



Review

# PDGF/PDGFR: A Possible Molecular Target in Scleroderma Fibrosis

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**Abstract:** Systemic sclerosis (SSc) is a clinically heterogeneous disorder of the connective tissue characterized by vascular alterations, immune/inflammatory manifestations, and organ fibrosis. SSc pathogenesis is complex and still poorly understood. Therefore, effective therapies are lacking and remain nonspecific and limited to disease symptoms. In the last few years, many molecular and cellular mediators of SSc fibrosis have been described, providing new potential options for targeted therapies. In this review: (i) we focused on the PDGF/PDGFR pathway as key signaling molecules in the development of tissue fibrosis; (ii) we highlighted the possible role of stimulatory anti-PDGFR $\alpha$  autoantibodies in the pathogenesis of SSc; (iii) we reported the most promising PDGF/PDGFR targeting therapies.



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## 1. Introduction

Systemic sclerosis (SSc) is a rare, immune-mediated, connective tissue disease characterized by progressive fibrosis of the skin and internal organs. The hallmarks of this disease are microvascular lesions, the production of autoantibodies, and increased deposition of extracellular matrix (ECM).

SSc predominantly affects the female sex and its progression and severity vary greatly amongst individuals. The variable extent of fibrosis of the skin, lungs, and gastrointestinal (GI) tract is the most frequent manifestation of the disease, with the leading causes of death being interstitial lung disease (ILD) and pulmonary hypertension (PAH) [1,2].

The pathogenesis of this complex systemic disease is not completely understood. The heterogeneity of SSc clinical manifestations likely reflects the interplay of multiple pathogenic pathways, some of which are well characterized but not clearly associated with disease subsets and clinical phenotypes [3,4]. Much recent progress has been made with the contribution of new experimental models and omics approaches [5–8]. Nevertheless, there are no specific targeted therapies approved, with the exception of ERAs [9,10] and nintedanib [11]. Optimal clinical management of SSc is, therefore, hampered by a lack of drugs.

To possibly fill this gap, this article reviews the role of PDGF and its receptor as a key signaling pro-fibrotic pathway and potential therapeutic target in SSc.

## 2. Platelet-Derived Growth Factor (PDGF)

Platelet-derived growth factor (PDGF) was identified more than three decades ago as a serum growth factor for fibroblasts, smooth muscle cells (SMCs), and glial cells [12].

Moreover, it is the major mitogen for connective tissue cells and certain other cell types [13]. PDGF stimulates cell survival, growth, and proliferation, but also changes cell shape and motility; it induces a reorganization of the actin filament system and stimulates chemotaxis, i.e., a directed cell movement toward a gradient of PDGF [14].

Four distinct PDGF polypeptide chains have been described, namely, the PDGF-A, PDGF-B, PDGF-C, and PDGF-D, which form four homodimers including PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD, as well as one heterodimer, PDGF-AB.

Different PDGF isoforms are generally produced by discrete populations of cells (i.e., platelets, endothelial, epithelial, glial, and inflammatory cells) [15], and act locally to drive different cellular responses [12].

PDGF expression in cultured cells is dynamic and responsive to a variety of stimuli, including hypoxia, thrombin, cytokines, and growth factors, including PDGF itself [12,16]. PDGF has an important role during embryonic development, functioning at the sites of epithelial–mesenchymal interactions during organogenesis, as well as during wound healing [13]. It also controls cell differentiation and stem cell pluripotency, along with mesenchymal stem cell (MSC) stroma formation and differentiation through negative regulation [17].

However, PDGF deregulation has been associated with several pathological conditions, such as atherosclerosis, organ fibrosis, and tumorigenesis [18,19].

### 3. PDGF Receptor (PDGFR)

The different isoforms of PDGF exert their biological effects on target cells by binding and activating PDGFR $\alpha$  and PDGFR $\beta$ , two structurally related class III tyrosine kinase receptors (RTKs) with different expression patterns and physiological roles. PDGFRs are transmembrane glycoproteins that include three kinds of dimers,  $\alpha\alpha$ ,  $\alpha\beta$ , and  $\beta\beta$ , which are composed of PDGF- $\alpha$  and PDGFR- $\beta$  subunits in a certain way. PDGF-A and PDGF-C predominantly bind to the PDGFR- $\alpha$  chain, PDGF-B binds to both - $\alpha$  and - $\beta$  chains, and PDGF-D binds to the  $\beta$  chain only.

PDGFR protein consists of an extracellular ligand-binding domain, a single transmembrane helix to pass structural/informational input from outside the cell, and an intracellular tyrosine kinase domain for the downstream transduction [20]. Downstream signal transduction pathways include phosphatidylinositol 2 kinase, MAPK, PI3K, Src family kinases, and phospholipase C $\gamma$  [21,22].

Compared to other RTKs, PDGFRs are featured by five immunoglobulin-like (Ig-like) domains in the extracellular segment (D1–D5), and a split kinases domain that contains an insert of variable length between the N- and C-terminals [23].

Two PDGFR genes (*PDGFRA* and *PDGFRB*), located on chromosomes 4 and 5 in humans and 5 and 18 in mice, respectively, are present [15].

PDGF receptors are tightly regulated by an auto-inhibitory allosteric conformation, resulting in very low basal activity in the absence of the ligand [24].

The best-known mechanism to activate PDGFRs is the PDGF-mediated (direct) activation mode, in which activated PDGFR tyrosine phosphorylates substrates engage signaling cascades that drive subsequent cellular responses [15]. For instance, both PDGFR $\alpha$  and PDGFR $\beta$  autophosphorylate and thereby create a binding site for SH2 domain-containing proteins such as phosphoinositide 3 kinase (PI3K). The relocation of PI3K to the plasma membrane increases its access to its lipid substrates and to co-activators such as activated Ras.

In different pathological conditions, PDGF-independent modes to activate PDGFRs were observed. For instance, growth factors outside of the PDGF family (non-PDGFs) were able to induce the PDGFR $\alpha$  indirect activation [25]. These non-PDGFs engage their own receptors and thereby trigger an intracellular signaling cascade that drives monomeric PDGFR $\alpha$  activation. It was discovered in the context of experimental PVR, where the pathogenesis depends on the prolonged activation of PDGFR $\alpha$ . VEGF enables indirect activation of PDGFR $\alpha$  by antagonizing PDGF-dependent activation of the receptor; removing VEGF

allows PDGF to block the non-PDGFs, indirectly inhibiting activation of PDGFR $\alpha$  and thereby enabling the survival of cells displaced into the vitreous.

Several factors induce PDGFR expression, including TGF- $\beta$ , estrogen, interleukin-1 $\alpha$  (IL-1 $\alpha$ ), basic fibroblast growth factor-2 (FGF-2), tumor necrosis factor- $\alpha$ , and lipopolysaccharide.

PDGFRs are primarily expressed on mesenchymal cells. PDGFR activation exerts mitogenic and chemoattractant effects, which determine the main biological roles of PDGF in development, physiological, and pathological processes, e.g., cardiovascular and renal development, wound healing, atherosclerosis, malignancies, or fibrosis [26–28].

#### 4. PDGF/PDGFR in Physiology and in Organ Fibrosis

Fibrosis is a pathological response to tissue wound healing that results in excessive formation and deposition of extracellular matrix (ECM) with significant architectural remodeling. It may occur in any organs and tissues; it may become irreversible with time and cause loss of organ function [17].

Multiple pathways are involved in the initiation and progression of organ fibrosis, with PDGF-signaling being one of the central mediators [29]. In fact, aberrant PDGFR signaling drives pathological responses in the principal cellular executors of fibrosis, i.e., mesenchymal stromal cells such as fibroblasts, pericytes, and myofibroblasts, in diverse fibrotic diseases [12], including cardiac and pulmonary fibrosis, liver cirrhosis, glomerulosclerosis, and systemic sclerosis [30–32].

PDGFs and their receptors contribute to normal heart development. Overexpression of human PDGF-A and PDGF-B genes in transgenic mice has a negative impact on cardiac development and function, resulting in isoform-specific fibrotic reactions and cardiac hypertrophy [33].

PDGFR $\alpha$  signaling promotes muscle development in growing embryos and angiogenesis in regenerating adult skeletal muscle. However, both increased PDGF ligand abundance and enhanced PDGFR $\alpha$  pathway activity cause pathological fibrosis, as seen in aged and diseased muscles. In mice, PDGFR $\alpha$  signaling regulates a population of muscle-resident fibro/adipogenic progenitors (FAPs) that play a supportive role in muscle regeneration but may also cause fibrosis when aberrantly regulated [34]. During muscle regeneration, FAPs produce an intronic variant of PDGFR $\alpha$ , which can inhibit PDGF signaling and prevent FAPs activation. Increasing the expression of this isoform limits fibrosis in mice.

#### 5. PDGF/PDGFR in Preclinical Models of Systemic Sclerosis

Several recent findings confirm previous evidence suggesting that the PDGF/PDGFR pathway plays an important role in the development and progression of fibrosis in systemic sclerosis, being implicated in the activation of SSc fibroblasts [35].

PDGFs and PDGFRs are upregulated in fibrotic dermal lesions of patients with scleroderma [36,37].

Moreover, elevated levels of PDGF-A and PDGF-B are found in bronchoalveolar lavage (BAL) fluid obtained from scleroderma patients [38].

Increased PDGFR $\alpha$  signaling in both embryo and adult mice leads to connective tissue hyperplasia and increased extracellular matrix deposition, resulting in progressive, chronic fibrosis in multiple organs [24]. Vice versa, RNA interference against PDGFR $\alpha$  mRNA inhibits in vitro transdifferentiation of SSc dermal fibroblasts into myofibroblasts [39].

MicroRNA miR-30b, which in turn suppresses PDGFR $\beta$  expression, is strongly down-regulated in serum and in the affected skin of SSc patients [40]. The blocking of PDGFR $\beta$  by miR-30b transfection in SSc dermal fibroblasts in vitro significantly reduces  $\alpha$ SMA and Col1A2 gene expression, inhibiting collagen synthesis and myofibroblast differentiation. Conversely, PDGFR $\beta$  activation by intradermal injection of PDGF-BB in mice significantly aggravates bleomycin-induced dermal thickening, vascular alterations, and monocyte/macrophage infiltration, exacerbating skin injury and fibrosis [41].

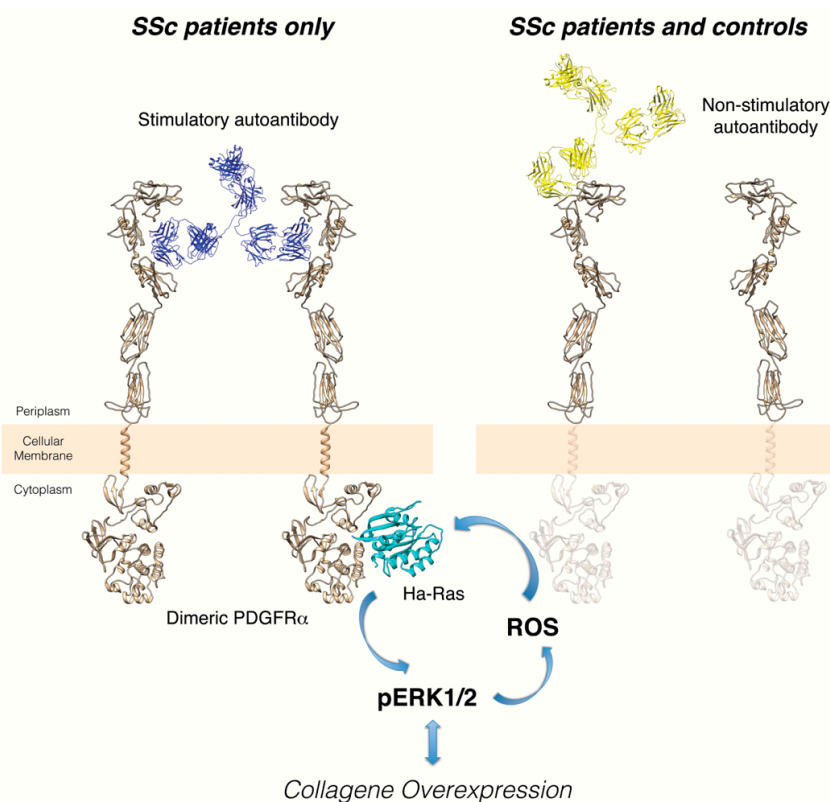
## 6. Anti-PDGFR $\alpha$ Autoantibodies in Systemic Sclerosis

Human PDGFR $\alpha$  is a target of the autoimmune response in systemic sclerosis. Stimulatory anti-PDGFR $\alpha$  autoantibodies were detected in the whole IgG purified from the serum of SSc patients [42]. Unlike other autoantibodies previously described in the literature [43], these autoantibodies possess a biological activity and may contribute, as pathogenic factors, to tissue damage. In fact, upon binding to the receptor on the fibroblast cell surface, they can abnormally activate it, selectively inducing the Ha-Ras-ERK1/2 signaling pathway and reactive oxygen species (ROS) production and stimulating type I collagen gene expression. Triggering this intracellular loop by specific autoantibodies against PDGFR $\alpha$  might sustain the myofibroblast phenotype conversion and the persistent profibrotic response of SSc fibroblasts, suggesting a causal role in the pathogenesis of scleroderma [44]. There are no studies indicating the mechanisms responsible for the generation of anti-PDGFR $\alpha$  autoantibodies. One of the following may be postulated: (i) anti-PDGFR $\alpha$  autoantibodies are the result of the general break of tolerance towards self-antigens, which is a hallmark of SSc disease; (ii) more specifically, PDGFR $\alpha$  peptides recognized by autoantibodies may be the result of intracellular splicing processes caused by external triggers such as viral agents, e.g., CMV [45].

To confirm the original discovery, the immune repertoire of one scleroderma patient was directly investigated [46]. Briefly, memory B cell clones specifically producing antibodies against the human PDGFR $\alpha$  were isolated from peripheral blood mononuclear cells (PBMCs); messenger RNA was extracted, antibody-heavy and light chain genes were PCR amplified and sequenced, and then cloned and recombined *in vitro* to obtain three novel human monoclonal antibodies. The latter replicated the anti-PDGFR $\alpha$  autoantibodies naturally generated by the scleroderma patient. Interestingly, the three monoclonals, differing only in the light chain, display distinct functional features, depending on their epitopes, i.e., the different binding sites on the human PDGFR $\alpha$ : the first one binds with very low affinity to a single linear amino acid sequence in the first PDGFR $\alpha$  extracellular domain, without any stimulatory effects on human fibroblasts *in vitro*; the second one binds a discontinuous epitope within the first and the second PDGFR $\alpha$  extracellular domains, and induces the generation of ROS but not of collagen; the third one binds with high affinity a discontinuous epitope within the second and third PDGFR $\alpha$  extracellular domains, largely overlapping with the PDGF binding site, and induces both ROS and collagen production [46,47].

Through a large conformational PDGFR $\alpha$  peptide library, the epitope of the stimulatory anti-PDGFR $\alpha$  monoclonal autoantibody, overlapping with the PDGF binding site, was further characterized. In particular, the receptor regions required for binding were dissected from the minimal regions of the receptor mediating the functional activity of the stimulatory anti-PDGFR $\alpha$  monoclonal autoantibody. Moreover, based on these data and by using synthetic peptides mimicking this epitope, it was possible to detect antibodies with the same binding features in the serum of other SSc patients, but not in the serum of healthy or pathologic (Primary Raynaud's Phenomenon, Systemic Lupus Erythematosus) individuals. On the contrary, non-stimulatory anti-PDGFR $\alpha$  autoantibodies were also found in a significant percentage of both control cohorts (Figure 1). These findings demonstrate that agonistic anti-PDGFR $\alpha$  autoantibodies directed toward stimulatory epitopes are SSc-specific [48].

This study is of particular interest for different reasons: (i) the existence of activating anti-PDGFR $\alpha$  autoantibodies in the memory B cells and serum of scleroderma patients has been confirmed, shedding light on the still-unclear pathogenesis of one of the most important autoimmune diseases; (ii) the study provides novel evidence about the involvement of PDGFR $\alpha$  in SSc pathogenesis, identifying potentially useful new therapeutic targets for this drug-orphan disease and other fibrotic disorders; (iii) the identification of selective immunodominant epitopes discriminating between SSc and control sera could contribute to developing novel diagnostic assays.



**Figure 1.** Distinct epitopes of stimulatory and non-stimulatory anti-PDGFR $\alpha$  autoantibodies. Left cartoon: the interaction of stimulatory autoantibody cloned from memory B cells of a scleroderma patient and the discontinuous, conformational epitope identified on the extracellular PDGFR $\alpha$  domain induces the intracellular signaling activation and collagen gene overexpression. The identification of this binding region of PDGFR $\alpha$  provides a possible target for new therapeutic strategies to block excessive collagen synthesis under pathologic conditions such as SSc. Right cartoon: binding of non-stimulatory autoantibodies cloned from memory B cells of the same scleroderma patient to a single linear epitope of extracellular PDGFR $\alpha$  domain does not induce receptor dimerization/activation. This non-agonistic autoantibody could reflect the natural autoimmunity, already observed in many healthy individuals, without any clear role in physiology or disease.

