Chimeric BCR-abl Messenger RNA as a Marker for Minimal Residual Disease in Patients Transplanted for Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia

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We correlated polymerase chain reaction (PCR)-detectable BCR-ABL fusion transcripts with cytogenetic status in 24 patients with acute lymphocytic leukemia (ALL). Of 10 Philadelphia chromosome negative (Ph−) patients, only one was found to exhibit a BCR-ABL fusion transcript. Fourteen patients with Ph+ ALL, including eight in clinical remission, exhibited PCR-detectable BCR-ABL rearrangements. A detectable Philadelphia chromosome was present in only five of the eight patients in clinical remission. Of the three cytogenetically negative, BCR-ABL-positive patients, two eventually succumbed to post-bone marrow transplantation (BMT) relapse. The third died of early transplant complications. Serial PCR analyses were performed on four Ph+ ALL patients in clinical remission who underwent allogeneic BMT. One patient who was PCR negative on post-BMT days 21 and 75 became PCR-positive on day 116 and died in relapse on day 154. One patient was weakly positive for BCR-ABL on day 23, negative on day 56, but died of transplant complications on day 124. Two patients exhibited no post-BMT BCR-ABL rearrangements and remain well on days 279 and 371. Our findings suggest that PCR analysis may be useful in the early identification of relapse in patients transplanted for Ph+ ALL.

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ELAPSE AFTER chemotherapy or bone marrow transplantation (BMT) is a problem for patients with acute lymphoblastic leukemia (ALL). Conventional morphologic examination or metaphase cytogenetics do not detect minimal residual disease in patients in clinical remission. In this report we apply the sensitive technique of the polymerase chain reaction (PCR) to the detection of leukemic cells in patients with Philadelphia chromosome positive (Ph+) ALL.

The Ph results from a reciprocal translocation between the long arms of chromosomes 9 and 22, [t(9;22)(q34;q11)]. It occurs in greater than 95% of patients with chronic myelogenous leukemia (CML) and 17% to 25% of those with adult ALL, as well as a lesser percentage of patients with juvenile ALL or acute myeloid leukemia (AML). Molecular analyses have established that the Ph translocation results in the joining of 3' sequences of the c-abl proto-oncogene on chromosome 9 to the 5' sequences of a gene designated BCR on chromosome 22 and gives rise to the production of aberrant c-abl-derived gene products that by as yet ill-defined mechanisms deregulate cell growth and differentiation. We use BCR to refer to the approximately 90-kb gene that contains a 5.8-kb region known as the breakpoint cluster region or bcr. The total number of exons in the BCR gene is currently unknown. Exon I and II refer to the first and second exons of the BCR gene while exons 2, 3, and 4 refer to a cluster of downstream exons within the bcr region. In CML, the majority of Ph breakpoints occur in introns within the restricted 5.8-kb genomic bcr region and produce a unique 8.5-kb bcr-ABL chimeric messenger RNA (mRNA) that is expressed as a 210-Kd fusion protein. In approximately 50% of patients with Ph+ ALL, joining of c-abl to the BCR gene occurs between exon I and exon II located 5' of the bcr region. These rearrangements give rise to a shorter 7.0-kb bcr-ABL mRNA and expression of a distinctive 190-Kd fusion protein. Both the 210-Kd and 190-Kd proteins possess enhanced tyrosine kinase activity compared with the unarranged 145-Kd c-abl gene product. Several authors have suggested that the structurally distinct BCR-ABL rearrangements seen in CML and ALL may ultimately determine the chronic or acute biologic behavior of Ph+ leukemias. Recently, retrovirus-mediated gene transfer of cloned p210 gene sequences has been used to construct a murine model system in which animals exhibit a myeloproliferative syndrome similar to that seen in humans.

Genomic analysis using Southern transfer and probes specific for the 5.8-kb bcr region has proven useful and specific for confirming the Ph translocation in most patients with CML but many patients with Ph+ ALL are negative by this technique. Direct demonstration of genomic rearrangements within the first large intron of BCR using standard Southern analysis is both time-consuming and expensive. Pulse field gel electrophoresis has been used successfully to detect rearrangements among Ph+ ALL patients. Genomic Southern analysis and pulse field electrophoresis lack the sensitivity that is required for the detection of minimal residual disease.

