome and more rational selection of therapy based on risk of treatment failure.

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