Preparation and Successful Engraftment of Purified CD34+ Bone Marrow Progenitor Cells in Patients With Non-Hodgkin's Lymphoma

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From September 1992 to January 1994, we evaluated the use of the CEPRATE SC stem cell concentrator (CellPro, Inc, Bothell, WA) to select CD34+ cells from the bone marrow (BM) of 25 patients with non-Hodgkin's lymphoma in complete remission. This system uses the biotinylated 12.8 IgM MoAb to select CD34+ cells. Cells are retained on an avidin column and detached by agitation. Fifteen patients have been transplanted with the CD34+ purified fraction. The CD34+ purified fraction of the 25 processed BMs contained a median of 0.54% of the original nucleated cells in a volume of 5 to 10 mL. The median concentration of CD34+ cells was 49% (range, 12% to 80%), and the median enrichment of CD34+ cells was 33-fold (range, 9- to 85-fold). This selected CD34+ fraction retained 60% (range, 15% to 95%) of late granulocyte-macrophage colony-forming units (CFU-GM), 55% (range, 12% to 99%) of early CFU-GM, and 31% (range, 2% to 100%) erythroid burst-forming units (BFU-E) corresponding to median enrichments of 22-fold (range, 1- to 71-fold), 19-fold (range, 2- to 58-fold), and 14-fold (range, 2- to 200-fold), respectively. There was a correlation between immune phenotypes and progenitor cells. In the initial buffy-coat fractions, the percentage of CD34+ cells was correlated to the cloning efficiency of both late CFU-GM ($P < .05$) and early CFU-GM ($P < .001$). In the final selected fraction, there was a correlation between the percentage of CD34+/CD33− and the cloning efficiency of early CFU-GM ($P < .05$) and between the percentage of CD34+/CD33+ and the cloning efficiency of late CFU-GM ($P < .05$). Lymphoma cells positive for t(14;18) were found by polymerase chain reaction in 9 of 14 buffy coats tested before CD34+ cell purification. In 8 cases, the CD34+-selected fraction was found to be negative, and the CD34− fraction was found to be positive. After cryopreservation, the recoveries of progenitor cells in the CD34+-purified fraction were 79% for late CFU-GM, 71% for early CFU-GM, and 73% for BFU-E. The 15 patients transplanted with the concentrated CD34+ fraction received a median dose of 1×10^6 CD34+ cells/kg (range, 0.3 to 2.96) and 10.62×10^4 early CFU-GM/kg (range, 0.92 to 25.55). Median days to recovery to 0.5×10^9/L neutrophils and 50×10^9/L platelets were days 15 (range, 10 to 33) and 23 (range, 11 to 68), respectively. The median numbers of red blood cell (RBC) units transfused was 8 (range, 4 to 19). The median number of platelet transfusions was 8 (range, 3 to 31). Neutrophil recovery was quicker in patients receiving the higher doses of BFU-E ($P = .05$); these patients also received fewer platelet transfusions ($P = .04$). One patient remains thrombocytopenic and platelet-dependent 6 months posttransplant. This patient received the lowest dose of CD34+ cells (0.3×10^6/kg). This suggests, as a general guideline, to avoid transplanting patients with doses of BM CD34+ cells below 0.5×10^6/kg. Of the 15 patients transplanted, 11 are presently alive in complete remission from 2 to 17 months posttransplant. This study provides further evidence that CD34+ stem cell concentrates can be used to reconstitute hemopoiesis and decrease the tumor contamination of the graft.

HIGH-DOSE INTENSIFICATION followed by transplantation of autologous bone marrow (ABMT) or peripheral blood stem cells is currently used to consolidate patients with non-Hodgkin's lymphoma (NHL). This approach has been shown to improve prognosis in patients with intermediate- or high-grade NHL when used in chemosensitive relapses[1,2] or in first remission (CR1).[3,4] Similarly, several ongoing studies are investigating the impact of high-dose intensification with stem cell rescue in early remission[5-8] of low-grade lymphoma.

In all instances, infusion of stem cells to NHL patients bears the intrinsic risk of also infusing tumor cells, even when tumor contamination of the graft has not been detected by histology. The traceable translocation t(14;18) has been detected by polymerase chain reaction (PCR) in BM and/or blood of more than 75% of the patients with low-grade follicular lymphomas.[9-11] This includes patients with localized disease (stage I and II) at presentation[11] and patients in complete remission (CR) after conventional chemotherapy.[10] Effective purging of the BM autograft has been shown to be correlated with a lower relapse rate.[12] Current methods of purging lymphoma cells in NHL have consisted of lysis by monoclonal antibodies (MoAbs) with complement[2] or incubation with a cyclophosphamide derivative, mafosfamide.[13-14] These techniques are cumbersome and potentially harmful to normal progenitors. Recent studies have shown that BM progenitor cells that bear the CD34 antigen can be isolated and concentrated by a biotin-avidin immunoadsorption method and are able to reconstitute hemopoiesis in animals[15-16] and patients with neuroblastoma or breast cancer.[17-18] Because lymphoma tumor cells do not bear the CD34 antigen,[19] positive selection can be proposed to NHL patients

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with several potential benefits including a reduction in the tumor contamination of the graft. Here, we report on the preparation of $CD34^+$ stem cell concentrates and the engraftment data as the conclusion of a phase-1 to -2 trial.

