Eradication of Polymerase Chain Reaction Detectable Immunoglobulin Gene Rearrangement in Non-Hodgkin’s Lymphoma Is Associated With Decreased Relapse After Autologous Bone Marrow Transplantation

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In B-cell non-Hodgkin’s lymphoma (NHL), as in other B-cell malignancies, clonal rearrangement of the third complementarity determining region (CDR III) of the immunoglobulin heavy chain gene (IgH) provides a useful marker for the detection of minimal residual disease (MRD) after treatment. To determine the clinical utility of IgH polymerase chain reaction (PCR), we analyzed peripheral blood (PB) and bone marrow (BM) samples from 25 patients with NHL with no PCR detectable chromosomal rearrangement who have undergone autologous bone marrow transplantation (ABMT). Patients with histologic bone marrow infiltration at the time of bone marrow harvest were selected for study since this provided us with diagnostic tissue samples. As an initial strategy DNA was amplified using consensus variable (VH) and joining (JH) region primers. In those cases failing to amplify using consensus region primers, PCR was performed using a panel of VH family-specific framework region 1 (FR1) primers. The clonal products were directly sequenced. From the V-N-D region nucleotide sequences, clone specific probes were constructed and used for subsequent detection of MRD. A clonal PCR product could be PCR amplified and directly sequenced in 19 (76%, 90% confidence interval 54%-86%) of these 25 patients, 8 with diffuse and 10 with follicular NHL. Eight of these 18 patients have relapsed after ABMT. All had detectable lymphoma cells before relapse and the sequence of the CDR III region at the time of relapse was identical to that obtained at the time of ABMT. All 10 patients who remain in complete remission from 18 to 36 months after ABMT had eradication of PCR detectable lymphoma cells after ABMT, although in three patients PCR detectable MRD was detected early after ABMT. We conclude that sequencing and the use of patient specific IgH CDR III oligonucleotides probes provides a simple and highly reliable method to determine the specificity of the IgH PCR technique. The clinical utility of this technique is demonstrated by the finding that eradication of PCR detectable lymphoma cells in these patients is associated with decreased relapse after ABMT (P = .0002).

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greater number of patients, they are less sensitive than the detection of fixed chromosomal translocations. PCR amplification of Ig gene rearrangements has been used to detect clonal B-cell proliferation. The utility of PCR amplification of the CDR III region in B-cell malignancies has been studied in large numbers of patients with acute lymphoblastic leukemia (ALL), myeloma, and chronic lymphocytic leukemia (CLL), but this technique has been used to assess clonality in relatively few patients with NHL. Moreover, the clinical utility of the detection of clonal rearrangements of IgH in NHL has not been established.

In the present study, we assess the detection of PCR detectable IgH rearrangements in patients with B-cell NHL who have undergone autologous bone marrow transplantation (ABMT). We examined the clinical utility of this technique in 25 NHL patients who had no PCR detectable bcl-2/IgH rearrangement. We demonstrate that this technique is feasible in 72% of patients studied. Moreover, we demonstrate in this patient population that high dose therapy and ABMT is capable of eradicating PCR detectable MRD and that this is associated with decreased relapse after ABMT.

**MATERIALS AND METHODS**

*Patients.* Patients with relapsed B-cell NHL were eligible for ABMT if they were up to 65 years of age and had chemosensitive disease. Chemosensitivity was determined by response to salvage therapy such that the largest nodal mass was less than 2 cm and the bone marrow (BM) was infiltrated with less than 20% of the intraradicular space. All patients had lymphoma that expressed CD20. Harvested autologous BM was purged using a cocktail of anti-B-cell monoclonal antibodies and complement. All patients received cyclophosphamide 60/mg/kg intravenously (IV) on each of two consecutive days followed by total body irradiation (TBI) to 12 Gy hyper-fractionated in 200 cGy fractions. Institutional Review Board approval was obtained and informed consent obtained in all cases. Patients were eligible for inclusion in the present study if they had no PCR detectable bcl-2/IgH rearrangement and if they had morphologic evidence of BM infiltration at the time of ABMT. This provided us with a diagnostic tissue sample containing residual lymphoma cells that could be used to PCR amplify and sequence the lymphomatous clone.

