Detection of Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia by Polymerase Chain Reaction: Possible Eradication of Minimal Residual Disease by Marrow Transplantation


Minimal residual disease (MRD) in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Phl ALL) who received allogeneic (n = 9) or autologous (n = 6) bone marrow transplantation (BMT) was evaluated by the polymerase chain reaction (PCR) for the bcr-abl transcript. Twelve patients received BMT at the time of hematologic and cytogenetic remission. However, MRD was detected in 8 of 10 patients evaluated. Seven patients, including three who had MRD before BMT, continue to have a disease-free survival 5 to 64 months after BMT. Twenty-one specimens obtained from these patients at various times after BMT did not show MRD. In three patients, MRD detected just before BMT seems to be eradicated by BMT protocol. The other eight patients developed cytogenetic or hematologic relapses 2 to 8 months after BMT. Seven of 14 samples from these patients demonstrated MRD, which preceded clinical relapse by 3 to 9 weeks. Thus, this technique for the detection of MRD appears to be useful for the more precise assessment of various antileukemia therapies and for early detection of leukemia recurrence.

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ONE MARROW transplantation (BMT) has been performed as one mode of cure-oriented therapy for high-risk acute lymphoblastic leukemia (ALL), such as Philadelphia chromosome-positive (Phl) ALL.4 Nevertheless, recurrence of the original disease remains one of the major obstacles for obtaining long-term survivals after BMT. Leukemia recurrence has been considered to originate from minimal residual disease (MRD) that escaped intensive chemoradiotherapy or posttransplant immune surveillance. As most patients receive BMT at the hematologic remission phase, it is not clear whether some of the recipients might have been cured by conventional chemotherapy. For such patients, BMT might be only hazardous. Therefore, more sensitive techniques for the detection of MRD, which cannot be diagnosed by conventional hematologic or cytogenetic analysis, may provide insight into improving the treatment strategy.

Recently, polymerase chain reaction (PCR) has become available for the detection of low levels of the chimeric bcr-abl transcripts in Phl leukemia patients.3 Phl chromosome-occurs in most cases of chronic myelogenous leukemia (CML) and in 10% to 30% of ALL cases, and results from a reciprocal translocation between chromosome 9q and 22q. This translocation involves the movement of the abl proto-oncogene on chromosome 9 to the breakpoint cluster region (bcr) gene on chromosome 22.4 This results in transcript of an 8.5-kb bcr-abl mRNA, which encodes a hybrid protein of 210 Kd (p210).5 Most of Phl ALL cases have a translocation site different from that of CML, and more 5' bcr exon (BCR) is used in the formation of the fused bcr-abl gene.6 This results in a smaller bcr-abl mRNA of 7 kb, encoding a 190-Kd protein (p190).7 To date, there have been several studies reporting that residual bcr-abl transcripts in CML can be detected by this technique after BMT.6,11 However, the clinical significance of the residual bcr-abl-positive clones after BMT is still controversial.

In Phl ALL, a report from our group suggested that residual bcr-abl transcript was directly correlated with hematologic relapse, although the number of patients studied was small.12 Here we extend our findings and report the clinical details and the results of PCR on 15 Phl ALL patients. In addition, we assessed their remission status before and after BMT more precisely. In some of these patients, MRD detectable after chemotherapy seems to be eradicated by BMT.

PATIENTS AND METHODS

The characteristics of 15 Phl ALL patients are given in Table 1. All had Phl chromosome at the time of diagnosis. The clinical diagnosis of Phl ALL was made according to the standard clinical findings (acute onset of leukemia, leukemia cells with lymphoblastic features, no cytogenetic abnormalities in the remission marrow, etc). Nine of 15 patients received allogeneic BMT, while the other six patients received autologous BMT because of the absence of suitable donors. In these six patients, five patients (cases 1, 9, 10, 13, and 15) with common ALL antigen (CALLA)-positive leukemia cells received their bone marrow that had been purged with mixture of three distinct CALLA monoclonal antibodies and baby rabbit complement as described previously.13 Case 2, whose leukemia cells were negative for CALLA antigen, received autologous marrow without purging. Preparative regimens for BMT are also shown in Table 1. Details of the BMT regimens have been reported previously.13,14 When the karyotypes of more than 20 metaphase cells were all normal, the patients were judged to be in cytogenetic...
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remission. All patients were in cytogenetic and hematologic remission at the time of the PCR study.

Thirteen patients (cases 1 through 13) were prospectively studied. After informed consent was obtained, fresh bone marrow samples were collected just before BMT and 1, 2, 3, and 12 months after BMT. The other two patients (cases 14 and 15) had p210 type expression studied several years after BMT. In addition, frozen leukemia cells at the time of initial diagnosis were used. Ph1-positive cell lines K 562 and MR26 (kindly provided by Dr J. Okamura, Kyushu Cancer Center, Fukuoka, Japan), which express the p210 or p190 type transcript, were used as positive controls in PCR after appropriate dilution. RNA extracted from the peripheral blood mononuclear cells of healthy volunteers' material was used as a negative control.

