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ORIGINAL ARTICLE

Mobilization, harvesting and selection of peripheral blood stem cells in patients with autoimmune diseases undergoing autologous hematopoietic stem cell transplantation

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Peripheral blood stem cells (PBSC) were mobilized in 130 patients with autoimmune diseases undergoing autologous hematopoietic stem cell transplantation using cyclophosphamide 2 g/m^2 and either granulocyte colony-stimulating factor (G-CSF) 5 mcg/kg/day (for systemic lupus erythematosus (SLE) and secondary progressive multiple sclerosis, SPMS) or G-CSF 10 mcg/kg/day (for relapsing remitting multiple sclerosis (RRMS), Crohn's disease (CD), systemic sclerosis (SSc), and other immune-mediated disorders). Mobilization-related mortality was 0.8% (one of 130) secondary to infection. Circulating peripheral blood (PB) CD34$^+$ cells/μl differed significantly by disease. Collected CD34$^+$ cells/kg/apheresis and overall collection efficiency was significantly better using Spectra apheresis device compared to the Fenwall CS3000 instrument. Patients with SLE and RRMS achieved the lowest and the highest CD34$^+$ cell yields, respectively. *Ex vivo* CD34$^+$ cell selection employing Isolex 300iv2.5 apparatus was significantly more efficient compared to CEPRATE CS device. Circulating PB CD34$^+$ cells/μl correlated positively with initial CD34$^+$ cells/kg/apheresis and enriched product CD34$^+$ cells/kg. Mean WBC and platelet engraftment (ANC $> 0.5 \times 10^9$/l and platelet count $> 20 \times 10^9$/l) occurred on days 9 and 11, respectively. Infused CD34$^+$ cell/kg dose showed significant direct correlation with faster white blood cell (WBC) and platelet engraftment. When adjusted for CD34$^+$ cell/kg dose, patients treated with a myeloablative regimen had significantly slower WBC and platelet recovery compared to non-myeloablative regimens.

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Introduction

Multiple studies have been performed investigating kinetics, safety and efficiency of autologous peripheral blood stem cell (PBSC) mobilization, harvesting and selection in patients with hematological diseases and solid tumor malignancies.[1–27] In single arm studies performed over the last decade, hematopoietic stem cell transplantation (HSCT) has been increasingly used to treat a variety of severe and refractory autoimmune and/or immune-mediated diseases.[28–32] As a result, HSCT for immune-mediated disorders has recently evolved into randomized controlled trials. Unlike HSCT for malignancy, to date there are limited reports investigating stem cell mobilization, harvesting, selection and engraftment in patients with immune-mediated disorders.[33–37] In this paper, we evaluate the features and outcomes of mobilization, harvesting and *ex vivo* selection of PBSC in 130 patients with a variety of autoimmune diseases. Besides safety and feasibility of PBSC collection in patients with autoimmune diseases, we estimated correlations among: (1) peripheral blood (PB) CD34$^+$ cell concentration and white blood cell (WBC), platelet and hemoglobin concentrations and collected and enriched product stem cell yields, (2) type of autoimmune disease and PB CD34$^+$ cell, WBC, platelet and hemoglobin concentrations, collected and enriched stem cell yields, collection efficiency, enriched product purity, CD34$^+$ cell recovery, and timing of hematopoietic recovery, (3) type of apheresis device and collected and enriched product stem cell yields, collection efficiency and enriched product purity, (4) type of *ex vivo* CD34$^+$ cell selection device and enriched product stem cell yields, purity, CD34$^+$ cell recovery and CD3$^+$ cell reduction, (5) gender and age and PBSC collection and selection parameters, (6) infused CD34$^+$ cell dose and timing of hematopoietic recovery and (7) conditioning regimen and timing of hematopoietic recovery.

Patients and methods

Patients

One hundred and thirty patients with a wide array of severe and refractory autoimmune diseases underwent PBSC

mobilization. All patients signed an informed consent before enrolling on an Institutional Review Board approved autoimmune disease-specific autologous HSCT protocol (Table 1). The mean age in our patient population was 34 (range 14–63) years old. Female to male ratio was 91 and 39 patients, respectively.

Stem cell collection

The goal number of $CD34^+$ cells available for infusion (either after selection or unmanipulated for protocols that did not require $CD34^+$ selection) was $>2.0 \times 10^6$/kg (optimal stem cell collection). Marginal stem cell collection is defined as $1.0–2.0 \times 10^6$ $CD34^+$ cells/kg available for infusion. Failed stem cell collection is defined as $<1.0 \times 10^6$ $CD34^+$ cells/kg available for infusion.

Stem cell yield is defined as $CD34^+$ cells/kg of patient weight in collected or enriched apheresis products. Collection efficiency is calculated as total $CD34^+$ cells obtained per apheresis $\times 100$ divided by PB $CD34^+$ cells/μl times processed blood volume (liter). Purity is defined as a percentage of $CD34^+$ cells in enriched apheresis product. $CD34^+$ cell recovery is defined as a percentage of $CD34^+$ cells recovered during selection of one apheresis product.

Bone marrow harvest (BMH)

Bone marrow harvests were performed under general anesthesia for the first two SPMS patients, but owing to poor yields all subsequent patients underwent PBSC collection as the initial stem cell harvesting procedure (Table 2). For patients in whom PBSC collection was unsuccessful in achieving an adequate number of $CD34^+$ cells (three patients) bone marrow harvests were performed as well.

Table 1 Patient characteristics

Autoimmune disease	*No. of patients*	*Gender (female/male)*	*Mean age (range), years*
SLE	50	43/7	31
MS:	43	20/23	38
SPMS	23	11/12	39
RRMS	20	9/11	37
CD	15	7/8	26
SSc	10	9/1	43
RA	3	3/0	46
MG	2	2/0	42
Systemic vasculitis:	4	4/0	32
Behcet's	2	2/0	31
Wegener's	1	1/0	26
Sjogren's	1	1/0	42
PF	1	1/0	30
RP	1	1/0	39
ARRON	1	1/0	50
Total	130	91/39	34 (14–63)

Abbreviations: ARRON = autoimmune-related retinopathy and optic neuropathy; CD = Crohn's disease; MG = myasthenia gravis; MS = multiple sclerosis; PF = pemphigus foliaceus; RA = rheumatoid arthritis; RP = relapsing polychondritis; RRMS = relapsing remitting multiple sclerosis; SLE = systemic lupus erythematosus; SPMS = secondary progressive multiple sclerosis; SSc = systemic sclerosis.

G-CSF PBSC mobilization

The first four patients with SPMS were mobilized with granulocyte colony-stimulating factor (G-CSF) 10 mcg/kg/day with leukapheresis beginning on day 4 of G-CSF (Table 2). Thereafter, owing to flare of MS during G-CSF mobilization in the fourth patient, all further patients were mobilized with cyclophosphamide (Cy) and G-CSF.

Cy and G-CSF PBSC mobilization

For 126 patients, PBSC were mobilized with Cy and G-CSF (Table 2). Patients were admitted to the hospital to receive intravenous (i.v.) Cy 2 g/m^2, infused over 1 h, through either a peripherally inserted central catheter (PICC) or peripheral line, and i.v. Mesna and hydration, given before, during and for 24 h after completion of Cy. To prevent nausea, patients were premedicated with dexamethasone 20 mg i.v., ondansetron 24 mg i.v., and lorazepam 1 mg i.v. Subcutaneous G-CSF was started 72 h after completion of Cy at 10 mcg/kg/day (5 mcg/kg/day of G-CSF was used for patients with SLE and 19 patients with SPMS) and continued daily usually as an outpatient procedure until the completion of apheresis. Simultaneously with G-CSF, patients received prophylactic oral levofloxacin 500 mg and fluconazole 400 mg daily.

Leukapheresis

Central venous catheter lines were placed via interventional radiology into the internal jugular vein on the morning of first apheresis. Stem cell harvesting was initiated when the WBC count reached 1.0×10^9/l and continued daily until the number of $CD34^+$ cells collected reached a goal of 2.0×10^6/kg (optimal collection), although a few patients ($N = 9$) were allowed to proceed to HSCT if at least 1.0×10^6 $CD34^+$ cells/kg were available for infusion (marginal collection). Either Fenwall CS3000 (Baxter, Deerfield, IL, USA) or Spectra (Cobe, Lakewood, CO, USA) apheresis devices were utilized. For patients who were severely ill, such as most patients with SLE and some with CD, daily G-CSF administration and leukapheresis procedure(s) were performed as an inpatient. Before leukapheresis, if necessary, packed red blood cells were transfused to maintain a hemoglobin concentration above 90 g/l. Platelet transfusions were given to maintain platelet count $>40 \times 10^9$/l. Red blood cell and platelet products were irradiated, cytomegalovirus safe and leukocyte depleted.

