Philadelphia chromosome-positive acute myelocytic leukemia with French-American-British M2 morphology, refractory to tyrosine kinase inhibitors

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We report a 19-year-old male with Philadelphia chromosome (Ph)-positive acute myelocytic leukemia (AML) showing the French-American-British M2 morphology. The karyotype was 46,XY,t(9;22)(q34;q11.2)[17]/46,XY[3] and real-time polymerase chain reaction detected chimeric mRNA consisting of the minor cluster of the BCR gene and the ABL oncogene. The patient failed to respond to treatment with either imatinib or dasatinib, and died of AML progression. Fluorescence in situ hybridization of interphase nuclei revealed that t(9;22)/Ph+ cells comprised a fraction of AML blasts, suggesting that the translocation was a secondary abnormality. It appeared that, in this case, t(9;22)/Ph and expression of the p190 BCR-ABL oncoprotein played a limited role in the development and progression of AML.

Keywords: Philadelphia chromosome, acute myelocytic leukemia, imatinib, dasatinib, fluorescence in situ hybridization

INTRODUCTION

The Philadelphia chromosome (Ph) is generated by the reciprocal translocation t(9;22)(q34;q11.2), involving the ABL oncogene on 9q34 and the BCR gene on 22q11.2. The translocation in BCR occurs within any of the major (M), minor (m), or micro-clusters, producing the chimeric BCR-ABL oncoprotein p210, p190, or p230, respectively. Although the Ph chromosome is primarily associated with chronic myelocytic leukemia (CML) and a fraction of acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML) carrying the Ph chromosome has been described; in one series, Ph-positive (Ph+) AML accounted for 0.35% of de novo AML and 0.48% of Ph+ leukemias. Ph+ AML is currently considered as a rare but separate disease category distinct from myeloid blast crisis of CML (CML-BC). It has been established that tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL oncoprotein are of value in the treatment of CML and Ph+ ALL. In contrast, limited information is available regarding the efficacy of TKIs for Ph+ AML, even though there have been sporadic case reports of a cytogenetic and molecular response and long-term remission being achieved. Here, we report a case of Ph+ AML and describe the clinical presentation and response to TKIs.

CASE REPORT

A 19-year-old male was admitted to Hyogo Prefectural Amagasaki Hospital with a diagnosis of acute leukemia. Four months before admission, anemia and thrombocytopenia had been detected during a medical examination at school. On examination, there was no surface lymphadenopathy or splenomegaly. The hemoglobin level was 8.3 g/dl, white blood cell count was 27,700/μl, and platelet count was 4.9×10^4/μl. The white cell differential was 1.0%
promyelocytes, 0.5% myelocytes, 0.5% metamyelocytes, 2.0% segmented neutrophils, 6.0% lymphocytes, 0.5% monocytes, and 89.5% blasts; the number of nuclear red blood cells was 34 per 100 white cells. Leukemic blasts were positive for myeloperoxidase on routine cytochemistry, while peroxidase-negative neutrophils were occasionally observed.

The bone marrow was hypercellular and contained 67.5% blasts, showing maturation to the stage of promyelocytes with azurophilic granules (Figure 1A). Some cells had Auer rods (Figure 1B). Eosinophils and their precursors comprised 1.7% nucleated cells. The remaining cells included 18.7% polychromatic erythroblasts showing a dysplastic appearance, although the degree of dysplasia was not marked (Figure 1C). There were many mitotic figures of erythroid-lineage cells (Figures 1B and C). Flow cytometry revealed that the blasts were positive for CD13, CD33, CD34, and HLA-DR, and lacked lymphoid-lineage markers. G-banding cytogenetic analysis revealed the Ph chromosome in 17 of the 20 metaphase cells analyzed; the karyotype was 46,XY,t(9;22)(q34;q11.2)[17]/46,XY[3] (Figure 2). Real-time polymerase chain reaction (PCR) detected chimeric mRNA consisting of m-BCR and ABL. A mutation assay within the BCR-ABL kinase domain was reported to be negative (PCR-Invader® Assay; BML, Inc.).

A diagnosis of AML of the French-American-British (FAB) M2 subtype was made. The patient underwent induction therapy consisting of cytarabine and idarubicin employing the 7 + 3 regimen (Figure 3). Two weeks after treatment, when cytogenetic results were obtained, the bone marrow was hypocellular, containing 11% blasts, while normal hematopoiesis was not found. We started imatinib at a dose of 400 mg daily, and then increased the dose to 600 mg daily. As the bone marrow showed reduced cellularity with an increased percentage of blasts after 15 days of imatinib treatment, we switched to dasatinib at a dose of 50 mg twice daily for 7 days, and then increased the dose to 70 mg twice daily for an additional 10 days. However, blasts in the blood and bone marrow rapidly increased (Figure 3). We finally withdrew...
treatment with TKIs and started salvage treatment with cytotoxic drugs. Unfortunately, the patient did not respond to high-dose cytarabine and died of AML progression 4 months after admission.

