

Transplantation of Highly Purified CD34⁺Thy-1⁺ Hematopoietic Stem Cells in Patients With Recurrent Indolent Non-Hodgkin's Lymphoma

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ABSTRACT

Purpose: To evaluate the results of high-dose chemotherapy and transplantation of highly purified "mobilized" peripheral blood CD34⁺Thy-1⁺ hematopoietic stem cells (HSCs) in patients with recurrent indolent non-Hodgkin's lymphoma (NHL) or mantle cell lymphoma (MCL).

Patients and Methods: Twenty-six patients with recurrent indolent NHL or MCL were mobilized with either granulocyte colony-stimulating factor (G-CSF) alone or cyclophosphamide plus G-CSF. Apheresis was performed, and the product was purified using the Isolex immunomagnetic positive CD34⁺ cell selection device initially and subsequent high-speed flow-cytometric cell sorting for the final purification of CD34⁺Thy-1⁺ HSCs. The patients received high-dose chemotherapy with BEAC (carmustine, etoposide, cytarabine, and cyclophosphamide) followed by transplantation with the purified HSCs in 2 dose cohorts (cohort 1: $\geq 5 \times 10^5$ viable and pure HSC/kg; cohort 2: $\geq 3 \times 10^5$ HSC/kg).

Results: We attempted to mobilize 26 patients with G-CSF alone. Six patients did not collect adequate cells with G-CSF alone; subsequent mobilization with cyclophosphamide plus G-CSF was attempted, but adequate CD34⁺Thy-1⁺ HSCs could not be collected on these 6 patients. Twenty patients underwent transplantation with the BEAC transplantation regimen followed by purified HSCs. Patients in cohort 1 engrafted at a median of day 12 to an absolute neutrophil count (ANC) $>500/\mu\text{L}$, a median of day 19 for platelet transfusion independence, and a median of day 20 for red blood cell transfusion independence. Patients in cohort 2 engrafted at a median of day 12 to an ANC $>500/\mu\text{L}$, a median of day 12 for platelet transfusion independence, and a median of day 12 for red blood cell transfusion independence. Fourteen of the 20 patients had significant infections reported at some point post-transplantation, including influenza, respiratory syncytial virus, pneumonitis, and *Pneumocystis carinii* pneumonia. With a median follow-up of 38 months, 8 of the 20 patients have had progressive lymphoma and 5 patients have died. The 3-year event-free survival is 55%, and overall survival is 78%.

Conclusions: CD34⁺Thy-1⁺ HSCs can be collected successfully from most lymphoma patients mobilized with G-CSF alone. The engraftment and disease outcomes in the patients in this small pilot study using these cells do not appear to be different from the outcomes of similar patients cited in the literature. However, the short- and long-term risks of infection were a concern in this patient population.

KEY WORDS

Hematopoietic stem cells • Non-Hodgkin's lymphoma • Mantle cell lymphoma • High-dose chemotherapy • Autologous stem cell transplantation

INTRODUCTION

Advanced-stage indolent non-Hodgkin's lymphoma (NHL) is typically characterized by high response rates but inevitable relapse [1,2]. High-dose chemotherapy and autologous stem cell transplantation have been used cautiously in

this patient population because of the possible toxicities of the procedure, the potential for long-term survival of patients undergoing standard therapy, and the unknown long-term outcomes of transplantation in these patients [3-5]. A potential concern with autografts in these patients is that a

high percentage have bone marrow and peripheral blood lymphomatous involvement [6]. In addition, the use of high-dose chemotherapy and transplantation has not produced long-term disease-free survival in patients with mantle cell lymphoma (MCL) who are in relapse [7].

In most studies, the use of mobilized peripheral blood progenitors in autologous transplantation has resulted in rapid, stable long-term engraftment [8,9]. The rapidity of the engraftment has been correlated with the CD34⁺ cell content of the graft [10,11]. Within the CD34⁺ cell population, considerable heterogeneity exists with respect to other markers [12].

The subpopulation of CD34⁺ cells necessary for long-term engraftment in humans is unknown. However, various CD34⁺ cell selection devices have been evaluated in an effort to positively select for the hematopoietic cells needed for engraftment while eliminating tumor cells, which are CD34⁻ [13,14]. The initial selection devices selected only for CD34⁺ cells, with no other fractionation. However, investigators subsequently identified a subpopulation of human cells with the cell surface phenotype of CD34⁺Thy-1⁺Lin⁻ that are found in both bone marrow and peripheral blood after mobilization [12,15,16]. Highly selected CD34⁺Thy-1⁺Lin⁻ stem cells have demonstrated multilineage potential according to in vitro and in vivo assays in SCID mice and in utero sheep models [17,18].

