Oliver Distler

CURRICULUM VITAE

Name:	Oliver Distler
Date of birth:	3.11.1968
Place of birth:	Nuremberg, Germany
Nationality:	Swiss and German
Business address:	Department of Rheumatology, University Hospital Zurich Gloriastrasse 25, 8091 Zurich, Switzerland Phone: +41 44 255 29 70 E-Mail: oliver.distler@usz.ch

Education:

1989–1996	Medical student at the University of Erlangen, Germany, and Duke University, North Carolina, USA
2004/2005	Specialization in Internal Medicine
2006	Specialization in Rheumatology
2012	Certificate of Advanced Studies in Healthcare Management, University of St. Gallen, Switzerland

Employment history including current position:

1996–1997	Intern, Department of Internal Medicine II, Bamberg, Germany
1997–1998	Resident, Department of Internal Medicine I, University of Regensburg, Germany
1998–2002	Fellow, Center of Experimental Rheumatology, University of Zurich, Switzerland
2002–2003	Fellow, Department of Clinical Immunology, University of Zurich, Switzerland
2004–2006	Fellow, Department of Rheumatology, University of Zurich, Switzerland
2006–2009	Attending Physician (Oberarzt), Department of Rheumatology, University of Zurich, Switzerland
2006–2013	Privatdozent, University of Zurich, Switzerland

2009–2016	Senior Attending Physician (Leitender Arzt) and Director Scleroderma Program, Department of Rheumatology, University of Zurich, Switzerland
2012	Visiting Professor, University of Gothenburg, Sweden
2013–2016	Professor ad personam of Inflammatory Rheumatology, University of Zurich, Switzerland
2015–2017	Adjunct Professor, University of Florence, Italy
2015	Visiting Professor, Stanford University, USA
2016	Full Professor of Rheumatology, University of Zurich, Switzerland

Current institutional responsibilities:

2016–	Professor, University of Zurich, Switzerland; Chairman Department of Rheumatology, University Hospital Zurich and Balgrist University Hospital, Switzerland
2018–	Head of Business Division, Trauma-Derma-Rheuma-Plastische Chirurgie und Notfallmedizin (TDR), University Hospital Zurich, Switzerland
Since 2018	Faculty Board Member, Faculty of Medicine, University of Zurich

Approved research projects
(currently running, O. Distler as PI or Co-PI):

2015–2018:	Sinergia-SNF (coordinating PI). Topic: Novel Imaging and Therapeutic Tools in Systemic Sclerosis.
2016–2018:	SNF Project Grant. Topic: Long non-coding RNAs in systemic sclerosis and other fibrotic diseases.
2016–2018:	Swisslife-Foundation. Topic: Development of systemic autoimmunity through Fra2 mediated regulatory T cell deficiency.
2016–2018:	Helmut Horten Foundation. Topic: The role of microRNA-125b in systemic sclerosis.
2016–2019:	SKINTEGRITY, Flagship Project UZH-ETH. Topic: Systemic sclerosis.

2017–2018:	Kurt und Senta-Herrmann-Foundation. Topic: Development of systemic autoimmunity through Fra2 mediated regulatory T cell deficiency.
2017–2018:	Baugarten Foundation. Topic: Einfluss von körpereigenen Knorpelabbauprodukten auf die Hämatopoese und deren Relevanz in schmerzhaft entzündlichen Knochenmarkveränderungen.
2017–2020:	Foundation for Research in Science and the Humanities at the UZH (STwF). Topic: The role the bromodomain proteins in arthritis susceptibility and synovial biology.
2018–2021	Personalized Health and Related Technologies – Swiss Personalized Health Network Grant. Topic: PRECISE: Identification of biomarkers and therapeutic targets in inflammatory disease immunotherapy by high-dimensional single cell analysis and cluster proteomics
2018–2022	Velux Foundation. Topic: Ageing without an aching spine: Biomarkers to guide treatment of Modic Changes.
2019–2021	Clinical Research Priority Program (CRPP) at the University of Zurich. Topic: Pain – from phenotypes to mechanisms.

Supervision of junior researchers:

Master, PhD and MD students: 16 Master/MD/PhD students between 1998 and 2019 (successfully completed Master/MD/PhD thesis);
Current: Fabian Brennecke, Stephan Baumgartner, Sebastian Burgener, Rucsandra Dobrota, Caroline Evers, Jasmin Hérnandez, Matthew Kassier, Simon Kuster, Dzulija Kiceva, Simon Kuster, Geraldine Lautenbach, Fiona Martin, Chantal Meier, Thomas Moser, Dominic Moret, Elena Pachera, Florian Renoux, Michal Rudnik, Chantal Rufer, Janine Schniering, Noah Schweizer, Mara Stellato, Kabriya Charles Thavaratnam, Michele Trussardi, Siim Uhtjärv, Luca Vernazza, Adam Wunderlin.

Postdocs and senior group leaders: 8 postdocs/senior group leaders between 2001 and 2017; Current: Przemyslaw Blyszczuk, Stefan Dudli, Mojca Frank-Bertoncelj, Suzana Jordan, Astrid Jüngel, Gabriela Kania, Anastasiia Kozlova, Emmanuel Karouzakis, Kerstin Klein, Britta Maurer, Michel Neidhart, Caroline Ospelt, Masaya Yokota.

Current teaching activities:

- Clinical examination course (MeF): Internal Medicine, 8 hours per year
- Human biology block course (MNF): Pathophysiology and molecular biology of vegetative systems, 2 x 1.5 weeks/year
- Basic clinical lecture series (MeF): Infectious diseases and immunology, 2 hours front lectures per year
- Advanced clinical lecture series (MeF): Differential diagnosis of the musculosceletal system Rheumatology, 8 hours front lectures/year

Current membership in panels, boards, etc.:

Since 2012	Editorial Board Annals of the Rheumatic Diseases
Since 2013	Secretary and Board Member of EUSTAR (EULAR Scleroderma Trials and Research Group)
Since 2014	President Scientific Committee Swiss Society of Rheumatology (SGR)
Since 2014	President and/or Co-President of Scientific Advisory Boards (SAB) of three randomized controlled clinical phase 2/3 registration trials in systemic sclerosis
Since 2014	Member of SAB of multiple additional phase 2/3 randomized controlled clinical trials and investigator-initiated proof of concept studies in systemic sclerosis
Since 2015	SAB GILS (Gruppo Italiano per la Lotta alla Sclerodermia) Foundation
Since 2016	Editorial Board Journal of Scleroderma and Related Disorders
Since 2016	SAB of the AbbVie Rheumatology Grant
Since 2016	SAB Member of the Hartmann Müller Foundation
Since 2016	Senate Member of SAMW (Swiss Academy of Medical Sciences), Representative of Medical Faculty, University of Zurich

