Review

Targeted immunotherapies in systemic sclerosis

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ABSTRACT

Systemic sclerosis (SSc) is a heterogeneous systemic disorder characterised by alterations of the microvasculature, disturbances of the immune system and massive deposition of collagen and other matrix substances in connective tissue. Recent genetic studies have underlined the importance of the autoimmune component of the disease. Biologic therapies target molecules involved in the mechanisms of the immune system, such as cytokines (TNF-α, IL-6), immune cells (B cells) or co-stimulation molecules (CTLA4), and are currently used in several autoimmune rheumatic diseases, in particular rheumatoid arthritis. These drugs provide an alternative to the existing treatment methods of disease-modifying anti-rheumatic drugs and other immunosuppressive medications.

Since some of the molecules targeted by biologic therapies are known to contribute to fibrosis in vitro or in animal models of experimental fibrosis, and considering that preliminary data are now available regarding the efficacy and safety of targeted immunotherapies in SSc, we aim to report with this review the results obtained in animals and humans for the biotherapies that have already been developed.

Introduction

Systemic sclerosis (SSc) is an orphan disease that is a member of the group of connective tissue diseases. It is a generalised ailment of the conjunctive tissue characterised by polyvisceral involvement. This disease distinguishes itself from other connective tissue diseases owing to an original pathologic process: it is marked by microvascular anomalies, initially functional and partly reversible, an inflammation with autoimmune participation which appears to be moderate and perhaps transitory, then fibroblastic activation leading to fibrosis.

SSc is a complex multifactorial disease with an implication of multiple players in its pathogenesis. It has been established that environmental factors are associated with the risk of contracting the disease. At the same time, convergent data have shown a genetic susceptibility. Candidate-gene or whole-genome genetic approaches have identified robust susceptibility factors that mostly belong to both innate and adaptive immunity regulation pathways (1). The presence of antinuclear autoantibodies (Auto-ab), some of which are specific to the disease, reinforces an autoimmune component of the disease. The immune character of SSc involves both T and B cells. Thus, one can observe a trans-endothelial migration of T cells with a phenotype that is activated on SSc cutaneous lesions. The major role of the T cell in SSc genesis is emphasised i) by the restriction of the T cells TCR repertory present in the cutaneous lesions of local oligoclonal T cell expansion, ii) the correlation observed between the activity/severity of the diffuse form of SSc and the serum level of soluble CTLA-4. The role of the B cells also appears to be important: involved in Auto-ab synthesis, the B cell plays a pivotal role in the physiopathology of cutaneous lesions and therefore in the murine model of SSc. Depletion in B cells reduces cutaneous fibrosis as well as the presence of Auto-ab. However, the pathogenicity of Auto-ab has not been demonstrated and recent results have shown that the weak or even negative effects of cyclophosphamide on this disease argue against the autoimmune character of the disease.

Moreover, on an individual and family level, there appears to be an aggregation of autoimmune pathologies during SSc with, in particular, an overrepresentation of diseases such as autoimmune thyroiditis, Sjögren's syndrome or primitive biliary cirrhosis.

All of these arguments suggest that the new treatments developed for diseases with autoimmune components and targeting immune pathways could be pertinent in this ailment. We propose to report the results obtained in animal and man for the biotherapies that have already been developed.

Animal models

Anti-TNF-α and systemic sclerosis experimental models

TNF-α is a proinflammatory cytokine produced by a great variety of cells such as macrophages, TCD4^{+} and CD8^{+} cells, NK and B cells, neutrophil polynuclear cells, endothelial cells and fibroblasts. This cytokine binds to two high-affinity receptors (TNFR-I and TNFR-II). The effects of TNF-α on the development of fibrosis are controversial. Studies initially showed that TNF-α inhibited *in vitro* the synthesis of types I and III collagen messenger RNA as well as fibronectin in cultured fibroblasts (2). TNF-α could also play an important part in the inhibitor role of T CD4^{+} cells on the production of collagen by dermal fibroblasts. This TNF-α inhibitor role could in part be mediated by NF-κB: the use of deficient fibroblasts for NEMO, an essential NF-κB modulator, prevents TNF-inhibitor effects on RNAm synthesis of COL1A2 (3). Similar results have been obtained by transfecting fibroblasts with a dominant negative IKKα. In addition to its direct inhibitor effect on collagen synthesis, TNF-α could also play an indirect role by interfering with the TGF-β signalling pathway. TNF-α could inhibit the signalling cascade of Smad proteins. This inhibition would be independent of Smad7 but rather would occur through the implementation of AP-1 transcription factor with TNF-α, a powerful inducer of c-Jun and JunB, two members of the AP1 family in the dermal fibroblasts. These proteins would form heterocomplexes with Smad3, a phenomenon that would reduce its binding to its specific response elements. Finally, TNF-α regulates the expression of enzymes degrading the extracellular matrix (ECM) and its inhibitors. In a dose-dependent manner, TNF-α induces RNAm and MMP-1 protein synthesis (matrix metalloproteinase-1), in particular, in dermal fibroblasts (4). Moreover, strong concentrations of TNF-α reduce the expression of TIMP-1 metalloproteinase inhibitor, favouring the degradation of matrix proteins and therefore reducing their accumulation (5).

