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Inhibition of PDGF VEGF and FGF signalling attenuates fibrosis

N I Chaudhary[1], G J Roth[2], F Hilberg[3], J Müller-Quernheim[4], A Prasse[4], G Zissel[4], A Schnapp[1§], and J E Park[1]

[1]Department of Pulmonary Research, [2]Department of Chemical Research, Boehringer Ingelheim Pharma GmbH & Co. KG, D-88937 Biberach an der Riss, Germany. [3]Department of Oncology Research, Boehringer Ingelheim Austria GmbH, Vienna, Austria. [4]Department of Pneumology, Medical Centre, University Hospital Freiburg, Germany

§ Corresponding author:
Dr Andreas Schnapp
Department of Pulmonary Research
Boehringer Ingelheim Pharma GmbH & Co. KG
Birkendorferstraße 65, D-88937, Biberach an der Riss
Germany

Email: andreas.schnapp@bc.boehringer-ingelheim.com
Phone : +49 (0)7351-54-5240
Fax : +49 (0)7351-54-5148

Short title:
Inhibition of fibrosis by BIBF1000

Abstract

Background: BIBF1000 is a small molecule inhibitor targeting the receptor kinases of PDGF, bFGF and VEGF, which have known roles in the pathogenesis of pulmonary fibrosis.

Methods: The anti-fibrotic potential of BIBF1000 was determined in a rat model of bleomycin-induced lung fibrosis and in an *ex vivo* fibroblast differentiation assay. Rats exposed to a single intra-tracheal injection of bleomycin were treated with BIBF1000 starting 10 days after bleomycin administration. To gauge for anti-fibrotic activity, collagen deposition and pro-fibrotic growth factor gene expression was analyzed in isolated lungs. Furthermore, the activity of BIBF1000 was compared to imatinib mesylate (combined PDGFR, c-kit, c-abl kinase inhibitor) and SB-431542 (TGFβ receptor I kinase inhibitor) in an *ex vivo* TGFβ-driven fibroblast to myofibroblast differentiation assay, performed in primary human bronchial fibroblasts.

Results: Treatment of rats with BIBF1000 resulted in attenuation of fibrosis as assessed by the reduction of collagen deposition and the inhibition of pro-fibrotic gene expression. In the cellular assay both SB-431542 and BIBF1000 showed dose-dependent inhibition of TGFβ-induced differentiation whereas imatinib mesylate was inactive.

Conclusions: BIBF1000, or related small molecules with a similar kinase inhibition profile, may represent a novel therapeutic approach for the treatment of IPF.

Key Words:
Bleomycin, Lung fibrosis, BIBF1000, imatinib mesylate

BACKGROUND

Fibrotic conditions can occur in all tissues but are especially prevalent in organs that have had frequent exposure to chemical and biological insults, for example the lung, skin, digestive tract, kidney and liver [1-3]. These conditions often compromise the normal function(s) of the organ and many fibrotic diseases are at least severely debilitating, if not life-threatening [4].

Fibroses of the lung represent a set of pathological changes which accompany a wide range of inflammatory conditions of the conducting airways. For instance, in patients with chronic obstructive pulmonary disease a patchy alveolar wall fibrosis with peribronchiolar distribution is present, whereas in patients with chronic asthma fibrosis is predominantly localised to the *lamina reticularis* resulting in a thickening of the basement membrane [5;6]. In both conditions a continuously on-going inflammation-repair cycle in the airways leads to permanent structural changes in the airway wall (remodelling) of which interstitial collagen fibrosis is the major component [7;8]. Similar etiologies have been observed in the liver [9]. In contrast to the fibrotic changes observed in COPD and asthma, in patients with diseases such as idiopathic pulmonary fibrosis (IPF) and acute respiratory distress syndrome (ARDS), the fibrotic changes are more severe and widely disseminated. In these diseases, fibrosis is associated with extreme morbidity and the clinical course is invariably one of gradual deterioration. Median length of survival from time of diagnosis varies between 2.5 and 3.5 yr [4;10].

Although the degree of pulmonary fibrosis differs between various lung diseases, there is evidence to suggest that the underlying pathophysiological mechanisms involved in the development may be similar across diseases. In all forms of pulmonary fibrosis, fibroblasts and myofibroblasts are the most predominant cells [11;12]. Both cell types become activated by growth factors secreted by the airway epithelium after the inflammatory damage [13;14]. Depending on the precise stimulatory milieu, fibroblasts transform to myofibroblasts or proliferate, resulting in areas of fibroblastic foci which are thought to be the sites of active extracellular matrix (ECM), collagen and fibronectin synthesis and which are regarded to the be leading edge of fibrosis [15;16].

The polypeptide mediators and growth factors believed to be pivotal for the fibrotic process include transforming growth factor beta (TGFβ), vascular endothelial growth

factor (VEGF), basic fibroblast growth factor 2 (bFGF-2), platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), chemokine ligand-18 (CCL18) and endothelin-1 (ET-1) [17-26]. Amongst these, TGFβ is believed to be a critical mediator of fibrogenesis, exerting immunologic actions, direct effects on structural cells involved in the synthesis of ECM, fibroblast proliferation, and the differentiation of fibroblasts into myofibroblasts [27;28]. Several preclinical studies have shown that inhibition of TGFβ-signalling results in attenuation of fibrosis in rodents [29-31], suggesting that drug-targeting of the TGFβ pathway could provide a useful therapeutic intervention in human fibrotic diseases including IPF. Unfortunately, TGFβ is a pleiotropic mediator and a number of reports have suggested that anti-TGFβ therapy may result in a number of unacceptable adverse effects [32;33], particularly, tumour promotion.

Another important fibrogenic mediator, PDGF, induces fibroblast chemotaxis, fibroblast proliferation, and promotes fibroblast-mediated tissue matrix contraction [34]. Furthermore, a number of fibrogenic mediators such as TGFβ, IL-1, TNF-α, bFGF and thrombin exhibit PDGF-dependent profibrotic activities [35-39]. Two isoforms of PDGF, namely PDGF-C and PDGF-D, are increased in expression during bleomycin-induced lung fibrosis and it has been shown that (PDGF) receptor tyrosine kinase inhibitors markedly attenuate radiation-induced pulmonary fibrosis [35;40;41;45].

Fibroblasts isolated from patients with moderate to severe asthma have the ability to transform into myofibroblasts after *in vitro* stimulation with TGFβ resulting in the secretion of VEGF, FGF, and ET-1 [42]. ET-1 is a known potent mitogen for smooth muscle cells and is thought to be responsible for the increased smooth muscle mass in patients with chronic inflammation of the lungs. VEGF as well as bFGF-2 are elevated in patients with asthma and are associated with increased vascularity [43;44]. Transfection of the soluble VEGF receptor (*sflt -1*) gene, resulted in attenuation of pulmonary fibrosis in a mouse model of bleomycin-induced pneumopathy, suggesting that an anti-VEGF approach might also offer a suitable anti-fibrotic therapy [18].

We have recently shown in the bleomycin-induced lung fibrosis model in rats that an initial inflammatory phase is followed by subsequent fibrosis. Depending on the treatment scheme, anti-inflammatory and anti-fibrotic activities of test compounds can be discriminated in this model [45]. Using this model, we showed that a prototype anti-inflammatory treatment (the oral steroid prednisolone) attenuated lung fibrosis when commenced at day 1, but had no efficacy if administered from day 10 onwards. In

contrast, treatment with a prototype anti-fibrotic compound (oral imatinib mesylate, a c-abl / c-kit / PDGFR kinase inhibitor) was effective, even when administered beginning at day 10, post-bleomycin treatment [45].

