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Philadelphia Chromosome-positive Chronic Myelogenous Leukemia with Deleted Fusion of BCR and ABL Genes

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In the great majority of patients with chronic myelogenous leukemia (CML) the reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11), resulting in the Philadelphia (Ph) chromosome produces fusion DNA sequences consisting of the 5′ part of the major breakpoint cluster region-1 (M-BCR-1) and the ABL protooncogene which encodes for the $P210^{BCR-ABL}$ phosphoprotein with tyrosine kinase activity implicated in the pathogenesis of CML. Molecular analysis was performed on 25 patients with Ph-positive CML using 2 breakpoint cluster region (*bcr*) probes within the M-BCR-1 DNA sequences, and two of them did not contain either detectable rearranged DNA homologous to the 5′ side *bcr* probe or ABL-related fusion mRNA. The chromosomal *in situ* hybridization technique revealed that these two Ph-positive CML cases did not carry DNAs homologous to the 5′ *bcr* or ABL probes on the Ph chromosome. Furthermore, one of the two Ph-positive CML cases did not show either rearranged DNA or regions homologous to the 3′ *bcr* probe on a 9q+ chromosome, while the other CML case showed a rearrangement detected by the 3′ *bcr* probe and transposition of the 3′ *bcr* homologous to the 9q+ chromosome. Thus, the possibility is raised that the BCR/ABL fusion DNA has been deleted in rare CML cases, and that the deletion possibly occurred in a stepwise manner following the formation of the Ph chromosome at any stage of the disease.

Key words: Chronic myeloid leukemia — Philadelphia translocation — Gene deletion

The Philadelphia (Ph) chromosome is a cytogenetic hallmark of chronic myelogenous leukemia (CML),[1] and this minute chromosome which results from the reciprocal translocation between chromosomes 9 and 22 is found in more than 90% of CML cases.[2] In Ph-positive CML, a part of the ABL protooncogene, normally located at 9q34, transposes onto a limited DNA sequence which was originally designated by Groffen *et al.* as a *bcr*,[3] resulting in formation of the BCR/ABL fusion gene.[4–6] It is known that BCR (breakpoint cluster region) gene extends for more than 100 kb and 3 additional BCR-related genes are also located on chromosome 22 at band q11.[7,8] The approximately 5.8 kb region within the BCR in which most Ph-positive CML show breakage, has recently been termed the major breakpoint cluster region-1 (M-BCR-1).[8] Although variation in the breakpoints within the M-BCR-1 in CML cases has been reported,[3,9] alternative splicing mechanisms act to form new chimeric mRNA species (8 to 8.5 kb BCR/ABL mRNA),[6,10,11] encoding novel $P210^{BCR-ABL}$ with tyrosine kinase activity that is thought to play a role in neoplastic transformation in Ph-positive CML.[12–15] Therefore, the most important genomic event in Ph-positive CML seems to be the transposition of the ABL gene into the broken M-BCR-1. On the other hand, a considerable number of patients with acute lymphoblastic leukemia (ALL) also manifest the Ph-chromosome,[16] and about 50% of them show breakage on chromosome 22 at the 3′ side of the first intron of the BCR gene[17–19]; this region is currently known as the minor breakpoint cluster region-1 (m-BCR-1).[8]

In the present study, we employed two different probes derived from the M-BCR-1 and ABL regions, in order to localize chromosome 22 breakpoint in 25 Ph-positive CML patients. In this analysis, we demonstrate that two CML patients do not have a rearrangement within the M-BCR-1 and also do not express aberrant ABL-related mRNA. In addition, these cases do not carry DNAs homologous to the 5′ *bcr* and ABL probes on the Ph chromosome, yet the disease persisted as CML, and the Ph chromosome remained.

MATERIALS AND METHODS

DNA probes The probes used in this study were a 1.1 kb *Hin*dIII/*Eco*RI digested 3′ *bcr* probe,[20] a 2.0 kb *Bgl*III/*Hin*dIII-digested 5′ *bcr* probe,[3,20] and a 1.8 kb *Eco*RI-digested ABL fragment from pc-*abl* 18.2,[20] which contains a part of the kinase domain and some 3′ ABL sequences. The 3′ and 5′ *bcr* probes used in this study can detect rearrangement within the M-BCR-1 DNA sequences.[3,21] The DNA probes were labeled with [^{32}P]dATPs for Southern and Northern blot analyses and with [^{3}H]dATPs (Amersham) for chromosomal *in*

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situ hybridization with a random priming method using the Klenow (large) fragment of DNA polymerase 1.[22] Unincorporated [^{32}P]dATPs or [^{3}H]dATPs were removed by a Sephadex G-75 column.