**DNA preparation.** Peripheral blood (PB) or BM mononuclear cells were centrifuged on a Ficoll/Hypaque gradient (Pharmacia, Uppsala, Sweden) for isolation. The cells were transferred to new tubes, centrifuged, lysed, and DNA extracted, as previously described.

**PCR amplification.** DNA extracted from BM harvest samples was initially amplified with consensus V (ACCCGGCC/TG GTGATTACTGT) and J region (ACCTGGGAGACCGTGACC) oligonucleotides. One-microgram target DNA was amplified in a reaction mixture of 100 μL volume comprising 2.5 μL of PCR Buffer II (Perkin Elmer-Cetus, Norwalk, CT, 100 mM Tris-HCl, pH 8.3, 500 mM KCl), 10 μL of MgCl2 2.5 mM/mL, 200 μM/mL of each of dATP, dCTP, dGTP, dTTP (Perkin Elmer), and 2.5 U of AmpliTaq DNA polymerase (Perkin Elmer). Samples were amplified at 94°C for 4 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds with a final single extension step at 72°C for 7 minutes. To minimize the number of nonspecific bands "hot start" PCR was carried out using AmpliFAX 100 beads (Perkin Elmer). All PCR reactions were carried out using the GeneAmp PCR 9600 (Cetus, Emoryville, CA). Standard precautions were taken to prevent contamination and false-positive results. All results were confirmed on at least three occasions. A 20 μL aliquot of PCR product was run on 2% agarose gel to determine the presence or absence of clonal PCR product. Positive amplification resulted in a sharp clonal band between 80 and 120 base pairs; amplification of normal blood or marrow resulted in a broad smear at approximately 120 base pairs.

Samples in which no satisfactory PCR product was obtained using the consensus primers were amplified using V region family-specific primers. Reaction mixtures for FR1 PCR were essentially identical to those used for CDR III amplification with the exception that all samples were amplified with seven different 5' primers (VH1-CTTCAGTGAAGGTCCTCTGACGG, VH2-TCCTGCGCTTGTTGAAAGCCACACA, VH3-GGTCCTGGAGACTCTCTGACTTGCA, VH4a-TCCGGAGACCTGTCCTCACTCTGCA, VH4b-CGCTGTCCTGGTTACCTCATCAG, VH5-GGAAAGCCCGGGGATTCTCTGAA, and VH6-CCTGTGCCATCTCCGGGACAGTG) and the consensus JH consensus primer. Samples were amplified as follows: a single denaturation step at 94°C for 4 minutes was followed by 30 cycles of 94°C for 1 minute, 62°C for 30 seconds, and 72°C for 30 seconds. This was followed by a final extension step at 72°C for 10 minutes. PCR product was assessed by electrophoresis of a 20 μL aliquot on a 2% agarose gel stained with ethidium bromide. Positive amplification resulted in a band of approximately 350 base pairs.

PCR amplification at the major breakpoint region (MBR) and minor cluster region (mcr) of the bcl-2/IgH rearrangement was performed as previously described.

**Sequence analysis of PCR products.** Following PCR amplification using CDR III or FR1 primers, 100 μL of product was analyzed by agarose gel electrophoresis and the PCR band was excised. After transferring the gel slice to a 1.5 mL microcentrifuge tube, the agarose was heated to 70°C and the PCR product was purified using Wizard PCR Prens (Promega Corp, Madison, WI). For CDR III PCR products the yield of the PCR product was calculated by assessment of optical density. A 200 ng aliquot was sequenced directly in both directions using the JH consensus primer and a nondegenerate 5' VH consensus sequencing primer (5'-ACGGCCCTGATTACGTGTG-3'). The 3' JH primer and 5' V region specific primers were used to directly sequence the FR1 PCR products. All samples were analyzed on an Applied Biosystems 373A Automated DNA Sequencer (Foster City, CA) using the dyeoxy sequencing method and Taq polymerase.

**Identification of V, D, and J segments.** V, D, and J region nucleotides were identified from within CDR III or FR1 sequences to construct patient-specific oligonucleotide probes. Sense and antisense DNA sequences were aligned and contiguous sequences generated using Sequencher software (Gene Codes Corporation, Ann Arbor, MI). Identification of germline V, D, and J regions was carried out using Blast network service (National Center for Biotechnology Information, Bethesda, MD) and FASTA (NIH GenBank database, Bethesda, MD). Comparisons were also made using Sequencher software comparing patient-specific FR1 and CDR III sequences with previously published V, D, and J sequences (National Center for Biotechnology Information).

**Designing patient specific probes.** From the unique V-N-D region identified by sequence analysis of the CDR III region, a patient specific oligonucleotide was designed. The aim was to design a probe of approximately 20 oligonucleotides. Oligonucleotides were synthesized using the cyanethyl phosphoramidite method using an ABI 394 DNA synthesizer.

3' End-labeling of patient-specific oligonucleotide probes. 3' End-labeling with digoxigenin of the patients specific probes was
used to confirm the specificity of the PCR reaction and for subsequent detection of MRD (Genius Systems; Boehringer Mannheim, Indianapolis, IN). The following reagents were added to 1 L patient-specific oligonucleotide (100 pmol): 4 mL 10× tailing buffer, 4 mL CoCl2 (25 mmol/L), 1 L Dige-ddUTP, 1 L terminal transferase, and 9 mL sterile water. The reaction was started by placing the mixture at 37°C and incubating for 15 minutes. The reaction was stopped by adding 2 mL of a glycerol and 200 mmol EDTA mix on ice. The labeled oligonucleotide was precipitated by addition of 2.5 L of 4 mol/L LiCl and 75 mL 100% chilled ethanol. The mix was incubated at -70°C for 30 minutes and centrifuged at 12,000g for 15 minutes. The pellet was washed with 70% chilled ethanol and centrifuged again for 5 minutes. The ethanol was removed, the pellet air dried and resuspended in 20 L of sterile water.

Probing with patient-specific oligonucleotides. Preparation of the membranes: 3 L of PCR product was applied onto positively charged nylon membranes (Boehringer Mannheim). After air drying, the membranes were incubated in denaturing buffer (NaCl 1.5 mol/L, NaOH 0.5 mol/L) for 1 minute, and in neutralization buffer (Tris 0.5 mol/L, NaCl 1.5 mol/L, EDTA 1 mmol/L) for 1 minute. The DNA was then UV fixed using the autocrosslink program of Stratagene TM 2400 (Stratagene, La Jolla, CA). For hybridization, a rotatory oven was used (Bellco Glass Co). The temperature of hybridization was chosen according to the melting temperature of the oligonucleotide (Tm) calculated for each patient. For each patient, 20 pmol of 3' end labeled oligonucleotide was added. The mixture was incubated at Tm-5°C for 1 minute and in neutralization buffer (Tris 100 mmol/L, pH 7.5) for 1 hour and in buffer 2 (0.1 mol/L Tris, 0.15 mol/L NaCl, pH 7.5) for 1 hour. Three microliters anti-digoxigenin antibody was added to 30 mL of buffer 2 and the membrane further incubated for 30 minutes. After two 15-minute washes in buffer 1, the membrane was laid on an acetate sheet. Lumiphos® (Boehringer Mannheim), was spread on the membrane, covered with a second acetate sheet, and incubated at room temperature for 1 minute. The excess Lumiphos was removed and the membrane exposed on an autoradiograph film (DuPont, Inc, Boston, MA) for 2 to 60 minutes.

Statistical methods. Descriptive statistical methods have been used. Exact binomial confidence intervals have been provided for all estimates of proportions. The Fisher exact test was used to compare binomial proportions.

RESULTS

Sensitivity of detection of IgH rearrangement. The level of sensitivity of amplification of IgH rearrangements was first determined using the lymphoma cell lines DHL-16, DHL-4, DHL-6, and RL. Since these cell lines also have PCR detectable bcl-2/IgH rearrangements, the sensitivity of amplification of IgH was compared with amplification of the bcl-2/IgH. The IgH rearrangement of the DHL-16 cell line could be PCR amplified using both the consensus CDR III primers and the FR1 family specific VH3 primer. The sequence of the IgH CDR III region from DHL-16 is shown in Fig 1. An oligonucleotide probe was designed from this sequence and used as a lymphoma specific oligonucleotide probe. The results of PCR amplification of serial dilutions of DHL-16 in normal PB mononuclear cells are shown in Fig 2. A sharp clonal product could be visualized up to a dilution of 10^-4. A polyclonal smear produced by amplification of normal B-cell IgH rearrangements was visualized at a higher molecular weight than the clonal product seen with DHL-16. Similar results were obtained amplifying serial dilutions of three other B-cell lymphoma cell lines, RL (one cell in 10^-4), DHL-6 (one cell in 10^-3), and DHL-4 (one cell in 10^-2) (data not shown). In contrast, amplification of the bcl-2/IgH mcr rearrangement allowed detection of one DHL-16 cell in 10^9 normal cells and similar levels of sensitivity were seen amplifying the bcl-2/IgH MBR rearrangements in RL and DHL-6. Probing with the specific oligonucleotide probe increased the level of sensitivity of detection to one cell in 10^6. There was no difference in sensitivity of detection using either the consensus CDR III primer or the VH3 family specific FR1 primer (data not shown). When the sequence specific oligonucleotide was used as the 5' primer in the second round of a semi-nested amplification PCR amplification, this increased the level of sensitivity of detection of this cell line to one cell in 10^5 (data not shown). Therefore, this technique appears to be approximately two logs less sensitive than the detection of the bcl-2/IgH rearrangement.

 Determination of CDR III sequence in patients with B-cell NHL. BM harvest samples from 25 patients were analyzed. These patients were selected since they had histologic evidence of BM infiltration at the time of ABMT but no PCR amplifiable bcl-2/IgH rearrangement. The characteristics of these 25 patients is shown in Table 1. Ten patients had diffuse lymphoma and 15 patients had follicular lymphoma. PCR amplification of the DNA extracts of the harvested BM was performed using the following strategy. DNA from each sample was first amplified using the consensus FR3 and JH primers. A clonal product could be obtained, and the amplified DNA directly sequenced successfully in 11 of these 25 patients (44%). Samples from the 14 patients that could not be amplified or sequenced using consensus FR3 primers were then amplified with the V family specific FR1 and JH primers. The lymphoma specific CDR III sequence could be obtained using this strategy in an additional six of these 14 patients (43%). Attempts to increase the yield using alternative FR1 and JH primers resulted in direct sequencing of a clonal product in only one additional patient. Therefore, using this combined strategy a CDR III sequence could be obtained in 18 of the 25 patients studied (72%, 95% confidence intervals, 54% to 86%), even when starting with a tissue source that contained a small percentage of contaminating lymphoma cells. The CDR III region could be amplified and sequenced in 8 of 10 patients (80%) with diffuse histology and in 10 of 15 patients (67%) with follicular lymphomas that did not express a PCR amplifiable bcl-2/IgH. There was no statistically significant difference in the rate of amplification and sequencing of the CDR III region in these two patient groups (P = .66 by the fisher exact test). Representative sequences from two patients with diffuse lymphoma (#D1 and #D6) and two patients with follicular lymphoma (#F1 and #F9) are shown in Fig 3. In this figure
Fig 1. Sequence of the IgH rearrangement of the cell line DHL-16. The identical CDR III region could be PCR amplified using the VH3 FR1 primer as well as the CDR III VH consensus primer and the JH consensus primer (underlined). The V, N-D-N, and J regions are shown and the sequence used for the design of a specific oligonucleotide probe is highlighted.

