Adult stem cell treatment of scleroderma

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Purpose of review

Provides an update of hematopoietic stem cell transplantation for systemic sclerosis from phase I/II studies and prospective randomized phase III trials, and introduces the concept of mesenchymal stem cells as potential therapy for autoimmune disease.

Recent findings

Around 170 transplanted systemic sclerosis patients are registered in Europe. Most received autologous, peripheral blood derived hematopoietic stem cell transplantation. Treatment-related mortality has fallen to 2.5% in the controlled trials compared with 12.5% in the first report in 2002. Over one-third of patients have experienced sustained remission. Two prospective randomized phase III studies are active: the Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial in Europe and the Scleroderma Cyclophosphamide Or Transplant (SCOT) trial in the USA. Both have similar selection criteria, endpoint and control arms, but the SCOT trial uses radiation and less cyclophosphamide. So far, no unexpected toxicity has occurred. Reports produced in the past 12 months show reduction of skin collagen and reversal of microvascular remodelling, years after transplant. Bone marrow-derived mesenchymal stem cells from systemic sclerosis patients show in-vitro immunomodulatory properties equal to healthy controls.

Summary

Hematopoietic stem cell transplantation is currently being tested in prospective randomized controlled trials and appears to 'reset' autoimmunity in systemic sclerosis. Mesenchymal stem cells may have an immunomodulatory role in autoimmune disease.

Keywords

autoimmune disease, hematopoietic stem cell, mesenchymal stem cell, scleroderma, systemic sclerosis, transplant

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Abbreviations

ASTIS	Autologous Stem cell Transplantation International Scleroderma
EBMT	European Group for Blood and Marrow Transplantation
EULAR	European League Against Rheumatism
GvHD	graft versus host disease
HSCT	hematopoietic stem cell transplantation
MSC	mesenchymal stem cell
SCOT	Scleroderma Cyclophosphamide Or Transplant
SSc	systemic sclerosis
TRM	transplant-related mortality

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Introduction

Immunosuppressive agents such as cyclophosphamide have long been used to treat autoimmune disease, but the dose is often limited by bone marrow suppression. Ten years ago several groups considered adopting the oncological approach of myeloablative therapy followed by haematological 'rescue' using either autologous or allogeneic hematopoietic stem cells to treat severe, therapy-resistant autoimmune disease. The concept was supported by animal model data [1], suggesting tolerance induction in a rat arthritis model and cases of patients receiving an hematopoietic stem cell transplantation (HSCT) for conventional indications and in whom a coincidental autoimmune disease was improved or eradicated [2].

After several international meetings [3,4], consensus guidelines were developed and the first published case of a patient receiving an HSCT as treatment for an autoimmune disease alone was published in October 1996 [5]. Since then, over 1000 patients have been transplanted for autoimmune disease, the majority within the context of phase I/II trials and more recently within phase III prospective randomized studies.

In the European Group for Blood and Marrow Transplantation (EBMT) and European League Against Rheumatism (EULAR) database, 136 systemic sclerosis (SSc) patients are registered.

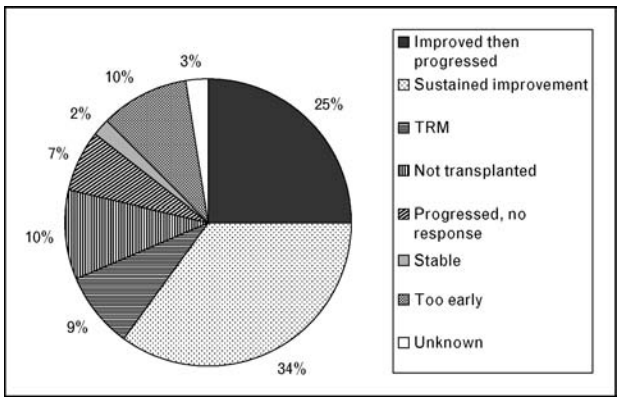
Results of phase I/II studies

All studies have used autologous hematopoietic stem cells.