Kawasaki et al proposed analyzing BCR-ABL breakpoint-specific mRNA using PCR amplification as a direct method for distinguishing different Ph rearrangements. This approach clearly identifies breakpoints between bcr exons 2 and 3 or exons 3 and 4 commonly seen in CML as well as rearrangements between BCR exon I and II. Directed amplification of target nucleic acids by reverse transcription (RT)/PCR provides a level of sensitivity unsurpassed by other currently available, molecular techniques. The RT/PCR technique has been widely used to investigate BCR-ABL rearrangements in patients with CML and Hooberman et al used the method to identify an unexpected
heterogeneity of chimeric mRNAs in patients with Ph+ ALL. We used RT/PCR to assay for BCR-abl rearrangements in patients with Ph+ ALL and report here the utility of this technique for the detection of minimal residual disease.

MATERIALS AND METHODS

Patients. We investigated leukemic cells that were either arbitrarily selected from a bank of cryopreserved specimens previously submitted to the Hematopathology Laboratory at the University of Washington's University Hospital or from patients who were evaluated for bone marrow transplantation at the Fred Hutchinson Cancer Research Center. Specimens were collected only after informed consent was obtained for protocols approved by our Institutional Review Board. A previous diagnosis of ALL, confirmed by review of the medical record, was based upon clinical data and morphologic, cytochemical, and immunologic studies of blood and marrow blast cells. Control cells included a Ph+ lymphoblastic cell line (ALL-1) (donated by Giovanni Rovera at the Wistar Institute in Philadelphia) derived from a 6-year-old female with ALL, harboring a t(9;22)(q34;q11) chromosomal translocation and no bcr rearrangement, and Ph+ K562 cells derived from a patient with CML and known to contain a breakpoint between bcr exon 3 and 4.23,25

Immunophenotyping and karyotyping. Immunophenotyping of Ficoll-Hypaque separated leukemic blasts from blood or marrow was performed using direct or indirect immunofluorescence by fluorescent microscopy or flow cytometry and included antibodies directed against terminal deoxynucleotidyl transferase (TdT), common leukocyte antigen (CALLA), and HLA-DR (Ia) as well as T- and B-cell–specific antigens. Routine cytogenetic analysis was performed on banded chromosome preparations from direct or overnight cultures of unstimulated marrow or blood.26

RNA isolation. Leukocytes from freshly drawn specimens were isolated by dextran sulphate separation and washed twice with ice-cold phosphate-buffered saline (PBS). Cryopreserved blood or marrow aspirate specimens containing 20% fetal bovine serum (FBS), 10% dimethyl sulfoxide (DMSO), and to 2 × 10^6 cells were rapidly thawed in a 37°C water bath and washed twice with 5 mL of PBS. Cryopreservation was found to give at least 50% cell viability after thawing as measured by trypan blue exclusion and to have no effect on subsequent RNA isolation. The pellet resulting from the final PBS wash was resuspended in denaturing solution and extracted according to the acid guanidinium thiocyanate-phenol-chloroform method of Chomczynski and Sacchi.27 RNA pellets were resuspended in 20 μL diethylpyrocarbonate (DEPC)-treated water and used directly for reverse transcription or stored at −80°C for later analysis. The initial analysis for each patient involved splitting of the RNA sample into two independent RT/PCR reactions: one for the detection of exon I-abl chimeric mRNA and a second for the detection of bcr exon 2 or 3-abl mRNA.

Reverse transcription/PCR amplification. RT/PCR amplification was performed according to modifications of the protocol used by Kawasaki et al.,26 except that 10 to 15 U of avian myeloblastosis RT (Boehringer Mannheim, Indianapolis, IN) were used and 10 U of RNAsin (Promega Biotec, Madison, WI) were added to the RT mix. Specific primers were synthesized on a 380B DNA synthesizer (Applied Biosystems, Foster City, CA) and were identical in sequence to those published by Kawasaki et al. The relative positions of the RT/PCR primers are illustrated in Fig 1.

Amplification of β 2-microglobulin mRNA was accomplished within the same reaction mixture as that used to identify bcr 2,3-abl rearrangements. β 2-microglobulin primer sequences were derived from published sequences.28 Primer 3' β 2 (5'-CCTCCATGAGTGC-TGCTTACATGTC) was added to the RT reaction to give a final concentration of 2.5 μmol/L. The final concentration of primer 3' β 2 and primer 5' β 2 (5'-ATGTCTCGCTCLGTGGCmAGCT-3') was 1 μmol/L in the amplification reaction.

