Bone Marrow Versus Peripheral Blood Progenitor Cells CD34 Selection in Patients With Non-Hodgkin’s Lymphomas: Different Levels of Tumor Cell Reduction. Implications for Autografting


Human CD34+ selected cells are able to reconstitute hematopoiesis in patients receiving myeloablative treatment. Although the role of reinfused tumor cells contaminating the grafts on the determination of postautograft relapses remains unclear, the major interest of CD34+ cell selection is to reduce the tumor contamination of the graft. This can be achieved if tumor cells do not express the CD34 antigen. We previously showed that this approach was effective with bone marrow (BM) collections in patients with non-Hodgkin’s lymphoma (NHL). Because peripheral blood progenitor cells (PBPC) allow faster hematologic recovery than BM and are expected to contain less tumor contamination, we have compared the results of CD34+ cell selection in 35 BM and 16 PBPC from 48 patients with NHL. The PBPC were collected after a course of chemotherapy followed by granulocyte colony-stimulating factor (G-CSF) administration. The data showed that the final CD34+ cell purity achieved with PBPC was higher than with BM (medians, 70% v 50%; P = .02). The CD34+ cell recovery was also better for PBPC (medians, 42% v 24%; P = .001). Tumor contamination was assessed by detection of BCL2/JH rearrangement using polymerase chain reaction (PCR) in 38 of 48 patients (22 BM, 16 PBPC). In addition, immunoglobulin heavy chain gene (Igh) rearrangements were investigated using PCR with consensus Igh primers. At harvesting, 10 of 22 BM and two of 16 PBPC contained BCL2/JH+ cells, one of 22 BM and 14 of 16 PBPC contained abnormal IgH+ cells (one PBPC contained both BCL2/JH+ and abnormal IgH+ cells) at harvesting. However, because lymphoma tissue specimens from patients at diagnosis were not available, the malignant character of IgH rearrangements could not be confirmed by sequencing and probing with allele-specific nucleotides.

IT HAS NOW BEEN clearly demonstrated that human CD34+ selected cells are able to reconstitute hematopoiesis in patients receiving myeloablative treatments.1-5 Besides the better tolerance resulting from the reduction in the number of autologous cells and consequently the total volume of dimethyl sulfoxide (DMSO) reinfused to the patient, the major interest of the CD34+ cell selection is to reduce tumor contamination of the graft in cases where tumor cells do not express the CD34 antigen. Based on previous data showing that lymphoma cells are not CD34+,6,7 we recently performed a pilot trial for the separation and reconstitution of CD34+ cells selected from bone marrow (BM) in patients with non-Hodgkin’s lymphoma (NHL).1 In this study, CD34+ cell selection was performed by immunoabsorption (Ceprate SC; Cellpro Inc, Bothell, WA) tumor contamination by detection of BCL2/JH rearrangements could not be confirmed by sequencing and probing with allele-specific nucleotides. After CD34+ cell selection, a reduction to below the level of detection of BCL2/JH+ cells of BM and PBPC was effective in seven of 12 informative selections. In contrast, a reduction to below the level of detection of abnormal IgH+ cells was effective in only three of 15 informative selections. However, the detection of cells with an abnormal IgH pattern in the context of chemotherapy plus G-CSF progenitor mobilization in patients with NHL and its correlation with actual tumor contamination needs further investigation.

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After selection of CD34+ cells, BCL2/JH+ cells were reduced to below the level of detection in seven of 10 BM, but not in the two PBPC positive products; in contrast, abnor-
mal IgH+ cells persisted in one of one BM and in 11 of 14
PBPC samples (one with both BCL2/JH+ and abnormal IgH+ cells). The discrepancy between tumor cell depletion ef-
ciency as evaluated by BCL2/JH+ and by abnormal IgH+ cells is a matter of concern. However, because samples of the initial lymphoma tumor were not available, it is unclear whether the detection of an abnormal IgH in the context of chemotherapuy plus G-CSF progenitor mobilization in pa-
patients with NHL was due to tumor cell contamination.

