Philadelphia-Negative (Ph-) Chronic Myeloid Leukemia (CML): Comparison With Ph+ CML and Chronic Myelomonocytic Leukemia

By P. Martiat, J.L. Michaux, and J. Rodhain for the Groupe Francais de Cytogenetique Hematologique

To better understand the Philadelphia-negative (Ph-) chronic myeloid leukemia (CML) and its relationships with Philadelphia-positive (Ph+) CML and chronic myelomonocytic leukemia (CMML), a study was undertaken by the Groupe Francais de Cytogenetique Hematologique. Thirty-five Ph- CML patients were investigated and compared with 55 chronic phase Ph+ CML and 100 CMML patients. There were 12 M-BCR positive (M-BCR+) and 23 M-BCR negative (M-BCR-) patients. No clinical or biologic differences were found between Ph+ and Ph-, M-BCR patients. In the Ph+ group, M-BCR+ and M-BCR- patients differed significantly in age (47.7 ± 6.6 v 67.0 ± 6.1 years, respectively; P = 0.01), leukocytosis (153.4 ± 135.1 v 58.5 ± 37.7 10^9/L, P = 0.002), relative monocytes (1.8% ± 1.2% v 5.6% ± 1.4%, P = 0.048), absolute basophilia (8.5 ± 9.7 v 0.9 ± 15.1 10^9/L, P = 0.001), percentage of immature myeloid precursors (IMP) in peripheral blood (29.0% ± 9.5% v 15.3% ± 8.1%, P = 0.001), and percentage of erythroblasts in bone marrow (BM) (6.5% ± 3.5% v 14.6% ± 3.6%, P = 0.001). Karyotypic abnormalities other than the Ph chromosome occurred in 0 of 12 M-BCR+ at diagnosis and 7 of 23 M-BCR- Ph- CML (P = 0.033). None of the 13 investigated BCR+ patients had detectable BCR/ABL transcripts using polymerase chain reaction (PCR) and none had an N-RAS mutation. Cytologic findings showed a marked morphologic difference between M-BCR+ and M-BCR- patients, especially in the monocytic lineage. Dysmyelopoietic features in CMML and M-BCR- patients were very similar, and the differences were of quantitative order only. Using four criteria (monocytosis, percentage of IMP, basophilia, and percentage of erythroblasts in BM), patients could be divided into typical and atypical CMML and this classification correlated well with molecular findings. We conclude that, while Ph+, M-BCR+, and Ph- CML are identical diseases, Ph-, M-BCR- CML, and CMML have many similarities and might be only different aspects of a same entity.

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Address reprint requests to P. Martiat, MD, Department of Hematology, Cliniques Universitaires Saint-Luc, UCL, Avenue Hippocrate 10, B-1200 Brussels, Belgium.

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exclude refractory anemia with ringed sideroblasts. BM biopsies, available in all but one patient at diagnosis, were reviewed to rule out idiopathic myelofibrosis.

**Cytogenetics.** Chromosome studies were performed on peripheral blood and BM cells cultured for 24 to 48 hours without mitogens. Metaphases were G-banded with Wright's stain or trypsin technique and R-banded with heating techniques. A minimum of 25 metaphases were analyzed and classified according to the ISCN guidelines. The karyotypes of all patients were first reviewed in subgroup meetings of the Groupe Français de Cytogénétique Hématologique (GFCH). Karyotypic abnormalities raising problems were reviewed a second time during general workshops of the same group.

DNA analysis. High molecular weight DNA was prepared and restriction digested with at least three restriction enzymes according to standard protocols. After electrophoresis on a 0.8% agarose gel and transfer to nylon membrane, the DNA was hybridized to either a probe covering most of the M-BCR sequences (universal probe) or to two probes 3' and 5' in M-BCR. In seven M-BCR patients, N-RAS mutations (codons 12, 13, and 61) were sought for using the polymerase chain reaction (PCR) and specific oligonucleotide hybridization according to the method described by Cogswell et al.

RNA analysis. Total RNA was extracted from samples of 13 M-BCR nonrearranged patients, reverse transcribed, and submitted to PCR for the detection of hybrid BCR-ABL transcripts (e,a, b,a,J, and b,a,u) as previously described by the participating centers.