Southern and Northern blot analyses Cellular DNA and RNA were extracted from heparinized bone marrow cells obtained from 25 Ph-positive CML patients using the urea lysis/cesium chloride method. For detection of M-BCR-1 rearrangement, DNA was digested by restriction enzymes (*Hin*dIII, *Bam*HI, or *Bgl*II) and electrophoresed through a horizontal agarose gel (0.8%). The electrophoresed DNA was denatured, neutralized, and transferred to a nylon membrane (Zeta-bind, Cuno). The filter was hybridized with the ^{32}P-labeled probe, washed, air-dried, and then exposed to Fuji X-ray film for various periods at −70°C with an intensifying screen.[23]

For Northern blot analysis, polyadenylated [poly (A)$^+$] RNA was purified by oligo(dT) cellulose column. Twenty μg of poly(A)$^+$ RNA was denatured in 1 *M* glyoxal, electrophoresed in 1.4% agarose gel, transferred to a nylon membrane, then UV-crosslinked, prehybridized, and hybridized to the labeled probe. After the hybridization, the procedure for autoradiography was the same as for the Southern hybridization technique.

Chromosomal *in situ* hybridization The localizations of DNAs homologous to the 3′ *bcr*, 5′ *bcr*, or ABL probes were identified with a chromosomal *in situ* hybridization technique, performed according to the method of Harper and Saunders,[24] with minor modifications.[25] Briefly, chromosomal DNA was treated with RNase (100 μg/ml in 2×SSC) at 37°C for 1 h, denatured using 70% formamide in 2×SCC at 70°C for 2 min, and then hybridized with the random-primer-labeled probes in a hybridization mixture (50% formamide, 10% dextran sulfate, sonicated salmon sperm DNA, 1×Denhardt's solution and 2×SSCP) at 41°C for 18 h. The processed preparations were washed and dehydrated in an ethanol series. The slides were exposed to Konica NR-M2 emulsion for 14 days at 4°C, developed, and stained using a sequential Hoechst 33258 and quinacrine mustard (Q-banding) method.[26]

Table I. Hematologic and Clinical Findings of Ph-positive Chronic Myelogenous Leukemia without Rearranged 5′ *bcr* and Aberrant 8.5 kb c-*abl* mRNA

	Case 1	Case 2	
Age/Sex[a]	24/M	61/F	
Phase[b]	chronic	chronic	blastic
Peripheral blood			
white blood cell count (/μl)	118,800	19,100	103,700
hemoglobin (g/liter)	113	119	74
platelet count (×10^4/μl)	36.4	94.2	79.4
blasts (%)	0	0	53.5
stab and segmented cells (%)	45	78	0
basophils (%)	9	2	17.5
Neutrophil alkaline phosphatase score[c]	2	149	31
Bone marrow			
nuclear cell count (×10^4/μl)	71.9	52.0	22.0
myeloid/erythroid cell ratio	24.0	11.4	123.0
blasts (%)	4.4	0	17.8
Splenomegaly (cm)[d]	3	6	14
Treatment[e]	α-interferon	Busulfan	DCMP
Duration of chronic phase (months)	16+	35	
Duration of blastic phase (months)	−		9
Chromosomes[f]	46,XY,tPh (25/25 cells)	46,XX,tPh (20/20 cells)	46,XX,tPh (12/13 cells) 46,XX,inv(3),tPh (1/13 cells)

a) Age/Sex: M, male; F, female.
b) Phase: chronic, chronic phase; blastic, blastic phase.
c) Neutrophil alkaline phosphatase score: normal range 180–300.
d) Splenomegaly: centimeters below the left costal margin.
e) Treatment: DCMP; daunorubicin, cytosine arabinoside, 6-marcaptopurine, and prednisolone.
f) Chromosomes: tPh, t(9;22)(q34;q11); inv(3), inv(3)(q21q26); numbers in parentheses indicate marrow metaphase cells.

RESULTS

All 25 patients studied were shown to have the standard Ph translocation, t(9;22)(q34;q11), and no patient had complex Ph translocation, unless "masked" Ph chromosomes were present. Southern blot analysis was carried out on 25 Ph-positive CML cases; 10 were examined during the blastic crisis, 14 during the chronic phase, and the remaining case was examined in both the chronic and blastic phases. Among the 25 CML patients, rearrangements within the M-BCR-1 DNA sequences were detected in 23 patients using the 1.1 kb 3′ and 2.0 kb 5′ *bcr* probes. The remaining two cases of Ph-positive CML did not show rearrangement within the M-BCR-1 as detected by the 5′ *bcr* probe. Hematological and clinical findings of these two CML cases are shown in Table I.

BCR rearrangement and ABL gene expression In case 1, DNA digested with *Bgl*II, *Bam*HI, or *Hin*dIII did not have aberrantly sized fragments homologous to the 5′ *bcr* probe (Fig. 1, lanes 1 (*Bgl*II digests) and 4 (*Bam*HI digests)). On the other hand, aberrant and germ line fragments hybridized to the 3′ *bcr* probe in *Bgl*II digests and *Bam*HI digests (Fig. 1, lanes 7 (*Bgl*II digests) and 10 (*Bam*HI digests)), suggesting that the breakage within M-BCR-1 which was detected by the 3′ *bcr* probe was present in this case. The breakpoint on a Ph chromosome detected by the 3′ *bcr* probe was mapped to within about 2.5 kb *Bam*HI/*Bgl*II M-BCR-1 and this breakage might be detected by using the 5′ *bcr* probe in the *Bgl*II-digested DNA, though the 5′ *bcr* probe actually did not hybridize to an abnormal fragment in *Bgl*II digests obtained from this case. The patient from whom the DNA was obtained was followed in the chronic phase as an out-patient, 16 months after the diagnosis of CML was made.

