The exciting future for scleroderma: What therapeutic pathways are on the horizon?

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Abstract

Emerging evidence shows that a complex interplay between cells and mediators as well as extracellular matrix factors are likely to underlie the development and persistence of fibrosis in systemic sclerosis. Similar processes may determine vasculopathy. This article reviews recent progress in understanding the way in which fibrosis become profibrotic and how the immune system, vascular and mesenchymal compartment interest in disease development. This growing understanding can be exploited in forward translation to identify new targets for therapy. Similarly, early phase trials are informing about pathogenic mechanisms in vivo and reverse translation for observational and randomized trials is allowing hypotheses to be developed and tested. As well as repurposing drugs that are already available, these studies are paving the way for the next generation of targeted therapeutics.

Introduction

There has been major progress in understanding pathogenic mechanisms in systemic sclerosis at a molecular and cellular level. This has permitted key pathways, mediators and cell types that may be central to the disease to be better characterized. The disease can be regarded conceptually as one of dysfunctional connective tissue repair. This may arise due to ongoing injurious mechanisms or could reflect inability to harness the physiological regulators of tissue repair and growth. It seems likely that most of the mechanisms and pathways involved in disease progression are also implicated in normal growth and repair of connective tissue. This adds extra challenges for therapeutics that modify the disease as they might also impair normal wound healing or connective tissue homeostasis. Since SSc is an autoimmune disease a key mechanism that drive the pathology is likely to be immune mediated damage or cellular stimulation. The emerging biology and targets that could be exploited therapeutically are summarized in this article.

Pathways and mechanisms with central roles in fibroblast activation

Fibroblasts as key effector cells of fibrotic tissue remodeling in SSc

Single cell OMIC techniques demonstrated that fibroblasts are a highly heterogeneous population of cells composing of several subpopulations with distinct gene expression profiles and functional roles. Recent work from the Lafyatis laboratory identified 10 subpopulations of fibroblasts in SSc skin [1]. Fibrotic tissues in SSc are characterized by shifts from resting and homeostatic fibroblast subpopulations to inflammatory and profibrotic subpopulations. The proportions of several profibrotic and proinflammatory subpopulations such as COL11A1+, COMP+, PRSS23/SFRP2+, SFRP4/SFRP2+ fibroblasts and CCL19+ fibroblasts positively correlated with clinical and histopathological parameters of skin fibrosis, whereas immature, homeostatic populations such as CXCL12+ and PI16+ fibroblasts inversely correlated with progression of skin fibrosis (Honglin Zhu et al, manuscript submitted). The profibrotic pathogenic subpopulations include so-called myofibroblasts. Myofibroblasts are defined by the expression of contractile proteins such as alpha-smooth muscle actin (αSMA) with the ability to contract tissue. Myofibroblasts release abundant amounts of extracellular matrix (ECM) as well as numerous soluble mediators that maintain a local profibrotic milieu. A variety of different cell types can acquire at least a partial myofibroblast phenotype and may thus contribute to the accumulation of myofibroblasts in fibrotic tissues. These precursor cells include resident fibroblasts (e.g. a subpopulation of resident fibroblasts with high mRNA levels of SFRP2 [1]) and cells of the vascular wall, such as pericytes, endothelial cells and smooth muscle cells,

but also epithelial cells, tissue-resident progenitor populations and bone-marrow-derived fibrocytes that reach fibrotic tissues via the blood stream [2].

The characterization of functionally distinct subpopulations of fibroblasts and myofibroblast precursors may have therapeutic implications. Specific targeting of pathogenic subpopulations may not only provide increased antifibrotic efficacy and may also limit adverse events of antifibrotic therapies as they would not affect homeostatic subpopulations required for homeostasis. However, the molecular mechanisms that promote the expansion of these pathogenic fibroblast subpopulations in SSc require further studies. We are just beginning to understand the transcriptional networks that are active in individual fibroblast subpopulations.

The transcription factor PU.1, a member of the ETS family of transcription factors encoded by the *SPI1* gene, coordinates pro-fibrotic gene expression programs in fibroblasts required for differentiation into profibrotic fibroblast subsets [3]. The expression of PU.1 is upregulated in a subset of profibrotic fibroblasts in SSc, but not in resting fibroblasts. Forced overexpression of PU.1 in proinflammatory and resting fibroblasts converts them into profibrotic fibroblasts with increased expression of contractile proteins and enhanced release of ECM. Vice versa, genetic inactivation of PU.1 or pharmacologic targeting with heterocyclic diamidines enables re-programming of fibrotic fibroblasts into homeostatic fibroblasts with antifibrotic effects across different organs [3]. Although heterocyclic diamidines effectively inhibit PU.1 in vitro and ameliorate fibrosis in murine models, further pharmacological refinement will be required to yield candidates for clinical trials.

The transcription factor Engrailed 1 (EN1), a member of the family of homeodomain-containing transcription factors, is also involved into the differentiation of resting fibroblasts into profibrotic fibroblast subsets in SSc. A subset of fibroblasts in the skin that expressed EN1 during development can give rise to a subpopulation of fibroblasts with high capacity for extracellular matrix (ECM) production that is required for scar formation [4, 5]. Moreover, EN1 amplifies the profibrotic effects of TGFβ in the skin of SSc patients. EN1 is induced in certain fibroblast subpopulations in a TGFβ / SMAD3-dependent manner, and in turn facilitates the transcription of a subset of pro-fibrotic TGFβ target genes to promote ROCK activation, cytoskeleton organization and fibroblast-to-myofibroblast transition. Knockdown of EN1 inhibited fibroblast-to-myofibroblast transition and ameliorated experimental skin fibrosis in murine models and human models of skin fibrosis. Pharmaceutical targeting of EN1 has not been investigated so far but might be achieved by cell permeable-peptides [6].

Nuclear receptors as potential targets for antifibrotic therapies

Nuclear receptors compose a superfamily of transcriptional regulators with 48 members. Several members of the nuclear receptor family have been implicated in the pathogenesis of SSc as they may regulate inflammatory processes, facilitate metabolic adaptation or regulate fibroblast activation. The role of selected nuclear receptors with future potential as therapeutic targets in SSc is discussed below.

NR4A1 (also referred to as Nur77, TR3) signaling is repressed by epigenetic and posttranslational mechanisms in SSc [7]. Under physiologic conditions with short-term upregulation of TGF-β, NR4A1 expression is induced and recruits a repressor complex comprising SP1, SIN3A, CoREST, LSD1, and HDAC1 to limit the transcription of profibrotic genes downstream of TGF-β [7]. However, in SSc and other fibrotic diseases, persistently high levels of TGF-β deactivate this feedback loop; prolonged stimulation of fibroblasts with TGF-β represses NR4A1 signaling by histone deacetylase-induced silencing and phosphorylation-induced inactivation of NR4A1 via AKT kinases [7, 8]. NR4A1 agonists prevent the inactivation of NR4A1 signaling, limit TGF-β-dependent fibroblast activation and exert antifibrotic effects in mouse models of SSc and other fibrotic diseases [7]. However, the pharmacologic profile of the NR4A1 agonist used in these studies (cytosporone B, a natural product) is not suitable for use in humans and the development of potent and selective agonists of NR4A1 remains challenging to date.

Vitamin D receptor (VDR, NR1I1) is also an antifibrotic receptor that could serve as a potential target for antifibrotic therapies. Activated VDR binds to phosphorylated SMAD3 to inhibit TGF-β-SMAD-signaling and fibroblast-to-myofibroblast transition [9]. In SSc, however, the expression of VDR is decreased in the skin of patients with SSc [9] and additionally vitamin D deficiency is common in SSc and other chronic diseases. The resulting downregulation of VDR signaling fosters fibroblast activation. VDR signaling can be activated by synthetic VDR agonists, which ameliorate TGF-β-induced fibroblast activation and tissue fibrosis. Multiple highly potent agonists of VDR are approved for clinical use and could be tested in SSc.

