Engraftment After High-Dose Therapy for Lymphoma With Low Doses of CD34+ Peripheral Blood Stem Cells and Ex Vivo Expanded Bone Marrow Cells

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ABSTRACT

Limitation in the numbers of collected CD34$^+$ cells in a peripheral blood stem cell collection can mean that an otherwise curable patient may not receive high-dose chemotherapy because of the risk of delayed engraftment or nonengraftment. We explored the feasibility of adding ex vivo expanded bone marrow cells to limited CD34$^+$ cell dose peripheral blood stem cells—either of which would be expected to produce delayed engraftment—to accelerate engraftment. Rapid granulocyte recovery was seen in all 5 patients. Rapid platelet recovery was seen in 3 patients but in 2 patients who had previously received fludarabine, delayed platelet recovery was seen (perhaps only coincidentally).

INTRODUCTION

The anticipation of delayed engraftment or failure to engraft after peripheral blood stem cell (PBSC) transplant is a contraindication to high-dose chemotherapy (HDCT), even though the patient's specific malignancy may be treatable with curative intent. The quantity and quality of PBSCs obtained after mobilization therapy may be inadequate to ensure prompt engraftment. Consequent prolonged pancytopenia after suboptimal-dose PBSC transplant may be too risky, in certain subpopulations of patients, because of high probabilities of infection, bleeding, and vital organ compromise.

There are a number of identifiable risk factors for potential poor engraftment after autologous PBSC transplant,[1] including older patient age, involvement of

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bone marrow by the malignant disease process, prior chemotherapeutic drug exposures (eg, fludarabine or nitrogen mustard), and poor mobilization of stem cells into the peripheral blood (ie, low numbers of CD34+ cells collected).[2,3] After the first attempt at PBSC collection has been considered poor, second attempts at mobilization have been tried.[4] Expansion of bone marrow cells (BMCs) ex vivo has been shown to be feasible on a large scale[5] and has resulted in successful hematopoietic engraftment.[6] The combination of expanded BMCs along with PBSCs may be advantageous to prevent delayed engraftment or failure to engraft.

After autologous PBSC transplants following HDCT when more than 2×10^6 CD34+ cells/kg are infused, engraftment is usually prompt, and when fewer than 2×10^6 CD34+ cells/kg are infused, engraftment may be delayed.[2,3,7] For patients with breast cancer receiving BMCs collected after granulocyte colony-stimulating factor (G-CSF) treatment and expanded ex vivo in the AastromReplicell, engraftment is disconcertingly slow.[8] However, when ex vivo expanded BMCs are infused along with very low doses of PBSCs ($<1\times10^6$ CD34+ cells/kg), the pace of engraftment is, surprisingly, nearly normal.[8]

We hypothesized that, for patients with Hodgkin's disease and non-Hodgkin's lymphoma (NHL) who have poor collections of PBSCs and other risk factors for poor engraftment, adding ex vivo expanded BMCs to suboptimal doses of PBSCs may improve the pace of engraftment after PBSC transplant.[9] This pilot feasibility study was performed at 2 institutions, the Roger Williams Medical Center (RWMC) in Providence, Rhode Island, and the Hackensack University Medical Center (HUMC) in Hackensack, New Jersey.

MATERIALS AND METHODS

All patients who participated in this phase 1/2 study gave written informed consent to participate in protocols that were approved and annually reviewed by the respective institutional review boards of RWMC and HUMC. Characteristics of the 5 patients and their diseases that form the basis of this report of preliminary data are given in Table 1. Patients were deemed eligible to enter this study if their first PBSC mobilization regimen was suboptimal because it yielded fewer than 1×10^6 CD34+ cells/kg body weight (Table 2).

At RWMC, the mobilization regimen for PBSCs, called CPG, was cyclophosphamide (total of 3500 mg/m^2 in 2 doses per day), prednisone (2 mg/kg per day for 4 days) and G-CSF (10 μg/kg per day starting on day 3 of chemotherapy). PBSCs were collected starting the day after the white blood cell count rose above 1000/μL. In poor PBSC mobilizers, in addition, G-CSF (alone) was given at 10 μg/kg per day for 3 days, after which PBSC collection commenced with continuation of daily G-CSF. At HUMC, the mobilization regimen for PBSCs was G-CSF alone at 16 μg/kg twice a day, commencing PBSC collection on the third day of G-CSF.