MATERIALS AND METHODS

Patients. A total of 48 patients included in this study were diag-
nosed with NHL (31 men and 17 women with a median age of 46
years (range, 27 to 60). Forty-three patients were stage III/IV and
5 were stage I/II; 35 patients had low-grade NHL (32 follicular and
the initial lymphoma tumor were not available, it is unclear were collected during hematopoietic recovery after treatment with

ciciency as evaluated by BCL2/JH

CD34 CELL SELECTION IN NON-HODGKIN’S LYMPHOMAS 2831

A total of 35 BM were harvested from 32 pa-

PBPC processing. PBPC were collected by two leukapherases from 16 patients using a Cobe Spectra apheresis machine. PBPC
were collected during hematopoietic recovery after treatment with
ACVBP (Adriamycin:75 mg/m2, day 1; Etoposide: 1,200 mg/m2, day 1;
Eldesine: 2 mg/m2, day 1 and day 5; Bleomycin: 10 mg, day 1 and
day 5; Prednisolone: 60 mg/m2, from day 1 to day 5) plus
G-CSF (5 mg/kg/d). For each apheresis, approximately 2.5 blood
volumes were processed for 3 hours through a dual-lumen central
venous catheter resulting in approximately a 200-mL collection. The
first apheresis was performed in the afternoon of the day when pa-

Flow cytometry analysis. The initial BC and CD34-selected
fractions were studied. MoAbs: fluorescein isothiocyanate (FITC)–
and phycoerythrin (PE)-anti CD34 (8G12), IgG1, PE-anti–CD33
(P67.6) IgG1, FITC-anti–HLA-DR (L243) IgG2a, PE–anti–CD38
 Franco), detoxiﬁed BSA 10 mg/mL and
Cobe Processor with 1 L of phosphate-buffered saline (PBS) (Braun
Laboratories-92; Boulogne, France). An aliquot containing 0.5 × 106
nucleated cells per kilogram of body weight (kg bw) was re-

BM processing. A total of 35 BM were harvested from 32 pa-

CellPro Inc) in 150 mL of PBS containing 1% autologous plasma
or, more recently, 0.1% human serum albumin (HSA; LFB, Orsay,
Francia). The treated cells were washed with PBS on the Cobe Pro-

Hematopoietic culture assays. Two types of culture assays were
used to quantitate late and early progenitors.

The assay for early colony-forming unit–granulocyte-macrophage
(CFU-GM) was performed according to our previously described

9

were eluted with 90 mL of PBS containing 10 U/mL of heparin and 4
mL of HSA. After centrifugation for 10 minutes at 500g, the cells
were resuspended in PBS containing 7.5% dimethyl sulfoxide (Braun
Laboratories) and 2% HSA to a ﬁnal volume of 4.5 mL. The cells
were then cryopreserved in at least two 5-mL vials (Nalgene, Red-
mond, WA), using a controlled-rate freezing method and stored at

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each culture. Granulocyte-macrophage colonies (CFU-GM: more than 50 cells) were scored on day 14.

The assay for late CFU-GM was performed in agar according to the technique of Pike and Robinson. The basic medium was McCoy’s 5A medium without serum (GIBCO-Biocult) supplemented with 30% heat-inactivated FCS. Colony-stimulating activity (CSA) was supplied by 10% human placenta conditioned medium. BC and CD34+ selected cells were seeded at 5 × 10^6/mL and 10^6/mL, respectively in medium containing equal volumes of 0.6% agar and 2× McCoy’s 5A medium to achieve a final serum concentration of 15%. Three 35 × 10-mm petri dishes (Greiner, Frickenhausen, Germany) were plated for each assay. The cultures were incubated for 10 days at 37°C in a humidified 5% CO2 atmosphere. The CFU-GM were scored on day 10.

Study of minimal residual disease (MRD) by PCR detection of IgH and BCL2/JH rearrangements. For molecular studies, the same number of cells, obtained from CD34+ and CD34- cell fractions and from unseparated cell fractions, was used. High molecular weight DNA was extracted according to standard methods.

