Coexistence of Inversion 16 and the Philadelphia Chromosome in Acute and Chronic Myeloid Leukemias

Report of Six Cases and Review of Literature

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Key Words: Chronic myelogenous leukemia; Acute myeloid leukemia; t(9;22); BCR-ABL; inv(16); CBFβ-MYH11

DOI: 10.1309/F0MX5CL8CEDY3W86

Abstract

We report 5 cases of chronic myelogenous leukemia (CML) and 1 case of acute myeloid leukemia (AML) with the dual presence of t(9;22) and inv(16). The 6 patients were 5 men and 1 woman with a median age of 42.5 years. All cases were BCR-ABL+ with p210 products detected in all CML cases and a p190 product detected in the AML case. An increase in bone marrow eosinophils was detected in 3 of 5 cases, and abnormal eosinophils were identified in these 3 cases. The CBFβ-MYH11 fusion gene was confirmed in all 3 CML cases and the 1 AML case tested, and this correlated with the presence of abnormal eosinophils with coarse basophilic granules. Of 5 patients with CML, 4 had a rapid transformation to myeloid accelerated phase of blast crisis. The coexistence of t(9;22) and inv(16) in CML seems to correlate with more rapid transformation.

The coexistence of the Philadelphia chromosome (Ph+) and an inversion of chromosome 16 [inv(16)] in patients with chronic myelogenous leukemia (CML) and acute myeloid leukemia (AML) is rare, and only limited numbers of cases are reported.1-7 However, the presence of chromosome 16 abnormalities, including inv(16)(p13q22) and t(16;16)(p13q12), in AML is relatively common and is found in 10% to 12% of all cases of AML. AML with the aforementioned chromosome 16 abnormalities has been defined as a distinctive morphologic subtype of AML8,9 and is designated as M4Eo in the French-American-British Cooperative Group classification and AML with inv(16)(p13q22) in the World Health Organization classification.10,11

Inversion of chromosome 16 usually involves the smooth muscle myosin heavy chain (MYH11) gene at 16p13 and the core binding factor β (CBFβ) gene at 16q12, forming a fusion gene identified as CBFβ-MYH11.12,13 The CBFβ is a heterodimeric transcription factor known to bind the enhancers of various murine leukemia viruses and similar motifs in the enhancers of T-cell receptor genes.14 AML with inv(16) is reported to predict a more favorable clinical course, with better response to chemotherapy and longer remission and survival.15,16

There also have been a few case reports of the coexistence of the inv(16) and Ph in patients with de novo AML and CML.1-3 Most of the de novo AMLs with chromosome abnormalities of t(9;22) and inv(16) have as favorable a prognosis as simple AML with inv(16) alone.17 However, the coexistence of t(9;22) and inv(16) in CML seemed to indicate an unfavorable prognosis in the limited number of reported cases,1,3,5 but the number of cases in the literature is small. In this report, we describe 5 cases of CML and 1 of AML with
the dual presence of t(9;22) and inv(16). An overview of similar cases reported in the literature also is given.

Materials and Methods

We identified 6 cases of Ph+/inv(16)+ leukemia from the cytogenetic files of the Division of Pathology, City of Hope National Medical Center, Duarte, CA, from January 1990 through July 2004. All available material, including peripheral blood smears, touch preparations, bone marrow aspirate smears and core biopsy specimens, cytochemical stains, immunophenotypic data, molecular findings, and clinical information also were reviewed, but all of these elements were not available for all cases. Cases of CML were further classified as chronic phase (CP), accelerated phase (AP), or blast crisis (BC) according to criteria of the World Health Organization classification of neoplastic diseases of hematopoietic and lymphoid tissue.11

Flow cytometric analysis was performed for immunophenotyping of involved peripheral blood or bone marrow in 4 cases using previously described methods. Briefly, flow cytometric analysis of bone marrow aspirate or peripheral blood specimens was performed, initially with an EPICS 5 (Coulter, Hialeah, FL) and later with a FACScan instrument (Becton Dickinson, Mountain View, CA). After mononuclear cell enrichment by centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ), the peripheral blood or bone marrow samples were studied for surface antigen expression using a panel of monoclonal antibodies. Blasts were gated using CD45 antigen expression and right-angle light scatter. Blasts were considered positive if 20% or more expressed an antigen. Isotype controls were used to determine positive and negative antigen expression.