Stimulatory autoantibodies against human PDGFR $\alpha$  have also been demonstrated to activate human smooth muscle cells *in vitro* and may thus contribute to the development of SSc vascular lesions [49]. In fact, upon incubation with PDGF or with agonistic anti-PDGFR $\alpha$  autoantibodies, but not with normal IgG, human pulmonary artery smooth muscle cells (HPASMC) displayed a pro-fibrotic synthetic phenotype as expressed by high levels of type-I collagen and myofibroblastic marker,  $\alpha$ -SMA and a higher growth rate and reduced expression of markers characteristic of the contractile phenotype such as smooth-muscle myosin heavy chain and smooth-muscle calponin.

Furthermore, the profibrotic role of stimulatory anti-PDGFR $\alpha$  autoantibodies was demonstrated also *in vivo* by intradermal injection in three-dimensional bioengineered skin samples, containing human keratinocytes and fibroblasts isolated from skin biopsies of healthy donors, grafted onto the back of SCID mice [50]. An agonistic anti-PDGFR $\alpha$  monoclonal antibody, but not the non-agonistic one, induced enhanced collagen deposition, increased fibroblast activation markers, and vascular alterations in the dermis of human skin equivalents, leading to scleroderma-like skin fibrosis.

## 7. Structural Analysis of PDGFR $\alpha$ and its Ligands

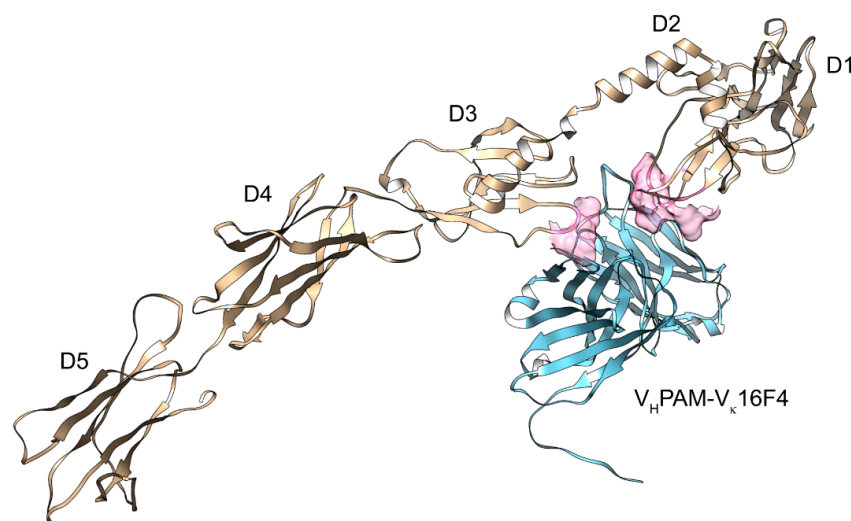
The three-dimensional structure of the PDGFR $\alpha$  extracellular domain, complexed or not with ligands, has been inferred from *in silico* predicted analyses, since a complete

crystallographic structure of this receptor is still missing. The only current available X-ray crystallographic structure (pdbID: 7lbf) of PDGFR $\alpha$ , although partial, was published by Kschonsak et al. in 2021 [51]. Here, the authors presented the molecular complex between PDGFR $\alpha$  and HCMV Trimer, involving four binding sites on the receptor: S1 and S2 placed into the D1, S3 into the D2, and S4 into the D3 domain.

However, a reliable and complete homology modelled structure of human PDGFR $\alpha$  extracellular domain is obtainable, as previously reported by us [46], using templates with relatively high structural and sequence similarities such as the crystal structures of human PDGFR $\beta$  complex (pdbID: 3mjg) [52], of the extracellular domain of human receptor tyrosine kinase, Kit (pdbID: 2e9w) [53], or of human vascular endothelial growth factor receptor 1, VEGFR-1 (pdbID: 5t89) [54].

Our study [46] confirmed the crystal structure of the PDGF/PDGFR $\beta$  complex by performing a molecular docking analysis, based on the rigid body protein–protein geometric approach between the homology modelled PDGFR $\alpha$  extracellular domain and the crystallographic structure of PDGF (pdbID: 1pdg) [55]. We found the same corresponding amino acids, included in the large cleft at the D2–D3 boundary for interaction with PDGF-B, as also described by Chen et al. [23]. The latter study reported that the replacement of these aromatic residues in PDGFR $\beta$  with non-aromatic residues in PDGFR $\alpha$  makes the ligand recognition surface of PDGFR $\alpha$  more adaptive to a wider variety of PDGF ligand surfaces. For example, the HCMV Trimer binds with high affinity and high selectivity to PDGFR $\alpha$ , but it does not bind to the closely related PDGFR $\beta$ .

In this regard, in the same study [46], we also reported the predicted, subsequently validated binding of the aforementioned monoclonal autoantibodies with the PDGFR $\alpha$ . Some predicted models (in particular, for the V<sub>H</sub>PAM–V $\kappa$ 16F4/PDGFR $\alpha$  complex) showed binding regions superimposable with the conformational and discontinuous site related to the binding of PDGF/PDGFR $\alpha$  (Figure 2), further confirming the previous adaptive hypothesis suggested by Chen et al. Moreover, additional models showed two other different binding sites/epitopes—one placed into the D1–D2 boundary (for the V<sub>H</sub>PAM–V $\lambda$ 16F4/PDGFR $\alpha$  complex) and the other one located onto the D1 domain (for the V<sub>H</sub>PAM–V $\kappa$ 13B8/PDGFR $\alpha$  complex)—that would produce partial or no responses into the cell. All these models were then confirmed by screening a conformational PDGFR $\alpha$  peptide library with these ligands by binding competition experiments performed on a surface plasmon resonance device, and by analysis of ROS production and type I collagen gene induction in human fibroblasts.



**Figure 2.** Molecular modeling of the V<sub>H</sub>PAM–V $\kappa$ 16F4 (light blue)/PDGFR $\alpha$  (gold) complex. The identification of the five immunoglobulin-like (Ig) domains in the extracellular segment (D1–D5) and the binding area (in pink surface) of the PDGF-B/PDGFR $\alpha$  complex are reported.

In addition, the crystal structure of the globular PDGFR $\alpha$  kinase domain, free or bound to small ligands/drugs (WQ-C-159, crenolanib, sunitinib, and imatinib), was well characterized [56]. Its structure shows a typical type III RTK composition incorporating a bilobal kinase, an activation loop, and a JM domain, and it is very similar to the structure of auto-inhibited FLT3 and c-Kit.

## 8. PDGF/PDGFR Targeting Approaches in Systemic Sclerosis

PDGF is a well-described mediator of fibrosis, and its modulation is a promising option in an anti-fibrotic treatment [17].

In general, three main approaches can be conceived to inhibit the PDGF/PDGFR pathway: (1) sequestering the ligand with neutralizing antibodies, or soluble extracellular fragments of the receptors acting as ligand traps/decoy receptors or DNA/RNA aptamers; (2) disrupting the interaction between the receptor and the ligand by blocking the receptor with receptor-specific antibodies or small molecule inhibitors; (3) using low molecular weight inhibitors to block the kinase activity of the PDGFR [57].

Table 1 summarizes the possible pharmacological targeting of the PDGF/PDGFR pathway.

**Table 1.** Drugs targeting PDGF/PDGFR and their clinical applications. Abbreviations: moAb, monoclonal antibody; IPF, idiopathic pulmonary fibrosis; SSc-ILD, systemic sclerosis-associated interstitial lung disease; PF-ILD, progressive fibrosing interstitial lung disease.

Drug Name	Mechanism of Action	Current Clinical Indications
MOR8457	PDGF-B blocking antibody	Pre-clinical use only
Olaratumab	Recombinant human IgG1 anti-PDGFR moAb	Withdrawn from market
Imatinib	Tyrosine kinase inhibitor	Chronic myeloid leukaemia (CML) Acute lymphoblastic leukaemia (ALL) Myelodysplastic/myeloproliferative diseases Hypereosinophilic syndrome (HES) Gastrointestinal stromal tumours (GIST) Dermatofibrosarcoma protuberans (DFSP)
Crenolanib besylate	Tyrosine kinase inhibitor	Orphan designation for acute myeloid leukaemia
Dasatinib	Tyrosine kinase inhibitor	Chronic myeloid leukaemia (CML) Acute lymphoblastic leukaemia (ALL)
Nilotinib	Tyrosine kinase inhibitor	Chronic myeloid leukaemia (CML)
Nintedanib	Tyrosine kinase inhibitor	IPF SSc-ILD PF-ILD

There are several studies focused on the efficacy of PDGF/PDGFR-blocking antibodies. Structural studies on the PDGF-B blocking antibody MOR8457 showed that it causes a conformational change of PDGF-B that prevents binding to PDGFR $\beta$  [58]. Moreover, Muse H. et al. discovered that a PDGF-AB neutralizing antibody prevented electromechanical remodeling of adult atrial myocytes in a co-culture model with a myofibroblast [59].

