CD34+ Cell Enrichment

Allogeneic bone marrow stem cell transplantation following CD34+ immunomagnetic enrichment in patients with inherited metabolic storage diseases

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Summary:

T-cell depletion is an essential step in reducing the risk of graft-versus-host disease (GVHD) in patients with inherited metabolic storage diseases (IMSD) undergoing hematopoietic stem cell transplantation. This goal can be achieved either by selective removal of T cells or by positive selection of CD34+ cells. Large-scale preparations of purified CD34+ cells from bone marrow products have not been extensively described. We report our results with bone marrow CD34+ cell enrichment using the CliniMACS system in eight children with IMSD. The median recovery of positively selected CD34+ cells was 46.2% with a purity of 97.5%, and a residual T cell content of 0.04×10^6 . A median of 5.5×10^6 /kg of CD34+ cells was infused. All patients engrafted at a median time of 12 days and none of the patients developed GVHD. This method is technically feasible and can be successfully used to transplant children with IMSD.

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when using unrelated donors, has been considered a major goal in this setting.

The most effective method of GVHD prophylaxis is depletion of T cells from the donor inoculum.⁵ Positive selection of hematopoietic stem cells expressing the CD34 antigen is one of the best methods of obtaining a T-lymphocyte-depleted product. Moreover, CD34+ selection allows a noteworthy reduction of B lymphocytes, thus reducing the risk of EBV-related lymphoproliferative disorders. Different techniques have been proposed to select CD34+ stem cells.⁶ It has been previously shown that the magnetic-activated cell separation technique is highly effective for clinical-scale preparation of CD34+ progenitor cells collected from leukapheresis products.^{7–9} Large-scale preparations of purified CD34+ cells from bone marrow (BM) products have been used and reported only occasionally, as the expected cell loss has been considered too high to safely perform HSCT, particularly in adult patients. Children with IMSD have relatively low weights and often receive BM from an unrelated donor. We report results using the CliniMACS system in BM CD34+ cell enrichment for allogeneic transplantation of children with these rare diseases.

Hematopoietic stem cell transplantation (HSCT) has become the standard treatment for selected patients with inherited metabolic storage diseases (IMSD).¹ Different patterns and degrees of central nervous system abnormalities are usually present in patients with IMSD and their progression could be worsened by the demyelinating effects of graft-versus-host disease (GVHD).² As the development of GVHD has been correlated with a poorer neuropsychological outcome,^{3,4} the prevention of GVHD, particularly

Patients and methods

From November 1999 to May 2002, 11 HSCTs were performed in nine patients with IMSD at San Gerardo Hospital in Monza, Italy. In the last eight HSCTs, T- and B-cell depletion was obtained using positively selected CD34+ cells with CliniMACS (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) on BM. Patient characteristics are summarized in Table 1. Conditioning included busulfan, cyclophosphamide (CY) and ATG; fludarabine was added in three cases, two undergoing second HSCT and one receiving a reduced CY dose because of cardiomyopathy. GVHD prophylaxis consisted of methylprednisolone and cyclosporin-A (CsA). The IRB approved this study and consent was obtained from parents.

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Table 1 Patient characteristics

Pt. no	Sex	Diagnosis	Age at HSCT (months)	HSCT	Weight (kg)	Donor	HLA mismatching ^a		Conditioning regimen
							Class I	DRB1	
1	M	GLD	76	1st	18	Unrelated, carrier	2 M	—	BuCyATG
2	F	MPS I-H	28	2nd	14	Unrelated, noncarrier	2 μ	1 μ	FluBuCyATG
3	M	MPS I-H	26	1st	10	Unrelated, noncarrier	1 μ	—	BuCyATG
4	F	MPS I-H	12	1st	16	Unrelated, noncarrier	—	—	BuCyATG
5	F	MPS I-H	20	1st	10	Related, noncarrier	1 M	—	FluBuCyATG
6	M	GLD	109	1st	36	Unrelated, noncarrier	1 M, 1 μ	—	BuCyATG
7	F	MPS I-H	34	2nd	13	Unrelated, noncarrier	1 M, 1 μ	—	FluBuCyATG
8	F	GLD	74	1st	26	Unrelated, noncarrier	—	—	BuCyATG

GLD = globoid cell leukodystrophy (Krabbe disease); MPS = mucopolysaccharidosis; M = major mismatching at molecular low-resolution typing; and μ = 'micro' mismatching at molecular high-resolution typing.

