Detection by Polymerase Chain Reaction of Residual Cells With the bcl-2 Translocation Is Associated With Increased Risk of Relapse After Autologous Bone Marrow Transplantation for B-Cell Lymphoma

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Although molecular biologic techniques can now detect minimal numbers of residual cancer cells in patients in complete clinical remission, the clinical significance of minimal residual disease has never been conclusively established. If the detection of minimal residual disease predicts which patients will relapse, then therapy could be altered based upon the detection of these cells. The t(14;18) can be detected by polymerase chain reaction (PCR) amplification in 50% of patients with B-cell non-Hodgkin’s lymphoma and allows detection of one lymphoma cell in up to 1 million normal cells. To determine the clinical significance of the detection of minimal residual lymphoma cells in the bone marrow (BM) PCR amplification was used to detect the presence of residual lymphoma cells after autologous BM transplantation (ABMT) in serial BM samples from 134 patients with B-cell lymphoma in whom a bcl-2 translocation could be detected. PCR analysis was performed on a total of 542 BM samples obtained while these patients were in complete remission. Disease-free survival was markedly increased in patients with no PCR-detectable lymphoma cells in the marrow compared with those in whom residual lymphoma cells were detected (P < .00001), and the presence of detectable lymphoma cells was associated with a 48-fold increase in the risk of relapse. Of the 77 patients (57%) with no PCR-detectable lymphoma cells in their most recent BM sample, none have relapsed. In contrast, all 33 patients (25%) who have relapsed had PCR-detectable lymphoma cells detected in their BM before clinical relapse occurred. In 19 patients (14%), residual lymphoma cells in the BM were detected early following transplantation and subsequently were no longer detectable, although these patients received no further therapy. In these patients, residual lymphoma cells may already have been irreversibly damaged by the high-dose therapy or an endogenous immune mechanism may be capable of eliminating residual lymphoma cells in some patients. Therefore, although the detection of minimal residual disease by PCR following ABMT in patients with lymphoma identifies those patients at high risk of relapse, the presence of residual minimal disease early after transplantation may not be associated with poor prognosis in a small subset of patients. Confirmatory studies will be required to determine more definitively the role of minimal disease detection to identify which patients require additional therapy.

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portance of eradicating minimal residual disease in the patient to achieve cure. For example, eight reported studies have examined the significance of PCR detection of minimal residual disease in almost 200 patients with chronic myeloid leukemia who have undergone allogeneic BMT but have yielded conflicting results.25-29 Five studies suggested that the persistence of leukemic cells either early after transplantation22,23 or later24-26 did not adversely affect prognosis. Three studies have reported an association between detection of residual lymphoma cells and subsequent relapse.27,29 The major shortcomings of these studies are the limited patient numbers and the short length of follow-up. Therefore, based on the available data, we are still unable to alter treatment based on PCR detection of neoplastic cells.

In the present study, we examined the significance of the detection of minimal residual lymphoma cells in serial BM samples obtained after ABMT from 134 patients with B-cell non-Hodgkin’s lymphoma with a documented PCR-detectable bcl-2 translocation. We show that the failure to achieve or maintain a complete remission as assessed by PCR analysis of BM predicts which patients will relapse. All patients who relapsed have PCR-detectable lymphoma cells in the BM prior to relapse irrespective of the site of relapse. Therefore, the detection of minimal residual disease by PCR following ABMT in patients with lymphoma identifies those patients at high risk of relapse and suggests that our therapeutic goal should be to eradicate all PCR-detectable lymphoma cells.

MATERIALS AND METHODS

Patients. One hundred thirty-four patients (82 male and 52 female) with B-cell non-Hodgkin’s lymphomas (NHLs) were studied. For inclusion in the present study, patients were required to have lymphoma with a PCR-detectable bcl-2 translocation and have remission BM samples available for analysis following ABMT. Immunophenotyping confirmed in all cases that the lymphoma cells expressed B1 (CD20). At the time of ABMT, all patients had hematopoietic disease, as reflected by achievement of minimal clinical disease after induction or salvage chemotherapy. Minimal disease was defined as either a complete remission or a partial remission to maximum tumor masses of 2 cm or less and BM infiltration of less than 20% of the intertrabecular space. At the time of ABMT, 55 patients (41%) were in complete remission (CR). However, 79 patients (59%) had achieved only a partial remission (PR) to protocol-eligible minimal disease, 59 of whom (44%) had morphologic evidence of BM infiltration. Ninety patients (67%) had low-grade and 43 patients (33%) had intermediate-grade histology, 16 of whom had a documented prior history of low-grade histology that had transformed to intermediate-grade histology by the time of high-dose therapy. One patient had high-grade lymphoma. Ninety-seven patients (72%) had a prior history of morphologic infiltration of the BM.

