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\(^{bcr-abl}\)). Recently, the absence of bcr rearrangement and expression of a distinct aberrant 190-kD abl protein (p190\(^{abl}\)) has been described in Ph-positive ALL, with the suggestion that the two abl variants may be pathogenetically associated with myeloid vs lymphoid leukemogenesis. Here we report that the genomic configuration and translation product of Ph-positive AML can be similar to that of Ph-negative 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\(^{bcr-abl}\), was also associated with AML-derived p190\(^{abl}\). Our current observations indicate that p190\(^{abl}\) 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\(^{abl}\) instead of p210\(^{bcr-abl}\) appears to be a characteristic of the acute rather than the chronic Ph-positive leukemic state.

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range, 40% to 225%). 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 OKT11 (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 chemotherapy procedures and for drawing blood.

Antibodies. Rabbit antibodies were made against the following peptides: the predicted hydrophobic v-abl sequence DEVE-KELGKRGTRGC-C (abl389-403); the predicted high-term potential domain of c-abl (Chou-Fasman predictive model for protein secondary structure, KENLALPSDENPN-C (abl)-3; the region DDESGLYGFNLN-C (bcr3) within bcr exon 3; and the NH2-terminal peptide sequence 1 to 17 derived from the Heisterkamp et al.5 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-BglII) 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.15 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-BglII) genomic bcr probe. Hybridization of blots of BamHI-, 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 BamHI-, 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 V1,12 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 p210(Ph+)- related 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 p190(Ph+) rather than p210(Ph+).12 The mRNA encoding p190 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 p210(Ph+) 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 p190(Ph+) of a Ph-positive bcr rearrangement–negative ALL patient.12 Similar to the situation in ALL,12 AML-derived p190(Ph+) is not detected by anti-bcr3 serum directed against bcr exon 3 (Fig 2A, lanes 9 and 10) or by anti-bcr serum directed against the most 5′ portion of the Heisterkamp bcr sequences12 (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 p210(Ph+). The p190(Ph+) of AML patient V1 is distinct from the 190,000–mol wt band of Ph-positive CML cells because anti-bcr serum clearly recog-
nizes the latter protein (Fig 2A, lanes 3 and 6). Of course, because of their similar mol wts, we cannot rule out the possibility that CML patients produce p190<sub>abl</sub> in addition to p190<sub>bcrid</sub>. Similarly, the 170,000-mol wt band in Ph-positive ALL cells (Fig 2B, lane 3) and the 160,000–mol wt protein in Ph-positive AML patient V1 (Fig 2B, lane 1) may also represent proteolytic fragments or additional translational modifications of the abl protein.

**DISCUSSION**

The end stage of CML, termed blast crisis, strongly resembles Ph-positive acute leukemia: the leukemic cells lose their capacity for terminal differentiation, and similar clinical characteristics and laboratory parameters are observed. Further, occasional chemotherapy-induced transition of Ph-positive ALL and AML to CML is well documented. 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. Our work<sup>17</sup> as well as that of other groups<sup>19</sup> has revealed a novel c-abl protein—p190<sub>bcrid</sub>—in Ph-positive, bcr rearrangement–negative ALL patients; p210<sub>bcrid</sub> is not expressed. In the current report, we demonstrate that the Ph tranlocation in AML can also be associated with a 22q11 break outside of the bcr locus and production of p190<sub>bcrid</sub> without an accompanying p210<sub>bcrid</sub>. Our data suggest that p190<sub>bcrid</sub> differs from p210<sub>bcrid</sub> by virtue of the absence of bcr sequences in the N-terminal of the former protein. Similar to the situation in ALL, AML-derived p190<sub>bcrid</sub> 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)<sup>22</sup> but fewer than 50% of Ph-positive acute leukemia patients have bcr rearrangement. Further, p210<sub>bcrid</sub> appears to be expressed in all chronic<sup>19</sup> and acute leukemia patients<sup>12</sup> with bcr rearrangement. Although the two molecular subgroups of Ph-positive acute leukemia have similar clinical and morphological features, their distinct genomic configurations may have biologic relevance. The presence of bcr rearrangement makes Ph-positive acute leukemia virtually indistinguishable from CML blast crisis<sup>26</sup> 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) p190<sub>bcrid</sub> without p210<sub>bcrid</sub> appears to be associated exclusively with 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 v acute leukemogenesis.

Despite the presence of p190<sub>bcrid</sub> instead p210<sub>bcrid</sub> 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<sup>10</sup> and as mentioned earlier, both subgroups resemble CML blast crisis. 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
phase, it seems likely that superimposed secondary genetic events are required. If expression of p190\(^{c-abl}\) without p210\(^{bcr}\) is associated with an acute leukemic state remarkably similar to CML blast crisis, is it possible that the production of p190\(^{c-abl}\) in addition to p210\(^{bcr}\) occurs during the course of CML and plays a role in blast transformation? Further analysis of c-abl expression in the nosological spectrum of Ph-positive hematologic malignancies is in progress and should help elucidate the role of molecular variants of this gene on leukemogenesis.

**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>
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<tbody>
<tr>
<td>CML</td>
<td>Groffen et al(^3)</td>
<td>17</td>
<td>17</td>
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<tr>
<td>Popoee et al(^8)</td>
<td>14</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Collins(^2)</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Shalad et al(^2)</td>
<td>72</td>
<td>70</td>
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</tr>
<tr>
<td>Totals</td>
<td></td>
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<td>ALL</td>
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<td>0</td>
</tr>
<tr>
<td>Erikson et al(^10)</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>de Klein et al(^11)</td>
<td>14</td>
<td>9</td>
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<td>Kurzrock et al(^12,24)</td>
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<tr>
<td>Clark et al(^13)</td>
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</tr>
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<td>Chan et al(^14)</td>
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<td>1</td>
<td></td>
</tr>
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<td>Totals</td>
<td></td>
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<td>14 (40)</td>
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<tr>
<td>AML</td>
<td>Erikson et al(^10)</td>
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<td>1</td>
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<tr>
<td>Kurzrock et al (current report)</td>
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<td>1</td>
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<tr>
<td>Totals</td>
<td></td>
<td>2</td>
<td>1 (50)</td>
</tr>
</tbody>
</table>

13. Clark SS, McLaughlin J, Crist WM, Champlin T, Witte ON: Unique forms of the abl tyrosine kinase distinguish Ph\(^1\)-positive CML from Ph\(^1\)-positive ALL. Science 235:85, 1987


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