This trial was a phase 1 study of highly purified CD34⁺Thy-1⁺ stem cells given back following high-dose chemotherapy in patients with recurrent NHL. The goals of this study were to determine the feasibility of isolating sufficient numbers of extensively purified CD34⁺Thy-1⁺ cells by high-speed fluorescence-activated cell sorting, to evaluate the degree of tumor cell depletion, to determine the engraftment of these highly selected cells, and to determine the immune reconstitution and short- and long-term complications in these transplantation patients.

PATIENTS AND METHODS

Patients

Patients at the University of Nebraska Medical Center were enrolled in this study between November 1996 and December 1997. Patients with NHL with the following histologies were eligible for enrollment in this trial: small lymphocytic lymphoma, follicular small cleaved lymphoma, follicular mixed small and large cell lymphoma, follicular large cell lymphoma, composite lymphoma with diffuse and follicular lymphoma (<50% large cells), MCL, and monocytoid B-cell lymphoma (marginal zone lymphoma). Patients were in first partial remission, second complete remission, or second partial remission following conventional induction and salvage chemotherapy. The patients had to have a demonstrated chemotherapy-sensitive response with $\geq 50\%$ reduction of the bidimensional tumor mass. Additionally, patients with MCL could have been in first complete remission for this protocol. The patients had to have a Karnofsky performance status $\geq 70\%$, with adequate cardiac, pulmonary, renal, and hepatic function and without evidence of active infection. The patients were adults with adequate hematologic function premobilization as defined by an absolute neutrophil count (ANC) $\geq 1.5 \times 10^9/L$ and a platelet

count $\geq 120 \times 10^9/L$. Results of serologic tests for hepatitis B and C and human immunodeficiency virus (HIV) were required to be negative. All patients signed informed consent forms approved by the Scientific Review Committee and the Institutional Review Board of the University of Nebraska Medical Center (Omaha, NE).

Screening Studies

Before mobilization, eligible patients were required to have a complete history and physical examination, complete blood count with differential and platelet count, radiologic assessment of the lymphoma, bone marrow biopsy and aspirate, pulmonary function assessments, ejection fraction evaluation, pregnancy test, coagulation profile, hepatitis and HIV testing, chemistry profile, and urinalysis. Patients' lymph node biopsies were also reviewed for confirmation of the diagnosis and for molecular analysis for bcl-1 (MCL) or bcl-2 or for complementarity-determining region 3 (CDR 3) analysis in the case of other histologic types.

Trial Design

This trial was an open-label, 2-cohort study with cell dose de-escalation, evaluating autologous transplantation of varying numbers of CD34⁺Thy-1⁺ HSCs. This dose de-escalation design was chosen in an effort to identify the minimum number of selected cells necessary for adequate and sustained engraftment that would allow for fewer aphereses. After confirmation of eligibility, the patients were mobilized with granulocyte colony-stimulating factor (G-CSF) alone. After 4 days of G-CSF, the patients had hematopoietic progenitors harvested by leukapheresis. The first cohort had a target of $\geq 5 \times 10^5$ HSC/kg, and the second had a target of $\geq 3 \times 10^5$ HSC/kg (Figure 1). The patients received the BEAC protocol (carmustine 300 mg/m² on day -7, etoposide 100 mg/m² intravenously [IV] twice daily on days -6 to -3, cytarabine 100 mg/m² IV twice daily on days -6 to -3, and cyclophosphamide 35 mg/kg IV once daily on days -6 to -3). The purified HSCs were administered on day 0 of the protocol.

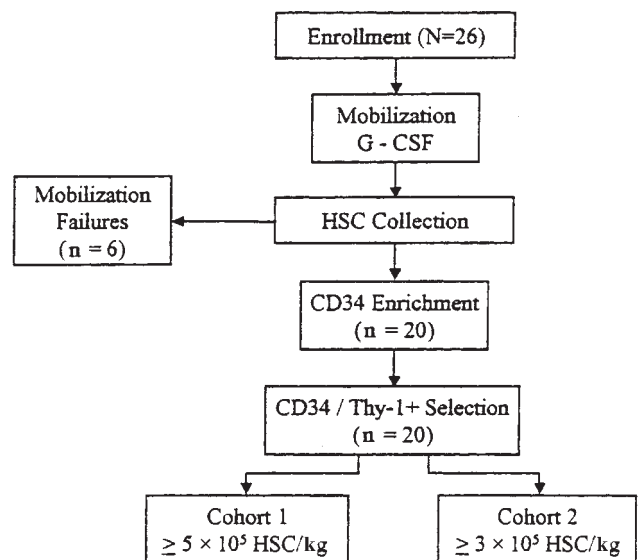


Figure 1. Trial design, by cohort assignment.

