

***bcr* REARRANGEMENT WITHOUT JUXTAPOSITION OF *c-abl*  
IN CHRONIC MYELOCYTIC LEUKEMIA**

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Chronic myelocytic leukemia (CML) is associated with the presence of a Philadelphia (Ph<sup>1</sup>) chromosome in 95% of cases (1). This cytogenetic hallmark is molecularly characterized by the translocation of the *c-abl* oncogene from chromosome 9 into the breakpoint cluster region (*bcr*) of chromosome 22 (2-5), and the subsequent transcription of a chimeric 8.5 kb *bcr/c-abl* RNA species within leukemic cells (6-8), which represents mRNA for an altered *c-abl* protein with associated tyrosine kinase activity (9). Ph<sup>1</sup>-negative CML, however, constitute a heterogeneous group of prognostically distinct disorders (10). Recently (11), my coworkers and I identified a Ph<sup>1</sup>-negative CML patient with a *c-abl/bcr* rearrangement; however, other patients lack this recombination (3). Here I report on the first Ph<sup>1</sup>-negative CML patient whose leukemic cells exhibit a rearrangement in the *bcr* gene without juxtaposition of *c-abl* sequences.

**Materials and Methods**

**Patients.** Patient 1 is a 62-yr-old male in the chronic phase of Ph<sup>1</sup>-positive CML. Patient 2 is a 48-yr-old male with splenomegaly; Hb, 107 g/liter; leukocytes,  $95 \times 10^9$  cells/liter (52% neutrophils, 1% eosinophils, 3% basophiles, 9% myeloblasts, 24% myelocytes, 8% lymphocytes, 3% monocytes);  $452 \times 10^9$  platelets/liter; bone marrow revealed increased myeloid lines without major maturation disturbance; repeated cytogenetic analysis showed a male karyotype without chromosomal aberrations; this patient is being treated with hydroxyurea and has been in chronic state for 28 mo.

**DNA Analysis.** Bone marrow DNA (15  $\mu$ g) obtained from both patients were digested with restriction enzymes (C. F. Boehringer & Soehne, Mannheim, Federal Republic of Germany), electrophoresed on a 0.7% agarose gel, blotted, and hybridized to a 2 kb Bgl II/Hind III 5' *bcr* probe and a 1.2 kb Hind III/Bgl II 3' *bcr* probe as described (11).  $\lambda$ -DNA were included as molecular weight standards (not shown). Specific activity of the probes was  $1-3.5 \times 10^8$  cpm/ $\mu$ g. After hybridization, filters were washed under high stringency (10% saline citrate, 65°C) and exposed to XAR-2 film (Eastman Kodak Co., Rochester, NY) for up to 3 d at -70°C with DuPont Lightening Plus intensifying screens (DuPont Instruments, Wilmington, DE).

**RNA Analysis.** RNA was isolated from bone marrow cells according to the LiCl/urea method (12); poly(A) RNA was obtained after two passages over oligo(dT) cellulose; 15  $\mu$ g of poly(A) RNA was electrophoresed on a 1.2% agarose gel in the presence of formaldehyde (13). After blotting, nitrocellulose filters (Schleicher and Schuell, Dassel, FRG) were first hybridized to a 0.6 kb Eco RI/Bam HI *c-abl* probe washed, and rehybridized to a 2 kb Bgl II/Hind III 5' *bcr* probe, as described (11). Filters were exposed

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to Kodak XAR-5 film using DuPont Lightning Plus intensifying screens for up to 6 d at  $-70^{\circ}\text{C}$ .