Autologous hematopoietic stem cell transplantation

The first 65 transplanted patients reported to the EBMT/EULAR database showed an improvement of 25% or more in the skin score (measured by the modified Rodnan method) in 70% of the patients, with a transplant-related

Figure 1 Clinical outcome in 57 systemic sclerosis patients followed up to 60 months following transplant



TRM, transplant-related mortality.

mortality (TRM) of 12.5% [6]. Several protocols were used, mostly either cyclophosphamide based (4 g/m^2 cyclophosphamide mobilization and cyclophosphamide 200 mg/kg body weight conditioning) or radiation (8 Gy/cyclophosphamide 120 mg/kg body weight). With further patient recruitment and longer term follow up, the TRM of the EBMT-registered patients fell, considered to be related to more careful patient selection and appropriate changes in the treatment regimens. Lung function tended to stabilize and renal function generally remained stable, but some factors were identified as potentially hazardous for HSCT (see below). A long-term follow up of this cohort showed an overall TRM of 8.5%, no further transplant-related deaths and trend to durable remissions (Fig. 1) [7]. Within controlled trials, the TRM has thus far been 2.5%. For this subset of SSc there is so far no proven disease-modifying therapy capable of controlling the disease.

Between 1997 and early 2005, 34 early, poor prognosis SSc patients enrolled in a pilot North American study. After an early protocol in eight patients (which had probable radiation-related toxicity) was modified, the next 25 patients underwent conditioning with 800 cGy total body irradiation (with lung shielding to 200 cGy), 120 mg/kg cyclophosphamide and 90 mg/kg equine ATG. After a median follow up of 4 years, an approximately 70% improvement in skin and 55% improvement in function were noted ($P < 0.0001$). Transplant-related mortality occurred in five patients [two from pulmonary toxicity before protocol modification – none have died of pulmonary toxicity since – one of Epstein–Barr virus-associated posttransplantation lymphoproliferative disorder, one with myelodysplastic syndrome, and one of multiorgan failure (15%)] [8,9].

Patient selection

Initially, most groups followed the consensus guidelines of 'life or organ threatening autoimmune disease refractory to conventional therapy and with sufficient reversible pathology to allow a decent quality of life after cessation of inflammation'. Such SSc patients have a 50% 5-year survival [10] and were therefore considered suitable candidates for such an aggressive treatment. The phase I/II experience showed that particular clinical features are associated with potential toxicity, for example patients with a mean pulmonary artery pressure above 50 mmHg by right heart catheterization tended not to tolerate neutropenic fever well due to circulatory compromise, and advanced cardiac or pulmonary disease was prone to deteriorate. This is due to a combination of direct organ toxicity, for example cyclophosphamide cardiac toxicity, and the hyperhydration required during mobilization and conditioning.

Cardiac assessment and monitoring is particularly important in HSCT for SSc [11••], since subclinical myocardial involvement is more frequent than suspected [12] and fatal ventricular tachyarrhythmia may occur. In general, it is thought that an ejection fraction above 40% is necessary to cope with a doubling of cardiac output over several days, as may occur with sepsis-associated pyrexia, and sufficient diastolic reserve to tolerate a 30% expansion of intravascular volume typically seen in hyperhydration. This is analogous to the reserve required to survive pregnancy [11••]. SSc as well as amyloidosis are both conditions associated with diastolic dysfunction and which may be treated with HSCT.

All patients with SSc who are potential HSCT candidates should undergo screening consisting of a standard 12-lead ECG, 24 h Holter monitor and, if coronary artery disease is suspected, coronary artery angiography or pulmonary hypertension, and right heart catheter exam. In addition, echocardiography should always be performed, and if the results suggest pulmonary artery hypertension, a right heart catheter study is required. This should include measuring the pulmonary capillary wedge pressure, which reflects the left ventricular function and should be less than 15 mmHg. This procedure may need to be repeated prior to conditioning in the European protocol in which cyclophosphamide is used during mobilization, as the patient may experience a deterioration after taking this drug. Based on one experience [11••], an implantable defibrillating device is suggested if nonsustained ventricular arrhythmias are present, since antiarrhythmic drugs are considered as adjunct therapy and will not prevent sudden cardiac death on their own.

Thoracic high-resolution computer tomography (HRCT) should be performed to assess interstitial lung disease, with a 'ground glass' pattern suggesting active alveolitis [13]. In addition, HRCT excludes unsuspected pulmonary emboli as the cause of pulmonary artery hypertension. This is particularly relevant if significant pulmonary

artery hypertension is present, and in fact, most patients are anticoagulated for this reason.

