



Special report

Collection of hematopoietic stem cells from patients with autoimmune diseases

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Summary:

We reviewed data from 24 transplant centers in Asia, Australia, Europe, and North America to determine the outcomes of stem cell collection including methods used, cell yields, effects on disease activity, and complications in patients with autoimmune diseases. Twenty-one unprimed bone marrow harvests and 174 peripheral blood stem cell mobilizations were performed on 187 patients. Disease indications were multiple sclerosis (76 patients), rheumatoid arthritis (37 patients), scleroderma (26 patients), systemic lupus erythematosus (19 patients), juvenile chronic arthritis (13 patients), idiopathic autoimmune thrombocytopenia (8 patients), Behcet's disease (3 patients), undifferentiated vasculitis (3 patients), polychondritis (1 patient) and polymyositis (1 patient). Bone marrow harvests were used in the Peoples Republic of China and preferred worldwide for children. PBSC mobilization was the preferred technique for adult stem cell collection in America, Australia, and Europe. Methods of PBSC mobilization included G-CSF (5, 10, or 16 $\mu\text{g}/\text{kg}/\text{day}$) or cyclophosphamide (2 or 4 g/m^2) with either G-CSF (5 or 10 $\mu\text{g}/\text{kg}/\text{day}$) or GM-CSF (5 $\mu\text{g}/\text{kg}/\text{day}$). Bone marrow harvests were without complications and did not affect disease activity. A combination of cyclophosphamide and G-CSF was more likely to ameliorate disease activity than G-CSF alone ($P < 0.001$). G-CSF alone was more likely to cause disease exacerbation than the combination of cyclophosphamide and G-CSF ($P = 0.003$). Three patients died as a result of cyclophosphamide-based stem cell collection (2.6% of patients mobilized with cyclophosphamide). When corrected for patient weight and apheresis volume, progenitor cell

yields tended to vary by underlying disease, prior medication history and mobilization regimen. Trends in the approaches to, and results of, progenitor cell mobilization are suggested by this survey. While cytokine-based mobilization appears less toxic, it is more likely to result in disease reactivation. Optimization with regard to cell yields and safety are likely to be disease-specific and prospective disease-specific studies of mobilization procedures appear warranted. *Bone Marrow Transplantation* (2001) 28, 1–12.

Keywords: hematopoietic stem cell transplantation; mobilization; autoimmune disease

High-dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation (HSCT) is an increasingly used treatment for severe autoimmune disorders.^{1–8} HSCT may induce remission or stabilization of otherwise refractory disease including multiple sclerosis (MS),^{9–13} rheumatoid arthritis (RA),^{9,14–18} juvenile chronic arthritis (JCA),¹⁹ scleroderma (SSc),^{20–22} systemic lupus (SLE),^{9,23–27} and others. The concept is to ablate the immune system and then allow regeneration of a new immune system from the hematopoietic stem cell compartment. The optimal method of collecting stem cells for autoimmune diseases is unknown and diverse methods for harvesting and processing hematopoietic stem cells are being investigated worldwide. The method of stem cell procurement, bone marrow harvest vs peripheral blood stem cell (PBSC) mobilization, could modify disease behavior and treatment results. Complications of stem cell collection may arise that are unique to a particular autoimmune disorder and may not be anticipated from experience in harvesting or mobilizing stem cells from patients with malignancies. Furthermore, collection of stem cells may also be influenced by the autoimmune process and by prior exposure to specific immunosuppressive or immune-modulating therapies. To evaluate current practice and outcomes of auto-

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logous hematopoietic stem cell collection, 24 centers from four continents provided retrospective data from patients diagnosed with an autoimmune disease.

Methods

Data collection

Twenty-five centers known to be enrolling patients with autoimmune diseases for HSCT were asked to submit information. Twenty-four centers responded to the survey. Data were restricted to outcomes of stem cell collection. The outcomes of transplantation were not evaluated in this study. All patients who started stem cell priming were included independent of whether they completed mobilization or proceeded to transplant. Demographics requested included: age, sex, disease, duration of disease, and medications within 2 weeks of mobilization, as well as all prior disease-related medications. Medication doses were generally either not known or unavailable from the reporting institution. Data on stem cell procurement included: marrow *vs* PBSC, type and dose of growth factor, type and dose of chemotherapy, CD34⁺ cells/kg collected, CD34⁺ cells/kg infused, method of CD34⁺ cell enrichment or lymphocyte depletion, number of apheresis procedures, and apheresis volume. Some centers did not select CD34⁺ cells and since the efficiency of CD34⁺ cell recovery varies by selection method, unless otherwise stated, CD34⁺ cell yield refers to the number of cells collected before selection. Outcome of apheresis or marrow harvest included any complications and/or effect on disease activity. To confirm accuracy, data from each center were submitted back to the investigating sites to be verified as accurate and correct by the investigator.

The Kurtzke Extended Disability Status Scale (EDSS) is a neurologic performance scale that varies by 0.5 point increments from 0 (normal) to 10 (death due to neurologic dysfunction).²⁸ For the purposes of this analysis, a flare of multiple sclerosis is defined as a 1.0 or greater increase in the EDSS. Criteria for improvement are defined as a 1.0 or greater decrease in the EDSS. A change in rheumatoid arthritis or juvenile chronic arthritis is defined as a 30% increase (disease flare) or 30% decrease (disease amelioration) from pre-mobilization baseline in two of the following: swollen joint count, tender joint count or pain score on a visual analog scale. A change in scleroderma is defined as a 25% increase (disease flare) or decline (disease amelioration) in the modified Rodnan skin scoring system.²⁹ Improvement of SLE is a decline in the systemic lupus erythematosus disease activity index (SLEDAI) by 5 or more points. Progression of SLE is an increase of the SLEDAI by 5 or more points.³⁰ Patients with idiopathic thrombocytopenic purpura (ITP) were given intravenous immunoglobulin and transfused with platelets prior to apheresis catheter placement. The apheresis procedure itself could acutely lower the platelet count, and patients went directly from mobilization into transplant. Therefore, for ITP, no definition of exacerbation or deterioration was applicable. Failure of marrow harvest or PBSC mobilization is defined as less than 2.0×10^6 CD34⁺ cells/kg (after

CD34⁺ cell selection or purging) and inability to proceed to transplant, as determined by the transplant center. Patients who failed mobilization were not included in analysis of stem cell yield. Marginal CD34⁺ cell collections are defined as less than 2.0×10^6 CD34⁺ cells/kg (after CD34⁺ cell selection or purging) but the patient still proceeded to transplant.

Statistical analysis

This paper summarizes a survey of 24 sites performing autologous hematopoietic stem cell transplants for autoimmune diseases. The data were collected via retrospective survey capturing most but not all sites known to be performing these procedures. Technical details of stem cell collection varied, including the apheresis instrument, flow rate, day of growth factor on which apheresis was initiated, post-chemotherapy rebound of the white blood cell count required to initiate apheresis, and target dose of CD34⁺ cells. Therefore, in terms of stem cell dose collected, our purpose is not to test clear-cut statistical hypotheses, but to examine the ongoing practices in the field and provide pilot data for future studies. Thus, for progenitor cell yield, we provide tables with raw data, proportions, and graphs but no statistical analysis. To address the question of toxicity or impact of mobilization on disease activity, we used Fisher exact test and confidence intervals for proportions. *P* values and confidence intervals should be taken as indicators of underlying variability and differences rather than tests of hypotheses.

