Detection of the Philadelphia Chromosome in Acute Lymphoblastic Leukemia by Pulsed-Field Gel Electrophoresis

By Arthur L. Hooberman, Charles M. Rubin, Kevin P. Barton, and Carol A. Westbrook

The Philadelphia (Ph1) chromosome is an acquired abnormality in the malignant cells of approximately 95% of patients with chronic myelogenous leukemia (CML), as well as approximately 20% of adults and 2% to 9% of children with acute lymphoblastic leukemia (ALL). It is the product of a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11). In CML, this translocation juxtaposes the 3' portion of the ABL protooncogene on chromosome 9 to the BCR gene on chromosome 22, resulting in a chimeric gene that is transcribed into an 8.5 kilobase mRNA, and translated into a novel protein of molecular weight 210,000 daltons, the p210BCR-ABL, which contains amino-terminal sequences derived from BCR and carboxy-terminal sequences derived from ABL. The BCR-ABL fusion gene, its mRNA, and its protein are present in the tumor cells of virtually all cases of CML; thus, detection of any one of these molecular species would represent a highly specific and sensitive method of diagnosis. As nearly all translocation breakpoints occur in a limited, 5.8 kilobase region, the BCR gene, 10 Southern hybridization analysis can easily be used to demonstrate translocations, or "bcr rearrangements." The detection of the Ph1 chromosome by identification of a bcrr rearrangement is a reliable and sensitive test that is rapidly gaining acceptance in the diagnosis and management of CML.

The presence of a Ph1 chromosome in ALL has important clinical implications because this subgroup of ALL patients has a significantly lower survival compared with ALL patients with normal karyotypes. Furthermore, a reliable means of detecting the Ph1 chromosome has a potential clinical usefulness in a disease such as ALL characterized by frequent remissions and relapses. However, the detection of the Ph1 chromosome in this disease is difficult for several reasons. First, cytogenetic analysis in ALL is more difficult than in CML due to relatively poor chromosome morphology and relatively low number of analyzable metaphase cells. Second, in ALL the percentage of metaphase cells with a Ph1 chromosome is variable, and therefore it may be missed if only a few metaphases are examined, in distinction to CML where virtually 100% of metaphase cells from the marrow contain a Ph1 chromosome. Third, a DNA probe assay based on Southern analysis is not as simple as in CML, as only about one-half of ALL patients have a bcrr rearrangement. This is because there are two distinct molecular subtypes of the Ph1 chromosome in ALL: those with a bcrr rearrangement (bcrr-positive), resulting in production of the p210BCR-ABL, as is found in CML, and those without a bcrr rearrangement (bcrr-negative). In the latter group, the translocation interrupts the BCR gene within the large intron 5' of the bcr, resulting in a protein of molecular weight 190,000 daltons, the p190BCR-ABL, smaller than the p210BCR-ABL seen in CML (Fig 1). Although these breakpoints often occur in a 10 kb region, the first intron is over 50 kb in extent, and Southern analysis will fail to detect some portion of these translocations. As this region is so large, analysis for translocation breakpoints might be possible with the technique of pulsed-field gel electrophoresis (PFGE). PFGE refers to any of a number of methods, including field-inversion gel electrophoresis (FIGE) and transverse alternating gel electrophoresis (TAFE), that use alternating pulses of current to separate the very large restriction fragments (100 to >1,000 kb) that are obtained by cleaving DNA with enzymes that cut infrequently. DNA from PFGE gels can be transferred to filters and hybridized with standard techniques as used in Southern blotting. As we have previously used this method successfully to demonstrate chromosome 22 translocation breakpoints in

From the Department of Medicine, Section of Hematology/Oncology and the Department of Pediatrics, Section of Hematology/Oncology, University of Chicago. Submitted February 17, 1989; accepted April 25, 1989.

Supported by ACS IN-41-29, the Richard L. Duchsossois Cancer Research Fund, NCI CA 47000-01, NCI CA 19266, NCI CA 42557, the University of Chicago Cancer Research Center, the Schweppie Foundation, the Louis Block Fund, and the Leukemia Research Foundation.

A.L.H. is a Bristol-Myers Cancer Research Fellow. C.M.R. is a Special Fellow of the Leukemia Society of America and a Pew Scholar in the Biomedical Sciences.

Address reprint requests to Carol A. Westbrook, MD, PhD, University of Chicago, 5841 S Maryland Ave, Box 420, Chicago, IL 60637.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1989 by Grune & Stratton, Inc.

