

## EXTENDED REPORT

## A phase I-II trial of autologous peripheral blood stem cell transplantation in the treatment of refractory autoimmune disease

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**Objectives:** To carry out a phase I-II trial to elucidate the feasibility and efficacy of high dose cyclophosphamide (CY) supported by autologous peripheral blood stem cell transplantation (PBSCT) in the treatment of severe and refractory autoimmune disease (AD).

**Methods:** Peripheral blood stem cells (PBSCs) were mobilised during haematological recovery after relatively high dose CY (2 g/m<sup>2</sup>) for 2 days, followed by administration of granulocyte colony stimulating factor. After collecting PBSCs—more than  $2 \times 10^6$  CD34+ cells/kg—by apheresis, CD34+ cells were immunologically selected and cryopreserved. Eight patients were enrolled—five had systemic sclerosis (SSc) alone, one had SSc with systemic lupus erythematosus, one amyopathic dermatomyositis (ADM), and one Wegener's granulomatosis (WG). All of the patients were treated with high dose CY (50 mg/kg) for 4 days and autologous PBSCT.

**Results:** Haematopoietic reconstitution was rapid and sustained. Toxicity due to the regimen included various infections such as pneumonia, sepsis, cystitis, herpes zoster, and acute heart failure. However, there was no treatment related mortality. Encouraging results were obtained after autologous PBSCT. Sclerosis of the skin was markedly improved in all of the patients with SSc. Interstitial pneumonia (IP), evaluated by PaO<sub>2</sub>, serum KL-6 levels, and pulmonary high resolution computed tomography, improved significantly. In a patient with ADM, severe and progressive IP also improved markedly. In a patient with WG, the size of the left orbital granuloma decreased substantially, resulting in reduction of the exophthalmos.

**Conclusions:** These observations suggest that high dose CY with autologous PBSCT is feasible and may be effective in the treatment of severe and refractory AD.

Although most patients with autoimmune disease (AD) have a relapsing, remitting or smouldering disease, some of them are damaged severely or fatally from the uncontrolled disease progression, and conventional treatments are not effective. The concept of high dose immunosuppressive treatment and autologous haematopoietic stem cell transplantation (HSCT) for AD is based on the finding that HSCT is effective for animal models of AD,<sup>1–3</sup> and that patients receiving autologous HSCT for treatment of malignant diseases can achieve long term remission of coincidental AD.<sup>4–6</sup>

Autologous HSCT as a treatment for AD was initiated in 1996, and more than 800 patients with AD have been treated.<sup>7</sup> Clinically significant responses were found in two thirds of the patients who received HSCT, and treatment related mortality (TRM) was reported to be relatively high (9%) in the early period until 2000.<sup>8</sup> The mechanism for inducing remission in AD is based not only on the eradication of autoreactive lymphocytes by an immunoablative pretransplant conditioning regimen but also on the correction of a dysregulated immune balance by newly developed lymphocytes derived from the haematopoietic stem cells transplanted.<sup>9</sup>

Many reports have examined the clinical results of autologous HSCT for AD. However, few studies provided detailed information about the effect of autologous HSCT on interstitial pneumonia (IP), which is often associated with AD. In the study by McSweeney *et al*, 19 patients with

systemic sclerosis (SSc) were treated with high dose immunosuppression followed by autologous HSCT, resulting in no significant changes in carbon monoxide transfer factor (Tlco) or vital capacity (VC) at 12 months after autologous HSCT.<sup>10</sup> We carried out a phase I-II trial to elucidate the feasibility and efficacy of high dose cyclophosphamide (CY) supported by autologous peripheral blood CD34 selected stem cell transplantation (PBSCT) in patients with severe and refractory AD. We report encouraging results obtained in eight patients, suggesting that high dose CY with autologous PBSCT may be effective for treatment of AD complicated by IP.

**Abbreviations:** A-aDO<sub>2</sub>, alveolar-arterial oxygen tension difference; AD, autoimmune disease; ADM, amyopathic dermatomyositis; ATG, antithymocyte globulin; CY, cyclophosphamide; HRCT, high resolution computed tomography; G-CSF, granulocyte-colony stimulating factor; HSCT, haematopoietic stem cell transplantation; IP, interstitial pneumonia; mRSS, modified Rodnan skin score; NHL, non-Hodgkin's lymphoma; NIH-CTC, National Cancer Institute-Common Toxicity Criteria; PaO<sub>2</sub>, arterial oxygen pressure; PBSCs, peripheral blood stem cells; PBSCT, peripheral blood stem cell transplantation; SSc, systemic sclerosis; TBI, total body irradiation; Tlco, carbon monoxide transfer factor; TRM, treatment related mortality; VC, vital capacity; WG, Wegener's granulomatosis