In Case 2, three different restriction enzymes (*Bam*HI, *Bgl*II, or *Hin*dIII) digested DNA containing only germ line fragments hybridized to the 5′ *bcr* probes (Fig. 1, lanes 2 (*Bgl*II-digested DNA at the chronic phase), 3 (at the blastic phase), 5 (*Bam*HI-digested DNA at the chronic phase), and 6 (at the blastic phase)). The 3′ *bcr* probe also did not detect rearrangement in DNA obtained from this case (Fig. 1, lanes 8, 9, 11 and 12). The

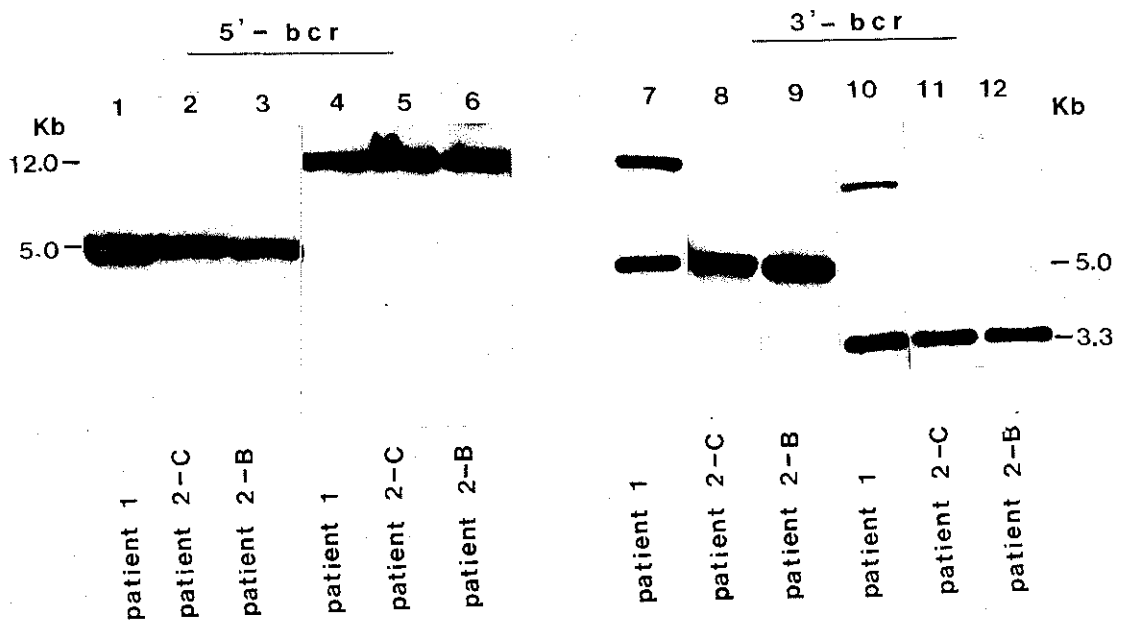

Fig. 1. Southern blot analyses using a 1.1 kb *Hin*dIII/*Eco*RI-digested 3′ *bcr* probe (right side) and a 2.0 kb *Bgl*II/*Hin*dIII-digested 5′ *bcr* probe (left side). DNA was extracted using the urea lysis/cesium chloride method, and digested by endonucleases (lane 1, *Bgl*II-digested DNA from Case 1; lane 2, *Bgl*II-digested DNA from Case 2 at the chronic phase; lane 3, *Bgl*II-digested DNA from Case 2 at the blastic phase; lane 4, *Bam*HI-digested DNA from Case 1; lane 5, *Bam*HI-digested DNA from Case 2 at the chronic phase; lane 6, *Bam*HI-digested DNA from Case 2 at the blastic phase). The 5′ *bcr* probe hybridized only to germ line DNA obtained from two cases (left side). The 3′ *bcr* probe detected rearrangement in Case 1, but not in Case 2 (lane 7, *Bgl*II-digested DNA from Case 1; lane 8, *Bgl*II-digested DNA from Case 2 at the chronic phase; lane 9, *Bgl*II-digested DNA from Case 2 at the blastic phase; lane 10, *Bam*HI-digested DNA from Case 1; lane 11, *Bam*HI-digested DNA from Case 2 at the chronic phase; and lane 12, *Bam*HI-digested DNA from Case 2 at the blastic phase).