Cytogenetic analysis was performed on peripheral blood or bone marrow specimens using standard techniques. GTG banding was used to identify the individual chromosomes. At least 20 metaphases were examined from each case. Cytogenetic nomenclature followed standard 1995 International System for Human Cytogenetic Nomenclature criteria. Fluorescence in situ hybridization (FISH) analyses were performed to confirm the presence of the Ph translocation and the inv(16)(p13q22) using residual cell pellets from the conventional cytogenetic studies. The LSI BCR-ABL dual-color, single fusion translocation probes and the LSI CBFB dual-color break-apart rearrangement probes were used in the FISH analysis according to the manufacturer’s instructions (Vysis, Downers Grove, IL). A minimum of 200 interphase cells was scored.

For molecular studies, RNA was extracted from available peripheral blood or bone marrow aspirate cells and was analyzed using the reverse transcriptase–polymerase chain reaction (RT-PCR) designed to detect the BCR-ABL fusion transcripts of the t(9;22) translocation or the CBFB-MYH11 fusion transcript of inv(16), as previously described.

Results

Clinical Features

Table 1 and Table 2 summarize the clinicopathologic features of the 6 patients with the coexistence of chromosome 16 inversions and the Ph. The 6 patients included 5 men and 1 woman with a median age of 42.5 years (range, 21-62 years). One patient (case 1) had CML-CP. All other patients with CML originally had CML-CP, but the disease rapidly progressed to CML-AP (case 2) or CML-BC (cases 3-5). One patient (case 6) had AML at diagnosis with no evidence of previous CML.

All patients had vague constitutional symptoms, including progressive fatigue and weight loss, localized or diffuse pain, low-grade fever, and anorexia. Physical examination revealed splenomegaly (5/6), hepatomegaly (4/6), and lymphadenopathy (3/6).

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Hemoglobin, g/dL (g/L)</th>
<th>Platelet Count, × 10^9/L</th>
<th>WBC Count, × 10^9/L</th>
<th>Neutrophils, %</th>
<th>Blasts, %</th>
<th>Blasts, %</th>
<th>Eosinophils, % (Abnormal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/21</td>
<td>CMLCP*</td>
<td>14.3 (143)</td>
<td>530</td>
<td>13.2</td>
<td>57</td>
<td>0</td>
<td>2</td>
<td>2 (No)</td>
</tr>
<tr>
<td>2/M/44</td>
<td>CMLCP→AP*</td>
<td>12.1 (121)</td>
<td>71</td>
<td>6.7</td>
<td>72</td>
<td>0</td>
<td>10</td>
<td>4 (No)</td>
</tr>
<tr>
<td>3/M/33</td>
<td>CMLCP→AP*→BC</td>
<td>9.6 (96)</td>
<td>26</td>
<td>39.9</td>
<td>37</td>
<td>26</td>
<td>27</td>
<td>12 (Yes)</td>
</tr>
<tr>
<td>4/M/41</td>
<td>CMLCP→BC</td>
<td>11.8 (118)</td>
<td>27</td>
<td>18.6</td>
<td>14</td>
<td>62</td>
<td>63</td>
<td>6 (Yes)</td>
</tr>
<tr>
<td>5/F/62</td>
<td>CMLCP→BC</td>
<td>10.5 (105)</td>
<td>36</td>
<td>126</td>
<td>11</td>
<td>52</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6/M/44</td>
<td>AML, M4Eo*</td>
<td>10.2 (102)</td>
<td>93</td>
<td>23.9</td>
<td>17</td>
<td>36</td>
<td>44.5</td>
<td>26.5 (Yes)</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; AP, accelerated phase; BC, blast crisis; CML, chronic myelogenous leukemia; CP, chronic phase; M4Eo, the French-American-British Cooperative Group classification M4 with eosinophils; NA, not available in reports.

* The phase in which cytogenetic studies were performed.