Results

Demographics

Twenty-four centers from the continents of Asia, Australia, Europe, and North America participated in this survey and provided data on 187 patients (Table 1). Diagnoses were: MS (76 patients), RA (37 patients), SSc (26 patients), SLE (19 patients), JCA (13 patients), ITP (8 patients), Behcets syndrome (3 patients), undifferentiated vasculitis (3 patients), polymyositis (1 patient) and polychondritis (1 patient) (Table 2). Overall, 66% of patients were female. The majority of patients were female in all disease categories except JCA (5 of 13 patients) and Behcets (1 of 3 patients). The mean age varied by disease: JCA (9 years old), SLE (29 years), ITP (34 years), and MS, SSc, and RA (39, 40 and 42 years, respectively). Disease duration varied between diseases from a mean of 3 to 10 years. The stem cell product was unmanipulated in 59 patients, although 37 of these 59 patients had '*in vivo*' purging by means of anti-thymocyte infusion. The majority of grafts were purified to remove lymphocytes. Positive enrichment for CD34⁺ cells was performed using either CEPRATE (CellPro, Bothel, WA, USA), Isolex (Nexell, Irvine, CA, USA), or CliniMACS (Miltenyi, Bergish Gladbach, Germany) cell separation systems. Negative selection was performed with T cell antibodies by e-rosetting or Nexel Isolex CD4/CD8 selection. A back-up stem cell source was cryopreserved for 28 patients.

Table 1 Institutions

<i>Location/Center</i>	<i>Disease (No. of patients)</i>	<i>Methods of ex vivo purifying CD34⁺ cells</i>
<i>Asia</i>		
Nanjing, China/University of Nanjing	SLE (3)	No purging
<i>Australia</i>		
Sydney, Australia/St Vincent's Hospital	RA (16)	No purging
<i>Europe</i>		
Antwerp, Belgium/Algemeen Ziekenhuis Middelheim	Behcet (1)	No purging
Berlin, Germany/Universitätsklinikum Campus Charite Mitte	SLE (3); SSc (3); PC (1)	Miltenyi CD34 ⁺ selection
Cagliari, Italy/Binaghi Hospital	MS (1)	No purging
Firenze, Italy/Azienda Ospedaliera Di Careggi	MS (2); SLE (1)	No purging
Genoa, Italy/Centro Trapianti Di Midollo Osseo, Ospedale San Martino	MS (4); SLE (1)	No purging
Heidelberg, Germany/University of Heidelberg,	Vasculitis (3); Behcets (2)	Miltenyi CD34 ⁺ selection
Leeds, UK/Leeds General Infirmary	RA (5), PM (1), SSc (1)	Isolex CD34 ⁺ positive and CD4/CD8 negative selection
Leiden, Netherlands/Leiden University Medical Center	RA (10), SSc (5)	Miltenyi CD34 ⁺ selection
London, UK/Royal Free Hospital	SSc (7)	CellPro CD34 ⁺ selection
Pescara, Italy/Ospedale Civile	MS (1)	No purging
Pisa, Italy/Chiari Hospital	MS (1)	No purging
Prague, Czech Republic/University Hospital Kralovske Vinohrady	MS (14)	No purge; CellPro CD34 ⁺ selection and CD2/CD3 mouse monoclonal antibody e-rosettes; Miltenyi CD34 ⁺ selection
Thessaloniki, Greece/George Papanicolaou General Hospital	MS (32)	No purge, CellPro CD34 ⁺ selection
Utrecht, Netherlands/Universitair Medisch Centrum	JCA (13), SLE (1)	Miltenyi CD34 ⁺ selection and CD2/CD3 mouse monoclonal antibody e-rosettes
<i>North America</i>		
Bethesda, Maryland/National Institutes of Health	ITP (8)	Isolex CD34 ⁺ selection
Chicago, Illinois/Northwestern University	RA (3), MS (8), SLE (9)	Cellpro CD34 ⁺ , Isolex CD34 ⁺ selection
Los Angeles, California/University of California	SSc (2)	Isolex CD34 ⁺ selection
Madison, Wisconsin/University of Wisconsin	RA (1)	CellPro CD34 ⁺ selection
Milwaukee, Wisconsin/Medical College of Wisconsin	MS (5)	CellPro CD34 ⁺ selection
Omaha, Nebraska/University of Nebraska	RA (2), MS (3)	No purge, Isolex CD34 ⁺ selection
San Diego, California/University of California at San Diego	SLE (1)	Isolex CD34 ⁺ selection
Seattle, Washington/Fred Hutchinson Research Cancer Center	MS (5); SSc (8)	Isolex CD34 ⁺ selection

ITP = idiopathic thrombotic thrombocytopenic purpura; JCA = juvenile chronic arthritis; MS = multiple sclerosis; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; SSc = scleroderma.

Table 2 Patient demographics

<i>Disease</i>	<i>No. of patients</i>	<i>Sex: female/male</i>	<i>% Female</i>	<i>Mean age in years (range)</i>	<i>Mean disease duration in years (range)</i>
MS	76	49/27	65	39 (21–59)	10 (0.8–30)
RA	37	27/10	73	42 (23–60)	10 (2–20)
SSc	26	18/8	69	40 (23–61)	3 (0.6–13)
SLE	19	14/5	74	29 (14–51)	7 (0.3–20)
JCA	13	5/8	38	9 (4–14)	6 (1–11)
ITP	8	6/2	75	34 (18–52)	13 (2–40)
Behcets	3	1/2	33	40 (32–49)	3 (1.2–4)
Undifferentiated vasculitis	3	2/1	66	46 (39–54)	4 (2–7)
Polymyositis	1	1/0	100	42	5
Polychondritis	1	1/0	100	41	3
Total	187	124/63	66		

Bone marrow harvest

Bone marrow harvest was the initial procedure to collect stem cells from 19 patients (2 with MS, 13 with JCA and 4 with SLE). Harvesting bone marrow was the preferred procedure for pediatric patients with JCA, and for adults suffering from lupus in the Peoples Republic of China. Marrow harvest was without complication and did not affect disease severity or activity. Failure to collect adequate numbers of CD34⁺ cells occurred in two patients, both of whom had multiple sclerosis. The number of CD34⁺ cells before *ex vivo* CD34⁺ enrichment was 1.9 and 1.98 × 10⁶/kg, respectively. Both patients had an adequate CD34⁺ cell collection after supplementing marrow with G-CSF mobilized PBSCs.

PBSC mobilization

Effect of mobilizing regimen on disease: Fifty-six patients (20 with MS, 16 with RA, 10 with scleroderma, 8 with ITP, 1 with SLE, and 1 with Behcets) received only G-CSF to mobilize peripheral blood stem cells (Table 3). While on G-CSF, disease flared in five patients. In two patients with MS, the extended status disability scale (EDSS) increased by 1.0 point during cytokine mobilization. Three patients with RA had a flare of disease activity with a greater than 30% increase in swollen joint count during cytokine mobilization. In two of these patients, disease exacerbation resolved without altering medications. In one patient, rheumatoid symptoms resolved with oral prednisone. While not considered an exacerbation of disease, two patients with SSc developed cutaneous telangiectasis and one developed skin edema and joint pain while on G-CSF.