Southern analysis of PCR products. PCR products (30 μL) were separated electrophoretically on a 3% Nusieve/0.3% Agarose (FMC Bioproducts, Rockland, ME) gel containing Hae III-digested Phi X174 double-stranded DNA markers and transferred to 0.45-μm Biotrace RP nylon membranes (Gelman Sciences, Ann Arbor, MI) according to the method of Southern et al. Membranes were UV-crosslinked using a UV Stratalinker 1800 (Stratagene, La Jolla, CA) and prehybridized for 4 hours at 50°C. Prehybridization and hybridization were conducted in the same

Fig 1. Schematic of possible BCR-abl fusion mRNAs found in patients with ALL. (A) and (B) represent amplification primers that yield a 200- or 125-bp PCR fragment depending on the presence or absence of bcr exon 3. (E) and (F) are amplification primers that yield a 307-bp PCR fragment in patients whose breakpoint involves BCR exon I. After amplification, the 200-, 125-, and 307-bp products are specifically detected by the diagnostic oligonucleotide probes (C), (D), and (G), respectively. Exons and primer binding sites are not drawn to scale.
buffer consisting of 0.72 mol/L NaCl, 0.04 mol/L NaHCO₃, 0.004 mol/L EDTA, 4.5% sodium dodecyl sulphate (SDS), and 2X Denhardt’s (0.04% Ficoll, 0.04% bovine serum albumin and 0.04% polyvinylpyrrolidone). End-labeled oligonucleotide probes were synthesized according to manufacturer’s recommendation using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and γ-[³²P]ATP at a specific activity of 3,000 mCi/mole (ICN Radiochemicals, Irvine, CA). Hybridization was performed overnight at 50°C using 1 to 2 × 10⁶ cpm of 5’-labeled probe per milliliter of hybridization buffer. The sequence of breakpoint specific oligonucleotide probes was as previously published (see Fig 1). Throughout the RT/PCR protocol, avoidance of false positives was accomplished by adhering to published recommendations.⁸⁻¹²

RESULTS

Assay sensitivity. The sensitivity of the PCR assay was determined by mixing experiments using the Ph+ ALL-1 cell line and normal blood leukocytes as shown in Fig 2A. One percent leukemic cells was easily detected after 2 hours of autoradiographic exposure (Fig 2A, bottom panel) and 0.01% leukemic cells could reliably be detected after 24 hours of exposure (Fig 2A, top panel). Normal blood leukocytes gave no detectable signal even after exposures as long as 48 hours. Similar mixing experiments using the CML-derived K562 cell line demonstrated a sensitivity of 0.001% (data not shown).

Amplification of β 2-microglobulin was used to eliminate the possibility of false-negative results. Cells from all marrow-derived lineages express this gene. The decision to use β 2-microglobulin as a control was based on this ubiquitous distribution. Preliminary experiments using β 2-microglobulin consistently demonstrated that this gene’s transcripts did not amplify more efficiently than those of rearranged BCR-abl. Thus, it is unlikely that BCR-abl rearrangements would be missed in the presence of a positive β 2-microglobulin signal. Amplification of β 2-microglobulin was performed within the same reaction as that used to generate the 200- or 125-bp bcr-abl PCR products. Figure 2B demonstrates that bcr exon 3-4 is efficiently amplified without interference by the co-amplification of β 2-microglobulin sequences.

Heterogeneity of BCR-abl RNA junctions. Splicing of messenger RNA transcripts from the region of the BCR-abl