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All patients with CML seemed to have some unfavorable features. One patient (case 1), although in CP, had central nervous system involvement at diagnosis and, therefore, received 4 cycles of intrathecal methotrexate therapy. Another patient (case 2) died of graft-vs-host disease at 18 months following allogeneic bone marrow transplantation (BMT). Two allogeneic BMTs in case 3 failed, and the patient died of extramedullary leukemia infiltration 6 months after the second BMT. In 2 patients (cases 4 and 5), the disease transformed to BC during treatment with imatinib mesylate or interferon alfa. In case 4, cytogenetic and molecular remission were achieved for 3.5 years after allogeneic BMT. Patient 5 decided against BMT and died of relapsed BC at 2.5 years after initial diagnosis. The patient with AML (case 6) was disease-free for 18 months following allogeneic peripheral blood stem cell transplantation but had posttransplantation red cell aplasia and died of presumed viral encephalitis (2 y after diagnosis).

### Peripheral Blood and Bone Marrow Findings

Except for case 1, anemia and thrombocytopenia were present in 5 cases. Myeloblasts confirmed by immunophenotyping were present in the peripheral blood of 3 patients with CML-BC and 1 with AML. The blasts all expressed CD13 and CD33. In addition, expression of CD34 in blasts was detected in cases 3 and 4 (CML-BC), and an aberrant expression of CD2 was present in case 6 (AML, M4Eo), respectively. All bone marrow samples were markedly hypercellular (80%-95%) with myeloid hyperplasia. An increase in bone marrow eosinophils (>5%) was detected in cases 3, 4, and 6 (range, 6%-26.5%) with available material, and abnormal eosinophils with coarse basophilic granules were identified in these 3 cases.

### Cytogenetic and Molecular Findings

Cytogenetic and molecular findings are summarized in Table 2. All patients demonstrated t(9;22)(q34.1;q11.2) and inv(16)(p13q22) at some point in their disease, but cytogenetic results were not available for all patients during CP. Of the 5 patients with CML, 2 had both abnormalities detected in CP samples, 2 in AP samples, and 1 in a BC sample. In case 2, karyotype studies on a splenectomy specimen during CP showed t(9;22) but no inv(16). One month later, his disease had progressed to AP and there was evidence of inv(16) and...
t(9;22). The CP specimen in case 4 showed both abnormalities, and the disease progressed to BC after 3 months; a specimen from that time also showed both abnormalities. Karyotype results from the CP specimens in cases 3 and 5 were not available for review, but the disease in both patients progressed to BC quickly (3 and 4 months, respectively), with t(9;22) and inv(16) present in the BC specimens and the AP specimen in case 3.

The BCR-ABL fusion transcript of the (9;22) translocation was detected by RT-PCR in all patients. A p210 fusion was detected in all patients with CML, and a p190 fusion was detected in case 6 (AML, M4Eo). The \( CBF\beta-MYH11 \) fusion gene was confirmed in 3 of 5 patients with CML (cases 3-5; assay not available for cases 1 and 2) and in the 1 patient with AML by RT-PCR and FISH. Abnormal eosinophils with coarse basophilic granules correlated with detection of a \( CBF\beta-MYH11 \) fusion. No material was available for FISH or RT-PCR assays for case 2. The FISH and RT-PCR assays performed in a representative case of CML-BC are illustrated in Image 2A and Figure 1A.

### Discussion

We describe 6 cases of a rare type of Ph+ leukemia with coexistent inv(16) as assessed by cytogenetic and molecular analysis. AML with inv(16) has been recognized as a distinct subtype of AML with a more favorable clinical outcome than most other types of AML.\(^{14}\) The coexistence of Ph and inv(16) is rare in AML and CML. A review of the literature revealed that most de novo AMLs with chromosome abnormalities of t(9;22) and inv(16) seem to have as favorable a prognosis as AML with the inv(16) alone (Table 3).\(^{23-26}\) The possibility of a blastic presentation of CML cannot be excluded entirely in such cases, but the presence of a p190 BCR-ABL fusion and the lack of basophilia or other background features of CML in our case suggests that this case represented a de novo AML. In contrast, the coexistence of t(9;22) and inv(16) in CML seems to indicate an unfavorable or uncertain prognosis (Table 4),\(^{1-5}\) and in 4 of our 5 patients