**RESULTS**

Patients. The records of 42 patients were addressed for inclusion. After morphologic review, two patients had to be excluded because of a peripheral blastosis greater than 5%. Four patients were reclassified as CMML. Another patient had to be excluded because cytogenetic data were not available at diagnosis.

Cytogenetics. No patient had evidence of standard or variant t(9;22). There were seven chromosomal abnormalities at diagnosis that are detailed in Table 1, part B. In three patients, the karyotype became abnormal during evolution of the disease. After classifying the patients into M-BCR+ and M-BCR-, the analysis showed that no M-BCR+ patient had karyotypic abnormalities at diagnosis (two became abnormal in crisis), whereas seven abnormal karyotypes were found at diagnosis in the M-BCR+ group (another patient also became abnormal in crisis) (Table 2).

DNA and RNA analysis. Twelve of 35 patients had a rearrangement within M-BCR and 23 were negative. Among the latter, 13 could be screened for the presence of a hybrid BCR/ABL transcript using PCR. PCR was performed to try to detect a rearrangement occurring within the BCR gene but downstream to M-BCR, which could lead to the formation of the typical message, as seen in a few Ph+ CML. None of them were shown to be positive. Among the seven M-BCR+ patients who were tested for possible N-RAS mutations, none was positive.

Clinical and morphologic studies. Clinical, biologic, and hematologic parameters were studied after classifying the patients into two (M-BCR+ and M-BCR-) groups and compared using the Mann-Whitney test. M-BCR+ patients were also compared with the 55 Ph+ CML patients, and the M-BCR- group with the 100 CMML patients.

The details of this comparison are shown in Table 3. The first comparison between Ph+, M-BCR+ and Ph-, M-BCR+ patients showed six parameters differing significantly: age (years): 47.7 ± 6.6 versus 67.0 ± 6.1, respectively, \( P = .001 \); leukocytosis: 153.4 10^9/L ± 135.1 versus 58.5 10^9/L ± 37.7, \( P = .002 \); relative monocytosis: 1.8% ± 1.2% versus 5.6% ± 2.8%, \( P = .048 \); absolute basophilia: 8.5 10^9/L ± 9.7 versus 0.9 10^9/L ± 1.5, \( P = .001 \); percentage of IMP in PB: 29.0% ± 9.5% versus 15.3% ± 8.1%, \( P = .001 \); and the percentage of erythroblasts in bone marrow: 6.5% ± 3.5% versus 14.6% ± 3.6%, \( P = .001 \). There were no differences with respect to spleen size, absolute monocytosis, hemoglobin level, platelet count, serum lysozyme level, and NAP score.

In Ph+, M-BCR+ patients, dysmyelopoiesis was mild. The most frequently observed abnormalities were a maturative defect of the granulocyte granules and nuclear abnormalities (nonlobulated nucleus) in the megakaryocytic lineage. The monocytic lineage did not show any maturative defect.

In the Ph+, M-BCR+ group, dysgranulopoiesis was more pronounced, nuclear abnormalities being associated with a maturative defect of the granules, and there were nuclear abnormalities in the monocytic series. Megakaryocytic lineage was less severely involved and the percentage of nuclear abnormalities was less than 15%. These observations are summarized in Table 4.

When comparing the Ph+ and Ph-, M-BCR+ patients, no significant differences with respect to the investigated parameters could be found.

Interestingly, the comparison between the Ph+, M-BCR- and the CML patients showed significant differences in relative (5.6% ± 1.4% vs 31.4% ± 19.2%, \( P < .001 \)) and absolute (3.3 10^9/L ± 2.1 ± 10.9 10^9/L ± 7.3, \( P = .036 \)) monocytes, absolute basophilia (0.9 10^9/L ± 1.5 ± 0.07 10^9/L ± 0.8, \( P < .001 \)), and percentage of IMP in PB (15.3% ± 8.1% vs 31.4% ± 2.3%, \( P = .001 \)). As far as age, sex ratio, spleen size, leukocytosis, platelet count, hemoglobin level, and percentage of BM erythroblasts were concerned, there were no differences between these two groups of patients. There were no qualitative differences for dysmyelopoiesis, but the fraction of dysplastic cells of the erythroid and megakaryocytic lineage was much more important in CMML, as was the number of patients involved.