$CD34^+$ cell selection

For 98 patients, leukapheresis products were enriched by positive selection using Isolex 300iv1.12 ($N = 2$), Isolex 300iv2.5 ($N = 78$) (Baxter, Deerfield, IL, USA) or CEPRATE CS ($N = 18$) (CellPro, Bothell, WA, USA) stem cell concentrator. For the rest (20 patients with RRMS, 10 patients with SSc, one patient with SLE, and one patient with autoimmune-related retinopathy and optic neuropathy (ARRON) syndrome), collected blood products were not manipulated. Stem cell products were cryopreserved in

Table 2 Mobilization parameters

Disease	*Mobilization regimen*	*No. of patients*	*Toxicity*			*>1 mobilization regimen*	*BMH*	*Marginal collection*[a]	*Failed mobilization*[b]
			Death	*Disease flare*	*Other*				
SLE	Cy2 + G5	50	1	3	1 – disseminated mucormycosis, 1 – CMV pneumonitis and esophageal candidiasis, 4 – Gram (+) bacteremia	3 (1 + BMH, 1 + BMH + G20, 1 + G10)	2 (both with marginal collection)	7	1 (patient who died from disseminated mucormycosis)
SPMS	Cy2 + G5 (first 4 patients G10 only)	23	0	1 (4th patient)	N/A	3 (2 with initial BMH by protocol, 1 + BMH + C4 + G10)	3 (2 – with initial BMH by protocol, 1 – who failed mobilization)	0	1 (patient with hx of cladribine tx; BMH, and 2nd mobilization with Cy 4 + G10)
RRMS	Cy2 + G10	20	0	0	N/A	0	0	0	0
CD	Cy2 + G10	15	0	0	N/A	0	0	1	0
SSc	Cy2 + G10	10	0	0	1 – fluid overload, 1 – severe myalgia and bone pain requiring narcotics	0	0	1	0
RA	Cy2 + G10	3	0	0	N/A	0	0	0	0
MG	Cy2 + G10	2	0	0	1 – transient liver transaminases elevation, 1 – coagulase (–) staph bacteremia related to indwelling plazmapheresis catheter	0	0	0	0
Vasculitis	Cy2 + G10	4	0	1 (Sjogren's ON)	1 – UE phlebitis	1 (+ G10)	0	0	0
PF	Cy2 + G10	1	0	0	N/A	0	0	0	0
RP	Cy2 + G10	1	0	0	N/A	0	0	0	0
ARRON	Cy2 + G10	1	0	0	N/A	0	0	0	0
Total		130	1	5	11	7	5	9	2

Abbreviations: ARRON = autoimmune-related retinopathy and optic neuropathy; BMH = bone marrow harvest; CD = Crohn's disease; Cy2 (Cy4) = cyclophosphamide at 2 (4) g/m2; G5 (G10, G20) = G-CSF at 5 (10, 20) mcg/kg/day; CMV = cytomegalovirus; MG = myasthenia gravis; N/A = not applicable; ON = optic neuritis; PF = pemphigus foliaceus; RA = rheumatoid arthritis; RP = relapsing polychondritis; RRMS = relapsing remitting multiple sclerosis; SLE = systemic lupus erythematosus; SPMS = secondary progressive multiple sclerosis; SSc = systemic sclerosis; UE = upper extremity.

[a]Marginal stem cell collection – $1.0–2.0 \times 10^6$ of CD34+ cells/kg available for infusion.

[b]Failed mobilization – $<1.0 \times 10^6$ of CD34+ cells/kg available for infusion.

liquid nitrogen for at least 2 weeks after collection until antimicrobial cultures per Food and Drug Administration (FDA) code of federal regulation (CFR) 610.12 were confirmed as sterile.

Conditioning regimens
All patients with SPMS received myeloablative conditioning regimen consisting of i.v. Cy (total dose of 120 mg/kg) and total body irradiation (TBI) (total dose of 1200 cGy). The rest were treated with non-myeloablative lymphoablative conditioning regimens by disease-specific protocols based on i.v. Cy (total dose of 200 mg/kg) and either i.v. equine anti-thymocyte globulin (ATG) (total dose of 90 mg/kg), rabbit ATG (total dose of 5.0–7.5 mg/kg), or CAMPATH-1 H (total dose of 20 mg).

Autologous HSCT
On day 0, $CD34^+$ selected cells ($N=95$) or unmanipulated PBSC ($N=31$) were infused through a PICC line. G-CSF 5 mcg/kg was given subcutaneously daily beginning day 0, and continued until the resolution of neutropenia (absolute neutrophil count (ANC) $>0.5\times10^9$/l). Packed red blood cells were transfused for hemoglobin concentration below 80 g/l. Platelet transfusions were given to maintain platelet count >10–30×10^9/l. Red blood cell and platelet products were irradiated, cytomegalovirus safe and leukocyte depleted.

WBC and platelet engraftment
WBC engraftment is defined as the first day of PB ANC $>0.5\times10^9$/l. Platelet engraftment is defined as the first day of platelet count $>20\times10^9$/l (or $>30\times10^9$/l for patients maintained at such a threshold).

Statistical analysis
Statistica (v.7) software (Tulsa, OK, USA) was used for basic descriptive analysis of data. Means, s.d., scatter and box plots were utilized for presentation of continuous variables by groups. *T*-test and analysis of variance were used to test for the difference in means when comparing two and more than two groups. In assessing correlation, we used 2D scatterplots with log 10 expression of variables and estimated correlation coefficient r and *P*-value for test H:$r=0$ presented. After log 10 transformation of continuous variables, we fit analysis of covariance (ANCOVA) model with disease groups defined by dummy variables, using reference cell coding, with SLE as reference group. In this way, we assessed whether regression lines among groups intersected, were parallel or identical, by testing for appropriate regression coefficients H:Beta = 0. S-PLUS (v.7.0, Insightful Co.) software was used for ANCOVA.

Results

Toxicity
PBSC mobilization regimens, apheresis and five BMH procedures were generally well tolerated in our autoimmune patient population. Mobilization-associated adverse events are shown in Table 2. One death occurred 2 weeks after PBSC collection. This patient with refractory SLE, who was severely immunosuppressed owing to chronic use of high-dose steroids, was found to have disseminated mucormycosis with lung and central nervous system involvement 7 days after stem cell harvesting when he presented with seizures. Besides this patient, there were another six infections diagnosed during the post-mobilization period: five patients with SLE (including four with Gram-positive bacteremia and one with CMV pneumonitis and esophageal candidiasis) and one patient with myasthenia gravis (MG) with coagulase negative staphylococcus bacteremia associated with indwelling plasmapheresis catheter use.

Four other grade I to III adverse events were observed during mobilization, including one patient with SSc who developed fluid overload requiring temporary oxygen therapy, another patient with SSc who required narcotics for musculoskeletal symptoms associated with G-CSF administration, one patient with MG who had transient liver function test abnormality, and one patient with vasculitis (Wegener's granulomatosis) who experienced upper extremity superficial phlebitis at a previous PICC site within 1 week of mobilization.

Five patients (4%) had exacerbation of their disease-related symptoms, including three patients with SLE who experienced worsening in their pulmonary condition, one patient with SPMS mobilized with G-CSF only, and one patient with neurovascular Sjogren's who had an attack of optic neuritis. All patients responded well to pulse-dose i.v. steroid therapy with improvement in condition to baseline level.

Suboptimal stem cell collection
A total of 130 patients underwent PBSC mobilization. One hundred and nineteen patients (91.5%) had successful collection (reached the goal of $CD34^+$ cells of $>2.0\times10^6$/kg). Mobilization regimens and complicated stem cell collections (including patients requiring BMH and/or additional mobilization regimen(s), patients who either failed or achieved only marginal stem cell collection) are shown in Table 2.

Marginal stem cell collection was achieved in nine patients (7%) (seven patients with SLE, one patient with SSc and one patient with CD). Three of them, all with SLE, required additional BMH procedure and/or a second mobilization regimen with G-CSF. Two patients (1.5%) failed stem cell collection: a patient with SLE and another patient with SPMS who was heavily pretreated with cladribine. The latter patient failed PBSC mobilization with Cy 2 g/m^2 and G-CSF 5 mcg/kg/day, required a BMH and additional mobilization regimen with Cy 4 g/m^2 and G-CSF 10 mcg/kg/day before being deemed not suitable for transplant.

