Bone Marrow Transplantation (2007) 40, 549–555

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ORIGINAL ARTICLE

# Autologous non-myeloablative hematopoietic stem cell transplantation in patients with systemic sclerosis

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**Autologous hematopoietic stem cell transplantation (HSCT) utilizing a myeloablative regimen containing total body irradiation has been performed in patients with systemic sclerosis (SSc), but with substantial toxicity. We, therefore, conducted a phase I non-myeloablative autologous HSCT study in 10 patients with SSc and poor prognostic features. PBSC were mobilized with CY and G-CSF. The PBSC graft was cryopreserved without manipulation and re-infused after the patient was treated with a non-myeloablative conditioning regimen of 200 mg/kg CY and 7.5 mg/kg rabbit antithymocyte globulin. There was a statistically significant improvement of modified Rodnan skin score whereas cardiac (ejection fraction, pulmonary arterial pressure), pulmonary function (DLCO) and renal function (creatinine) remained stable without significant change. One patient with advanced disease died 2 years after the transplant from progressive disease. After median follow-up of 25.5 months, the overall and progression-free survival rates are 90 and 70% respectively. Autologous HSCT utilizing a non-myeloablative conditioning regimen appears to result in improved skin flexibility similar to a myeloablative TBI containing regimen, but without the toxicity and risks associated with TBI.**

*Bone Marrow Transplantation* (2007) **40,** 549–555; doi:10.1038/sj.bmt.1705782; published online 23 July 2007
**Keywords:** systemic sclerosis; autologous hematopoietic stem cell transplantation; clinical trial

## Introduction

Systemic sclerosis (SSc) is a multi-system disease of unknown etiology and highly complex pathogenesis.[1–3] It is characterized by evidence of autoimmunity and inflammation, diffuse small vessel vasculopathy and progressive fibrosis of the skin and internal organs. Morbidity and mortality are high, and to date no treatment has been shown to be effective as a disease-modifying agent in a randomized controlled trial. Leading causes of death in patients with SSc include pulmonary fibrosis, pulmonary arterial hypertension (PAH), cardiac failure, arrhythmias and renal crisis. Skin induration, joint contractures, digital ischemia due to Raynaud's phenomenon, gastrointestinal failure and soft tissue calcifications contribute significantly to morbidity.

Owing to the lack of effective conventional treatments, autologous hematopoietic stem cell transplantation (HSCT) is being offered as a therapeutic option for patients with SSc and poor prognostic features.[4–9] Autologous HSCT is a two-step procedure involving a conditioning regimen to eliminate self-reactive lymphocytes followed by infusion of autologous hematopoietic stem cells (HSC). There are currently two different strategies to the design of conditioning regimens for SSc.[10] One approach is to utilize a TBI containing myeloablative regimen that is similar to the regimen employed in HSCT for hematological malignancy.[10] The other approach is to use a non-myeloablative non-radiation regimen that specifically targets lymphocytes (lymphoablative regimen).[10] Following a non-myeloablative regimen, re-infusion of HSC is not necessary for hematopoietic reconstitution but is performed as a safety measure to shorten the duration of neutropenia induced by the conditioning regimen.[11]

After HSCT using a TBI-based myeloablative conditioning regimen, the efficacy data in terms of modified Rodnan skin score (mRSS) appear promising;[6,8,9] however, substantial treatment-related toxicity including mortality and treatment-related deterioration of internal organ (lung and kidney) function and radiation-related myelodysplastic syndrome/leukemia has tempered enthusiasm for this approach.[9,10,12] In contrast, non-myeloablative non-radiation containing autologous HSCT regimens have been utilized to treat safely both systemic lupus erythematosus[13] and type I diabetes mellitus.[14] For comparison, we now report the toxicity and treatment-related mortality (TRM) using a non-myeloablative regimen in ten SSc patients with poor prognostic features. The preliminary efficacy data

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Received 4 April 2007; revised 14 May 2007; accepted 31 May 2007; published online 23 July 2007

were collected primarily through serial measurements of organ specific outcomes including mRSS,[15] pulmonary, cardiac and renal function.