**DISCUSSION**

Our study confirms the similarity of Ph+ and Ph- M-BCR+ CML, which is in good agreement with previously published reports focusing on the correlation between molecular and cytologic findings.
**Table 1. Clinical, Cytogenetic, and Molecular Characteristics of the 35 Ph- CML Patients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Initials</th>
<th>Sex</th>
<th>Age (mo)</th>
<th>Cytogenetics* (no. of cells)</th>
<th>BCR/ABL mRNA Mut</th>
<th>N-RAS Mutation</th>
<th>Evolution</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. M-BCR+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C.M.</td>
<td>F</td>
<td>56</td>
<td>[33] 46, XX</td>
<td>+</td>
<td>+</td>
<td>ND†</td>
<td>10+‡</td>
</tr>
<tr>
<td>2</td>
<td>F.I.</td>
<td>M</td>
<td>50</td>
<td>[47] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>18+</td>
</tr>
<tr>
<td>3</td>
<td>M.M.</td>
<td>M</td>
<td>30</td>
<td>[46] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>BMT 7.88; 42+</td>
</tr>
<tr>
<td>4</td>
<td>M.B.</td>
<td>M</td>
<td>36</td>
<td>[36] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>8 in blastic crisis</td>
</tr>
<tr>
<td>5</td>
<td>L.F.</td>
<td>F</td>
<td>58</td>
<td>B, [37] 46, XX</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>36 Nonhematologic cause</td>
</tr>
<tr>
<td>6</td>
<td>M.P.</td>
<td>M</td>
<td>51</td>
<td>B, [29] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>131 Nonhematologic cause</td>
</tr>
<tr>
<td>7</td>
<td>G.A.</td>
<td>F</td>
<td>59</td>
<td>M [25] 46, XX</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>17 In blastic crisis</td>
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<tr>
<td>B. M-BCR+</td>
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<tr>
<td>8</td>
<td>G.D.</td>
<td>M</td>
<td>31</td>
<td>B, [149] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>29+</td>
</tr>
<tr>
<td>9</td>
<td>B.M.</td>
<td>M</td>
<td>56</td>
<td>B, [45] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>20+</td>
</tr>
<tr>
<td>10</td>
<td>A.G.</td>
<td>F</td>
<td>42</td>
<td>M [33] 46, XX</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>3+</td>
</tr>
<tr>
<td>11</td>
<td>K.J.</td>
<td>M</td>
<td>35</td>
<td>B, [108] 46, XY</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>BMT 8.89; 58+</td>
</tr>
<tr>
<td>12</td>
<td>J.V.D.W.</td>
<td>M</td>
<td>23</td>
<td>B, [44] 46, XY</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>13 In lymphatic blastic crisis</td>
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</table>

**B, peripheral blood; M, bone marrow.**

†ND, not done.

‡10+, still alive after . . . months.

* B, peripheral blood; M, bone marrow.

+ ND, not done.
The comparison between Ph-, M-BCR+ and Ph-, M-BCR- patients shows that these two disorders are different entities: they differ significantly in age, total WBC count, relative monocytosis, absolute basophilia, percentage of immature precursors in peripheral blood, and percentage of erythroblasts in BM (a finding that is equivalent to the lower myeloid:erythroid ratio already reported by Ezdinli et al). NAP score was not helpful in our experience and this is in agreement with the findings of Kantarjian et al.

In their study, Shepherd et al. found that three entities (typical CML or CGL, atypical CML, and CMML) could be defined using morphologic criteria: morphology of granulocytes, monocytes, absolute basophil count, and the number of mature and immature granulocytes in PB. When we tried to apply these criteria to our series (Table 5), we found a good, though not perfect, correlation between the morphologic features and the molecular findings: four M-BCR+ patients satisfied the CGL criteria while the atypical CML (19 patients) group contained only M-BCR- patients; this is in agreement with two previously published studies. However, when we added the percentage of erythroblasts in bone marrow to these criteria (<15% for typical CML) we found a perfect agreement between the molecular findings and the cytologic features. Several groups have already focused on that correlation, but with some differences. In these studies, there remained a few patients who had either typical CML features without...
M-BCR rearrangement or atypical features with M-BCR rearrangement. In the study by Shepherd et al, dysgranulopoiesis played a major role in the classification of typical CML, atypical CML and CMML, a finding that we were unable to confirm, and they did not emphasize the role of the myeloid:erythroid ratio that we found significantly correlated with the molecular pattern. The absence of predictive value of dysgranulopoiesis in our study is worth mentioning, as is the fact that in the group of 55 Ph⁺ CML investigated in parallel, moderate dysgranulopoiesis of up to 50% of the cells or more was not unusual. Finally, this study shows that although there are a few (four) M-BCR⁻ patients who are by multiple parameters undistinguishable from typical CML, as reported by Kurzrock et al and Selleri et al, a careful examination of the myeloid:erythroid ratio in BM may help to reclassify them as atypical CML. Future studies of the underlying molecular abnormalities in this little subset of patients will certainly be of great interest, as these studies can give clues to mechanisms involved in the generation of CML.