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Fig 2. (A) Sensitivity of RT/PCR detection of BCR-abl mRNA. Normal peripheral blood leukocytes and leukemic cells from the Ph+ cell ALL-1 were mixed in varying percentages and RT and amplification performed as described in Materials and Methods. Lanes 1 through 5 contain 100%, 10%, 1%, 0.1%, and 0.01% leukemic cells; lane 6 contains 100% normal leukocytes. Hybridization was with radiolabeled oligonucleotide probe G and autoradiographic exposure was for 24 hours (top) or 2 hours (bottom). (B) Multiplexed amplification of bcr exon 3-4 junction and β 2-microglobulin. Varying numbers of leukocytes from a patient with Ph+ chronic phase CML were mixed with normal donor leukocytes to give a final cell number of 10⁸. RNA was isolated and RT/PCR performed with bcr exon 3-4 primers and β 2-microglobulin exon 1-2 primers within the same reaction mixture. Lanes 1 through 5 contain 100%, 50%, 25%, 10%, and 1% Ph+ leukemic cells. Lane 6 contains 100% normal donor leukocytes. The top panel shows an autoradiographic exposure after Southern transfer and hybridization with a bcr exon 3-4 specific oligonucleotide (probe C). The bottom panel shows an ethidium bromide stained gel containing bands corresponding to the 370-bp β 2-microglobulin-specific PCR fragment.
translocation in patients with ALL may result in a variety of PCR fragments, as shown in Fig 3, top and middle panels. The top panel in Fig 3 shows the results of amplification to detect the 307-bp fragment corresponding to a BCR exon I-ab1 rearrangement, and the middle panel shows the results for duplicate RNA samples analyzed for bcr exon 3-ab1 (200-bp fragment) or bcr exon 2-ab1 (125-bp fragment). Most specimens demonstrate a single, predominant product, as seen for patients 3, 6, 7, 8, 11, and 12, but occasionally patients show two (Fig 3, patient 5) or even three PCR products (Fig 3, patient 13). Minor, spurious bands not corresponding in size to any of the three possible BCR-ab1 junctional PCR fragments do occur as in Fig 3 (patients 6, 7, 12, and 13), but always appear to accompany major bands of greater signal intensity and of the predicted size. Such anomalies most likely result from partial degradation of the amplified PCR product in instances when the additional signal is smaller than the major band or, as suggested by Lange et al, from an alternative primer binding site when the secondary signal is larger in size.

With the exception of one patient (see below), we have consistently found leukocytes from patients with Ph– ALL to be negative by the RT/PCR technique as shown in Fig 3 (patients 17, 18, and 21). Likewise, we failed to detect BCR-ab1 rearrangements in a survey of leukocytes from six normal donors as shown for three donors in Fig 3 (lanes N1, N2, and N3). The possibility of false-negative results for these nonrearranged specimens was eliminated by the concomitant successful amplification of β 2-microglobulin mRNA, as shown in Fig 3, bottom panel. A control sample containing no RNA (Fig 3, lane W) was negative for all BCR-ab1 rearrangements and β 2-microglobulin.

Results with patients. We used RT/PCR to evaluate cells from 24 patients with ALL for the presence or absence of specific BCR-ab1 rearrangements. Leukemic cells were all positive for TdT and exhibited the phenotypic characteris-

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**Fig 3.** Heterogeneity of BCR-ab1 PCR products in patients with Ph+ ALL. Lane numbers refer to patients listed in Table 1. Identical lane numbers among the panels represent replicate analyses of single patient specimens. RNA was divided into two portions and RT and amplified with either BCR exon I-ab1-specific primers E and F (top) or bcr exon 3-ab1 primers A and B (middle). Reactions with bcr exon 3-ab1 primers for Ph– specimens also contained β 2-microglobulin–specific primers (bottom). The top two panels represent autoradiographic exposures of Southern transferred gels probed with oligonucleotide G (top) or a combination of C and D (middle). Specific junctional rearrangements are identified by probe-positive fragments migrating at 307 bp (BCR exon I-ab1), 200 bp (bcr exon 3-ab1), or 125 bp (bcr exon 2-ab1). The bottom panel is an ethidium bromide stained gel showing amplification of β 2-microglobulin mRNA to yield a 370-bp fragment. Lane M contains labeled Hae III-digested Phi X174 marker DNA. Lanes 6 through 11, BM specimens from patients with Ph+ ALL. Lanes 18, 21, and 17, BM specimens from patients with Ph– ALL. Lanes N1 through N3, peripheral blood leukocytes from normal donors. Lane W, “No RNA” negative control. Lanes A1 and A2, RNA from 10^6 and 10^7 positive control cells from the Ph+ ALL-1 cell line. Lanes C1 and C2, RNA from 10^6 and 10^7 leukocytes from a Ph+ patient with chronic phase CML. The oblique position of the band in lane A2 is a gel artifact.
tics listed in Table 1. Cells from 14 patients contained cytogenetically demonstrable Ph chromosomes at the time of our study or documented previously in their medical records (see Table 1). Cytogenetic abnormalities in this group of patients as well as cytogenetic abnormalities present in 10 Ph- patients are as listed in Table 1. The majority (8 of 14) of Ph+ patients possessed rearrangements between BCR exon I and exon II and the c-ab1 gene. RNA from five Ph+ patients contained single bcr exon 3- and bcr exon 2-ab1 rearrangements and one patient (patient 11) demonstrated a single bcr exon 2-ab1 rearrangement. Three patients (8, 10, and 13) demonstrated additional exon 3-ab1 breakpoint products within the bcr region. Cells from patient 13 contained both bcr exon 3- and bcr exon 2-ab1

