

# High-Dose Immunosuppressive Therapy and Autologous Hematopoietic Stem Cell Transplantation for Treatment of Severe Systemic Sclerosis

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Systemic sclerosis is an autoimmune disease characterized by progressive vascular damage (Raynaud's phenomenon and digital ulcers, hypertensive renal failure, cardiomyopathy, pulmonary hypertension) and organ fibrosis (skin thickening, pulmonary fibrosis, gastrointestinal dysmotility, myocardial fibrosis). Early in the disease process, signs of inflammation are frequently present (fatigue, edema, tendon friction rubs, arthritis, myositis, pericarditis). Two subtypes are defined by extent of skin involvement: limited cutaneous systemic sclerosis with skin thickening confined to the distal extremities and face often preceded by years of Raynaud's phenomenon and diffuse cutaneous systemic sclerosis with skin thickening involving the entire extremity and torso frequently associated with visceral organ involvements.<sup>1</sup>

The most severe cases are identified by rapid advancement of skin thickening and early internal organ damage during the first 5 years of onset. The 5- and 10- year survival rates for those with rapid onset of signs and symptoms is estimated to be 50% and 38%.<sup>2,3</sup> Although pulmonary involvement may be documented in the majority of patients with systemic sclerosis (with either limited or diffuse skin thickening), progression to end stage lung disease is variable. In patients with early symptomatic pulmonary or cardiac involvement the 5-year survival is decreased to 33%.<sup>4</sup>

Until recently, systemic sclerosis was considered an untreatable disease.<sup>5</sup> Pharmacotherapy focused on management of symptoms related to specific organ involvement, eg, calcium channel blockers and vasodilator therapy for Raynaud's and proton pump inhibitors for esophageal reflux secondary to esophageal dysmotility. Angiotensin-converting enzyme (ACE) inhibitors were the first class of drug to demonstrate an improvement in mortality. Acute scleroderma hypertensive renal disease was nearly 100% fatal within 6 months before the development and use of ACE inhibitors to treat this complication.<sup>5,6</sup> Pulmonary artery hypertension (PAH) is a rare but often fatal complication of

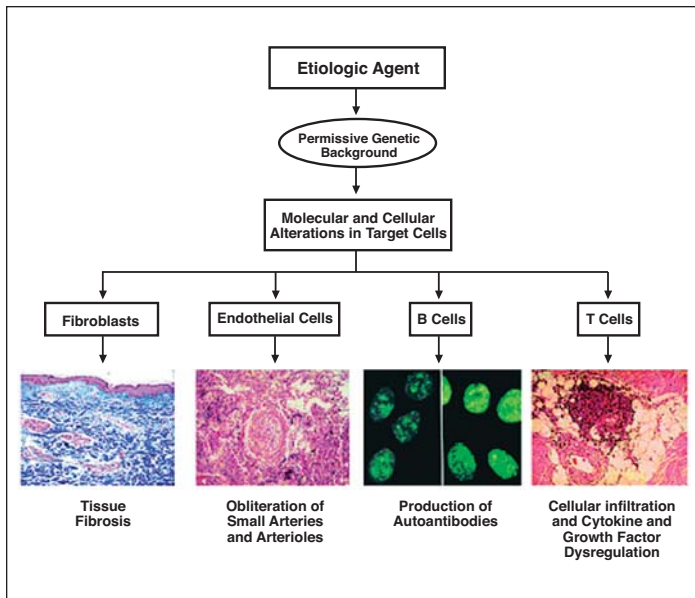
systemic sclerosis, more commonly associated with the limited cutaneous subset. PAH is no longer considered an untreatable complication of systemic sclerosis as studies with prostaglandins (intravenous, subcutaneous, or inhaled), antiendothelial receptor blockers, and phosphodiesterase inhibitors have demonstrated improved quality of life, function, and survival in systemic sclerosis patients with PAH.<sup>7-13</sup>

Despite these successes most pharmacotherapeutic intervention to treat the disease remains empiric. Efforts to document efficacy have been disappointing when therapies are tested in controlled trials.<sup>14-28</sup> Many of these trials were doomed to failure given the heterogeneous nature of disease expression and too often patients had late disease with established fibrosis that would not necessarily be amenable to the treatments tried. A multicenter Phase II clinical trial comparing oral Type I bovine collagen as a toleragen to placebo evaluating effect on the modified Rodnan skin score in diffuse cutaneous systemic sclerosis has completed enrollment though results have not yet been reported. Based on the up regulation of endothelin binding sites seen in systemic sclerosis lung fibrosis, the antiendothelin receptor bosentan is under evaluation in a double-blind, randomized, placebo-controlled, multicenter study. Information on these two novel therapies for treatment of systemic sclerosis manifestations (skin and lung) can be found on the Scleroderma Clinical Trials Consortium Web site: <http://www.sctc-online.org>.

