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C O N F I D E N T I A L

A Phase II Multi-Center Study of High-Dose Cyclophosphamide and Antithymocyte Globulin Followed by Autologous Hematopoietic Cell Transplantation with Post Transplant Maintenance for the Treatment of Systemic Sclerosis

Short Title: Scleroderma Treatment with Autologous Transplant (STAT) Study

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Study Sponsor: Scleroderma Research Foundation

The Principal Investigator from each site should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the coordinating center at Fred Hutchinson Cancer Research Center.

I confirm that I have read the above protocol and the corresponding consent form in the latest version. I understand them, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry – E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

As the Principal Investigator, I agree to conduct "A Phase II Multi-Center Study of High-Dose Cyclophosphamide and Antithymocyte Globulin Followed by Autologous Hematopoietic Cell Transplantation with Post Transplant Maintenance for the Treatment of Systemic Sclerosis." I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the protocol chairs.

_____
Principal Investigator (Print)

_____
Principal Investigator Signature

_____
Date

FLOW DIAGRAM OF PROTOCOL

Select 30 Subjects by Screening

↓

HDIT transplantation
(30 Subjects)

↓

Stem Cell Mobilization ≥ $2.5x10^6$ CD34 cells/kg

↓

Transplant Regimen	Day									
	-5	-4	-3	-2	-1	0	+1	+3	+5	+60
IV Methylprednisolone (1 mg/kg, prior to ATGAM)	X		X		X		X	X	X	
ATGAM (15 mg/kg/day)	X		X		X		X	X	X	
CY (50 mg/kg/day)	X	X	X	X						
Mesna (50 mg/kg/day) or Continuous Bladder Irrigation	X	X	X	X						
Hydration (50-200 cc/hour)	X	X	X	X						
Autologous HCT (≥2.5 x 10^6 CD34+ cells/kg)						X				
MMF mainenance up to 2 grams daily 2+ months-2 years post										X

↓

Subjects will have follow-up 28 days after infusion of autologous hematopoietic cell graft. Subjects will have safety labs according to the study schedule in addition to physical exams for the first 12 weeks after treatment and then at 6 months, then annually x 5 years.

Disease assessments for endpoint evaluation will be conducted at 4, 12, and 26 weeks, and then annually after autologous HCT. The visits will consist of some or all of the following evaluations, (as detailed in Section 6 and Appendices E and F): physical exam, 6MWT, SGRQ, SF-36, LVEF, PFTs (DLCO & FVC), high resolution chest CT scan, CBC w/diff, comprehensive metabolic panel, renal function measures, CPK and additional pulmonary and cardiac evaluations if clinically indicated. Maintenace therapy with mycophenolate mofetil will be started at 2-3 months after autologous HCT and continued for 2 years. Subjects will be followed for 5 years after treatment.

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1. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1. Systemic Sclerosis: Rationale for study

Systemic sclerosis (SSc) is a collagen vascular disease resulting from autoimmune mechanisms. SSc is a disabling and fatal disease with no proven highly effective therapies and a median survival in a subset of patients with diffuse cutaneous involvement of 5 years. This is a phase II clinical trial to investigate the use of high-dose cyclophosphamide (CY) and anti-thymocyte globulin followed by autologous hematopoietic cell transplantation (HCT) without CD34-selection for SSc. Patients with less advanced disease than those currently eligible for the SCOT clinical trial, including skin only disease, will be eligible since treatment is expected to be well tolerated with potential benefits of improved skin score and prevention of internal organ dysfunction which may not be reversible.

1.1.1. Epidemiology, Diagnosis and Clinical Course of Systemic Sclerosis

Systemic sclerosis is an autoimmune disease in which the skin, lungs, heart, gastrointestinal tract and kidneys are major targets for progressive and often relentless fibrosis.[1] This autoimmune disorder is quite variable in its manifestations and can be categorized into two primary subsets: diffuse cutaneous and limited cutaneous. As compared to limited cutaneous SSc, diffuse cutaneous SSc usually has involvement of internal organs, more rapid onset, and a decreased survival and, therefore, will be the focus of the current study. Marked visceral involvement is often present within 5 years of the first symptom.

1.1.1.1. Epidemiology of SSc

SSc is an uncommon disorder, and virtually all of the descriptive epidemiology is from studies of patients admitted to hospital or attending academic institutions. The disease is rare in childhood, and its incidence increases steadily among adults and peaks in the mid-40s to the early 50s. The disease disproportionately afflicts females more than males (3F:1M), especially during childbearing years.[1] In a community study from 1985-86, the point prevalence of SSc was 253-290 per million. The incidence (new cases/year) was reported as 14.1 per million.[2] SSc occurs most frequently and severely in young black women and Choctaw Native Americans, but otherwise there are no prominent racial differences. The disease occurs more frequently in first-degree relatives of patients with SSc, although the absolute risk is low, varying from 1 to 1.6%.[3] Environmental or occupational exposures to agents such as vinyl chloride, benzene derivatives, L-tryptophan, silica, and organic solvents are thought to play a role in the development of the disease.[4]

1.1.1.2. Pathogenesis of SSc

The pathogenesis of the fibrous and vascular lesions remains poorly characterized. A subset of skin fibroblasts from SSc are activated and synthesize increased quantities of fibronectin, proteoglycan core proteins and collagens types I and III.[5] It is believed that these fibroblasts are activated *in vivo* by cytokines derived from lymphocytes and monocytes/macrophages recruited during the associated inflammatory process. Organ fibrosis is the dominant manifestation of the disease and is likely to be a later event resulting from inflammation and vascular injury. Vascular abnormalities associated with SSc include 1) vasomotor instability (Raynaud's phenomenon) with repeated transient interruption of perfusion in digits and internal organs, 2) microvascular abnormalities with proliferative intimal arterial lesions and obliteration of the vessels leading to chronic ischemia, and 3) intravascular pathology such as plugging of vessels secondary to abnormal red cell deformation, increased platelet activity and

enhanced thrombus formation.[1,6,7] The vascular abnormalities are thought to be related to endothelial damage occurring secondary to both cytokine and non-immune cell-mediated cytotoxicity.[8,9] In particular, endothelin-1, a potent mitogen both for fibroblasts and smooth muscle cells, is overproduced by serum monocytes of patients with SSc and by lung tissue macrophages of patients with primary pulmonary hypertension, potentially causing both fibrotic events and vasospasm.[10,11] Studies of cell-mediated immune toxicity have shown that peripheral blood mononuclear cells can kill endothelial cells when co-cultured in the presence of sera, possibly mediated by IL-2[1]. Microchimerism, the stable presence of small populations of allogeneic cells resulting from maternal-fetal cell traffic during pregnancy, has also been implicated as a possible etiologic factor in disease development.[12]

1.1.1.3. Clinical manifestations of SSc

In the early stages of cutaneous SSc, mononuclear cells are present in the dermis, particularly around blood vessels.[7] Secreted monokines and lymphokines may be central in the further evolution of the disease. The cellular infiltrate is eventually replaced by fibrosis that may potentially extend deep into the connective tissue to surround tendons, muscle bundles and joint capsules. The clinical features of SSc bear similarities to other autoimmune disease such as systemic lupus erythematosus (SLE) and dermatomyositis with some patients reported to have overlap syndromes.[1] In addition, there are familial associations of SSc with other autoimmune diseases such as rheumatoid arthritis and SLE. The association of SSc clinical subtypes and antibody subsets with certain class II HLA alleles known to vary among different ethnic groups lends more support to the concept that the pathogenesis is immune-related for this disease.[13]

There are a varied but large number of autoantibodies present in the serum of patients including antibodies against well-defined target epitopes. Three mutually exclusive SSc autoantibodies specific for the centromere, topoisomerase I (Scl-70), and RNA Polymerase III are particularly important.[14,15] The anticentromere antibody appears early in the disease and is primarily found in the group with limited cutaneous systemic sclerosis. In contrast, Scl-70 is found in 30-40% of patients with diffuse cutaneous SSc.[16,17] RNA Polymerase III is a marker for diffuse disease and renal crisis. Antinuclear antibodies occur in 95% of SSc patients. In a mouse model of SSc (tight skin mouse) in which both skin fibrosis and autoantibodies develop, similar topoisomerase I epitopes exist to autoantibodies from SSc patients.[18] Adoptive transfer of immunocompetent cells as bone marrow or T and B lymphocytes was sufficient in this model for activation of collagen synthesis leading to skin fibrosis in recipient mice.

The clinical manifestations of SSc are variable and may be limited in extent or diffuse and severe. Patients with limited cutaneous SSc present with a history of Raynaud's phenomenon for years, involvement of acral skin (hand, face, feet and forearms), skin calcifications, telangiectasia and occasionally pulmonary hypertension with or without interstitial lung disease. Seventy percent to 80% of these patients are positive for the anti-centromere antibody. Diffuse cutaneous SSc is more severe and is associated with Raynaud's phenomenon, both truncal and acral skin involvement, tendon friction rubs, early and significant incidence of interstitial lung disease, renal failure, diffuse gastrointestinal disease and myocardial involvement. The anti-centromere antibody is usually absent and 30-40% of patients will be positive for Scl-70 (specific for topoisomerase I). Nailfold capillaroscopy demonstrates capillary dilatation and destruction.

The sudden development of taut hidebound skin proximal to the metacarpophalangeal joints with adherence to deeper structures causes limitation of movement and contractures, profoundly affecting the quality of life of these patients. In long-term survivors, the fibrosis may regress with epidermal thinning and loss of hair follicles and sweat glands. SSc commonly involves the gastrointestinal tract. Over 90%

of patients have esophageal hypomotility with clinical manifestations of dysphagia and esophageal reflux. Small bowel disease with hypomotility and bacterial overgrowth can lead to malabsorption and a significant wasting syndrome. Total parenteral nutrition may be required. Atony and hypomotility of the colon may result in significant constipation, occasional obstruction, or anal incontinence.

The three major features of involvement of the cardiovascular system are: 1) myocardial fibrosis associated with left ventricular dysfunction and dysrhythmias; 2) pericardial effusion, including symptomatic pericarditis and cardiac tamponade; and 3) pulmonary hypertension with right ventricular hypertrophy and progressive respiratory insufficiency. Pulmonary hypertension is more common in patients with limited SSc, and it is attributed to marked intimal and medial hyperplasia of the pulmonary arteries during the fibrotic stage. Interstitial lung disease is typical in diffuse SSc and characterized by alveolitis that slowly evolves into fibrosis of the interstitium, which is usually irreversible with current therapy. In its early phases, the inflammatory process is assumed to be partially reversible by immunosuppressive treatment. As shown by studies with bronchoalveolar lavage, alveolitis is characterized by an increase in the yield of neutrophils and eosinophils in the lavage fluid. Most cells in the presence of alveolitis are still alveolar macrophages.[19,20] SSc patients with persistent alveolitis may have greater deterioration in their lung function than those patients with negative studies.

Renal disease or "renal crisis" is a severe complication of SSc. The administration of high-dose corticosteroids may predispose to this complication.[21] Renal crisis is clinically defined by the new onset of severe hypertension with a rapid increase in serum creatinine, microangiopathic anemia, or both. Multiorgan involvement, particularly heart and lung, is common and early death may occur. The characteristic renal lesion is an intimal proliferative lesion in the small arteries causing a reduction in renal plasma flow, and abnormal red cell deformation. Schistocytes may be seen on a peripheral blood smear. Onset of renal failure may be sudden from acute renal cortical ischemia or infarction and may be secondary to a hypertensive event. Incipient renal failure might be suggested with a CrCl < 60 ml/min or a decrease of >20 ml/min especially if associated with proteinuria or the new onset of hypertension. Angiotensin converting enzyme (ACE) inhibitors should be started at this time with the hope that this intervention might reduce the risk of the emergence of the full-blown syndrome. Patients with early diffuse SSc are at greatest risk of developing renal crisis and should be encouraged to monitor their blood pressure regularly. Prompt and aggressive treatment with ACE inhibitors has been shown to decrease the need for dialysis in SSC patients with renal crisis. However, early deaths (mean time of 3 months) occurred in 19% of patients.[22]

1.1.1.4. Prognostic factors in SSc

Several studies have attempted to identify early high-risk features that predict poor survival. In 1991, a report of 264 patients with SSc who were entered into the multicenter Scleroderma Criteria Cooperative Study from 1973-77 demonstrated a steady and rapid decrease in the survival rate for the entire cohort. Survival was less than 80% at 2 years, 50% at 8.5 years and 30% at 12 years. Renal, cardiac, pulmonary and gastrointestinal involvement in SSc predicted a further reduction in survival. Different combinations of variables led to different survival rates. In a more recent study of 706 verified SSc cases, median survival was 11 years. Again, renal, cardiac, pulmonary, and GI involvement in SSc predicted a further reduction in survival.[23] Other studies have confirmed the poor-risk features of SSc when there is internal organ involvement.[24-29] Skin involvement has also been found to correlate with survival, thus representing a useful clinical predictor of outcome. SSc patients with a modified Rodnan skin score of >15 have demonstrated reduced survival when compared to patients with lower skin score,[30] and another study has shown that improvement of skin score in early SSc patients correlates with improved survival.[31] A long-term follow-up study of 164 patients also identified proximal sclerosis, trunk skin

involvement and the presence of the Scl-70 autoantibody in addition to pulmonary and/or heart involvement as indicators of poor outcome.[32] A follow-up study of 953 SSc patients carried out at the University of Pittsburgh between 1972 and 1995 showed that patients with severe organ involvement had a 9-year cumulative survival rate of 38% compared with 72% in patients without such involvement.[33] Severe internal organ involvement occurred within the first 3 years from diagnosis in most of the cases. In particular, severe skin and renal disease occurred early in 70% of the patients, and lung disease in approximately 55% of cases. Both cardiac and renal disease were the leading cause of death in the first 5-years, whereas lung disease was the principal cause of mortality in the second 5-year span of the study.

Because severe disease manifestations tend to appear early in the course of the disease, efforts have been made to identify poor outcome measures as early as the first patient evaluation. A recent study showed that among different clinical variables, the presence of proteinuria, ESR>25 mm/hr and DLCO<70% at first presentation represented an accurate estimate of high-risk 5-year mortality on multivariate analysis in a population of 280 patients.[34] Patients with pulmonary disease as part of their systemic sclerosis often harbor alveolitis characterized by an increase in the yield of the total number of cells obtained during bronchoalveolar lavage with most cells being alveolar macrophages with some eosinophils, neutrophils, and lymphocytes. Patients with persistent alveolitis while on therapy have continued deterioration in their lung function compared to those patients with negative studies.[19,20] The introduction of standardized scales to measure scleroderma disease activity, damage and severity have helped to identify patients with the lowest possible risk/benefit ratio of treatment and have provided adequate follow-up parameters for treatment comparisons.[35] Patients with high disease severity and activity and mild-modcerate organ injury are ideal candidates for more aggressive treatments. A recent study demonstrated the reliability of a modified Medsger severity scale in predicting survival after a mean follow-up of 7.7 years.[36] Best entry predictors of survival were younger age, higher skin score, lower glomerular filtration rates, EKG abnormalities, and pulmonary function as assessed by lung capacity and static compliance. In a more recent study by EULAR, a high skin score >30 alone or proteinuria were associated with a high mortality.[37]

1.1.2. Current Therapy

1.1.2.1. Cyclophosphamide

In the Scleroderma Lung study (SLS), 12 months of daily oral cyclophosphamide was reported to be superior to placebo in slowing the rate of progression of pulmonary fibrosis in SSc.[38] Eligible subjects were those with ≤ 7 years of disease, who had pulmonary fibrosis and/or ground glass opacities on computerized tomography (CT) lung scans or those with active alveolitis as defined by BAL cell count and differential. Except for a sustained impact on dyspnea, all of these effects waned and were no longer apparent at 24 months.[39] In this study, individuals on cyclophosphamide also had a modest improvement in skin thickness score compared with the placebo group at the 12-month time points. The degree of skin improvement, however, was not as dramatic as that seen in the pilot hematopoietic cell transplantation (HCT) trial. Although therapeutic effects were modest, this study was important for demonstrating that SSc was responsive to immunosuppressive therapy.

Although not approved for this indication, cyclophosphamide has largely become a standard of care for individuals with early and severe SSc, particularly those with pulmonary involvement. For those who fail cyclophosphamide, due to either inefficacy or intolerable side effects, there are no controlled, prospective trials that demonstrate safety and efficacy of any other options for therapy in this disease.

1.1.2.2. Mycophenolate mofetil

Multiple uncontrolled clinical studies regarding mycophenolate mofetil (MMF) suggest that this drug is a reasonable alternative to cyclophosphamide. MMF has been shown to be effective in inducing remission in lupus nephritis with less toxicity than cyclophosphamide, the mainstay of therapy for lupus nephritis for more than 20 years.[40-42] As rheumatologists have become more accustomed to treating their lupus patients with MMF, they have turned to this drug to assess activity in SSc patients.

There is a strong rationale for using MMF in scleroderma. MMF is the ester prodrug of mycophenolic acid (MPA) and strongly inhibits both T- and B-lymphocyte proliferation and is now widely used in the prevention of acute and chronic allograft rejection.[43] Recent evidence also suggests that MMF is capable of inhibiting the proliferation of non-immune cells including smooth muscle cells, renal tubular cells, mesangial cells, and fibroblasts. MPA has been shown to be antifibrotic in animal models of renal fibrosis, suggesting that MMF may be effective in preventing fibrosis as well as providing immunosuppression. MMF is supplied as both an oral and intravenous formulation. The most common adverse effects associated with MMF are reversible myelosuppression, infections secondary to immunosuppression and gastrointestinal toxicity.

Regarding human studies directly relevant to use of MMF for scleroderma, Stratton et al.[44] reported a pilot study of anti-thymocyte globulin (ATG) and MMF in 13 SSc patients with recent-onset dcSSc. These subjects received ATG for 5 days, followed by MMF for 12 months. There was an improvement in skin score and apparent stability of systemic disease during the study period, and this combination of ATG and MMF appeared safe. Serum sickness developed in five patients after ATG treatment but was controlled by corticosteroid therapy, whereas MMF was well tolerated.

Liossis et al.[45] reported results of five consecutive dcSSc patients with recent-onset alveolitis treated with MMF alone. Pulmonary function test parameters, particularly forced vital capacity (FVC) and diffusing capacity in liters of carbon monoxide (DLCO), improved. Ground glass opacities on CT scan cleared in three patients and were reduced in a fourth, and cough and dyspnea also improved. A study of MMF by Swigris et al.[46] described improvement in percent predicted values of FVC and DLCO on average in their 28 patients with interstitial lung disease related to connective tissue diseases. Of the 28 patients studied, nine had scleroderma. They concluded that MMF was safe and well tolerated in this group of patients. In a more recent study by Nihtyanova et al., the clinical records of 109 patients treated with MMF and 63 control subjects receiving other immunosuppressive drugs were retrospectively reviewed.[47] The MMF and control groups were well matched in terms of basic demographic and clinical parameters. Treatment with MMF was very well tolerated. Of all patients, 12% experienced adverse reactions, with gastrointestinal tract disturbances and infections being most frequent. MMF was discontinued due to disease stabilization in 9%, side effects in 8%, and no effect on the disease activity in 14% of the patients. There was a significantly lower frequency of clinically significant pulmonary fibrosis in the MMF-treated cohort ($P = 0.037$) and significantly better 5-yr survival from disease onset and from commencement of treatment ($P = 0.027$ and $P = 0.012$, respectively). There was no significant difference between the two groups in terms of modified Rodnan skin score and forced vital capacity (FVC) change. These studies of MMF in systemic sclerosis demonstrated that it was very well tolerated and appeared to be at least as effective as the other current therapies for dcSSc. In other small studies similar results were also observed.[48]

Le et al. published a retrospective study of SSc patients on MMF compared to historical controls and noted improvement in skin at 12 months associated with improvement in severity scores and quality of life measures, as well as stable pulmonary function.[49] MMF, based on this experience in SSc as well

as in transplant, will be used as maintenance therapy after high-dose immunochemotherapy and autologous HCT in this proposal.[50,51]

1.1.2.3. Rituximab

Another immunomodulatory agent, rituximab, has been FDA-approved for treatment of patients with lymphoma and rheumatoid arthritis (RA).[52,53] Rituximab is a genetically engineered chimeric/ murine monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. The Fab domain of rituximab binds to the CD20 antigen on the B lymphocytes, and the Fc domain recruits immune effector functions to mediate B cell lysis in vitro. Possible mechanisms of cell lysis include complement-dependent cytotoxicity and antibody-dependent cell mediated toxicity.[54-59] Randomized clinical trials showed that the combination of rituximab with methotrexate (MTX) is superior than single agent therapy for rheumatoid arthritis (RA).[52,56,60] Additionally, there was improvement in patient related outcomes such as HAQ-DI, patient global VAS, fatigue, disability, and quality of life for RA and other immune-mediated diseases.[55,61-67]

The experience with rituximab for the treatment of patients with SSc is more limited but it is now being more commonly used because of efficacy in other autoimmune disorders and a low risk of adverse effects. There is one recent report of 15 patients with SSc who were treated with rituximab.[68] All patients were enrolled in the clinical trial within 18 months of first non-Raynaud's symptom. Patients were treated with rituximab 1000 mg intravenously x 2 doses separated by 2 weeks. No patient experienced a severe adverse event. There was not a significant change in the skin score from baseline. The average baseline mRSS in these patients was 20.6 ± 4.4 with the average score at 6 months of 20.2 ± 5.5 and at 12 months of 21.1 ± 5.2 (P = 0.82 and P = 0.83 respectively). Rituximab in this patient population did not appear to have a beneficial effect on the skin but none of the patients showed new or progressive pulmonary disease by HRCT or any signs of progressive cardiac disease and had stable ejection fractions and findings on electrocardiograms during follow-up. Additionally, none of the patients experienced renal crisis or symptoms suggesting progressive gastrointestinal disease. More recently, 2 other studies of rituximab for SSc demonstrated potential efficacy for skin disease in diffuse cutaneous SSc.[69,70] A significant decrease in skin score and an improvement in FVC and DLCO was observed. Although further studies are required, stability or improvement has been observed in SSc patients treated with rituximab.

1.1.2.4. Imatinib

Imatinib mesylate is a small molecule tyrosine kinase inhibitor effective at blocking both TGFß and PDGF signaling pathways. It has shown potent anti-fibrotic effects in pre-clinical models, including the bleomycin murine model. Other clinically available c-Abl-kinase inhibitors with similar effects in pre-clinical fibrosis models include dasatinib and nilotinib. These c-Abl-kinase inhibitors have been well tolerated in oncological diseases such as bcr-Abl-positive chronic myelogenous leukemia, with relatively rare severe side-effects, but frequent mild side-effects. Case reports and an open-label trial suggest efficacy of imatinib in diffuse SSc, though adverse events are common.[71] Initial clinical trials with imatinib for the treatment of fibrosis in SSc are in progress, with one controlled and multiple uncontrolled trials ongoing. In one as yet unreported phase 1/2a study (n=20), patients received imatinib for 1 year (Daniel Furst, Maureen Mayes; personal communication). There was a trend towards an improvement of FVC% of 1.74% ($p > 0.05$) and mRSS of 3.9 units ($p < 0.001$). Seven patients (35%) had to stop imatinib becase of adverse events. More results from larger clinical trials will elucidate the role of tyrosine kinase inhibition in the treatment of this disease.[72]

1.2. Summary of Pre-Clinical and Clinical Studies of High-Dose Immunosuppressive Therapy for Autoimmune Diseases including Systemic Sclerosis

The previous experience using high-dose immunosuppressive therapy and autologous HCT for severe autoimmune diseases will be reviewed. No studies have yet investigated the addition of immunosuppressive maintenance therapy posttransplant to reduce the risk of disease progression.

1.2.1. High-Dose Therapy and Autologous Hematopoietic Cell Transplantation

High-dose immunosuppressive therapy with autologous stem cell transplantation may be effective for the control of severe autoimmune diseases because the preparative regimen will profoundly suppress the host immune system. Preclinical studies in mouse models of autoimmune disease have demonstrated effective control of autoimmunity by congenic, autologous, or allogeneic marrow transplantation after a cytotoxic preparative regimen.[73,74] Pilot studies of HDIT followed by HCT have been conducted for various autoimmune diseases. High-dose immunosuppressive therapy with autologous stem cell rescue offers a comparatively safe and flexible approach to myelosuppression and intensive immunosuppression. High-dose immunosuppressive therapy with autologous stem cell rescue could be offered to suitable subjects up to 70 years of age. In SSc, conventional therapy as used to date has been minimally effective. The use of intensive chemotherapy with stem cell rescue offers an opportunity to deliver maximally tolerated myelosuppression and immunosuppression to test response of SSc to such treatment. High-dose therapy followed by autografting may also "reset" the immune system resulting in control of SSc if immune-mediated events contribute to the disease process.

1.2.2. Results of Studies of HDIT Transplantation in Autoimmune Diseases Other than Systemic Sclerosis

Earlier clinical studies in subjects who had blood cancers or aplastic anemia together with autoimmune diseases have demonstrated that allogeneic and autologous HCT may be effective for treating human autoimmune diseases.[75] Since that time HDIT has been applied to various autoimmune diseases. Aplastic anemia is considered an immune-mediated disorder, and the survival probability is 85-90% at 4 years after allogeneic marrow transplantation.[76] Aplastic anemia may also respond to high-dose cyclophosphamide. A joint EBMT /ABMTR registry analysis included 73 patients with rheumatoid arthritis who underwent autologous HCT after high-dose cyclophosphamide as a single agent.[77] Most of these patients were treated before the widespread use of anti-TNF agents. While the response in RA was promising with 68% of patients maintaining an ACR 50 response at some point post transplant, the introduction of TNF blocking agents with their favorable toxicity profiles and significant therapeutic benefit reduced the interest in HCT as a treatment for RA. High dose chemotherapy followed by autologous HCT has been utilized for the treatment of patients with severe SLE. In a series of 50 subjects with organ or life threatening disease, overall 5 year survival was 84% and the probability of disease-free survival at 5 years was 50%.[78] In a multi-center retrospective study of 53 patients with SLE, 5-year disease-free survival was 55%, a number similar to the study described above.[79] Twenty-two patients with severe refractory polyarticular or systemic JIA underwent high dose chemotherapy followed by autologous HCT in a prospective phase II clinical trial. Fifteen of twenty subjects experienced a prolonged complete or partial response; five experienced relapse of their disease. Overall survival at 5 years was 82% and disease free survival censored for relapse and death was 36%.[80] Multiple single arm trials have been conducted in MS with varying conditioning regimens which in general reveal stabilization of disease.[81-84] To improve outcomes after treatment, clinical trials are now being done in patients with less advanced disease before evolution to the secondary progressive type of MS. The use of HDIT transplantation has been reported in other autoimmune diseases including vasculitis[85] and Crohn's disease.[86]

1.2.3. Pre-clinical Studies of HDIT-Transplantation in SSc

No studies are available that evaluate the feasibility of immune ablation and bone marrow or stem cell transplantation in animal models of SSc.

1.2.4. Clinical Studies of HDIT-Transplantation in SSc

High-dose immunosuppressive therapy followed by autologous transplantation in SSc patients is supported by a number of clinical studies conducted with SSc patients as well as studies conducted with other severe autoimmune diseases.

1.2.4.1. Results of Fred Hutchinson Cancer Research Center Protocol 1019

A pilot study was designed to evaluate the safety and potential efficacy of HDIT using total body irradiation (TBI) 800 cGy, cyclophosphamide (CY) 120 mg/kg, and equine antithymocyte globulin (ATGAM) 90 mg/kg followed by infusion of lymphocyte-depleted (CD34-selected) autologous peripheral blood stem cells (PBSCs). Initial results for the first 19 subjects enrolled at 5 medical centers nationally were published.[87] The summary below was based on an update describing the results of 34 subjects followed through 2005.[88]

Granulocyte colony stimulating factor (G-CSF) was well tolerated without major disease activation and was effective for PBSC mobilization, with 33 subjects having sufficient PBSC for transplant collected with a single course of G-CSF and 1 patient requiring 2 courses of G-CSF. Five subjects (15%) experienced mild skin inflammation during G-CSF administration that was thought to be mild disease activation related to G-CSF; inflammation resolved with completion of the G-CSF administration.

While the conditioning therapy was well tolerated by most subjects, who experienced only mild regimen-related toxicities, eight subjects (23%) developed fatal treatment-related complications (7 early and 1 late). Two subjects with pre-transplant SSc lung disease (DLCO 46% and 52%), developed progressive pneumonitis at 14 days and 2 months post-transplant, respectively. The similarity of the two cases prompted shielding of the lungs to 200 cGy during TBI administration. No similar cases were observed in 26 subsequent subjects who received TBI with lung shielding. A 3rd subject developed post-transplant lymphoproliferative disorder (PTLD) and died of multi-organ failure. Because of this observation and a similar case in a subject who received HDIT and CD34-selected stem cell infusion for multiple sclerosis,[89] the protocol was modified and patients were carefully followed. A treatment plan with rituximab was put into place if there was evidence of EBV reactivation. In the current protocol, TBI will not be used in the high-dose regimen and the autologous hematopoietic cell graft will not be CD34-selected which will limit the risks for lung injury and EBV-associated PTLD. The 4th 5th and 6th subjects had rapidly progressive renal disease and multi-organ failure and then expired at <1, 4 and 6 months after transplantation. The 4th patient with pre-existing renal and heart disease developed renal failure and died from multi-organ failure within 1 month post-transplant without evidence of renal crisis. The 5th and 6th subjects had renal crisis and progressive renal failure by 3 months, and died of multi-organ failure at 4 and 6 months post-transplant. A seventh subject with scleroderma heart and an abnormal pre-transplant LVEF (47%) had a fatal cardiac arrest 22 days post-transplant. Patients treated in this clinical trial of high-dose therapy for SSc will be carefully screened to exclude patients with abnormal kidney or heart function. An 8th subject died from complications of a myelodysplastic syndrome (MDS) at 76 months post-transplant. Clonal chromosomal abnormalities were evident in a pre-transplant sample of CD34+ cells consistent with the MDS clone and indicated pre-existing disease. In the randomized clinical trial of HDIT followed by autologous HCT sponsored by the NIH (SCOT), there had been 1 death attributed to treatment as of late 2009.[90] The decrease in treatment-related

mortality after HDIT followed by autologous HCT for SSc is likely the result of better patient selection and modifications to the treatment regimen.

Figure 1 shows the Kaplan-Meier overall and progression-free survival curves for the study cohort. In addition to the eight treatment-related deaths, four subjects have died from disease progression. The Kaplan-Meier estimate of the 5-year survival for all subjects based on the data shown in Figure 1 is 64%. Mortality for two cohorts, without lung shielding and with lung shielding were similar. When the analysis was performed on patients from the pilot study who would have met the eligibility criteria or being treated as on SCOT (except for the renal shielding; $n = 22$), the overall survival was 91% and 78% at 3 and 5 years post-transplant respectively.

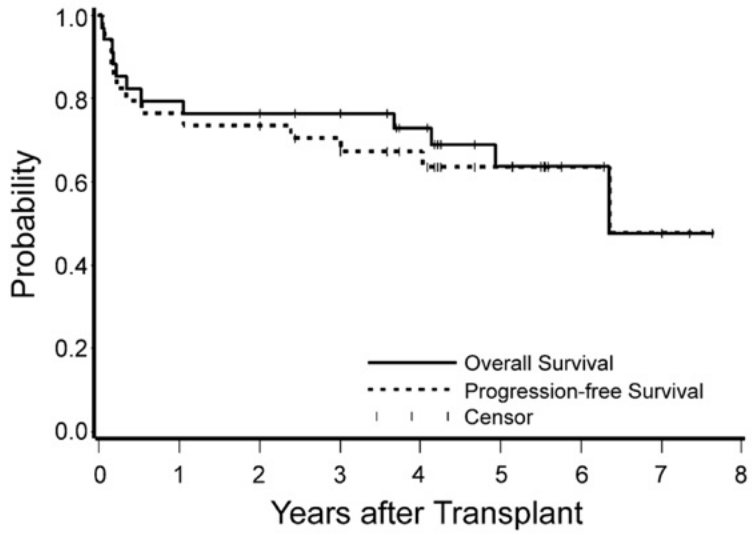

Figure 1. Survival and progression-free survival after high-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for systemic sclerosis (FHCRC Protocol 1019). The estimated probability of survival and progression-free survival at 5 years was 64%. Tick marks represent censored observations.

Outcomes of various response parameters after HDIT and autologous HCT for SSc are summarized in **Table 1**.

Of the 34 patients enrolled in the study, 27 (79%) survived 1 year and were evaluable for response. Summarized in Table 1 are changes from baseline to the last evaluation within various time windows for each of the clinical parameters that were assessed. The mean decrease in mRSS (baseline: 30.12) and mHAQ score (baseline: 1.85) at final evaluation was 22.08 (-70.3%) and 1.03 (-55%), respectively (both $P < 0.0001$). There was also a statistically significant linear decrease in mRSS and mHAQ scores over time (both $P < 0.0001$, GEE; **Figures 2A and B**). At last follow-up, an mRSS of 0–5 was observed in 11 patients. Marked improvement of mRSS was also associated with improvement of the dermal fibrosis in 8/10 patients for whom biopsies were available from both before and after HDIT (mean decrease 19.6 (95% CI 12.6 to 26.6, $P = 0.0001$)) (**Figure 3**). There was a mean decrease in the dermal fibrosis grade of 3.1 (95% CI 2.2 to 4.0, $P < 0.0001$) from a mean baseline grade of 4.3. Using all available skin biopsies ($n = 40$) including from patients without samples from both before and after HDIT, a strong correlation was noted between the dermal fibrosis grades and the mRSS ($r = 0.62$, $P < 0.0001$). Also noted in the subset of patients with skin biopsies before and after HDIT was an improvement in the SSc-associated vasculopathy.[91]

Table 1. Changes in Organ Function, mRSS, and mHAQ-DI after HDIT Transplantation (FHCRC Protocol 1019)

Assessments	Mean Baseline (*n* = 34)	Years 1-2; Difference From Baseline*	Years 3-4; Difference From Baseline*	Years 5-8 Difference From Baseline*	Final Evaluation;† Difference From Baseline
mRSS (0-51)	30.12	-17.56 (-20.72, -14.40; *P* <.0001) (*n* = 25)	-21.24 (-25.42, -17.05; *P* <.0001) (*n* = 21)	-21.82 (-25.88, -17.74; *P* <.0001) (*n* = 11)	-22.08 (-25.71, -18.45; *P* <.0001) (*n* = 25)
mHAQ (0-3)	1.85	-1.26 (-1.60, -0.92; *P* <.0001) (*n* = 23)	-1.08 (-1.53, -0.64; *P* <.0001) (*n* = 20)	-1.50 (-1.92, -1.09; *P* <.0001) (*n* = 11)	-1.03 (-1.40, -0.66; *P* <.0001) (*n* = 26)
$DLCO_{adj}$ (%)	60.09	-1.37 (-5.64, 4.98; *P* = .60) (*n* = 27)	-3.70 (-9.86, 4.90; *P* = .31) (*n* = 23)	-2.27 (-9.69, 5.15; *P* = .51) (*n* = 11)	-6.04 (-12.08, 0.002; *P* = .05) (*n* = 27)
FVC (%)	71.53	4.48 (=0.14, 9.10; *P* = .06) (*n* = 27)	2.09 (-5.17, 9.34; *P* = .56) (*n* = 23)	10.36 (3.52, 17.20; *P* = .007) (*n* = 11)	2.11 (-4.27, 8.49; *P* = .50) (*n* = 27)
Creatinine (mg/dL)	0.78	0.26 (0.04, 0.47; *P* = .02) (*n* = 26)	0.25 (0.07, 0.44; *P* = .009) (*n* = 23)	0.13 (0.04, 0.21; *P* = .008) (*n* = 11)	0.25 (0.10, 0.40; *P* = .003) (*n* = 27)
Ejection Fraction (%)	63.24	-0.21 (-3.60, 3.19; *P* = .90) (*n* = 24)	-2.84 (-5.80, 0.11; *P* = .06) (*n* = 19)	-3.85 (-7.22, -0.48; *P* = .03) (*n* = 11)	-2.37 (-4.80, 0.07; *P* = .06) (*n* = 27)

*Represents mean difference between baseline and last evaluation for 27 patients who survived >1 year in the indicated period. Confidence intervals (95%) are contained within parentheses. Each patient was compared with his own baseline result.

†Represents mean difference between baseline and final evaluation after HDIT.

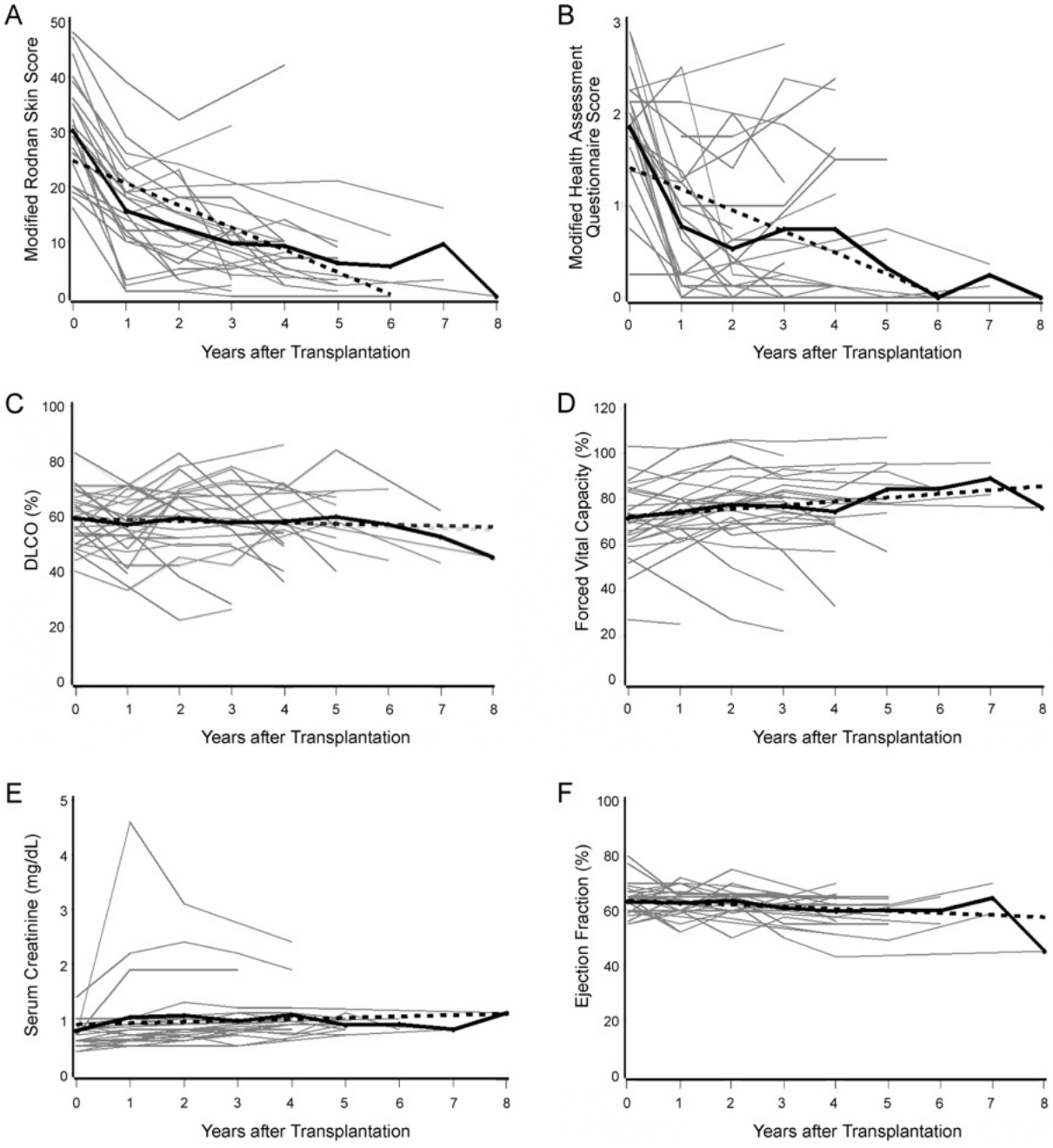

Figure 2. Outcome of mRSS, mHAQ, DLCO, FVC, Serum Creatinine and Cardiac Ejection Fraction Over Time After HDIT and Autologous Hematopoietic Cell Transplantation, (FHCRC Protocol 1019). A determination was made whether a parameter value was statistically significantly increasing or decreasing over time using a generalized estimating equation (GEE) model. The bold black solid line represents the mean value over time for the parameter of interest. The bold black dotted line represents an estimate of the modeled linear relationship between the parameter value and time and summarizes the results of the GEE models. The gray solid lines are parameter values for individual patients.

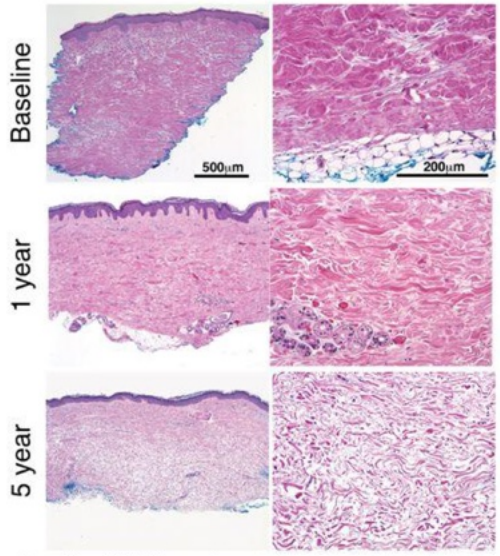

Figure 3: Resolution of dermal fibrosis after HDIT and autologous HCT. Full thickness skin biopsies from patient after high-dose immunosuppressive therapy and autologous hematopoietic cell transplantation. Skin biopsies collected at baseline and then at 1 and 5 years after HDIT were histologically evaluated (H&E). The skin biopsies performed after HDIT were performed at a site adjacent to the baseline skin biopsy. The biopsies in the left-hand column were taken at 5x original magnification and those in the left hand column were taken at 20x original magnification. All sections were stained with H&E. **A)** Skin biopsy was obtained before HDIT and autologous HCT. Pan-dermal sclerosis from the dermal-epidermal border to the hypodermis (subcutaneous fat) was observed. The epidermis is mildly acanthotic (thickened) with loss of rete ridges. The reticular dermis is replaced by dense compact collagen without normal fascicular bundles or dermal appendages. This pretransplant skin biopsy was determined as grade 5 dermal fibrosis. The thickness of the dermis was measured at >2 mm. **B)** In the higher power magnification, the straightened dermal-subcutaneous border demonstrates the abnormal, densely packed, homogenized collagen. **C)** The skin biopsy at 1 year after HDIT was determined to be a grade 2 dermal fibrosis and has less fibrosis than at baseline. The low-power magnification view shows crowded collagen fascicles with focal areas of residual thickened bundles. **D)** A higher power view of the 1-year skin biopsy from C shows thin and collagen bundles admixed with residual thick straightened hypereosinophilic collagen bundles without dense homogenization at baseline. Residual eccrine unit lacks any surrounding adipose tissue. **E)** The skin biopsy at 5 years shows complete resolution of the dermal fibrosis (grade 0) with a reduction in the thickness of the dermis from baseline to 1 mm. The collagen bands in the dermis are thin with a relative increase in the intervening extracellular matrix (space between the collagen bands). The dermal-epidermal border remains straightened with loss of rete ridges. **F)** A higher power view of collagen in lower reticular dermis demonstrates the change to thin wavy bundles separated by increased ground substance.

The mean increase in FVC between baseline and final evaluation was 2.11 % (P = 0.50) and $DLCO_{adj}$ decreased by an average of 6.04% (P = 0.05). Considering all values across time, there was a statistically significant increase in FVC (increase of 1.66 per year (95% CI 0.37 to 2.96), P = 0.01, GEE; **Figure 2C**) and $DLCO_{adj}$ values were decreased, but this did not achieve statistical significance (decrease of 0.36 per year [95% CI 1.42 to -0.69], P = 0.50, GEE; **Figure 2D**). The HRCT scans of the chest were centrally reviewed in 21 of the evaluable patients. No significant changes were observed after HDIT except for the 6 patients with reactivation of disease in the lungs. In 6 of 7 patients who survived ≥ 1 year and were centrally reviewed, 'ground-glass' abnormalities at baseline decreased and evolved to interstitial fibrosis, and in the other patient the findings remained unchanged. Activation of pulmonary disease requiring therapy was detected in 6 subjects, 4 of whom eventually died with lung progression. There was no statistically significant change in the ANA titer (P = 0.26). Of 11 patients who had a positive test for anti-Scl-70, two had become negative at last follow-up (6 and 8 years).

The data also showed significant reductions in DLCO but not FVC at 3 months after transplant (not shown). This early decrement is attributed to effects of high-dose cytotoxic therapy, as it is consistent with changes in DLCO observed in patients with hematologic malignancy who received a preparative regimen for allogeneic bone marrow transplant that included 1000 to 1,575 cGy TBI. The FHCRC SSc subjects and the oncology patients had a normalization of DLCO over the subsequent year. In the

FHCRC study, there were no statistically significant difference in changes of DLCO between the subjects who received lung shielding and those who did not, suggesting that CY chemotherapy may have affected DLCO values.

In the FHCRC study, dermal fibrosis significantly decreased confirming that if the disease process is controlled, skin re-modeling will occur.[88] FVC increased but this improvement was more limited compared to the skin and might have resulted from the improvement in the skin score.

HDIT transplantation induced profound lymphocytopenia. Recovery to normal values was the fastest for NK cells, intermediate for B cells and CD8 T-cells, and the slowest for CD4 T-cells. Whereas the tempo of recovery of NK cells and B cells was similar to that reported after autografting using unmodified PBSC, recovery of CD4 and CD8 T-cells appeared to be slower[87,92] and was mostly from cells with a memory phenotype.

Disease activation/progression was observed in 10 of the 27 evaluable patients who survived at least one year. Disease activation/progression occurred in lung (n=6), heart (n=1) or in other systems (n=3) that required DMARD treatment at a median of 1 (range, 1-6) year after treatment. Four of the 6 patients with disease activation/progression died at a median of 4 (range, 1-5 years) after transplant.

1.2.5. Other Reports of HDIT Transplantation in Systemic Sclerosis

Initial case reports documented major responses after HDIT transplantation. These reports have been supplemented by summaries of the EBMT registry experience and reports from the French/Dutch multi-center trial and Northwestern University.[93-96]

1.2.5.1. Review of European Bone Marrow Transplant Registry experience

In 2001, Binks et al. reported data on HDIT followed by autologous HCT for SSc from the EBMT registry and this was later updated in 2004 by Farge et al.[95,97] Of 57 patients, 50 were evaluable for response with a follow-up of at least 6 months. A partial (n = 32) or complete response (n = 14) was observed in 92% of patients at a median follow-up of 22 months. No response was seen in 8% (n = 4). 35% of patients with an initial response relapsed within 10 months after transplant. The treatment-related mortality was 8.7% and mortality from disease progression was 14%. At 5 years, progression probability was 48% and estimated survival was 72%. Based in part on the observed responses, the Autologous Stem cell Transplantation International Scleroderma (ASTIS) randomized clinical trial was developed and is being conducted in 20 centers in Europe.

1.2.5.2. French multi-center study

Farge et al. have reported outcomes in a series of 12 subjects, the only other significant series of SSc subjects treated on a single protocol.[93] An additional 6 subjects were enrolled but not treated because of patient refusal or delays that prevented eligibility. Amongst these 6 subjects, 3 died and 3 progressed to end-stage organ failure with follow-ups of 3-10 months. The eligibility criteria for the study required diagnosis of severe SSc of less than 4 years duration with internal organ involvement affecting heart, lungs, GI tract, or kidneys. While the study had a core protocol, there were some variations in the treatment methods across institutions. Peripheral blood stem cells were mobilized with CY 2 g/m^2 x 2 days followed by G-CSF at 5 µg/kg/day. Apheresis products were CD34-selected with the Isolex 300i device (Baxter, Deerfield IL). The conditioning regimen utilized cyclophosphamide at a dose of 50 mg/kg/day for 4 days. Anti-lymphocyte globulin (ALG) was administered prior to CY if PBSC or CD34$^+$ selection failed.

During the mobilization phase, 9 subjects developed infectious episodes requiring antibiotic treatment. Eleven of 12 subjects who underwent mobilization received HDIT transplantation and 1 refused. At 18 months (range 1-26 months), 8 of 11 subjects showed evidence of disease response at some time after HCT. However, 4 (36%) transplant recipients died, including 1 from treatment toxicity and 3 from disease progression, and 4 additional subjects had progressive or non-responsive disease requiring systemic immunosuppressive therapy between 6 and 12 months post-transplant. Systematic evaluations of lung functions were not reported. Based on graphs from the manuscript, 4 of 9 subjects had evidence of disease activity documented by increasing HAQ scores after initial stabilization or response. Similarly, disease progression was evident in 6 of 10 subjects who had increasing skin scores after HDIT transplantation. The relatively poor response may have resulted from selecting patients with advanced disease. In a follow-up report describing the combined Dutch and French experience, survival without relapse or progression resulting in major organ dysfunction was 64.3% at 5 years.[94] For those patients with at least a 6-month follow-up after HDIT, the Kaplan-Meier estimate of survival was 96.2%.

1.2.5.3. Northwestern experience

The Northwestern group has now treated 35 SSc patients with high-dose cyclophosphamide and ATG. In there first report of 10 patients, progression-free survival was 70% and overall survival was 90% at +2 years.[98] One patient with advanced SSc died from disease progression at +2 years. In a follow-up, a total of 37 patients had received HDIT.[96]They observed that patients with a baseline DCLO <40% had a treatment-related mortality of 27% (4/15 patients) whereas there were no deaths among those patients with a DLCO >40% (0/22 patients). The major reason for treatment-related mortality was cardiac events. Another patient had died at 4 years follow-up from disease progression. With a mean follow-up of 24 (6-60) months, the mRSS and FVC improved significantly and DLCO remained stable. The Kaplan-Meier estimated survival and event-free survival at 3 and 5 years was 74% and 68% respectively. SSc patients in the current protocol will be required to have a DLCO >40% to be included in this clinical trial of HDIT followed by autologous HCT.

In a follow-up, the Northwestern group reported the results of a randomized phase 2 clinical trial (n=19).[99] They showed that in patients treated high-dose immunoschemotherapy followed by autologous HCT (n=10), skin scores and FVC were significantly improved at 1 year compared to a group of patients who received monthly pulse cyclophosphamide for 6 months (n=9). The value of this study was limited because follow-up was only 1 year but does indicate that high-dose immunochemotherapy and autologous HCT has a favorable short-term effect on disease progression.

1.3. Known and Potential Risks and Benefits

1.3.1. High-Dose Immunosuppressive Therapy

The use of cyclophosphamide/ATGAM may cause acute side effects, including the following: nausea and vomiting, diarrhea, oral mucositis, alopecia, pancytopenia, immunodeficiency, infections, bleeding, and acute failure of lungs, liver, kidneys, and heart. Late side effects include infertility, osteoporosis, secondary malignancies, and myelodysplastic syndromes. The mortality risk from high-dose therapy may be as high as 5% over the first 3 months. Potential benefits from HDIT transplantation include reversal or stabilization of manifestations of SSc and prolongation of survival. Side effects specific to the individual agents used in this protocol are summarized below. There is an increased risk of infectious complications after HDT, and therefore an active infection prophylaxis regimen has been

described (Protocol Section 5.3). Specific toxicity management guidelines for each of these therapies are found in Protocol Section 5.5.

1.3.1.1. Predictive value of pre-transplant pulmonary function testing on mortality risk of HDT

Pulmonary damage is an important life-threatening toxicity of allogeneic and autologous transplantation. Pulmonary complications may arise through direct organ damage from radiation or from certain cytotoxic agents that are associated with higher risks of lung damage. Agents such as TBI, bischloroethyl-nitrosourea (BCNU), busulfan, and cyclophosphamide (CY), which have been used in transplants for autoimmune diseases and malignancy, are capable of causing lung toxicity.

Baseline lung function testing has been associated with mortality risk when using conventional transplant regimens in subjects with malignancy. A low DLCO score did not appear to increase significantly the risk of respiratory failure or non-relapse mortality.[100] Reduced diffusing capacity values are common after high-dose therapy, particularly in the first 6 to 12 months, with a trend towards recovery thereafter.[101]

While reduced DLCO, forced vital capacity (FVC), and arterial-oxygen gradients may predict increased risk from transplantation, they are also predictive of a relatively advanced state of SSc with poor survival. Despite the reduced baseline lung function in many SSc subjects, it appears that their lungs can tolerate the proposed regimen based on previous clinical trials of high-dose immunosuppressive therapy and autologous HCT for SSc. In the randomized clinical trial of high-dose immunosuppressive therapy for SSc (SCOT), it was reported that there was only 1 treatment-related death.[90] It was observed that mortality was greater in SSc patients with DLCO $\leq$40%.[96] Most previous studies of HDIT for SSc have used a lower limit of the DLCO of 45% for eligibility so this will remain as the lower limit for this study.

1.3.1.2. EBV serology and post-transplant surveillance for EBV reactivation

Epstein-Barr virus reactivation and/or infection, with the associated risk of PTLD, is an unlikely complication of high-dose chemotherapy and transplantation of an unmanipulated autologous stem cell graft for malignancies. However, risk factors for EBV reactivation or infection following HDIT and autologous HCT for autoimmune diseases include profound chemotherapy-induced general immunosuppression and suppression of T-cell function due to ATG in the regimen. Epstein-Barr virus-PTLDs are a spectrum of B cell hyperproliferative states including infectious mononucleosis-like illness, monoclonal malignancies such as B cell and occasionally T cell lymphomas, and can prove fatal even after autologous HCT.[89,102]

To determine the rate of EBV reactivation and/or infection for subjects enrolled in this protocol for HDIT and HCT, subjects will be monitored prospectively through Day 100 post-transplant. Laboratory testing will include assessment of EBV serology and viral load in the peripheral blood by EBV PCR amplification. Subjects who develop a viral load of > 1000 copies per mL plasma will receive further individualized monitoring and/or therapy (see section 5.3.5.3).

1.3.2. G-CSF

Granulocyte colony stimulating factor is a growth factor given to boost granulocyte counts. It has been administered to subjects with primary or acquired granulocyte deficiencies. Most frequently it is used to aid granulocyte recovery after drug-induced myelosuppression, including conventional cytotoxic chemotherapy for cancer, and also after marrow transplantation. Granulocyte colony stimulating factor is produced by recombinant DNA technology and acts by binding to specific receptors on hematopoietic

cells. It stimulates proliferation, differentiation, and some functional activities of granulocytes and their precursors. It is administered by either subcutaneous (SC) injection or intravenous (IV) infusion. The elimination half-life is approximately 3.5 hours. Primary toxicities of G-CSF are usually mild and include flu-like symptoms such as myalgia and bone pain. There have been reports that G-CSF may cause flares of some autoimmune diseases. Although no randomized studies have been done, when G-CSF has been used in combination with cyclophosphamide or prednisone, it has been well-tolerated. Systemic sclerosis subjects have exhibited transient edematous or telangiectatic changes in the skin during mobilization with G-CSF but they have otherwise tolerated the G-CSF well without the use of cyclophosphamide or prednisone.

1.3.3. Cyclophosphamide

Cyclophosphamide is an alkylating agent used to treat a variety of malignancies. Because it has potent immunosuppressive properties, cyclophosphamide is used both in subjects undergoing allogeneic marrow transplants and to treat severe autoimmune diseases. It is an alkylating agent that requires hepatic metabolism to active metabolites phosphoramide mustard and acrolein, which react with nucleophilic groups. The half-life of the parent compound is 5.3 hours in adults, and the half-life of the major metabolite, phosphoramide mustard, is 8.5 hours. Liver or renal dysfunction will lead to prolonged serum half-life. The major dose-limiting side effect at high doses is cardiac necrosis. Hemorrhagic cystitis can occur and is mediated by the acrolein metabolite; it can be prevented by co-administration of Mesna or by continuous bladder irrigation. Other side effects include nausea, vomiting, alopecia, myelosuppression, infertility, secondary malignancies, and syndrome of inappropriate antidiuretic hormone secretion (SIADH). Ovarian failure occurred in 60%-70% of females over 26 years of age who received high-dose CY. It would be expected the risk might increase as the age of pre-menopausal women increases.

1.3.4. Antithymocyte Globulin

Equine antithymocyte globulin (ATGAM) will be administered at a dose of 15 mg/kg of recipient body weight IV 6 times on days -5, -3, -1, +1, +3, and +5. Subjects with a positive skin test or a hypersensitivity reaction to ATGAM will be eligible to receive rabbit ATG (Thymoglobulin). Thymoglobulin will be administered at a dose of 0.5, 1.0, 1.5, 1.5, 1.5 and 1.5 mg/kg of recipient body weight on days -5, -3, -1, +1, +3 and +5 respectively (total dose- 7.5 mg/kg). Methylprednisolone is given as premedication for each ATGAM dose to protect against ATG-induced serum sickness. Side effects include fever and chills; skin rash and itching, usually prevented by or controlled with antihistamines and concomitant administration of corticosteroids; hypotension; wheezing; anaphylaxis; platelet and white blood cell (WBC) count depression; serum sickness with severe skin rashes, mouth, and vaginal sores; joint pain and swelling; and kidney damage.

1.3.5. Mycophenolate Mofetil

MMF inhibits inosine monophosphate dehydrogenase and has been shown to deplete guanosine nucleotides, thereby suppressing T- and B-cell proliferation and promoting apoptosis of monocytes and other inflammatory cells. The end result is an inhibition of cell-mediated immunity and antibody formation.[103] Because of its immunosuppressive properties and its favorable safety profile, MMF is indicated for the prevention of organ transplant rejection and is frequently used to treat autoimmune inflammatory conditions such as lupus nephritis. MMF has also been shown to decrease mRNA for IL-6 and TGF-β in renal biopsies from patients undergoing acute rejection.[104] These effects are particularly relevant to SSc, in which increased TGF-β may play a central pathogenetic role. The principal adverse reactions associated with the administration of MMF include diarrhea, leukopenia, sepsis, vomiting, and

there is evidence of a higher frequency of opportunistic infections. Phlebitis and thrombosis have been reported with intravenous administration.

1.4. Rationale for Clinical Trial of High-Dose Immunosuppression with Cyclophosphamide and ATGAM Followed by Transplantation of Unmanipulated CD34-selected Graft for SSc

Systemic sclerosis is a collagen vascular disease characterized by a fibrosis in several different organ systems. Oral cyclophosphamide has been shown to have a very modest therapeutic effect but was not sustained at 2 years.[38] However, this study's results indicated that SSc may be responsive to immunosuppressive therapy and serves as a rationale for an approach which intensifies immunosuppression. It is proposed that high-dose therapy followed by autologous HCT will reduce the degree of inflammation in the lung and other involved organ systems and may have a persistent modulatory effect on the immune-mediated disease process. Responses have been promising in pilot studies, and there are no other highly effective immunomodulatory therapies yet for SSc. Randomized clinical trials of high-dose immunosuppressive therapy followed by autologous HCT for SSc are being conducted in Europe (ASTIS) and North America (SCOT). The SCOT clinical trial is scheduled to complete accrual on March 31, 2011. In SCOT, the high-dose immunosuppressive therapy regimen consists of TBI, cyclophosphamide (120 mg/kg) and ATGAM. Both ASTIS and SCOT use CD34-selected autologous hematopoietic stem cell grafts after high-dose therapy. In the current proposal, we will evaluate the use of high-dose cyclophosphamide (200 mg/kg) in combination with ATGAM but without TBI. The pilot European studies and the pilot study at Northwestern University have used high-dose cyclophosphamide in combination with ATG but without TBI for high-dose immunosuppression and have described responses.[93-96,98] High-dose immunosuppression without TBI may decrease toxicity associated with the regimen and reduce some of the complexity and cost associated with treatment, although the deletion of TBI may result in more recurrences.. The autologous hematopoietic cell graft will not be CD34-selected in this proposal. Based on registry data from EBMT, CD34-selection to T-deplete the hematopoietic cell grafts was not associated with a strong treatment effect.[105] Pilot studies of high-dose immunosuppressive regimens followed by transplantation of unmanipulated grafts have been associated with significant response rates in SSc and other autoimmune diseases.[82,96,98] Maintenance therapy will be given up to 2 years after transplant with MMF to reduce the risk of disease progression.

1.4.1. Rationale for the Patient Population Selection: Inclusion Criteria

Subjects will be included who have a poor prognosis but less advanced disease involving only skin
Patients with diffuse cutaneous SSc and internal organ involvement have a poor prognosis (see Section 1.1.1.4). The inclusion criteria for the current clinical trial will include the similar criteria as that used for the SCOT clinical trial. These criteria were selected to identify a subset of SSc patients with an expected 5-year survival of approximately 50%. Patients were required to be no longer than 5 years from the onset of first non-Raynaud's symptoms. This requirement in the SCOT clinical trial was included because in many patients the disease is less active after 4-5 years. However since SSc may still be active beyond 5 years in some cases, patients will be eligible for the study at 6 and 7 years from the onset of first non-Raynaud's symptoms if SSc disease progression is documented. Other criteria that have been associated with a poor prognosis are diffuse scleroderma with disease duration $\leq$ 2 years since development of first sign of skin thickening plus mRSS $\geq$ 20 plus ESR >25 mm/1st hour and/or Hb < 11 g/dL, not explained by causes other than active scleroderma.[34] This last criteria is comparable to an inclusion criteria used in the European randomized clinical trial (ASTIS).

We are also proposing to include patients with less advanced disease, earlier to prevent irreversible organ damage and to improve outcomes from high-dose therapy. These will be patients with a high mRSS (≥ 30) and with internal organ involvement but not sufficiently severe to be eligible for the study without the high skin score. Patients with less advanced disease are included in the study since high-dose immunosuppression without TBI and without CD34 selecting the autologous hematopoietic cell grafts will be done and is expected to reduce the risks associated with this treatment. A report in 2010 from EULAR has shown that patients with high skin score ≥ 30, FVC<80%, and proteinuria were associated with a high SSc-related mortality.[96] However, all patients in the study will have failed to respond to front-line disease modifying therapy, either cyclophosphamide or mycophenolate mofetil (see section 4.1).

Subjects will be less than or equal ***to the age of 70.***
For the purpose of this study, patients less than or equal to 70 years of age will be included based on knowledge that autologous transplants are performed without compromising safety in an older population including patients in their seventies. In the scleroderma lung study of oral cyclophosphamide versus placebo, patients into their seventies were included. The major concern of including older patients is the potential for increased treatment-related mortality in the older age group. Data to address this concern were obtained from CIBMTR in an analysis of age related mortality after autologous transplant. Patients with multiple myeloma and lymphoma, the two biggest disease groups, were used for the analysis. A total of 3689 patients > 65 years have been reported to the registry of whom 67% were aged 65–69 years and 67% were male. Sixty percent had multiple myeloma, 38% non-Hodgkin lymphoma and 2% Hodgkin's lymphoma. Eighty-two percent of the transplants were done from 2000 until the present illustrating the increased application in older patients in recent years. For comparison there were 40,290 transplants in patients ≤ 65 years of which 38% were for myeloma, 44% for non-Hodgkin lymphoma and 18% for Hodgkin's lymphoma. The overall day 30 and day 100 mortalities were slightly higher in the older group aged > 65 but the overall mortality risk still remained quite low with these procedures (**Table 2**). For patients > 65 the mortality related to disease progression was 32%, slightly lower than the 41% in the younger age group, and indicating a day 100 treatment mortality of approximately 5.3%. We believe these data support upper age limit in the protocol to 69 years.

Table 2: Early Mortality in Patients >65 years and ≤ 65 years.

Age	>65 years	≤65 years
30 day mortality (95%CI)	3.08% (2.54%–3.67%)	1.97% (1.84%–2.11%)
100 day mortality (95% CI)	7.75% (6.90%–8.64%)	6.17% (5.93%–6.41%)

1.4.2. Rationale for Mobilization Strategy with G-CSF Alone

Since G-CSF has been associated with flares of autoimmune diseases, most transplant groups are now mobilizing hematopoietic stem cells with the combination of G-CSF and cyclophosphamide or prednisone.[81,106,107] No significant complications were observed mobilizing SSc patients with lung disease using G-CSF alone in the FHCRC pilot study and in the SCOT clinical trial.[88] Since adding cyclophosphamide to the mobilization regimen will increase the risk of infections, patients in this clinical trial will be mobilized with G-CSF alone.

1.4.3. Rationale for Choice of Treatment Regimen

This protocol will use a preparative regimen of ATGAM 90 mg/kg, and cyclophosphamide 200 mg/kg followed by transplantation with an unmanipulated hematopoietic cell graft. The paragraphs below first present a rationale for the elements of the preparative regimen followed by the rationale for the use of an unmanipulated autologous PBSC graft.

A combination of cyclophosphamide and ATGAM has been one of the most commonly used regimens for high-dose immunosuppressive therapy followed by autologous HCT for autoimmune diseases including systemic sclerosis. Transplant programs have experience with this regimen since it is used for conditioning patients with aplastic anemia before allogeneic HCT. This regimen has been well-tolerated by patients with several different autoimmune diseases. Importantly the regimen has been well-tolerated in patients with systemic sclerosis with pulmonary fibrosis. The combination of cyclophosphamide and ATGAM has been shown to be efficacious in inducing remission of refractory or severe autoimmune diseases in phase 2 clinical trials. Pre- and post-transplant ATGAM will be administered in a further attempt to deplete T cells that survive the preparative regimen and are infused with the autologous hematopoietic cell graft.

Because this regimen may be more immunosuppressive than conventional autografting regimens, an increased incidence of infectious complications is anticipated as compared to conventional treatment. Important potentially life-threatening infections include cytomegalovirus (CMV) and EBV-related PTLD. Infection prophylaxis for transplant recipients is based on prevailing practice for hematopoietic allograft recipients, where immunosuppression is more severe and opportunistic infections occur more frequently than after conventional autografts. Patients are closely followed in transplant programs for the development of opportunistic infections and most will be effectively prevented or treated. Infections were infrequent beyond 6 months after transplant.[92]

1.4.4. Rationale for Maintenance Therapy with Mycophenolate Mofetil

In the pilot study conducted by the Seattle Consortium, 10 of 27 evaluable patients (37%) had disease activation/progression at a median of 1.0 (1.0-6.0) years after transplant.[88] EBMT observed that 35% of patients had relapsed at 10 (2.2-48.7) months after high-dose therapy and autologous HCT.[95] At 5 years, progression probability was 48%. Vonk et al observed that 28% of patients progressed at 2.7 (2.0-4.0) years.[94] This is consistent with other studies of high-dose immnochemotherapy and autologous HCT for other severe autoimmune diseases in which 30-50% of patients will have disease progression. MMF has been used now by many groups for the treatment of SSc and although no studies have shown that it prevents disease progression, there is sufficient data suggesting that it can be used safely in the population of patients. MMF has also been used extensively now after allogeneic HCT for prevention of graft-versus-host disease and except for hematological and gastrointestinal complications which are reversible on dose adjustment, it has also been used safely and is effective. Based on the experience after allogeneic HCT, it is expected that MMF will have a satisfactory safety profile after high-dose immunochemotherapy and autologous HCT. It is proposed that the dose of MMF will be 1.0 gram orally twice daily which is the dose and schedule approved for solid organ transplantation and the most common dose use after allogeneic HCT. We have proposed to start maintenance therapy at 2-3 months after autologous HCT at a time when the regimen-related toxicity of the regimen has resolved and continue until the 2-year anniversary of the transplant. The decision to discontinue maintenance therapy with MMF beyond 2 years will be made by each site rheumatologist. Maintenance therapy for this duration of time would serve to cover that period during which most patients will have disease

activation/progression. For those patients who would have been destined to have disease activation/progression after 2 years, it is proposed that maintenance therapy may further delay or prevent disease activation/progression because of a potential modulating effect when administered posttransplant.

1.5. Rationale and Background for the Mechanistic Studies

1.5.1. Assessment of Immune Reconstitution by Flow Cytometry

Changes in the immune regulatory environment including an increase in T regulatory cells may explain responses of autoimmune diseases in recipients after high-dose immunosuppression followed by autologous HCT. Therefore, we will monitor immune reconstitution after HCT by serial flow cytometry studies.

Natural killer (NK) cell counts recovered by 1 month and were followed by recovery of B and CD8+ T-cell counts by 6-12 months after HDIT. There was a slower recovery of CD4+ T cell counts, which were low-normal by 2 years.[92] Immune recovery at 2 years after HDIT was associated with increasing thymic-derived naïve CD4+ T cells.[108] It was also observed that there was an increase in T-cell receptor excision circles (TRECs; a marker for recent thymic emigrants) in CD4+ T cells at 1 and 2 years after HDIT. There was a steady decrease over time in CD4+ central memory T cells. CD4+ effector memory cells were relatively increased at 6 months after HDIT likely from homeostatic proliferation but had recovered to normal levels by 2 years. There were no significant changes in the CD8+ T cell subsets. There was an increase in regulatory T cells, and broader clonal diversity than was present before HDIT.[108,109] In association with the increased levels of naïve CD4+ T cells, there was hypertrophy of the thymus at 1 and 2 years compared to baseline especially in the younger patients (less than 43 years of age). This evidence suggested a thymic origin for the recovery of the CD4+ T-cell repertoire after HDIT and autologous HCT. Even though B-cell counts were very low in the first 3 months after HDIT, median serum levels of immunoglobulin G specific for tetanus toxoid, *Hemophilus influenzae* and *Streptococcus pneumoniae,* were normal. The clinical responses to HDIT that have persisted for 2 or more years may have resulted from these late immunomodulatory effects especially evident in the CD4+ T cell compartment.

We will determine the levels of T cell subsets including regulatory T cells and their association with disease activity or remission especially while recipients are mixed chimeras early after NMT.

1.5.2. Microarray Studies of the Skin

In scleroderma, the extent of skin involvement is positively correlated with the severity of internal organ involvement and is also predictive of greater morbidity and mortality.[33] Mechanisms that are responsible for initiation of this fibrotic response, persistence of excess matrix, and resolution following treatment are poorly understood. In the past, one of the barriers to understanding these mechanisms has been the lack of highly effective therapies so that pre- and post treatment comparisons could be done. The goal of this study is to identify disease-related pathways in scleroderma by investigating differences in gene expression before and after treatment (prior to therapy and at 1, 2 and 5 years) using skin biopsy samples. Skin thickening is the hallmark of scleroderma and is due to accumulation of collagen and other matrix materials whose production is increased by pro-fibrotic cytokines.[110] Skin is also an organ in which the disease-associated vasculopathy is evident. Gene expression patterns will be identified and

correlated with outcome in particular organ systems (skin and lung), as well as overall outcome (time to death or organ system failure).

We had previously shown that histopathology of skin biopsies done pre- and post-high dose chemotherapy followed by autologous stem cell rescue showed resolution of skin fibrosis and return to a more normal histology.[88] It is the purpose of this study to investigate gene expression profiles in skin biopsy sections performed pre- and post-transplant. Pre- and post-therapy gene expression profiles in skin will be compared to identify pathways that are responsible for the fibrotic response in scleroderma. This should provide a better understanding of important disease mechanisms, should identify biomarkers that could be useful to predict patient outcomes and response to therapy, and should also identify additional and perhaps novel targets for future therapeutic approaches.

Microarray analysis has been particularly valuable in the field of oncology, where investigators have been able to correlate gene expression in tumor samples and peripheral blood with disease outcomes in various types of cancers.[111-115] The expression patterns of certain genes might thus be considered as biomarkers for disease or disease activity. In SLE for example, it has been shown that expression patterns of a group of interferon-inducible genes identifies a subgroup of patients with a significantly higher prevalence of renal disease and worse lupus disease activity/damage scores.[116] Preliminary microarray data has been obtained on skin from patients with SSc.[117] Biopsy samples obtained from patients with SSc had a robust and distinctive gene profile, with 1,800 qualifiers distinguishing normal skin from SSc skin at a significant level. The SSc phenotype was the major driver of sample clusters, independent of origin. Alterations in transforming growth factor and Wnt pathways, extracellular matrix proteins, and the CCN family were prominent. Explanted fibroblasts from SSc biopsy samples showed a far smaller subset of changes that were relatively variable between samples, suggesting that either nonfibroblast cell types or other aspects of the dermal milieu are required for full expression of the SSc phenotype.

We will attempt to determine an ideal biomarker for SSc that will include these characteristics: identifies early stages of disease, is indicative of disease prognosis, and correlates well with progression and response to therapy. Although invasive, skin biopsies are an obvious source of valuable information since skin is a major target organ in SSc and is almost always involved early in the disease, particularly for those with diffuse cutaneous involvement. Moreover, studies have shown that skin biopsies are a much richer source of potentially informative biomarkers than cultured fibroblasts.[117] Skin can be obtained and measured in a minimally invasive way and can be measured repeatedly over time. Microarray studies of the skin will be correlated with the results of the immunohistopathology studies of the skin.

1.5.3. Immunohistopathology Studies of the Skin

The goal of this mechanistic study is to follow the course of angiogenic regeneration and other changes in the skin after high-dose immunosuppression followed by autologous HCT. The skin is a target of the disease process in SSc and understanding the changes associated with skin re-modeling may be informative of disease mechanisms. Therefore, skin biopsies will be used to follow the time course of angiogenic regeneration.

While inflammation does exist in the skin of patients with scleroderma, it is not the most characteristic feature. Instead, there are three histological features in the skin that define scleroderma: fibrosis, intimal thickening, and rarefaction of capillaries. Focusing on the last of these, it is axiomatic that an avascular tissue cannot heal. In work already done in skin biopsies from a limited number of

patients after HDIT and autologous HCT, it has been observed that capillaries in the skin regenerate.[91] Further, there was increased expression of alpha interferon in the scleroderma skin, as well as changes in expression of markers implicated in angiogenesis including VE-cadherin, a molecule required to form tubes, and RGS5, a molecule that marks the pericytes believed to inhibit branching in angiogenesis.

Capillary malformation, not rarefaction, has been demonstrated repeatedly in patients with SSc using nail bed microscopy. Presumably the malformed vessels, as with telangiectasia seen in scleroderma, reflect elevated levels of VEGF in scleroderma tissue made ischemic due to intimal hyperplasia and vasospasm. The malformed vessels formed by excess VEGF are known to be labile; however, this is not widely appreciated. The regeneration of blood vessels in the skin is very likely a response to a reduction in alpha interferon that occurs following HDIT and autologous transplantation since alpha interferon is known to be very anti-angiogenic. Although to date new capillaries were seen in this preliminary work, the signs of active regeneration such as endothelial replication have not been seen. This may be an issue with the sampling schedule. It is also not known whether or how the vascular changes correlate with other clinical signs of improvement or with serum parameters.

The hypothesis proposed for this mechanistic study is that local generation of alpha interferon, combined with the sensitizing effect of excess VEGF present due to vasospasm, are responsible for capillary rarefaction. To evaluate the effect of high-dose immunosuppression followed by autologous HCT on skin re-modeling, biopsies will be stained to detect cell replication as well as expression of the markers described above including interferon alpha, VE-cadherin, and RGS5; smooth muscle alpha actinin, CD123, and VCAM will be used to assess the inflammatory status of the vessel walls and the availability of antigen processing dendritic cells able to make interferon. CD31 will be used to define capillaries since this marker did not disappear from the residual vessels during rarefaction. Skin biopsies will be performed before and after transplantation and the results correlated with those from the skin microarray studies, serum cytokine analysis and blood endothelial progenitor cell (EPC) analysis.

1.5.4. Vascular Studies

The loss of blood vessels (small arteries, capillaries and venules) in the skin and other organs is the dominant feature of SSc-associated vasculopathy. Injuries to blood vessels, such as those resulting from the presence of circulating anti-endothelial cell antibodies clearly contribute to the disruption of the homeostasis of the vascular tree in SSc patients. The loss of capacity for the repair of blood vessels—repair that requires the availability of endothelial progenitor cell (EPC) arising from the bone marrow—however, is also likely to play an important role in the pathogenesis of vessel loss in SSc.

We propose to assess certain cytokines potentially relevant to SSc at baseline and following study therapy. SSc is characterized by the activation of the immune system with production of inflammatory mediators, including autoantibodies and proinflammatory cytokines. Anti-endothelial cell antibodies (AECA) are associated with endothelial damage.[118] Multiple cytokine, chemokine, and growth factor blood levels may be involved in the pathogenesis of SSc including INFγ, IL-2, IL-4, IL-6, IL-13, IL-15, MCP-1, VEGF and bFGF. Of particular relevance to this proposal, are VEGF and basic fibroblast growth factor (bFGF). VEGF and bFGF are endothelial cell survival factors that activate two distinct signaling pathways and protect from apoptosis signals.[119] VEGF is also a potent stimulator for EPC mobilization from the bone marrow. Higher serum levels of VEGF have been detected in patients with SSc relative to healthy subjects.[120,121] Interestingly, the elevated VEGF levels did not enhance angiogenesis in patients with SSc, consistent with a lack of EPCs being the explanation for defective angiogenesis and vascular lesion formation in the disease. bFGF promotes the regeneration of capillary-

rich granulation tissue. However, only low levels of bFGF were detected in the sera of limited number of SSc patients.[122] Importantly, topical application of recombinant human bFGF successfully treated patients having therapy-resistant, chronic leg ulcers due to SSc.[123] Using a SCID mouse vein graft model, infusion of human lineage negative umbilical cord blood results in paracrine secretion of human bFGF and protects vein graft endothelial cells from apoptosis. Hence, it will be of interest to measure serum levels of VEGF at baseline and following study therapy. We hypothesize that VEGF levels will be decreased following high-dose immunosuppression followed by autologous HCT. Blood and serum samples will be stored so that other questions related to the effects of autologous HCT on recovery of SSc-related vasculopathy can be addressed.

1.6. Statement of Good Clinical Practice

Compliance with Good Clinical Practice (GCP) guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in the International Conference on Harmonisation (ICH) document "Guidance for Industry – E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. By signing this protocol, the investigator agrees to adhere to these requirements. The study (protocol, informed consent and investigator CV and credentials) should be reviewed and approved by the Institutional Review Board (IRB) or ethics committee. Changes to the protocol will be initiated by the study team, approved by the IRB at the Fred Hutchinson Cancer Center (FHCC), and then reviewed by the DSMB. Subjects must sign written informed consent prior to mobilization and transplant.

The investigators and institutions affiliated with this study will agree to trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing the source documents.

2. STUDY OBJECTIVES AND PURPOSE

2.1. Hypothesis

Time to clinical progression of disease as determined by serial testing of pulmonary, cardiac, renal function will be delayed in subjects after HDIT followed by autologous HCT and maintenance therapy with MMF for 2 years resulting in an improved EFS .

2.2. Primary Objective

The primary objective is to evaluate the safety and potential efficacy of HDIT followed by autologous HCT (without CD34-selection) and maintenance therapy with MMF in SSc patients by evaluating the effects on event-free survival (EFS) at 5 years posttransplant.
Event-free survival will be defined as survival without significant organ damage (as defined in Section 3.1, Study Endpoints). An event based on lung dysfunction must be documented on at least 2 occasions ≥ 3 months apart.

2.3. Secondary Objectives

1. To evaluate safety of HDIT followed by autologous HCT as determined by regimen-related toxicities, infectious complications, treatment-related mortality, overall total mortality, and time to engraftment.
2. To evaluate treatment effect on disease activation/progression.
3. To evaluate health-related quality of life (HRQOL) using Short Form 36 (SF-36), the St. George's Respiratory Questionnaire (SGRQ), the modified scleroderma health assessment questionnaire (SHAQ), and PROMIS v 1.0 measures.
4. To assess work productivity (Work Productivity Survey) and health care utilization (using UCSD healthcare utilization).

3. STUDY DESIGN

3.1. Study Endpoints

3.1.1. Description of Primary Endpoints

The **primary endpoint** for this study is event-free survival at 5 years. An event is defined as:

a. Death.

or

b. Respiratory failure as defined one of the following three criteria, each sustained for ≥ 3 months without explanations indicative of causation other than disease progression: *a)* a demonstrated decrease of > 30% in DLCO[a] or a decrease of > 20% in FVC[b] measured as an actual difference in percent predicted units; *b)* resting arterial $pO_2 < 60$ mmHg or $pCO_2 > 50$ mmHg without supplemental oxygen; *c)* resting O_2 saturation of < 88% as determined by forehead pulse oximeter. (Note that the primary measure for the respiratory failure endpoint will be the annual measurements of DLCO and FVC obtained by pulmonary function tests at the Transplant Centers, with the resting arterial pO_2 and pCO_2 or resting O_2 saturation used as alternatives should the pulmonary function tests be unavailable.) Measurements of the primary pulmonary endpoints will start from 6 months after treatment since a transient pulmonary dysfunction may occur as a result of the transplant regimen.

or

c. Renal failure, as defined by chronic dialysis ≥ 6 months or kidney transplantation.

or

d. The occurrence of cardiomyopathy, confirmed by clinical CHF (New York Class III or IV), or LVEF < 30% by echocardiogram, sustained for at least 3 months despite therapy.

3.1.2. Description of Secondary Endpoints

The secondary endpoints fall into 4 classes: safety measurements, disease response, disease activation/progression and quality of life.

a. Time to treatment-failure. Treatment-failure is an event as defined in the primary endpoint (Section 3.1.1). The time to treatment-failure will be defined as the time interval between transplant (day 0) and the initial visit at which death or the qualifying event first occurs.

b. Pulmonary function: Using DLCO and FVC, improvement is indicated by an increase of > 15% in DLCO or > 10% in FVC (actual change in % predicted units from baseline) sustained for ≥ 3 months (on two determinations) during 5 years after treatment. DLCO and FVC will be analyzed separately, each as an ordinal outcome.

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[a] DLCO change = DLCO at assessment ((% predicted) – DLCO at baseline (% predicted)). Adjusted DLCO will be used based upon a subject's hemoglobin <13 or >17 gm/dL and altitude adjustments. Hemoglobin will be adjusted per the Cotes (1972) formula, and percent predicted per the Crapo Morris equation.

[2] FVC change = FVC at assessment ((% predicted) – FVC at baseline (% predicted))

c. HRQOL measured by the SF-36, the PROMIS-29 v 1.0, the SHAQ, and the SGRQ. The PROMIS-29 assesses physical functioning, anxiety, depressive symptoms, fatigue, sleep disturbance, satisfaction with social roles, and pain. Scores will be evaluated at set time points post-HCT and compared to the baseline evaluations.

d. Change in renal function over time as measured by serum creatinine.

e. Change in cardiac function as measured by ejection fraction on echocardiogram (percentage).

f. Treatment-related mortality as defined by death occurring at any time after start of mobilization procedure to day +90 after autologous HCT and definitely or probably resulting from treatment given in the study. Study investigators will make a determination about the cause of death.

g. All-cause mortality is defined as any death.

h. Regimen-related toxicities are defined as adverse events (AEs) ≥ Grade 3 and assessed by the investigator as 1 of the following:

    i. Unrelated, unlikely, or possibly related to treatment

    ii. Probably related to treatment

    iii. Definitely related to treatment

i. Infectious complications will be captured as a secondary safety endpoint for this study. The definition of infectious complications can be found in Appendix A.

j. Initiation of putative disease-modifying antirheumatic drugs (DMARDS) to modify disease. Subjects are not expected to receive additional disease-modifying therapy for SSc in the absence of disease progression or activity. A decision to initiate disease-modifying therapy other than that specified in the protocol will be considered as an indication of disease progression or activity and thus fulfills this secondary endpoint. In general, this would include the administration of any therapy (drugs, biologics, or any other treatments) clearly given for the purpose of treating the underlying SSc. This will include any FDA-approved agents and experimental agents not currently available but that become available during the period of the trial. Permitted concomitant therapies are summarized in Protocol Section 5.4.1. Systemic corticosteroids given at > 10 mg/day (prednisone or prednisone equivalent) without clearly defined non-SSc indications or methotrexate (MTX) given for non-arthritis indications will be considered DMARDs.

k. Health care utilization as assessed by UCSD Healthcare Utilization surveys. UCSD healthcare utilization is a self-report instrument that asks the patient about outpatient and inpatient visits, prescription and non-prescription medications, any surgeries, and major medical expenses during the last 3 months. Scores will be evaluated at specific time points after HCT and compared to the baseline evaluations.

l. Work productivity Survey (WPS): The survey is based on self-report, with a recall period of one month and consists of 9 questions. The first question assesses employment status, type of job for the employed (non-manual, manual or mixed manual/non-manual) and the status of those unemployed (homemaker, retired, student, unable to work due to SSc, unable to work due to non-SSc health problems, or other, i.e. volunteer). The next 3 questions apply only to employed patients and assess *absenteeism* (full days of work missed due to SSc), *presenteeism* (days with work productivity reduced by ≥50%), and how much SSc interfered with work productivity on a

scale of 0-10, where 0 = "no interference" and 10 = "complete interference." WPS was recently validated in SSc by the Singh et al using the UCLA Scleroderma Quality of Life cohort. Scores will be evaluated at set time points post-HCT and compared to the baseline evaluations.

Secondary endpoints identified by examining physicians that are subject to clinical interpretation may be submitted to a masked independent review committee at the end of the study for validation of the endpoint assessment. Endpoints that may be reviewed include, but are not limited to, mortality causation and acute exacerbations.

3.1.3. Description of Tertiary Endpoints

Tertiary endpoints are the observations made in ancillary studies of effect on skin, vasculopathy, fibrosis and immune response/recovery.

3.1.4. Description of Study Design

This prospective, open-label, multi-center, 1-arm, Phase II clinical trial will enroll 30 subjects with SSc. Subjects will be accrued over a 3-year period and receive high-dose therapy with autologous stem cell rescue followed by MMF as maintenance therapy for 2 years. The initial treatment period will be approximately 3 months. Subjects will be evaluated over a period of 5 years after autologous HCT.

4. SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects with diffuse cutaneous SSc will be recruited to participate in this study. Subjects of all genders, races, and ethnicities who are 70 year of age or less and meet eligibility criteria will participate in this study. Rheumatologists will conduct clinical assessments to determine whether patients are potentially eligible. If so, they will be referred to the transplant center to complete screening. An eligibility checklist will be completed following the Baseline Screening Visit to determine eligibility.

Written informed consent must be obtained prior to the subject undergoing any study-related procedures.

Prior treatment with cyclophosphamide, glucocorticoids, MMF, MTX or other immunosuppressive agents does not exclude subjects from eligibility for the protocol.

4.1. Subject Inclusion Criteria

1. Patients with SSc as defined by the American College of Rheumatology (Appendix W) with diffuse cutaneous disease (except Group 5) at risk of disease progression.
2. Age less than or equal to 70 years.
3. Patients must have failed a prior ≥ 4-month course of either MMF/Myfortic or cyclophosphamide before being eligible for the study (determined at ≥ 1 week before start of mobilization). "Failure" is defined as evidence of disease progression or absence of improvement. The response to prior MMF or cyclophosphamide will be assessed by the participating site study rheumatologist.
4. Patients must meet eligibility in at least 1 of the following 6 groups:

Group 1: Patients must have 1) both a and b below; and 2) either c or d.

a. Diffuse cutaneous scleroderma as defined by skin thickening proximal to the elbows and knees and/or involving the torso in addition to distal extremity involvement. A skin score will be obtained but not used to determine eligibility; (Appendix B).

b. Duration of systemic sclerosis ≤ 7 years from the onset of first non-Raynaud's symptom. For those patients with disease activity between 5-7 years from the onset of first non-Raynaud's symptom, recent progression or activity of disease must be documented.

c. Presence of SSc-related pulmonary disease with FVC <80% or hemoglobin-adjusted DLCO[c] < 70% of predicted AND evidence of alveolitis or SSc-related interstitial lung disease by high-resolution chest CT scan and/or by BAL. Intersitial lung disease may be nonspecific interstitial pneumonia (NSIP) or usual interstitial pneumonia (UIP). A bronchoalveolar lavage (BAL) should be done to confirm the findings of alveolitis only if the high resolution CT scan (HRCT) fails to show findings typically associated with systemic sclerosis changes (ground glass, NSIP, UIP, SSc related interstitial lung disease). Alveolitis by BAL cell count will be defined based on a BAL cell differential count (>3% neutrophils and/or >2% eosinophils) from any lavaged lobe.

d. History of SSc-related renal disease that may not be active at the time of screening. Stable serum creatinine must be documented for a minimum of 3 months post-renal crisis at the time of

[c] Adjusted DLCO will be used based upon a subject's hemoglobin < 13 or > 17 gm/dL and altitude adjustments. Hemoglobin will be adjusted per the Cotes (1972) formula, and percent predicted per the Crapo Morris equation.

the baseline visit. History of scleroderma hypertensive renal crisis is included in this criterion, and is defined as follows:

History of new-onset hypertension based on any of the following (measurements must be repeated and confirmed at least 2 hours apart within 3 days of first event-associated observation, with a change from baseline*):

- SBP ≥ 140 mmHg
- DBP ≥ 90 mmHg
- Rise in SBP ≥ 30 mmHg compared to baseline
- Rise in DBP ≥ 20 mmHg compared to baseline

*Historical baseline blood pressure of the subject.

AND

One of the following 5 laboratory criteria:

- Increase of ≥ 50 % above baseline in serum creatinine*
- Proteinuria: ≥ 2+ by dipstick confirmed by protein:creatinine ratio > 2.5
- Hematuria: ≥ 2+ by dipstick or > 10 RBCs/HPF (without menstruation)
- Thrombocytopenia: < 100,000 plts/mm3
- Hemolysis: by blood smear or increased reticulocyte count

*Historical baseline serum creatinine of the subject.

The above definition of SSc hypertensive renal crisis is independent of whether concomitant anti-hypertensive medications are used.

Subjects who present with solely skin and renal disease in the absence of other organ involvement, except classic SSc renal crisis as described above and including non-hypertensive renal crisis, must see a nephrologist to confirm that their renal disease is secondary to only SSc.

Note: Subjects may be re-screened if they fail to meet inclusion criteria on initial evaluation.

Group 2: Progressive pulmonary disease as defined by a decrease in the FVC or DLCO-adjusted by 10 or 15 percent or greater, respectively, from a prior FVC or DLCO-adjusted in the previous 18-month period. Patients will have diffuse cutaneous disease and may have both FVC and $DLCO_{corr}$ ≥ 70% at screening for the study. Patients must also have evidence of alveolitis as defined by abnormal chest CT or BAL.

Group 3: Diffuse scleroderma with disease duration ≤ 2 years since development of first sign of skin thickening plus modified Rodnan skin score ≥ 25 plus either 1) ESR > 25 mm/1st hour and/or Hb < 11 g/dL, not explained by causes other than active scleroderma or 2) lung involvement (either FVC or DLCO <80% and evidence of interstitial lung disease by CT scan or alveolitis by BAL).

Group 4: Diffuse scleroderma with disease duration ≤ 2 years and skin score ≥ 30.

Group 5: Limited cutaneous scleroderma and SSc-related pulmonary disease with FVC <80% or hemoglobin-adjusted DLCO[d] < 70% of predicted AND evidence of alveolitis/ interstitial lung disease by high-resolution chest CT scan and/or by BAL. Intersitial lung disease may be nonspecific interstitial pneumonia (NSIP) or usual interstitial pneumonia (UIP).A bronchoalveolar lavage (BAL) should be done to confirm the findings of alveolitis only if the high resolution CT scan (HRCT) fails to show findings typically associated with systemic sclerosis changes (ground glass, NSIP, UIP, SSc related interstitial lung disease). Alveolitis by BAL cell count will be defined based on a BAL cell differential count (>3% neutrophils and/or >2% eosinophils) from any lavaged lobe.

Group 6: Progressive gastrointestinal disease as defined by all of the following items:

a. Disease duration of scleroderma ≤ 2 years.
b. Documented severe malabsorption syndrome requiring nutritional support. Severe malabsorption syndrome is ≥10% weight loss and on total parenteral nutrition (TPN) or enteral feedings.
c. High score on distention/ bloating scale (>=1.60 out of 3.00) on GI questionnaire (Appendix J, questions 9-12).

4.2. Subject Exclusion Criteria

Subjects who meet any of the following criteria are disqualified from enrollment in the study:

1. Subjects with pulmonary, cardiac, hepatic, or renal impairment that would limit their ability to receive cytoreductive therapy and compromise their survival. This includes, but is not restricted to, subjects with any of the following:
    a. Pulmonary dysfunction defined as:
        i. Severe pulmonary dysfunction with (1) a hemoglobin corrected DLCO[e] <40% of predicted at the Baseline Screening visit, or (3) FVC < 45% of predicted at the Baseline Screening visit, or
        ii. pO_2 < 70 mmHg or pCO_2 ≥ 45 mmHg without supplemental oxygen, or
        iii. O_2 saturation < 92% at rest without supplemental oxygen as measured by forehead pulse oximeter.
    b. Significant pulmonary artery hypertension (PAH) defined as:
        i. Peak systolic pulmonary artery pressure >50 mmHg by resting echocardiogram will require right heart catheterization. If PAP is not evaluable on echocardiogram due to lack of a Tricuspid regurgitant jet, then normal anatomy and function as evidenced by normal right atrium and ventricle size, shape and wall thickness and septum shape must be documented to rule-out PAH. Otherwise, right heart cathertization is indicated. Prior history of PAH but controlled with medications will not exclude patients from the protocol. PAH is considered controlled with

[d] Adjusted DLCO will be used based upon a subject's hemoglobin < 13 or > 17 gm/dL and altitude adjustments. Hemoglobin will be adjusted per the Cotes (1972) formula, and percent predicted per the Crapo Morris equation.
[e] Adjusted DLCO will be used based upon a subject's hemoglobin < 13 or > 17 gm/dL and altitude adjustments. Hemoglobin will be adjusted per the Cotes (1972) formula, and percent predicted per the Crapo Morris equation.

medications if peak systolic pulmonary artery pressure is <45 mmHg or mean pulmonary artery pressure by right heart catheterization is ≤30 mmHg at rest.

ii. Mean pulmonary artery pressure by right heart catheterization exceeding 30 mmHg at rest. If mean pulmonary artery pressure is elevated and pulmonary vascular resistance and transpulmoanry gradient are normal then the patient is eligible for the protocol.

iii. New York Heart Association (NYHA/ World Health Organization, Appendix C) Class III or IV.

c. Cardiac: Uncontrolled clinically significant arrhythmias; clinical evidence of significant CHF (NYHA Class III or IV, Appendix D); LVEF < 50% by echocardiogram.

N.B. History/presence of arrhythmia (even controlled) on chemical anti-arrhythmic(s) must have cardiac consult to ensure the subject could safely proceed with protocol requirements.

d. Significant renal pathology defined as:

i. Estimated CrCl < 40 mL/min (using Cockcroft-Gault formula based on actual body weight[f]) **and** serum creatinine > 2.0 mg/dL; OR

ii. Active, untreated SSc renal crisis at the time of enrollment.

Presence of nephrotic range proteinuria (defined as ≥ 3.5 gms/24 hours, or protein:creatinine ratio ≥ 3.5), active urinary sediment, urinary RBCs > 25 perHPF, or red cell casts require further investigation by a nephrologist to rule out glomerulonephritis, overlap syndromes, or other causes of renal disease in all subjects. Subjects with glomerulonephritis or overlap syndromes will be excluded.

e. Hepatic: Active hepatitis (ALT, AST, or bilirubin > 2 times the ULN) or evidence of moderate to severe periportal fibrosis by liver biopsy.

2. Active or clinically significant Gastric Antral Vascular Ectasia (GAVE, "watermelon stomach").

Subjects must receive treatment outside the study, and may then be re-screened.

3. Unwilling or unable to discontinue disallowed DMARDs for treatment of SSc prior to mobilization .

4. History or presence of a 2nd autoimmune disease requiring immunosuppressive therapy that has substantial risk of immunosuppressive treatment beyond transplant with the following exceptions:

a. History and/or presence of Sjogren's Syndrome is allowed.

b. Stable myositis (A history of myositis that is clinically stable as defined by lack of progressive proximal muscle weakness and a stable or decreasing CPK <3x ULN) is allowed.

c. The presence of anti-ds-DNA without clinical systemic lupus erythematosus in a patient with a diagnosis of otherwise "pure" SSc is allowed.

[f] Cockcroft-Gault Formula: CrCl= [(140-age) x weight in kg] / [72 x serum creatinine], in women (x 0.85)

d. Concomitant rheumatoid arthritis without extra-articular disease characteristic of rheumatoid arthritis is allowed.

5. Active uncontrolled infection that would be a contraindication to safe use of high-dose therapy.
6. Positive study for Hepatitis B surface antigen or Hepatitis B or C confirmed by PCR.
7. Positive serology for human immunodeficiency virus (HIV).
8. Hematologic abnormalities as defined below (per peripheral blood counts):
   a. ANC < 1500 cells/μL, or
   b. Platelets < 100,000 cells/μL, or
   c. Hematocrit < 27%, or
   d. Hemoglobin < 9.0 g/dL
9. Malignancy within the 2 years prior to entry in study, excluding adequately treated squamous cell skin cancer, basal cell carcinoma, and carcinoma in situ. Treatment must have been completed (with the exception of hormonal therapy for breast cancer) with cure/remission status verified for at least 2 years prior to entry in this study.
10. Presence of other comorbid illnesses with an estimated median life expectancy < 5 years.
11. Evidence of myelodysplasia (MDS). Subjects with history of receiving any prior chemotherapy and/or radiotherapy for the treatment of malignant disease, history of greater than 2 months total prior cyclophosphamide for any condition (regardless of dose and route) and/or subjects presenting with abnormal peripheral blood counts require unilateral bone marrow aspiration for pathology, flow cytometry, cytogenetics, and FISH MDS panel (per institutional profile) to rule out MDS.
12. Pregnancy.
13. Inability to give voluntary informed consent.
14. Unwilling to use contraceptive methods for at least 15 months after starting treatment.
15. History of smoking tobacco (or other related/ herbal products) in the prior 3 months.
16. History of prior autologous hematopoietic cell transplantation.

4.3. Subject Withdrawal Criteria

4.3.1. Description of Subject Completion

Subjects are considered to have completed the study if they have undergone transplantation and have completed 5 years of follow-up.

4.3.2. Withdrawal of Individual Subjects

The investigator(s) will make every reasonable effort to keep each subject in the study through the year-5 visit. If a subject withdraws from the study, the reason for withdrawal must be documented in the study chart. Possible reasons for withdrawal include:

- Patient's preference
- Adverse experiences

- Protocol deviation, including non-compliance
- Subject lost to follow-up
- Discretion of investigator(s)

Subjects shall be withdrawn from the study immediately if any of the following occur:

- The subject or subject's guardian requests withdrawal from the study.
- The investigator believes it is in the best interest of the subject.
- There is clinically significant deterioration of the subject's medical status that warrants termination from the study.
- There are clinically significant abnormal laboratory results that warrant termination from the study.

4.3.3. Withdrawal of Subject from Study Following Adverse Events

The site investigator must apply his/her clinical judgment to determine if an adverse event (AE) is of sufficient severity to require that the subject should immediately be withdrawn from the study. If the withdrawal from the study is due to an AE, the subject should be given appropriate care under medical supervision until the symptoms of the AE resolve or his/her condition becomes stable. Subsequent review by the DSMB, the FHCC IRB or the Steering Committee may also result in the suspension of further trial treatments at a site. The DSMB retains the authority to suspend additional enrollment and treatments for the entire study as applicable.

A subject may also voluntarily withdraw from treatment. If voluntary withdrawal is requested, or if withdrawal occurs for any reason, the subject should be asked to continue (at least limited) scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable.

4.3.4. Procedures for Handling Withdrawals

In case of early withdrawal, subjects should be asked to continue (at least limited) scheduled evaluations and to complete an end-of study evaluation which includes all scheduled exams, procedures, and laboratory tests as if it is the year-5 visit; and should be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable. If the subject is unwilling or unable to complete visits, he/she should be asked to return for the end-of-study visit and then should be followed through telephone calls by the site study coordinator.

Subjects who do not complete study therapy will continue to be followed to the extent possible and asked to provide the same data as subjects who did not withdraw.

4.3.5. Subject Replacement

Subjects will not be replaced once treated. Subjects will be encouraged to continue in more limited follow-up even if they drop out.

5. TREATMENT OF SUBJECTS

Subjects must commence high-dose therapy within 3 weeks of completing PBSC collection unless there are new medical contraindications. If new medical contraindications occur during this time period, the subject's status will be reviewed by the Steering Committee prior to initiating therapy. Approval by this Committee is required for initiation of procedures beyond this window, and recommendations for

repeating specific baseline evaluations may be made on a case-by-case basis. Maintenance therapy with MMF will be started between 2 and 3 months after transplant when regimen-related toxicities are expected to be resolved.

5.1. Treatment Regimen: Mobilization, High-Dose Therapy and Autologous Hematopoietic Cell Transplantation

Subjects will undergo high-dose therapy with cyclophosphamide and ATGAM. Subjects will then be infused with autologous hematopoietic cell graft. The procedures for this treatment are detailed below.

5.1.1. G-CSF Stem Cell Mobilization and Preparation

Patients should have a double or triple lumen tunneled apheresis catheter placed that will also be used for transplant.

It is recommended to stop immunosuppression or cytotoxic agents at least 1 week in advance of initiating mobilization.

Subjects will receive G-CSF for mobilization of hematopoietic stem cells. G-CSF 16 µg/kg/day will be administered subcutaneously (**Table 3**). Prednisone up to 0.5 mg/kg/day may be given during each day of G-CSF and for up to 5 days after G-CSF completion to help prevent flare of autoimmune disease based on the clinical recommendation/ discretion of the site Rheumatologist.

Table 3. Schedule of Stem Cell Mobilization with G-CSF

Mobilization Regimen	Mobilization Day				
	M1	M2	M3	M4	M5 & subsequent days
G-CSF (16 µg/kg/day)	X	X	X	X	If more than one apheresis
CBC & differential	X	X	X	X	X
CD34+ cell count				X*	X*
Apheresis				X	X

*CD34+ peripheral blood cell count may be obtained according to institutional standard practice. If CD34+ peripheral blood cell count is obtained, it is recommended to evaluate the assay result prior to the clinical decision to perform the apheresis collection on that day.

Peripheral blood mononuclear cells will be collected by continuous flow blood cell centrifugation (apheresis or leukapheresis). Whole blood apheresis will be performed according to the institutional standard of practice. Procedures will be performed by qualified staff with a physician immediately available.

Typically ACD-A will be used for anticoagulation during the apheresis procedure. Side effects include transient hypocalcemia, with resultant mild perioral and/or peripheral paresthesias, a sensation of coldness, and occasional skeletal muscle irritability. Symptoms are generally controlled with administration of calcium-containing antacid, IV infusion of $CaCl_2$, and/or decreasing the flow rate of the procedure. Rare (≤ 5%) vasovagal episodes are managed with fluid administration and postural manipulation.

A minimum target dose of CD34+ cells ≥ 2.5 x 10^6/kg will be collected. The preferred target cell dose per institutional standard clinical practice is 5.0 x10^6/kg. In some cases, only 1 collection procedure will be needed; however, apheresis may continue daily for up to 5 days until the target is reached. If collection on several successive days is required, there is an increased risk for transient reduction of hematocrit and thrombocytopenia following the procedures.

If the first cycle of mobilization and collection is insufficient, one additional mobilization with up to 5 days of apheresis may be performed to collect the target CD34+ cell dose. The regimen for the second cycle of mobilization, if needed, will consist of either repeat G-CSF mobilization (if the stored product from the first cycle has CD34+ cells ≥ 1.5 and < 2.5 x 10^6/kg), or mobilization with a cyclophosphamide-based regimen (if < 1.5 x 10^6/kg CD34+ cells has been stored), as described below (Section 5.1.3). Patients difficult to mobilize after the first mobilization will be managed in consultation with their attending physician and the Principal Investigator to achieve adequate CD34+ cell collection with a second cycle of mobilization.

The autologous hematopoietic cell graft will be cryopreserved using controlled rate freezing with DMSO as cryoprotectant for later reinfusion. Aseptic quality-controlled methods will be used; procedures will be available for review in the cell processing laboratory.

On the day of transplantation, the graft will be infused per individual transplant center standard of practice, with verification of identity according to institutional practice for blood component transfusion.

Potential reinfusion toxicities attributable to DMSO include headache, nausea/vomiting, flushing, mild cardiac effects such as arrhythmia, and hypotension. Rarely, side effects of DMSO include anaphylactic-type reinfusion reactions.

5.1.2. Plerixafor plus G-CSF for mobilization of Autologous Peripheral Blood Stem Cells (PBSC)

For subjects for whom it is difficult to mobilize PBSC with G-CSF alone (collection of <2 x 10^6/kg CD34+ cells) have the option to receive plerixafor combined with G-CSF for mobilization of hematopoietic stem cells. This treatment is an alternative to the cyclophosphamide-based mobilization regmen (see 5.1.3). The decision to use plerixafor vs. cyclophosphamide will be based on the individual treating physician. Institutional standard practice guidelines for the use of plerixafor will be followed. The standard dose of plerixafor is 240mcg/kg (not to exceed 40mg/day) given subcutaneously in the evening prior to planned apheresis. Please refer to appendix V for additional suggested guidelines for the use of plerixafor (based on FHCC standard guidelines for plerixafor).

5.1.3. Cyclophosphamide-Based Mobilization Regimen (Subjects Difficult to Mobilize)

Subjects difficult to mobilize with G-CSF alone (< 1.5 x 10^6/kg CD34+ cells) may receive cyclophosphamide followed by G-CSF for mobilization of hematopoietic stem cells. Cyclophosphamide 2.0 g/m^2 will be administered according to institutional practice for two days in succession. Mesna 2.0 g/m^2 will be administered concurrently either IV or orally per institutional practice. Urine output of ≥ 100 mL/hour should be maintained. Subjects will be observed for arrhythmias, cardiac failure, and hematuria following infusion of cyclophosphamide. Premedication with anti-emetics will be administered pre- and post-chemotherapy according to institutional practice. G-CSF 10 µg/kg/day subcutaneously (SC) will be started 72 hours after completion of cyclophosphamide administration. Antibiotic prophylaxis should be administered for severe neurtopenia until resolution of neutropenia

(ANC > 500 cells/μL). Patients must be monitored closely for sepsis during the neutropenic phase of mobilization. When the WBC count is ≥ 1000 cells per μL, peripheral blood CD34+ counts will be evaluated the following morning. For most patients, this will occur after 4 to 7 doses of G-CSF. The mobilization regimen and schedule are shown below in Table 4. For safety, there must be at least a 3 week window between administration of cyclophosphamide for mobilization and administration of cyclophosphamide for conditioning pre-transplant.

Table 4. Cyclophosphamide-Based Mobilization Schedule (For Subjects Difficult to Mobilize)

Mobilization Regimen	Mobilization Day							
	M1	M2	M3	M4	M5	M6	M7	M8 & Subsequent days
CY (2 g/m^2)	X	X						
G-CSF (10 μg/kg/d)					X	X	X	If more than one apheresis
CBC & differential	X	X	X	X	X	X	X	X
Apheresis								X Performed when peripheral CD34+ count is sufficient

5.1.4. HDIT Conditioning

Initiation of high-dose therapy must begin within 3 weeks of completing mobilization. Subjects will be treated with ATGAM 90 mg/kg, and cyclophosphamide 200 mg/kg. The conditioning regimen and transplantation schedule is described in **Table 5**.

Table 5. Schedule for HDIT Conditioning and Autologous HCT

Conditioning Regimen	Day									
	-5	-4	-3	-2	-1	0	+1	+3	+5	+60
IV Methylprednisolone (1 mg/kg, prior to ATGAM) [1]	X		X		X		X	X	X	
ATGAM Skin Test (Prior to ATGAM)	X									
ATGAM (15 mg/kg/day) [2]	X		X		X		X	X	X	
CY (50 mg/kg/day)	X	X	X	X						
Mesna (50 mg/kg/day) or Continuous Bladder Irrigation	X	X	X	X						
Hydration (50-200 cc/hour)	X	X	X	X						
Infusion of Autologous Hematopoietic Cell Graft (≥ 2.5 x 10^6 CD34+ cells/kg)						X				
MMF maintenance therapy up to 2 Grams daily; start at 2-3 months post transplant until 2 years post										X

1 Subjects who do not receive either ATGAM or Thymoglobulin because of a positive skin test (skin test only for ATGAM) or allergic reaction will receive corticosteroids (methylprednisolone or prednisone) at a daily single dose of 0.5 mg/kg/day from Day -5 – Day +21 (or from when the ATG is discontinued)
2 Subjects who have a positive skin test or allergic to ATGAM will be eligible to receive Thymoglobulin.

Subjects will receive the conditioning regimen as inpatients and remain hospitalized until recovery of an ANC to > 500 cells/µL for at least 2 days. Although most patients are expected to be hospitalized until recovery of an ANC to > 500 cells/µL for at least 2 days, the requirement for hospitalization will be determined by the institute's standard of practice. In the event of severe side effects of chemotherapy or neutropenic fever, subjects will be hospitalized immediately. Subjects who become neutropenic (ANC < 500 cells/µL) in OPD should receive prophylactic oral or IV antibacterial treatment until the neutropenia resolves.

5.1.4.1. Cyclophosphamide before transplant

Cyclophosphamide will be administered at a dosage of 50 mg/kg on each of 4 successive days (refer to Table 5, for the dosing schedule). If subject actual body weight is less than/equal to the ideal body weight (IBW), then the actual body weight will be used to calculate drug dosing. When actual body weight is greater than IBW, then adjusted ideal body weight will be used. The formulas for calculating IBW and adjusted IBW are found below in Table 6.

Table 6. Formulas for Calculating Ideal Body Weight for the Dose of Cyclophosphamide

Ideal Body Weight	Men = 50 + 2.3 [Height (in) - 60]
	Women = 45.5 + 2.3 [Height (in) - 60]
Adjusted Ideal Body Weight	= IBW + [0.40 x (Actual Body Weight - IBW)]

Adjusted ideal body weight will be used for obese subjects. Cyclophosphamide will be administered as an IV infusion over 1-2 hours. Bladder protection with either Mesna and/or continuous bladder irrigation via a urinary catheter will be undertaken according to institutional SOPs for high-dose cyclophosphamide in all cases. Antiemetics should be given prophylactically per the standard practice of the institution for subjects undergoing high-dose chemoradiotherapy.

5.1.4.2. Antithymocyte globulin

Equine antithymocyte globulin (ATGAM) will be administered at a dose of 15 mg/kg IV (based on actual weight) on Day -5, -3, -1, +1, +3, and +5. Methylprednisolone will be given at a dose of 1 mg/kg/day (actual body weight) as premedication 1 hour prior to each ATGAM dose. After completing the 6 ATGAM doses, methylprednisolone or prednisone 0.5 mg/kg/day will be given as a single dose from Day +6 to Day +21, and then tapered until Day 37 (downward daily dosage adjustment to 75% on Day 22, 50% on Day 27, 25% on Day 32 and 0 on Day 37). Subjects will be administered the ATGAM skin test prior to treatment. Subjects with a positive skin test performed per institutional practice (i.e., a local skin reaction of 10 mm or greater with a wheal or erythema, or both) will not receive ATGAM. Subjects who have a positive skin test or allergic to ATGAM will be eligible to receive Thymoglobulin. Thymoglobulin will be administered at a dose of 0.5, 1.0, 1.5, 1.5, 1.5 and 1.5 mg/kg of recipient body weight on days -5, -3, -1, +1, +3 and +5 respectively (total dose- 7.5 mg/kg). If ATGAM is stopped because of allergy or severe infusional reactions, the Thymoglobulin dose will be based on the preceding schedule for the day that Thymoglobulin administration is started (i.e. day -3 give 1.0 mg/kg but for day -1 and after, give 1.5 mg/kg). Subjects who do not receive either ATGAM or Thymoglobulin because of

a positive skin test (skin test only for ATGAM) or allergic reaction will receive corticosteroids (methylprednisolone or prednisone) at a daily single dose of 0.5 mg/kg/day from Day -5 – Day +21 (or from when the ATG is discontinued), and then tapered until Day 37 (downward daily dosage adjustment to 75% on Day 22, 50% on Day 27, 25% on Day 32 and 0 on Day 37).

5.1.5. Peripheral Blood Stem Cell Infusion

Infusion of cryopreserved autologous PBSCs will be performed 36 to 48 hours following the last cyclophosphamide dose. The treating physician should refer to the individual institution's standard practice policy manual for infusion guidelines.

On the day of transplant, the graft will be infused per individual transplant center standard of practice, with verification of identity according to institutional practice for blood component transfusion.

Potential reinfusion toxicities attributable to DMSO include headache, nausea/vomiting, flushing, mild cardiac effects such as arrhythmia, and hypotension. Rarely, side effects of DMSO include anaphylactic-type reinfusion reactions.

5.1.6. Maintenace Therapy with Mycophenolate Mofetil

5.1.6.1. Initial Dosing.

Mycophenolate mofetil will be intiated between 2-3 months after autologous HCT when all regimen-related toxicities are expected to have resolved. Patients will be instructed to take 500 mg MMF p.o. b.i.d. initially and continue for 1 month. After 1 month, if MMF is tolerated well, the dose will be increased to 1000 mg twice daily. Patients who do not tolerate 1000 mg twice daily may have the dose decreased at any time to a tolerable dose. Attempts will be made to re-institute MMF at the target dose once the reason for the dose decrease has resolved. Myfortic may be used as an alternative for MMF. Myfortic doses equivalent to MMF will be used. For the purpose of this study, 720 mg (360 mg + 360 mg tablets) of Myfortic is equivalent to 1000 mg (two 500 mg tablets) of MMF.

5.1.6.2. Rules for adjusting the dose of Mycophenolate Mofetil.

The following abnormalities and laboratory tests require MMF discontinuation, either temporary (until normalization or identification of the etiology for the abnormality) or permanent:

- WBC <2500, or <1000 neutrophils
- Platelet count <100,000.
- Serum creatinine >2.0 mg/dl, or increase in
- Serum creatinine of >50% over baseline, or decrease of creatinine clearance to <45 ml/min (corrected) in the absence of other etiology.
- Pregnancy, or breast feeding.
- Ongoing infection whose management would be significantly compromised by MMF.
- Adverse experience felt by the investigator to be clinically significant and requiring drug discontinuation.
- Malignant hypertension: BP ≥160/110 on two occasions at least 12 hrs apart, and one of the following abnormalities: proteinuria, hematuria (unrelated to menses) or casts, evidence of microangiopathic hemolytic anemia, or renal insufficiency (serum creatinine > upper limits of normal).
- Significant worsening of gastrointestinal symptoms including anorexia, nausea, vomiting or diarrhea.

If any of the above toxicity occurs, the dose of MMF will be altered as follows:

- For ANC <1000, platelet count <100,000, or clinical toxicity judged by the investigator to be severe but dose-related: Stop MMF until ANC >1500, platelets >100,000, or clinical side effects have ceased. At that point re-introduce MMF at 1 capsule daily for 1 week, 1 capsule b.i.d. for 1 week, and then a dose 1 capsule lower than the dose causing side effects. Follow-up should be every 1-2 weeks, as clinically indicated, until the investigator is satisfied that it is safe to return to the protocol-defined dosing schedule.
- For less severe adverse events (e.g., dyspepsia, diarrhea) not responding to concomitant medications, MMF is to be discontinued until the adverse event disappears. At that point, MMF can be re-started at one-half the original dose. The patient can return to the full dose or one capsule less than the full dose, as clinically indicated, after 2 wks at the half dose of MMF.
- For surgery or infections requiring antibiotics or hospitalization, the MMF should be discontinued until the infection is cleared and the patient is off antibiotics for two weeks after surgery.
- If GI intolerance recurs on mycophenolate mofetil then treatment with equivalent dosing schedule of Myfortic is permitted (see above for dosing schedule). Patients will be instructed to initiate Myfortic at 360 mg p.o. b.i.d. and continue for 1 month. After 1 month, if Myfortic is well tolerated, the Myfortic dose will be increased to 720 mg p.o. twice daily.
-

The decision to discontinue maintenance therapy with MMF beyond 2 years will be made by each site rheumatologist.

5.1.7. Other Supportive Care

5.1.7.1. Corticosteroids

Methylprednisolone 1 mg/kg/day (actual body weight) will be administered concomitantly with ATGAM infusions. From then on, methylprednisolone or prednisone 0.5 mg/kg/day will be given as a single dose from Day +6 to Day +21, and then tapered until Day 37 (downward daily dosage adjustment to 75% on Day 22, 50% on Day 27, 25% on Day 32 and 0 on Day 37). If subjects do not receive ATGAM, they will receive corticosteroids (methylprednisolone or prednisone) at a daily single dose of 0.5 mg/kg/day from Day -5 – Day +21, and then tapered until Day 37 (downward daily dosage adjustment to 75% on Day 22, 50% on Day 27, 25% on Day 32 and 0 on Day 37).

5.1.7.2. Growth factors

It is recommended that subjects will receive G-CSF (5 μg/kg/day) from Day +5 to the time of sustained neutrophil engraftment (ANC > 1,000 cells/μL) for 3 consecutive days or > 5000 cells/μL for 1 day. Alternatively, G-CSF may be given as per institutional standard practice. G-CSF may be held based on the discretion of the treating physician in the case of toxicity.

5.1.7.3. Outpatient treatment

Subjects will receive the conditioning regimen as an inpatient. Although most patients are expected to be hospitalized until recovery of an ANC to > 500 cells/μL for at least 2 days, the requirement for hospitalization will be determined by the institute's standard of practice. Subjects who become neutropenic (ANC < 500 cells/μL) in the OPD should receive prophylactic oral or IV antibacterial treatment until neutropenia resolves. Refer to Section 5.3 for infection prophylaxis guidelines.

5.1.7.4. ACE Inhibitors

Angiotensin-converting enzyme inhibitors will be used from the start of conditioning through day 60. The ACE inhibitor will be lisinopril 2.5-20 mg/day or an equivalent/comparable agent, with the exception of captopril, which is contraindicated for transplant subjects. It is recommended that the lowest renal prophylactic dose should be started and titrated as necessary with the ideal target goal that systolic blood pressure be maintained between approximately 90 and 110 mmHg. Nephrology and Rheumatology consultants may vary treatment guidelines for hypertension based on the clinical status of the patient.Patients intolerant to ACE inhibitors may receive an angiotensin II receptor blocker (ARB). Valsartan (Diovan) may be given orally at 40-160 mg/day as tolerated. There may be situations in which ACE inhibitors may not be indicated and in these cases patients should be followed closely and treated if blood pressure increases.

Management and treatment of pediatric hypertension will be supported in consultation with pediatric renal team.

5.2. Transfusion Guidelines

5.2.1. Transfusion Guidelines after the High-Dose Immunosuppressive Therapy and Autologous HCT

Due to the degree of regimen-induced anemia and thrombocytopenia and subsequent risk of bleeding, supportive care will be required immediately following transplantation during the recovery phase. All aspects of supportive care will be in keeping with individual institutional SOPs and in keeping with the guidance found below.

5.2.1.1. Transfusion support

All blood products except the autologous PBSC graft should be irradiated to 2500 cGy until two years post-transplant. CMV seronegative patients are to receive CMV negative blood products or alternatively (leukocyte-poor) PALL filtered blood products. Prior to administration of blood products, patients may be medicated with Benadryl and acetaminophen to prevent febrile reactions.

Red Cells: For Hb < 9.0 g/dL (Hct < 27), or per transplant center local policy, transfuse 1 to 2 units irradiated ABO/Rh matched units.

Platelets: Platelets are to be administered per transplant center local policy. HLA-matched platelets may be required in individuals with poor responses to random donor transfusions.

Additional platelet transfusions can be administered based on the clinical judgment of the treating physician. For overt hemorrhage, maintain platelet counts greater than 50 x 10^9/L or per transplant center institutional standard. Patients may receive single donor or random platelets per institutional practice.

5.3. Infection Prophylaxis Guidelines

An increased incidence of infectious complications as compared to conventional treatment is anticipated. Important potentially life-threatening infections include CMV and EBV-related PTLD. Recommendations for infection prophylaxis are based on prevailing practice for hematopoietic allograft recipients. All persons must wash their hands before and after leaving the patient's room as intervention

and prevention measures for infection. Patients should receive an "immunosuppressed" diet in the hospital.

5.3.1. Screening and Surveillance

The general prevention methods for infection control and surveillance are described below.

5.3.1.1. Infection control

1. Oral hygiene will consist of a dental evaluation that should be obtained before referral and needed repair and dental procedures performed before transplant to eliminate abscesses and resolve dental caries. In addition, teeth are to be cleaned twice daily with soft-tipped brushes and necrotic debris removed with QID cleansing and debridement per institutional practice during transplant. Care per institutional practice should be employed to prevent candida.

5.3.1.2. Screening and surveillance

1. Screening for respiratory viruses (RSV, parainfluenza, and influenza) with nasal wash and throat swab for DFA, culture, or PCR (the latter is the most sensitive indicator) should be done. Screening should be done after mobilization but prior to initiating conditioning regimen. If positive, transplant should be delayed until repeat cultures/PCR are negative. Subjects with URI symptoms after transplant should be re-screened as above.
2. Determination of IgG serostatus for CMV, EBV, HSV and VZV should be obtained before the start of transplant. CMV pp 65 antigenemia or CMV DNA (by PCR or hybrid capture) should be obtained before transplant.
3. Quantitative serum IgG, IgM, and IgA levels should be obtained before the start of transplant with repeated levels at approximately Weeks 12, 26 and 52 post-transplant or as indicated by the institutional practice of the transplant center.

5.3.2. Bacterial Prophylaxis

1. For neutropenia with ANC < 500 cells/μL, the investigator will administer broad-spectrum intravenous antibiotic coverage per local institutional policy. Subjects with febrile neutropenia will be readmitted as necessary for evaluation and administration of broad-spectrum antibiotic treatment.
2. If the serum IgG levels are less than 400 mg/dL, administer IV immumoglobulin (IVIg) 500 mg/kg every 1-3 weeks and monitor serum trough IgG levels.

5.3.3. PCP Prophylaxis

Prophylaxis against *pneumocystis jiroveci* pneumonia (PCP) should follow institutional standards. Prophylaxis should start as pre-transplant loading and resume at the time of engraftment and continue until day 365 post-transplant or longer if receiving corticosteroid therapy. The preferred prophylaxis is:

1. Trimethoprim-sulfamethoxazole (TMP-SMX, bactrim) will be the first-line agent, given orally as 1 double strength tablet BID on days -9 to -2 pre-transplant. After engraftment, TMP-SMX is given as 1 double strength tablet BID on 2 days per week until Day 365 (or longer if on prednisone > 10 mg/day).

1. Oral dapsone (100 mg/QD) should be given to SSc subjects as the second-line agent if allergic or resistant to TMP-SMX. All dapsone recipients will be screened for G6PD deficiency before administration.

If the subject is unable to take TMP-SMX because of a history of sulfa drug sensitivity, and dapsone is not indicated, IV or aerosolized pentamidine can be used at the discretion of the physician. The dose of IV pentamidine is 4 mg/kg/month.

2. Atovaquone or other therapy as consistent with local transplant center standard of practice may be administered as 3rd line agent.

5.3.4. Fungal Prophylaxis

Transplant patients should receive oral or IV fluconazole (400 mg/QD), given from the start of conditioning to Day 75 post-transplant, to prevent fluconazole-susceptible *Candida* species. Centers may broaden antifungal prophylaxis per local institution policy.

5.3.5. Viral Prophylaxis

5.3.5.1. Herpes simplex virus (HSV)

Subjects with HSV seropositive test will be administered acyclovir (ACV) or equivalent per institutional standards from the start of conditioning until Day 30 after transplant (for VZV seropositive patients, see section 5.3.5.4). Dosing per present FHCC Standard Practice Guidelines are as follows: oral ACV (800 mg BID), oral Valacyclovir (500 mg BID), or IV ACV at 250 mg/m^2 BID. Individuals given gancyclovir (GCV), foscarnet, cidofovir, or valacyclovir for CMV prevention or treatment should not receive concurrent ACV.

5.3.5.2. Cytomegalovirus (CMV)

Surveillance for CMV and an early treatment strategy has changed the indicence of CMV disease. This manageament should be accomplished through standard institutional practice, or as described below.

1. Subjects who are CMV seronegative will be monitored for CMV antigenemia or CMV DNA weekly until Day 60.
2. Subjects who are CMV seropositive with no evidence of reactivation will be monitored for CMV pp65 antigenemia or CMV DNA assays (by PCR or hybrid capture) before transplant, weekly after transplant until Day 100, and then every 2 weeks until Day 180. Subjects with reactivation of CMV should be monitored weekly after transplant until at least Day 100, and longer if they remain antigen and/or DNA positive or are on steroids. Weekly monitoring should continue until they are antigen and/or PCR negative for at least 1 month, and are clinically stable and off GCV and steroids. Monitoring should then continue every 2 weeks through Day 365 if necessary.
3. CMV reactivation of disease will be treated according to institutional practice.
4. GCV dose adjustments: Adjust dose for white count and creatinine. If the absolute neutrophil counts decrease to 1000/mm^3, give G-CSF and hold drug. If GCV cannot be tolerated, IV foscarnet is the recommended second-line agent (recommended 60 mg/kg q 12 hours for the first 7 days then 90-120 mg/kg q 24 hours) until Day 100 after transplant. Dose adjustments may be done according to local institutional standards of practice for treatment and monitoring, which may include use of oral agents such as valganciclovir.
5. Persistent CMV DNA or antigenemia for 1-2 weeks on GCV prophylaxis does not necessarily mean treatment failure. However, if quantitative levels increase after more than 3 weeks of continuous treatment, it is recommended that GCV be stopped and IV foscarnet

(recommended 90 mg/kg BID for the first 7 days then 90-120 mg/kg QD until Day 100) or alternative treatment per institutional standard practice should be given.

5.3.5.3. Epstein Barr virus (EBV)

Patients undergoing autologous HCT for autoimmune disorders may have an increased risk for EBV reactivation. Surveillance for EBV is described below.

1. At baseline, serology for antibodies, including antibody titers, to EBV viral capsid antigen and/or nuclear antigen will be collected.
2. Pretransplant EBV PCR amplification will be performed to evaluate viral load in the peripheral blood for individual subjects when baseline EBV serology is negative.
3. Epstein-Barr virus monitoring will be done by EBV PCR, performed weekly from Day +14 and twice per week, if evidence of rising copy number, through Day 100 (Week 12) post-transplant. EBV monitoring by PCR will be done every other week from Day 101 until Month 6. If there is evidence of a rising copy number, the frequency of the monitoring will be increased to once or twice a week.
4. Subjects who develop a viral load of > 1000 copies per mL plasma will receive further individualized monitoring and/or therapy.
5. If the subject develops EBV reactivation, he or she will be treated with preemptive Rituximab 375 mg/m^2 IV weekly x 4 weeks or on an EBV reactivation protocol as per local institution practice. Subjects who develop EBV reactivation will also undergo flow cytometry of the peripheral blood and CT or MRI imaging of chest, abdomen, and pelvis to assess the development of lymphadenopathy, organomegaly, or other evidence of the development of PTLD. Whenever possible, the clinical diagnosis of PTLD should be confirmed by tissue biopsy.

5.3.5.4. Varicella-zoster virus (VZV)

Varicella-Zoster virus seropositive subjects will be treated with Acyclovir from the start of conditioning until Day 365 or until day of discontinuation of maintenance MMF/Myfortic, whichever is greater, to prevent VZV development or according to institutional guidelines. Acyclovir may be administered as tolerated orally as ACV (800 mg BID), oral valacyclovir (500 mg BID), or IV ACV (250 mg/m^2 BID). Individuals developing a VZV infection will be treated according to institutional guidelines. An acceptable treatment regimen is IV ACV (500 mg/m^2 TID) until 2 days past the date all lesions have crusted. Individuals exposed to an infected individual will be managed as per local ID policy.

5.3.6. Immunizations after Autologous Hematopoietic Cell Transplantation

Immunizations will be administered per institutional standard practice.

5.4. Permitted and Prohibited Concomitant Treatments

5.4.1. Permitted Concomitant Treatments

All subjects are permitted to take the following concomitant therapy:

1. Proton pump inhibitors, prokinetic agents, or antacids.
2. The use of non-steroidal anti-inflammatory drugs (NSAIDs) or analgesics.

3. The use of antihypertensives for treatment of hypertension.
4. Medications for treatment of CHF (e.g., diuretics, ACE inhibitors, etc).
5. The use of phosphodiesterase inhibitors for treatment of sexual dysfunction or for digital ulcers is allowed, as long as the criteria that exclude pulmonary artery hypertension (PAH) per Exclusion Criterion 4.2.1.b.*i* are met, and there is no previous documented history of PAH. The use of prostacycline analogues and endothelin receptor antagonists for digital ulcers is allowed, as long as criteria excluding PAH per the Exclusion Criterion 4.2.1.b.*i* are met, and there is no previous documented history of PAH.

The treatment of any illness unrelated to the subject's SSc should be initiated and continued per the appropriate clinical practice.

5.4.2. Prohibited Concomitant Medications

All subjects are to have access to any care deemed medically necessary, but administration of the following medications will be protocol deviations:

1. All disallowed medications should be discontinued by time of mobilization. In general, the administration of any therapy (drugs, biologics, or any other treatments) clearly given for the purpose of treating the underlying SSc is prohibited except for MMF. This includes drugs currently investigated in clinical trials, daily corticosteroids, azathioprine, N-acetyl cysteine, agents used for treatment of PAH.
2. Captopril, which contains a sulfa-moiety, has been associated with neutropenia and is therefore excluded.

Other medications not listed in the permitted concomitant medication, Protocol Section 5.4.1, may be contraindicated for the treatment therapy. Please refer to institutional guidelines for use of other concomitant medications and confer with the protocol chair(s) if necessary.

5.5. Supportive Care

5.5.1. Supportive Care Related to High-Dose Therapy and Autologous Transplantation

5.5.1.1. High-dose cytotoxic therapy

The use of high-dose cyclophosphamide/ATGAM may cause acute side effects including the following: nausea and vomiting; diarrhea; painful mucositis; alopecia; pancytopenia; immunodeficiency, infections; bleeding; and acute failure of lungs, liver, kidneys, and heart. Late side effects include cataracts, hypothyroidism, infertility, osteoporosis, secondary malignancies, and myelodysplastic syndromes. Other potential effects of the procedure include psychosocial problems and depression.

Management of these toxicities will be dictated by the standard of practice of the participating institution.

Because this regimen may more immunosuppressive than conventional autografting regimens, an increased incidence of infectious complications as compared to conventional treatment is anticipated. Important potentially life-threatening infections include CMV disease and EBV-related PTLD. Infection prophylaxis for recipients after high-dose therapy and autologous HCT is based on prevailing practice for hematopoietic allograft recipients, where immunosuppression is more severe and opportunistic infections occur more frequently than after conventional autografts. Prophylaxis and treatment of

infections will be dictated by the standard of practice of the participating institution, per the guidance described in Protocol Section 5.3.

5.5.1.1.1. Management of hematologic toxicity

Due to the degree of regimen-induced anemia and thrombocytopenia and subsequent risk of bleeding, supportive care will be required immediately following transplantation during the recovery phase. All aspects of care will be in keeping with individual institutional SOPs, and the guidance found below and also in Protocol Section 5.2.

5.5.1.1.2. Management of anemia

The hematocrit will be maintained with packed red cell transfusions as indicated. Refer to Protocol Section 5.2 for guidelines specific to management of anemia and transfusion support.

5.5.1.1.3. Management of thrombocytopenia

The platelet count will be maintained at ≥ 20,000/mm^3 or per local transplant center institutional policy. HLA-matched platelets may be required in individuals with poor responses to random donor transfusions. Refer to Protocol Section 5.2 for guidelines specific to management of thrombocytopenia and transfusion support.

5.5.1.1.4. Management of neutropenia

Subjects will receive G-CSF (5 µg/kg/day) intravenously from Day +5 until the time of sustained neutrophil engraftment (ANC > 1,000 cells/µL) for 3 consecutive days or > 5000 cells/µL for 1 day. MMF should be held until recovery of neutrophil counts as described in section 5.1.5.2. Determine if PCP prophylaxis should be changed from Bactrim to an alternative agent.

Side effects specific to the individual agents used in this protocol are summarized below.

5.5.1.2. Pulmonary and other toxicities after high-dose therapy

As indicated in the summary of data from FHCRC Protocol 1019 (Protocol Section 1.2.4.1) (high-dose therapy and autologous HCT for SSc) there were transient reductions in DLCO but not FVC at 3 months after transplant. Diffusion in liters of carbon monoxide reductions was also less at 1 year and returned to baseline at 2 years. As a review of the literature demonstrates, reduced diffusing capacity values are common, particularly in the first 6 to 12 months after high-dose therapy, with a trend towards recovery.

5.5.1.3. G-CSF

Potential primary toxicities of G-CSF are usually mild and include flu-like symptoms such as myalgia and bone pain. There have been reports that G-CSF may cause flares of some autoimmune diseases. SSc subjects have exhibited transient edematous or telangiectatic changes in the skin. Management of these toxicities will be dictated by the standard of practice of the participating institution.

5.5.1.4. Cyclophosphamide

The major dose-limiting side effect at high doses is cardiac necrosis. Hemorrhagic cystitis can occur and is mediated by the acrolein metabolite. Hemorrhagic cystitis can be prevented by co-administration of Mesna. Bladder protection with either Mesna or continuous bladder irrigation via a urinary catheter will be undertaken according to institutional SOPs for high-dose cyclophosphamide in all cases. Other potential acute side effects including nausea, vomiting, alopecia, myelosuppression, and SIADH, will be managed by the standard of practice of the participating institution. High-dose cyclophosphamide is also

associated with cardiac toxicity especially in patients with pre-existing cardiac disorders. Patients will be carefully screened for cardiac disorders and excluded from the study if present.

5.5.1.5. Equine antithymocyte globulin (ATGAM)

Subjects will be administered the ATGAM skin test prior to treatment. Subjects with a positive skin test will not receive ATGAM. Rabbit ATG will be considered instead of ATGAM if there is a positive skin test. Methylprednisolone or equivalent is given as premedication for each ATGAM dose. Potential side effects include fever and chills; skin rash and itching, usually prevented by or controlled with antihistamines and concomitant administration of corticosteroids; hypotension; wheezing; anaphylaxis; platelet and white cell count depression; serum sickness with severe skin rashes, mouth and vaginal sores, joint pain and swelling; and kidney damage. Management of these effects is dictated by the standard of practice of the participating institution.

5.5.1.6. Mycophenolate mofetil (MMF) or Mycophenolic acid (Myfortic)

Oversuppression of the immune system can increase susceptibility to infection, including opportunistic infections, fatal infections, and sepsis. Severe neutropenia [ANC $<0.5 \times 10^3/\mu L$] developed in up to 2.0% of renal, up to 2.8% of cardiac, and up to 3.6% of hepatic transplant patients receiving MMF 3 g daily. Cases of pure red cell aplasia have been reported in patients treated with MMF in combination with other immunosuppressive agents. The mechanism for mycophenolate mofetil induced PRCA is unknown. Gastrointestinal bleeding (requiring hospitalization) has been observed in approximately 3% of renal, in 1.7% of cardiac, and in 5.4% of hepatic transplant patients treated with MMF 3 g daily. In pediatric renal transplant patients, 5/148 cases of gastrointestinal bleeding (requiring hospitalization) were observed. Gastrointestinal perforations have rarely been observed. Most patients receiving MMF were also receiving other drugs known to be associated with these complications. Patients receiving immunosuppressive regimens involving combinations of drugs, including MMF or Myfortic, as part of an immunosuppressive regimen are at increased risk of developing lymphomas and other malignancies, particularly of the skin. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. For women of childbearing potential, use of MMF/ Myfortic in pregnancy is associated with an increased risk of first trimester pregnancy loss and an increased risk of birth defects so effective contraception must be used during treatment.

5.5.1.7. Management of delayed recovery of blood counts

A bone marrow aspirate should be performed between Days 28 to 35 if criteria for graft failure are met. If the bone marrow is hypoplastic, supportive care and careful clinical observation is warranted, and marrow aspirates and biopsy should be obtained as clinically indicated to further assess engraftment.

5.5.1.8. Management of post-transplant lymphoproliferative disorder

Post-transplant lymphoproliferative disorder (PTLD) is a rare, potentially fatal complication of hematopoietic stem cell transplantation. The risk of developing PTLD appears to be related to the intensity of the preparative regimen and the use of CD34-selection of the autologous hematopoietic cell graft. One SSc patient in FHCRC Protocol 1019 developed PTLD. Subjects treated in this protocol are not undergoing CD34-selection and the high-dose cyclophosphamide is less intense than high-dose regimen administered to the SSc patients in FHCRC 1019. However, if PTLD does occur, it will be treated according to the standard of practice of the participating institution.

5.5.1.9. Management of engraftment syndrome

Engraftment syndrome is a fever and skin rash associated in time with the return of peripheral blood myeloid cells after hematopoietic stem cell transplantation in the absence of infection. Pulmonary infiltrates and other manifestations of systemic disease may also be present. It has been seen in transplant for both treatment of hematologic malignancies and for treatment of autoimmune diseases such as MS. Patients will receive posttransplant corticosteroids to reduce the risk of the engraftment syndrome. If engraftment syndrome does occur, it will be treated according to the standard of practice of the participating institution.

5.5.2. Management of Worsening Pulmonary Function Related to Disease Progression

Patients will be evaluated to ascertain that the decrement in pulmonary function tests are related to scleroderma lung disease progression and not due to superimposed complications. Appropriate clinical evaluations and management will include chest radiographs to rule out pneumothorax, HRCT to rule out new changes attributable to change in pulmonary function tests (e.g. new ground-glass changes, pneumonia, lung neoplasm) and other evaluations directed by the clinical status of the patient. This will include ruling out deep venous thrombosis, pulmonary embolus and worsening of cardiac function or pulmonary hypertension.

5.5.3. Hypertension Management in Systemic Sclerosis Transplant Patients

A response to changes in BP is essential to management of SSc patients after transplant. A baseline BP and serum creatinine must be determined prior to patients receiving conditioning therapy. Monitoring for hypertension will be relative to each patient's baseline. Baseline BP is determined by taking BP on 2 separate occasions more than 48 hours following the initiation of the ACE inhibitor. Determine baseline serum creatinine with labs drawn prior to the initiation of conditioning and while patient is on the ACE inhibitor.

Hypertension will be defined as blood pressure:

- >140/90 systolic/diastolic measured on 2 successive occasions at least one hour apart;

or

- if baseline systolic blood pressure was less than 120 mm Hg, increase of SBP >20 mm Hg and DBP >10 mm Hg above baseline on 2 successive occasions at least one hour apart, or sustained increase in SBP of 15 mm Hg over 24 hour period.

Mild hypertension will operationally be defined as:

- SBP <20-30 mm Hg above baseline

and

- SBP <150 mm Hg and one of:
  - serum creatinine less than 0.4 mg/dL above baseline
  - absence of microangiopathy (red cell fragmentation on peripheral smear, elevation in LDH in absence of other explanation, thrombocytopenia without other explanation)
  - absence of hypertensive encephalopathy or retinopathy

Moderate hypertension will operationally be defined as:

- SBP >20-30 mm Hg above baseline

or

- SBP >150 mm Hg and:
  - increase in serum creatinine >0.4 mg/dL above baseline

- absence of microangiopathy (red cell fragmentation on peripheral smear, elevation in LDH in absence of other explanation, thrombocytopenia without other explanation)
- absence of hypertensive encephalopathy or retinopathy

Severe hypertension will operationally be defined as an increase in SBP or DBP associated with hypertensive encephalopathy or retinopathy +/- significant microangiopathy with worsening renal function.

Pediatric hypertension will be assessed and monitored by pediatric nephrologist.

Treatment

Pediatric hypertension will be treated by pediatric nephrologist.

Adult patients:

- Mild hypertension treatment in the absence of active GI disease (e.g. infectious gastroenteritis, graft vs. host disease involving gut, scleroderma-related diarrhea or constipation):

  Increase the dose of oral lisinopril by 2.5 mg/day every 2 days until target blood pressure is reached. The maximum lisinopril dose is 80 mg/day.

- Moderate hypertension treatment, or mild hypertension in the presence of active GI disease:

  IV enalaprilat 0.625 to 1.25 mg (recommend 0.625 mg dose unless pt over 80 kg) over 5-10 minutes. Additional doses of 0.625 to 1.25 mg may be administered every 6 hours as needed to control blood pressure, with initial target of returning systolic blood pressure at least half way to baseline level.

- Severe (no BP improvement within one hour):
  Treat initially as outlined for moderate hypertension and consult Nephrology. Administer a second dose IV enalaprilat (0.625 to 1.25 mg.) if severe and no response to initial dose within one hour. Evaluate if patients require admission to intensive care bed. Inform Rheumatology of change in patient status.

Subsequent doses of enalaprilat should be given every 12-24 hours, starting with half the administered daily dose every 12 hours IV, with anticipated decreasing dose as levels of the drug increase over time. The usual half-life of enalaprilat is 11 hours, i.e. steady state anticipated after about 2-3 days and has a renal clearance. Onset of action is usually within 15 minutes with the peak effect of the first dose up to 4 hours after administration. The duration of action is 12-24 hours. Doses as high as 5 mg/dose every 6 hours have been tolerated for up to 36 hours. Serum creatinine and potassium require monitoring. Although serum creatinine may increase while taking ACE inhibitors, maintaining a normal blood pressure is a higher priority in the setting of scleroderma renal hypertensive crisis. A calcium channel blocker such as amlodipine may be used as an adjunct, but ACE-inhibitors are essential for effective management of hypertensive crisis in scleroderma patients. Nephrology and Rheumatology consultants may vary treatment for hypertension based on the clinical status of the patient.