MATERIALS AND METHODS

From September 1992 to January 1994, purified $CD34^+$ fractions were prepared from the BM of 25 patients with NHL, harvested in preparation for ABMT. Fifteen patients have been transplanted.

BM harvested, CD34 concentration, and cryopreservation. Under general anesthesia, BM was harvested in heparinized flask (heparin, 100 IU/mL; Roche, Nevilly/Seine, France) from the posterior iliac crests. In all patients, 0.5×10^8 nucleated cells/kg was stored unprocessed to serve as a back-up. A buffy coat was obtained from the second part by centrifugation using a COBE 2991 blood cell processor (COBE Laboratories, Lakewood, CO) at 3,000 rpm for 10 minutes. The product was washed twice with 1,000 mL of phosphate-buffered saline (PBS; Dulbecco's without Ca^{++} and Mg^{++}; GIBCO, Paisley, Scotland) and concentrated in a 150-mL final volume of PBS with 0.1% human serum albumin (HSA). The cell suspension containing a median of 2×10^{10} cells (1×10^{10} to 5.2×10^{10}) was then incubated with 20 μg/mL of the biotinylated 12.8 MoAb[20] for 25 minutes at room temperature. After washing with PBS to remove any antibody excess, this antibody-sensitized buffy-coat fraction was filtered through the Ceprate avidin column (Ceprate Stem Cell Concentrator; CellPro, Bothell, WA). In this system, the $CD34^+$ cells link to the avidin-coated polyacrylamide beads through an "avidin-biotin–anti-CD34 antibody–$CD34^+$ cell" complex. After washing the column with 300 mL of PBS-HSA 1% to remove nonspecifically bound cells, the $CD34^+$ cells were released from the avidin beads by mechanical agitation and recovered with 90 mL of PBS-HSA 1% supplemented with 10 IU/mL heparin. Aliquots were taken from both $CD34^-$ and $CD34^+$ fractions for flow cytometric analysis and progenitor cell culture assays. The selected $CD34^+$ suspension was centrifuged for 10 minutes at 500*g* and resuspended in 4.5-mL aliquots of PBS medium (GIBCO) with 7.5% dimethylsulfoxide and 4% HSA. This suspension was then cryopreserved in liquid nitrogen using controlled-rate freezing and stored at −196°C[21] to be reinfused later into the patient.

Flow cytometric analysis. The initial buffy-coat and $CD34^+$ fractions were analyzed on a flow cytometer (FACSort; Becton Dickinson, Lincoln Park, NJ). Double labeling was used in the $CD34^+$-selected fraction to evaluate the following phenotypes: CD34/CD33, CD34/CD38, CD34/HLA DR, CD34/CD19, and CD34/CD2. Because of the low concentration of this specific cell population, the initial buffy coat could be reliably analyzed only for the percentage of $CD34^+$ cells. A half-million buffy-coat cells and 10^5 $CD34^+$-selected cells were incubated with 10 μL fluorescein- and phycoerythrin-labeled antibodies in 100 μL PBS for 20 minutes at 4°C. After two washes with PBS 1% bovine serum albumin (BSA), the cells were fixed with 500 μL of PBS 1% paraformaldehyde solution. To define the exact percentage of $CD34^+$ cells in the two fractions, we compared the dot-plot of side scatter and fluorochrome green or red with the double-labeled negative control and with the cell control. A total of 1×10^4 to 2×10^4 cells were analyzed . We analyzed the cell phenotypes in the selected fraction as follows: $CD34^+/CD33^-$, $CD34^+$/HLA DR^-, $CD34^+/CD38^-$ for early progenitor cells; and $CD34^+/CD33^+$ for late progenitor cells.

Hematopoïetic culture assays. We used two types of culture assays to quantitate late and early progenitors as described below.

The assay for early granulocyte-macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) was performed according to the technique of Eaves and Eaves.[22] Briefly, 5×10^4 mononuclear cells (MNCs) were plated in 35-mm petri dishes in 1-mL aliquots of Iscove's modified Dulbecco's medium containing 30% fetal calf serum (GIBCO), 10 mg/mL detoxified BSA (Sigma, St Louis, MO), and 0.92% methylcellulose (Flucka, Buchs, Switzerland). Cultures were stimulated with a mixture of human recombinant growth factors: interleukin-3 (10 ng/mL; Sandoz, Basel, Switzerland), granulocyte colony-stimulating factor (G-CSF; 10 ng/mL; Amgen, Thousand Oaks, CA), granulocyte-macrophage CSF (GM-CSF; 10 ng/mL; Schering-Plough, Kenilworth, NJ), erythropoietin (3 U/mL; Boehringer, Mannheim, Germany), and stem cell factor (SCF; 100 ng/mL; Immunex, Seattle, WA). Dishes were incubated at 37°C in a humidified 5% CO_2 atmosphere. Three dishes were plated for each culture. Granulocyte-macrophage colonies (CFU-GM; more than 50 cells) and erythroblastic colonies (BFU-E) were scored on day 14.