BM samples. BM aspirates and biopsies were obtained at the time of initial evaluation and at the time of BM harvest to assess BM infiltration. These samples were used to document that the lymphoma expressed the bcl-2 translocation.20 Immunologic purging of the harvested autologous BM was performed using a cocktail of anti-B-cell monoclonal antibodies and complement as previously described.30 Samples of the BM following immunologic purging were available for analysis in 121 patients. BM biopsy and aspirate samples were obtained at the time of follow-up clinic visits at 6 and 12 months after transplantation and yearly thereafter until the time of documented clinical relapse of disease. A total of 542 remission follow-up BM samples were available for analysis. This ranged from 1 to 12 samples per patient. All BM samples obtained before January 1990 were cryopreserved. Normal BM was obtained from healthy volunteers. BM samples were obtained only after the human protection committee of our institution gave permission and the patients gave informed consent. Genomic DNA was isolated by cell lysis with nonionic detergents and proteinase K (Sigma Co, St Louis, MO).31 DNA was extracted from the cell lines DHL-6 (a gift from Dr A. Epstein, University of Southern California, Los Angeles) and RL (a gift from Dr W. Urba, NIH, Biologic Response Modifiers Branch, Frederick, MD). Both cell lines contain a PCR-amplifiable bcl-2 translocation and were used as positive controls.

PCR. PCR amplifications at the major14,16 and minor25 breakpoint regions of the bcl-2/lgH translocation were performed using nested oligonucleotides as previously described.30 In brief, samples containing 1 µg of genomic DNA were amplified for 25 cycles in a Perkin Elmer–Cetus thermal cycler (Cetus, Emoryville, CA) in a 50-µL final volume containing 50 mmol/L potassium chloride, 10 mmol/L TRIS hydrochloride, 2.25 mmol/L magnesium chloride, 0.01% gelatin, 200 nmol/L oligonucleotide primers, 200 µmol/L of each of deoxyadenosine triphosphate (dATP), deoxyctydine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP), 1.5 U Taq polymerase (Cetus). Reamplification for 30 cycles of a 5-µL aliquot of the initially amplified mixture was performed in a 50-µL final volume using oligonucleotide primers internal to the original primers. Aliquots of the final reaction product were analyzed by electrophoresis in 4% agarose gel (NuSieve, FMC, Rockland, ME) containing ethidium bromide and visualized under UV light. DNA was blotted onto Zeta-probe blotting membranes (Bio-Rad, Richmond, CA) and bcl-2–specific DNA detected by hybridization for 8 hours with 32P-labeled oligonucleotide probes. Oligonucleotides were radiolabeled with [γ-32P]ATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Standard precautions against cross-contamination of amplified material were taken.33 With each amplification, a weak positive control consisting of DNA from a 10-2 dilution of the cell lines DHL-6 or RL in normal BM cells and a negative control consisting of PCR buffers containing heat-inactivated proteinase K was performed. Each sample was analyzed at least three times at each breakpoint site. In addition, in samples with no detectable PCR product, PCR amplification was repeated using oligonucleotide primers specific for the V region of the gene encoding the human B-cell activation antigen B7 and confirmed that PCR-amplifiable DNA was present in each of the samples analyzed. PCR analysis was performed by investigators who were blinded about clinical outcome or whether a bcl-2 translocation had been previously identified in that patient. BM samples were therefore analyzed by PCR from all patients irrespective of whether a bcl-2 translocation had been documented in their diagnostic tissue.

Statistical analysis. The relationships between the patients’ characteristics at transplantation and time to relapse were assessed using the log-rank test.34 The results of PCR analysis of the BM samples following high-dose therapy were categorized, and the relationship with time to relapse assessed using the log-rank test. Relationships between baseline characteristics and the presence of residual lymphoma in the BM after high-dose therapy were assessed using Fisher’s exact test.35 Stratified log-rank tests were performed to assess the significance of variables thought to be prognostic of a longer time to relapse. The curves for disease-free survival were estimated according to the method of Kaplan and Meier36 and compared by the log-rank test. Cox proportional hazards regression was used to estimate the effect of covariates on the risk of relapse.37
RESULTS

