Frequency and Prognostic Significance of HRX Rearrangements in Infant Acute Lymphoblastic Leukemia: A Pediatric Oncology Group Study

By Jeffrey E. Rubnitz, Michael P. Link, Jonathan J. Shuster, Andrew J. Carroll, Nasrollah Hakami, Lawrence S. Frankel, D. Jeanetta Pullen, and Michael L. Cleary

Chromosome band 11q23, the location of the HRX gene, is a site of recurrent translocations in human malignancies. Infants with acute lymphocytic leukemia (ALL) commonly have 11q23 translocations and have an especially poor prognosis despite intensive chemotherapy. We analyzed 96 cases of infant ALL treated on three consecutive Pediatric Oncology Group protocols to determine the frequency and prognostic significance of molecular rearrangements of HRX. Overall, 78 cases (81%) had HRX rearrangements detected by Southern blot analysis performed with a single HRX cDNA probe, whereas 18 cases (19%) had germline HRX. Of the 78 cases with HRX rearrangements, only 50 had abnormalities of 11q23 detected cytogenetically. Molecular abnormalities of HRX were associated with early treatment failure and a very poor outcome. Estimated event-free survival for patients with HRX rearrangements was 19% (SE, 7%) at 3 years, compared with 46% (SE, 17%) for patients with germline HRX (P = .033 by the two-sided logrank test). Therefore, infants with ALL and molecular abnormalities of HRX represent a group with an extremely high rate of failure who clearly need innovative or experimental treatment. Furthermore, cytogenetic analysis alone failed to detect 36% of HRX rearrangements, suggesting that molecular analysis be performed on all infants with ALL to identify this group of high-risk patients.

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ACUTE LYMPHOBLASTIC leukemia (ALL) in infants less than 1 year of age has an extremely poor prognosis despite intensive multiagent chemotherapy.1,2 In a recent Pediatric Oncology Group (POG) therapeutic trial for infant ALL (POG protocol no. 8493), 76 of 82 infants achieved remission, but 55 subsequently relapsed, resulting in an actuarial event-free survival (EFS) of only 27% at 4 years.3 At diagnosis, infants have significantly higher white blood cell (WBC) counts, a higher incidence of organomegaly, and an increased frequency of central nervous system leukemia compared with that for older children with ALL.4,5 Infant leukemia is also characterized by a common ALL antigen-negative phenotype in a majority of cases and a high frequency of translocations involving chromosome band 11q23.6-12 The most common translocation, t(4;11)(q21;q23), has been detected in 30% to 50% of cases by standard cytogenetic techniques.

The gene, designated HRX,13,14 ALL-1,15 or HTRX,16 that is disrupted by 11q23 translocations has been cloned and characterized. It codes for a predicted 431-kD protein that contains putative DNA-binding motifs and shares homology with the Drosophila trithorax protein. 11q23 translocations interrupt HRX and fuse nearly identical portions of HRX to the ENL gene17,18 in t(11;19), to the FEL/AF-1 gene17,19 in t(4;11), and to the AF-9 gene19 in t(9;11) leukemias to create fusion proteins with features of chimeric transcription factors. Genomic breakpoints from various 11q23 leukemias cluster in a restricted 8-kb region of HRX, and DNA probes from this cluster region can detect HRX rearrangements on Southern blot analysis.13,20

Recent studies using molecular analysis have shown that HRX rearrangements are common in infant leukemia and are associated with a poor prognosis. In an analysis of 30 cases of infant ALL treated on Children’s Cancer Group (CCG) protocols, Chen et al21 found that 21 patients with molecular rearrangements of HRX had a projected 15% EFS, compared with an 80% EFS for the 9 patients with germline HRX. Similarly, Cimino et al22 detected HRX rearrangements in 12 of 15 infants with ALL. Of these 12 patients, 11 died of leukemia, whereas the 3 patients with germline HRX remained in complete remission. The present study was undertaken to determine if these results would be applicable to a larger group of patients treated on consecutive POG protocols. Our results indicate that 81% of infants with ALL have molecular rearrangements of HRX, including many that were missed by cytogenetic analysis alone. However, in contrast to the CCG study, we did not identify a subgroup of infants with a very good prognosis. Although infants with ALL and germline HRX had a significantly better survival than those with rearranged HRX, they still fared quite poorly compared with older children with standard-risk ALL.

MATERIALS AND METHODS

Patient specimens. Cryopreserved bone marrow (BM) specimens were available from diagnosis for 96 of 163 infants with ALL treated on POG protocols no. 8398 (open 1983 to 1984), 8493 (open 1984 to 1989), and 9107 (open 1991 to 1993). Briefly, these protocols each included a four-drug induction regimen (VPCA) consisting of vincristine, prednisone, cyclophosphamide, and cytarabine as well as central nervous system prophylaxis with intrathecal chemotherapy (methotrexate, hydrocortisone, and cytarabine). Postinduction therapy consisted of rotating combinations of agents, including teniposide/cytarabine, 6-mercaptopurine/methotrexate, and VPCA (POG no. 8493); the addition of cytarabine/daunomycin to the above combinations on POG no. 8398; and etoposide/cytarabine, methotrexate/6-mercaptopurine, VPCA, and high-dose cytarabine/daunomycin on POG no. 9107.