One hundred and seventeen patients were mobilized with

cyclophosphamide (either 2.0 or 4.0 g/m²) and a growth factor (either G-CSF or GM-CSF) (Table 3). Disease improved in 26 patients. Although not defined as improvement, another five patients with MS had a decline in their EDSS by 0.5 points, and while not considered an exacerbation, the EDSS of two patients with MS increased by 0.5 points, and one patient had a seizure.

The combination of cyclophosphamide and growth factor was more likely to improve disease activity compared to G-CSF alone (*P* < 0.001). Similarly, G-CSF alone was more likely to cause disease exacerbation compared to mobilization with cyclophosphamide and G-CSF (*P* = 0.003).

Toxicity of mobilization: The only non-disease-related toxicities reported for patients mobilized with G-CSF were chest pain of unclear etiology, transient elevation of transaminases, joint pain, myalgia, and a dysfunctional central venous catheter interrupting apheresis (Table 4). No patient was reported to develop fever or splenomegaly.

Of patients mobilized with cyclophosphamide and growth factors, all had transient neutropenia, and nine developed fever while neutropenic. Documented infections occurred in four patients. Two patients (one with RA and one with Behcets) developed uncomplicated bacteremia. One patient with RA developed hidradenitis. A patient with lupus died of disseminated mucormycosis. Seizures occurred in one patient with MS (idiopathic) and in the lupus patient (cerebral mucor) who died of mucormycosis. At one center, seven patients were mobilized with cyclophosphamide and GM-CSF which was changed to G-CSF when hypotension occurred following injection of GM-CSF.

Three patients (1.6%) overall, including three of 117 cyclo-

Table 3 Effect of cyclophosphamide and/or growth factor mobilization on disease activity

Mobilization	Disease	No. of patients	No. of patients improved	No. of patients worse
G-CSF	MS ^d	20	0	2 by 1.0 or > points
G-CSF	RA	16	0	3
G-CSF	SSc	10	0	0
G-CSF	ITP ^c	8 ^c	0	0
G-CSF	SLE	1	0	0
G-CSF	Behcets	1	0	0
Total		56	0	5
Cyclophosphamide and G-CSF or GM-CSF	MS ^d	58	5 – by 1.0 points ^a	0 ^b
Cyclophosphamide and G-CSF	RA	21	12	0
Cyclophosphamide and G-CSF	SSc	16	2	0
Cyclophosphamide and G-CSF	SLE	14	4	0
Cyclophosphamide and G-CSF	Behcets	3	2	0
Cyclophosphamide and G-CSF	Vasculitis	2	1	0
Cyclophosphamide and G-CSF	Polymyositis	2	0	0
Cyclophosphamide and G-CSF	Polychondritis	1	0	0
Methotrexate and G-CSF	Vasculitis	1	0	0
Total		117	26	0

^aAlthough not defined as improvement, five patients had a decrease in the EDSS by 0.5 points.

^bAlthough not defined as a flare, two patients had an increase of 0.5 EDSS points following mobilization with 4.0 g/m² cyclophosphamide and 10 μg/kg G-CSF.

^cEight ITP patients underwent mobilization but one was mobilized twice, first with 10 μg/kg then with 15 μg/kg G-CSF.

^dTwo patients with MS were mobilized twice using different procedures (G-CSF vs G-CSF and cyclophosphamide) with the second mobilization. G-CSF = granulocyte colony-stimulating factor; GM-CSF granulocyte/macrophage colony-stimulating factor.

Table 4 Toxicity of mobilization

Disease	Mobilization regimen	No. of patients	Death	No. of patients with toxicities other than disease flare
MS ^b	G10	12	0	0
	G16	8	0	0
	Cy2 + G10	3	0	0
	Cy4 + G5	20	0	1 – grand mal seizure
	Cy4 + G7	4	0	0
	Cy4 + G10	24	0	0
	Cy4 + GM	7	0	1 – hypotension
RA	G5 or G10	16	0	8 – myalgias
	Cy2 + G10	11	0	2 – fever/neutropenia; 1 abdominal pain, 1 myalgia
	Cy4 + G10	10	0	5 – fever/neutropenia, 1 streptococcus bacteremia 1 – hydradenitis
SSc	G16	10	0	1 joint pain, 2 with new telangiectasia
	Cy2 + G10	4	0	0
	Cy4 + G10	12	2	2 died – 1 from myocardial infarct, 1 from pulmonary hemorrhage
SLE	G5	1	0	0
	Cy2 + G10	12	1	1 death from cerebral mucormycosis, 1 CMV pneumonitis, 2 intubation and dialysis for volume overload and pulmonary edema, 1 pericardial effusion and arthralgias
ITP ^a	Cy4 + G5	2	0	0
	G15	1	0	0
	G10	8	0	1 – chest pain that required ICU admission but was attributed to sternal bone pain 1 – Dysfunctional central line interrupting apheresis
Behcets	G5	1	0	0
	Cyc4 + G5	2	0	0
	Cyc4 + G10	1	0	1 not collected due to gram-negative sepsis – resolved
Vasculitis	Cyc4 + G5	1	0	0
	Cyc2 + G5	1	0	0
	MTX + G5	1	0	0
	Cy2 + G10	1	0	0
Polymyositis	Cy2 + G10	1	0	0
Polychondritis	Cy2 + G10	1	0	0

^aEight ITP patients underwent mobilization but one was mobilized twice, first with 10 $\mu\text{g}/\text{kg}$ then with 15 $\mu\text{g}/\text{kg}$ G-CSF.

^bTwo patients with MS were mobilized twice using different procedures (G-CSF vs G-CSF and cyclophosphamide) with the second mobilization. Neutropenia is not considered a toxicity.

C2 = cyclophosphamide at 2.0 g/m^2 ; C4 = cyclophosphamide at 4.0 g/m^2 ; G5 = G-CSF 5 $\mu\text{g}/\text{kg}/\text{day}$; G10 = G-CSF 10 $\mu\text{g}/\text{kg}/\text{day}$; G16 = G-CSF 16 $\mu\text{g}/\text{kg}/\text{day}$.

phosphamide recipients, died as a result of mobilization. Although not statistically significant, mortality risk tended to be higher in SSc (2/26) and lupus (1/19) compared to MS (0/76), RA (0/37), or JCA (0/13). The SSc mortality was mobilization related. Both patients were mobilized with 4.0 g/m^2 of cyclophosphamide. One died of cyclophosphamide-related alveolar hemorrhage. The other died of a myocardial infarction. A patient with lupus mobilized with 2.0 g/m^2 of cyclophosphamide died from infection (mucormycosis), which presented as a seizure 1 week after mobilization. The difference in mortality between mobilization with G-CSF (0/56) vs cyclophosphamide and growth factor (3/117) was not statistically significant ($P = 0.4$).