**Table 1** Patient profile

| Patient No | Diagnosis | Sex | Age (years) | PS | mRSS | Major disorders associated with AD | VC/Tlco (%) | Auto-antibody | Prior treatment | Follow up (months) |
|------------|-----------|-----|-------------|----|------|------------------------------------|-------------|---------------|-----------------|--------------------|
| 1          | SSc+SLE   | F   | 54          | 2  | 16   | IP, digital ulcer                  | 58/51       | Anti-Scl-70   | St, CY          | 33                 |
| 2          | SSc       | M   | 55          | 2  | 15   | IP                                 | 65/47       | Anti-Scl-70   | St              | 25                 |
| 3          | SSc       | M   | 58          | 2  | 31   | IP                                 | 63/44       | –             | St              | 22                 |
| 4          | SSc       | F   | 54          | 1  | 26   | IP                                 | 73/60       | Anti-Scl-70   | St              | 20                 |
| 5          | SSc       | F   | 53          | 1  | 28   | IP                                 | 74/29       | Anti-Scl-70   | St              | 17                 |
| 6          | SSc       | F   | 48          | 2  | 32   | IP                                 | 77/25       | Anti-Scl-70   | St, CY, CsA     | 13                 |
| 7          | ADM       | F   | 54          | 2  |      | IP                                 | 50/50       | –             | St, CY, CsA     | 31                 |
| 8          | WG        | M   | 21          | 1  |      | Exophthalmos                       |             | Anti-PR-3     | St, CY, CsA     | 16                 |

PS, performance status; mRSS, modified Rodnan skin score for systemic sclerosis; AD, autoimmune disease; F, female; M, male; SSc, systemic sclerosis; IP, interstitial pneumonia; ADM, amyopathic dermatomyositis; WG, Wegener's granulomatosis; PR3, proteinase 3; St, corticosteroids; CY, cyclophosphamide; CsA, ciclosporin A.

## PATIENTS AND METHODS

### Protocol

The protocol of this phase I-II clinical trial was approved by the ethics committee of Kyushu University Hospital. Written informed consent was obtained from all patients.

### Patients and eligibility

Patients aged between 16 and 65 years were eligible at the time of pretransplant evaluation. Patient eligibility depended on a diagnosis of AD. All of the patients were followed up for at least 12 months after transplantation for the evaluation of treatment outcomes.

Patients with SSc were eligible when they had severe diffuse SSc that had rapidly developed over the previous 4 years. They also had to have at least one of the following: (a) pulmonary involvement including VC or Tlco <70% predicted or arterial oxygen pressure (Pao<sub>2</sub>) at room temperature below 70 mm Hg and evidence of interstitial lung disease defined by pulmonary high resolution computed tomography (HRCT); (b) cardiac disease, which was reversible congestive heart failure or significant arrhythmia; and (c) renal involvement such as hypertension, persistent urine analysis abnormalities, microangiopathic haemolytic anaemia, and renal insufficiency.

Patients with limited scleroderma were considered eligible when progressive and life threatening IP was present.

Patients with amyopathic dermatomyositis (ADM) were eligible when they had the following criteria: (a) clinical diagnosis of ADM by the criteria reported<sup>11</sup>; (b) progressive and life threatening IP that was refractory to conventional immunosuppressive treatment.

Patients with Wegener's granulomatosis (WG) were eligible when they had the following criteria: (a) clinical diagnosis of WG by the criteria reported<sup>12</sup>; (b) vasculitis or granuloma causing severe organ damage that was refractory to conventional immunosuppressive treatment.

### Exclusion criteria

Patients were excluded from the study when they had uncontrolled arrhythmia, heart failure with left ventricular ejection fraction (LVEF) <50%, mean pulmonary artery pressure >50 mm Hg, Tlco <20% predicted, and creatinine clearance below 40 ml/min/m<sup>2</sup>.

### Peripheral blood stem cell (PBSC) mobilisation, CD34 cell selection, and autologous PBSC

PBSCs were mobilised during haematological recovery after relatively high dose CY (2 g/m<sup>2</sup>) for 2 days followed by administration of recombinant human granulocyte-colony stimulating factor (G-CSF, filgrastim; Kirin Brewery, Tokyo, Japan) at a dose of 10 µg/kg as previously described.<sup>13</sup> After collecting PBSCs to obtain 2×10<sup>6</sup> CD34+ cells/kg or more by apheresis, CD34+ cells were positively selected using immunomagnetic beads with an anti-CD34 monoclonal antibody (CliniMACS, Miltenyi Biotec, Germany). Mobilisation of PBSCs was repeated when 2×10<sup>6</sup> CD34+ cells/kg were not obtained. For pretransplant conditioning, high dose CY (50 mg/kg) was given for 4 days from day –5 to –2. After transplantation of frozen-thawed CD34+ cells on day 0, G-CSF was administered from day 1. Acyclovir (250 mg/day, from day 1 to 18), ciprofloxacin (600 mg/day from day –7 to –1), fluconazole (400 mg/day from day –7 to 14, 200 mg/day from day 15 to 100), trimethoprim-sulfamethoxazole (1920 mg/day, from day –14 to –2 and 1920 mg/day, twice a week from day 30 to 100) were prophylactically given as previously described.<sup>13</sup>

