Expression of c-abl in Philadelphia-Positive Acute Myelogenous Leukemia

By Razelle Kurzrock, Mordechai Shtalrid, Moshe Talpaz, William S. Kloetzer, and Jordan U. Gutterman

The identical cytogenetic marker, t(9;22)(q34;q11) (Philadelphia [Ph] translocation), is found in approximately 90%, 20%, and 2% of adult patients with chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), and acute myelogenous leukemia (AML), respectively. In CML, the molecular events resulting from the Ph translocation include a break within the bcr locus on chromosome 22, transfer of the c-abl protooncogene from chromosome 9 to 22, and formation of an aberrant 210-kD bcr-abl fusion protein (p210\textsuperscript{abl}). Recently, the absence of bcr rearrangement and expression of a distinct aberrant 190-kD abl protein (p190\textsuperscript{abl}) has been described in Ph-positive ALL, with the suggestion that the two abl variants may be pathogenetically associated with myeloid v lymphoid leukemogenesis. Here we report that the genomic configuration and translation product of Ph-positive AML can be similar to that of Ph-positive ALL: the break at 22q11 may occur outside the 5.8 kb bcr region and result in expression of a 190-kD abl protein lacking these bcr sequences. Phosphokinase enzymatic activity, a fundamental property of p210\textsuperscript{abl}, was also associated with AML-derived p190\textsuperscript{abl}. Our current observations indicate that p190\textsuperscript{abl} can be found in cells of lymphoid or myeloid lineage and is therefore unlikely to play a specific role in the development of lymphoid leukemias. Formation of p190\textsuperscript{abl} instead of p210\textsuperscript{abl} appears to be a characteristic of the acute rather than the chronic Ph-positive leukemic state.

© 1987 by Grune & Stratton, Inc.

From the Departments of Clinical Immunology and Biological Therapy and Hematology, M.D. Anderson Hospital and Tumor Institute at Houston; and the Johnson and Johnson Biotechnology Center at San Diego.


Dr Gutterman is a Senior Clayton Foundation Investigator.

Address reprint requests to Razelle Kurzrock, M.D., M.D. Anderson Hospital and Tumor Institute, Department of Clinical Immunology and Biological Therapy, P.O. Box 41, 1515 Holcombe, Houston, TX 77030.

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. § 1734 solely to indicate this fact.

Case report. In May 1986, patient VI presented with Ph-positive AML. Her past medical history included a diagnosis of carcinoma of the right breast in 1965. She underwent mastectomy and postoperative chest wall irradiation (3,000 rad). In 1970, a left breast carcinoma was found and subsequently removed by mastectomy. She returned to M.D. Anderson Hospital and Tumor Institute every 4 to 6 months for follow-up evaluation. As part of each visit, a CBC was done. In January 1986, the blood counts were normal: WBC count, 6 \times 10^9/\mu L with a normal differential count; hemoglobin, 12.7 g/dL; platelet count, 226 \times 10^9/\mu L. At the time of her routine return appointment in May 1986, she felt well. There was no evidence of recurrent breast cancer. Surprisingly, laboratory tests revealed a grossly elevated WBC count of 179 \times 10^9/\mu L with 94% blasts. Her hemoglobin content was 8.1 g/dL; platelet count, 28 \times 10^9/\mu L; and leukocyte alkaline phosphatase score, 5% (normal...
C-ABL IN Ph+ AML

A bone marrow aspirate and biopsy specimen revealed a cellularity of 95% with 84% blasts. There was no peripheral blood or bone marrow basophilia. The majority of the bone marrow cells stained positive for myeloperoxidase, consistent with a diagnosis of AML. Reactivity with monoclonal antibody against the differentiation marker My4 was about 50% positive. Cell surface markers for OKTI I (T cell surface marker) and surface immunoglobulin (B cell marker) were negative. These results as well as morphological analysis of the bone marrow by light and electron microscopy indicated a diagnosis of M4 (myelomonocytic) subtype of AML (French-American-British classification). Karyotype analysis (Giemsa-stained chromosomes) revealed a hyperdiploid (51 to 87 chromosomes) Ph-positive clone in 25 of 25 (100%) bone marrow metaphases. The patient was treated with high-dose cytosine arabinoside and achieved complete hematologic remission after one course. However, the aberrant cytogenetic clone persisted in 10% of the cells. The patient remains in hematologic remission at 6 months. Blood for molecular studies was obtained immediately before initiating chemotherapy and stored at -70°C until analysis. Informed consent, in accordance with institutional guidelines, was obtained for chemotherapeutic procedures and for drawing blood.

