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REVIEW ARTICLE

Does the Breakpoint Within the Major Breakpoint Cluster Region (M-bcr) Influence the Duration of the Chronic Phase in Chronic Myeloid Leukemia? An Analytical Comparison of Current Literature

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CHRONIC MYELOGENOUS leukemia (CML) is a clonal myeloproliferative disorder of a pluripotent stem cell precursor of myeloid, erythroid, megakaryocytic, and B-lymphoid cells. The disease is characterized by the presence, in at least 95% of the cases, of a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11), which results in a $22q^-$ or Philadelphia (Ph^1) chromosome and a $9q^+$ chromosome.[1] The signs and symptoms of the initial phase of the disease, usually called the chronic phase, often develop insidiously and include tiredness, splenomegaly, anaemia, and leukocytosis. The white blood cell (WBC) count can reach 200×10^9/L, usually consisting of myeloid cells at all stages of differentiation, although the mature cell types predominate.[2] After a median duration of the chronic phase of 42 months, a change in the clinical course of the disease occurs.[2] This period is usually called the acute phase or blast crisis, due to the presence of maturation-arrested myeloblasts and promyelocytes, often exceeding 30%, in the peripheral blood. Blast crises can occur as one of two general types, myeloid and lymphoid, with the latter occurring in 20% to 30% of patients. Patients in blast crisis are usually less responsive to chemotherapy and often develop additional cytogenetic abnormalities (eg, a second Ph^1, trisomy 8 or isochromosome 17q), indicative of a clonal expansion of a genetically evolving population of Ph^1-positive cells.[2] The median survival from diagnosis of blast crisis is approximately 4 months.

MOLECULAR CONSEQUENCES OF THE PHILADELPHIA CHROMOSOME

At the molecular level, the (9;22)(q34;q11) translocation resulting in the Philadelphia (Ph^1) chromosome involves the movement of the majority of the ABL proto-oncogene from chromosome 9 to become contiguous with the 5′ portion of the BCR gene on chromosome 22. The breakpoint on chromosome 9 can occur over a region of 200 kb or more, and is usually located in the intron 5′ of exon 2.[3] Occasionally, ABL exons 1a and 1b are also translocated to chromosome 22, but their transcripts are spliced out of the mature mRNA. In Ph^1-positive CML, and about half of the Ph^1-positive acute lymphoblastic leukemia (ALL) and acute nonlymphoblastic leukemia (ANLL) (thought to be acute phase CMLs that were not diagnosed in chronic phase), the breakpoint in the BCR gene occurs within a relatively short, 5.8-kb sequence called the major breakpoint cluster region (M-bcr).[4] This region encompasses four exons (exons 12 through 15, but often referred to as exons b1 through b4) (Fig 1). In about half of the cases of Ph^1-negative CML, the translocation is masked at a karyotypic level, but the molecular rearrangement has still occurred.[5]

Transcription of this chimeric BCR-ABL gene results in a hybrid mRNA that is translated into a hybrid p210 protein. Both the mRNA and protein are unique to M-bcr–positive cells. Most breakpoints occur between exons b2 and b3 or between b3 and b4 and the chimeric gene may therefore either include or exclude BCR exon b3. Thus, two distinct species of mRNA may be encoded by this form of BCR-ABL gene. A gene from which BCR exon b3 has been excluded encodes an mRNA in which BCR exon b2 is spliced to ABL exon 2 (a b2-a2 spliced mRNA). A gene that includes BCR exon b3 may give rise to either an mRNA in which exon b3 is spliced to ABL exon 2 (a b3-a2 spliced mRNA) or, by an alternative splicing mechanism, a b2-a2 spliced mRNA.[5] The two types of hybrid mRNA and protein differ in size by 75 bases and 25 amino acids, respectively (Fig 2).

A CORRELATION BETWEEN MOLECULAR AND CLINICAL ASPECTS OF CML?