## Methods

*Study design*

The study was designed primarily to investigate the toxicity and TRM of autologous HSCT utilizing a non-myeloablative regimen of high-dose CY and rabbit antithymocyte globulin (rATG). Preliminary efficacy data were collected and compared to related studies using a myeloablative approach. Primary end points were TRM, engraftment and HSC mobilization. Secondary end points were survival and disease status by mRSS, and cardiac, renal and pulmonary function.

*Patient selection*

Patients enrolled represented 10 consecutive patients who were evaluated for transplant at our center and met criteria for entry into the study. Inclusion criteria were a diagnosis of diffuse systemic sclerosis with an mRSS $\geqslant 14$ and evidence of internal organ involvement (gastrointestinal, pulmonary, renal or cardiac). Patients read and signed an informed consent under an Institutional Review Board approved protocol. No patient with SSc was enrolled who only had a skin score greater than 14 (also required visceral organ involvement). Since pulmonary disease independent of skin involvement carries a poor prognosis,[16] a revision of entry criteria was made after the study began to accommodate a single patient with disabling lung disease, who did not meet one of the original entry criteria of a skin score greater than 14. All patients had undergone complete blood counts (CBC), a comprehensive chemistry panel, an autoantibody profile, an mRSS measurement, a cardiac evaluation including a pulmonary artery pressure measurement by Doppler echocardiogram or right heart catheterization, a high-resolution chest computerized tomography (HRCT) and pulmonary function studies (PFT) within 3 months before the transplant. Contraindications for HSCT were total lung capacity (TLC) $<45\%$, left ventricular ejection fraction (LVEF) $<40\%$, or pulmonary artery systolic pressure $>45$ mm Hg.

*Hematopoietic stem cell procurement*

PBSC were utilized as a source of HSC. PBSC were mobilized with CY 2 g/m$^2$ and G-CSF 10 mcg/kg/day, beginning 72 h following completion of the CY administration. A leukapheresis was initiated when the white blood cell count rebounded to more than 1000/mcl and continued daily until the target CD34+ cell count ($2.0 \times 10^6$/kg) was achieved. The PBSC graft was not manipulated and cryopreserved until the date of transplantation (re-infusion).

*Conditioning regimen*

The conditioning regimen consisted of CY 50 mg/kg/day on days $-5$, $-4$, $-3$ and $-2$ (total 200 mg/kg), and rATG 1.5 mg/kg/day on days $-5$, $-4$, $-3$, $-2$ and $-1$ (total 7.5 mg/kg). Mesna was administered along with CY to prevent hemorrhagic cystitis and methylprednisolone 1.0 g/day was administered before each dose of rATG to prevent an infusion reaction and serum sickness. PBSC were infused intravenously on day 0, 48 h following the last dose of CY. G-CSF 5 mcg/kg/day was started on day 0 and continued until an ANC reached 500/mcl.

*Supportive care*

Patients were treated on a high-efficiency particulate air filtered transplant unit. For the prevention of renal crisis that can be triggered by high-dose corticosteroids during the transplant period, an angiotensin converting enzyme (ACE) inhibitor, lisinopril was used routinely as tolerated. Low microbial diet, oral levofloxacin 500 mg daily, fluconazole 400 mg daily, valacyclovir 500 mg three times daily and aerosolized pentamidine 300 mg were started on admission. Levofloxacin was discontinued and intravenous cefepime 2 g every 8 h was started when the neutrophil count dropped to less than 500/mcl. Cefepime was stopped upon neutrophil recovery. Valacyclovir 500 mg twice daily and fluconazole 400 mg once daily were continued for 12 and 6 months post-transplant, respectively. Trimethoprim/sulfamethoxazole double strength (160 mg/800 mg) three times weekly was started upon recovery of hematopoietic engraftment and continued for 6 months post-transplant. Hb levels and platelet counts were maintained above 8 g/dl and 10 000/mcl, respectively, with leukoreduced, irradiated and CMV-safe blood transfusions. All immunosuppressive and disease modifying agents were discontinued upon HSC procurement, except systemic corticosteroids that were tapered over 2–6 months.