^aAll but two patients (No 4 and 8) were mismatched for at least one DPB1 locus.

BM harvests were processed within 24 h of collection, and ACD-A (Baxter, Deerfield, IL, USA) was added to each bag of BM at 10% w/v; Plasmasteril (6% hydroxyethyl starch) at 450 000 MW (Fresenius, Stans, CH) was then added at 1:6 – 1:8 w/w to enhance erythrocyte sedimentation. After 90 min, both plasma and buffy coat (BC) were collected and underwent a first centrifugation (1500 g for 15 min), and then plasma excess was removed and BC underwent a washing step after having been resuspended to 500 ml with Dulbecco's phosphate-buffered saline containing 1% (v/v) human serum albumin (DPBS-HSA); this step was followed by a second centrifugation (200 g for 15 min). Washed cells were subsequently resuspended to 100 ml with DPBS-HSA containing 7.5 ml of CliniMACS CD34 reagent (Miltenyi Biotec) and incubated for 30 min at room temperature under gentle agitation. After two further wash/centrifugation steps as above, nucleated cells (NC) were resuspended in 100–250 ml of DPBS-HSA and the bag was then connected to the CliniMACS device. This is a clinical grade closed system that automatically performs a CD34+ cell selection from up to 60×10^9 NC containing up to 600×10^6 CD34+ cells. Briefly, CD34-positive hematopoietic stem cells labeled with superparamagnetic iron-dextran particles conjugated to a CD34 antibody (QBEND 10) are captured by a selection column placed in a magnetic separation unit. After a series of washing steps, the purified cells are eluted and sent to a collection bag, while unlabeled cells (negative fraction) are collected in a waste bag. The whole selection procedure takes 75–90 min.

WBC counts were performed using the SE 9500 automatic device (Dasit Spa, Cornaredo, Italy) in basal BM specimen from each BM bag, after BC collection and in both positive and negative cell fractions obtained after the selection procedure. Phenotypic expression of surface molecules was determined by direct labeling using standard methodology. Cells were incubated with either fluorescein isothiocyanate or phycoerythrin-conjugated anti-CD34 (Becton Dickinson, San Jose, CA, USA), CD3, CD19, CD16-56, CD45, CD14 (ImmunoQuality, Groningen, The Netherlands) monoclonal antibodies. At least 30 000 cells were acquired with a FACScan™ flow cytometer (Becton Dickinson), and analysis was performed using the Cell Quest™ software program (Becton Dickinson). Enzyme (galactocerebrosidase or alpha-L-iduronidase) activity^{10–12} in peripheral blood WBC and plasma were assayed at day

+21 and then once a month during the first year and once every 3 months during the second year after HSCT, using fluorogenic artificial substrates. Chimerism was assessed at the same time using semiquantitative variable nucleotide tandem repeats (VNTR) polymorphism evaluation of monocytes.¹³ Patients with mixed chimerism or after second HSCT were monitored monthly also during the second year.

Results

The results of BM manipulation including enrichment and recovery of CD34+ cells as well as T- and B-cell depletion are shown in Table 2. Procedures were performed with a median starting cellularity of 16920.4×10^6 NC. After erythrocyte sedimentation, the median cellularity of the BCs was 12992.8×10^6 NC, and the median recovery of CD34+ cells was 77.1%. After the selection step, the median cellularity was 99.3×10^6 NC, with a purity of 97.5%; the median recovery of CD34+ cells was 65.1 and 46.2 % compared with the BC and the unprocessed BM, respectively. The final T-cell reduction was 4 logs. Residual CD19+ cells were 25.4×10^6 including both CD19+/CD34+ precursor B cells and CD19+/CD34+ B lymphocytes. T (CD3+) and B (CD19+) cell losses were similar, 100 and 95.5%, respectively.

