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# Fine Mapping of Chromosome 22 Breakpoints within the Breakpoint Cluster Region (*bcr*) Implies a Role for bcr Exon 3 in Determining Disease Duration in Chronic Myeloid Leukemia

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## Summary

**The chromosomal translocation that fuses the *phl* gene with the c-*abl* proto-oncogene appears to be a pivotal step in the pathogenesis of some leukemias. In chronic myeloid leukemia (CML) the breakage within the *phl* gene is largely confined to a 5.8-kb segment referred to as the breakpoint cluster region (*bcr*). To determine whether the presence of specific *bcr* exons on the Philadelphia chromosome has any clinical significance, we have analyzed the *bcr* breakpoints in 134 patients with CML. As many as five probes were used in this analysis, including a synthetic oligonucleotide probe homologous to the *bcr* exon 3 (*phl* exon 14) region. The distribution of breakpoints indicates that, in fact, breakage is largely confined to a 3.1-kb segment lying between *bcr* exon 2 and exon 4 (*phl* exons 13–15). In 61 CML patients analyzed within 1 year of diagnosis, the distribution of breakpoints appeared to be random within the 3.1-kb region. However, a significant excess of 5′ breakpoints was observed in the total population studied, consistent with previous data showing that patients with 3′ breakpoints have shorter disease durations. Analysis using the *bcr* exon 3 sequence probe indicated it was probably the presence or absence of *bcr* exon 3 on the Philadelphia chromosome that accounts for some of the variability in disease duration seen in CML. The data suggest that the *phl/abl* protein product may influence the timing of the onset of blast crisis and imply a continuing role for this protein during the evolution of the disease.**

## Introduction

The reciprocal translocation t(9;22)(q34;q11) is found in 90%–95% of patients with chronic myeloid leukemia (CML) (Nowell and Hungerford 1960; Rowley 1973), approximately 20% of patients with acute lymphocytic leukemia (ALL) (Propp and Lizzi 1970; Priest et al. 1980; Sandberg et al. 1980), and in occasional cases of acute myelogenous leukemia (AML) (Whang-Peng et al. 1970). These are referred to as the Philadelphia (Ph) chromosome–positive leukemias. Studies at the molecular level have shown that breakage on chromosome 9 occurs at the 5′ end of the c-*abl* cellular oncogene (Heisterkamp et al. 1983; Bernards et al. 1987) and that the breakage on chromosome 22 arises in a gene referred to as "*bcr* gene," "*phl*," or "BCR" (Heisterkamp et al. 1985; Stam et al. 1987; Gale and Goldman 1988). The *phl* gene is of unknown function (Collins et al. 1987) and is thought to be composed of 130 kb with 21 exons (Heisterkamp et al. 1988). As a result of the translocation, a *phl/abl* fusion protein is produced that has a tyrosine kinase activity that differs from that of the normal *abl* protein product (Konopka et al. 1984).

In CML, the breakage within the *phl* gene is confined to a segment referred to as the breakpoint cluster region (*bcr*), originally defined as a 5.8-kb segment that included four small exons (usually separately numbered 1–4 and equivalent to *phl* exons 12–15) (Groffen et al.

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1984). Approximately half of the Ph chromosome–positive ALL specimens and some Ph chromosome–positive AML specimens show chromosome 22 breakpoints in the same region, the remainder probably having breakpoints confined to the first *phl* intron (Fainstein et al. 1987; Heisterkamp et al. 1988; Hermans et al. 1988). Breakage on chromosome 9 appears to be consistently 5′ of the c-*abl* second exon (Bernards et al. 1987). The *phl/abl* protein products in CML patients are clearly different from those produced by some ALL patients, reflecting the various chromosome 22 breakpoints (Clark et al. 1987). However, the precise role that these proteins (referred to as p210 and p190, respectively) may play to account for the differing phenotype of the various hematologic disorders remains undetermined (Kurzrock et al. 1988).

Within the group of patients with CML, small differences in the p210 protein can also exist, determined by the precise breakpoint within *bcr*. Since the *bcr* includes four exons, the inclusion or exclusion of these various coding sequences in the *bcr/abl* chimeric DNA could potentially give p210 proteins with differing properties and could result in different clinical courses of the disease. Evidence that breakpoint location may be related to disease heterogeneity comes from studies that have compared disease duration for patients with 5′ versus 3′ breakpoints and associated 3′ breakpoints with poorer prognosis (Eisenberg et al. 1988; Mills et al. 1988). Other investigators have, however, failed to observe any difference in disease duration for patients with breaks in particular parts of the *bcr* (Dreazen et al. 1988; Shtalrid et al. 1988).

