Early View

Review

Potential of Nintedanib in Treatment of Progressive Fibrosing Interstitial Lung Diseases

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Take-home message

Non-clinical studies suggest that nintedanib, an approved treatment for idiopathic pulmonary fibrosis, may have anti-fibrotic effects in other interstitial lung diseases with a progressive fibrosing phenotype.

Abstract

A proportion of patients with fibrosing interstitial lung diseases (ILDs) develop a progressive phenotype characterised by decline in lung function, worsening quality of life and early mortality. Other than idiopathic pulmonary fibrosis (IPF), there are no approved drugs for fibrosing ILDs and a poor evidence base to support current treatments. Fibrosing ILDs with a progressive phenotype show commonalities in clinical behaviour and in the pathogenic mechanisms that drive disease worsening. Nintedanib is an intracellular inhibitor of tyrosine kinases that has been approved for treatment of IPF and has recently been shown to reduce the rate of lung function decline in patients with ILD associated with systemic sclerosis (SSc-ILD). *In vitro* data demonstrate that nintedanib inhibits several steps in the initiation and progression of lung fibrosis, including the release of pro-inflammatory and pro-fibrotic mediators, migration and differentiation of fibrocytes and fibroblasts, and deposition of extracellular matrix. Nintedanib also inhibits the proliferation of vascular cells. Studies in animal models with features of fibrosing ILDs such as IPF, SSc-ILD, rheumatoid arthritis-ILD, hypersensitivity pneumonitis and silicosis demonstrate that nintedanib has anti-fibrotic activity irrespective of the trigger for the lung pathology. This suggests that nintedanib inhibits fundamental processes in the pathogenesis of fibrosis. A trial of nintedanib in patients with progressive fibrosing ILDs other than IPF (INBUILD) will report results in 2019.

Interstitial lung diseases (ILDs) comprise a large and varied group of diseases that generally affect the interstitium, the connective tissue stroma that separates the epithelial and endothelial barriers in the lungs. ILDs may have a known cause; for example, they may be a manifestation of an autoimmune disease or a result of sensitisation to an inhaled antigen. However, the ILDs also include several diseases of unknown cause [1]. Idiopathic pulmonary fibrosis (IPF) is one of the most common types of ILD. Although the course of IPF is variable, IPF is, by definition, a progressive fibrosing disease that ultimately results in respiratory failure [2]. A proportion of patients with certain other types of ILD also develop a progressive fibrosing phenotype associated with decline in lung function, worsening symptoms and quality of life, and early mortality [3]. These include idiopathic non-specific interstitial pneumonia [4], ILDs associated with autoimmune diseases such as rheumatoid arthritis (RA-ILD) [5] and systemic sclerosis (SSc-ILD) [6], chronic hypersensitivity pneumonitis (HP) [7], chronic sarcoidosis [8], exposure-related ILDs such as silicosis [9], and unclassifiable IIP [10]. At present, the goal of treatment for fibrosing ILDs with a progressive phenotype is to slow decline in lung function. However, IPF is the only form of fibrosing ILD for which approved drug therapies are available. The treatment of patients with other types of progressive fibrosing ILD is based largely on immunosuppression, which, other than in SSc-ILD, is not supported by a robust evidence base, leaving patients with a high unmet need for efficacious treatment options [11, 12].

Pathogenesis of fibrosing ILDs with progressive phenotype

Several mechanisms are known to be involved in the pathogenesis and progression of all fibrosing ILDs (Figure). All ILDs are believed to be triggered by repetitive chronic epithelial or vascular injuries, or by granulomatous inflammation, which lead to cell destruction and, in the case of fibrotic disease, to unregulated repair [13-15]. Fibroblasts, the main effector cells in fibrosing ILDs, are drawn from different sources to the site of injury. Resident interstitial fibroblasts proliferate and migrate [16], fibrocytes from the circulation invade [17], and epithelial cells [18] and pericytes [19] acquire features usually associated with fibroblasts. Ultimately these fibroblasts are activated to become myofibroblasts, which secrete excessive amounts of extracellular matrix, resulting in increased tissue stiffness and loss of function of the alveolar tissue [20]. In addition, macrophages and lymphocytes are recruited to the site of injury and release pro-fibrotic mediators that further promote fibroblast activation [16]. In a feed-forward loop, the increased lung tissue stiffness further activates and stimulates fibroblasts, in a process known as mechanostimulation, to drive self-sustaining and progressive lung fibrosis [21].

Nintedanib

Nintedanib has been approved for the treatment of IPF in many countries, including Europe and the US. The standard dosing in adults is 150 mg twice daily (bid). Exposure at steady state after standard oral dosing in patients with IPF is about 59-74 nmol/L (Cmax) [22,23].

Nintedanib is a small molecule tyrosine kinase inhibitor that targets the receptors platelet-derived growth factor receptor (PDGFR) α and β, fibroblast growth factor receptor (FGFR) 1-3, and vascular endothelial growth factor receptor (VEGFR) 1-3 [24]. Nintedanib binds competitively to the adenosine triphosphate (ATP) binding pocket of these receptors, thereby blocking intracellular signalling [24]. Nintedanib also inhibits the Src family kinase Lck (lymphocyte-specific tyrosine-protein kinase), CSF1R (colony stimulating factor 1 receptor), and 20 other kinases with IC_{50} values below 100 nM [24,25]. The impact of enzymatic inhibition of most of these kinases on cellular functions has not yet been explored.

Mechanisms of action of nintedanib in pulmonary fibrosis

In vitro data have demonstrated that nintedanib inhibits a number of steps in the initiation and progression of lung fibrosis that might have relevance to the treatment of patients with progressive fibrosing ILDs.

1) Nintedanib exerts anti-inflammatory activity, attenuating the initiation and progression of fibrosis.

On activation, T cells release pro-inflammatory and pro-fibrotic mediators that are believed to initiate and enhance the progression of fibrosis in autoimmune diseases [26,27]. The lymphocyte-specific protein tyrosine kinase (Lck) is activated after T cell stimulation and is required for T cell proliferation and interleukin (IL)-2 production [28-31]. At an enzymatic level, nintedanib inhibits the activity of Lck with an IC_{50} of 16 nM [24]. In addition, nintedanib inhibits the release of mediators including IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13 and IFNγ by human peripheral blood mononuclear cells or T cells [32]. The potency of nintedanib is within the range of exposure observed in patients with IPF treated with the standard dose of 150 mg bid, suggesting that it is feasible that treatment with nintedanib would modulate these mediators during clinical use of the drug.

CCL18, a marker of M2 macrophage polarization, has been associated with disease progression in patients with fibrosing ILDs including IPF, SSc and RA [33-36]. It has been demonstrated that nintedanib prevents pro-fibrotic macrophage polarization. When macrophages differentiated from human THP-1 cells exposed to a cocktail of IL-4/IL-13 or IL-4/IL-13 and IL-6 were treated with nintedanib at a concentration of 100 nM, the release of CCL18 was significantly reduced [37]. It is

assumed that the inhibitory activity of nintedanib on CSF1R, which has been demonstrated at an enzymatic level with an IC_{50} of 5 nM [25], is at least partly responsible for this reduction. A recent study showed that nintedanib (0.01 to 1 µM) prevented the CSF1-induced phosphorylation of CSF1R, with a significant decrease in production of CCL2, adhesion of macrophages, and polarization of macrophages to alternative M2a macrophages [38]. Nintedanib has also been shown to inhibit alternative activation of monocytes. At a concentration of 100 nM, nintedanib significantly reduced M2 polarization of human macrophages incubated with macrophage-CSF, IL-4 and IL-13, as shown by changes in the mean fluorescence intensity of CD163 and CD206, while expression of M1 markers did not change [39].

2) Nintedanib inhibits fundamental processes of progressive lung fibrosis

Fibrocytes are a source of lung fibroblasts in fibrosing ILDs and are believed to promote fibrosis [40]. Nintedanib has been shown to attenuate the migration of fibrocytes, their differentiation to fibroblasts, and the triggering of fibroblast proliferation by cultured fibrocytes [41]. Activated interstitial fibroblasts are the key effector cells in the pathogenesis of fibrosing ILDs. Nintedanib inhibits fibroblast proliferation induced by stimuli such as PDGF, FGF, VEGF [42,43] and serum [44]. Nintedanib has also been shown to reduce the motility of lung fibroblasts from patients with IPF [45] and to inhibit the contraction of human lung fibroblasts in collagen gels stimulated with PDGF [46]. These effects are not limited to the lung: studies conducted on dermal fibroblasts from patients with SSc showed that nintedanib reduced their PDGF- and TGF-β-induced proliferation and migration [47].

Nintedanib inhibits TGFβ-induced fibroblast to myofibroblast transformation [48] and the release of collagen from TGFβ-stimulated lung fibroblasts [37]. Upon TGFβ stimulation, pericytes, microvascular mural cells of mesenchymal origin, may achieve features of myofibroblasts and form fibroblastic foci. Nintedanib has been shown to inhibit deposition of fibronectin and collagen I by pericytes with myofibroblast features when they are cultured on decellularized lung matrix [49]. Further, nintedanib reduces the enzymatic activity and secretion of matrix metalloproteinase (MMP)-2 and tissue inhibitor of MMP-2 by primary lung fibroblasts from patients with IPF [42] and increases MMP8, MMP9 and MMP13 expression, and MMP2 and MMP9 activity, in pericytes activated with TGF-β1 [49]. Outside the lung, nintedanib reduced myofibroblast differentiation and collagen release from dermal fibroblasts from patients with SSc [47].

3) Nintedanib targets cells of the lung vasculature

The potential impact of attenuating vascular remodeling in patients with IPF and other fibrosing ILDs remains controversial [50,51]. Nintedanib inhibits the proliferation of several vascular cell types in the lung, including endothelial cells, pericytes and smooth muscle cells [24,52]. Nintedanib also

inhibits the proliferation of human pulmonary artery vascular smooth muscle cells at basal conditions and on stimulation with PDGF [39]. Taken together, the experimental evidence supports nintedanib having effects on vascular cells; however, whether these effects add to its anti-fibrotic activity remains to be elucidated.

Studies of nintedanib in animal models of pulmonary fibrosis

Nintedanib has been explored in several animal models with features of fibrosing ILDs such as IPF, SSc-ILD, RA-ILD, chronic HP and silicosis. In these animal models, nintedanib was administered in a therapeutic manner, *i.e.*, after the onset of substantial fibrosis. Nintedanib demonstrated anti-fibrotic activity irrespective of the trigger that caused the fibrotic lung pathology, supporting the hypothesis that nintedanib inhibits fundamental processes in the pathogenesis of fibrosis. In addition to anti-fibrotic efficacy, nintedanib demonstrated anti-inflammatory activity and effects on the vascular compartment. In some animal models, nintedanib also showed effects on aspects of the disease other than fibrosing ILD, such as on heart fibrosis in a model of SSc-ILD, and on airway hyper-reactivity and inflammation in a model showing features of chronic HP. An overview of the different models is given in the Table.

