Analysis of Breakpoints Within the bcr Gene and Their Correlation With the Clinical Course of Philadelphia-Positive Chronic Myelogenous Leukemia

By Mordechai Shtalrid, Moshe Talpaz, Razelle Kurzrock, Hagop Kantarjian, Jose Trujillo, Jordan Gutterman, Galina Yoffe, and Mark Blick

Chronic myelogenous leukemia (CML) is characterized by a reciprocal translocation between chromosomes 9 and 22. The breakpoints on chromosome 22 are clustered within a 5.8-kilobase (kb) DNA fragment known as the breakpoint cluster region (bcr), which encodes part of a functionally active gene. We analyzed the bcr in DNAs from 108 consecutive, unselected Philadelphia chromosome-positive CML patients by Southern blot and determined five restriction enzyme fragments within which breaks occur on chromosome 22. The exact sublocalization was determined in the DNA of 100 patients. It was found to be within the 5.8-kb bcr in 99 patients and outside the bcr in only one. Within the bcr, most of the breakpoints occurred in fragments 1, 2, and 3.

Overall, laboratory and clinical features of CML did not correlate with specific breakpoint fragments, but chronic-phase duration was longer in patients with a breakpoint in fragment 2 of the bcr. Large 3' bcr deletions were found in nine patients but did not influence clinical outcome. DNA from one of six patients analyzed both during chronic phase and blast crisis showed an additional aberrant fragment, which suggested that a second abnormal clone developed in blast crisis.

© 1988 by Grune & Stratton, Inc.
RESULTS

Sublocalization of the \textit{bcr} breakpoint. We subdivided the \textit{bcr} gene into defined restriction endonuclease fragments (Fig 1). Representative examples of Southern blots for breaks within each fragment are displayed in Fig 2. As shown in Fig 2, lane C, breaks within fragment 1 (F1) resulted in rearranged and germline fragments when using probe A and restriction enzymes \textit{EcoRI} and \textit{BglII} but only germline fragments when using \textit{HindIII} and \textit{BamH}I. For breaks in F2 (Fig 2, lane D), we detected rearranged fragments when using probe A and restriction enzymes \textit{EcoRI}, \textit{BglII}, and \textit{BamH}I but not with \textit{HindIII}. In a similar fashion, we defined the specific breakpoint fragments for each DNA sample. Rearrangements were confirmed by the demonstration of rearranged fragments with both probes or with two different restriction enzymes.

DNA from 108 consecutive, unselected \textit{Ph}1-positive CML patients was analyzed by Southern blot. In all 108 DNA samples, the \textit{bcr} gene was rearranged. The location of the breakpoint within the \textit{bcr} gene, as defined in Fig 1, was determined in 100 patients. In eight patients, the \textit{bcr} gene was rearranged, but the exact fragment within which the breakpoint occurred, as defined by Fig 1, could not be determined.

The distribution of the patients on the basis of clinical stage and correlated with \textit{bcr} breakpoint fragments is presented in Table 1. In the majority of patients, the DNA breakpoints occurred within fragments 1, 2, or 3 of the gene (93%). The breakpoint occurred in fragment 0 in only one patient and in fragment 4 in six patients. Thirty-eight patients were examined at the time of blastic crisis, 24 at myeloid blastic crisis, seven in lymphatic, and seven in undifferentiated. There was no difference in the distribution of those patient samples within the breakpoint fragments when comparing chronic phase to blastic crisis and when comparing different histological types of blastic crisis.

Clinical correlations. The correlation between the site of the \textit{bcr} breakpoint fragment and the patients’ clinical and hematologic features is summarized in Table 2. To standardize our analysis, we compared only the laboratory and clinical features obtained at the time of diagnosis, when all patients were in the chronic phase of CML. Because M.D. Anderson Hospital is a referral institution, presenting features for some patients were sometimes unknown, and these patients were excluded from our statistical analysis.

There was no difference in the initial presenting laboratory values for hemoglobin, WBC count, or platelet count when comparing patients in groups F1 through F4. Clinical features such as age, sex, and palpable spleen were similar among the groups. There was a trend for longer duration of the chronic phase in patients with breakpoints in F2 compared with all other groups (68 vs 59 months, \(P = .06\)) as well as when comparing with the individual subsets of patients with breakpoints in F1 (68 vs 43 months, \(P = .07\)) or in F3 (68 vs 51 months, \(P = .04\)). However, there was no statistical difference in the duration of the chronic phase in patients with breakpoints in F1 and F2 compared with F3 and F4 (59 vs 59 months, \(P = .4\)).