*Assessment of outcomes*

Neutrophil engraftment was defined as the first day of 3 consecutive days with an ANC $\geqslant 500$/mcl. Platelet engraftment was defined as the first day of 3 consecutive days with a platelet count $\geqslant 20\,000$/mcl without a transfusion.

Each patient was asked to return to this center at six and twelve months following transplant and then yearly thereafter. Evaluation at those dates included: a history and physical examination, review of systems, medication usage, history of hospitalizations, infections, a mRSS, an echocardiogram with Doppler study, PFT, a HRCT, a renal function measurement, CBC, a chemistry panel, ESR, antinuclear antibodies (ANA) and SCL-70 antibody.

## Results

*Patient demographics and pretransplant disease manifestations*

Of the 10 patients, seven Caucasian, one mixed Caucasian and Eskimo, one African American and one Hispanic; nine females and one male; and a median age 46.5 (range 19–56) years old were enrolled. The median mRSS was 30.5 (range 4–41). Only a single patient had a mRSS less than 23. All patients had pulmonary involvement defined by either restricted ventilatory defect with decreased carbon monoxide lung diffusion capacity (DLCO) on PFT or interstitial lung disease consistent with scleroderma lung

**Table 1** Demographics of patients and disease manifestations before transplant

| *Age/sex* | *DD* | *mRSS* | *Previous therapies* | *HRCT* | *DLCO (%)* | *EF (%)* | *PAP (mmHg)* | *EKG* | *CMV* | *SCL-70* |
|---|---|---|---|---|---|---|---|---|---|---|
| 56/F | 36 | 26 | Etanercept, PNCL, CS, MTX, oral Cy | IL | 48 | 60 | 23 | NSTC | + | – |
| 58/F | 12 | 31 | MTX, PNCL | IL | 86 | 58 | 33 | NSTC, LV | + | – |
| 56/F | 47 | 31 | Minocycline, PNCL, pulse Cy | IL | 57 | 70 | 36 | NSTC, LV | – | – |
| 19/F | 35 | 24 | Type I collagen | Normal | 59 | 55 | — | NSTC | + | + |
| 55/F | 51 | 30 | MTX | IL | 48 | 55 | 25 | LV, LAHB, CCR | + | + |
| 33/F | 56 | 36 | HQ, leflunomide, inflix, etanercet | Normal | 48 | 65 | 29 | IRBBB, NSTC | + | + |
| 42/F | 34 | 23 | HQ, MTX, CS, oral Cy | IL | 64 | 55 | 28 | Normal | + | – |
| 28/F | 48 | 39 | HQ, leflunomide, MTX, CS | IL | 41 | 55 | 13 | RAD | – | – |
| 45/M | 35 | 4 | CS, hyoscyamine, oral Cy | IL | 29 | 65 | 29 | RVH | – | – |
| 48/F | 10 | 41 | MMF, HQ, topical diflorasone | IL | 78 | 68 | 23 | Normal | – | – |

Abbreviations: CCR = counter-clockwise rotation; CMV = anti-cytomegalovirus IgG antibody; CS = systemic corticosteroids; DD = disease duration (months); DLCO = carbon monoxide lung diffusion capacity; EF = cardiac ejection fraction; EKG = electrocardiogram findings; HRCT = high-resolution chest computerized tomography findings; HQ = hydroxychloroquin; IL = interstitial lung disease; Inflix = infliximab; IRBBB = incomplete right bundle branch block; LAD = left axis deviation; LAHB = left anterior hemi-block; LV = low voltage; MMF = mycophenolate mofetil; mRSS = modified Rodnan skin score; NSTC = non-specific T-wave changes; PAP = pulmonary arterial pressure; PNCL = D-penicillamine; RAD = right-axis deviation; RVH = right ventricular hypertrophy.

disease on a HRCT. The median disease duration (onset of the first non-Raynaud's symptoms to transplant) was 35.5 (range 10–56) months. Gastrointestinal involvement was present in all patients as defined by presence of significant gastroesophageal reflux, severe constipation and malabsorption, dilated esophagus on CT scan or positive fibrosis on a biopsy specimen. None had significant renal involvement, but four had trace proteinuria. Eight out of ten patients had abnormal EKG findings. Six out of ten patients had significant joint contractures and pain. All patients had Raynaud's phenomenon. Treatments before the transplant are listed in Table 1. SCL-70 antibody was positive in three patients. Mean ESR was 43 mm/h (range 5–110).