Over the past 2 to 3 years several studies[6-17] have attempted to determine whether the site of the breakpoint

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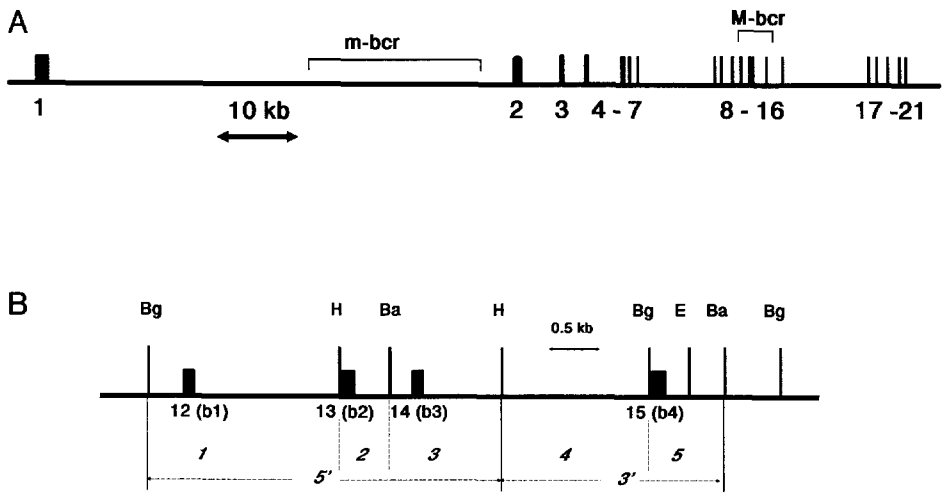


Fig 1. (A) A schematic diagram of the BCR gene on chromosome 22. M-bcr is the major breakpoint cluster region in which breakpoints are located in CML and 50% of Ph¹-positive acute leukemias, m-bcr is the minor breakpoint cluster region in which breakpoints occur in 50% of Ph¹-positive acute leukemias. (B) Diagram of the M-bcr, indicating the zones (delineated by dashed lines) and regions (5′ = zones 1 through 3, 3′ = zones 4 and 5) in which a breakpoint could occur. Solid black boxes indicate exons. H, *Hind* III; Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI.

within the M-bcr can be correlated with any clinical parameter observed during the course of the disease, in particular, the onset of blast crisis. These studies have produced conflicting results: some reports have indicated a correlation between the site of the breakpoint and the average duration of the chronic phase, while others have not. It is the intention of this review to discuss those reports that have studied the relationship between breakpoint and chronic phase duration in an attempt to determine the cause of these differing conclusions.

A summary of the technical and clinical details obtained from 12 reports are shown in Tables 1 and 2 and Fig 3. Wherever possible details presented in the reports have been converted to a common form or have been recalculated to allow the maximum degree of comparability.

The first indication that a breakpoint within M-bcr may have a correlation with a clinical parameter was by Schaefer-Rego et al,[6] who reported a strong correlation between a breakpoint in the 5′ region of M-bcr and patients in chronic phase. All 17 chronic phase patients had breaks clustered in the 5′ region, while eight of nine patients studied in blast crisis had breakpoints at the 3′ end of M-bcr. From this study it was concluded that only patients with a 3′ breakpoint within the M-bcr progressed to blast crisis. An excess of 3′ breakpoints in patients in blast crisis was also seen by Eisenberg et al,[9] but was less striking than that reported by Schaefer-Rego et al.[6] If patients with a 3′ breakpoint progress more rapidly to blast crisis, then it would be expected that, at any one time, more 3′ breakpoints would be seen in the blast crisis population. However, later investigators[7,11,12] could not find any correlation between the site of the breakpoint and the phase of the disease.

Since then the relationship between the site of the breakpoint within M-bcr and the duration of the chronic phase has been examined by several groups. Two of the studies,[8,9] on 22 and 68 patients, respectively, indicated that those patients with a break in the 5′ portion of the M-bcr had an average duration of chronic phase that was 1.7- to 4-fold longer than those patients with a breakpoint in the 3′ region. These findings have been confirmed by updated studies[13,14] on augmented groups of patients (80 and 129, respectively). In these cases, the average duration of chronic phase for patients with a 5′ breakpoint was 42.3 to 55 months while that for 3′ breakpoint patients was 24 to 25 months. Although Przepiorka[10] did report that 5′ breakpoint patients had a longer chronic phase than 3′ patients, the difference was not statistically significant, possibly due to the sample size. A later report by Ogawa et al[12] presented data from which it was possible to calculate that those patients with a 3′ breakpoint had an average chronic phase of 36 months, while of the two patients with a 5′ breakpoint, one had relapsed after 48 months and the other was only 3 months into chronic phase.

In contrast, other studies[7,10,15-17] on samples of between 25 and 99 patients indicated no statistical difference in chronic phase duration between patients with a 3′ or 5′ breakpoint.