Antisera. Rabbit antisera were made against the following peptides: the predicted hydrophobic v- abl sequence DEV-KELGKRTRGGC (abl389-403); the predicted high-term potential domain of c- abl (Chou-Fasman predictive model for protein secondary structure, KENNLAPSENIDN-C (abl-3)); the region DDESPGGLGFLNV-C (bcr3) within bcr exon 3; and the NH2-terminal peptide sequence 1 to 17 derived from the Heisterkamp et al15 bcr sequence (bcr1). The methodology has been previously reported in detail by Kloetzer et al.7

DNA analysis. DNA was prepared by cell lysis, proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. DNA was analyzed by restriction enzyme digestion, Southern blotting, and hybridization of filters to nick-translated probes. The methodology was identical to that previously used in our laboratory.12 A 3', 1.2-kb bcr (HindIII-BgII) plasmid probe (Oncogene Sciences, Inc, Mineola, NY)7 and a 5', 0.45-kb (EcoRI/PstI fragment) bcr CDNA probe (donated by E. Canaan)14 were used.

Immune complex kinase assays. Proteins from blood samples were analyzed by the immune complex kinase assay (ICKA) under identical conditions to those previously described by our laboratory.12 K562 cells (CML erythroid blast crisis cell line) were assayed as controls.

RESULTS

Analysis of leukemia cells for bcr rearrangement. To determine whether bcr rearrangement was present in patient samples, we performed Southern blotting and hybridization with a 3', 1.2-kb (HindIII-BgII) genomic bcr probe. Hybridization of plasmid DNA with restriction enzymes BamHl-, BglII, HindIII-, and EcoRI-digested DNA did not reveal rearrangement within bcr in Ph-positive AML patient V1 (Fig 1, even lanes). In contrast, Fig 1 (odd lanes) illustrates rearranged bands in BamHl-, BglII, and EcoRI-digested, Ph-positive, CML-derived DNA. Because a DNA deletion in the segment encompassing the sequence of the 3' bcr probe could result in a false-negative result for bcr rearrangement in AML patient V1,16 we probed the filters with a 5' (EcoRI/PstI fragment) bcr probe.16 Rearrangement in bcr was not observed (data not shown).

Analysis of c-abl protein expression. To study c-abl protein expression, we perform ICKAs. This assay can detect abl-related proteins because they are enzymatically active as phosphokinases. Consistent with our previously published results,5,12 anti- abl389-403 serum and anti-bcr3 serum detected p210bcr-abl in extracts from the K562 CML blast crisis cell line (Fig 2A, lanes 1 and 3). To demonstrate that p210 is bcr-abl specific, we preincubated each of the antisera with the peptide against which they were made (Fig 2A, lanes 2 and 4). This procedure results in blocking of antisera-specific bands but not nonspecific background phosphorylation. To date, we have studied fresh samples from 16 Ph-positive CML blast crisis patients (myeloid blast crisis, ten patients; lymphoid, five patients; undifferentiated, one patient); all patients produced p210bcr (Fig 2A, lanes 5 and 6; data not shown).19 We have recently demonstrated that a Ph-positive ALL patient without bcr rearrangement expresses a distinct aberrant p190bcr-abl rather than p210bcr-abl.12 The mRNA encoding p190bcr lacks at least some bcr sequences and is smaller (7.4 kb) than the 8.5-kb CML-derived bcr-abl transcript.12 As shown in Fig 2A (lanes 7 and 9), bcr rearrangement–negative AML patients V1 does not produce p210bcr-abl despite the presence of the Ph translocation; however, a 190-kD abl-related protein is detected by two anti-abl sera (Fig 2A, lane 7, and Fig 2B, lane 1). Figure 2B (lanes 1 and 3) demonstrates that the abl-related product of AML patient V1 comigrates with the p190bcr-abl of a Ph-positive bcr rearrangement–negative ALL patient.12 Similar to the situation in ALL,12 AML-derived p190bcr-abl is not detected by anti-bcr3 serum directed against bcr exon 3 (Fig 2A, lanes 9 and 10) or by anti-bcr1 serum directed against the most 5' portion of the Heisterkamp bcr sequences15 (data not shown).

A 190,000–molecular weight (mol wt) band is also observed in K562 cells (Fig 2A, lanes 1 and 3). This band may represent a second translational product of bcr-abl or a proteolytic fragment of p210bcr-abl. The p190bcr-abl of AML patient V1 is distinct from the 190,000–mol wt band of Ph-positive CML cells because anti-bcr serum clearly recog-
antiserum used was and 2. Ph-positive.


AML patient. Antisera used were as follows: lanes 1

Ph-negative

1 0. anti-bcr3. Blocking and antisera with cognate peptide was performed in lanes 2. 4. 8. 1 0. and 1 2. (B) Cells used were as follows: lanes 1

chronic phase. Even so, it seems likely that at least some

on these observations, it has been postulated that the disease

which escaped diagnosis during its early, often asymptomatic

actually represents the blast transformation stage of CML,

Further, occasional chemotherapy-induced transition of Ph-

positive ALL and AML to CML is well documented.2' Based

on these observations, it has been postulated that the disease

of some patients presenting with Ph-positive acute leukemia

actually represents the blast transformation stage of CML,

which escaped diagnosis during its early, often asymptomatic chronic phase. Even so, it seems likely that at least some

ALL and AML patients with t(9;22) have a bona fide acute presentation. Because Ph-positive acute leukemia and CML

are similar but retain some distinct features, it may be that related but not identical molecular events are involved in their pathogenesis.

