Chapter 7

Biological Therapy in Systemic Sclerosis

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Additional information is available at the end of the chapter

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Abstract

Systemic sclerosis is the autoimmune connective tissue disease with the highest morbidity and mortality, through the combination of inflammation, vasculopathy and fibrosis leading to severe internal organ involvement. Currently, there are no approved disease-modifying therapies, and treatment is based on organ-specific treatment and broad immunosuppression, with disappointing long-term results in most cases. Recent research has helped to improve knowledge of the pathogenesis of systemic sclerosis and to optimize treatment based on specific physiopathological targets, and a new era of biological agents in systemic sclerosis has now begun. Promising results are emerging from targeting specific cytokine signalling, especially IL-6, and cellular subpopulations such as B cells, with anti-CD20 therapy, and T-cells, with inhibition of T-cell co-stimulation. Other approaches under evaluation are based on the modulation of profibrotic pathways by anti-TGF-β agents. In this chapter, we discuss the available evidence to support the use of each biological agent in systemic sclerosis based on data from basic and translational research and on results from clinical studies.

Keywords: systemic sclerosis, biological therapy, cytokines, immune dysfunction, targeted treatment

1. Introduction

Systemic sclerosis (SSc) is a rare multisystem connective tissue disease in which inflammation, fibrosis, vasculopathy and autoimmunity are the principal features leading to diverse organ-based injuries [1]. The complex physiopathology, the substantial clinical heterogeneity, the unknown determinants of organ involvement and severity and the different individual outcomes that are frequently independent of therapeutic interventions make SSc one of the most challenging autoimmune diseases to treat [2–4].

With increasing understanding of the mechanisms and the targets underlying the immune dysregulation in many autoimmune connective tissue diseases, substantial therapeutic advances have been achieved in the past few years, including the development of biological therapies [5]. Biological agents have indeed significantly changed the prognosis of different conditions not only in rheumatology [rheumatoid arthritis (RA), seronegative spondyloarthropathies and systemic lupus erythematous (SLE)] but also in neurological, dermatological and gastrointestinal diseases [6–8].

The available treatment approved for SSc is still directed towards organ-associated manifestations, with cyclophosphamide and mycophenolate mofetil being the main therapeutic options. While these treatments may reduce the progression of organ damage, especially cardiac and lung disease, their effects are often not sustained [3, 4]. Therefore, the use of targeted therapy such as biological agents, acting on the central pathways of the disease, could be considered as a true disease-modify treatment for SSc, halting fibrosis and preventing disease progression [5].

In this chapter, we provide an update of the biological agents available for clinical treatment of SSc. First, we briefly review the pathogenic pathways that help clarify the targets for such therapeutic agents, followed by an overview of the available evidence for the use of each biological agent in SSc.

2. Main physiopathological mechanisms with specific treatment potential in systemic sclerosis

Vasculopathy, inflammation, autoimmunity and fibrosis are the basis of SSc pathophysiology. However, the relative contribution of each of these processes varies, leading to protean manifestations and clinical phenotypes. Hence, the use of specific targeted therapies in SSc is complicated by the heterogeneous nature of the disease across patients [2, 3]. Recently, a more integrated model based on the interplay between all these processes has been suggested, with sequential immune activation and vasculopathy leading to fibrosis, although the primary triggering event is still unknown (**Figure 1**) [4, 9].

Immune activation is thought to be an early event, with circulating and tissue activated CD4+ T-cells, production of T helper 2 (Th2) cell-derived cytokines (interleukins 4, 10 and 13) and subsequent skin infiltration by macrophages. Differentiation of T helper 17 (Th17) lymphocytes may also be an important process in SSc, promoting fibrosis [9, 10]. Gourh et al. analysed the levels of 13 cytokines in 444 SSc patients and compared with 216 healthy donors. They found that SSc patients had higher plasma levels of tumour necrosis factor-α (TNF-α) ($p < 0.0001$) and interleukin-6 (IL-6) ($p < 0.001$), and lower levels of interleukin-23 (IL-23) ($p = 0.014$). Additionally, cytokine profiles differed in SSc patients based on the presence of specific autoantibodies, with IL-6 being elevated in patients with positive anti-topoisomerase I and anti-RNA polymerase antibodies, but not in anti-centromere-positive patients. Moreover, disease duration also influenced cytokines levels: IL-6 and TNF-α were significantly elevated in SSc patients with disease duration between 0 and 10 years, with no difference compared with healthy controls for disease duration of more than 10 years. In contrast, levels of interleukin-10

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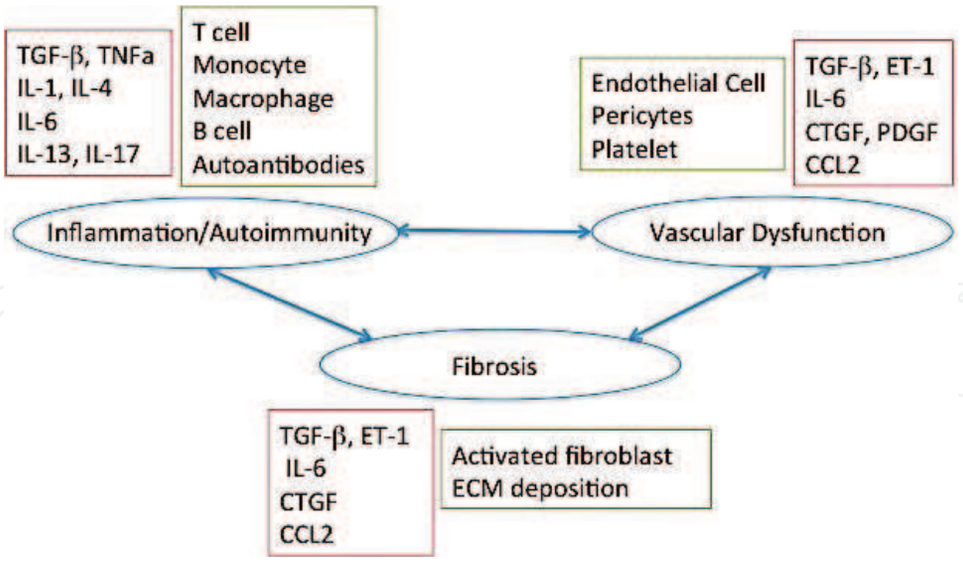

Figure 1. Integrated pathogenesis and target molecules in systemic sclerosis. Abbreviations: CCL2, chemokine ligand 2; CTGF, connective tissue growth factor; ECM, extracellular matrix; ET-1, endothelin-1; IL-1, interleukin-1; IL-4, interleukin-4; IL-6, interleukin-6; IL-13, interleukin-13; IL-17, interleukin-17; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β and TNF-α, tumour necrosis factor α.

(IL-10), interleukin-5 (IL-5) and interferon-γ (IFN-γ) were significantly increased in later stages of the disease (>10 years), compared with healthy controls [10].

Although immune activation is more common in the early disease phases, it persists in later stages, with B-cells promoting not only the production of antibodies but also interfering with antigen presentation, T-cell response and cytokine secretion, mostly through the secretion of IL-6 and transforming growth factor (TGF-β). In the tight skin mouse (tsk) model of SSc, B-cell depletion leads to suppression of skin fibrosis and downregulation of Th2 cytokines, suggesting a key role of B-cells in SSc pathogenesis and providing a link between autoimmunity and fibrosis [11].

Vascular dysfunction and remodelling can also occur early in SSc, resulting from disrupted and inappropriate repair processes following endothelial injury, with upregulation of angiogenic factors including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), endothelin-1 (ET-1), TGF-β and chemokine ligand 2/chemoattractant protein 1 (CCL2/MCP-1) [9]. These processes lead to a reduction in the number of capillaries and a narrowing of the vessels, impairing blood flow and resulting in tissue hypoxia. The abnormal vascular repair may initiate uncontrolled and persistent tissue remodelling, culminating in fibrosis [12].

Among all the cytokines found to be up-regulated in SSc, especially in the skin and lung, TGF-β is a potent stimulator of extracellular matrix production and has been widely studied and reviewed [13]. It plays an important role in wound healing and tissue repair, and an aberrant regulation of TBG-β is associated with inherited conditions, such as hereditary haemorrhagic telangiectasia, familial pulmonary hypertension and Marfan syndrome, and with fibrotic diseases, such as liver cirrhosis and idiopathic pulmonary fibrosis. In SSc, TBG-β plays a major role in fibroblast activation and differentiation and contributes to altered extracellular matrix deposition, creating a persistent state of activation. Additionally, TGF-β influences

endothelial cells and the production of anti- and pro-angiogenic factors (increasing VEGF and ET-1 expression and downregulating inducible nitric oxide synthase), hence contributing to the complex vascular phenotype seen in SSc patients, with proliferative and obliterative vascular lesions occurring simultaneously [12, 13].

Many of these molecules are potential targets for biological therapy in SSc. Indeed, many of these cytokines are known to correlate with specific organ-based complications in SSc. In the study by Gourh et al. described previously, higher serum levels of IL-6 were associated with interstitial lung disease (ILD) and pulmonary hypertension (PH), whereas patients with increased serum levels of TNF-α were more likely to have SSc renal crisis. IL-6 was also associated with higher total skin scores and IL-17 with arthritis [10].

The main effects of the biological agents are (**Table 1**) [7, 8]:

1. Neutralization of pro-inflammatory cytokines
2. Blockage of co-stimulation between antigen-presenting cells and T-cells
3. B-cell depletion

Most of these biological agents are already approved for the treatment of many autoimmune diseases, and for some of them, there are on-going clinical trials in SSc, based on pre-clinical work showing the relevance of each of their respective targets in the pathogenesis of SSc.

In the following section, we will review the available evidence to support the use of each biological agent in SSc, based on data from basic and translational research and on results from clinical studies. At the end of this chapter, **Tables 2** and **3** summarize the reported experience from observational studies and clinical trials for each biological agent in SSc.

Biological agent	Biological target
Infliximab, etanercept, adalimumab, golimumab	TNF-α
Tocilizumab	IL-6
Rilonacept	IL-1
Ixekizumab	IL-17
Brodalumab	IL-17 receptor
Tralokinumab	IL-13
Fresolimumab	TGF-β
Abatacept	CTLA-4 (T-cell)
Rituximab	CD20 (B-cell)
Belimumab	BAAF (B-cell)

Abbreviations: BAAF, B-cell activating factor; CTLA-4, cytotoxic T-lymphocyte-associated protein-4; IL-1, interleukin-1; IL-6, interleukin-6; IL-13, interleukin-13; IL-17, interleukin-17; TGF-β, transforming growth factor β and TNF-α, tumour necrosis factor α.

Table 1. Potential targets for current biological therapies in SSc.

Biological agent	First author, Year [Ref]	Study type	Patients, *n*	Organ involvement	Treatment, dose and duration	Outcome
Tocilizumab	Shima, 2010 [18]	CS	2 dcSSc (patients A and B)	ILD (A) SRC (B)	8 mg/Kg/ monthly Duration: 6 months	mRSS reduction: 27→13 (A); 26→20 (B) Internal organ stable (A,B)
	Elhai, 2013 [19]	CHS	15 SSc	Polyarthritis (*n* = 15)	8 mg/Kg/ monthly Duration: 5 months	DAS-28 score improvement (*n* = 10): 5.2→2.8 Stopped due to inefficacy (*n* = 2)
	Fernandes das Neves, 2015 [20]	CS	2 dcSSc (patients A and B) 1 lcSSc (patient C)	ILD (A,B,C)	8 mg/Kg/ monthly Duration: 6 months	mRSS reduction: 17→11(A); 41→25(B); 7→5(C) ILD: stable (A,B); progression (C) Patient VAS score reduction: 70→40(A); 70→30(B); 60→10(C)
Anti-TNF-α	Distler, 2011 [25]	CS	65 SSc	ILD* Polyarthritis* Myositis*	INF (*n* = 30) ETA (*n* = 29) ADA (*n* = 6) Duration: not specified	Organ involvement: improvement (*n* = 48) - stable (*n* = 10) - worsened (*n* = 7) – mainly ILD
	Allanore, 2006 [27]	CR	1 lcSSc	ILD and polyarthritis	ADA (40 mg sc/every 2 weeks) Duration: 7 months	DAS-28 score improvement: 7.8→5.1 ILD progression Dead from respiratory failure
Abatacept	Elhai, 2013 [19]	CHS	12 SSc	Polyarthritis (*n* = 12) Myositis (*n* = 7)	10 mg/Kg/ monthly Duration: variable 11–18 months	DAS-28 score improvement (*n* = 12) Median score: 5.2→2.8 Myopathy stable (*n* = 7)
	Paoli, 2014 [49]	CS	4 dcSSc (patients A, B, C and D)	Extensive skin fibrosis (A, B, C, D) ILD (A,B)	500–750 mg/ dose at weeks 0,2 and 4, then every 4 week Duration: variable 12–15 months	mRSS reduction: 37→8 (A); 32→20 (B); 35→17 (C); 30→15 (D) ILD regression (B)
Rituximab	Daoussis, 2012 [63]	CHS	8 dcSSc	ILD (*n* = 8)	375 mg/m^2 at baseline, and at 6, 12 and 18 months	At 24 months: ILD improvement (*n* = 8) mRSS reduction (mean score): 13.5 ± 12.42→4.87 ± 0.83

Abbreviations: CS, case series; CHS, cohort study; CR, case report; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; ILD, interstitial lung disease, mRSS, modified Rodnan skin score; SRC, scleroderma renal crisis; TCZ, tocilizumab, RTX, rituximab; ADA, adalimumab; ETA, Etanercept; INF, infliximab; VAS, visual analogue scale and DAS-28, Disease Activity Score for 28 joints.

*Number of patients not specified.

Table 2. Observational studies and case series reporting the experience of biological therapy in SSc.

Biological agent	First author, year [Ref]	Study type	Patients, *n*	Control Y/N (n)	Treatment	Study duration	Aim/primary end-point	Outcome
Tocilizumab	Khanna, 2016 [21]	RCT	87 dcSSc	Y (n = 44)	162 mg sc weekly	48 weeks (followed by open-label weekly TCZ for 48 weeks)	Change in mRSS at week 24	Reduction in mRSS at w24: TCZ −3.92 vs placebo −1.22, (−2.70, p = 0.0915) Fewer patients on TCZ with FVC decline vs placebo (w24, p = 0.009, w48 p = 0.03)
Infliximab	Denton, 2009 [24]	OL	16 dcSSc	N	5 mg/Kg at week 0, 2,6, 14 and 22	26 weeks	Safety assessment and potential efficacy	No change in mRSS Frequent AEs (19 treatment-related)
Metelimumab (CAT-129)	Denton, 2007 [46]	RCT	45 dcSSc	Y (n = 11)	0.5, 5 or 10 mg/Kg every 6 weeks	24 weeks	Safety assessment	No change in mRSS AEs more frequent in treatment group
Fresolimumab	Rice, 2015 [47]	OL	15 dcSSc	N	2 × 1 mg/kg (4 weeks apart), or 1 × 5 mg/Kg	24 weeks	Safety assessment Effect on TGF-β responsive skin gene expression	Downregulation of all TGF-β skin gene expression Reduction in mRSS: w11 (−6, p = 0.0005); w17 (−9, p = 0.0024) Frequent bleeding events (n = 11)
Abatacept	Chakravarty, 2011 [50]	RCT	10 dcSSc	Y (n = 3)	500–750 mg/dose at week 0, 2 and 4, then every 4 weeks	24 weeks	Safety assessment and change in mRSS at week 24	Reduction in mRSS vs placebo: −8.6 vs −2.3, p = 0.059 No serious AEs
	Chakravarty, 2015 [51]	RCT	10 dcSSc	Y (n = 3)	500–750 mg/dose at week 0, 2 and 4, then every 4 weeks	24 weeks	Safety assessment and potential efficacy Effect on skin gene expression	Reduction in mRSS vs placebo: −9.8, p = 0.0014 Decreased inflammatory-related intrinsic skin gene expression

Biological agent	First author, year [Ref]	Study type	Patients, *n*	Control Y/N (n)	Treatment	Study duration	Aim/primary end-point	Outcome
Rituximab	Bosello, 2010 [59]	OL	9 dcSSc	N	1000 mg day 1 and 15 (with 100 mg MPDN each)	36 months	Safety assessment and change in mRSS and serum IL-6 levels	At 6 months: Reduction in mRSS: 21.1 ± 9.0 to 12.0 ± 6.1, $p = 0.001$ Decrease in serum IL-6 ($p = 0.02$) No significant change in FVC or DLCO
	Smith, 2010 [60]	OL	8 dcSSc	N	1000 mg day 1 and 15 (with 100 mg MPDN each)	24 weeks	Safety and potential efficacy assessment	At w24 Reduction in mRSS: 24.8 ± 3.4–14.3 ± 3.5, $p < 0.001$ No significant change in FVC or DLCO Decrease in myofibroblast score on skin biopsy
	Lafyatis, 2009 [61]	OL	15 dcSSc	N	1000 mg day 1 and 15	12 months	Safety and potential efficacy assessment Change in mRSS	No significant change in mRSS or in FVC or DLCO at 6 months
	Jordan, 2015 [62]	MCNCC	35 dcSSc 11 lcSSc	Y ($n = 25$)	Variable most received 1000 mg at day 1 and 15	Variable: 7 months (5–9)	Change in mRSS	At 7 months: RTX group vs baseline: Reduction in mRSS 14.4 ± 1.5 vs 18.1 ± 1.6 v $p = 0.0002$ no decline in FVC ($p = 0.5$), improvement in DLCO ($p = 0.03$) Control group: decline in FVC ($p = 0.01$)
	Daoussis, 2010 [64]	RCT	8 dcSSc	Y ($n = 6$)	375 mg/m^2 weekly 4 weeks, at 0 and 24 weeks	48 weeks	Efficacy assessment	At 48 weeks RTX group vs baseline: Reduction in mRSS 8.37 ± 6.45 vs 13.5 ± 6.84, $p < 0.001$ Improvement in FVC ($p = 0.0018$) and DLCO ($p = 0.017$)
	Melissaropoulos, 2015 [65]	OL	30 dcSSc	N	At 0, 6, 12 and 18 months*	7 years	Long term efficacy and safety assessment	Treatment vs baseline: Lung function improvement FVC: 86.2 ± 5.5 vs 78.2 ± 3.6, $p = 0.018$; DLCO: 62.4 ± 4.5 vs 57.3 ± 3.2, $p = 0.012$ Reduction in mRSS, ($p < 0.001$) 5 deaths not proven to be treatment-related

Abbreviations: RCT, randomized controlled trial; OL, open label; MCNCC, multicentre nested case-control; Y, yes; N, no; sc, subcutaneous; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; TCZ, tocilizumab; INF, infliximab; RTX, rituximab; AEs, adverse events; IL-6, interleukin-6 and MPDN, methylprednisolone.

*Dose not specified.

Table 3. Clinical trials investigating the efficacy of biological therapy in SSc.

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3. Targeting pathogenic processes in systemic sclerosis

3.1. Targeting cytokines

3.1.1. Interleukin-6

Multiple lines of evidence indicate that IL-6 is a critical interleukin in SSc. Animal models have confirmed this hypothesis. Saito et al. demonstrated that in the bleomycin mouse model, the IL-6 knockout (KO) variety had reduced fibrotic bleomycin-induced lung fibrosis, and a significant decrease in the total inflammatory cell count, namely macrophages and neutrophils, in bronchoalveolar lavage fluid, compared with the wild type (WT) mice ($p < 0.05$). Moreover, lung tissue pathology, on day 21 after bleomycin treatment, showed significant fibrotic changes with increased collagen content in WT mice compared with IL-6 KO mice ($p < 0.05$) [14]. Kitaba et al. obtained similar results with IL-6 KO bleomycin mice having reduced dermal fibrosis comparing with the WT mice [15].

In a recent work by Khan et al., serum IL-6 levels were determined in 39 patients with diffuse cutaneous SSc (dcSSc), 29 patients with limited cutaneous SSc (lcSSc) and 15 healthy controls. Serum IL-6 levels were higher in dcSSc patients, especially in the subgroup with thrombocytosis and elevated C-reactive protein levels, compared with dcSSc with normal platelets count, lcSSc and controls ($p < 0.001$). IL-6 expression was higher in dermal fibroblasts and endothelial cells especially in patients with early dcSSc (<3 years since disease onset) [16].

The relationship between serum IL-6 levels and clinical outcomes was also addressed. Serum IL-6 levels positively correlated with modified Rodnan skin score (mRSS) at the time of disease onset (Spearman's $p = 0.514$, $p = 0.001$). In addition, serum IL-6 levels at presentation also correlated with the mRSS at 36-month follow-up (Spearman's $p = 0.795$, $p < 0.001$), which may reflect a predictive role for IL-6 levels regarding the extent of skin involvement in early diffuse patients. Furthermore, these authors also showed that higher levels of IL-6 (≥10 pg/mL) at disease presentation predicted mortality in dcSSc, with a 15-year survival of 30% in patients with high IL-6 levels vs 93% survival among patients with low IL-6 serum levels ($p = 0.021$). These results reinforce and define a subset of SSc patients in which IL-6 plays a relevant role and support the rationale for specific anti-IL6 therapeutics in SSc [16].

Tocilizumab is a humanized anti-IL6 receptor antibody that binds to both the soluble and the membrane bound IL-6 receptor, and is approved for the treatment of rheumatoid arthritis amongst other immune-mediated diseases [17]. The first report of SSc patients treated with tocilizumab was published in 2010, demonstrating its benefits in two dcSSc patients, one with lung fibrosis and the other with renal crisis. Monthly treatment with tocilizumab for 6 months reduced skin sclerosis in both patients, with skin biopsies showing a reduced number of cells in the dermis and vascular walls, and reduced the immunohistochemical staining for α-smooth muscle actin (SMA) after treatment. Internal organ involvement remained stable, namely lung and renal impairment [18]. Other cases were subsequently reported, reinforcing the role of tocilizumab as a valuable treatment option in dcSSc, improving skin thickness, halting lung fibrosis and reducing the patient's Global Disease Activity scores [19, 20].

In a European League Against Rheumatism (EULAR) Systemic Sclerosis Trials and Research Group (EUSTAR) observational study, 15 SSc patients with refractory polyarthritis and myopathy were treated with tocilizumab for 5 months, with significant improvement in the 28-joint count Disease Activity Score in 10 patients. No significant change was seen in lung or skin involvement [19].

Another mini-series evaluated the effect of tocilizumab in three dcSSc patients with interstitial lung disease. After treatment for 6 months, the skin score improved in all three patients, and the patients' global assessment also improved. In two patients, there was a halt in the progression of lung fibrosis, on lung high-resolution CT scan (HRCT) and lung function tests [20].

The first clinical trial assessing the effects of subcutaneous tocilizumab in dcSSc (faSScinate) was done in 35 hospitals in five different countries (Canada, France, Germany, United Kingdom and United States of America) and was published in 2015. In this phase II study, 87 patients with early dcSSc (disease duration ≤5 years) were enrolled, 43 patients were treated with tocilizumab and 44 with placebo for 48 weeks. The change in the mRSS at 24 weeks was −3.92 in the tocilizumab group and −1.22 in the placebo group (difference of −2.70, 95% CI −5.85 to 0.45, $p = 0.0915$). Also, fewer patients assigned for tocilizumab treatment had an absolute decline of forced vital capacity (FVC) of more than 10%, compared with patients treated with placebo [21]. Given these encouraging results, there is an on-going 2-year randomized double-blinded controlled clinical trial, across 120 global study sites, which will assess the efficacy and safety of subcutaneous tocilizumab in early dcSSc. The primary outcome measure is the change in mRSS at 48 weeks [22].

3.1.2. Tumour necrosis factor α

Tumour necrosis factor α (TNF-α) is a pro-inflammatory cytokine that plays an important role in the pathogenesis of many autoimmune diseases. Anti-TNF-α agents are approved for the treatment of rheumatoid arthritis, seronegative spondyloarthropathies and inflammatory bowel diseases [7]. Contrasting with its well-proven role in other inflammatory diseases, the relevance in SSc and in profibrotic conditions is still conflicting.

Some animal models have shown a benefit from TNF-α blockade. In a study using the bleomycin mouse model, treatment with etanercept resulted in a significant reduction in dermal sclerosis compared with bleomycin mice not treated with etanercept (dermal thickness 85.83 ± 12.8 vs 126.4 ± 31.3 μm, respectively, $p < 0.05$). Histopathological analysis of the skin revealed a reduction in the collagen content and in infiltrating myofibroblasts (reduced levels of tissue hydroxyproline and α-SMA, $p < 0.05$) and reduced levels of TGF-β (514.4 ± 62,6 vs 716.9 ± 49.1, treated mice vs non-treated, $p < 0.05$) [23].

The efficacy of anti-TNF-α agents was also assessed in SSc patients. In an open-label study, 16 patients with dcSSc were treated with infliximab 5 mg/kg (five infusions at weeks 0, 2, 6, 14 and 22). There was no significant improvement in the mRSS, but there was a trend towards a decline between the peak score at 6 weeks (OR 29, 95% CI 11–44) and the 22-week time point (OR 17, 95% CI 6–46, $p = 0.10$). A limiting factor was the frequency of adverse events (total of 127, with 21 considered serious events and 19 definitively related to infliximab treatment). Eight patients (50%) discontinued infliximab prematurely [24].

In addition, a EUSTAR consensus meeting on the role of anti-TNF-α therapy in SSc concluded that its use in SSc should be discouraged, although it can be considered for patients with inflammatory arthritis overlap. These conclusions were based on a review of data on anti-TNF-α therapy from 79 EUSTAR centres. From a total of 65 patients treated with TNF-α inhibitors, 48 patients (74%) improved, but their main clinical manifestation was inflammatory arthritis. In seven patients (11%), the disease worsened mostly due to progression of lung fibrosis, and in two patients (15%), there was no change in the outcome [25].

Furthermore, there is evidence in the literature of progression of lung fibrosis in patients with rheumatoid arthritis, mainly related to lung alveolitis after treatment with TNF-α antagonists [26], and in the last few years, some similar cases in SSc have been reported. Recently, Allanore et al. reported a case of a patient with a 5-year diagnosis of lcSSc with established interstitial lung disease with relatively well-preserved lung function (FVC of 72% predicted and diffusing capacity for carbon monoxide (DLCO) of 52%), treated with azathioprine and with progressively disabling inflammatory polyarthritis. Treatment with adalimumab was started with improvement in the articular symptoms, but worsening of the lung disease after 6 months of treatment, requiring long-term oxygen therapy and resulting in death from respiratory failure [27].

Due to these reports, anti-TNF-α therapy is now seldom used in SSc patients, based on recommendations by experts and despite the absence of randomized controlled trials.

3.1.3. Interleukin-1

The role of interleukin-1 (IL-1) in the pathogenesis of SSc has been addressed in a few studies. The IL-1 role in proliferation and collagen production of fibroblasts is well established. After IL-1 stimulation, normal fibroblasts produce various cytokines, including IL-6, IL-8, TNF-α and PDGF. IL-1α is elevated in the serum of SSc patients, and it is constitutively produced by SSc fibroblasts, as demonstrated by Maekawa et al. [28]. In this study, IL-1α levels were significantly higher in SSc patients compared with healthy controls ($p = 0.0017$), although the stratification of SSc patients according to disease subsets (limited vs diffuse) did not show a significant difference in serum IL-1α levels. Furthermore, Kawaguchi et al. demonstrated that inhibition of IL-1α in SSc fibroblasts resulted in decreased fibroblast proliferation, decreased IL-6 production and procollagen synthesis, whilst overexpression of IL-1α in normal fibroblasts resulted in an SSc phenotype fibroblast [29].

Considering these results, a phase I/II double-blinded placebo-controlled trial is on-going in patients with early dcSSc in order to assess the effects of rilonacept (IL-1 Trap), an anti-IL-1 antibody, currently used for autoinflammatory syndromes such as cryopyrin-associated periodic syndromes. In this study, the primary outcome is the 4-gene biomarker expression in the skin, and the secondary outcome is the reduction of skin thickness measured by mRSS. Farina et al. previously described the 4-gene biomarker as highly predictive of mRSS in patients with early dcSSc. It includes two TFG-β-regulated genes: cartilage oligomeric matrix protein (COMP) and thrombospondin-1 (THS1), and two interferon (IFN)-regulated genes: interferon-induced 44 (IFI44) and sialoadhesin (SIG1). The central hypothesis is that if IL-1 is a key cytokine leading to fibrosis in SSc, its inhibition will downregulate the expression of the 4-gene biomarker in the skin [30, 31].

3.1.4. Interleukin-13

Substantial experimental evidence has shown that interleukin-13 (IL-13) has a significant role in SSc as a profibrotic cytokine that interplays with other mediators such as TGF-β [2, 6, 32].

Fichtner-Feigl et al. have recently defined the pathway of IL-13-induced fibrosis via TGF-β in an *in vivo* bleomycin model of fibrosis. Firstly, this process involves the induction of a cell-surface IL-13 receptor (IL-13Rα_2) by IL-13 and TNF-α. In a second phase, IL-13 signalling through this receptor induces activation of the TGFB1 promoter. Prevention of IL-13Rα_2 expression via IL13Rα_2 gene silencing or blockade of the IL-13Rα_2 signalling leads to marked downregulation of TGF-β production and collagen deposition in bleomycin-induced lung fibrosis [32].

Fuschiotti et al. analysed the ability of SSc patients' CD4+ and CD8+ T-cells to produce cytokines following *in vitro* activation. There was a dysregulation in IL-13 production by effector CD8+ T-cells in SSc, not present in the healthy control group or in rheumatoid arthritis patients ($p < 0.001$). This dysregulated IL-13 production also correlated with the extent of skin fibrosis, with dcSSc showing higher levels of IL-13 compared with lcSSc ($p < 0.01$) [33].

Supporting this work, Bleperio et al. also concluded that in the bleomycin-induced mouse model, IL-13 levels were elevated and its neutralisation led to attenuation of bleomycin-induced pulmonary fibrosis and to a decrease in C10 levels (a chemotactic factor for mononuclear phagocytes) [34].

Moreover, in another work from Fuschiotti et al., circulating IL-13 producing CD8+ T-cells in SSc expressed skin-homing receptors and induced a profibrotic phenotype in normal fibroblasts which was inhibited by an anti-IL13 antibody. The histopathological analysis showed a dermal inflammatory infiltration of CD8+ T-cells and the IL-13 accumulation was higher in the early phases of the disease (duration <3 years) [35].

Tralokinumab is an interleukin-13 neutralising humanized monoclonal antibody used in uncontrolled severe asthma. It seems to be particularly effective in the subgroup of patients with higher baseline levels of periostin, an IL-13-induced protein in the airways, which is also elevated in SSc patients [36]. Yang et al. showed that after bleomycin treatment, WT mice had marked cutaneous fibrosis, increased expression of periostin and increased numbers of myofibroblasts, while these changes were not seen in periostin−/− mice (PN−/−). Moreover, fibroblasts of PN−/− mice showed reduced expression of α-SMA and procollagen type-Iα1 induced by TGF-β. Also, SSc patients had elevated expression of periostin in the skin compared with healthy controls [37]. Tralokinumab is currently under evaluation in a phase II dose-ranging study for patients with idiopathic pulmonary fibrosis [38].

Altogether, there is significant research supporting a possible role for tralokinumab in SSc treatment, especially in patients with interstitial lung disease.

3.1.5. Interleukin-17

Interleukin-17 (IL-17) is a cytokine secreted by a distinct T-cell subset called T helper 17 (TH17) cells, which leads to activation of fibroblasts and subsequent secretion of pro-inflammatory

cytokines such as IL-6 and IL-8, and to an increased surface expression of Intercellular Adhesion Molecule 1 (ICAM-1). In a work by Kurasawa et al., IL-17 was found to be overexpressed in peripheral blood cells and in lymphocytes from the skin and lungs of SSc patients, compared with samples from patients with systemic lupus erythematosus, autoimmune inflammatory myositis and healthy donors. Furthermore, IL-17 overproduction significantly correlated with an early disease stage and an enhanced proliferation of fibroblasts and IL-1 production in SSc patients [39]. Altered IL-17 expression in SSc has also been reported elsewhere, with studies documenting a decrease in regulatory T-cell (Treg) levels, as well as a functional deficiency, accompanied by an increase in CD4+CD25+FoxP3+ T-cells and IL-17 levels, potentially causing an immune imbalance between Treg and Th17 cells [40, 41].

Recently, studies have started looking at several biological agents that target IL-17 (ixekizumab) and its receptor (brodalumab), especially in psoriatic arthritis, but currently there are no data available on its use in SSc [42].

3.2. Targeting morphogenic regulators

3.2.1. Transforming growth factor-β

Transforming growth factor-β (TGF-β) has been recognised as the central mediator of fibrosis in SSc and the persistence of TBG-β signalling is a key feature in SSc pathogenesis [43]. TGF-β is secreted from monocytes, lymphocytes and fibroblasts in the latent form and sequestered in the extracellular matrix. In its active form, TGF-β promotes collagen gene expression and sustained fibrosis via SMAD (mothers against decapentaplegic) 2/3 signalling and epithelial-mesenchymal transition through a SMAD-independent pathway [44, 45].

Examining the role of TGF-β in fibrosis, Sargent et al. assessed TGF-β responsive gene expression in the skin of SSc patients. This TGF-β responsive signature was found only in patients with the diffuse subset and correlated with higher mRSS and with a higher prevalence of interstitial lung disease. There was no association between disease duration, specific autoantibodies or any other clinical manifestation and TGF-β-expression gene signature [43].

The blockade of TGF-β pathways has been shown to prevent collagen synthesis in SSc fibroblasts. Ihn et al. evaluated the levels of active and latent TGF-β and its receptor from cultured dermal fibroblasts of 10 patients with early dcSSc (<2 years disease duration). They also examined the expression of human α2(I)-collagen messenger RNA (mRNA) and its transcriptional activity both before and after blocking TGF-β signalling with anti-TGF-β antibodies or with a TGF-β anti-sense oligonucleotide. SSc fibroblasts produced an equivalent amount of TGF-β as control fibroblasts (0.381 ± 0.031 vs 0.435 ± 0.082, respectively), although SSc fibroblasts had a significantly increased expression of TGF-β receptors ($p < 0.01$). Furthermore, the blockage of TGF-β signalling resulted in a dose-dependent decrease of α2(I)-collagen mRNA expression [44].

Given the role of TGF-β in SSc pathogenesis and the availability of commercial anti-TGF-β antibodies, some recent trials tested their safety and efficacy in the treatment of SSc.

In a multi-centre randomised placebo-controlled trial, 45 early dcSSc patients were treated with CAT-192 (Metelimumab), a recombinant human antibody that neutralises active human

TGF-β1 (doses of 0.5, 5 or 10 mg/kg, in a total of four administrations, every 6 weeks). The primary endpoint was to evaluate safety and tolerability of CAT-192; secondary outcomes included mRSS, Systemic Sclerosis Health Assessment Questionnaire, organ-based manifestations, levels of collagen propeptides (N propeptide and type I and type III collagen) and skin levels of mRNA for procollagen I and III, TGF-β1 and TGF-β2. No changes in mRSS or in any other clinical variables assessed were documented, including the levels of biomarkers in the serum and the mRNA analysis of skin biopsies, independently of the dose used. Moreover, there were more serious adverse events, including deaths, than in the placebo group (four deaths in the treatment group vs zero in the placebo group) [46].

Contrasting with CAT-192 that has a weak neutralising effect and is monospecific for TGF-β1, fresolimumab is a high-affinity neutralising antibody that targets all three TGF-β isoforms. In an open-label trial, 15 patients with early dcSSc were divided in two groups and treated with fresolimumab (either two infusions of 1 mg/kg 4 weeks apart, or one infusion of 5 mg/kg). The skin expression of TGF-β-regulated genes was assessed, as well as skin thickening using mRSS. At weeks 3 and 7 after treatment, there was a downregulation of all the TGF-β-regulated biomarker genes studied, namely thrombospondin-1 (THBS1), cartilage oligomeric protein (COMP), Serpin Family E Member 1 (SERPINE1) and connective tissue growth factor (CTGF) in both groups. There was also a parallel improvement in mRSS, with a mean change in mRSS in both groups of −6 ($p = 0.0005$) and −9.5 ($p = 0.0024$), at weeks 11 and 17, respectively, compared with baseline. SMA staining on skin biopsies also decreased significantly ($p < 0.01$, comparing week 4 to baseline). Regarding safety, one patient died of congestive heart failure 12 weeks after fresolimumab treatment. The most significant adverse events were bleeding episodes (11 patients, two of them with gastrointestinal bleeding) and anaemia in 10 patients (66.7%) [47]. By considering these results and fresolimumab's effect as an antifibrotic agent, further studies are needed to determine the safety profile of fresolimumab and its effects on organ involvement in SSc.

3.3. Targeting of cellular subpopulations

3.3.1. T-cells

As described previously, activated T-cells play an important role in SSc pathogenesis. Abatacept is a soluble fusion protein consisting of an extracellular domain of the human cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), linked to the modified fragment crystallizable (FC) region of IgG1. It inhibits T-cell activation by binding CD80/CD86 on antigen presenting cells, blocking its interaction with CD28 on T-cells. It is currently approved for the treatment of rheumatoid arthritis [7].

In mouse models of bleomycin-induced dermal fibrosis and chronic graft-vs-host disease (early and inflammatory stages of SSc), abatacept prevented skin fibrosis and it was also effective in treating established fibrosis. Activated T-cells, B-cells and monocytes infiltrating the skin were reduced, along with IL-6 and IL-10 levels. However, abatacept did not have any efficacy reducing dermal fibrosis in Tsk-1 mice (inflammatory-independent mouse model of SSc) reinforcing the concept that inhibition of T-cell response by abatacept can only prevent and reduce inflammation-driven dermal fibrosis. Moreover, abatacept did not protect

against bleomycin-induced dermal fibrosis in CB17-SCID mice, further supporting the idea that T-cells have a role in the anti-fibrotic effect of abatacept [48].

Some case reports and observational studies have shown the benefit of abatacept in SSc patients. In a small clinical report of four patients with dcSSc refractory to conventional therapy, abatacept was administered intravenously at weeks 0, 2 and 4, and then every 4 weeks. All four patients had severe progression of skin thickness, and two of them had interstitial lung disease under conventional therapy (cyclophosphamide and low-dose prednisolone). After adding abatacept, a decrease in mRSS was achieved in all patients (average of 1.3 units/month) and lung function improved 6 months after starting abatacept. Furthermore, these effects were sustained even after tapering the other treatments [49]. In an observational EUSTAR study in 20 SSc patients with refractory polyarthritis and myositis, treatment with abatacept for a mean of eleven months showed benefits in polyarthritis with a reduction of the 28-joint count Disease Activity Score but it showed no efficacy in muscle involvement. There was also no difference in mRSS, but only half of these patients had dcSSc and the mean baseline mRSS was only 5 [19].

In addition, Chakravarty et al. carried out a pilot study in 10 early dcSSc patients (seven treated with abatacept at 0, 2, 4 and every 4 weeks, for 24 weeks; three patients treated with placebo). Compared to placebo, patients treated with abatacept showed a greater improvement in absolute mRSS (−8.6 vs −2.3, $p = 0.059$) and a higher mean percentage of change in mRSS (−33% vs −6.2%, respectively, $p = 0.31$). No changes in the other variables were documented, namely in lung function tests. There were no serious adverse events [50].

Recently, the same group performed a randomised placebo-controlled clinical trial to evaluate the effect of abatacept and to assess safety outcomes in 10 SSc patients (seven treated with abatacept for 24 weeks and three with placebo). Improvement in mRSS was defined as ≥30% from baseline; skin biopsies were obtained for differential gene expression and intrinsic gene expression subset assignment (inflammatory signature, fibroproliferative signature and normal-like signature). This included biopsies from four healthy controls [51].

