Cyclophosphamide as disease-modifying therapy for scleroderma: pros and cons

Cyclophosphamide is widely used to treat severe manifestations of numerous autoimmune rheumatic diseases including systemic sclerosis. Based on recent data, immunological and inflammatory activation remain the primary therapeutic targets in systemic sclerosis. Other effective therapies targeting the fibrotic mechanisms have not been developed despite the increased understanding of the key pathogenic pathways. The two pivotal studies of cyclophosphamide in scleroderma-associated interstitial lung disease have rejuvenated interest in this chemotherapeutic agent in this disease. Although the studies suggest that the benefit is modest, identification of appropriate patients is central to management to harness its maximal potential clinical efficacy. This article provides a timely evaluation of the benefits and drawbacks of cyclophosphamide in systemic sclerosis.

**KEYWORDS:** cyclophosphamide  hemorrhaic cystitis  interstitial lung disease  malignancy  systemic sclerosis

Systemic sclerosis (SSc; scleroderma) is an autoimmune connective tissue disorder characterized by microvasculopathy, excessive collagen deposition and autoimmunity. It can affect virtually any organ with the skin, heart, lungs, kidneys and gastrointestinal systems most commonly involved. Early studies have indicated that patients with early active diffuse cutaneous SSc (dcSSc) and significant visceral involvement have a significant mortality of 50% at 5 years [1]. By contrast, recent studies have indicated that 5-year survival amongst patients with early active dcSSc has improved over the last decade. However, approximately half of these patients will either succumb to their illness or develop major internal organ involvement within the first 3 years following onset of their disease [2].

There are many aspects of SSc that support the hypothesis that it is an immune-mediated disease and might therefore respond to immunosuppressive therapy [3]. It is therefore relevant and timely to review the current available literature regarding the use of cyclophosphamide (CYC) in SSc, in particular in the organ-based management of complications related to this disease.

**Mechanisms of action**

Cyclophosphamide, a nitrogen mustard, is an alkylating agent from the oxazophosphorine group (Figure 1). It is one of the most potent immunosuppressive drugs available. First described in 1958 by Arnold and Bourseaux, it is widely used as a chemotherapeutic agent. It undergoes extensive metabolism via the cytochrome-P450 enzymatic system with phosphoramidase mustard and acrolein as the main active and inactive metabolites, respectively. The former undergoes spontaneous degradation whereas the latter is excreted into the urine. These compounds crosslink DNA by adding an alkyl group (C \(_{2n+1}\)) to the guanine base of DNA, at the number seven nitrogen atom of the imidazole ring. This induces inhibition of DNA replication, leading to cell death. CYC exerts its cytotoxic effect on both resting and dividing lymphocytes. Its precise mechanisms in treatment of autoimmune diseases are not well established. In patients with rheumatoid arthritis, CYC has been shown to suppress T-helper cell functions with prolonged reduction of B cells due to the slower rate of recovery of B lymphocytes from an alkylating agent [4].

**Lung**

Interstitial lung disease (ILD) is the second most common visceral complication among SSc patients, after esophageal involvement. Owing to advances in the management of renal complications, lung disease including ILD and pulmonary arterial hypertension (PAH) has emerged as the leading cause of mortality in SSc, with up to 30% of deaths directly attributable to lung fibrosis. Approximately 42% of patients with SSc-ILD will die of disease progression within 10 years of diagnosis, with the most rapid decline in lung function occurring in the first 3 years of disease [5]. Although the mechanisms underlying lung fibrosis are unclear, recent developments...
in the etiopathogenesis of ILD have proposed the role of epithelial cell injury, endoplasmic reticulum stress, Wnt signaling and the extra-mesenchymal origin of fibroblasts including epithelial–mesenchymal transition and fibrocytes [6–9]. Depending on the detection method, the prevalence of ILD in SSC ranges from 25 to 90%. Some studies suggest that ILD is more common among patients with dcSSC than limited cutaneous SSC (lcSSC): in a 2003 study, lung function tests demonstrated a restrictive pattern in 23% of patients with lcSSC and in 40% of those with dcSSC [10].

The autoantibody profile may predict development of ILD; anti-topoisomerase I, anti-U11/U12, anti-Pm/Scl and anti-Th/To antibodies are associated with an increased risk, whereas anticentromere antibodies confer relative protection from SSC-associated pulmonary fibrosis [11]. However, the autoantibody specificity or extent of skin involvement does not influence the severity of lung fibrosis [12]. The most frequently noted histopathological finding is nonspecific interstitial pneumonitis (76% of patients, with isolated ground-glass pattern of opacification on high-resolution computed tomography [HRCT]) followed by usual interstitial pneumonia (13% of patients generally associated with a reticulon HRCT pattern) [13]. Common clinical tools to evaluate lung disease in SSC include pulmonary function tests and HRCT of the chest.

There are several ways to assess the degree of fibrosis on HRCT: most commonly, a visual score is used. Some authors divide each lung into three zones and then score the extent of parenchymal abnormality in each zone on a scale of 0–4 [14]. An alternative staging system has been proposed in which minimal and severe parenchymal disease on HRCT is set at a threshold of 20% [15]. Recently, a quantitative lung fibrosis score based on a computer-aided diagnosis system has been formulated. These methods are potentially useful for objective measurements of fibrosis and should be subjected to clinical trials of interventions in ILD [16].

Not all patients with deterioration in pulmonary function tests have a progressive disease: 30% of patients develop progressive lung disease and only 16% will develop severe lung fibrosis (defined as forced vital capacity [FVC] <55%) [12].

A variety of immunosuppressive agents have been evaluated as potential disease-modifying therapies in SSC-ILD, although only a small number of randomized controlled therapeutic trials have been performed in patients with SSC-related ILD. Many prospective observational studies have examined the utility of CYC as a potential treatment of SSC-ILD; studies with appropriate length of follow-up and adequate outcome assessments are listed in Table 1 [17–33]. Whilst some of the earlier studies demonstrated improvement in lung function parameters, not all subsequent studies confirm these observations. The rationale of its use in SSC-ILD is based on current concepts of disease pathogenesis: endothelial damage promotes local inflammatory response and this is thought to precede parenchymal fibrosis [17]. There are two double-blind randomized controlled trials (RCTs) that examined the efficacy of CYC in SSC-related ILD. Although both trials compared CYC with placebo, there were important differences in the trial design in terms of route of administration and type of concurrent therapy in the active treatment arm. The Sclerodema Lung Study (SLS) was the first RCT to demonstrate the effectiveness of oral CYC (1 mg/kg) in 158 patients with symptomatic SSC-ILD at the end of the 1-year treatment period [34]. The study design defined active SSC-ILD based on abnormal bronchoalveolar lavage (>3% neutrophils and/or >2% eosinophils) and/or ground-glass opacification on HRCT. Moreover, inclusion criteria included a FVC between 45 and 85% of the predicted value and grade 2 exertional dyspnea on the Magnitude of Task component of the Mahler Modified Dyspnea Index. Patients from both major subsets of SSC with disease duration of no more than 7 years from the onset of non-Raynaud’s phenomenon were included. Compared with placebo, the benefits of oral CYC were modest: 2.53 and 4.09% improvement in percentage predicted FVC (95% CI: 0.28–4.79; p < 0.03) and total lung capacity (95% CI: 0.49–7.65; p = 0.026), respectively, at 12 months. These results were paralleled with improvement in fibrosis scores on HRCT scans and patient-related outcomes including skin thickening, breathlessness,
### Table 1. Prospective observational studies evaluating cyclophosphamide efficacy in the treatment of systemic sclerosis-related interstitial lung disease.

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>Study design</th>
<th>Primary end points</th>
<th>Treatment regimen</th>
<th>Treatment length (months)</th>
<th>Follow-up (months)</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>18</td>
<td>Prospective, controlled</td>
<td>FVC, DLCO, MRSS</td>
<td>iv. CYC 1 g/m² monthly ± PDN (60–10 mg/day)</td>
<td>12</td>
<td>36</td>
<td>No changes in FVC and DLCO in both groups after 1 year No differences in FVC and DLCO between two groups</td>
<td>[18]</td>
</tr>
<tr>
<td>2009</td>
<td>36</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>iv. CYC monthly + PDN 1 mg/kg os tapered to 7.5 mg/day</td>
<td>6</td>
<td>6</td>
<td>4.15% improvement in mean FVC (p = 0.069) 5.66% improvement in mean DLCO (p = 0.27)</td>
<td>[19]</td>
</tr>
<tr>
<td>2008</td>
<td>10</td>
<td>Prospective, observational</td>
<td>FVC, DLCO, HRTCT</td>
<td>iv. CYC 500–700 mg/m²</td>
<td>6–24</td>
<td>36</td>
<td>No changes in FVC, DLCO, HRCT appearance</td>
<td>[20]</td>
</tr>
<tr>
<td>2008</td>
<td>27</td>
<td>Retrospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>iv. CYC 600 mg/m² monthly for 6 months then AZA for 18 months</td>
<td>24</td>
<td>24</td>
<td>22% of patients improved 29.6% were stable 48.2% worsened</td>
<td>[21]</td>
</tr>
<tr>
<td>2007</td>
<td>33</td>
<td>Prospective, uncontrolled</td>
<td>DLCO</td>
<td>Oral CYC 2 mg/kg/day + PDN 25 mg/day in the first 3 months, then 5 mg for 9 months</td>
<td>12</td>
<td>12</td>
<td>FVC stable at 6 and 12 months DLCO stable at 6 month and significantly increased at 12 month</td>
<td>[22]</td>
</tr>
<tr>
<td>2007</td>
<td>13</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>iv. CYC 750–1000 mg/m² monthly + MPDN 1 g iv. for 12 months then bimonthly for 12–18 months</td>
<td>24–30</td>
<td>48</td>
<td>66% of patients improved or stabilized in DLCO (at 24 but not at 48 months) and in FVC (at 24 and 48 months)</td>
<td>[23]</td>
</tr>
<tr>
<td>2006</td>
<td>13</td>
<td>Prospective, uncontrolled</td>
<td>DLCO</td>
<td>iv. CYC 500 mg/m² weekly for 2 weeks and then monthly + low-dose PDN</td>
<td>12</td>
<td>12</td>
<td>DLCO significantly improved at 6 (from 58.5% at baseline to 61%) and 12 months (71%) FVC stable at 6 and 12 months</td>
<td>[24]</td>
</tr>
<tr>
<td>2006</td>
<td>19</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>iv. CYC 500 mg/m² monthly</td>
<td>6</td>
<td>7</td>
<td>Significant improvement of DLCO (p = 0.04) FVC change from 86.6 to 89.2%</td>
<td>[25]</td>
</tr>
<tr>
<td>2004</td>
<td>16</td>
<td>Retrospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>iv. CYC 750 mg/m² every 3 weeks + MPDN 125 mg every 3 weeks</td>
<td>5</td>
<td>6</td>
<td>At 6 months, modest improvement in the FVC (+2.7%; p = 0.08) and DLCO (+2.2%; p = 0.08)</td>
<td>[26]</td>
</tr>
<tr>
<td>2002</td>
<td>14</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO, HRTCT</td>
<td>iv. CYC 15 mg/kg + iv. MPDN 10 mg/kg monthly</td>
<td>6</td>
<td>6</td>
<td>No changes in FVC and DLCO 13% improvement in HRCT appearance</td>
<td>[27]</td>
</tr>
<tr>
<td>2002</td>
<td>23</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO, HRTCT, BAL</td>
<td>iv. CYC 1 g/m² monthly + PDN 25 mg/day</td>
<td>6</td>
<td>6</td>
<td>No significant FVC changes in FVC and DLCO No significant changes in BAL and HRCT scores</td>
<td>[28]</td>
</tr>
<tr>
<td>2002</td>
<td>28</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>iv. CYC 750 mg/m² monthly for 6 months then bimonthly for 6 more months + PDN 1 mg/kg for 4 weeks or PDN &lt;10 mg/day</td>
<td>12</td>
<td>12</td>
<td>Low-dose steroid group: no improvement for any end point High-dose steroid group: improvement in HRCT score (+5.7%), FVC (+14.4%) and DLCO (+7.3%)</td>
<td>[29]</td>
</tr>
<tr>
<td>2000</td>
<td>103</td>
<td>Retrospective</td>
<td>FVC, DLCO</td>
<td>Oral CYC 2 mg/kg/day or iv. CYC 800–14,400 mg monthly or no treatment</td>
<td>6–9</td>
<td>16</td>
<td>Stabilization or increase in FVC (72%) and DLCO (49%) in CYC-treated patients, decrease in FVC (71.1%) and DLCO (9.6%) in untreated patients</td>
<td>[17]</td>
</tr>
</tbody>
</table>

AZA: Azathioprine; BAL: Bronchoalveolar lavage; CYC: Cyclophosphamide; DLCO: Diffusion lung capacity for carbon monoxide; FVC: Forced vital capacity; HRCT: High-resolution computed tomography; iv.: Intravenous; MPDN: Methylprednisolone; MRSS: Modified Rodnan skin score; PDN: Prednisolone.
function and health-related quality-of-life measures. Notably, effects on lung function continued to increase for an additional 6 months after CYC discontinuation, although benefit for dyspnea score persisted to the final assessment. Interestingly, patients with more severe restriction (FVC <70% predicted) had a greater difference in percentage FVC predicted at 12 and 18 months between CYC and placebo groups than was observed when the entire cohort was analyzed. Conversely, subjects with less severe disease at baseline (FVC >70% predicted) had decidedly smaller treatment-related differences.

In support of these results, a second RCT, Fibrosing Alveolitis in Scleroderma Trial (FAST), was designed to examine the efficacy of a 6-month regimen of low-dose prednisolone (20 mg on alternate days) associated with monthly intravenous infusion of CYC (600 mg/m²) followed by oral azathioprine (2.5 mg/kg/day) as maintenance treatment in 22 patients with active SSc-ILD. This regimen was compared with placebo infusion and oral placebo in a 23-patient control group. The results were considered to be consistent with the SLS with a 4.19% reduction of loss in FVC (95% CI: 0.57–8.95; P = 0.08) at 1 year. These results did not achieve statistical significance, although the prespecified coprimary end point of BMI-adjusted FVC change was significant (95% CI: -0.14–9.38; P = 0.04) [36].

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<th>Outcome</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>1999</td>
<td>16</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO, HRCT</td>
<td>iv. CYC 750 mg/m² monthly or oral CYC 2–2.5 mg/kg/day + PDN 10 mg/day</td>
<td>12</td>
<td>12</td>
<td>In both groups, HRCT disease extent decrease or remained stable</td>
<td>[30]</td>
</tr>
<tr>
<td>1998</td>
<td>5</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO, HRCT</td>
<td>iv. CYC 1 g monthly</td>
<td>12</td>
<td>12</td>
<td>7% increase in FVC 12% decrease in DLCO No change in HRCT</td>
<td>[33]</td>
</tr>
<tr>
<td>1994</td>
<td>18</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>Oral CYC (2–2.5 mg/kg/day) + PDN 30 mg/day</td>
<td>12</td>
<td>12</td>
<td>Improvement of FVC in 78% of patients No improvement in DLCO</td>
<td>[32]</td>
</tr>
<tr>
<td>1993</td>
<td>14</td>
<td>Prospective, uncontrolled</td>
<td>FVC, DLCO</td>
<td>Oral CYC 1–2 mg/kg/day + PDN &lt;10 mg/day</td>
<td>24</td>
<td>24</td>
<td>Improvement of mean FVC (63.6 vs 51.4%) No improvement in DLCO</td>
<td>[33]</td>
</tr>
</tbody>
</table>

AZA: Azathioprine; BAL: Bronchoalveolar lavage; CYC: Cyclophosphamide; DLCO: Diffusion lung capacity for carbon monoxide; FVC: Forced vital capacity; HRCT: High-resolution computed tomography; iv.: Intravenous; MPDN: Methylprednisolone; MRSS: Modified Rodnan skin score; PDN: Prednisolone.
them the standard of care with CYC. This could have led to underestimation of the true therapeutic effect of CYC in this context. For example, in the SLS, the baseline FVC exceeded 70% predicted in approximately half of the cohort and only 19 (13.1%) of 145 patients lost at least 10% of FVC within 1 year of treatment. In addition, the treatment effect with selective decline in FVC in the placebo group was largely observed in patients with severe disease as evident by extensive fibrosis on HRCT. This is also supported by histological observation that inflammatory abnormalities are only present in less than 20% of cases of SSc-ILD in a large series.

It is also now appreciated that unlike idiopathic lung fibrosis, SSc-ILD is slowly progressive in the majority of patients. In a third RCT with bosentan in SSc-ILD, there was minimal change in measures of lung function with a reduction of FVC by less than 2% in all patients [37]. The lack of clear response to CYC was also observed in both the SLS and FAST. It is therefore suggested that immunosuppression with CYC should aim to stabilize the disease. The lack of durability in its clinical effect of FVC in the SLS also suggests that a prolonged course may be required, in particular in those with progressive disease notwithstanding the toxicity profile of CYC. The question then remains as to which patients with SSc-ILD might benefit from CYC. As previously discussed, Goh et al. recently proposed a simple algorithm to classify subsets of SSc-ILD as mild or extensive ILD. A semiquantitative evaluation of disease extent on HRCT (as defined by presence of groundglass opacity or fibrosis on visual scanning) was demonstrated to be reproducible between ILD extent scores of less than 10% and greater than 20% lung involvement. If the HRCT assessment is inconclusive, the distinction between mild and extensive disease is determined using a FVC threshold of 70% predicted. Such staging strategy aims to identify those who are likely to progress, and immunosuppression with CYC will be justified for these selected patients. In the majority of patients with stable disease, close observation is warranted. This approach has been formalized in a proposed simple severity staging system (the United Kingdom Raynauds and Scleroderma Association [UKRSA] staging score) that is supported by data from a well-characterized cohort and congruent with the results of post hoc analyses of the SLS dataset [15].

Two recent meta-analyses evaluated data from observational studies, including the pivotal studies by Tashkin et al. and Hoyles et al. [38,39]. Nannini et al. reported on six observational studies with the additional RCT trial comparing azathioprine and CYC in the treatment of dSSc. Nannini reported that there was no apparent effect of the administration route on the outcome and there was no apparent increased risk of adverse events with CYC therapy. However, a subgroup analysis of the observational studies demonstrated statistically significant improvement compared with baseline to both predicted FVC and diffusion lung capacity for carbon monoxide values. However, most of the studies included in the meta-analysis were small and statistically weak.

Interestingly, Khanna et al. used a decision analytical model to evaluate the tradeoffs between the risks and benefits of oral CYC in the SLS and they concluded that unless the patients are treated early for the SSc-ILD, oral CYC was not a good treatment choice [40]. However, in most studies, CYC has been shown to prevent further decline in lung function, if not improving lung function parameters and survival. Therefore, these results should be considered promising and clinically significant even if not statistically powerful. In support of this, preliminary studies indicated that serum concentrations of procollagen type III amino-terminal peptide as a marker of type III collagen fibrogenesis and a sensitive serological marker for fibrosis are reduced in response to CYC [32,41]. In a separate study, elevated levels of procollagen type III amino-terminal peptide were shown to predict mortality in SSC [42].

Given the limited efficacy of CYC in a selected group of SSc patients, it is hoped that the algorithm suggested by Goh et al. would help in targeting appropriate patients to receive this treatment [15]. Interestingly, Furuya recently proposed that measurement of bone marrow-derived endothelial precursor cells (EPCs) may help to predict the effectiveness of CYC in SSc-ILD [43]. Bone marrow EPCs have been hypothesized to contribute to tissue repair by homing to the site of injury, replacing the injured vascular endothelium and promoting regeneration processes [44]. In a prospective study of 12 patients with SSc-ILD who receive monthly low-dose intravenous CYC over 24 weeks, mobilization of EPCs after the first dose was observed in a selected subset of patients. Compared with the group without an increase in EPCs, this group with increased EPCs were less likely to progress to develop end-stage lung disease, although the difference did not meet statistical significance. The authors also reported improvement in markers of endothelial activation, E-selectin and VEGF. These results, although interesting, need to be confirmed in a
larger cohort of patients in light of the ongoing debate on the significance of EPCs in etiopathogenesis of SSc. In contrast to the promising role of CYC in SSc-ILD, CYC has not been shown to be effective in SSc-associated PAH (SSc-PAH) [45]. In a subgroup analysis of patients with SSc-PAH, the pulmonary arterial systolic pressure increased 6 months following completion of CYC [46].

High-dose CYC with hemopoietic stem cell transplant
Increased understanding of the immunological mechanisms responsible for SSc has made immunosuppressive treatments such as intravenous CYC and bone marrow transplant novel therapeutic options. As a central component of autologous hematopoietic stem cell transplantation (HSCT) regimens, administration of high-dose CYC (1.5–4 g/m²) is aimed at depleting peripheral blood cells, in particular autoreactive T lymphocytes [47]. A recent analysis of the Dutch–French database of 26 patients treated with HSCT reported a clinical response in over 80% of patients and the event-free survival (defined as survival without mortality, relapse or disease progression with major organ dysfunction) was 64% at 5 years and 57% at 7 years [48].

Interestingly, the immunomodulatory effect of HSCT extends to reversal of the fibrotic process and amelioration of the microvasculopathy [49,50]. The reversal of the vascular abnormalities is associated with normalization of vascular endothelial cadherin and antiangiogenic IFN-α [51,52].

There are three long-term trials investigating the safety and efficacy of HSCT in SSc, of which the results are awaited. It is anticipated that the results will delineate the potential role of CYC as a major immunosuppressive agent in SSc.

Skin
The skin in the initial stage of SSc is characterized by symmetrical swelling of the distal fingers and hands. As the disease progresses in the diffuse subset, progressive changes extend proximally, with an inflammatory stage evolving to a more fibrotic phenotype with loss of the subcutaneous tissue, sweat glands and other skin appendages (Figure 2).

Dau reported gradual softening of the affected skin and relaxation of contractures in response to plasmapheresis combined with immunosuppressive drug therapy including CYC [53]. Histologically, there was less prominent dermal collagen with increased ground substance in the skin biopsies taken from adjacent sites before and after plasmapheresis [53].

Several open-label uncontrolled studies have investigated the efficacy of CYC in SSc-related ILD, which have utilized skin score as a secondary end point. Domiciano reported improvement of modified Rodnan skin score (MRSS) with a combination of intravenous CYC and oral prednisolone (14.9 ± 12.6 to 9.0 ± 9.9; p = 0.02; n = 9) at 1 year compared with those treated with CYC alone (24.5 ± 13.4 to 22.4 ± 12.5; p = 0.72; n = 9). The authors also reported that the MRSS remained stable for a 3-year period following 1 year of treatment [48]. However, approximately half of the patients who received the combination treatment had limited disease and, therefore, this may have masked any true potential benefit of CYC. Valentini, on the other hand, reported that in a cohort of 12 patients with dcSSc, median MRSS improved from 23 at baseline to 10 at 1 year (p = 0.002) with intravenous CYC (total cumulative dose 7.5 g) and low-dose prednisolone (10 mg/day) [24].

Griffiths evaluated the use of intravenous CYC with intravenous methylprednisolone given at 3-weekly intervals for the first three pulses and at 4-weekly intervals for a further three pulses. Comparison of MRSS pre- and post-treatment revealed a 35% improvement from median score of 17 at baseline to 13 after completion of treatment (p < 0.006) [27]. Pakas treated 28 patients with dcSSc and lcSSc with monthly intravenous pulses of CYC for 6 months and bimonthly pulses for a further 6 months. In total, 16 patients received high-dose prednisolone (1 mg/kg/day for 4 weeks, reduced by 5 mg/day on alternating days fortnightly) and the remaining 12 received a low dose of less than 10 mg/day. At the end of the 12 months, no significant difference was observed in MRSS in the group treated with low-dose prednisolone, whereas a significant reduction in the skin score was demonstrated in the group who received high-dose prednisolone [29]. Nadashkevich reported a statistically significant effect of oral CYC in a group of 30 patients treated over 18 months with a mean change in MRSS of -9.47, compared with 0.2 in a similar number of patients who received azathioprine [54].

The recent SLS provided further support for the use of CYC in SSc. Analysis of the 12-month data indicated that a statistically significant difference in MRSS between the groups was demonstrated only among the patients who had dcSSc. The mean MRSS in the active arm fell from 21.7 ± 10.1 to 15.9 ± 11.0, whereas it showed very little change in the placebo arm, in which it was 20.2 ± 9.3 at baseline and 19.1 ± 11.2 at 12 months (p < 0.01). However, like its effect on
FVC, the treatment advantage on skin disease was not sustained beyond 18 months ($p = 0.23$). The magnitude of reduction in MRSS as reported in the SLS (mean difference of -5.3) and other studies meets the minimally important difference estimates for MRSS improvement (range: 3.2–5.3) as determined using the data from the randomized controlled penicillamine study [55]. This would suggest that that CYC may be useful in treating skin disease in SSc although the longer-term immunosuppression treatment may be required to maintain treatment benefit, and the role of corticosteroids needs to be clarified in future studies.

**Heart**

The heart is one of the major organs involved in SSc, with a reported prevalence of 10% of clinically evident primary cardiac disease [56]. It predominantly affects the patients with anti-Scl70 antibody-associated dSSc, in particular those with rapid progression of skin disease [57]. It is often occult; however, when clinically evident, it is recognized as a poor prognostic factor contributing significantly to mortality [58]. Any parts of the heart involving the myocardium, coronary arteries, pericardium and the conduction system may be affected. As a consequence, the clinical manifestations are diverse, including myocarditis, myocardial fibrosis, restrictive cardiomyopathy, systolic and/or diastolic dysfunction, cardiac failure, valvular regurgitation, coronary artery disease, conduction system abnormalities, arrhythmias and pericardial disease [59]. Classically, myocarditis is clinically defined as an increase in cardiac enzymes associated with impaired ventricular function. In addition, there may be coexistent peripheral myositis [60].

Doppler echocardiography usually shows a depressed cardiac function, while ECG may demonstrate ST- and T-wave abnormalities. Cardiac MRI is increasingly recognized as the gold standard for accurate assessment of myocarditis, as it allows a noninvasive quantitative and morphological evaluation of fibrotic myocardium compared with viable tissue. Moreover, it is extremely useful in the assessment of response to immunosuppressive therapy. Myocardial tissue biopsy on the other hand is reserved for indeterminate cases [61].

The management of most SSc-associated cardiac complications parallels the protocols established for similar clinical events occurring outside the context of SSc, with immunosuppression reserved for inflammation-driven myocardial damage. There are few anecdotal reports regarding the use of CYC in SSc-related myocarditis: in three patients, the combination of CYC with methylprednisolone followed by maintenance immunosuppressive therapy including azathioprine or cyclosporine indicates that CYC may be considered to halt the progression of myocardial involvement [62–64].

**Gastrointestinal involvement**

The observation that vascular disease, such as nailfold capillary abnormalities, may be reversed in response to intravenous CYC and bone marrow transplant suggests that immunological mechanisms may mediate the microvasculopathy...
in SSc. High-dose CYC immunosuppression may reset the dysregulated immune system by restoring the imbalance in the inflammatory cellular milieu that ultimately leads to vascular dysfunction. Consistent with this, recent case reports have demonstrated that gastric antral vascular ectasia (GAVE) characterized by aberrant capillary dilatation with chronic inflammatory infiltrate improved with intravenous CYC [65,66]. The cases described were transfusion dependent and refractory to conventional therapy including endoscopic laser coagulation or sclerotherapy treatments. CYC was administered for treatment of coexisting severe organ complications such as lung fibrosis and the authors reported clinical and endoscopic improvement in the gastric vasculopathy. The close temporal relationship between immunosuppression and resolution of GAVE supports an autoimmune-mediated vascular gastropathy, although these observations are clearly uncontrolled without a direct comparative cohort of SSc-GAVE patients not receiving immunosuppressive therapy. It is also noteworthy that there is no evidence to suggest that similar responses may occur in other forms of SSc vasculopathy such as renal crisis. Moreover, there is good evidence to indicate that immunosuppression does not benefit SSc-PAH [67]. Further controlled studies are required to evaluate the potential benefit of immunomodulating therapies for GAVE-associated complications.

**Adverse effects**

A critical consideration when interpreting any potential benefit of treatment with CYC is the need for a thorough assessment of its associated risks. As discussed, the SLS and FAST have provided robust evidence that lung function and other aspects of SSc may respond to CYC, but overall treatment effect appears modest and may not be justified by the considerable toxicity of CYC.

The route of administration is a major determinant of risk of toxicity with CYC. Oral CYC in particular is frequently associated with adverse effects that require either a dosage adjustment or treatment interruption. In an early study by Akesson et al., oral CYC (2–2.5 mg/kg/day) was administered over 1 year but the median daily dose was reduced by at least 70% in the majority of patients due to neutropenia and thrombocytopenia [32,55]. An increased risk of malignancy has been reported among patients treated with CYC, with leukemia and tumors affecting the urinary tract most commonly reported. Acrolein, the inactive metabolite of CYC, which is excreted and concentrated in the urine, is thought to cause hemorrhagic cystitis. Current available data suggest that antecedent hemorrhagic cystitis that develops during CYC treatment is associated with an increased risk of bladder cancers, predominantly transitional cell carcinomas and some cases of sarcoma. Interestingly, even within the first year of CYC in the SLS, one case of each carcinoma *in situ* of the bladder, squamous-cell carcinoma of the vulva and angiosarcoma of the scalp were reported. Radii estimated a relative risk of malignancy of 1.5 among patients with severe rheumatoid arthritis treated with CYC followed-up over 20 years [68].

Moreover, patients with SSc may have an inherent risk of malignancy, in particular lung and breast cancers. Lung cancer for example has been described to develop in the context of severe lung fibrosis. How CYC will modify this risk is unclear but the risk of malignancy with oral daily CYC occurs in a dose-dependent and/or duration-dependent manner. Hematological malignancies are usually associated with large cumulative doses of CYC (between 80 and 120 g) [69]. In the SLS, the daily CYC regimen would equate to cumulative doses ranging from 20 to 70 g. There were a greater number of patients withdrawing from the active treatment arm as compared with placebo due to adverse events or serious adverse events. Interestingly, RNA polymerase I/III autoantibodies were recently shown to be strongly associated with malignancy that occurred contemporaneously with onset of SSc, suggesting that this antibody may drive the immune response in this group of patients [70]. Other SSc-specific antibodies including anti-Scl70 antibody have also been associated with a range of cancers, including lung cancer, although the significance of these results needs validation in a larger cohort with non-SSc cancer controls. Periodic high-dose intravenous CYC (1–2 g/month) is generally thought to be associated with a reduced risk of adverse effects. However, Silver et al. had to temporarily delay the treatment in three out of 14 patients [55], and Airò had to taper the dose in two out of 16 patients [26]. In the FAST, which had a mean CYC of 6 g over a 6-month period, serious adverse events occurred at similar rates in both groups and only two patients in the active treatment arm withdrew (one due to nausea and the second for reasons unrelated to CYC). Moreover, no episodes of hemorrhagic cystitis were reported in this study. In order to avoid bladder toxicity, CYC is administered in association with aggressive hydration (3–4 l daily) and mesna, which
assists to neutralize CYC metabolite acrolein and promote urinary excretion of cysteine. It is noteworthy that hydration and regular bladder evacuation cannot be assured with the daily oral dosing regimen. On the other hand, the current literature indicates that for periodic intravenous CYC, there is a paucity of evidence on the efficacy of mesna in prevention of cystitis, largely because of the low baseline risk of cystitis with this regimen. Antiemetics (usually 5HT3 receptor antagonists and steroids) may help to prevent gastrointestinal symptoms. CYC toxicity should be monitored with a full blood count and urinalysis on days 10 and 14 after each infusion, in order to adjust further doses or stop treatment if any side effect occurs[71]. CYC should be managed with caution in patients with hepatic and renal impairment and dosage adjustment may be needed. CYC-induced amenorrhea associated with decreased estrogen and increased gonadotropin in women and oligo- or azoospermia associated with normal testosterone in men have also been reported. Importantly, the effect of CYC on sterility is dependent on the age of patients, dose, duration of treatment and pretreatment state of gonadal function. In patients with breast cancer, the mean cumulative dose of CYC required to produce gonadal failure in women in their 20s, 30s and 40s were 20, 10 and 5 g, respectively[72]. In some centers, combined estroprogestinic therapy is used in premenopausal women in order to protect ovarian function, although there is no clear consensus among experts.