### Treatment outcome

The modified Rodnan skin score (mRSS) was used to evaluate the improvement of skin sclerosis in patients with SSc.<sup>14</sup> Arterial blood gas at room temperature, a pulmonary function test, pulmonary HRCT, and serological examinations were used to evaluate the effect of high dose CY on IP. HRCT scans were graded and scored blinded according to the relative amount of ground glass opacity and reticular infiltrates as follows: 1 = pure ground glass; 2 = ground glass more than reticular; 3 = ground glass equals reticular; 4 = reticular more than ground glass; 5 = pure reticular.<sup>15</sup> The lower grade indicates more active inflammation in this system. Regimen related toxicity was determined and graded according to the National Cancer Institute-Common Toxicity Criteria (NIH-CTC) version 2. Cytomegalovirus antigenaemia was determined as previously described.<sup>16</sup>

**Table 2** Apheresis and CD34+ selection in eight patients

| Characteristic                   | Median (range)    |
|----------------------------------|-------------------|
| <i>Apheresis</i>                 |                   |
| Number of apheresis/patient      | 2 (1–4)           |
| Total cells ×10 <sup>9</sup>     | 22.3 (8.7–90.4)   |
| CD34+ (%)                        | 2.06 (0.11–4.72)  |
| CD34+ ×10 <sup>7</sup>           | 36.8 (10.1–161.1) |
| CD34+ ×10 <sup>6</sup> /kg       | 7.61 (2.06–35.80) |
| <i>CD34+ selection</i>           |                   |
| Total cells ×10 <sup>7</sup>     | 33.4 (11.6–95.0)  |
| Total cells ×10 <sup>6</sup> /kg | 5.11 (2.34–21.11) |
| CD34+ ×10 <sup>7</sup>           | 28.0 (10.1–94.3)  |
| CD34+ ×10 <sup>6</sup> /kg       | 4.90 (2.10–21.10) |
| CD3+ ×10 <sup>5</sup>            | 5.78 (0–15.00)    |
| CD3+ ×10 <sup>4</sup> /kg        | 0.91 (0–2.95)     |
| Purity (%)                       | 96.4 (87.0–99.3)  |
| Yield (%)                        | 75.7 (58.6–100.0) |

**Table 3** Number of reinfused cells and haematological recovery

| Patient No | Number of reinfused CD34+ cells ( $\times 10^6$ /kg) | Number of reinfused CD3+ cells ( $\times 10^4$ /kg) | ANC $>0.5 \times 10^9$ /l (days) | Platelet $>50 \times 10^9$ /l (days) | Interval between PBSC harvest and PBSCT (days) |
|------------|--|---|----------------------------------|--------------------------------------|--|
| 1          | 8.4  | 0.33  | 9                                | 20                                   | 27   |
| 2          | 4.9  | 0.27  | 9                                | 10                                   | 64   |
| 3          | 2.2  | 2.95  | 10                               | 12                                   | 39   |
| 4          | 2.1  | 1.71  | 11                               | 16                                   | 87   |
| 5          | 7.2  | 13.00   | 13                               | 9                                    | 51   |
| 6          | 4.0  | 2.35  | 11                               | 11                                   | 355  |
| 7          | 4.9  | 0.50  | 8                                | 10                                   | 31   |
| 8          | 5.0  | 0.52  | 13                               | 11                                   | 50   |

ANC, absolute neutrophil count; PBSC, peripheral blood stem cell; PBSCT, peripheral blood stem cell transplantation.

### Statistical analysis

Wilcoxon's signed rank test was used for statistical analysis of the data.

## RESULTS

### Patients

Eight patients (three male, five female) with a median age of 54 years (range 21–58) were studied (table 1). Patients 1–6 were diagnosed as diffuse SSc. Patient 1 had had systemic lupus erythematosus (SLE) for 22 years and SSc for 2 years. She had progressive IP and severe digital ulcers due to SSc while the SLE was inactive. Patients 2, 3, 5, 6 (SSc), and 7 (ADM) also developed severe and progressive IP. Patient 4 had mild IP. Patients 3, 4, 5, and 6 showed severe skin sclerosis. Patient 3 had been in complete remission of non-Hodgkin's lymphoma (NHL) for 1 year and he was considered to be eligible. Patient 8 (WG) presented with severe exophthalmos due to a granuloma, which was 18 mm in diameter and located in the upper lateral region of the left orbit affecting the superior rectus muscle. He needed monthly steroid pulse therapy to prevent further growth of the granuloma. Eastern Cooperative Oncology Group performance status<sup>17</sup> was  $<3$  in all patients. Anti-Scl-70 antibody was positive in 5/6 patients with SSc. CY and ciclosporin A were used in four and three of the patients, respectively. All patients were treated with corticosteroids, and the median duration of follow up was 21 months (range 13–33). Results are reported as of February 2005.