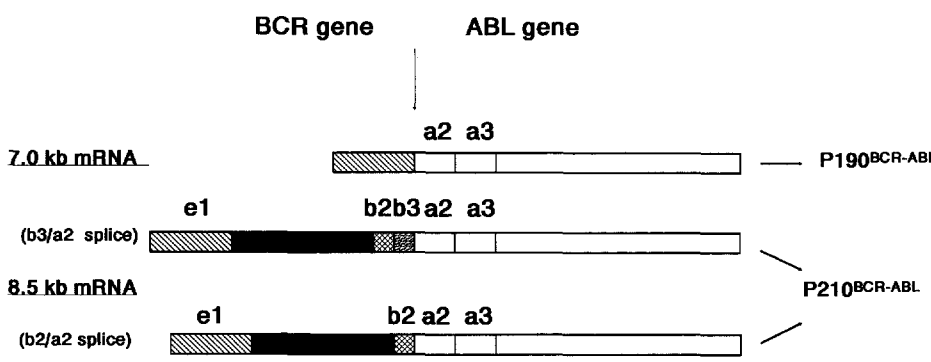


Fig 2. A schematic diagram of the types of splicing that could occur in the hybrid BCR-ABL mRNA. In some acute leukemias with breakpoints in m-bcr the resultant RNA consists of BCR exon 1 (e1) spliced to ABL exon 2 (a2). Depending on the site of the breakpoint in M-bcr the hybrid RNA could consist of BCR exon 13 (b2) spliced to ABL exon 2 (known as a b2-a2 splice) or BCR exon 14 (b3) spliced to ABL exon 2 (a b3-a2 splice).

Table 1. Comparison of BCR Breakpoint Data, Duration of Chronic Phase, Age, Sex, and Therapy in 12 Studies

Reference	Correlation With C.P. Duration	Distribution of Breakpoints: Zone (%) 1+2	3	4	5	Region (%) 5′	3′	Total No. Studied	Median Duration of C.P. (mo): Zone 1+2	3	4	5	Region 5′	3′	All	Therapy	Median Age (y): 5′	3′	All	Sex Ratio (male/female): 5′	3′	All
6	Yes	31	27	42	0	58	42	26	0/8‡	1/7§	30	—	1/15§	30	36	—	—	—	—	—	—	—
7	No	24	33	36	6	57	42	99	43	68	51	69	59	59	59	IFN	39-45‡‡	40-41‡‡	39-45‡‡	1.6	2.5	1.9
8*	Yes	23	32	41	4	55	45	22	35	55	8.5	0/1	47	12	33	HU/BU	55	44	49	1.0	1.0	1.0
9†	Yes	—	—	—	—	71	29	68	—	—	—	—	50.6	30.2	42	—	—	—	—	—	—	—
10	No	—	—	—	—	56	44	25	—	—	—	—	33	22	—	—	—	—	—	—	—	—
11	Not done	(-55-)		34	11	55	45	44	—	—	—	—	—	—	—	—	34	37	36	3.8	1.5	2.1
12	Not done	—	13	80	7	13	87	15	—	2/2‖	36	1/1¶	36	36	36	—	28	45	40	1.0	1.6	1.5
13*	Yes	25	34	37	4	59	41	80	55	51	24	1/3**	55	25	43	HU/BU	44	45	45	0.8	1.3	1.0
14†	Yes	31	33	36	1	64	36	129	—	—	—	—	42.3	24	—	—	42 h	44.5§§	43 h	1.5	1.8	1.6
15	No	35	23	35	8	58	42	66	—	—	—	—	37	44	44	BU	42	39	41	1.7	1.5	1.7
16	No	20	44	33	2	64	36	81	44	47	58	2/2††	45.5	52.5	—	BU/HU	36.5-38.6	38	36.5-38.6	2.1	2.0	2.0
17	No	18	36	47	0	54	47	45	—	—	—	—	34	39	—	—	43-52	40	40-52	1.0	2.0	1.4
Average		26	31	39	4	55	45	58	44	55	35	69	44	34	42	—	41	41	41	1.6	1.7	1.6

Abbreviations: BU, busulphan; HU, hydroxyurea; IFN, α-interferon; C.P., chronic phase.
*The patients in ref 8 are a subset of those in ref 13.
†The patients in ref 9 are a subset of those in ref 14.
‡Ratio indicates none of eight patients had progressed to blast crisis.
§Progressed to blast crisis (b.c.) after 60 months.
‖ Progressed to b.c. after 3 and 48 months.
¶Progressed to b.c. after 24 months.
**Progressed to b.c. after 27 months.
††Progressed to b.c. after 16 and 54 months.
‡‡Median age only given for individual zones.
§§Mean age.