### *HSC (PBSC) procurement, engraftment, transfusion requirement and discharge day*

There were no HSC procurement-related disease exacerbations. The only infection occurring during HSC procurement was clostridium difficile colitis that resolved with metronidazole. The median number of leukaphereses was 1 (range 1–2). The median CD34+ and CD3+ cell counts were $7.38 \times 10^6$/kg (range 2.35–14.7) and $2.14 \times 10^8$/kg (range 0.41–6.83), respectively. The median days of neutrophil and platelet engraftment were nine (range 7–11) and nine (range 0–14), respectively. Three patients never developed platelet counts less than 38 000/mcl. The median number of packed red blood cell and single donor platelet transfusions were 4.5 (range 2–9) and 3 (range 0–6), respectively. Four patients did not need a platelet transfusion. The median day of hospital discharge after PBSC reinfusion was 12.5 (range 8–21). Engraftment was prompt and complete and none developed late cytopenias.

### *Autologous non-myeloablative HSCT toxicity*

*Infections.* Five out of nine patients developed neutropenic fever with only one positive blood culture that was considered a contamination (Diphtheroids). No patient developed evidence of sepsis manifest as hypotension or impaired organ function during the transplant admission. One patient developed clostridium difficile colitis treated with metronidazole. Six out of ten patients were positive for CMV IgG which indicates pretransplant exposure. Despite absence of post-transplant surveillance CMV cultures, no CMV disease occurred after PBSC transplantation. No culture positive early or late bacterial, fungal or viral infections were documented. However, one patient was diagnosed with culture negative pulmonary infiltrate on day 20 that resolved with antibiotics.

*Cardiopulmonary toxicity.* Four patients developed fluid overload treated with diuretics. These four patients also received transient supplemental oxygen via nasal cannula. One of them had slight elevation of a cardiac enzyme, troponin-I (peak 11.48 ng/ml) on day +1 without additional sequelae. No specific treatment was required. The troponin leak was considered secondary to acute left ventricular failure due to combination of hyper-hydration and pre-existing sub-clinical diastolic dysfunction.

*Renal toxicity.* One patient who had not received prophylactic ACE inhibitor therapy (because of baseline systolic blood pressure was less than 90 mm Hg), developed acute renal failure 3 weeks after the transplant. A renal biopsy revealed findings consistent with scleroderma renal crisis and an ACE inhibitor was started. After 8 months of hemodialysis, renal function improved and hemodialysis was discontinued. Since then, the patient has been off dialysis for 12 months with stabilization of the renal function with a creatinine of 0.9 g/dl (creatinine clearance 56 ml/min/1.73 $m^2$).

*Other toxicities.* Chemotherapy-related nausea, vomiting, diarrhea, asthenia and mild liver enzyme elevation, occurred in most of the patients and were controlled with conservative measures. rATG was complicated by fever and rash in two patients. G-CSF-related mild bone pain occurred in most of the patients around the time of engraftment. One patient developed engraftment syndrome[17] characterized by fever, pulmonary edema, arthralgias and skin rash that improved with corticosteroids and

diuretics. One patient developed melena that was secondary to intestinal telangiectasia (watermelon stomach) a known complication of SSc.[18] One patient sustained a mandibular fracture after an accidental fall in the patient's room.

*Survival*

There was no treatment-related mortality. At median follow-up of 25.5 months (range 5–40), nine out of ten patients are alive. One patient died suddenly 24 months after the transplant. No autopsy was performed.

