Relationship of bcr Breakpoint to Chronic Phase Duration, Survival, and Blast Crisis Lineage in Chronic Myelogenous Leukemia Patients Presenting in Early Chronic Phase

By Stephan W. Morris, Lambert Daniel, C.M.I. Ahmed, Anthony Elias, and Paul Lebowitz

Strong evidence implicates fusion of control elements and 5' sequences of the bcr gene of chromosome 22 with 3' sequences of the c-abi gene of chromosome 9 in the pathogenesis of Ph-positive and certain cases of Ph-negative chronic myelogenous leukemia (CML). Since this fusion gene gives rise to a chimeric tyrosine protein kinase with transforming potential, and since the bcr exon contribution to this chimeric protein is variable, the question has arisen as to whether bcr breakpoint location and bcr exon contribution could influence the clinical course of CML. Prior studies have yielded conflicting results on this point. Here we have looked, in a manner approximating a prospective analysis, at the relation of bcr breakpoint localization to the duration of chronic phase, total survival, and blast crisis phenotype in 81 patients presenting in the chronic phase of CML. We have found no significant differences in chronic phase duration or total survival among patients with breakpoints in the three major subregions of a breakpoint cluster region within the bcr gene. These findings indicate that chronic phase duration and total survival cannot be predicted from bcr breakpoint for CML patients presenting in chronic phase and suggest that unknown oncogenic events determining the onset of blast crisis are the prime determinants of prognosis. Combined analysis of blast crisis cell lineage in our patients and patients presented in a previous study has revealed an overall ratio of myeloid:lymphoid (M:L) crisis of 3.4:1, but a striking predominance of myeloid crisis in patients with breakpoints in subregion 2 (M:L of 9:1), and a lower than expected M:L ratio (1.6:1) among patients with breakpoints in subregion 3 (P for subregion 2 versus 3 = .012; subregions 0.1, 1.2 versus 3 = .012; subregions 0.1, 0.3 versus 2 = .032). The molecular basis for this divergence from the anticipated M:L ratio in patients with breakpoints in bcr subregions 2 and 3 is unknown.

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STUDIES DONE OVER the past several years strongly implicate fusion of control elements and 5' sequences of the truncated bcr gene of chromosome 22 with 3' sequences of the truncated c-abi gene of chromosome 9 in the genesis of Ph-positive and certain cases of Ph-negative chronic myelogenous leukemia (CML).1-14 Whereas chimeric mRNA and protein products of the fused bcr-c-abi gene in different patients appear to carry the identical c-abi information,2 they may carry different bcr sequences depending upon the specific intron within a breakpoint cluster region (bcr) where cleavage occurs,3-4 alternative splicing,8 and possible alterations of genomic sequences occurring during cleavage and fusion.15 Since the protein produced by the fused bcr-c-abi gene is an active tyrosine protein kinase10-12 implicated in cellular transformation,13-14 one may question whether variability in the clinical course among patients with CML is related to the variable contribution of bcr sequences to the protein kinase product.

A number of recent studies have addressed this question, but with confusing and seemingly contradictory results. On the one hand, there are three studies that suggest that patients with breakpoints in 5' subregions of the bcr have a more benign disease course than patients with 3' breakpoints. These include two small studies16,17 that suggest that 5' breakpoints (located 5' of the HindIII site separating bcr subregions 2 and 3 in Fig 1) are significantly more common in chronic phase patients, while 3' breakpoints (located 3' of this HindIII site) are more common in patients in crisis and accelerated phase, and two small studies18,19 showing significantly longer chronic phase durations in patients with 5' breakpoints. In contrast, there are two studies suggesting that breakpoint location has little or no influence on disease course: one large study18 showing no significant differences in chronic phase duration or in presentation in chronic versus crisis phases among patients with breakpoints in various bcr subregions and a smaller study20,21 showing no relation between bcr breakpoint location and bcr exonic contribution to bcr-c-abi mRNA and chronic phase duration.