In contrast with the above data, other studies have suggested a profibrotic role of TNF-α on murine intestinal myofibroblasts (6). An increase in collagen synthesis and a reduction in MMP-2 activity have been observed in these cells. These effects would be mediated by the type II TNF-α receptor (TNFRII) and could suggest tissue specificity regarding the effects of TNF-α. Another TNF-α profibrotic mechanism could be through the indirect induction of TGF-β: incubation of a fibroblastic line (3T3 cells) with TNF-α induced TGF-β mRNA and protein synthesis. This TGF-β induction by TNF-α was blocked by specific inhibitors of ERK (7). Nevertheless, the effects of this TGF-β induction on collagen synthesis have not been studied and it is not certain that this TGF-β induction is sufficient to overcome the inhibitor effect of TNF-α on collagen synthesis. Overall, this TGF-β induction by TNF-α could represent a mechanism that compensates for the inhibition of Smad signalling by TNF-α. TNF-α could also inhibit collagen phagocytosis by fibroblasts and could therefore increase its accumulation (8, 9). Animal studies appear to more suggest a profibrotic role for TNF-α. The use of TNF-α antagonists or deficient mice for TNF-α receptor led to the prevention of dermal and pulmonary fibrosis. These antifibrotic effects were particularly observed in murine models of dermal and pulmonary fibrosis induced by bleomycin, reflecting the early inflammatory stages of SSc. An increase in serum and cutaneous concentrations of TNF-α was observed in mice treated with subcutaneous bleomycin compared with mice injected with NaCl (10, 11). A study reported the effects of etanercept in the mouse model of bleomycin-induced dermal fibrosis (11). In that study, in addition to daily subcutaneous injections of bleomycin (10μg) or PBS, the mice received intraperitoneal injections of etanercept (100 μg 3 times per week) or PBS. Etanercept injections provided a significant reduction in dermal thickness, local production of collagen, estimated by the amount of hydroxyproline as well as the number of myofibroblasts, quantified by immunohistochemistry after staining for α-smooth muscle actin (αSMA). A trend towards a reduction in proinflammatory cytokine serum concentrations (TNF-α and interleukine-6 [IL-6]) was also observed. In the same manner, other studies have reported that the use of TNF-α antagonists (antibody perfusion neutralising TNF-α or the use of recombinant soluble TNFRI) can reduce ECM accumulation in the pulmonary fibrosis model induced by bleomycin (12, 13).

There is therefore discordance between the data obtained *in vitro*, more in favour of an antifibrotic effect by TNF-α, and animal studies in which TNF-α inhibition led to prevention of fibrosis, particularly in the fibrosis model induced by bleomycin. The difference between *in vitro* and *in vivo* data may be explained by the high inflammatory component of this murine model. It is therefore possible that the role of TNF-α in the inflammatory response induced by bleomycin that led to fibrosis exceeded the direct antifibrotic effect by TNF-α on the fibroblasts. Thus, it is difficult to make conclusions given these different results vis-à-vis the exact role of TNF-α in fibrosis. The clinical results appear more in support of an antifibrotic role for TNF-α following the description of cases reported on the progression of fibrosis under anti-TNF-α (14).

B cells, anti-CD20 and systemic sclerosis experimental models

The role of B cells and the effects of their depletion have especially been studied in the tight-skin mouse model (tsk-1), reflecting the late stages of SSc. This SSc murine model is characterised by extensive dermal and hypodermal fibrosis, independent of inflammatory processes or reactions, provoked by endogenous fibroblast activation, which produce an exaggerated quantity of collagen and matrix proteins. This model is also characterised by immunologic anomalies including the

production of different Auto-ab that are specific or not specific to SSc, such as those directed against topoisomerase-I (15). The potential role of B cells has particularly been studied in this model. B cells from tsk-1 mouse have an activated profile that is characterised by a cascade of intracellular signalling mediated by CD19 which is absent in B cells from pa/pa control mice (16). In fact, an increase of 45% in CD19 phosphorylation has been observed in tsk-1 mouse B cells compared with control lymphocytes. The cytoplasmic calcium response, generated by CD19 binding, was also increased in tsk-1 mouse B cells. Inversely, CD19 invalidation made it possible to eliminate the B cells hyperactivated phenotype and significantly reduce dermal fibrosis.

Another study used transgenic mice for C19 and studied the activation profile of B cells (17). In these mice, there was a significant increase in CD19 phosphorylation and increased production of Auto-ab and in particular, of anti-topoisomerase I (an increase of 7.9 to 20 times compared with that of the control mice). Nevertheless, despite B cell activation, dermal and hypodermal fibrosis was not increased compared with that of the control mice. Parallel to the increase of CD19 phosphorylation, a reduction was observed in CD22 phosphorylation, a negative regulator of B receptor (BCR) reducing the activation of B cells. Antibodies neutralising CD22 were also detected in tsk-1 mice (18). Tsk-1 mouse B cells were therefore producing Auto-ab that favoured their own activation.

Thus, there appears to be a link between B cell activation and the development of fibrosis in this model. In order to confirm this, 3-day-old tsk-1 mice were treated with rituximab, enabling B-cells depletion (19). This depletion caused a 43% reduction in cutaneous fibrosis compared with the control mice, associated with a significant reduction in Auto-ab levels. On the other hand, the same treatment applied to 56-day-old mice with established fibrosis did not obtain a significant reduction in cutaneous thickness. These data suggest that role of lymphocytes is more important in the early phase of the disease but less

so at the stage of established fibrosis. This contribution to the development of cutaneous fibrosis appears to be indirect given the absence of B cells in the skin of tsk-1 mice.

