The search for the perfect animal model discloses the importance of biological targets for the treatment of systemic sclerosis

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The pathogenesis of systemic sclerosis (SSc) has been intensely investigated, and the scientific community is unravelling a detailed dissection of the molecular pathways leading to the fibrotic process. Additionally, a wealth of brilliant in vivo studies on several animal models has shown the capacity of several drugs to temper the fibrotic process. However, to date, the translation of these data in the clinic with intervention trials failed to demonstrate any consistent efficacy in ameliorating tissue fibrosis or modifying the evolution of SSc. The discrepancy between the extremely promising in vitro and in vivo results, and the otherwise disappointing results of clinical studies is striking, and it is now inevitably dampening the enthusiasm on the potentially direct translation of research into clinical progress. Moreover, it is pushing the community to raise an increasing number of doubts on the use of the current preclinical models.

The studies on the use of tyrosine kinase inhibitors (TKI) nilotinib and imatinib for skin fibrosis are a clear example of the contrast between extremely promising in vitro and in vivo data, and the poor data obtained in the clinical setting. Indeed, in vitro data on TGF-β and PDGF signalling have built a strong rationale for the use of TKI as antifibrotic agents in SSc. Furthermore, extensive in vivo studies on bleomycin-treated mice and TSK mice, widely used animal models of SSc, showed brilliant results of nilotinib and imatinib in preventing skin fibrosis and improving fibrosis in different organs. Unfortunately, both TKIs failed to demonstrate consistent clinical results in SSc patients in open label and controlled trials.

An interesting manuscript from Maurer et al, published in the December issue of *Ann Rheum Dis*, describes the effort to understand the reasons of this discrepancy through a series of simple but elegant experiments based on a reverse translational approach. Specifically, the authors compare the levels of activation of the biological targets of imatinib and nilotinib (namely, p-c-abl and p-PDGFR-β) in parallel on SSc skin biopsies, and in three different animal models: bleomycin-induced skin fibrosis, TSK1 and Fra2 mice. The first two models have been used in preclinical studies for both TKIs, giving impressive data on the control of fibrosis, whereas the Fra2 is a newly described animal model for SSc, developed by the same authors. It is interesting to remark that skin biopsies from different animal models had different numbers of cells expressing the drug targets, for example, phosphorylated form of c-abl and PDGFBR. The authors show that TSK and bleomycin-treated mice had a very high level of activation of both targets, and that the treatment with the TKI induced, as expected, a drastic reduction of p-c-abl and p-PDGFRβ-positive cells. Accordingly, skin thickness, the content of extracellular matrix and the number of myofibroblasts decreased at the levels detected in controls following treatment with TKI. On the contrary, Fra2 mice showed a lower level of activation of molecular targets of the TKIs and when treated with either drug. Skin thickness, the extracellular matrix content and the myofibroblast count were not different from untreated mice. Additionally, perhaps the most important results of the manuscript concern the levels of activation of c-abl and PDGFBR in SSc skin. While patients with limited cutaneous subset showed very low levels of both targets, patients with diffuse cutaneous subset showed a very heterogeneous level of p-c-abl and p-PDGFRβ ranging from near to healthy control to very high levels. These data may explain the inconsistent results of clinical studies and perhaps suggest that these latter could have been predicted according to the activation status of the drug targets in SSc skin. The authors suggest also that, given the heterogeneous level of activation of c-abl and PDGFBR in SSc skin, the results on animal models showing very high level of drug target activation should have been considered more cautiously as potentially informative for predicting response to treatment with TKIs in SSc. The authors conclude that levels of target activation may predict, at least in this case, biological and clinical response to treatment, and suggest that these levels should, therefore, be considered when interpreting preclinical data.

The work of Maurer et al has several merits. First, it clearly shows a new pathway for the future, suggesting to evaluate the levels of target activation before translating the in vivo data to clinical trials design. Second, it suggests that the scientific community should carefully interpret the preclinical results obtained in the current available animal models before translating them into the clinical setting. Third, it raises the awareness that a complete and predictive animal model for SSc is still lacking. Fourth, it describes the heterogeneity of target activation in SSc skin and, therefore, suggests the need for stratification of SSc patients according to drug target activation when planning treatment. In this case, it would be very interesting to determine, retrospectively, whether there is a correlation between drug target activation and clinical response to TKI in the SSc patients. If this were to be true, levels of target activation may drive a stratified medical approach to a therapeutic intervention characterised by the choice of different drugs according to the target activation of the tissues. Fifth, it suggests that also in SSc, biological markers might be used to measure the biological outcome in the evaluation of drug efficacy.

In the preclinical setting it is clear that, while there are several animal models mimicking quite closely some features of the human disease, the antifibrotic effects observed in these mice have been, in general, poorly predictive of response to therapy in humans. For this reason, the in vivo results obtained in preclinical research should be interpreted with caution and potentially might include the analysis of target activation to determine the efficiency of translation into humans.

It is clear now that the current animal models that are widely used for preclinical studies in SSc, may carry a bias for the direct translation in clinical settings if not properly interpreted. This is not in contrast with the experience accrued on inflammatory arthritis where studies on animal models failed to predict the
success of TNF inhibition. Hence, the search for other animal models of SSc is an important task that the community has to seriously consider. In this perspective, newly characterised models, such as the Fra2 models described in the same manuscript from Mauer et al., or the knock-out of uPAR, obtained from data developed in human SSc and translated into animals,7 surely deserve further attention as potentially useful preclinical animal models.

In the hunt for the perfect animal model, we should also consider the interesting observation that SSc has never been observed in any other animal, not even chimps. This raises the possibility that the difficulties encountered so far may be the consequence of SSc being a merely human-specific condition.9

Nevertheless, given the awareness that SSc is more than a fibrotic condition with a major vascular component,9 animal models that recapitulate the inflammatory vasculopathy and the fibrotic reaction should be the ‘reference points’ in deeming as clinically predictive any preclinical study. In this view, it might not be a coincidence that the Fra2 mice, which develop vasculopathy and skin fibrosis, were more similar to human SSc in levels of target activation and clinical results following TK inhibition.

This means also that the aims of the experimental and clinical studies should significantly change and move from the attempt to reverse fibrosis to prevent fibrosis through the modulation of inflammation and vasculopathy, thus influencing the early phase of fibrotic reaction and avoiding the evolution to mature fibrosis.

Apparently, the search for a perfect animal model of SSc may seem like the search for the Holy Grail, a perhaps unreachable ‘myth’ to which many scientists of our community are devoting their scientific life. We must now admit that the huge efforts spent in trying to revert established fibrosis have been a failure, demonstrated by the impossibility to translate in the clinical setting the promising experimental data. Maurer et al. have now shed light on how animal model data should be translated in the clinic, and also suggested the importance of pursuing a comprehensive approach in the treatment of scleroderma. It seems now necessary to move from a lost myth, the reversal of fibrosis, to a new more realistic aim oriented toward the modulation or the prevention of inflammation in the early phase of the mechanisms leading to the generation of a mature fibrosis. Our community is obviously facing now the challenge of finding new avenues for the discovery of effective treatments, and finally deny the 20-year-old but sadly still irrefutable observation that ‘Everything or nothing seems to work in SSc’.10

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REFERENCES