**Fluorescence in situ hybridization (FISH) of interphase nuclei**

To confirm the t(9;22) and BCR-ABL gene fusion, the bone marrow aspirates at presentation and peripheral blood at progression were subjected to FISH analysis of interphase nuclei. These two materials contained over 70% blasts, as determined by flow cytometric analysis (Table 1). The probe set was designed to generate one fusion signal for t(9;22) involving M-BCR and two signals for that involving m-BCR. Consistent with the data of real-time PCR, interphase cells showed the m-BCR signal patterns. However, the percentages of positive cells were significantly lower than those of blasts (Table 1), indicating that t(9;22)/Ph+ cells comprised a fraction of blasts. The discrepancy of positivity between G-banding and FISH of the bone marrow at presentation suggests that t(9;22)/Ph+ cells may have had an advantage in terms of proliferation in vitro over negative cells.

**DISCUSSION**

Here, we described an adolescent patient who developed Ph+ AML showing the FAB-M2 morphology. A literature review shows a wide variety of morphology and antigen expression of Ph+ AML, ranging from FAB-M0 to M7,3,4,7-10 except for M3; the M2 type was observed in 3 of 11,2 and 2 of 16 cases with Ph+ AML.1 In the present case, the presence of Auer rods, maturation to the stage of promyelocytes, positive staining for myeloperoxidase, and the lack of lymphoid markers clearly indicated that the leukemia cells had purely myeloid features.

![Figure 3. Clinical course and response to treatment. The treatment consisted of idarubicin (IDR, 12 mg/m² for 3 days), cytarabine (AraC, 100 mg/m², continuous infusion for 7 days), imatinib (400 mg or 600 mg, daily), and dasatinib (50 mg or 70 mg, twice daily). The numbers of white blood cells (WBC) and blasts in the peripheral blood (per µl, plotted on a logarithmic scale), and the percentage of blasts in the bone marrow are shown. The copy number of m-BCR-ABL transcripts was assayed by real-time PCR (BML, Inc.).](image-url)

<table>
<thead>
<tr>
<th>Table 1. t(9;22)(q34;q11.2)/Ph+ blasts</th>
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<tr>
<td><strong>Material</strong></td>
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<tr>
<td><strong>Morphology</strong></td>
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<tr>
<td>At presentation</td>
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<tr>
<td>At progression</td>
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*By examination of May-Giemsa-stained smear slides under microscopy
**By means of CD45-gating method
Controversy has existed about whether Ph+ AML represents a true acute leukemia or a presentation of CML-BC.3-4 The current case can be convincingly differentiated from CML-BC, as there were no clinical and laboratory features of CML, such as splenomegaly and blood or marrow basophilia; there were no additional cytogenetic abnormalities common to CML-BC; and t(9;22)(q34;q11.2) involved m-BCR encoding the p190-type fusion protein instead of p210 detected in 99% of CML cases.2-4 The coexistence of normal metaphases along with Ph+ metaphases at diagnosis favors the diagnosis of Ph+ AML to CML-BC.3

A literature review found a total of 8 cases with Ph+ AML that showed a long-term response to the treatment of TKIs (Table 2).7-13 The age range was 48 to 67 and the male to female ratio was 6 to 2. Morphology and antigen expression of leukemia cells were variable. The BCR-ABL gene fusion occurred within both M- and m-BCR. Five cases had additional cytogenetic abnormalities. The patients were treated with TKI alone or in combination with other cytotoxic chemotherapeutic agents, leading to a durable cytogenetic or molecular response. However, there seems to be no common clinical/genetic features among these cases that can predict a favorable response to TKIs. Because the current patient failed to respond to treatment with not only imatinib but also dasatinib, which is 325-fold more potent against cells expressing wild-type BCR-ABL than imatinib,1,5 and because there were no mutations in the BCR-ABL kinase domain known to be associated with TKI resistance,1 the lack of a response to TKIs is likely to be attributable to a BCR-ABL-unrelated mechanism of resistance. We found that only a fraction of non-dividing AML cells were positive for t(9;22), as evidenced by interphase FISH analysis. Thus, although primary genetic abnormality could not be identified on conventional chromosomal analysis, t(9;22) was most likely a secondary abnormality. On the other hand, the dysplastic appearance of erythroid-lineage cells and the presence of peroxidase-negative neutrophils suggest that myelodysplastic syndrome may have preceded the development of florid AML. Indeed, there are reports of AML in which Ph appeared following a pre-existing myeloid neoplasm lack-