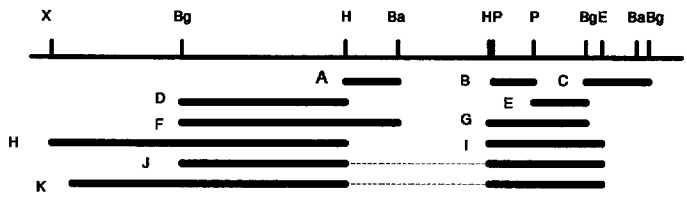
One study[7] reported that the average chronic phase durations of patients with a 3′ or a 5′ breakpoint were the same (59 months). Three reports[15-17] have shown an inversion of the correlation between chronic phase duration and site of the breakpoint by reporting that 3′ breakpoint patients had a longer chronic phase than 5′ breakpoint patients.

If the average duration of the chronic phase of all the patients in each study is examined, it can be observed that these range from 33 to 59 months. The overall median durations of the chronic phase in those series of patients for whom a correlation between the site of the breakpoint within the M-bcr and chronic phase duration was reported

Table 2. Patient Selection, Sample Type, and Postanalysis Selection

Reference	Study Area	Patient Selection (no. studied)	Sample Type	Postanalysis Selection
6	New York	Not discussed (26)	PB	Not discussed
7	Houston	Patients referred to MD Anderson (108)	PB and BM	Patients whose presenting features where unknown excluded from statistical analysis
8*	Great Britian	Samples sent for BCR analysis (22)	PB	One patient excluded due to lack of clinical detail
9†	New York	Specimens referred to BCR analysis (68)	PB and BM	Not discussed
10	Pittsburgh	Not discussed (25)	Not discussed	Not discussed
11	Malaysia	Hospital CGL research study (44)	PB	Not discussed
12	Osaka	Not discussed (15)	PB	Not discussed
13*	West Scotland and U.K.	Samples sent for BCR analysis (80)	PB and BM	Not discussed
14†	New York	Specimens referred for BCR analysis (129)	PB and BM	Not discussed
15	London	Referred to specialist center (91)	Cryopreserved buffy coats	91 samples readily available, 69 patients with clinical details, 2 treated with BMT, 67 fully analyzed
16	Boston	Patients for whom leukapheresed material available (118)	Leukapheresed blood	81/118 patients with adequate clinical and molecular information
17	Taiwan	Patients enrolled in a cytogenetic study (77)	Cryopreserved cells	45 Ph^+ patients analyzed for whom cells were available

Abbreviations: PB, peripheral blood; BM, bone marrow; BMT, bone marrow transplantation.
*The patients in ref 8 are a subset of those in ref 13.
†The patients in ref 9 are a subset of those in ref 14.



STUDY	Bam HI	Bgl II	Hind III	Eco RI	Xba I	Probes used
6	x	x	x	x	x	A, C, D, I
7	x	x	x	x	x	G, K
8	x	x	x			K, B
9	x	x	x		x	F, D, G
10			x	combined x		G
11	x	x	x			G
12	x	x	x	x		A, B, H
13	x	x	x	x		B, D
14	x	x	x			D, F, G
15	x	x	x			D, E, G
16	x	x	x			D, G
17	x	x	x			G, J

Fig 3. The different probes and the types of restriction enzymes used in these studies to determine the site of the breakpoint within the M-bcr. Probes are indicated by solid lines (labeled A through K), dotted lines indicate repetitive sequences deleted from probes J and K. X, *Xba*I; Bg, *Bgl*II; H, *Hin*dIII; Ba, *Bam*HI; P, *Pst*I; E, *Eco*RI.

were 36,[6] 43,[13] and 24 to 42.3[14] months. Because the average duration of CML chronic phase is between 36 and 42 months,[2] those studies that reported a correlation between the site of the breakpoint and the average length of chronic phase appear to have comprised representative groups of patients. On the other hand, the chronic phase durations reported by those investigators who did not observe this correlation were 59,[7] 44,[15] 45.5 to 52.5,[16] and 34 to 39[17] months. Thus, three of these four studies comprised patients who had, on average, a longer chronic phase than usual, suggesting that some degree of selection of patients with a less aggressive disease had occurred. In another study[10] comprising patients with an overall shorter chronic phase (22 to 33 months) than the usual average,[2] it was found that those with a 5′ breakpoint had longer chronic phases than those with a 3′ breakpoint, although the difference was not statistically significant.