Five patients underwent BMH. Two patients with SPMS had BMH performed as the initial stem cell collection procedure. Both harvests were inadequate and supplemented with PBSC collections with G-CSF 10 mcg/kg/day. Three patients (two with SLE with eventual marginal collection and one with MS who eventually failed mobilization) required an additional BMH procedure as described above.

One patient with vasculitis (Sjogren's syndrome) underwent a second mobilization with G-CSF 10 mcg/kg/day before $>2.0\times10^6$/kg of CD34$^+$ cells were obtained.

Pre-collection PB variables

Table 3A shows mean values of PB pre-collection variables (PB CD34$^+$ cell percentage, PB CD34$^+$ cells/μl, PB WBC/μl, PB platelets/μl, and PB hemoglobin/μl) for each disease. The highest mean PB WBC/μl and PB CD34$^+$ cells/μl were achieved in patients with RRMS, the lowest were in patients with SLE and SSc. Mean PB CD34$^+$ cell percentage was the highest in patients with RRMS, and lowest in patients with SSc and SPMS. As two different mobilization regimens were applied to distinct diseases, we analyzed difference in mean PB CD34$^+$ cells/μl by mobilization regimen and further analyzed by disease within each regimen. Figure 1a shows that mean PB CD34$^+$/μl is 146 in patients mobilized utilizing Cy 2 g/m^2 plus G-CSF 10 mcg/kg/day (vasculitis, CD, SSc and RRMS) which is significantly higher ($P=0.003$) than mean PB CD34$^+$/μl of 56 in patients mobilized with Cy 2 g/m^2 plus G-CSF 5 mcg/kg/day (SLE and SPMS). Within the latter group, SLE and SPMS, there is a statistically significant difference ($P=0.03$) in means of circulating PB CD34$^+$/μl (52 and 96, respectively) (Figure 1b). Patients mobilized with Cy 2 g/m^2 plus G-CSF 10 mcg/kg/day showed differences in mean PB CD34$^+$/μl among diseases as well, the highest achieved in patients with RRMS (283), followed by CD (105), vasculitis (78) and SSc (52), $P=0.004$ for differences among diseases (Figure 1c).

Leukapheresis

Forty-one patients underwent stem cell collection with Fenwall CS3000, 80 patients with Spectra and for nine patients both apheresis devices were utilized.

The mean number of apheresis sessions per patient was 1.8 (range 1–10). Patients with SLE required the largest mean number of apheresis sessions (2.5) comparing to patients with vasculitis (2.0), CD (1.9), SPMS and rheumatoid arthritis (RA) (1.7 each), SSc (1.6) and RRMS (1.0) (Table 3b). Mean collection efficiency was 43%. The mean number of CD34$^+$ cells/kg in each apheresis unit was 6.27×10^6/kg. Yields (means) of collected CD34$^+$ cells/kg, CD3$^+$ cells/kg, as well as mononuclear cells (MNC)/kg per apheresis for each disease depending on apheresis device used are shown in Table 3B. Overall, mean MNC/kg, CD34$^+$ cells/kg and CD3$^+$ cells/kg were lower within the same disease when employing CS3000 apheresis device compared to the Cobe Spectra. In addition, SLE and RRMS had the lowest and highest yields, respectively, achieved per apheresis session.

When analyzing all diseases together, Spectra apheresis device was superior to Fenwall CS3000 in: total mean number of apheresis per patient required (1.7 versus 2.3) ($P=0.01$) (Figure 2a), mean number of collected CD34$^+$ cells/kg/apheresis (7.4×10^6/kg versus 4.62×10^6/kg) ($P=0.006$) (Figure 2b), collected MNC/kg/apheresis (5.97×10^8/kg versus 3.35×10^8/kg) ($P<0.01$) (Figure 2c) and overall collection efficiency (46 versus 29%) ($P=0.003$) (Figure 2d). Apheresis machine also influenced further product selection properties such as purity (81% versus 69%) ($P<0.01$) (Figure 2e) and recovery of CD34$^+$ cells (64% versus 59%) (not statistically significant) (Figure 2f).

CD34$^+$ cell selection

Ninety-eight patients underwent *ex vivo* CD34$^+$ cell selection with CEPRATE CS ($N=18$), Isolex 300iv1.12 ($N=2$) or Isolex 300iv2.5 ($N=78$) stem cell concentrator.

For all diseases, mean numbers of enriched CD34$^+$ cells/kg and CD3$^+$ cells/kg were 3.12×10^6 and 6.33×10^4/kg, respectively (Table 3c). The mean purity of selected products was 75%. The mean recovery of CD34$^+$ cells was 62%. CD3$^+$ cell reduction averaged 3.8 logs.

Patients with SLE and SPMS had *ex vivo* CD34$^+$ cell selection performed by both devices (whereas for the other diseases either CellPro or Isolex were used), and our analysis shows higher means for enriched product purity and CD3$^+$ cell reduction when employing Isolex 2.5 device compared to CellPro. Patients with SLE achieved the lowest enriched product CD34$^+$ cells/kg with either selection device correlating with the lowest initial product CD34$^+$ cell yields obtained for patients with SLE. Overall, Isolex 300iv2.5 selection device was superior to CEPRATE CS in: achieved mean number of enriched CD34$^+$ cells/kg/apheresis (3.63×10^6 versus 1.07×10^6/kg) ($P<0.01$) (Figure 3a), purity of selected product (82 versus 43%) ($P<0.01$) (Figure 3b), recovery of CD34$^+$ cells (64 versus 47%) (not statistically significant) (Figure 3c), and T-cell log reduction (4.2 versus 2.5) ($P<0.01$) (Figure 3d) (Isolex300iv1.12 was excluded from analysis because of small ($N=2$) size).

Correlations among PB, apheresis and CD34$^+$ cell selection variables

Statistically significant ($P<0.05$) correlations were observed between PB CD34$^+$ cells/μl and PB WBC/μl, PB platelets/μl, PB hemoglobin/μl (not shown), and total number of apheresis per patient required ($r=-0.59$) (Figure 4a). Additionally, PB WBC/μl and PB platelets/μl correlated with total number of apheresis per patient performed, collected numbers of MNC/kg/apheresis, CD3$^+$ cells/kg/apheresis, and CD34$^+$ cells/kg/apheresis (not shown). There was a positive correlation between PB hemoglobin/μl and collected numbers of CD3$^+$ cells/kg/apheresis and CD34$^+$ cells/kg/apheresis (not shown).

A strong positive correlation was observed between PB CD34$^+$ cells/μl and collected product CD34$^+$ cells/kg ($r=0.82$, $P<0.001$) (Figure 4b) as well as enriched product CD34$^+$ cells/kg ($r=0.72$, $P<0.001$) (Figure 4c). We found a strong positive correlation between PB CD34$^+$ cell % and collected product CD34$^+$ cell % ($r=0.84$, $P<0.001$) (Figure 4d). Purity of selected product showed moderate positive correlation with both PB CD34$^+$ cell % ($r=0.58$, $P<0.001$) and collected product CD34$^+$ cell % ($r=0.51$, $P<0.001$) (Figures 4e and 4f).

Hematopoietic recovery

The mean number of infused CD34$^+$ cells was 7.16×10^6/kg. The highest mean number of CD34$^+$ cells/kg was

Table 3 Means of mobilization, apheresis, CD34+ cell selection variables and WBC and platelet engraftment by disease

