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Stem Cell Therapies in Systemic Sclerosis: An Updated Review

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Abstract

Systemic sclerosis systemic is a systemic autoimmune disease that ultimately leads to severe damage and failure of multiple body organs. The disease is associated with significant morbidity and mortality as well as poor response to the available immunosuppressive therapies. Stem cells particularly hematopoietic and mesenchymal stem cells have recently been shown to be not only safe but also efficacious in the treatment of patients with systemic sclerosis. Autologous transplantation of hematopoietic stem cells has become the standard of care for patients with severe systemic sclerosis. Mesenchymal stem cells, alone or in combination with other therapies, have been shown to be effective in the treatment of certain forms and complications of systemic sclerosis such as chronic sclerodermatous graft versus host disease. The review will discuss the various aspects of systemic sclerosis and it will highlight the role of hematopoietic as well as mesenchymal stem cells in the treatment of systemic sclerosis.

Keywords

Systemic Sclerosis; Stem Cell Therapies; Hematopoietic Stem Cells; Mesenchymal Stem Cells; Autologous Transplantation

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Introduction to Systemic Sclerosis

Scleroderma or Systemic Sclerosis (SSc) is a chronic systemic autoimmune disease characterized by widespread obliterative microvasculopathy, activation of the immune system with production of autoantibodies, and variable degrees of inflammation and tissue fibrosis that ultimately lead to severe damage and failure of multiple body organs including: skin, blood vessels, lungs, heart, kidneys, Gastrointestinal Tract (GIT), and musculoskeletal system [1-4]. Scleroderma patients have defects in the number and function of circulating endothelial cells. Bone Marrow (BM)-circulating endothelial progenitor cells play a key role in blood vessel repair and neovascularization. Therefore, manipulating the production, function, and differentiation of circulating progenitor cells may represent a new therapeutic modality for treating scleroderma [5]. The Chitinase-like protein (Chi 3L1) is associated with enhanced inflammatory and fibrotic processes and the presence of progenitor cells expressing Chi 3L1 in the skin of patients with SSc appears to play a role in the initiation of the disease process [6].

Etiology and Associations of SSc

The etiology of SSc is multifactorial. The various factors that are involved in the etiology and associations of SSc are shown in Table 1 [7-16]. However, there are familial aggregates of SSc [13]. Polymorphisms of the major histocompatibility complex Human Leukocyte Antigen (HLA) particularly class II HLA have been associated with disease development [7,12,13]. Specific genes are involved particularly: STAT4, BANK1, IRF5, IRF8, TNFSF4, Interleukin (IL)-23R, IL-12RB1, IL-12RB2, ATG5, fibrillin1, MIF173, PTPN22, and BLK genes [7,9,10].

In a systematic review and a meta-analysis that included 34 published studies, the following results were obtained [8]:

1. The confirmed risk factors included: female gender, age between 45 and 64 years, positive family history, and exposure to silica
2. Microchimerism and exposure to organic solvents were controversial associations
3. There was negative correlation with alcohol consumption, cigarette smoking and infectious agents

1	Unknown etiology
2	Autoimmune disorder associated with connective tissue diseases
3	Genetic susceptibility
4	Environmental exposure: vinylchloride, and organic solvents
5	Silicone breast implants
6	Infectious agents:
a	Cytomegalovirus
b	Epstein Barr virus
c	Parvovirus B-19
d	Lyme disease
7	Medications: bleomycin, cocaine and pentazocine
8	Trauma
9	Radiation
10	Chronic graft versus host disease following allogeneic hematopoietic stem cell transplantation

Table 1: Etiology and associations of systemic sclerosis.

Subtypes of SSc

SSc has the following subsets or subtypes [17-20]:

1. Limited cutaneous SSc
2. Diffuse cutaneous SSc
3. Intermediate cutaneous SSc
4. SSc Sine Scleroderma

Although CREST syndrome (Calcinosis, Raynaud's phenomenon, Esophageal hypomotility, Sclerodactyly, Telangiectasia) is most confined to a single subset of SSc, it is classified as a variant of limited cutaneous SSc and may be considered as intermediate cutaneous SSc [18,20]. Localized forms of scleroderma include linear scleroderma and morphea [18]. Raynaud's phenomenon and scleroderma, hardening of the skin, are the clinical hallmarks of SSc [18].

Chronic Sclerodermatous Graft Versus Host Disease (GVHD)

Sclerodermatous chronic GVHD is a form of chronic GVHD that is characterized by involvement of skin and subcutaneous tissues or fascia without significant visceral involvement [15]. Manifestations of sclerodermatous chronic GVHD include: (1) marked cutaneous sclerosis with skin induration or atrophy, and (2) Sjogren's syndrome with xerostomia and xerophthalmia [16,21,22]. However, sclerodermatous chronic GVHD is believed to be an autoimmune disease or process [15]. Similarities between chronic GVHD and scleroderma include: chronic fibrosis including skin fibrosis; immunological abnormalities including autoantibodies, and involvement of mast cells [23,24]. Differences between chronic GVHD and scleroderma include: the type of collagen laid down and its precise location;

distribution of inner organ involvement; absence of Raynaud's phenomenon in chronic GVHD; lack of ischemic changes in digital extremities in chronic GVHD; and the superficial dermal microvessels have no capillary refractoriness and no loss of endothelial cell specific markers in chronic GVHD [23,24].

The use of DNA microarray technology in murine models of sclerodermatous chronic GVHD has shown that histological development of cutaneous scleroderma is accompanied by upregulated expression of many chemokines and their receptors [25]. In patients with sclerodermatous chronic GVHD, antibodies against platelet derived growth factor receptor have been reported [26]. Sclerodermatous chronic GVHD is usually refractory to steroid therapy but may benefit from tyrosine kinase inhibitors such as imatinib [26].

The factors that are associated with high incidence of sclerosis include: mobilized blood cell grafts; conditioning therapy with total body irradiation; and allografts obtained from Human Leukocyte Antigen (HLA)-identical sibling donors, while low incidence of sclerosis is associated with: use of HLA-mismatched donors; and use of major ABO-mismatched donors [16,27]. Patients with chronic GVHD have high incidence of sclerosis, which can cause disability but does not affect mortality or recurrence of malignancy in patients with chronic GVHD [27]. Sclerosis occurs in up to 20% of patients by 3 years after starting systemic therapy for chronic GVHD. Fibrotic manifestations of chronic GVHD include: Bronchiolitis Obliterans (BO) as well as skin thickening and fibrosis [14].

Studies in murine models of sclerodermatous GVHD have shown that:

1. The main pathological changes in the skin and lungs are: monocyte infiltration, upregulation of cutaneous collagen messenger RNA
2. Sclerodermatous GVHD can be prevented by the administration of Transforming Growth Factor- Beta 1 (TGF- β 1) neutralizing antibodies which can effectively block the influx of monocyte/macrophage and T-cells into the skin and prevent new collagen synthesis by abrogation of the upregulation of TGF- β 1 [28,29]. Fibrosis in sclerodermatous GVHD is driven by production of TGF- β 1 by mononuclear cells [29]

Clinical Manifestations and Complications of SSc

The most typical symptoms of SSc emerge from dermal and vascular lesions [30]. Pulmonary fibrosis and pulmonary artery hypertension are the leading causes of death in patients with SSc as pulmonary complications of SSc are one of the most challenging complications of SSc [18,31]. GIT complications are the most frequent internal complications of SSc [32]. The clinical manifestations and the systemic complications of SSc are included in Table 2 [2,18,31-37]. The differential diagnosis of SSc is shown in Table 3 [38-41].

1	Cardiovascular	• Heart block, arrhythmias, and other conduction disturbances
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		<ul style="list-style-type: none"> • Diastolic dysfunction, left ventricular and congestive cardiac failure • Myocarditis, cardiac fibrosis and valvular abnormalities • Pericardial effusions, vasospasm and ischemic heart disease • Raynaud's phenomenon, digital ischemia and digital ulcers
2	Gastrointestinal	<ul style="list-style-type: none"> • Gastroesophageal reflux disease with dysphagia and regurgitation • Gastric antral vascular ectasia • Barrett esophagus, esophageal strictures and carcinoma of esophagus • Small intestinal bacterial overgrowth and intestinal malabsorption • Chronic large intestinal pseudo-obstruction • Xerostomia, gastroparesis and delayed gastric emptying • Fecal incontinence due to loss of internal anal sphincter function
3	Genito-urinary	<ul style="list-style-type: none"> • Sexual dysfunction
4	Musculoskeletal	<ul style="list-style-type: none"> • Muscle weakness and atrophy, myopathy and myositis • Puffy hands and fingers and shortening of distal phalanges • Flexion contractures and restriction of joint movement • Tendon friction rubs and tenosynovitis • Arthritis and erosive arthropathy
5	Pulmonary	<ul style="list-style-type: none"> • Pulmonary fibrosis, interstitial pneumonitis and interstitial lung disease • Pulmonary arterial hypertension and restrictive ventilatory defects • Pulmonary infiltrates and ground-glass opacities on chest X-ray
6	Renal	<ul style="list-style-type: none"> • Renal crisis, proteinuria and azotemia
7	Skin	<ul style="list-style-type: none"> • Calcinosis, shortening of distal phalanges. telangiectasia, pruritis and thick skin tags • Hypopigmentation and hyperpigmentation • Tight skin leading to: mask-line face and narrowing of mouth aperture
8	Central Nervous System	<ul style="list-style-type: none"> • Transient ischemic attack and stroke • Headache, dizziness, aphasia and visual disturbances • Anxiety, depression, convulsions and coma

Table 2: Systemic complications of systemic sclerosis.

S. No.	Differential Diagnosis
1	Lichen sclerosis

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2	Morphea form of basal cell carcinoma
3	Post-radiation morphea
4	Vaccination-associated morphea
5	Injection of silica, paraffin and vitamin-K
6	Eosinophilic fascitis
7	Porphyria
8	Amyloidosis
9	Lipodermatosclerosis
10	Diabetic cheiroarthropathy/cheiroarthritis
11	Scleromyxedema
12	Nephrogenic systemic fibrosis
13	Mucinosis associated with thyroid disorders
14	Scleroderma adultorum Buschke

Table 3: Differential diagnosis of systemic sclerosis.

Treatment of SSc

SSc is a disease which is associated with significant morbidity and mortality with relatively small benefits from immunosuppressive therapies [42]. In SSc, it is difficult to develop effective disease-modifying agents and to perform randomized controlled clinical trials due to the following reasons: the clinical heterogeneity of the disease; the scarcity of objective tools to evaluate SSc; the extent of organ involvement; and the rapid progression of the disease [43,44].

So far, there is no curative or standard effective therapy for SSc [1,3]. Treatment of SSc is aimed at: improving the Quality of Life (QoL), minimizing specific organ involvement, and prevention of life-threatening complications [3]. However, the management of SSc is multidisciplinary and requires a holistic approach [2]. The available therapeutic options for SSc are shown in Table 4 [1,2,4].

1	Treatment of Symptoms and Complications	• Antimicrobials for infections
		• Proton pump inhibitors for gastrointestinal complications

		<ul style="list-style-type: none"> • Angiotensin-converting enzyme inhibitors and calcium channel blockers for hypertension
2	Immunosuppressive Therapies	<ul style="list-style-type: none"> • Cyclophosphamide, azathioprine, methotrexate and mycophenolate mofetil
3	Biological Therapies	<ul style="list-style-type: none"> • Intravenous immunoglobulins and abatacept • Monoclonal antibodies such as: rituximab, tocilizumab, belimumab, inebilizumab and fresolimumab • Systemic targeted therapies including <ul style="list-style-type: none"> - Rapamycin - Tyrosine kinase inhibitors: imatinib, dasatinib, nilotinib
4	Stem Cell and Organ Transplantation	<ul style="list-style-type: none"> • Autologous hematopoietic stem cell transplantation for early diffuse systemic sclerosis
		<ul style="list-style-type: none"> • Solid organ transplantation: kidney and lung transplantation

Table 4: Available therapeutic modalities for systemic sclerosis.

Stem Cell and Their Role in the Therapy of SSc

Stem cells are a subset of biological cells in the human body that are capable of self-renewal, tissue repair, differentiation, and division into different cell lineages [45-47]. Based on their origin and potency, stem cells are classified into either embryonic and adult stem cells or unipotent, oligopotent, totipotent, multipotent, and pluripotent stem cells [45,46,48,49]. Mesenchymal Stem Cells (MSCs) are heterogenous adult multipotent stromal cells that can be isolated from various sources including: BM, Peripheral Blood (PB), Umbilical Cord Blood (UCB), and Adipose Tissue (AT) [50,51]. They have certain immunomodulatory, immunosuppressive, and antimicrobial properties that enable them to have several clinical applications including: their role in transplantation of Hematopoietic Stem Cells (HSCs) as well as regenerative medicine and treatment of autoimmune disorders [50,51].

HSCs which arise during embryogenesis sustain the production of all blood cells in humans throughout their lifetime [52-56]. They are the only cells within the hematopoietic system that possess the ability of multipotency and self-renewal [54-57]. The local BM microenvironment or the niche provides HSCs with the crucial and indispensable factors that are needed for self-renewal and differentiation [53,56,57]. HSCs are widely used for transplantation and gene therapy and there are 3 main sources of HSCs for HSC Transplantation (HSCT): UCB, BM, and PB [54,57]. Both HSCs and MSCs have been successfully used in the treatment of SSc [58].

MSCs in SSc

In recent years, the therapeutic efficacy of MSCs has been demonstrated in several preclinical animal models and is being evaluated in human clinical trials [59]. The roles of oxidative environment and crosstalk with other cells such as fibroblasts and endothelial cells on the functions of MSCs have been reported [59]. MSCs obtained from AT and BM have been used in the treatment of SSc using Intravenous (IV) and subcutaneous routes of administration [58-60].

Transplantation of a combination of autologous AT-MSCs and hyaluronic acid solution has been shown to cause significant improvement in the skin tightening without causing complications [60]. Autologous as well as allogeneic MSC transplantations have been used in the treatment of SSc [58,59]. MSCs could target the main features of the disease: vascular system (vasculopathy), tissue fibrosis, and immune system abnormalities [58]. The proangiogenic, immunomodulatory, anti-inflammatory, and immunosuppressive properties of MSCs make them ideal candidates for targeted cell-based therapies in SSc to restore the functions of the immune system [58,59]. The main outcomes of the clinical trials performed in patients with SSc include [59]:

1. Improvement in perfusion, Raynaud's phenomenon, and mouth opening
2. Improvement in skin elasticity
3. Reduction in pain
4. Reduction in skin ulcerations

In patients with SSc, the combination of plasmapheresis and allogeneic MSC transplantation could produce sustained benefit in terms of: improvement in lung function, improvement in radiological features, and significant reduction in serological markers including: anti-Scl 70 antibody titers [61].

Digital ulcers represent a frequent manifestation of vasculopathy in patients with SSc [62]. However, currently there is no proven therapeutic strategy to promote healing of these ulcers. The MANUS (Mesenchymal Stromal Cells for Angiogenesis and Neovascularization in Digital Ulcers of Systemic Sclerosis) is the first double blind randomized controlled trial to assess safety and potential efficacy of MSCs in digital ulcers. In this on-going clinical trial, the study group arm will receive intramuscular MSCs while the control group will receive placebo [62]. In patients with SSc, MSCs obtained from BM and AT show upregulation of specific micro-RNAs (miRNAs) and downregulation of other miRNAs and the involved miRNAs exhibit profibrotic behavior. So, the miRNA profile or signature of MSCs may play a possible therapeutic role in treating patients with SSc [63]. In a murine model, repeated exposure of hypochloride resulted in a multistage process that leads to skin fibrosis. Infusion of MSCs during the process of fibrogenesis may halt the fibrotic process due to the great plasticity of MSCs [64].

Although some functional properties of MSCs could be affected upon culture with the serum of patients, MSCs can adapt to the oxidative environment and exert their therapeutic effects [65]. Recent evidences suggest that Extracellular Vesicles (ECVs) and exosomes play a role in

the 3 main pathogenetic aspects of SSc: immunity, vascular damage, and fibrosis. Exosomes, which can be used as biological carriers, may have a potential role in the diagnostics, prognostics, and therapeutics in patients with SSc [66]. MSCs obtained from patients with SSc retain considerable immunosuppressive properties and a normal ability to generate functional T regulatory cells. However, evidence of their senescence does not represent a limitation for their potential use in cellular therapy and regenerative medicine to target scleroderma [67]. In 2 patients with refractory, progressive SSc, the combination of; plasmapheresis, rituximab, and IV MSCs derived from UCB resulted in clinical improvement lasting for more than 1 year [68]. In experimental models, it has been shown that the activity of inducible nitrous oxide synthase plays a crucial role in the antifibrotic activity of MSCs [64].

Transplantation of MSCs results in transferring miRNAs to the stem cells of the recipient in order to ameliorate osteopenia by recruiting non-coding RNA pathways [69]. MSCs obtained from patients with SSc may still harbor or retain some disease-specific abnormalities such as: intrinsic deregulation of vascular smooth muscle and myelofibroblastic transformation [70].

MSCs in Chronic sclerodermatous GVHD

Four patients with sclerodermatous GVHD received MSCs that were expanded ex-vivo from unrelated donors and administered by intra-BM injection [71]. Results of the study showed [71]:

1. Dramatic reversal of helper T-lymphocyte 1 (Th1) cells to Th2 cells i.e. increase in Th1 and decrease in Th2 cells
2. Gradual improvement in symptoms in all 4 patients. The authors concluded that MSC therapy in chronic GVHD was safe and beneficial

A prospective multicenter study that included 81 patients with BO following allogeneic HSCT randomized into [72]:

1. Group A: 49 patients receiving azithromycin, prednisolone and MSCs
2. Group B: 32 patients receiving azithromycin and prednisolone without MSCs

Responses were encountered in 71% of group A and 44% of group B patients. Three years Overall Survival (OS) was 70.6% in group A and 58.2% in group B. There were no significant differences in the rates of infections or leukemia relapse between the 2 groups of patients. The authors concluded that MSC therapy was safe and effective in the treatment of BO encountered following allogeneic HSCT and that clinical improvement was accompanied by significant increase in IL-10 producing CD5+ B cells [72].

HSCT in SSc

Allogeneic HSCT has been performed in patients with SSc, but due to the high toxicity and Treatment-Related Mortality (TRM) most centers have focused on autologous HSCT because

of its significantly lower organ toxicity and TRM [73]. Three randomized controlled clinical trials comprising 250 patients and comparing autologous HSCT to cyclophosphamide in the treatment of patients with SSc showed superior outcomes; in terms of longer OS and Event-Free Survival (EFS), improvement in skin condition and lung function, and decreased rates of disease progression; in recipients of autologous HSCT compared to patients receiving Cyclophosphamide (CPM) alone [74-76]. Details of these 3 randomized clinical trials are shown in Table 5 [74-76]. The positive outcomes of autologous HSCT in the 3 randomized controlled clinical trials were confirmed by the results of systematic reviews and meta-analyses that included other similar studies. Hence, two years ago, the American Society of Blood and Marrow Transplantation (ASBMT) issued a position statement that recommended autologous HSCT as the standard of care for patients with severe SSc [77-79].

The Randomized Clinical Trials and Details of the Clinical Trials	ASTIS (Autologous stem cell transplantation international scleroderma)	ASSIST(American scleroderma stem cell versus immune suppression trial)	SCOT (Scleroderma: cyclophosphamide or transplantation)
Authors, Journal, and Year of Publication	van Laar JM, et al. JAMA 2014	Burt RK, et al. Lancet 2011	Sullivan KM, et al. NEJM 2018
Type of trial, Centers and Country of Origin of Trial	Phase III randomized multicenter trial 29 centers in 10 countries The Netherlands	Phase II randomized single center USA	Phase III randomized multicenter [26 sites] USA
Number of Patients in the Study and in the Study Arms	156 Autologous HSCT (79 patients) versus CPM (77 patients)	19 Autologous HSCT (10 patients) versus CPM (9 patients)	75 Autologous HSCT (36 patients) versus CPM (39 patients)
Conditioning Therapy and Graft	Lymphoablative conditioning [CPM, rabbit ATG] CD34+ cell selection	Lymphoablative conditioning [CPM and rabbit ATG] No graft manipulation or CD34+ cell selection	Myeloablative conditioning [CPM, ATG, and TBI] CD34+ cell selection
Basis of the Clinical Trial	Based on experience with similar regimens at: Saint Louis hospital in France and Leiden University Medical Center in the Netherlands. No cross over to HSCT if failure on control arm	Based on phase I experience with 10 systemic sclerosis patients treated at North-Western University in USA	Based on phase I study of 33 patients mostly performed at the Fred Hutchinson Cancer Center in USA. No cross over to HSCT if failure is encountered on control arm

Results	<p>(1) 1 year: - Events: 13 (16.5%) in HSCT arm versus 10.4% in CPM arm - TRM: 8 patients in HSCT arm versus 0 patients in CPM arm.</p> <p>(2) 2 years: 14 events (17.7%) in HSCT arm versus 14 events (18.2%) in CPM arm. (3) 4 years: 15 events (19%) in HSCT arm versus 20 events (26%) in CPM arm</p>	<p>(1) At 12 months: all patients in HSCT arm improved and none experienced disease progression, while none of CPM arm patients improved and 8 patients had disease progression so 7 of them were switched to HSCT.</p> <p>(2) At 24 months: 11 HSCT patients had improvement in skin and lung condition</p>	<p>(1) EFS: - At 54 months: 79% in HSCT arm versus 50% in CPM arm with p value of 0.02 - At 72 months: 74% in HSCT arm versus 47% in CPM arm with p value of 0.03.</p> <p>(2) OS at 72 months: 86% in HSCT arm versus 51% in CPM arm with p value of 0.02</p> <p>(3) TRM: - At 54 months: 3% in HSCT arm versus 0.00 % in CPM arm - At 72 months: 6% in HSCT arm versus 0.00% in CPM arm</p>
Conclusions	HSCT arm had superior EFS and OS compared to CPM arm	Non-myeloablative autologous HSCT improved skin condition and pulmonary function for up to 2 years	Myeloablative autologous HSCT achieved long-term benefits including improved EFS and OS in patients with scleroderma but at the cost of increased expected toxicity
<p>HSCT: Hematopoietic Stem Cell Transplantation; ATG: Antithymocyte Globulin; CPM: Cyclophosphamide; TBI: Total Body Irradiation; TRM: Treatment Related Mortality; EFS: Event Free Survival; OS: Overall Survival</p>			

Table 5: Details of the randomized controlled clinical trials on autologous HSCT in systemic sclerosis.

In a single center retrospective comparison-case control from historical cohort, Del papa N, et al., compared autologous HSCT in patients with rapidly progressive cutaneous SSc to conventional therapy with 18 patients in HSCT arm and 36 patients in the conventional therapy arm [80]. Patients in the autologous HSCT arm had mobilization with granulocyte-colony stimulating factor and CPM. Peripheral stem cell collection was performed using

leukapheresis. The product was enriched with CD34+ cells. Conditioning therapy was composed of: CPM and ATG in addition to hydration, mesna, and methylprednisolone. Infection prophylaxis was composed of: ciprofloxacin, acyclovir and fluconazole. The conventional therapy was composed of CPM, prednisolone or methylprednisolone, azathioprine, and methotrexate. The selection criteria for autologous HSCT included: active disease, severe skin involvement, non-severe heart involvement, and non-severe lung involvement. Results of the clinical trial showed:

1. TRM less than 5.6%
2. Significantly better outcomes in the HSCT arm compared to the conventional therapy arm in terms of OS, stabilization of lung function, as well as reduction in skin thickening and disease activity.

The authors concluded that autologous HSCT in rapidly progressive SSc had superior outcomes compared to conventional therapies [80].

Several clinical studies on autologous HSCT in patients with SSc have shown improvement in: skin condition, organ function particularly lung and vascular system, and long-term survival despite the toxicity and relatively high TRM encountered in some of these trials [81-87]. Complications of autologous HSCT in patients with SSc include [81-89]:

1. TRM and organ toxicity particularly cardiotoxicity
2. Gonadal failure
3. Various infections
4. Autoimmune diseases such as myasthenia gravis

According to European Blood and Marrow Transplantation data, in patients with severe SSc, non-myeloablative conditioning therapy followed by autologous HSCT has been shown to be effective as durable responses with acceptable TRM have been encountered in two thirds of patients [90]. Additionally, the ASBMT has recommended that SSc should be considered a standard of care indication for autologous HSCT based on the evaluation of high-dose chemotherapy followed by autologous HSCT for the treatment of SSc in: several observational and retrospective studies, at least 4 randomized controlled clinical trials, as well as several systematic reviews and meta-analyses [74-86,91,92].