### PBSC mobilisation and CD34+ cell selection

PBSCs were collected by apheresis after CY plus G-CSF-induced mobilisation in all patients as previously described.<sup>13</sup> The median of the total number of CD34+ cells collected was  $7.61 \times 10^6$ /kg (range 2.06–35.80) after apheresis (table 2).

CD34+ cell selection was performed using CliniMACS. Purity and yield of the CD34+ cells selected were 96.4% (range 87.0–99.3) and 75.7% (range 58.6–100), respectively. Mobilisation was repeated in patient 4 because an insufficient number of CD34+ cells ( $2 \times 10^6$ /kg) were collected after the initial mobilisation.

### Autologous PBSCT

All the patients received autologous transplantation of frozen-thawed CD34+ cells after pretransplant conditioning with high dose CY. The median numbers of CD34+ and CD3+ cells infused were  $4.92 \times 10^6$ /kg (range 2.1–8.4) and  $1.17 \times 10^4$ /kg (range 0.27–13.0), respectively (table 3). All the patients achieved rapid haematopoietic engraftment. Median days to an absolute neutrophil count  $>0.5 \times 10^9$ /l and a platelet count  $>50 \times 10^9$ /l were 10.5 (range 8–13) and 11.5 (range 9–20), respectively. The interval between PBSC harvest and PBSCT was a median of 50.5 days (range 27–355).

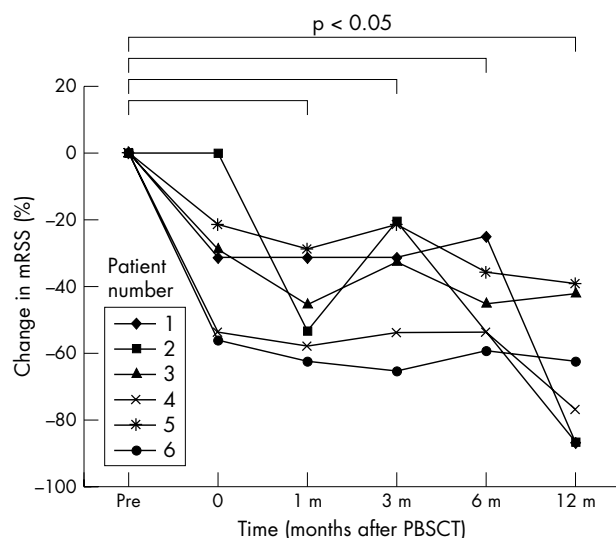
### Toxicity

Patients 1, 6, and 7 developed post-transplant infections and showed grade 3–4 toxicity according to NCI-CTC (table 4). Patient 1 also developed pneumonia of unknown cause and adenoviral cystitis, and patient 6 showed positive blood cultures for *Streptococcus mitis* in addition to adenoviral cystitis. Adenoviral cystitis was successfully treated with cidofovir. Patient 7 had positive blood cultures for *Listeria monocytogenes*. Four patients developed herpes zoster with grade 2–3 toxicity around 12 months after transplantation. Five patients showed cytomegaloviral antigenaemia. Epstein-Barr titres were not checked in this study. Patient 1 had ventricular arrhythmia, and patient 4 showed ST depression in ECG during intravenous administration of CY. Patient 6 developed acute heart failure, requiring temporary intubation

**Table 4** Toxicity (NCI)

| Patient No | Infection   | CMV antigenaemia | Cardiovascular        | Haemorrhage     | Pulmonary   | Gastro-intestinal | Hepatic                 |
|------------|---|------------------|-----------------------|-----------------|-------------|-------------------|-------------------------|
| 1          | Pneumonia (3) cystitis, adenovirus (3)                    | + (2)            | VPCs (3)              | –               | –           | Nausea (2)        | Raised transaminase (1) |
| 2          | –   | –                | –                     | GI bleeding (3) | –           | (1)               | (1)                     |
| 3          | HZ (3)  | + (1)            | –                     | –               | –           | (2)               | (1)                     |
| 4          | HZ (3)  | + (1)            | Cardiac ischaemia (2) | –               | –           | (2)               | (2)                     |
| 5          | HZ (3)  | –                | –                     | –               | Hypoxia (3) | (1)               | –                       |
| 6          | Sepsis, <i>Strep. mitis</i> (3), cystitis, adenovirus (3) | + (1)            | CHF (4)               | –               | –           | (2)               | (3)                     |
| 7          | Sepsis, <i>Listeria monocytogenes</i> (3), HZ (2)         | + (2)            | –                     | –               | –           | (2)               | (2)                     |
| 8          | –   | –                | –                     | –               | –           | (1)               | (2)                     |

NCI, National Cancer Institute; CMV, cytomegalovirus; HZ, herpes zoster; *Strep*, *Streptococcus*; VPC, ventricular premature capture; GI, gastrointestinal; CHF, congestive heart failure; (number), grade of toxicity.