Specificity of hybridization using patient-specific oligonucleotide probe. Specific oligonucleotide probes were constructed for each patient. We next determined the specificity of these oligonucleotide probes for the PCR product amplified from each patient studied, to ensure that the probes would not cross-hybridize with clonal products from one patient to another or with the polyclonal products of normal B cells. Specificity was demonstrated by the finding that there was no cross reaction between the patient specific probe and the clonal product obtained by amplification of other patient lymphoma samples or the polyclonal product obtained by amplification of normal PB B cells. Specificity was demonstrated by the finding that there was no cross reaction between the patient specific probe and the clonal product obtained by amplification of other patient lymphoma samples or the polyclonal product obtained by amplification of IgH from normal peripheral blood mononuclear cells. Representative results obtained on patient #D5 shown in Fig 4. A clonal product was visualized on agarose gel electrophoresis on all follow-up samples obtained from this patient. The probe unique for this patient hybridized with all of the clonal PCR products amplified from this patient, demonstrating that this represented the lymphomatous clone. Sequencing of follow-up samples from this patient demonstrated the identical sequence of the clonal product obtained in each sample analyzed. This further demonstrates that the sequence obtained in the BM harvest sample represents the lymphomatous clone and confirms the specificity of this technique. However, when the patient-specific oligonucleotide probe #D5 was used to hybridize clonal PCR products from other patients, the cell line DHL-16 or the polyclonal product obtained after amplification of normal PB B cell, there was no cross hybridization. This result demonstrates that we are able to follow a specific clone over time with this technique, and that there is no cross reactivity with the PCR products derived from other clones.

Sequence of IgH CDR III at BM harvest and at relapse. We sought to determine the sequence of the CDR III product at the time of ABMT, during follow-up, and at the time of relapse in these patients to determine whether the CDR III sequence was stable or whether there was clonal variation over time, as has been observed in the more immature B-cell malignancies. This is an important issue since we demonstrate that the patient specific probe can only detect the clonal product identified with that specific sequence. Eight patients in whom the CDR III region could be sequenced have relapsed to date. In all but one patient studied, the sequence obtained at each time point after ABMT or at

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Fig 2. PCR amplification of serial dilutions of the DHL-16 cell line in normal PB mononuclear cells. (A) Ethidium bromide stained agarose gel electrophoresis of PCR product obtained using the FR3 consensus primers. (B) Dot blot hybridization of PCR product probed with the lymphoma specific oligonucleotide probe outlined in Fig 1. (C) Ethidium bromide stained agarose gel electrophoresis of PCR product obtained using nested PCR amplification of the bcl-2/IgH mcr rearrangement.
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Histology</th>
<th>Sex</th>
<th>Age at ABMT</th>
<th>BM Involvement at ABMT</th>
<th>Primers</th>
<th>V Family</th>
<th>Status</th>
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<tbody>
<tr>
<td>D1</td>
<td>MC</td>
<td>M</td>
<td>46</td>
<td>&lt;5%</td>
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</tr>
<tr>
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<tr>
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<td>DSC</td>
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<td>--</td>
<td>--</td>
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<td>+</td>
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</tr>
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<td>DSC</td>
<td>M</td>
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<td>CCR 24 mo</td>
</tr>
<tr>
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<td>DMC</td>
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<td>DLC</td>
<td>F</td>
<td>53</td>
<td>&lt;5%</td>
<td>--</td>
<td>--</td>
<td>CCR 24 mo</td>
</tr>
<tr>
<td>D10</td>
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<td>M</td>
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<td>CCR 36 mo</td>
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<tr>
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<td>&lt;5%</td>
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<tr>
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</tr>
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<td>+</td>
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<td>CCR 25 mo</td>
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Abbreviations: D, diffuse histology; F, follicular histology; MC, mantle cell lymphoma; DLC, diffuse large cell lymphoma; DMC, diffuse mixed cell lymphoma; DSC, diffuse small cell lymphoma; FSC, follicular small cell lymphoma; FMC, follicular mixed cell lymphoma; CCR, continuous complete remission.

Relapse demonstrated the identical sequence, again confirming that the sequence obtained at the time of initial evaluation represented the lymphomatous clone. In one patient (#D1) three different clonal products could be isolated and sequenced suggesting that this patient had oligoclonal disease. Oligoclonality in lymphoma has been described previously. Additional samples are being sought from this patient and further analysis will be performed in the future to attempt to determine whether these clones were present from the time of initial diagnosis and whether these clones evolved by clonal evolution or arose independently.