Other nuclear receptors that have been implicated into the pathogenesis of SSc include the constitutive androstane receptor (CAR)/NR1I3, the liver X receptors (LXRs) and the pregnane X receptor (PXR)/NR1I2. CAR is thought to directly regulate fibroblast activation, whereas LXR and PXR may rather regulate the release of profibrotic mediators from macrophages or T cells, respectively. CAR is a profibrotic nuclear receptor. Its activation by treatment with CAR agonists fosters activation of canonical TGFβ signaling and exacerbates experimental fibrosis [10]. In contrast, LXRs limit macrophage activation and cytokine release and treatment with LXR agonists reduces macrophage influx and IL-6 release in murine models of SSc [11]. As for LXR, PXR activation also did not demonstrate direct inhibitory effects on fibroblasts, but

inhibited the release of IL-13 from Th2 cells to ameliorate fibrosis in inflammatory mouse models of SSc [12].

Targeting the reactivation of developmental pathways in SSc

Several lines of evidence in complementary models demonstrate that hedgehog- and WNT signaling are central pathways of fibroblast activation in SSc and other fibrotic diseases [13-22]. These pathways are essentially required for embryonic development and are thus referred to as developmental pathways. After embryonic development, these pathways are silenced in most cell types except rapidly cycling stem cells. However, hedgehog signaling and WNT signaling can be reactivated upon injury to promote proliferation and differentiation of target cells. Persistent activation of those pathways in fibroblasts drive fibroblast-to-myofibroblast differentiation and fibrotic tissue remodeling.

The expression of the ligand sonic hedgehog (SHH) is upregulated in the skin of patients with SSc with consistent accumulation of the downstream transcription factor GLI2 [23, 24]. Moreover, SHH levels are increased in the blood of SSc patients [23]. This upregulation might at least in part be mediated by TGF-β as TGF-β induces the expression of SHH and of GLI2 in fibroblasts [24]. Activation of hedgehog signaling stimulates fibroblast-to-myofibroblast transition and promotes experimental skin fibrosis [24], whereas pharmacologic or genetic inactivation of hedgehog signaling e.g. by selective genetic or pharmacologic inactivation of GLI2 or by treatment with inhibitors of the receptor Smoothened ameliorates experimental fibrosis in murine models of SSc [25].

β-catenin-dependent WNT signaling, also referred to as canonical WNT signaling, is active in and multiple other fibrotic diseases. Activation of canonical WNT signaling occurs as a consequence of deregulation on multiple levels with upregulation of WNT proteins, downregulation of endogenous WNT inhibitors and by transcriptional synergism with other profibrotic mediators [17, 18, 26-30]. As for hedgehog signaling, TGF-β can activate canonical WNT signaling, i. p. by epigenetic downregulation of the expression of endogenous WNT antagonists such as dickkopf-1 (DKK1) or secreted frizzled-related protein-1 (SFRP1) [31-33]. Canonical WNT signaling is sufficient and required for fibrotic tissue remodeling and targeted inhibition of WNT signaling exerts potent antifibrotic effects in various preclinical models of SSc and other fibrotic diseases [3, 17, 21, 22, 26, 28, 31, 34-40].

Despite their crucial role in embryonic development and stem cell maintenance, hedgehog and WNT signaling are both assessable for pharmacologic intervention. For hedgehog signaling, Smoothened inhibitors are already in clinical use for neoplastic diseases and GLI2 inhibitors are clinical development [41]. Compounds with WNT inhibitory activity such as pyrvinium are

also in clinical use and more selective and potent WNT inhibitors such as porcupine- or tankyrase inhibitors are in clinical development. Indeed, clinical trials with WNT targeting strategies are currently in preparation for interstitial lung diseases and are also discussed for sclerodermatous chronic graft-versus-host diseases. However, potential concerns of these approaches may include toxicity associated with impaired regeneration of stem cells upon long-term treatment. Given the crucial roles of WNT and hedgehog signaling in stem cell regeneration, specific strategies might be required to minimize the effects on the stem cell compartment associated with long-term use.

Epigenetic changes and the establishment of a profibrotic tissue memory

The chronic profibrotic milieu in affected tissues of SSc patients induces epigenetic modifications in fibroblasts[42-44]. These epigenetic modifications consolidate an activated myofibroblast phenotype and render them at least in part independent of external stimuli. Epigenetic modifications establish self-sustaining activation loops to promote chronic fibroblast activation and progressive fibrotic tissue remodeling in SSc. This is best evidenced by the activated phenotype of SSc fibroblasts even upon long-term culture: Fibroblasts explanted from fibrotic skin of SSc patients exert a myofibroblast-like phenotype with increased expression of contractile proteins and enhanced release of collagen, which persists for several passages *in vitro*. Epigenetic modifications are critical to maintain the profibrotic phenotype of SSc fibroblasts. This stabilization of an activated phenotype by epigenetic modifications is often referred to as profibrotic tissue memory. The tissue memory is encoded by a complex pattern of different epigenetic alterations. Epigenetic alterations including DNA methylation, histone acetylation, histone methylation, bromodomain (BRD) dependent regulation and non-coding RNAs such as microRNAs (miRNAs) or long non-coding RNAs (lncRNAs) are well established as drivers of progressive fibrotic tissue remodeling in SSc, but also in other fibrotic diseases [45-52] [48, 53]. Selected examples and potential approaches for therapeutic intervention are discussed below.

DNA methylation

DNA can be methylated at position C5 of the pyrimidine ring of cytosine by a family of three DNA methyltransferases (DNMTs): DNMT1, DNMT3A and DNMT3B [54]. Methylation of cytosine residues generates binding sites for methyl-CpG-binding domain (MBD) proteins, in particular when methylated cytosine residues are clustered in so-called CpG islands. Binding of MBD proteins promotes the recruitment of repressor complexes to silence transcription of

the associated genes [55]. Several studies demonstrated a role of altered DNA methylation in fibrotic diseases including SSc [35, 48, 56-58]. The first and best studied target regulated by DNA methylation in SSc is the Friend leukemia integration factor 1 (FLI1) gene, which encodes for a transcription factor of the ETS family [48, 59, 60]. FLI1 limits TGF-β-signaling to inhibit fibroblast activation under homeostatic conditions [61]. However, in the profibrotic milieu of SSc, FLI1 expression and activity are repressed by epigenetic and posttranslational mechanisms. TGF-β induces DNA methylation of the FLI1 promoter to silence its expression and also promotes FLI1 degradation via PKCδ-mediated phosphorylation [62]. Moreover, DNMT-induced silencing of the suppressor of cytokine signaling 3 (SOCS3) facilitates prolonged activation of JAK2 / STAT3 signaling to facilitate TGF-β-induced fibroblast activation (PMID: 31990678). Aberrant DNA methylation also facilitates activation of canonical WNT signaling by silencing of the endogenous WNT antagonists Dickkopf-1 (DKK1) and secreted frizzled-related protein 1 (SFRP1) [35]. Treatment with the DNMT inhibitor 5-aza-2'-deoxycytidine (5aza), which is in clinical use for myelodysplastic syndromes, has exerted antifibrotic effects in murine models of SSc and other fibrotic diseases [35, 46, 63]. As treatment with 5aza is relatively well tolerated, 5aza may offer potential for testing in clinical trials in SSc.

Histone acetylation

Histone modifications include acetylation and methylation at various sites. First evidence for a role of histone modulations in the pathogenesis of SSc was provided by the observation that treatment with histone deacetylation inhibitors reduced the activation of SSc fibroblasts and ameliorated bleomycin-induced skin fibrosis [64]. As HDAC inhibitors such as SAHA are in clinical use for malignant diseases, targeting of HDACs may offer therapeutic potential for SSc.

Follow-up studies revealed that the expression of the profibrotic transcription factor PU.1 (see above) is also controlled by a complex network of epigenetic mechanisms that include histone modifications [3]. In resting fibroblasts, PU.1 expression is silenced and the promoter and the upstream regulatory element of the PU.1 locus is dominated by repressive H3K9me3 and H3K27me3 marks. In fibrotic environments, however, the upstream regulatory element of the PU.1 locus becomes permissive with increased H3K27 acetylation and loss of H3K9me3 and H3K27me3. These epigenetic alterations at the PU.1 locus promote expression of PU.1 protein in fibrotic fibroblasts. As discussed above, PU.1 inhibitors with improved pharmacological profile are currently in development.