The present study looks at the clinical course of CML in relation to bcr cleavage site, but differs from prior studies in being restricted to patients presenting in early chronic phase and in being carried out in a manner closely approximating a prospective study. We chose to confine ourselves to patients presenting in early chronic phase since these patients may stand the best chance of cure from bone marrow transplantation, and a means to reliably predict a good versus a poor prognosis would aid in determining the optimal time for.

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2035
translocation in individual patients. Our study demonstrates no significant correlation between bcr breakpoint location and either chronic phase duration or survival. However, it has unexpectedly revealed a significant relationship between blast crisis lineage and bcr breakpoint location.

MATERIALS AND METHODS

Patient material. One hundred-eighteen CML patients who underwent leukapheresis at the Dana Farber Cancer Institute (Boston, MA) for possible autologous transfusion from 1977 to 1982, or whose leukapheresed blood was stored at Dana Farber Cancer Institute (DFCI) for autologous transfusion during the same 6-year period, served as subjects for this study. All patients were in the chronic phase of disease when leukapheresed, most were within 2 to 3 months of diagnosis, and all were without evidence of acceleration. Patients were treated only with hydroxyurea or busulfan before onset of the accelerated stage of disease; none received interferon, whose influence on disease course was questioned in an earlier study. Patients and their clinical histories were unknown to investigators carrying out bcr breakpoint analyses.

Analyses of bcr breakpoint. High molecular weight leukemic cell DNA was obtained by lysis of leukapheresed cells with sodium dodecyl sulfate (SDS) and proteinase K, extraction with phenol-chloroform and chloroform, and precipitation with ethanol and spooling of DNA strands. DNAs were digested with restriction enzymes Bgl II, BamHI, and Hind III, electrophoresed in 3% dextran sulfate, and transferred to nylon filters (GeneScreen Plus; New England Nuclear, Boston, MA) in 0.4 N NaOH. Hybridizations were done according to Groffen et al.7

Fig 1. Schematic diagram of the breakpoint cluster region (bor) of the bcr gene indicating five subregions (0 through 4), restriction sites bounding subregions, exons, and probes used to identify subregions of bcr cleavage. Numbering of subregions according to Groffen et al.7

Subregions I Ex1 Ex2 Ex3 Ex4

Bcr Bg H B H Bg B

III

1 kb

II were restested with a "universal" probe (Transprobe, Oncogene Sciences) that identifies breakpoints in greater than 98% of Ph-positive CML patients. However, all six were found to have no demonstrable breakpoint.

Clinical information. After the completion of molecular studies, relevant clinical information was sought for each patient with a demonstrable bcr breakpoint from hospital charts and/or by telephone calls to physicians. In this way, we attempted to correlate bcr breakpoints with clinical course in a manner approximating a prospective analysis. Of the 118 patients studied, adequate clinical and molecular information was obtained on 81. The remainder were excluded from the study for the following reasons: clinical information was unavailable or inadequate on 21, bone marrow transplantation was done in early chronic phase on seven; three died relatively soon after diagnosis in chronic phase of unrelated causes, five were found to be Ph-negative, bcr-negative, and one was found to be bcr-negative although Ph-positive.

Information on red blood cell (RBC), white blood cell (WBC), and platelet counts, white cell differential, presence or absence of splenomegaly, appearance of bone marrow and Ph chromosome status was available at diagnosis on all 81 patients presented. Eight of these patients were alive in stable chronic phase as of April, 1989, while 69 had entered blast crisis and succumbed. The four remaining patients succumbed before reaching blast crisis. One who died 74 months post-diagnosis due to an unrelated disease was considered to have a chronic phase duration of 74+ months. The other three died in advanced accelerated phase from infection and complications of pancytopenia and splenectomy, respectively. Chronic phase duration in these patients was considered to be time from diagnosis to death from accelerated phase complications.

A diagnosis of blast crisis was made in most of the 69 patients entering crisis on the basis of a bone marrow aspirate showing blasts (plus promyelocytes for myeloid crisis) of greater than 30%. For those patients in whom crisis was diagnosed without a bone marrow aspirate being done, a diagnosis of crisis was accepted on the basis of (1) peripheral WBC and organomegaly unresponsive to hydroxyurea or busulfan and (2) a WBC differential showing greater than 30% blasts with (3) support in most cases from the presence of chromosomal abnormalities in addition to the Ph chromosome and/or the appearance of discrete tumor masses.