0006-4971/89/7403-0028$3.00/0


1101
In these cases, peripheral blood or bone marrow cells that had been collected during periods of acute leukemia and that had been frozen in 10% DMSO at −170°C were available from eight patients. Very high molecular weight DNA suitable for PFGE could be prepared on seven of the eight.

**Cell lines.** BV173 was established from the malignant cells of a patient in the blast phase of CML. It contains three copies of the Ph1 chromosome and produces the p210BCR-ABL. SUP-B13 was established from the cells of a child with Ph1-positive ALL. It contains one copy of the Ph1 chromosome and produces the p190BCR-ABL. Control cell lines used were RCH-ACV, MOLT4, MOLTI6, and MOLTI7, which were derived from lymphoid malignancies; none of these have a Ph1 chromosome.

**Preparation and digestion of DNA.** DNA of very high molecular weight was prepared by embedding 1.6 × 106 diploid cells in 50 μL 0.5% low gelling temperature agarose plugs to yield 10 μg DNA per plug. The plugs were then treated with 1% n-lauryl sarcosine and 1 mg/mL proteinase K at 50°C for 48 hours to lyse the cells and purify the DNA, followed by extensive dialysis against TE buffer at 4°C.

**Southern hybridization and DNA probes.** Restriction enzyme digestion was carried out directly on the agarose plugs, rather than on DNA in solution, using buffer conditions recommended by the enzyme manufacturer, with an excess of restriction enzyme (40 to 100 units per plug). The plugs were then placed in 0.8% agarose gels with phage lambda multimers and intact yeast chromosomes used as size markers. Initially, Southern blots were prepared according to standard methods and hybridized under high-stringency conditions, as previously described. DNA probes were labeled with 32P by the random primer method of Feinberg and Vogelstein. The ABL probe used was pHabl-5, a 0.85 kb BamHI-HindIII fragment containing sequences from ABL exon 1b. This recognizes a 175 kb Norl fragment containing most of the first intron of ABL, the region in which the majority of the CML translocations occur. The following chromosome 22 probes were used: 3′-bcr, a 1.3-kb HindIII-EcoRI fragment containing sequences from bcr exon 4; 5′-bcr, a 0.6-kb HindIII-BglII fragment of bcr containing sequences from bcr exon 2; and pAE-B-2.3, a 2.3-kb EcoRI-BamHI fragment that was cloned from the 9;22 translocation junction of the SUP-B13 cell line.

**Pulsed field gel electrophoresis.** PFGE was performed according to procedures described previously. Briefly, restriction digestions were carried out directly on the agarose plugs, which were then placed in 0.8% agarose gels with phage lambda multimers and intact yeast chromosomes used as size markers. Initially, the PFGE separations were performed using FIGE by the method of Carle et al27 and run for 24 hours at 12°C using a 200 volt inverting field. The switching cycle increased linearly from 0.9 seconds forward/0.3 seconds reverse to 60 seconds forward/20 seconds reverse while maintaining a 3:1, forward/reverse ratio, as described elsewhere. Later, the separations were performed with vertical PFGE using a TAFE apparatus (Geneline/Beckman, Palo Alto, CA). The initial 30 minutes were run at 330 volts and 180 mA with four-second alternating pulses. The next 18 hours were run at 250 volts and 140 mA. Pulse times during the 18 hour run were 20 seconds for the first six hours, followed by 40 seconds for the next six hours, followed by 60 seconds for the last six hours. Following electrophoresis, the gels were treated successively with 0.25 N HCl for 20 minutes, 0.4 N NaOH/0.6 mol/L NaCl for 90 minutes, and 1.5 mol/L NaCl/0.5 mol/L Tris-HCl (pH 7.5) for 60 minutes. The DNA was then

---

**METHODS**

**Patient selection.** The population consisted of cases of acute leukemia referred to the University of Chicago Hematology/Oncology Cytogenetics Laboratory. In these cases, excess bone marrow or blood not needed for cytogenetic analysis is routinely stored. Fifteen patients were identified who presented with an initial clinical picture consistent with acute lymphoblastic leukemia referred to the University of Chicago Hematology/Oncology Cytogenetics Laboratory. This group of patients with Ph1-positive ALL was investigated to detect the Ph1 chromosome that may become a useful adjunct to cytogenetics in ALL. We believe that this method will eventually have clinical utility for the detection of other chromosomal translocations in other malignancies.