Deletions of the \textit{bcr}. As demonstrated in Figs 2 and 3 (panel B1), chromosomal breaks and subsequent translocations that occur within the DNA sequences used as a probe result in two rearranged fragments: one fragment representing 22 q− sequences and one, 9q+ sequences.\(^{34}\) In the 57 samples in which the breakpoint occurred within F1 and F2 when using probe B and the \textit{Bcl}I and \textit{Bgl}II restriction enzymes...
ANALYSIS OF BREAKPOINTS WITHIN THE bcr GENE

We have previously been interested in the monitoring of breakpoints within the bcr region for two reasons. First, as CML patients progress, certain criteria were not known for all patients. Numbers in parentheses indicate the number of patients in whom this parameter was known to us at the time of diagnosis.

...enzymes, we detected rearranged fragments in all instances. We then reprobed these same blots with probe A and detected a single, rearranged band in 48 instances, thus suggesting that probe A was detecting the 9q+ sequences as expected. In nine of the 57 instances in which we detected no rearranged band with probe A, we assume that the 3' bcr sequences, which should have translocated to the 9q+ chromosome, were deleted. In one additional instance, we detected 5' breakpoint and 3' breakpoint. We assume that the region between these two breakpoints was deleted (case I0, Table 3). There were no unique clinical or laboratory features in this group of patients (Table 3).

Among the 36 DNA samples in which the breaks occurred within fragment 3 when we used probes A and B and BamHI, BglII, HindIII, or EcoRI (Fig 2, lane E), we detected two rearranged fragments in 33 instances, thus suggesting that in these instances we were detecting both 22q- and 9q+ sequences. In those three of 36 instances in which we detected only one rearranged fragment, we assume that 3' bcr sequences were deleted. Because we did not have a separate probe 3' to fragment 3, we could not test this directly.

Progression. To determine whether or not the breakpoint within the bcr gene changes as CML patients progress from chronic phase to blastic crisis, we studied six patients during both chronic phase and acute blastic crisis. There was no change in the breakpoints in five patients, and one patient showed an additional aberrant band only with EcoRI digestion and both probes (Fig 3, lane C). A summary of certain laboratory and clinical features of these patients is given in Table 4.

DISCUSSION

The breaks on chromosome 9, band q34, in Ph1-positive CML patients are spread over 200 kb 5' to the second ("common") abl exon.21,22 Despite this wide variability at the DNA level, we and others have demonstrated that the mRNA product always includes this second ("common") abl exon, which provides the acceptor splice site for 5' bcr sequences.23,24,25 Our data in 100 consecutive unselected patients with Ph1-positive CML confirmed that, in contrast to the breaks on chromosome 9, the breaks on chromosome 22, band q11, are clustered within a restricted 5.8 kb bcr.24,25 Every case had its own specific break within both the abl and bcr genes, thereby resulting in wide heterogeneity at the DNA level. The breakpoints are located within introns.26,27 Within bcr, the breaks are located mainly between exons 2 and 3, between exons 3 and 4, or between 4 and 5. From this analysis it is clear that for the bcr portion of the hybrid bcr/abl mRNA, the splice donor side varies. In >24% of the cases, the breaks on chromosome 22 occur so that the "second" bcr exon provides the splice donor site, in >36%, the "third" bcr exon provides this site. After translation at least two different chimeric proteins are synthesized, which may affect the phenotype and the characteristics of the disease.27

Our study was undertaken to determine whether this molecular heterogeneity correlated with the clinical or laboratory features of CML. There was no correlation with sex; age; splenomegaly; or degree of anemia, leukocytosis, or thrombocytosis. However, there was a trend for longer duration of the chronic phase in patients who had group 2 than in groups 1 and 3 in which it was similar. This finding is interesting because groups 1 and 3 are homogeneous groups with respect to the exclusion or inclusion of bcr exon 3 within the chimeric bcr/abl mRNA. These data suggest that bcr exon 3 does not influence the clinical course of Ph1-positive CML. In contrast, the localization of the breakpoint in the intron between exons 2 and 3 may possibly influence the duration of chronic phase. Because the efficiency of splicing is in part dependent on the 5' splice region within introns,28 perhaps translocations within this region diminish the efficiency of splicing and formation of the hybrid mRNA in CML. The observed differences in chronic-phase duration according to the bcr breakpoints are, however, marginal and should be interpreted cautiously and confirmed in larger population studies.