*Disease response*

*Modified Rodnan skin score.* For nine patients who have been followed for more than 12 months, all nine patients demonstrated mRSS improvement within 12 months. Two patients had recurrence of skin thickening and tightening at 12 and 24 months, respectively. In these two patients the skin score subsequently improved in one and stabilization was achieved in the other following treatment with mycophenolate mofetil (Figure 1). Compared to pretransplant baseline mRSS, the post-transplant skin scores improved significantly with *P*-values of 0.008 at 6 months, 0.004 at 12 months and 0.06 at 2 years.

*Cardiac.* Cardiac function as monitored by ejection fraction and pulmonary artery systolic pressure remained unchanged following transplant. No patient developed new cardiac symptoms of dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, or angina and no patient required new cardiac medications.

*Pulmonary.* Pulmonary function as monitored by vital capacity, total lung capacity and DLCO adjusted for hemoglobin generally remained stable between pre- and post-transplant evaluations (Figures 2a, b and c). Eight of the ten patients had pretransplant HRCT findings consistent with interstitial lung disease. Both patients without radiological manifestations of SSc had restricted ventilatory defects with decreased DLCO on pulmonary function tests consistent with subradiological SSc lung involvement. One patient with a negative pretransplant HRCT, despite improvement of the mRSS, subsequently developed radiographic findings and declining pulmonary function tests consistent with presumed disease progression, and expired 24 months post-transplant. The other patient without pretransplant radiological evidence of interstitial lung disease has continued to have normal HRCT for 1 year since transplant. Of the eight patients with pretransplant radiological pulmonary involvement, one patient with extensive reticulonodular opacities without ground-glass patterns on the HRCT showed dramatic improvement on both HRCT and pulmonary function tests. The HRCT findings of all other patients remained unchanged after the transplant. Two patients whose disease have recurred, had unchanged HRCT for 24 months in one, and the other patient had decreased interstitial markings on the HRCT in 6 months and then increased (worsening) at 12 months correlating with other features of disease activity such as improvement and then worsening of the mRSS, ESR and ANA.

*Renal.* Five out of ten patients had trace positive protein in dipstick test pretransplant. Trace proteinuria resolved in three out of the five positive patients post-transplant.