Mobilization

The patients were mobilized with G-CSF (Neupogen; Amgen, Thousand Oaks, CA) alone at 24 (g/kg per day subcutaneously. The dosage of 24 µg/kg per day of G-CSF was chosen because it was believed a higher dose might optimize the collections. On the day of the fourth injection of G-CSF, daily apheresis was begun with 12- to 20-L exchanges on the COBE Spectra apheresis machine (Gambro BCT, Denver, CO). The product was shipped to SyStemix (Palo Alto, CA) for cell processing. After successful collection of the purified HSC product, collection of unmodified peripheral blood progenitors from the patients continued, to obtain a backup preparation with $\geq 1.5 \times 10^6$ CD34⁺ cells/kg. If adequate purified HSCs could not be collected, an attempt at chemotherapy mobilization was made. The 6 patients from whom adequate purified HSCs could not be collected received cyclophosphamide, 4 g/m² IV, followed by G-CSF at 10 µg/kg subcutaneously. When the white blood cell count started to recover from the nadir, an attempt at re-collection was made. We were still unable to collect a purified HSC product from these 6 patients by this method. However, these patients were all able to undergo transplantation using the unmodified HSCs.

Stem Cell Isolation and Cryopreservation

Mononuclear cells collected by apheresis were diluted to a final concentration of $< 5 \times 10^7$ cells/mL in transport medium before shipment to SyStemix. At SyStemix, each patient sample was analyzed for cell number, viability, and phenotype and was either processed immediately or held overnight at room temperature. CD34⁺Thy-1⁺ HSCs were initially purified using the Isolex (Baxter, Irvine, CA) immunomagnetic positive selection device to enrich for CD34⁺ cells, with subsequent high-speed flow-cytometric cell sorting for the final purification of CD34⁺Thy-1⁺ HSCs.

The immunomagnetic CD34 selection process consisted of the following: (1) labeling cells with an anti-CD34 monoclonal antibody (MoAb); (2) mixing cells with paramagnetic beads coated with sheep antimouse immunoglobulin G (IgG), with CD34⁺ cells forming rosettes with the beads; (3) attaching the rosettes to a magnet and washing CD34⁻ cells from the system; and (4) releasing the cells from the beads using a peptide mimotope that competes with the MoAb binding site of the CD34 molecule. Residual erythrocytes, if any, were lysed with 150 mmol/L ammonium chloride lysis buffer, pH 7.5, for 5 minutes at 4°C. Before staining, the cells were incubated with 0.1% human immunoglobulin (Gammimune; Bayer, Berkeley, CA) for 10 minutes at 4°C to block nonspecific binding. The cells were stained at a concentration of 2×10^7 cells/mL for 20 minutes at 4°C with SyStemix-manufactured MoAbs that recognize CD34 and Thy-1 antigens (2.5 µg/mL CD34 sulforhodamine, 2.5 µg/mL Thy-1 biotin). SyStemix's fluoresceinated anti-CD34 MoAb differed from the antibody used in the immunomagnetic selection device. Without washing, streptavidin (Societa Prodotti Antibiotici, Milan, Italy) was added to the cells at a final concentration of 0.15 mg/mL and incubated for an additional 20 minutes. Cells were washed twice in cold phosphate-buffered saline (PBS) with 1% human serum albumin (HSA) (Alpha Therapeutics, Los Angeles, CA), resuspended to 2×10^7 cells/mL in 5 µg/mL phycoery-

thrin (PE)-biotin (SyStemix) and incubated for 20 minutes at 4°C. In preparation for sorting, the cells were washed once with cold PBS/1% HSA, resuspended to 2×10^7 cells/mL in PBS/1% HSA plus 100 U/mL deoxyribonuclease (DNase) (Benzonase; Nycomed, Copenhagen, Denmark), and kept at a temperature of 2°C to 8°C.

The HSCs were sorted as previously described using SyStemix's proprietary dual-laser, fluorescence-activated, high-speed clinical cell sorter [19]. PE was excited by an argon laser emitting 488 nm light, and SRG was excited by a rhodamine 6-G dye laser emitting 590 nm light. Forward- and side-scatter sort windows were set to exclude very small or very large cells and cell clusters. Selection criteria for CD34 cells were based on the fluorescence of unstained cells (autofluorescence), and the selection criteria for Thy-1 (PE) were based on the background fluorescence of cells stained with anti-Thy-1 biotin and biotin-PE but lacking streptavidin. Cells were sorted at a rate of 15,000 to 20,000 events per second. The CD34⁺Thy-1⁺ cells were sorted directly into a culture tube containing a small volume of SyStemix's proprietary culture medium and were stored at a temperature of 2°C to 8°C until cryopreservation.

The purified HSCs were washed free of sorter sheath fluid, counted with a hemocytometer, and resuspended to a maximum of 4×10^7 cells/mL in SyStemix-manufactured proprietary cryopreservation medium containing 2% hetastarch, 4% HSA, and 7.5% dimethyl sulfoxide (DMSO) as cryoprotectants. The cells were divided into aliquots and placed into 2 or more cryovials. The cells were frozen using a programmable step-down freezer with a freezing profile optimized for HSC survival. The step-down freezer was programmed to freeze at -1°C/min until the sample reached a temperature of -45°C and then to freeze at -10°C/min until the sample reached a temperature of -140°C. After the product temperature reached -140°C, the vials were transferred to the liquid phase of a liquid nitrogen cryostorage tank. Once the target dose was achieved and all products were released, the HSC infusion products were shipped to the clinical site in a liquid nitrogen shipper (CP65; Taylor-Wharton Scientific, Harrisburg, PA) and returned to the liquid phase of liquid nitrogen until the time of infusion.