Cytogenetic analysis. Greater than 90% of specimens were ana-

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lyzed by the POG reference cytogenetics laboratory in Birmingham, AL as previously described.26

**HRX rearrangement analysis.** High molecular weight DNA was extracted, digested with restriction endonucleases, and analyzed by Southern blot analysis as previously described.23 All DNAs were initially digested with BamHI. Those showing only germline HRX were also digested with HindIII. A 0.86-kb BamHI fragment of the HRX cDNA, designated probe B9, was previously shown to span the fusion sites of chimeric transcripts from t(11;19) and t(4;11) translocations and was used as the hybridization probe.23

**Statistical methods.** Differences in EFS were compared using the two-sided logrank test.23 Actuarial survival curves were constructed by the method of Kaplan-Meier,23 using standard errors of Peto and coworkers.27

**RESULTS**

A total of 96 cases of infant ALL treated on three consecutive POG protocols was analyzed for HRX rearrangements. Overall, 78 cases (81%) had HRX rearrangements detected by molecular analysis (Table 1). Of these, 77 were detected with BamHI-digested DNA, whereas 1 case required digestion with HindIII to detect the rearranged band. Of the 78 cases, 50 (64%) had abnormalities of 11q23 detected cytogenetically. The remaining 28 cases with HRX rearrangements included 17 with normal or unsuccessful karyotypes, 4 cases in which no sample was available for cytogenetics, and 7 with cytogenetic abnormalities not involving chromosome 11. As expected, the most common translocation was t(4;11) (36 of 50 cases). In addition, rearrangements of 11q23 with at least seven other translocation partners were observed (Table 2).

Only 18 of the 96 cases analyzed had germline HRX. These included 7 with abnormal karyotypes not involving chromosome 11, 8 normal or unsuccessful karyotypes, 1 case unavailable for cytogenetics, 1 case containing a t(11;15) (q23;q22) translocation, and 1 case showing a t(4;11) translocation (Table 2). The single case containing germline HRX and a t(4;11) underwent a second central review and was again found to have a t(4;11)(q21;q23) karyotype that was indistinguishable from the t(4;11) karyotypes observed in the HRX rearranged cases. Thus, of 52 cases with 11q23 abnormalities detected cytogenetically, 50 had molecular rearrangements of HRX.

Clinical features of the 96 cases analyzed include a median age of 0.4 years (range, 0.0 to 1.0) for patients with rearranged HRX and 0.6 years (range, 0.0 to 0.9) for those with germline HRX (Table 1). Median WBC counts at diagnosis were 173 × 10^9/L (range, 6 to 1676) versus 52 × 10^9/L (range, 3 to 450) for patients with and without HRX rearrangements. Of the 78 patients with HRX rearrangements, 42 were girls, compared with 6 of the 18 germline patients. There were 2 cases of T-cell leukemia, both of which had germline HRX, and 2 cases of B-cell leukemia, both with rearranged HRX. Detailed immunophenotyping data will be published elsewhere.2

Molecular abnormalities of HRX were associated with early treatment failure and a very poor outcome, with 41 of the 78 patients with HRX rearrangements failing during the first year of therapy (Fig 1). At 3 years, the estimated EFS for patients with HRX rearrangements was 19% (SE, 7%), compared with 46% (SE, 17%) for patients with germline HRX (P = .033, by the two-sided logrank test). There were a total of 55 treatment failures in the rearranged HRX group, including 45 relapses, 1 induction failure, 1 toxic death, 2 infectious deaths, and 6 failures from other causes. Among the 18 patients with germline HRX, there were 8 failures, all of whom suffered relapse of their leukemia. Infants with ALL and HRX rearrangements clearly had a dismal prognosis that was significantly worse than infants with germline HRX.

**DISCUSSION**

Using a single HRX cDNA probe, molecular rearrangements of HRX were detected in 78 of 96 cases (81%) of infant ALL selected only by availability of diagnostic BM samples. HRX rearrangements were detected in 50 of 52 cases previously shown to have 11q23 abnormalities by standard cytogenetic analysis. The only exceptions were a patient with a t(11;15)(q23;q22) translocation and a patient with a t(4;11)(q21;q23) translocation who had germline HRX on Southern blot analysis of DNA digested with three separate restriction enzymes. It is possible that these 2 patients did in fact have HRX rearrangements that were outside of the common breakpoint cluster,26 or that they had germline HRX with rearrangements of nearby genes. HRX rearrangements were found in 7 cases with cytogenetic abnormalities not involving chromosome 11 and in 21 cases with normal, un-

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**Table 1. Analysis of 96 Infants With ALL**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Rearranged HRX</th>
<th>Germline HRX</th>
</tr>
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<tbody>
<tr>
<td>Cases</td>
<td>78</td>
<td>18</td>
</tr>
<tr>
<td>Cyto 11q23 abn</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Other cyto abn</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Unsuccessful cyto</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Sample unavailable</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Median WBC × 10^9/L (range)</th>
<th>173 (6-1676)</th>
<th>52 (3-450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in yr (range)</td>
<td>0.4 (0.0-1.0)</td>
<td>0.6 (0.0-0.9)</td>
</tr>
</tbody>
</table>

Abbreviations: cyto, cytogenetic; abn, abnormality.