## Pathophysiology of Systemic Sclerosis

Lacking a specific etiology there is no unifying hypothesis to explain the varied clinical manifestations of systemic sclerosis. The development of systemic sclerosis is believed to be due to an interaction of an as yet unidentified environmental exposure(s) (infectious and/or noninfectious) in a genetically susceptible individual. Endothelial and immune activation cause endothelial damage, fibroblast proliferation and collagen synthesis resulting in dysfunction of various end organs.<sup>29</sup> Abnormalities of three cell types: fibroblasts, endothelial cells, and cells of the immune system, especially T and B lymphocytes, have been identified as primal to the development of the clinical and pathologic expression

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**Figure 1. General overview of pathogenesis of systemic sclerosis. (Copyright © 2004, *Annals of Internal Medicine*. All rights reserved.)**

of clinical disease (Figure 1).<sup>30</sup> In diffuse cutaneous systemic sclerosis, organ fibrosis is the most characteristic clinical finding. However, fibrosis is considered a late manifestation and occurs as a consequence of immune activity and vascular injury. An initial immune-mediated process is hypothesized to trigger endothelial injury and fibroblast activation.

Vascular dysfunction occurs early and is characterized by vasomotor instability and impaired vasodilatation. As the disease progresses, proliferative intimal arterial lesions and eventual obliteration of vessels lead to chronic ischemia. Perivascular activated T cells are present in small blood vessels and secrete transforming growth factor-beta (TGF-beta, which injures endothelial cells inducing expression of MHC class I and II antigens and adhesion ligand intercellular adhesion molecule-1 (ICAM-1). TGF-beta also up regulates connective tissue growth factor, resulting in increased extracellular matrix components and platelet-derived growth factor. Endothelial cell injury may also result from cytotoxic factors present in serum,<sup>31,32</sup> or by serum IgG antibodies causing antibody-dependent cell-mediated cytotoxicity.<sup>33,34</sup>

The evidence for autoimmune activity is supported by several observations, including the presence of systemic sclerosis findings in overlap syndromes characterized more clearly as autoimmune, such as systemic lupus erythematosus and the familial associations with other autoimmune connective tissue diseases. Additional recognition of an autoimmune process is supported by similarities of human graft-versus-host disease and the occurrence of scleroderma-like changes in experimental murine graft-versus-host disease.<sup>35</sup> The presence of circulating autoantibodies to a variety of nuclear antigens is an obvious laboratory manifestation of autoimmunity. Although these antibodies are useful diagnostically, and can help predict the probable pattern of organ involvement, severity, and disease progression, they do not appear to be involved directly in pathogenesis.<sup>36,37</sup>

The presence of T cells in affected skin in early disease and the

**Table 1. Evidence for Autoimmune Activity in Systemic Sclerosis.**

Antibody-dependent cellular cytotoxicity against fibroblasts and endothelium
Activated endothelium in early disease moderates intracellular adhesion molecules and promotes immunological chemotaxis
Activated T cells in lung parenchyma and alveolar fluid
Activated T cells in skin in early disease
Activated fibroblasts
High prevalence of disease-specific antinuclear antibodies
Genetic array data

severity and progression of skin sclerosis correlates with the extent of lymphocytic infiltration. In the skin, the majority of the mononuclear cells are CD4+ T cells, express the activation marker MHC class II antigen DR, and appear to be oligoclonal, consistent with an antigen-driven response. These T cells produce cytokines that can stimulate fibroblast collagen production.