Failed mobilization (Table 5): Eight patients (4 with MS, 3 with SLE, and 1 with ITP) failed PBSC mobilization, since the number of stem cells collected after selection was considered inadequate to proceed to transplant. Two of 56 patients (1 with MS, 1 with ITP) failed mobilization with G-CSF alone. Subsequently, one was successfully

mobilized with cyclophosphamide (2.0 g/m^2) and G-CSF (10 $\mu\text{g}/\text{kg}$). The other was successfully mobilized by increasing the dose of G-CSF from 10 to 15 $\mu\text{g}/\text{kg}/\text{day}$. Six of 117 patients (3 with MS, 3 with SLE) failed cyclophosphamide mobilization. In two cases, supplemental marrow harvests were successful to achieve adequate collections. In one case, after cyclophosphamide mobilization failed, the patient was successfully mobilized with G-CSF (10 $\mu\text{g}/\text{kg}$) alone. The mobilization failure rate between G-CSF and cyclophosphamide combined with G-CSF was not statistically different.

There were eight additional patients who had marginal CD34⁺ cell collections, defined as less than 2.0×10^6 CD34⁺ cells/kg, but who proceeded to transplant. In these cases, the transplant conditioning regimens were not myeloablative, and consequently the transplant center deemed the collection adequate. The total number of marginal or failed mobilizations by disease was MS (7/78), RA (3/36), SSc (0/26), SLE (5/12), and ITP (1/9). A difficult mobilization (failed or marginal stem cell collection) tended to be more likely for SLE and least likely for SSc.

Table 5 Mobilization failure

Disease	Mobilization regimen	Marginal PBSC collection ^b (CD34 ⁺ cells × 10 ⁶ /kg/ infused)	Failed mobilization ^c	Total marginal or failed mobilization
MS ^d	G10	0/12	1/12	
	G16	0/8	0/8	
	Cy2 + G10	0/3	0/3	
	Cy4 + G5	0/20	1/20	
	Cy4 + G7	0/4	0/4	
	Cy4 + G10	2/24 (1.22, 1.29)	2/24	
	Cy2 + GM	1/7 (1.87)	0/7	
	Total	3/78	4/78	7/78
RA	G5	0/8	0/8	
	G10	0/8	0/8	
	Cy2 + G10	3/11 (1.8, 1.6, 0.8)	0/11	
	Cy4 + G10	0/10	0/10	
	Total	3/37	0/37	3/37
SSc	G16	0/10	0/10	
	Cy2 + G10	0/4	0/4	
	Cy4 + G10	0/12	0/12	
	Total	0/26	0/26	0/26
SLE	G5	1/1 (1.24)	0/1	
	Cy2 + G10	1/12 (1.9)	3/12	
	Cy4 + G5	0/2	0/2	
	Total	2/15	3/15	5/15
ITP	G10	0/8	1/8	
	G15	0/1	0/1	
	Total	0/9	1/9	1/9
Behcets ^a	G5	0/1	0/1	
	Cyc4 + G5*	0/2*	0/2	
	Total	0/3	0/3	0/3
Vasculitis	Cy2 + G5	0/1	0/1	
	Cy4 + G5	0/1	0/1	
	MTX + G5	0/1	0/1	
	Total	0/3	0/3	0/3
Polymyositis	Gy2 + G10	0/1	0/1	0/1
Polychondritis	Cy2 + G10	0/1	0/1	0/1
Total		8/173	8/173	16/173

^aOne patient with Behcets received cyclophosphamide-based mobilization but did not undergo apheresis due to sepsis. That attempt at collection was not included in this table since it is unknown whether that patient would have had an adequate progenitor cell recovery.

^bMarginal stem cell collection = less than 2.0×10^6 CD34⁺ cells/kg after CD34 selection but patient proceeded to transplant.

^cDefinition of failure – not enough CD34⁺ cells to proceed to transplant – regardless of success from a second mobilization or marrow harvest procedure.

^dTwo patients with MS were mobilized twice using different procedures (G-CSF vs G-CSF and cyclophosphamide) with the second mobilization.

Effect of age, gender, and disease duration

CD34⁺ progenitor cell recovery did not vary by age, gender, or disease duration.

Effect of regimen on CD34⁺ cell yield

When all diseases are analyzed either together or separately, increasing G-CSF dose increased stem cell recovery (Figure 1). The addition of cyclophosphamide tended to further increase progenitor cell yield. There was a small improvement in CD34⁺ cell yield when cyclophosphamide was increased from 2.0 g/m² and 4.0 g/m². Only seven patients, all with MS, were mobilized with cyclophosphamide and GM-CSF. The progenitor cell recovery for cyclophosphamide combined with GM-CSF tended to be lower than for patients mobilized with cyclophosphamide and G-CSF (Figure 1).

Effect of disease on CD34⁺ cell yield

When CD34⁺ cell yield was evaluated by disease (Figure 2), stem cell yield was highest for SSc and lowest for ITP and SLE. Since this difference could be related to differences used to mobilize stem cells, results were separated by mobilization using G-CSF alone vs G-CSF combined with either low- or high-dose cyclophosphamide (Figure 2). The results reveal a trend for higher CD34⁺ recovery in patients with SSc and lower CD34⁺ yield in patients with SLE independent of mobilizing method.

Effect of medications on stem cell collection

Multiple sclerosis: Only patients with MS had received prior interferon-β. No patient was taking interferon within at least 2 weeks of starting mobilization. The total duration of interferon-β exposure (which may have been given intermittently or continuously) may affect stem cell recovery. For patients who received more than 2 years of interferon-