The median number of CD34+ cells infused was 5.5×10^6 /kg. To favor engraftment, CD3+ cells were added back to the selected product in order to infuse $0.3\text{--}0.5 \times 10^6$ /kg CD3+ cells. In patients undergoing second HSCT, the CD3+ add-back goal was $1 \pm 0.2 \times 10^6$ /kg.

Rapid hematologic engraftment was observed in all patients (Table 3). Three patients (patients 3, 4 and 5) with mucopolysaccharidosis type I-H (MPS I-H) later developed mixed chimerism (at +120, +90 and +60, respectively). At day +21 seven patients had normal enzyme activity; a globoid cell leukodystrophy patients's galactocerebrosidase activity was equal to that of the carrier donor. Enzyme activity remained stable in all patients, unless donor chimerism dropped below 10% (ie rejection) which occurred in patient 5. No patients developed acute or chronic GVHD.

Table 2 Cell composition of unmanipulated bone marrow, buffy coat and positively selected product

	Bone marrow	Buffy coat ^a	Selected CD34+ product	
Total nucleated cells × 10 ⁶	16920.4	12992.8	99.3	
	8816.8–28232.1	5020.0–24156.3	12.9–233.3	
CD34+ cells × 10 ⁶	216.7	149.4	96.4	
	61.7–336.0	31.6–289.8	10.7–227.8	
CD34+ cells (%)	1.0	1.2	97.5	
	0.7–1.4	0.6–1.5	83.0–99.0	
CD34+ cell recovery (%)	—	77.1	65.1 ^b	46.2 ^c
		51.2–91.9	33.9–87.9	17.3–77.1
CD3+ cells × 10 ⁶	2106.4	1265.1	0.04	
	564.3–3487.7	137.0–3019.2	0.01–0.12	
CD3+ cells (%)	9.4	9.66	0.05	
	5.6–18.1	2.7–13.5	0.02–0.1	
CD3+ loss (%)	—	28.3	100.0 ^b	100.0 ^c
		0.0–75.7	99.9–100.0	99.9–100.0
CD19+ cells × 10 ⁶	618.2	435.2	25.4	
	220.4–1125.3	48.2–785.3	2.3–39.5	
CD19+ cells (%)	3.33	2.46	18.87	
	1.92–7.21	0.96–5.12	5.7–34.6	
CD19+ loss (%)	—	47.9	95.5 ^b	97.7 ^c
		1.1–78.0	91.1–100.0	92.7–100.0

Data are shown as median and range.

^aAfter HES sedimentation.^bCompared to the buffy coat.^cCompared to the initial bone marrow.**Table 3** Composition of the infused product, time of engraftment and status of patients

Pt. no	Composition of the graft				Days from graft infusion		Status
	TNC × 10 ⁶ /kg	CD34+ × 10 ⁶ /kg	CD3+ × 10 ⁶ /kg	CD19+ × 10 ⁶ /kg ^a	ANC > 500/mm ³	Platelets > 30 × 10 ⁹ /l	
1	6.0	5.3	0.4	0.1	13	12	100% donor; alive, + 708 days
2	7.0	5.5	1.0	0.5	12	21	100% donor; alive, + 580 ^b days
3	9.0	8.9	0.4	0.2	13	19	15% donor; alive, + 502 days
4	14.0	12.6	0.3	0.2	11	12	40% donor; alive, + 467 days
5	2.1	2.0	0.3	<0.1	12	16	Rejection, + 186 days deceased, + 369 ^c days
6	6.0	3.0	0.4	<0.1	14	16	100% donor alive, + 384 days
7	14.8	7.6	0.9	2.4	11	14	100% donor alive, + 145 ^b days
8	9.2	5.6	0.3	<0.1	12	16	100% donor alive, + 68 days
Median	8	5.5	0.4	0.15	12	16	

^aincluding CD19+/CD34+ cells^bFrom second HSCT.^cBecause of immune thrombocytopenia, immune hemolysis and lung hemorrhage.