**In Situ Hybridization.** Chromosomes obtained from bone marrow were prepared according to standard techniques. After RNase treatment, chromosomes were denatured in 70% formamide/2 $\times$  standard saline citrate (SSC) at  $70^{\circ}\text{C}$  for 2 min, rinsed in 2 $\times$  SSC, and dehydrated. The probe, a 1:1 mixture of human 3' 1.1 kb Hind III/Eco RI and 5' 0.6 kb Bam HI *c-abl* plasmids (3), was labeled by nick-translation using [ $^3\text{H}$ ]dCTP and [ $^3\text{H}$ ]dTTP (New England Nuclear, Boston, MA) to a specific activity of  $0.8 \times 10^7$  cpm/ $\mu\text{g}$ . The probe was denatured for 5 min at  $70^{\circ}\text{C}$  at a concentration of 0.2  $\mu\text{g}/\text{ml}$  in 50% formamide/2 $\times$  SSC/10% dextran sulfate added to the chromosomes, and hybridized for 10 h at  $37^{\circ}\text{C}$ ; slides were rinsed in three changes of 50% formamide/2 $\times$  SSC at  $39^{\circ}\text{C}$ , followed by 6 h washing in 2 $\times$  SSC at room temperature, exposed to Kodak NTB2 emulsion, for 15 d at  $4^{\circ}\text{C}$ , developed, and stained with quinacrine mustard as described (11).

### Results

Southern blot analysis revealed a rearrangement within the *bcr* gene in leukemic cells of a Ph<sup>1</sup>-negative CML patient. Hybridization to 5' *bcr* sequences detects a 5 kb germline band together with a 3.4 kb rearranged fragment (Fig. 1, lane 2). A Bgl II polymorphism appears to be an unlikely explanation of this result, since digests using different restriction enzymes (Bam HI, Eco RI, and Hind III) demonstrated a similar pattern (not shown). Hybridization to a 1.2 kb Hind III/Bgl II 3' *bcr* probe also identified an additional autoradiographic band. These results are comparable to the data obtained in Ph<sup>1</sup>-positive CML (e.g., Fig. 1, lane 1).

To investigate a possible involvement of *c-abl* sequences in this genomic alteration, I performed in situ hybridization studies of *c-abl* sequences to metaphase chromosomes of the Ph<sup>1</sup>-negative CML patient (Table I). Distribution of silver grains obtained from analysis of 42 metaphases was uniform and at random on all chromosomes except the specific signal ( $P < 0.01$ ) on chromosome 9. Analysis by Poisson distribution, with the number of grains per chromosome adjusted for the relative size of the band in a 400-band idiogram (15), revealed highly significant ( $P \ll 10^{-7}$ ) grain accumulation at band 9q34. Thus, in contrast to all Ph<sup>1</sup>-positive CML patients investigated so far (2-4), in situ hybridization studies failed to detect a translocation of *c-abl* sequences in leukemic cells of this patient.

To elucidate in some more detail the consequences of these results I performed

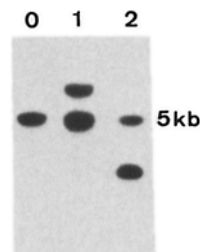


FIGURE 1. Southern blot analysis of *bcr* sequences of human placenta DNA (lane 0) and bone marrow DNA from two leukemic patients (lanes 1 and 2). DNA (15  $\mu\text{g}$ ) were digested with Bgl II, electrophoresed on a 0.7% agarose gel, blotted, and hybridized to a Bgl II/Hind III 5' *bcr* probe that detects a 5 kb normal *bcr* fragment.

TABLE I  
Results of *In Situ Hybridization to Human c-abl Probes*

Chromosome	Grains observed	Grains expected
1	9	17.5
2	20	17.0
3	9	14.1
4	7	13.5
5	15	12.9
6	3	12.2
7	14	11.2
8	8	10.3
9	42*	9.6
10	7	9.4
11	6	9.6
12	10	9.5
13	6	7.5
14	8	7.3
15	9	6.8
16	3	6.3
17	5	6.0
18	7	5.7
19	1	4.4
20	2	4.8
21	5	3.3
22	1	3.5
X	3	10.9
Y	2	3.7

Number of grains expected according to DNA content (14). Analysis by Poisson distribution demonstrate a highly significant grain accumulation at band 9q34. 31 grains on region 9q34.