Interestingly, some ALL patients carrying a t(9;22), which is cytogenetically indistinguishable from that found in CML, do not have bcr rearrangement.9'11 Our work12 as well as that of other groups10,14 has revealed a novel c-abl protein—p1900′—in Ph-positive, bcr rearrangement-negative ALL patients; p2100′ is not expressed. In the current report, we demonstrate that the Ph translocation in AML can also be associated with a 22q11 break outside of the bcr locus and production of p1900′ without an accompanying p2100′. Our data suggest that p1900′ differs from p2100′ by virtue of the absence of bcr sequences in the N-terminal of the former protein. Similar to the situation in ALL,12 AML-derived p1900′ is enzymatically active as a protein kinase, an observation consistent with a direct role for this aberrant protein in the malignant process. A literature review (Table 1) revealed that about 95% of Ph-positive CML patients (including at least 20 blast crisis patients)22 but fewer than 50% of Ph-positive acute leukemia patients have bcr rearrangement. Further, p2100′ appears to be expressed in all chronic9 and acute leukemia patients12 with bcr rearrangement. Although the two molecular subgroups of Ph-positive acute leukemia have similar clinical and morphological features,10,11 their distinct genomic configurations may have biologic relevance. The presence of bcr rearrangement makes Ph-positive acute leukemia virtually indistinguishable from CML blast crisis20 and suggests a single disease state, with the acute leukemia representing the blast transformation phase of subclinical CML. Conversely, Ph-positive bcr rearrangement-negative ALL and AML may reflect true de novo acute leukemia as evidenced by the following: (a) bcr rearrangement negativity is almost never found in CML and (b) p1900′ without p2100′ appears to be associated exclusively with the the acute leukemia phenotype. This concept is supported by our current observations because a preceding undetected CML state can be ruled out in our Ph-positive, bcr rearrangement-negative AML patient because of the fortuitous availability of normal blood counts obtained 4 months before diagnosis. Analysis of additional patients will be needed to strengthen the generality of these concepts. However, our data suggest that the presence or absence of proximal bcr sequences attached to c-abl may modulate this gene's tumorigenic potential for chronic c acute leukemogenesis.

Despite the presence of p1900′ instead p2100′, the subgroup of bcr rearrangement-negative, Ph-positive acute leukemia patients bears a close clinical resemblance to bcr rearrangement–positive, Ph-positive acute leukemia patients,10,11 and as mentioned earlier, both subgroups resemble CML blast crisis.20 The molecular events leading to the inevitable malignant evolution of CML from a chronic to an acute leukemia phenotype remain largely unexplored; however, because bcr rearrangement is found in the early benign

**REFERENCES**


### Table 1. Summary of Studies Analyzing Ph-Positive Leukemia Patients for bcr Rearrangement

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>Source</th>
<th>No. of Patients Analyzed</th>
<th>No. of Patients With bcr Rearrangement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>Groffen et al&lt;sup&gt;3&lt;/sup&gt;</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Popnose et al&lt;sup&gt;10&lt;/sup&gt;</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Collins&lt;sup&gt;73&lt;/sup&gt;</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Shalirid et al&lt;sup&gt;23&lt;/sup&gt;</td>
<td>72</td>
<td>70</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td><strong>123</strong></td>
<td><strong>117 (95)</strong></td>
</tr>
<tr>
<td>ALL</td>
<td>Rodenhuis et al&lt;sup&gt;8&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Erikson et al&lt;sup&gt;10&lt;/sup&gt;</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>de Klein et al&lt;sup&gt;11&lt;/sup&gt;</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Kurzrock et al&lt;sup&gt;12,24&lt;/sup&gt;</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Clark et al&lt;sup&gt;13&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chan et al&lt;sup&gt;14&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td><strong>35</strong></td>
<td><strong>14 (40)</strong></td>
</tr>
<tr>
<td>AML</td>
<td>Erikson et al&lt;sup&gt;10&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kurzrock et al (current report)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td><strong>2</strong></td>
<td><strong>1 (50)</strong></td>
</tr>
</tbody>
</table>


Expression of c-abl in Philadelphia-positive acute myelogenous leukemia

R Kurzrock, M Shtalrid, M Talpaz, WS Kloetzer and JU Gutterman

Updated information and services can be found at:
http://www.bloodjournal.org/content/70/5/1584.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml