Hematopoietic Stem Cell Transplantation for Scleroderma: Effective Immunomodulatory Therapy for Patients with Pulmonary Involvement

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Could a greater miracle take place
than for us to look through each
other’s eyes for an instant?

Henry David Thoreau

Introduction

As seen from both patients’ and physicians’ points of view, there is wide agreement that systemic sclerosis (SSc) is one of the most morbid and mortal of the autoimmune disorders. In this review, we will follow the evolution of a new approach to its treatment. Support for using hematopoietic stem cell therapy (HSCT) for SSc arose from seminal studies of genetic and antigen-induced experimental models of autoimmune disease which demonstrated that high-dose immunosuppression followed by either allogeneic (same species) or autologous (self) marrow transplants could prevent and even reverse damage from autoimmune diseases. Three decades after these initial preclinical observations, our understanding of the therapeutic potential of immune restoration following autologous HSCT has deepened and the clinical evidence for its application in scleroderma has broadened.

In this review we will examine the outcome of conventional therapy of scleroderma lung disease, detail the techniques, toxicities and results of HSCT for SSc and explore the biology of immune restoration following autologous transplantation. We will compare the design and outcomes of randomized trials comparing HSCT with cyclophosphamide (CYC) and formulate criteria for the timely referral of patients with scleroderma lung disease for HSCT.
Pathogenesis of Scleroderma

The heterogeneous manifestations of SSc are the result of three primary pathogenic processes: vasculopathy, inflammation and fibrosis. As with the other connective tissue diseases, there is no single genetic abnormality conferring susceptibility to the disease; however, a permissive genetic environment is likely necessary for initiation of disease. For example, there is an increased relative risk of SSc in first degree relatives of patients with the condition and a number of human leukocyte antigens are associated with SSc, several of which correlate with specific ethnic populations or clinical features (1). Genomic analyses can differentiate normal from scleroderma skin and disclose associated fibroinflammatory and interferon (IFN)-associated gene signatures (2, 3). Among the chemokines, CXCL4 has been shown to predict the risk and progression of SSc (4). Epigenetic modification, including DNA methylation, histone modification and microRNA expression also appear to play roles in the pathophysiology of SSc.

Phenotypic changes in SSc are driven by vascular damage, immune dysregulation, fibroblast activation and collagen deposition (1). In a permissive genetic environment, increased circulating factors such as endothelin-1 (ET-1) promote vasoconstriction and injury to the endothelium. Vascular injury leads to a cascade of inflammatory cytokines, growth factors and reactive oxygen species which propagate tissue damage. Platelet derived growth factor can promote complement directed endothelial cell injury, and aberrant transforming growth factor (TGF)-beta and ET-1 signaling appear essential for myofibroblast activation and profibrotic signaling.
Mortality in Scleroderma

While SSc is a rare disease with an incidence of only 20 patients per million adults per year, it disproportionally impacts women in their 30’s to 50’s and is both debilitating and deadly. Independent risk factors for mortality in SSc include the presence of anti-topoisomerase I antibodies as well as pulmonary, renal or cardiac organ involvement. Rapidity of progression of skin thickness has been shown to predict internal organ involvement and mortality (5). Meta-analysis of 40 years of publications comprising 2,691 patients with SSc has provided Standardized Mortality Ratios (SMRs, the ratio of deaths compared to the expected deaths in the general population matched for age and gender) for the disease. Analysis showed that the pooled SMR for SSc was 3.5 (95% CI, 3.0-4.1) with little apparent change over the past four decades (6). Similarly, a 15 year follow-up of 398 patients with SSc enrolled between 1995 and 1999 at the Royal Free Hospital showed an overall SMR of 3.8 (ie, 280% greater mortality than in the general population) (7). Diffuse cutaneous scleroderma had a worse outcome than limited disease and females with diffuse cutaneous SSc had a remarkable SMR of 7.1. Organ (often lung) involvement denoted a much poorer prognosis compared to patients without organ complications (7). Scleroderma (either limited or diffuse) with organ involvement remains a progressively fatal disease without evidence of a survival plateau (Figure 1).