Since 2017 Board Member, SCQM Foundation (Swiss Clinical Quality Management in Rheumatic Diseases)

Active memberships in scientific societies, renowned academies:

Since 2016: Member Walter-Siegenthaler-Gesellschaft für Fortschritte in der Inneren Medizin

Organisation of conferences:
Involved in the organization of 19 conferences, including chair or member of scientific organization committee:

- Co-Chair and Co-organizer of the congress «Controversies in connective tissue diseases», Florence 2007, Zurich 2008 and London 2009
- Co-Chair and Co-organization of the congress «Expert Meeting on pulmonary hypertension», Frankfurt 2010
- Steering committee Member of the Systemic Sclerosis World Congress, Florence 2010, Madrid 2012, Rome 2014, Lisbon 2016, Bordeaux 2018
- International Advisory Board for the Annual European Workshop of Rheumatology Research (EWRR) since 2011
- Program Committee Member of the Annual European League against Rheumatism (EULAR) congress Berlin 2012, London 2013 and Paris 2014
- President Annual Meeting of the Swiss Society of Rheumatology (SGR) 2014–2018
- Program Committee of the EUSTAR educational course on systemic sclerosis since 2015

Major prizes, awards, fellowships:

2001: Young Investigator Research Career Award of the University of Zurich
2002: European Workshop of Rheumatology Young Investigators Award
2002: Academic Exchange Travel Award of ACR and EULAR
2003: Abbott Award Basic Science
2005: Warnery Award of the Swiss Society for Rheumatology
2009: Pfizer Award Rheumatology/Clinical Immunology

SELECTED PUBLICATIONS

1. **Distler O,** Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, Raghu G, Sauter W, Girard M, Alves M, Clerisme-Beaty E, Stowasser S, Tetzlaff K, Kuwana M, Maher TM; SENSCIS Trial Investigators. Nintedanib for Systemic Sclerosis-Associated Interstitial Lung Disease. N Engl J Med. **2019** Jun 27;380(26):2518–2528.

2. Kozlova A, Pachera E, Maurer B, Jüngel A, Distler JHW, Kania G, **Distler O.** MicroRNA-125b Regulates Fibroblast Apoptosis Proliferation in Systemic Sclerosis. Arthritis Rheumatol. **2019** [Epub ahead of print]

3. Becker M, Graf N, Sauter R, Allanore Y, Curram J, Denton CP, Khanna D, Matucci-Cerinic M, de Oliveira Pena J, Pope JE, **Distler O;** EUSTAR Collaborators; EUSTAR Collaborators. Predictors of disease worsening defined by progression of organ damage in diffuse systemic sclerosis: a European Scleroderma Trials and Research (EUSTAR) analysis. Ann Rheum Dis. **2019** Sep;78(9):1242–1248.

4. Wu W, Jordan S, Graf N, de Oliveira Pena J, Curram J, Allanore Y, Matucci-Cerinic M, Pope JE, Denton CP, Khanna D, **Distler O;** EUSTAR Collaborators. Progressive skin fibrosis is associated with a decline in lung function and worse survival in patients with diffuse cutaneous systemic sclerosis in the European Scleroderma Trials and Research (EUSTAR) cohort. Ann Rheum Dis. 78(5):648–656, **2019**

5. Wu W, Jordan S, Becker MO, Dobrota R, Maurer B, Fretheim H, Ye S, Siegert E, Allanore Y, Hoffmann-Vold AM, **Distler O.** Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model. Ann Rheum Dis. **2018** [Epub ahead of print]

6. Allanore Y*, **Distler O*,** Jagerschmidt A, Illiano S, Ledein L, Boitier E, Agueusop I, Denton CP, Khanna D. Double-blind, Randomized, 8-week Placebo-controlled followed by a 16-week open label extension study, with the LPA1 receptor antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis. Arthritis Rheumatol. **2018** [Epub ahead of print] * contributed equally

7. Iwamoto N, Vettori S, Maurer B, Brock M, Pachera E, Jüngel A, Calcagni M, Gay RE, Whitfield ML, Distler JH, Gay S, **Distler O.** Downregulation of miR-193b in systemic sclerosis regulates the proliferative vasculopathy by urokinase-type plasminogen activator expression. Ann Rheum Dis **2016;** 75(1):303–310. Impact Factor: 12.384

8. Maurer B, Graf N, Michel BA, Müller-Ladner U, Czirják L, Denton CP, Tyndall A, Metzig C, Lanius V, Khanna D, **Distler O;** on behalf of EUSTAR co-authors. Prediction of worsening of skin fibrosis in patients with diffuse cutaneous systemic sclerosis using the EUSTAR database. Ann Rheum Dis. **2015;** 74(6):1124–31. Impact Factor: 12.384

9. Jordan S, Distler JH, Maurer B, Huscher D, van Laar JM, Allanore Y, **Distler O;** on behalf of the EUSTAR Rituximab study group. Effects and safety of rituximab in systemic sclerosis: an analysis from the European Scleroderma Trial and Research (EUSTAR) group. Ann Rheum Dis **2015;** 74(6):1188–94. Impact Factor: 12.384

10. Frauenfelder T, Winklehner A, Nguyen TD, Dobrota R, Baumueller S, Maurer B, **Distler O.** Screening for interstitial lung disease in systemic sclerosis: performance of high-resolution CT with limited number of slices: a prospective study. Ann Rheum Dis **2014;** 73(12):2069–73. Impact Factor: 12.384

DEVELOPING TARGETED THERAPIES IN SYSTEMIC SCLEROSIS: FROM BENCH TO BEDSIDE

Oliver Distler[1]

Summary

Systemic sclerosis (SSc) is a rare, chronic autoimmune disease with multi-organ involvement. Its pathophysiology includes early activation of the immune system, microvascular changes and activation of fibroblasts, ultimately leading to fibrosis and tissue ischemia. SSc puts a high morbidity and mortality on the patients, and there is a high-unmet need for effective therapies in SSc. In recent years, we were able to establish a platform to identify and develop novel molecular targeted therapies for SSc using newly developed and characterized in vitro and in vivo models of SSc. In this overview, the characterization of serotonin signaling is given as an example for a comprehensive preclinical portfolio to allow informed translation into clinical applications. Using this example, the design of biomarker driven proof of concept studies is shown that provides further justification to develop targeted therapies into phase 2 and 3 trials. We could also contribute to the improved design of advanced clinical studies in SSc by modeling clinical trials and their inclusion and exclusion criteria from the worldwide largest patient registry EUSTAR. Very recently, one of the targeted therapies, Nintedanib, which we could help to develop, showed positive effects in a very large, placebo controlled clinical trial for patients with SSc-interstitial lung disease (ILD). The FDA has now approved Nintedanib as the first treatment for patients with SSc-ILD.