Repeated donor lymphocyte infusions (DLI), at escalating doses, from 1 × 10⁶/kg up to 1 × 10⁸/kg CD3+ cells, every 3–4 weeks, were unsuccessfully attempted in two patients: patient 5 showed complete autologous reconstitution (+ 186) and died 6 months later (1 year after HSCT) of lung hemorrhage related to immune thrombocytopenia during DLI (*n* = 8); chimerism declined, despite seven DLIs, in patient 3.

The follow-up after HSCT is too short (ie 68–708 days) to evaluate the impact of HSCT on the neurological and neuropsychological outcome; however, all survivors including those with mixed chimerism are stable or improved.

Discussion

The MACS technology can be used successfully to obtain a high recovery of CD34+ cells without a significant number

of contaminating T cells in both small-scale laboratory and large-scale clinical practices.^{9,14,15} Schumm *et al*⁹ showed the feasibility of the CliniMACS device for CD34+ cell isolation in both allogeneic and autologous settings, although they reported only one procedure using BM. We isolated CD34+ cells from eight allogeneic BM donors using the CliniMACS system. The recovery of BM CD34+ cells obtained in our procedures was lower compared to that obtained, with the same technology, in large series of leukapheresis products.^{8,9} This could be because of the different cellular composition and density of BM in comparison with the mobilized leukapheresis. However, the efficiency of CliniMACS in terms of CD34+ purity and T-cell reduction was comparable with that observed in previous studies.^{7–9}

The aim of HSCT in this setting is not simply to improve survival with normal enzyme activity, but also to stabilize or enhance neuropsychological function thereby promoting

quality of life and patient independence. Therefore, prevention of the potential demyelinating effects because of toxicity and/or GVHD should be considered relevant for these patients. Irradiation was not included in the conditioning to avoid potential CNS toxicity. Our method of graft manipulation was effective in preventing GVHD in all patients.

Besides GVHD, another major problem is the high rate of partial or complete autologous recovery, which, for unknown reasons, seems to occur frequently in patients with MPS I-H, and has been only partially attributed to T-cell depletion.^{3,4,16,17} Relatively normal immune function, insufficient myeloablative and/or immunosuppressive therapy, or the interaction between glycosaminoglycans and the marrow microenvironment have been suggested as possible explanations. A second HSCT is frequently needed and well tolerated in MPS I-H patients. This information should be provided to the family during initial discussions. We cannot exclude that T-cell depletion may have caused the initial engraftment failures in our patients. The extensive T-cell depletion achieved after positive selection was felt to be an excessive risk for graft failure. Therefore, our strategy included add back of an adjusted T-cell dose to favor engraftment without excessively increasing the risk of GVHD.¹⁸ CsA after HSCT was continued both as GVHD prophylaxis and as prevention of graft failure.

The CliniMACS procedure, usually applied to leukapheretic products, was also feasible for unrelated donor BM HSCT in these children. The absolute number of CD34+ cells in the final product cannot be considered adequate for adult recipients or for a haplo-identical HSCT where a higher purified CD34+ cell number with extensive T-cell depletion would be preferable. However, in the haplo-identical setting this can be easily achieved with the CliniMACS technology, since stem cells are usually leukapheresed from a related donor.

In conclusion, our data demonstrate the technical feasibility and reproducibility of the CliniMACS system in the isolation of purified CD34+ cells from BM. This method can be used successfully to transplant children with IMSD.

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