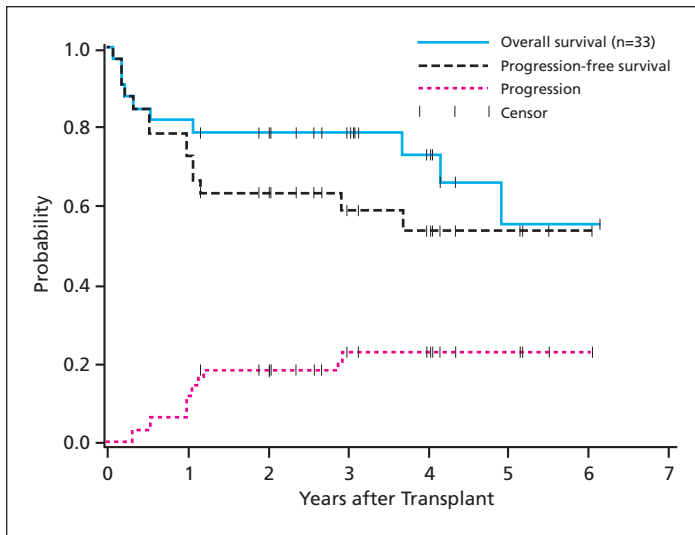
In the peripheral blood the proportions and absolute numbers of C4+CD45RA+ (suppressor-inducer T cells) and CD8+/CDI1b (suppressor T cells) are decreased consistent with impaired balance between immunoregulatory T cell populations. Peripheral blood T cells in systemic sclerosis express interleukin-2 receptor (IL-2R) on their membranes and serum from systemic sclerosis patients has higher levels of soluble IL-2R consistent with activation.<sup>38,39</sup> Activated T cells express adhesion ligands that promote egress from the blood vessel to the tissues. The integrin lymphocyte function-associated antigen-1 (LFA-1) is one of the cell surface receptors with increased expression on T cells that promotes adhesion to fibroblasts by interaction with its counter receptor intercellular adhesion molecules ICAM-1, ICAM-2, and ICAM-3.

T cells are also important in the pathogenesis of systemic sclerosis interstitial lung disease.<sup>40</sup> Histologic examinations of lung tissue and bronchoalveolar lavage fluid confirm high levels of CD+8 and  $\gamma/\delta$  T cells. These cells are oligoclonal and have increased expression of type 2 (Th2) cytokines and Il-4 and Il-5 messenger RNA compared to normal controls. The production of these Th2 cytokines by CD+8 cells in alveolar fluid predicts a greater decline in lung function.<sup>41</sup>

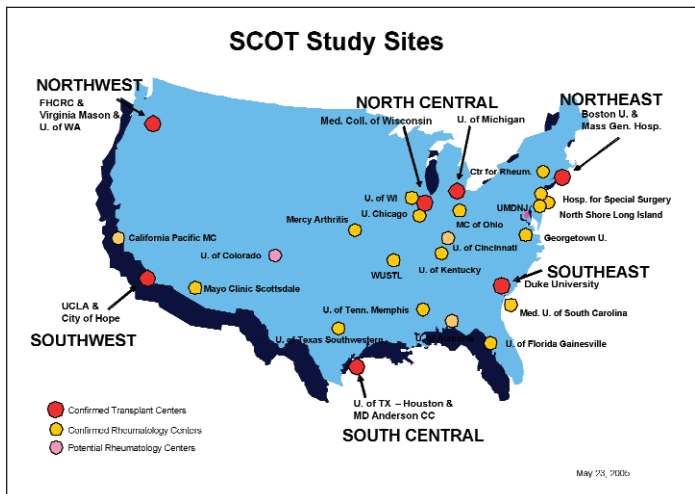
### Immunosuppressive Therapy as Treatment for Systemic Sclerosis

The increasing recognition of systemic sclerosis as an autoimmune disorder (Table 1) is the basis for immunosuppressive therapy.<sup>35</sup> Lung fibrosis without cardiac or renal disease is now identified as the most common cause of death in systemic sclerosis patients. Patients with diffuse cutaneous systemic sclerosis and symptomatic pulmonary involvement without cardiac or renal disease have a median survival of 78 months.<sup>42</sup> The presence of inflammatory cells found in bronchoalveolar lavage fluid and from open lung biopsies has lent credence that treatment of "active" alveolitis may be amenable to immunosuppressive therapy.

(continued on page 8)



**Figure 2. Summary of survival in Fred Hutchinson Cancer Research Center (FHCRC) Protocol 1019.**



**Figure 3. Scleroderma Cyclophosphamide or Transplant (SCOT) trial transplant and rheumatology study sites.**

py. A trial of total lymphoid irradiation did not prove sustained benefit when 12 patients were equally randomized to treatment versus observation. However, neither was irradiation found to be excessively toxic in the active treatment arm.<sup>20</sup> Improvement of alveolitis and stabilization of lung function have been demonstrated in uncontrolled trials with cyclophosphamide.<sup>43-51</sup> Preliminary data from the Scleroderma Lung Study were presented at the International Conference of the American Thoracic Society in San Diego on May 25, 2005. Systemic scleroderma patients with active alveolitis were randomized to receive either oral cyclophosphamide or placebo for one year. For the first time a small but statistically significant stabilization of lung function was found, as was an improved quality of life at one year. A European trial evaluating monthly intravenous cyclophosphamide for treatment of active alveolitis in systemic sclerosis patients with interstitial lung disease completed its first year and at the same American Thoracic Society meeting in June 2005 one of the investigators reported benefits on forced vital capacity by cyclophosphamide compared to placebo.

## Immunoablation and Stem Cell Transplantation in Severe Autoimmune Disease

The concept of resetting the autoimmunostat for treating severe autoimmune disease has evolved over the past decade. That bone marrow transplantation may be effective against human autoimmune disease was noted in aplastic anemia, a hematologic autoimmune disease caused by immunological suppression of the bone marrow. HLA-identical marrow transplantations are now routine practice in transplantations centers since the 1980s.<sup>52</sup> Preclinical studies of high-dose immunosuppressive therapy followed by allogeneic and later autologous hematopoietic stem cell transplantation (HSCT) in antigen-induced animal models of autoimmune disease encouraged application of this therapy to human autoimmune disease.<sup>53</sup> In contrast, syngeneic bone marrow transplantation in autoimmune animal models that develop generalized autoimmunity (eg, the model of systemic lupus erythematosus in NZBxNZW F1 mice) or organ-specific autoimmunity (eg, the model of diabetes mellitus in NOD mice) did not prevent disease expression. The effectiveness of allogeneic transplantation in such models suggests that the hematopoietic stem cells are the source of the autoimmunity in that animal model.<sup>54</sup>

If development of clinical autoimmune disease was purely based on genetic make-up, predisposition would reside in hematopoietic stem cells and autologous HSCT would provide at best temporary antiinflammatory benefit from immunosuppression as seen in autoimmune-prone animal models. Most theories of the development of autoimmune disease in genetically susceptible individuals include exposure to an unidentified environmental trigger, infectious or noninfectious.<sup>55,56</sup> The difficulty of identifying the environmental trigger is consistent with a latent period between exposure and expression of clinical disease. The rationale for high-dose immunosuppressive therapy with autologous HSCT is to “time shift” the course of the clinical autoimmune disease to an earlier period, thereby restoring self-tolerance. To be successful, this rationale assumes that response to repeat exposure to even self-antigens will differ and not result in reexpression of clinical autoimmunity.<sup>57</sup>

Low-dose immunosuppressive therapy has demonstrated some benefit, although it is often the absence of progression rather than improvement that is considered the positive outcome. Evidence that intensive chemoradiotherapy followed by immune reconstitution with “naïve” stem cells is beneficial in patients with severe autoimmune disease was observed when patients with pre-existing autoimmune disease received allogeneic bone marrow transplantation for marrow failure or malignancy.<sup>58,59</sup> The need for HLA-identical matched donor stem cells and the risk of graft-versus-host disease have limited the role of allogeneic stem cell transplantation in autoimmune diseases. The rationale for autologous HSCT relies on the effect of high-dose immunosuppressive therapy on T cell recovery. Non-T cell immune recovery is more rapid than T cell recovery. Three months after high-dose immunosuppressive therapy, CD3 cells normalize while the numbers of CD4 cells remain reduced and an inverted CD4/CD8 cell ratio persists for 12 months. There is a predominance of CD45RO+ cells, a deficiency of naïve CD45RA+ cells, and restriction of the T cell repertoire in adult patients. With experience, protocols of