The assay for late CFU-GM was performed in agar according to the technique of Pike and Robinson.[23] The basic medium was McCoy's 5A medium without serum (GIBCO-Biocult, Paisley, Scotland) supplemented with 30% heat-inactivated fetal calf serum. Colony-stimulating activity was supplied by 10% human placenta-conditioned medium. Buffy-coat and concentrated $CD34^+$ cells were seeded at 5×10^4/mL and 10^3/mL, respectively, of 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 (Greuner, Frickenhausen, Germany) were plated for each assay. The cultures were incubated for 10 days at 37°C in a humidified 5% CO_2 atmosphere. The CFU-GM were scored on day 10.

Detection of residual disease. Bcl-2 rearrangement at the major breakpoint region (MBR) was performed according to a slightly modified technique described by Gribben et al.[10] Briefly, DNA was extracted using a standard procedure, heated to 96°C for 10 minutes to destroy proteinase-K activity. Each sample was amplified by PCR using nested oligonucleotide primers as follows. PCR was performed in a 50-μL final volume using 1.5 μg of DNA; 200 nmol/L oligonucleotide primers; 200 mmol/L each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxytyrosine triphosphate; 1.5 U Taq polymerase (Cetus, Emeryville, CA) in PCR buffer (50 mmol/L KCL, 10 mmol/L Tris-Cl, 2.25 mmol/L Mg Cl_2, 0.01% BSA). The initial amplification was performed for 25 cycles in a Perkin Elmer-Cetus (Norwalk, CT) thermal cycler with the oligonucleotides 5′-CAGCCTTGAAACATTGATGG-3′ for the MBR and 5′-ACCTGAGGAGACGGTGACC-3′ for the J_H consensus region. Each cycle was performed with 1 minute of denaturation at 94°C, 1 minute of annealing at 56°C for the MBR amplification, and 1 minute of extension at 72°C. The final extension period was extended to 10 minutes.

Reamplification of a 5-μL aliquot of this amplified mixture was performed for 30 cycles using oligonucleotide primers internal to the original primers, 5′-TATGGTGGTTTGACCTTTAG-3′ for the MBR and 5′-ACCAGGGTCCCTTGCCCCA-3′ for the J_H consensus region with 1 minute of denaturation at 94°C, 1 minute of annealing at 58°C, and 1 minute of extension at 72°C. The final extension period was again extended to 10 minutes. Aliquots of this final reaction were analyzed by electrophoresis and visualized by staining with ethidium bromide under UV light. Then, the reamplified DNA was blotted by alkaline transfer onto Hybond N^+ membranes (Amersham, Buckinghamshire, UK) and bcl-2–specific DNA was detected by hybridization overnight with ^{32}P-labeled oligonucleotide probes, 5′CCCTCCTGCCCTCCTTCCG-3′ for the MBR. Oligonucleotides were radiolabeled with (γ^{32}P) adenosine triphosphate using T4 polynucleotide kinase (Boehringer) according to the manufacturer's instructions.

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 included in each experiment. Each sample was analyzed at least twice. The limit of sensitivity of the technique, optimized by using

serial dilutions of the RL cell line in normal BM MNCs, was approximatively 1 tumor cell in 10^4 normal cells.

High-dose chemotherapy and CD34+ stem cells reinfusion. From September 1992 to January 1994, 15 patients were transplanted with their autologous CD34+-selected stem cells. This represents all consecutive lymphoma patients autografted in our institution during this 16-month period. Age, sex, classification of lymphoma, initial staging, and status at time of transplant are described in Table 5. The median age of the population was 45 years (range, 31 to 57 years), and the sex ratio (M/F) was 1.5. A total of 11 patients had follicular lymphoma including 1 in transformation, 2 had intermediate-grade, and 2 high-grade lymphoma, including 1 CD30 (Ki1)-positive T-cell lymphoma. At initial diagnosis, 2 patients were stage I/II, and 13 were stage III/IV with detected BM infiltration by biopsy in 7 of them, all low-grade (64% of all follicular lymphomas). At time of BM harvesting, no patient had tumor BM infiltration detected on pathologic examination of BM aspiration and biopsy. The pretransplant intensification regimen was BEAM consisting of 300 mg/m^2 BCNU on day −7, 200 mg/m^2 etoposide intravenously, and 200 mg/m^2 cytosine-arabinoside by continuous infusion from day −6 to day −3, and 140 mg/m^2 high-dose melphalan intravenously on day −2. The frozen CD34+ fraction was rapidly thawed, diluted in 30 mL of heparinized (10 IU/mL) TC 199 medium and infused via a central venous line on day 0. Human recombinant G-CSF (Neupogen, 5 μg/kg; Amgen) was administered intravenously from day 0 to recovery of neutrophil count to over 10^9/L.