We describe detailed studies mapping chromosome 22 breakpoints in 134 patients with CML. These patients were studied with as many as five *bcr* probes to provide fine mapping of breakpoint locations. We show that in CML the breakage on chromosome 22 is largely confined to a region approximately 3.1 kb in size that surrounds *bcr* exon 3 (*phl* exon 14). The initial distribution of breakpoints in patients with newly diagnosed CML appears to be random within this region. Our preliminary observation that patients with 3′ *bcr* breakpoints have shorter disease duration (Eisenberg et al. 1988) is extended to show that it is probably the presence or absence of *bcr* exon 3 (*phl* exon 14) on the Ph chromosome that accounts for at least some of the variability in disease duration seen in CML.

## Material and Methods

Blood and/or bone marrow specimens were obtained from 134 patients with CML. Nuclei were isolated by adding 4 vol blood lysis buffer (0.32 M sucrose, 10 mM Tris HCl pH 7.6, 5 mM MgCl, 1% Triton X 100) and were digested with Proteinase K (100 μg/ml) with 1% SDS in a DNA lysis buffer (10 mM Tris HCl pH 7.4, 10 mM NaCl, 10 mM EDTA). DNA samples were purified by extracting them twice with phenol, once with phenol/chloroform/isoamyl alcohol (25:24:1), and once with chloroform/isoamyl alcohol (24:1), followed by dialyzing them overnight in a 1,000-fold excess of TE buffer (10 mM Tris HCl pH 7.4, 1 mM EDTA). DNA was digested according to instructions provided by the restriction-enzyme suppliers by using a 20-fold excess of *Bam*HI, *Bgl*II, or *Hin*dIII and approximately 1 μg DNA checked for complete digestion by electrophoresis on 0.8% agarose for 1–2 h at 6 V/cm. Five micrograms of each digested DNA sample were electrophoresed for approximately 20 h at 1.2 V/cm. As references, negative control DNAs and molecular-weight markers were run on the same gel. The electrophoresis buffer was TAN (40 mM Tris HCl pH 7.9, 4 mM sodium acetate, 1 mM EDTA). Southern blotting involved denaturation of the gel DNA in 0.5 N NaOH, 0.5 M NaCl; neutralization in 0.5 N Tris HCl pH 7.4 with 2.5 NaCl; and transfer with 6 × SSC (0.9 M NaCl plus 0.09 M sodium citrate) onto 0.45-μm MSI nylon membranes (Micron Separations, Inc.). Nylon filters were rinsed in 2 × SSC, dried, baked at 80°C, and exposed to UV light. Prehybridization (3–20 h) and hybridization (3–20 h) were at 65°C or 58°C with 10% PEG, 5 × SSPE (0.75 M NaCl, 0.5 N $NaH_2PO_4$, 5 mM EDTA), 1 mg heparin/ml, 2% SDS, and 250 μg herring testes DNA/ml (Sigma).

The following DNA probes were used: a 1.2-kb *Hin*dIII/*Bgl*II 3′ *bcr* probe (Groffen et al. 1984) (Oncogene Science, Manhasset, NY), a 2.5-kb *Bgl*II/*Bam*HI 5′ *bcr* probe (Eisenberg et al. 1988), a 1.9-kb *Bgl*II/*Hin*dIII *bcr* probe (Benn et al. 1987), a 4.5-kb *phl/bcr* probe that spanned the entire *bcr* sequence but excluded the internal *Hin*dIII/*Hin*dIII segment (Blennerhassett et al. 1988) (*phl/bcr*-3; Oncogene Science), and a specifically designed oligonucleotide probe consisting of 75 bases of *bcr* exon 3 (phl exon 14) sequence together with an additional 24 bases of the intron immediately 5′ of this exon (Heisterkamp et al. 1985) and synthesized for us by Research Genetics, Inc. (Huntsville). Probes were labeled by the random primer technique (Feinberg and Vogelstein 1984) to a specific activity $>5 \times 10^8$ cpm/μg for the recombinant probes and $>5 \times 10^6$ for the oligonucleotide probe. Following hybridization, nylon filters were washed in four

changes of 2 × SSC and four changes of 0.1 × SSC at 65°C or 58°C. Autoradiography was carried out using X-ray film (X-omat; Kodak) with DuPont Lightening-Plus intensifying screens. Interpretation of autoradiographs was as described elsewhere (Eisenberg et al. 1988). Wherever possible, all analyses were carried out in duplicate.

Criteria for establishing accelerated or blast-crisis CML were as described by Karanas and Silver (1968). Except for the analysis of the distribution of breaks in newly diagnosed CML, duration of disease was determined from diagnosis to the most recent laboratory referral. The newly diagnosed group of patients consisted of those patients analyzed for breakpoint location within 1 year of disease diagnosis. This latter group of patients were considered separately to determine the breakpoint distribution in a population where the effects of differential patient survival would be relatively small. The statistical significance of differences in disease durations were calculated using the Kaplan-Meier product-limit survival estimate and were compared through the Mantel-Cox statistic (Kaplan and Meier 1958). Differences in the relative frequencies of breakpoints within different zones of the *bcr* were compared by contingency tables and were analyzed by the $\chi^2$ statistic.