1) Chemical trigger: Bleomycin-induced lung fibrosis

In rodents, intratracheal administration of bleomycin elicits severe epithelial damage, pulmonary inflammation, a massive fibrotic response in the lung and irregular vascularization. In three animal models of bleomycin-induced lung fibrosis, nintedanib demonstrated antifibrotic, anti-inflammatory and vascular remodeling activity [43,48,53]. Antifibrotic activity was demonstrated through significant reductions in lung tissue density determined by micro computed tomography, Ashcroft score, protein expression of inhibitor of metalloproteinases (TIMP)-1, mRNA expression of transforming growth factor (TGF)-β1 and procollagen 1, and the collagen content of lung tissue, as well as by an improvement in lung function, as determined by static compliance calculated from pressure volume loops. In addition, nintedanib showed anti-inflammatory activity, including attenuation of the accumulation of lymphocytes in bronchoalveolar lavage fluid (BALF) and a reduction in IL-1β and chemokine CXCL1/KC. In a mouse model of bleomycin-induced lung fibrosis, nintedanib normalized the distorted vascular architecture and inhibited proliferation of alveolar macrophages and alveolar epithelial type II cells [53].

2) Environmental trigger: Silica crystal-induced lung fibrosis

Intratracheal application of silica crystals in mice results in ongoing lung inflammation and a progressive fibrotic response resembling features of silicosis. In this model, nintedanib reduced lung

inflammation and granuloma formation and reduced neutrophil and lymphocyte numbers in the BALF and IL-1β, CXCL1/KC and IL-6 levels in lung tissue homogenates. Anti-fibrotic activity of nintedanib was demonstrated by a reduction in fibrosis in lung histology and reduced collagen levels in lung tissue [48].

3) Allergic trigger: Ovalbumin-induced fibrotic lung remodeling

In mice, chronic allergic stimulation of the lung with ovalbumin results in features like airway hyper-reactivity, lung inflammation and goblet cell hyperplasia, and ultimately also in airway remodelling with fibrosis, resembling not only asthma, but also aspects of chronic HP. In this model, treatment with nintedanib led to a significant reduction in airway hyper-reactivity and a reduction in the hydroxyproline content of the lungs, indicating anti-fibrotic activity [54].

4) Immunological trigger: Zymosan-induced arthritis and lung fibrosis in SKG mice

In SKG mice (which contain a Zap-70 mutation resulting in auto-reactive T-cells), administration of zymosan induces an arthritis pathology. Arthritis score increases for 6 weeks after zymosan administration, remains high up to week 10 and then slowly decreases. At least 20% of SKG mice treated with zymosan develop lung fibrosis, reflected in an increase in lung collagen (hydroxyproline) content at week 10, which increases further until week 16. Treatment of SKG mice with nintedanib in weeks 5–11 after zymosan administration has been shown to attenuate arthritis pathology with no effect on lung fibrosis, while nintedanib treatment in weeks 10–16 led to a reduction in collagen deposition in the lungs. Nintedanib seemed to trigger a slight but selective inflammation in the lung; the impact of this with respect to the fibrosis is unknown [55].

5) Vascular trigger: Vascular lesions, skin, lung and heart fibrosis in Fra-2 +/- mice

Transgenic overexpression of fos-related-antigen-2 (Fra2) in mice results in development of microvascular disease and pulmonary hypertension. Fra2 transgenic mice develop progressive fulminant skin fibrosis with dermal thickening, increased hydroxyproline content and an increase in myofibroblasts. Fibrosis in these animals also affects the lung. In this model, treatment with nintedanib from an age of 9 to 16 weeks effectively attenuated fibrosis in the skin and lung, with a near normalization of dermal thickness, a reduction in the hydroxyproline content of the skin and lungs, and reduced myofibroblast counts in both organs. Further, nintedanib prominently inhibited pulmonary arterial hypertension-like changes and reduced the proliferation of pulmonary vascular smooth muscle cells, the thickening of vascular walls of pulmonary arteries, and the number of occluded vessels. In the skin, nintedanib reduced apoptosis in dermal microvascular endothelial cells,

increased the number of endothelial cells (a marker of vascularization) and prevented the increase in pro-fibrotic M2 polarized macrophages [39].

6) Additional animal models

Experiments in other animal models have demonstrated the activity of nintedanib on fundamental processes of fibrosis. Nintedanib has shown anti-fibrotic activity in mouse models of skin fibrosis [47], radiation-induced lung fibrosis [56], polyhexamethylene guanidine phosphate-induced lung fibrosis [57], renal fibrosis [58], liver fibrosis [59] and muscle fibrosis [60].

Clinical investigation of nintedanib in patients with progressive fibrosing ILDs

Based on the above evidence from non-clinical studies that nintedanib inhibits fundamental processes of progressive fibrosis, irrespective of the trigger, and the established efficacy of nintedanib in slowing disease progression in patients with IPF [61,62], nintedanib has been investigated as a potential treatment for other fibrosing ILDs with a progressive phenotype. The SENSCIS®trial (NCT02597933) investigated the efficacy and safety of nintedanib versus placebo in patients with SSc-ILD, an extent of fibrotic disease ≥10% on HRCT, and onset of SSc 7 years before screening [63]. Nintedanib significantly reduced the annual rate of decline in FVC assessed over 52 weeks (primary endpoint) (-52.4 versus -93.3 mL/year). The rate of FVC decline was lower in patients with SSc-ILD in the SENSCIS trial than in patients with IPF in the INPULSIS trials [62]but the relative reduction in the rate of FVC decline observed with nintedanib versus placebo was similar (44% and 49%, respectively). The INBUILD® trial (NCT02999178) assessed the efficacy and safety of nintedanib 150 mg bid versus placebo in patients with a physician-diagnosed ILD other than IPF, who have features of diffuse fibrosing lung disease of >10% extent on HRCT [64]. To be eligible to enter the INBUILD® trial, patients had to meet criteria for disease progression in the two years prior to screening, based on decline in FVC, worsening of respiratory symptoms, or an increased extent of fibrotic changes on HRCT. As in the SENSCIS trial, the primary endpoint is the annual rate of decline in FVC (mL/year) assessed over 52 weeks. There are two co-primary analysis populations: all patients and patients with a usual interstitial pneumonia (UIP)-like fibrotic pattern on HRCT; patients with ILD and a UIP-like pattern on HRCT have been shown in a number of studies to be at greater risk of FVC decline than patients with other patterns on HRCT [5,10,65].   The results of the INBUILD® trial will be reported in 2019.

Conclusions

Fibrosing ILDs with a progressive phenotype show commonalities both in clinical behaviour and in the pathogenic mechanisms that drive their progression. Nintedanib is an approved treatment for IPF

and has recently been shown to reduce the rate of progression of ILD in patients with SSc-ILD. Data from *in vitro* and *in vivo* studies have shown that nintedanib inhibits a number of steps in the initiation and progression of lung fibrosis. Further, nintedanib has demonstrated anti-fibrotic effects in several animal models with features of fibrosing ILDs, irrespective of the trigger that caused the fibrotic lung pathology. A large clinical trial of nintedanib in patients with progressive fibrosing ILDs other than IPF will shortly report results.

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Disclosures

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Table   Overview of non-clinical exploration of nintedanib in animal models of lung fibrosis

Model	Bleomycin-induced lung fibrosis in mice [43,53]		Silica-induced lung fibrosis in mice [43]		Bleomycin-induced lung fibrosis in rats [43]		Skin and lung fibrosis in Fra-2 mice [39]	Chronic allergen-induced lung inflammation and remodeling in mice [54]	Rheumatoid arthritis and lung fibrosis in SKG mice [55]	
Trigger	Chemical: bleomycin		Environmental: silica crystals		Chemical: bleomycin		Vascular: Fra-2 transgene	Allergic: ovalbumin	Immunological: zymosan	
Model characteristics	Epithelial cell injury-induced lung inflammation and fibrosis		Ongoing epithelial cell injury-induced lung inflammation and progressive fibrosis		Epithelial cell injury-induced lung inflammation and fibrosis		Resembles aspects of skin and progressive lung fibrosis in SSc including microvascular disease and pulmonary hypertension	Airway hyper-reactivity, lung inflammation and remodeling with goblet cell hyperplasia, lung fibrosis and airway smooth muscle hypertrophy	Resembles aspects of arthritic joint inflammation and progressive lung fibrosis	
Treatment*	Preventive day 0-14	Therapeutic day 7-21 day 7–19	Preventive day 0-30	Therapeutic day 10-30$	Preventive day 0-21	Therapeutic day 10-21	Therapeutic week 10-16	Chronic starting at day 35 for 3 months	Early week 5-11	Late week 10-16

