Detection of Minimal Residual Disease by Polymerase Chain Reaction in Philadelphia Chromosome-Positive Chronic Myelogenous Leukemia Following Interferon Therapy

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The significance of the polymerase chain reaction (PCR) in the detection of minimal residual disease in Philadelphia chromosome (Ph')-positive chronic myelogenous leukemia (CML) following interferon therapy was investigated. Forty remission blood samples obtained at various remission time points from 29 patients in complete cytogenetic remission were analyzed. All 40 samples showed minimal residual Ph'-positive cells by PCR: 22 in remission for less than 12 months, 12 in remission for 12 to 24 months, four in remission for 25 to 60 months, and two in remission for more than 60 months. Of these 29 patients, seven relapsed at 4, 6, 9, 14, 17, 19, and 50 months after their first PCR-positivity during remission. One developed extramedullary myelopoiesis at 49 months after PCR-positivity. The remaining 21 patients remained in complete hematologic and cytogenetic remission with median follow-up of 13 months (range, 4 to 36 months) after PCR analysis. These findings indicate that PCR-positivity is not associated with immediate disease recurrence. Long-term follow-up is essential to determine the relevance of PCR-positivity, since late recurrence is observed in our study.

MATERIALS AND METHODS

Patient characterization and sample procurement. Twenty-nine patients who were diagnosed with Ph'-positive CML and who had achieved complete clinical and cytogenetic remission for at least 6 months entered this study. Of these 29 patients, 19 were treated with interferon-alpha alone, four were treated with interferon-alpha and hydrea, and the remaining six were treated with a combination of interferon-alpha and interferon-gamma as previously described. Forty peripheral blood samples were obtained at various time points of complete cytogenetic remission ranging from 3 to 68 months. The blood samples were subjected to PCR analysis. Bone marrow samples were obtained simultaneously for karyotypic analysis. Approval was obtained from the Institutional Review Board for these studies. Informed consent was provided according to the declaration of Helsinki.

Cytogenetic analysis. Periodic karyotypic analyses were performed as previously described in the bone marrow samples every 3 to 6 months. At least 20 metaphases were examined in each analysis. Complete cytogenetic remission was defined by the complete absence of Ph'-positive cells in at least two consecutive studies 3 months apart. Disease recurrence was defined by reappearance of Ph'-positive cells with or without changes in hematologic examination.

PCR amplification of the chimeric BCR/abl transcripts. Detection of the chimeric BCR/abl mRNAs by PCR was performed as we previously described. In brief, an antisense oligonucleotide, primer abl(-), derived from the sequence of the second exon of the c-abl oncogene was used to synthesize the first strand cDNAs from the total RNA samples. Another oligonucleotide derived from the second exon of the bcr region, primer bcr(+), was then added along with Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT) and PCR buffer. PCR was performed in a Thermocycler (Perkin-Elmer Cetus) for 50 cycles by a modification of the method described previously. Ten microliters of PCR amplified products was loaded onto a 3% NuSieve agarose gel and size fractionated at 60 V for 5 hours and then transferred to a nylon membrane after denatur-
DETECTION OF MRD BY PCR IN CML

The membrane was hybridized with a mixture of 5'-end radiolabeled probes L-6 and K-28, which were derived from the sequences at the BCR/abl junctions of L-6 (b2/a2) and K-28 (b3/a2) mRNAs, respectively. The membrane was then washed with 2x SSPE (sodium chloride/sodium phosphate/ethylene-diamine tetraacetate)/0.1% sodium dodecyl sulfate (SDS) at room temperature for 60 minutes and at 60°C for another 15 minutes. Autoradiography was performed against a single intensifying screen at −70°C for 4 to 48 hours. Special precautions to prevent contamination while performing PCR were undertaken as previously described. Furthermore, PCR was performed in duplicate to ensure the consistency of the results. In the presence of L-6 (b2/a2) mRNA, a cDNA fragment of 80 bp was detected. In the presence of K-28 (b3/a2) mRNA, a 155-bp fragment was detected. Since the PCR was performed in duplicate, discordant results were observed occasionally. In such cases, we repeated the analysis by adding another oligonucleotide primer c-ab1(+), 5'-TTGGAGATCT-GCCCTGAAGCT-3' (derived from the exon la of the c-ab1 gene), in our PCR reaction to coamplify the c-ab1, as well as the BCR/abl transcripts in the same PCR reaction. The amplified c-ab1 fragment was expected to be 110 bp in size. The amplification of the c-ab1 fragment allowed us to indirectly evaluate the integrity of our RNA samples and the efficiency of PCR, which in turn avoided false-negative results.

RESULTS

Forty remission blood samples were analyzed by PCR for the presence of minimal residual chimeric BCR/abl transcripts. These samples were obtained at various time points of complete cytogenetic remission: 22 in remission for less than 12 months, 12 in remission for 12 to 24 months, four in remission for 25 to 60 months, and two in remission for more than 60 months. All of these 40 samples (from 29 patients) showed PCR-positivity with various degrees of intensity (Fig 1 and Table 1). Of these 29 patients, there was heterogeneity in the type of chimeric messages detected: 17 had an L-6 (b2/a2) message, 11 had a K-28 (b3/a2) message, and one had both messages (Fig 1 and Table 1). Sequential follow-up samples during remission were available in eight patients (Table 1, patients no. 6, 9, 15, 17, 20, 23, 24, and 26). None of these eight patients had conversion of PCR-positivity to PCR-negativity or changes in the type of chimeric messages during remission. Of the 29 patients, seven (four with L-6, two with K-28, and one with both messages) relapsed at 4, 6, 9, 14, 17, 19, and 50 months after their first PCR-positivity with the duration of remission lasting for 7, 9, 33, 17, 20, 24, and 53 months, respectively (Table 1, patients no. 21, 29, 2, 28, 6, 5, 17, respectively). One (with L-6 message) developed hepatosplenomegaly with extramedullary myelopoiesis at 49 months after the first PCR-positivity (Table 1, patient 13). The remaining 21 patients (12 with L-6 and nine with K-28) remained in complete hematologic and cytogenetic remission with a median follow-up of 13 months (range, 4 to 36 months) after their first PCR analysis. Strikingly, two samples from patient 15 obtained at 65 and 68 months of remission, respectively, showed persistent PCR-positivity even though interferon treatment had been discontinued for more than 24 months.