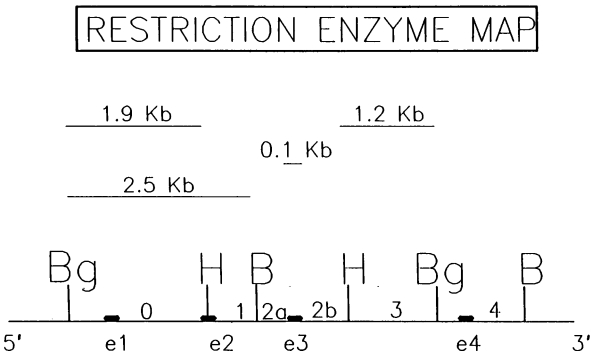


**Figure 1** Restriction-enzyme map of the breakpoint cluster region (bcr). Bg = *Blg*II; H = *Hin*dIII; B = *Bam*HI. e1–e4 denote the *bcr* exons equivalent to *phl* exons 12–15 (based on the characterization of the gene by Heisterkamp et al. [1988]). 0, 1, 2a, 2b, 3, and 4 denote subregions of the *bcr*. Solid lines above the map denote *bcr* probes used.

## Results

The location of chromosome 22 breakpoints was studied in a total of 134 patients with *bcr* rearrangement–positive CML. This included 113 patients with Ph chromosome–positive CML, 8 patients with Ph chromosome–negative CML, and 13 patients of unknown karyotype. Results for some of these patients have been presented elsewhere (Benn et al. 1987, 1988*a;* Eisenberg et al. 1988; Weinstein et al. 1988). Our initial analysis of chromosome 22 breakpoints primarily utilized a 1.2-kb *Hin*dIII/*Bgl*II probe from the 3′ end of *bcr* and a 2.5-kb *Bgl*II/*Bam*HI probe from the 5′ end of *bcr*. These probes have previously been shown to be efficient for the detection of rearrangement with the *bcr* and to allow assignment of breaks to four zones within the *bcr* (0 + 1 combined, 2, 3, and 4 of fig. 1) (Eisenberg et al. 1988).

To resolve between zones 0 and 1, nylon filters carrying *Hin*dIII- or *Bam*HI-digested DNA were rehybridized to the 1.9-kb *Bgl*II/*Hin*dIII probe (fig. 1). Rearrangement within region 0 should result in DNA fragments of sizes that differ from those seen for the germ-line alleles with both *Hin*dIII- and *Bam*HI-digested DNA. Rearrangement within region 1 should result in detection of DNA fragments that differ from the germ-line allele for *Bam*HI- but not *Hin*dIII-digested DNA. This analysis was carried out in 27 cases with breakpoints at the 5′ end of the *bcr*. In each case the results indicated that breakage was in region 1 and not in region 0. In one additional case both *Bam*HI- and *Hin*dIII-digested DNA failed to show the presence of rearrangement with the 1.9-kb probe. In that case the original assignment of the breakpoint had been made solely on the basis of the results obtained with the 1.2-kb *Hin*dIII/*Bgl*II probe, and it is possible that comigration of DNA fragments corresponding to rearranged and germ-line alleles may have been present in one (or more) of the DNA digests.

To distinguish between breaks arising 5′ or 3′ of *bcr* exon 3 (*phl* exon 14) in zone 2 (fig. 1), a sequence consisting of 75 bases of exon 3 (plus an additional 24 bases immediately 5′ of this exon) was synthesized and used as a DNA probe. The analysis was carried out on cases that had breakpoints of uncertain location (relative to exon 3) by using nylon filters with *Bgl*II-, *Bam*HI-, and *Hin*dIII-digested DNA. The resulting autoradiographs were compared with those obtained with either the 2.5-kb *Bgl*II/*Bam*HI probe or the 1.2-kb *Hin*dIII/*Bgl*II probe to determine whether breakage was 5′ or 3′ of exon 3 (fig. 2). Five breakpoints were thought to lie 5′ of exon 3, and 26 breakpoints were assigned 3′ of exon 3. In two cases no rearrangement was detectable with the exon 3 probe, probably indicating that there were deletions of the exon 3 sequences. In one case two rearrangement bands were detected, indica-