The routine use of angiotensin converting enzyme (ACE) inhibitors during the transplant period has been subject to debate, but several reports of acute renovascular crises in patients not taking these agents have prompted some groups to routinely use them. In the Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial it is mandatory during the treatment period and generally continued indefinitely afterwards. In the Scleroderma Cyclophosphamide Or Transplant (SCOT) trial, ACE inhibitors are required during the 2 months when steroids are utilized. The combination of hyperhydration and pulses of glucocorticosteroids during the ATG infusion may increase the risk of scleroderma renal crisis.

Outcome from phase I/II studies

There are now significant numbers of SSc patients followed for 10 years in which major regression of skin thickening has occurred as well as stabilization of lung function. All groups have noted that the first positive impact on disease is often improved quality of life and scleroderma health assessment questionnaires, which include parameters such as physical function, vitality and fatigue. Studies of immune reconstitution have been few, with one study up to 1 year following transplant confirming the known prolonged impairment of the naïve T-cell compartment with no significant difference between responders and nonresponders. A similar study in multiple sclerosis patients in remission 2 years following transplant showed a rejuvenation of the T-cell repertoire, with no relapse [14]. This indicates that the clinical benefit is not simply dependent on prolonged immunosuppression through lymphopenia.

Similar data are evolving concerning morphological changes induced by HSCT. One group has reported remodelling and reduction of skin collagen following autologous HSCT in eight of 10 patients [15••] and another following both autologous and allogeneic HSCT [9,16]. Several reports of improvement of nail fold capillaroscopy changes following autologous HSCT indicate a more profound impact on tissue and matrix function than simply transient antiinflammatory and immunosuppressive effects (M. Matucci-Cerinic, personal communication).

Prospective randomized trials

Figures 2 and 3 outline two ongoing studies: the ASTIS trial in Europe [17,18] and the SCOT trial in the USA. Both trials are similar in their selection criteria, primary outcome and control arms, but differ in the transplant regimen. ASTIS uses cyclophosphamide 200 mg/kg body weight and rabbit ATG, SCOT uses cyclophosphamide 120 mg/kg body weight, equine ATG and radiation 800 cGy (with shielding of the lungs to 200 cGy and renal shielding as well). The positive effect of cyclophosphamide alone (the control arm) on SSc was established in randomized placebo-controlled trials after initiation of both studies [19,20], confirming clinical practice and experience. Thus all patients in both trials receive active treatment.

ASTIS is run under the auspices of the EBMT and EULAR and started randomizing 5 years ago. As of May 2007, 92 patients have been randomized, 44 to transplant and 48 to control. So far, no unexpected toxicity has been observed with one 'probable' transplant-related death occurring due to progressive cardiac failure. The independent safety committee adjudicated that no protocol violation had occurred and that there is no reason to change the protocol. It is planned to include a total of 120 patients.

The 226-patient SCOT trial is supported by the National Institutes of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases and is currently recruiting. Twenty six patients have been randomized and 17 patients are undergoing testing for qualification. Thus far, there has been no mortality and there is no unexpected toxicity.

Both studies include extensive mechanistic studies relating to immune reconstitution, skin biopsy immunohistology, collagen and vascular remodelling, broncheoalveolar lavage cellular components and high-resolution computed tomography lung changes. An extensive biobank of serum, DNA and skin biopsy will facilitate further mechanistic studies, in particular, relating objective biomarkers to disease activity.