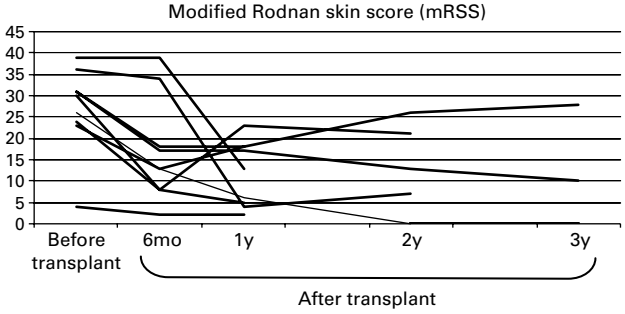


**Figure 1** Modified Rodnan skin score (mRSS) before and serially after the transplant.

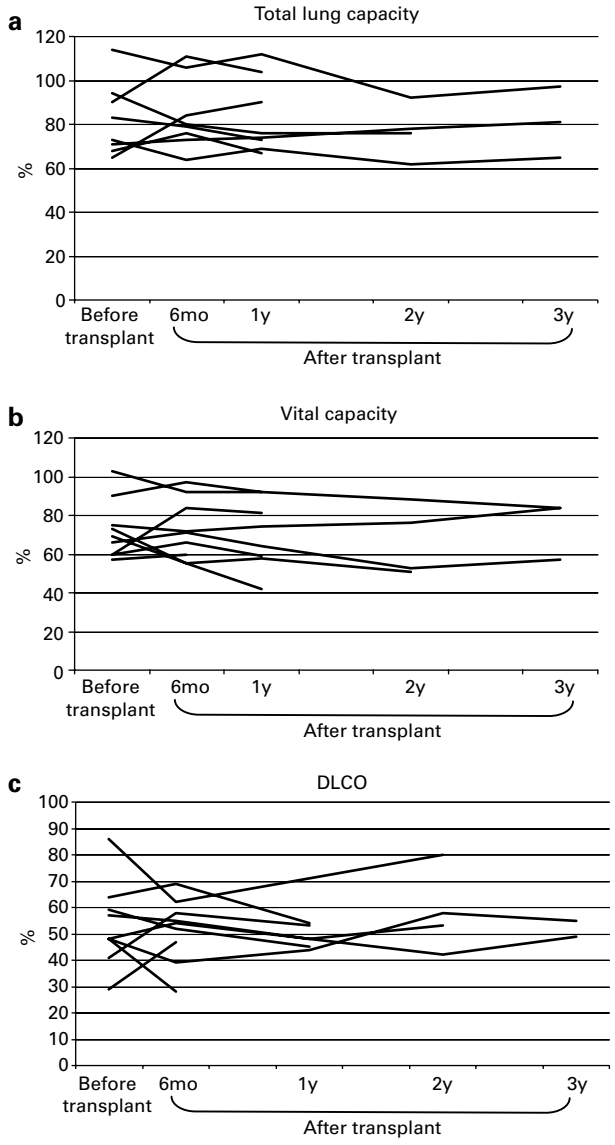


**Figure 2** (**a**) Total lung capacity, (**b**) vital capacity and (**c**) decreased carbon monoxide lung diffusion capacity before and serially after the transplant.

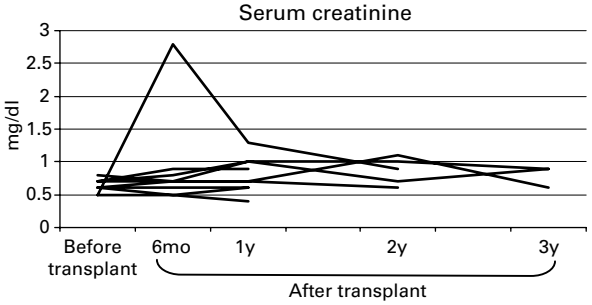


**Figure 3** Serum creatinine before and serially after the transplant.

Creatinine remained unchanged in all patients except the one who was not on an ACE inhibitor and developed scleroderma renal crises with subsequent complete resolution (Figure 3).

*Sedimentation rate and serology.* Laboratory parameters including, ESR and SCL-70 did not correlate with improvement of mRSS. Anti-nuclear antibody levels fell in patients with clinical improvement and remained low except for two patients who experienced relapse of skin tightness.

## Discussion

Although various treatments (glucocorticoids, chlorambucil, MTX, azathioprine, 5-FU, ATG, mycophenolate mofetil, interferon-$\alpha$, interferon-$\gamma$, cyclosporine A, D-penicillamine and extracorporeal photopheresis) have been used to treat SSc,[19–30] currently there is no known therapy that can change the natural history of SSc. Immune suppression with CY at 1–2.5 mg/kg/day orally (with low dose corticosteroids) or 750–1000 mg/m$^2$ intravenously monthly for 6–12 months has been reported to be effective in treatment of scleroderma alveolitis,[31–38] but the benefit to other features and survival advantage are not clear. Further intensification of immune suppression using autologous PBSC transplant support for SSc has been performed worldwide for the past several years. This therapy has, in some studies, been complicated by high treatment-related morbidity and mortality.[6,8,9]

A previously reported American study, performed predominantly at the Fred Hutchinson Research Cancer Center (FHRCC) (Seattle, WA, USA) utilized a myeloablative regimen of CY, TBI and equine ATG (eATG). Similar to our non-myeloablative PBSC transplant, the FHRCC myeloablative transplant regimen demonstrated improvement in skin score (mRSS) in the majority of patients. However, in that study despite adding lung shielding during TBI, mortality has been high. In reports from the FHRCC study, mortality, the majority of which was treatment related, in serial publications was reported as 16% or 3 of 19 patients,[6] then as 30% or 10 of 33 patients[8] and finally as 35% or 12 of 34 patients.[9] To diminish treatment related toxicity and based on the rationale of lymphoablation, we have proposed that autologous HSCT for autoimmune diseases should be performed using non-myeloablative conditioning.[10] Herein, we tested the hypothesis that compared to a myeloablative TBI containing regimen for scleroderma, similar improvements in skin score, but with less toxicity may be achieved with a non-myeloablative regimen.