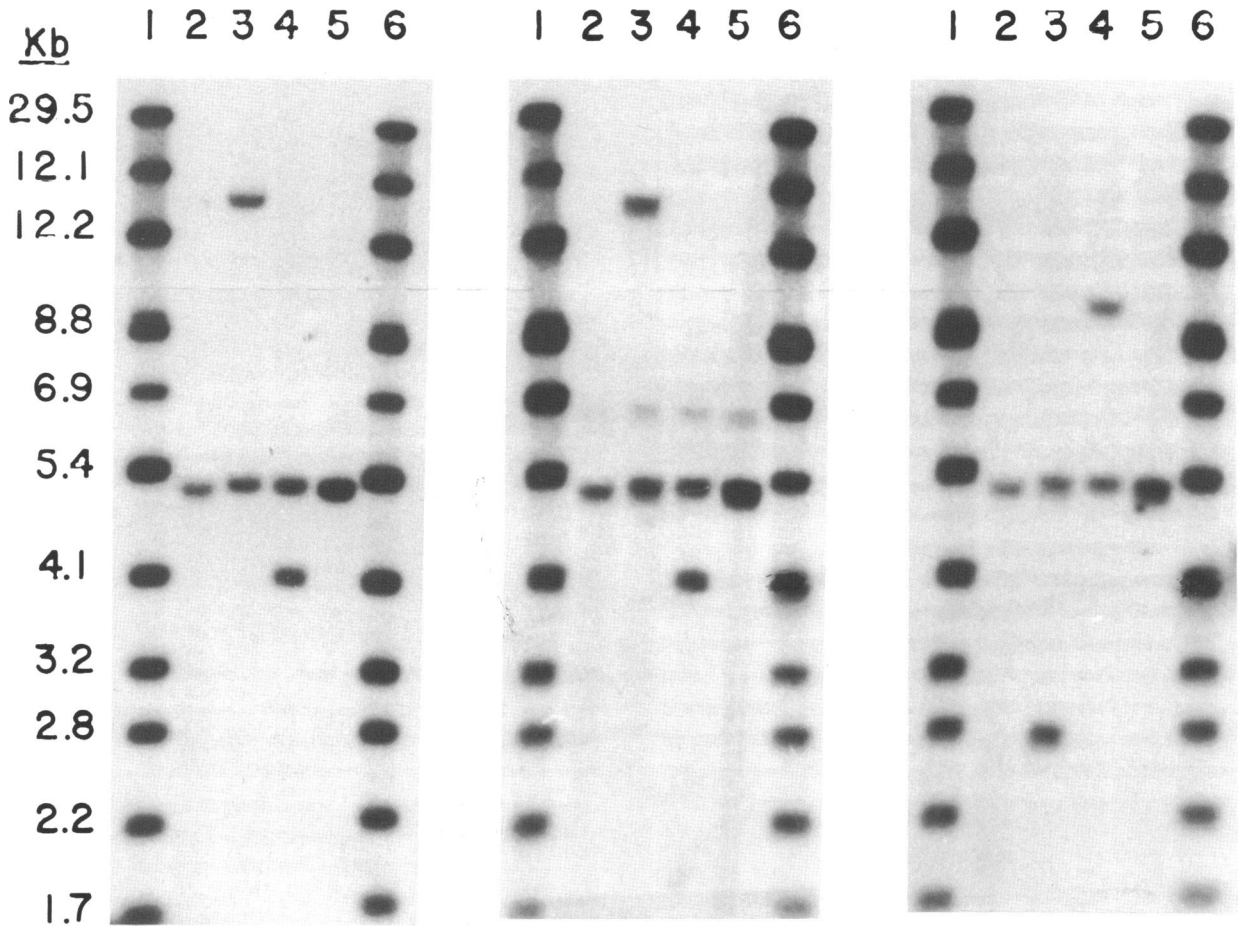


**Figure 2** Sublocation of breakpoints in zone 2. *A,* Autoradiograph using the 2.5-kb 5′ *bcr* probe. *B,* Autoradiograph using the 0.1-kb exon 3 *bcr* probe. *C,* Autoradiograph using the 1.2-kb 3′ bcr probe. Lanes 1 and lanes 6, Molecular-weight markers; Lanes 2 and lanes 5, Control DNA with no *bcr* rearrangement. Lanes 3 and lanes 4, DNA from two patients with *bcr* rearrangements. Since the exon 3 probe detects fragments identical in size to that seen with the 5′ *bcr* probe, the breakpoint in these two cases must lie 3′ of the exon 3. DNA was digested with the restriction enzyme *Bgl*II. All probes show a common 5.0-kb DNA fragment corresponding to the unrearranged allele, and, in addition, the *bcr* exon 3 probe detects an additional weakly hybridizing 6.8-kb DNA fragment because of the reduced stringency of hybridization.

tive of a breakpoint within the probe homologous sequence (fig. 3).

With *Bgl*II-digested DNA and either a 5′ or a 3′ probe, rearrangement could be detected in 133 of the 134 cases. The one exceptional case showed rearrangement with *Bam*HI-digested DNA analyzed with the 1.2-kb 3′ *bcr* probe. This case was reanalyzed with the *phl/bcr*-3 probe (Oncogene Sciences) which includes additional zone 4 sequences. With this latter probe, rearrangement was detectable as two additional autoradiograph bands on *Bam*HI digestion (i.e., sequences both 5′ and 3′ of the breakpoint were detectable). With *Bgl*II digestion, only one additional autoradiograph band corresponding to the rearranged allele could be detected. This result suggested that the breakpoint may have been very close to the 3′ *Bgl*II site in the *bcr*, although the alternative possibility of comigration of two similarly sized DNA fragments could not be entirely excluded.