Patients also had an unmodified backup collection performed with $> 1.5 \times 10^6$ CD34⁺ cells/kg collected and cryopreserved by standard methods at the University of Nebraska Medical Center.

Minimal Residual Disease Studies

Patients with indolent lymphomas had their initial lymphoma tissue block analyzed for the presence of bcl-2 or IgH gene rearrangements by polymerase chain reaction (PCR) analysis. In addition, the patients with MCL had their tissue block analyzed for bcl-1 rearrangements. If patients were found to have one of these abnormalities in their original tissue, their stem cell products before and after processing were examined by TaqMan Real-Time PCR. In this assay, the TaqDNA polymerase cleaves a doubly labeled fluorogenic probe. These nonextendable probes (TaqMan DNA probes; Roche Molecular Systems, Pleasanton, CA), designed to hybridize internally to the PCR primers, are labeled at the 5' end with a reporter dye (ie, FAM, 6-carboxyfluorescein; emission 518 nm). In addition, the 3' end of the probe, which

Table 1. Patient Characteristics*

Characteristic	Cohort 1 (n = 10)	Cohort 2 (n = 10)	All (N = 20)
Age, y, median (range)	52 (36-63)	50 (35-63)	51 (35-63)
Sex % male	60%	60%	60%
Diagnosis			
Follicular	7 (70%)	5 (50%)	12 (60%)
Marginal zone	1 (10%)	0	1 (5%)
Mantle cell	2 (20%)	4 (40%)	6 (30%)
Small lymphocytic	0	1 (10%)	1 (5%)
Number of prior regimens			
1-2	7 (70%)	9 (90%)	16 (80%)
3+	3 (30%)	1 (10%)	4 (20%)
Disease status at treatment			
PIF, chemosensitive	4 (40%)	7 (70%)	11 (55%)
First relapse	5 (50%)	2 (20%)	7 (35%)
CR1/CR2	1 (10%)	1 (10%)	2 (10%)
Bone marrow involved	2 (20%)	7 (70%)	9 (45%)
Elevated LDH	6 (60%)	3 (30%)	9 (45%)

*PIF indicates primary induction failure; CR, complete remission; LDH, lactic dehydrogenase.

is labeled with TAMRA (6-carboxytetramethyl-rhodamine), is phosphorylated to prevent extension of the probe during the PCR. Fluorescence of the intact probe is quenched mainly by Forster-type energy transfer while the nuclease degradation of the probe results in an increase in reporter dye fluorescence. This is proportional to the concentration of template in the PCR [20]. This real time-based assay has a detection limit of 1/50,000 cells or 0.002%, with quantitation by 10/50,000 cells [21].

High-Dose Therapy and Stem Cell Infusion

After the appropriate HSC and unmodified backup hematopoietic stem cell collections were obtained, the patients received the BEAC regimen (carmustine 300 mg/m² on day -7, etoposide 100 mg/m² twice daily on days -6 to -3, cytarabine 100 mg/m² twice daily on days -6 to -3, cyclophosphamide 35 mg/kg on day -2). On day 0, the HSCs were thawed in a 37°C water bath in the stem cell processing lab at the University of Nebraska Medical Center. The cells were slowly diluted 2 times in SyStemix sterile formulation medium and transferred to a 30-mL syringe. A cell count with a trypan blue viability test was performed using a hemocytometer on a 100-μL sample of the thawed product. As a result of previous human engraftment experience, a postthaw CD34⁺Thy-1⁺ HSC viability <80% required immediate infusion of the backup unselected graft as an alternative.

Following HSC infusion, G-CSF was begun on day 0 at a dosage of 5 μg/kg per day and continued until the ANC exceeded 2000/μL for 4 consecutive days. Packed irradiated red blood cells were transfused when a patient's hemoglobin measured <8.0 g/dL, and irradiated platelets were transfused when a patient's platelet count was <20,000/μL, or as clinically indicated. Antibiotics, including antibacterial, antifungal, and antiviral agents, were administered according to institutional policy. Patient follow-up included daily blood counts until recovery and then as clinically indicated and/or twice weekly until day 60 posttransplantation. Peripheral blood

absolute numbers of CD3, CD4, CD8, and CD56 cells as well as immunoglobulin levels were evaluated at baseline, on days 28, 60, and 100, and at 1-year posttransplantation.

