CONCISE REPORT

Molecular Abnormalities of **bcr** and **c-abl** in Chronic Myelogenous Leukemia Associated With a Long Chronic Phase

By Orna Dreazen, Michael Berman, and Robert Peter Gale

Median duration of the chronic phase of chronic myelogenous leukemia (CML) is 3 years; <20% of patients have a chronic phase >7 years. It is unknown whether the length of chronic phase is stochastic or is predetermined for each patient. Since molecular abnormalities of **bcr** and **c-abl** occur in CML, we sought to determine whether there were differences in **bcr** and **c-abl** translocation or transcription in individuals with long v short chronic phase. These studies were performed in six patients with CML in whom chronic phase was 7+ to 26 years and 20 patients in whom chronic phase was <7 years. All patients had translocation of **c-abl** to within **bcr**. The distribution of breakpoints in **bcr** were similar in both groups. Transcription of the chimeric **bcr/c-abl** mRNA was comparable. These data suggest that changes in **bcr** or **c-abl** alone do not determine the duration of chronic phase in CML; other factors are likely involved.

**The Clinical Course** of chronic myelogenous leukemia (CML) can be divided into two phases; chronic and acute. Chronic phase is characterized by accumulation of mature myeloid cells; median duration is 3 years in most studies. The acute phase, in contrast, is characterized by a loss of cell maturation and is frequently fatal within 6 to 12 months. Many patients have an intermediate or accelerated phase. Since acute phase CML is generally resistant to therapy, the duration of chronic phase is the major determinant of survival. Although most patients with CML enter acute phase within 7 years, approximately 20% have a longer chronic phase. In <5% of patients CML continues in chronic phase for >10 years.

There are two major hypotheses to account for the variability of duration of chronic phase in CML. One, the stochastic hypothesis, is that CML patients are relatively homogeneous each with a 25% annual risk of entering acute phase. The nonstochastic hypothesis is that each patient has a predetermined duration of chronic phase and that the 25% annual risk of conversion reflects the average of individually predetermined values.

The Ph1 chromosome, which results from a reciprocal translocation between 9 and 22 [t(9;22)] is the cytogenetic hallmark of CML. In individuals with CML and the Ph1 chromosome, the **c-abl** protooncogene is translocated from chromosome 9 to within the breakpoint cluster region (**bcr**) gene on chromosome 22. The chimeric **bcr/c-abl** gene formed by this translocation consists of the 5' **bcr** sequences linked to most or all of the **c-abl** gene in a tail-to-head configuration.

The chimeric gene is transcribed and translated into a chimeric **bcr/c-abl** protein, designated P210*bcrcabl* with enhanced tyrosine kinase activity.

One possible explanation of the rare patients with long chronic phase is that they have a molecular abnormality of **bcr** and/or **c-abl** distinct from typical patients with CML. To test this hypothesis we studied six patients with CML in chronic phase for >7 years. The data were compared with those of 20 patients with a chronic phase for <7 years. Our data indicate molecular abnormalities similar to patients with briefer chronic phase and suggest that other factors likely play a role.

METHODS

**Patients.** Requests were made to several physicians specializing in CML throughout the United States to identify individuals with chronic phase longer than 7 years. Six such patients were identified. The precise number of patients screened is unknown but estimated to exceed 100. Three patients were studied in chronic phase (10042, 10043, 10058) and three in accelerated or acute phase (10037, 10044, 10070). Blood and/or bone marrow samples were obtained by standard techniques. In one patient (10037) leukemia cells were obtained from the spleen following splenectomy for hypersplenism. Controls were 20 individuals previously or concurrently studied in our laboratory. All of these individuals were in chronic phase for <7 years; whether some of these individuals will remain in chronic phase for >7 years is unknown but is likely to be <20%. Written informed consent, approved by the UCLA institutional review board, was obtained from the participants.

**DNA studies.** Leukemia cells were isolated from peripheral blood or bone marrow by Ficoll-hypaque density centrifugation. High molecular weight DNA was prepared by standard techniques. DNA was treated with *ScaI, BglII, EcoRI*, or *HindIII* restriction endonucleases and analyzed by Southern blotting using a 4 kilobase pair (kb) **bcr** cDNA probe (provided by E. Canaani and B. Lifshitz) or a 1.2 kb **bcr** genomic probe (Oncogene Science, Mineola, NY).

**RNA studies.** RNA was prepared using the urea/lithium chloride method. Polyadenylated messenger RNA was isolated by purification on oligo-dT-cellulose columns. R**NA**se protection analysis for the alternative **bcr/abl** junctions has been described. RNA probes were synthesized by standard techniques in a SP6 vector (Promega Biotec, Madison, WI).

**RESULTS**

**Patients.** Clinical and laboratory details at the time of diagnosis of the six long chronic phase patients are indicated.

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and in three between
bcr
breaks in
bcr
in all six patients with a long chronic phase (Table 2 and Fig 1). In one, the break occurred between bcr exons “2” and “3” and in three between bcr exons “3” and “4.” In two patients the location of the bcr break could not be mapped. Similar breaks in bcr were detected in all 20 patients with more typical duration chronic phase CML. In the 19 evaluable cases, in four instances it was between bcr exons “2” and “3” and in 15 it was between bcr exons “3” and “4.” This distribution of bcr breakpoints is similar between the two groups.