Recent reports have shown that some patients with Ph1-positive acute lymphoblastic leukemia (ALL) have translocations to other not yet defined regions of chromosome 22.24,29,30 This observation suggests that sequences other than the usual 5.8-kb bcr fused to c-abl may influence the

<table>
<thead>
<tr>
<th>bcr Breakpoint Fragment</th>
<th>No. of Patients</th>
<th>Age (Median)</th>
<th>Sex (F/M)</th>
<th>Palatable Spleen</th>
<th>Anemia*</th>
<th>WBC (x 10^9/L) (Median)</th>
<th>Platelet Count (&gt;450 x 10^9/L)</th>
<th>Chronic Phase Duration (Median in mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>24</td>
<td>39</td>
<td>8/16</td>
<td>12 (17)†</td>
<td>7 (20)</td>
<td>113 (20)</td>
<td>7 (21)</td>
<td>43</td>
</tr>
<tr>
<td>F2</td>
<td>33</td>
<td>43</td>
<td>14/19</td>
<td>20 (24)</td>
<td>8 (21)</td>
<td>153 (23)</td>
<td>14 (20)</td>
<td>69</td>
</tr>
<tr>
<td>F3</td>
<td>36</td>
<td>41</td>
<td>10/26</td>
<td>17 (24)</td>
<td>12 (22)</td>
<td>113 (26)</td>
<td>16 (26)</td>
<td>51</td>
</tr>
<tr>
<td>F4</td>
<td>6</td>
<td>40</td>
<td>2/4</td>
<td>5 (6)</td>
<td>1 (1)</td>
<td>210 (5)</td>
<td>4 (6)</td>
<td>69</td>
</tr>
</tbody>
</table>

*Anemia: males, hemoglobin <12 g/dL; females, hemoglobin <11 g/dL.
†Certain criteria were not known for all patients. Numbers in parentheses indicate the number of patients in whom this parameter was known to us at the time of diagnosis.
histological type of leukemia and the aggressiveness of the disease. In our study, no linkage was found between the histological type of bladder cancer and location of the 3′ bcr breakpoint in samples from Ph′-positive CML patients. A recent report suggests a strong correlation between 3′ bcr breakpoints and bladder cancer of CML as well as a chronic phase of shorter duration. In our study, no statistical difference was found between the chronic-phase duration in patients with bcr breakpoints in F1 and F2 (5′ bcr) and F3 and F4 (3′ bcr). Most of our patients were given α-interferon rather than chemotherapy, and this difference in therapy might account for the discrepancies between our and the other study findings. Likewise, patients with bladder cancer had a distribution of bcr breakpoints similar to that seen in the chronic phase patients.

Several studies have demonstrated that 3′ bcr deletions occur in sequences that form part of the 9q + chromosome. Popenoe et al found large deletions in four of 14 patients with Ph′-positive CML and suggested that more proximal deletions occurred in four other patients. In the CML cell line K562, 3′ bcr sequences are also deleted. In contrast, de-Klein et al, found only small deletions in chromosomes from patients with Ph′-positive CML. Our analysis suggests that large deletions occur in 10% to 20% of patients and are independent of the site of breaks within the bcr. There were no unique features associated with these Ph′-positive CML samples. An even higher incidence of 3′ bcr deletions was found in patients with Ph′-positive ALL. Deletions probably occur at the time of translocation and may be involved in the mechanism of Ph′ chromosome recombination. Because of deletions and overlapping fragments, the use of a single 3′ bcr probe and a single restriction enzyme is not sufficient to detect all Ph′-positive CML bcr rearrangements. We suggest the use of a large 5′ bcr probe and at least two restriction enzymes (BglII, BclI).

To determine whether a new clone of cells develops when chronic-phase progresses to bladder crisis, we analyzed the DNA of six patients in both chronic phase and bladder crisis.