Scleroderma Lung Disease

While multiple target tissues may be damaged, pulmonary involvement is the leading cause of death in SSc (8). Detection of scleroderma associated interstitial lung disease (ILD) requires both
pulmonary function testing (PFT) and high-resolution computer tomographic (HRCT) imaging of the lung (9). In a systematic review of 20 publications comprising 1,524 patients, DLCO (diffusion capacity of the lung for carbon monoxide) was the most consistent predictor of mortality in scleroderma lung disease while the extent of disease on HRCT imaging was an independent predictor for overall mortality and ILD progression (10). Pulmonary fibrosis by HRCT and either an FVC (forced vital capacity) < 80% predicted or a decline of > 10% in the FVC or a decline of > 15% in the DLCO on serial measures constitute accepted indications for treatment (11). However, lung fibrosis is rarely reversible with conventional therapies.

While uncontrolled case series report varying responses to several disease modifying antirheumatic drugs (DMARDs) used alone or in combination, only CYC given for one year has been shown to be of benefit when compared to placebo administration (12). In the Scleroderma Lung Study (SLS) the degree of FVC benefit of CYC was modest (2.5% mean difference in the adjusted 12-month FVC between the arms), peaked at 6 months off therapy and was lost by 12 months off treatment (13). In a recently completed second SLS study, there was comparable stabilization and improvement in FVC with a 2-year course of mycophenolate mofetil (MMF) when compared to a 1-year course of CYC (14). Moreover, MMF appeared better tolerated than CYC. Ongoing other studies are exploring agents which inhibit fibrosis, TGF beta or Wnt signaling and B-cell activity.

Several issues emerge in review of the published literature of treatment of scleroderma lung disease. Other than the aforementioned SLS, most trials are comprised of less than 30 participants and can suffer from selection and publication bias, while studies demonstrating...
ineffectiveness may be under-reported. Most studies measure the change in percent predicted FVC, DLCO and/or changes on HRCT scans while others also report disease activity measures such as the modified Rodnan Skin Score (mRSS) and health assessment questionnaire (HAQ) scores. Few studies compare the treatment group with a control. Outside of CYC, MMF has been the next most commonly studied drug and doses of 2-3g daily were generally associated with stability of pulmonary function tests. However, many studies lasted only 6-12 months and a longer treatment course and follow-up may be necessary.

Animal Models of Autoimmune Diseases Treated with Myeloablation and HSCT

Preclinical models of autoimmune disease published in the 1980’s demonstrated a remarkable ability to prevent or reverse autoimmune disease following lymphomyeloablative conditioning and either allogeneic or autologous bone marrow transplantation (BMT). This efficacy was postulated due to the effects of total body irradiation (TBI) ablating the host autoreactive immune repertoire which was then replaced with normal allogeneic or autologous stem cells in the absence of the original antigenic trigger of the autoimmune disease. Genetic murine models of diabetes mellitus showed that TBI (which ablates both resting and dividing lymphoid immune cells) and allogeneic BMT could prevent subsequent development of the autoimmune disease (15). Importantly, established organ involvement in the genetic diabetes model (loss of islet cells in the pancreas) or in the lupus model (presence of immune complexes in the kidney) could be reversed after TBI and allogeneic BMT. Similar effects in a rat model of antigen-induced arthritis were observed after TBI and autologous BMT (16). In these animal experiments, myeloablative doses of TBI and CYC were significantly more effective than nonablative CYC
alone in producing sustained elimination of the autoimmune disease following autologous BMT (17).

**Initial Clinical Experience of HSCT for Autoimmune Diseases**

By 1997 several teams had published clinical designs for HSCT for autoimmune disorders (18, 19). In the rare situation of autoimmune disease co-existing with a hematologic malignancy, myeloablative allogeneic BMT appeared to offer long-term remission of both diseases (20). The first report of an individual undergoing successful autologous HSCT for SSc was published in 1997 (21). Shortly thereafter, European registry experience reported dramatic improvement in dermal fibrosis after autologous HSCT in SSc (22). By 2009, the number of reported patients with SSc treated with autologous HSCT had reached 224 individuals in Europe (23) and 97 in the North/South America (24). In these registry reports of transplants for autoimmune diseases, scleroderma was second only to multiple sclerosis as an indication for autologous HSCT.

**The Process of Autologous HSCT**

Spurred by research involving ionizing radiation following World-War II, the first successful human autologous HSCT was performed in 1957. Once comprehensive worldwide registries of HSCT became available, records showed that over one million individuals with malignant and non-malignant diseases had received autologous (58%) or allogeneic (42%) hematopoietic cell transplants between January 2006 and December 2014 in 1516 centers in 75 countries (25). During this 9 - year period, there were 1,162 transplants for autoimmune diseases: 1,058 autologous and 104 allogeneic.
HSCT is a multistep process involving: 1) hematopoietic cell mobilization, cell harvesting, selection and cryopreservation; 2) preparative conditioning with chemotherapy +/- irradiation; 3) infusion of the stem cell graft; and 4) supportive care after the transplant (26, 27). Mobilization of CD34+ hematopoietic stem cells from the marrow to the bloodstream is promoted by administration of hematopoietic growth factors such as granulocyte-colony stimulating factor (G-CSF) with or without CYC priming. Circulating blood stem cells are then collected by leukapheresis often in a one day collection. To further purify stem cells and remove differentiated lymphoid or other contaminating cells, positive stem cell selection by CD34+ isolation techniques (fluorescence or magnet-activated cell sorting) can be used before cryopreservation of the autologous cells (27). Once thawed, cells are infused intravenously and home to the marrow. These hematopoietic stem cells are able to repopulate and differentiate into erythrocytes, and megakaryocytes, and immune cells. Mediators of innate immunity, such as macrophages, granulocytes, and NK-cells repopulate and function early after HSCT. Adaptive immune cells, including effector and regulatory B and T cells also recover early while full function and immune memory return more gradually (28).

Multiple regimens have been employed to prepare patients for transplant, ranging from nonmyeloablative conditioning (CYC 200 mg/kg) to fully lymphomyeloablative regimens (high-dose chemotherapy +/- TBI). For the less intensive regimens, most patients can be cared for in outpatient “day hospitals” while residing in apartments near the transplant center. Others remain inpatient until blood counts have engrafted. Sustained granulocyte recovery occurs 10 to 12 days after transplant and most patients return home shortly thereafter. While at the center, family or friends assist as accompanying caregivers, a vital role in the recovery of the patient.
Complications associated with autologous HSCT include preparative regimen-related organ toxicity, opportunistic infections, infertility, recurrence of disease, and secondary malignancies (29). Autologous transplant-related mortality is approximately 3-5% for patients with myeloma and lymphoma and approximately 5-10% for those with scleroderma (23, 27, 30, 31).

Transplant is an intensive procedure with significant side effects including nausea, vomiting, diarrhea and oral mucositis which usually resolve with blood count recovery. Organ toxicity may be due to the preparative regimen and any underlying organ damage from SSc. Acute kidney injury reports led to several scleroderma-specific renal protective regimens (32, 33).

Gastrointestinal endoscopy has revealed gastric antral vascular ectasia (GAVE) in 22% of scleroderma patients screened before HSCT (34). Coagulation therapy of the GAVE lesions has been important in preventing hemorrhage during thrombocytopenia. All referred patients with scleroderma ILD will have diminished PFTs. For safety, most centers exclude individuals with FVC or DLCO < 45% predicted, although the lower limits are difficult to assess given other comorbidities (35). Conditioning regimens include varying doses of CYC which can be associated with acute cardiotoxicity which appears dose and schedule dependent. At a normally tolerated CYC dose of 200mg/kg given before transplant, cardiac deaths have been reported which raises the question of additional comorbidity from existing SSc heart disease (30).

Opportunistic infections are the leading cause of mortality after HSCT for autoimmune diseases (23). While neutrophils recover within two weeks of transplant other components of immune recovery may take up to 6-24 months (28). That said, total infections and serious infections
(ie, those treated as an inpatient) cluster within the first month after autologous HSCT. In four time intervals (day 0-30, days 31-180, days 181-365 and 366-730), the rates of total/serious infections were 5.75/4.90; 0.94/0.54; 0.29/0.05; and 0.16/0.08, respectively, among 56 individuals prepared with CYC, TBI, and ATG before autologous HSCT for autoimmune disease (28). Prevention of infection after transplant is becoming increasingly refined since the introduction of sensitive PCR assays for a number of viral pathogens (36).

**Phase I and II Trials of Autologous HSCT for Patients with SSc**

While allogeneic HSCT for autoimmune disease has been reported, most centers have focused on autologous transplant where fewer complications are observed. Consensus conferences were held in Basel and Seattle to establish frameworks for clinical trials and reporting (18, 19). Follow-on studies refined the pretransplant conditioning regimens. Protocols enrolled high-risk scleroderma patients with internal organ involvement but for safety reasons excluded individuals with severely compromised organ function (37). Results of initial pilot trials demonstrated dramatic improvement in skin scores and quality of life measures with stabilization of pulmonary function (22, 23, 38). Figure 2 depicts outcomes in 34 individuals with SSc with up to 8 year follow-up after a phase II trial of myeloablative autologous HSCT (38). Before transplant, mean measures for the cohort included a mRSS score of 30.1, a modified HAQ score of 1.85, FVC of 71% predicted and DLCO of 60% predicted. Over time, there were significant and durable improvements in mRSS (A) and HAQ (D), with stabilization/modest improvement in FVC (B) and DLCO (C).
Phase III Trials of Autologous HSCT for Patients with SSc

While encouraging, pilot studies underscored the need to control for potential selection or reporting biases by conducting prospective, randomized clinical trials comparing HSCT to CYC (the only DMARD shown of benefit in a controlled trial of scleroderma lung disease). Results from the ASSIST (39) and, ASTIS (31) randomized clinical trials have been published while the SCOT randomized trial results will be available in late 2016 after the last enrolled subject is evaluated at the 54th month primary endpoint. Table 1 details these three controlled studies. As shown, there are both similarities (inclusion and exclusion criteria) and differences (primary endpoints, preparative regimens, stem cell mobilization and selection techniques and length of follow-up) across the trials. Most randomized subjects had scleroderma internal organ involvement (predominately lung) although the ASTIS trial enrolled late in the study high-risk skin-only subjects (5, 31). High-dose CYC was employed for both stem cell mobilization and preparative conditioning in the nonmyeloablative ASSIST and ASTIS trials. In contrast, the SCOT trial mobilizes with G-CSF alone, uses a lower dose of pretransplant CYC, and employs TBI conditioning which is fully lymphomyeloablative. A significantly higher dose of CYC before transplant may account for reported cardiotoxicity in the nonmyeloablative scleroderma trials (30). In the single center ASSIST trial, all patients in the CYC arm failed to respond to treatment and all HSCT subjects improved. Criticism has been voiced concerning the small number of patients, short duration of follow-up and cross-over design (40).
Figure 3 depicts overall survival and event-free survival (survival free of organ failure) in the multicenter ASTIS trial. As expected, mortality was higher in the first year after transplant. The curves crossed at 2 years and thereafter HSCT was statistically superior to CYC for both event-free survival and overall survival (31). Table 2 presents morbidity outcomes in the ASTIS study. Rodnan scores, FVC, total lung capacity (TLC), disability ratings, and physical functioning were all significantly improved after HSCT compared to pretreatment assessments and these improvements were superior to similar serial assessments after CYC. Compared to their baseline tests, HSCT recipients had improvement over time in FVC and TLC; conversely, CYC recipients had worsening in pulmonary function over time. The difference in pre and post-transplant DLCO values for the two groups did not statistically differ. As expected, there were more grade 3 adverse events with HSCT but there were no significant differences in grade 4 events.

The Immunomodulatory Effects of Autologous HSCT

Since SSc is an aberrant activation of the adaptive and innate immune systems, several immunologic factors have been cited in examining these improved outcomes with HSCT. In both allogeneic and autologous HSCT, stem cell rescue allows delivery of very high doses of potentially disease-attenuating immunosuppression to patients with autoimmune disease. Allogeneic transplants provide a new immune system from a normal stem cell donor (15, 20). However, clinical experience with allogeneic transplants for autoimmune disease is too limited to afford comparisons with autologous HSCT in regard to long-term control of the autoreactive disease.
Autologous HSCT could “reset” the host immune system to a point in time when the antigenic triggers of autoimmunity were not present (41). Illustrative of this point, is the fact that pre-HSCT immunity wanes and often disappears after autologous HSCT. In recipients of TBI conditioning and autologous HSCT, the T-cell receptor (TCR) repertoire diversity was shown to normalize after lymphoablation and autologous transplant (42). Thymopoiesis renewal in adult recipients of autologous HSCT is also evidenced by thymic regrowth on imaging, a broadened diversity of the TCR Vβ repertoire among memory cells, and recovery of normal CD4+ T-cells. Conversely, individuals who did not respond to autologous transplant had less T-cell diversity early after immune recovery (43). Patients with SSc have diminished regulatory T-cell (Treg) proportions. Restoration of human Tregs has also been observed after autologous HSCT (44). In a murine congenic BMT model of proteoglycan-induced arthritis, the genetic background of the transplanted bone marrow was identical between host and donor except for the congenic T-cell marker. Treg pools of donor origin were shown to reconstitute a stable, tolerant immune repertoire (45). After human HSCT for juvenile idiopathic arthritis and dermatomyositis, renewal of regulatory T-cells was noted after autologous HSCT with evidence of a more diverse Treg TCR compartment (45, 46). Figure 4A and 4B illustrate this immune restoration after autologous HSCT.

**Future Directions**

Further explorations of genomic, immunologic, and cell signaling pathways in SSc are currently underway through mechanistic studies associated with the SCOT trial. For example, compared to age and gender matched healthy volunteers, pre randomization peripheral blood samples from SCOT patients showed differentially expressed genes (DEGs), mostly of the IFN signaling
pathways. When compared to their baseline samples, HSCT recipients exhibited significant declines in the IFN transcript score at month 26. In contrast, CYC recipients had no change over time (47). These genomic findings could further support the notion that autologous HCT can be associated with restoration of immune homeostasis.

Altered B cell homeostasis and high levels of B cell activating factor (BAFF) have been noted in patients with SSc (48). Patients treated with autologous HSCT have robust B cell recovery associated with rapid decline in BAFF levels. In addition to a diverse peripheral B cell pool that can potentially outcompete for BAFF, regulatory B cells likely arise after autologous HSCT. IL-10 producing B10 cells have been found to be critical for SSc attenuation in murine models.

Taken together, these data suggest that immune tolerance can be induced with HSCT. Figure 4C and 4D present a working model of how autologous HSCT results in immune tolerance/ immune homeostasis.

**Indications for Autologous HSCT in Scleroderma Lung Disease**

Based upon the above data, the following abridged criteria broadly serve as indications for referral for HSCT in patients with SSc.

**Inclusions:**

- Diffuse SSc with internal organ involvement
- Age < 65 years
- Disease duration < 5 years
- Rodnan skin score > 15
• Early pulmonary involvement by HRCT and PFTs with:
  • FVC or Hgb-adjusted DLCO between 80% - 45% predicted or
  • Decline in FVC >10% or DLCO > 15% on serial testing or
  • Failure to respond to initial therapy on serial PFT monitoring

Exclusions:
• FVC or Hgb-adjusted DLCO <45% predicted
• Pulmonary artery hypertension
• Cardiac insufficiency/ involvement with SSc
• Renal insufficiency
• Prior CYC > 6 months duration

Patient and Physician Choice of Treatment

Barriers to the application of HSCT for autoimmune disease include restrictions in insurance support and physician referral (41). Health insurer support for scleroderma transplants currently face fewer restrictions because more individuals now have health insurance coverage and two published randomized trials have shown significant clinical benefit with HSCT (31, 39). Physician referral preferences can also be a barrier as witnessed by patients’ self-referral for transplant consults. Patients wish to learn of the risks as well as the potential long-term gains with HSCT. However, rheumatologists may be unfamiliar with the details of a treatment outside their specialty or be concerned about the associated risk.
Concern for premature mortality from SSc is the major reason individuals seek out consultation for stem cell transplant. A new sense of urgency in scleroderma lung disease can be likened to the evolution of thinking in rheumatoid arthritis (RA). Once premature mortality in RA was demonstrated (SMRs of 1.2-1.7), a chronic autoimmune disorder became an urgent disease for the rheumatologist (49). If such urgency has been true in RA (with mortality rates 20%-70% greater than the general population), then it is even more pressing in scleroderma lung disease with mortality 250%-280% greater than expected (6, 7).

While DMARDS and antifibrotic agents may demonstrate initial improvement in PFTs, the key consideration for both patients and physicians is the effect on the premature mortality associated with scleroderma lung disease. Since the ASTIS trial has demonstrated improved long-term survival and event-free survival after HSCT, consideration for stem cell transplant referral is a reasonable action but requires close PFT monitoring before pulmonary compromise becomes too severe for safe transplant. In such an urgent and evolving situation, it is critical that the practitioner offers timely referral to a transplant center for evaluation and discussion of the techniques, toxicities and outcomes of HSCT. Without referral, patients will be without the knowledge and context to make informed decisions. Ultimately, this shared decision making is a partnership of physician and patient to make the right choice of treatment in light of the individual’s situation and values (50).