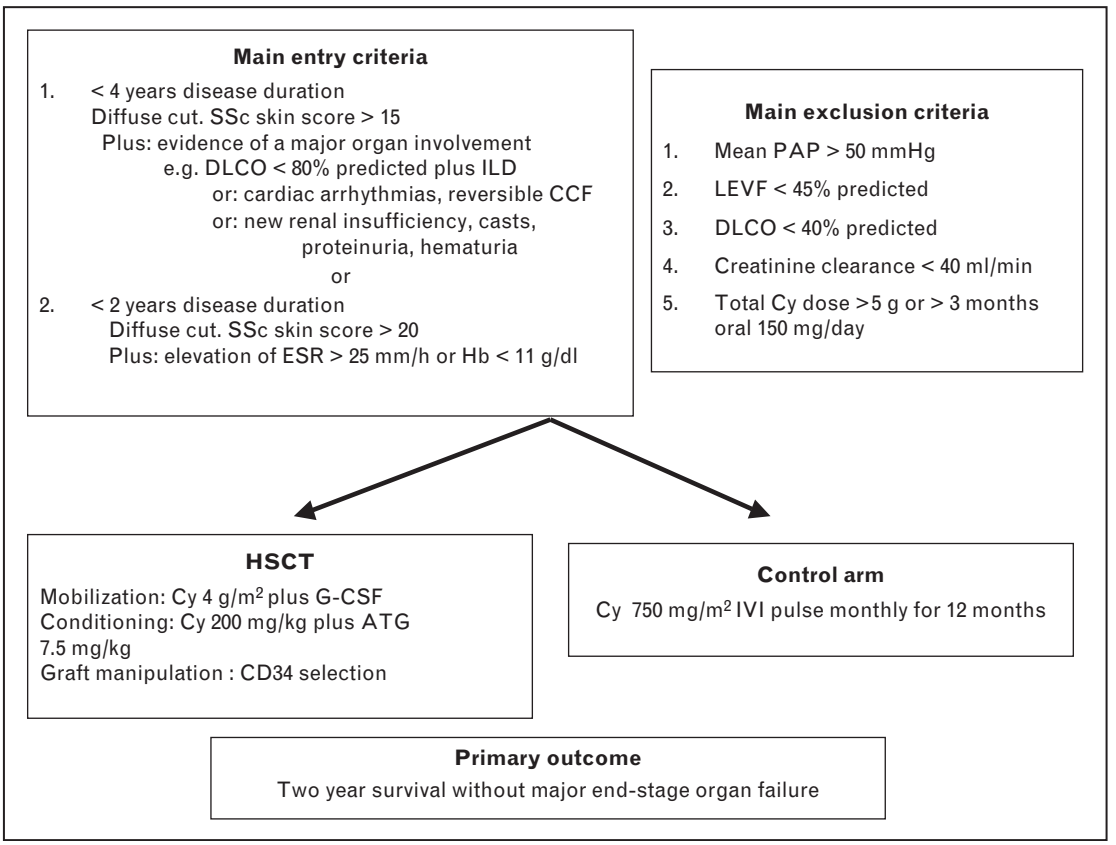
Allogeneic hematopoietic stem cell transplantation

Less is known about this more toxic treatment option, personal communication (GvHD) being a known complication. Three cases are reported, all receiving a reduced intensity nonmyeloablative HSCT [16,21]. In two, a successful response was seen and in a third, TRM occurred at about 1 year from complications relating to acute GvHD. A multidisciplinary consensus group recently published guidelines for allogeneic HSCT in autoimmune disease in general [22].

Mesenchymal stem cells

MSCs are multipotent cells capable of differentiating *in vitro* and *in vivo* to different MSC lineages, including adipose tissue, bone, cartilage and myelosupportive stroma [23–26]. MSCs are found in bone marrow, skeletal muscle, adipose tissue, synovial membranes and other connective tissues of human adults [27–30]. Although controversy exists as to how best to identify MSCs, they seem best defined by using a combination of phenotypic markers and

Figure 2 The Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial



CCF, congestive cardiac failure; Cy, cyclophosphamide; DLCO, carbon monoxide lung diffusion; ESR, erythrocyte sedimentation rate; G-CSF, granulocyte colony-stimulating factor; HSCT, hematopoietic stem cell transplantation; ILD, interstitial lung disease; IVI, intravenous infusion; PAP, pulmonary artery pressure; SSC, systemic sclerosis. Reproduced with permission from [18].