Moreover, histone acetylation at H4K16 has recently been shown to modulate the outcome of fibrotic diseases by fine-tuning autophagy [65]. Autophagy describes the catabolic cellular

process of degradation of unnecessary or dysfunctional cellular organelles in particular during starvation or in response to cellular stress [66]. However, components of the autophagy machinery are also involved in unconventional secretion of proteins [67-69]. Autophagy is activated in a TGFβ-dependent manner in SSc fibroblasts [65]. TGFβ represses the expression of the H4K16 histone acetyltransferase MYST1 via SMAD3-dependent mechanisms to promote the expression of core components of the autophagy machinery. The resulting increase the autophagic flux induces activation of human dermal fibroblasts and fibrosis in murine skin and lungs. Re-establishment of the epigenetic control of autophagy by forced expression of MYST1 in fibroblasts impairs myofibroblast differentiation and ameliorates experimental dermal and pulmonary fibrosis. However, pharmaceutical approaches to selectively promote MYST1 activation are currently not available and further studies are required how to best transfer these findings from bench to bedside.

Other epigenetic modifications: miRNAs and BRDs

miRNAs are small non-coding RNAs. Binding of miRNAs to their respective target mRNAs promotes degradation of target mRNAs [70]. More than 50 miRNAs have been implicated in the pathogenesis of fibrotic diseases [70]. Most of these miRNAs are expressed in a highly cell-specific and/or context-specific manner. However, miR-21 and miR-29 are broadly expressed miRNAs that have been implicated in fibrotic remodeling of multiple organs and might thus be particularly relevant for a multi-systemic disease such as SSc. TGF-β induces the expression of miR-21, which in turn downregulates SMAD7 to promote canonical TGF-β signaling[71]. Antagomirs against miR-21 attenuated experimental pulmonary, myocardial and renal fibrosis [71-73]. In contrast to miR-21, miR-29 is an antifibrotic miRNA that is downregulated in fibrotic diseases including SSc. miR-29 inhibits the translation of multiple collagen genes and of several enzymes involved in ECM turnover [74]. miR-29 mimics may thus offer potential for the treatment of SSc and other fibrotic diseases. Of note, miRNA-based therapies are currently evaluated for the treatment of cardiac fibrosis.

Bromodomain proteins (BRDs) bind acetylated lysines in histone tails and regulate gene transcription by the recruitment of molecular partners. BRDs have recently been implicated in aberrant fibroblast activation in SSc. Chromatin accessibility and transcriptome profiling revealed constitutive activation of a TGF-β2 enhancer in SSc fibroblasts [75]. The constitutive activation of this enhancer required BRD4 and targeted inhibition of BRD4 reduced the TGFB2 enhancer activity and the expression of profibrotic TGF-β2 target genes. Moreover, small molecule inhibitors of BRD2/4 ameliorated experimental fibrosis in preclinical models of SSc (unpublished data). BRD inhibitors are actively investigated in multiple clinical programs for

malignant diseases. Although these studies faced obstacles including toxicity, modified application schemes, e.g. with intermittent dosing, may limit concerns and offer opportunities for BRD inhibitors in fibrotic diseases.

Extracellular ligand-receptor targeting of fibrosis in systemic sclerosis

Targeting the extracellular space in systemic sclerosis

Biological therapies have transformed outcomes in immune mediated inflammatory diseases by harnessing the specificity and binding affinity of antibody molecules or soluble receptors to trap or block the biological effects of pathogenic cytokines. This was first demonstrated in rheumatoid arthritis using monoclonal antibodies that bound to TNFalpha and later with recombinant receptors fused to IgG heavy chains or other molecules. This approach has proven safe and effective and has been applied to many different clinical settings for TNFalpha and other ligands. As well as providing effective therapeutics this has provided important insight into pathogenesis and permitted exciting reverse translational studies.

Some of the established biological agents have been applied to different diseases including systemic sclerosis and have demonstrated some efficacy. This has included targeting cell types as well as cytokines and growth factors.

In addition to targeting cells and cytokines or growth factors though binding to ligand or receptor there is the possibility to bind other extracellular or matricellular proteins and this is an area of current investigations. Systemic sclerosis represents dysfunction or dysregulated connective tissue repair, and this may be normalised by modifying the cellular microenvironment through binding to extracellular proteins that are important regulators of fibroblast activation and differentiation as outline earlier in this review article.

Cytokines, growth factors, and matrix proteins in SSc pathogenesis

Cellular networks and fibroblasts activation by cytokines and ECM proteins

The hallmark pathological processes in systemic sclerosis are fibrosis and structural vasculopathy. These are likely to reflect the processes that are normally recruited for tissue repair and wound healing. A plausible explanation for the detrimental changes that develop and persist in SSc is that these usually coordinated and self-limiting biological processes occur excessively and are not appropriately resolved. This is likely to reflect imbalance between profibrotic and pro-resolution pathways and mediators. Much of this regulation is likely to occur

in the extracellular space and involve cytokines, growth factors their receptors and associated matricellular proteins. It is notable that many of these entities are identified as hallmarks of the SSc disease phenotype in recent gene and protein expression studies [76]. It is therefore logical to target these extracellular proteins using biological therapeutics or small molecule inhibitors. Some of the candidates that have been tested or are emerging in SSc are summarised below.

Targeting cytokines and receptors

TNFalpha.

Although targeting TNFalpha was enormously effective as a treatment for inflammatory disease including rheumatoid arthritis, seronegative spondylarthritis and inflammatory bowel disease it has not been shown to be very effective in fibrotic disease [77]. This may reflect that it is a less critical driver in diseases that are less characterised by persistent inflammation or that pathways are redundant. A review of use did not suggest major benefit and a small open label clinical trial pointed towards only modest improvements that did not reach statistical significance [78].

IL6

Although also considered a proinflammatory mediator, IL6 was first defined as a lymphocyte regulator especially important for B cells [79]. It appears to have a much broader role and it was reported that patients with elevated IL6 levels in the circulation had a worse outcome [80]. In addition, the markers for elevated IL6 activity including acute phase markers appeared to predict poor outcome in several observational studies [81]. The availability of tocilizumab as an inhibitor of both cis and trans signalling was attractive for clinical evaluation and a phase 2 trials (faSScinate) was supportive [82]. The subsequent phase 3 trial that included milder skin disease showed only a trend of benefit for skin but a very convincing signal for clinically meaningful impact on lung fibrosis [83]. Indeed, the worsening of lung function over 48 weeks was essentially prevented at a group level in the trial. This led to FDA approval for tocilizumab as a treatment to reduce worsening of lung function in SSc. Parallel mechanistic studies form the phase 2 trial highlighted that the activated phenotype of explant cultured fibroblasts could eb almost entirely reversed after 6 months treatment with tocilizumab [84]. This is notable as the clinical impact on skin was just a trend of benefit suggesting that other mechanisms or fibroblast populations may be important in determining the outcome and progression of skin fibrosis but that in early-stage lung fibrosis in SSc the population of fibroblasts that are attenuated but tocilizumab are critical.

IL4/IL13

There are multiple ways in which IL4 and IL13 may be implicated in pathogenesis of SSc [85]. This included effects on fibroblast, perhaps in concert with TGFbeta and driven by IL13 as well as key roles related to immune cell activation [86]. In addition, these cytokines are critical for macrophage polarisation an in vitro can induce and M2 like phenotype that is considered pro-fibrotic [87]. It is encouraging that a phase 2 trial of romilkimab, a novel bispecific antibody targeting IL4 and IL13 was positive with greater improvement in mRSS and some encouraging signals in other relevant endpoints [88]. This requires further confirmation.

Targeting the adaptive immune system

Autologous stem cell transplantation (ASCT) as maximal immunosuppressive therapy has shown to improve skin sclerosis, quality of live and predicted forced vital capacity, a surrogate marker for interstitial lung disease and/or lung fibrosis, and 5-years survival rate. So far, this is the most effective therapy in SSc as shown by three different studies. However, as recently elucidated for patients fulfilling the criteria to be eligible for ASCT, overall, 10-years survival by ASCT is nowadays equivalent to the current best clinical practice indicating that less aggressive therapies are currently successfully been applied [89]. Transfer of peripheral blood mononuclear cells (PBMCs) from SSc patients induce interstitial lung disease as well as inflammation of other organs, which is not present when PBMCs were transferred from rituximab treated patients [90]. Those studies indicate an important role of B cells at least in the early inflammatory part of SSc. In line with this, a recent placebo-controlled phase II study on patients with early diffuse SSc patients revealed improvement of both the predicted FVC and skin fibrosis as assessed by the modified Rodnan skin score (mRSS) [91].

As well as the attenuation of agonist antibodies that may be pathogenic. there is now convincing support for targeting B cells in SSc. This is based upon biological studies of the disease that highlight the potential role of abnormal B cells, especially transitional cell populations that may demonstrate a failure of peripheral tolerance [92].

