Rheumatology 2008;47:v12–v13 doi:10.1093/rheumatology/ken277

# Criteria to select molecular targets for anti-fibrotic therapy

**J. H. W. Distler[1] and O. Distler[2]**

Tissue fibrosis is a major cause of morbidity and mortality in SSc. An increasing number of promising molecular targets for anti-fibrotic therapies have been described recently. However, the number of patients eligible for clinical trials is limited in SSc. The present article discusses criteria to select the most promising molecular targets for clinical trials in SSc. Based on consensus among experts, important criteria for the selection of molecular-based therapies were as follows: First, there should be strong experimental evidence that targeting the molecule of interest inhibits fibrosis. Optimally, the anti-fibrotic effects should be confirmed in at least two complementary animal models of SSc. Second, inhibitors of the molecule of interest should be clinically available. Third, clinical experience with the drug of interest in other diseases hastens the initiation of clinical trials and reduces the risk of unexpected side-effects. Finally, funding for clinical trials with the drug of interest in SSc should be available. We propose that the priority of novel targets for evaluation in clinical trials in SSc might be selected based on these consensus criteria.

**Key words:** Systemic sclerosis, Fibrosis, Fibroblasts, Tyrosine kinase inhibitors, Molecular therapies.

The pathogenesis of tissue fibrosis is multifactorial, but fibrosis is mainly caused by an overproduction of extracellular matrix by activated fibroblasts/myofibroblasts. The resulting accumulation of extracellular matrix proteins disrupts the physiological tissue structure and often leads to organ dysfunction. Tissue fibrosis is a major cause of morbidity and mortality in SSc, but specific anti-fibrotic therapies are not yet available for clinical use. Thus, the development of novel anti-fibrotic therapies is a major goal in SSc research.

Lately, a number of promising molecular targets for the treatment of fibrosis in SSc have been characterized. This includes Abelson kinase (Abl) [1, 2], steroid receptor coactivator (Src) kinases [3], Rho-associated kinases (ROCK) [4], histone deacetylases (HDACs) [5], monocyte chemoattractant protein-1 (MCP-1, CCL2) [6], adenosine A2A receptors [7] and proteasomal degradation [8]. In addition, TGF-$\beta$, connective tissue growth factor (CTGF), PDGFs and ET-1 are well-established key players in the pathogenesis of SSc. Their mechanisms of action, their role in fibrosis and their level of evidence as important molecular factors driving fibrosis are described in other contributions in this supplement. Interestingly, it is a common feature of many of these profibrotic molecules that inhibitors are already in clinical use for other indications (e.g. ET-receptor antagonists, imatinib mesylate and other c-Abl inhibitors, the Rho-kinase inhibitor fasudil) or are in clinical development (e.g. TGF-$\beta$ inhibitors, PDGF inhibitors, HDAC inhibitors and others).

Thus, therapies for many of these promising molecular targets are already available for clinical trials or will be available in the near future. According to established guidelines, the potentially disease-modifying drugs should be tested in patients with early dcSSc because they are at greater risk for the development of progressive skin involvement, severe organ damage and death [9]. At best, studies should be designed as randomized, double-blind, placebo-controlled trials and should have a reasonable observation period of at least 1 yr. These rather strict requirements together with the increasing number of available interventions create a specific problem: SSc is an orphan disease with a limited number of patients available for clinical trials, in particular when patients with early dcSSc are targeted. Thus, it will not be feasible to test all molecular-targeted therapies at the same time, and there is the necessity to select those from the list of potentially disease-modifying agents that are most promising and should be tested first in clinical trials.

However, it is unclear according to which criteria such a priority list should be established. It was thus the aim of this exercise to create consensus on such criteria among SSc experts. Thirty-five scientists from Europe and the United States with expertise in basic and/or clinical science met at a face to face meeting in Florence, Italy, in October 2007. Potential criteria were first discussed in a breakout session. The preliminary consensus from the breakout session was then presented to the remaining group. In the case of disagreement it was solved by discussion. The group finally agreed to the criteria presented below and in Table 1. It needs to be emphasized that these criteria focus on molecular targets, while other approaches such as cell-based therapies and unspecific immunosuppressive therapies, were not addressed.

## Efficacy in preclinical models

Demonstration of the efficacy of the drug of interest in preclinical models of fibrosis was defined as the most important criterion. The level of evidence of an anti-fibrotic effect in preclinical models can be categorized into three levels (level C to level A). In general, it is a prerequisite for all drugs that the pathway of interest is activated and operative in the human disease. This can be best proven in biosamples from SSc patients, in which tissue samples are most meaningful. In addition, the expert group agreed that an optimal molecular target should play a key role in the immunological, vascular as well as fibrotic pathogenesis of SSc.

Drugs with Evidence Level C have been demonstrated to be anti-fibrotic *in vitro* only. Optimally, the mechanisms of action of novel compounds should be determined including downstream molecules and intracellular signalling pathways. However, the results from these studies are limited by their *in vitro* nature, and a positive finding on the *in vitro* level does not necessarily mean that it can be confirmed *in vivo* [10].