Analysis of data. Values for recoveries of progenitor cells in cultures (in percentage) were obtained by dividing the absolute numbers of progenitor cells in the final selected fraction by the corresponding values in the initial buffy-coat fraction. The values for enrichment (fold) of CD34+ cells were obtained by (1) dividing the percentage of CD34+ cells in the final selected fraction by the corresponding values in the buffy-coat fraction (CD34+ enrichment) and (2) dividing the cloning efficiency in the final selected fractions (colonies/10^5 MNCs plated) by the cloning efficiency in the buffy-coat fractions (CFU-GM enrichment). Values reported are median and range. Studies on correlation between populations, defined by immunophenotyping and by cultures, were performed using the nonparametric Spearman test. Linear regression curves were established using the least square method with the Statview program. Comparisons of groups were performed by the χ^2 and Student's *t*-test. For the clinical part of the study, the endpoints were the kinetics of hematologic recovery defined as the day to recovery of neutrophils over 0.5 × 10^9/L and the day to recovery of platelets over 50 × 10^9/L; the amount of blood support expressed in the number of RBC units transfused and the number of platelet transfusions. The nonparametric Spearman test was used in search of a correlation among the kinetics of engraftment, transfusion of RBCs or platelets, and the dose of progenitor cells infused.

RESULTS

In vitro studies. BM samples obtained from 25 patients with NHL were processed to yield a CD34+ stem cell concentrate. Results of cell phenotype analysis and culture assays are reported in Table 1.

In the initial buffy coat, the concentration in CD34+ cells varied from 0.44% to 7.2%, with a median value of 0.9%. The median number of progenitor cells in cultures expressed per 10^5 MNCs plated were 122 for late CFU-GM, 267 for early CFU-GM, and 172 for BFU-E. In the CD34+-selected fraction, the median concentration of CD34+ cells was 49%, with a range of 12% to 80%. The enrichment in CD34+ cells was 33-fold (range, 9- to 85-fold). Analysis of subpopulations indicated that the CD34+ population was a mixture of immature (CD34+/CD33−; 17%) and mature progenitors (CD34+/CD33+; 21.5%) with very few putative multipotent progenitors corresponding to the CD34+/HLA DR− or CD34+/CD38− phenotype (0.9% and 1.5%, respectively). Interestingly, lymphoid progenitors corresponding to the CD34+/CD19+ phenotype accounted for a median of 11% of the overall cell population.

Table 1. Progenitor and Tumor Cells Evaluations In BM Collections Before and After CD34+ Cell Concentration

	Initial Fraction	Purified Fraction
Immunophenotype (%)*		
CD34+	0.9 (0.44-7.2)	49 (12-80)
CD34+CD33−	—	17 (2.5-48)
CD34+CD33+	—	21.5 (5-58)
CD34+CD19+	—	11 (1-69)
CD34+CD2+	—	5.8 (0.8-44)
CD34+HLA DR−	—	0.9 (0.4-3)
CD34+CD38−	—	1.5 (0.1-3.5)
Cultures (colonies/10^5 MNCs plated)†		
Early CFU-GM	267 (65-950)	4,762 (400-20,750)
Late CFU-GM	122 (11-426)	2,570 (100-11,400)
BFU-E	172 (3-950)	1,900 (100-12,500)
Recovery postfreezing (%)‡		
Early CFU-GM	44 (24-100)	71 (30-100)
Late CFU-GM	59 (30-100)	79 (18-100)
BFU-E	70 (43-100)	73 (19-100)

The values are given as the median with the range in parentheses.

* N = 25.

† N = 25. Early CFU-GM and BFU-E were grown on methylcellulose with addition of 5 cytokines (stem cell factor, interleukin-3, G-CSF, GM-CSF, erythropoietin); late CFU-GM were grown on agar with placenta-conditioned medium.

‡ N = 14.

The median values for progenitor cells expressed per 10^5 MNCs plated in culture were 2,570 for late CFU-GM, 4,762 for early CFU-GM, and 1,900 for BFU-E. Overall, the CD34-selected fraction (recovery) contained a median of 60% (range, 15% to 95%) late CFU-GM, 55% (range, 12% to 99%) early CFU-GM, and 31% (range, 2% to 100%) BFU-E, which corresponds to median enrichments of 22-fold (range, 1- to 71-fold), 19-fold (range, 2- to 58-fold), and 14-fold (range, 2- to 200-fold), respectively.

In the initial buffy-coat fractions, there was a correlation between the percentage of CD34+ cells and the cloning efficiency of both late CFU-GM ($P < .05$) and early CFU-GM ($P < .001$) progenitors (Fig 1A and B).

In the selected fraction, the analysis of subsets by phenotypes showed a correlation between the percentage of CD34+/CD33− and the cloning efficiency of early CFU-GM ($P < .05$) (Fig 2A). There was also a correlation between the percentage of CD34+/CD33+ and the cloning efficiency of late CFU-GM ($P < .05$; Fig 2B). There was no correlation between the CD34+/CD38− and CD34+/HLA DR− cell populations and the cloning efficiencies of early or late progenitors.