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Introduction: Systemic sclerosis

Systemic sclerosis (SSc) is a rare chronic autoimmune disease of unknown etiology. It has the highest case-related mortality among the rheumatic diseases and puts a major morbidity on the patients and their relatives. As many other autoimmune diseases, the large majority of patients with SSc are females with a ratio of females to males from approximately 7 to 1. It can affect all ages including children, but onset of the disease peaks around an age of 40–60 years (1).

The complexity of SSc is among other reasons based on its diverse and heterogeneous organ involvements. It frequently involves the skin, the gastrointestinal tract and the lungs, but can also involve the heart, the kidneys and a variety of other organs (Figure 1). Organ manifestations peak at different times during the disease course, can show continuous progression as more frequently seen in the lungs, but can also show a stable disease or even spontaneous regression such as for skin fibrosis. Moreover, the individual disease course is highly variable and difficult to predict on the individual patient level. Disease severity can range from subclinical immunological and minor microvascular involvement to rapidly progressive disease with immediate involvement of major organs leading to death within several months (1).

This variability in the disease course and the individual heterogeneity makes clinical trial design in SSc extremely challenging. Accordingly, while a variety of immunosuppressive drugs is used in clinical routine despite limited evidence, approved therapies for SSc were until very recently not available and the large majority of randomized controlled clinical trials were negative not reaching statistical significance for their primary endpoints. The only exception is autologous hematopoietic stem cell transplantation, which has been shown to have favorable long-term effects in three large randomized controlled trials. However, its applicability is limited by its treatment related mortality, which is reaching up to 10%. It is therefore only recommended for a selected, rapidly progressive patient population at high risk of mortality, and should be only applied in specialized centers with experience in this treatment for patients with SSc.

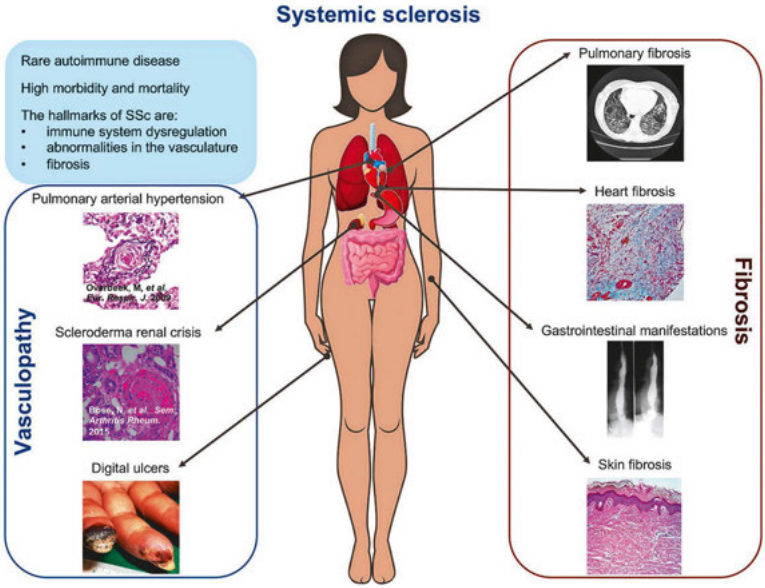

Figure 1: Major clinical manifestations and pathological hallmarks in SSc.

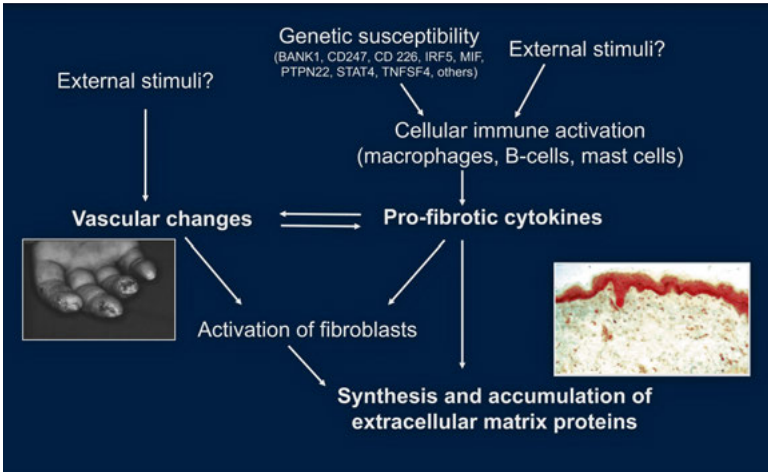

Figure 2: Vasculopathy, inflammation and fibrosis: A bird-eye view on major pathogenic steps in SSc. *Modified from (1)*

Part of the limited progress that has been made in finding treatments for SSc is due to the limited understanding of its pathophysiology. The pathological hallmarks of SSc are abnormalities of the microvasculature and immune system eventually leading to the activation of fibroblasts and fibrosis of affected organs. Major pathophysiological steps are summarized in Figure 2.

The etiology of SSc is complex and might be based on an overall minor genetic susceptibility. Interestingly, genetic polymorphisms linked to this susceptibility are located largely in genes involved in the immune regulation and overlap with other major autoimmune diseases. This raises the hypothesis that the triggering events are involving an immune activation, which might then be the first step in the earliest stages of SSc. While these triggering events are unknown, several hypothesis have been proposed such as viral infections or environmental factors.

More importantly, there has been advance in the identification of factors driving key pathophysiological processes in SSc. In general, immune dysregulation and microvasculopathy are early manifestation in the pathogenesis of SSc. The events contributing to vascular injury include endothelial cell activation and apoptosis, increased vascular permeability leading to leukocytes infiltration and perivascular inflammation, and breakdown of capillaries. Small arteries show proliferation and activation of vascular smooth muscle cells leading to vessel wall fibrosis, lumen narrowing and vessel obliteration. These processes overall result in ischemia and chronic tissue hypoxia. Immune dysregulation is characterized by the presence of perivascular inflammatory infiltrates consisting of activated T cells, macrophages and mast cells in the affected tissues and SSc-specific autoantibodies in the circulation (1).

The pathologically probably most important process in the pathophysiology of SSc is fibrosis. In fibrosis, the functional tissue is replaced with a collagen rich, stiff connective tissue leading to dysfunction and failure of affected organs. The links to vascular injury and immune dysregulation are poorly understood, but particularly the induction of cytokine and growth factor production in inflammatory cells and resident tissue cells appear to play a major role. Interstitial fibroblasts are important effector cells in this process. While proliferation is rarely detected in fibrotic tis-

sues of SSc patients, a major hallmark of SSc fibroblasts is the differentiation into highly activated myofibroblasts. In addition to resident interstitial fibroblasts, myofibroblasts can transdifferentiate from a variety of additional cells such as monocytes, pericytes, fibrocytes, and epithelial cells. Activated myofibroblasts are characterized by contractile alpha smooth muscle actin (α-SMA) and release large amounts of extracellular matrix components (ECM) such as collagens and fibronectin (1).