Baseline evaluation prior to HSCT should include comprehensive medical history and physical examination; laboratory tests including complete blood count and differential, renal and hepatic profiles, viral and hepatitis screens, chest X-ray, electrocardiogram, echocardiogram; and cardiac magnetic resonance imaging [77,93]. Ideal candidates for autologous HSCT in patients with SSc include [78,91,94]:

1. Non-smokers
2. Non-responders to standard immunosuppressive therapies
3. Patients with cutaneous SSc within 4-5 years from the diagnosis with mild to moderate organ involvement particularly interstitial lung disease
4. Patients having limited cutaneous SSc with progressive visceral involvement

5. Patients with high-risk SSc experiencing severe lung and diffuse skin involvement at an early stage of the disease

Exclusion criteria for autologous HSCT in patients with SSc include [77,93]:

1. Age > 65 years
2. Pregnancy
3. Drug or alcohol abuse
4. Active psychiatric illness
5. Inability to provide informed consent
6. Active infection such as human immunodeficiency virus, human T-cell lymphotropic virus types 1 and 2, and hepatitis B and C virus infections
7. Malignancy, myelodysplastic syndrome, or serious hematological disorder
8. End-stage organ failure or severe organ dysfunction particularly liver, heart, lungs, and kidneys

Mechanisms of action of autologous HSCT in patients with SSc are unknown. However, proposed mechanisms include: ablation or reduction of the aberrant immune cells, particularly those in the T-cell compartment, followed by re-establishment of immunological tolerance or reconstitution of a new immune system that is self-tolerant [77,78,94,95]. In patients with SSc, the mechanism of action of autologous HSCT may not be solely immunological [62]. Generally, immunological changes encountered after autologous HSCT in SSc do not necessarily reflect the clinical responses [62]. Profibrotic cytokines and even autoantibodies hardly appear to be influenced by autologous HSCT in patients with SSc [96].

Autologous HSCT induces long-lasting alterations in B-cell homeostasis or in the B-cell compartment such as the increase production of IL-10 [97]. Clinical improvement or response following autologous HSCT in patients with SSc is associated with coordinated thymic and bone marrow rebounds manifested by increase counts of the newly generated T and B regulatory cells encountered after HSCT [98]. In patients with SSc, unlike conventional therapies, autologous HSCT leads to normalization or significant alterations of disease-related molecular signatures such as: decrease in interferon- α , reduction in neutrophil module, and increase in cytotoxic/natural killer cell module [99,100]. Compared to unmanipulated autologous grafts, CD34+ selected autologous HSCT may produce favorable effects on skin fibrosis and lung function. Therefore, use of CD34+ selected autologous HSCT with high-dose CPM conditioning therapy may offer excellent outcomes [101,102]. In patients with SSc receiving autologous HSCT with purified CD34+ cells, Th1/Th2 ratio has been reported to be significantly increased for at least 3 years following autologous HSCT [101].

Conclusions and Future Directions

Compared to standard therapy, autologous HSCT in patients with SSc has the following benefits [80,88,91,93,103]:

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1. Significant improvement in organ function
2. Significant improvement in the QoL
3. Remarkable improvement in long-term survival

Autologous HSCT is an effective therapeutic modality in selected patients with early diffuse cutaneous SSc, despite the expected toxicities and the relatively high TRM due to major organ involvement by the disease [104]. Better selection of candidates for HSCT and the use of safer transplant conditioning regimens may improve the outcome of autologous HSCT in patients with SSc [105]. Autologous HSCT in SSc should be performed by expert multidisciplinary teams in specialized transplant centers in order to optimize the safety and efficacy of the procedure [73]. More clinical studies, preferably multicenter randomized controlled trials, should be performed on MSC transplantation in specific forms and complications of SSc. In addition, not only the doses and routes of administration, but also the timing of MSC transplantation as well as the other required therapies should be determined.

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