On the basis of the above results, the overall distribution of breakpoints indicated that breakage was largely, perhaps exclusively, confined to regions 1, 2, 3, and the first approximately 0.1 kb of region 4. This entire region, comprising approximately 3.1 kb, showed an overall higher frequency of involvement at the 5′ end. To examine statistically the distribution of the breakpoints within the 3.1-kb segment, the region was divided

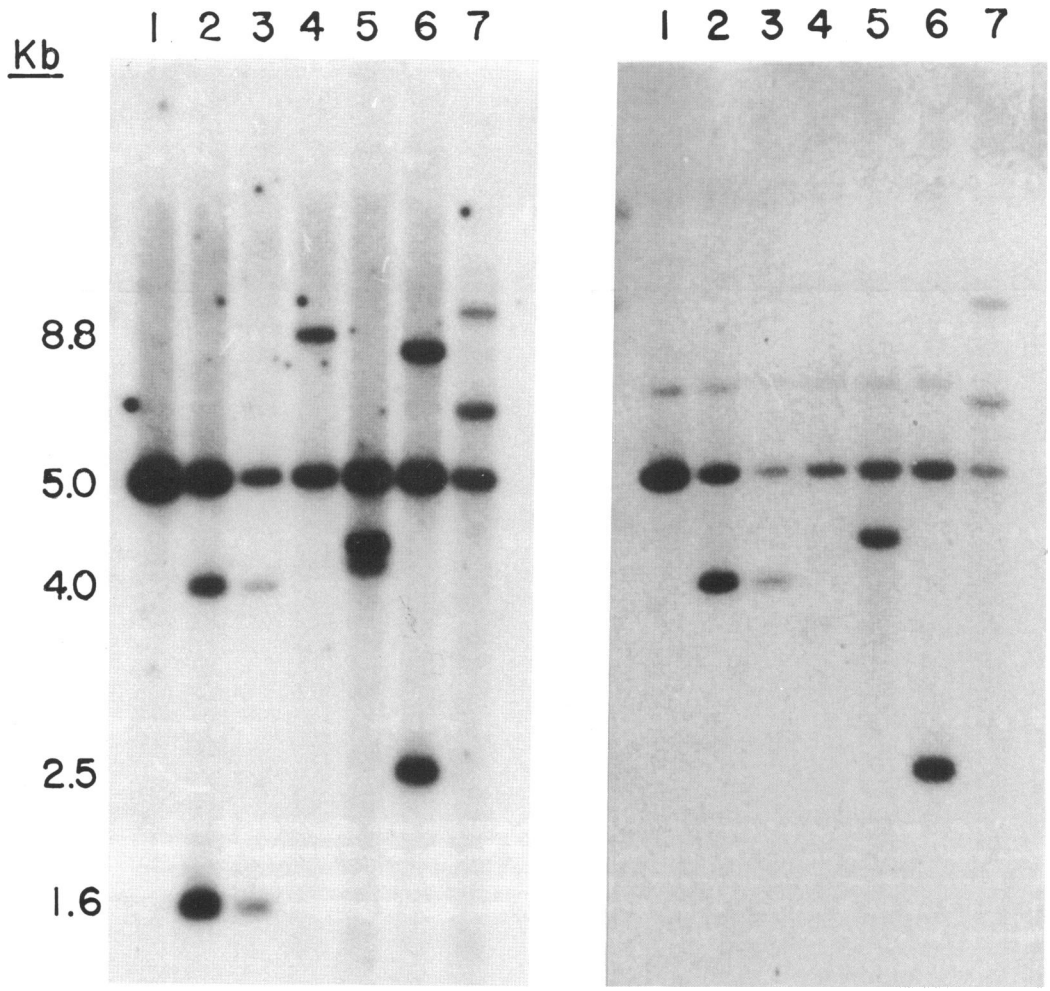


**Figure 3** Identification of a breakpoint within *bcr* exon 3. *A*, DNA hybridized with the 1.9-kb 5′ *bcr* probe and the 1.2-kb 3′ *bcr* probe combined. *B*, the same samples hybridized with the *bcr* exon 3 probe. DNA was digested with the restriction enzyme *Bgl*II. Lane 1, Control DNA. Lanes 2–7, Samples from patients with CML. In lane 4 only one DNA fragment corresponding to rearrangement is detectable, possibly because of deletion of *bcr* sequences. In lane 7 the *bcr* exon 3 probe detects two rearrangement products, indicating that the breakpoint lies within *bcr* exon 3.