(A)	*Mobilization regimen*	*PB CD34+ cell %*	*PB CD34+ cells/μl*	*PB WBC/μl*	*PB Plt/μl*	*PB Hb/μl*		
SLE	Cy2+G5	0.45	52	22	103	10		
SPMS	Cy2+G5	0.27	96	38	180	12		
RA	Cy2+G10	N/A	N/A	49	257	N/A		
Vasculitis	Cy2+G10	0.31	78	32	169	11		
CD	Cy2+G10	0.36	105	37	227	11		
SSc	Cy2+G10	0.25	52	29	193	10		
RRMS	Cy2+G10	0.55	283	52	201	13		
Average	—	0.41	99	31	154	11		
(B)	*Apheresis device*	*A-CD34+ cell %*	*A-CD34+/kg/apheresis (E+6)*	*A-MNC/kg/apheresis (E+8)*	*A-CD3+ cell %*	*A-CD3+/kg/apheresis (E+8)*	*CE*	*Total no. of apheresis/patient*
SLE	CS3000	1.36	2.83	2.26	30.8	0.74	27	2.5
	Spectra	1.36	4.91	5.07	15.6	0.86	53	
SPMS	CS3000	1.39	6.32	4.34	24.2	1.09	32	1.7
RA	CS3000	0.46	1.92	4.24	51	2.16	N/A	1.7
	Spectra	0.73	5.82	7.15	33	2.36	N/A	
Vasculitis	Spectra	0.88	9.22	7.4	24.3	1.87	47	2
CD	CS3000	1.96	4.11	2.42	28.8	0.6	38	1.9
	Spectra	1.24	7.13	7.21	29.7	1.68	34	
SSc	Spectra	0.66	4.3	N/A	23.8	1.67	42	1.6
RRMS	Spectra	1.6	16.3	N/A	27.6	2.98	38	1
Average	—	1.31	6.27	4.72	23.9	1.28	43	1.9
(C)	*Selection device*	*E-CD34+ cell % (purity)*	*E-CD34+/kg/apheresis (E+6)*	*CD34+ cell recovery (%)*	*E-MNC/kg/apher (E+6)*	*E-CD3+ cell %*	*E-CD3+/kg/apheresis (E+4)*	*Log CD3+ cell reduction*
SLE	Cellpro	43	0.58	49	1.6	16	18.2	2.4
	Isolex 2.5	82	2.86	62	10.2	0.3	0.8	4.2
SPMS	Cellpro	40	1.19	67	2.7	12	31.4	2.6
	Isolex 2.5	84	4.47	62	5.2	0.4	2.1	3.9
RA	Cellpro	49	2.36	53	4.3	9.8	37.1	2.8
Vasculitis	Isolex 2.5	75	4.46	65	5.1	0.2	0.6	4.5
CD	Isolex 2.5	82	4.07	70	4.6	0.1	0.6	4.4
Average	—	75	3.12	62	7.2	2.7	6.3	3.8
(D)	*Conditioning regimen*	*I-CD34+ cells/kg (E+6)*	*WBC engraftment (days)*	*Plt engraftment (days)*				
SLE	NM	5.78	9	11				
SPMS	M	4.88	11	16				
RA	NM	3.93	9	6				
Vasculitis	NM	6.86	11	9				
CD	NM	6.7	9	8				
SSc	NM	6.45	9	8				
RRMS	NM	13.6	9	8				
Average	—	7.16	9	11				

Abbreviations: A = initial product (collected by apheresis); CE = collection efficiency; CD = Crohn's disease; Cy2+G5 = cyclophosphamide 2 g/m^2 plus G-CSF 5 mcg/kg/day; Cy2+G10 = cyclophosphamide 2 g/m^2 plus G-CSF 10 mcg/kg/day; E = enriched product; Hb = hemoglobin; I = infused; M = myeloablative; MNC = mononuclear cells; NM = non-myeloablative; PB = peripheral blood; Plt = platelets; RA = rheumatoid arthritis; RRMS = relapsing remitting multiple sclerosis; SLE = systemic lupus erythematosus; SPMS = secondary progressive multiple sclerosis; SSc = systemic sclerosis; WBC = white blood cells.

infused to patients with RRMS, the lowest to patients with RA and SPMS (Table 3D). Mean (median, range) WBC and platelet engraftment was observed on days 9 (9, 7–15) and 11 (11, 6–30), respectively. Patients with SPMS demonstrated an average WBC and platelet engraftment on days 11 and 16, respectively. In fact, all diseases had the same mean duration of neutropenia (9 days), except patients with SPMS and vasculitis (11 days). Patients with SPMS sustained mean platelet counts $<20\times10^9$/l the longest (16 days), whereas patients with RA engrafted platelets on average day 6 (Table 3D). Twelve patients did not develop a platelet count $<20\times10^9$/l. Nine patients with marginal collection ($CD34^+$ cells $<2.0\times10^6$/kg) showed mean (median) engraftment on days 9 (10) and 11 (13), for WBC and platelets, respectively.

For all diseases, we found a statistically significant negative correlation between dose of infused $CD34^+$ cell/kg and duration of neutropenia ($r=-0.27$, $P=0.002$)

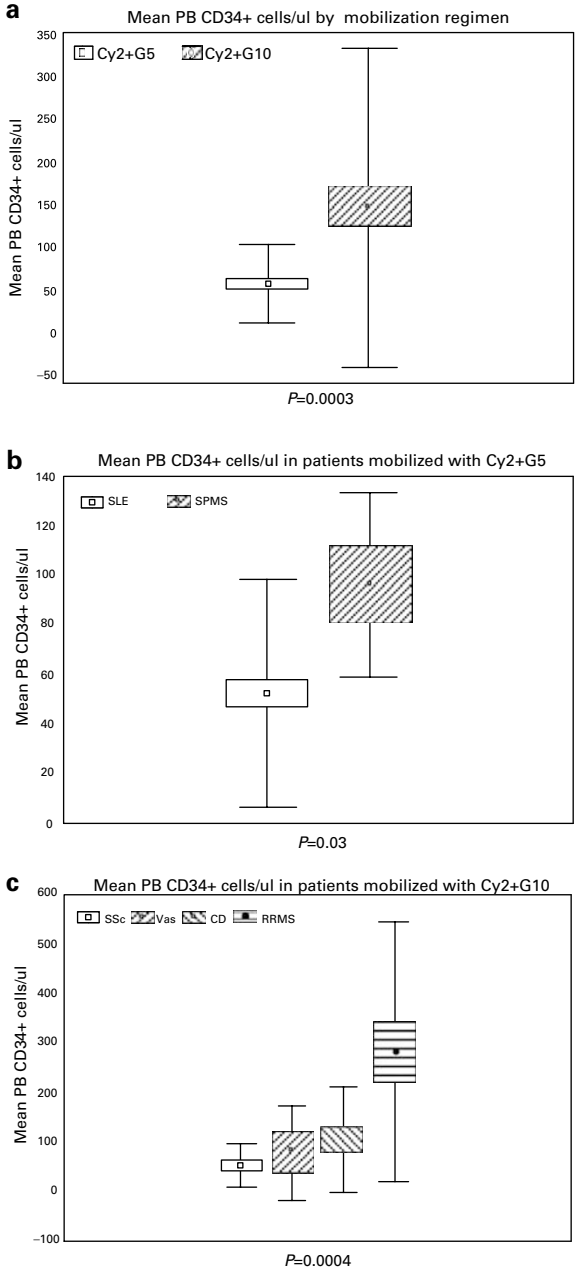

Figure 1 Mean PB $CD34^+$ cells/μl by mobilization regimen and disease. PB – peripheral blood, Cy2 + G5 – patients mobilized with cyclophosphamide 2 g/m^2 plus G-CSF 5 mcg/kg/day, Cy2 + G10 – patients mobilized with cyclophosphamide 2 g/m^2 plus G-CSF 10 mcg/kg/day, SLE – systemic lupus erythematosus, SPMS – secondary progressive multiple sclerosis, SSc – systemic sclerosis, Vas – vasculitis, CD – Crohn's disease, RRMS – relapsing remitting multiple sclerosis.

(Figure 4g). Similar negative correlation existed between infused $CD34^+$ cell/kg dose and days until platelet engraftment ($r = -0.34$, $P = 0.0002$) (Figure 4h).

We analyzed the influence of type of disease (SLE, SPMS, CD, SSc and RRMS) on the timing of WBC and platelet recovery using multiple regression (ANCOVA) model with SLE as a reference disease. When adjusted for infused $CD34^+$ cell/kg dose, we found no statistically significant differences among diseases for days until WBC engraftment except for SPMS group (Beta = 0.06, $P < 0.001$). When adjusted for infused $CD34^+$ cell dose, SPMS patients had a more prolonged neutropenic interval. Analysis of disease effect on platelet recovery, when adjusted for infused $CD34^+$ cell dose, revealed SPMS, CD and RRMS patients to differ from SLE (Beta = 0.1, −0.09, −0.12 with $P = 0.0005$, 0.02, 0.0009, for above diseases, respectively). These results reflect a more prolonged period until platelet engraftment in patients with SPMS and a shortened period in patients with CD and RRMS when compared to patients with SLE infused with the same $CD34^+$ cell dose.

The group of patients with SPMS was treated with a myeloablative regimen whereas all other patients received non-myeloablative conditioning regimens. When adjusted for infused $CD34^+$ cell/kg dose, the group of patients with SPMS compared to all other patient groups showed an even more significant delay in WBC as well as platelet engraftment (Beta = 0.4, $P < 0.001$ and Beta = 0.42, $P < 0.001$, for WBC and platelets, respectively).

Influence of gender and age

We performed a restricted gender-specific analysis within the MS group as most of our male patients had a diagnosis of MS. No statistically significant differences were found between genders in any PBSC mobilization, collection or enriched product parameters. Equally, age was not a significant factor affecting mobilization efficiency.

