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CD34$^+$ Hematopoietic Progenitor Cell Selection of Bone Marrow Grafts for Autologous Transplantation in Pediatric Patients

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ABSTRACT

CD34$^+$-selection of hematopoietic grafts for patients undergoing autologous hematopoietic stem cell transplantation (HSCT) is frequently used to obtain a tumor-free graft. The majority of published experience is with peripheral blood stem cell (PBSC) products; only scant information has been published on bone marrow (BM) grafts. We reviewed our experience using CD34$^+$ selection of BM grafts in children undergoing autologous BM transplantation. After obtaining institutional approval, we performed a retrospective review of the medical records of patients who underwent autologous stem cell collection at St. Jude. From January 1, 1999, to December 31, 2003, 373 patients underwent autologous HSCT; 131 received marrow grafts, 237 received PBSC grafts, and 5 received a combination. Seventeen patients underwent BM harvests for CD34$^+$ selection of their stem cell grafts. Sixteen patients received 19 CD34 purified grafts processed on the Isolex 300i Magnetic Cell Selection System® device. Four patients were not included in the engraftment analysis as 1 did not receive the collected product, 1 received a tandem product, and 2 received products that were composed of 2 or 3 combined purified products. Following selection, marrow grafts contained a median of 1.4 × 10^6 CD34$^+$ cells/kg (range: 0.09-8.3 × 10^6/kg) and a median of 0.014 ×10^8 total nucleated cell cells/kg (range: 0.001-0.09 × 10^8/kg). The median CD34% recovery was 30.9% (range: 9.3%-57.1%), with the median CD34 purity being 95.5% (range: 62.2%-98.8%). All patients engrafted. The median time to absolute neutrophil count ≥500/mm^3 was 19 days (range: 12-35 days), and to platelet recovery was 28 days (range 18-37 days). No patient died from transplant-related complications. Our study demonstrates that CD34$^+$-selection of marrow grafts is feasible, and these grafts are able to successfully reconstitute hematopoiesis in patients undergoing autologous BMT.

KEY WORDS

Autologous bone marrow transplantation • Isolex® • Lymphoma • Neuroblastoma • CD34$^+$-selection

INTRODUCTION

CD34 positive (CD34$^+$) selection of hematopoietic progenitor cells has been used to obtain highly purified tumor-free grafts in patients with malignancies who require autologous hematopoietic stem cell transplantation (HSCT) [1-3]. CD34$^+$ selection serves as a measure of tumor purging, provided the tumor cells do not express the CD34 antigen [4, 5]. The only commercially available methodology in the United States to perform this procedure is the Isolex 300i Magnetic Cell Selection System® device (Baxter, Deerfield, IL) (Isolex®) [6]. This device uses a murine antibody to the human CD34 antigen to select hematopoietic cells expressing the CD34 antigen. The specific licensed use of the Isolex® device is for CD34$^+$ selection of hematopoietic stem cells from autologous

peripheral blood of patients with malignancies. The majority of published literature has described its use in processing peripheral blood stem cell (PBSC) grafts of patients with breast cancer, multiple myeloma, and lymphoma [2,7-10]. $CD34^+$-selected grafts have reproducibly resulted in acceptable neutrophil and platelet engraftment in these circumstances [1]. In pediatrics, this methodology is used to purify PBSC grafts of patients undergoing autologous HSCT for malignancies with a tendency to metastasize to the marrow, such as neuroblastoma, lymphoma, and other solid tumors [5, 11, 12]. Unfortunately, many patients are unable to have sufficient numbers of peripheral blood stem cells collected for HSCT, whereas other patients may be too small to undergo apheresis. In these situations, a bone marrow (BM) harvest is indicated. However, BM grafts are reported to have a higher frequency of tumor contamination than peripheral blood grafts [10]. In addition, unmanipulated marrow grafts are composed of larger volumes and thus have larger dimethylsulfoxide (DMSO) content than PBSC grafts. The DMSO present in the graft can cause cardiovascular adverse events such as hypertension and bradycardia. Although $CD34^+$ selection may decrease the graft tumor content, it will decrease graft volume, allowing for smaller amounts of DMSO for cryopreservation, and hopefully fewer adverse events. At our institution, we have used the Isolex® device to purify both peripheral blood and BM grafts for pediatric and adolescent patients undergoing autologous HSCT. In this article, we describe our institutional experience using $CD34^+$ selection of marrow grafts from patients undergoing autologous BM transplant (BMT). To our knowledge, this is the first published series describing $CD34^+$ selection involving autologous BM grafts.

MATERIALS AND METHODS

Patients

We performed a retrospective, St. Jude institutional review board-approved study on BM grafts that were processed on the Isolex® device. Between January 1, 1999, and December 31, 2003, 373 autologous HSCT procedures were performed at St. Jude Children's Research Hospital. Categorically, 237 received only PBSC grafts, 131 received only marrow grafts, and 5 received both peripheral blood and marrow grafts. Of the patients receiving the 131 marrow grafts, diagnoses included brain tumors (n = 81 grafts), neuroblastoma (n = 31 grafts), Hodgkin lymphoma (n = 5 grafts), sarcoma (n = 4 grafts), non-Hodgkin's lymphoma (NHL) (n = 3 grafts), Wilm's tumor (n = 2 grafts), germ cell tumor (n = 2 grafts), severe combined immunodeficiency (n = 2 grafts), and hepatoblastoma (n = 1 graft). Seventeen patients had marrow grafts processed on the Isolex® device, comprising 20 marrow products. Thirteen patients received this product as the sole graft. All 20 products were included in the processing analysis. Four patients were excluded from the engraftment and survival data: (a) 1 patient did not receive his autologous product and later underwent an allogeneic HSCT, (b) 1 received a tandem transplant, (c) 1 received a combination of 2 selected grafts, and (d) 1 received a combination graft composed of 1 PBSC and 3 BM products, all selected. No patient received growth factors immediately prior to the marrow harvest; all patients had steady-state BM grafts collected. These patients underwent a BM harvest because they weighed <10 kg (n = 2) or had failed PBSC mobilization (n = 15). We compared these patients to 7 patients who received a single, unmanipulated, autologous BM infusion and had a similar diagnosis as those who received a single, Isolex® device processed autologous BM graft. Those who received tandem transplants, those who received a non-Isolex® device manipulated graft, or who had a diagnosis other than neuroblastoma or NHL were excluded from the data analysis.

Graft Procurement

Patients underwent harvest when the marrow was morphologically free of tumor. After a physician obtained informed consent from the legal guardian, the patient underwent the BM harvest in the operating room under sterile conditions with general anesthesia. The goal was to collect a BM graft that contained $\geq 1 \times 10^8$ total nucleated cell (TNC)/kg with a maximum collection volume of 20% total blood volume or 15 mL/kg, whichever was greater. If insufficient cells were collected with 1 harvest, subsequent ones were performed prior to the initiation of the transplant conditioning regimen. The decision to collect more marrow stem cells was made by the patient's transplant physician and the laboratory medical director after completion of the processing procedure and the analysis of the $CD34^+$ enriched product.

Graft Processing

The manufacturer provided guidelines for $CD34^+$ selection using the Isolex® device (Version 2.5). According to manufacturer's instructions, the starting material, the peripheral blood progenitor cell/mononuclear cell product, was to contain $\leq 8.0 \times 10^{10}$ nucleated cells and <35 mL of red blood cells. In our study, the same limits were applied to the processing of BM cells on the Isolex® device. Grafts were processed on the day of collection.

BM Preparation

Bone marrow products were processed within the Stem Cell Processing section of the Human Applica-

tions Laboratory according to standard operating procedures of the laboratory. Each product was initially assessed for volume, cell count, mononuclear cell content, hematocrit, and CD34 content. To minimize the amount of cell loss prior to CD34 selection, an inverted centrifugation procedure (400 g for 10 minutes) rather than density gradient separation was performed. Red blood cells were carefully withdrawn from the products to target a final red blood cell content of ≤35 mL. Following red blood cell removal, final RBC content resulted in a mean volume of 21.5 mL (range: 12.0-28.1 mL). The maximum loading volume for the Isolex® device is 1000 mL, with a maximum product hematocrit being 5.0%. Each product was diluted with Isolex Working Buffer (Dulbecco's phosphate-buffered saline supplemented with 1.0% human serum albumin and 0.48% sodium citrate [w/v]).

CD34 Selection

The Isolex 300i Magnetic Cell Selection System® is an automated system employing a microprocessor-controlled instrument, dedicated reagents, and disposable tubing set. The cells are sensitized with a mouse antihuman monoclonal antibody directed against the CD34 antigen. The $CD34^+$ cell-antibody complexes are then incubated with immunomagnetic beads (Dynabeads® M-450 Sheep anti-Mouse IgG, Dynal Biotech, ASA, California), which are coated with sheep antimouse antibody (Ab), resulting in bead-Ab-$CD34^+$ cell complexes. The complexes are isolated using the primary magnet of the Isolex® device. Through a releasing agent, a peptide resembling CD34 antigen, the $CD34^+$ cells are released from the bead, washed, and collected in the final processing step on the Isolex® device while the beads remain attached to the magnet.

CD34 Positive Fraction

Each $CD34^+$ product was concentrated by centrifugation to remove the Isolex Working Buffer and resuspended with Plasma-Lyte 148 and 5% Dextrose with 5% human serum albumin according to standard operating procedures of the Human Applications Laboratory. The final product was assessed for cell count, viability, CD34 content, and sterility. All products were cryopreserved with 10% DMSO in a control rate freezer and stored in liquid nitrogen until being thawed for infusion.

Graft Tumor Analysis

If possible, an aliquot of the graft was obtained prior to and following CD34 selection for tumor assessment. Samples from neuroblastoma patients were analyzed for the presence of tyrosine hydroxylase, synaptophysin, and dopamine decarboxylase by reverse transcriptase polymerase chain reaction (RT-PCR). The sensitivity of the assay was 1:100,000 cells for tyrosine hydroxylase and dopamine decarboxylase, and 1:1,000 cells for synaptophysin.

Transplant Care and Patient Management

All patients were transplanted on St. Jude institutional review board-approved protocols, and this retrospective study was also approved by the St. Jude institutional review board. The patient with NHL received total body irradiation (TBI) 12 Gy and etopophos 60 mg/kg. Patients with neuroblastoma received 1 of 2 regimens: (a) cyclophosphamide 375 mg/m^2 with targeted dosing of topotecan [13], or (b) melphalan 140 mg/m^2 with 16 targeted doses of busulfan [14]. Cryopreserved stem cell products were infused by intravenous push through a central venous catheter at a minimum of 24 hours after the last dose for chemotherapy. Patients received irradiated, single donor, leukocyte-reduced blood products. Packed red blood cells were transfused in a volume of 10 cc/kg to maintain hemoglobin ≥8 g/dL and platelet apheresis products were transfused to maintain platelets ≥20,000/mm^3. All patients received granulocyte-colony stimulating factors (G-CSF) 5 μg/kg/day after stem cell infusion until absolute neutrophil count (ANC) ≥2000/mm^3 for 2 consecutive days. Neutrophil engraftment was defined as ANC ≥500/mm^3 for 3 consecutive days. Platelet engraftment was defined as platelet count ≥20,000/mm^3 and ≥50,000/mm^3 for 7 consecutive days without transfusion support, respectively. Time to lymphocyte recovery (absolute lymphocyte count ≥500/mm^3) from the time of HSCT was also collected. All patients received prophylaxis for *Pneumocystis carinii* pneumonia; antifungal and antiviral prophylaxes were not routinely administered. Adverse events were coded according to National Cancer Institute Common Toxicity Criteria, version 2.0 [15].

RESULTS

Patient Characteristics

The 17 patients in this study who had marrow grafts collected were a median age of 4 years (range: 2-18 years) at the time of collection. Seven patients were males, and diagnoses included neuroblastoma (n = 15), and NHL (n = 2). One NHL patient had extensive bone metastasis, including the pelvic region, and the second NHL patient had previous morphologic BM involvement. Thus, to reduce the concern of tumor contamination, products from these 2 patients were processed on the Isolex® device.

During this same time period, 5 patients with neuroblastoma and 2 with NHL received unmanipulated, autologous BM grafts as a single product after a single collection.

Table 1. *Graft Characteristics of the 20 Bone Marrow Collections Obtained Prior to Processing, Including TNC and CD34 Cell Dose/kg, CD34%, Preselection Collection Volumes, and Hematocrit*