For IgH rearrangement analysis, PCR was performed in a 50 μL final volume using 1 μg of DNA, 600 mMol/L oligonucleotide primers, 200 mMol/L each of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP), 400 mMol/L of deoxythymidine triphosphate (dUTP), 2.5 U Taq polymerase (Cetus, Emeryville, CA), MgCl2, 500 mMol/L in PCR buffer (500 mMol/L KCL, 100 mMol/L Tris HCl, pH 8.3). One unit of the enzyme Uracil DNA glycosylase (UDG) was also added to avoid carryover contamination from previous PCR products. The amplification was performed for 40 cycles in a Perkin Elmer Cetus thermal cycler with the consensus oligonucleotides 5’-ACCTGAGGAGACGGTGACC-3’ (FR4) located on the 3’ end of the JH segment and 5’-ACACGGC(CT)(GC)TGTATTACTGT-3’ (FR3) located on the 3’ end of FR3 of the VH segment. Aliquots of the PCR together with a molecular weight marker (FXI74RF/HoeIII; GIBCO-BRL, Cergy Pontoise, France) were analyzed by vertical electrophoresis in 6% polyacrylamide gel in Tris borate electrophoresis buffer. The size of the expected band was 80 to 110 bp. The sensitivity of this PCR technique, determined by serial dilutions of a IgH-positive B-acute lymphoblastic leukemia (B-ALL) cell line in normal BM mononuclear cells followed by subsequent extraction of DNA, was approximately 5 in 10^3 cells for a final number of 10^3 cells.

In 17 cases (8 BM, 9 PBPC), independently extracted DNA samples were amplified as above using a 5’ fluorescein-labeled FR3 consensus primer and a FR4 consensus primer. This technique modified from Shiach et al allows analysis of PCR products with a fluorescent automated laser DNA sequencer (ALF, Pharmacia LKB Biotechnology, Uppsala, Sweden). Electrophoresis was achieved on a 6% long ranger gel (Bioprobe, Paris, France) containing 8 mol/L urea in a 0.6× Tris-borate-EDTA buffer). Areas of the peaks were determined using the integration software Smart Manager (Pharmacia).

The amplification of the BCL2/JH rearrangement at the major breakpoint region (MBR) was performed by nested PCR according to a technique previously described by us and others. For each experiment, a positive control consisting of DNA from RL cells, a lymphoma cell line with a t(14;18) translocation and a negative control consisting of the PCR buffer with heat-inactivated proteinase K was performed. Each sample was analyzed at least twice. The sensitivity of the nested PCR, determined by serial dilutions of BCL2/JH+ RL cells in normal BM mononuclear cells followed by subsequent extraction of DNA, was approximately 1 in 10^3 cells for a final number of 10^6 cells. No increase in sensitivity was obtained by hybridization with an internal probe.

**Statistical analysis.** Results of cell processing are given as medians and ranges. The statistical significance of observed differences between BM and PBPC was assessed using the Mann-Whitney U-test. Correlations between initial CD34+ cell content and final CD34+ cell purity of BM and PBPC after processing were calculated using the Spearman correlation test.

### RESULTS

**Cell Recovery.** A total of 35 BMs from 32 patients were collected (patients 18, 24, and 27 were harvested twice). After removing a back-up marrow containing 0.5 × 10^8 nucleated cells/kg bw, a median of 2.1 × 10^6 nucleated cells (corresponding to 3.1 × 10^6 nucleated cells/kg bw) containing 0.9% (range, 0.2 to 7.2) CD34+ cells were incubated with 3 mg of the 12.8 anti-CD34 antibody and then processed on the Cephrate SC column (Table 1). The adsorbed CD34+ selected cell fraction contained 0.5% (range, 0.1% to 1.3%) of the original nucleated cells, with a CD34+ cell purity of 50% (range, 13 to 81). A median of 0.7 × 10^8 CD34+ cell/kg bw (range, 0.1 to 3.0) was present in the cryopreserved selected fractions.