In this study, we used BIBF1000, a prototypical small molecule inhibitor selective for the family of VEGF, FGF, and PDGF receptor tyrosine kinases [46] and studied its activities in the aforementioned therapeutic bleomycin model and in an *ex vivo* assay of pulmonary fibrosis. We show that BIBF1000 attenuates fibrosis *in vivo* and inhibits the differentiation of fibroblasts to myofibroblasts *in vitro*, indicating that this class of compounds may be useful for the treatment of IPF while avoiding the possible adverse effects of direct TGFβ inhibition.

Materials and Methods

Compounds

Imatinib mesylate (Novartis, Basel, Switzerland) and bleomycin sulfate (HEXAL, Holzkirchen, Germany) were purchased from a local pharmacy. BIBF1000 [46] was synthesized by the department of chemistry, Boehringer Ingelheim. SB-431542 [47] is available from Sigma-Aldrich (Schnelldorf, Germany). Recombinant TGFβ_1 (Serotec, Raleigh, North Carolina, USA) and TGFβ_2 (Sigma-Aldrich, Schnelldorf, Germany) were diluted with sterile water and stored in siliconized tubes (Eppendorf, Hamburg, Germany).

Bleomycin administration and treatment protocol

All experiments were performed in accordance with German guidelines for animal welfare and were approved by the responsible authorities.

A dose of 2.2 mg/kg bleomycin sulfate was determined to be efficacious in establishing interstitial pulmonary fibrosis [45]. At day zero, male Wistar rats (10 per group) were intratracheally injected with bleomycin sulfate in 300 μl saline using a catheter (0.5 mm internal diameter, 1.0 mm external diameter) through the nasal passage. To determine the fully effective dose of BIBF1000 rats treated with 2.2 mg/kg bleomycin, were treated with BIBF1000 (10, 30, and 50 mg/kg in 1 ml 0.1% Natrosol) from day 0 to day 21 and fibrotic markers were analyzed in lungs isolated at day 21. 50 mg/kg was the most efficacious dose showing a complete inhibition of bleomycin-induced fibrosis. At none of the applied doses the animals showed any signs of toxicological side effects.

For the experiments described in this manuscript, BIBF1000 (50 mg/kg) was orally administered once daily from day 10 to day 21, after which the rats were sacrified and the lungs excised. As controls, rats were treated on day 0 with saline only (= control group), or rats treated with bleomycin received vehicle alone from days 10 - 21 (= bleomycin group). The degree of fibrosis was analyzed again by gene expression profiling and histology of the excised lungs.

Histology

Histology was performed as described before [45]. Collagen deposition was assessed using Masson's Trichrome staining as previously described [45;48] .

Total RNA extraction and synthesis of cDNA

The total RNA extraction and synthesis of cDNA was carried out using the methods we have previously published [45].

Investigation of gene expression using real time PCR

Gene expression was investigated using the methods we have previously published [45]. Primers for the 18S endogenous control and TGFβ_1 were purchased as pre-developed assay reagent kits (PDAR; Applied Biosystems, California, USA), whereas primers and probes for pro-collagen I, connective tissue growth factor (CTGF) and fibronectin were designed using PrimerExpress™ (Applied Biosystems, California, USA). At least one of the primers or probes in each set overlapped an intron / exon junction, thus eliminating the possibility of amplifying any contaminating genomic DNA in the cDNA sample. The following primer and probe sequences were used: **RAT Fibronectin:** forward **(F):** 5'-GAT GCC GAT CAG AAG TTT GGA-3', reverse **(R):** 5'-TCG TTG GTC GTG CAG ATC TC-3', probe **(Pr):** 5'-FAM-CTG CCC AAT GGC TGC CCA TGA- 3'TAMRA; **RAT Pro-Collagen: F:** 5'-CAG ACT GGC AAC CTG AAG AAG TC-3', **R:** 5'-TCG CCC CTG AGC TCG AT-3', **Pr:** 5'-FAM-CTG CTC CTC CAG GGC TCC AAC GA-3'TAMRA; **RAT CTGF: F:** 5'-CGC CAA CCG CAA GAT TG-3', **R:** 5'-TAC ACG GAC CCA CCG AAG AC-3', **Pr:** 5'-FAM-CGT GTG CAC TGC CAA AGA TGG TGC-3' TAMRA; **Human CTGF: F:** 5'-GCG GCT TAC CGA CTG GAA-3', **R:** 5'-GGA CCA GGC AGT TGG CTC TA-3', **Pr:** 5'-FAM- CAC GTT TGG CCC AGA CCC AAC TAT GA- 3' TAMRA; **Human α-SMA: F:** 5'-GAC AGC TAC GTG GGT GAC GAA-3', **R:** 5'-TTT TCC ATG TCG TCC CAG TTG-3', **Pr:** 5'-FAM-TGA CCC TGA AGT ACC CGA TAG AAC ATG GC-3' TAMRA.

Gene expression investigation of primary fibroblast cultures from patients with fibrotic lung disease

CCD25 lung fibroblasts were purchased from ECACC European Collection of Cell Cultures (Porton Down, Salisbury, Wiltshire; UK). Fibroblasts were obtained from outgrowths of transbronchial biopsy material taken from patients with lung fibrosis at the University Hospital Freiburg (for patient information, see table 2). The study received ethics approval from the appropriate hospital authorities and all patients underwent a process of informed consent. Fresh bronchial biopsies were placed on a 15 cm Petri-dish pre-coated with collagen I (Sigma-Aldrich, Schnelldorf, Germany) in

culture medium (RPMI + 1 % Glutamine + 1 % penicillin / streptomycin + 15 % FCS; Invitrogen, Karlsruhe, Germany). After 21 days, cells were trypsinized and re-cultured in 75 cm^2 tissue culture flasks.

For the fibroblast differentiation assay cells were seeded at a density of 3 x 10^5 cells. Serum-free medium was added 24 h before TGFβ_2 (0.4 nM) and the inhibitors (used at concentrations of 30 nM, 100 nM, 300 nM, 1 μM and 3 μM). After 72 h cells were lysed with 500 μl of Trizol (Invitrogen, Karlsruhe, Germany) and the cell lysate was stored at -80 °C until further analysis.

Immunofluorescent detection of α-SMA as a marker for myofibroblasts

Fibroblasts seeded on 8 well chamber slides at a density of 5 x 10^4 cells per well were incubated in serum free RPMI medium for 24 h. Inhibitors (3 μM) were added 30 min before addition of TGFβ_2 (0.4 nM). After 72 h the medium was removed and the slides were fixed. Detection of αSMA was performed by incubation with a monoclonal anti-αSMA antibody (Sigma-Aldrich, Schnelldorf, Germany; diluted 1:100 with PBS) and a FITC conjugated rabbit anti-mouse antibody (DAKO, Glostrup, Denmark) (diluted 1:500 in PBS). The slides were cover-slipped using a mixture of propidium iodide (DAKO, Glostrup, Denmark) and mounted with MOWIOL (Calbiochem, San Diego, USA).