into three parts—a 0.6-kb segment from the *Hin*dIII restriction-enzyme site to the *Bam*HI site (zone 1; fig. 1), a 1.2-kb segment from the *Bam*HI site to the *Hin*dIII site (zones 2a and 2b), and a 1.3-kb segment from the *Hin*dIII site to *bcr* exon 4 (zone 3 and the first 0.1 kb of zone 4). The number of breakpoints observed in each of the three segments was compared with that expected assuming that the number of breakpoints present was proportional to the size of the segment and assuming that all breaks assigned to regions 0 and 1 were in fact located in region 1 (fig. 4). For 61 patients analyzed within 1 year of disease diagnosis (newly diagnosed cases), the distribution of breakpoints within the 3.1-kb segment showed no statistically significant difference from the expected distribution ($\chi^2 = 0.7$, $P > .05$) on the basis of the size of each zone. In contrast, for the total patient population in this study, a statistically significant excess number of breakpoints were found in zone 1, compared with the number of breakpoints in the remaining segments of the *bcr* ($\chi^2 = 12.4$, $P < .001$).

The finding of an excess of 5′ breakpoints in the total patient population (in contrast to the patients analyzed within 1 year of diagnosis) is consistent with the concept of preferential loss, over time, of patients with 3′ breakpoints (Eisenberg et al. 1988; Mills et al. 1988).

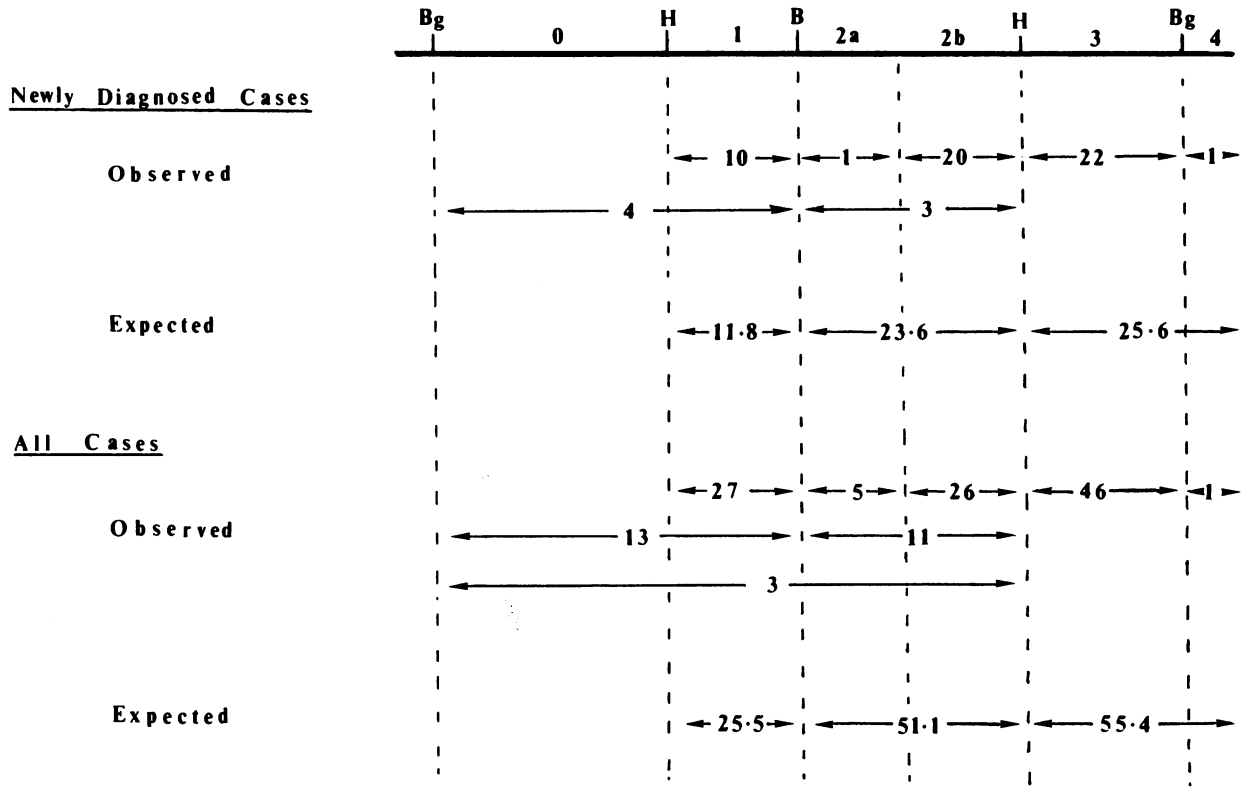


**Figure 4** The observed and expected distribution of breakpoints in relation to the physical map of the *bcr* for newly diagnosed CML patients (analyzed within 1 year of diagnosis) and in all patients studied. Two patients with uncertain or multiple breakpoint locations were excluded. The expected distributions were calculated based on the assumptions that all breaks occur within an approximately 3.1-kb region surrounding *bcr* exon 3 and that the number of breaks expected in each zone would be proportional to its size (see text). Map abbreviations and zone designations are as explained in the legend to fig. 1.