**Figure 1** Evaluation of the modified Rodnan skin score (mRSS). The serial skin score data are presented for six patients with SSc. The proportional change from baseline measurement was calculated for each patient at each available time point. The x axis is not drawn to scale. Data obtained before mobilisation and just before conditioning are shown as "Pre" and "0" respectively.

after mobilisation. Patient 2 was complicated by grade 3 bleeding from an intestinal ulcer due to a non-steroidal anti-inflammatory drug during mobilisation. Patient 5 showed hypoxia due to transient worsening of IP shortly after administration of G-CSF. All the patients had grade 1–2 nausea and seven patients showed grade 1–3 hepatic toxicity. Twelve months after autologous PBSCT, patient 3 experienced a relapse of the NHL, which was successfully treated to reinstate complete remission by chemotherapy including rituximab. All the patients are alive with performance status 1 or 2.

## CLINICAL OUTCOME

### SSc

Figure 1 shows post-transplant changes in the mRSS for patients with SSc. A decline in skin score is considered significant if it is >25% of the baseline or >10% of the maximum skin score. When this definition is used, 6/6 (100%) patients showed significant improvement. The mean skin scores at 1, 3, 6, and 12 months post-transplant were significantly less than those before mobilisation ( $p < 0.05$ ). Five out of six patients showed an improvement in the skin score after mobilisation before pretransplant conditioning, although it was not statistically significant. Reincreases in skin score were not seen in any of the three patients who were followed up for 18 months or more after autologous PBSCT (data not shown).

To investigate the effect of autologous PBSCT on IP, blood gas analysis, a pulmonary function test, and pulmonary HRCT were performed at 3 and 12 months after transplantation. Figure 2A shows that  $P_{aO_2}$  was significantly increased from the median value of 66.5 mm Hg (range 51–88.7) before transplantation to 78.3 mm Hg (69.7–102) and 83.2 mm Hg (72.6–93.2) at 3 and 12 months after transplantation, respectively. Improvement of alveolar-arterial oxygen tension difference ( $A-aDO_2$ ) was also seen in four patients at 12 months (fig 2B). The VC was improved in four and five patients at 3 and 12 months, respectively (fig 2C). Improvement of  $Tl_{CO}$  was seen in only one patient (fig 2D). Serum levels of KL-6, a marker for IP,<sup>18</sup> significantly decreased from the median value, 1823 U/ml (range

1080–2988) before transplantation to 890 U/ml (740–1962) and 989 U/ml (532–1273) at 3 and 12 months after transplantation, respectively (fig 2E). The ground glass opacity markedly regressed in all of the patients, although reticular infiltrates remained essentially unaffected after transplantation (fig 3), resulting in significant improvement of pulmonary HRCT grading from the median value of 2.5 (range 2–3) before transplantation to 4 (range 3–4) at 12 months after transplantation (fig 2F).

### ADM

Skin lesions had resolved by conventional immunosuppressive treatment before mobilisation.  $P_{aO_2}$  was increased from 65.6 mm Hg before transplantation to 87.8 and 83.9 mm Hg at 3 and 12 months after transplantation, respectively. VC increased from 52.6% to 59.3% and 74.1% of the predicted value at 3 and 12 months, respectively. KL-6 decreased from 3280 IU/ml before transplantation to 1020 and 425 IU/ml at 3 and 12 months after transplantation, respectively. Both the ground glass opacity and the reticular infiltrates were markedly improved in pulmonary HRCT at 12 months post-transplant. The clinical course of this case has been described in detail elsewhere.<sup>19</sup>

### WG

The size of the left orbital granuloma markedly decreased, resulting in an improvement of the exophthalmos, and regrowth of the granuloma has not been seen. Monthly steroid pulse therapy was not necessary to maintain this remission state. A serum level of proteinase 3 (PR3)-anti neutrophil cytoplasmic antibodies (ANCA) decreased from 72 IU/ml before transplantation to 39 IU/ml at 3 months after transplantation. However, it increased again to 157 IU/ml 12 months after transplantation.