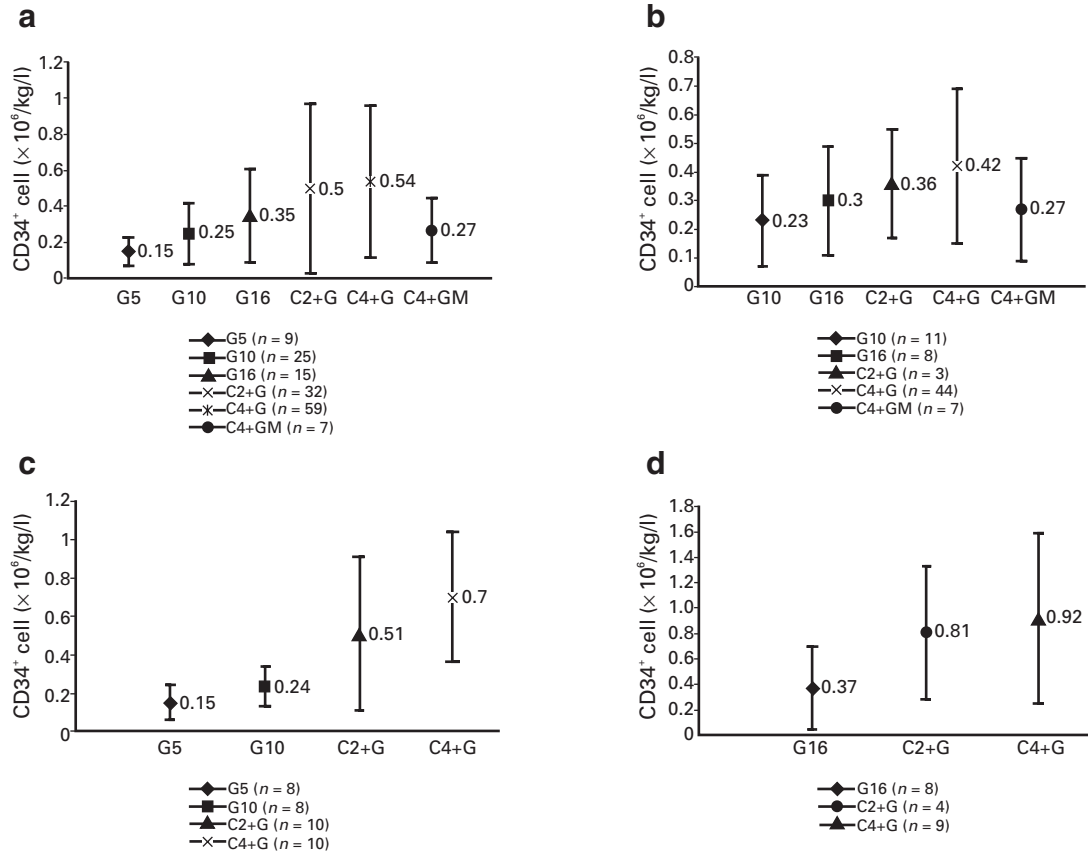


Figure 1 CD34⁺ cell yield (10⁶/kg/l) by mobilization regimen for (a) all diseases, (b) multiple sclerosis, (c) rheumatoid arthritis, (d) scleroderma. C2, cyclophosphamide at 2.0 g/m², C4, cyclophosphamide at 4.0 g/m²; G5, G-CSF 5 μg/kg/day, G10, G-CSF 10 μg/kg/day; G16, G-CSF 16 μg/kg/day; GM, GM-CSF; MS, multiple sclerosis; RA, rheumatoid arthritis; SSc, scleroderma. All CD34⁺ yields are based on number of CD34⁺ cells (10⁶/kg/l) collected before CD34⁺ enrichment.

β treatment, CD34⁺ cell recovery tended to be lower than for patients who received less than 2 years of interferon-β (Figure 3). Prior therapy for MS usually involved low doses of oral daily prednisone or short intermittent courses of i.v. corticosteroids with a rapid taper. There was no difference in CD34⁺ cell recovery between patients on corticosteroids within 2 weeks of mobilization, those who had never received corticosteroids, or those who had received prior corticosteroids (Figure 3).

Rheumatoid arthritis: For RA, almost all patients had received prior methotrexate or gold. Patients who received gold or methotrexate within 2 weeks of mobilization may have diminished CD34⁺ yield (Figure 3).

Systemic lupus erythematosus: For patients with SLE, virtually all patients had received corticosteroids and cyclophosphamide. Corticosteroid dose often varied significantly over a period of years and detailed records were not available. Therefore, CD34⁺ cell recovery was analyzed based on prednisone dose at time of mobilization. Two patients were on <20 mg/day; four were on 20–40 mg/day; two were on 40–60 mg/day; one was on 60–80 mg/day; and six were on >80 mg/day. No apparent difference in CD34⁺ yield and prednisone dose at time of mobilization was

present. Total prior cyclophosphamide dose was less than 5 g/m² in five patients; 5–10 g/m² in four; 10–20 g/m² in one; and >20 g/m² in three. There was no apparent correlation in total cyclophosphamide exposure and progenitor cell recovery, although the number of evaluable patients was small.

Scleroderma: For SSc, there was no one immune suppressive medication that was common to a majority of patients. In this small series, stem cell collection did not appear to be influenced by prior exposure to methotrexate, cyclosporine, cyclophosphamide, penicillamine, or prednisone.

Discussion

To date, little literature exists on the methods used for mobilizing hematopoietic progenitor cells from patients with autoimmune diseases. Approaches developed for mobilization have been based on those used to mobilize progenitor cells from either normal donors or autologous transplant recipients with malignancies. For these patients, cytokine mobilization is generally considered safer and less toxic than chemotherapy-based techniques. Since chemotherapy is not given to normal donors, allogeneic stem cells are

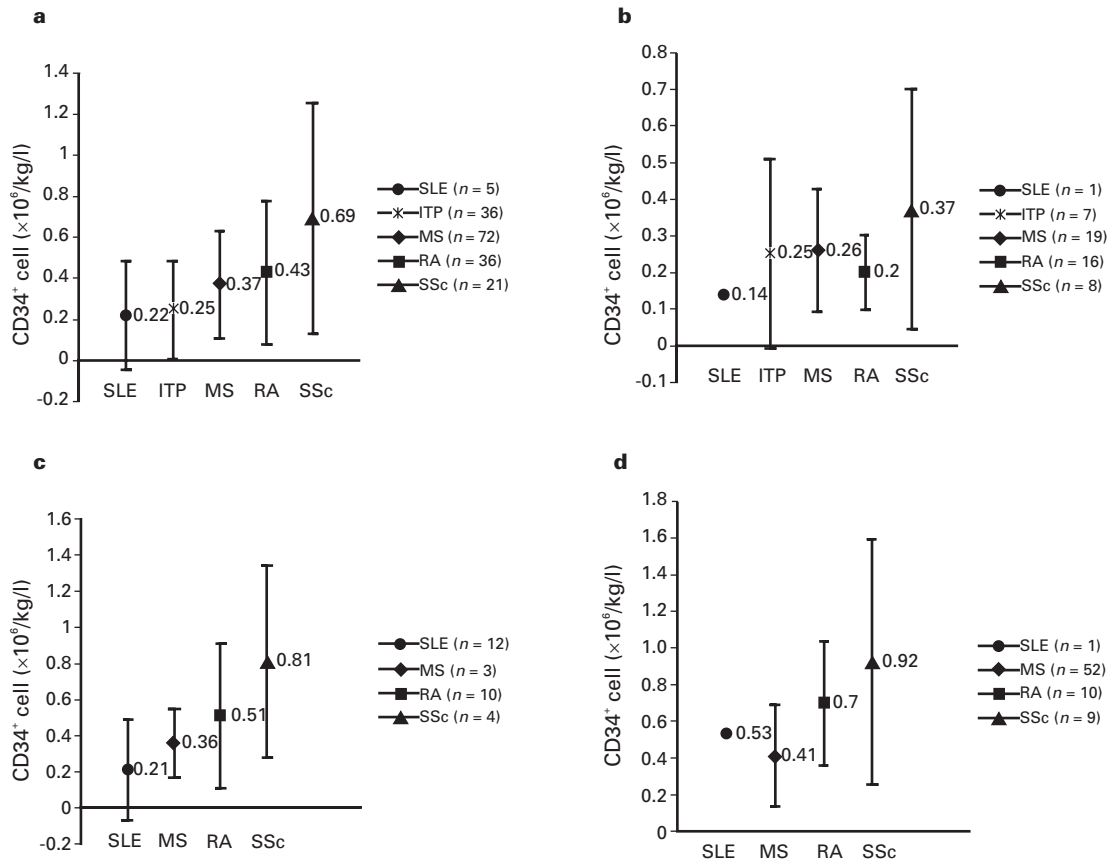


Figure 2 CD34⁺ cell yield (10⁶/kg/l) by disease and mobilization regimen for (a) all diseases and all regimens, (b) G-CSF mobilization for all diseases, (c) cyclophosphamide (2 g/m²) for all diseases, (d) cyclophosphamide (4 g/m²) for all diseases. ITP, idiopathic thrombotic thrombocytopenic purpura; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, scleroderma. All CD34⁺ yields are based on number of CD34⁺ cells (10⁶/kg/l) collected before CD34⁺ enrichment.

mobilized with cytokines alone.³¹ This survey was performed to evaluate the current practice for collection of progenitor cells from patients with autoimmune diseases. A variety of approaches were found and while there are limitations to this analysis due to the retrospective nature of the study, some interesting and potentially important trends were evident.