Taken together, while there is an increasing knowledge about factors and processes driving the pathophysiology of SSc and fibrotic diseases in general, this has not led to major breakthroughs in the treatment of this devastating disease until very recently. The research program from my group therefore spans from preclinical to clinical science, which includes the following parts:

(1) A preclinical program focusing on the identification of key molecules and intracellular signaling cascades that are driving the disease process;

(2) The characterization of animal models and 3D cellular systems, which can be used for proof of principle targeting;

(3) A translational and clinical program with emphasis on precision medicine and phase 2/3 clinical trial design.

Some of the major findings are summarized on the following pages.

Targeting TGF-β associated pathways as a treatment principle: Serotonin as an example

TGF-β: Several cytokines have been characterized to drive the activation of fibroblasts. TGF-β is been considered by many researchers as a main factor in many different fibrotic diseases including SSc. The circumstantial evidence for a key role of TGF-β in SSc includes the presence of a strong TGF-β-activated gene signature in skin biopsies from patients with SSc. Immunohistochemical analysis show that expression of TGF-β is increased in SSc skin. Moreover, elevated levels of TGF-β receptors (TGFβR) on SSc fibroblasts suggest an ongoing autocrine stimulation to maintain the activated phenotype even in absence of exogenous stimuli. Microarray studies showed that signatures of TGF-β activated

pathways occurred exclusively in a subset of skin biopsies from patients with diffuse cutaneous SSc. However, direct targeting of TGF-β has been challenging and is linked to a meaningful toxicity because of its general widespread physiological role. Thus, an alternative and possibly more promising strategy for the treatment of fibrotic diseases might be the targeting of less commonly involved and therefore less toxic downstream pathways and pathways that are associated with TGF-β.

Proof of principle for serotonin as a potential anti-fibrotic target: In this regard, 5-hydroxytryptamine (5-HT; serotonin) is a molecule, which is stored in platelets in the circulation. It is released upon activation of platelets. Because of the microvascular changes in SSc, which lead to a disturbed blood flow and consequently to an activation of platelets, many platelet derived factors including 5-HT have been found elevated in the circulation of SSc patients. We hypothesized that 5-HT might be involved in the activation of fibroblasts, and might provide a link between the microvascular changes, platelet activation and tissue fibrosis in SSc (2). The key findings of this study are mentioned here and are also summarized in (3).

First, we could show that dermal fibroblasts from SSc patients and healthy subjects, which were stimulated with 5-HT, increased the mRNA and protein of different extracellular matrix proteins including collagen type I alpha 1 (COL1A1) in dose-dependently. Doses used for the stimulations were in the range of those detected in biological fluids (Figure 3).

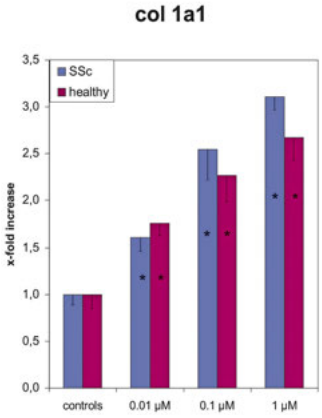

Figure 3: Serotonin (5-HT) induces the release of extracellular matrix proteins such as collagen I (col1A1) from cultured dermal fibroblasts. *Adapted from (2).*

There are seven 5-HT receptors, 5-HT1 to 5-HT7, by which the cellular effects of 5-HT are mediated. We found that three different 5-HT receptors were expressed by dermal fibroblasts: 5-HT1B, 5-HT2A, and 5-HT2B. The mRNA levels of 5-HT2B were slightly upregulated in SSc fibroblasts as compared to healthy controls (143 ± 17%), while 5-HT1B and 5-HT2A were not different. Inhibition of 5-HT2, but not 5-HT1 by selective chemical inhibitors decreased the mRNA and protein levels of COL 1A1, COL 1A2 and fibronectin-1. Additional inhibition experiments using siRNA knock-down revealed that this effect was specific for 5-HT2B, but not for 5-HT2A. These results suggested that 5-HT2B plays a crucial role for the synthesis of extracellular matrix proteins in dermal fibroblasts.

Immunohistochemistry in fibrotic skin biopsies of SSc patients and normal skin of healthy individuals supported these results. Expression of 5-HT2B was increased in fibrotic tissue as compared with unaffected tissue from healthy controls. Double staining with the fibroblast-specific marker prolyl-4-hydroxylase-beta confirmed that 5-HT2B was mostly expressed by dermal fibroblasts.

We suspected indirect mechanisms for the pro-fibrotic effects, because the effects of 5-HT on collagen synthesis were delayed. Thus, we investigated, whether TGF-β might be the second mediator of 5-HT signaling. Indeed, 5-HT increased dose-dependently the mRNA levels of TGF-β1 in SSc fibroblasts. Furthermore, 5-HT induced in a time-dependent manner the nuclear levels of phospho-Smad3, the intracellular mediator of TGF-β signaling. Inhibition of TGF-β1 by neutralizing antibodies abrogated the pro-fibrotic effects of 5-HT on the expression of COL1A1, COL A2 and fibronectin-1, suggesting that the serotonin effects are mediated by TGF-β.

Next, we used animal models to proof our in vitro findings. Injection of bleomycin intradermally into mice potently stimulated the expression of 5-HT2B and induced dermal fibrosis (4). The 5-HT2 inhibitors terguride and cyproheptadine as well as the selective 5-HT2B inhibitor SB 204741 efficiently prevented and treated bleomycin-induced dermal thickening, collagen content and myofibroblast counts. The anti-fibrotic effects of 5-HT2 inhibition were further tested in a therapeutic approach using a

modification of the bleomycin model. To complement these pharmacological inhibitions, we used 5-HT2B -deficient mice. 5-HT2B-/- mice were almost completely protected from bleomycin-induced dermal fibrosis, while no spontaneous fibrosis was observed in these mice. Similar effects were seen in another less inflammatory mouse model of fibrosis, the tight skin 1 (Tsk-1) model. In this genetic model hypodermal fibrosis occurs, which was no longer seen when the Tsk-1 mice were crossed with 5-HT2B-/- mice.