Table II. *In situ* Hybridization of 3′- and 5′-*bcr* and c-*abl* Probes to Leukemia Metaphase from Two Patients with a t(9;22)(q34;q11)

Patients	Probes	Total number of cells analyzed	Number of labeled sites	Normal 9	Band 9q34	9q+	9q+ breakpoint	Normal 22	Band 22q11	22q−	22q− breakpoint
1	3′-*bcr*	100	114	3(2.6%)	0	13(11.4%)	12(10.5%)*	12(10.5%)	10(8.8%)*	0	0
	5′-*bcr*	75	91	1(1.1%)	0	1(1.1%)	0	10(11.0%)	9(9.9%)*	0	0
	c-*abl*	100	133	15(11.3%)	13(9.8%)*	2(1.5%)	0	0	0	0	0
2	3′-*bcr*	75	87	2(2.3%)	0	2(2.3%)	0	12(13.8%)	12(13.8%)*	0	0
	5′-*bcr*	75	98	2(2.0%)	0	1(1.0%)	0	13(13.3%)	12(12.2%)*	1(1.0%)	0
	c-*abl*	75	103	15(14.6%)	13(12.6%)*	3(2.9%)	0	2(1.9%)	0	0	0

Asterisks indicate significant difference from expectation (Poission distribution) $P>0.0005$.
Metaphases observed were obtained at chronic phases from both patients.

DNA obtained from this patient had no rearrangement within M-BCR-1 in either the chronic or blastic phase. Thus, we conclude that in this patient, there was no rearrangement within the M-BCR-1 DNA sequences, and that this molecular feature did not change with disease progression.

We next performed Northern hybridization on poly $(A)^+$ RNAs obtained from these two patients. Both of them contained 6 and 7 kb bands hybridized to the 1.8 kb ABL probe, though no 8.5 kb BCR/ABL mRNA was detected in either case, which was in keeping with the results obtained by the Southern hybridization (data not shown). On the other hand, poly$(A)^+$ RNA obtained from a CML-derived cell line, K562, exhibited an aberrant 8.5 kb fragment which hybridized to the ABL probe.

Localization of BCR and ABL genes To determine the breakpoint on chromosome 22 in these two cases of Ph-positive CML, chromosome *in situ* hybridization was carried out on bone marrow cells obtained at the chronic phase. The localizations of DNAs homologous to the 3′ and 5′ *bcr* probes, and ABL probes are demonstrated in Table II and Fig. 2.

Case 1 showed sequences homologous to the 3′ *bcr* probe on a normal chromosome 22 at band q11 (8.8%), as well as the 9q34 region of a 9q+ chromosome

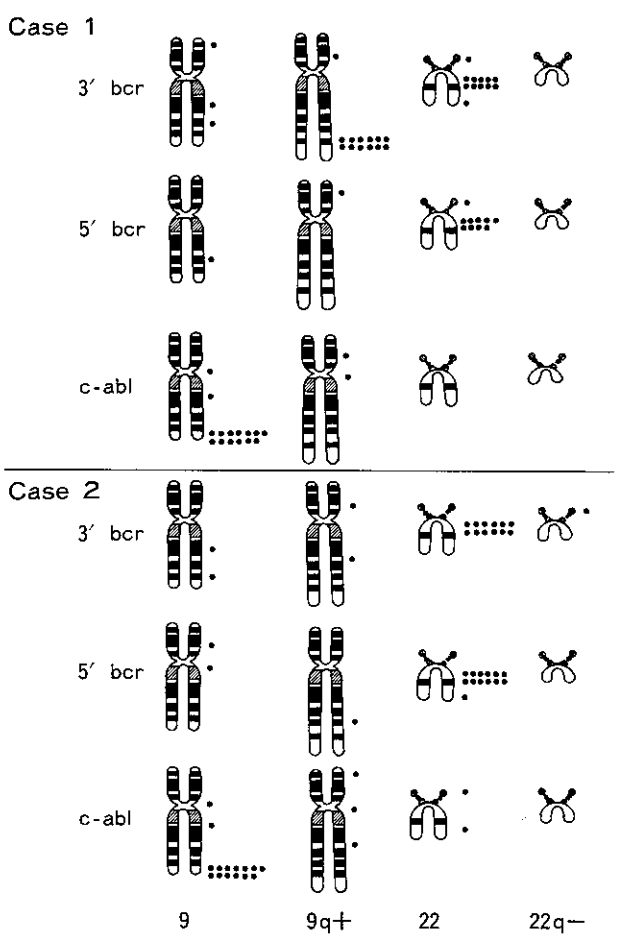

Fig. 2. Schematic representation of clustered grains hybridized to the 3′ and 5′ *bcr* probes and the ABL probe. In Case 1, 9.9% of grains hybridized to the 5′ *bcr* probe were present at 22q11 of normal chromosome 22, 8.8% of grains hybridized to the 3′ *bcr* probe at 22q11 of a normal chromosome 22 and 11.4% of grains hybridized to the 3′ *bcr* at 9q34 of a 9q+ chromosome. Further, 9.8% of grains hybridized to the ABL probe clustered at band 9q34 of a normal chromosome 9. In Case 2, 12.2% of grains hybridized to the 5′ *bcr* probe clustered at band 22q11 of a normal chromosome 22, 13.8% of grains hybridized to the 3′ *bcr* probe at 22q11 of a normal chromosome 22, and 12.6% of grains hybridized to the ABL probe at 9q34 of a normal chromosome 9, indicating that transposition of these genes was not detected in this case.