Statistical Analysis/Engraftment Evaluation

Neutrophil engraftment was defined as the first of 3 consecutive days that the patient achieved an ANC >500/μL. Platelet engraftment was defined as the first of 7 consecutive days of an unsupported platelet count >20,000/μL. Red blood cell transfusion independence was reported as the first of 30 consecutive days that the hemoglobin measured >8.0 g/dL. Complete response (CR) was defined as the disappearance of all clinical and radiographic evidence of disease (<1.5 cm) at the day +100 evaluation. A CR undetermined (CRu) was defined as some residual computed tomographic abnormalities at the day +100 evaluation that were not conclusively active disease. A partial response (PR) was defined as a ≥50% reduction in the bidimensional measurements at the end of therapy. Overall survival was defined as the time from transplantation to death from any cause. Event-free survival was defined as the time from transplantation to disease progression or death from any cause. Survival times and event-free survival distributions were calculated using the Kaplan-Meier method [22]. Comparisons of these time-to-event distributions were made using the log-rank test [23].

RESULTS

Patient Characteristics

Twenty-six patients were enrolled in this study, with 20 going on to receive high-dose BEAC and SyStemix-selected HSC transplants. Patient characteristics by cohort are shown in Table 1. The median age of all patients was 51 years (range, 35-63 years), with 60% of the patients being male. Patients were receiving transplants for recurrent follicular lymphoma (n = 12), recurrent small lymphocytic lymphoma (n = 1), recurrent marginal zone lymphoma (n = 1), or MCL (n = 6). Eleven (55%) of the 20 patients were undergoing transplantation as primary induction failures with chemosensitive disease. Seven of the patients were undergoing transplantation in first relapse with chemosensitive or minimal disease. The other patients were in complete remission at the time of transplantation. The majority of patients had received fewer than 3 prior chemotherapy regimens. The other patient characteristics are noted in Table 1.

Mobilization and HSC Collection

Once they were determined to be transplantation candidates, patients were mobilized with G-CSF at 24 μg/kg per day; on the fourth day, patients were initiated on stem cell collections as described. Four patients required 2 collections, 11 required 3 collections, and 5 required 4 collections to obtain the minimum CD34⁺Thy-1⁺ cell count to proceed with transplantation. Following the minimum collection of CD34⁺Thy-1⁺ cells, the patients underwent a backup unmodified collection of ≥1.5 × 10⁶ CD34⁺ cells/kg. Six patients did not achieve the minimum CD34⁺Thy-1⁺ cell dose and underwent a second mobilization attempt with cyclophosphamide at 4 g/m² followed by G-CSF, with collection of cells as the patients recovered the neutrophil count. None of these

Table 2. Engraftment, by Cohort*

Engraftment	Cohort 1	Cohort 2	All
Days to ANC >500/ μ L, median (range)	12 (10-13)	12 (9-14)	12 (9-14)
Days to ANC >2000/ μ L, median (range)	15 (13-19)	14.5 (12-20)	14.5 (12-20)
Days to RBC independence, median (range)	20 (15-25)	11.5 (12-30)	15 (12-30)
Days to platelet independence, median (range)	19 (11-65)	12 (11-20)	14.5 (11-65)

*RBC indicates red blood cell.

6 patients with mobilization failures were able to obtain an adequate CD34⁺Thy-1⁺ cell count with the second attempt at chemo/cytokine mobilization. However, all 6 of these patients did go on to transplantation with unselected stem cell products and demonstrated normal engraftment.

Once obtained, mononuclear cells collected by apheresis were diluted to a final concentration of $<5 \times 10^7$ cells/mL in proprietary transport medium before shipment to SyStemix. At SyStemix, each patient's sample was analyzed for cell number, viability, and phenotype and was processed immediately using the Isolex immunomagnetic positive selection device to enrich for CD34⁺ cells with subsequent high-speed flow-cytometric cell sorting for the final purification of CD34⁺Thy-1⁺ HSCs. The median number of viable HSCs cryopreserved was 5.3×10^5 CD34⁺Thy-1⁺ cells/kg in cohort 1 and 4.9×10^5 CD34⁺Thy-1⁺ cells/kg in cohort 2. The purity of the CD34⁺Thy-1⁺ cells after sorting exceeded 90% in all cases, with a median purity of 93%. The cells were shipped back to the clinical site for infusion after the BEAC regimen, as discussed.

Hematopoietic Stem Cell Reconstitution

Neutrophil recovery was rapid in most patients, with a median time to reach an ANC >500/ μ L of 12 days (range,

9-14 days). The median time to reach an ANC >2000/ μ L was 14.5 days (range, 12-20 days). The median time to red blood cell transfusion independence was 15 days (range, 12-30 days). The median time to platelet transfusion independence was 14.5 days (range, 11-65 days). Engraftment times by cohort are listed in Table 2. In the range of cell doses infused, there was no clear effect of the cell dose on engraftment in the patients (Figure 2). The patients received a median of 6 units of red blood cells during transplantation (range, 2-29 units) and a median of 10 transfusions of single-donor platelets (range, 4-44 transfusions). No patients required their backup unmodified hematopoietic stem cells to be infused because of poor engraftment.