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<th>Patient</th>
<th>Sex/Age</th>
<th>Clinical Status</th>
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<th>Karyotype</th>
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<td>BCR exon I-ab1+bcr exon 3-ab1+bcr exon 2-ab1</td>
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<td>46,XY(70%)/48,XY,-7,-12,t(7;12), (qter-&gt;7cen::12cen-&gt;12qter) (19%)</td>
<td>Negative</td>
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</table>

*Additional clonal and/or nonclonal cytogenetic abnormalities not involving 9q or 22q were present in some cells.
†The diagnostic cytogenetic results for these patients with normal karyotypes at the time of pretransplant evaluation were as follows: patient 11, 46,XX/46,XX,t(9;22)(q34;q11); patient 12, 46,XY(9%)/47,XY,+t(9;22)(q34;q11.2),t(10;14)(q24,q11)(91%); patient 14, 46,XX,t(9;22)(q34;q11); patient 23, 46,XX,multiple nonclonal chromosome breaks.
‡Host and donor cells seen at relapse after BMT.
§Multiple complex clonal abnormalities not involving 9q or 22q were present.
products in addition to BCR exon I-ab1. Cytogenetic analysis of two of the three patients with multiple PCR products showed additional Ph chromosomes [+der(22)]. Although cytogenetic analysis of cells from the initial presentation of patient 23 showed multiple, nonclonal chromosome breaks without evidence of a Ph chromosome, we consistently have found bcr exon 2-ab1 rearrangements after PCR analysis of BM specimens from this patient. Because our pretransplant evaluation of this patient showed a histologically and cytogenetically normal BM, it was not possible for us to confirm the original Ph− cytogenetic findings.

**PCR detection of BCR-ab1 mRNA in ALL patients in clinical remission.** Of 14 Ph+ patients, eight were judged to be in remission on the basis of hematologic and clinical criteria. Specimens from these patients were collected at the time of marrow storage for future autologous infusion (patients 8, 11, and 12), or during pretransplant evaluation for allogeneic family (patients 9, 10, 13, and 14) or unrelated donor (patient 6) transplantation. The RT/PCR technique showed evidence of residual Ph positivity in all eight of these patients. Cytogenetic analysis (Table 1) showed a normal karyotype in three (patients 11, 12, and 14) but was positive for the Ph chromosome in the remaining five remission patients. Of the three BCR-ab1 positive, Ph− patients, two died of post-BMT relapse. The third succumbed to transplant-related complications.

**BCR-ab1 PCR fragments as a marker for relapse in patients after transplant.** We assayed marrow specimens collected from four patients at various times after transplantation (Fig 4). Patient 10 showed positive BCR exon I-ab1 and bcr exon 3-ab1 PCR products in two independent pretransplant specimens. Both these specimens were also positive by cytogenetic analysis. After allogeneic transplantation, marrow specimens were obtained on days 55 and 72. Although β 2-microglobulin RNA was detected in both these posttransplant samplings, no evidence of either BCR-ab1 PCR products could be detected. Patient 13 showed no BCR-ab1 rearrangement on days 56 and 84 after demonstration of a bcr exon 3-ab1 200-bp PCR fragment in a pretransplant marrow specimen. Figure 4, patient 9 illustrates a case in which a weak bcr exon 3-ab1 signal was detected on posttransplant day 23, but no rearrangement was detectable in a subsequent specimen from posttransplant day 56. In Fig 4, patient 6, however, a BCR exon I-ab1 PCR product was detected on day 116 following two consecutive negative specimens on posttransplant days 21 and 75.

**DISCUSSION**

Our findings of PCR positivity in cells of all eight patients in clinical remission is not surprising given this technique's sensitivity and the knowledge that the majority of adult Ph+ ALL patients eventually relapse after chemotherapy. Four patients received allogeneic transplants. Patient 9 died of transplant complications on day 123. Patients 10 and 13 are currently well and free of disease at posttransplant days 371 and 279, respectively. Patient 14 relapsed 115 days after transplant. Patient 8 was evaluated for autologous marrow storage, did not undergo transplantation, and relapsed 457 days after evaluation. Two patients...