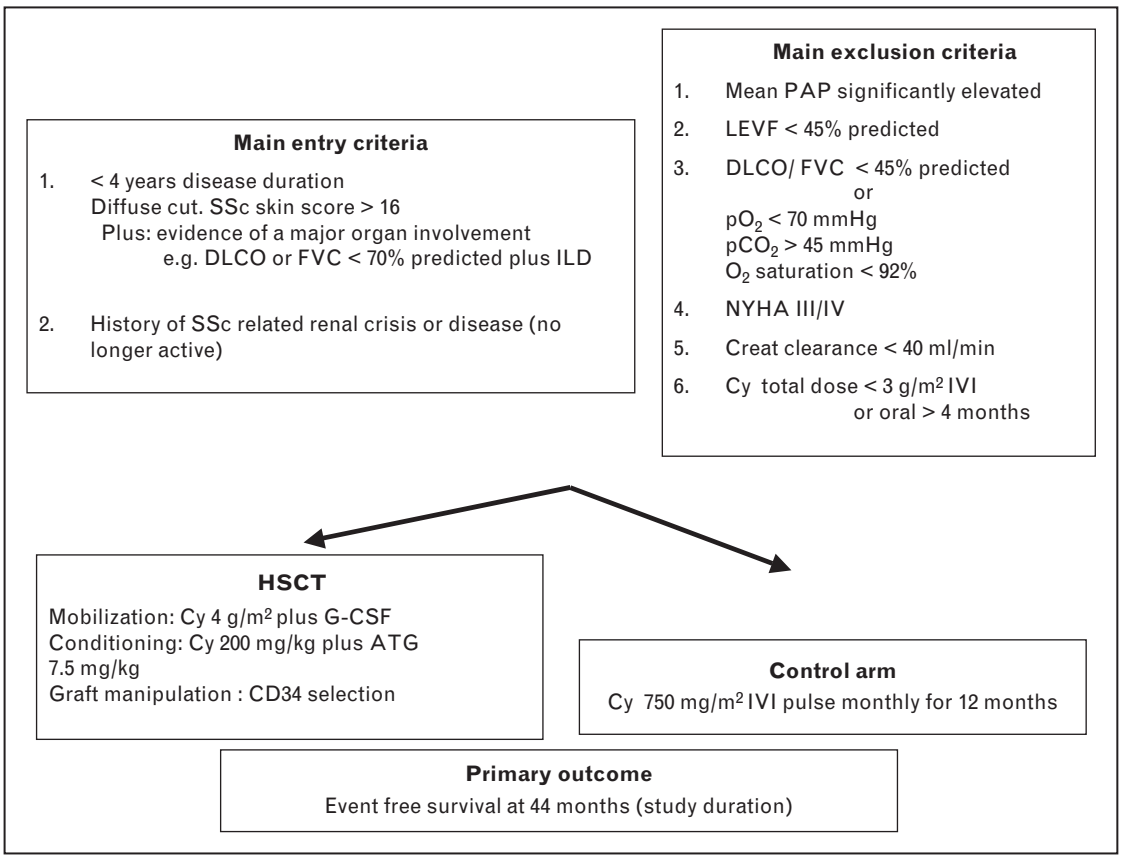
functional properties. Controversy still exists over the in-vivo phenotype of MSCs: however, ex-vivo expanded MSCs do not express the hematopoietic markers CD14, CD34, CD45 and major histocompatibility (MHC) class II [26,31]. In addition to their multipotentiality, they can be identified as cells that stain positive for CD73, CD90 and CD105, and by flow cytometry [25,26,31–33].

In vitro, MSCs have vast proliferative potential, can clonally regenerate, and can give rise to differentiated progeny. They also exhibit antiproliferative and antiinflammatory properties *in vitro* and *in vivo*, making them candidates for treatment of acute inflammatory autoimmune disease [34••]. Regardless of whether or not MSCs are true stem cells, the clinical benefit from MSCs may not require sustained engraftment of large numbers of cells. It is possible that the therapeutic benefit is due to MSCs homing to inflamed tissue and the release of local cytokines and growth factors resulting in a local antiproliferative and immunomodulatory effects.

MSCs were originally thought to be immunoprivileged, in that they did not induce lymphocyte proliferation when cocultured with allogeneic lymphocytes and were not targets for CD8+ cytotoxic lymphocytes or KIR-ligand mismatched natural killer (NK) cells [35–38]. Recent data, however, suggest that in a nonimmunosuppressed host, allogeneic MSCs will be eliminated [39] and that allogeneic MSCs under some circumstances may be targeted by NK cells [40].