Phospho-SMAD-2 ELISA

HaCat cells (CLS Cell Lines Service; Eppelheim, Germany) seeded into a 96 well microtiter plate at a concentration of 3 x 10^4 cell per well were incubated for two days. Following an incubation in serum-free medium for 3 h, the compounds, dissolved in medium containing 10 % dimethyl sulfoxide (DMSO), were added up to a final concentration of 50 μM and TGFβ1 (5 ng/ml) was added to the appropriate wells 15 min later. After incubation for 30 min cells were lysed with 120 μl 10 X lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 1 mM PMSF). Lysates were stored at -80 °C. To perform the phospho-SMAD-2 ELISA, a monoclonal anti-SMAD 2/3 antibody (Upstate, Dundee, UK; diluted 1:250) was coated on the surface of a 96 well plate (Nunc F8 Maxisorp) and incubated with the lysates at room temperature for 90 min. A rabbit polyclonal anti-phospho-Smad2 antibody (Upstate, Dundee, UK) was added to the bound material

and immunocomplexes were detected by addition of an alkaline phosphatase labeled mouse anti-rabbit antibody (Dako, Glostrup, Denmark) using p-Nitrophenyl Phosphate (pNPP; Upstate, Dundee, UK) as substrate. The plate was incubated in the dark at 37 $^{\circ}$C and the optical density of the signal was measured at 406 nm with an ELISA plate reader (Tecan Genios Plus, Tecan, Männedorf, Switzerland).

Determining the IC50 values for TGFβRI and TGFβRII kinase inhibition

The inhibitory actions of SB-431542, imatinib mesylate and BIBF1000 on the kinase activity of TGFβRI and TGFβRII were determined using the Promega Kinase-Glo™ kit (Promega, Mannheim, Germany) according to the manufacturer's protocol.

Statistics

All statistical analyses were carried out using GraphPad Prism V 4.02 software (GraphPad, California, USA). Comparisons were made using a non-parametric T-test (Mann-Whitney U test) and a significant value was considered to be $p \leq 0.05$. On all graphs,* signifies a significance level of $p \leq 0.05$, ** signifies a significance level of $p \leq 0.01$ and *** signifies a significance level of $p \leq 0.001$.

Results

The effect of BIBF1000 on the development of fibrosis in a therapeutic rat bleomycin model

BIBF1000 (figure 1) was identified as a selective inhibitor of the family of VEGF, PDGF, and FGF receptor tyrosine kinases [46]. To test whether BIBF1000 would exert anti-fibrotic activity in lung fibrosis, the compound was tested in a rat bleomycin model. BIBF1000 was used at its fully effective dose (50 mg/kg) in a therapeutic setting [45] with daily oral treatment from days 10 to day 21 post bleomycin administration. As controls, groups of rats received saline instead of bleomycin (saline group), or animals treated with bleomycin received vehicle only (bleomycin group). After 22 days, animals were sacrificed and the level of fibrosis was determined by gene expression profiling of TGFβ_1, procollagen-I, fibronectin, and CTGF of isolated lung tissue. As shown in figure 2, the gene expression of these factors is very low in the saline-treated control group and is increased after bleomycin treatment. In rats exposed to bleomycin, treatment with 50 mg/kg BIBF1000 from day 10 to day 21 resulted in expression levels comparable to those observed in rats treated with saline alone.

To address the deposition of collagens at the protein level, lung sections obtained at day 22 were stained with Masson´s Trichrome. As shown in figure 3, collagen deposition, as indicated by blue staining, is weak in the saline-treated control group. In contrast, rats treated with bleomycin alone showed extensive pulmonary fibrosis in the interstitial spaces. Fibrosis was strongly attenuated when bleomycin-treated rats received 50 mg/kg BIBF1000, with collagen staining levels comparable to the rats treated with saline.

TGFβ-stimulated myofibroblast formation is inhibited by BIBF1000 in vitro

It had been previously shown that stimulation of primary fibroblasts with TGFβ induces fibroblast proliferation and differentiation into myofibroblasts [49]. To determine whether BIBF1000 would influence the TGFβ-mediated induction of myofibroblasts, primary fibroblasts obtained from outgrowths of transbronchial biopsies (table 1) were treated with 0.4 nM TGFβ_2 for 72 h in the absence or presence of BIBF1000. Furthermore, SB-431542, reported to be a potent and selective inhibitor of the TGFβ superfamily of kinases [47;50] was included as a reference. The differentiation of fibroblasts to myofibroblasts by TGFβ_2 was determined by assessing the expression

of α smooth muscle actin (αSMA) and connective tissue growth factor (CTGF). As shown in figure 4 A and B, cells treated for 72 h with TGFβ$_2$ display a robust staining for αSMA, suggesting that differentiation into myofibroblasts had taken place. In contrast, both BIBF1000 and SB-431542 blocked the differentiation into myofibroblasts as seen by the absence of αSMA staining (figure 4 C and D). To quantify the effects of BIBF1000 and SB-431542, expression of αSMA was determined by real time PCR. As shown in figure 5, both BIBF1000 and SB-431542 inhibited αSMA gene expression (as well as CTGF gene expression, data not shown) in a concentration-dependent manner in three primary fibroblast cultures and in CCD25 lung fibroblasts.

Since BIBF1000 showed a weak inhibition of the isolated TGFβ-receptor I kinase (table 2), we asked whether the cellular activities mediated by BIBF1000 could be accounted for by direct inhibition of TGFβRI. We therefore established a quantitative ELISA assay for the detection of phospho-SMAD2 (an immediate downstream target of TGFβRI) as a marker for the intracellular activity of TGFβRI. HaCat cells were stimulated with TGFβ for 30 min in the presence or absence of BIBF1000 and the amount of phosphorylated SMAD2 was determined after lysis of the cells. Again, SB-431542 was used as a positive control. As shown in figure 5 B, treatment with BIBF1000, at concentrations exceeding those needed to inhibit TGFβ-mediated fibroblast differentiation, did not block the TGFβ-induced phosphorylation of SMAD2, whereas treatment with SB-431542 abrogated the phosphorylation of SMAD2 in a concentration-dependent manner (figure 5 B). Therefore, we surmise that BIBF1000 is blocking other cellular pathway(s) needed to induce and/or maintain the myofibroblast phenotype without directly interfering with SMAD-dependent TGFβ signalling.

Previously, it has been shown, that imatinib mesylate exerts anti-fibrotic activity in bleomycin-induced lung fibrosis model [41;45]. We were therefore interested in studying the effects of imatinib mesylate on the TGFβ-mediated differentiation of primary fibroblasts to myofibroblasts and on the TGFβ-mediated phosphorylation of SMAD2. As shown in figure 4 E and 5 A, imatinib mesylate did not block TGFβ-induced αSMA expression at the protein or mRNA level in primary fibroblasts nor did it influence the TGFβ-induced phosphorylation of SMAD2 in HaCat cells (figure 5 B).

Discussion

The use of different treatment regimes in the bleomycin model may prove a valuable method by which drugs with true anti-fibrotic potential can be identified and investigated [41;45]. In the present study, we tested BIBF1000, previously identified as an inhibitor of the receptor tyrosine kinases for VEGF, FGF, and PDGF, and show that BIBF1000 is attenuating established lung fibrosis in an *in vivo* setting. Furthermore, the compound blocked TGFβ-mediated differentiation of human primary lung fibroblasts isolated from lung fibrosis patients.

Inhibition of the pathways regulated by CTGF, IGF-I, VEGF, FGF, PDGF and TGFβ have been suggested to provide novel therapeutic approaches to the treatment of fibrosis associated with chronic lung diseases. As discussed earlier, each of these growth factors has distinctive roles in the pathophysiology of fibrosis and many are induced by TGFβ. However, the relative contribution of each of these pathways for the pathogenesis of lung fibrosis remains obscure and may furthermore depend on the specific stage and the type of the fibrotic disease. TGFβ is the most potent pro-fibrotic growth factor known and it has been shown that interference with the TGFβ-pathway will attenuate fibrosis of different origin [30;51;53]. However, direct inhibition of TGFβ-signalling, e.g. via small-molecule inhibition of TGFβ receptor kinases, may not offer a viable therapeutic option due to the pleiotropic functions of this growth factor which suggest that a number of side effects, including especially SMAD-dependent promotion of tumour formation, might be associated with a long-term anti-TGFβ-treatment [31-33]. These concerns are particularly important in light of the dramatically increased lung cancer rates seen in IPF patients [54;55]. It was therefore interesting to note that BIBF1000 was able to block TGFβ-mediated differentation of primary fibroblasts isolated from normal lung and from patients with fibrotic lung diseases in the absence of inhibition of the TGFβ receptor kinases. This suggests that fibroblasts transform to myofibroblasts through the actions of TGFβ via downstream factor(s) which are inhibited by BIBF1000. Since differentiation of fibroblasts to myofibroblasts is a phenomenon seen in fibroblasts isolated from normal lung and from a number of different diseases including asthma [13;56], liver cirrhosis [57], renal fibrosis [58], sarcoidosis, IPF, and UIP, BIBF1000 or related compounds may be of general utility in a number of fibrotic diseases.

It has been shown that c-abl is a SMAD-independent signalling molecule downstream of TGFβ required for morphological transformation and expression of extracellular

matrix [59]. Although we and others have previously shown that imatinib mesylate (a PDGFR / c-abl / c-kit inhibitor) is efficacious in the bleomycin-induced lung fibrosis model [41;45;59], little effect on the differentiation of fibroblasts was observed following treatment with imatinib mesylate, indicating that neither PDGF nor c-abl (nor the combination) are the sole mediators of the differentiation process. As shown by global expression profiling [60], more than 100 genes play a role in TGFβ-mediated fibroblast-myofibroblast differentiation. Future cell culture experiments comparing gene expression profiles with the inhibitors described here could provide important clues about the mechanism of TGFβ-mediated fibroblast-myofibroblast differentiation.

Since BIBF1000 is an inhibitor of the receptor tyrosine kinases for PDGF, FGF, and VEGF it is tempting to speculate that the concerted inhibition of several pro-fibrotic factors is required for its anti-fibrotic activity. PDGF is believed to play a role in the pathogenesis of fibrotic disease by stimulating fibroblast chemotaxis, fibroblast proliferation, and by promoting fibroblast-mediated matrix contraction [61]. Furthermore, PDGF is important in inducing the secretion of growth factors and ECM components in fibroblasts [19] and it induces proliferation and the production of fibronectin of both normal and fibrotic lung fibroblasts. Interestingly, PDGF did not have any effect on the production of interstitial collagens, again, supporting the hypothesis that the concerted action of several factors may be required to induce all aspects of fibrosis. Basic FGF (bFGF or FGF-2), is released by activated fibroblasts and damaged epithelial cells during remodelling processes associated with bronchial asthma [14, 62-64] and it stimulates the proliferation and fibronectin production of human lung fibroblasts. Furthermore, TGFβ$_1$-induced proliferation of fibroblasts is mediated through the release of extracellular FGF-2 since FGF-2-blocking antibodies inhibited the proliferation of fibroblasts [19;62]. Finally, it has been shown that both PDGF and FGF-2 are important factors in the migration of myofibroblasts [65], suggesting that blockade of both pathways might be required to interfere with myofibroblasts.

The function of angiogenesis and of pro-angiogenic factors like VEGF for the pathophysiology of pulmonary fibrosis is currently not understood. Neovascularization with anastamoses between the systemic and pulmonary vasculature is apparent at sites of fibrosis [44;66]. However, a regional heterogeneity of the vascularization in IPF patients has been reported and it has been proposed that this heterogeneity may on the one site support fibroproliferation but may block on the other site normal repair mechanisms [66]. Although the exact site and mechanism of the neovascularization

remains controversial, it is tempting to speculate that angiogenesis may play a role in IPF, acute respiratory distress syndrome (ARDS) and other lung fibroses, and that the use of VEGF inhibitors might attenuate these processes.

We presume that the combined VEGFR, FGFR and PDGFR inhibition of BIBF1000 is acting in a concerted manner to control fibrosis. Of course we cannot rule out the possibility that inhibitory effects of some, as yet, unidentified targets of BIBF1000 may also play a role in this process.

Conclusion

In summary, our data suggest that BIBF1000, or a molecule with a similar kinase inhibition profile, may present a novel therapeutic opportunity to treat IPF. Its distinctive inhibitory profile is uniquely capable of preventing fibroblast-myofibroblast differentiation, a crucial step in the establishment of fibrosis, without directly affecting SMAD signalling. Ultimately, only clinical trials in IPF and other fibrotic diseases will show whether such compounds can stop or slow the inexorable course of this invariably fatal disease.

Competing interests

The authors declare that have no competing interests.

Authors contributions

First author: NIC

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Reference List

1. Dacic S, Yousem SA: **Histologic classification of idiopathic chronic interstitial pneumonias**. *Am.J.Respir.Cell Mol.Biol.* 2003, 29: S5-S9

2. Eddy AA: **Molecular insights into renal interstitial fibrosis**. *J.Am.Soc.Nephrol.* 1996, 7: 2495-2508

3. Wynn TA: **Fibrotic disease and the T(H)1/T(H)2 paradigm.** *Nat.Rev.Immunol.* 2004, 4: 583-594

4. Nadrous HF, Myers JL, Decker PA, Ryu JH: **Idiopathic pulmonary fibrosis in patients younger than 50 years.** *Mayo Clin.Proc.* 2005, 80: 37-40

5. Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR: **Myofibroblasts and subepithelial fibrosis in bronchial asthma.** *Am.J.Respir.Cell Mol.Biol.* 1990, 3: 507-511

6. Roche WR, Beasley R, Williams JH, Holgate ST: **Subepithelial fibrosis in the bronchi of asthmatics.** *Lancet* 1989,1: 520-524

7. Holgate ST, Lackie PM, Davies DE, Roche WR, Walls AF: **The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma.** *Clin.Exp.Allergy* 1999, 29 Suppl 2: 90-95

8. Jeffery PK: **Remodeling in asthma and chronic obstructive lung disease.** *Am.J.Respir.Crit Care Med.* 2001, 164: S28-S38

9. Elsharkawy AM, Oakley F, Mann DA: **The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis.** *Apoptosis* 2005, 10: 927-939

10. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am.J.Respir.Crit Care Med.* 2002, 165: 277-304

11. Uhal BD, Joshi I, True AL, Mundle S, Raza A, Pardo A, Selman M: **Fibroblasts isolated after fibrotic lung injury induce apoptosis of alveolar epithelial cells in vitro.** *Am.J.Physiol* 1995, 269: L819-L828

12. Ramos C, Montano M, Garcia-Alvarez J, Ruiz V, Uhal BD, Selman M, Pardo A: **Fibroblasts from idiopathic pulmonary fibrosis and normal lungs differ in growth rate, apoptosis, and tissue inhibitor of metalloproteinases expression.** *Am.J.Respir.Cell Mol.Biol.* 2001, 24: 591-598

13. Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL: **Epithelial-mesenchymal interactions in the pathogenesis of asthma.** *J.Allergy Clin.Immunol.* 2000, 105: 193-204

14. Zhang S, Smartt H, Holgate ST, Roche WR: **Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: an in vitro co-culture model of airway remodelling in asthma.** *Lab Invest* 1999, 79: 395-405