### Table 3. Clinical Characteristics at the Time of Diagnosis of Ph′-Positive CML Patients With bcr Deletions

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Spleen (cm)</th>
<th>Hemoglobin* (g/dL)</th>
<th>WBC (x 10^9/L)</th>
<th>Platelets (x 10^11/L)</th>
<th>Basophils (%)</th>
<th>Time From Diagnosis to bcr Breakdown (mo)</th>
<th>Duration of Chronic Phase (mo)</th>
<th>Karyotype at Time of bcr Breakdown</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63/M</td>
<td>+</td>
<td>N</td>
<td>114</td>
<td>NA</td>
<td>NA</td>
<td>2</td>
<td>3</td>
<td>Ph′, 95%</td>
<td>Died after undifferentiated BC</td>
</tr>
<tr>
<td>2</td>
<td>50/M</td>
<td>Tip</td>
<td>N</td>
<td>105</td>
<td>760</td>
<td>4</td>
<td>68</td>
<td>68</td>
<td>Ph′, 100%</td>
<td>Died after myeloid BC</td>
</tr>
<tr>
<td>3</td>
<td>29/M</td>
<td>25 L</td>
<td>153</td>
<td>540</td>
<td>2</td>
<td>1</td>
<td>24+</td>
<td></td>
<td>Ph′, 100%</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>4</td>
<td>33/M</td>
<td>0 N</td>
<td>34</td>
<td>834</td>
<td>1</td>
<td>6</td>
<td>42+</td>
<td></td>
<td>Ph′, 95%</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>5</td>
<td>58/F</td>
<td>+ NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
<td>12+</td>
<td>Ph′, 100%</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>6</td>
<td>32/M</td>
<td>NA</td>
<td>NA</td>
<td>84</td>
<td>84+</td>
<td>3</td>
<td>9+</td>
<td></td>
<td>Ph′, 100%</td>
<td>Accelerated phase</td>
</tr>
<tr>
<td>7</td>
<td>22/F</td>
<td>12 L</td>
<td>NA</td>
<td>1,300</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
<td>Ph′, 100%</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>8</td>
<td>65/F</td>
<td>15 L</td>
<td>112</td>
<td>690</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
<td>Clonal evolution†</td>
<td>Died due to undifferentiated BC</td>
</tr>
<tr>
<td>9</td>
<td>27/M</td>
<td>0 N</td>
<td>160</td>
<td>350</td>
<td>5</td>
<td>8</td>
<td>12+</td>
<td></td>
<td>Ph′, 100%</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>10</td>
<td>29/M</td>
<td>20 L</td>
<td>291</td>
<td>336</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td>Ph′, 75%</td>
<td>Died after lymphatic BC</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; BC, blast crisis.

* N, normal; L, low: male, <12 g/dL; female, <11 g/dL.
† Clonal evolution [46xx,t(3p-;4q+), t(9q+;22q-)], 55%; [46xx,t(9q+;22q-)], 40%. Hyperdiploid cells with additional Ph′ and other changes, 5%.
Only one patient showed an additional aberrant bcr gene band. Bartram et al found a new chromosome rearrangement in one of four patients in blastic crisis and a deletion of bcr/c-abl sequences in another. However, such cases are rare among Ph'-positive CML patients. Despite the major development in our understanding of genetic events associated with CML pathogenesis, little is known about Ph'-positive CML evolution from a chronic to a more advanced stage. We cannot clearly demonstrate changes within the bcr-abl gene to account for disease evolution in the majority of the cases we examined. However, this does not rule out that more 5' secondary bcr-abl changes are associated with disease evolution or that (b) minor changes (point mutations) not detected by the methodologies used by us contribute to disease progression. At this point, a more likely explanation is that activation of additional genes (on chromosomes 8, 22, and 17) cooperate with the bcr-abl gene to lead toward a more aggressive disease stage.

ACKNOWLEDGMENT

The authors would like to thank Oncogene Science, Inc., for the bcr probe (Ph'-bcr/3) and Rima Aivazian for the excellent secretarial support.

REFERENCES

5. Groffen J, Stephenson JR, Heisterkamp N, deKlein A, Bartram CR, Grosveld G: Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 36:93, 1984


30. Clark SS, McLaughlin J, Crist WM, Champlin R, Witte ON: Unique forms of the \( abl \) tyrosine kinase distinguish \( Ph^1 \)-positive CML from \( Ph^1 \)-positive ALL. Science 235:85, 1987


32. Poponeo DW, Shaefer-Rego K, Mears JG, Bank A, Leibowitz D: Frequent and extensive deletion during the 9,22 translocation in CML. Blood, 5:1123, 1986


Analysis of breakpoints within the bcr gene and their correlation with the clinical course of Philadelphia-positive chronic myelogenous leukemia

M Shtalrid, M Talpaz, R Kurzrock, H Kantarjian, J Trujillo, J Gutterman, G Yoffe and M Blick

Updated information and services can be found at: http://www.bloodjournal.org/content/72/2/485.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml