Advances in disease-specific treatment of systemic sclerosis

Systemic sclerosis is a heterogeneous autoimmune connective tissue disorder characterized by humoral and cellular immune dysregulation, vascular dysfunction and a prominent cutaneous and visceral organ fibrosis. While there have been great strides in managing organ-specific involvement, disease-specific therapy has remained elusive. Small open-label pilot studies have typically given good results, while the more uncommon randomized, double-blind, placebo-controlled trials have not. We review the disease-specific therapies studied to date, with an eye to future therapies.

KEYWORDS: fibrosis, scleroderma, systemic sclerosis, therapy, treatment

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Scleroderma is a disease characterized by excessive collagen deposition in the skin, blood vessels and visceral organs. Derived from the Greek words 'skleros' (meaning hard or indurated) and 'derma' (skin), Hippocrates is credited with the first depiction of this condition in approximately 400 BC. Physically debilitating, scleroderma is associated with a five- to eight-fold increase in mortality over that of the general population [1]. Despite significant disease burden, no single therapeutic agent has ever been proven to be universally efficacious. This is due to the complex nature of the disease, which includes interplay between various immune cells as well as dysfunction at the vascular endothelial level. The net effect is uncontrolled inflammation along with abundant collagen production and deposition. The following article is intended to provide a brief review of the underlying pathology of scleroderma, and to discuss current, as well as potential future targets of treatment.

Background

Scleroderma is characterized mainly by the following cellular events: a humoral response with autoantibody production, activation of cellular immunity with inflammation and subsequent excess collagen production and deposition [2,3]. Molecular changes in the vascular endothelium precede the development of clinical symptoms, and may represent the earliest step in the scleroderma disease cascade. Carvalho *et al.* discovered the presence of IgG anti-endothelial cell autoantibodies in scleroderma patients [4]. *In vitro*, anti-endothelial cell autoantibodies have subsequently been shown to result in endothelial cell apoptosis and upregulated production of vascular endothelial cell surface adhesion molecules, such as intercellular adhesion molecule-1, vascular cell adhesion molecule and selectins. High levels of these and other adhesion molecules cause leukocyte activation, and have been found in the tissues of organs affected with systemic sclerosis (SSc) [5–7].

As mentioned previously, immune cell interplay is central in scleroderma, particularly TGF-β. This cytokine is released by many different cell types including macrophages, and plays an important role in fibroblast activation [8]. Presently, there are three known isoforms of TGF-β that bind to corresponding TGF-β receptors on the fibroblast cell surface, which in turn, stimulate the SMAD protein cascade, resulting in collagen gene transcription [9,10]. Furthermore, there is evidence that TGF-β receptors are present in greater numbers on fibroblasts collected from SSc patients, a finding that yields more credence to the significant role of TGF-β in scleroderma [11].

Other proteins that have received a lot of attention in scleroderma research include endothelin-1, connective tissue growth factor and PDGF. Endothelins produced by endothelial cells act as potent vasoconstrictors. They also possess the ability to upregulate myofibroblast activity and have significant profibrotic effects [12,13]. An intriguing finding is that TGF-β is a potent inducer of endothelin-1 gene transcription, possibly linking both cytokines in scleroderma pathogenesis [14,15]. TGF-β also promotes production of connective tissue growth factor, which in turn activates fibroblasts to secrete excess collagen characteristic of SSc [16]. SSc patients may possess other antibodies

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that are capable of stimulating PDGF receptors, inducing tyrosine kinase phosphorylation and subsequent Type 1 collagen production [17].

No therapeutic consensus exists in scleroderma treatment, given the complexity of known cellular interactions in addition to the many molecular pathways yet to be uncovered. To date, SSc disease-specific management can be subdivided into three categories: disease-modifying agents, targeted therapy and antifibrotic drugs.

Disease-specific management

Disease-modifying therapy

The largest category of therapy primarily consists of nonspecific immunosuppressive agents. A cytotoxic alkylating agent, cyclophosphamide (CYC), has been the mainstay treatment for active interstitial lung disease (ILD) in a variety of collagen vascular disorders. Since 1993, the efficacy of CYC in scleroderma ILD has been evaluated, in retrospective and subsequent randomized, controlled trials. The Scleroderma Lung Study consisted of 158 patients treated with oral CYC for at least 6 months. At 1 year, there was a statistically significant, though clinically modest, improvement in the primary end point – forced vital capacity (FVC). These findings persisted at 24 months in 113 patients [18]. Yiannopoulos *et al.* demonstrated stabilization rather than improvement of ILD with intravenous CYC treatment in 13 patients, with the least response observed in the subgroup of patients with the more compromised pulmonary function studies [19]. By contrast, in the Fibrosing Alveolitis in Scleroderma Trial, 45 scleroderma patients treated with prednisolone and CYC infusions showed no statistically significant improvement in ILD [20,21]. Other clinical areas examined have included CYC effect on SSc skin. The Scleroderma Lung Study showed significant improvement in skin scores of diffuse systemic sclerosis (d-SSc) patients at 12 months [21]. In 2003, Calguneri *et al.* treated 27 patients with early d-SSc with an oral CYC and prednisolone combination. Again, a significant effect was observed in terms of skin scores in addition to improvement in FVC and diffusing lung capacity (DLCO) [22]. Most recently, in an open-label, uncontrolled trial with six early d-SSc patients, high-dose intravenous CYC (50 mg/kg) administered over a 4-day period resulted in clinically significant skin score improvement. The same trial also had one death, perhaps illustrating the inherent danger of such an aggressive regimen [23]. However, other data, including a trial by Pakas *et al.*, indicated a conflicting lack of CYC efficacy in the treatment of skin fibrosis [24]. A more recent meta-analysis included three randomized, controlled trials and six prospective observational studies. Main outcomes consisted of DLCO and FVC. While modest improvements in DLCO and FVC were obtained, no clinically significant effect was seen, as defined by an improvement of at least 10% of the predicted value of each measure [25].

One treatment that appears promising in the treatment of scleroderma is an inosine monophosphate dehydrogenase inhibitor, mycophenolate mofetil (MMF). MMF interferes with lymphocyte proliferation and causes apoptosis of clonal T cells [26]. In one open-label trial, after receiving a combination of antithymocyte globulin for 5 days and MMF for 1 year, 13 scleroderma patients demonstrated benefit in skin scores [27]. The study was limited by small patient numbers and lack of a placebo arm; however, it paved the way to further clinical research into the utility of this agent. A retrospective analysis of 172 scleroderma patients examined morbidity and mortality as the primary end points. Secondary parameters included the therapeutic effect of MMF on cardiac, pulmonary, renal, gastrointestinal and musculoskeletal manifestations. At 5 years of treatment, there was a 10% survival benefit with MMF. No skin score improvement was observed [28]. Several other clinical studies have demonstrated MMF efficacy specifically in the treatment of ILD, especially in terms of DLCO, total lung capacity (TLC) and FVC [29,30].

Another commonly employed antirheumatic agent is methotrexate (MTX). To date, there are two randomized, controlled trials of MTX utilized in SSc. In 1996, 29 SSc patients were assigned to receive either MTX (n = 17) or placebo (n = 12). End points included improvements in: total skin score by 30% or more, DLCO by 15% or more, or visual analogue scale of general well-being by 30% or more. There was a statistically significant effect on total skin score and visual analogue scale in the group receiving MTX [31]. A later multicenter study consisted of 71 patients with d-SSc, where 35 were assigned MTX and 36 received placebo. End points included skin scores, DLCO and physician global assessment. At the conclusion of the study at 12 months, the MTX group demonstrated a trend in skin scores improvement, but not in DLCO or physician global assessment. The investigators concluded that there was no significant evidence for MTX efficacy in early d-SSc [32].

SSc treatment data with two other immunosuppressive agents, ciclosporin A and tacrolimus, were provided by Clements *et al.* and Morton and Powell, respectively. Ciclosporin A efficacy was evaluated in terms of skin thickening, pulmonary and cardiac involvement. Despite mild improvement in skin thickness, significant nephrotoxicity and lack of therapeutic response on visceral involvement precluded further use of ciclosporin in SSc [33]. Tacrolimus showed similar clinical effects, with fewer adverse events [34].

The same clinical investigators that studied the utility of ciclosporin A in scleroderma conducted a randomized, controlled trial, comparing the efficacy of weekly 15 mg MTX with oral sirolimus in the treatment of skin fibrosis. Sirolimus is believed to inhibit IL-2 dependent T-cell proliferation. Equal improvement was observed in both the MTX and sirolimus groups in terms of the primary end point, which was the modified Rodnan skin score (MRSS) scores. One must note that this comparison trial may have been underpowered to detect a between-group difference [35].

Previous research has suggested that intravenous immunoglobulin therapy (IVIg) can be an effective and relatively safe treatment for certain immune-mediated conditions. One of the proposed mechanisms for IVIg utility is through activation of the FcγRIIb receptor, which inhibits autoantibody-mediated inflammation [36]. In a pilot study involving seven women with SSc, IVIg was used to treat severe joint involvement refractory to MTX and CYC [37]. After 6 months of therapy with IVIg at 2 mg/kg of body weight, significant improvement was seen in the majority of patients as measured by the visual analogue scale, health assessment questionnaire and Dreiser Algo-Functional Index evaluation of hand joint function. Likewise, MRSS scores also improved after IVIg treatment, suggesting that this agent could be used in future studies to counteract skin fibrosis.

More recently, two large trials were designed to determine the effectiveness and safety of autologous stem cell transplants in SSc, the Scleroderma Cyclophosphamide Or Transplantation (SCOT) trial and the Autologous Stem cell Transplantation International Scleroderma trial (ASTIS). Subjects enrolled in both the ASTIS and SCOT trials share extensive skin disease (MRSS ≥ 16), along with significant visceral involvement [38,39]. Preliminary data from the SCOT trial suggest that autologous stem cell transplants may be very effective in the treatment of skin fibrosis in SSc [40].

Targeted immunotherapy

One of the first prototypes of targeted immunotherapy was a polyclonal IgG antibody derived from animals immunized to human thymocyte. Antithymocyte globulin causes T-cell depletion. In one open-label trial, ten patients with early d-SSc disease were treated with intravenous antithymocyte globulin at 10 mg/kg/day for 5 days and followed for 12 months. Main outcomes included change in the Rodnan skin score (at least 25%) or improvement in DLCO or FVC. No significant lung or skin improvement was found [41]. Again, the previously described combination of antithymocyte globulin followed by MMF did produce some skin improvement [27].

McKown *et al.* postulated that, perhaps through the introduction of a low dose of antigen from affected tissues in SSc, T-cell autoimmune activity may be suppressed. This idea of 'self-tolerance' induction was tested through oral administration of bovine Type 1 collagen to 17 patients with SSc. All patients received 1 year of therapy with statistically significant 6- and 12-month improvements in all of the outcomes including MRSS, pulmonary function tests and a modified health assessment questionnaire [42]. More recently, in a placebo-controlled trial of 168 patients, oral bovine collagen was no better than placebo at 12 months on the MRSS [43].

TNF-α inhibition has been gaining popularity in treatment of rheumatic diseases including rheumatoid and psoriatic arthritis. In a pilot study, etanercept was administered to ten patients with d-SSc at 25 mg, twice weekly. At 6 months, there was improvement in digital ulcers and skin scores, while pulmonary function remained unchanged [44]. In another open-label study, 16 patients with early active skin involvement were administered 5 mg/kg infliximab at 0, 2, 6, 14 and 22 weeks. Close to half of the subjects developed infusion reactions, with only 66% of patients completing the last infusion, and the mean MRSS was not statistically different at 26 weeks [45].

To date, aside from a few case reports, only one trial has examined the efficacy of rituximab in the treatment of SSc. Rituximab selectively depletes CD-20 expressing B lymphocytes. In an analysis of 13 patients with early SSc treated with two 1000 mg infusions of rituximab on consecutive weeks, minimal change in MRSS was observed at 6 and 12 months [46,47].

Anti-fibrotic therapy

The prototypical and still widely studied agent in this category is D-penicillamine (D-pen). D-pen has immunomodulatory properties and interferes with intra- and inter-molecular collagen crosslinks of newly formed collagen [48,49]. A recent retrospective analysis conducted at our institution evaluated 84 patients with early, d-SSc, who received at least 3 months of daily treatment with D-pen. Results, consistent with many previous trials, indicated a statistically significant reduction in MRSS scores and improvement of renal, cardiac and pulmonary function [50,51]. By contrast, an earlier randomized, double-blind, controlled trial examined the efficacy of high-dose versus low-dose D-pen. At 24 months, no statistically significant benefit in skin scores, renal crisis or mortality was achieved, along with considerably more (80%) adverse effects at higher doses [52].

Both IFN-α and IFN-γ have been studied in the treatment of SSc. In a randomized trial, IFN-α or placebo was administered to 35 patients with early scleroderma. Outcomes included MRSS, pre- and post-treatment skin biopsy, along with renal, lung and cardiac function. No significant improvement was observed in any of the above categories [53]. Another randomized, controlled study involved 44 patients, with 27 patients receiving treatment with IFN-γ three-times weekly for 12 months, while the remaining subjects were assigned to the control group. A modest beneficial effect was observed in terms of skin scores, but the study did not reach statistical significance. Moreover, treatment with this agent appeared to carry significant morbidity, as evidenced by a high drop out rate in the treatment group [54].

Relaxin, a protein secreted by the corpus luteum during pregnancy, has been shown to have antifibrotic effects *in vitro*, including inhibition of new collagen formation. In a multicenter, randomized, placebo-controlled trial, the efficacy of recombinant human relaxin was examined on MRSS, the health assessment questionnaire and pulmonary disease in SSc. Relaxin doses consisted of 25 and 100 µg/kg of body weight. After daily subcutaneous injections over a 24-week period, the 25 µg/kg arm demonstrated significant improvement in terms of the MRSS, as well as in the functional status of the patients. Interestingly, higher doses of relaxin were not effective [55]. A larger follow-up trial of 239 patients treated with low-dose relaxin versus placebo showed no difference in MRSS [56].

The conversation about antifibrotic therapy would not be complete without a discussion concerning one additional treatment option for SSc skin involvement – phototherapy with ultraviolet irradiation. Ultraviolet irradiation is believed to exert its activity through reduction of procollagen synthesis and production of immunosuppressive cytokines like IL-10 [57]. In a large multicenter, placebo-controlled trial, 64 patients with early SSc (less than 2 years in duration) were randomly assigned to receive either active or sham photophoresis. When compared with baseline at 6 and 12 months, statistically significant improvement in skin scores and joint contractures was observed. However, no clinical significance was observed in the treatment arm when it was compared with placebo, and this is likely to be a reflection of the study's inadequate statistical power [58].

As mentioned previously, TGF-β and its role in fibrosis has received a lot of recent attention in the SSc literature. CAT-192, a recombinant human monoclonal antibody against active human TGF-β1, was evaluated in a recent randomized, placebo-controlled trial of 45 patients with early active SSc. Despite a positive trend, no significant improvement in MRSS scores was observed [59].

Future perspective

Future therapeutic options for SSc are constantly expanding based on a better understanding of the pathogenesis of this disorder. Currently, trials are ongoing evaluating the efficacy and safety of a tyrosine kinase inhibitor, imatinib mesylate. We previously mentioned that patients with scleroderma have serum autoantibodies that stimulate the platelet-derived growth factor receptor (PDGFR), activating collagen gene expression. In one study, Baroni *et al.* demonstrated that PDGFR autoantibodies were found in the sera of all scleroderma subjects. Stimulation of PDGFR results in tyrosine phosphorylation and reactive oxygen species accumulation [17]. *In vitro*, imatinib efficiently reduced PDGF- and TGF-β-stimulated production of extracellular molecules in SSc dermal fibroblasts [60]. Clinical efficacy and safety of imatinib in SSc remain unknown. Other avenues of therapeutic research being evaluated as disease-specific treatment of scleroderma include antibodies to connective tissue growth factor, phosphodiesterase inhibitors, endothelin receptor blockers and gene targeting.

Conclusion

Organ-specific therapy has, in the past three decades, improved morbidity and mortality from scleroderma renal crisis and has provided options in the treatment of pulmonary arterial hypertension and ILD. While there have been great strides in managing organ-specific involvement in SSc, disease-specific therapy has remained elusive. Open-label pilot studies have typically produced good results in the treatment of SSc, while the more robust randomized, double-blind, placebo control trials have not. Unfortunately, while technically superior with less scientific bias, randomized, placebo-controlled trials are rare in scleroderma due to the low prevalence of this condition. Furthermore, the heterogeneity of the disease and the need to recruit patients in these larger studies may have caused the recruitment of a not-so-homogenous group of patients, causing the results of these studies to be diluted down. The need for a clearly defined group of SSc patients who would be considered for treatment studies has been looked at and may help avoid nonsignificance in therapeutic trials of SSc [61].

In summary, scleroderma treatment continues to evolve as we learn more about the pathogenesis of this unusual disease. As the field of pharmacogenomics continues to develop, in the future it may be possible to predict exactly which patient will respond to which treatment. This would allow for the development of individually tailored therapy that addresse the heterogeneity of SSc clinical manifestations. Until that time, basic and clinical research will continue to pursue the elusive scleroderma 'cure'.

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Executive summary

- Systemic sclerosis is an autoimmune connective tissue disorder characterized by humoral and cellular dysregulation, vascular dysfunction and organ fibrosis including skin.
- Autoimmune-mediated vasculopathy may represent the earliest step in disease pathogenesis and, therefore, acts as a major target for many disease-specific treatments.
- Molecules of particular interest in scleroderma include TGF-β, endothelin-1, connective tissue growth factor and PDGF.
- Treatment of systemic sclerosis is classically divided into disease-modifying therapy, targeted immunotherapy and antifibrotic agents.
- Disease-modifying therapy consists of nonspecific immunosuppressive agents, including some of the following: cyclophosphamide, mycophenolate mofetil and methotrexate.
- Targeted immunotherapy utilized in systemic sclerosis contains antithymocyte globulin, and requires self-tolerance through oral administration of bovine Type 1 collagen and various biologic agents.
- D-penicillamine, interferon, relaxin and ultraviolet irradiation comprise the bulk of antifibrotic treatment options.
- At present, some investigators are evaluating the efficacy and safety of bone marrow transplantation, tyrosine kinase inhibitors and antibodies against TGF-β in the treatment of scleroderma.
- Future therapeutic options are constantly expanding based on our understanding of pathophysiology.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Steen VD, Medsger TA: Changes in causes of death in systemic sclerosis, 1972–2002. *Ann. Rheum. Dis.* 66(7), 940–944 (2007).

2 Jimenez SA, Hitraya E, Varga J: Pathogenesis of scleroderma. Collagen. *Rheum. Dis. Clin. North Am.* 22(4), 647–674 (1996).

3 Jimenez SA, Derk CT: Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. *Ann. Intern. Med.* 140(1), 37–50 (2004).

▪ **Detailed review of pathophysiology.**

4 Carvalho D, Savage CO, Black CM, Pearson JD: IgG antiendothelial cell autoantibodies from scleroderma patients induce leukocyte adhesion to human vascular endothelial cells *in vitro*. Induction of adhesion molecule expression and involvement of endothelium-derived cytokines. *J. Clin. Invest.* 97(1), 111–119 (1996).

5 Gruschwitz MS, Hornstein OP, von den Driesch P: Correlation of soluble adhesion molecules in the peripheral blood of scleroderma patients with their *in situ* expression and with disease activity. *Arthritis Rheum.* 38, 184–189 (1995).

6 Stummvoll GH, Aringer M, Grisar J *et al.*: Increased transendothelial migration of scleroderma lymphocytes. *Ann. Rheum. Dis.* 63(5), 569–574 (2004).

7 Ahmed SS, Tan FK, Arnett FC, Jin L, Geng YJ: Induction of apoptosis and fibrillin 1 expression in human dermal endothelial cells by scleroderma sera containing anti-endothelial cell antibodies. *Arthritis Rheum.* 54(7), 2250–2262 (2006).

8 Khalil N, Bereznay O, Sporn M, Greenberg AH: Macrophage production of transforming growth factor β and fibroblast collagen synthesis in chronic pulmonary inflammation. *J. Exp. Med.* 170(3), 727–737 (1989).

- **Review of pathophysiology of pulmonary fibrosis.**

9 Varga J: Expression and regulation of intracellular SMAD signaling in scleroderma skin fibroblasts. *Arthritis Rheum.* 46, 1703–1713 (2002).

10 Jimenez SA, Derk CT: Statins and the vasculopathy of systemic sclerosis: potential therapeutic agents? *Autoimmun. Rev.* 5(1), 25–32 (2006).

11 Kawakami T, Ihn H, Xu W *et al.*: Increased transcriptional activities of transforming growth factor β receptors in scleroderma fibroblasts. *J. Invest. Dermatol.* 110(1), 47–51 (1998).

12 Shi-Wen X, Chen Y, Denton CP *et al.*: Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/PI3 kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts. *Mol. Biol. Cell* 15, 2707–2719 (2004).

13 Shi-Wen X, Denton CP, Dashwood MR *et al.*: Fibroblast matrix gene expression and connective tissue remodeling: role of endothelin-1. *J. Invest. Dermatol.* 116, 417–425 (2001).

14 Lee SD, Lee DS, Chun YG *et al.*: Transforming growth factor-β 1 induces endothelin-1 in a bovine pulmonary artery endothelial cell line and rat lungs via cAMP. *Pulm. Pharmacol. Ther.* 13, 257–265 (2000).

15 Kurihara H, Yoshizumi M, Sugiyama T *et al.*: Transforming growth factor-β stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 159, 1435–1440 (1989).

16 Leask A, Holmes A, Abraham DJ: Connective tissue growth factor: a new and important player in the pathogenesis of fibrosis. *Curr. Rheumatol. Rep.* 4(2), 136–142 (2002).

- **Concise review of connective tissue growth factor.**

17 Baroni SS, Santillo M, Bevilacqua F *et al.*: Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N. Engl. J. Med.* 354(25), 2667–2676 (2006).

- **A unique autoimmune-based pathogenesis of scleroderma.**

18 Tashkin DP, Elashoff R, Clements PJ; the Scleroderma Lung Study Research Group: Cyclophosphamide versus placebo in scleroderma lung disease. *N. Engl. J. Med.* 354 (25), 2655–2666 (2006).

▪▪ **Landmark study of the use of oral cyclophosphamide in scleroderma lung disease.**

19 Yiannopoulos G, Pastromas V, Antonopoulos I *et al.*: Combination of intravenous pulses of cyclophosphamide and methylprednisolone in patients with systemic sclerosis and interstitial lung disease. *Rheumatol. Int.* 27(4), 357–361 (2007).

20 Hoyles RK, Ellis RW, Herrick AL *et al.*: Fibrosing Alveolitis in Scleroderma Trial (FAST) – a multi-centre prospective randomised double-blind placebo-controlled trial. Presented at: *The American College of Rheumatology Annual Meeting.* San Diego, CA, USA, 13–17 November, 2005.

21 Hoyles RK, Ellis RW, Wellsbury J *et al.*: A multicenter, prospective, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral azathioprine for the treatment of pulmonary fibrosis in scleroderma. *Arthritis Rheum.* 54(12), 3962–3970 (2006).

▪▪ **Well-designed study of intravenous cyclophosphamide for scleroderma.**

22 Calguneri M, Apras S, Ozbalkan Z *et al.*: The efficacy of oral cyclophosphamide plus prednisolone in early diffuse systemic sclerosis. *Clin. Rheumatol.* 22(4–5), 289–294 (2003).

23 Tehlirian CV, Hummers LK, White B, Brodsky RA, Wigley FM: High-dose cyclophosphamide without stem cell rescue in scleroderma. *Ann. Rheum. Dis.* 67(6), 775–781 (2008).

24 Pakas I, Ioannidis JP, Malagari K, Skopouli N, Moutsopoulos HM, Vlachoyiannopoulos PG: Cyclophosphamide with low or high dose prednisolone for systemic sclerosis lung disease. *J. Rheumatol.* 29(2), 298–304 (2002).

25 Nannini C, West CP, Erwin PJ, Matteson EL: Effects of cyclophosphamide on pulmonary function in patients with scleroderma and interstitial lung disease: a systematic review and meta-analysis of randomized controlled trials and observational prospective cohort studies. *Arthritis Res. Ther.* 10(5), R124 (2008).

- **Metanalysis of all well-designed studies of cyclophosphamide for scleroderma lung disease.**

26 Allison AC: Mechanisms of action of mycophenolate mofetil. *Lupus* 14 (Suppl. 1), S2–S8 (2005).

27 Stratton RJ, Wilson H, Black CM: Pilot study of anti-thymocyte globulin plus mycophenolate mofetil in recent-onset diffuse scleroderma. *Rheumatology* 40(1), 84–88 (2001).

28 Nihtyanova SI, Brough GM, Black CM, Denton CP: Mycophenolate mofetil in diffuse cutaneous systemic sclerosis – a retrospective analysis. *Rheumatology* 46(3), 442–445 (2007).

- **Well-designed retrospective study of mycophenolate mofetil for scleroderma.**

29 Liossis SNC, Bounas A, Andonopoulos AP: Mycophenolate mofetil as first-line treatment improves clinically evident early scleroderma lung disease. *Rheumatology* 45, 1005–1008 (2006).

30 Swigris JJ, Olson AL, Fischer A *et al.*: Mycophenolate mofetil is safe, well tolerated, and preserves lung function in patients with connective tissue disease-related interstitial lung disease. *Chest* 130(1), 30–36 (2006).

31 van den Hoogen FH, Boerbooms AM, Swaak AJ, Rasker JJ, van Lier HJ, van de Putte LB: Comparison of methotrexate with placebo in the treatment of systemic sclerosis: a 24 week randomized double-blind trial, followed by a 24 week observational trial. *Br. J. Rheumatol.* 35(4), 364–372 (1996).

▪▪ **Well-designed study of methotrexate use in scleroderma.**

32 Pope JE, Bellamy N, Seibold JR *et al.*: A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma. *Arthritis Rheum.* 44(6), 1351–1358 (2001).

▪▪ **Large well-designed study of methotrexate in scleroderma.**

33 Clements PJ, Lachenbruch PA, Sterz M *et al.*: Cyclosporine in systemic sclerosis. Results of a forty-eight-week open safety study in ten patients. *Arthritis Rheum.* 36(1), 75–83 (1993).

34 Morton SJ, Powell RJ: Cyclosporin and tacrolimus: their use in a routine clinical setting for scleroderma. *Rheumatology* 39(8), 865–869 (2000).

35 Clements PJ, Khanna D, Burger C, Danovitch G, Furst DE: Rapamycin vs methotrexate in early diffuse systemic sclerosis: a 48 week randomized single-blind pilot safety study. Presented at: *American College of Rheumatology Annual Meeting.* Washington, DC, USA, 13–17 November 2006.

36 Ballow M: Practical considerations for the use of IGIV therapy. *Am. J. Health Pharm.* 62(16 Suppl. 3), S12–S18 (2005).

37 Nacci F, Righi A, Conforti ML *et al.*: Intravenous immunoglobulins improve the function and ameliorate joint involvement in systemic sclerosis: a pilot study. *Ann. Rheum. Dis.* 66(7), 977–979 (2007).

38 Van Laar JM, Farge D, Tyndall A: Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial: hope on the horizon for patients with severe systemic sclerosis. *Ann. Rheum. Dis.* 64(10), 1515 (2005).

39 McSweeney PA, Nash RA, Sullivan KM *et al.*: High-dose immunosuppressive therapy for severe systemic sclerosis: initial outcomes. *Blood* 100(5), 1602–1610 (2002).

40 Tyndall A, Furst DE: Adult stem cell treatment of scleroderma. *Curr. Opin. Rheumatol.* 19(6), 604–610 (2007).

41 Matteson EL, Shbeeb MI, McCarthy TG, Calamia KT, Mertz LE, Goronzy JJ: Pilot study of antithymocyte globulin in systemic sclerosis. *Arthritis Rheum.* 39(7), 1132–1137 (1996).

42 McKown KM, Carbone LD, Bustillo J, Seyer JM, Kang AH, Postlethwaite AE: Induction of immune tolerance to human Type I collagen in patients with systemic sclerosis by oral administration of bovine Type I collagen. *Arthritis Rheum.* 43, 1054–1061 (2000).

43 Postlethwaite AE, Wong WK, Clements P *et al.*: A multicenter, randomized, double-blind, placebo-controlled trial of oral Type I collagen treatment in patients with diffuse cutaneous systemic sclerosis: oral Type I collagen does not improve skin in all patients, but may improve skin in late phase disease. *Arthritis Rheum.* 58(6), 1810–1822 (2008).

▪▪ **Landmark study of the use of Type I bovine collagen to induce tolerance in scleroderma.**

44 Ellman MH, MacDonald PA, Hayes FA: Etanercept as treatment for diffuse scleroderma: a pilot study. *Arthritis Rheum.* 43(Suppl.), S392 (Abstract) (2000).

45 Denton CP, Englehart M, Trede N *et al.*: An open-label pilot study of infliximab therapy in diffuse cutaneous systemic sclerosis. Presented at: *American College of Rheumatology Annual Scientific Meeting*. Washington DC, USA, 10–15 November, 2006.

46 Anandarajah AP: The American College of Rheumatology Annual Scientific Meeting 2006: advances in the treatment of connective tissue diseases. *Future Rheumatol.* 2(4), 379–384 (2007).

47 Lafyatis R, Kissin E, Merkel PA *et al.*: Rituximab treatment for patients with diffuse cutaneous systemic sclerosis – a Phase I study. Presented at: *The American College of Rheumatology Annual Meeting*. Washington DC, USA, 10–15 November, 2006.

48 Lipsky PE: Immunosuppression by D-penicillamine *in vitro*. Inhibition of human T lymphocyte proliferation by copper- or ceruloplasmin-dependent generation of hydrogen peroxide and protection by monocytes. *J. Clin. Invest.* 73, 53–65 (1984).

49 Nimni ME, Deshmukh K, Gerth N: Collagen defect induced by penicillamine. *Nature New Biol.* 240, 220–221 (1972).

50 Derk CT, Huaman G, Jimenez SA: A retrospective randomly selected cohort study of D-penicillamine treatment in rapidly progressive diffuse cutaneous systemic sclerosis of recent onset. *Br. J. Dermatol.* 158(5), 1063–1068 (2008).

51 Steen VD, Medsger TA Jr, Rodnan GP: D-Penicillamine therapy in progressive systemic sclerosis (scleroderma): a retrospective analysis. *Ann. Intern. Med.* 97(5), 652–659 (1982).

52 Clements PJ, Furst DE, Wong WK *et al.*: High-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis: analysis of a 2-year, double-blind, randomized, controlled clinical trial. *Arthritis Rheum.* 42(6), 1194–1203 (1999).

▪▪ **Landmark study of low- vs high-dose D-peniccilamine for the treatment of scleroderma.**

53 Black CM, Silman AJ, Herrick AI *et al.*: Interferon-α does not improve outcome at one year in patients with diffuse cutaneous scleroderma: results of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* 42(2), 299–305 (1999).

54 Grassegger A, Schuler G, Hessenberger G *et al.*: Interferon-γ in the treatment of systemic sclerosis: a randomized controlled multicentre trial. *Br. J. Dermatol.* 139(4), 639–648 (1998).

55 Seibold JR, Korn JH, Simms R *et al.*: Recombinant human relaxin in the treatment of scleroderma. A randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* 132(11), 871–879 (2000).

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56 Erikson MS, Unemori EN: Relaxin clinical trials in systemic sclerosis. In: *Relaxin: Proceedings of the Third International Conference on Relaxin and Related Peptides.* Tregear GW (Ed). Kluwer, Dordrecht, The Netherlands, 373–382 (2001).

57 Sunderkötter C, Kuhn A, Hunzelmann N, Beissert S: Phototherapy: a promising treatment option for skin sclerosis in scleroderma? *Rheumatology* 45(3), iii52–iii54 (2006).

58 Knobler R, French L, Kim Y *et al.*: A randomized, double-blind, placebo-controlled trial of photopheresis in systemic sclerosis. *J. Am. Acad. Dermatol.* 54(5), 793–799 (2006).

59 Denton CP, Khanna D, Emery P *et al.*: Recombinant human anti-transforming growth factor β1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled Phase I/II trial of CAT-192. *Arthritis Rheum.* 56(1), 323–333 (2007).

60 Distler JH, Jüngel A, Huber LC *et al.*: Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. *Arthritis Rheum.* 56(1), 311–322 (2007).

61 White B, Bauer EA, Goldshmith LA *et al.*: Guidelines for clinical trials in systemic sclerosis (scleroderma). I. Disease-modifying intervention. The American College of Rheumatology Committee on design and outcomes in clinical trials in systemic sclerosis. *Arthritis Rheum.* 38(3), 351–360 (1995).