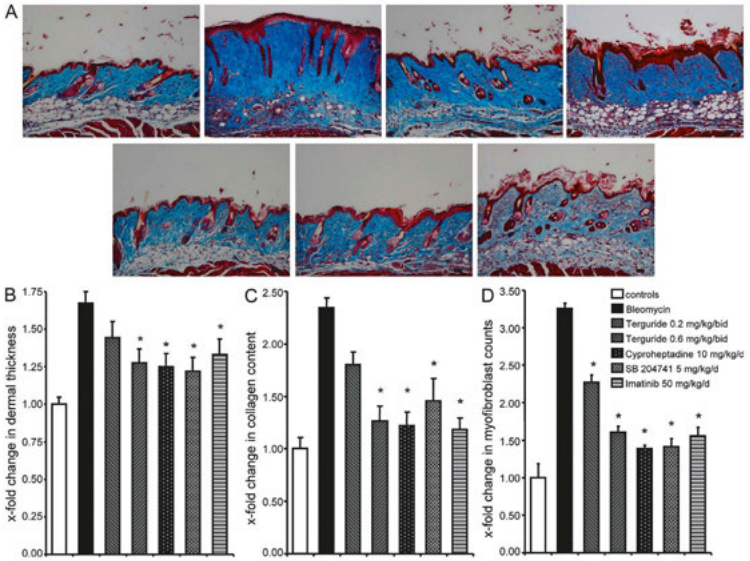

Figure 4: Inhibition of serotonin signaling by different 5-HT2 inhibitors prevents and treats bleomycin-induced skin fibrosis. *A: skin sections. Top left to bottom right: control mice, bleomycin mice untreated, bleomycin treated with tergurid low dose, bleomycin mice untreated, bleomycin treated with tergurid low dose, bleomycin treated with cyproheptadine, bleomycin treated with SB 204741. B-D: quantification with different read-outs. Taken from (2).*

To confirm the link between platelet activation and increased 5-HT/5-HT2B signaling in fibrosis, we used mice deficient for tryptophan hydroxylase (TPH) 1, which is the key enzyme for the synthesis of 5-HT in

platelets, and challenged them with intradermal injections of bleomycin. Experiments with TPH 1 deficient mice further underlined the important role of platelet-derived 5-HT in experimental fibrosis. Dermal fibrosis in bleomycin-challenged TPH1-/- mice was strongly decreased compared with bleomycin-challenged TPH1+/+ mice. The concept derived from these and other studies is summarized in Figure 5.

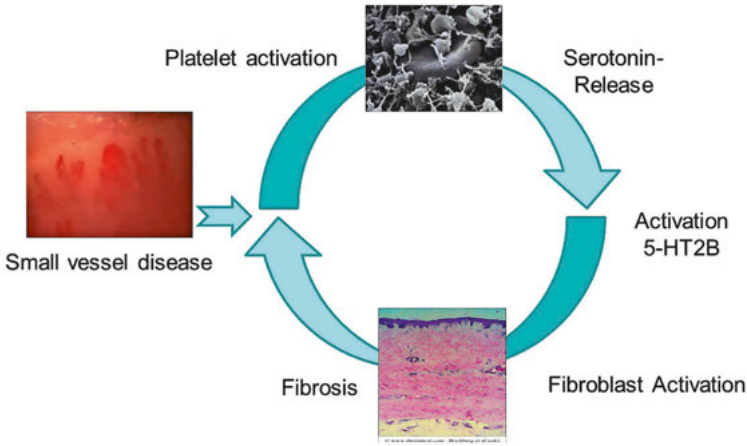

Figure 5: The vicious circle of serotonin signaling in SSc. *Small vessel disease leads to platelet activation, releasing serotonin (5-HT). 5-HT activates fibroblasts via 5-HT2B receptors in a TGF-β depended manner, which increases the release of extracellular matrix proteins eventually contributing to fibrosis. Fibrosis increases the distance of cells to blood vessels leading to hypoxia, which further enhances the microvessel disturbance seen in SSc, resulting in further platelet activation.*

Translation into clinical application: In a next step, we aimed to translate these findings into a proof of concept phase 2 clinical study (5). In this investigator-initiated two-center study using the pan 5HT2 inhibitor terguride, the primary endpoint was safety. Serious adverse events (SAEs) and AEs were coded using MedDRA. Primary efficacy endpoints included changes of pre-defined skin biopsy biomarkers over the three months treatment period. Secondary efficacy endpoints were change of mRSS and lung function parameters. Main inclusion criteria were fulfillment of ACR classification criteria for SSc and the more severe, progressive sub-

type of SSc, diffuse cutaneous SSc (dcSSc). Patients with end-stage organ involvement and treatment with potentially disease modifying agents including immunosuppressives were excluded. Patients were treated with Terguride at up to 3 mg/d p.o. or standard of care (post hoc control) for three months. The study was externally monitored.

We were able to recruit twelve patients into the Terguride group and six patients into the control group. The primary endpoints, skin biopsy biomarkers, showed a consistent and statistically significant down-regulation compared to the control group (Figure 6) for dermal thickness, myofibroblast counts and mRNA levels of col1a1, col1a2. In addition, the Lafyatis 4-gene biomarker set (COMP, THSP-1, SIGLEC-1, IFI-44), which is a validated biomarker set to predict the progression of skin fibrosis, was also changed. This was accompanied by a reduction in mRSS of –32.3% versus baseline in the Terguride group versus stable values in the control group ($p < 0.05$). Lung function parameters did not change significantly. Overall, 33 adverse events (n=27 mild and n=6 moderate) and one serious adverse event (pyelonephritis, not related) occurred in the Terguride group, most often consisting of nausea and vomiting (9% and 13% of patients respectively).

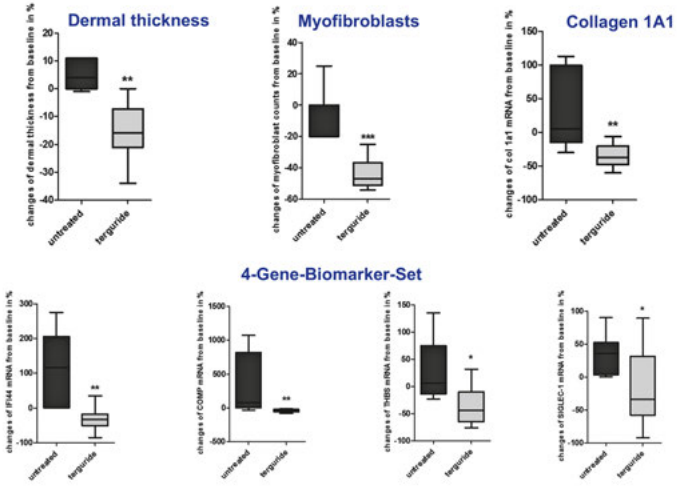

Figure 6: *Biomarker results of the proof of concept study with Terguride in 12 patients and 6 controls. Biomarkers were measured in skin biopsies taken from patients at baseline and 3 months.*