In this analysis, G-CSF was the only cytokine used as the sole agent to mobilize CD34⁺ cells. G-CSF alone caused disease exacerbation in five patients. For two patients with MS, the flare caused a marked deterioration in neurologic performance. For three patients with RA, the flares were transient exacerbation of joint symptoms that responded to corticosteroids. Severity and consequences of disease flare may depend on underlying disease and its specific organ involvement. An increase in disease activity with only G-CSF mobilization in patients with MS has been previously reported from a center not included in this survey.³² An Australian study has reported flares of RA in patients receiving G-CSF for progenitor cell mobilization.³³ In contrast, one report in patients with RA and another in patients with SSc conclude that G-CSF may be given without a flare.^{34,35} The patients with RA were pre-treated with corticosteroids which may have prevented disease exacerbation.

In this series, we found a difference in the risk of disease

flare between patients mobilized with G-CSF alone vs combined cyclophosphamide and G-CSF. In contrast to G-CSF, the combination of chemotherapy and G-CSF not only protected against disease flares but tended to ameliorate autoimmune disease activity. The effect of cyclophosphamide on at least transient amelioration of disease may be understated in this survey since many patients proceeded directly from mobilization to transplant without an intervening time interval to assess improvement. Whether other immune suppressive agents such as high-dose corticosteroids could have the same protective effect is under investigation (R Nash, unpublished). Active disease, in contrast to quiescent disease, may be more likely to flare during mobilization. MRI gadolinium enhancement in MS, or swollen joint count and sedimentation rate in RA, could be used as markers of active disease. Correlation of disease activity with mobilization-related flare may be prudent for prospective randomized trials.

The results of this survey raise some concerns about using G-CSF to mobilize stem cells in patients with autoimmune diseases that involve vital organs. The mechanism of G-CSF-related disease flare is unknown. G-CSF may alter cytokine and adhesion or homing signals with complex interactions upon inflammatory pathways.³⁶⁻⁴²

While cyclophosphamide-based mobilization ameliorated disease activity, it was associated with more frequent

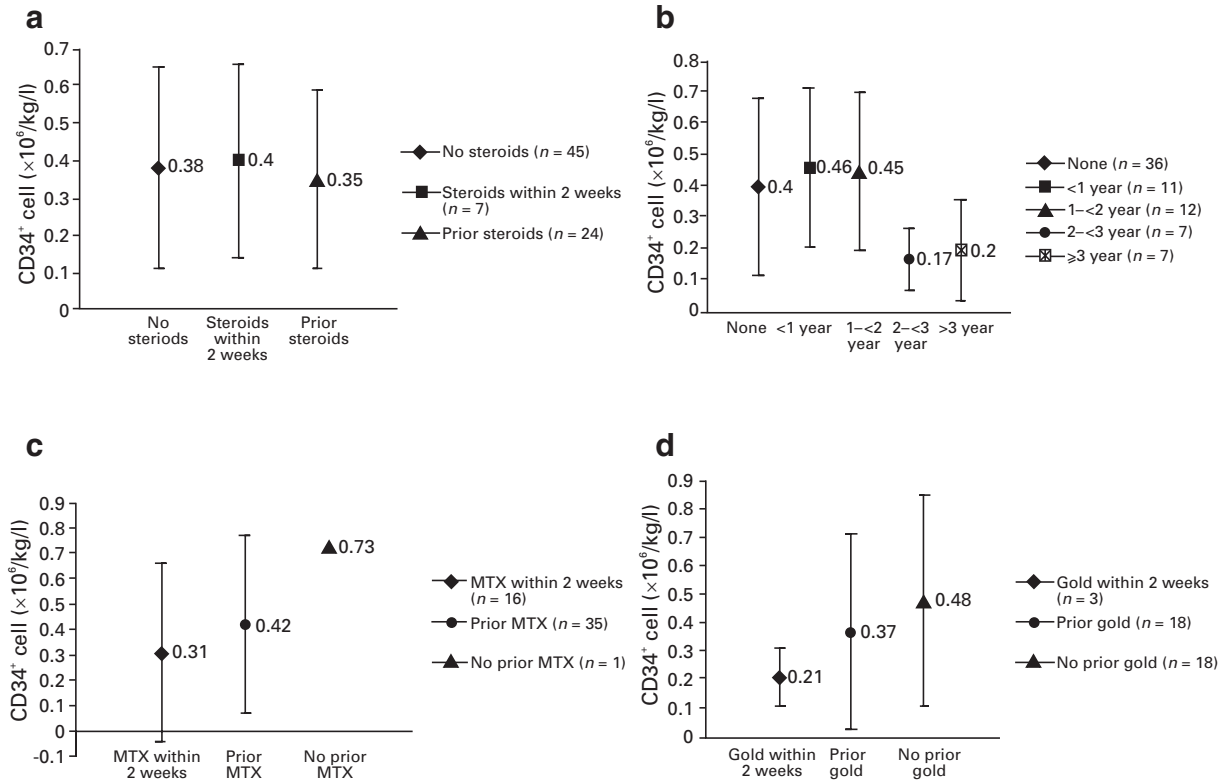


Figure 3 Impact of medications on CD34⁺ yield (10⁶/kg/l): (a) corticosteroid exposure in multiple sclerosis; (b) duration of interferon exposure in multiple sclerosis, (c) methotrexate exposure in rheumatoid arthritis, (d) gold exposure in rheumatoid arthritis. MS, multiple sclerosis; MTX, methotrexate; RA, rheumatoid arthritis. All CD34⁺ yields are based on number of CD34⁺ cells (10⁶/kg/l) collected before CD34⁺ enrichment.

and severe non-disease-related complications. These included neutropenia with fever, bacteremia and fungemia, and treatment-related toxicity. No patient receiving G-CSF developed an infection or died. Four patients mobilized with cyclophosphamide developed an infection and one patient with SLE on chronic high-dose corticosteroids died from disseminated mucormycosis despite being neutropenic for only 2 days. Two patients with SSc, both of whom received 4.0 g/m² of cyclophosphamide, died from chemotherapy-related complications (alveolar hemorrhage and myocardial infarction). Chemotherapy doses designed to mobilize stem cells from patients with malignant diseases may not necessarily be the best regimen for patients with autoimmune diseases. Patients with malignancy who have end organ dysfunction are usually ineligible for high-dose chemotherapy. Patients with an autoimmune disease and visceral organ failure may still be eligible for transplant if the organ failure is deemed disease-related. For patients with autoimmune visceral organ dysfunction, especially for patients with SSc, lower doses of cyclophosphamide (2.0 g/m²) may be equally effective and safer than higher doses (4.0 g/m²). Mobilization using cyclophosphamide at 4.0 g/m² has been reported to cause fatal and abrupt bleeding in a patient with Evans syndrome.⁴³ This was presumed secondary to suppression of compensated hyperactive hematopoiesis without an immediate decrease in peripheral antibody-mediated platelet destruction. PBSC mobilization may need to be tailored according to disease and disease stage in order to avoid mobilization-related mortality.