Minimal residual disease detection after ABMT. The results obtained at the time of and after ABMT in the 18 patients in whom the CDR III region could be sequenced are shown in Fig 5. Since patients were selected on the basis of histologic evidence of BM infiltration at the time of ABMT, it is hardly surprising that PCR detectable disease was seen in all cases at the time of BM harvest. After immu-
nologic purging PCR detectable lymphoma was detected in all samples tested from the patients with diffuse histologies. PCR detectable lymphoma was not found after immunologic purging in five of the 10 samples (50%) from the patients with follicular lymphoma. There was a significant difference in the efficacy of purging in these two patient groups ($P = .04$ by the fisher exact test). Although PCR amplification of the CDR III region is less sensitive than amplification of the bcl-2/IgH, the result in patients with follicular lymphoma is in keeping with our findings in patients with a PCR amplifiable bcl-2/IgH. In these patients with follicular lymphoma, purging of PCR detectable lymphoma cells was successful in approximately 50% of patients tested. Of the 13 patients in whom PCR detectable lymphoma cells were detected after immunologic purging, 7 patients (54%) have relapsed to date. Of the five patients in whom PCR detectable lymphoma cells could be detected after immunologic purging, one patient (#F5) has relapsed. There is no statistical difference in the outcome of those patients who remain PCR positive after purging compared with those whose marrow becomes PCR negative ($P = .31$ by fisher exact test). Whether this is a result of the small number of patients studied or represents a difference in outcome in those patients with a t(14;18) compared with those patients without such a translocation clearly requires further study in a larger cohort of patients. These studies are ongoing in our laboratory.

On serial sampling after ABMT, PCR detectable lymphoma cells could be detected in all PB and BM samples available from five patients with diffuse lymphoma (#D1, #D2, #D5, #D7, and #D8). Of note, all five of these patients subsequently relapsed. PCR detectable lymphoma cells were not detected after ABMT in three patients (#D3, #D6, and D10). Although follow-up after ABMT is short (21, 24, and 36 months after ABMT), none of these patients have relapsed to date. In the patients with follicular lymphoma, PCR detected lymphoma cells in each sample analyzed after ABMT in three patients (#F3, #F5, and F9) and all three of these patients have relapsed. PCR detected lymphoma cells early after ABMT in three additional patients (#F1, #F10, and #F15). However, no lymphoma cells were detected on later follow-up samples from these patients although none received any additional therapy after ABMT. Whether these represent patients in whom DNA from nonviable lymphoma cells was obtained at the time of sampling or whether other mechanisms are involved in the eradication of these lymphoma cells over time is not clear. However, this pattern of result has been seen in our previous studies of patients with the bcl-2/IgH rearrangement. Irrespective of the mechanism of eradication of PCR detectable lymphoma, none of these patients have relapsed. PCR detectable lymphoma cells could not be found at any time after ABMT in the remaining four patients (#F2, #F7, #F8, and #F14). None of these patients have relapsed. Therefore, detection of MRD invariably preceded clinical evidence of relapse in each of the eight patients who have relapsed, whereas in the 10 patients in whom high dose therapy eradicated PCR detectable lymphoma cells, no relapses have occurred to date ($P = .00002$).

**DISCUSSION**

In the present study we report that PCR analysis and direct sequencing of the CDR III region of the IgH gene allow subsequent detection of MRD. We isolated DNA from the BM of patients with minimal histologic evidence of infiltration with lymphoma and demonstrate that it is possible to obtain the sequence of the CDR III region of the IgH gene in 72% of patients studied, even when the diagnostic tissue sample contained relatively low levels of tumor contamination. From this sequence a patient-specific oligonucleotide probe could be designed for the subsequent detection of minimal residual disease. Immunologic purging was capable of eradicating PCR detectable lymphoma cells in 50% of the patients with follicular lymphoma. Purging was signifi-
cantly less successful in patients with diffuse histology in whom residual cells were detected after purging in all cases studied. Moreover, we demonstrate that even in patients who have BM infiltration at the time of BM harvest, high dose therapy and ABMT were capable of eradicating PCR detectable lymphoma cells in 10 of the 18 patients (55%). Eradication of detectable MRD is associated with decreased relapse after ABMT \( P = 0.0002 \).