DISTRIBUTION OF BREAKPOINTS WITHIN M-BCR

Ten of the 12 reports[6-8,11-17] presented the distribution of breakpoints by zone of the M-bcr. The percentage of breakpoints occurring in zone 1 + 2, 3, 4, and 5 in each study is shown in Table 1. Overall, an average of 26% of the breaks occurred in zones 1 + 2, 31% in zone 3, 39% in zone 4, and 4% in zone 5. Most of the studies were in relatively close agreement with this distribution. However, Ogawa et al[12] reported that 80% of the patients in their study had a breakpoint within zone 4. The overall distribution of breakpoints between the two regions was 55% for 5′ breakpoint patients and 45% for the 3′ breakpoint patients. With the exception of the Japanese study,[12] it is remarkable how uniform the reported zonal and regional distribution of breakpoint sites were, despite the wide variation in geographic location.

PROBES AND RESTRICTION ENZYME DIGESTS

Figure 3 shows the locations of the probes and the restriction enzymes used in these studies. Eleven different probes have been used, with 10 of the 12 studies using at least two probes, usually a 3′ and a 5′ probe. None of the probes had homology to the *Bam*HI-*Hin*dIII fragment due to the presence of a repeated sequence in that fragment. Two studies[10,11] used only a 3′ probe that covered a portion of the M-bcr often deleted during the translocation[18]; consequently, some patients may have been omitted from these studies because they were wrongly classified as M-bcr negative. The majority of the studies used *Bam*HI, *Bgl*II, and *Hin*dIII to localize the site of the breakpoint. While some studies made use of additional enzymes, this would not improve the 5′/3′ resolution of the site of the breakpoint and cannot account for any of the differences between these reports.

CRITERIA FOR DIAGNOSIS OF CHRONIC AND ACUTE PHASES

Most of the studies did not refer to the manner in which the diagnosis of CML was made, other than by stating that all the patients were selected as being Ph[1]-positive CML in chronic phase of the disease. Diagnoses in many of the studies had been made at a hospital or institution different from that in which the bcr analysis was performed. The diagnosis of CML can often be difficult due to the insidious nature of the symptoms, and in some cases can only be established following the cytogenetic identification of a Ph[1] chromosome. While the onset of blast crisis can be diagnosed relatively easily, the precise time of the onset of the chronic phase is more difficult to determine. Therefore, the exact length of the chronic phase may depend on variations in the aggressiveness of the disease between individual patients and, thus, on the nature of symptoms when the patients first present at the clinic or hospital. It is possible that better screening procedures, perhaps related to medicals for insurance policies or to socioeconomic factors, have enabled some groups of patients to be diagnosed earlier than others.

PATIENT SELECTION

Patients were entered into the various studies either by the diagnosing hospital or by referral to a specialist center.

In those reports from which this information can be gleaned, it would seem that some studies[8,9,11-14,17] analyzed samples obtained from local hospitals at, or soon after, diagnosis. In three other cases,[7,15,16] patients were referred to a central hospital from which samples (blood, bone marrow, cryopreserved, or leukapheresed material) were obtained and subsequently analyzed. In these cases, the time between diagnosis and sample collection was not stated in one report,[7] but was mostly within 2 to 3 months in the second[15] and within 4 weeks in 49 of 67 of the patients, and between 5 and 113 months (median 16 months) for the remainder, in the third.[16] Furthermore, these reports[7,15,16] did not find a correlation between the site of the breakpoint and duration of the chronic phase.