Variables such as apheresis machine, flow rate, day of starting apheresis, and target cell dose may affect stem cell recovery and could not be accounted for in this retrospective survey. Since the percentage of circulating CD34⁺ cells peaks and then declines with continued daily G-CSF administration, the optimal day for initiating apheresis may be improved by monitoring peripheral blood CD34⁺ cell count. Peak CD34⁺ cell counts in the blood were either not monitored or unavailable. Some centers targeted collected cell dose to include an unmanipulated back-up graft in case of graft failure from the CD34⁺ enriched product. Altering the total target cell dose collected may result in a decline of progenitor cell recovery with each consecutive day of apheresis. For these reasons, statistical analysis was omitted on progenitor cell recovery.

Despite these limitations, when correlated for apheresis volume and patient weight, some trends in progenitor cell recovery emerged which are consistent with PBSC mobilization in patients with malignancies. For instance, consistent with PBSC collection in patients with cancer, increasing the dose of G-CSF from 5 to 10 to 16 μg/kg increased stem cell yield. Also, conforming with the experience in cancer, the addition of cyclophosphamide to G-CSF further increased CD34⁺ cell recovery. While higher dose cyclophosphamide (4.0 g/m²) increased stem cell recovery compared to low-dose cyclophosphamide (2.0 g/m²), the benefit in CD34⁺ cell yield was small.

Hematopoietic stem cell yields may be affected by prior medications. In multiple sclerosis, exposure to interferon-

β therapy for more than 2 years tended to diminish CD34⁺ cell recovery. This is consistent with studies in cancer patients that correlated prior interferon- α treatment with poor PBSC collections.⁴⁴ The anti-rheumatic medications, methotrexate and gold, which are myelosuppressive drugs, may adversely affect CD34⁺ cell yield (Figure 3).

Hematopoietic stem cell yield may also vary by disease. Stem cell yield may be dependent on a disease-specific cytokine and chemokine milieu, as well as adhesion molecules and the bone marrow microenvironment.^{45–52} When corrected for mobilizing regimen, weight and apheresis volume, patients with SSc had the best cell recovery. Patients with RA, MS, and ITP had similar but lower progenitor cell yields. Patients with SLE had the lowest CD34⁺ cell recovery. Similarly, failed mobilization was most likely to occur in lupus. SLE was the only disease previously treated with high doses of intravenous cyclophosphamide. This could have diminished stem cell yield compared to other diseases. However, within the group of patients with lupus, cyclophosphamide exposure did not impact upon progenitor cell recovery.

Differences in disease flare risks, progenitor cell yields, and other mobilization toxicities may be not only disease-specific but depend on underlying organ-specific involvement. Therefore, the optimal stem cell collection procedure may vary by disease. In order to minimize mobilization-related morbidity, a uniform approach to collection of stem cells for all autoimmune diseases may not be prudent. While a statistical analysis was not practical due to variability in apheresis techniques, these data indicate caution in clinical trial design as well as the need for disease-specific prospective mobilization trials.

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References

- Burt RK, Burns WH, Miller SD. Bone marrow transplantation for multiple sclerosis: returning to Pandora's box. *Immunol Today* 1997; **18**: 559–561.
- Burt RK. BMT for severe autoimmune diseases: an idea whose time has come. *Oncology* 1997; **11**: 1001–1014; 1017, discussion 1018–1024.
- Snowden JA, Brooks PM, Biggs JC. Haemopoietic stem cell transplantation for autoimmune diseases. *Br J Haematol* 1997; **99**: 9–22.
- Tyndall A, Gratwohl A. Hemopoietic blood and marrow transplants in the treatment of severe autoimmune disease. *Curr Opin Hematol* 1997; **4**: 390–394.
- Ikehara S. Bone marrow transplantation for autoimmune diseases. *Acta Haematologica* 1998; **99**: 116–132.
- Marmont AM. Stem cell transplantation for severe autoimmune diseases: progress and problems. *Haematologica* 1998; **83**: 733–743.
- Burt RK, Traynor A. Hematopoietic stem cell therapy of autoimmune diseases. *Curr Opin Hematol* 1998; **5**: 472–477.
- Marmont AM. New horizons in the treatment of autoimmune diseases: immunoablation and stem cell transplantation. *Annu Rev Med* 2000; **51**: 115–134.
- Burt RK, Traynor AE, Pope R *et al*. Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. *Blood* 1998; **92**: 3505–3514.
- Burt RK, Traynor AE, Cohen B *et al*. T cell-depleted autologous hematopoietic stem cell transplantation for multiple sclerosis: report on the first three patients. *Bone Marrow Transplant* 1998; **21**: 537–541.
- Fassas A, Anagnostopoulos A, Kazis A *et al*. Peripheral blood stem cell transplantation in the treatment of progressive multiple sclerosis: first result of a pilot study. *Bone Marrow Transplant* 1997; **20**: 631–638.
- Kozak T, Havrdova E, Pit'ha J *et al*. High-dose immunosuppressive therapy with PBPC support in the treatment of poor risk multiple sclerosis. *Bone Marrow Transplant* 2000; **25**: 525–531.
- Fassas A, Anagnostopoulos A, Kazis A *et al*. Autologous stem cell transplantation in progressive multiple sclerosis – an interim analysis of efficacy. *J Clin Immunol* 2000; **20**: 24–30.
- Burt RK, Georganas C, Schroeder J *et al*. Autologous hematopoietic stem cell transplantation in refractory rheumatoid arthritis: sustained response in two of four patients. *Arthr Rheum* 1999; **42**: 2281–2285.
- Snowden JA, Biggs JC, Milliken ST *et al*. A phase I/II dose escalation study of intensified cyclophosphamide and autologous blood stem cell rescue in severe, active rheumatoid arthritis. *Arthr Rheum* 1999; **42**: 2286–2292.
- Durez P, Toungouz M, Schandene L *et al*. Remission and immune reconstitution after T-cell-depleted stem-cell transplantation for rheumatoid arthritis. *Lancet* 1998; **352**: 881.
- McCull G, Kohsaka H, Szer J, Wicks I. High-dose chemotherapy and syngeneic hemopoietic stem-cell transplantation for severe, seronegative rheumatoid arthritis. *Ann Intern Med* 1999; **131**: 507–509.
- Joske DJ, Ma DT, Langlands DR, Owen ET. Autologous bone-marrow transplantation for rheumatoid arthritis. *Lancet* 1997; **350**: 337–338.
- Wulffraat N, van Royen A, Bierings M *et al*. Autologous haematopoietic stem-cell transplantation in four patients with refractory juvenile chronic arthritis. *Lancet* 1999; **353**: 550–553.
- Martini A, Maccario R, Ravelli A *et al*. Marked and sustained improvement two years after autologous stem cell transplantation in a girl with systemic sclerosis. *Arthr Rheum* 1999; **42**: 807–811.
- Tyndall A, Black C, Finke J *et al*. Treatment of systemic sclerosis with autologous haematopoietic stem cell transplantation. *Lancet* 1997; **349**: 254.
- Rosen O, Theil A, Massenkeil G *et al*. Autologous stem cell transplantation in refractory autoimmune diseases after *ex vivo* depletion of mononuclear cells. *Arthritis Res* 2000; **2**: 327–336.
- Burt RK, Traynor A, Ramsey-Goldman R. Hematopoietic stem-cell transplantation for systemic lupus erythematosus. *New Engl J Med* 1997; **337**: 1777–1778.
- Fouillard L, Gorin NC, Laporte JP *et al*. Control of severe systemic lupus erythematosus after high-dose immunosuppressive therapy and transplantation of CD34⁺ purified autologous stem cells from peripheral blood. *Lupus* 1999; **8**: 320–323.
- Musso M, Porretto F, Crescimanno A *et al*. Autologous peripheral blood stem and progenitor (CD34⁺) cell transplantation for systemic lupus erythematosus complicated by Evans syndrome. *Lupus* 1998; **7**: 492–494.
- Marmont AM, van Lint MT, Gualandi F, Bacigalupo A. Auto-