Discussion

Our data of 130 patients with autoimmune diseases undergoing autologous HSCT is the first large single center analysis of stem cell mobilization in patients with immune-mediated diseases. The only previous comprehensive report performed by Burt *et al*.[37] was a summary of stem cell collection in 187 patients with various autoimmune diseases from 24 transplant centers throughout the world, and was hindered by absence of patient PB $CD34^+$ cells/μl levels and lack of standardized mobilization regimens and apheresis instruments and techniques.

It has been previously reported that mobilization of PBSC using growth factors without chemotherapy may be associated with disease flare.[34,38–40] At our center, only 4 of 130 patients (all with SPMS) were mobilized with G-CSF alone. As the fourth patient experienced neurological deterioration, all subsequent patients received Cy 2 g/m^2 in addition to G-CSF. Among these patients, only four cases of mild disease exacerbation were observed, with quick return to baseline or complete resolution of the symptoms after high-dose steroid therapy. Eleven patients, all receiving Cy, experienced peri-mobilization complications. Six patients with SLE developed infectious complications (mostly gram positive bacteremia), however, one patient with SLE died from disseminated mucormycosis. Another infection was documented in a patient with MG who was highly immunocompromised from prolonged plasmapheresis and immune suppressive drugs. Culture

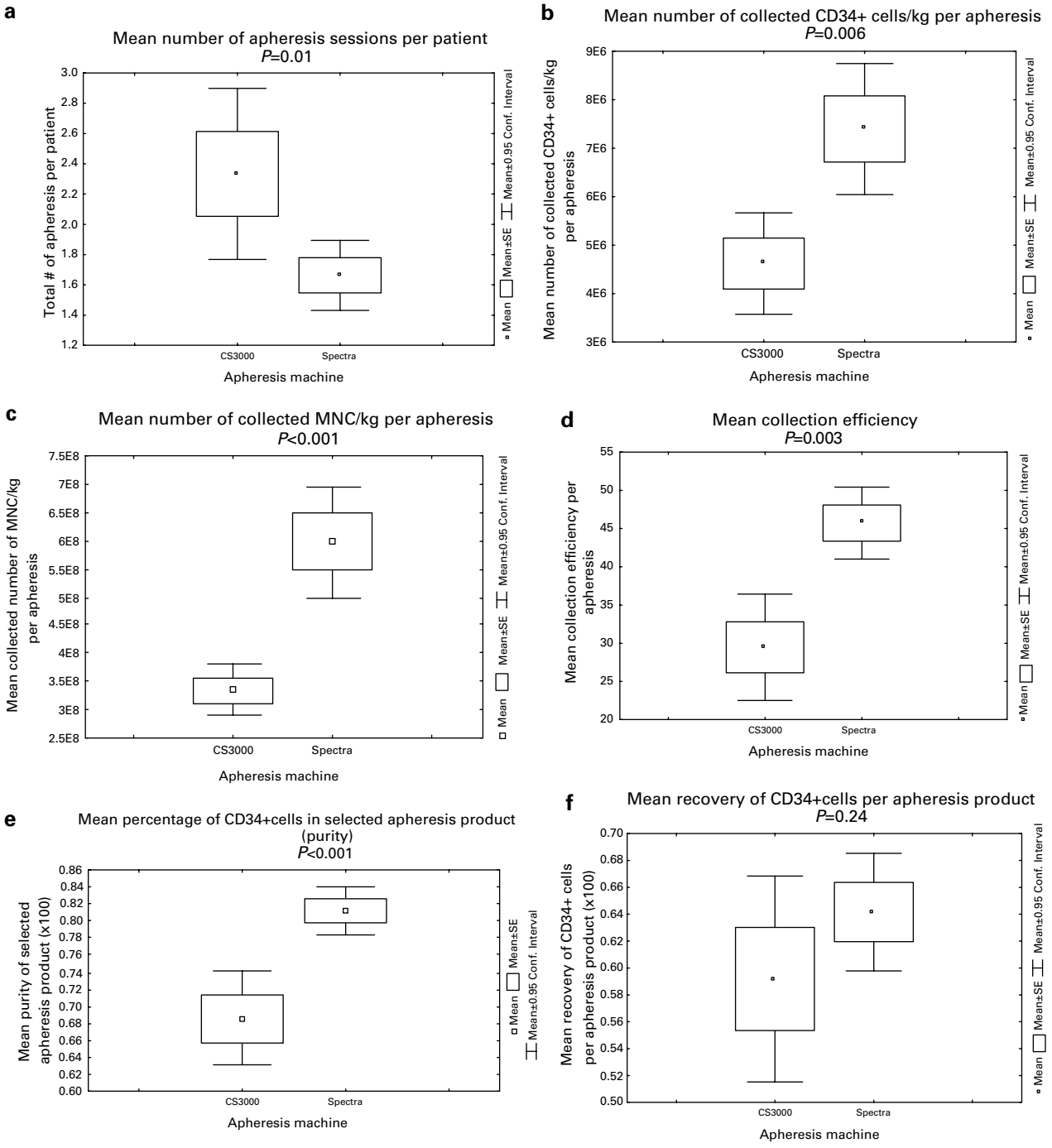

Figure 2 Apheresis device performance.

negative neutropenic fever, although common, especially in patients with CD, was transient and easily controlled with antibiotic therapy. Patients with SSc are prone to cardiopulmonary decompensation which can be exacerbated by hyperhydration and/or cytokine release during mobilization. One patient with SSc required repeated admission to hospital for pulmonary edema which was successfully managed with supplemental oxygen and diuretics. None of our SSc patients developed life-threatening cardiopulmonary complications which were reported to occur in patients mobilized using a higher (4 g/m^2) Cy dose.[37]

Our analysis revealed statistically significant differences between different autoimmune diseases in ability to mobilize CD34$^+$ cells into the peripheral blood (PB CD34$^+$ cells/μl). However, as two different mobilizing regimens (Cy 2 g/m^2 plus G-CSF 5 mcg/kg/day versus Cy 2 g/m^2 plus G-CSF 10 mcg/kg/day) were applied to distinct diseases, we cannot determine whether differences in PB CD34$^+$ cells/μl were secondary to differences in mobiliza-

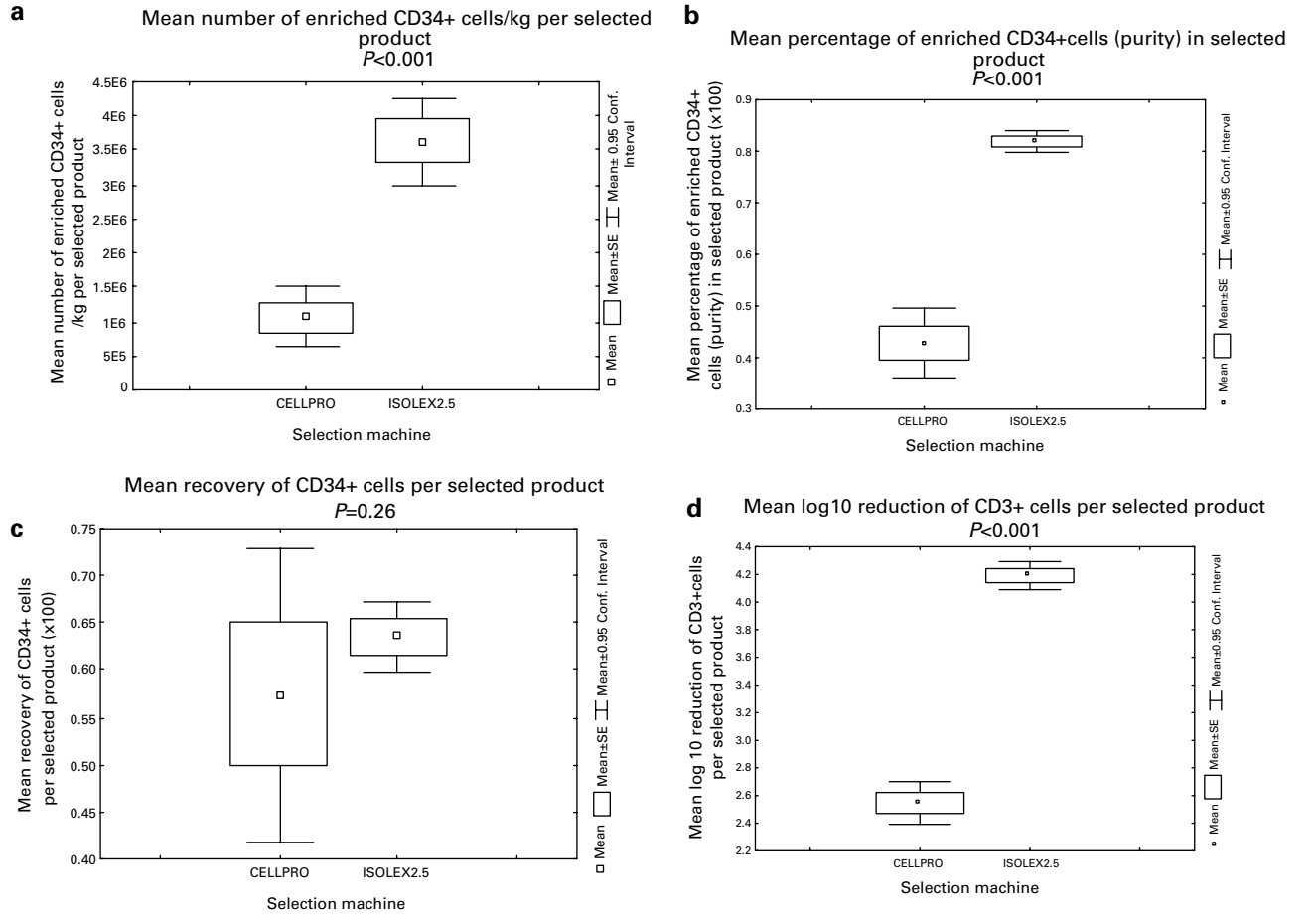

Figure 3 Selection device performance.