Adequate nonselected cells $\geq 1.5 \times 10^6$ CD34⁺ cells/kg were collected from the 6 patients from whom adequate CD34⁺Thy-1⁺Lin⁻ cells could not be collected despite 2 mobilizations. These patients received a standard transplant with these cells, and their engraftment was as follows: Time to ANC >500/ μ L, 12 days; platelet independence >20,000/ μ L, 14 days; and red blood cell transfusion independence, 15 days. These engraftment times are not significantly different from the engraftment times of the patients receiving the SyStemix-selected products or of patients treated previously with unselected stem cells at our institution [24].

Immune Reconstitution and Infections

Immunoglobulin levels and T-cell reconstitution by cohort are shown in Table 3. CD4⁺ reconstitution was variable, with patients at day +100 posttransplantation having a median of 120 and 106 CD4⁺ cells/ μ L in cohorts 1 and 2, respectively. These results are slightly lower than the levels in NHL patients who received transplants at our center with unselected stem cell products [25]. Levels of IgM, IgG, and IgA were also depressed in several patients in the posttransplantation period. Significant clinical infections in the immediate posttransplantation period included 2 gram-negative rod sepsis syndromes, 1 episode of influenza and respiratory syncytial virus (RSV) requiring prolonged

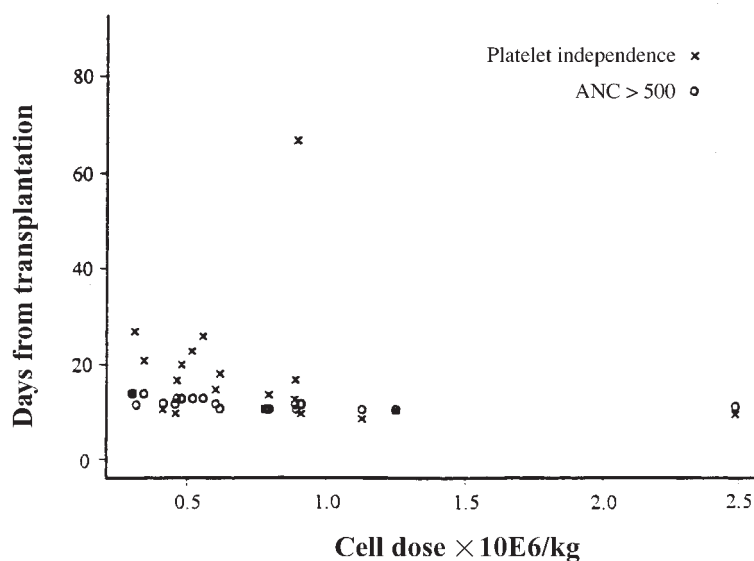


Figure 2. Engraftment as a function of HSC dose.

Table 3. Immunoglobulin and T-Cell Reconstitution After Transplantation (Median Values)

	Day 28	Day 60	Day 100	Day 365
Cohort 1 (normal values)				
CD3/ μ L (603-2849)	122	588	419	795
CD4/ μ L (312-2064)	33	241	120	159
CD56/ μ L (18-578)	161	97	89	78
CD8/ μ L (113-1272)	214	1058	318	648
IgM, mg (40-214)	32	32	66	68
IgG, mg (680-1530)	823	677	882	873
IgA, mg (75-374)	99	68	117	93
Cohort 2				
CD3/ μ L	141	581	448	630
CD4/ μ L	48	147	106	213
CD56/ μ L	79	156	73	86
CD8/ μ L	97	440	301	413
IgM, mg	36	34	57	99
IgG, mg	707	629	785	941
IgA, mg	109	105	127	152

intubation in a patient, and 1 episode of RSV requiring brief intubation. Later (>100 days posttransplantation), there were 5 cases of varicella zoster, 1 episode of *Pneumocystis pneumonia*, 1 episode of systemic candidiasis, and 2 episodes of interstitial pneumonitis without a specific pathogen being identified, requiring intubation.

Toxicity

The acute and chronic infectious complications are as outlined above. Additional toxicities seen in the transplant recipients included temporary renal insufficiency requiring dialysis in 1 patient, seizures associated with the influenza and RSV infections in 1 patient, and congestive heart failure, which was treated medically, in 1 patient. There were no toxic deaths in the patients within the first 100 days posttransplantation. Nine of the 20 patients have failed, most as a result of progressive disease ($n = 8$). Five patients have died, 4 of progressive disease and 1 of pulmonary fibrosis at 17 months posttransplantation.

Minimal Residual Disease Studies

Six patients were found to have minimal residual disease in their apheresis product prior to processing. Four patients with follicular lymphoma had a positive finding for the bcl-2 rearrangement, and 2 patients with MCL had a positive finding for the bcl-1 rearrangement by PCR. The patients had a >3.26- to >5.68-fold reduction (log 10) with the CD34⁺Thy-1⁺ cell sorting process (Table 4).