**PBPC.** Two leukaphereses were collected from consecutive days from 16 patients. They were mixed and a BC prepared and processed on the Cephrate SC column. The BC contained a median of 2.4 × 10^10 nucleated cells (corresponding to 3.8 × 10^9 nucleated cells/kg bw) with 1.1% (range, 0.4 to 2.8) CD34+ cell content (Table 1). After Cephrate SC processing, a total of 0.9% (range, 0.3% to 3.1%) of the cells were recovered in the adsorbed fraction, with 70% (range, 16% to 99%) CD34+ cell purity. From the 16 processes, only three resulted in a final purity less than 50%. The median enrichment produced by the column (final/initial % CD34+) was 45-fold (range, 9 to 140). The CD34+ cell adsorbed fraction contained a median of 2.6 × 10^7 late CFU-GM/kg (50% median recovery; range, 4 to 150) and a median of 2.8 × 10^7 early CFU-GM/kg (49% median recovery; range, 6 to 135).

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<tbody>
<tr>
<td></td>
<td>BM (n = 35)</td>
<td></td>
<td>PBPC (n = 16)</td>
<td></td>
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<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
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<tr>
<td><strong>BC</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NC (×10^6)</td>
<td>92.1</td>
<td>(1.5-2.2)</td>
<td>2.4</td>
<td>(1.2-5.5)</td>
</tr>
<tr>
<td>% CD34+</td>
<td>0.9%</td>
<td>(0.2-7.2)</td>
<td>1.1%</td>
<td>(0.4-2.8)</td>
</tr>
<tr>
<td><strong>Selected fractions</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC/kg (×10^6)</td>
<td>1.6</td>
<td>(0.3-4.1)</td>
<td>3.1</td>
<td>(0.7-7.5)</td>
</tr>
<tr>
<td>NC recovery</td>
<td>0.5%</td>
<td>(0.1-1.3)</td>
<td>0.9%</td>
<td>(0.3-3.1)</td>
</tr>
<tr>
<td>% CD34+</td>
<td>50%</td>
<td>(13-81)</td>
<td>70%</td>
<td>(16-99)</td>
</tr>
<tr>
<td>Enrichment</td>
<td>×45</td>
<td>(9-140)</td>
<td>×70</td>
<td>(12-123)</td>
</tr>
<tr>
<td>CD34+ cell/kg (×10^6)</td>
<td>0.7</td>
<td>(0.1-3.0)</td>
<td>2.0</td>
<td>(0.1-6.6)</td>
</tr>
</tbody>
</table>

Abbreviations: NC, nucleated cells; BC, buffy coats.
Ceprate SC were only 14% and 28% for late and early CFU-GM, respectively.

**BM versus PBPC.** Taken together (BM and PBSC), the data on CD34+ cell selection showed a correlation ($P = .003$) between the final CD34+ cell purity and the initial CD34+ cell content of the processed BCs (Fig 1): the higher the initial % CD34+ cells, the higher the final purity. The CD34+ cell recovery was higher in PBPC than in marrow (42% vs 24%; $P = .001$). In contrast, the median recoveries post-Ceprate SC were lower in PBPC than in BM for late and early CFU-GM ($P = .001$ and $P = .05$, respectively). When late and early CFU-GM were plotted together (Fig 2), the slopes of the curves were different for BM and PBPC selected fractions. These data suggest that CD34 cells selected from BM and PBPC products were different.

**Flow Cytometry Analysis**

The immunophenotyping analysis of the CD34+ cell selected fractions either from BM or PBSC showed that they were highly heterogeneous for the CD33, CD38, HLA-DR, CD117, CD2, and CD19 antigens (Fig 3). The CD34+ cell selected fractions from PBPC contained a higher proportion of HLA-DR+, CD38+, and CD33+ cells than those from BM products. The median proportion of CD19+ cells was 14% (range, 1 to 43) and 2% (range, 0.4 to 51) in fractions selected from BM and PBPC, respectively, with medians of 14% (1 to 43) and 1.6% (0.3 to 5.0) of CD34+/CD19+ cells for BM and PBPC, respectively. It is noteworthy that in several instances almost all CD19+ cells were CD34+.

The double staining showed that the proportion of CD34+/CD33+, CD34+/CD38+, and CD34+/HLA-DR+ cells (corresponding to the more immature cells) was low both in BM (respective medians 15%, 0.7%, 1.0%) and PBPC (respective medians, 3.3%, 0.3%, 1.3%).