Conclusions

While there are often multiple disabling manifestations of SSc, lung disease is the major cause of death. Although conventional DMARDS show modest short-term benefit, the
The published literature of SSc shows a pooled SMR of 3.5 (250% increase in mortality) with little change over decades of reporting. Hence, premature mortality remains of urgent concern to patients and providers.

The basis for recommending stem cell transplant in SSc arose from insightful animal experiments showing HSCT could prevent and reverse organ damage in genetic and antigen-induced models of autoimmune disease. Subsequent laboratory studies have revealed that benefit arises, in part, from the reinfused autologous stem cells restoring thymopoiesis, broadening diversity of the TCR repertoire, renewing regulatory T-cells and altering disease signatures of differentially expressed genes. In the absence of the original antigenic triggers, immune homeostasis is restored and self-tolerance can return.

But do the benefits of autologous HSCT outweigh the risks? Likely not in autoimmune diseases with low associated mortality, but for scleroderma lung disease the benefits appear compelling. Replicated in multiple phase II reports from Europe and the US, dramatic and durable improvements in skin fibrosis and quality of life measures have been observed along with stabilization of PFTs. Three prospective, randomized clinical trials in patients with SSc and internal organ involvement have compared autologous HSCT treatment to high-dose IV pulse CYC given for up to 12 months. The SCOT trial is still following all subjects through the 54th month primary endpoint, but as detailed above the ASSIST and ASTIS randomized trials have been completed and both report statistically significant clinical benefits after stem cell transplantation.
Given the morbidity and inexorable mortality of SSc with organ involvement, it appears reasonable to consider consultation for HSCT treatment and listings of experienced scleroderma research and transplant centers are available online (www.sclerodermatrial.org/announce/sitelist.html and www.scleroderma.org/site/PageServer?pagename=patient_centers_list#.Vry-ObQrJeg).

In the choice of transplant, patients will be accepting more upfront mortality risk for long-term gain; hence, they must be carefully informed of risks and fully participate in the decision. Criteria for referral to the HSCT center are given above but close monitoring of pulmonary function is required for timely referral. During the transplant consultation, the procedures and toxicities are detailed along with the evidence supporting HSCT as treatment for scleroderma lung disease. Some patients will accept transplant and others will decline. Not surprisingly, such critical decisions about treatment, as Thoreau observed, can best be reached as patients and practitioners look through each other’s eyes, if only for an instant.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article and revising it critically for important intellectual content, and all authors approved the final version to be published.
REFERENCES


contribute to its pathogenesis through high BAFF gene expression and high collagen synthesis. Ann Rheum Dis. 2015; 0:1-7


Table 1. Randomized Trials of HSCT in SSc

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<td>SSc duration (years)</td>
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**Progression of SSc or No Response**

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**Overall Mortality**

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<th>HSCT</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Mortality</td>
<td>0/9</td>
<td>0/10</td>
<td>30/77</td>
<td>19/79</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

A. American Scleroderma Stem Cell versus Immune Suppression Trial (39)
B. Autologous Stem Cell Transplantation International Scleroderma (31)
C. Scleroderma: Cyclophosphamide or Transplantation (ongoing)
D. 17 Rheumatology and 8 Transplant Centers
E. Insurance denials for clinical trial coverage often precluded randomization
F. By cardiac echo, other values by right heart catheterization
G. Lung and Kidney shielded to 200 cGy transmission (33)

**Abbreviations:**
ATG, Anti-thymocyte Globulin
BAL, Bronchoalveolar Lavage
CYC, Cyclophosphamide
DLCO, Diffusion Capacity of the Lung for Carbon Monoxide
FVC, Forced Vital Capacity
G-CSF, Granulocyte Colony Stimulating Factor
HRCT, High Resolution Chest Tomography
HSCT, Hematopoietic Stem Cell Transplant
ILD, Interstitial Lung Disease
IV, Intravenous
LVEF, Left Ventricular Ejection Fraction
PAP, Pulmonary Artery Pressure
NA, not available
SSc, Systemic Sclerosis
TBI, Total Body Irradiation
TLC, Total Lung Capacity
Table 2. ASTIS Treatment Responses: Change in the area under the time response curve from baseline to year 2 follow-up (A)