Patient Outcomes

At a median follow-up of 38 months (range, 35-49 months), 8 patients had progressed and 1 patient had died of pulmonary fibrosis at 17 months posttransplantation. This patient had a preexisting scleroderma-type syndrome that appeared to accelerate after transplantation, with manifestations of pulmonary fibrosis. The responses in the patients in the trial are CR/CRu in 14 (70%) and PR in 5 (25%). Overall survival in all patients is 78% at 36 months posttransplantation (Figure 3). Event-free survival in all patients is 55% at 36 months, with no difference seen by dose cohort

(Figure 4). Additionally, no difference in event-free survival was seen by presence or absence of minimal residual disease in the transplantation product. Sites of relapse included nodal areas ($n = 6$), pulmonary parenchyma ($n = 1$), and 1 unknown site. All nodal areas were sites of previous disease; the pulmonary parenchyma was a new site of disease.

DISCUSSION

The use of high-dose chemotherapy and autologous transplantation for patients with recurrent indolent NHL is somewhat controversial because no survival advantage over standard therapy has been documented [3-5]. In addition, the use of high-dose chemotherapy and autologous transplantation has not led to long-term disease-free survival in most patients with relapsed MCL [7]. Concerns of lymphoma contamination of the stem cell product and the possible reinfusion of these cells and its contribution to relapse have led to many clinical trials involving purging of cell products [4,6]. In addition, positive selection of CD34⁺ cells has now been evaluated in several clinical trials evaluating a cell avidin CD34⁺ selection device that releases CD34⁺ cells by mechanical agitation [14]. Positive selection using an immunomagnetic bead device has also been evaluated in clinical trials [13]. Both devices have been reported to allow adequate and sustained engraftment with a decrease in the tumor cell contamination of the graft. The CD34⁺ selection device has been compared to unselected transplantation in patients with multiple myeloma and found to have equivalent clinical outcomes [26]. In the current clinical trial evaluating CD34⁺Thy-1⁺Lin⁻ cells, a highly selected stem cell product is produced as an avenue toward further decreasing tumor contamination while still allowing rapid engraftment of the hematopoietic and immune systems posttransplantation.

This study demonstrated the feasibility and safety of harvesting unmodified hematopoietic progenitors with long-distance shipment to a high-speed cell sorting facility (SyStemix) and subsequent successful transplantation and engraftment with these cells in patients receiving high-dose chemotherapy and HSC transplants for follicular NHL or MCL. Previous phase 2 studies have also been reported in patients undergoing transplantation for breast cancer and multiple myeloma [19,26,27]. In the current trial, 20 of 26 patients with relapsed or high-risk first complete remission NHL were able to have adequate numbers of hematopoietic stem cells harvested using G-CSF alone to have a minimum number of CD34⁺Thy-1⁺ cells collected. In the 6 patients

Table 4. Minimal Residual Disease Reduction, by CD34⁺Thy-1⁺ Cell Sorting*

Patient No.	PCR	Apheresis, No. of Tumor Cells	CD34 ⁺ †	CD34 ⁺ Thy-1 ⁺ †
105	bcl-1	7.44×10^6	NA	>4.32
111	bcl-2	8.19×10^5	>2.78	>3.26
119	bcl-2	1.05×10^7	3.6	>4.14
121	bcl-1	1.17×10^8	NA	>5.65
125	bcl-2	1.21×10^7	3.95	>5.68
126	bcl-2	4.51×10^7	5.07	>5.61

*NA indicates not available.

†Absolute fold reduction (log 10).

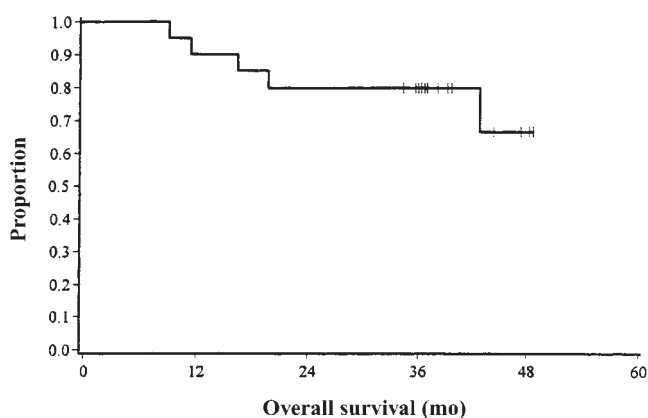


Figure 3. Overall survival of patients receiving transplants.

from whom adequate cells could not be collected by mobilization with cytokine alone, adequate cells also could not be collected with cyclophosphamide plus cytokine mobilization.