DISCUSSION

PCR is extremely sensitive in detecting minimal residual Ph'-positive cells in complete cytogenetic responders. However, the clinical and biological significance of PCR-positivity has yet to be explored. To address this specific question, we studied a large number of complete cytogenetic responders following interferon therapy with long-term clinical follow-up.

We observed PCR-positivity in 40 of 40 samples obtained at various time points of complete clinical and cytogenetic remission, suggesting that interferon therapy does not completely eradicate Ph'-positive cells. Interestingly, two of
we noted that disease recurrence in some patients (as late
whether the minimal residual Ph'-positive cells can be kept
extra medullary myelopoiesis. Cytogenetic analysis was not performed
on the biopsy sample to determine the presence
of these samples were analyzed at the time when the patient
had been in remission for more than 60 months (patient 15,
Table 1). This suggests that minimal residual Ph'-positive
cells can be present for a very long time without the
imminent possibility of disease recurrence. Nevertheless,
we noted that disease recurrence in some patients (as late
as 50 months after the first PCR-positivity) eventually
occurred. This finding raises an interesting question as to
whether the minimal residual Ph'-positive cells can be kept
quiescent for a long time and then reactivated at a later
time.

Twenty-one of the 29 patients we studied remained in
complete hematologic and cytogenetic remission with me-
dian follow-up of 13 months (range, 4 to 36 months) after
their first PCR-positivity. Since we have observed late
disease recurrence, it will not be surprising that at least
some of these patients will eventually relapse after long-
term clinical follow-up. It is not unreasonable to hypothe-
size that a patient with a high percentage of actively
proliferating Ph'-positive cells will relapse earlier. There-
fore, in the future it will be more meaningful to know if
PCR-positivity is observed in dividing cells (cells sorted in
the S phase of the cell cycle) or in cells with self-renewal
capability (cells harvested by colony assay). Such a bio-
directed PCR assay may help define the proliferative
potential of the residual Ph'-positive cells in the remission
samples and eventually help predict disease recurrence in
an early stage.

PCR has also been applied to the detection of minimal
residual disease in Ph'-positive CML following allogeneic
bone marrow transplant. The results have been contradic-
tory.20-23 Gabert et al reported that 11 of 12 patients in
complete cytogenetic remission showed minimal residual
disease and three patients had been well for more than 5
years after transplant.20 However, lower frequencies of
PCR-positivity have been observed by other investiga-
tors.21,22 Recently, Hughes et al reported that 18 complete
cytophenetic responders at 8 months to 8 years posttrans-
plant were all PCR-negative and suggested that PCR
results might have prognostic significance.24 However, Pignon
et al reported that 16 of 17 patients studied were PCR-
positive.25 Snyder et al observed 13 PCR-positivities in 19
patients in remission after transplant and only one patient
had relapsed.26 There could be many possible explanations
for these discrepancies, including that the PCR assay has
not yet been standardized. Different investigators are using
different PCR programs, different PCR primers, different
numbers of PCR cycles, and different methods to avoid
cross-contamination. Since long-term clinical follow-up af-
ter PCR analysis is not available, the clinical significance of
these observations remains unclear. Questions should be
addressed in the future are: do the PCR-negative patients
remain in remission and do the PCR-positive patients
eventually relapse? Since we have observed late recurrence
several years after PCR-positivity, long-term clinical fol-
low-up is important to accurately access the significance of
PCR assay.

Table 1. Correlation of PCR Analysis With Clinical Outcome in Complete Cytogenetic Responders After Interferon Therapy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Duration of CR From PCR Analysis (mo)</th>
<th>Duration of Follow-Up After PCR Analysis (mo)</th>
<th>Clinical Status at the Latest Follow-Up</th>
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<tr>
<td>Patients with the L-6 (b2/a2) message</td>
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<td></td>
<td></td>
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<tr>
<td>1</td>
<td>21</td>
<td>17</td>
<td>Remission, off treatment</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>9</td>
<td>Relapse, on treatment</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>16</td>
<td>Remission, on treatment</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>19</td>
<td>Remission, on treatment</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>19</td>
<td>Relapse, on treatment</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>17</td>
<td>Remission, on treatment</td>
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<td>7</td>
<td>10</td>
<td>10</td>
<td>Relapse, on treatment</td>
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<tr>
<td>8</td>
<td>3</td>
<td>4</td>
<td>Remission, on treatment</td>
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<td>10</td>
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<td>Remission, on treatment</td>
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<td>Remission, on treatment</td>
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<tr>
<td>29</td>
<td>3</td>
<td>6</td>
<td>Relapse, on treatment</td>
</tr>
</tbody>
</table>

*The patient developed hepatosplenomegaly. A liver biopsy showed extramedullary myelopoiesis. Cytogenetic analysis was not performed on the biopsy sample to determine the presence of Ph'.

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