Identification of patients with bcl-2 translocation. At the time of analysis, 249 patients with B-cell NHLs had been treated at our institution with an identical protocol that included total body irradiation, high-dose cyclophosphamide, and immunologic purging of autologous BM before transplantation. The criteria for inclusion in the present study were that patients had lymphoma with a documented PCR-detectable bcl-2 translocation, that follow-up marrow samples were available for analysis, and that the patients were clinically disease free and the BM was histopathologically negative when the BM samples were obtained. The bcl-2 translocation was detected in the diagnostic tissue of 149 patients. Fifteen patients had a documented bcl-2 translocation but were not evaluable for the present study. Two of these patients died during the transplant period, seven patients had insufficient follow-up (less than 6 months after transplantation), and no samples were available for analysis from two further patients. Four additional patients, all reinfused with marrow containing residual lymphoma cells after purging, relapsed early after high-dose therapy before any follow-up BM samples were obtained. Samples from 134 patients were therefore evaluated. The bcl-2 translocation involved the major breakpoint region in 105 patients (79%) and the minor cluster region in 29 patients (21%).

Sensitivity and specificity of detection of residual lymphoma. BM biopsies and aspirates were obtained at the time of follow-up clinic visits after ABMT and DNA extracted. Nested PCR amplification was performed at both the major and minor breakpoint regions of the bcl-2 translocation to assess whether residual lymphoma cells were present. Each marrow sample was analyzed for the detection of minimal residual disease at least three times in a blinded manner and the identical result was obtained on each determination. Because the aim of the present study was to assess the clinical significance of minimal disease detection, only those BM samples that were documented to be free of histopathologic evidence of lymphomatous infiltration and obtained when the patient was staged to remain in clinical CR were included in the present analysis. Of the patients in whom a bcl-2 translocation was previously documented, a total of 542 BM aspirate and biopsy samples were obtained when the patients were assessed to be in clinical CR and the marrow samples had no morphologic evidence of infiltration with lymphoma. A total of 614 BM samples were analyzed from patients with B-cell NHLs without a detectable bcl-2 translocation and these provided negative control samples. PCR analyses of these BM samples were consistently negative. This suggests that false-positive results were unlikely throughout this study.

Residual lymphoma in the marrow. In the present study, all 134 patients had PCR-detectable lymphoma cells in the BM at the time of BM harvest following induction or salvage chemotherapy. After BMT, no residual lymphoma cells were detected in 381 of the 542 (70%) remission marrow samples available for analysis. Three distinct patterns of results were obtained. In 58 patients (43%), no residual lymphoma cells were detected in any of the 256 marrow samples available. Conversely, 35 patients (26%) had PCR-detectable residual lymphoma cells in all of the 77 follow-up BM samples analyzed from this group. Therefore, BM samples from 93 patients (69%) either contained no residual lymphoma in any sample or contained residual lymphoma in every sample analyzed. Of the remaining 41 patients (31%), residual lymphoma cells were detected in some, but not all, of the 209 BM samples analyzed.

Association of residual lymphoma in the marrow and relapse. Disease-free survival was very strongly associated with the detection of residual lymphoma cells in the BM after ABMT (P < .00001) (Fig 1). In those 58 patients with no detectable lymphoma cells in the marrow at any time following transplantation, no relapses have occurred to date. Data on two of these patients were censored 24 and 48 months after transplantation. Both died of causes unrelated to transplantation and were found to have no lymphoma on gross or microscopic examination at autopsy. In contrast, in the 35 patients who had consistently detectable residual lymphoma cells in the marrow samples, 25 patients have relapsed to date. The median disease-free survival for these 35 patients was only 15 months, and, as a result, only 77 BM samples were obtained before overt relapse occurred. In the 41 patients with residual lymphoma cells detected in some, but not all, the BM samples analyzed, 8 have relapsed. These patients have an intermediate prognosis compared with those who had no detectable lymphoma cells and those with detectable lymphoma cells in all BM samples analyzed.

Detection of residual lymphoma changes with time in some patients. Three distinct patterns of results were identified for the 41 patients who had lymphoma cells detected in some, but not all, marrow samples. There were 19 patients (14%) in whom residual lymphoma cells were detected in the BM in the early post-transplant period, but subsequent samples contained no detectable lymphoma.