15. Cosgrove GP, Brown KK, Schiemann WP, Serls AE, Parr JE, Geraci MW, Schwarz MI, Cool CD, Worthen GS: **Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: a role in aberrant angiogenesis.** *Am.J.Respir.Crit Care Med.* 2004, 170: 242-251

16. Crystal RG, Bitterman PB, Mossman B, Schwarz MI, Sheppard D, Almasy L, Chapman HA, Friedman SL, King TE Jr., Leinwand LA, Liotta L, Martin GR, Schwartz DA, Schultz GS, Wagner CR, Musson RA: **Future research directions in idiopathic pulmonary fibrosis: summary of a National Heart, Lung, and Blood Institute working group.** *Am.J.Respir.Crit Care Med.* 2002, 166: 236-246

17. Fehrenbach H, Haase M, Kasper M, Koslowski R, Schuh D, Muller M: **Alterations in the immunohistochemical distribution patterns of vascular endothelial growth factor receptors Flk1 and Flt1 in bleomycin-induced rat lung fibrosis.** *Virchows Arch.* 1999, 435: 20-31

18. Hamada N, Kuwano K, Yamada M, Hagimoto N, Hiasa K, Egashira K, Nakashima N, Maeyama T, Yoshimi M, Nakanishi Y: **Anti-vascular endothelial growth factor gene therapy attenuates lung injury and fibrosis in mice.** *J.Immunol.* 2005, 175: 1224-1231

19. Hetzel M, Bachem M, Anders D, Trischler G, Faehling M: **Different effects of growth factors on proliferation and matrix production of normal and fibrotic human lung fibroblasts.** *Lung* 2005, 183: 225-237

20. Howell DC, Goldsack NR, Marshall RP, McAnulty RJ, Starke R, Purdy G, Laurent GJ, Chambers RC: **Direct thrombin inhibition reduces lung collagen, accumulation, and connective tissue growth factor mRNA levels in bleomycin-induced pulmonary fibrosis.** *Am.J.Pathol.* 2001, 159: 1383-1395

21. Krein PM, Winston BW: **Roles for insulin-like growth factor I and transforming growth factor-beta in fibrotic lung disease.** *Chest* 2002, 122: 289S-293S

22. Lappi-Blanco E, Soini Y, Kinnula V, Paakko P: **VEGF and bFGF are highly expressed in intraluminal fibromyxoid lesions in bronchiolitis obliterans organizing pneumonia.** *J.Pathol.* 2002, 196: 220-227

23. Lasky JA, Ortiz LA, Tonthat B, Hoyle GW, Corti M, Athas G, Lungarella G, Brody A, Friedman M: **Connective tissue growth factor mRNA expression is upregulated in bleomycin-induced lung fibrosis.** *Am.J.Physiol* 1998, 275: L365-L371

24. Masamune A, Satoh M, Kikuta K, Suzuki N, Shimosegawa T: **Endothelin-1 stimulates contraction and migration of rat pancreatic stellate cells.** *World J.Gastroenterol.* 2005, 11: 6144-6151

25. Ogata T, Miyauchi T, Irukayama-Tomobe Y, Takanashi M, Goto K, Yamaguchi I: **The Peroxisome Proliferator-activated Receptor alpha Activator Fenofibrate Inhibits Endothelin-1-induced Cardiac Fibroblast Proliferation.** *J.Cardiovasc.Pharmacol.* 2004, 44 Suppl 1: S279-S282

26. Prasse A, Pechkovsky DV, Toews GB, Jungraithmayr W, Kollert F, Goldmann T, Vollmer E, Muller-Quernheim J, Zissel G: **A Vicious Circle of Alveolar Macrophages and Fibroblasts Perpetuates Pulmonary Fibrosis via CCL18.** *Am.J.Respir.Crit Care Med.* 2006

27. Coker RK, Laurent GJ, Shahzeidi S, Lympany PA, du Bois RM, Jeffery PK, McAnulty RJ: **Transforming growth factors-beta 1, -beta 2, and -beta 3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis.** *Am.J.Pathol.* 1997, 150: 981-991

28. McAnulty RJ, Campa JS, Cambrey AD, Laurent GJ: **The effect of transforming growth factor beta on rates of procollagen synthesis and degradation in vitro.** *Biochim.Biophys.Acta* 1991, 1091: 231-235

29. Bonniaud P, Margetts PJ, Kolb M, Schroeder JA, Kapoun AM, Damm D, Murphy A, Chakravarty S, Dugar S, Higgins L, Protter AA, Gauldie J: **Progressive Transforming Growth Factor{β}1-induced Lung Fibrosis Is Blocked by an Orally Active ALK5 Kinase Inhibitor.** *Am.J.Respir.Crit Care Med.* 2005, 171: 889-898.

30. de Gouville AC, Huet S: **Inhibition of ALK5 as a New Approach to Treat Liver Fibrotic Diseases.** *Drug News Perspect* 2006, 19(2): 85-90

31. Laping NJ, Grygielko E, Frazier K, Harling J, Osborn RR, Underwood D, Portis M, Walker CL: **ALK5 Kinase Inhibitors as Potential Treatment for Fibrosis and Uterine Fibroids.** Keystone Symposia Therapeutic Strategies based on TGF beta or antagonists. 2005, Ref Type: Abstract

32. Tang B, Vu M, Booker T, Santner SJ, Miller FR, Anver MR, Wakefield LM: **TGF-beta switches from tumor suppressor to prometastatic factor in a model of breast cancer progression.** *J.Clin.Invest* 2003, 112: 1116-1124

33. Wakefield LM, Roberts AB: **TGF-beta signaling: positive and negative effects on tumorigenesis.** *Curr.Opin.Genet.Dev.* 2002, 12: 22-29

34. Bonner JC: **Regulation of PDGF and its receptors in fibrotic diseases.** *Cytokine Growth Factor Rev.* 2004, 15: 255-273

35. Abdollahi A, Li M, Ping G, Plathow C, Domhan S, Kiessling F, Lee LB, McMahon G, Grone HJ, Lipson KE, Huber PE: **Inhibition of platelet-derived growth factor signaling attenuates pulmonary fibrosis.** *J.Exp.Med.* 2005, 201: 925-935

36. Battegay EJ, Raines EW, Seifert RA, Bowen-Pope DF, Ross R: **TGF-beta induces bimodal proliferation of connective tissue cells via complex control of an autocrine PDGF loop.** *Cell* 1990, 63: 515-524

37. Battegay EJ, Raines EW, Colbert T, Ross R: **TNF-alpha stimulation of fibroblast proliferation. Dependence on platelet-derived growth factor (PDGF) secretion and alteration of PDGF receptor expression.** *J.Immunol.* 1995, 154: 6040-6047

38. Raines EW, Dower SK, Ross R: **Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA.** *Science* 1989, 243: 393-396

39. Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J: **Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis.** *J.Clin.Invest* 2001, 107: 1529-1536

40. Zhuo Y, Zhang J, Laboy M, Lasky JA: **Modulation of PDGF-C and PDGF-D expression during bleomycin-induced lung fibrosis.** *Am.J.Physiol Lung Cell Mol.Physiol* 2004, 286: L182-L188

41. Aono Y, Nishioka Y, Inayama M, Ugai M, Kishi J, Uehara H, Izumi K, Sone S: **Imatinib as a Novel Antifibrotic Agent in Bleomycin-induced Pulmonary Fibrosis in Mice.** *Am.J.Respir.Crit. Care Med.* 2005, 171: 1279-1285