In addition to blocking the ligand, there are several strategies that block the receptor.

PDGFR-specific neutralizing antibodies block signaling through either PDGFR $\alpha$  or PDGFR $\beta$  receptors, reducing PDGF-AA and PDGF-BB-induced collagen gel remodeling and migration in human dermal and lung fibroblasts in vitro [60].

Olaratumab is a recombinant human IgG1 anti-PDGFR $\alpha$  monoclonal antibody that binds specifically to PDGFR $\alpha$ , blocking PDGF-AA, PDGF-BB, and PDGF-CC binding and receptor activation. Through this mechanism, it reduces the proliferation of several tumor models both in vitro and in vivo [61,62]. Olaratumab was initially evaluated and preliminarily approved for the treatment of patients with advanced soft-tissue sarcomas but, following the disappointing results of the phase III study, approval for clinical use was withdrawn [63].

To date, the most effective approach to decreasing PDGFR signaling is to block its enzymatic activity; the inhibition of kinase activity of PDGFRs is based on multi-targeted small molecules, called tyrosine kinase inhibitors (TKI). TKIs are not selective for the PDGFR but their mechanism of action encompasses several other kinases such as, for example, c-KIT and Abl for imatinib or FGFR and VEGFR for nintedanib [63].

Imatinib was the first TKI developed and approved for clinical use in hematologic cancers [64]. Current indications for imatinib include hematologic neoplasms (such as chronic myeloid leukemia and acute lymphoblastic leukemia) and gastrointestinal stromal tumors (GIST). Imatinib is a potent inhibitor of TK phosphorylation that prevents the binding of ATP to the target kinase. Imatinib inhibits collagen and ECM production induced by PDGF, but also, through a non-canonical pathway, by TFG- $\beta$  [65].

Several studies tested the potential use of imatinib as an antifibrotic drug for the treatment of dermal fibrosis in systemic sclerosis. In preliminary studies, treatment with imatinib reduced dermal thickness and prevented the differentiation of fibroblasts into myofibroblasts, both in vitro and in vivo [66,67].

Following the preclinical results, several clinical studies evaluated imatinib use in patients with systemic sclerosis [68]. Although encouraging results were reported by several uncontrolled studies, clinical outcomes were highly variable and often limited by important toxicity. Subsequent randomized and controlled trials yielded mostly negative results, reporting that imatinib use was not associated with improvement of cutaneous involvement, although it may determine the stabilization of lung function [65,69].

As recently observed by Harrach et al. [70], the reason for insufficient clinical responses to imatinib therapy in SSc patients may be partly attributable to the reduced drug transporter expression, lowering the intracellular imatinib concentrations and thus the therapeutic outcome. In fact, PDGF significantly reduced imatinib influx into SSc fibroblasts by decreasing gene transcription and subsequent protein expression of the transporter MATE1, whereas the transport process in healthy fibroblasts remained unaffected. Because MATE1 expression is regulated by Notch signaling, the blockade of the deregulated Notch signaling network normalized the transporter function of MATE1, presumably rescuing the decreased imatinib efficacy in SSc fibroblasts.

Crenolanib besylate is a selective inhibitor of type III tyrosine kinases with higher sensitivity for PDGFR $\alpha$  than PDGFR $\beta$  [71]. It can block, in a dose-dependent manner, PDGFR $\alpha$  and PDGFR $\beta$  phosphorylation upon stimulation with PDGF-AA and/or PDGF-BB in SSc and healthy control dermal fibroblast and can inhibit their proliferation and migration in vitro. A recent study demonstrated that crenolanib abrogates both the inflammation and ECM deposition in dermal fibroblasts in vitro [72]. They first identified in the skin and circulation of SSc patients a strong increase of CXCL4, a chemokine that activates monocytes, and M2 macrophages inducing the release of PDGF-BB. CXCL4/PDGF-BB signaling axis in these cells can induce both a pro-inflammatory and a pro-fibrotic phenotype in dermal fibroblasts through the release of IL6 and IL8. Inhibiting the PDGF receptors using crenolanib or siRNA knockdown prevented the inflammatory and fibrogenic phenotype induced by human recombinant PDGF-BB and CXCL4-conditioned macrophage supernatant.