To examine the question of disease durations in more detail, time from initial diagnosis to laboratory analysis was compared for patients with breakpoints in zones 0, 1, and 2a combined versus those with breakpoints in zones 2b and 3 combined (i.e., 5′; vs. 3′; of *bcr* exon 3). For 41 patients with 5′ breakpoints the average disease duration was 42.3 mo (range 0–192 mo), while for 60 patients with 3′ breakpoints the average disease duration was 24.0 mo (range 0–115 mo). These two groups of patients differed significantly from each other when compared by life-table analysis using the Kaplan-Meier product-limit technique and compared by the Mantel-Cox statistic ($\chi^2$ = 7.1, $P$ = .009).

When patients with breaks in zone 2b were similarly compared with those in zone 3, no significant difference in disease duration was noted ($\chi^2$ = 0.59, $P >$ .05). For 23 patients with breaks in zone 2b the average disease duration was 27.8 mo (range 0–115 mo), while for 36 patients with zone 3 breakpoints the average disease duration was 22.1 mo (range 0–91 mo).

Information on progression to accelerated phase or blast crisis was available for only 26 patients. For 10 patients with zones 0, 1, or 2a breakpoints the average chronic-phase disease duration was 56.1 mo (range 2–105 mo). A shorter chronic-phase disease duration was seen for 16 patients with breakpoints in zones 2b or 3, the average disease duration being 36.6 mo (range 6–86 mo). The difference in chronic-phase disease duration for these two groups was not statistically significant ($P$ = .14), possibly because of the lack of statistical power related to the small sample size.

## Discussion

The *bcr* has been defined as a 5.8-kb region of chromosome 22 preferentially involved in exchanges with

chromosome 9 in patients with CML (Groffen et al. 1984). We have attempted to define more accurately the region of breakage by using as many as five *bcr* probes and several restriction enzymes. The purpose of these studies has been to determine whether breakpoint location is related to the clinical course of the disease.

At the 5′ end of the *bcr* no breakpoint could be conclusively assigned to the first 1.9-kb segment (zone 0; fig. 1). In a series of 28 patients with breakpoints known to be located in the most 5′ 2.5-kb segment of the *bcr,* 27 had breakpoints located 3′ of the *Hin*dIII site that separates zone 0 from zone 1. Since *bcr* exon 1 (*phl* exon 12) and part of *bcr* exon 2 (*phl* exon 13) are located in zone 0, these data strongly suggest that these two exons are usually present on the derivative (Ph) chromosome 22 following translocation. The frequent inclusion of *bcr* exons 1 and 2 on the Ph chromosome is consistent with results on RNA from cells studied either by an RNA protection assay (Shtivelman et al. 1986, 1987) or by polymerase chain reaction (PCR) amplification of cDNA (Dobrovic et al. 1988; Hermans et al. 1988; Kawasaki et al. 1988; Lee et al. 1988*b*).

Only one breakpoint was assigned to the most distal 0.8-kb segment at the 3′ end of *bcr* (zone 4; fig. 1). In this case the rearrangement products observed by digesting DNA with various restriction enzymes and applying different probes suggested that the breakpoint was located close to the *Bgl*II site at the 5′ end of this zone. The paucity of breakpoints assigned to this zone and the sublocalization of the one breakpoint within this zone suggest that *bcr* exon 4 (*phl* exon 15) is usually not present on the Ph chromosome. The limited data currently available on the size of cDNA products detected following PCR analyses are also consistent with suggestion that *bcr* exon 4 (*phl* exon 15) is rarely present on the Ph chromosome (Dobrovic et al. 1988; Hermans et al. 1988; Kawasaki et al; Lee et al. 1988*b*).

Thus, all the breakpoints observed in the present study were thought to be within a 3.1-kb sequence that is bounded by *bcr* exon 2 (*phl* exon 13) and *bcr* exon 4 (*phl* exon 15). A number of studies have assigned exceptional CML chromosome 22 breakpoints either more 5′ or more 3′ of the 3.1-kb sequence described above (Bartram et al. 1987; Selleri et al. 1987; Saglio et al. 1988; Shtalrid et al. 1988). Comparison of the data from these various studies is complicated by the use of different DNA probes which have varying specificity and sensitivity for detecting rearrangement. Polymorphisms (Benn et al. 1988*b*; Kato et al. 1988) and comigration of DNA fragments (Eisenberg et al. 1988) can complicate the assignment of breakpoints. Cases with breakpoints outside the 3.1-kb *bcr* sequence, if confirmed, may prove to be of considerable value in further understanding the consequences of subtle differences in the *phl/abl* fusion product.