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Table 1. Patient and Disease Characteristics*

			Lymphoma			
Patient	*Age, y*	*Sex*	*Histologic Type*	*Relevant Characteristic*	*Shown in Bone Marrow Biopsy*	*Prior Therapy With Fludarabine or Nitrogen Mustard*
1	54	F	NHL	Intermediate Grade	Yes	Yes
2	69	M	NHL	Intermediate Grade	No	No
3	69	F	NHL	Low Grade	Yes	Yes
4	64	M	NHL	Low Grade	Yes	No
5	34	M	HD	Primary refractory	No	Yes

**HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma.*

At RWMC, the high-dose chemotherapy regimen, called CTC, consisted of cyclophosphamide (6000 mg/m^2), thiotepa (500 mg/m^2), and carboplatin (800 mg/m^2) in 3 divided doses per day. The PBSC infusion was given 48–96 hours after the conclusion of chemotherapy, and G-CSF (5 µg/kg per day) was begun 1 day after the PBSC infusion. At HUMC, the high-dose chemotherapy regimen, called CBVA, was cyclophosphamide (90 mg/kg), BCNU (600 mg/m^2), VP-16 (etoposide) (1600 mg/m^2), and cytosine arabinoside (Ara-C) (15 mg/m^2). The PBSC infusion was given 48 hours after the final dose of chemotherapy, and G-CSF (10 µg/kg per day) was begun 1 day after the PBSC infusion.

The clinical end points measured were, for myeloid engraftment, the first day of 3 consecutive days that the absolute granulocyte count (AGC) exceeded 500/µL and, for megakaryocytic engraftment, the first day of 3 consecutive days that the platelet count (PLT) exceeded 20,000/µL without platelet transfusion support.

Ex vivo marrow expansion was performed as follows. Upon bone marrow biopsy, if the cellularity was at least 20%, then a small marrow harvest (SMH)

Table 2. Stem Cell Harvests and Engraftment Times*

Patient	*CD34$^+$ PBSCs After CPG, ×10^6/kg*	*CD34$^+$ PBSCs After G-CSF, ×10^6/kg*	*Expanded BMCs, ×10^7/kg*	*First Day of ANC >500/µL*	*First Day of PLT >20,000/µL*
1	0.3	1.0	3.0	14	>120
2	0.04	2.3	2.6	10	16
3	0.4	0.5	6.6	14	>120
4	—	0.8	1.0	13	20
5	0.4	0.6	1.0	10	16

**ANC, absolute neutrophil count; BMC, bone marrow cell; CPG, cyclophosphamide, prednisone, G-CSF; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral blood stem cell; PLT, platelet count.*

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(80 mL bone marrow) was collected into heparinized syringes. The SMH was transported at 4°C to Progenitor Cell Therapy (Hackensack, NJ). Cells were cryopreserved using 10% dimethylsulfoxide (DMSO) (Research Industries) and stored at –135°C until use. On day –12 of transplant, the SMH cells were thawed and washed using a Gentran (Baxter) and Pulmozyme (Genentech) solution. Washed SMH cells were inoculated at a seeding density of 366 to 500×10^6 cells into each of 3 cell cassettes. Inoculates were cultured at 37°C for 12 days in the AastromReplicell using Iscove's modified Dulbecco's medium supplemented with fetal bovine serum, horse serum, hydrocortisone (Complete Medium; Aastrom BioSciences), erythropoietin (Amgen or Ortho Biotech), flt3-ligand (Immunex), PIXY321 (Immunex), L-glutamine (Gibco), gentamycin (Gibco), and vancomycin (Eli Lilly). Medium perfusion started on day 3 of culture. On the day of transplant, the expanded cells were released from the growth surface of the cell cassettes using trypsin, then washed, pooled, and transported to the transplant center at 4°C. The ex vivo expanded cells were reinfused at least 2 hours before the thawing and reinfusion of PBSCs (which were considered to be a suboptimal dose).

RESULTS

Five patients were enrolled in this feasibility study. All had at least 2 and up to 4 risk factors for poor engraftment (Table 1), all had poor collections of PBSCs after their first attempt at mobilization (Table 2), and all had SMH expanded ex vivo (Table 3). For these 5 patients, ex vivo BMC expansion (Table 4) resulted on the average in a 60% increase in nucleated cells; a 75% reduction in $CD34^+$, $CD3^-$, $CD11b^-$, $CD15^-$, $CD20^-$, $glyA^-$ cells ($CD34^+$ lineage-negative hematopoietic progenitors); a 13-fold increase in $CD13^+$, $CD3^-$, $CD11b^-$, $CD14^-$, $CD20^-$, $glyA^-$ cells ($CD13^+$ myeloid progenitors); and a 6.2-fold increase in colony-forming units-granulocyte/macrophage (CFU-GM). All 5 patients had prompt AGC recovery (median, day 13) after limited-dose PBSCs and expanded BMCs were infused (Table 2). Three patients had prompt PLT recovery, but 2 patients had delayed PLT recovery. The median time of PLT recovery was day 20. In the only patient studied, $CD20^+$ cells were depleted from the ex vivo expanded BMC. It is worthy of note that 4 patients had sufficient hematopoietic reserve after the combination transplant to tolerate subsequent immunoconsolidation therapy with anti-CD20 antibody ($n = 3$) or extensive field irradiation therapy ($n = 1$).