The purity of the selected fraction was higher for initial buffy-coat fractions, with a CD34 concentration above the median 0.9% value (59% *v* 32%; $P < .01$).

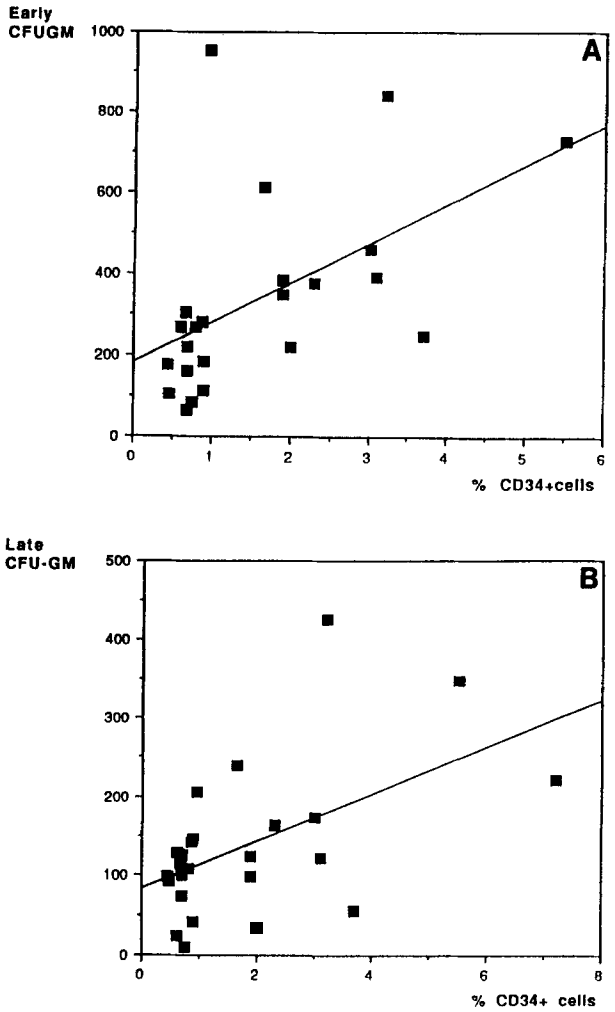

Fig 1. Correlations in the initial preselected BM buffy-coat fraction between progenitor cell evaluations by immunophenotyping (% CD34$^+$) and by cultures (colonies/10^5 MNCs plated). (A) Early CFU-GM (n = 22); y = 178 + 97x, R = 0.53; $P < .01$. (B) Late CFU-GM (n = 25); y = 82 + 30x, R = 0.53; $P < .01$.

The cryopreservation efficiencies in the purified CD34$^+$ fractions were available for patients that received transplants. Recoveries of early and late CFU-GM and BFU-E after thawing were identical to those obtained with frozen-thawed initial fractions (buffy-coats; see Table 1).

At a time when tumor contamination was not detected by routine pathologic examination of aspiration and biopsy in any of these patients, lymphoma cells positive for t(14;18) were found by PCR in 9 (8 patients) of 20 buffy-coats (19 patients, including 14 with follicular NHL) tested before CD34$^+$-cell purification. In 8 cases, the CD34$^+$-selected fraction was found to be negative, and the CD34$^-$ fraction was found to be positive. Interestingly, this includes 2 BM collections in 1 patient (first collection insufficient for a safe graft by conventional criteria in use in our institution) leading to 2 buffy coats separately processed; the two procedures gave the same results showing twice effective purging. In one case, the CD34$^+$ fraction still contained t(14;18) tumor cells (Table 2).

Clinical data. The number of progenitor cells infused, kinetics of engraftment and blood support for all patients are shown on Table 3.

All patients engrafted successfully on neutrophils with a median to recovery to 0.5 × 10^9/L of 15 days (range, 10 to 33). One patient was not evaluable for platelet recovery because he died of tumor progression on day 30. Of the 14 evaluable patients, 13 recovered a platelet count over 50 × 10^9/L at a median of 23 days (range, 11 to 68). One patient is still thrombocytopenic and supported by platelet transfusion at 6 months. This patient received the lowest dose of CD34$^+$ cells (0.3 × 10^6/kg); this also corresponded to the lowest doses of early CFU-GM (0.92 × 10^4/kg) and BFU-E (0.84 × 10^4/kg). The median number of transfused RBC units and the median number of platelet transfusions was 8 (range, 4 to 19) and 8 (range, 3 to 31+), respectively, with the two extremes (19 RBC units and 31$^+$ platelet transfusions) for the patient receiving the lowest doses of CD34$^+$-selected cells. There was a trend for more rapid recovery