Interesting preliminary results have also been obtained with the use of B cells survival factor (BLyS/BAFF), which improved the cutaneous fibrosis and reduced Auto-ab production (20). Overall, these results therefore suggest that B cells have a key role in the fibrotic process of this animal model.

The role of B cells has also been studied in a fibrosis model induced by bleomycin. Subcutaneous administration of bleomycin in mice invalidated for CD19 led to a reduction in their dermal thickness compared with mice expressing CD19 and injected with bleomycin. Similar results were obtained in a pulmonary fibrosis model induced by bleomycin with histologic reduction of fibrotic lesions (21). It is of interest to note that CD19 expression correlated with the number of B cells in the bronchoalveolar lavage (BAL): in particular, CD19 invalidation inhibited the accumulation of B cells in the BAL. B cells therefore appeared to also play a role in this more inflammatory model.

Interleukin-6 (IL-6) and systemic sclerosis experimental models

Interleukin-6 (IL-6) is a protein that is implicated in the acute phase of inflammation. In particular, it stimulates the hepatic synthesis of proteins in the acute phase of inflammation such as the C-reactive protein (CRP). Data in the literature suggest that IL-6 is involved in SSc and fibrosis (22). First of all, there is an increase in serum concentrations and IL-6 protein expression in the damaged skin of patients with SSc, in particular, in the endothelial cells and dermal fibroblasts. This increase in IL-6 production could be induced by the IL-1α secreted by the dermal fibroblasts (23). The dermal fibroblasts in patients with SSc secrete up to 30 times more IL-6 than the dermal fibroblasts in healthy controls (24). IL-6 is also at the origin of the excessive production of matrix proteins, in particular, collagen, and a proliferation of sclerodermal fibroblasts (25). These properties would

be the result of fibroblast autocrine regulation by IL-6 (26). Moreover, inhibition of the response to IL-6 by the antibodies neutralising this cytokine lead to a reduction in collagen synthesis by the dermal fibroblasts (23).

In vivo data have confirmed these results. There is an increase in IL-6 expression in the dermal fibrosis models induced by bleomycin and tsk-1 mice (10, 27).

Several studies have also shown the efficacy of various IL-6 inhibition strategies by passive or active immunisation in the dermal fibrosis induced by inflammation. Two studies, including one performed by our team, showed that administration of an antibody blocking the IL-6 receptor (MR16-1) prevented the development of dermal fibrosis induced by bleomycin (25, Desallais et al., manuscript submitted). In the mice with subcutaneous injections of bleomycin, MR16-1 treatment significantly reduced their dermal thickness compared with that of mice with injections of bleomycin coupled with the administration of a control antibody. A reduction both in the local production of collagen in the dermis and in the number of myofibroblasts infiltrating the dermis was also observed in the mice treated with MR16-1 (27). Similar results were obtained with MR16-1 in a murine model of a graft-versus-host reaction, another fibrosis model induced by an inflammatory process (28). On the other hand, MR16-1 treatment did not prevent the development of fibrosis in the tsk-1 mouse model, which is characteristic of the late and non-inflammatory phases of the disease (Desallais *et al.*, manuscript submitted). Since the use of anticytokine monoclonal antibodies has several disadvantages including primary and secondary resistance phenomena, we also tested an innovative alternative approach by active immunisation against a peptide derived from a murine IL-6 fibrosis model induced by bleomycin and compared its effects with those of MR16-1. This active immunisation procedure had antifibrotic effects close to those observed with MR16-1 on the reduction of dermal thickness, local production of collagen and the number of myofibroblasts infiltrating

the dermis. Together, these results demonstrated the interest of targeting IL-6 in SSc. The transfer of these results to man is awaited and given the results obtained in the fibrosis models induced by bleomycin and tsk-1, it would appear to be pertinent as a priority to first target patients in the early and inflammatory phase of the disease (29). Active anti-IL-6 immunisation also appears to be a promising strategy which could lead to original and innovative therapeutic perspectives for SSc.

The role of IL-6 has also been recently suggested by the development of a new SSc murine model, which appears to be IL-6 dependent. This induced model is based on subcutaneous injections of recombinant topoisomerase associated with Freund's complete adjuvant. These injections result in the development of extensive dermal fibrosis associated with pulmonary fibrosis and the presence of anti-topoisomerase-I antibodies (30). Large concentrations of IL-6, TGF-β1 and IL-17 have been observed in this model. The inhibition of IL-6 production led in this model to an improvement in pulmonary and dermal fibrosis lesions, in association with a reduction in the number of TH2 and TH17 cells, suggesting that IL-6 plays a key role in the immunologic anomalies of this model.

All of these data obtained *in vitro* and in SSc animal models therefore indicate a potential link between TNF-α, B cells, IL-6 and fibrosis. However, SSc is a complex disease that is only partially reproduced by these models and the previously reported results therefore cannot be extrapolated to the human disease.

Imatinib and systemic sclerosis experimental models
Imatinib is a tyrosine kinase inhibitor with demonstrated activity against c-Abl, c-kit and the PDGF receptor (PDGFR). Imatinib also targets TGF-β through Abelson tyrosine kinase (c-Abl), which is an important molecule downstream in the TGF-β pathway. In fact, the production of matrix proteins induced by TGB-β is significantly diminished in cells deficient in c-Abl. In a dose-dependent manner, imatinib in-

hibits collagen and fibronectin synthesis through the dermal fibroblasts in patients with SSc (31). Imatinib treatment results in dramatic changes in the expression of genes involved in fibrosis, cardiovascular disease, inflammation, and lipid and cholesterol metabolism in SSc fibroblasts but has only modest effects in control fibroblasts (32). Imatinib has also demonstrated antifibrotic properties *in vivo* by preventing the development of experimental dermal fibrosis induced by bleomycin and the development of hypodermal fibrosis in the tsk-1 mouse model (33). At doses of 50 mg/kg/day and 150 mg/kg/day, imatinib inhibited fibroblast differentiation in myofibroblasts and significantly reduced the synthesis and accumulation of extracellular matrix in damaged mouse skin. Imatinib has also shown preventive efficacy in other preclinical fibrosis models, in particular, pulmonary, renal and hepatic fibrosis (34).

In addition to its preventive action, imatinib can also induce a regression in established dermal fibrosis induced by bleomycin (33). In this model, the mice received subcutaneous injections of bleomycin for 6 weeks and were treated in parallel with imatinib during the last 3 weeks. The use of imatinib in this model not only stopped the progression of fibrosis, but it also induced a regression of dermal fibrosis at a lower level than that observed after the first 3 weeks of treatment with bleomycin.

Other Abl tyrosine kinase and PDGF receptor inhibitors such as dasatinib and nilotinib have demonstrated antifibrotic in vitro and in vivo and could therefore appear to be interesting candidates for the treatment of fibrosis (30).

The first human experiments
Anti-TNF-α
Trials of certain anti-TNF-α on SSc stem from the observation of certain forms of SSc that overlap with connective tissue diseases which respond to anti-TNF-α with, in particular, articular involvement. In addition, anti-TNF-α have been beneficial in certain inflammatory bowel diseases which imply, like SSc, a fibrosis and healing process following inflammatory stimuli. Pulmonary data from animal studies have

also suggested the key role of TNF-α. However, *in vitro*, other aspects are in contrast with the hypotheses cited above because TNF-α may negatively regulate profibrotic factors and act directly on some proteolytic enzymes. There have also been observations of immune activation in some diseases including, for example, multiple sclerosis under anti-TNF-α treatment.

Eighteen patients with an active articular form (18 women, mean age: 44 years) were treated with etanercept 50 mg/week for 2 to 66 months (mean: 30 weeks) (35). Three patients had positive anti-RNP antibodies, 8 had positive rheumatoid factors and 3 had positive anti-CCP antibodies. Concomitant treatments included NSAIDs (18 patients), methotrexate (15 patients, mean dose: 12 mg/week), low-dose prednisone (9 patients) and hydroxychlroroquine (5 patients). In that open study, 15 out of 18 patients (83%) were considered as responders with a decrease in inflammatory articular signs. The mean HAQ score decreased from 1.08±0.70 to 0.74±0.56 (*p*=0.13). Otherwise, the Rodnan skin score decreased from 6.63±6.35 to 3.94±2.38 (*p*=0.12). As for pulmonary tests, the DLCO value decreased by 5.1% (IC95% -10.4 to +0.18) and forced capacity by -1.4% (IC95% -5.8 to +2.9) which resembles the natural evolution of the disease. Finally, there were no opportunistic infections or deaths attributed to treatment. One patient discontinued treatment following a lupus-like reaction and another patient stopped treatment owing to a strong progression in interstitial pulmonary involvement.

Infliximab administration was evaluated in a 26-week open study on 16 patients with diffuse cutaneous forms receiving 5 perfusions at 5 mg/kg (36). They were patients with recent progression of cutaneous involvement. Exclusion criteria were neoplastic or infectious episodes, the use of DMARDS or cardiac (LVEF <50%) or severe pulmonary involvement (FVC <55%). Mean age was 48 years and duration of the disease was 16±20 months. No change in cutaneous fibrosis was observed: a median value of 22 (range: 6–48) at week 26 *versus* 26 (range: 11–45) at the beginning. Seven patients discontinued treatment

following severe allergic reactions. Exploratory serum doses have suggested a moderate decrease in products derived from collagen III and collagen I. A dermal marker study of TGF-β on cutaneous biopsies did not reveal clear change during the treatment phase.

While the effects on the parameters studied and the tested methodologies appear to be modest, it should be noted that cases of worsening pulmonary fibrosis like that have been observed elsewhere in rheumatoid polyarthritis complicated by pulmonary fibrosis which should call for caution in the use of these molecules for SSc (14, 37). Questions also remain open on the long-term tolerance of anti-TNF-α in SSc (38). Following a survey on clinical practice, the EUSTAR group recommended not using these products outside of possible clinical trials (39).

Anti-CD20

Rituximab is a chimeric monoclonal anti-CD20 antibody found on mature B cells and but not on plasmocytes. It is a glycosylated immunoglobulin associating, on one hand, the constant regions of human IgG1 and, on the other hand, the variable regions of light and heavy chains of murine origin. In association with methotrexate, it is indicated for the treatment of active severe rheumatoid arthritis in adult patients who have presented an inadequate response or intolerance to disease-modifying therapies including at least an anti-TNF-α. In this indication, it has demonstrated symptomatic and structural effects limiting the progression or articular erosion. Its tolerance profile is satisfactory – close to that of other biotherapies. By extension, it has also been evaluated for disseminated erythematous lupus. While phase II trials have revealed good tolerance and encouraging clinical results, phase III trials have not confirmed its place in this disease, be it a cutaneo-articular approach or targeting renal involvement. Just as for anti-TNF-α, the existence of an overlap syndrome, the genetic proximity of various autoimmune diseases and finally, preclinical data have led to phase II trials in systemic sclerosis. The results are shown in Table I (40-44).

A EUSTAR group observational study tends to confirm these promising data. The methodology consists of a comparison between patients receiving RTX as standard care with patients included in the group's database and not receiving this molecule. From 25 patients treated with rituximab, a greater reduction in skin score was observed after 6 months of follow-up. The variation was from 26.6±1.4 to 20.3+1.8 in the rituximab group, which corresponded to a variation of -24.0±5.2% *versus* -7.5±4.3 (p=0.02) in the control group (paired for age, gender, duration of the disease). Encouraging data were also observed for pulmonary involvement (45, 46).

Overall, these results point to the need for a randomised study *versus* placebo in order to analyse both the dermatologic and pulmonary effects in a context

Table I. Results of available trials regarding the efficacy and safety of rituximab in systemic sclerosis.

Smith *et al*. (40)	n=8 9-30 months of disease duration mostly diffuse cutaneous SSc	1000 mg RTX at days 1 and 15 + 100 mg methylprednisolone 5/8 + MTX	At week 24, reduction of skin score from 24.8 (3.4) to 14.3 (3.5) (40% improvement)	SSc-DAS score at 6 months: 4.5 (1.5-7.5) to 1.0 (0.0-2.0) HAQ-DI at 6 months: 1.3 (0.8-2.1) to 1.1 (0.3-2.1)	2 serious side effects not related to RTX (one myocardial infarction and one unexplained fever)
(41)		New treatment at week 26 and 28	Stability of Rodnan skin score at 2 years: 13.8 (5.6)	Stability of SSc-DAS score at 2 years: 2.1 (0.0-5.5) Stability of HAQ-DI at 2 years: 1.3 (0.4-2.0)	At 2 years, 3 additional serious side effects (one infection of digital ulcer, one sepsis and one episode of hyperventilation)
Lafyatis *et al*. (42)	n=15 dcSSc 1st symptoms <18 months	1000 mg RTX at days 1 and 15	No change of the skin score at 6 months: -0.37 (-14.5; +14) (20.6 to 20.2)	No change of DLCO (79.7±8.3 to 77.8±7.5), FVC (89.2±10.8 to 92.7±10.3) and HAQ : 0.67±0.32 to 0.64±0.36	Infusion reaction: 47% 1 urinary tract infection 1 dental abscess 1 prostatic cancer
Bosello *et al*. (43)	n=9 diffuse cutaneous SSc Cutaneous progression despite CYC Disease duration: 49±73 months	1000 mg RTX at days 1 and 15 + 100 mg methylprednisolone 2/9 + MTX	Skin score: 21.1±9 to 12.0 ±6.1 (6 months) and 7.0±4.0 (12 months) (global improvement 57%)	SSc-DAS 10.5±3.2 to 7.2±2.8 (6 months) and 6.2±2.8 (12 months) SSc-HAQ: 0.9±0.7 to 0.4±0.5 (6 months) and 0.3±0.7 (12 months) No change of DLCO and FVC	1 breast cancer
Daoussis *et al*. (44)	n=14 dcSSc Disease duration >5 years Topoisomerase-I antibodies + and presence of ILD	Randomisation 8 *vs.* 6 No placebo 4 X 375 mg/m2/s at inclusion and at 6 months	Improvement of 1/ FVC: + 10.2% *vs.* decrease of 5.0% in the control group 2/ DLCO: + 19.5% vs. decrease of 7.5% in the control group 3/ skin score: 39.2% decrease vs. 20.1% decrease in the control group	HAQ: 0.69 (0.3-1.25) to 0.31 (0.12-0.69) at 12 months in the RTX group vs. non significant change in the control group 0.31 (0.1-0.9) to 0.125 (0.1-0.4)	1 infection requesting IV antibiotics

that appears to be reassuring for safe use. A French phase III study versus placebo called RECOVER is currently being performed. It is evaluating the efficacy of rituximab for SSc inflammatory articular involvement (principal judgment criterion) and will also provide more precise information on the dermatologic and pulmonary effects of this treatment.

Interleukin 6
In spite of the abundance of i*n vitro* and animal study data, few data are available concerning interleukin 6 targeting. An open study reported favourable evolution of the skin in 2 cases (47). The EUSTAR group collected observations that revealed a tendency toward articular improvement in cases of polyarthritis under tocilizumab during refractory SSc without, however, favourable signs of dermatologic or pulmonary involvement but with a rather weak decline. There was decrease in the Disease Activity Score (DAS)-28 of 1.8 ± 1.5 (2.8 ± 0.6 before the last perfusion administered at 6 months *versus* 4.6 ± 1.0 at the beginning, $p=0.02$) with a decrease in the painful joint count of 5 (4.2 ± 4.3 *vs*. 9.2 ± 8.6) and in the swollen joint count of 0.5 (3.0 ± 4.4 *vs*. 3.5 ± 3). A good EULAR response was observed in 3 out of 5 patients. On the other hand, neither the Rodnan score (12 ± 9.6 *vs*. 13.7 ± 7.8, $p=0.6$) nor the HAQ score (1.2 ± 0.4 *vs*. 1.7 ± 0.9, $p=0.8$), nor the spirometric test results were significantly influenced. There were no signs of poor tolerance (48). A phase III study called FaSScinate is currently being performed. It is evaluating the efficacy of tocilizumab on SSc cutaneous involvement in recent and evolving diffuse cutaneous form.