tion regimens utilized or internal disease effect on mobilization parameters. However, for both SLE and SPMS patients, the same mobilization regimen (Cy 2 g/m^2 plus G-CSF 5 mcg/kg/day) was used and significantly higher PB CD34$^+$ cells/μl were achieved in SPMS compared to SLE suggesting a disease or prior therapy effect on ability to mobilize CD34$^+$ cells into the blood. Similarly, for diseases in which stem cells were mobilized with Cy 2 g/m^2 plus G-CSF 10 mcg/kg/day, RRMS had significantly higher PB CD34$^+$ cells/μl compared to SSc, vasculitis and CD, again suggesting an independent disease effect. Various malignant diseases have been shown to differ in PBSC mobilization capacity because of multiple factors associated with pathogenesis of disease. Significant history of chemotherapy especially with alkylating agents has been widely shown to decrease bone marrow stem cell compartment reserve.[41–43] Some studies have suggested significant bone marrow involvement by disease to be an important factor negatively impacting on mobilization of CD34$^+$ cells.[44,45] In autoimmune disorders, further studies will be necessary to determine if disease-associated cytokines and chemokines and/or medication history affects the number of stem cells mobilized into the PB.

We found no statistically significant differences between gender or age on any mobilization, collection or enriched product parameters. In a patient population with malignancies, influence of age and sex of the patient on PBSC yield have been largely controversial.[27]

This study demonstrates the importance of high PB CD34$^+$ cell counts for high collected blood product CD34$^+$ cell yields, an observation reported in malignancies by multiple investigators.[1,8,46,47] In fact, our results show that PB CD34$^+$ cell concentration highly correlated with initial as well as selected product CD34$^+$ cell yields, and PB CD34%, initial product CD34% and enriched product purity correlated well with each other. Optimal apheresis and *ex vivo* selection of CD34$^+$ cells depends on adequate PB CD34$^+$ cell concentration. Low PB CD34$^+$ cell concentration at the time of apheresis results in poor recovery of CD34$^+$ cells after apheresis and/or *ex vivo* selection. This supports the significance of achieving high PB CD34$^+$ cell counts to determine the optimal time for initiation of leukapheresis in order to achieve the best collection parameters with minimal number of apheresis procedures.

Consistent with previous reports,[26,48,49] we found statistically significant differences in apheresis and selection device efficiency. Compared to CS3000, the Spectra apheresis device demonstrated the best performance and should be continuously employed for stem cell collection and processing in patients with autoimmune diseases undergoing HSCT. The Isolex 2.5 stem cell selection device

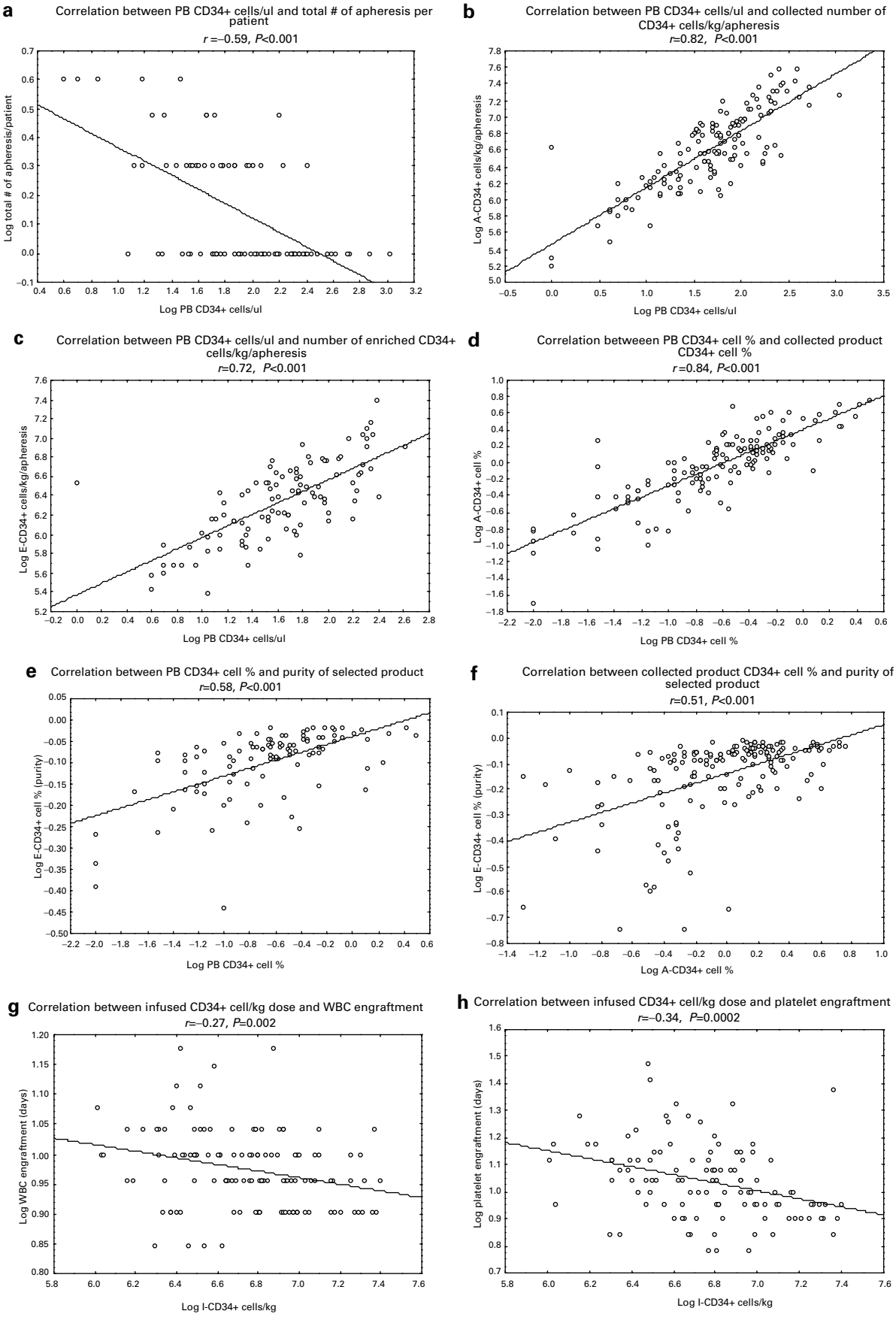

Figure 4 Correlation variables. E – enriched product, A – initial product (collected by apheresis), I – infused.

was superior to the CellPro apparatus. However, as the CellPro CEPRATE system is no longer commercially available, future studies on *ex vivo* selection of $CD34^+$ cells will need to compare the Baxter Isolex to Miltenyi CliniMACS devices.[50,51]

Although with no universal agreement, recommended optimal $CD34^+$ cell dose ($2–5 \times 10^6$/kg) for successful engraftment after autologous myeloablative HSCT for patients with malignancies has been accepted by most clinicians.[7,20,25,52] Non-myeloablative chemotherapy used in most of our patients carries virtually no risk of non-engraftment after autologous HSCT. Indeed, nine patients with suboptimal collection ($CD34^+$ cells $< 2.0 \times 10^6$/kg), all treated with non-myeloablative chemotherapy, showed only slightly delayed hematopoietic recovery. However, our findings demonstrate a correlation between infused $CD34^+$ cell dose and faster WBC and platelet engraftment supporting the role of autologous stem cell re-infusion after high-dose chemotherapy in shortening the period of neutropenia and critical thrombocytopenia. Studies in patients with malignancies undergoing myeloablative HSCT investigating $CD34^+$ cell dose influence on hematopoietic engraftment have shown that higher doses of transplanted stem cells provide a clinical benefit, particularly significant for faster platelet recovery.[7,20]