<table>
<thead>
<tr>
<th></th>
<th>AUC, mean</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSCT</td>
<td>CYC</td>
<td>Difference</td>
<td>P-Value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(N=67)</td>
<td>(N=64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodnan skin score</td>
<td>-19.9</td>
<td>-8.8</td>
<td>11.1</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>+6.3</td>
<td>-2.8</td>
<td>-9.1</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>TLC (% predicted)</td>
<td>+5.1</td>
<td>-1.3%</td>
<td>-6.4</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>DLCO (% predicted)</td>
<td>-4.7</td>
<td>-4.1</td>
<td>0.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HAQ – DI</td>
<td>-0.58</td>
<td>-0.19</td>
<td>0.39</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>SF36 (physical)</td>
<td>10.1</td>
<td>4.0</td>
<td>-6.1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Grade 3 AE</td>
<td>38</td>
<td>20</td>
<td></td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Grade 4 AE</td>
<td>29</td>
<td>21</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

(A) Van Laar, JAMA 2014; 311: 2490 (31).

**Abbreviations:** AE: Adverse Event; AUC, Area Under the Curve; CYC, Cyclophosphamide; HAQ-DI, Health Assessment Questionnaire-Disability Index; HSCT, Hematopoietic Stem Cell Transplant; NS, Not Significant; OF, Organ Failure; SF36, 36 items Short Form Health Survey
Legends to Figures

Figure 1. Survival of patients with limited (L) or diffuse (D) cutaneous systemic sclerosis (cSSc) with internal organ complications from the Royal Free Hospital experience (illustration adapted from 7).

Figure 2. Results after TBI, CYC ATG preparative conditioning and CD4\(^+\) 34 selected autologous HSCT in 34 patients with severe scleroderma: A, Rodnan skin score; B, Forced Vital Capacity; C, DLCO; D, Quality of life measures (mHAQ). Dark solid lines denote the mean and dashed lines represent the generalized estimating equation (GEE). Gray lines represent individual patient values (38).

Figure 3. Results from the ASTIS trial comparing CD4\(^+\) 34 selected autologous HSCT after conditioning with CYC and ATG compared with results after 12 months CYC (controls). Left panel, event-free survival (survival free of organ failure). Right panel, overall survival (31).

Figure 4. Immune homeostasis following autologous HSCT.

Panels A and B: T cell receptor (TCR) diversity in Tregulatory (Tr) cells restored after autologous HSCT in patient in A with autoimmune disease and 4 healthy controls (HC) in B. The number (N) of different TCR sequences per sample and diversity (D, where 0 = none and 1 = maximum) are illustrated pre and post transplant (45, illustration adapted from 46).

Panels C and D: C. Outcompetition of non-autoreactive lymphocytes result in death of autoreactive clones. IL-2 and B cell Activating Factor (BAFF) increase after
lymphomyeloablation (1) Increased proportions of regulatory T and B cells, both known to suppress or kill effector T and B (2). D. Supranormal numbers of B cells and activated regulatory cells eliminate autoreactive clones during immune recovery. The ‘surge’ in cell numbers abates as immune homeostasis is achieved.
Survival rate vs. Time to death (months)

- IcSSc - with complications
- dcSSc - with complications

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Surviving at 60 mos</th>
<th>Surviving at 120 mos</th>
<th>Surviving at 180 mos</th>
<th>Surviving at 216 mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>L - with complications</td>
<td>56</td>
<td>48</td>
<td>44</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>D - with complications</td>
<td>89</td>
<td>75</td>
<td>59</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Group</th>
<th>Number at risk</th>
<th>Number of patients dying</th>
</tr>
</thead>
<tbody>
<tr>
<td>L - with complications</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>D - with complications</td>
<td>89</td>
<td>75</td>
</tr>
</tbody>
</table>

338x190mm (300 x 300 DPI)
Figure 2. Results after TBI, CYC ATG preparative conditioning and CD4+ 34 selected autologous HSCT in 34 patients with severe scleroderma: A, Rodnan skin score; B, Forced Vital Capacity; C, DLCO; D, Quality of life measures (mHAQ). Dark solid lines denote the mean and dashed lines represent the generalized estimating equation (GEE). Gray lines represent individual patient values (38).
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