Table 2. Literature review of successful treatment of Ph+ AML with tyrosine kinase inhibitors (TKIs)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age/sex</th>
<th>Morphology/phenotype</th>
<th>Breakpoint</th>
<th>Additional cytogenetic abnormalities</th>
<th>TKIs</th>
<th>Concurrent chemotherapy</th>
<th>Outcome/duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamaguchi12</td>
<td>51/M</td>
<td>FAB-M2</td>
<td>M-BCR</td>
<td>ND</td>
<td>Imatinib</td>
<td>Cytarabine, ocfosphere</td>
<td>CCyR/&gt;1Y</td>
</tr>
<tr>
<td>Ito7</td>
<td>64/M</td>
<td>FAB-M4, mixed-lineage?</td>
<td>m-BCR</td>
<td>Three-way translocation</td>
<td>Imatinib</td>
<td>Cytarabine</td>
<td>CCyR/&gt;1Y</td>
</tr>
<tr>
<td>Kondo8</td>
<td>67/M</td>
<td>AML with trilineage dysplasia</td>
<td>M-BCR</td>
<td>None</td>
<td>Imatinib</td>
<td>Idarubicin, cytarabine</td>
<td>CMR/&gt;6M</td>
</tr>
<tr>
<td>Lazarevic13</td>
<td>73/M</td>
<td>ND</td>
<td>M-BCR</td>
<td>ND</td>
<td>Imatinib</td>
<td>Amsacrine, cytarabine, etoposide</td>
<td>CMR/&gt;19M</td>
</tr>
<tr>
<td>Lazarevic13</td>
<td>63/M</td>
<td>Mixed lineage?</td>
<td>m-BCR</td>
<td>del(10), del(22)(9;10;22)</td>
<td>Imatinib</td>
<td>Daunorubicin, cytarabine</td>
<td>MCyR/&gt;1Y</td>
</tr>
<tr>
<td>Pompett10</td>
<td>52/F</td>
<td>FAB-M6</td>
<td>M-BCR (e13a2)</td>
<td>t(9;21;22), t(10;21)</td>
<td>Imatinib</td>
<td>None</td>
<td>CMR/&gt;4Y</td>
</tr>
<tr>
<td>Ritchie11</td>
<td>53/F</td>
<td>Panmyelosis</td>
<td>e6a2</td>
<td>-del(9)</td>
<td>Imatinib, dasatinib</td>
<td>Idarubicin, cytarabine</td>
<td>CMR/&gt;18M</td>
</tr>
<tr>
<td>Papageorgiou9</td>
<td>48/M</td>
<td>FAB-M7</td>
<td>M-BCR</td>
<td>del(4)(t;1;4), t(5;11), del(6)(p21) +8, +17</td>
<td>Dasatinib</td>
<td>None</td>
<td>CMR/18M</td>
</tr>
</tbody>
</table>

Abbreviations: CCyR, complete cytogenetic response; MCyR, major cytogenetic response; CMR, complete molecular response; ND, not described.
ing t(9;22).\textsuperscript{3,4} It seems that, in this case, t(9;22) and the expression of p190 BCR-ABL oncoprotein may not be as crucial in the development and progression of leukemia as those in CML and Ph+ ALL. This case report suggests that treatment with TKIs may not be warranted in all Ph+ AML cases.

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REFERENCES
FAB分類M2の形態を示したフィラデルフィア染色体陽性急性骨髄性白血病の1例

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症例: 19歳男性。労作時息切れ、全身倦怠感、発熱などを自覚し近医受診、血液検査で白血球増多が認められたため、急性白血病を疑われて紹介入院となった。表在性リンパ節腫脹なし、肝腫脅なし。

検査結果: 白血球27,700/μl、白血病細胞89.5%、ヘモグロビン8.3 g/dl、血小板4.9×10⁴/μl（前医で赤血球・血小板輸血後）、LDH1,300 IU/l。骨髄は過形成で芽球から顆粒の豊富な前骨髄球レベルに分化した白血病細胞を65.7%認めた。ペルオキシダーゼ染色陽性、Auer小体陽性で、FAB分類ではM2に該当した。フローサイトメトリー検査では、CD13+, CD33+, CD34+, HLA-DR-で、リンパ球系のマーカーは陰性。染色体検査は46, XY、t(9;22)(q34;q11) [17]/46, XY [3]。間期核FISHではBCR/ABL融合シグナル22.5%陽性(minor BCRパターン)、キメラmRNAはminor BCR/ABL1×10⁵copies/μgRNAであった。

治療経過: イダマイシン＋シタラビンによる寛解導入療法を行ったが白血病細胞が残存した。上記結果が判明した後、イマチニブの投与を開始したところ骨髄中の白血病細胞は増加傾向を示した。次いで、ダサチニブに変更したが白血病細胞はさらに増加した。チロシンキナーゼ阻害薬による治療は断念し、高用量シタラビンに変更したが治療に反応せず入院後4か月で死亡した。

考察: 間期核FISHでt(9;22)/Ph陽性細胞は白血病細胞の一部を占めるに過ぎなかったことから、この染色体転座は二次的な異常であった可能性が高い。本例において、t(9;22)/Phとp190 BCR/ABL蛋白の発現が、AMLの発症・進行に果たした役割は限定的であったと考えられた。

キーワード: 急性骨髄性白血病, Ph染色体, BCR/ABL, チロシンキナーゼ阻害薬