Table 1. Criteria to determine the priority of novel compounds for clinical trials

| Criteria to determine the priority of novel compounds for clinical trials |
|---|
| (1) Evidence for anti-fibrotic effects in preclinical models |
| Level C: *in vitro* data |
| Level B: SSc animal model |
| Level A: Several SSc animal models |
| (2) Availability of the inhibitor for clinical trials |
| (3) Clinical experience |
| (4) Availability of funding |

[1]Department for Internal Medicine 3 and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany and [2]Center of Experimental Rheumatology and Center of Integrative Human Physiology, Rheumaklinik, University Hospital Zurich, Zurich, Switzerland.

Submitted 30 April 2008; accepted 19 June 2008.

Correspondence to: O. Distler, Department of Rheumatology, Center of Experimental Rheumatology, University Hospital Zurich, Gloriastr. 25, CH-8091 Zurich, Switzerland. E-mail: Oliver.Distler@usz.ch

Thus, for Evidence Level B, the anti-fibrotic effects of the drug of interest should in addition be proven *in vivo* in an animal model of SSc. During recent years, a number of animal models have been established for SSc. This includes among others the mouse models of bleomycin-induced dermal fibrosis, sclerodermatous chronic graft *vs* host disease, tight skin-1 mice and $TBR1^{CA}$ Cre-ER mice, Fra-2 transgenic mice as well the UCD 200 chicken model. These animal models cover different aspects and mechanisms of SSc pathogenesis. For instance, in the mouse model of bleomycin-induced dermal fibrosis, repeated injections of bleomycin, a profibrotic chemotherapeutic drug, in defined areas of the upper back induces a localized dermal fibrosis. The underlying molecular mechanisms are similar to that observed in SSc with infiltration of T cells and macrophages in lesional skin and TGF-$\beta$ and PDGF-dependent activation of resident fibroblasts. In contrast, $TBR1^{CA}$ Cre-ER mice are less dependent on inflammatory infiltrates. This model is based on the expression of a constitutively active TGF-$\beta$ receptor 1, which is selectively induced in fibroblasts upon feeding of 4-hydroxytamoxifen. The post-natal induction of TGF-$\beta$ signalling leads to an SSc-like disease with progressive dermal fibrosis and a vasculopathy with fibrotic thickening of the walls of smaller vessels. These examples illustrate that while all models are useful for specific aspects of SSc, none of them fully reflects the human pathogenesis.

Drugs that fulfil Evidence Level A should therefore be tested in at least two of the animal models mentioned earlier addressing different aspects of the disease. At best, the anti-fibrotic effects should also be shown in different organs involved in SSc, e.g. the lungs and skin, because the profibrotic mechanisms might differ substantially between organs.

## Availability of drugs

Besides efficacy, another important criterion is that the inhibitor of interest is either already available or will become available soon for clinical trials. Therapies that are just entering early clinical development would delay the onset of clinical trials and thus have a lower priority for evaluation in clinical trials compared with drugs that are already available.

## Clinical experience

Furthermore, clinical experience with the drug of interest is desirable and increases the priority of the drug for evaluation in clinical trials. Previous experience with the drug in other diseases reduces the risk of unexpected side-effects and enhances the safety for SSc patients. Also, experiences in other diseases help to avoid lack of efficacy of the drug on its target and pharmacokinetic problems.

## Funding

Finally, large-scale randomized controlled trials with an acceptable level of quality are only feasible when funding is available. Most often, funding for large-scale clinical trials nowadays comes from pharmaceutical industry. With the definition of orphan diseases and its related financial advantages, the interest of pharmaceutical companies in rare diseases such as SSc will increase in the near future. In addition, national and international funding agencies support clinical studies that are independent from industry.

### Rheumatology key messages

- An increasing number of promising molecular targets for the treatment of fibrosis in SSc have been characterized.
- Inhibitors of these key molecules are clinically available and await testing in clinical trials in SSc.
- However, the number of patients eligible for clinical trials in SSc (e.g. patients with early diffuse disease) is limited.
- Therefore, experts developed criteria to develop a priority list of the most promising molecular targets for anti-fibrotic therapies that should be first tested in clinical trials.
- The criteria included: (i) evidence level for anti-fibrotic effects in preclinical models; (ii) availability of drugs; (iii) clinical experience in other indications; and (iv) availability of funding.

## Acknowledgements

Dr Jörg Distler would like to acknowledge the grants they have received–ELAN grant 53410022 of the University Erlangen-Nuremberg and grant A20 of the Interdisciplinary Center of Clinical Research (IZKF) in Erlangen and Career Support Award of Medicine of the Ernst Jung Foundation.

*Supplement*: This paper forms part of the supplement entitled 'Update in systemic sclerosis'. This supplement was supported by an unrestricted grant from Encysive.

*Disclosure statement*: O.D. has received research grants and/or served as a consultant for Encysive, Actelion, Ergonex and Array Biopharma. J.H.W.D. has received materials from Novartis and Bristol-Myers Squibb, financial support from Novartis and speaker fees from Actelion and Encysive.

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