**Conclusion**

Cyclophosphamide is well established as a potential treatment for severe manifestations of SSC. Recent data point towards limited efficacy in SSC-ILD with positive outcomes in lung function, anatomical parameters and patient-related health outcomes. However, there is a lack of durability of its treatment effect beyond 18 months. The results also support the concept that CYC is able to stabilize disease in those with severe lung fibrosis. It is important to be mindful of the potential toxicity of CYC and to consider the balance of therapeutic benefits and potential risks of CYC. Its long-term safety remains one of the important issues that needs to be further documented with current regimens or with other novel therapies to minimize the occurrence of side effects.

**Future perspective**

Cyclophosphamide remains an important immunomodulatory treatment for specific organ-based complication of SSc. Current evidence suggests a lack of durability of treatment effect and it is likely that the search for more specific targeted therapies is necessary to address the multifaceted aspects of this disease. Despite the growing interest in other novel therapeutic agents in this disease, CYC is unlikely to be consigned to the status of historical interest any time soon. It is envisaged that future work is necessary in areas of cohort enrichment with selection of patients who are at greater risk of progression, in addition to targeting specific stages of the disease to favorably alter the risk/benefit ratio of CYC. Given that CYC is now regarded as standard of care for progressive lung fibrosis, future clinical trials with novel agents should be compared with CYC. Moreover, to reach clinical relevance and therapeutic usefulness, careful considerations should be given for new treatments to be assessed over a period beyond 12 months and a maintenance regimen with reduced doses of CYC after 1 year of treatment at full dose. The latter regimen of CYC dose is used in many chemotherapy protocols in oncology and could be potentially feasible in SSC.

Several key elements should be considered in the design of future clinical trials in SSC-ILD. The lung function parameters may display very high inter- and intra-patient variability. It is therefore useful to adopt a nonstandard statistical analysis approach to include covariates to reduce treatment effect variability. Moreover, difficulties in patients’ recruitment and a significant dropout rate at 24 months are usually encountered.

Consistent with this, a follow-up trial to SLS, SLS-2, comparing mycophenolate mofetil over 2 years with CYC, has recently commenced recruitment[101]. An alternative to change of FVC as an end point measure of clinical response of CYC in lung fibrosis includes disease progression-free survival to link surrogate markers with important outcomes such as survival. Further research should be undertaken to extend the preliminary data on the role of clinically applicable biomarkers in evaluation of patients, particularly in RCTs. For example, future studies, including the ongoing SLS-2, will collect baseline and serial serum markers to determine whether biomarker expression may identify those patients who are likely to respond to CYC.

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**Executive summary**

- Despite the improved survival among patients with the serious form of scleroderma (systemic sclerosis; SSc), diffuse cutaneous SSc (dcSSc), over the last decade, there is a significant morbidity and mortality in the early stage of this disease.

**Mechanisms of action**

- Cyclophosphamide (CYC) is one of the major immunosuppressive agents used for management of serious connective tissue diseases including SSc.
- Its precise mechanisms of immunosuppressive effects are not completely understood.

**Lung involvement**

- There is substantial evidence to suggest that CYC, in selected cases of progressive lung fibrosis, may improve lung function and health-related outcome measures.
- Stability of lung fibrosis, rather than regression of the fibrotic process, should be the aim of immunosuppression.

**High-dose CYC with hemopoietic stem cell transplant**

- Hematopoietic stem cell transplantation (HSCT) aims to reset immunity and restore immune regulation and tolerance in SSc.
- Clinical trials comparing immunoablation and autologous HSCT with high-dose CYC in patients with early dcSSc at high risk of mortality are currently underway.

**Skin**

- Evidence for the efficacy of CYC in skin disease among patients with dcSSc is largely derived from uncontrolled studies with limited evidence from the Scleroderma Lung Study (SLS) trial.
- The reduction in skin activity meets the minimally important difference estimate for modified Rodnan skin score improvement and this suggests that the treatment effect may be clinically relevant.

**Heart**

- Cardiac involvement heralds a poor prognosis in SSc.
- Cardiac MRI is a useful tool for assessment of cardiac involvement in SSc.
- There is anecdotal evidence to suggest that CYC may be helpful in SSc-related myocarditis.

**Gastrointestinal involvement**

- The microvasculopathy and immune dysregulation may partly contribute to the gastric antral vascular ectasia in SSc.
- Few case reports suggest that CYC may be useful to treat the vascular abnormalities in the GI tract but this will need to be confirmed in future studies.

**Adverse effects**

- The benefit of CYC in management of SSc has to be balanced with the risks related to its toxicity.
- The risk of toxicity with CYC is determined by its route of administration, cumulative dose and duration of treatment.

**Conclusion**

- Recent studies have confirmed the efficacy of CYC in the management of patients with significant lung fibrosis in SSc.
- The lack of durability of its therapeutic benefits and its associated toxicity may potentially limit its role as an effective treatment in SSc.
- It is anticipated that the results of the current HSCT will help to further delineate the role of CYC as a major immunosuppressive agent in SSc.

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