Several second-generation TKI have been developed following the discovery of imatinib. They have been mostly approved for clinical use in patients with chronic myeloid leukemia but have also been evaluated for SSc. In general, second-generation TKI have a broader spectrum of TK targets, encompassing not only PDGFR but also other important downstream signaling pathways [65].

Dasatinib has been evaluated in a small cohort of patients with diffuse SSc in an open-label uncontrolled study, failing to show any significant improvement in skin and lung outcomes [73]. Of note, the patients were treated only for 6 months and then followed up for 18 months. Moreover, an unexpected adverse effect of dasatinib administration was the induction of pulmonary hypertension.

Nilotinib is another second-generation TKI that has been evaluated in a small open-label study enrolling 10 SSc patients, demonstrating not only potential clinical efficacy on



cutaneous involvement (although the study was not adequately controlled) but also the ability to induce a significant downregulation of the expression of genes involved in the PDGFR and TGFBR pathways [74]. The onset of QT interval prolongation in 20% of the study participants was a potential adverse effect of nilotinib administration in SSc patients.

Nintedanib is a highly selective TKI targeting PDGF, VEGF, and FGF pathways [75] but, due to its action on other kinases such as Src, MAPK, and ERK, it also inhibits downstream TGF- $\beta$  pathways [63].

Initially developed in the oncology field, it has subsequently been extensively evaluated for its action in fibrotic diseases [65].

Nintedanib was the first TKI to be approved for the treatment of idiopathic pulmonary fibrosis (IPF) after the INPULSIS trial showed unequivocally that it was effective in halting disease progression as measured by the rate of decline in forced vital capacity (FVC) [76].

Based on preclinical evidence showing that nintedanib was able to inhibit the TGF $\beta$ -induced myofibroblast differentiation in preclinical models of SSc [77] and following the success of nintedanib in IPF trials, two large studies evaluating its efficacy on lung involvement in SSc (SSc-ILD) and in other fibrotic lung diseases with a progressive phenotype (PF-ILD) were conducted in recent years.

In the SENSCIS trial that randomized 663 SSc-ILD patients, at 52 weeks the adjusted rate of decline in the FVC was lower using nintedanib instead of placebo, for a between-group difference of 107 mL per year [11]. Of note, despite its efficacy on lung outcomes, nintedanib treatment did not improve skin fibrosis in the SENSCIS trial.

Numerous post-hoc and subgroup analyses of the SENSCIS study suggest that nintedanib efficacy is consistent across the disease spectrum, regardless of predicted %FVC, disease duration, the extent of fibrotic involvement on HRCT, autoantibody status, or SSc [78].

As already mentioned, the subsequent INBUILD study evaluated 663 patients with ILD associated with a variety of systemic diseases, including SSc-ILD [79]. Importantly, patients could be enrolled if they showed features of ILD progression (i.e., a relative decline in the FVC of at least 10% of the predicted value, a relative decline in the FVC of 5% to less than 10% of the predicted value and worsening of respiratory symptoms or an increased extent of fibrosis on high-resolution CT or worsening of respiratory symptoms and an increased extent of fibrosis). The INBUILD study confirmed the remarkable efficacy of nintedanib in slowing the functional decline in patients at high risk of progression, as well as its good safety profile, across all spectra of associated diseases [80].

Following the success of these trials, both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved nintedanib for use in SSc-ILD and PF-ILD.5.

## 9. Conclusions

In this article, we critically reviewed several lines of scientific evidence supporting the notion that PDGF/PDGFR molecular interaction and signaling pathway thereof may play a role in SSc-related fibrosis, affecting multiple organs of the affected individuals. This notion encourages the research of novel therapeutic strategies directed toward this molecular target, which will require aptly designed new animal models for preclinical drug validation and new assays for SSc patient stratification. Indeed, it is entirely possible that upregulation of this signaling pathway is not common to all SSc disease subsets or that this signaling pathway is selectively modulated in distinct disease subsets. Future personalized medicine for SSc patients may benefit from a solution to these issues.

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