DISCUSSION

It is attractive to believe that any source of hematopoietic progenitor cells that are limited in number (ie, contain a low number of $CD34^+$ cells) may be expanded ex vivo and produce prompt and durable engraftment. One such example is

Table 3. Expanded Bone Marrow Cell Doses*

Patient	*Nucleated BMCs, ×10⁷/kg*	*CD34⁺Lin⁻, ×10⁶/kg†*	*CD13⁺LinB⁻, ×10⁶/kg‡*	*CFU-GM, ×10⁵/kg*
1	3.0	0.02	4.67	0.81
2	2.6	0.02	7.43	0.82
3	6.6	0.04	10.1	2.54
4	1.0	0.01	2.25	0.16
5	1.0	0.01	1.65	0.02
Mean	2.8	0.02	5.22	0.87

**BMC, bone marrow cell; CFU-GM, colony-forming unit-granulocyte/macrophage; †Lin⁻ = CD3⁻, CD11b⁻, CD15⁻, CD20⁻, and glyA⁻; ‡LinB⁻ = CD3⁻, CD11b⁻, CD14⁻, CD20⁻, and glyA⁻.*

umbilical cord blood.[10] It is also attractive to believe that, if the first mobilization of $CD34^+$ cells is insufficient to ensure rapid engraftment, it would be advantageous to collect more hematopoietic progenitor cells from the same source (the blood) or an alternative source (the marrow) to exceed a critical value of $CD34^+$ cell content when the 2 collections are added together. Unfortunately, this does not always ensure prompt engraftment. When mobilization of a critical number of $CD34^+$ cells into a blood-derived stem cell collection does not occur, merely exceeding the critical number with a second collection of hematopoietic progenitor cells derived from the bone marrow does not always ensure prompt engraftment.[11] As seen in breast cancer patients, ex vivo expanded BMCs alone did not ensure prompt engraftment, but adding ex vivo expanded BMCs to limited numbers of PBSCs was advantageous in preventing delayed engraftment.[8] Perhaps the same would be true in lymphoma patients, providing proof for the nascent principle.

From the observations reported in this communication, it is reasonable to conclude that it is feasible to harvest and expand a small amount of bone marrow even from patients with NHL who have disease in their marrow. In patients receiving suboptimal doses of PBSCs plus expanded BMCs, prompt granulocyte

Table 4. Bone Marrow Cell Fold Expansion*

Patient	*Nucleated BMCs*	*CD34⁺Lin⁻ Cells†*	*CD13⁺LinB⁻ Cells‡*	*CFU-GM*
1	2.0	0.19	13.2	9.2
2	1.4	0.62	10.4	8.0
3	1.8	0.27	13.17	8.4
4	1.0	0.10	8.82	4.8
5	0.6	0.08	18.0	0.6
Mean	1.4	0.25	12.72	6.2

**BMC, bone marrow cell; CFU-GM, colony-forming unit-granulocyte/macrophage; †Lin⁻ = CD3⁻, CD11b⁻, CD15⁻, CD20⁻, and glyA⁻; ‡LinB⁻ = CD3⁻, CD11b⁻, CD14⁻, CD20⁻, and glyA⁻.*

recovery was seen in all patients, but delayed platelet recovery was seen in the 2 NHL patients with prior exposure to fludarabine (perhaps only coincidentally). These observations lead us to conclude that poor PBSC-mobilizing patients may still be candidates for curative intent therapy with high-dose chemotherapy as long as both small marrow harvest and an ex vivo expansion of BMCs can be performed.

At this point, only speculation can be made as to what cell population in the expanded bone marrow is responsible for the hastening of engraftment of PBSCs with limited $CD34^+$ cell content. One school of thought is that it is the additional $CD34^+$ cells. We, however, favor the hypothesis that it is microenvironmental progenitor cells that are responsible.[12,13]

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