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**Table 2. Overview of Scleroderma Cyclophosphamide or Transplant (SCOT) Trial.**

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**Primary study end point**

- “Event-free” survival at 44 months after randomization; events are defined as:
    - Death
    - Respiratory failure
    - Chronic renal dialysis
    - Cardiomyopathy (NYHA heart failure class III or IV) or left ventricular ejection fraction <30% by echocardiography sustained for 3 months
- 

**Eligibility criteria**

- Subjects with poor prognosis systemic sclerosis characterized by extensive skin involvement (modified Rodnan skin score  $\geq 16$ ) and early internal organ involvement will be recruited to participate
- 

**Inclusion criteria**

- Age 18 to 65 years
  - Systemic sclerosis as defined by American College of Rheumatology criteria
  - Modified Rodnan skin score  $\geq 16$  verified on two separate occasions  $\geq 1$  day and  $< 28$  days apart
  - Duration of systemic sclerosis  $\leq 4$  years from onset of first non-Raynaud’s symptom
  - One of following two: (1) systemic sclerosis-related pulmonary disease with forced vital capacity or hemoglobin-adjusted diffusing capacity of carbon monoxide  $< 70\%$  and evidence of alveolitis by high-resolution chest CT scan or bronchoalveolar lavage; or (2) history of systemic sclerosis-related renal crisis or disease, not active at time of screening
- 

**Exclusion criteria**

(more fully detailed at <http://www.sclerodermatrial.org>)

- Pulmonary, cardiac, hepatic, or renal impairment of degree that would limit therapy and compromise survival
  - Active gastric antral vascular ectasia (GAVE, “watermelon stomach”)
  - Previous treatment with cyclophosphamide
  - Steroid therapy:  $> 10$  mg/day prednisone or equivalent within 30 days prior to randomization
  - Unwilling or unable to discontinue disease-modifying antirheumatic drugs for treatment of systemic sclerosis
  - History or presence of overlap syndrome
  - Active uncontrolled infection
  - Positive serology for hepatitis B or C or HIV
  - Absolute neutrophil count  $< 1500$  cells/ $\mu$ L, platelets  $< 120,000$  cells/ $\mu$ L, hematocrit  $< 27\%$ , or hemoglobin  $< 9.0$  g/dL
  - Malignancy within previous 2 years, excluding treated skin cancer and carcinoma *in situ*
  - Myelodysplasia
  - Comorbid illnesses with estimated median life expectancy  $< 5$  years
  - Uncontrolled hypertension
  - History of hypersensitivity to murine or *E. coli* proteins
  - Pregnancy or unwilling to use contraceptive methods for at least 15 months after starting treatment
  - History of substance abuse within last 5 years
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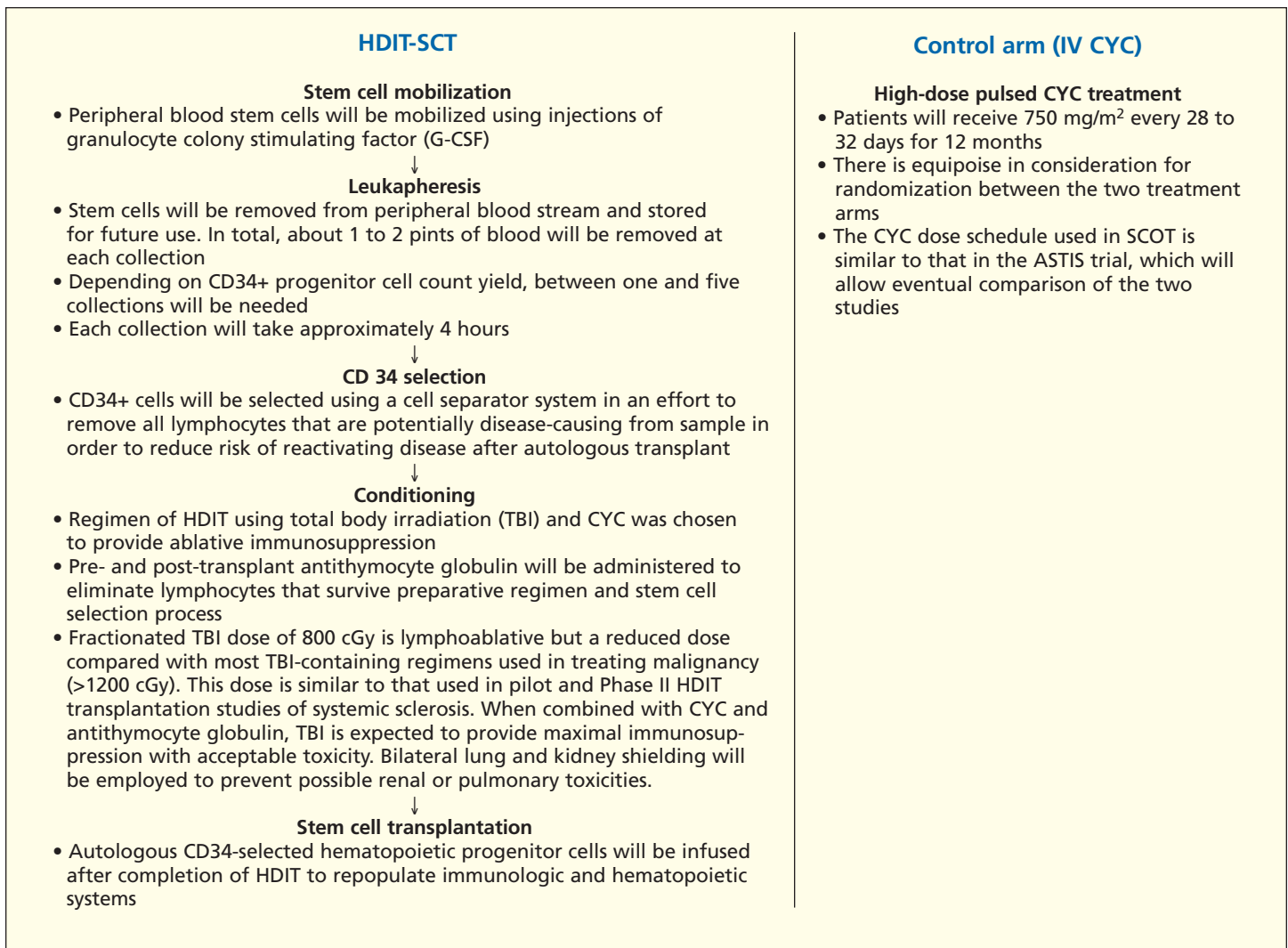
HSCT in autoimmune disease have now come to use CD34+ cell selection and antithymocyte globulin therapy at the time of stem cell reinfusion, for an even greater degree of immunosuppression.<sup>57</sup>