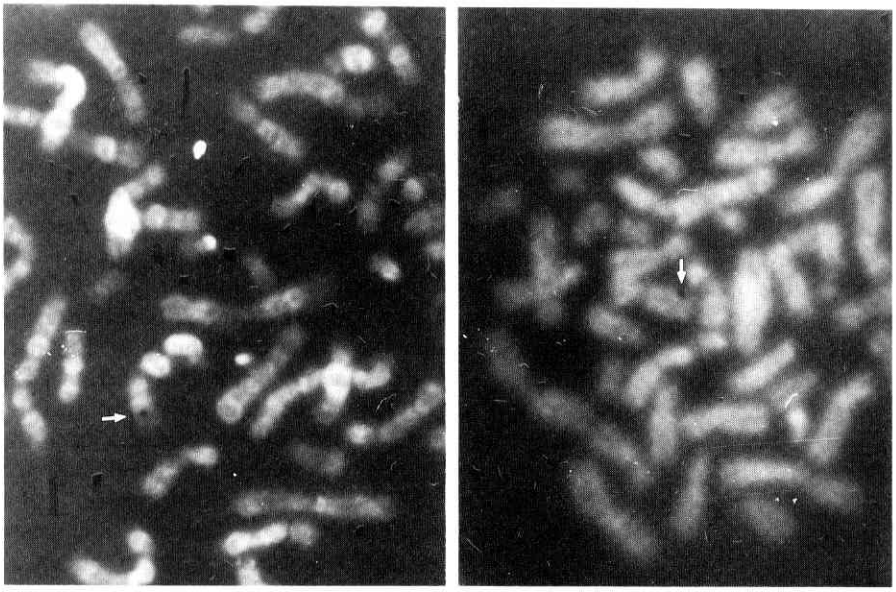

Fig. 3. Localization of DNAs homologous to the ^{3}H-labeled 3′ *bcr* probe by chromosomal *in situ* hybridization. These photographs of Ph-positive metaphase cells obtained from Case 1 show homology at band q34 of 9q+ chromosomes. Silver grains were identified on Q-banded chromosomes using a double-staining method and analyzed under a fluorescence microscope.

(10.5%)(Figs. 2 and 3). On the other hand, the ABL and 5′ *bcr* probes did not cluster on the Ph chromosome or on other chromosomal segments. Rather, the ABL probe hybridized to sequences on a normal chromosome 9 at band q34, and the 5′ *bcr* was homologous to the q11 band on a normal chromosome 22 (Table II and Fig. 2), indicating that the 3′ portion of M-BCR-1 was transposed to the 9q+ chromosome at the q34 region, although DNAs homologous to the 5′ *bcr* and ABL probe were not present on the Ph chromosome.

Case 2 showed that the 5′ *bcr* probe was homologous to sequences on a normal chromosome 22 at the q11 region (12.2%) and ABL on a normal chromosome 9 (12.6%), but neither probe had homologous regions on the Ph chromosome. The 3′ *bcr* sequences were also clustered only on the normal chromosome 22 at band q11 (13.8%), but not on the Ph or on the 9q+ chromosome (Table II and Fig. 2).

DISCUSSION

In this study, we have encountered two CML cases which did not have either DNA rearrangement as detected by the 5′ *bcr* probe nor ABL-related fusion mRNA. The chromosome *in situ* hybridization results for these two cases demonstrated that the 5′ portion of the M-BCR-1 sequences, which usually remains on chromosome 22 in Ph-positive CML cells, was not present either on a Ph chromosome nor on a 9q+ chromosome, indicating that the breakpoint did not occur outside the M-BCR-1, and the 5′ portion of M-BCR-1 and the ABL genes were completely missing from the Ph chromosome.

The deletion of M-BCR-1 DNA sequences homologous to the 3′ *bcr* probe has been found in some Ph-positive CML cases.[27-30] Popenoe *et al.* reported that 4 out of 14 Ph-positive CML cases did not show abnormal bands hybridizing to the 3′ *bcr* probe, while they did find rearranged M-BCR-1 as detected by a 5′ *bcr* probe.[27] However, they also found 4 cases in which no rearrangement within the M-BCR-1 was detected, suggesting the possibility of variable deletions in the M-BCR-1 DNA sequences during the Ph translocation process.[27] Deletion of the 3′ portion of M-BCR-1 was also found in a K562 CML-derived cell line,[31] and if complete, deletion in this region does not interfere with the production of chimeric BCR/ABL mRNA. Furthermore, Shtalrid *et al.* recently reported that there was no unique clinical or laboratory finding in Ph-positive CML cases with a deletion of the 3′ side of M-BCR-1.[30]

When the breakage on chromosome 22 occurs within the 5th intron of BCR and the 3′ side of the broken DNA sequences is simultaneously deleted, Southern analysis using the 5′ and 3′ *bcr* probes could fail to detect the change. However, in such a case, the chromosomal *in situ* hybridization technique will reveal 5′ *bcr* probe homology to Ph chromosomal DNA. However, the Ph chromosome from the two CML cases presented here showed no sequence homology to the 5′ *bcr* probe.