**Evaluation of MRD**

The MRD in BM and PBPC before and after Ceprate SC selection was assessed in 38 of 48 patients by detection of cells containing the BCL2/JH rearrangement (within the major breakpoint region of the BCL2). Abnormal IgH rearrangement patterns in the FR3/FR4 region were also investigated by detection of monoclonal or oligoclonal IgH bands using electrophoresis.

**Detection of MRD before CD34 Selection**

**BM.** From the 32 patients whose BM was harvested, 22 (18 of 22 with follicular lymphoma) were tested for the presence of cells carrying BCL2/JH and/or IgH rearrangements. At harvesting, 10 of 22 had BCL2/JH+ cells in the BM and 1 of 22 had abnormal IgH+ cells in the BM (Table 2 and Figs 4 and 5). It is noteworthy that among these 11 patients with positive MRD (BCL2/JH or IgH rearrangement) in BM, 10 of 11 had a follicular lymphoma with BM involvement at diagnosis as detected by histopathology (Table 2).

**PBPC.** All of the 16 PBPC products were tested for BCL2/JH and IgH rearrangements: at harvesting, one of 16 (patient 45) had BCL2/JH+ cells, 13 of 16 had abnormal IgH cells, and one of 16 (patient 47) had BCL2/JH+ and abnormal IgH cells (Table 2 and Figs 4 and 5) at harvesting. Only PBPC of patient 33 lacked both markers. These results indicate that a high proportion of patients had PBPC with an abnormal IgH pattern and hence potentially tumor contamination, despite the fact that the number of CD19+ cells (corresponding to the more immature cells) was low both in BM and PBPC selected fractions. These data suggest that CD34 cells selected from BM and PBPC products were different.

**Fig 1. Correlation between CD34+ cell content (%) in BC and CD34+ cell selected fractions from BM (●) and PBPC (▲) samples.**

**Fig 2. Correlation between CD34+ cell recovery and early CFU-GM recovery in BM (●) and PBPC (▲) selected fractions.**
2 of 9 PBPC (nos. 33 and 45) from patients with NHL and the control cells, which were all found negative with the standard technique, exhibited no predominant peak. Therefore, the results of automated scanning paralleled those obtained by observation of photographs after ethidium bromide staining, suggesting the actual presence of cells with an abnormal IgH pattern in the samples from NHL patients.

**Detection of MRD After CD34 Selection**

After selection of CD34+ cells, BCL2/JH+ cells were still detected in 3 of 10 BM (patients nos. 14, 28, and 32) and in 2 of 2 initially positive PBPC (patients 45 and 47) (Table 2 and Figs 4 and 5). It is noteworthy that the BM of patient 24, which was harvested twice after a 3-month interval, was found both times to be BCL2/JH+ and twice negative after CD34 selection.

Abnormal IgH+ cells persisted in 1 of 1 BM (patient 25) and in 11 of 14 initially positive PBPC (1 with both BCL2/JH+ and abnormal IgH+ cells) (Table 2). It is also of interest that none of the samples that were MRD-negative at harvesting became positive after CD34+ cell selection, indicating that the processing did not result in an increased concentration of tumor cells.

In summary, CD34+ cell selection led to a reduction of BCL2/JH+ cells to below the level of detection in 7 of 12 (58%) of the initially positive BM and PBPC harvests. This reduction was not linked to the final CD34+ cell purity (Table 2). For instance, the BM of patient no. 19, which became BCL2/JH+ after CD34+ cell selection, had a 28% CD34+ cell purity, while the BM of patient no. 32, which remained BCL2/JH+, had a 69% CD34+ cell purity. In contrast, a reduction to below the level of detection of abnormal IgH+ cells was not effective in 12 of 15 harvests (one BM, 11 PBPC).