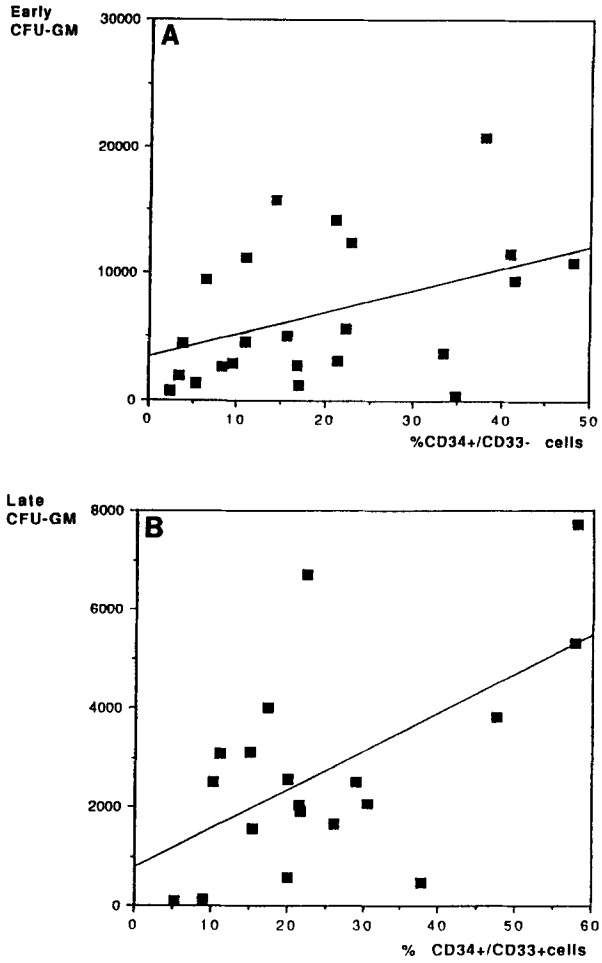

Fig 2. Correlation in the final enriched CD34$^+$ fraction between progenitor cell evaluations by immunophenotyping and by cultures (10^5 MNCs plated). (A) Percentage of CD34$^+$/CD33$^-$ and early CFU-GM (n = 23); y = 3,355 + 174x, R = 0.43; $P < .05$. (B) Percentage of CD34$^+$/CD33$^+$ and late CFU-GM (n = 19); y = 765 + 78x, R = 0.58; $P < .05$.

Table 2. Evaluation of Minimal Residual Disease in the CD34+ Concentrated BM Fraction for Patients Found bcl-2 JH-Positive by Nested PCR (9 Procedures for 8 Patients)

BM Histology			BM Collection		
At Initial Disease	At BM Collection	Clinical Status at BM Collection	BC	CD 34− Fraction	CD 34+ Fraction
−	−	PR chemoresistant	+	+	−
+	−	CR1	+	+	+
+	−	PR1	+	+	−
+	−	PR1	+	+	−
+	−	CR2	+	+	−
+	−	PR chemoresistant	+	+	−
−	−	CR2	+	+	−
+	−	PR chemoresistant*	+	+	−

The sensitivity of the PCR assay was 1 tumor cell detected per 10^4 cells.

Abbreviations: CR1, first CR; CR2, second CR; PR, partial remission; BC, buffy coat.

* This patient had 2 marrow collections, and 2 buffy-coats separately processed, leading twice to the results showed. He was transplanted with both CD34+ concentrates.

of neutrophils in patients receiving the higher doses of CD34+ cells ($P = .11$) or early CFU-GM ($P = .10$). Neutrophil engraftment was more rapid in patients receiving the higher doses of BFU-E ($P = .05$; $R = -0.45$). These patients also received fewer platelet transfusions ($P = .04$). Table 4 indicates the kinetics of recovery of early CFU-GM and BFU-E in BM and peripheral blood.

Of the 15 patients, 11 are presently alive in CR from 2 to 17 months posttransplant. One patient transplanted while his tumor was in relapse died on day 30 posttransplantation from tumor progression. Three patients in CR posttransplant have relapsed at 5, 8, and 10 months (Table 5).

DISCUSSION

This study reports results of a phase-I to -II trial designed to test the feasibility of using CD34+ stem cell ABMT after the administration of a myeloablative regimen for consolidation in patients with NHL.

The role of high-dose consolidation with the BEAM regimen or the combination of cyclophosphamide and total body irradiation has been clearly established in patients with chemosensitive relapse.[1,2] This protocol is presently being tested in our institution and in others for patients in first remission, regardless of the grade, including patients with low grade (follicular lymphoma).[5-8,14] The population of patients in the present study included all consecutive NHL patients autografted in our institution in the 16-month period of the trial. Eleven patients had follicular NHL, and four patients had high- or intermediate-grade lymphoma, of whom 3 were beyond CR1 and 1 in CR1, who was included in view of having a very poor prognosis (anaplastic large cell T lymphoma CD30+ with lymph node and cutaneous infiltration). Indeed, this last patient relapsed posttransplant.