Information on blast crisis lineage was available for 49 of the 69 patients reaching crisis. Ten were identified as having a lymphoid phenotype, all on the basis of lymphoid morphology of blasts (including absence of primary myeloid granules) together with common acute lymphoblastic leukemia antigen (CALLA), terminal deoxynucleotidyl transferase (TdT), lymphoid surface marker, and/or periodic-acid Schiff (PAS) positivity and negativity for myeloid markers, myeloid dysplasia, and Pelger-Huet abnormality. Thirty-six were identified as having myeloid crisis: 25 on the basis of myeloid morphology of blasts plus either myeloid dysplasia and/or Pelger-Huet abnormality; and the remaining 11 were classified as having either myeloid or lymphoid crisis and were thought to have an undifferentiated crisis phenotype.

Statistical analyses. Median values at diagnosis for age and WBC and platelet counts of patients with breakpoints falling in different bcr subregions were analyzed for significant differences by a Kruskal-Wallis test for continuous variables. Sex and the presence of a Ph chromosome were compared by a chi-squared test. Patient data with high or low bcr breakpoint values were compared by two-tailed Student's t test. Other statistical analyses were performed by the non-parametric methods of M. H. Peto and co-workers. Mean values of the logarithm (base 10) of white blood cell (WBC) and platelet counts of patients with breakpoints falling in subregions were compared by the non-parametric methods of M. H. Peto and co-workers.
or absence of symptoms, anemia, and splenomegaly at diagnosis were compared for patients with breakpoints in different bcr subregions by a Mantel-Haenszel \( \chi^2 \) test for categorical variables. Chronic phase duration and survival for patients with breakpoints in different bcr subregions were compared by computerized Kaplan-Meier life table analysis, followed by use of Mantel-Cox analyses to test significance. The statistical significance of lymphoid versus myeloid blast crisis frequencies in patients with breakpoints in different bcr subregions was determined by calculating risk ratios and then carrying out a Fischer exact test for our data and that of Shtalrid et al.\(^9\) individually, and \( \chi^2 \) analysis for the combined data.

**RESULTS**

**Localization of bcr breakpoints:** Clinical parameters at diagnosis in relation to breakpoint localization. Of our 81 patients with adequate information for clinical-molecular correlations, breakpoints were demonstrated in bcr subregions 0-1 in 14, subregion 2 in 29, subregion 3 in 27, and subregion 4 in 2, using probe I for detection. Nine patients failed to demonstrate any breakpoint using this probe, indicating deletion of all, or virtually all, sequences within bcr subregion 3. However, studies with probe II revealed breakpoints in subregions 0-1 in two and, in subregion 2, in seven of these nine. Thus, a total of 16 patients were identified with breakpoints in subregions 0-1 and 36 with breakpoints in subregion 2.

No significant differences were noted among patients with breakpoints falling in bcr subregions, 0-1, 2, and 3, and in patients with deletions in subregion 3 with respect to sex, age, WBC and platelet levels, and presence or absence of symptoms, anemia, and splenomegaly at diagnosis (Table 1). The two patients with breakpoints in subregion 4 were not considered in these comparisons. Data relating to prognosis (eg, percentage marrow blasts, percentage peripheral basophils, cytogenetic abnormalities other than the Ph chromosome, marrow fibrosis) were not available on an adequate number of patients at diagnosis to allow comparison of prognosis among patients with breakpoints falling in different bcr subregions.