Based on these promising safety and biomarker results, a phase 3 placebo-controlled one year study was designed and discussed with both the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Terguride is not serotonin receptor specific, but also targets adrenergic and dopaminergic receptors, which are largely responsible for the observed adverse events described above. Terguride had been approved for the treatment of hyperprolactinemia in some countries. Thus, SSc was a repositioning of this drug and had a reasonable toxicity profile in this indication, but not in SSc. This led FDA to request additional phase 1 studies. Unfortunately, these phase 1 studies revealed potential cardiovascular risk with increases in blood pressure and heart rate in certain subgroups of volunteers. As a result, the clinical Terguride program was not continued for SSc. Because these unwanted adverse events were not caused by the serotonin-receptor targeting, but the unrelated adrenergic and dopaminergic receptors targeted by Tergruide, we are currently developing in cooperation with start companies specific small molecule inhibitors specifically targeting 5HT2.

Development of new animal models in SSc

Animal models are an important part of pre-clinical drug development in SSc and fibrotic diseases. While none of these animal models fully reflects the human disease, they are giving important insights into the various mechanisms being operative in SSc patients. Activated pathways are highly heterogeneous in SSc patients. Consequently, a positive proof of concept study in a specific animal model with an activation of certain, specific pathways does definitely not guarantee a positive clinical trial and translation into the clinic. However, when several fibrotic animal models with diverse mechanisms are showing positive effects in the prevention and treatment of fibrosis, the likelihood of a positive clinical trial in humans is increasing. Considering the major burden for patients of the large and long (placebo-controlled) clinical trials that are required in SSc, and considering the major costs of such trials, any effort that decreases the likelihood of negative trials is worthwhile. Thus, a wide variety of fibrotic animal models fosters drug development in SSc despite its well-accepted limitations. In addition, 3D skin models with primary cells from SSc patients reflecting the fibrotic environment in the skin are another

important tool for screening of targeted therapies. They allow for high-throughput screening and minimize the burden for living animals. These 3D skin models are currently in development and in optimization in our laboratories. The following chapter focuses on the Fra-2 tg mouse model as an example of a new model for fibrotic autoimmune diseases such as SSc. The overall importance of this model for studies in SSc is also summarized in (6).

The Fra-2 transgenic mice: Fra-2 tg mice were initially described by the group of Erwin Wagner as a model that develops a severe form of lung fibrosis (7). Fra-2 belongs to the family of AP-1 (activator protein-1) transcription factors consisting of members of the Jun (c-Jun, JunB, JunD) and the Fos family (c-Fos, FosB, Fra-1, Fra-2). In the Fra-2 tg mouse model, the murine Fra-2 gene is expressed under the control of the ubiquitous major histocompatibility complex class (MHC) I antigen H2Kb promoter. In Fra-2 tg mice, the mRNA of the transgene is detectable in various tissues, but the extent and distribution of protein expression varies depending on the insertion of the transgene into the genome in the founder animals, depending on the background of the mice and possibly because of additional, unidentified factors. In our lab, we are in the meantime using a newly generated mouse model of Fra-2 with the same MHC I promotor, which shows a slightly different, more autoimmune and somewhat less fibrotic phenotype than described in the following paragraphs.

We could show in our initial studies on the Fra-2 tg mouse model that Fra-2 tg mice displayed several features of the peripheral microvasculopathy characteristic for human SSc (8). In the skin of both Fra-2 tg mice and SSc patients, but not in controls, Fra-2 protein was predominantly expressed in vascular structures. Whereas Fra-2 tg mice did not differ from wt mice at an age of 9 weeks, starting from week 12, a significant decrease in capillary density occurred. Increased perivascular inflammatory infiltrates characteristic for human SSc were also present in the skin of 9-week-old Fra-2 tg mice compared to wt mice, but not in older mice. The rarefication of capillaries in Fra-2 tg mice paralleled the development of skin fibrosis. Starting from 12 weeks, Fra-2 tg mice showed a time-dependent increase of dermal thickness due to accumulation of extracellular matrix that became even more pronounced at 16 weeks.

Endothelial cell (EC) apoptosis is considered one of the earliest events in the development of vascular lesions in human SSc. Interestingly, in Fra-2 tg mice, apoptosis of dermal EC at 9 weeks preceded the development of microangiopathy and skin fibrosis suggesting that similar mechanisms as in the human disease are important in this model. Using the variety of functional in vitro experiments, we could show that the expression of Fra-2 induced apoptosis, and inhibited proliferation, migration and tube formation capacity of human microvascular endothelial cells. Clinical manifestation of peripheral microvasculopathy such as ulcers or tissue necrosis did, however, not occur during the 16-week observation period in mice. Despite this lack of clinical vascular manifestations, it is until today the only model that develops both peripheral fibrotic and vascular changes reflecting what is seen in human SSc.

This is also underlined by changes in the lungs in Fra-2 tg mice. The two major manifestations in the human disease are pulmonary arterial hypertension (PAH) and interstitial lung fibrosis (ILD). Lung manifestations are the most frequent cause of SSc-related death and are therefore of major importance for treatment studies. Fra-2 tg mice develop both of these features (9). In our analysis, the phenotype of ILD in Fra-2 tg mice resembled features of human NSIP, whereas fibroblastic foci, and honeycombing, associated with UIP, were rarely detectable. NSIP is the predominant feature of SSc-ILD. ILD occurred after the pulmonary vascular features described below but let to sacrifice of the mice at week 16.

In one of our analysis, we focused on the pulmonary vascular manifestations of the Fra-2 tg mice and compared them to histological changes seen in human PAH. Fra-2 tg mice displayed several features that are considered to be more common in SSc-associated PAH than in idiopathic PAH (IPAH). This included intimal thickening with mainly concentric laminar lesions, medial hypertrophy, perivascular inflammatory infiltrates, adventitial fibrosis, and lung fibrosis with interstitial inflammatory infiltrates (Figure 7). Histological features underrepresented in SSc-PAH were also rarely detectable in Fra-2 tg mice including concentric and eccentric non-laminar lesions. Complex lesions such as plexiform and thrombotic lesions, which occur more often in IPAH were not observed. Different from human SSc-PH, pulmonary occlusive venopathy was not detectable in Fra-2 tg mice. Later experiments proofed by right

heart catheterization that Fra-2 tg mice have strongly increased right atrial pressures and increased pulmonary artery pressures.