![Image 1A and B. Chronic myelogenous leukemia with the dual presence of t(9;22) and inv(16) showing increased abnormal eosinophils with dysplastic basophilic granules (A, Wright-Giemsa, ×1,000; B, Wright-Giemsa, ×1,000).](https://academic.oup.com/ajcp/article-abstract/125/2/260/1759891)

![Image 2A and B. Fluorescence in situ hybridization analysis was performed using the BCR-ABL dual-color, single-fusion translocation probes. The translocation shows a fused red-green signal (adjacent to stars).](https://academic.oup.com/ajcp/article-abstract/125/2/260/1759891)
with CML, the disease progressed rapidly to AP or BC within 1 to 4 months of diagnosis.

Since Asou et al described the first case of Ph+ CML with inv(16) in 1992, only 8 other fully reported cases of CML with these chromosome abnormalities have been described in the literature (Table 4). All previously described patients with CML were men with an age range from 21 to 78 years. These CML cases with inv(16) also tended to show frequent transformation and rapid clinical progression. The majority of these cases showed an increase of myelomonocytic cells and abnormal eosinophils with dysplastic granules in the bone marrow; however, a case of CML-CP transformed to lymphoid BC (positive for CD19, CD34, and HLA-DR and negative for CD13 and CD33) with acquisition of additional inv(16) also was reported. Two younger patients (ages 21 and 22 years) similar to our case 1 (21 years old) did well following bone marrow transplantation. The rest of the previously reported patients had a more unfavorable outcome.

Recently, 5 additional cases of CML with inv(16) were reported in abstract form. Although the details of these cases

Figure 1 Real-time reverse transcriptase–polymerase chain reaction analysis was used to identify the CBFβ-MYH11 transcripts of inv(16)(q22p13). The polymerase chain reaction cycles (x-axis) are plotted against the fluorescence intensity (y-axis).

Table 3 Previously Reported Cases of Ph+ Acute Myeloid Leukemia* With inv(16)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sex/Age (y)</th>
<th>Hemoglobin, g/dL (g/L)</th>
<th>Platelet Count, × 10^9/L</th>
<th>WBC Count, × 10^9/L</th>
<th>Neutrophil Count, %</th>
<th>Blasts, %</th>
<th>Eosinophils, % (Abnormal)</th>
<th>Cytogenetic Findings</th>
<th>Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siddiqui et al7</td>
<td>M/23</td>
<td>9.2 (92)</td>
<td>99</td>
<td>22.6</td>
<td>36</td>
<td>14</td>
<td>21</td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Alive, 36 mo</td>
</tr>
<tr>
<td>Preudhomme et al17</td>
<td>M/64</td>
<td>10.2 (102)</td>
<td>57</td>
<td>34.6</td>
<td>15</td>
<td>21</td>
<td>32</td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Alive, 13 mo</td>
</tr>
<tr>
<td>Marcucci et al22</td>
<td>M/64</td>
<td>8.5 (85)</td>
<td>50</td>
<td>200</td>
<td>1</td>
<td>78</td>
<td>76</td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Alive, 7 mo</td>
</tr>
<tr>
<td>Li and Hayhoe24</td>
<td>M/39</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>&gt; 30</td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Alive, 38 mo</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Died, 15 mo</td>
</tr>
<tr>
<td>Miura et al26</td>
<td>M/25</td>
<td>11.7 (117)</td>
<td>11</td>
<td>34.7</td>
<td>29</td>
<td>13</td>
<td>50</td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Died, 7 mo</td>
</tr>
<tr>
<td>Miura et al26</td>
<td>M/40</td>
<td>6.4 (64)</td>
<td>18</td>
<td>28.9</td>
<td>NA</td>
<td>59</td>
<td>36</td>
<td>46,XY(t;9;22)(q34;q11) inv(16)(p13q22)</td>
<td>Alive, 27 mo</td>
</tr>
</tbody>
</table>

*All cases were M4 with eosinophils in the French-American-British Cooperative Group classification.