---

**Fig 1.** Two molecular subtypes of the Philadelphia chromosome in ALL. (A) bcr-positive. The chromosome 22 breakpoints occur within the 5.8 kb bcr and produce a bcr rearrangement on Southern analysis. This subtype is identical to the translocations seen in CML. (B) bcr-negative subtype. Chromosome 22 breakpoints occur over a variable region 5′ of the bcr, and give rise to gene products that are distinct from those seen in CML. Southern analysis does not detect bcr rearrangements in this group. The diagram represents the structure of the genes at the regions surrounding the fusion of chromosome 9 and 22 sequences at the translocation breakpoint on the 22q-, or Philadelphia chromosome. Exons are depicted as boxes above the line; sequences from chromosome 22 are shown by closed boxes, sequences from chromosome 9 are shown by open boxes.
DETECTION OF PH' CHROMOSOME IN ALL

transferred to GeneScreen Plus membrane (New England Nuclear, Boston) according to the manufacturer’s recommendations, hybridized under high stringency conditions, and then autoradiographed using the same procedures as described for standard Southern hybridizations.

RESULTS

Patient population. The clinical features of the seven patients studied are shown in Table 1. The median age of the group was 33 years old, with a range from 6 to 52; two were children with ages of 6 and 12 years. All presented with an acute leukemia felt to be ALL based on morphologic appearance, negative myeloperoxidase staining, and positive terminal deoxynucleotidyltransferase staining. Following induction chemotherapy, two patients, no. 3 and 5, had their clinical diagnosis changed to “CML in lymphoid blast phase.” This retrospective change was undoubtedly biased by the original cytogenetic findings, and was based on the fact that their remission bone marrow biopsy specimens were hypercellular, had an increased myeloid:erythroid ratio with a shift toward immature forms, and retained a Ph' chromosome. Both patients’ courses consisted of several recurrences of an acute lymphoid leukemia with intervening remissions of several months duration before their eventual deaths from refractory acute leukemia.

Standard Southern analysis for translocations within BCR. To determine if a translocation occurred within the bcr, DNA isolated from the seven patient samples was digested with the restriction enzymes BglII, HindIII, BamHI, and EcoRI, electrophoresed, and hybridized to the 5'-bcr probe. As there are occasional deletions in this region that lead to a false-negative result with this probe,36 the blots without rearrangements were then re-hybridized to the 3'-bcr probe. These probes and enzymes are sufficient to detect breakpoints within bcr in most cases of CML. These studies demonstrated a rearrangement within bcr in two patients, no. 1 and 3 (Fig 2), and no rearrangements in the other five (Table 2). We also studied a region of the first intron of BCR where we have previously demonstrated that the SUP-B13 cell line has a chromosome 22 translocation. This is approximately 50 kb upstream of bcr.37 We used a probe, pAEB-2.3, which had been cloned from the SUP-B13 translocation junction, on standard Southern blots to determine if other breakpoints cluster in this region. This probe detects germ-line BamHI restriction fragments of 4.6 and 5.6 kb due to a 1.0-kb deletion/insertion polymorphism.33 Only one patient, no. 4, has a rearranged band seen with this probe with BamHI, which demonstrates a chromosome 22 breakpoint within 4 kb of the SUP-B13 breakpoint (Fig 3). This patient has been reported previously.30

Analysis with PFGE. The BCR gene was analyzed by hybridization of NotI digests of DNA separated by PFGE, using as probes either 3'-bcr or pAEB-2.3. When these probes are hybridized to PFGE blots containing DNA from control ALL cells or lymphoid cell lines without a Ph' chromosome, germine fragments of 450 and/or 1,600 kb are observed. This is due to a polymorphic NotI site upstream of the BCR gene.35 Hybridization of these probes to the NotI digests of DNA isolated from the bone marrow samples of the seven Ph'-positive ALL patients in this study revealed the presence of rearrangements in all seven (Fig 4, Table 3). These blots were re-hybridized to the pHabl-5' probe (Table 3). Rearranged bands were seen in all patients studied.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Presenting Dx</th>
<th>Dx After Rx</th>
<th>Response (mo)</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>M</td>
<td>ALL</td>
<td>ALL</td>
<td>CR (2)</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>M</td>
<td>ALL</td>
<td>ALL</td>
<td>CR (5)</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>M</td>
<td>ALL</td>
<td>CML-LBP</td>
<td>CR (12)</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>M</td>
<td>ALL</td>
<td>ALL</td>
<td>No CR</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>F</td>
<td>ALL</td>
<td>CML-LBP</td>
<td>CR (9)</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>F</td>
<td>ALL</td>
<td>ALL</td>
<td>No CR</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>M</td>
<td>ALL</td>
<td>ALL</td>
<td>CR (12)</td>
<td>14</td>
</tr>
</tbody>
</table>

Abbreviations: Dx, diagnosis; Rx, induction chemotherapy; CML-LBP, chronic myelogenous leukemia in lymphoid blast phase; CR, complete remission after induction chemotherapy; No CR, complete remission never obtained.