- logous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. *Lupus* 1997; **6**: 545–548.
- 27 Traynor A, Schroeder J, Rosa R *et al*. Treatment of severe lupus erythematosus with high-dose intense chemotherapy and hematopoietic stem cell transplantation: a phase I study. *Lancet* 2000; **356**: 701–707.
- 28 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology* 1983; **33**: 1444–1452.
- 29 Clements PJ, Lachenbruch PA, Seibold JR *et al*. Skin thickness score in systemic sclerosis: an assessment interobserver variability in 3 independent studies. *J Rheumatol* 1993; **20**: 1892–1896.
- 30 Bombardier C, Gladman DD, Urowitz MB *et al*. Derivation of the SLEDAI: a disease activity index for lupus patients. *Arthr Rheum* 1992; **35**: 630–640.
- 31 Cleaver SA, Goldman JM. Use of G-CSF to mobilise PBSC in normal healthy donors – an international survey. *Bone Marrow Transplant* 1998; **21** (Suppl. 3): S29–31.
- 32 Openshaw H, Stuve O, Antel JP *et al*. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology* 2000; **54**: 2147–2150.
- 33 Snowden JA, Biggs JC, Milliken ST *et al*. A randomised, blinded, placebo-controlled, dose escalation study of the tolerability and efficacy of filgrastim for haemopoietic stem cell mobilisation in patients with severe active rheumatoid arthritis. *Bone Marrow Transplant* 1998; **22**: 1035–1041.
- 34 Locatelli F, Perotti C, Torretta L *et al*. Mobilization and selection of peripheral blood hematopoietic progenitors in children with systemic sclerosis. *Haematologica* 1999; **84**: 839–843.
- 35 McGonagle D, Rawstron A, Richards S *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.
- 36 Arpinati M, Green CL, Heimfeld S *et al*. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* 2000; **95**: 2484–2490.
- 37 Reddy V, Hill GR, Pan L *et al*. G-CSF modulates cytokine profile of dendritic cells and decreases acute graft-versus-host disease through effects on the donor rather than the recipient. *Transplantation* 2000; **69**: 691–693.
- 38 Reyes E, Garcia-Castro I, Esquivel F *et al*. Granulocyte colony-stimulating factor (G-CSF) transiently suppresses mitogen-stimulated T-cell proliferative response. *Br J Cancer* 1999; **80**: 229–235.
- 39 Rondelli D, Raspadori D, Anasetti C *et al*. Alloantigen presenting capacity, T cell alloreactivity and NK function of G-CSF-mobilized peripheral blood cells. *Bone Marrow Transplant* 1998; **22**: 631–637.
- 40 Hartung T. Immunomodulation by colony-stimulating factors. *Rev Physiol Biochem Pharmacol* 1999; **136**: 1–164.
- 41 Hartung T, Doecke WD, Bundschuh D *et al*. Effect of filgrastim treatment on inflammatory cytokines and lymphocyte functions. *Clin Pharmacol Ther* 1999; **66**: 415–424.
- 42 Bellucci R, De Propriis MS, Buccisano F *et al*. Modulation of VLA-4 and L-selectin expression on normal CD34+ cells during mobilization with G-CSF. *Bone Marrow Transplant* 1999; **23**: 1–8.
- 43 Martino R, Sureda A, Brunet S. Peripheral blood stem cell mobilization in refractory autoimmune Evans syndrome: a cautionary case report. *Bone Marrow Transplant* 1997; **20**: 521.
- 44 Singhal S, Mehta J, Desikan K *et al*. Collection of peripheral blood stem cells after a preceding autograft: unfavorable effect of prior interferon-alpha therapy. *Bone Marrow Transplant* 1999; **24**: 13–27.
- 45 Klingemann HG, Eaves CJ, Barnett MJ *et al*. Transplantation of patients with high risk acute myeloid leukemia in first remission with autologous marrow cultured in interleukin-2 followed by interleukin-2 administration. *Bone Marrow Transplant* 1994; **14**: 389–396.
- 46 Feng CS, Ng MH, Szeto RS, Li EK. Bone marrow findings in lupus patients with pancytopenia. *Pathology* 1991; **23**: 5–7.
- 47 Otsuka T, Nagasawa K, Harada M, Niho Y. Bone marrow microenvironment of patients with systemic lupus erythematosus. *J Rheumatol* 1993; **20**: 967–971.
- 48 Koch AE, Kronfeld-Harrington LB, Szekanecz Z *et al*. *In situ* expression of cytokines and cellular adhesion molecules in the skin of patients with systemic sclerosis. Their role in early and late disease. *Pathobiology* 1993; **61**: 239–246.
- 49 Hasegawa M, Sato S, Takehara K. Augmented production of chemokines (monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1alpha (MIP-1alpha) and MIP-1beta) in patients with systemic sclerosis: MCP-1 and MIP-1alpha may be involved in the development of pulmonary fibrosis. *Clin Exp Immunol* 1999; **117**: 159–165.
- 50 Veale DJ, Kirk G, McLaren M, Belch JJ. Clinical implications of soluble intercellular adhesion molecule-1 levels in systemic sclerosis. *Br J Rheumatol* 1998; **3**: 1227–1228.
- 51 Papayannopoulou T. Hematopoietic stem/progenitor cell mobilization. A continuing quest for etiologic mechanisms. *Ann NY Acad Sci* 1999; **872**: 187–197.
- 52 Broxmeyer HE, Kim CH, Cooper SH *et al*. Effects of CC, CXC, C, and CX3C chemokines on proliferation of myeloid progenitor cells, and insights into SDF-1-induced chemotaxis of progenitors. *Ann NY Acad Sci* 1999; **872**: 142–162.

Appendix

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