The putative role of agonistic autoantibodies in pathogenesis have now been further appreciated, having first been suggested some years previously [93]. Transfer for IgG purified from SSc patients induced interstitial lung disease and obliterative vasculopathy in mice suggesting the potent role of antibodies in the development of inflammation and vasculopathy in SSc [94]. In vitro, purified IgG from SSc patients induced an inflammatory and fibrotic proteome in monocytic cells lines and induced proteins show associations with the mRSS of the corresponding donors. Specifically, antibodies induced several cytokines and chemokines known to be important biomarkers for ILD in SSc [95]. The transfer of fibrotic signals by IgG from SSc patients also induced a fibrotic transcriptome and proteome in fibroblasts also

supporting the ability of IgG to induce disease mechanisms [96]. Indeed, very recent studies identified antibodies directed to the angiotensin receptor type-1 (AT1R) as causative factors for interstitial lung disease and skin fibrosis (Yue). The generation of these abs require T cell help also supporting the use of immunosuppressive therapies in the therapy of SSc. In vitro, these abs induced TGFß ad adhesion molecules in endothelial cells, collagen-1 in fibroblasts and CCL18 or other cytokines in monocytes. AT1R abs often correlate with antibodies directed to the endothelin receptor type-1 (ETAR).

Other natural regulatory antibodies such as antibodies against the thrombin receptor 1 (PAR-1) were recently show to biological activity which further supports the potential for targeting antibodies therapeutically**.**

The use of B cell depleting treatments is further supported by a multi-centre cohort analysis [97] and by a recent meta-analysis [98].

Costimulatory molecules

Abatacept, a fusion protein composed of the Fc region of the immunoglobulin IgG1 fused to the extracellular domain of CTLA-4 has been tested in a small phase 2 trial and showed only a trend of benefit at a group level [99]. However certain subgroups of patients appeared to be driving the effect of abatacept on mRSS and this was especially in those patients with an inflammatory gene expression signature in their skin biopsy [100]. More encouragingly, all patients showed a meaningful impact on HAQ-DI. This was a phase 2 study, but it seems likely that if a phase 3 trial confirmed the results for HAQ-DI that this is a treatment that might be very helpful for some patients with SSc.

Future candidates for targeted therapy in the extracellular compartment

There are a number or promising future candidates for targeting extracellular proteins and ligands. These are likely to emerge based on current studies and preclinical and supportive translational data.

OSM

Despite strong theoretical rationale [101], a well-conducted phase IIa trial of anti-OSM has recently been completed and unfortunately did not show evidence of benefit in SSc based upon prespecified markers of biological or clinical effect. This was an early-stage study that was primarily testing the safety of the antibody and there was meaningful impact on

haematological parameters including anaemia and thrombocytopenia I some cases. On this basis it is not being pursued further as a treatment for systemic sclerosis [102, paper in press].

CTGF

This prototypic member of the CCN family of matricellular proteins has long been a hallmark marker of the profibrotic SSc fibroblast phenotype [103]. It appears to promote or augment fibrosis and may work as a downstream mediator or co-factor related to TGFbeta [104]. There have bene encouraging phase 2 data for targeting CTGF in IPF and in the future it may be a promising target in SSc [105].

S100A4

This protein was originally identified as a fibroblast expressed protein marker (FSP-1) based on differential display data for cells undergoing epithelial-mesenchymal transition [106]. It is now clear that this protein is a member of the S100 protein family [107] is expressed by several cell types and that it is an important extracellular matrix protein that can activate innate immune pathways [108]. Promising preclinical data support its potential as a target for treatment for fibrosis and in SSc [109].

Interferon

An elevated IFN signature is seen in SSc as well as many other rheumatic diseases [110]. Although not present in all patients it seems to be associated with poor outcome and with an overlap phenotype [111]. Since targeting IFN using anifrolumab is now an approved treatment for SLE [112] and because early phase studies showed attenuation often IFN signature in SSc patients this is a potential treatment for evaluation in SSc [113].

TGFbeta

This profibrotic master regulator is an obvious candidate for ligand directed therapy in SSc and other rooms of fibrosis [114]. However, the very broad roles in connective tissue and immunological development and homeostasis together with potential roles in protecting for neoplastic transformation have raised legitimate concerns about targeting TGFbeta and the balance between risk and benefit [115]. The first trials using weak monospecific antibody against TGFbeta1 suggested safe but showed no signal of efficacy [116]. An open label study of fresolimumab was more encouraging [117]. There are several ongoing trials evaluating TGFbeta directed antibodies or ligand traps. The recent promising data for PAH targeting active pathways provide support for the potential of this approach [118] and the results of ongoing trials are eagerly awaited.

Reverse translation and insights into pathogenesis

Trials of targeted treatment offers a unique platform for experimental medicine studies as was shown for tocilizumab and these give real insights into treatment mechanism, target engagement and biology of SSc. There have been exciting results from studies and insights that may allow more targeted or stratified approaches in future trials to advance clinical trial development and pave the way for a more stratified approach to clinical practice.

Preclinical models

It has proven challenging to use preclinical models to conform treatment benefit, but they are very valuable in highlighting clinical potential and identifying the stage and type of SSc that may be most informative in a subsequent clinical trial.

Concluding remarks

It is an exciting time for SSc therapeutics because the benefits of background treatments are being established and so it is possible to design trials that add more targeted approaches on top of standard immunosuppression. The trials or nintedanib and romilkimab are excellent examples of this approach and it has been helpful in confirming the benefits from standard treatments as well as additive value of new drugs. The powerful effect of HSCT provides a gold standard of what can be achieved therapeutically, albeit with a mortality that has high morbidity and treatment related mortality. In addition, it is unsuitable for many cases and indeed those most in need are excluded from current trail protocols despite having a demonstrably worse outcome than those that are eligible. In the future it is a legitimate goal to achieve the therapeutic impact of HSCT through combination targeted therapies with much less treatment related toxicity. Emerging data suggest that an individual approach may be rewired and that tools such as skin subset, disease duration and ANA profile as well as skin biopsy intrinsic subset or peripheral blood IFN signature may be valuable tools in developing an optical approach to treatment for SSc.