42. Richter A, Puddicombe SM, Lordan JL, Bucchieri F, Wilson SJ, Djukanovic R, Dent G, Holgate ST, Davies DE: **The contribution of interleukin (IL)-4 and IL-13 to the epithelial-mesenchymal trophic unit in asthma.** *Am.J.Respir.Cell Mol.Biol.* 2001, 25: 385-391

43. Hoshino M, Takahashi M, Aoike N: **Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis.** *J.Allergy Clin.Immunol.* 2001, 107: 295-301

44. Turner-Warwick M: **Precapillary systemic-pulmonary anastomoses.** *Thorax* 1963, 18: 225-237

45. Chaudhary NI, Schnapp A, Park JE: **Pharmacological Differentiation of Inflammation and Fibrosis in the Rat Bleomycin Model.** *Am.J.Respir.Crit Care Med* 2006, 173: 769-776

46. Bisping G, Kropff M, Wenning D, Dreyer B, Bessonov S, Hilberg F, Roth GJ, Munzert G, Stefanic M, Stelljes M, Scheffold C, Muller-Tidow C, Liebisch P, Lang N, Tchinda J, Serve HL, Mesters RM, Berdel WE, Kienast J: **Targeting receptor kinases by a novel indolinone derivative in multiple myeloma: abrogation of stroma-derived interleukin-6 secretion and induction of apoptosis in cytogenetically defined subgroups.** *Blood* 2005, 107: 2079-2089

47. Laping NJ, Grygielko E, Mathur A, Butter S, Bomberger J, Tweed C, Martin W, Fornwald J, Lehr R, Harling J, Gaster L, Callahan JF, Olson BA: **Inhibition of transforming growth factor (TGF)-beta1-induced extracellular matrix with a novel inhibitor of the TGF-beta type I receptor kinase activity: SB-431542.** *Mol.Pharmacol.* 2002, 62: 58-64.

48. Yi ES, Lee H, Yin S, Piguet P, Sarosi I, Kaufmann S, Tarpley J, Wang NS, Ulich TR: **Platelet-derived growth factor causes pulmonary cell proliferation and collagen deposition in vivo.** *Am.J.Pathol.* 1996, 149: 539-548

49. Hashimoto S, Gon Y, Takeshita I, Matsumoto K, Maruoka S, Horie T: **Transforming growth Factor-beta1 induces phenotypic modulation of human lung fibroblasts to myofibroblast through a c-Jun-NH2-terminal kinase-dependent pathway.** *Am.J.Respir.Crit Care Med.* 2001, 163: 152-157

50. Matsuyama S, Iwadate M, Kondo M, Saitoh M, Hanyu A, Shimizu K, Aburatani H, Mishima HK, Imamura T, Miyazono K, Miyazawa K: **SB-431542 and Gleevec inhibit transforming growth factor-beta-induced proliferation of human osteosarcoma cells.** *Cancer Res.* 2003, 63: 7791-7798

51. Li Y, Azuma A, Usuki J, Abe S, Matsuda K, Sunazuka T, Shimizu T, Hirata Y, Inagaki H, Kawada T, Takahashi S, Kudoh S, Omura S: **EM703 improves bleomycin-induced pulmonary fibrosis in mice by the inhibition of TGF-beta signaling in lung fibroblasts.** *Respir.Res.* 2006, 7: 16

52. Santiago B, Gutierrez-Canas I, Dotor J. Palao G, Lasarte JJ, Ruiz J, Prieto J, Borras-Cuesta F, Pablos JL: **Topical application of a peptide inhibitor of transforming growth factor-beta1 ameliorates bleomycin-induced skin fibrosis.** *J.Invest Dermatol.* 2005, 125: 450-455

53. Pittet JF, Griffiths MJ, Geiser T, Kaminski N, Dalton SL, Huang X, Brown LA, Gotwals PJ, Koteliansky VE, Matthay MA, Sheppard D: **TGF-beta is a critical mediator of acute lung injury.** *J.Clin.Invest* 2001, 107: 1537-1544

54. Kishi K, Homma S, Kurosaki A, Motoi N, Yoshimura K: **High-resolution computed tomography findings of lung cancer associated with idiopathic pulmonary fibrosis.** *J.Comput.Assist.Tomogr.* 2006, 30: 95-99

55. Park J, Kim DS, Shim TS, Lim CM, Koh Y, Lee SD, Kim WS, Kim WD, Lee JS, Song KS: **Lung cancer in patients with idiopathic pulmonary fibrosis.** *Eur.Respir.J.* 2001, 17: 1216-1219

56. Chaudhary NI, Richter A, Roche WR, Powell RM, Hamilton L, Holgate ST., Davies DE: **The role of autocrine growth factor in the proliferation of asthmatic (myo)fibroblasts.** *Am.J.Respir.Crit Care Med.* 2003, 165[8], A78 Ref Type: Abstract

57. Guyot C, Lepreux S, Combe C, Doudnikoff E, Bioulac-Sage P, Balabaud C, Desmouliere A: **Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved.** *Int.J.Biochem.Cell Biol.* 2006, 38: 135-151

58. Qi W, Chen X, Poronnik P, Pollock CA: **The renal cortical fibroblast in renal tubulointerstitial fibrosis.** *Int.J.Biochem.Cell Biol.* 2006, 38: 1-5

59. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, Leof EB: **Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis.** *J.Clin.Invest* 2004, 114: 1308-1316

60. Chambers RC, Leoni P, Kaminski N, Laurent GJ, Heller RA: **Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching.** *Am.J.Pathol.* 2003, 162: 533-546

61. Levitzki A: **PDGF receptor kinase inhibitors for the treatment of PDGF driven diseases.** *Cytokine Growth Factor Rev.* 2004, 15: 229-235

62. Khalil N, Xu YD, O'Connor R, Duronio V: **Proliferation of pulmonary interstitial fibroblasts is mediated by transforming growth factor-beta1-induced release of extracellular fibroblast growth factor-2 and phosphorylation of p38 MAPK and JNK.** *J.Biol.Chem.* 2005, 280: 43000-43009

63. Jones SG, Morrisey K, Williams JD, Phillips AO: **TGF-beta1 stimulates the release of pre-formed bFGF from renal proximal tubular cells.** *Kidney Int.* 1999, 56: 83-91

64. Vlodavsky I, Folkman J, Sullivan R, Fridman R, Ishai-Michaeli R, Sasse J, Klagsbrun M: **Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix.** *Proc.Natl.Acad.Sci.U.S.A* 1987, 84: 2292-2296

65. Kilarski WW, Jura N, Gerwins P: **An ex vivo model for functional studies of myofibroblasts.** *Lab Invest* 2005, 85: 643-654

66. Keane MP: **Angiogenesis and pulmonary fibrosis: feast or famine?** *Am.J.Respir.Crit Care Med.* 2004, 170: 207-209

Figures

Figure 1

Chemical structure of BIBF1000

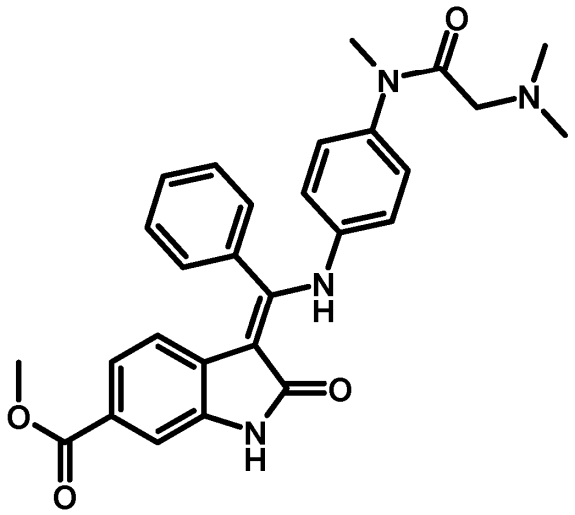

Figure 1

Figure 2:

BIBF1000 inhibits the gene expression of pro-fibrotic marker genes in the rat bleomycin model. Rats (10 animals per group) were treated either with saline or Bleomycin on day 0. Treatment with vehicle or BIBF1000 (p.o.; 50 mg/kg) commenced at day 10 and was continued daily until day 21. On day 22, rats were sacrificed and a part of the left lung lobe was processed for RNA extraction. The gene expression levels of TGFß$_1$, Procollagen I, Fibronectin, and CTGF were determined by quantitative RT-PCR. The gene expression for each gene is indicated relative to endogenous 18S RNA control. Values are given as fold induction. The bars represent median values. Statistics were determined by use of a Mann-Whitney U test (*** p = 0.001).