The baseline characteristics of our patients were similar to those studied with the FHRCC myeloablative regimen. All but one of our patients have diffuse disease which was early ($<5$ years duration in all patients, $<4$ years in 7). The median skin score at entry was nearly identical (30 vs 30.5). The major difference between the FHRCC myeloablative and our current non-myeloablative transplant regimens used for SSc is the use of TBI.[10]

Owing to the concerns over the use of TBI in the conditioning regimen,[10,12] as well as the rationale for transplant, that is myeloablation vs lymphoablation, we used a non-myeloablative regimen based on dose escalation of CY, an agent with previously reported efficacy for SSc. Since this regimen is non-myeloablative, hematopoietic reconstitution may occur without hematopoietic stem cell re-infusion. The reason for collection and re-infusion of PBSC in this study is to shorten the duration of CY-induced neutropenia and thus decrease the risk of infection.[11] Our data suggest that a non-myeloablative transplant regimen may have less adverse effects than was seen in a myeloablative regimen containing TBI while producing similar skin score (mRSS) improvement. However, this is a controversial area and only time will prove the superiority of one regimen over another.

We experienced one death post-transplant due to disease progression. Despite the improvement of mRSS and the preservation of internal organ function, the patient's functional status did not improve. We also experienced the development of normotensive scleroderma renal crises in one patient. Owing to the low systolic blood pressure, ACE inhibitor was not used prophylactically in this patient. Whether high-dose corticosteroids used with transplant triggered the scleroderma renal crises is unknown.[39] The patient was already at high-risk of developing scleroderma renal crises because of the early diffuse disease.

Our results in terms of treatment-related safety are similar to a non-myeloablative randomized PBSC transplant trial termed ASTIS (Autologous Stem Cell Transplantation International Scleroderma) that is ongoing in Europe.[40] The European ASTIS trial uses a non-myeloablative conditioning regimen of CY, and rATG, along with CD34+ selection. To date, the ASTIS trial has enrolled 65 patients and no treatment-related deaths have occurred (verbal communication, January, 2006, Dominique Farge, Paris). There is no available published data from the SCOT (Scleroderma: CY or Transplant) trial which is the current active randomized trial using a TBI containing myeloablative transplant regimen. Our results indicate that non-myeloablative HSCT can be safely performed and results in improved mRSS, similar to myeloablative regimens but with less apparent toxicity. Observations of skin score improvement in uncontrolled trials must always be interpreted cautiously since skin improvement may occur in scleroderma spontaneously. This study forms the basis for ASSIST (American Scleroderma Stem Cell Transplantation vs Immune Suppression Trial/ NCT 00278525, www.clinicaltrials.gov) of non-myeloablative

autologous HSCT vs conventional pulse CY in patients with SSc and poor prognostic features. The need for CD34+ selection, that is T-cell depletion, of the graft remains unclear. It will, therefore, be instructive to compare the ASTIS and ASSIST trials that use identical non-myeloablative conditioning agents with and without CD34+ selection, respectively.

## Conclusion

Long-term observations in these various transplant trials will be necessary to judge the true efficacy of these treatments in reducing morbidity and mortality in SSc. Meanwhile, the intensity and rationale of the treatment regimen demands ongoing vigilance with regard to both short-term and long-term side effects. Major differences between the therapeutic arms of SCOT, ASTIS and ASSIST could dictate very different outcomes from the application of hematopoietic stem cell transplantation to this deadly and disabling disease. Rheumatologists who refer patients and transplant physicians who perform these studies should be aware that important differences exist between the current trials. The full significance of these differences is a story not yet fully told. Meanwhile there will hopefully continue to be an evolution in HSCT for autoimmune diseases towards safer and better-tolerated approaches.

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