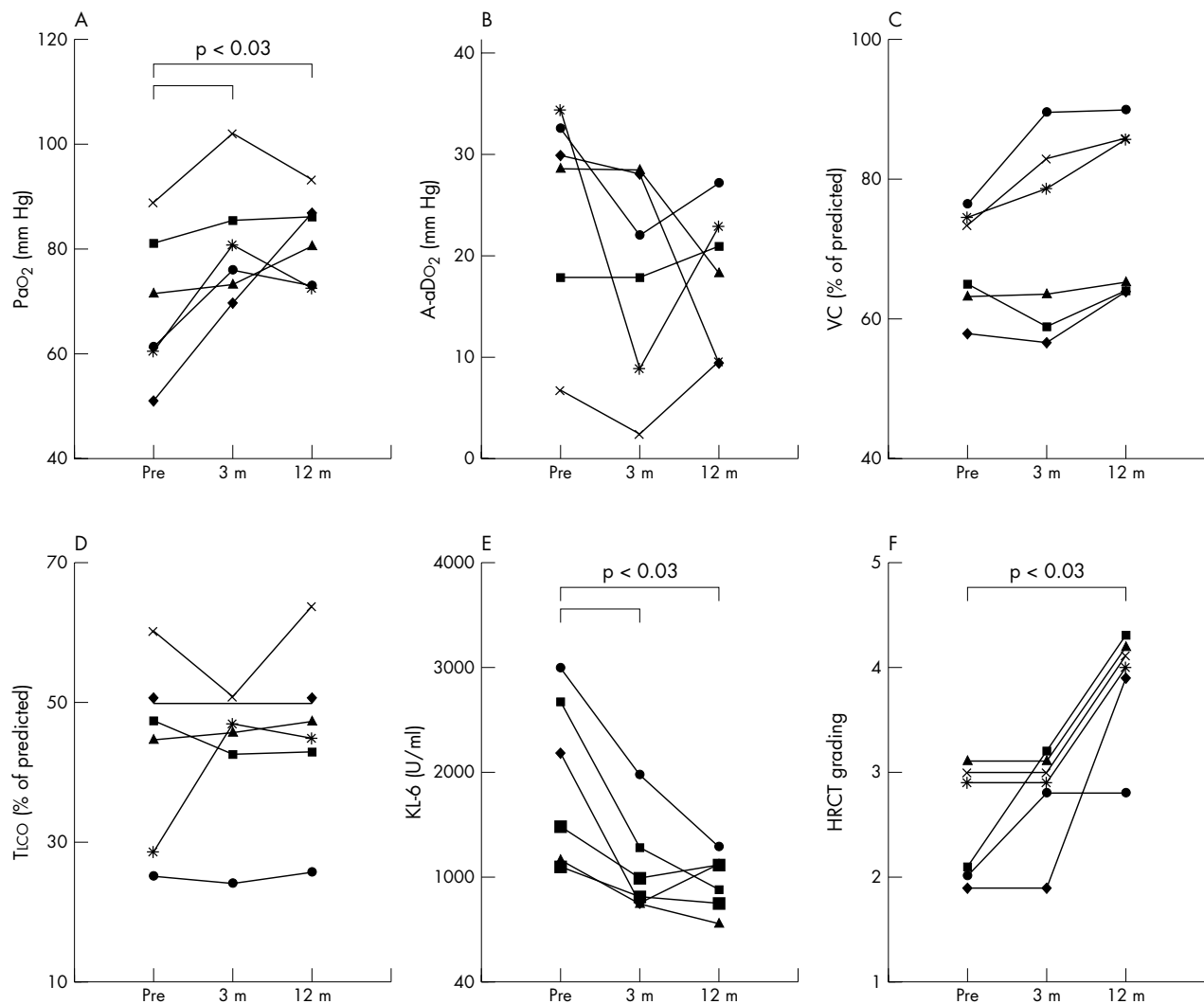
## DISCUSSION

In this study, we demonstrated that high dose CY with autologous PBSCT was feasible and effective in the treatment of refractory AD. For patients with SSc, we first showed that high dose CY and autologous PBSCT had favourable effects not only on skin sclerosis but also on IP. We thought our patient with WG was probably the first to have been treated with high dose CY and autologous PBSCT. However, during the revision of this manuscript a similar case was reported in the *Annals*.<sup>19a</sup>

We used a combination of high dose CY and G-CSF to mobilise a sufficient number of PBSC without the disease flare, although G-CSF alone was able to mobilise PBSC.<sup>10</sup> Flares of AD when G-CSF is used have been reported in rheumatoid arthritis,<sup>20</sup> multiple sclerosis,<sup>21</sup> and SSc.<sup>22</sup> In the European trial for SSc, the use of CY+G-CSF (84% of the cases) was preferred rather than G-CSF alone (10.7%).<sup>23</sup> In our trial, one patient had to repeat the mobilisation because an insufficient number of PBSC were obtained by the initial mobilisation. In another study, one of 12 PBSC mobilisations failed with the same protocol and autologous bone marrow transplantation was subsequently performed instead.<sup>24</sup>

We used immunological selection of CD34+ cells from PBSC harvests to minimise the risk of reinfusing autoreactive lymphocytes.<sup>25</sup> The selection device (CliniMACS) permitted good yield and purity of CD34+ cells with few contaminated T cells. In a study of patients with malignancy and concomitant AD, a high rate of recurrent AD was seen when unmanipulated autografs were used.<sup>26</sup> In the European phase I-II trial for SSc, 47/55 (85%) patients received CD34+ selection.<sup>23</sup> On the other hand, a randomised trial of 31 patients with rheumatoid arthritis comparing T cell depleted v unmanipulated autologous PBSCT after high dose CY (200 mg/kg) without additional T cell purging agents failed to





**Figure 2** Evaluation of variables associated with IP in six patients with SSc before mobilisation, and at 3 and 12 months after autologous PBSCT. (A) PaO<sub>2</sub> at room temperature; (B) A-aDO<sub>2</sub>; (C) VC; (D) TlCO; (E) KL-6; (F) pulmonary HRCT grading. The x axis shows time. Data obtained before mobilisation and just before conditioning are shown as "Pre" and "0" respectively. m, month.