Oligonucleotides used in this study were synthesized by an Applied Biosystems 391A DNA synthesizer (Applied Biosystems, Foster City, CA). Many sets of primers were used for detection of fused transcripts: bcr2 (exon 2)-abl2 and bcr2-ab13 (for p210 type transcripts), and BCR (more 5' bcr exon)-abl1 and BCR-abl13 (for p190 type transcript). Sequences of mainly used primers and junctional probes for Southern analysis were as follows: primer “bcr2" (abl exon 2), 5'-GCTGAAGGGCTTCTTCCT-3'; primer “bcr3" (abl exon 3), 5'-ATTCCGTGACCATCAATAA-3'; primer “BCR" (more 5' bcr exon), 5'-GCTGAAAGTCAGATGCTATC-3'; and junctional probes “bcr2-ab12": 5'-GCTGAAAGGGCTTCTTCCTTCTCTTATTAAGCAGCTATTGC-3'; primer “bcr3-ab12," 5'-GCTGAAAGGGCTTCTTCCTTCTCTTATTAAGCAGCTATTGC-3'; and “BCR-ab12,” 5'-GCTGAAAGGGCTTCTTCCTTCTCTTATTAAGCAGCTATTGC-3'. To confirm the quality of RNA, amplification of β2-microglobulin was used with primer “BMT" (exon 1): 5'-TTCTCGCTCTGGCCCTTAGC-3', and primer “BM2" (exon 2): 5'CCCACCTAAAACTTGTTGGGAC-3'.

Total RNA was prepared by the acid guanidinium thiocyanate/phenol/chloroform method. Reverse transcription and PCR amplification was performed according to the previously described methods. 15 PCR was performed for 35 cycles using a Perkin Elmer Cetus DNA Thermal Cycler (Emeryville, CA) (denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and then extension at 72°C for 2 minutes). For detection of MRD, PCR products were analyzed by Southern hybridization with a junctional probe that had been 5' end-labeled with 32P-ATP. Details of this procedure was also described previously. 15

Using this approach, we could detect a single Ph1-positive cell in 10⁹ normal cells. Precautions to prevent the cross-contamination of the amplified material were taken according to the recommendations of Kwok and Higuchi. 16 All experiments were first performed with negative controls at all steps of the experiment to ensure the absence of cross-contamination. If all results were negative, the same procedure was repeated with a diluted positive control.

RESULTS

To confirm the existence of bcr-abl transcripts, frozen cells from all patients stored at initial diagnosis were examined by PCR. Twelve patients were found to have p190 type transcript (BCR-abl2, 128 bp) and the other three patients (cases 8, 12, and 15) had p210 type expression (bcr2-abl2, 61 bp in one patient and bcr3-abl2, 136 bp in two) (Table 1).

Results of clinical course and PCR analysis are shown in Fig 1. Twelve patients were proved to be in complete remission by standard hematologic and cytogenetic criteria just before BMT. Ten patients in remission were examined for the presence of MRD using fresh bone marrow cells. Fused bcr-abl transcript was detected in eight patients (Fig 2, A and C).

Seven patients (cases 9 through 15) are alive and in complete remission 5 to 64 months after BMT. Six of these patients received BMT during their first remission and one in his third remission; however, three patients (cases 10, 11, and 13) had MRD just before BMT. After BMT, bcr-abl transcripts were not detected by PCR in 21 samples obtained from these seven disease-free survivors (Fig 1). The other eight patients (cases 1 through 8) suffered hematologic or cytogenetic relapses (clinical relapse) 2 to 8 months after BMT, and they died 4 to 14 months after their BMT. All but two (cases 2 and 4) of these patients were negative for MRD by PCR analysis shortly after BMT. However, five patients (cases 1, 3, 5, 6, and 7) eventually expressed bcr-abl transcript detected by PCR (molecular

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**Table 1. Characteristics of 15 Patients With Ph1 ALL**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Immunophenotype</th>
<th>Status at BMT (cytogenetic studies, Ph1/metaphases)</th>
<th>BMT</th>
<th>Preparatory Regimen</th>
<th>Results of PCR at Initial Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/F</td>
<td>CALLA</td>
<td>2CR (0/20)</td>
<td>Autologous*</td>
<td>L-PAM + TBI</td>
<td>BCR-abl2</td>
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<td>CA + CY + TBI</td>
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<tr>
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<td>CA + CY + TBI</td>
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</tr>
<tr>
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<td>2REL (8/20)</td>
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<td>CY + BU</td>
<td>BCR-abl2</td>
</tr>
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</tr>
<tr>
<td>8</td>
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</tr>
<tr>
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<td>1CR (0/20)</td>
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<td>L-PAM + TBI</td>
<td>BCR-abl2</td>
</tr>
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</tbody>
</table>

Abbreviations: CR, complete remission; REL, relapse; CA, cytosine arabinoside (8 mg/m²); CY, cyclophosphamide (120 mg/kg); TBI, total body irradiation (10-12 Gy, fractionation); BU, busulfan (16 mg/kg); L-PAM, melphalan (120 mg/m²).