Murine models had previously demonstrated that highly enriched populations of HSCs are capable of engrafting lethally irradiated animals with cells that behave similarly to bone marrow-derived cells [28]. The 3 previous studies in humans in patients with breast cancer and multiple myeloma demonstrated excellent neutrophil engraftment in most patients, with some concern about delayed platelet engraftment in 1 of the trials in patients with multiple myeloma [19]. In the study by Tricot et al. [19], 10 patients with multiple myeloma underwent stem cell collection with cyclophosphamide and granulocyte-macrophage CSF. These cells then were sent for the high-speed cell sorting of CD34⁺Thy-1⁺ cells. Nine of the 10 patients met the minimum criteria of 7.2×10^5 cells/kg and received transplants using high-dose melphalan. Eight of the 9 had successful engraftments; however, the median times to an ANC $>500/\mu\text{L}$ of 16 days and a platelet count $>50,000/\mu\text{L}$ of 39 days were substantially delayed compared with the median times achieved by historical control patients receiving unmanipulated HSC grafts [19]. One patient required infusion of the reserve unmanipulated HSCs because of lack of engraftment at day +28.

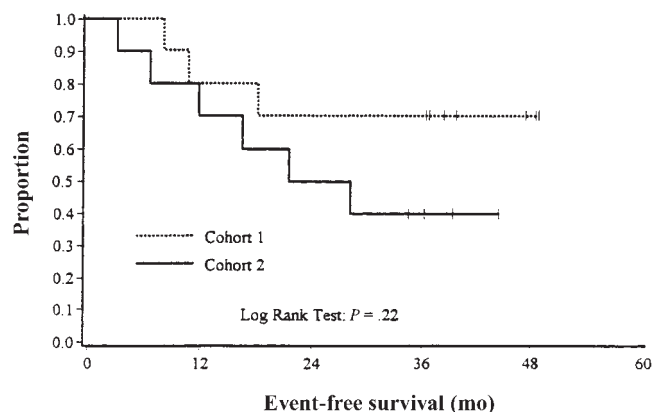


Figure 4. Event-free survival of patients receiving transplants, by dose cohort.

A more recent publication, by Michallet et al. [29], evaluated 23 patients with multiple myeloma who received melphalan and total body irradiation with CD34⁺Thy-1⁺-selected cells as the graft source. In this study, patients who received $>0.8 \times 10^6$ CD34⁺Thy-1⁺ HSC/kg had prompt engraftment, with a median time to an ANC $>500/\mu\text{L}$ of 10 days and a median time to a platelet count $>50,000/\mu\text{L}$ of 13 days. Additionally, prompt engraftment was reported by Negrin et al. [27] in 22 patients with breast cancer undergoing high-dose chemotherapy with carmustine, cisplatin, and cyclophosphamide and CD34⁺Thy-1⁺-selected cells. The median time to reach an ANC $>500/\mu\text{L}$ was 10 days, and the median time to reach a platelet count $>50,000/\mu\text{L}$ was 17 days. Engraftment in our study was prompt for neutrophils $>500/\mu\text{L}$ at a median of 12 days and satisfactory as well for platelet transfusion independence at a median of 14.5 days.

A concern with the highly selected stem cell products has been delayed immune reconstitution and the possibility of infectious complications as a result. In the 2 myeloma studies, the authors felt that compared with similar patients receiving unselected HSC transplants, the patients in the SyStemix trials experienced more short- and long-term infectious complications. In the study by Michallet et al., pulmonary aspergillosis and cytomegalovirus and varicella zoster infections were seen in a high percentage of patients who received $<0.8 \times 10^6$ HSC/kg [29]. The study with breast cancer patients demonstrated a delayed time to engraftment of CD4⁺ and CD8⁺ cells but no major infectious complications [27]. Our current study of the SyStemix-isolated cells for reconstitution for patients with NHL also demonstrates some delays in CD4⁺ and CD8⁺ engraftment and some unanticipated viral infections. This increase in viral infections has also been reported in patients undergoing CD34⁺ selection by other techniques [30].

An additional object of this trial was to evaluate the possible effects of decreasing the minimal residual disease in the hematopoietic stem cell product infused in the hope that such a decrease would reduce the potential relapse rate in patients. Out of 20 patients who received transplants, 6 had an identified minimal residual disease marker in their apheresis product. An overall >3 to 5 log reduction of tumor cells was identified with the CD34⁺Thy-1⁺ selection process in these patients. However, in this small group of patients, this reduction did not appear to influence the outcome. In the breast cancer trial, all apheresis products were able to have successful log depletions of tumor cells [27]. The multiple myeloma studies also demonstrated successful tumor depletion [19,29].

This study has demonstrated the feasibility of isolation of CD34⁺Thy-1⁺ stem cells by high-speed cell sorting after long-distance shipment of the product. In addition, tumor depletion of the product was demonstrated. Rapid neutrophil and platelet engraftment were seen in the majority of patients, with no requirement for the backup stem cell product to be infused. However, delayed immunologic reconstitution and a slightly higher than expected incidence of certain types of opportunistic infections remain a concern. It is unknown whether this approach will make a difference in the long-term outcomes of patients receiving high-dose chemotherapy and stem cell transplantation for lymphoma.

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