Selleri *et al.* described results similar to ours from molecular analysis of two Ph-positive CML patients.[32] They found three cases without detectable DNA rearrangement within the M-BCR-1 sequences, two of which did not have a chimeric BCR/ABL mRNA, and they postulated that the breakpoint occurred outside the

M-BCR-1 in some Ph-positive CML cases.[32] Other investigators also reported that a few Ph-positive CML cases showed rearrangements outside the M-BCR-1.[9, 30, 31, 33, 34] Saglio *et al.* reported that seven out of 80 CML cases in the chronic phase showed breakpoints falling outside the M-BCR-1; two of which had breaks upstream to the M-BCR-1,[33] while Shtalrid *et al.* found one out of 100 CML cases in which breakage on chromosome 22 occurred at the 5′ side of the M-BCR-1.[30] However, it is difficult to conclude from these results whether the break on a Ph chromosome occurred outside the M-BCR-1 or whether the fused BCR/ABL gene was deleted, without using chromosomal *in situ* hybridization techniques. The reason for this is that if a deletion occurs after the formation of a BCR/ABL fusion gene and the deleted portion includes the broken 5′ side of the M-BCR-1, the results of Southern blot analysis might be mistakenly interpreted as indicating that the break occurred outside the M-BCR-1. When the breakage on a Ph chromosome occurs within the 1st intron of BCR, DNA homologous to the 5′ *bcr* probe should move to a 9q+ chromosome in Ph-positive cells. On the other hand, when DNA sequences on a Ph chromosome including the broken 5′ *bcr* are deleted, DNA hybridized to the 5′ *bcr* probe might not be present either on a 9q+ chromosome or on a Ph chromosome. Thus, at least in our two Ph-positive CML cases, the results of Southern blot analysis, showing that only germ line fragments were homologous to the 5′ *bcr* probe, do not unequivocally indicate that the break on a Ph chromosome occurred outside the M-BCR-1.

The observation by chromosomal *in situ* hybridization that the ABL probe did not hybridized to the Ph chromosome in these CML patients, strongly suggests that the breakpoints on chromosome 22 do not occur at the first intron of BCR. Even when a breakpoint on a chromosome 22 occurred upstream of M-BCR-1 as in case of Ph-positive ALL,[17–19] the ABL probe should hybridize to DNAs which remain on the Ph chromosome. As mentioned above, rare Ph-positive CML patients who had breakpoints located within a short distance from the 5′ part of the M-BCR-1 have been described,[30, 33] but their breakpoint locations do not occur as far upstream as those observed in Ph-positive ALL.[17–19] However, molecular changes in the two Ph-positive CML cases presented here are probably different from those observed in Ph-positive ALL or in those rare cases of Ph-positive CML. Furthermore, not only DNAs hybridized to the 5′ *bcr* probe but also the ABL gene were simultaneously absent from the Ph chromosome in these cases, indicating that those genes may have been deleted after the formation of the BCR/ABL fusion gene, rather than being deleted independently.

Recently, Bartram *et al.* reported a Ph-positive CML case with a deletion of the 5′ end of the rearranged M-BCR-1 with simultaneous absence of the aberrant ABL-related mRNA.[35] They demonstrated the deletion of the fused DNA sequences which might occur following the formation of the usual BCR/ABL fusion using chromosomal *in situ* hybridization techniques. Furthermore, Bartram *et al.* suggested that the deletion of a fused BCR/ABL DNA sequence might have modulating effects on the clinical course of the disease, since the Ph-positive CML patient presented by them survived more than 14 months in the acute blastic phase.[35] Our Case 2 also survived the acute megakaryoblastic phase for 9 months following a chronic phase of about 35 months (Table I),[36] which is in accordance with the description by Bartram *et al.*[35] Thus, the cases presented by Bartram *et al.* and those reported here might indicate that deletion of the fused BCR/ABL gene in Ph-positive CML has some effects on clinical features, especially on prolongation of the blastic phase.

One possibility for the deletion of the 5′ portion of the M-BCR-1 sequences and the ABL gene from a Ph chromosome in our two CML patients might be that the deletion occurred subsequent to the BCR/ABL fusion. However, the results of this study strongly suggest that the fused DNA sequences could disappear during any phase of Ph-positive CML. This further suggests the possibility that the fused BCR/ABL sequences and an aberrant ABL-related message might not be necessary for maintenace of the disease, again supporting the observation by Bartram *et al.*[35, 37]