While re-analyzing our data[13] for possible causes of the disagreement with other studies, we tested the effect of sequentially eliminating data from those patients who had entered an acute phase within 3, 6, 9, or 12 months from diagnosis. Surprisingly, if those patients who entered crisis within 9 months (n = 9) were eliminated, the average duration of chronic phase of those patients with a 3′ breakpoint increased from 25 months to 40 months; similarly, the chronic phase duration for patients with a 5′ breakpoint increased from 55 months to 64 months. The overall duration of chronic phase was now 53 months (compared with 43 months), ie, longer than the usually accepted median of 36 to 42 months. Moreover, the ratio between chronic phase durations of 5′ and 3′ breakpoint patients decreased from 2.2 to 1.6. The statistical significance of the difference, using a log-rank test, also decreased from $P < .001$ to $P < .05$. This result suggests that those studies that failed to observe such a correlation may not have included rapidly progressing patients, either because in a retrospective analysis their clinical data may be more difficult to obtain, or because such patients may not have been referred to a secondary center for treatment.

Because none of the reports based on data from patients referred to specialist centers show any correlation between the clinical course of the disease and the site of the breakpoint, it is pertinent to ask whether some unstated reason for the referral of the patients has a significant influence, eg, were they patients whose clinical symptoms suggested they might be candidates for bone marrow transplantation? In this regard, it is perhaps pertinent to note that one report[16] described only data from specially preselected patients, viz, a group with abnormally high WBC counts for which treatment by leukapheresis had been deemed appropriate.

SAMPLE MATERIAL

The types of samples analyzed in the various studies can be divided into two broad categories: fresh samples or stored samples.

In the first category, some samples were obtained from patients either from within their own hospital[7,11] or from a specific area or group of hospitals,[13] or they were sent to a diagnostic company for BCR breakpoint analysis.[14] Most of these samples were obtained at presentation. Furthermore, the majority of these samples appeared to have been supplied for molecular analysis by clinicians outwith the group making the analysis, and it was they who decided whether samples from an individual patient should be studied for BCR rearrangement. Thus, it is possible that at least some of the patients selected were those with a more aggressive disease, or with unusual features that made diagnosis more difficult.

The second category depended on the availability of leukapheresed, cryopreserved, or other material from CML patients that had been accumulated over a period of time.[15-17] The criteria for the initial storage of these samples were not discussed. It may have been the hospital policy to store material at diagnosis or at a later stage of the disease, or indeed it may simply have been that enough material was available for storage. In some instances, peripheral blood leukocytes from chronic phase were cryopreserved for retransfusion at a later stage in the disease, and some samples may have been unavailable for analysis as they would have been used for treatment.

Therefore, samples from CML patients were accumulated into the studies either by random sampling, usually after referral from a third party on the basis of unspecified criteria, or by the availability of material stored for unspecified reasons.

POSTANALYSIS SELECTION

In the majority of studies a further selection of the data was made, usually based on the availability of clinical details. This was particularly noticeable in those studies in which the analysis had been performed on stored material. For example, in one study[16] 118 leukapheresed samples were initially available; however, adequate clinical details were available on only 81 (21 excluded due to inadequate information, 7 had bone marrow transplantation [BMT], 3 died of causes other than blast crisis, 6 were M-bcr rearrangement-negative). Other cases of postanalysis selection were also reported: 22 patients analyzed, 21 with clinical details[8]; cryopreserved material from 91 patients, clinical data available from referring clinicians in 67 patients[15]; 70 patients with Ph^1-positive CML, cells available for BCR analysis in 52, 47 analyzed, chronic phase duration data available for 37 patients.[17] Other studies did not indicate whether any postanalysis selection of data was made.

The extent of postanalysis selection does not in itself explain the differences between those groups that reported a correlation with breakpoint and chronic phase duration and those that did not. The three studies that reported that (in contrast to other earlier studies) 3′ breakpoint patients had a longer (albeit not statistically significant) chronic phase than 5′ breakpoint patients all applied the highest degree of postanalysis selection.[15-17] However, it is possible that those patients for whom clinical details were not available and were thus excluded from further analysis were also those patients that had relapsed quickly and from whom follow-up information would be harder to obtain.

THERAPY

For three series of patients, four of the reports[8,13,15,16] indicated that the initial therapy used was hydroxyurea

and/or busulphan. Only one report[7] indicated that all patients were treated with α-interferon (α-IFN), although α-IFN was used as a secondary therapy on a minority (10% and 7.5%) of patients in two additional studies.[13,15] Again, no relationship could be easily discerned between the type of therapy used and the presence or absence of a correlation between the breakpoint site and duration of chronic phase.