In-vitro results indicate that MSCs possess immunosuppressive properties. Rodent, baboon and human MSCs suppress T and B-cell lymphocyte proliferation in mixed lymphocyte cultures or when induced by mitogens and antibodies, in a dose-dependent fashion [35,37,38,41–45]. The suppression is MHC independent and in human cell cultures, the magnitude of suppression is not significantly reduced when the MSCs are separated from the lymphocytes in transwells, indicating that cell–cell contact is not required [35,38,46]. The mechanisms underlying the immunosuppressive effect remain to be clarified. Various factors produced by MSCs, including hepatocyte growth factor, transforming growth factor-β1 [38], prostaglandin E2 [47], indoleamine 2,3-deoxygenase [48] and inducible nitric oxide synthetase [49] have been implicated as

Figure 3 The Scleroderma Cyclophosphamide Or Transplant (SCOT) trial



Cy, cyclophosphamide; DLCO, carbon monoxide lung diffusion; FVC, forced vital capacity; G-CSF, granulocyte colony-stimulating factor; ILD, interstitial lung disease; IVI, intravenous infusion; NYHA, New York Heart Association; PAP, pulmonary artery pressure; SSC, systemic sclerosis.

responsible for reduced lymphocyte proliferation. Clearly, a major antiproliferative mechanism in lymphocytes is arrest of the cell cycle in G0/G1 [45].

An immunosuppressive effect of MSC *in vivo* was first suggested in a baboon model, in which infusion of ex-vivo expanded donor or third-party MSCs delayed the time to rejection of histoincompatible skin grafts [43]. MSCs also downregulate bleomycin-induced lung inflammation and fibrosis in murine models, if given early (but not late) after the induction [50]. A similar effect was seen in a murine hepatic fibrosis model (carbon tetrachloride induced) using a MSC line bearing the fetal liver kinase-1 marker [51]. Tissue protective effects of MSCs were also seen in a rat kidney model of ischaemia/ reperfusion injury in which syngeneic MSCs but not fibroblasts were used [52].

Autologous bone marrow-derived MSCs have been shown to be highly antiproliferative to activated T cells from normal individuals and autoimmune (rheumatoid arthritis, SSc, Sjoegrens, systemic lupus erythematosus) patients [53••], and in SSc patients these MSCs were normal with respect to proliferation, clonogenicity and differentiation [54].

Animal models of autoimmunity

In the two experimental autoimmune encephalomyelitis murine models both clinical and histological improvement occurred. The responses were dependant on the timing of MSC treatment – the earlier the better – and the effects were reversed with interleukin-2 treatment, indicating that anergy rather than apoptosis had occurred [55,56]. In a murine model of arthritis, however, collagen-induced arthritis was not improved by the addition of MSCs and the in-vitro immunosuppressive effects were reversed by the addition of tumor necrosis factor-α. MSCs were not found in the joints [57]. A second murine arthritis model, however, showed a positive outcome [58].

Mesenchymal stem cells and human experience

Ex-vivo-expanded allogeneic MSCs have been infused in several phase I studies [59–63]. No adverse events during or after MSC infusion have been observed and no ectopic tissue formation has been noted. After infusion, MSCs remain in the circulation for no more than an hour [62].

Although durable stromal cell chimerism has been difficult to establish, low levels of engrafted MSCs have been detected in several tissues [60,63,64].

Infusion of haploidentical MSCs to a patient with steroid-resistant severe acute GvHD of the gut and liver promptly improved liver values and intestinal function [65]. Upon discontinuation of cyclosporine, acute GvHD recurred but was still responsive to a second MSC infusion. Lymphocytes from the patient, when investigated on multiple occasions after MSC infusion, continued to proliferate against lymphocytes derived from the haploidentical MSC donor in coculture experiments. This suggests an immunosuppressive effect of MSCs *in vivo*, rather than a development of tolerance.

The EBMT is currently planning protocols for prevention and treatment of acute GvHD using MSCs, through the Developmental Sub-Committee (W. Fibbe, K. Le Blanc, personal communication).

Conclusion

In conclusion, HSCT for SSc has advanced to the stage of two international prospective randomized controlled trials which should determine if this aggressive form of therapy may 'reset' an autoaggressive immune system and benefit patients with severe, poor prognosis SSC. During the past several years the potential use of MSCs as immunomodulating agents is being explored (e.g. in the acute GvHD setting in which a positive effect seems possible with little or no acute toxicity). Preliminary results suggest that bone marrow-derived MSCs from SSc patients exhibit effective in-vitro antiproliferative effects on lymphocytes.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 653).

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