**Table 2. HRX Status in Infant Leukemias With 11q23 Cytogenetic Alterations**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Rearranged HRX</th>
<th>Germline HRX</th>
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</thead>
<tbody>
<tr>
<td>Total cases</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>t(4;11)*</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>t(11;19)(p23;13)</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>t(1;11)(p34;q23)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>t(10;11)(p21;q13)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>t(10;11)(p12;q23)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>t(11;15)(q23;q22)</td>
<td>0</td>
<td>1</td>
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<tr>
<td>t(10;11)(p11;q23)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>t(10;11)(q15;14)(p15;14.2q25;q11)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>inv(5;11)(q31;23q13)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>del(11)(q23)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Breakpoints were t(4;11)(q21;q23) or not determined.
† Presumed to be 11q23 translocation.
successfult, or undetermined karyotypes. Molecular analysis, which was successfully performed on all samples obtained, was thus able to detect 28 cases of HRX rearrangements that were missed by or unavailable for cytogenetic analysis. Cytogenetic studies in newly diagnosed cases of ALL are often complicated by insufficient BM specimens and low mitotic index, particularly in infants. These results are consistent with two earlier studies that showed a high frequency of HRX rearrangements in infant leukemia, including cases without cytogenetically detected 11q23 abnormalities. Chen et al.24 found molecular HRX rearrangements in 21 of 30 cases (70%), whereas Cimino et al.25 found rearrangements in 12 of 15 cases (80%) of infant ALL. Taken together, these studies strongly support the use of molecular techniques for the routine diagnostic characterization of all infants with ALL. Furthermore, infants with molecular abnormalities at diagnosis could then be analyzed both during and after treatment for the presence of minimal residual disease.

The present study also confirms and extends earlier studies showing a poor outcome for infants with ALL and HRX rearrangements. Chen et al.24 observed an EFS of 15% at 4 years for 21 patients with HRX rearrangements treated on CCG protocols, whereas 11 of 12 patients with HRX rearrangements died in the analysis of Cimino et al.25 Whereas these studies were limited by small sample size, we were able to analyze 60% of all cases of infant ALL treated on POG protocols over the past 10 years. Our results show an EFS of 19% at 3 years for 78 cases containing HRX rearrangements treated on three consecutive POG protocols using intensive multiagent chemotherapy. Although our cases were analyzed retrospectively, the dismal outcome of HRX patients in this large study strongly suggests that these infants represent a particularly high-risk group of patients who are candidates for innovative or experimental therapy.

The uniformly poor prognosis of infants with ALL and HRX rearrangements, despite differences in therapy between treatment centers (POG vs CCG) or differences in POG therapies over the past 10 years, further argues for more aggressive therapy for these patients.

Historically, ALL in infancy has had a very poor prognosis, with long-term survival in the range of 20% to 40%. More recently, the studies of Chen et al.24 and Cimino et al.25 suggest that, for the first time, a subgroup of infants with ALL and a favorable prognosis could be identified by molecular analysis. They propose that the presence of germline HRX may be a prognostic factor that predicts a good outcome. However, the current analysis did not identify a subgroup of infants with such a good prognosis. Although the 18 patients with germline HRX had significantly better survival than those with nongermline HRX (46% vs 19% EFS at 3 years), they still fared quite poorly compared with older children with standard-risk ALL. Differences between our study and others may reflect statistical variation resulting from small sample size, differences in treatment, or other prognostic factors not yet identified. The results presented here indicate that infants with ALL and germline HRX likely comprise an intermediate-risk group that also requires improved therapy.

Although cases were selected only by the availability of diagnostic BM specimens, it is possible that a selection bias still occurred. However, this appears to be highly unlikely because patients with and without diagnostic BM (and thus HRX data) had equally poor outcomes. Overall, the 96 patients analyzed here had a 3-year EFS of 25% (SE, 7%) versus a 3-year EFS of 31% (SE, 8%) for 49 infants with no HRX data (P = .48, by the two-sided logrank test; data not shown).

Nearly all HRX breakpoints can be detected with the B9 probe and are, thus, clustered within an 8-kb region of HRX between exons 5 and 11. As a result, these translocations fuse nearly identical amino-terminal portions of the Hrx protein (containing A-T hooks thought to be involved in DNA binding) to various recipient proteins. Recently, three of these partner genes (FEUAF-4, ENL, and, AF-9) have been cloned and shown to code for proteins rich in serine and proline residues. Biologic studies currently in progress should further clarify the functional properties of Hrx fusion proteins and help to elucidate their role in malignant transformation.

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