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REFERENCES

1) Nowell, P. C. and Hungerford, D. A. A minute chromosome in human chronic granulocytic leukemia. *Science,* **132,** 1197 (1960).
2) Rowley, J. D. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature,* **243,** 290–293 (1973).
3) Groffen, J., Stephenson, J. R., Heisterkamp, N., de Klein, A., Bartram, C. R. and Grosveld, G. Philadelphia chromosomal breakpoints are clustered within a limited region, *bcr* on chromosome 22. *Cell,* **36,** 93–99 (1984).
4) De Klein, A., Geurts van Kessel, A., Grosveld, G., Bartram, C. R., Hagemeijer, A., Bootsma, D., Spurr, N. K., Heisterkamp, N., Groffen, J. and Stephenson, J. R. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature,* **300,** 765–767 (1982).
5) Heisterkamp, N., Stephenson, J. R., Groffen, J., Hansen, P. F., de Klein, A., Bartram, C. R. and Grosveld, G. Localization of the c-*abl* oncogene adjacent to a translocation breakpoint in chronic myelocytic leukaemia. *Nature,* **306,** 239–242 (1983).
6) Stam, K., Heisterkamp, N., Grosveld, G., de Klein, A., Verma, R. S., Coleman, M., Dosik, H. and Groffen, J. Evidence of a new chimeric *bcr*/c-*abl* mRNA in patients with chronic myelocytic leukemia and the Philadelphia chromosome. *N. Engl. J. Med.,* **313,** 1429–1432 (1985).
7) Croce, C. M., Huebner, K., Isobe, M., Fainstein, E., Lifshitz, B., Shtivelman, E. and Canaani, E. Mapping of four distinct BCR-related loci to chromosome region 22q11: order of BCR loci relative to chronic myelogenous leukemia and acute lymphoblastic leukemia breakpoint. *Proc. Natl. Acad. Sci. USA,* **84,** 7174–7178 (1987).
8) Gale, R. P. and Goldman, J. M. Rapid progress in chronic myelogenous leukemia. *Leukemia,* **2,** 321–324 (1988).
9) Leibowitz, D., Schaefer-Rego, K., Popenoe, D. W., Mears, J. G. and Bank, A. Variable breakpoints on the Philadelphia chromosome in chronic myelogenous leukemia. *Blood,* **66,** 243–245 (1985).
10) Canaani, E., Gale, R. P., Stender-Saltz, D., Berrebi, A., Aghai, E. and Januszewicz, E. Altered transcription of an oncogene in chronic myeloid leukaemia. *Lancet,* **1,** 593–595 (1984).
11) Shtivelman, E., Lifshitz, B., Gale, R. P. and Canaani, E. Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukemia. *Nature,* **315,** 550–554 (1985).
12) Kurzrock, R., Kloetzer, W. S., Talpaz, M., Blick, M., Walters, R., Arlinghaus, R. B. and Gutterman, J. U. Identification of molecular variants of p210*bcr-abl* in chronic myelogenous leukemia. *Blood,* **70,** 233–236 (1987).
13) Konopka, J. B., Watanabe, S. M. and Witte, O. N. An alteration of the human c-*abl* protein in K562 leukemia cells unmasks associated tyrosine kinase activity. *Cell,* **37,** 1035–1042 (1984).
14) Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N. and Baltimore, D. The chronic myelogenous leukemia-specific p210 protein is the product of the *bcr/abl* hybrid gene. *Science,* **233,** 212–214 (1986).
15) Kloetzer, W., Kurzrock, R., Smith, L., Talpaz, M., Spiller, M., Gutterman, J. and Arlinfhaus, R. The human cellular *abl* gene product in the chronic myelogenous leukemia cell line K562 has an associated tyrosine protein kinase activity. *Virology,* **140,** 230–238 (1985).
16) Bloomfield, C. D., Peterson, L. C., Yunis, J. J. and Brunning, R. D. The Philadelphia chromosome (Ph^1) in adults presenting with acute leukaemia: a comparison of Ph^1+ and Ph^1− patients. *Br. J. Haematol.,* **36,** 347–358 (1977).
17) Erikson, J., Griffin, C. A., ar-Rushdi, A., Valtieri, M., Hoxie, J., Finan, J., Emanuel, B. S., Rovera, G., Nowell, P. C. and Croce, C. M. Heterogeneity of chromosome 22 breakpoint in Philadelphia-positive (Ph+) acute lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA,* **83,** 1807–1811 (1986).
18) Kurzrock, R., Shtarlrid, M., Gutterman, J. U., Koller, C. A., Walters, R., Trujillo, J. M. and Talpaz, M. Molecular analysis of chromosome breakpoints in adult Philadelphia-positive acute lymphoblastic leukaemia. *Br. J. Haematol.,* **67,** 55–60 (1987).
19) Secker-Walker, L. M., Cooke, H. M. G., Browett, P. J., Shippey, C. A., Norton, J. D., Coustan-Smith, E. and Hoffbrand, A. V. Variable Philadelphia breakpoints and potential lineage restriction of bcr rearrangement in acute lymphoblastic leukemia. *Blood,* **72,** 784–791 (1988).
20) Bartram, C. R., Kleihauer, E., de Klein, A., Grosveld, G., Teyssier, J. R., Heisterkamp, N. and Groffen, J. c-*abl* and *bcr* are rearranged in a Ph^1− negative CML patient. *EMBO J.,* **4,** 683–686 (1985).
21) Ohyashiki, K., Ohyashiki, J. H., Kinniburgh, A. J., Rowe, J., Miller, K. B., Raza, A., Preisler, H. D. and Sandberg, A. A. Transposition of breakpoint cluster region (3′ *bcr*) in CML cells with variant Philadelphia translocations. *Cancer Genet. Cytogenet.,* **26,** 105–115 (1987).
22) Feinberg, A. P. and Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.,* **132,** 6–13 (1983).
23) Church, G. M. and Gilbert, W. Genomic sequencing. *Proc. Natl. Acad. Sci. USA,* **81,** 1991–1995 (1984).
24) Harper, M. E. and Saunders, G. F. Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. *Chromosoma,* **83,** 431–439 (1981).
25) Ohyashiki, J. H., Ohyashiki, K., Sandberg, A. A., Minowada, J. and Kinniburgh, A. J. Human-*fms* gene is retained in acute lymphoblastic leukemia cells with del(5)(q32). *Cancer Genet. Cytogenet.,* **25,** 341–350 (1987).
26) Yoshida, M. C., Ikeuchi, T. and Sasaki, M. Differential staining of parental chromosomes in interspecific cell hybrids with a combined quinacrine and 33258 Hoechst