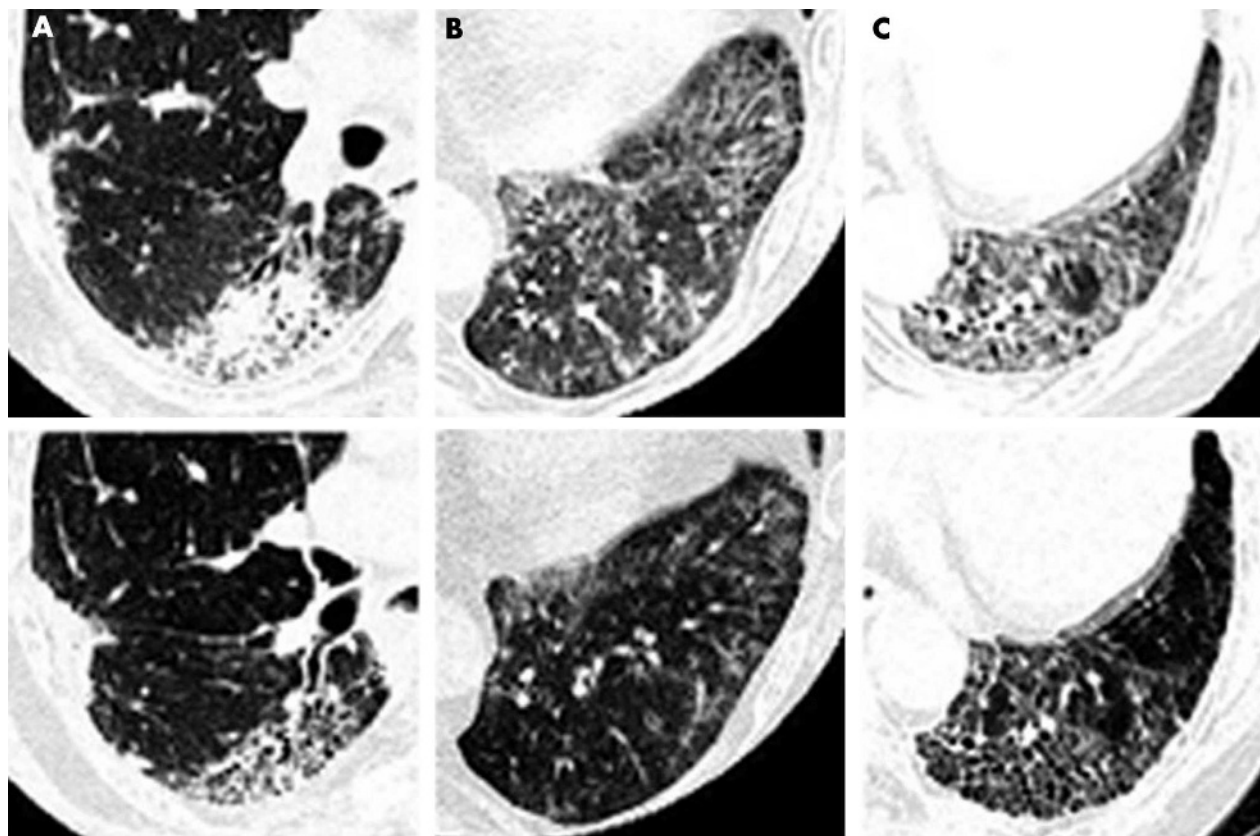
demonstrate significant differences between the two groups.<sup>27</sup> The usefulness of T cell depletion should also be investigated carefully in patients with other AD.

Six of the eight patients had infectious episodes. Viral infections were more common than bacterial infections. Other toxicity included cardiac toxicity of CY and temporary exacerbation of IP by G-CSF. Patient 6 developed acute heart failure, requiring temporary intubation after mobilisation, which might be due to the combination of viral myocarditis and cardiac toxicity of CY. She recovered from heart failure and received autologous PBSCT 1 year later. Patient 3 had a relapse of NHL. The relation between autologous PBSCT and the relapse of NHL was not clear because autologous PBSCT was also used for treatment of NHL. There was no TRM in our study, whereas an early European study and the study by McSweeney *et al* reported that the overall TRM was 9% and 15.8%, respectively.<sup>8-10</sup> One of the most important toxicities was cardiotoxicity, possibly related to direct CY toxicity and hyperhydration. The patient selection was reported to be important to reduce TRM<sup>23</sup> and a full cardiological assessment before transplantation was recommended by the European group.<sup>28</sup> Careful patient selection, especially in the light of cardiac function evaluation, which is often

underestimated in patients with severe rheumatic diseases, may have allowed us to avoid TRM.