**Relation of bcr breakpoint subregion to chronic phase duration.** Figure 2 and Table 2 summarize the relation of bcr breakpoint localization to duration of chronic phase in our patients. The range for the durations of chronic phase for patients with breakpoints in bcr subregions 0-1, 2, and 3 was very similar, with shortest chronic phase durations in each subregion from 7 to 11 months and the longest from 120 to 127 + months. Similarly, the mean durations of chronic phase in patients with bcr breakpoints in these subregions were not significantly different: means of 61.1, 58.2, and 58.6 months for patients with breakpoints in subregions 0-1, 2, and 3, respectively. In contrast, there appeared to be a trend to longer median chronic phase durations as breakpoints proceeded from subregions 0-1 (median 44 months) through subregion 2 (median 47 months) to subregion 3 (median 58 months). The reason for the differences in median time to crisis in the three subregions studied is apparent from Fig 2; that is, there appeared to be some clustering of patients with chronic phase durations below the median in subregions 0-1 and 2, while the distribution of chronic phase durations in patients in subregion 3 represented a more nearly normal

<table>
<thead>
<tr>
<th>Bcr Breakpoint Subregion</th>
<th>Deletions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
</tr>
</tbody>
</table>

**Table 1. Clinical and Hematologic Parameters at Diagnosis in CML Patients With Breakpoints in Different bcr Subregions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0-1</th>
<th>2</th>
<th>3</th>
<th>Del 3†</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>16</td>
<td>36</td>
<td>27</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age (median yr)</td>
<td>38.6</td>
<td>36.5</td>
<td>38.0</td>
<td>33.0</td>
<td>.67*</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>7/9</td>
<td>10/26</td>
<td>9/18</td>
<td>0/9</td>
<td>.09†</td>
</tr>
<tr>
<td>Symptoms (N/Y/U)*</td>
<td>3/3/10</td>
<td>1/8/27</td>
<td>8/4/15</td>
<td>1/17</td>
<td>.78†</td>
</tr>
<tr>
<td>Splenomegaly (N/Y/U)*</td>
<td>2/7/7</td>
<td>2/18/16</td>
<td>2/13/12</td>
<td>2/2/5</td>
<td>.53†</td>
</tr>
<tr>
<td>Anemia (N/Y/U)*</td>
<td>2/6/8</td>
<td>2/9/25</td>
<td>3/5/19</td>
<td>2/2/5</td>
<td>.37†</td>
</tr>
<tr>
<td>WBC Count (× 10^9/µL), median (n)</td>
<td>142.5 (12)</td>
<td>204.0 (21)</td>
<td>170.0 (19)</td>
<td>124.5 (4)</td>
<td>.19*</td>
</tr>
<tr>
<td>Platelets (× 10^9/µL), median (n)</td>
<td>259.0 (7)</td>
<td>420.0 (13)</td>
<td>412.5 (12)</td>
<td>220.0 (3)</td>
<td>.90*</td>
</tr>
</tbody>
</table>

Abbreviations: N/Y/U, no/yes/unknown; Del 3, patients with deletions in subregion 3.

*Kruskal-Wallis test.

†Mantel-Haenszel \( \chi^2 \) test.
Table 2. Relation of Chronic Phase Duration to Breakpoint Subregion

<table>
<thead>
<tr>
<th>Bcr Subregion</th>
<th>0-1*</th>
<th>2*</th>
<th>3*</th>
<th>Del 3 (No Del)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>16</td>
<td>36</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>Mean (mos)</td>
<td>61.1</td>
<td>58.2</td>
<td>58.6</td>
<td>66.8</td>
</tr>
<tr>
<td>Median (mos)</td>
<td>44</td>
<td>44</td>
<td>58</td>
<td>66</td>
</tr>
</tbody>
</table>

Abbreviations: Del 3, patients with deletions in subregion 3; no del, patients with breakpoints falling in indicated bcr subregions who lack deletions in subregion 3.

*Total number of patients with breakpoints in indicated bcr subregions.

distribution about the median. Comparison of mean and median chronic phase durations and Kaplan-Meier life table plots for patients with breakpoints in subregions 0-1, 2, and 3 did not reveal statistically significant differences. Although chronic phase duration (and survival in subsequent section) are shown for the two patients with breakpoints in subregion 3, statistical comparisons with patients in other groups could not be considered meaningful and are not presented. However, on comparing chronic phase durations of patients with breakpoints falling in subregions 0-1 and 2 versus subregions 3 and 4, the 5' and 3' subregions referred to in other studies, there were no significant differences (Fig 3).