The strategy outlined for the amplification and direct sequencing of the IgH gene was designed to be relatively simple and rapid so that cohorts of patients could be followed serially from the time of ABMT. This strategy was successful in sequencing the amplified product in 80% of the patients with diffuse NHL and in 67% of patients with follicular lymphoma. Somatic mutations of the IgH region are common in follicular lymphoma\(^4\) and may result in decreased binding of the consensus primers used for amplification.\(^5\) In addition, since there is differential VH gene family usage in different B cell malignancies, the consensus primers used here may not be the best primers to amplify follicular lymphoma.\(^4,5\) Use of alternative primers did not increase the yield significantly. We are currently assessing whether sequencing of diagnostic lymph node biopsy material will increase the yield in patients with NHL.

We have demonstrated previously in patients with lymphoma with bcl-2/IgH translocations, that immunologic purging of autologous harvested BM was capable of eradicating PCR detectable disease in a subset of patients.\(^3,7\) We show now that similar results are observed in patients with follicular lymphoma who do not have a PCR amplifiable bcl-2/IgH rearrangement. In contrast, no patients with diffuse lymphoma were successfully purged of PCR detectable lymphoma cells following immunologic purging although patient numbers are small. Clearly, additional patients will have to be studied to determine if this decreased efficacy of purging in the diffuse histology patients compared with those patients with follicular histology \( P = 0.004 \) is maintained. As can be seen in Table 1, there was no quantitative difference in the lymphomatous infiltration present at the time of BM harvest in the patients with diffuse histology compared with those with follicular histology, nor was there any difference observed in the expression of the targeted antigens on their lymphoma cells (data not shown). However, there may be differences of antigen expression on the clonogenic lymphoma cells in these patient subgroups or patients with diffuse lymphoma may be intrinsically more resistant to immunologic purging using complement. Irrespective of the mechanism, the efficacy of purging in these patients is clearly not optimal and ongoing studies are directed toward modification of the purging procedures to increase the efficacy of purging. The techniques described here will allow us to assess whether such modifications to the purging procedure will result in depletion of PCR detectable lymphoma cells.\(^4,4\)

The CDR III sequence appears stable over time in patients with NHL and allows us to use the patient specific probe for the analysis of consecutive follow-up samples for the detection of MRD. This is in contrast to previous studies on B-cell ALL.\(^45-48\) We have also shown previously that eradication of PCR detectable lymphoma was associated with prolonged disease free survival after ABMT in patients with PCR amplifiable bcl-2/IgH rearrangement.\(^3\) We now demonstrate that this also appears to be the case in patients with NHL without bcl-2/IgH translocations. As performed here, the assessment of MRD using PCR of CDR III appears to be two or more logs less sensitive than PCR of the bcl-2/IgH. We chose to perform this relatively simple procedure using consensus primers rather than using patient specific oligonucleotides to assess the utility of this technique in following cohorts of patients through experimental treatment approaches. Of note, even using this less sensitive technique, all patients who relapsed had prior evidence of PCR detectable disease suggesting that greater sensitivity may not be required to assess MRD in these patients. The finding that all patients with NHL who relapsed had evidence of PCR detectable lymphoma cells in the BM is of particular interest since PB and BM provide the only readily available tissue sources to assess MRD in these patients. However, all the patients in the present study were selected because of their BM infiltration. It is certainly possible that patients with NHL could relapse at nodal or extranodal sites without evidence of lymphomatous infiltration in the BM. Whether patients who never had BM infiltration will have evidence of PCR detectable MRD in PB or BM remains to be determined and is currently under investigation in prospective studies at our own and other centers.

In conclusion, in this pilot study we demonstrate that PCR amplification of the IgH CDR III region is feasible in patients with NHL. This technique can be used to assess the clinical significance of MRD detection after experimental treatment approaches. These preliminary results suggest that eradication of PCR detectable MRD is associated with decreased relapse after ABMT and suggest that our therapeutic goal should be to eradicate PCR detectable lymphoma cells for cure.

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Eradication of polymerase chain reaction detectable immunoglobulin gene rearrangement in non-Hodgkin's lymphoma is associated with decreased relapse after autologous bone marrow transplantation

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