\* 31 grains on region 9q34.

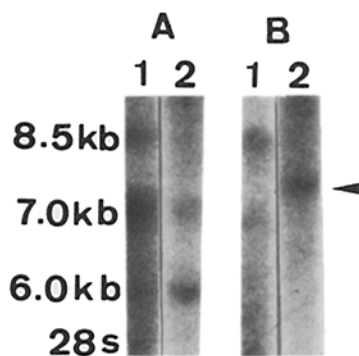


FIGURE 2. Northern blot analysis of poly(A) RNA obtained from patients 1 and 2. The blot was first hybridized to a *c-abl* probe (A), washed, and rehybridized to 5' *bcr* sequences (B). Arrow points to a novel 7.3 kb *bcr* RNA species in leukemic cells of patient 2. Molecular weight standards are not shown.

an RNA analysis. Northern blots hybridized to *c-abl* sequences showed 6 and 7 kb normal *abl* RNA species, as well as the fused 8.5 kb transcript in Ph<sup>1</sup>-positive CML (Fig. 2A, lane 1); in the Ph<sup>1</sup>-negative CML patient, only normal *c-abl* species were visible (Fig. 2A, lane 2). Rehybridization of the filters to 5' *bcr* sequences, however, led to the detection of a novel 7.3 kb *bcr* transcript in leukemic cells of this individual (Fig. 2B, lane 2) replacing a 6.7 kb normal *bcr* transcript (8); the 8.5 kb chimeric RNA species (7, 8) demonstrated in Ph<sup>1</sup>-positive CML (Fig. 2B, lane 1).

### Discussion

Molecular analysis of the Ph<sup>1</sup> translocation in CML led to the identification of the *bcr* gene on chromosome 22. A modification of the *c-abl* protein by N-terminal substitution of *bcr* sequences appears to be a crucial event in the development of Ph<sup>1</sup>-positive CML. Chronic myelocytic leukemia without a Ph<sup>1</sup> chromosome comprises a heterogeneous group of related disorders. Recent evidence (3, 11) suggests that a subset of cytogenetic Ph<sup>1</sup>-negative CML patients may in fact belong to the molecularly defined entity of Ph<sup>1</sup>-positive CML, while leukemic cells of other Ph<sup>1</sup>-negative CML patients lack a *c-abl/bcr* rearrangement.

Here I report on yet another type of genomic recombination, i.e., a *bcr* rearrangement without involvement of *c-abl* sequences. Data obtained by both in situ hybridization studies and Northern blotting demonstrate that this oncogene is neither translocated nor abnormally transcribed in the Ph<sup>1</sup>-negative CML patient described here. However, a rearrangement within *bcr* leads to a replacement of normal 4.5 and 6.7 kb *bcr* RNA species (7) by a novel 7.3 kb transcript in leukemic cells of this individual.

Chronic myelocytic leukemia is a clonal disease of pluripotent hematopoietic stem cells, and the expression of *bcr* may be closely associated with this cell lineage. However, the normal function of the *bcr*-encoded product is still unknown. The detection of a rearrangement within *bcr* per se is in all likelihood not a sufficient explanation for the development of myeloid leukemia. As for the patient described herein, it may be possible that *bcr* sequences are fused with other, yet unknown sequences, instead of *c-abl*. Molecular cloning and detailed analysis of the novel *bcr* transcript may substantiate this speculation. However, the demonstration of heterogeneity among Ph<sup>1</sup>-negative CML cases detected by *bcr* and *c-abl* sequences may finally contribute to a novel subclassification of this poorly defined group of leukemias based on molecular, morphological, and clinical features.

### Summary

Southern blot analysis detected a *bcr* gene rearrangement within leukemic cells of a Philadelphia chromosome-negative chronic myelocytic leukemia (CML) patient that led to transcription of a novel 7.3 kb *bcr* RNA species. Participation of the *c-abl* oncogene in this genomic recombination could be ruled out by in situ hybridization studies and Northern blot analysis.

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