Effects of nintedanib									
Lung fibrosis↓ Lung inflammation↓ IL-1β↓ TIMP-1↓ BALF lymphocytes↓	Lung fibrosis↓ Ashcroft score↓ Lung tissue density↓ Cstat↑ Lung inflammation↓ Lung collagen↓ IL-1β↓ TIMP-1↓ BALF lymphocytes↓ vascularisation↓	Lung fibrosis↓ Lung inflammation↓ Lung collagen↓ IL-1β↓ KC↓ TIMP-1↓ BALF neutrophils + lymphocytes↓	Lung fibrosis↓ Lung inflammation↓ Lung collagen↓ IL-1β↓ IL-6↓ KC↓ TIMP-1↓ BALF neutrophils + lymphocytes↓	Ashcroft score ↓ TGFβ mRNA↓ Procollagen-1 mRNA↓	Ashcroft score ↓ TGFβ mRNA↓ Procollagen-1 mRNA↓	Skin and lung myofibroblast count↓ Dermal thickness↓ Hydroxyproline↓ ECM↓ Vessel wall thickness↓ Occluded vessels↓ VSMC↓ MVEC apoptosis↑	Airway hyper-reactivity Total cells in BALF↓ Ovalbumin-specific IgE↓ IL-4, IL5, IL-13↓ Vascularization↓ Goblet cell hyperplasia↓ Airway smooth muscle area↓ Hydroxyproline↓	Arthritic score↓ Lung tissue B220+ B-cells↑ CD103+ dendritic cells↑ Monocytes↓ Neutrophils↑	Hydroxyproline↓ BALF lymphocytes and neutrophils↑ Lung tissue inflammatory macrophages↑

↓: significant reduction (independent of dose); ↑: significant increase (independent of dose)

BALF, bronchoalveolar lavage fluid; Cstat, static lung compliance; ECM, extracellular matrix; IgE, immunoglobulin E; IL-1β; interleukin-1β; IL-4, -5, -6, -13, interleukin-4, 5, -6, -13; KC, chemokine CXCL1/KC; MVEC, microvascular endothelial cells; mRNA, messenger RNA; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; VSMC, vascular smooth muscle cells.

*"Preventive" indicates that the administration of nintedanib started simultaneously with the pathogenic trigger. "Therapeutic" indicates that the administration of nintedanib began after the onset of substantial fibrosis. The days/weeks listed indicate when nintedanib was administered relative to the pathogenic trigger. $^{\$}$An effect of nintedanib was shown for treatment from day 10-30. Treatment only from day 20-30 was less effective.

Legend to figure

In fibrosing ILDs, tissue damage may occur at the alveolar epithelial and/or microvascular endothelial sites. Fibroblasts are recruited from resident fibroblasts, circulating fibrocytes, and epithelial cells and fibrocytes undergoing epithelial mesenchymal transition (EMT). Growth factors are released by the damaged epithelium and endothelium and leucocytes are recruited. Mononuclear cells and T cells release profibrotic mediators. Activated fibroblasts transition to myofibroblasts (fibroblast to myofibroblast transition or FMT), which release excessive amounts of ECM. This results in increased tissue stiffness, which further stimulates fibroblast activation, leading to self-sustaining fibrosis.

bFGF; basic fibroblast growth factor, ECM; extracellular matrix, EMT; epithelial mesenchymal transition, FMT; fibroblast to myofibroblast transition, PDGF; platelet-derived growth factor, VEGF; vascular endothelial growth factor.