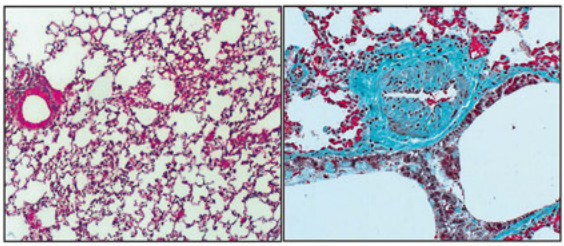

Figure 7: *Pulmonary vascular lesions in Fra-2 tg mice resembling PAH. Left: wild-type control. Right: Fra-2 tg mice showing strongly thickened pulmonary vessels and obliterated vessels (green).*

We next wanted to know whether Fra-2 tg mice are sensitive to change over treatment and can thus be used for proof of concept treatment studies. Our previous studies had shown that PDGF-BB and its phosphorylated (=activated) receptor was increased in the vascular lesions of Fra-2 tg mice. By applying the tyrosine kinase inhibitor nilotinib, which targets the PDGF-R pathway, an almost complete prevention of vascular remodeling and lung fibrosis was observed in Fra-2 tg mice.

Taken together, the Fra-2 tg mouse model resembles both peripheral and internal organ features of both the fibrotic and vascular phenotype of human SSc. As Fra-2 is also overexpressed in the human disease, it is an attractive model to study the pathophysiology of SSc. It is nowadays one the most frequently used proof of concept models to test and screen for potential targeted therapies in SSc.

Improvement of clinical trial design in SSc

Our laboratories where involved in the preclinical characterizations of a number of potential molecular targeted therapies as outlined for serotonin above. Some of them passed the quality tests of efficacy and were proceeded to clinical testing in phase 2 and 3 studies.

The most frequently chosen primary endpoint for clinical trials in SSc has been skin fibrosis as measured by the modified Rodnan skin score (mRSS), a semi-quantitative measure of skin fibrosis on the whole body. However, clinical studies had frequently failed in SSc in the past. One of the potential reasons was the fact that in all previous clinical trials, the mRSS showed a spontaneous improvement on the group level in the placebo groups, indicating that skin fibrosis had already peaked at inclusion into the clinical trials. From a molecular targeting point of view, this suggested that the previous trials could have been negative because the targeted pathways were no longer active during regression of skin fibrosis and thus targeting them could not lead to clinical effects. All attempts to enrich for patients showing progression of skin fibrosis had failed in the past.

In this situation, we had the opportunity to take advantage of the European Scleroderma Trials and Research Group (EUSTAR) registry. This patient registry was founded in 2004 with our group in Zurich being one of the founding members. As of today, this registry contains more than 16 000 SSc patients from more than 200 centers across the world with an extensive clinical characterization collected in the registry. The majority of patients are followed longitudinally with annual visits. It is by far the largest registry worldwide in this rare disease and a rich source of scientific analysis of the clinical course of SSc with over 60 clinical research publications. I am currently the elected chairperson of EUSTAR.

To allow enrichment of patients with progressive skin fibrosis by modifying inclusion criteria into phase 2/3 trials, we mimicked the typical population included into these trials (diffuse cutaneous SSc, dcSSc) and the lengths of those trials (12 months) from the registry (10). Patients with these features were included into the analysis and logistic regression was performed to identify independent clinical features at baseline that can predict patients with progression at 12 months follow up.

A total of 637 dcSSc patients were eligible. Univariate analyses identified joint synovitis, short disease duration (≤15 months), short disease duration in females/patients without creatine kinase (CK) elevation, low baseline mRSS (≤22/51), and absence of esophageal symptoms as potential predictors for progressive skin fibrosis. In the multivariate analysis,

by employing combinations of the predictors, 17 models with varying prediction success were generated, allowing cohort enrichment from 9.7% progressive patients in the whole cohort to 44.4% in the optimized enrichment cohort. Using a second validation cohort of 188 dcSSc patients, short disease duration, low baseline mRSS and joint synovitis were confirmed as independent predictors of progressive skin fibrosis within 1 year resulting in a 4.5-fold increased prediction success rate. An example how the prediction models can be used for clinical trial design is shown in Table 1.

Model	Included prediction markers	Prediction success (%)
1	Joint synovitis	20/122 (16.4)
2	Disease duration	22/126 (17.5)
3	Joint synovitis, disease duration	9/31 (29.0)
4	Joint synovitis, disease duration, gender, interaction between short disease duration and gender	9/25 (36.0)
5	Joint synovitis, disease duration, gender, interaction between short disease duration and gender, oesophageal symptoms	9/25 (36.0)
6	MRSS at baseline	52/412 (12.6)
7	MRSS at baseline, joint synovitis	18/88 (20.5)
8	MRSS at baseline, disease duration	20/84 (23.8)
9	MRSS at baseline, joint synovitis, disease duration	8/22 (36.4)
10	MRSS at baseline, joint synovitis, disease duration, gender, interaction between short disease duration and gender	8/18 (44.4)

Table 1: Clinical features identified from the EUSTAR registry to predict worsening of skin fibrosis. *Progression without enrichment approximately 9%, total number of patients in the derivation analysis 537. For example, in model 1, using joint synovitis as an inclusion criterion, 122/537 can be included into the clinical trial, and progression increases from 9% to 16% under standard of care. An optimal enrichment from 9% to 44% can be achieved with model 10 and a combination of low MRSS at baseline, joint synovitis, disease duration, and short disease duration as inclusion criteria. However, only 18/537 are fulfilling all these inclusion criteria questioning the feasibility of such a theoretical study. Adapted from (10).*

In a follow-up study, we used a similar approach to define criteria predicting the improvement in skin fibrosis in diffuse cutaneous SSc (11). This analysis was aimed to inform exclusion criteria of a clinical trial to avoid inclusion of patients with spontaneous regression of skin fibrosis. From the 919 EUSTAR patients included in this analysis, 218 (24%) improved and 95 (10%) progressed. Eleven candidate predictors for skin improvement were analyzed. Using logistic regression with bootstrap validation, the final model identified high baseline mRSS and absence of tendon friction rubs as independent predictors of skin improvement (Figure 8). The baseline mRSS was the strongest predictor of skin improvement, independent of disease duration. An upper threshold between 18 and 25 performed best in enriching for progressors over regressors.