technique. *Proc. Jpn. Acad.*, **51**, 184–187 (1975).
27) Popenoe, D. W., Schaefer-Rego, K., Mears, J. G., Bank, A. and Leibowitz, D. Frequent and extensive deletion during the 9, 22 translocation in CML. *Blood*, **68**, 1123–1128 (1986).
28) Hirosawa, S., Aoki, N., Matsushime, H. and Shibuya, M. Undetectable *bcr-abl* rearrangements in some CML patients are due to a deletion mutation in the *bcr* gene. *Am. J. Hematol.*, **28**, 33–36 (1988).
29) De Klein, A., Hagemeijer, A., Bartram, C. R., Houwen, R., Hoefsloot, L., Carbonell, F., Chan, L., Barnett, M., Greaves, M., Kleihauer, E., Heisterkamp, N., Groffen, J. and Grosveld, G. *bcr* rearrangement and translocation of c-*abl* oncogene in Philadelphia-positive acute lymphoblastic leukemia. *Blood*, **68**, 1369–1375 (1986).
30) Shtalrid, M., Talpaz, M., Kurzrock, R., Kantarjian, H., Trujillo, J., Gutterman, J., Yoffe, G. and Blick, M. Analysis of breakpoints within the *bcr* gene and their correlation with the clinical course in Philadelphia-positive chronic myelogenous leukemia. *Blood*, **72**, 485–490 (1988).
31) Grosveld, G., Verwoerd, T., Van Agthoven, T., de Klein, A., Ramachandran, K. L., Heisterkamp, N., Stam, K. and Groffen, J. The chronic myelocytic cell line K562 contains a breakpoint in *bcr* and produces a chimeric *bcr/c-abl* transcript. *Mol. Cell. Biol.*, **6**, 607–616 (1986).
32) Selleri, L., Narni, F., Emilia, G., Colo, A., Zucchini, P., Venturelli, D., Donelli, A., Torelli, U. and Torelli, G. Philadelphia-positive chronic myeloid leukemia with a chromosome 22 breakpoint outside the breakpoint cluster region. *Blood*, **70**, 1659–1664 (1987).
33) Saglio, G., Guerrasio, A., Tassinari, A., Ponzetto, C., Zaccaria, A., Testoni, P., Celso, G., Rege-Cambrin, G., Serra, A., Pegoraro, L., Avanzi, G. C., Attadia, V., Falda, M. and Gavosto, F. Variability of the molecular defects corresponding to the presence of a Philadelphia chromosome in human hematologic melignancies. *Blood*, **72**, 1203–1208 (1988).
34) Blennerhassett, G. T., Furth, M. E., Anderson, A., Burns, J. P., Chaganti, R. S. K., Blick, M., Talpaz, M., Dev, V. G., Chan, L. C., Wiedemann, M., Greaves, M. F., Hagemeijer, A., van der Plas, D., Skuse, G., Wang, N. and Stam, K. Clinical evaluation of a DNA probe assay for the Philadelphia (Ph^1) translocation in chronic myelogenous leukemia. *Leukemia*, **2**, 648–657 (1988).
35) Bartram, C. R., Janssen, J. W. G., Becher, R., De Klein, A. and Grosveld, G. Persistence of chronic myelocytic leukemia despite deletion of rearranged *bcr/c-abl* sequences in blast crisis. *J. Exp. Med.*, **164**, 1389–1396 (1986).
36) Ohyashiki, K., Ohyashiki, J. H., Hojo, H., Ohtaka, M., Toyama, K., Sugita, K., Nakazawa, S., Sugiura, K., Nakazawa, K., Nagasawa, T., Enomoto, Y. and Watanabe, Y. Cytogenetic findings in adults acute leukemia and myeloproliferative disorders with an involvement of megakaryocyte lineage. *Cancer*, in press.
37) Bartram, C. R., De Klein, A., Hagemeijer, A., Carbonell, F., Kleihauser, E. and Grosveld, G. Additional c-*abl/bcr* rearrangements in a CML patient exhibiting two Ph chromosomes during blast crisis. *Leuk. Res.*, **10**, 221–225 (1986).