There was a trend towards improvement in mRSS in the treatment group at week 24, when compared to the baseline (−8.6 ± 7.5, $p = 0.0625$), but not to the placebo group (−2.3 ± 15, $p = 0.75$). When accounting for repeated measures over the eight visits of the study, there was a significant difference between the two groups, with a decrease estimate of −9.8 (95% CI −16.7 ± 3.0, $p = 0.0014$) in mRSS. Five of the patients in the treatment group and one in the placebo group showed a decrease in mRSS ≥ 30%. Adverse events were similar in both groups, with one serious adverse event in the treatment group (supraventricular tachycardia), considered to be unrelated to the study drug and the patient completed the study. Patients who improved with abatacept mapped to the inflammatory intrinsic subset of skin gene expression at baseline and showed decreased gene expression after treatment, mainly in genes related to CD28 T-cell co-stimulation, whereas non-improvers and the placebo group showed stable or reverse inflammatory gene expression [51].

Given these data, the same group is currently conducting a larger multi-centre placebo-controlled trial for subcutaneous abatacept in early dcSSc. In addition to safety assessment, the primary outcome of this trial is variation in mRSS [52].

3.3.2. B-cells

The findings of pre-clinical and clinical works suggest an important role for B-cells in the physiopathology of SSc. SSc patients have an altered blood B-cell homeostasis, with studies showing expanded naive B-cells, activated but diminished memory B-cells, and chronic hyper-reactivity of memory B-cells [53, 54]. Additionally, DNA microarrays of gene expression patterns in skin biopsies of dcSSc patients have revealed a B-lymphocyte signature when compared to healthy controls, and this was present in both affected and unaffected skin [55].

B-cell infiltration was also found in lung biopsies of SSc patients with interstitial lung disease (ILD). Lafyatis et al. analysed pulmonary tissue of 11 patients with dcSSc (four with non-specific interstitial pneumonia and seven with a usual interstitial pneumonia pattern). Tissue was stained for CD20, CD3 and CD68 and compared with lung biopsies from four healthy controls. Lung tissue from SSc patients showed a variable but intense infiltration of B-cells arranged in lymphoid aggregates as well as in a diffuse pattern. Staining tended to be more intense in patients with the usual interstitial pneumonia (UIP) pattern, but the correlation was not statistically significant, possibly due to the small sample size. These data suggest that B-cells could be important in the pathogenesis of ILD in SSc, and considering the variability of B-cell infiltration, anti-CD20 therapy can be a therapeutic option at least in a subset of patients with SSc [56].

Contributing to probable relevancy of B-cells in SSc, serum levels of B-cell activating factor (BAAF) were also shown to be elevated in SSc patients, correlating with the extent of skin fibrosis. BAAF is a member of the tumour necrosis factor (TNF) superfamily and plays an important role in the survival and maturation of B-cells. It influences all stages of B-cell differentiation, from development, selection and homeostasis of naive primary B-cells to the maintenance of long-lived bone marrow plasma cells. BAAF excess rescues self-reactive B-cells from anergy, contributing to autoimmunity. Matsushita et al. examined BAAF levels in the serum of 21 SSc patients (both diffuse and limited subsets) and related the results to clinical features of the patients. Serum BAAF levels were significantly higher in patients with SSc when compared with healthy controls [median 1.26 ng/mL (0.32 − 1.37) vs 0.78 ng/mL (0.39.1.37), respectively, $p < 0.001$], and patients with dcSSc had higher levels than lcSSc ($p < 0.05$). BAAF levels in SSc correlated positively with mRSS ($r = 0.415$, $p < 0.005$). A longitudinal study of BAAF levels for 6 years classified SSc patients as follows: 7 patients had decreased BAAF levels, 11 had unchanged levels and 3 had increased levels. Decreased BAAF levels were associated with a significant decrease in mRSS from baseline to 2 years (36%, $p < 0.05$), 4 years (45%, $p < 0.05$) and 6 years (54%, $p < 0.05$). In the three patients in whom BAAF levels remained high, there was no change in mRSS, and new onset/worsening of internal organ involvement (renal crisis and deterioration of ILD) was documented. In the group in whom BAAF levels remained unchanged, mRSS tended to decrease, but it was not statistically significant [57].

The levels of BAAF receptor were also higher in SSc compared to controls (mean 81 ± 40 vs 43 ± 7, $p < 0.05$). Moreover, BAAF mRNA expression in affected skin from SSc patients was significantly upregulated in early SSc patients (disease duration <3 years) compared with late SSc patients (>6 years disease duration) and normal controls ($p < 0.005$ and $p < 0.0001$, respectively). The expression of BAAF mRNA in the skin of late SSC and normal controls was not significantly different. To evaluate the role of BAAF in B-cell function of SSc, B-cells

were stimulated with BAAF and the amounts of IL-6 and IgG were determined. SSc B-cells produced 38% more IL-6 and 35% more IgG than B-cells from healthy controls [57].

B-cell depletion has been proven to be effective in reversing skin fibrosis both in animal models of SSc and in small case series and clinical trials. Studies in the tsk mouse model showed that B-cell depletion by an anti-CD20 antibody inhibited the development of autoimmunity and skin fibrosis when initiated early in the course of disease. In a study by Hasegawa et al., B-cell depletion using an anti-mouse CD20 monoclonal antibody before (at day 3 after birth) and after disease development (day 56) resulted in reduced skin fibrosis, decreased autoantibody generation and decreased levels of hypergammaglobulinemia in new born mice, but not in adult mice with established disease [58].

Rituximab is a chimeric anti-CD20 monoclonal antibody largely used in rheumatology for the treatment of RA, SLE and more recently for Anti-neutrophil cytoplasmic antibody (ANCA)-positive vasculitis [7]. In an open-label trial, Bosello et al. treated nine dcSSc patients with rituximab, and analysed long-term safety, skin score and serum levels of IL-6. After treatment, at 6 months, there was an improvement in mRSS from 21.1 ± 9.0 to 12.0 ± 6.1 ($p = 0.001$), with a median decrease of 43.3% (range from 21.1 to 64.0%), a parallel fall in IL-6 serum levels (from 3.7 ± 5.3 to 0.6 ± 0.9 pg/mL, $p = 0.02$) and an improvement in the Health Assessment Questionnaire (HAQ) score (from 0.9 ± 0.7 to 0.4 ± 0.5, $p = 0.01$). The FVC and DLCO levels showed no significant difference at follow-up compared with baseline. Nevertheless, none of the patients had a clinically significant reduction in FVC values (>10%). Moreover, none of the patients had new or progressive cardiac involvement, renal crisis or symptoms suggesting progressive gastrointestinal disease [59].

Smith et al. obtained similar results in a 24-week open-label clinical trial in eight dcSSc patients. Rituximab induced effective B-cell depletion in all patients and improved the skin score from 24.8 ± 3.4 to 14.3 ± 3.5 ($p < 0.001$) at 24 weeks. Parameters of internal organ involvement remained stable, namely lung function tests. Histopathological study of the skin revealed a decrease in the mean hyalinised collagen score from 60 ± 6.5 to 7.1 ± 7.2 ($p < 0.0001$) and abolished myofibroblast positivity [4/7 vs 0/8, (χ^2 test $p = 0.013$)] [60].