### High-Dose Immunosuppressive Therapy and Autologous HSCT

More patients with systemic sclerosis have undergone autologous HSCT than have patients with rheumatoid arthritis or systemic lupus erythematosus. This reflects the poor prognosis for an identifiable subset of systemic sclerosis patients and the absence of effective therapy. Initial case reports of benefit have been substantiated by cumulative experience in the European Bone Marrow Transplantation (EBMT) registry,<sup>60</sup> the French multicenter trial,<sup>61</sup> and the Fred Hutchinson Cancer Research Center (FHRC) Protocol 1019.<sup>62</sup> In contrast to patients with hemato-

logic malignancies undergoing HSCT, eligible systemic sclerosis patients by definition have underlying organ damage that poses increased risk for transplant-related mortality.

In the EBMT registry, initial transplant-related mortality was high. With experience, protocol modifications (addition of cyclophosphamide 4 g/m<sup>2</sup> to G-CSF for mobilization, CD34+ purging, and conditioning regimens of cyclophosphamide plus anti-T cell antibodies) and better patient selection, transplant-related mortality improved from 27% to 8.7%.<sup>63</sup> Of the 57 systemic sclerosis patients entered into this prospective registry, 19 were available for 24-month assessment. Fifteen (79%) had a  $> 25\%$  decline in initial modified Rodnan skin score. Pulmonary function, as measured by diffusing capacity of carbon monoxide and forced vital capacity, remained stable in a majority. There were no instances of systemic sclerosis renal crisis. Although either complete or partial remission as assessed by local investi-



**Figure 4. High-dose immunotherapy with stem cell transplant (HDIT-SCT) vs control (intravenous cyclophosphamide, IV CYC).**

gators was seen in 92% of patients, a 35 % relapse rate was seen within 1 year. Eight (14%) of the 57 died of disease progression. Transplant-related mortality reported at 8.7% included death from sepsis in two, one CNS bleed, one with interstitial pneumonitis, and one from diffuse alveolar hemorrhage. Death from disease progression, transplant-related causes, or relapse was 23% at a mean of 12 months. Overall two thirds of the patients experienced an initial clinical response not previously seen for any other therapeutic intervention in severe systemic sclerosis.