Cytogenetics was also of some help in discriminating these two groups of patients: while none of the Ph⁻, M-BCR⁻ patients had karyotypic abnormalities at diagnosis, 7 of 23 M-BCR⁻ had an abnormal karyotype. This difference was statistically significant. This frequency of abnormalities also is in good agreement with most of reported data. In their study of four Ph⁻, M-BCR⁺ patients, van der Plas et al found two abnormalities that could be considered Ph variant translocations, while in the two remaining cases karyotype was normal at diagnosis and the abnormalities occurred later on in the evolution, as usual in Ph⁺ CML. The study by Kantarjian et al does not segregate the abnormalities according to the molecular status.

The most striking finding to us was the similarity of the Ph⁻, M-BCR⁻ patients and the 100 CMML patients used for comparison. They were identical as far as age, sex ratio, spleen size, survival, hemoglobin, platelet count, and myeloid:erythroid ratio were concerned. Due to the heterogeneity of the therapies used, survival data have only an indicative value. Of course, there were significant differences (total WBC count, basophilia, high percentage of IMP in PB and monocytosis), but the study itself by its design could have produced these differences. While the 100 CMML patients were selected only on the basis of the FAB criteria for myelodysplastic syndromes (MDS), the criteria of this Ph-CML study required a high percentage of granulocytes (which excludes the possibility for the monocytosis to be as high as in unselected CMML) and the presence of IMP in PB (which obviously results in a higher percentage than in the CML group). In our study on CMML, we found a significant percentage of patients having characteristics (high number of IMP and basophils in PB) classically related to typical CML. Although not systematically investigated in all patients, it seems worth mentioning that 2 of 10 M-BCR⁻ patients had a monoclonal peak, a finding that has been reported in CMML. The frequency and type of cytogenetic abnormalities were very similar to what is described in CMML, except that no deletion of chromosome 7 (7q⁻ or 7) was found in this study. Two patients (nos. 24 and 30) had an abnormality of the long arm of chromosome 5 (5q31), one of which, [t(5;12)(q31;p12-13)], has been reported only once in a CMML case. Taking all these facts into account, we indulge on speculating that CMML and so-called Ph⁻ atypical CML might be two aspects of the same disorder, with a tendency for atypical CML to express in a more balanced way between the granulocytic and the monocytic lineage. This opinion is supported by two other studies: Cogswell et al also speculated that atypical CML could be regarded as a subgroup of CMML, and Kurzrock et al reported that the evolution of M-BCR⁻ patients was quite close to what could be expected in CMML.

In conclusion, our study confirms the good correlation between molecular, clinical, and morphologic findings in Ph⁻ CML, especially if the myeloid:erythroid ratio in BM is taken into account, and also raises the question of whether Ph⁻, M-BCR⁻ CML should continue to be regarded as an entity distinct from CMML.

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Appendix. Groupe Français de Cytogénétique Hématologique

Chairman: J. Tanzer, Poitiers
Secretary: P. Bernard, Bordeaux

Centers
St-Luc: UCL, Brussels, Belgium
Centre de Génétique: UCL
Center Human Genetics: KUL, Leuven, Belgium
CHU St Antoine 1: Paris, France
CHU St Antoine 2: Paris, France

Names of Participants
A. Delannoy, A. Ferrant, P. Martiat, J.L. Michaux, J. Rodhain
C. Verellen
J.J. Cassiman, C. Mecucci, M. Stul, H. Van den Berghe
J. Van den Akker, Ch. Perrot
N. Smadja, M. Krulik

(Continued on following page)
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