*Purging with anti-CALLA antibodies.
Fig 1. Clinical course and PCR results in the 15 Ph1 ALL patients. (●), PCR-positive; (○), PCR-negative; (□), presence of leukemia cytogenetically or hematologically; (■), absence of leukemia cytogenetically and hematologically. M, months; ND, not done; †, dead.

relapse) (Fig 2B). Median interval between PCR positivity and clinical relapse was 4 weeks. One patient (case 8) relapsed hematologically without a prior positive PCR result (Figs 1 and 2C). To eliminate the possibility of false-negative result, amplification of β2-microglobulin was used. All samples demonstrated correct amplification, confirming the presence of intact RNA (Fig 2, B and C).

In 13 patients (cases 1 through 13) studied prospectively, the value of PCR results to predict relapse was examined. Of 33 PCR analyses, 7 were positive and 26 were negative. PCR positivity was well related with clinical relapse in the near future. Conversely, negative results did not always mean cure of disease. Seven negative PCR analysis were eventually related with clinical relapse. PCR results between 1 and 12 months after BMT had significant correlation with clinical relapse (Table 2).

**DISCUSSION**

In the present study, we have used PCR as a tool for monitoring MRD in Ph1 ALL patients who received BMT. Most patients who had achieved cytogenetic and hematologic remission after conventional chemotherapy still had bcr-abl-positive clones by PCR analysis. If this is an indicator for future clinical relapse, conventional chemotherapy appears to be inadequate to eradicate all of the leukemic cells and to achieve a durable remission in most patients. Among 11 patients who still had a Ph1 clone by cytogenetic or PCR analysis just before BMT, nine were negative for the bcr-abl transcripts shortly after BMT. Therefore, our BMT regimen was able to reduce the level of leukemic cells to less than 1 in 10⁶ (our PCR technique limit). Six patients have disease-free survival of more than 12 months without the sign of molecular relapse. They are thought to be cured by BMT because all clinical relapses occurred within 8 months after BMT in this study. In three patients (cases 10, 11, and 13), MRD detected by PCR before BMT seems to be eradicated by transplant procedures. These data support the current concept that Ph1
ALL patients belong to a high-risk group and that BMT should be considered as the first-line therapy for them.\(^7\)

Eight patients had clinical relapse 2 to 8 months after BMT. However, six of these eight patients had been PCR-negative shortly after BMT. In these patients, amount should be considered as the first-line therapy for them.\(^7\)

Detection of the \(bcr-abl\) transcripts by PCR preceded clinical relapse by 3 to 9 weeks. This would indicate that molecular relapse after BMT correlates with clinical relapse in Ph1 ALL, as we have previously suggested.\(^7\)

Furthermore, five patients were initially negative by PCR analysis but turned positive 1 to 3 months later. Thus, leukemia cells in Ph1 ALL patients appear to have highly proliferative capacity.

It is interesting to note that the significance of positive \(bcr-abl\) transcripts by PCR after BMT in CML patients appears to be different from that in Ph1 ALL patients. Despite persistent \(bcr-abl\) expression, positive PCR analysis is not always an early sign of clinical relapse in CML.\(^4\)\(^\text{1}\)

Blood cells from nine patients with CML in the chronic phase were studied 3 to 6 months after BMT and six were PCR-positive; three were negative on subsequent studies and all six patients remain in remission 9 to 18 months after BMT.\(^1\)

These distinct observations in Ph1 ALL and CML may be interpreted as follows. The tumor burden in patients with CML in the chronic phase is much greater than that of ALL patients in remission state, and some CML leukemic clones tend to survive beyond preconditioning, which results in MRD detected by PCR. Yet, these CML clones tend to grow very slowly\(^4\) and, therefore, do not appear to predict hematologic relapse in the near future. In contrast, Ph1 ALL clones may possess high proliferative potential, and the existence of minimal residual Ph1 clones correlates with imminent clinical relapse.

Among six patients who received autologous BMT, four (cases 1, 2, 10, and 13) were proved to have MRD before BMT. Three patients who received purged bone marrow continue to have long-term survival whereas the other patient (case 2) who received unpurged marrow developed clinical relapse. It would be interesting to know if MRD was removed by our purging protocol. Further study is needed to evaluate the efficacy of our bone marrow purging protocol.

In one report, 10 Ph1 ALL patients received allogeneic BMT, and six were alive and well 6 to 30 months (median 19 months) after BMT.\(^9\) Four patients died with transplant-related complications. However, in our study patients died only from recurrence of leukemia. The reason of these distinct results is unclear but it may come from, in part, the difference of BMT protocol and, in part, the difference of median age of patients studied (19 years in this study \& 28 years in the previous report).

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