Transplantation of concentrated CD34+ stem cells, rather than infusion of autologous BM or leukapheresis products, has the advantage of reducing volume, which facilitates storage and decreases the amount of dimethylsulfoxide and cell lysis products. This decreased volume has been shown to reduce cardiovascular side effects (specifically, maximal heart rate and blood pressure) that are observed with standard BM infusion.[24] More importantly, the risk of infusing tumor cells while performing an autograft has led to the development of multiple techniques aimed at the removal or destruction of occult tumor cells that potentially contaminate the graft. The level of toxicity of these methods (referred to as negative selection) toward the normal stem cell compartment ensuring engraftment is variable but potentially high, espe-

Table 3. CD34-Purified Stem Cell Transplantation: Progenitor Cells Infused, Kinetics of Engraftment, and Blood Support (n = 15)

Concentrated CD34+ Graft				Recovery to (d)		Blood Support (total no. of)	
CD34+ (%)	CD34+ (10^6/kg)	Early CFU-GM (10^4/kg)	BFU-E (10^4/kg)	Neutrophils >0.5 (10^9/L)	Platelets >50 (10^9/L)	RBC Units	Platelet Transfusions
66	1.12	—	—	16	23	8	8
59	1.84	19.9	6.63	25	68	10	25
51	0.66	11.2	6.14	12	13	4	4
50	0.58	10.45	3.66	20	42	10	13
68	2.20	16.95	16.62	13	18	6	8
72	2.96	25.55	8.34	12	NE	10	20
72	1.59	10.62	9.17	11	27	8	9
58	1.48	13.17	5.96	10	11	4	3
40	0.55	10.9	3.78	17	40	16	20
30	0.41	9.45	3.74	15	15	4	4
64	1.29	7.79	0.80	17	25	6	5
27	0.30	0.92	0.62	33	NR	19	31+
34	0.69	8.97	1.28	12	20	6	7
52	0.38	3.36	0.90	16	19	4	4
59	0.89	3.46	0.70	14	29	8	7
58 (27-72)*	1 (0.3-2.96)	10.62 (0.92-25.55)	3.76 (16.62-0.70)	15 (10-33)	23 (11-68)	8 (4-19)	8 (3-31+)

Abbreviations: NR, not reached; NE, nonevaluable.

* Values shown at the bottom of each column are the median with the range in parentheses.

Table 4. Kinetics of Recovery of Progenitor Cells Postinfusion of CD34⁺ Concentrates

	Day Post-ABMT			
	7	14	21	28
BM				
Early CFU-GM	7 (0-1,300)	270 (0-9,100)	3,392 (0-3,600)	335 (0-15,800)
BFU-E	2.5 (0-400)	52 (0-860)	424 (0-10,700)	176 (0-11,800)
Peripheral blood				
Early CFU-GM	0.15 (0-1.6)	1.85 (0-456)	52 (0.5-309)	32 (0.5-410)
BFU-E	0 (0-1.7)	3 (0-278)	30 (0-252)	21 (0-668)

Values are the median number of progenitor cells per milliliter with the range in parentheses (n = 14).

cially when cyclophosphamide derivatives are used.[7] Positive selection of CD34⁺ stem cells reduces the risk of damaging stem cells and, if needed, would facilitate further manipulation of the graft for various purposes such as gene transfer. With the notable exception of patients with acute leukemias, most hematologic tumors do not express the CD34 antigen on their surface. In particular, of 36 NHL tumor samples (25 large cell, 11 follicular), which one of us (R.J.B.) previously tested for reactivity with the antibody 12.8, none expressed the CD34 antigen.[19] Low-grade follicular lymphoma is a particular challenge because it generally presents as a disseminated disease with a very high BM and blood tumor infiltration rate, which is easily detectable by molecular biology.[10-12] Furthermore, although controversial, a recent analysis of the outcome has shown lower relapse rates posttransplant in patients with follicular lymphomas and the traceable bcl-2 rearrangement, after effective purging of the BM by a cocktail of MoAbs.[12]

Several techniques have been developed to concentrate CD34⁺ stem cells for autografting. All of these techniques rely first on the recognition of these cells through their immunophenotype, CD34⁺ alone[25,26] or, for instance, CD34⁺ Thy1⁺[27] or CD34⁺/CD38⁻.[28] Afterwards, the selection of cells is performed using panning,[25] immunomagnetic beads,[26] highly efficient cell sorting,[27] or the CellPro Ceprate device used in the current study. Considerable in vitro data has been reported on the CD34⁺ and the CD34⁻ subpopulations obtained with these different approaches. These results indicate that the CD34⁺ fraction is heterogeneous and contains both mature committed progenitors (CFU-GM, BFU-E), ensuring early recovery from aplasia, and immature progenitors, ensuring long lasting hemopoietic reconstitution. Successful engraftment has been obtained in several animal models, including monkeys, with the CD34⁺ fraction[15,16] but not with the CD34⁻ fraction. In humans, consistent engraftment in a large series of patients has been reported with the biotin-avidin immunoadsorption technique. The simplicity of this technique makes it easy to use in a hematology laboratory. In a comparative study of engraftment kinetics in 44 patients with breast cancer who received ablative chemotherapy followed by infusion of CD34⁺ concentrates from BM with or without infusion of growth factors, Shpall et al[18] have reported median days to neutrophil recovery to 0.5 $\times$ 10^9/L of 23 days with BM alone, of 16 days when adding GM-CSF, and of 10 days when adding G-CSF posttransplant.