Significant improvement in the skin score of >25% after autologous PBSCT occurred in all of the patients with SSc. In the European study and the study by McSweeney *et al*, it occurred in 69% and 100% of the patients transplanted, respectively.<sup>10-29</sup> The mechanism for the effect of autologous PBSCT on skin sclerosis may be due to intensive immunosuppression and immune reconstitution. In the European study and the study by McSweeney *et al*, 35% and 0% of the patients with an initial response relapsed during 20 and 14.7 months of median follow up after transplantation, respectively.<sup>10-23</sup> A longer follow up is necessary to assess the response duration of skin sclerosis in our trial.

Improvement of IP has been demonstrated in patients with SSc and ADM, whereas pulmonary function remained unaffected in other studies.<sup>10-23-29</sup> In this study, PaO<sub>2</sub>, KL-6, and pulmonary HRCT grading were significantly improved, while TlCO values showed no significant change. KL-6 is a high molecular weight glycoprotein recently identified in humans as MUC1 mucin. It is a useful marker in the evaluation of disease activity not only of idiopathic pulmonary fibrosis but also of IP associated with collagen vascular



**Figure 3** Pulmonary HRCT in patients with SSc. Upper panel, before mobilisation; lower panel, 12 months after autologous PBSCT. (A) Patient 1; (B) patient 2, (C) patient 4.

disease.<sup>30</sup> VC was increased in 5/6 patients with SSc, although it was not statistically significant. McSweeney *et al* treated 19 patients with SSc with high dose CY, total body irradiation (TBI), and antithymocyte globulin (ATG) followed by autologous PBSCT. They reported a significant decrease in the Tlco values at 3 months but not at 12 months and no significant change in the VC at 3 or 12 months after autologous PBSCT.<sup>10</sup>

Because IP was not evaluated with respect to Pao<sub>2</sub>, KL-6, or pulmonary HRCT grading in previous studies, the improvement of IP might have been undetectable. Selection biases of patients may be another reason; we might have selected patients with more active IP without honeycombing, whereas more patients with inactive and stable IP might have been selected in other studies. A different treatment regimen, especially the use of TBI, might be responsible for the different results. It is reasonable to suppose that high dose CY and autologous PBSCT could provide favourable effects on the IP of patients with SSc because intravenous pulse CY was reported to be effective for IP in patients with collagen vascular diseases including SSc.<sup>15, 31</sup> In this study, improvement of Tlco was not seen, as it was in previous studies, despite the improvement of KL-6 and pulmonary HRCT grading. Because Tlco reflects not only interstitial lesions but also microvascular lesions of the lung,<sup>32</sup> periphery distributed microvascular impairment of the lung due to SSc may not have improved after autologous PBSCT compared with the improvement of interstitial lesions, resulting in the absence of an improved Tlco.

In this study, a patient with WG receiving autologous PBSCT was described. In the European study, three patients with WG receiving autologous PBSCT were listed, but the treatment outcome was not described.<sup>8, 33</sup> In our case, G-CSF

in combination with CY did not cause a disease flare, and high dose CY with autologous PBSCT produced long term remission for more than 16 months.

We did not incorporate ATG into the conditioning regimen. Although ATG is believed to be useful for deleting the residual T cells and is often used in the other settings,<sup>10, 23, 29</sup> its usefulness has not been fully proved. Because we obtained significant clinical responses and considerable susceptibility to infections when treating with CY alone, ATG did not seem to be necessary. We did not incorporate TBI for a similar reason. Although our conditioning regimen was less intense than high dose CY with ATG and/or TBI, initial clinical responses were comparable, at least at the 12 month follow up. It is important to look at the response duration of our regimen in comparison with those of more immunosuppressive regimens. A randomised controlled trial will be necessary to assess the usefulness of ATG and/or TBI.

Most of the patients showed an improvement in disease activity after high dose CY and G-CSF for PBSC mobilisation before pretransplant conditioning, as shown in a previous study.<sup>34</sup> Haematopoietic stem cells express high levels of aldehyde dehydrogenase, an enzyme responsible for cellular resistance to CY. Hence, high dose CY should have strong effects on fully differentiated and aggressive autoreactive lymphocytes and allow immune reconstitution by newly developed lymphocytes from CY resistant haematopoietic stem cells.<sup>35</sup>

In conclusion, the present phase I-II study demonstrated that high dose CY with autologous PBSCT is feasible and effective in the treatment of refractory AD. We first demonstrated the clinical effects of high dose CY with autologous PBSCT on IP of SSc and on granuloma of WG. A prospective study with longer follow up time and more

patients will be necessary to assess the efficacy of this treatment modality in AD.

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