			Starting Dose Values/kg				
Product ID#:		Recipient Weight (kg)	TNC $\times 10^8$	CD34 $\times 10^6$	Starting % CD34	Pre-Isolex Diluted Vol.	Pre-Isolex HCT
1		12.1	3.76	15.87	4.22	500.62	4.0
2		10.7	2.03	7.08	3.49	500.00	3.9
3		19.6	1.77	5.21	2.95	505.00	3.7
4		85.2	1.5	4.44	2.97	760.00	3.7
5		19.7	2.94	7.03	2.79	550.00	3.4
6		87.3	2.95	5.07	1.78	640.00	3.9
7		90.1	0.41	0.39	0.96	699.80	3.8
8		10.8	2.82	11.79	4.18	600.00	2.0
9		24.6	2.91	13.74	4.72	699.50	2.9
10		19.8	4.11	3.82	0.93	700.00	3.9
11		86.7	5.07	2.89	0.58	837.00	2.4
12		18.3	2.6	3.56	1.37	700.00	3.2
13		15.1	0.83	1.76	2.11	901.00	1.6
14		14.7	2.43	5.27	2.17	605.00	2.8
15		10.7	4.95	8.12	1.64	500.00	2.8
16		13.2	3.68	7.8	2.12	624.00	2.6
17		15.9	5.62	5.51	0.98	904.40	3.0
18		10.8	4.5	25.52	5.67	604.30	2.5
19		12.2	4.4	17.86	4.06	618.20	2.5
20		18.6	1.79	6.06	3.39	750.50	2.5
	Mean	29.8	3.05	7.94	2.65	659.97	3.0
	Median	17.1	2.93	5.79	2.48	632.00	3.0
	St. Dev.	29.8	1.44	6.16	1.43	125.57	0.7
	Range						
	Low	10.7	0.41	0.39	0.58	500.00	1.6
	High	90.1	5.62	25.52	5.67	901.00	4.0

Information regarding the mean, median, standard deviation, and ranges is also provided for each column.
TNC indicates total nucleated cell count; HCT, hematocrit.

Graft Characteristics

Characteristics of the 20 grafts prior to CD34 selection are described in Table 1. Prior to selection, the BM grafts contained a median of 5.8×10^6 $CD34^+$/kg (range: 0.4-25.5 $\times$ 10^6/kg) and 2.9×10^8 TNC/kg (range: 0.4-5.6 $\times$ 10^8/kg). The median CD34% prior to selection was 2.48% (range: 0.58%-5.67%). The median diluted product volume was 632 mL (range: 500-901 mL), and the median preselection hematocrit was 3.0% (range: 1.6%-4.0%).

Following $CD34^+$ selection, marrow grafts contained a median of 1.4×10^6 $CD34^+$ cells/kg (range: 0.09-8.3 $\times$ 10^6/kg) and a median of 0.01×10^8 TNC cells/kg (range: 0.001-0.09 $\times$ 10^8/kg). The median graft purity was 95.5% (range: 62.2%-98.9%), with 16 grafts having a CD34 purity >90%. The median CD34% recovery was 30.9% (range: 9.3%-57.1%). The median volume of each cryopreserved BM infusion was 34 mL (range: 9.2-37.6 mL) (Table 2). The patient who underwent a tandem procedure required only 1 BM harvest to obtain a sufficient number of $CD34^+$ hematopoietic stem cells. One patient required 3 (product numbers 4, 6, and 7) and another required 2 (product numbers 3 and 5) harvests to obtain a sufficient number of progenitor cells to proceed to autologous HSCT. All patients had appropriate doses of stem cells cryopreserved prior to the initiation of the conditioning regimen. For the 13 patients who received a single product, the median $CD34^+$ cell count of these infused grafts was 2.5×10^6 cells/kg (range: 05-8.3 $\times$ 10^6 cells/kg). In comparison, those who received unmanipulated BM grafts had a median $CD34^+$ cell count of 2.6×10^6 cells/kg (range: 0.7-10.4 $\times$ 10^6 cells/kg).

Graft Tumor Content

After collection and prior to processing, 7 samples were not tested for residual tumor content, 3 were insufficient for analysis, and 10 were tested and found to have no detectable disease. After processing, 8 samples were not evaluated for residual tumor, and the 12 samples tested did not have detectable disease. Four products were collected from the 2 patients with NHL. For the remaining 16 products from neuroblastoma patients, 4 had insufficient cell count to evaluate the product for residual tumor. This data was not used to predict relapse rate, as it had to be interpreted with extreme caution as normal cells also express tyrosine hydroxylase, synaptophysin, and dopamine decarboxylase (S. Shurtleff, personal communication). This method did not correlate with risk of relapse. Of the 12 neuroblastoma

Table 2. *Graft Characteristics of the 20 Bone Marrow Collections after CD34 Selection, Including the Final TNC and CD34 Cell Dose/kg, the Final CD34%, the Percentage of CD34 Cells Recovered from the Processing, and the Volume Infused*