Our study provides further evidence that CD34⁺ cell concentrates are capable of reconstituting hematopoiesis in patients receiving myeloablative therapy. The study also provides data on several practical aspects regarding the preparation of the concentrates. A first observation was that the purity and recovery of CD34⁺ cells varied from patient

Table 5. Characteristics and Outcome of Patients Transplanted With Purified CD34⁺ BM Cells (n = 15)

Age/Sex	Classification	Initial Staging	Status at Transplant	Outcome at (mo)
52/F	Follicular	IV m+	CR1	AW 17+
41/F	Intermediate	II	PR2	Relapse 5
44/M	High, T, Ki1⁺	IV cutaneous	CR1	Relapse 10
31/M	Follicular	III	PR1	AW 16+
42/M	Follicular	IV m+	CR1	Relapse 8
56/M	High	IV m+	In relapse	Relapse, death, 1
54/M	Intermediate	II	PR3	AW 10+
45/F	Follicular transformed	IV m+	CR1	AW 12+
57/M	Follicular	IV	PR2	AW 6+
33/F	Follicular	I	CR2	AW 10+
55/M	Follicular	IV m+	CR1	AW 9+
50/F	Follicular	IV m+	PR1	AW 6+
41/F	Follicular	IV m+	CR2	AW 5+
46/F	Follicular	III	CR2	AW 3+
42/M	Follicular	III	PR1	AW 2+

Abbreviations: m+, BM involvement; PR1, first partial remission; PR2, second PR; PR3, third PR; AW, alive and well.

to patient. Our results are similar to those observed by others using the CellPro CEPRATE System[17,18] and other techniques.[29] These results are probably related to our observation that the purity of the selected fraction was related to the concentration of $CD34^+$ cells before selection.

The correlation of $CD34^+$ cells by immunophenotyping to early and late CFU-GM by culture both in the buffy-coat fraction before selection and in the $CD34^+$ selected cells is consistent with prior studies. Furthermore, the finding in the latter of a linear correlation linking the $CD34^+/CD33^-$ subset to early CFU-GM and the $CD34^+/CD33^+$ subset to late CFU-GM is not unexpected. It confirms that the $CD34^+$ cell content of the purified fraction, in part, reflects its potential to reconstitute hemopoiesis. When looking at the kinetics of engraftment, patients receiving the lowest doses of $CD34^+$ cells tended to engraft more slowly both on neutrophils and platelets. The only patient who had not recovered platelets at 6 months posttransplant received the lowest dose (0.3×10^6 of $CD34^+$ cells/kg), which also translated into the lowest doses in CFU-GM and BFU-E. The absence of a statistically significant correlation between engraftment and the number of $CD34^+$ cells may result from the limited number of patients transplanted. Further transplants with low doses of $CD34^+$ cells would be necessary to establish a safety threshold, which, for ethical reasons, will never be done. Therefore, we have established a general guideline to only transplant patients with doses of BM $CD34^+$ cells above 0.5×10^6/kg. This safeguard comes as an addition rather than a replacement of our previously established minimum threshold of 10^4 late CFU-GM/kg.

The detection of the bcl-2 translocation in BM buffy coats from 8 of 19 patients (meaning 8 of 14 with follicular lymphoma (57%) and none with concomitant detectable tumor infiltration by conventional cytology and histology) is a confirmation of the high rate of tumor contamination in BM previously reported in patients at all stages of disease, even those in remission, using molecular biologic technique.[10-12] In preclinical experiments, we (R.J.B.) have used normal BM contaminated with tumor cells from either the HSB2 or the DAUDI lymphoma cell lines; by fluorescence microscopy to detect tumor cells, we have been able to show a 3-logarithm reduction in the enriched cell population compared with BM before separation.[19] In the present study, tumor contamination assessable through nested PCR at the 10^{-4} level was no longer detected in 8 of the 9 $CD34^+$-purified fractions. In the clinical setting, these results indicate that $CD34^+$ cell purification may reduce signicantly the tumor load of the infused graft. However, further evaluation is needed on larger series of patients.

This phase-1 to -2 study did not address the question of the value of early ABMT in NHL or follicular NHL, nor was it designed to assess the value of positive selection of BM in this context. These results do show that $CD34^+$ progenitor cell concentrates contain less contamination with tumor cells and retain full engraftment capacity in patients with NHL.

Ongoing laboratory experiments using cell lines show that up to a 4-logarithm reduction in tumor contamination is attainable when using two successive passages on the avidin column after reloading with the anti-CD34 antibody. Further studies are obviously needed to better define high-dose therapy management of follicular lymphomas and to compare it with conventional management.

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