† BCR-ABL confirmed by Southern blot analysis.

NA, not available in reports.

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are limited, the cases also were associated with rapid progression, and 3 of 5 died of disease, with fairly limited follow-up periods for the survivors. The previous reports of CML with t(9;22) and inv(16) provide only karyotype data, and this is the first report of cases with molecular characterization of the cytogenetic abnormalities.

We report an additional 6 well-documented cases of Ph+ leukemia with inv(16). The presence of CBFβ-MYH11 fusion transcripts was confirmed in all 4 cases tested by RT-PCR and FISH. Similar to previously reported cases, some cases did not have abnormal eosinophils, a feature that usually is associated with inv(16). The detection of abnormal eosinophils with dysplastic basophilic granules correlated fairly well with detection of a CBFβ-MYH11 fusion, but they were not seen in all cases. However, even in AML with inv(16), abnormal eosinophils may not be apparent in all cases. In addition, in all 3 patients with CML with confirmed CBFβ-MYH11 fusions, the disease progressed rapidly to BC, suggesting that this abnormality may signal disease progression or more aggressive disease.

Based on the data from our CML cases and previous reports, we suggest that patients with CML carrying and expressing both BCR-ABL and CBFβ-MYH11 fusion genes may have a rare subtype of CML that is more likely to progress rapidly to AP or BC. The dual presence of the CBFβ-MYH11 and BCR-ABL fusion gene products could have an important role in early transformation of CML. Further studies using molecular and cell culture techniques are needed to answer the paradox of why inv(16)-associated AML has a relatively good prognosis, but its occurrence with the Ph in CML results in a rapidly progressive disease. Furthermore, real-time RT-PCR techniques that will quantitate the CBFβ-MYH11 transcripts of inv(16)(q22p13) can be used to study and follow up patients with Ph+ CML with inv(16) who are treated with transplantation, tyrosine kinase inhibitors, or interferon.

NOTE: Since submission of this study, further details of the cases described by Merzianu et al have been published (Merzianu M, Medeiros LJ, Cortes J, et al. inv(16)(p13q22) in chronic myelogenous leukemia in blast phase: a clinicopathologic, cytogenetic, and molecular study of 5 cases. Am J Clin Pathol. 2005;124:807-814).

From the Divisions of 1Pathology and 2Hematology and Stem Cell Transplantation, City of Hope National Medical Center, Duarte.

Table 4
Previously Reported Cases of Ph+ CML With inv(16)†

<table>
<thead>
<tr>
<th>Reference/</th>
<th>Peripheral Blood</th>
<th>Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/ Age (y)</td>
<td>Hemoglobin, g/dL (g/L)</td>
<td>Platelet Count, x 10^9/L</td>
</tr>
<tr>
<td>Asou et al1</td>
<td>M/51</td>
<td>CMLAP → CML-BC</td>
</tr>
<tr>
<td>Evers et al2</td>
<td>M/39</td>
<td>CMLCP → CML-BC</td>
</tr>
<tr>
<td>Enright et al3</td>
<td>M/22</td>
<td>CMLCP → CML-BC</td>
</tr>
<tr>
<td>Heim et al4</td>
<td>M/78</td>
<td>CMLCP → CML-BC</td>
</tr>
<tr>
<td>Myint et al5</td>
<td>M/29</td>
<td>CML-BC → CML-CP</td>
</tr>
<tr>
<td>Colovic et al6</td>
<td>M/58</td>
<td>CMLCP → CML-AP</td>
</tr>
<tr>
<td>Tsuboi et al7,8</td>
<td>M/44</td>
<td>CML-AP → CML-BC (B-lymphoid)</td>
</tr>
</tbody>
</table>

AP, accelerated phase; BC, blast crisis; CML, chronic myelogenous leukemia; CP, chronic phase; NA, not available in reports.
† Five additional cases are reported in abstract form in Merzianu et al.28
† BCR-ABL+ by Southern blot analysis.
† CBFβ-MYH11+ by fluorescence in situ hybridization.
References