Fig 1. Actuarial probability of disease-free survival after ABMT in 134 patients with B-cell non-Hodgkin’s lymphoma. PCR neg denotes the patients in whom PCR did not detect any residual lymphoma after transplantation; PCR pos denotes the patients in whom PCR detected residual lymphoma in all samples; and Mixed Responses denotes the patients in whom PCR detected residual lymphoma in only some of the samples analyzed. All BM samples analyzed were obtained while patients remained in complete clinical remission.
None of these patients received additional therapy after transplantation. In 15 of these patients, residual lymphoma cells were detected only within the first year of follow-up, and no patients had residual detectable lymphoma cells by 2 years after ABMT. No relapses have occurred in these 19 patients to date (Fig 2). Conversely, 14 patients (10%) had no detectable lymphoma cells in the BM in the early post-transplant period, but residual lymphoma cells were detected in later samples. The earliest detection of residual lymphoma in the BM of these patients ranged from 11 to 47 months following ABMT. Six of these patients have relapsed, and in all cases, PCR detected residual lymphoma cells in the BM before relapse. The period from detection of residual lymphoma to overt relapse ranged from 3 to 32 months. In the final eight patients (6%), residual detectable lymphoma cells were detected at the time of each follow-up but not in all of the marrow samples obtained at each clinic visit. To date, two relapses have occurred in these patients.

At the time of analysis, there were 77 patients (57%) with no detectable residual lymphoma cells in the BM, including 58 patients who never had detectable lymphoma cells and 19 patients who had residual lymphoma detectable only within the first 2 years following transplant. To date, none of these patients has relapsed. Conversely, PCR analysis detected residual lymphoma cells in the BM before relapse in all 33 patients who have clinically relapsed after ABMT. Only one patient has remained disease free for a prolonged period with consistently detectable lymphoma cells in the marrow.

To determine the prognostic significance of these observations, a proportional hazards model was constructed using the result of the PCR analysis of the marrow sample as an explanatory variable that varied over time. The presence of detectable lymphoma cells after ABMT was associated with a 48-fold increase in the risk of relapse. In this model, once lymphoma cells are no longer detectable, the prognosis of the 19 patients who had detectable lymphoma cells only within the first 2 years following transplantation is not different from that of patients who never had detectable lymphoma cells in the marrow.

**Association of residual lymphoma with clinical characteristics.** In the 134 patients with a documented bcl-2 translocation, a number of baseline characteristics were identified as prognostic indicators on univariate analysis ($P < .05$). These included (1) disease status (complete partial remission) at the time of high-dose therapy; (2) morphologic BM involvement at the time of harvest; (3) histology, and (4) the presence of PCR-detectable lymphoma cells in the harvested marrow after purging. We examined whether the presence of residual lymphoma cells following autologous transplantation was associated with any of these clinical characteristics.

The clinical characteristics of patients within the groups categorized as consistently PCR negative (N = 58), consistently PCR positive (N = 35), or mixed (N = 41) are shown in Table 1. There were no significant associations between these categories with respect to histologic grade of tumor, previous history of BM infiltration, or BM infiltration at the time of BM transplantation. However, significantly more patients were in CR at the time of transplantation within the group of 58 patients with no residual lymphoma cells detectable after transplantation ($P = .0496$). In addition, there was a very strong association between patients whose marrow contained no residual lymphoma cells after immunologic purging and those patients in whom no lymphoma cells

<table>
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<th>Characteristic</th>
<th>Negative (N = 58)</th>
<th>Positive (N = 35)</th>
<th>Mixed (N = 41)</th>
<th>Total</th>
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<td>26</td>
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<td>43</td>
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<td><strong>Status at marrow transplantation</strong></td>
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<td></td>
<td></td>
</tr>
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<td>25</td>
<td>18</td>
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<td>2</td>
<td>23</td>
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<td>Positive</td>
<td>11</td>
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<td>55</td>
</tr>
<tr>
<td>No sample</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

![Table 1. Characteristics of Patients With B-Cell Non-Hodgkin’s Lymphoma and bcl-2 Translocation According to the Result of PCR Analysis After Transplantation](image)

Fig 2. Disease-free survival after transplantation in 41 patients with B-cell lymphoma in whom residual lymphoma was detected in only some of the samples analyzed after transplantation. PCR pos — neg denotes those patients in whom residual lymphoma cells were detected early after transplantation but were not detectable in later samples; PCR neg — pos denotes the patients in whom residual lymphoma cells were not detected early after transplantation but were detectable in later samples; and PCR pos denotes the patients in whom residual lymphoma cells were detected in some, but not all, of multiple samples obtained at the time of each clinic visit. All BM samples analyzed were obtained while patients remained in complete clinical remission.
were detected in the BM after high-dose therapy ($P < .0001$).