**Discussion**

PBPC have become the prevalent source of stem cells for hematologic rescue in patients given high-dose chemotherapy for the treatment of solid tumors and hematologic malignancies. The major advantage of PBPC transplants over BM is a faster hematologic recovery. Similarly, faster kinetics have been observed with CD34+ progenitors selected from BM and in 2 of 2 initially positive PBPC fractions. However, the final CD34+ cell purity was higher in PBPC selected fractions than in BM (70% vs 50%), as well as the CD34+ cell recovery (42% vs 24%). The heterogeneous results obtained in CD34+ cell purity with BM (range, 13% to 81%) were in part due to the low initial CD34+ cell concentration, but were also probably due to the regularly high hematocrit of the BM BC. We think that a high hematocrit combined with a low initial CD34+ cell content, likely impeded in some instances a good capture of CD34+ cells by the column. Indeed, in independent experiments, where
Table 2. MRD as Assessed by Detection of Cells Bearing a BCL2 and/or a IgH Gene Rearrangement on BM (patients 11 to 32) and PBPC (patients 34 to 48) Before (buffy coat) and After CD34 Selection (selected)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Histology at Diagnosis</th>
<th>BM Involvement</th>
<th>MRD Phenotype</th>
<th>Phenotype</th>
<th>CD34</th>
<th>CD19</th>
<th>CD34</th>
<th>CD19</th>
<th>CD34/CD19</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>BCL2/JH</td>
<td>IgH</td>
<td>BC</td>
<td>Selected</td>
<td>BC</td>
<td>Selected</td>
<td>(%)</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<td>14</td>
<td>F</td>
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Abbreviations: MRD, minimal residual disease; F, follicular; HG, high grade; DIFF, diffuse; ND, not done.

we have performed a preliminary step of Ficoll-Hypaque separation on BM BC, the CD34+ cell purity of the selected fractions reached levels obtained with PBPC (M. Lopez, unpublished results, June 1995).

The content of the more immature cell populations (CD34+/CD33-, CD34+/HLA-DR-, CD34+/CD38-) in the selected fractions was low in both BM and PBPC samples. However, the median number of CD34+/CD117+ and CD34+/CD38+ cells was significantly higher in BM than in PBPC selected fractions. It is noteworthy that in the selected CD34+ cell fractions, medians of 14% and 1.6% of CD34+/CD19+ cells remained present in BM and PBPC, respec-

![Fig 4. PCR detection of BCL2/JH rearrangement.](image-url)
BM was collected in patients in steady state and PBPC were collected after a course of chemotherapy followed by G-CSF administration, we first hypothesized that we were dealing with false positivity emerging from a G-CSF disturbance of the hematopoietic equilibrium. Therefore, we tested samples from eight patients with solid tumors who received a course of chemotherapy followed by G-CSF and none were positive, suggesting that the presence of these abnormal cells is restricted to NHL samples. However, because lymphoma tissue specimens from patients at diagnosis were not available, the malignant character of the observed IgH rearrangements could not be confirmed by sequencing and probing with allele-specific nucleotides. In addition, we ignored whether these cells were already present before PBPC mobilization. Hence, a correlation between the detection of cells

![Fig 5. PCR detection of IgH rearrangement. Four representative results from unprocessed and processed BM and PBPC are shown. Left upper panel: Data from patient 25 (persistence of the IgH rearrangement in the CD34⁺ cell fraction of BM). Right upper panel: Data from patients 33 (negativity of the IgH rearrangement in the three fractions) and 37 (persistence of the IgH rearrangement in the CD34⁺ cell fraction of PBPC). Lower panel: Data from patient 41 (disappearance of the IgH rearrangement in the CD34⁺ cell fraction of PBPC). Control, B-ALL cells; Control, normal PB lymphocytes; BC, DNA extracted from buffy coat (unprocessed BM or unprocessed PBPC); CD34⁺, DNA extracted from the unadsorbed CD34⁺ cell fraction; CD34⁻, DNA extracted from the adsorbed CD34⁻ cell fraction; MW, molecular weight markers. Arrows show the specific abnormal JH rearranged band.]

![Fig 6. IgH electrophoretograms produced by gene scanning software of PCR-amplified DNA extracts from unprocessed BM and PBPC products and from various control samples. Lines 1 to 8, samples 21, 22, and 24 to 28 from BM of NHL patients. Lines 3 and 4 corresponded to BM samples of patient 24 collected at a 3-month interval. Line 9, control BM of a healthy donor (HD). Lines 10 to 18, samples 33 to 35, 37, 39, 41 to 43, and 45 from PBPC of NHL patients. Lines 19 to 26, control samples from various solid tumors. Line 27, control blood from a healthy donor (HD). Abnormal peaks are indicated by an arrow (→). Please note that in the ethidium bromide staining technique, samples 21, 22, 24, 26, 27, 28, 33, 45, and the controls were found negative, while samples 25, 34, 35, 37, 39, 41, 42, and 43 were found positive.]

tively. However, there was no correlation between the proportion of these residual CD34⁻/CD19⁺ cells and the presence of cells with BCL2/JH marker in the selected fractions.