Table 2. Southern Analysis for bcr Rearrangement

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Rearrangements Using bcr Probes (5'-bcr/3'-bcr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HindIII</td>
</tr>
<tr>
<td>1</td>
<td>-/+</td>
</tr>
<tr>
<td>2</td>
<td>-/-</td>
</tr>
<tr>
<td>3</td>
<td>+/-</td>
</tr>
<tr>
<td>4</td>
<td>-/-</td>
</tr>
<tr>
<td>5</td>
<td>-/-</td>
</tr>
<tr>
<td>6</td>
<td>-/-</td>
</tr>
<tr>
<td>7</td>
<td>ND/ND</td>
</tr>
</tbody>
</table>

Abbreviations: +, rearrangement; -, no rearrangement; ND, not done.

![Fig 2. Southern analysis for bcr rearrangements. (A) Southern blot of DNA from control cell line (c) and patients no. 1 and 6 digested with HindIII, and then hybridized to 3'-bcr probe. (B) Southern blot of DNA from control cell line (c) and patients no. 4 and 3 digested with EcoRI, and then hybridized to 3'-bcr probe. Rearranged bands are shown by arrows. Size markers are in kilobases.](image-url)
Fig 3. Southern analysis for rearrangements with pAEB-2.3. Southern blot of DNA from control cell line (c) and patients no. 6, 4, and 5 digested with BamHI and hybridized to pAEB-2.3. The rearranged band is shown by the arrow. Size markers are in kilobases.

Table 3. Sizes of NotI Restriction Fragments

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>3'-bcr</th>
<th>pHabl-5'</th>
<th>pAEB-2.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>1</td>
<td>1,600</td>
<td>375</td>
<td>175</td>
</tr>
<tr>
<td>2</td>
<td>450</td>
<td>400</td>
<td>175</td>
</tr>
<tr>
<td>3</td>
<td>450</td>
<td>335</td>
<td>175</td>
</tr>
<tr>
<td>4</td>
<td>1,600</td>
<td>470</td>
<td>175</td>
</tr>
<tr>
<td>5</td>
<td>450</td>
<td>350</td>
<td>175</td>
</tr>
<tr>
<td>6</td>
<td>450</td>
<td>460</td>
<td>175</td>
</tr>
<tr>
<td>7</td>
<td>450</td>
<td>380</td>
<td>175</td>
</tr>
</tbody>
</table>

All sizes are given in kilobases.

Abbreviations: G, germline fragment size; R, rearranged fragment size.

demonstrating that the breakpoints all fall within the first intron of ABL. As Table 3 shows, in each case the rearranged band seen with the 3'-bcr probe cross-hybridized to the pHabl-5' probe, demonstrating a fusion of the 3' part of BCR to the 5' part of ABL; an example of this is shown in Fig 5A and B. While in all cases we are seeing evidence of the t(9;22), what is being detected by the 3'-bcr and pHabl-5' probes is the 9q+ chromosome, which is the reciprocal translocation partner of the Ph', or 22q-, chromosome. The fusion of the 5' part of BCR to the 3' part of ABL, which gives rise to the p210BCR-ABL and the p190BCR-ABL, actually occurs on the Ph1 chromosome; however, this is difficult to demonstrate directly using NotI because the rearranged bands are very large and difficult to resolve from the 1,600-kb germline BCR band. Also, as there is a NotI site between most ABL breakpoints and the available chromosome 9 probes, the demonstration of linkage of ABL to BCR on the Ph1 chromosome is difficult. Use of other infrequent cutting restriction enzymes is also problematic. The only enzyme for which a restriction map is available is SfiI, and it generates too many small fragments in this region for it to be useful.

These blots were then re-hybridized to pAEB-2.3 (Table 3). An example of this is shown in Fig 5C. Patients no. 1, 2, and 3 had rearranged bands seen that were different in size than those seen with the 3'-bcr probe, indicating that the chromosome 22 breakpoint is between 3'-bcr and pAEB-2.3.