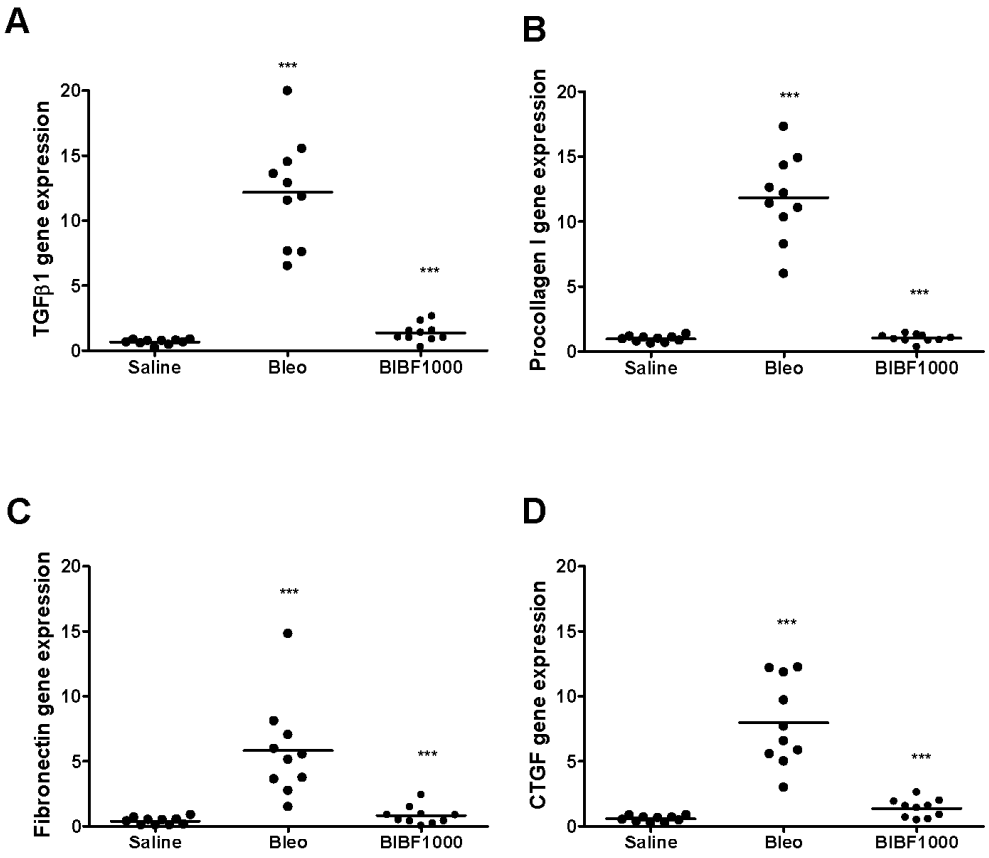

Figure 2

Figure 3

Collagen staining of representative lung sections. Rats (10 animals per group) were treated either with saline or Bleomycin on day 0. Treatment with vehicle or BIBF1000 (p. o.; 50 mg/kg) commenced on day 10 and was continued daily until day 21. On day 22, rats were sacrificed and the lungs were fixed with paraformaldehyde,

prior to paraffin embedding. Sections (4 μM) were stained with Masson's Trichrome stain. Muscle and cells are stained red, nuclei are stained black and collagens are stained blue. Three representative photomicrographs are shown for each of the groups. Magnification x 250.

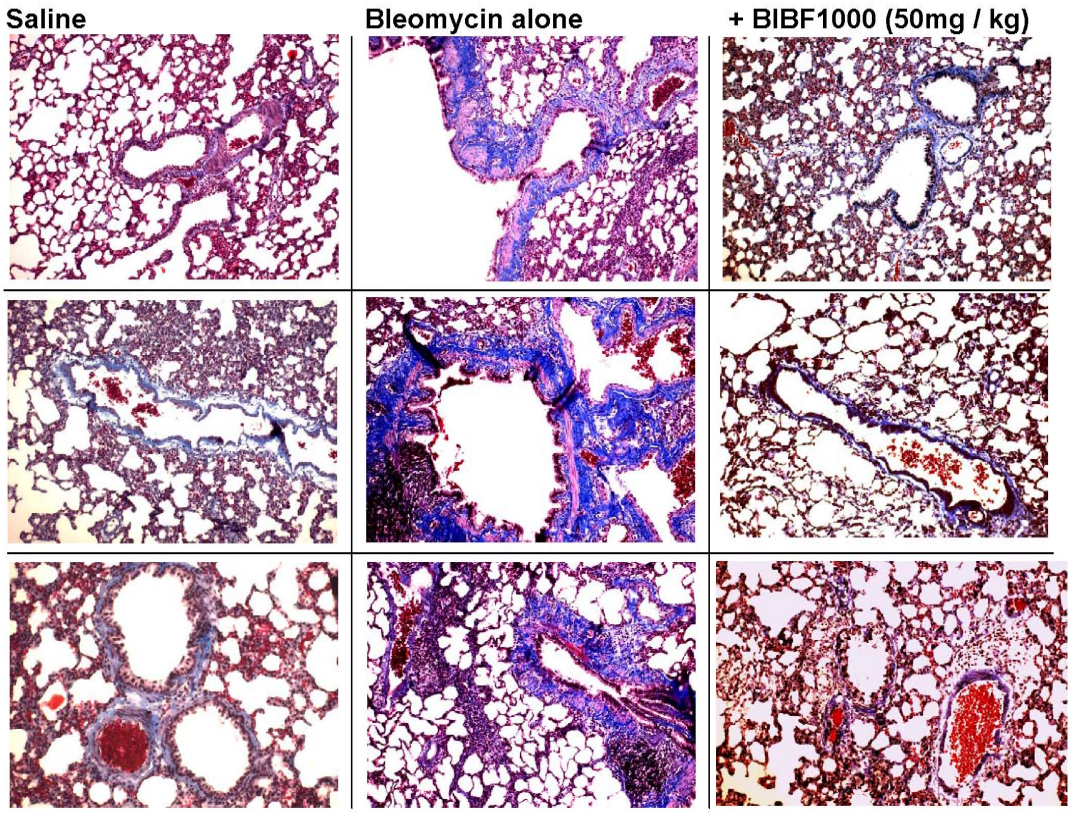

Figure 3

Figure 4

BIBF1000 blocks TGFß-mediated differentiation of fibroblasts.