AGE AT PRESENTATION

The median age of the patients was stated in six reports,[7,11,12,15-17] and the median or mean later obtained from the investigators of three others.[8,13,14] It has been reported[2] that the median age of onset of CML is 50 years. In Table 1 it can be seen that the median age of diagnosis of patients in the various studies ranged from 36.5 to 49 years; thus, all of the studies had a younger population than the quoted median. However, of those groups of patients for whom the ages at diagnosis were available, two of the three oldest median ages at diagnosis[13,14] were also those that reported a correlation between the site of the breakpoint and the average duration of the chronic phase. In contrast, all the studies with patients of younger median age did not observe such a correlation.[7,11,12,15,16] As there is evidence for a longer survival in younger patients,[2] it is possible that those studies with groups of younger patients may be biased because of the naturally greater survival rate of these patients.

We have re-analyzed the data presented by Mills et al[13] on the basis of patients' age at diagnosis. The chronic phase duration of 35 patients who were older than the median age at diagnosis was 40 months for patients with a 5′ breakpoint compared to 21 months for those with a 3′ breakpoint. However, analysis of data from 45 patients whose age at diagnosis was less than the median resulted in an average chronic phase of 55 months for 3′ breakpoint patients, but no median duration of chronic phase could be calculated for patients with a 5′ breakpoint because 78% remained in chronic phase at 72 months. Therefore, it would seem that a population of younger CML patients remains in chronic phase longer.

It would seem that the age distribution in many of these studies might indicate some degree of preselection, presumably unintentional, on the part of the researchers indicating that most, if not all, of the studies were performed on a nonrepresentative population of CML patients.

SEX

The sex ratio is shown in Table 1. The ratio varies from 2.4 males/females to 0.98. However, it has been reported that there is no sex bias in the incidence of CML.[2] This result may again suggest that some studies at least have comprised a nonrandom group of patients.

CONCLUSIONS

The direct comparison of these reports was difficult due to the different ways in which the data were presented. However, from the comparisons we have made, it would seem that several variables may be responsible for the disparity between the conclusions drawn in these reports. These variables are all related to the extent to which each of the populations of patients for whom sufficient data are reported is truly random, and as such it is very difficult to distinguish among them, or to assess their relative importance, as they are interrelated to various extents. For example, younger patients tend to survive longer, and they are more likely to be referred to a secondary center for more aggressive treatment. It is these factors that appear to separate those studies that did not observe a correlation between the site of breakpoint in the M-bcr and the average duration of the chronic phase from those that did.

Variations in the length of the chronic phase of CMLs with 5′ or 3′ breakpoints indicate that the location of the breakpoint within the M-bcr is only one factor determining the length of the chronic phase, of greater importance in some cases than in others. The site of the breakpoint is only an indicator of the type of splice site present in the hybrid BCR-ABL mRNA, and recent reports have suggested a correlation between the type of mRNA expressed and the disease phase[19] or duration of chronic phase.[20] However, this cannot explain different conclusions regarding the relationship between the location of the breakpoint and the duration of chronic phase in different groups of patients unless, as this analysis suggests, there are as yet unrecognized, systematic differences in the other factor(s) between the groups. The only clear way in which a definitive answer to the question of whether the site of the breakpoint within the M-bcr is related to any clinical feature of CML, including the duration of chronic phase, is by a truly prospective study in which all patients presenting with CML, regardless of age and sex, are analyzed for the site of the breakpoint and subsequently observed through their disease until eventual death. Even then, it may be difficult to allow for variations in the period between initial onset and first clinical presentation. Nevertheless, the question is important enough to warrant this extensive study for, if there is a correlation, it will not only be useful in a clinical context but also give some indication as to the mechanism of action of the BCR-ABL oncogene in the pathogenesis of CML.

NOTE ADDED IN PROOF

Since the acceptance of this comparative literature review, two further reports on the relationship between M-bcr breakpoints and chronic phase duration have been published. Tefferi et al (*Leukemia* 4:839, 1990) failed to observe a correlation on data obtained from 62 patients in the Minnesota and Wisconsin areas. However, Tanaka and Kamada (*Acta Haematol Japonica* 53:1559, 1990), in a study of 100 patients in the Hiroshima area, reported that patients with a breakpoint within zone 3 had a significantly shorter chronic phase. These reports emphasize the need for a truly prospective study.