In addition, our analysis demonstrates that independent of infused $CD34^+$ cell dose, significantly slower WBC and platelet engraftment occurs after myeloablative conditioning regimens compared to non-myeloablative regimens for immune-mediated disorders. The conditioning regimen used to eliminate self-reactive lymphocytes within the patient has been designed depending on investigator to either specifically target lymphocytes (lymphoablative i.e. non-myeloablative regimen) or to destroy the entire hematopoietic bone marrow compartment (myeloablative regimen).[32] Nevertheless, the goal of autologous HSCT for autoimmune diseases is to generate new self-tolerant lymphocytes after elimination of self- or auto-reactive lymphocytes (i.e. lymphoablation), rather than ablate and reconstitute the entire hematopoietic compartment myeloablation). Although non-myeloablative regimens are often considered safer owing to lower organ toxicity, the more rapid engraftment following non-myeloablative HSCT demonstrated herein also supports the safety of non-myeloablative regimens compared to myeloablative regimens. In cancers, autologous HSCT regimens are designed to be myeloablative as the rationale is to destroy leukemic cancer-causing stem cells. Therefore, in malignancies, there are no reports comparing engraftment after non-myeloablative compared to myeloablative regimens for autologous HSCT. Following allogeneic HSCT for malignancies, only a few retrospective studies have reported on engraftment interval which generally report earlier WBC and/or platelet recovery using reduced intensity regimens compared to conventional myeloablative protocols.[53,54]

In summary, our retrospective study indicates the mobilization of PBSC may be performed safely without significant risk of disease exacerbation using cyclophosphamide and G-CSF mobilization but that astute and close observation and prophylaxis for infectious complications is prudent with immune-mediate diseases. Circulating post-mobilization PB $CD34^+$ cell numbers vary between autoimmune diseases. We found a strong statistically significant correlation between PB $CD34^+$ cell counts and collected and enriched $CD34^+$ cell yields. $CD34^+$ cell collection from the PB is influenced by the apheresis device employed, and *ex vivo* $CD34^+$ cell selection efficiency is affected by selection device used. We observed a statistically significant correlation between infused $CD34^+$ cell dose and faster WBC and platelet recovery. Finally, myeloablative autologous HSCT regimens even when corrected for infused $CD34^+$ cell dose have a longer time to both platelet and WBC engraftment compared to lymphoablative but non-myeloablative regimens.

Acknowledgements

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References

1 Fruehauf S, Haas R, Conradt C, Murea S, Witt B, Mohle R *et al*. Peripheral blood progenitor cell (PBPC) counts during steady-state hematopoiesis allow to estimate the yield of mobilized PBPC after filgrastim (R-metHuG-CSF)-supported cytotoxic chemotherapy. *Blood* 1995; **85**: 2619–2626.
2 Roberts AW, Begley CG, Grigg AP, Basser RL. Do steady-state peripheral blood progenitor cell (PBPC) counts predict the yield of PBPC mobilized by filgrastim alone? *Blood* 1995; **86**: 2451.
3 Husson B, Ravoet C, Dehon M, Wallef G, Hougardy N, Delannoy A. Predictive value of the steady-state peripheral blood progenitor cell (PBPC) counts for the yield of PBPC collected by leukapheresis after mobilization by granulocyte colony-stimulating factor (G-CSF) alone or chemotherapy and G-CSF. *Blood* 1996; **87**: 3526–3528.
4 Breems DA, van Hennik PB, Kusadasi N, Boudewijn A, Cornelissen JJ, Sonneveld P *et al*. Individual stem cell quality in leukapheresis products is related to the number of mobilized stem cells. *Blood* 1996; **87**: 5370–5378.
5 Koc ON, Gerson SL, Cooper BW, Laughlin M, Meyerson H, Kutteh L *et al*. Randomized cross-over trial of progenitor-cell mobilization: high-dose cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF) versus granulocyte-macrophage colony-stimulating factor plus G-CSF. *J Clin Oncol* 2000; **18**: 1824–1830.
6 Akard L. Optimum methods to mobilize stem cells. *J Clin Oncol* 2000; **18**: 3063.
7 Chabannon C, Le Corroller A-G, Viret F, Eillen C, Faucher C, Moatti J-P *et al*. Cost-effectiveness of repeated aphereses in poor mobilizers undergoing high-dose chemotherapy and autologous hematopoietic cell transplantation. *Leukemia* 2003; **17**: 811–820.
8 Haas R, Mohle R, Fruhauf S, Goldschmidt H, Witt B, Flentje M *et al*. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. *Blood* 1994; **83**: 3787–3794.
9 Narayanasami U, Kanteti R, Morelli J, Klekar A, Al-Olama A, Keating C *et al*. Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. *Blood* 2001; **98**: 2059–2064.