References

1. Tabib T, Huang M, Morse N, Papazoglou A, Behera R, Jia M, et al. Myofibroblast transcriptome indicates SFRP2(hi) fibroblast progenitors in systemic sclerosis skin. Nature communications. 2021 Jul 19; 12(1):4384.
2. McAnulty RJ. Fibroblasts and myofibroblasts: their source, function and role in disease. The international journal of biochemistry & cell biology. 2007; 39(4):666-671.
3. Wohlfahrt T, Rauber S, Uebe S, Luber M, Soare A, Ekici A, et al. PU.1 controls fibroblast polarization and tissue fibrosis. Nature. 2019 Feb; 566(7744):344-349.
4. Rinkevich Y, Walmsley GG, Hu MS, Maan ZN, Newman AM, Drukker M, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science. 2015 Apr 17; 348(6232):aaa2151.
5. Jiang D, Correa-Gallegos D, Christ S, Stefanska A, Liu J, Ramesh P, et al. Two succeeding fibroblastic lineages drive dermal development and the transition from regeneration to scarring. Nat Cell Biol. 2018 Apr; 20(4):422-431.
6. Beltran AS, Graves LM, Blancafort P. Novel role of Engrailed 1 as a prosurvival transcription factor in basal-like breast cancer and engineering of interference peptides block its oncogenic function. Oncogene. 2014 Sep 25; 33(39):4767-4777.
7. Palumbo-Zerr K, Zerr P, Distler A, Fliehr J, Mancuso R, Huang JG, et al. Orphan nuclear receptor NR4A1 regulates transforming growth factor-beta signaling and fibrosis. Nature medicine. 2015 Feb; 21(2):62-70.
8. Chen HZ, Liu QF, Li L, Wang WJ, Yao LM, Yang M, et al. The orphan receptor TR3 suppresses intestinal tumorigenesis in mice by downregulating Wnt signalling. Gut. 2012 May; 61(5):714-724.
9. Zerr P, Vollath S, Palumbo-Zerr K, Tomcik M, Huang JG, Distler A, et al. Vitamin D receptor regulates TGF-beta signalling in systemic sclerosis. Annals of the Rheumatic Diseases. 2015 Mar; 74(3).
10. Avouac J, Palumbo-Zerr K, Ruzehaji N, Tomcik M, Zerr P, Dees C, et al. The nuclear receptor constitutive androstane receptor/NR1I3 enhances the profibrotic effects of transforming growth factor beta and contributes to the development of experimental dermal fibrosis. Arthritis & rheumatology. 2014 Nov; 66(11):3140-3150.
11. Beyer C, Huang J, Beer J, Zhang Y, Palumbo-Zerr K, Zerr P, et al. Activation of liver X receptors inhibits experimental fibrosis by interfering with interleukin-6 release from macrophages. Ann Rheum Dis. 2015 Jun; 74(6):1317-1324.
12. Beyer C, Skapenko A, Distler A, Dees C, Reichert H, Munoz L, et al. Activation of pregnane X receptor inhibits experimental dermal fibrosis. Ann Rheum Dis. 2013 Apr; 72(4):621-625.
13. Horn A, Kireva T, Palumbo-Zerr K, Dees C, Tomcik M, Cordazzo C, et al. Inhibition of hedgehog signalling prevents experimental fibrosis and induces regression of established fibrosis. Ann Rheum Dis. 2012 May; 71(5):785-789.
14. Lam AP, Flozak AS, Russell S, Wei J, Jain M, Mutlu GM, et al. Nuclear beta-catenin is increased in systemic sclerosis pulmonary fibrosis and promotes lung fibroblast migration and proliferation. Am J Respir Cell Mol Biol. 2011 Nov; 45(5):915-922.
15. Horn A, Palumbo K, Cordazzo C, Dees C, Akhmetshina A, Tomcik M, et al. Hedgehog signaling controls fibroblast activation and tissue fibrosis in systemic sclerosis. Arthritis Rheum. 2012 Aug; 64(8):2724-2733.
16. Dees C, Tomcik M, Zerr P, Akhmetshina A, Horn A, Palumbo K, et al. Notch signalling regulates fibroblast activation and collagen release in systemic sclerosis. Ann Rheum Dis. 2011 Jul; 70(7):1304-1310.
17. He W, Dai C, Li Y, Zeng G, Monga SP, Liu Y. Wnt/beta-catenin signaling promotes renal interstitial fibrosis. J Am Soc Nephrol. 2009 Apr; 20(4):765-776.

18. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A, et al. WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. J Clin Invest. 2009 Apr; 119(4):772-787.
19. Guan S, Zhou J. Frizzled-7 mediates TGF-beta-induced pulmonary fibrosis by transmitting non-canonical Wnt signaling. Experimental cell research. 2017 Oct 01; 359(1):226-234.
20. Saito A, Nagase T. Hippo and TGF-beta interplay in the lung field. Am J Physiol Lung Cell Mol Physiol. 2015 Oct 15; 309(8):L756-767.
21. Burgy O, Konigshoff M. The WNT signaling pathways in wound healing and fibrosis. Matrix biology : journal of the International Society for Matrix Biology. 2018 Aug; 68-69:67-80.
22. Zhang Y, Shen L, Dreißigacker K, Zhu H, Trinh-Minh T, Meng X, et al. Targeting of canonical WNT signaling ameliorates experimental sclerodermatous chronic graft-versus-host disease. Blood. 2021 Apr 29; 137(17):2403-2416.
23. Beyer C, Huscher D, Ramming A, Bergmann C, Avouac J, Guiducci S, et al. Elevated serum levels of sonic hedgehog are associated with fibrotic and vascular manifestations in systemic sclerosis. Ann Rheum Dis. 2017 May 11.
24. Horn A, Palumbo K, Cordazzo C, Dees C, Akhmetshina A, Tomcik M, et al. Hedgehog signaling controls fibroblast activation and tissue fibrosis in systemic sclerosis. Arthritis and Rheumatism. 2012 Aug; 64(8):2724-2733.
25. Liang R, Šumová B, Cordazzo C, Mallano T, Zhang Y, Wohlfahrt T, et al. The transcription factor GLI2 as a downstream mediator of transforming growth factor-β-induced fibroblast activation in SSc. Ann Rheum Dis. 2017 Apr; 76(4):756-764.
26. Wei J, Melichian D, Komura K, Hinchcliff M, Lam AP, Lafyatis R, et al. Canonical Wnt Signaling Induces Skin Fibrosis and Subcutaneous Lipoatrophy A Novel Mouse Model for Scleroderma? Arthritis and Rheumatism. 2011 Jun; 63(6):1707-1717.
27. Konigshoff M, Balsara N, Pfaff EM, Kramer M, Chrobak I, Seeger W, et al. Functional Wnt Signaling Is Increased in Idiopathic Pulmonary Fibrosis. PLoS One. 2008 May; 3(5).
28. Cheng JH, She HY, Han YP, Wang JH, Xiong SG, Asahina K, et al. Wnt antagonism inhibits hepatic stellate cell activation and liver fibrosis. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2008 Jan; 294(1):G39-G49.
29. He W, Zhang LN, Ni AG, Zhang ZP, Mirotsou M, Mao L, et al. Exogenously administered secreted frizzled related protein 2 (Sfrp2) reduces fibrosis and improves cardiac function in a rat model of myocardial infarction. Proceedings of the National Academy of Sciences of the United States of America. 2010 Dec; 107(49):21110-21115.
30. Trensz F, Haroun S, Cloutier A, Richter MV, Grenier G. A muscle resident cell population promotes fibrosis in hindlimb skeletal muscles of mdx mice through the Wnt canonical pathway. American Journal of Physiology-Cell Physiology. 2010 Nov; 299(5):C939-C947.
31. Akhmetshina A, Palumbo K, Dees C, Bergmann C, Venalis P, Zerr P, et al. Activation of canonical Wnt signalling is required for TGF-beta-mediated fibrosis. Nature Communications. 2012 Mar; 3.
32. Chen JH, Chen WLK, Sider KL, Yip CYY, Simmons CA. beta-Catenin Mediates Mechanically Regulated, Transforming Growth Factor-beta 1-Induced Myofibroblast Differentiation of Aortic Valve Interstitial Cells. Arteriosclerosis Thrombosis and Vascular Biology. 2011 Mar; 31(3):590-597.
33. Sato M. Upregulation of the Wnt/beta-catenin pathway induced by transforming growth factor-beta in hypertrophic scars and keloids. Acta Dermato-Venereologica. 2006; 86(4):300-307.
34. Chen CW, Beyer C, Liu J, Maier C, Li C, Trinh-Minh T, et al. Pharmacological inhibition of porcupine induces regression of experimental skin fibrosis by targeting Wnt signalling. Annals of the Rheumatic Diseases. 2017 Apr; 76(4).
35. Dees C, Schlottmann I, Funke R, Distler A, Palumbo-Zerr K, Zerr P, et al. The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. Ann Rheum Dis. 2014 Jun; 73(6):1232-1239.

36. Beyer C, Reichert H, Akan H, Mallano T, Schramm A, Dees C, et al. Blockade of canonical Wnt signalling ameliorates experimental dermal fibrosis. Annals of the Rheumatic Diseases. 2013 Jul; 72(7):1255-1258.
37. Beyer C, Schramm A, Akhmetshina A, Dees C, Kireva T, Gelse K, et al. beta-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. Annals of the Rheumatic Diseases. 2012 May; 71(5):761-767.
38. Bergmann C, Akhmetshina A, Dees C, Palumbo K, Zerr P, Beyer C, et al. Inhibition of glycogen synthase kinase 3 beta induces dermal fibrosis by activation of the canonical Wnt pathway. Annals of the Rheumatic Diseases. 2011 Dec; 70(12):2191-2198.
39. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007 Aug; 317(5839):807-810.
40. Wei J, Fang F, Lam AP, Sargent JL, Hamburg E, Hinchcliff ME, et al. Wnt/beta-catenin signaling is hyperactivated in systemic sclerosis and induces Smad-dependent fibrotic responses in mesenchymal cells. Arthritis and Rheumatism. 2012 Aug; 64(8):2734-2745.
41. Rimkus TK, Carpenter RL, Qasem S, Chan M, Lo HW. Targeting the Sonic Hedgehog Signaling Pathway: Review of Smoothened and GLI Inhibitors. Cancers. 2016 Feb; 8(2).
42. Watson CJ, Collier P, Tea I, Neary R, Watson JA, Robinson C, et al. Hypoxia-induced epigenetic modifications are associated with cardiac tissue fibrosis and the development of a myofibroblast-like phenotype. Human molecular genetics. 2014 Apr 15; 23(8):2176-2188.
43. Beyer C, Schett G, Gay S, Distler O, Distler JH. Hypoxia. Hypoxia in the pathogenesis of systemic sclerosis. Arthritis Res Ther. 2009; 11(2):220.
44. Parker MW, Rossi D, Peterson M, Smith K, Sikstrom K, White ES, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. J Clin Invest. 2014 Apr; 124(4):1622-1635.
45. Mann J, Oakley F, Akiboye F, Elsharkawy A, Thorne AW, Mann DA. Regulation of myofibroblast transdifferentiation by DNA methylation and MeCP2: implications for wound healing and fibrogenesis. Cell Death Differ. 2007 Feb; 14(2):275-285.
46. Bechtel W, McGoohan S, Zeisberg EM, Muller GA, Kalbacher H, Salant DJ, et al. Methylation determines fibroblast activation and fibrogenesis in the kidney. Nat Med. 2010 May; 16(5):544-550.
47. King TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet. 2011 Dec; 378(9807):1949-1961.
48. Wang Y, Fan PS, Kahaleh B. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. Arthritis Rheum. 2006 Jul; 54(7):2271-2279.
49. Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I, et al. TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. Nature Cell Biology. 2009 Jul; 11(7):881-U263.
50. Montgomery RL, Yu G, Latimer PA, Stack C, Robinson K, Dalby CM, et al. MicroRNA mimicry blocks pulmonary fibrosis. EMBO molecular medicine. 2014 Oct; 6(10):1347-1356.
51. Zeisberg EM, Zeisberg M. The role of promoter hypermethylation in fibroblast activation and fibrogenesis. J Pathol. 2013 Jan; 229(2):264-273.
52. Mann J, Mann DA. Epigenetic regulation of wound healing and fibrosis. Current opinion in rheumatology. 2013 Jan; 25(1):101-107.
53. Altorok N, Tsou PS, Coit P, Khanna D, Sawalha AH. Genome-wide DNA methylation analysis in dermal fibroblasts from patients with diffuse and limited systemic sclerosis reveals common and subset-specific DNA methylation aberrancies. Ann Rheum Dis. 2015 Aug; 74(8):1612-1620.
54. Razin A, Riggs AD. DNA methylation and gene function. Science. 1980 Nov 7; 210(4470):604-610.
55. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature. 1998 May 28; 393(6683):386-389.

56. Chen X, Li WX, Chen Y, Li XF, Li HD, Huang HM, et al. Suppression of SUN2 by DNA methylation is associated with HSCs activation and hepatic fibrosis. Cell death & disease. 2018 Oct 3; 9(10):1021.
57. Sanders YY, Pardo A, Selman M, Nuovo GJ, Tollefsbol TO, Siegal GP, et al. Thy-1 promoter hypermethylation: a novel epigenetic pathogenic mechanism in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2008 Nov; 39(5):610-618.
58. Zhang Y, Potter S, Chen CW, Liang R, Gelse K, Ludolph I, et al. Poly(ADP-ribose) polymerase-1 regulates fibroblast activation in systemic sclerosis. Ann Rheum Dis. 2018 May; 77(5):744-751.
59. Noda S, Asano Y, Nishimura S, Taniguchi T, Fujiu K, Manabe I, et al. Simultaneous downregulation of KLF5 and Fli1 is a key feature underlying systemic sclerosis. Nature communications. 2014 Dec 12; 5:5797.
60. Asano Y, Bujor AM, Trojanowska M. The impact of Fli1 deficiency on the pathogenesis of systemic sclerosis. Journal of Dermatological Science. 2010 Sep; 59(3):153-162.
61. Asano Y, Trojanowska M. Fli1 represses transcription of the human alpha2(I) collagen gene by recruitment of the HDAC1/p300 complex. PLoS One. 2013; 8(9):e74930.
62. Asano Y, Czuwara J, Trojanowska M. Transforming growth factor-beta regulates DNA binding activity of transcription factor fli1 by p300/CREB-binding protein-associated factor-dependent acetylation. Journal of Biological Chemistry. 2007 Nov; 282(48):34672-34683.
63. Zhao S, Cao M, Wu H, Hu Y, Xue X. 5-aza-2'-deoxycytidine Inhibits the Proliferation of Lung Fibroblasts in Neonatal Rats Exposed to Hyperoxia. Pediatrics and neonatology. 2017 Apr; 58(2):122-127.
64. Huber LC, Distler JH, Moritz F, Hemmatazad H, Hauser T, Michel BA, et al. Trichostatin A prevents the accumulation of extracellular matrix in a mouse model of bleomycin-induced skin fibrosis. Arthritis Rheum. 2007 Aug; 56(8):2755-2764.
65. Zehender A, Li YN, Lin NY, Stefanica A, Nüchel J, Chen CW, et al. TGFβ promotes fibrosis by MYST1-dependent epigenetic regulation of autophagy. Nature communications. 2021 Jul 20; 12(1):4404.
66. Wang CW, Klionsky DJ. The molecular mechanism of autophagy. Mol Med. 2003 Mar-Apr; 9(3-4):65-76.
67. Deretic V, Jiang S, Dupont N. Autophagy intersections with conventional and unconventional secretion in tissue development, remodeling and inflammation. Trends in cell biology. 2012 Aug; 22(8):397-406.
68. Ponpuak M, Mandell MA, Kimura T, Chauhan S, Cleyrat C, Deretic V. Secretory autophagy. Curr Opin Cell Biol. 2015 Aug; 35:106-116.
69. Guo H, Chitiprolu M, Roncevic L, Javalet C, Hemming FJ, Trung MT, et al. Atg5 Disassociates the V(1)V(0)-ATPase to Promote Exosome Production and Tumor Metastasis Independent of Canonical Macroautophagy. Dev Cell. 2017 Dec 18; 43(6):716-730.e717.
70. Vettori S, Gay S, Distler O. Role of MicroRNAs in Fibrosis. The open rheumatology journal. 2012; 6:130-139.
71. Liu G, Friggeri A, Yang YP, Milosevic J, Ding QA, Thannickal VJ, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. Journal of Experimental Medicine. 2010 Aug; 207(8):1589-1597.
72. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature. 2008 Dec; 456(7224):980-U983.
73. Zhong X, Chung ACK, Chen HY, Meng XM, Lan HY. Smad3-Mediated Upregulation of miR-21 Promotes Renal Fibrosis. Journal of the American Society of Nephrology. 2011 Sep; 22(9):1668-1681.
74. Maurer B, Stanczyk J, Jungel A, Akhmetshina A, Trenkmann M, Brock M, et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. Arthritis Rheum. 2010 Jun; 62(6):1733-1743.

75. Shin JY, Beckett JD, Bagirzadeh R, Creamer TJ, Shah AA, McMahan Z, et al. Epigenetic activation and memory at a TGFB2 enhancer in systemic sclerosis. Sci Transl Med. 2019 Jun 19; 11(497).

76. Clark KEN, Campochiaro C, Csomor E, Taylor A, Nevin K, Galwey N, Morse MA, Singh J, Teo YV, Ong VH, Derrett-Smith E, Wisniacki N, Flint SM, Denton CP. Molecular basis for clinical diversity between autoantibody subsets in diffuse cutaneous systemic sclerosis. Ann Rheum Dis. 2021 Dec;80(12):1584-1593. doi: 10.1136/annrheumdis-2021-220402. Epub 2021 Jul 6. PMID: 34230031.77. Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, Lee SR, Yang SH. The Role of Tumor Necrosis Factor Alpha (TNF-α) in Autoimmune Disease and Current TNF-α Inhibitors in Therapeutics. Int J Mol Sci. 2021 Mar 8;22(5):2719. doi: 10.3390/ijms22052719. PMID: 33800290; PMCID: PMC7962638.

77. Denton CP, Engelhart M, Tvede N, Wilson H, Khan K, Shiwen X, Carreira PE, Diaz Gonzalez F, Black CM, van den Hoogen FH. An open-label pilot study of infliximab therapy in diffuse cutaneous systemic sclerosis. Ann Rheum Dis. 2009 Sep;68(9):1433-9. doi: 10.1136/ard.2008.096123. Epub 2008 Sep 9. PMID: 18782794.

78. Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR, Kishimoto T. Translating IL-6 biology into effective treatments. Nat Rev Rheumatol. 2020 Jun;16(6):335-345. doi: 10.1038/s41584-020-0419-z. Epub 2020 Apr 23. PMID: 32327746; PMCID: PMC7178926.

79. O'Reilly S, Cant R, Ciechomska M, van Laar JM. Interleukin-6: a new therapeutic target in systemic sclerosis? Clin Transl Immunology. 2013 Apr 12;2(4):e4. doi: 10.1038/cti.2013.2. PMID: 25505952; PMCID: PMC4232056.

80. Khan K, Xu S, Nihtyanova S, Derrett-Smith E, Abraham D, Denton CP, Ong VH. Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. Ann Rheum Dis. 2012 Jul;71(7):1235-42. doi: 10.1136/annrheumdis-2011-200955. Epub 2012 May 14. PMID: 22586157.

81. Muangchant C, Pope JE. The significance of interleukin-6 and C-reactive protein in systemic sclerosis: a systematic literature review. Clin Exp Rheumatol. 2013 Mar-Apr;31(2 Suppl 76):122-34. Epub 2013 Jul 23. PMID: 23910616.

82. Khanna D, Denton CP, Jahreis A, van Laar JM, Frech TM, Anderson ME, Baron M, Chung L, Fierlbeck G, Lakshminarayanan S, Allanore Y, Pope JE, Riemekasten G, Steen V, Müller-Ladner U, Lafyatis R, Stifano G, Spotswood H, Chen-Harris H, Dziadek S, Morimoto A, Sornasse T, Siegel J, Furst DE. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. Lancet. 2016 Jun 25;387(10038):2630-2640. doi: .1016/S0140-6736(16)00232-4. Epub 2016 May 5.

83. Khanna D, Lin CJF, Furst DE, Goldin J, Kim G, Kuwana M, Allanore Y, Matucci-Cerinic M, Distler O, Shima Y, van Laar JM, Spotswood H, Wagner B, Siegel J, Jahreis A, Denton CP; focuSSced investigators. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Respir Med. 2020 Oct;8(10):963-974. doi: 10.1016/S2213-2600(20)30318-0. Epub 2020 Aug 28. Erratum in: Lancet Respir Med. 2020 Oct;8(10):e75.

84. Denton CP, Ong VH, Xu S, Chen-Harris H, Modrusan Z, Lafyatis R, Khanna D, Jahreis A, Siegel J, Sornasse T. Therapeutic interleukin-6 blockade reverses transforming growth factor-beta pathway activation in dermal fibroblasts: insights from the faSScinate clinical trial in systemic sclerosis. Ann Rheum Dis. 2018 Sep;77(9):1362-1371. doi: 10.1136/annrheumdis-2018-213031.

85. Nguyen JK, Austin E, Huang A, Mamalis A, Jagdeo J. The IL-4/IL-13 axis in skin fibrosis and scarring: mechanistic concepts and therapeutic targets. Arch Dermatol Res. 2020

Mar;312(2):81-92. doi: 10.1007/s00403-019-01972-3. Epub 2019Sep 6. PMID: 31493000; PMCID: PMC7008089.

86. O'Reilly S. Role of interleukin-13 in fibrosis, particularly systemic sclerosis. Biofactors. 2013 Nov-Dec;39(6):593-6. doi: 10.1002/biof.1117. Epub 2013 Jul 25. PMID: 23893513.

87. Lescoat A, Lecureur V, Varga J. Contribution of monocytes and macrophages to the pathogenesis of systemic sclerosis: recent insights and therapeutic implications. Curr Opin Rheumatol. 2021 Nov 1;33(6):463-470. doi: 10.1097/BOR.0000000000000835. PMID: 34506339.

88. Allanore Y, Wung P, Soubrane C, Esperet C, Marrache F, Bejuit R, Lahmar A,Khanna D, Denton CP; Investigators. A randomised, double-blind, placebo-controlled, 24-week, phase II, proof-of-concept study of romilkimab (SAR156597) in early diffuse cutaneous systemic sclerosis. Ann Rheum Dis. 2020 Dec;79(12):1600-1607. doi: 10.1136/annrheumdis-2020-218447. Epub 2020 Sep 22. PMID: 32963047; PMCID: PMC7677494.

89. Spierings J, Nihtyanova SI, Derrett-Smith E, Clark KEN, van Laar JM, Ong V, Denton CP. Outcomes linked to eligibility for stem cell transplantation trials in diffuse cutaneous systemic sclerosis. Rheumatology (Oxford). 2022 May 5;61(5):1948-1956. doi: 10.1093/rheumatology/keab604. PMID: 34314500; PMCID: PMC9071533.

90. Yue X, Petersen F, Shu Y, Kasper B, Magatsin JDT, Ahmadi M, Yin J, Wax J, Wang X, Heidecke H, Lamprecht P, Müller A, Yu X, Riemekasten G. Transfer of PBMC From SSc Patients Induces Autoantibodies and Systemic Inflammation in Rag2-/-/IL2rg-/- Mice. Front Immunol. 2021 Jun 23;12:677970. doi: 10.3389/fimmu.2021.677970. PMID: 34248959; PMCID: PMC8261241.Ebata [19]

91. Ebata, S., Yoshizaki, A., Oba, K., Kashiwabara, K., Ueda, K., Uemura, Y., Watadani, T., Fukasawa, T., Miura, S., Yoshizaki-Ogawa, A., Asano, Y., Okiyama, N., Kodera, M., Hasegawa, M., & Sato, S. (2021). Safety and efficacy of rituximab in systemic sclerosis (DESIRES): a double-blind, investigator-initiated, randomised, placebo-controlled trial. The Lancet Rheumatology, 3(7), e489–e497. https://doi.org/10.1016/S2665-9913(21)00107-7

92. Taher TE, Ong VH, Bystrom J, Hillion S, Simon Q, Denton CP, Pers JO, Abraham DJ, Mageed RA. Association of Defective Regulation of Autoreactive Interleukin-6-Producing Transitional B Lymphocytes With Disease in Patients With Systemic Sclerosis. Arthritis Rheumatol. 2018 Mar;70(3):450-461. doi: 10.1002/art.40390. Epub 2018 Feb 6. PMID: 29193892.93.

93. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Avvedimento EV, Gabrielli A. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med. 2006 Jun 22;354(25):2667-76. doi: 10.1056/NEJMoa052955. PMID: 16790699.

94. Becker MO, Kill A, Kutsche M, Guenther J, Rose A, Tabeling C, Witzenrath M, Kühl AA, Heidecke H, Ghofrani HA, Tiede H, Schermuly RT, Nickel N, Hoeper MM, Lukitsch I, Gollasch M, Kuebler WM, Bock S, Burmester GR, Dragun D, Riemekasten G. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. Am J Respir Crit Care Med. 2014 Oct 1;190(7):808-17. doi: 10.1164/rccm.201403-0442OC. PMID: 25181620.

95. Prasse A, Müller-Quernheim J. Non-invasive biomarkers in pulmonary fibrosis. Respirology. 2009 Aug;14(6):788-95. doi: 10.1111/j.1440-1843.2009.01600.x. PMID: 19703061.

96. Kill A, Riemekasten G. Functional autoantibodies in systemic sclerosis pathogenesis. Curr Rheumatol Rep. 2015 May;17(5):34. doi: 10.1007/s11926-015-0505-4. PMID: 25876754.

97. Jordan S, Distler JH, Maurer B, Huscher D, van Laar JM, Allanore Y, Distler O; 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 Jun;74(6):1188-94. doi: 10.1136/annrheumdis-2013-204522. Epub 2014 Jan 17. PMID: 24442885.

98. Moradzadeh M, Aghaei M, Mehrbakhsh Z, Arab-Bafrani Z, Abdollahi N. Efficacy and safety of rituximab therapy in patients with systemic sclerosis disease (SSc): systematic review and meta-analysis. Clin Rheumatol. 2021 Oct;40(10):3897-3918. doi: 10.1007/s10067-021-05698-4. Epub 2021 Apr 2. PMID: 33796953.