On the basis of the conclusion that breakpoints are confined to the 3.1-kb *bcr* bounded by exon 2 and 4, we observed that the breakpoints appeared to be randomly distributed within the 3.1-kb segment for 61 CML patients analyzed within 1 year of diagnosis. These patients were analyzed separately to determine whether there appeared to be any specificity in breakpoints for a population in which the effect of differential patient survival would be minimal. Breakpoint site specificity has been noted for other tumor-associated chromosome rearrangements with sequence homology and recombinase-specific sequences present in the vicinity of the breakpoints. (Baer et al. 1985; Tsujimoto et al. 1985; Denny et al. 1986; Mengle-Gaw et al. 1988; Russo et al. 1989). There is little known homology between *bcr* and c-*abl* sequences (Heisterkamp et al. 1985), and the translocation would appear to be a random event with ultimate detection presumably based solely on the occurrence of an exchange that has a phenotypic effect. Complex translocations and Ph chromosome–negative CML that involve chromosome loci in addition to *bcr* and c-*abl* may differ, with respect to breakpoint distribution, from cases with simple (9;22) translocations (Dubé et al., in press). Some sequence-specific preferential breakage may also occur for the group of acute leukemias which have exchanges involving the *phl* intron 1 and the c-*abl* gene. Recent data have shown that for this group of acute leukemias, breakage appears to be confined to a small segment of the first intron rather than being distributed throughout the intron (Chen et al. 1988; Heisterkamp et al. 1988; Denny et al. 1989).

While the subgroup of patients with relatively newly diagnosed CML showed no breakpoint specificity within the 3.1-kb region, the distribution of breakpoints in the total CML patient population showed a statistically significant excess of 5′ breakpoints. This presumably reflects the previously noted shorter disease duration for patients with 3′ breakpoints (Eisenberg et al. 1988; Mills et al. 1988). Previous studies separated patients into two groups; (1) those with breakpoints 5′ of the *Hin*dIII site separating zones 2 and 3 and (2) those with breakpoints 3′ of this *Hin*dIII site. The most likely explanation for the observed difference would be the presence or absence of *bcr* exon 3 (*phl* exon 14) on the Ph chromosome, although the possibility that

an intron sequence that might affect the level of expression (as has been observed for c-Ha-*ras* [Cohen and Levinson 1988]) could not be excluded. To resolve this question we carried out additional analyses with a probe specific for *bcr* exon 3 (*phl* exon 14), which allows the subdivision of patients on the basis of the presence or absence of the exon 3 sequence on the Ph chromosome. The analysis showed that patients with Ph chromosomes containing *bcr* exon 3 had a statistically significantly shorter disease duration than did those patients without *bcr* exon 3. In contrast, no statistically significant difference exists between those patients with breakpoints immediately 3′ of *bcr* exon 3 (zone 2b) and those patients with breakpoints farther downstream (zone 3). These results strongly implicate *bcr* exon 3 in determining disease duration.

Some exceptional patients with 3′ breakpoints clearly have long disease durations (Dreazen et al. 1988; Nowell et al. 1988), while a 5′ breakpoint does not necessarily indicate a long survival. Patients with 3′ breakpoints can produce mRNA identical to that produced by patients with 5′ breakpoints, as a result of alternate splicing (Shtivelman et al. 1986, 1987). Thus, some patients with 3′ breakpoints could behave like those with 5′ breakpoints, and it will be of interest to study the types of mRNA produced in patients with long disease durations. Very preliminary data have been presented that indicate that the type of transcripts produced could be more variable after disease progression (Lee et al. 1988*a*). Mapping breakpoints, mRNA analysis, or a combination of these approaches may prove to be of considerable value in identifying patients with a high likelihood for early blast transformation.

Two studies failed to show any difference in the distribution of breakpoints in patients with various disease durations (Dreazen et al. 1988; Shtalrid et al. 1988), and the extent of the difference in disease duration associated with a 5′ versus a 3′ breakpoint is somewhat different in each of the two previously published cases (Eisenberg et al. 1988; Mills et al. 1988). The inclusion of newly diagnosed cases which minimize the extent of any difference in the two groups, the presence of atypical cases of the type described above, and the inclusion of patients on widely different therapies could account for this variability. To accurately assess the significance of a 5′ versus a 3′ breakpoint, disease duration to blast crisis needs to be determined in a prospective study of patients with newly diagnosed CML.

Since most patients who develop CML will eventually die in the acute phase of the disease, it is reasonable to conclude that the observed shorter disease duration for patients with 3′ breakpoints reflects earlier progression to blast crisis. For patients who had progressed to acute disease we observed a shorter duration of chronic-phase disease for patients with 3′ breakpoints (average 36.6 mo) than was observed in patients with 5′ breakpoints (average 56.1 mo), although the difference was not statistically significant. This may simply reflect the lack of statistical power related to the small number of patients followed to accelerated phase or blast crisis. The suggestion that the *phl/abl* protein product may influence the timing for the onset of acute disease implies a continuing role for this protein during the evolution of the disease.

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