Patients with deletions in subregion 3 whose breakpoint mapped in subregions 0-1 and 2 demonstrated mean and median chronic phase durations (66.8 and 66 months) greater than those noted for patients without deletions mapping in subregions 0-1, 2, and 3. However, as in prior studies, these differences were not statistically significant. If one subtracts the chronic phase duration data for deletion patients with breaks in subregions 0-1 and 2 from the total number of patients with breakpoints in these subregions, the trend toward shorter chronic phase duration in patients with breakpoints in subregions 0-1 also becomes more noticeable, but is still not statistically significant.

Relation of bcr breakpoint subregion to survival. Figure 4 and Table 3 relate bcr breakpoint localization to total survival in our patients. Of the 81 patients on whom chronic phase duration data are presented, 80 are included in the survival data, the sole exception being a single patient who underwent allogenic bone marrow transplantation in blast crisis and remains free of disease 92 months post-transplantation. As for the data on chronic phase duration, there were no statistically significant differences in mean and median survivals and life table plots for patients with breakpoints in bcr subregions 0-1, 2, and 3, and patients with deletions in subregion 3. This was expected since total survival is closely tied to chronic phase duration.

Relation of blast crisis lineage to bcr breakpoint subregion. Table 4 presents our data, that of Shtalrid et al, and the combined data on myeloid versus lymphoid blast crisis in patients with breakpoints in bcr subregions 0-1, 2, and 3. The two sets of data are comparable, showing a greater than expected frequency of myeloid crisis in patients with breakpoints in subregion 2 and a greater than expected frequency of lymphoid crisis in patients with breakpoints in

Fig 4. Relation of survival to subregion of bcr breakpoint in 80 patients included in study. Patients are same as in Fig 2 except for exclusion of a single patient in subregion 2 with allogeneic transplantation in blast crisis, resulting in long-term survival. □ indicates patients dead of CML; ○, patients alive in chronic phase as of April, 1989 and one patient dying of unrelated cause during chronic phase; and △, patients with deletions of subregion 3 sequences with breakpoints in subregions 0-1 and 2. Mean and median values are indicated by μ and m, respectively.

Fig 3. Kaplan-Meier plot comparing chronic phase duration in patients with breakpoints in bcr subregions 0-1 and 2 versus subregions 3 and 4.
subregion 3. However, the results of neither study reached statistical significance, at least in part because of inadequate numbers of patients.

In contrast, when the data from the two studies were combined, the ratio of myeloid to lymphoid (M:L) crisis for patients with breaks in subregion 2 (9:1) was significantly increased with respect to the ratio for the remaining patients with breaks falling in subregions 0 to 1 and 3 \((P = .032)\), while the M:L ratio for patients with breaks in subregion 3 (1.6:1) was significantly decreased with respect to patients with breaks in subregions 0 to 1 and 2 \((P = .012)\). Moreover, when the M:L ratios for patients with breaks falling in subregions 2 and 3 were compared directly, the difference was also highly significant \((P = .012)\). These results suggest that (1) patients with breakpoints in bcr subregion 2 have an approximately 2.5-fold greater risk than the total CML population presenting in chronic phase for developing myeloid blast crisis, (2) patients with breakpoints in subregion 3 have an approximately twofold greater risk of developing lymphoid crisis than the total population of patients with chronic phase CML, and (3) patients with breakpoints in subregion 2 have about 5.6-fold greater risk of developing myeloid crisis than patients with breaks in subregion 3.

**DISCUSSION**

We have found that duration of chronic phase and total survival in CML patients presenting in early chronic phase are not significantly influenced by bcr breakpoint. This conclusion is in agreement with the results of Shtivelman et al\(^1\) on 99 patients, also based on breakpoint location, and those of Shtalrid et al\(^2\) and Dreazen et al\(^3\) on 23 patients based on analysis of both breakpoint location and alternatively spliced bcr-abl mRNAs. It does not agree with the study of Mills et al\(^4\) on 22 patients, which indicated a fourfold longer chronic phase duration for patients with breakpoints in bcr subregions 0, 1, and 2 versus subregions 3 and 4, or with that of Eisenberg et al\(^5\) on 26 patients, indicating 50- versus 30-month chronic phase durations for patients with 5' and 3' breakpoints, respectively. It is difficult to understand the basis for these differing results. However, the three studies demonstrating lack of a relationship between bcr breakpoint and chronic phase duration gain strength from the large number of total patients studied, comparable conclusions being reached on the basis of both DNA and mRNA analyses, and the performance of our study in a manner approximating a prospective study. For these reasons, we believe that lack of a correlation between breakpoint location and chronic phase duration, at least for patients diagnosed in early chronic phase, is a valid conclusion.