99. Khanna D, Spino C, Johnson S, Chung L, Whitfield ML, Denton CP, Berrocal V, Franks J, Mehta B, Molitor J, Steen VD, Lafyatis R, Simms RW, Gill A, Kafaja S, Frech TM, Hsu V, Domsic RT, Pope JE, Gordon JK, Mayes MD, Schiopu E, Young A, Sandorfi N, Park J, Hant FN, Bernstein EJ, Chatterjee S, Castelino FV, Ajam A, Wang Y, Wood T, Allanore Y, Matucci-Cerinic M, Distler O, Singer O, Bush E, Fox DA, Furst DE. Abatacept in Early Diffuse Cutaneous Systemic Sclerosis: Results of a Phase II Investigator-Initiated, Multicenter, Double-Blind, Randomized, Placebo-Controlled Trial. Arthritis Rheumatol. 2020 Jan;72(1):125-136. doi: 10.1002/art.41055. Epub 2019 Dec 10. PMID: 31342624; PMCID: PMC6935399.

100. 100. Chung L, Spino C, McLain R, Johnson SR, Denton CP, Molitor JA, Steen VD, Lafyatis R, Simms RW, Kafaja S, Frech TM, Hsu V, Domsic RT, Pope JE, Gordon JK, Mayes MD, Sandorfi N, Hant FN, Bernstein EJ, Chatterjee S, Castelino FV, Ajam A, Allanore Y, Matucci-Cerinic M, Whitfield ML, Distler O, Singer O, Young A, Nagaraja V, Fox DA, Furst DE, Khanna D. Safety and efficacy of abatacept in early diffuse cutaneous systemic sclerosis (ASSET): open-label extension of a phase 2, double-blind randomised trial. Lancet Rheumatol. 2020 Dec;2(12):e743-e753. doi: 10.1016/s2665-9913(20)30237-x. Epub 2020 Oct 19. PMID: 34966900; PMCID: PMC8713509.

101. Duncan MR, Hasan A, Berman B. Oncostatin M stimulates collagen and glycosaminoglycan production by cultured normal dermal fibroblasts: insensitivity of sclerodermal and keloidal fibroblasts. J Invest Dermatol. 1995 Jan;104(1):128-33. doi: 10.1111/1523-1747.ep12613623. PMID: 7798630.

102. Denton CP et al Biological and clinical insights from a randomised phase II study of an anti-oncostatin M monoclonal antibody in systemic sclerosis, Rheumatology 2022 (in press)

103. Abraham D. Connective tissue growth factor: growth factor, matricellular organizer, fibrotic biomarker or molecular target for anti-fibrotic therapy in SSc? Rheumatology (Oxford). 2008 Oct;47 Suppl 5:v8-9. doi: 10.1093/rheumatology/ken278. PMID: 18784153.

104. Parada C, Li J, Iwata J, Suzuki A, Chai Y. CTGF mediates Smad-dependent transforming growth factor β signaling to regulate mesenchymal cell proliferation during palate development. Mol Cell Biol. 2013 Sep;33(17):3482-93. doi: 10.1128/MCB.00615-13. Epub 2013 Jul 1. PMID: 23816882; PMCID: PMC3753855.

105. Richeldi L, Fernández Pérez ER, Costabel U, Albera C, Lederer DJ, Flaherty KR, Ettinger N, Perez R, Scholand MB, Goldin J, Peony Yu KH, Neff T, Porter S, Zhong M, Gorina E, Kouchakji E, Raghu G. Pamrevlumab, an anti-connective tissuegrowth factor therapy, for idiopathic pulmonary fibrosis (PRAISE): a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Respir Med. 2020 Jan;8(1):25-33. doi: 10.1016/S2213-2600(19)30262-0. Epub 2019 Sep 28. PMID: 31575509.

106. Rossini M, Cheunsuchon B, Donnert E, Ma LJ, Thomas JW, Neilson EG, Fogo AB. Immunolocalization of fibroblast growth factor-1 (FGF-1), its receptor (FGFR-1), and fibroblast-specific protein-1 (FSP-1) in inflammatory renal disease. Kidney Int. 2005 Dec;68(6):2621-8. doi: 10.1111/j.1523-1755.2005.00734.x. PMID: 16316338.

107. Schneider M, Hansen JL, Sheikh SP. S100A4: a common mediator of epithelial-mesenchymal transition, fibrosis and regeneration in diseases? J Mol Med (Berl). 2008 May;86(5):507-22. doi: 10.1007/s00109-007-0301-3. Epub 2008 Mar 6. PMID: 18322670.

108. Li Z, Li Y, Liu S, Qin Z. Extracellular S100A4 as a key player in fibrotic diseases. J Cell Mol Med. 2020 Jun;24(11):5973-5983. doi: 10.1111/jcmm.15259. Epub 2020 Apr 19. PMID: 32307910; PMCID: PMC7294136.

109. Tomcik M, Palumbo-Zerr K, Zerr P, Avouac J, Dees C, Sumova B, Distler A, Beyer C, Cerezo LA, Becvar R, Distler O, Grigorian M, Schett G, Senolt L, Distler JH. S100A4 amplifies TGF-β-induced fibroblast activation in systemic sclerosis. Ann Rheum Dis. 2015 Sep;74(9):1748-55. doi: 10.1136/annrheumdis-2013-204516. Epub 2014 Apr 7. PMID: 24709861.

110. Wu M, Assassi S. The role of type 1 interferon in systemic sclerosis. Front Immunol. 2013 Sep 6;4:266. doi: 10.3389/fimmu.2013.00266. PMID: 24046769; PMCID: PMC3764426.

111. Moinzadeh P, Frommolt P, Franitza M, Toliat MR, Becker K, Nürnberg P, Nihtyanova SI, Ahrazoglu M, Belz D, Hunzelmann N, Abraham D, Ong VH, Mouthon L, Hesselstrand R, Denton CP, Krieg T. Whole blood gene expression profiling distinguishes systemic sclerosis-overlap syndromes from other subsets. J Eur Acad Dermatol Venereol. 2020 May;34(5):e236-e238. doi: 10.1111/jdv.16198. Epub 2020 Feb 19. PMID: 31945216.

112. Koh JWH, Ng CH, Tay SH. Biologics targeting type I interferons in SLE: A meta-analysis and systematic review of randomised controlled trials. Lupus. 2020 Dec;29(14):1845-1853. doi: 10.1177/0961203320959702. Epub 2020 Sep 22. PMID: 32960720.

113. Ciechomska M, Skalska U. Targeting interferons as a strategy for systemic sclerosis treatment. Immunol Lett. 2018 Mar;195:45-54. doi: 10.1016/j.imlet.2017.10.011. Epub 2017 Oct 26. PMID: 29106987.

114. Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-β) isoforms in wound healing and fibrosis. Wound Repair Regen. 2016 Mar;24(2):215-22. doi: 10.1111/wrr.12398. Epub 2016 Mar 2. PMID: 26704519.

115. 94. Peng D, Fu M, Wang M, Wei Y, Wei X. Targeting TGF-β signal transduction for fibrosis and cancer therapy. Mol Cancer. 2022 Apr 23;21(1):104. doi: 10.1186/s12943-022-01569-x. PMID: 35461253; PMCID: PMC9033932.

116. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, Silliman N, Streisand J, Powell J, Akesson A, Coppock J, Hoogen Fv, Herrick A, Mayes MD, Veale D, Haas J, Ledbetter S, Korn JH, Black CM, Seibold JR; Cat-192 Study Group; Scleroderma Clinical Trials Consortium. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. 2007 Jan;56(1):323-33. doi: 10.1002/art.22289. PMID: 17195236.

117. Rice LM, Padilla CM, McLaughlin SR, Mathes A, Ziemek J, Goummih S, Nakerakanti S, York M, Farina G, Whitfield ML, Spiera RF, Christmann RB, Gordon JK, Weinberg J, Simms RW, Lafyatis R. Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. J Clin Invest. 2015 Jul 1;125(7):2795-807. doi: 10.1172/JCI77958. Epub 2015 Jun 22. PMID: 26098215; PMCID: PMC4563675.

118. Humbert M, McLaughlin V, Gibbs JSR, Gomberg-Maitland M, Hoeper MM, Preston IR, Souza R, Waxman A, Escribano Subias P, Feldman J, Meyer G, Montani D, Olsson KM, Manimaran S, Barnes J, Linde PG, de Oliveira Pena J, Badesch DB; PULSAR Trial Investigators. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. N Engl J Med. 2021 Apr 1;384(13):1204-1215. doi: 10.1056/NEJMoa2024277. PMID: 33789009.