Fibroblasts obtained from from biopsies of patients with fibrotic lung disease were cultured on collagen I coated chamber slides for 72 h in (**A**) serum free medium (SFM) alone, (**B**) SFM + 0.4 nM TGFβ2, (**C**) SFM + 0.4 nM TGFβ2 + 5 μM SB-431542, (**D**) SFM + 0.4 nM TGFβ2 + 5 μM BIBF1000, or (**E**) SFM + 0.4 nM TGFβ2 + 5 μM imatinib mesylate. αSMA filaments (green) were detected with a monoclonal antibody and visualised with a fluorescein conjugated rabbit anti-mouse antibody. Magnification x 400.

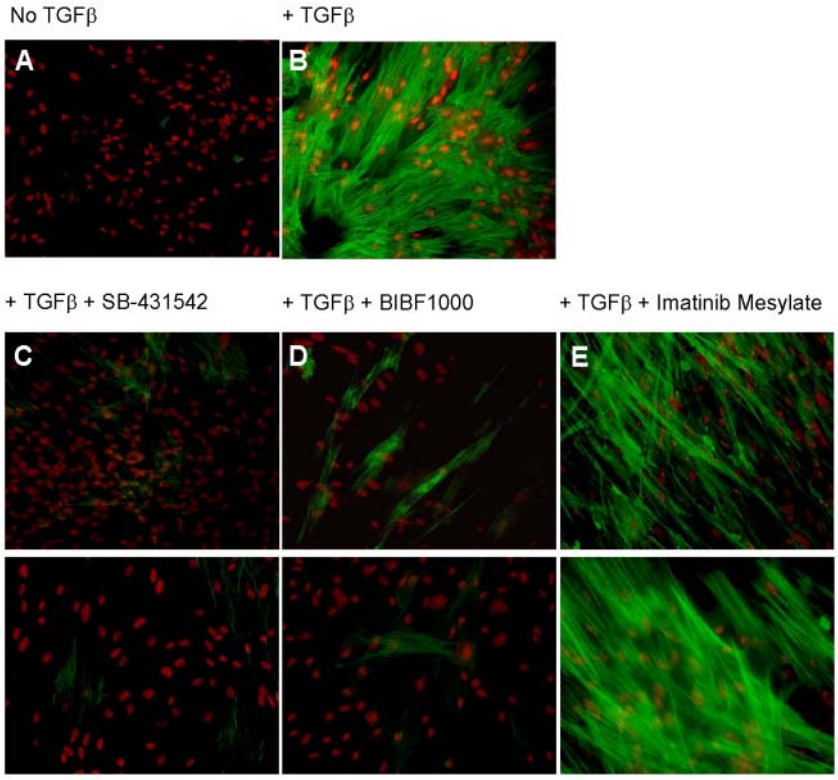

Figure 4

Figure 5

Activity of BIBF1000, imatinib mesylate, and SB-431542 on TGFß$_2$-induced α-smooth muscle gene expression and on TGFß-mediated SMAD phosphorylation. (A) Primary fibroblasts lines isolated from bronchial biopsies of three patients with lung fibrosis (# 2217, 2272, 2278) and the primary lung fibroblast line CCD25 were incubated with TGFβ$_2$ in the presence of SB-431542, BIBF1000 and imatinib mesylate at concentrations ranging from 0 - 30 μM. After for 72 h the gene expression levels of αSMA were determined by quantitative RT-PCR, normalised relative to endogenous 18 S RNA. Data are presented as % of gene expression compared to DMSO alone. **(B)** HaCat cells were incubated in serum free medium with the respective inhibitors to final concentrations ranging from 0 to 50 μM. After 15 min, 5 ng/ml TGFβ$_1$ was added and incubation was continued for 30 min before the cells were lysed. The amount of phosphorylated Smad2 was determined by ELISA. 100% corresponds to the phosphorylation of Smad2 after stimulation with 5 ng/ml TGFβ$_1$.

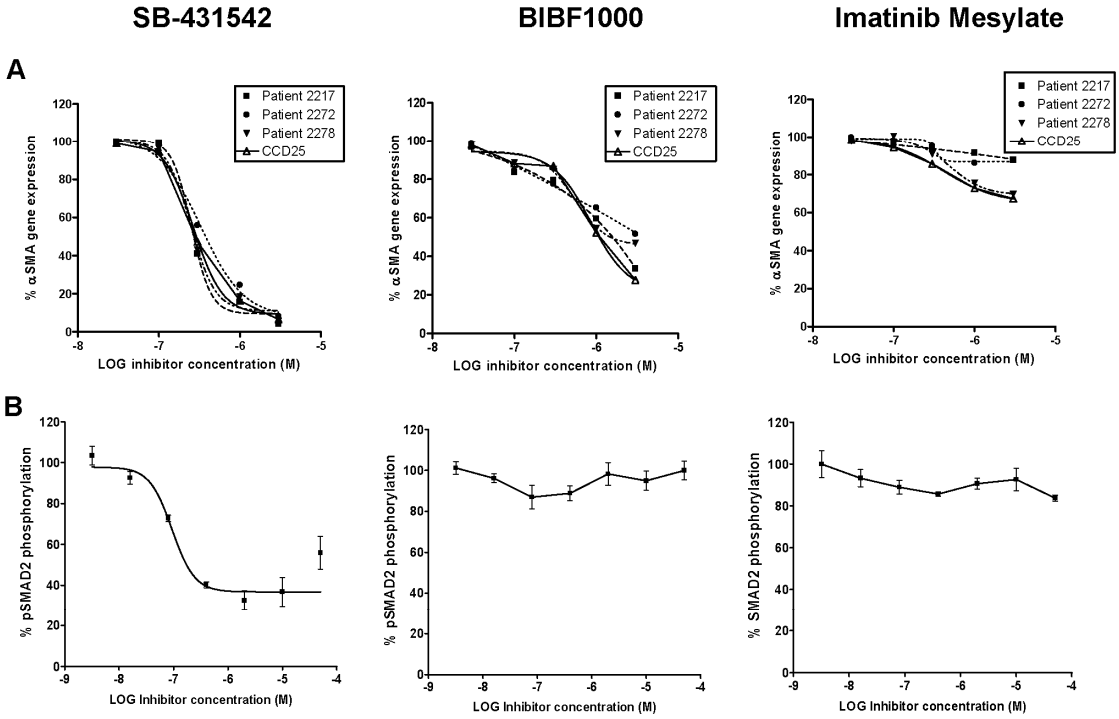
SB-431542
BIBF1000
Imatinib Mesylate
A
B
Patient 2217
Patient 2272
Patient 2278
CCD25
% αSMA gene expression
LOG inhibitor concentration (M)
% pSMAD2 phosphorylation
% SMAD2 phosphorylation
LOG Inhibitor concentration (M)

Figure 5

Tables

Table 1: Patient information from cultured primary bronchial fibroblasts

Patient/ cell line	Sex	Age	Diagnosis	Smoker
2217	F	40	Sarcoidosis	No
2272	F	75	Idiopathic lung fibrosis	No
2278	M	56	Fibrosis / Usual Interstitial Pneumonia	No
CCD25	M	7	Glioma (normal lung)	No

Table 2: The IC50 values for BIBF1000, Imatinib Mesylate and SB-431542 against TGFβ receptors I & II

Inhibitor	IC_{50}	
Imatinib Mesylate	TGFβ RI TGFβ RII	> 50 μM > 50 μM
BIBF1000	TGFβ RI TGFβ RII	1.6 μM > 50 μM
SB-431542	TGFβ RI TGFβ RII	125 nM 4.2 μM