It should be noted that the data of Mills et al\(^6\) and Eisenberg et al\(^7\) that indicate longer chronic phase durations in patients with 5' versus 3' bcr breakpoints provide support for the aforementioned findings of Schaefer-Regó et al\(^8\) on nine patients in blast crisis and Eisenberg et al\(^9\) on 15 patients in crisis or accelerated phase, which suggest that 5' breakpoints are significantly more common in patients in chronic phase, while 3' breakpoints are more common in patients in accelerated or crisis phases. Moreover, in the present study, we may have missed a subset of patients with early conversion to blast crisis and 3' breakpoints, since patients with evidence of aggressive disease were excluded from the therapeutic autotransfusion program that was our source of CML cells. However, Shtalrid et al\(^10\) were unable to detect a significant correlation between crisis and 3' breakpoints among 38 patients studied in crisis. Because of this conflicting evidence, it seems imprudent to draw conclusions at this time as to whether a subset of patients exist with aggressive disease, early conversion to crisis, and predominantly 3' bcr breakpoints. Further study on this point will be required.

Our conclusion that bcr breakpoint subregion does not influence the duration of chronic phase or survival in CML patients presenting in chronic phase does not preclude significantly longer or shorter chronic phase durations among patients with breakpoints in specific bcr introns, as opposed to the presently constructed subregions that, except for subregion 3, include sequences on both sides of specific exons. The fact that clusters of our patients with breaks in subregions 0 to 1 and 2 demonstrated short chronic phase durations suggests that this possibility may exist. Furthermore, it does not preclude the possibility that more refined mRNA studies that account for possibilities of alternative splicing not previously investigated could reveal subsets of patients with significantly better or worse prognosis than the entire population of CML patients.

Two important corollaries follow from our conclusion that chronic phase duration and survival are uninfluenced by bcr breakpoint site. First, breakpoint location cannot be used as a prognostic indicator as once hoped. The tremendous range of

**Table 3. Relation of Survival to bcr Breakpoint Subregion**

<table>
<thead>
<tr>
<th>bcr Subregion</th>
<th>0-1</th>
<th>2</th>
<th>3</th>
<th>Deletion 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>16</td>
<td>35</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>Mean (mos)</td>
<td>64</td>
<td>65.8</td>
<td>63.5</td>
<td>71.4</td>
</tr>
<tr>
<td>Median (mos)</td>
<td>47</td>
<td>54</td>
<td>68</td>
<td>70</td>
</tr>
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</table>

**Table 4. Myeloid v Lymphoid Blast Crisis in Relation to bcr Breakpoint Subregion**

<table>
<thead>
<tr>
<th>bcr Subregion</th>
<th>No. Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>M</td>
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<tr>
<td>Present study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, 1, 2, 3</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>0-1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Shtalrid et al(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, 1, 2, 3</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>0-1</td>
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<td>6</td>
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<td>8</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>10</td>
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<tr>
<td>Combined data</td>
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<td>2</td>
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<tr>
<td>3</td>
<td>11</td>
<td>18</td>
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chronic phase duration alone among patients with breakpoints in each bcrl subregion makes drawing prognostic conclusions very risky. Secondly, if length of chronic phase among patients presenting in chronic phase is unrelated to the bcr-c-ab1 oncogenic hit, it almost certainly is related to a subsequent oncogenic event that results in the development of blast crisis. Still, the bcr-c-ab1 event may be required for the development of crisis, although it does not always appear to be required for its maintenance.