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**Fig 4.** Bcr-ab1 mRNA as a marker for relapse following transplantation. Each panel shows pretransplant and posttransplant results of the RT/PCR assay for BCR-ab1 and β 2-microglobulin mRNA and simultaneous cytogenetic analysis. Posttransplant specimens from patients 10 and 13 failed to show Ph+ mRNA and are consistent with continued remission. Patient 9 shows a faint Ph+ signal at day 23, but a subsequent day 56 specimen was negative. Patient 6 illustrates a case of clinical relapse on day 116 following two negative samplings on days 21 and 75. Although the day 116 signal shown above partially obscures the day 75 lane, an independent exposure of the day 75 analysis confirmed a negative result.
received autologous transplants. Patient 11 died of infectious complications on posttransplant day 25, and patient 12 relapsed 150 days after transplant. Patient 12 had been negative for the Ph chromosome at the time of PCR positivity (Table 1). Patient 6 relapsed on day 116 after receiving an unrelated donor transplant (see Fig 4). Thus, of eight BCR-abl-positive patients, four relapsed, two died of transplant complications, and two remain free of disease.

Our results demonstrating three of 14 patients with at least two BCR-abl rearrangements suggest that multiple chimeric transcripts are not infrequent among patients with Ph+ ALL. Hooberman et al2 and Shannon et al3 both previously reported finding ALL- and CML-type mRNAs in a single patient. Use of multiple BCR splice acceptor sequences by the abl gene's exon II in leukemic cells possessing a single genomic BCR rearrangement may account for such findings.24 Such alternative splicing has been offered as an explanation for the progression from chronic phase to blast crisis in patients with CML. This proposal is controversial and the biologic importance of two genomic BCR rearrangements in a Ph+ ALL patient appears clinically and molecularly to represent an overlap of the classical forms of Ph+ ALL and CML.

In one posttransplant patient we detected a BCR-abl rearrangement at day 23 (patient 9) that was not associated with clinical relapse. A subsequent day 56 specimen was negative and follow-up analyses were not possible because this patient died on day 123 of severe, acute graft-versus-host disease. Evidence of residual recipient cells during the immediate posttransplant period has previously been documented by several methods. Thomas et al36 first drew attention to transient cytogenetic positivity in transplanted CML patients who showed no clinical signs of relapse. Subsequently, Durnam et al,37 using a Y-chromosome-specific in situ hybridization assay, showed that host cells were detectable in marrows of 74% of transplant patients at posttransplant day 14. Using an identical assay, Przepiorka et al38 demonstrated a plateau of host blood mononuclear cells within the first 4 weeks after transplantation. Whether weak PCR positivity immediately following BMT is due to true residual disease or simply detection of nonmitotic recipient cells can be determined only by repeat testing and clinical follow-up.

Previous studies have demonstrated the use of RT/PCR as an adjunct to cytogenetics in the diagnosis of chronic phase CML,1821,39 and several investigators have used this technique to study patients transplanted for CML.23,32,40,41 Results from these latter studies showed 0% to 94% PCR positivity depending on a variety of parameters, including assay sensitivity, use of T-cell depletion of donor marrow, and time of testing after transplantation. Clearly, BMT may not eliminate all Ph+ CML cells, and the prognostic significance of molecular relapse in these patients remains to be established. In patients with Ph+ ALL, BCR-abl mRNA expression may be a more specific marker of leukemic relapse. BCR-abl rearrangement on posttransplant day 116 in patient 6 (Fig 4) was accompanied by 5% to 10% blasts on histopathologic examination and eight of 18 metaphases positive for the Ph chromosome on cytogenetic analysis. This patient's two negative results on days 21 and 75 suggest too few cells were present for PCR detection. These negative findings may also be explained by sampling inconsistencies arising from focal posttransplant relapse. Moreover, the interval between detectable minimal residual disease and relapse in patients with ALL may be relatively short and quite different from the more gradual relapse observed in patients after transplant for CML.36,42

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Chimeric BCR-abl messenger RNA as a marker for minimal residual disease in patients transplanted for Philadelphia chromosome-positive acute lymphoblastic leukemia

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