	Final Dose Values/kg				
Product ID#:	TNC $\times 10^8$	CD34 $\times 10^6$	Final % CD 34	% CD34 Recovery	Volume Infused (mL)
1	0.041	4.03	98.08	29.8	17.0
2	0.012	0.76	62.24	11.8	9.2
3	0.011	1.10	98.42	21.1	18.2
4	0.006	0.63	96.88	14.1	20.0
5	0.014	1.39	96.71	21.3	19.5
6	0.006	0.46	83.44	9.3	19.8
7	0.001	0.09	91.1	23.1	10.0
8	0.054	4.97	92.05	42.9	37.0
9	0.026	2.47	94.81	18.6	36.0
10	0.014	1.28	91.49	35.6	36.0
11	0.006	0.59	97.74	38.3	Not infused
12	0.014	1.35	96.18	40.4	34.0
13	0.007	0.51	72.68	29.0	34.0
14	0.017	1.59	93.34	31.9	32.0
15	0.039	3.82	97.92	57.1	36.0
16	0.025	2.36	94.27	32.2	35.0
17	0.028	2.51	88.86	46.1	36.0
18	0.090	8.30	97.83	39.0	37.6
19	0.047	4.66	98.79	33.3	36.0
20	0.010	1.17	97.63	23.8	37.0
Mean	0.023	2.20	92.023	29.93	28.44
Median	0.014	1.37	95.495	30.86	34.00
St. Dev.	0.022	2.04	9.40156	12.26	10.02
Range					
Low	0.001	0.09	62.24	9.30	9.2
High	0.090	8.30	98.88	57.10	37.6

One patient who had undergone collection did not receive the CD34 selected product. Information regarding the mean, median, standard deviation, and ranges is also provided for each column.

TNC indicates total nucleated cell count.

patients, 7 (58.3%) have experienced disease relapse. Of the 4 neuroblastoma products not tested, 2 of the 3 patients are currently alive and the third patient died of disease.

Clinical Outcome

Sixteen patients received processed products and none experienced adverse reactions during the stem cell infusion. All 13 patients who received nontandem, single selected BM products engrafted. No adverse reactions to the cryopreservative, DMSO occurred. The median time to neutrophil engraftment was 19 days (range: 12-35 days). The median time to lymphocyte recovery was 31 days (range: 16-346 days) from HSCT. The median times to platelet count of 20,000/mm^3 and 50,000/mm^3 were 28 days (range: 18-37 days) and 29 days (range: 20-44 days), respectively. The median times to last red blood cell and platelet transfusion were 25 days (range: 15-182 days) and 21 days (range: 11-279 days) after HSCT, respectively. Five patients experienced infectious complications; 2 with *Staphylococcus* species bacteremia (days +1 and +79) and 3 with diagnostic imaging consistent with pneumonia, 1 presumed to be Candida (days +9, +11, and +39). No patient died from regimen-related toxicity, infection, or hemorrhage. Ten patients had disease recurrence at a median of 324 days (range: 169-528 days) following HSCT. One of these patients remains alive >3.5 years after HSCT, and 9 have died, all of recurrent disease. Six patients are alive disease free, at a median of 1291 days (range: 1127-1570 days) after autologous HSCT.

In comparison, 7 patients (5 neuroblastoma and 2 NHL) received nonselected BM grafts during this time period. One patient experienced hypertension and sinus bradycardia secondary to the DMSO. The median time to neutrophil engraftment was 18 days (range: 10-44), and the median time for lymphocyte recovery for 6 patients was 37 days (range: 17-56 days). Five patients had a platelet count of 20,000/mm^3 at a median of 25 days (range: 17-91 days), and 4 patients had a platelet count of 50,000/mm^3 at a median of 23 days after HSCT. Two patients did not have platelet engraftment by day 100 after HSCT, and another did not have a platelet count of 50,000/mm^3 by this time point. Two patients had infectious complications after the initiation of conditioning: adenovirus in stool (1 patient, day +3) and oral herpes simplex virus (1 patient, day +6). No patient died from regimen-related toxicity, infection, or hemor-

rhage. Currently 6 patients are alive after autologous HSCT and 1 died of progressive disease.

DISCUSSION

This study demonstrates that $CD34^+$ selection of BM grafts on the Isolex 300i Magnetic Cell Selection System® is feasible for pediatric and adolescent patients. No detectable tumor content and prompt neutrophil and platelet engraftment can be achieved with this processed graft. Although great cell loss during processing occurs, engraftment is not compromised, as all of our study patients recovered neutrophil and platelet counts in a timely manner. Furthermore, no adverse effects occurred secondary to the DMSO, and few patients had documented infections, all occurring prior to day 100 after BMT and resolving with appropriate therapy.