Surprisingly, the recoveries of early and late CFU-GM progenitors were higher with BM than with PBPC, in contrast with the CD34⁺ cell recoveries. This could mean that in selected PBPC, a proportion of CD34⁺ cells were not clonogenic and therefore not involved in the CFU-GM recoveries.

Concerning the tumor cell reduction in the BM by the CD34⁺ cell selection procedure, our results with the BCL2/JH detection were in agreement with our preliminary results: from 10 initially positive BM, only 3 remained positive after Ceprate SC processing. As expected from previous reports,⁷ the BCL2/JH⁺ cell contamination was lower in PBPC collections than in BM; at harvesting, 2 of 16 PBPC only contained BCL2/JH⁺ (1 with both BCL2/JH⁺ and IgH⁺ cells). Taking BM and PBPC products together, the BCL2/JH⁺ marker was present in 12 before Ceprate SC processing, and still found in 5 after processing. The BCL2/JH⁺ cell reduction was not correlated with the final CD34⁺ cell purity of the selected fractions.

Concerning the detection of cells bearing an abnormal IgH pattern, contrasting results were observed between BM and PBPC before CD34⁺ cell selection: only 1 of 25 BM, but 14 of 16 PBPC contained abnormal IgH⁺ cells. Because
CD34 CELL SELECTION IN NON-HODGKIN’S LYMPHOMAS

with an abnormal IgH pattern and the presence of tumor cells remains to be established. However, because a risk of mobilizing tumor cells into the circulation by chemotherapy and growth factor treatment was already shown for solid tumors and myelomas,\textsuperscript{20-22} the persistence of these abnormal cells in the majority of the CD34\textsuperscript{+} cells selected from PBPC raises concern. Indeed, it has been recently shown\textsuperscript{23} that after autologous BM transplantation (ABMT), eradication of PCR detectable IgH rearrangement is associated with decreased relapse in patients with NHL.

Whether lymphoma cells bear the CD34 antigen is still a matter of discussion. Berenson et al\textsuperscript{8} initially reported that lymphoma cells do not express CD34. But recently, Macintyre et al\textsuperscript{24} sorted CD34\textsuperscript{+/CD19} and CD34\textsuperscript{+/CD19} cells from marrow samples of patients with follicular lymphoma at diagnosis or at progressive disease, with tumor contamination by histopathology and demonstrated by PCR that 3 of 3 of the CD34\textsuperscript{+/CD19} samples were positive for BCL2/JH rearrangement, while 4 of 5 of the CD34\textsuperscript{+/CD19} samples were negative. Although their number of samples studied was low, this questioned the potentiality of CD34\textsuperscript{+} cell selection alone to deplete adequately lymphoma cells from BM or PBPC. However, in our experience, there was no correlation between the presence of tumor cells as detected by BCL2/JH rearrangement and the number of CD34\textsuperscript{+}/CD19\textsuperscript{+} cells in the BM and PBPC products of NHL patients (the majority of them being in complete or partial remission at the time of harvesting, without BM involvement by histopathology).

In conclusion, our data indicate that CD34\textsuperscript{+} cell selection using an avidin-biotin immunoadsorption technique for hematopoietic stem cell products in NHL patients resulted in better CD34\textsuperscript{+} cell purity and recovery with PBPC. However, the significance of the high proportion of cells with an abnormal IgH pattern in PBPC and their persistence after CD34\textsuperscript{+} cell selection call for additional investigations to understand whether the detection of IgH rearrangement in the context of PB mobilization in NHL means either tumor contamination or, if so, tumor cell mobilization.

ACKNOWLEDGMENT

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Bone Marrow Versus Peripheral Blood Progenitor Cells CD34 Selection in Patients With Non-Hodgkin's Lymphomas: Different Levels of Tumor Cell Reduction. Implications for Autografting