Fig 4. PFGE analysis for rearrangement of chromosome 22. PFGE analysis of DNA from seven patients digested with NotI, electrophoresed on either TAFE (patients no. 3, 4, 6, and 7) or FIGE apparatus (patients no. 1, 2, and 5), transferred and hybridized as described to either 3'-bcr (patients no. 1, 2, 5, and 7) or pAEB-2.3 (patients no. 3, 4, and 6). Size markers are in kilobases.
DETECTION OF PH\(^+\) CHROMOSOME IN ALL

Fig 5. PFGE analysis for rearrangements of chromosomes 9 and 22. PFGE analysis of DNA from patients no. 3 and 4 digested with Nod, electrophoresed on TAFE apparatus, transferred and hybridized as described to the following probes: (A) 3'-bcr, (B) pHabl-5', and (C) pAEB-2.3. Rearranged bands are shown by arrows. Size markers are in kilobases.

(Patient no. 1 had only one band seen with this probe. As we have demonstrated that this patient has a breakpoint within the bcr, we presume this represents a rearranged band of a size difficult to resolve from the germline band under these conditions.) Patient no. 4 previously was seen to have a chromosome 22 breakpoint in the region of pAEB-2.3. Patients no. 5, 6, and 7 had rearranged bands seen with pAEB-2.3 that were the same size as those seen with 3'-bcr, indicating that the translocation breakpoints are upstream of both pAEB-2.3 and 3'-bcr. The sizes of the rearranged bands seen with both the 3'-bcr and pAEB-2.3 probes in patients no. 5, 6, and 7 were 350 kb, 460 kb, and 380 kb, respectively. Based on what is known about the BCR gene, we can construct a map of it as shown in Fig 6.\(^{18,19}\) As the distance from pAEB-2.3 to the 3' Nod site is 350 kb, and as the first intron of BCR is a minimum of 20 kb 5' of pAEB-2.3, rearranged Nod restriction fragments that are 350 to 370 kb in size and include pAEB-2.3 must be associated with a breakpoint in the first intron of BCR. Patient no. 5 has a rearranged band of 350 kb, which is evidence that the translocation is in the first intron of BCR. Patient no. 7 has a rearranged band of 380 kb. This represents a chromosome 22

![Fig 6. Location of translocation breakpoints within the BCR gene. The top line is a map of the BCR gene on chromosome 22.\(^{22}\) Exons are shown as dark boxes above the line, and restriction sites are shown by vertical lines: N, Nod; E, EcoRI. The exons within the BCR are numbered 1-6.\(^{22}\) The most 5' known exon of the BCR gene is indicated here as the first exon, to be distinguished from exon 1 of the bcr. The locations of the probes used for this study are indicated by the arrows. The middle line shows relative distances within the BCR gene, based on our PFGE mapping.\(^{18,19}\) Below the map are shown the regions of chromosome 22 breakpoints for the individual patients examined in this series, identified to the right by patient number.](/).
breakpoint that can occur in an area that includes the first intron of \( BCR \) and up to 10 kb 5' of \( BCR \). Patient no. 6 has a rearranged band of 460 kb; similarly, the rearrangement occurred either within the \( BCR \) gene or up to 110 kb upstream of it. Thus, of the seven cases of Ph'-positive ALL we studied, five definitely have breakpoints within the \( BCR \) gene, while in two it is either within the \( BCR \) gene or a short distance upstream of it. The location of all chromosome 22 breakpoints is shown at the bottom of Fig 6.

**DISCUSSION**

Our study of seven patients with Ph'-positive ALL reveals that only two patients had DNA rearrangements detectable by Southern analysis of the \( bcr \) similar to those seen in CML. On the other hand, all seven patients had rearrangements of the \( ABL \) gene, and all seven had rearrangements either within or very close to the \( BCR \) gene, as detected by PFGE. Thus, the PFGE method was not only equally sensitive as cytogenetics in this small sample, but also gives additional information regarding molecular structure of the Ph' chromosome that is beyond the resolution of cytogenetics. Based on this limited series, we can suggest that PFGE analysis of DNA digested with one enzyme (\( NcoI \)) and two probes (from \( BCR \) and \( ABL \)) will be as useful for the diagnosis of the Ph' chromosome in ALL as Southern blot analysis for \( bcr \) rearrangement is for the diagnosis of CML.