10 Deliliers GL, Annaloro C, Marconi M, Soligo D, Morandi P, Luchesini C *et al*. Harvesting of autologous blood stem cells after a mobilising regimen with low-dose cyclophosphamide. *Leuk Lymph* 2002; **43**: 1957–1960.
11 Mollee P, Pereira D, Nagy T, Song K, Saragosa R, Keating A *et al*. Cyclophosphamide, etoposide and G- CSF to mobilize peripheral blood stem cells for autologous stem cell transplantation in patients with lymphoma. *Bone Marrow Tranpslant* 2002; **30**: 272–278.
12 Petzer AL, Hochenburger E, Haun M, Duba HC, Grunewald K, Hoflehner E *et al*. High-dose hydroxyurea plus G-CSF mobilize BCR-ABL-negative progenitor cells (CFC, LTC-IC) into the blood of newly diagnosed CML patients at any time of hematopoietic regeneration. *J Hematother Stem Cell Res* 2002; **11**: 293–300.
13 Weaver CH, Birch R, Greco FA, Schwartzberg L, McAneny B, Moore M *et al*. Mobilization and harvesting of peripheral blood stem cells: randomized evaluations of different doses of filgrastim. *Br J Haematol* 1998; **100**: 338–347.
14 Yanovich S, Mitsky P, Cornetta K, Maziarz RT, Rosenfeld C, Krause DS *et al*. Transplantation of $CD34^+$ peripheral blood cells selected using a fully automated immunomagnetic system in patients with high-risk breast cancer: results of a prospective randomized multicenter clinical trial. *Bone Marrow Transplant* 2000; **25**: 1165–1174.
15 Meehan KR, Slack R, Gehan E, Herscowitz HB, Areman EM, Ebadi M *et al*. Mobilization of peripheral blood stem cells with paclitaxel and rhG-CSF in high-risk breast cancer patients. *J Hematother Stem Cell Res* 2002; **11**: 415–421.
16 Prosper F, Sola C, Hornedo J, Arbona C, Menendez P, Orfao A *et al*. Mobilization of peripheral blood progenitor cells with a combination of cyclophosphamide, r-metHuCSF and filgrastim in patients with breast cancer previously treated with chemotherapy. *Leukemia* 2003; **17**: 437–441.
17 Martin-Murea S, Voso MT, Hohaus S, Pforsich M, Fruehauf S, Golschmidt H *et al*. The dose of granulocyte colony-stimulating factor administered following cytotoxic chemotherapy is not related to the rebound level of circulating $CD34^+$ haematopoietic progenitor cells during marrow recovery. *Br J Haematol* 1998; **101**: 582–585.
18 Morris CL, Siegel E, Barlogie B, Cottler-Fox M, Lin P, Fassas A *et al*. Mobilization of $CD34^+$ cells in elderly patients (>70 years) with multiple myeloma: influence of age, prior therapy, platelet count and mobilization regimen. *Br J Haematol* 2003; **120**: 413–423.
19 Sevilla J, Gonzalez-Vicent M, Madero L, Garcia-Sanchez F, Diaz MA. Granulocyte colony-stimulating factor alone at 12 μg/kg twice a day for 4 days for peripheral blood progenitor cell priming in pediatric patients. *Bone Marrow Transplant* 2002; **30**: 417–420.
20 Ketterer N, Salles G, Raba M, Espinouse D, Sonet A, Tremisi P *et al*. High $CD34^+$ cell counts decrease hematologic toxicity of autologous peripheral blood progenitor cell transplantation. *Blood* 1998; **91**: 3148–3155.
21 Gordon PR, Leimig T, Mueller I, Babarin-Dorner A, Holladay MA, Houston J *et al*. A large-scale method for T cell depletion: towards graft engineering of mobilized peripheral blood stem cells. *Bone Marrow Transplant* 2002; **30**: 69–74.
22 Dunbar CE, Cottler-Fox M, O'Shaughnessy JA, Doren S, Carter C, Berenson R *et al*. Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. *Blood* 1995; **85**: 3048–3057.
23 De Boer F, Drager AM, Van Haperen MJAM, Van der Wall E, Kessler F, Huijgens PC *et al*. The phenotypic profile of CD34-positive peripheral blood stem cells in different mobilization regimens. *Br J Haematol* 2000; **111**: 1138–1144.
24 Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for $CD34^+$ cell determination by flow cytometry. *J Hematother* 1996; **5**: 213–226.
25 Shpall EJ, Champlin R, Glaspy JA. Effect of $CD34^+$ peripheral blood progenitor cell dose on hematopoietic recovery. *Biol Blood Marrow Transplant* 1998; **4**: 84–92.
26 Gryn J, Shadduck RK, Lister J, Zeigler ZR, Raymond JM. Factors affecting purification of $CD34^+$ peripheral blood stem cells using Baxter Isolex 300i. *J Hematother Stem Cell Res* 2002; **11**: 719–730.
27 Fruehauf S, Seggewiss R. It's moving day: factors affecting peripheral blood stem cell mobilization and strategies for improvement (review). *Br J Haematol* 2003; **122**: 360–375.
28 Burt RK, Verda L, Oyama Y, Statkute L, Slavin S. Non-myeloablative stem cell transplantation for autoimmune diseases. *Springer Semin Immunopathol* 2004; **26**: 57–69.
29 Sykes M, Nikolic B. Treatment of severe autoimmune disease by stem-cell transplantation. *Nature* 2005; **435**: 620–627.
30 Popat U, Krance R. Haematopoietic stem cell transplantation for autoimmune disorders: the American perspective. *Br J Haematol* 2004; **126**: 637–649.
31 Hough RE, Snowden JA, Wulffraat NM. Haemopoietic stem cell transplantation in autoimmune diseases: a European perspective. *Br J Haematol* 2005; **128**: 432–459.
32 Burt RK, Marmont A, Oyama Y, Slavin S, Arnold R, Hiepe F *et al*. Randomized controlled trials of autologous hematopoietic stem cell transplantation for autoimmune diseases: the evolution from myeloablative to lymphoablative transplant regimens. *Arthr Rheum* 2006; **54**: 3750–3760.
33 McGonagle D, Rawstron A, Richards S, Isaacs J, Bird H, Jack A *et al*. A phase 1 study to address the safety and efficacy of granulocyte colony-stimulating factor for the mobilization of hematopoietic progenitor cells in active rheumatoid arthritis. *Arthr Rheum* 1997; **40**: 1838–1842.
34 Snowden JA, Biggs JC, Miliken ST, Fuller A, Staniforth D, Passuello F *et al*. A randomized, blinded, placebo-controlled, dose escalation study of the tolerability and efficacy of filgrastim for hematopoietic stem cell mobilization in patients with severe active rheumatoid arthritis. *Bone Marrow Transplant* 1998; **22**: 1035–1041.
35 Breban M, Dougados M, Picard F, Zompi S, Marolleau J-P, Bocaccio C *et al*. Intensified-dose (4 g/m^2) cyclophosphamide and granulocyte colony-stimulating factor administration for hematopoietic stem cell mobilization in refractory rheumatoid arthritis. *Arthr Rheum* 1999; **42**: 2275–2280.
36 Locatelli F, Perotti C, Torretta L, Maccario R, Montagna D, Ravelli A *et al*. Mobilization and selection of peripheral blood hematopoietic progenitors in children with systemic sclerosis. *Haematologica* 1999; **84**: 839–843.
37 Burt RK, Fassas A, Snowden JA, van Laar JM, Kozak T, Wulffraat NM *et al*. Collection of hematopoietic stem cells from patients with autoimmune diseases. *Bone Marrow Transplant* 2001; **28**: 1–12.
38 Openshaw H, Stuve O, Antel JP, Nash R, Lund BT, Weiner LP *et al*. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology* 2000; **54**: 2147–2150.
39 Euler HH, Harten P, Zeuner RA, Schwab UM. Recombinant human granulocyte colony stimulating factor in patients with systemic lupus erythematosus associated neutropenia and refractory infections. *J Rheumatol* 1997; **24**: 2153–2157.
40 Gottenberg JE, Roux S, Desmoulins F, Clerc D, Mariette X. Granulocyte colony-stimulating factor therapy resulting in a flare of systemic lupus erythematosus: comment on the article by Yang and Hamilton. *Arthr Rheum* 2001; **44**: 2458–2460.

41 Hohaus S, Martin H, Wassmann B, Egerer G, Haus U, Farber L *et al*. Recombinant human granulocyte and granulocyte-macrophage colony-stimulating factor (G-CSF and GM-CSF) administered following cytotoxic chemotherapy have a similar ability to mobilize peripheral blood stem cells. *Bone Marrow Transplant* 1998; **22**: 625–630.
42 Ketterer N, Salles G, Moullet I, Dumontet C, ElJaafari-Corbin A, Tremisi P *et al*. Factors associated with successful mobilization of peripheral blood progenitor cells in 200 patients with lymphoid malignancies. *Br J Haematol* 1998; **103**: 235–242.
43 D'Arena G, Musto P, Di Mauro L, Cascavilla N, Iacono ND, Scalzulli PR *et al*. Predictive parameters for mobilized peripheral blood $CD34^+$ progenitor cell collection in patients with hematological malignancies. *Am J Hematol* 1998; **58**: 255–262.
44 Demirer T, Buckner CD, Storer B, Lilleby K, Rowley S, Clift R *et al*. Effect of different chemotherapy regimens on peripheral-blood stem-cell collections in patients with breast cancer receiving granulocyte colony-stimulating factor. *J Clin Oncol* 1997; **15**: 684–690.
45 Tarella C, Zallio F, Caracciolo D, Cherasco C, Bondesan P, Gavarotti P *et al*. Hematopoietic progenitor cell mobilization and harvest following an intensive chemotherapy debulking in indolent lymphoma patients. *Stem Cells* 1999; **17**: 55–61.
46 Sautois B, Fraipont V, Baudoux E, Fassotte MF, Hermanne JP, Jerusalem G *et al*. Peripheral blood progenitor cell collections in cancer patients: analysis of factors affecting the yields. *Haematologica* 1999; **84**: 342–349.
47 Kudo Y, Minegishi M, Saito N, Itoh T, Fushimi J, Takahashi H *et al*. The absolute number of peripheral blood $CD34^+$ cells predicts a timing for apheresis and progenitor cell yield in patients with hematologic malignancies and solid tumors. *Tohoku J Exp Med* 2003; **199**: 111–118.
48 Mehta J, Singhal S, Gordon L, Tallman M, Williams S, Luyun R *et al*. Cobe Spectra is superior to Fenwal CS 3000 Plus for collection of hematopoietic stem cells. *Bone Marrow Transplantation* 2002; **29**: 563–567.
49 Hitzler WE, Wolf S, Runkel S, Kunz-Kostomanolakis M. Comparison of intermittent- and continuous-flow cell separators for the collection of autologous peripheral blood progenitor cells in patients with hematologic malignancies. *Transfusion* 2001; **41**: 1562–1566.
50 Despres D, Flohr T, Uppenkamp M, Baldus M, Hoffmann M, Huber C *et al*. $CD34^+$ cell enrichment for autologous peripheral blood stem cell transplantation by use of the CliniMACS device. *J Hematother Stem Cell Res* 2000; **9**: 557–564.
51 Schumm M, Lang P, Taylor G, Kuci S, Klingebiel T, Buhring HJ *et al*. Isolation of highly purified autologous and allogeneic peripheral $CD34^+$ cells using the CliniMACS device. *J Hematother* 1999; **8**: 209–218.
52 Weaver C, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L *et al*. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995; **86**: 3961–3969.
53 Kim I, Yoon SS, Lee K-H, Keam B, Kim TM, Kim J-S *et al*. Comparative outcomes of reduced intensity and myeloablative allogeneic hematopoietic stem cell transplantation in patients under 50 with hematologic malignancies. *Clin Transplant* 2006; **20**: 496–503.
54 Vela-Ojeda J, Garcia-Ruiz Esparza MA, Tripp-Villanueva F, Ayala-Sanchez M, Delgado-Lamas JL, Garces-Ruiz O *et al*. Allogeneic peripheral blood stem cell transplantation using reduced intensity versus myeloablative conditioning regimens for the treatment of leukemia. *Stem Cells Dev* 2004; **13**: 571–578.