Imatinib
Promising results with imatinib in preclinical pulmonary and dermal models have led to trials of this molecule in man. The first open trial performed by an American team studied the tolerance and efficacy of 400 mg/day of imatinib in 30 patients with a diffuse cutaneous form of SSc (49). Twenty-four patients completed the 12 months of treatment. In terms of tolerance, 358 side effects

were recorded during the study period including 24 severe side effects (2 linked to the treatment) and 171 side effects (73% grade 1, 25% grade 2 and 2% grade 3) linked to imatinib. The most frequent side effect was oedema which occurred in 80% of the patients. Treatment with furosemide was necessary in 60% of the patients presenting oedema. As for efficacy, the Rodnan score decreased by 6.6 points (22.4%) in 12 months ($p=0.001$) and forced vital capacity improved by 6.4% ($p=0.008$). The DLCO and quality-of-life scores remained stable. Histologic analysis of cutaneous biopsies before and after treatment showed a decrease in dermal thickness and an improvement in cutaneous morphology during treatment. Unfortunately, these first results were not confirmed in two randomised trials versus placebo. The first Canadian trial performed over 6 months in patients with a diffuse cutaneous form of SSc was discontinued after inclusion of 10 patients due to poor tolerance of the treatment. The majority of side effects observed (oedema, nausea diarrhoea, cramps, fatigue, anaemia) occurred during the first week of treatment and reappeared during reintroduction of the treatment despite a 50% reduction in the dosage (200 mg/day instead of 400 mg/day) (50). Given the small number of patients included in this trial, no conclusion on imatinib efficacy could be made. A French multicentre phase II double-blind randomised trial also compared imatinib with a placebo (51). Its principal aim was to evaluate dermal fibrosis measured by modified Rodnan skin score. This trial included 28 patients (25 patients with SSc and 3 with morphea lesions on more than 20% of their body surface). At 6 months, no difference was noted in skin score between the active and placebo groups. In the same manner, no difference was noted for secondary outcomes including skin thickness estimated on cutaneous biopsies, quality-of-life and DLCO/AV. Imatinib tolerance was also in question since 4 patients in the active group (versus only 1 in the placebo group) had to discontinue treatment owing to side effects. Side effects were more frequent in the imatinib group (n

= 53) than in the placebo group (n = 39) with, in particular, a greater incidence of oedema in the active group (17% *vs*. 5%). New randomised trials with greater power are awaited in order to confirm these disappointing results. In addition to the absence of demonstrated efficacy, the imatinib tolerance issues reported in all of the trials could be a major obstacle to its use for SSc.

Other targets
The EUSTAR group has collected observations on the effects of abatacept on articular and muscular involvement in SSc (48). Like tocilizumab, favourable effects on inflammatory articular involvement were obtained after 11 months of treatment with abatacept having a good EULAR response in 6 out of 11 patients. However, no muscular improvement was noted and no modification in cutaneous and pulmonary involvement was observed. A phase II study on the diffuse cutaneous form has just finished in the USA.
Owing to the possible implication of interleukin-2 and the results obtained in the context of graft *versus* host disease, basiliximab, an anti-CD25 monoclonal antibody, was the object of a pilot study (52). Ten patients with a diffuse and evolving cutaneous form received a total of 6 monthly perfusions at a dose of 20 mg. The median Rodnan skin score had decreased from 26/51 to 11/51 in week 68 ($p=0.015$) and the mean forced vital capacity increased from 82.1% to 88.4% (week 44, $p=0.078$) with, however, no change for the DLCO value. Tolerance was satisfactory with, nevertheless, 4 out of 10 patients complaining of nausea, fatigue, cutaneous signs and general weakness and for one patient, a respiratory infectious syndrome. A larger phase III study will now be necessary.
Rilonacept (IL-1 Trap) is in the development phase with, for now, a current study on the biomarkers of dermatologic involvement. Based on the same principal as an anti-TGF-β monoclonal antibody, fresolimumab is being evaluated for the biomarkers of dermal fibrosis in a phase II study.
Belimumab is also being evaluated in a preliminary study.

Conclusion

Systemic sclerosis is beginning to be studied for the possible benefits of targeted immunity therapies. Overall, anti-TNF-α does not appear to have a place in the treatment of this disease especially owing to the risk of pulmonary aggravation. On the other hand, anti-CD20 and tocilizumab are being evaluated. The same difficulties are posed as in lupus, namely, the outcome measure to apply as well as the subgroup to target in a heterogeneous disease as much for visceral involvement as for progression over time. For the moment, these products cannot be used for current treatment but the results of ongoing studies are awaited with impatience for a disease with therapeutic options that remain limited.

References

1. ROMANO E, MANETTI M, GUIDUCCI S *et al.*: The genetics of systemic sclerosis: an update. *Clin Exp Rheumatol* 2011; 29 (Suppl. 65): S75-86.
2. MAUVIEL A, DAIREAUX M, REDINI F *et al.*: Tumor necrosis factor inhibits collagen and fibronectin synthesis in human dermal fibroblasts. *FEBS Lett* 1988; 236: 47-52.
3. VERRECCHIA F, WAGNER EF, MAUVIEL A: Distinct involvement of the Jun-N-terminal kinase and NF-kappaB pathways in the repression of the human COL1A2 gene by TNF-alpha. *EMBO reports* 2002; 3: 1069-74.
4. DAYER JM, BEUTLER B, CERAMI A: Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. *J Exp Med* 1985; 162: 2163-8.
5. ITO A, SATO T, IGA T *et al.*: Tumor necrosis factor bifunctionally regulates matrix metalloproteinases and tissue inhibitor of metalloproteinases (TIMP) production by human fibroblasts. *FEBS Lett* 1990; 269: 93-5.
6. THEISS AL, SIMMONS JG, JOBIN C *et al.*: Tumor necrosis factor (TNF) alpha increases collagen accumulation and proliferation in intestinal myofibroblasts via TNF receptor 2. *J Biol Chem* 2005; 280: 36099-109.
7. SULLIVAN DE, FERRIS M, POCIASK D *et al.*: Tumor necrosis factor-alpha induces transforming growth factor-beta1 expression in lung fibroblasts through the extracellular signal-regulated kinase pathway. *Am J Respir Cell Mol Biol* 2005; 32: 342-9.
8. MCCULLOCH CA, KNOWLES GC: Deficiencies in collagen phagocytosis by human fibroblasts in vitro: a mechanism for fibrosis? *J Cell Physiol* 1993; 155: 461-71.
9. CHOU DH, LEE W, MCCULLOCH CA: TNF-alpha inactivation of collagen receptors: implications for fibroblast function and fibrosis. *J Immunol* 1996; 156: 4354-62.
10. AVOUAC J, FURNROHR BG, TOMCIK M *et al.*: Inactivation of the transcription factor STAT-4 prevents inflammation-driven fibrosis in animal models of systemic sclerosis. *Arthritis Rheum* 2011; 63: 800-9.
11. KOCA SS, ISIK A, OZERCAN IH *et al.*: Effectiveness of etanercept in bleomycin-induced experimental scleroderma. *Rheumatology* (Oxford) 2008; 47: 172-5.
12. PIGUET PF, COLLART MA, GRAU GE *et al.*: Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature* 1990; 344: 245-7.
13. PIGUET PF, VESIN C: Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 1994; 7: 515-8.
14. ALLANORE Y, DEVOS-FRANCOIS G, CARAMELLA C *et al.*: Fatal exacerbation of fibrosing alveolitis associated with systemic sclerosis in a patient treated with adalimumab. *Ann Rheum Dis* 2006; 65: 834-5.
15. SAITO S, KASTURI K, BONA C: Genetic and immunologic features associated with scleroderma-like syndrome of TSK mice. *Curr Rheumatol Rep* 1999; 1: 34-7.
16. SAITO E, FUJIMOTO M, HASEGAWA M *et al.*: CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in the tight-skin mouse. *J Clin Invest* 2002; 109: 1453-62.
17. ASANO N, FUJIMOTO M, YAZAWA N *et al.*: B Lymphocyte signaling established by the CD19/CD22 loop regulates autoimmunity in the tight-skin mouse. *Am J Pathol* 2004; 165: 641-50.
18. ODAKA M, HASEGAWA M, HAMAGUCHI Y *et al.*: Autoantibody-mediated regulation of B cell responses by functional anti-CD22 autoantibodies in patients with systemic sclerosis. *Clin Exp Immunol* 2010; 159: 176-84.
19. HASEGAWA M, HAMAGUCHI Y, YANABA K *et al.*: B-lymphocyte depletion reduces skin fibrosis and autoimmunity in the tight-skin mouse model for systemic sclerosis. *Am J Pathol* 2006; 169: 954-66.
20. MATSUSHITA T, FUJIMOTO M, HASEGAWA M *et al.*: Elevated serum APRIL levels in patients with systemic sclerosis: distinct profiles of systemic sclerosis categorized by APRIL and BAFF. *J Rheumatol* 2007; 34: 2056-62.
21. KOMURA K, YANABA K, HORIKAWA M *et al.*: CD19 regulates the development of bleomycin-induced pulmonary fibrosis in a mouse model. *Arthritis Rheum* 2008; 58: 3574-84.
22. MUANGCHANT C, POPE JE: The significance of interleukin-6 and C-reactive protein in systemic sclerosis: a systematic literature review. *Clin Exp Rheumatol* 2013; 31: 122-34.
23. KAWAGUCHI Y, HARA M, WRIGHT TM: Endogenous IL-1alpha from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. *J Clin Invest* 1999; 103: 1253-60.
24. KADONO T, KIKUCHI K, IHN H *et al.*: Increased production of interleukin 6 and interleukin 8 in scleroderma fibroblasts. *J Rheumatol* 1998; 25: 296-301.
25. ALLANORE Y, SAAD M, DIEUDE P *et al.*: Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. *PLoS Genet* 2011; 7: e1002091.
26. ATAMAS SP: Complex cytokine regulation of tissue fibrosis. *Life Sci* 2002; 72: 631-43.
27. KITABA S, MUROTA H, TERAO M *et al.*: Blockade of interleukin-6 receptor alleviates disease in mouse model of scleroderma. *Am J Pathol* 2012; 180: 165-76.
28. LE HUU D, MATSUSHITA T, JIN G *et al.*: IL-6 blockade attenuates the development of murine sclerodermatous chronic graft-versus-host disease. *J Invest Dermatol* 2012; 132: 2752-61.
29. FRECH TM, SHANMUGAM VK, SHAH AA *et al.*: Treatment of early diffuse systemic sclerosis skin disease. *Clin Exp Rheumatol* 2013; 31: 166-71.
30. YOSHIZAKI A, YANABA K, OGAWA A *et al.*: Immunization with DNA topoisomerase I and Freund's complete adjuvant induces skin and lung fibrosis and autoimmunity via interleukin-6 signaling. *Arthritis Rheum* 2011; 63: 3575-85.
31. DISTLER JH, JUNGEL A, HUBER LC *et al.*: Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. *Arthritis Rheum* 2007; 56: 311-22.
32. HINCHCLIFF M, HUANG CC, ISHIDA W *et al.*: Imatinib mesylate causes genome-wide transcriptional changes in systemic sclerosis fibroblasts in vitro. *Clin Exp Rheumatol* 2012; 30 (Suppl. 71): S86-96.
33. AKHMETSHINA A, VENALIS P, DEES C *et al.*: Treatment with imatinib prevents fibrosis in different preclinical models of systemic sclerosis and induces regression of established fibrosis. *Arthritis Rheum* 2009; 60: 219-24.
34. DISTLER JH, DISTLER O: Imatinib as a novel therapeutic approach for fibrotic disorders. *Rheumatology* (Oxford) 2009; 48: 2-4.
35. LAM GK, HUMMERS LK, WOODS A *et al.*: Efficacy and safety of etanercept in the treatment of scleroderma-associated joint disease. *J Rheumatol* 2007; 34: 1636-7.
36. DENTON CP, ENGELHART M, TVEDE N *et al.*: An open-label pilot study of infliximab therapy in diffuse cutaneous systemic sclerosis. *Ann Rheum Dis* 2009; 68: 1433-9.
37. RAMOS-CASALS M, PEREZ-ALVAREZ R, PEREZ-DE-LIS M *et al.*: Pulmonary disorders induced by monoclonal antibodies in patients with rheumatologic autoimmune diseases. *Am J Med* 2011; 124: 386-94.
38. OMAIR MA, PHUMETHUM V, JOHNSON SR: Long-term safety and effectiveness of tumour necrosis factor inhibitors in systemic sclerosis patients with inflammatory arthritis. *Clin Exp Rheumatol* 2012; 30: S55-9.
39. DISTLER J, JORDAN S, AIRO P *et al.*: Is there a role for TNFα antagonists in the treatment of SSc? - EUSTAR expert consensus development using the Delphi technique. *Clin Exp Rheumatol* 2011; 29 (Suppl. 65): S40-5.
40. SMITH V, VAN PRAET JT, VANDOOREN B *et al.*: Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. *Ann Rheum Dis* 2010; 69: 193-7.
41. SMITH V, PIETTE Y, VAN PRAET JT *et al.*: Two-year results of an open pilot study of a 2-treatment course with rituximab in patients with early systemic sclerosis with diffuse skin involvement. *J Rheumatol* 2013; 40: 52-7.
42. LAFYATIS R, KISSIN E, YORK M *et al.*: B cell depletion with rituximab in patients with dif-