RNA studies. A chimeric bcr/c-abl mRNA was detected in the four long-term chronic phase patients tested (Table 2 and Fig 2). In one patient we detected a junction between bcr exon “2” and c-abl exon II and in two between bcr exon “3” and c-abl exon II. In one patient both junctions were detected consistent with a break after bcr exon “3” with alternate splicing. These results are consistent with the bcr breakpoints determined by Southern analyses. In a study of 20 individuals with typical duration CML, four had a junction between bcr exon “2” and c-abl exon II and 17 between bcr exon “3” and c-abl exon II; eight had both junctions. In one patient no chimeric mRNA was detected. This difference is not significant.

DISCUSSION

We studied six patients with Ph+ chromosome positive chronic phase CML for 7 to 26 years. Patients’ cells were analyzed for rearrangement of bcr and for presence of bcr/abl chimeric mRNA. All patients had bcr gene rearrangement and the four studied had a chimeric bcr/abl mRNA. The distribution of bcr breaks and chimeric bcr/ c-abl mRNA were similar to those observed in 20 patients with more typical duration chronic phase CML. 14

Three of the 6 long-term patients were studied when they had more advanced CML; it is possible that their results might have differed had they been studied in chronic phase. This is unlikely, however, since results in these three did not differ from the three individuals studied while in chronic phase. More importantly, we have previously shown both in patient populations and in five individuals that transition from chronic to acute phase is not associated with detectable

Table 1. Clinical and Laboratory Features of CML at Diagnosis in Patients With a Long Chronic Phase

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Spleen*</th>
<th>Hemoglobin (g/dL)</th>
<th>WBC (x 10^9/L)</th>
<th>Platelets</th>
<th>LAP</th>
<th>Therapy</th>
<th>Duration (y)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>10037</td>
<td>30</td>
<td>M</td>
<td>+</td>
<td>15.8</td>
<td>80</td>
<td>Adq.</td>
<td>–</td>
<td>BU, HU</td>
<td>26</td>
</tr>
<tr>
<td>10042</td>
<td>56</td>
<td>M</td>
<td>–</td>
<td>10.3</td>
<td>19</td>
<td>274</td>
<td>–</td>
<td>BU</td>
<td>11+</td>
</tr>
<tr>
<td>10043</td>
<td>38</td>
<td>M</td>
<td>+</td>
<td>11.6</td>
<td>30</td>
<td>173</td>
<td>–</td>
<td>BU, HU</td>
<td>9+</td>
</tr>
<tr>
<td>10044</td>
<td>58</td>
<td>F</td>
<td>+</td>
<td>13.2</td>
<td>27</td>
<td>472</td>
<td>–</td>
<td>BU, HU</td>
<td>8</td>
</tr>
<tr>
<td>10058</td>
<td>36</td>
<td>F</td>
<td>–</td>
<td>12.6</td>
<td>33</td>
<td>830</td>
<td>–</td>
<td>BU, HU</td>
<td>7+</td>
</tr>
<tr>
<td>10070</td>
<td>28</td>
<td>M</td>
<td>–</td>
<td>16.6</td>
<td>59</td>
<td>259</td>
<td>–</td>
<td>BU, HU</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: UPN, unique patient number; LAP, leukocyte alkaline phosphatase; BU, myleran (busulfan); HU, hydroxyurea.

* +, Indicates splenic enlargement.
† +, Indicates still in chronic phase.

Table 2. Molecular Analysis of Long-Term Survivors of CML

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>bcr Rearrangement</th>
<th>bcr/abl mRNA Junction</th>
</tr>
</thead>
<tbody>
<tr>
<td>10037</td>
<td>+</td>
<td>&quot;2&quot;-II</td>
</tr>
<tr>
<td>10042</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>10043</td>
<td>+</td>
<td>&quot;3&quot;-III</td>
</tr>
<tr>
<td>10044</td>
<td>+</td>
<td>&quot;2&quot;-II; &quot;3&quot;-III</td>
</tr>
<tr>
<td>10058</td>
<td>+</td>
<td>&quot;3&quot;-III</td>
</tr>
<tr>
<td>10070</td>
<td>+</td>
<td>NT</td>
</tr>
</tbody>
</table>

Abbreviation: NT, not tested.

"2" and "3" refer to exons of the bcr region of the bcr gene. II refers to exon II of c-abl.

Fig 1. Southern blot analysis indicating rearrangement of bcr in two patients. Patients’ DNA was digested with BglII, separated on 0.7% agarose gel and hybridized to a 1.2 kbp genomic bcr probe. (A) Patient 10042. (B) patient 10043. (C) normal control. Arrows indicate the rearranged bands in patients DNA.

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Fig 2. RNase protection assay showing one possible bcr/abl chimeric mRNA. A 450 b chimeric probe would be protected only by patients mRNA in which bcr exon "3" is joined to c-abl exon II. The 278 b and 172 b fragments are protected by normal transcripts from the mRNA bcr or c-abl genes. (A) Mspl digest of pBR322 DNA, numbers indicate size in bases; (B) patient 10044; (C) patient 10043; (D) normal control. In patients B and C the 450 b chimeric probe is protected indicating the bcr exon "3" to c-abl exon II splice. The normal control protects only the 278 b and 172 b portions of the probe; the intact chimeric probe (450 b) is not protected.

changes in expression of the chimeric bcr/abl gene (unpublished data).

Based on these data we conclude that the duration of chronic phase in patients with CML is not related solely to abnormalities of the bcr or abl genes; other factors are likely involved.

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