In another small pilot study, Lafyatis et al. assessed rituximab safety, clinical efficacy and resulting skin B-cell and autoantibody depletion in 15 patients with early dcSSc (less than 18 months prior to trial entry). The mRSS did not change significantly between baseline and 6 months. Similarly, both predicted FVC and DLCO remained unchanged at 6 months after treatment. Nevertheless, none of the patients showed evidence of progression of major organ involvement. Circulating B-cell depletion was achieved in all patients at 3 months, with recovery at 6–12 months. Also, most patients showed complete depletion of skin B-cells, with an average quantification per specimen at baseline of 10.4, compared with 3.4 at 6 months. Autoantibody levels declined modestly but the changes were not consistent during follow-up [61].

In a large observational case-control series of 63 SSc patients (both diffuse and limited) from the EUSTAR cohort, mRSS decreased significantly from 18.1 ± 1.6 to 14.4 ± 1.5 ($p = 0.0002$) and there was also a significant decrease in the mean percentage of change from baseline of −15.0 ± 5.3% ($p = 0.008$) on follow-up at 7 months. Furthermore, in patients with SSc-related

ILD, FVC was stable after treatment, compared with baseline (60 ± 2.4 vs 61.3 ± 4.1%, $p = 0.5$), whereas DLCO significantly improved (41.1 ± 2.8 vs 44.8 ± 2.7%, $p = 0.03$). In contrast, matched controls from the EUSTAR database showed a decline in FVC in both the mean percentage of predicted value ($p = 0.02$) and in absolute change ($p = 0.01$) [62].

Focusing on lung fibrosis, Daoussis et al. studied eight dcSSc patients with ILD treated with rituximab, during a 2-year follow-up [63]. Similarly to previous results from a smaller scale study from the same group [64], there was a significant increase from baseline in both FVC (mean 68.13 ± 19.69 vs 75 ± 19.73, $p = 0.0018$) and DLCO (mean 52.25 ± 20.71 vs 62 ± 23.21, $p = 0.017$), reinforcing the benefits of rituximab treatment in SSc-associated ILD. In a larger population of 30 patients, the same group performed a 7-year follow-up open-label multicentre study, with results supporting the benefits of continuous rituximab treatment for skin involvement and SSc-related ILD. Patients were treated at baseline, 6, 12 and 18 months. Both FVC and DLCO improved after 2 years (mean FVC: 86.2 ± 5.5 vs 78.2 ± 3.6, $p = 0.018$; mean DLCO: 62.4 ± 4.5 vs 57.3 ± 3.2, $p = 0.012$, treatment vs baseline, respectively). There was a trend towards stabilisation after 2 years of treatment (mean FVC 84.3 ± 6.5 at 5 years, $p = 0.04$). However, in three patients who had an initial improvement or stabilisation of lung function with continuous treatment, there was deterioration in the lung function tests after 3 years of rituximab cessation and they did not respond to retreatment. Corroborating previous results, an early improvement in mRSS was also achieved at all time-points compared with baseline ($p < 0.001$) [65].

Considering the role of B-cells in SSc and positive results associated with its depletion, several clinical trials are on-going with rituximab and belimumab, an anti-B-lymphocyte stimulator monoclonal antibody used in patients with SLE, but no preliminary results are yet available [66].

In the study Rituximab in Systemic Sclerosis (RECOVER) the primary outcome is the treatment of polyarthritis (through analogy with rheumatoid arthritis), and the secondary outcomes are improvements in mRSS, lung function tests and quality of life [67].

Another on-going trial aims to validate an infusion protocol-based treatment for early diffuse SSc with rituximab, using a dose of 1000 mg intravenously, at day 1 and 15 and then at week 26–28, together with a corticosteroid regimen consisting of intravenous methylprednisolone 100 mg, 30 minutes prior to both infusions. Clinical variables will be assessed after 28 weeks, namely survival rate, presence of heart and lung failure and antibody titres [68].

Based on pre-clinical data and anecdotal reports, which suggest that modulation of the immune system may be an effective strategy for treating pulmonary arterial hypertension in SSc, patient recruitment is currently underway for a randomised double-blinded placebo-controlled trial (phase II) to evaluate the effects of rituximab on this severe manifestation of the disease. The primary outcome is the change in pulmonary vascular resistance measured by right heart catheterisation, assessed at week 24. Patients will still receive concurrent stable-dose standard therapy with a prostanoid, endothelin receptor antagonist and/or phosphodiesterase-5 inhibitor. In a sub-study, cardiac magnetic resonance will be used to evaluate changes in right ventricular end diastolic volume index and stroke volume, as measures of right ventricular function [69].

4. Conclusions

The interplay of vasculopathy, fibrosis, inflammation and autoimmunity makes SSc a complex disease. The growing understanding of its pathogenesis has increased the identification of potential targets, and thereby the use of specific therapies such as biological agents. Although most studies and clinical trials are restricted to a subgroup of SSc patients, mainly with the early diffuse cutaneous subset, the results are encouraging for some biologicals, especially B-cell depleting agents and anti-IL6 drugs for skin and lung involvement.

While tocilizumab has shown benefits in reducing skin fibrosis and halting progression of interstitial lung disease, data on rituximab are more consistent in relation to improvement of lung fibrosis, at least in early disease stages, although the benefits in later phases are not clear.

Apart from anti-IL6 antagonists, other biologicals targeting cytokines are not yet valuable options for SSc treatment. All three cytokines, IL-17, IL-13 and IL-1, are relevant in fibrosis mechanisms, but the clinical utility of their antagonists in SSc is still unknown. Anti-TNF-α therapy can be beneficial in a highly selected subgroup of patients with inflammatory arthritis overlap, although its use is not recommended for SSc, due to concerns related to worsening interstitial lung disease.

Other therapeutic approaches such as T-cell target therapy (abatacept) have had good results, although limited to skin fibrosis and arthritis, and without any proven benefits for SSc organ-based complications. Additionally, despite the key role of TGF-β in the pathogenesis of SSc, clinical use of TGF-β antagonists did not show any benefits regarding internal organ involvement, and there were serious treatment-related adverse events.

Although some of the results from clinical trials in SSc are compelling, primary outcomes mostly address only skin fibrosis and lung disease, probably reflecting the lack of validated internal organ assessment outcome measures, especially for gastrointestinal and cardiac complications. Nevertheless, randomised clinical trials are necessary to validate new therapies as the standard of care in clinical practice. Hence, apart from better insights into disease mechanisms, which will help to identify new therapeutic targets, improvement in clinical trial design, with better cohort definition and updating of traditional clinical end-point assessment, will provide more robust results to inform the clinical applicability of biological therapies in SSc.

Based on the complexity of SSc pathogenesis and early indicators from the studies up to date, it seems likely that future treatment approaches may be built around targeting multiple pathways simultaneously. This may means combined therapy using both biologicals and small molecule inhibitors, such as tyrosine kinases, although issues related to adverse events and cost would have to be addressed.

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