In the United States, a parallel Phase II study was conducted and results were recently published.<sup>64</sup> In contrast to the EBMT registry, patients received total-body irradiation prior to transplant to promote maximum immunosuppression in addition to G-CSF for mobilization, CD 34 purging, and cyclophosphamide 120 mg/kg, and antithymocyte globulin. The safety of total-body irradiation has improved as lung and kidney shielding has become standard. The 3-year summary experience reported improvement in modified Rodnan skin score by a median of 49% (n = 24) at 12 months and a median of 79% (n = 10) at 36 months post transplant. Functional improvement as measured by the modified Health Assessment Questionnaire Disability Index improved by a

median of 57.6% at 12 months and 72.8% at 36 months. Lung function (diffusing capacity of carbon monoxide and forced vital capacity), left ventricular ejection fraction, and serum creatinine remained stable. Transplant-related mortality was 8.7% after lung shielding, with no irradiation mortality noted in the 25 patients who received lung shielding. One death was due to Epstein-Barr virus-related lymphoproliferative disorder. By 3 years, a 23% mortality rate was reported from either transplant-related mortality or disease progression. Fifteen percent was attributed to disease progression (**Figure 2**).

In comparing the European regimen, which used higher doses of cyclophosphamide, and the US regimen, which used less cyclophosphamide but included total-body irradiation with lung shielding, there were no striking differences in mortality. The reported mortality rates following stem cell transplantation compare favorably to the projected 5-year mortality rate of approximately 50% for patients with early diffuse systemic sclerosis with renal and/or lung involvement who are left untreated. Encouraged by these results, a Phase III, prospective, randomized, controlled trial, the Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial (<http://www.astis-trial.com>) is being



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conducted under the auspices of the European League Against Rheumatism (EULAR) and EBMT. Results of the ASTIS trial are not expected until 2009.

In the United States the Scleroderma Cyclophosphamide or Transplant (SCOT) trial, a prospective, randomized, clinical trial approved by the FDA and supported by the NIH has begun. Enrollment of 226 subjects randomly assigned to either high-dose immunosuppressive therapy with autologous stem cell transplantation or monthly pulsed intravenous cyclophosphamide is planned over the next 3 years. A map of transplant centers and participating rheumatology centers is found in **Figure 3** and an overview of the study protocol is provided in **Table 2** and **Figure 4**. Although early experiences with stem cell transplantation in systemic sclerosis showed a higher than expected transplant-related mortality, more stringent eligibility requirements and modification of treatment regimens are anticipated to reduce mortality risk and improve outcome overall.

Both the ASTIS and SCOT trials have similar inclusion and exclusion criteria with nearly identical follow-up and end points. In both trials patients will be randomized to the transplant arm or the control arm (monthly intravenous cyclophosphamide for one year). The dose of 750 mg/m<sup>2</sup> for 12 monthly cycles is approximately twice the dose of cyclophosphamide given as part of the HSCT arm and was chosen to strengthen equipoise between the two regimens. The results of these parallel studies will provide sound clinical data to evaluate the optimal treatment regimen for

high-dose immunosuppressive therapy with autologous HSCT as well as to assess the efficacy of high-dose immunosuppressive therapy without HSCT in patients with early severe systemic sclerosis when immunomanipulation is proposed to be most effective.

Finally, the SCOT trial represents a unique opportunity to broaden our understanding of the pathogenesis of systemic sclerosis and its response to high-dose immunosuppressive therapy. Several mechanistic studies are proposed to improve understanding of the role of T cells in systemic sclerosis with lung disease, the molecular mechanisms of fibrosis and the role of circulating endothelial progenitor cells. Additional information on this pivotal study for patients and physicians can be found at <http://www.sclerodermatrial.org>. Study contact numbers are listed on the Web site.

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