**Detection of minimal residual lymphoma in the BM is the most significant factor predicting for relapse.** The 55 patients in complete remission at the time of ABMT had improved disease-free survival compared with the 79 patients who achieved only a partial remission (Log rank test, $P = .002$) (Fig 3A). However, of the 55 patients in complete remission, disease-free survival of the 24 patients who had no residual detectable lymphoma cells in the BM was increased compared with that of the nine patients who had residual lymphoma cells detected in all samples or the 22 patients who had residual lymphoma cells detected in some BM samples ($P < .001$) (Fig 3B). Similarly, disease-free survival for the 79 patients in partial remission was improved in the 34 patients who had no residual lymphoma cells in the BM after high-dose therapy as compared with the 26 patients with residual lymphoma cells detected in all samples or the 19 patients with residual lymphoma cells in some of the BM samples analyzed ($P < .001$) (Fig 3C).

The 66 patients with no PCR-detectable lymphoma cells after immunologic purging had increased disease-free survival compared with the 55 patients who had residual detectable lymphoma cells after purging and the 13 patients in whom no postpurging sample was available for analysis ($P < .00002$) (Fig 4A). Residual lymphoma cells were never detected in the follow-up BM of 41 patients of the 66 patients with no residual lymphoma cells present after immunologic purging. The disease-free survival of these 41 patients was increased compared with survival in the 2 patients who had residual lymphoma cells detected in all samples and the 23 patients who had residual lymphoma cells detected in some BM samples ($P = .0007$) (Fig 4B). Similarly, of the 55 patients in whom residual lymphoma cells were detected in the marrow after immunologic purging, disease-free survival was improved in the 11 patients who had no residual detectable lymphoma cells in BM samples obtained after transplantation as compared with that of the 31 patients with residual detectable lymphoma cells in all samples and the 13 patients with residual detectable lymphoma cells in some of the BM samples analyzed ($P = .00003$) (Fig 4C). The 11 patients who had no detectable lymphoma cells after being reinfused with marrow containing residual lymphoma may represent patients who were reinfused with cells containing the bcl-2 translocation that were not clonogenic or indicates that reinfused lymphoma cells were eradicated by endogenous immune mechanisms before the first follow-up marrow samples were obtained.

**Analysis of PCR status of BM following purging and after transplant predicts for disease-free survival.** Because the PCR status of the harvested marrow after purging and the PCR status of BM following transplantation were both predictive of relapse, we attempted to construct a statistical model that might be clinically useful in defining patients with the highest likelihood of relapse. As stated above, the risk of relapse for a patient who had PCR-detectable lymphoma cells in the BM at any given time after transplantation was 48 times the risk of a patient with no PCR-detectable lymphoma cells. In spite of the power of this result, this model was not helpful in identifying sufficiently early which patients would relapse. In the present study, the median time to relapse was only 14.2 months. Our goal was therefore to formulate a predictive model based on variables available early following transplantation, including pretransplant characteristics. The study protocols required follow-up BM samples at 6 months after high-dose therapy. In
practice, these samples were obtained up to 9 months following high-dose therapy because of scheduling difficulties, and BM samples were obtained in 36 patients during this period but were not cryopreserved and therefore were not available for PCR analysis. We classified patients as to whether all samples obtained within this 9-month period after high-dose therapy were negative (N = 51) or whether lymphoma cells were detected in any sample analyzed during that period (N = 47). The final model included two composite variables: (1) whether residual lymphoma cells were detected during the first 9 months and whether a follow-up sample was available; and (2) whether residual lymphoma cells were detected after immunologic purging and whether a postlysis sample was available. Table 2 shows the instantaneous risk of relapse in each of these categories. The final model predicted that patients with residual lymphoma cells detected in both the postpurging sample and in any follow-up sample within the first 9 months have a 19.5 (95% confidence interval 5.7 to 66) times greater risk of relapse than patients with no detectable lymphoma cells in either samples. No additional patient characteristic variables significantly improved the power of this model.