fuse cutaneous systemic sclerosis. *Arthritis Rheum* 2009; 60: 578-83.

43. BOSELLO S, DE SANTIS M, LAMA G *et al*.: B cell depletion in diffuse progressive systemic sclerosis: safety, skin score modification and IL-6 modulation in an up to thirty-six months follow-up open-label trial. *Arthritis Res Ther* 2010; 12: R54.

44. DAOUSSIS D, LIOSSIS SN, TSAMANDAS AC *et al*.: Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. *Rheumatology* (Oxford) 2010; 49: 271-80.

45. DAOUSSIS D, LIOSSIS SN, TSAMANDAS AC *et al*.: Effect of long-term treatment with rituximab on pulmonary function and skin fibrosis in patients with diffuse systemic sclerosis. *Clin Exp Rheumatol* 2012; 30 (Suppl. 71): S17-22.

46. JORDAN S, DISTLER J, MAURER B *et al*.: Rituximab improves skin fibrosis and prevents worsening of lung function in patients with systemic sclerosis in the EUSTAR cohort. *Ann Rheum Dis* 2012; 71: 403.

47. SHIMA Y, KUWAHARA Y, MUROTA H *et al*.: The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. *Rheumatology* (Oxford) 2010; 49: 2408-12.

48. ELHAI M, MEUNIER M, MATUCCI-CERINIC M *et al*.: Outcomes of patients with systemic sclerosis-associated polyarthritis and myopathy treated with tocilizumab or abatacept: a EUSTAR observational study. *Ann Rheum Dis* 2013; 72: 1217-20.

49. SPIERA RF, GORDON JK, MERSTEN JN *et al*.: Imatinib mesylate (Gleevec) in the treatment of diffuse cutaneous systemic sclerosis: results of a 1-year, phase IIa, single-arm, open-label clinical trial. *Ann Rheum Dis* 2011; 70: 1003-9.

50. POPE J, MCBAIN D, WATSON S *et al*.: Imatinib in active diffuse cutaneous systemic sclerosis: Results of a six-month, randomized, double-blind- placebo-controlled, proof-of-concept pilot study at a single center. *Arthritis Rheum* 2011; 63: 3547-51.

51. PREY S, EZZEDINE K, DOUSSAU A *et al*.: Imatinib mesylate in scleroderma-associated diffuse skin fibrosis: a phase II multicentre randomized double-blinded controlled trial. *Br J Dermatol* 2012; 167: 1138-44.

52. BECKER MO, BRUCKNER C, SCHERER HU *et al*.: The monoclonal anti-CD25 antibody basiliximab for the treatment of progressive systemic sclerosis: an open-label study. *Ann Rheum Dis* 2011; 70: 1340-1.