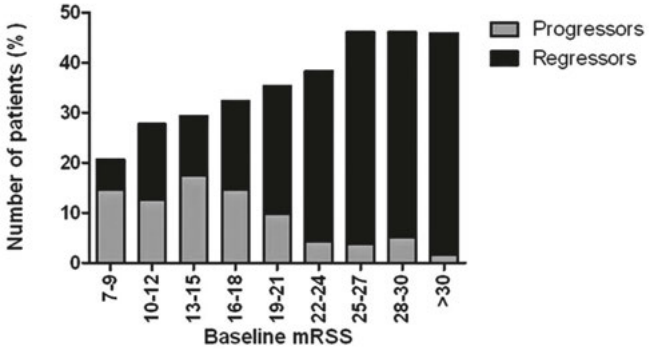

Figure 8: Percentage of progressors and regressors of skin fibrosis based on the baseline level of mRSS. *With higher baseline levels of mRSS, regression is more likely to occur. Adapted from (11).*

While some of these inclusion criteria had already been applied in previous studies, the consideration of less advanced skin fibrosis in very early diffuse SSc as an inclusion criterion to allow for progression was a new strategy that we subsequently applied to some of the phase 2/3 trials (Figure 8). Indeed, as for example seen in the Rise-SSc study with riociguat (submitted, presented at conferences), this strategy of evidence-based definitions of inclusion and exclusion criteria was success-

ful, leading to a remarkable increase in the percentage of patients with progression of skin fibrosis.

Nintedanib – the first FDA approved therapy for SSc-ILD

These and other findings contributed to the design of a very large international randomized placebo-controlled trial with the multi-tyrosine kinase inhibitor nintedanib for SSc-ILD. As for serotonin described above, our laboratories were providing the preclinical portfolio to characterize nintedanib as a potential anti-fibrotic treatment in SSc. Different animal models as well as in vitro experiments consistently showed strong anti-fibrotic effects in both the skin and lung, but also modulated the inflammatory response in the SSc models. Nintedanib could also prevent the onset of PH and vascular remodeling in the Fra-2 tg mouse model, thus providing a strong rationale for clinical testing in SSc.

Nintedanib is an orally available small molecule intracellular inhibitor of tyrosine kinases, targeting among other tyrosine kinase receptors VEGFR, PDGFR, and FGFR. It is an approved treatment for idiopathic pulmonary fibrosis (IPF), which has both similarities and differences to SSc-ILD. We conducted the SENSCIS trial, a randomized, double blind, placebo-controlled trial to investigate the efficacy and safety of nintedanib in patients with SSc-ILD (Figure 9) (12).

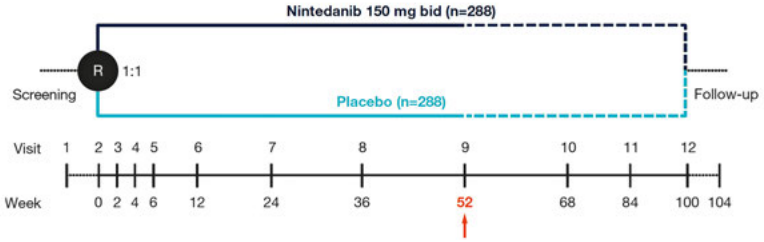

Figure 9: *Design of the SENSCIS trial, a one-year clinical trial with nintedanib in patients with SSc-ILD. Patients remained on blinded treatment until the last patient had reached week 52 but for no longer than 100 weeks.*

This study with 576 patients recruited from more than 30 countries and more than 170 centers in 5 continents was industry-sponsored, but coordinated from Zurich as one of the two coordinating centers. Patients with SSc with an onset of the first non-Raynaud's symptom within the past 7 years and a high-resolution computed tomographic scan that showed fibrosis affecting at least 10% of the lungs were randomly assigned, in a 1:1 ratio, to receive 150 mg of nintedanib, administered orally twice daily, or placebo. The primary endpoint was the annual rate of decline in forced vital capacity (FVC), assessed over a 52-week period. Key secondary endpoints were absolute changes from baseline in the mRSS and in the total score on the St. George's Respiratory Questionnaire (SGRQ) at week 52.

Of the 576 patients receiving at least one dose of nintedanib or placebo, 51.9% had diffuse cutaneous systemic sclerosis, and 48.4% were receiving mycophenolate at baseline. As the inclusion and exclusion criteria were designed rather liberal, the population was largely representative of a typical SSc-ILD population. In the primary endpoint analysis, the adjusted annual rate of change in FVC was −52.4 ml per year in the nintedanib group and −93.3 ml per year in the placebo group (difference, 41.0 ml per year; 95% confidence interval [CI], 2.9 to 79.0; P = 0.04, Figure 10). Sensitivity analyses based on multiple imputation for missing data yielded P values for the primary endpoint ranging from 0.06 to 0.10.

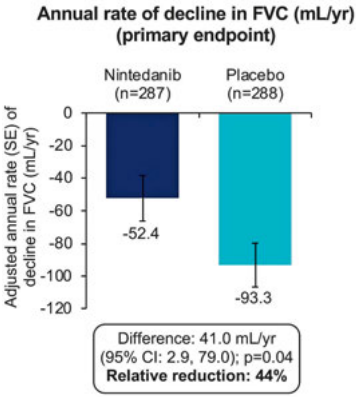

Figure 10: Annual rate of decline of FVC in patients with SSc-ILD treated with nintedanib or placebo. *Adapted from (12).*

The change from baseline in the modified Rodnan skin score and the total score on the SGRQ at week 52 did not differ significantly between the trial groups, with differences of −0.21 (95% CI, −0.94 to 0.53; P = 0.58) and 1.69 (95% CI, −0.73 to 4.12 [not adjusted for multiple comparisons]), respectively. Diarrhea, the most common adverse event, as already seen in the IPF trials, was reported in 75.7% of the patients in the nintedanib group and in 31.6% of those in the placebo group. This rate of adverse events was numerically higher than in the IPF, but exactly in the same range when baseline GI symptoms characteristic for disease involvement in SSc already existing at baseline were subtracted.

Taken together, these results showed that the annual rate of decline in FVC in patients with SSc-ILD was lower with nintedanib than with placebo. However, no clinical benefit of nintedanib was observed for skin fibrosis. These results led very recently (September 2019) to the approval of nintedanib for patients with SSc-ILD by the FDA and is thus the first FDA approved targeted therapy for SSc-ILD. Decisions about registrations in European and Asian countries are foreseen in 2020.

Outlook

While the registration of the first targeted treatment for SSc-ILD might be a breakthrough discovery, it is by far not addressing all unmet needs in SSc. Nintedanib is able to slow progression of SSc-ILD by about 50% in the clinical trial. We still do not have therapies that can halt the progression of fibrosis or even revert it. Precision medicine, the application of specific drugs only to those patients, who will show a response to the specific treatments, e.g. because the targeted pathways are activated, is still far away from clinical practice. Many non-vascular, non-fibrotic manifestations such as fatigue, calcinosis or GI-involvement, greatly affecting patients' daily life when present, are still poorly understood, are not addressed with the novel upcoming therapies and remain largely untreated. The ultimate goal, to prevent onset of the disease rather than treating it when it is present will require a much-improved understanding of the earliest disease stages, which is a focus of current research efforts.

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