Our work also suggests that bcr breakpoint location plays a role in the determination of blast crisis lineage. This suggestion comes from combining data from our study and the earlier study of Shalirid et al., neither of which alone included sufficient patients to draw statistically significant conclusions. In brief, the combined data indicated an M:L blast crisis ratio of 3.4:1 in a total of 75 patients, in line with the historical ratio; an approximately 2.5-fold increase in this ratio among 30 patients with breakpoints falling within subregion 2; and a decrease of about 50% among 29 patients with breakpoints falling within subregion 3. In other words, the frequency of myeloid crisis in patients with bcr breakpoints in subregion 2 was approximately 2.5-fold the expected, while the frequency of lymphoid crisis in patients with breakpoints in subregion 3 was approximately double that expected. Although the finding that bcr breakpoint location influences blast crisis lineage was unexpected, it appears to be valid in view of comparable supporting data in two independent studies and a significant difference (P = .012) in the M:L ratio of patients with breakpoints in subregions 2 and 3. However, since this is the initial report of an association between bcr breakpoint location and crisis lineage, it will be important to see if additional studies support our present findings. It should also be noted that since myeloid and lymphoid crises are seen in patients with breakpoints in all three of the major bcr subregions, other factors besides bcr breakpoint location must also play an important role.

The entire question of determination of blast crisis cell lineage in CML is complex, making it extremely difficult to consider in any detail possible mechanisms by which breakpoints in bcr subregions 2 and 3 could differentially affect crisis lineage. At the target cell level it is unclear whether myeloid and lymphoid crisis arise when a bcr-c-ab1-positive pluripotent stem cell sustains a second oncogenic hit, one effect of which is to confer either an acute myelo- or lymphoblastic leukemic phenotype, or whether myeloid and lymphoid crises arise when myeloid- and lymphoid-committed stem cells derived from the bcr-c-ab1-positive pluripotent stem cell sustain a second oncogenic hit. Although both mechanisms are feasible and different mechanisms may be operative in different patients, lack of information on the cellular target for crisis makes it especially difficult to focus on a role for bcr-c-ab1. Second, both the normal bcr gene and the CML bcr-c-ab1 gene are expressed in myeloid and lymphoid lines, and the normal gene is also expressed in a wide variety of other cell types. Moreover, until recently, there was no precedent for a mechanism of tissue-specific expression for a gene with widespread expression, and it was thus difficult to envision a mechanism that would permit either selective or enhanced bcr-c-ab1 expression in a single cell line during the development of crisis. However, it has now been shown that the housekeeping gene, porphobilinogen deaminase, which is expressed in a wide variety of cell types, contains an erythroid-specific promoter that binds two specific transcription factors. Thus, a model does exist for transcriptional control of genes with both general and tissue-specific expression. Third, there is a dearth of information available on bcr gene control elements, making it difficult to conceptualize possible mechanisms of tissue-specific expression. This difficulty would seem to be increased by the fact that the bcr lies in the center of the bcr gene and a single intron spans bcr subregions 2 and 3. However, it is now clear that in certain genes, control elements, including tissue-specific enhancers, may lie within introns.

Thus, for bcr-c-ab1 one can envision loss or damage of a lymphoid or myeloid enhancer or sequences involved in differential splicing, resulting in preferential expression of bcr-c-ab1 mRNA or an alternatively spliced bcr-c-ab1 mRNA in either lymphoid or myeloid cells. Further confusing the issue of tissue-specific expression of the bcr-c-ab1 gene is the recent finding that a bcr-c-ab1 cDNA obtained from the erythromyeloblastoid blast crisis cell line K562 induces only lymphoid hyperplasia in long-term cultures of both lymphoid- and myeloid-enriched precursor cells. In summary, it is difficult to envision how bcr-c-ab1 could be differentially expressed in myeloid versus lymphoid cells in the development of crisis, unless bcr-c-ab1 itself undergoes further structural alteration, an interacting transcriptional factor undergoes alteration, or a cellular target of its protein undergoes alteration. Evidence to support any of these possibilities awaits further study.

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Relationship of bcr breakpoint to chronic phase duration, survival, and blast crisis lineage in chronic myelogenous leukemia patients presenting in early chronic phase [see comments]

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