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REFERENCES

1. Rowley JD: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243:290, 1973

2. Silver RT: Chronic myeloid leukemia. A perspective of the clinical and biologic issues of the chronic phase. Hematol Oncol Clin North Am 4:319, 1990

3. Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G: Structural organisation of the bcr gene and its role in the Ph^1 translocation. Nature 315:750, 1985

4. Blennerhassett GT, Furth ME, Anderson A, Burns JP, Chaganti RSK, Blick M, Talpaz M, Dev VG, Chan LC, Weidemann LM, Greaves MF, Hagemeijer A, van der Plas D, Skuse G, Wang N, Stam K: Clinical evaluation of a DNA probe assay for the Philadelphia (Ph^1) translocation in chronic myelogenous leukemia. Leukemia 2:648, 1988

5. Shtivelman E, Lifshitz B, Gale RP: Fused transcripts of *abl* and *bcr* genes in chronic myelogenous leukaemia. Nature 315:550, 1985

6. Schaefer-Rego K, Dudek H, Popenoe D, Arlin Z, Mears JG, Bank A, Leibowitz D: CML patients in blast crisis have breakpoints localized to a specific region of the BCR. Blood 70:448, 1987

7. Shtalrid M, Talpaz M, Kurzrock R, Kantarjian H, Trujillo J, Gutterman J, Yoffe G, Blick M: Analysis of breakpoints within the *bcr* and their correlation with the clinical course of Philadelphia-positive chronic myelogenous leukemia. Blood 72:485, 1988

8. Mills KI, MacKenzie ED, Birnie GD: The site of breakpoint within the bcr is a prognostic factor in Philadelphia-positive CML patients. Blood 72:1237, 1988

9. Eisenberg A, Silver R, Soper L, Arlin Z, Coleman M, Bernhardt B, Benn P: The location of breakpoints within the breakpoint cluster region (bcr) of chromosome 22 in chronic myeloid leukemia. Leukemia 2:642, 1988

10. Przepiorka D: Breakpoint zone of bcr in chronic myelogenous leukemia does not correlate with disease phase or prognosis. Cancer Genet Cytogenet 36:117, 1988

11. Dyck JA, Bosco JJ: Clinical stage of chronic granulocytic leukaemia and BCR breakpoint location in South-East Asian patients. Br J Haematol 72:64, 1989

12. Ogawa H, Sugiyama H, Soma T, Massaocka T, Kishimoto S: No correlation between locations of bcr breakpoints and clinical states in Ph^1-positive CML patients. Leukemia 3:492, 1989

13. Mills KI, Hynds SA, Burnett AK, MacKenzie ED, Birnie GD: Further evidence that the site of the breakpoint in the major breakpoint cluster region (M-bcr) may be a prognostic indicator. Leukemia 3:837, 1989

14. Grossman A, Silver RT, Arlin Z, Coleman M, Camposano E, Gascon P, Benn PA: Fine mapping of chromosome 22 breakpoints within the breakpoint cluster region (bcr) implies a role for exon 3 in determining disease duration in chronic myeloid leukemia. Am J Hum Genet 45:729, 1989

15. Jaubert J, Martiat P, Dowding C, Ifrah N, Goldman JM: The position of the M-bcr breakpoint does not predict the duration of the chronic phase or survival in chronic myeloid leukaemia. Br J Haematol 74:30, 1990

16. Morris SW, Daniel L, Ahmed CMI, Elian A, Lebowitz P: Relationship of the bcr breakpoint to chronic phase duration, survival and blast crisis lineage in chronic myelogenous leukemia patients presenting in early chronic phase. Blood 75:2035, 1990

17. Tein HF, Wang CH, Chen YC, Shen MC, Wu HS, Lee FY, Chuang SM, Liu CH: Chromosome and bcr rearrangement in chronic myelogenous leukaemia and their correlation with clinical states and prognosis of the disease. Br J Haematol 75:469, 1990

18. Popenoe D, Schaefer-Rego K, Mears JG, Bank A, Leibowitz D: Frequent and extensive deletion during the 9;22 translocation in CML. Blood 68:1123, 1986

19. Morgan GJ, Hernandez A, Chan LC, Hughes T, Martiat P, Weidemann LM: The role of alternative splicing patterns of BCR/ABL transcripts in the generation of the blast crisis of chronic myeloid leukaemia. Br J Haematol 76:33, 1990

20. Dobrovic A, Hardingham J, Kotasek D, Sage RE, Wan JH, Morley AA, Seshadri R, Januszewicz EH: Correlation of molecular breakpoints and the duration of chronic phase in chronic myeloid leukemia. Hum Genet 47:A6, 1990 (abstr)