DISCUSSION

No study to date has conclusively determined for any malignancy whether the eradication of minimal residual
PCR DETECTION OF RESIDUAL LYMPHOMA AFTER ABMT

Table 2. Instantaneous Risk of Relapse After BMT of Patients With B-Cell Non-Hodgkin's Lymphoma

<table>
<thead>
<tr>
<th>Result of PCR After Purging</th>
<th>Within 9 mo</th>
<th>Risk of Relapse</th>
<th>95% Confidence Intervals</th>
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<tbody>
<tr>
<td>Negative</td>
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<td>1</td>
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</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>4.9</td>
<td>1.8, 13.4</td>
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<tr>
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<td>Negative</td>
<td>4</td>
<td>1.6, 9.7</td>
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<tr>
<td>Positive</td>
<td>Positive</td>
<td>19.5</td>
<td>5.8, 66.0</td>
</tr>
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</table>

Irrespective of the mechanism, once these patients eventually had complete resolution of residual lymphoma cells in their BM, they had an excellent prognosis as none has relapsed.

In the present study, there are 24 patients with detectable lymphoma cells in their marrow for varying lengths of time who have not relapsed to date. A major question is whether all such patients with PCR-detectable disease will ultimately relapse. In a previous study, PCR analysis of the bcl-2 translocation detected lymphoma cells in peripheral blood samples of six of eight patients with advanced-stage follicular lymphoma in very long-term disease-free remission. This suggests that the presence of the bcl-2 translocation alone is not sufficient to induce lymphomatous relapse. However, it is difficult to generalize from this small patient subgroup because less than 1% of patients with advanced-stage follicular lymphoma remain disease free for this length of time. In addition, because serial samples were not analyzed, it is not known for how long these patients had detectable circulating lymphoma cells. In contrast, the results of the present study suggest that virtually every patient with consistently PCR-detectable lymphoma cells after BM transplantation will relapse. Although we examined serial BM samples from 134 patients, we identified only a single patient who was a long-term disease-free survivor despite the presence of PCR-detectable lymphoma cells in the marrow.

We have previously shown that the detection of residual lymphoma in the BM after immunologic purging has prognostic significance, and this finding was confirmed in this study where it resulted in a 7.5-fold risk of relapse. Although we are attempting to improve the efficacy of purging, we do not know whether this will result in improved disease-free survival. The hypothesis that the reinfusion of lymphoma cells in autologous marrow would contribute to subsequent relapse was only one possible interpretation of the results of our previous study. Therefore, we were unwilling to add systemic therapy after transplantation based solely on the results of purging. The present study would argue that it is biologically more relevant and statistically more significant to detect residual lymphoma cells in the patient and thereby extend our ability to select which patients require additional treatment. By combining the status of the marrow postpurging with the presence of residual disease in the patient, we can now more confidently predict which patients will relapse (relative risk 19.5).

Can we now alter our treatment based on PCR detection of lymphoma cells in the marrow following ABMT and treat patients who remain in complete clinical remission at a time when their tumor burden is low? We identified 57 patients (42%) who would have merited additional therapy. This subgroup includes the 35 patients whose marrow always contained PCR-detectable lymphoma cells as well as the 8 patients whose marrow had PCR-detectable lymphoma at every time point, although not in every specimen. In addition, the 14 patients whose marrow was PCR negative and then became consistently PCR positive could also have received additional treatment at the time of conversion. We are confident that the 58 patients (43%) who re-
main PCR negative should not receive additional treatment until lymphoma cells are detected by PCR in the marrow. The remaining 19 patients (15%) whose residual lymphoma cells resolved more slowly after transplantation are more problematic. If we had elected to treat all patients whose marrow harbors residual lymphoma cells early post-transplant, we would have overtreated these 19 patients with potential significant toxicity. In patients who have residual cells early after transplantation, the PCR status of the BM after immunologic purging before transplantation may contribute to our clinical management. By examining marrow status after immunologic purging as an additional prognostic variable, 10 of these 19 patients were identified as having marrow that contained no residual lymphoma cells after purging. By observing patients and not administering additional therapy until two sequential BM samples contained residual lymphoma cells, we would have spared them additional treatment. Unfortunately, nine patients (7%) remain who would still have been treated inappropriately.

This is the first study that suggests that molecular detection of disease can contribute to subsequent clinical management, and the results provide compelling evidence that it is necessary to eliminate all residual lymphoma cells from the BM. The cure of this disease will require the development of therapeutic strategies to eradicate minimal residual disease. To this end, in addition to more intensive induction and high-dose ablative chemotherapy, we and others are administering posttransplant therapy in the form of immunotoxins or cytokines. In future studies, the prognostic value of detecting minimal residual disease in the marrow may allow us to determine rapidly whether such therapeutic modifications are likely to be of clinical benefit.

REFERENCES

16. Cleary ML, Sklar J: Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci USA 81:593, 1985
24. Gabert J, Lalive M, Maraninchi D, Thuret I, Carcassone Y,
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