Recently another method of detection of the Ph' chromosome has been described based on the polymerase chain reaction. Our application of this method to a group of 11 patients with Ph'-positive ALL, including several patients in this series, suggested a high false negative rate: two of 11 cases had no detectable fusion mRNA, including patient no. 4 of this series. Although the polymerase chain reaction is clearly more sensitive in ability to detect minimal disease, in our hands it is not as reliable as PFGE. The two methods may provide complementary information in the clinical management of Ph'-positive ALL. We believe that the PFGE technique will eventually have general applicability as an adjunct to cytogenetics for the detection of the other leukemia-associated translocations in addition to the \( t(9;22) \) as probes become available that can detect these anomalies.

The ability to separate patients into subgroups on the basis of a biologically relevant variable, such as the molecular origin of the leukemia, will have increasingly important clinical implications. For example, the presence of the Ph' chromosome in ALL is in itself a predictor of a poor prognosis, and might be helpful in making therapeutic decisions. One must be cautious, however, in using the results of molecular studies for patient management until the appropriate clinical-molecular correlations have been made in a well-controlled, prospective fashion. Thus, the clinical significance of the presence or absence of a \( bcr \) rearrangement in Ph'-positive ALL is not known. It has been suggested that Ph'-positive ALL patients with a \( bcr \) rearrangement have CML that has presented during blast crisis with a lymphoid phenotype following a silent chronic phase, and that the patients without a \( bcr \) rearrangement have de novo ALL.

There is no clinical data available to support this contention, however, nor does our study clarify this issue. In fact, some of our cases merely reflect the strong clinical bias that is associated with the detection of a Ph' chromosome. For example, patient no. 5 had a retrospective change of diagnosis to CML, yet the molecular findings were not consistent with the production of the \( p210^{BCR-ABL} \) that is characteristic of CML because the downstream exons of the \( bcr \) are not present on the Ph' chromosome. This case illustrates the difficulty of retrospective diagnosis of Ph'-positive ALL: there is yet no good standard by which to decide whether a patient has CML in lymphoid blast phase or de novo ALL at the time of presentation, or even whether such distinctions imply different prognoses or clinical course. We cannot rely on the molecular findings to make these distinctions until prospective clinical correlations have been made.

It is likely that many factors interact to produce the variety of hematologic malignancies that are associated with a Ph' chromosome. One factor may be related simply to the nature of the cell of origin of the malignancy. Another factor may be acquired mutations in the cells in addition to a Ph' chromosome. This study demonstrates that we now have the ability to investigate one factor, the structure of the Ph' chromosome, on a more fundamental level than has previously been possible. We have shown that the two distinct molecular subtypes of the Ph' chromosome that exist in ALL can be detected using PFGE. The identification of these subtypes may have a role in the diagnosis of leukemia, and these methods may find a role in the clinical management of patients with leukemia. Further studies with large numbers of patients should enable us to correlate the clinical features of these leukemias with the molecular abnormalities that underlie them. This increased understanding should translate into improvements in our ability to both diagnose and treat these diseases.

**ACKNOWLEDGMENT**

We are indebted to Dr David Leibowitz for the \( 3'-bcr \) and \( 5'-bcr \) probes, Dr Michelle Le Beau for providing cytogenetic analysis and cells from the patients, Michelle Rebelsky for expert assistance with pulsed-field gels, Dr Carol Cech for technical advice on the use of the TAFE system, Dr Janet Rowley for helpful advice and encouragement, and Dr Harvey Golomb for overall support and enthusiasm for this work.

**REFERENCES**

DETECTION OF PH\textsuperscript{1} CHROMOSOME IN ALL


13. Bloomfield CD, Peterson LC, Yunis JJ, Brunning RD: The Philadelphia chromosome (Ph\textsuperscript{1}) in adults presenting with acute leukemia: A comparison of Ph\textsuperscript{1}+ and Ph\textsuperscript{1}− patients. Br J Haematol 36:347, 1977


21. Clark SS, McLaughlin J, Crist WM, Champlin R, Witte ON: Unique forms of the abl tyrosine kinase distinguish Ph\textsuperscript{1}-positive CML from Ph\textsuperscript{1}-positive ALL. Science 235:85, 1987


30. Popenoe DW, Schaffer-Rego K, Mears JG, Banks A, Leibowitz D: Frequent and extensive deletion during the 9,22 translocation in CML. Blood 68:1123, 1986


Detection of the Philadelphia chromosome in acute lymphoblastic leukemia by pulsed-field gel electrophoresis

AL Hooberman, CM Rubin, KP Barton and CA Westbrook