Several other immunoselection devices besides the Isolex® device have been used for the purification of $CD34^+$ stem cells from BM and peripheral blood, including Ceprate™ (Cellpro, Inc., Bothell, WA) and the CliniMACS® (Miltenyi Biotech, Bergisch Gladback, Germany) [6, 16, 17]. Although the Ceprate™ is no longer commercially available, these systems have been compared to each other. Although the CliniMACS® tends to achieve a purer $CD34^+$ product, 1 study demonstrated that the Isolex® processed product had a greater functional capacity in culture [17]. However, all 3 systems did demonstrate that neutrophil and platelet engraftment could be obtained after infusing a CD34-enriched graft [16].

Time to engraftment with CD34 selected marrow grafts was slower than observed with unmanipulated PBSC grafts [18, 19], yet, they were similar to the times reported for unmanipulated marrow grafts [19]. Although neutrophil engraftment was similar to historic controls for disease and time period, platelet engraftment was likely slower in our patients because BM was harvested once the marrow was morphologically free of tumor. Thus, some patients received additional courses of chemotherapy prior to marrow collection, which resulted in cumulative marrow damage, leading to a damaged stem cell pool, a well-described phenomenon. Furthermore, we observed no fatal infectious or hemorrhagic complications.

A single BM harvest was sufficient for all but 2 patients to obtain adequate hematopoietic progenitor cells for transplantation. There was a wide range of recoveries of $CD34^+$ cells from the selection, which may result, in part, from the small numbers of heavily pretreated patients in our series. On average, the median recovery was 30.9% (range: 9.3%-57.1%) (Table 2). This figure is substantially less than reported from $CD34^+$ selections of PBSC products processed on the Isolex® device (58.4 ± 19.2%) [6]. Review of the literature reveals that the $CD34^+$ cell recovery from mobilized peripheral blood stem cells processed on the Isolex® device is quite variable [20-23]. One potential reason for the wide range of $CD34^+$ yield is the starting red blood cell volume and hematocrit. Those starting products that have a lower red cell volume tend to have a higher CD34 yield [21, 23]. Loading too many CD34 cells onto the processing machine without enough antibody or magnetic beads may also be a limiting factor and decrease the yield of the product [21]. Furthermore, in preliminary studies in our cell-processing laboratory, we observed that the peptide releasing agent was not optimized to release the beads from CD34 cells in BM grafts compared to PBSC grafts. This was an unexpected observation, and confirmed upon communications with the manufacturer. We investigated this issue and confirmed the presence of many $CD34^+$ cells attached to the beads after the completion of the processing. An enzymatic-based releasing procedure had been used prior to the current use of the peptide releasing agent; the beads were released from these residual cells with the enzyme treatment (P. Eldridge, personal communication). Although the cell loss did not prevent the availability of an adequate stem cell graft for most patients, this may be a potential concern, as some patients may require multiple harvests or an additional PBSC collection.

The grafts infused in our series had no evidence of tumor contamination after processing. We cannot solely attribute this finding to the graft processing. Patient selection and the timing of the marrow harvest may have played a role as we required a morphologically negative marrow. Potentially, one could hypothesize that purging may not have been necessary as the marrow was already tumor free. However, gene-marking studies have demonstrated that marrow harvested from neuroblastoma patients in morphologic remission does contribute to disease recurrence after autologous HSCT [24]. In addition, our assay may not have been sensitive enough to identify extremely small amounts of tumor. Moreover, of the 12 patients tested for minimal residual disease after selection, 7 patients have relapsed versus the 1 in the nonselected group. Thus, we do not have any evidence that this selection method acts as an additional purging process to help prevent relapse disease.

As multiple collections were required for 2 patients because of low cell dose yield, it may be important in the future to harvest marrow earlier irrespective of tumor content if $CD34^+$-selection is performed, with close analysis of the processed graft for tumor contamination. Collecting and processing cells earlier in the patient's treatment course may result in an improved hematopoietic cell content and higher cell dose, providing close analysis of the processed graft for tumor contamination is undertaken.

Finally, alternative methodologies, such as the CliniMACS® device, which have lower rates of cell loss, should be explored. Novel hematopoietic progenitor cell markers such as CD133, which has a more restricted tissue expression than CD34, should also be evaluated for their role in tumor purging within the context of a clinical trial.

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