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Galectin-3 levels are elevated following nintedanib treatment

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Abstract

Background and Aims: Idiopathic pulmonary fibrosis (IPF) is a common and severe form of pulmonary fibrosis. Nintedanib, a triple angiokinase inhibitor, is approved for treating IPF. Galectin 3 (Gal-3) activates a variety of profibrotic processes. Currently, the Gal-3 inhibitor TD139 is being tested in phase II clinical trials. Since this treatment is given 'on top' of nintedanib, it is important to estimate its effect on Gal-3 levels. Therefore, we evaluated the impact of nintedanib on Gal-3 expression using both *in vitro* and *in vivo* models, in addition to serum samples from patients with IPF .

Methods: Gal-3 levels were evaluated in IPF and control tissue samples, primary human lung fibroblasts (HLFs) following nintedanib treatment (10–100 nM, quantitative polymerase chain reaction), and in a silica-induced fibrosis mouse model with/without nintedanib (0.021–0.21 mg/kg) by immunohistochemistry. In addition, Gal-3 levels were analyzed in serum samples from 41 patients with interstitial lung disease patients with/without nintedanib treatment by ELISA.

Results: Nintedanib addition to HLFs resulted in significant elevations in Gal-3, phospho-signal transducer and activator of transcription 3 (pSTAT3), as well as IL-8 mRNA levels ($p < 0.05$). Gal-3 expression was higher in samples from IPF patients compared with non-IPF controls at the protein and mRNA levels ($p < 0.05$). In the *in vivo* mouse model, Gal-3 levels were increased following fibrosis induction and even further increased with the addition of nintedanib, mostly in macrophages ($p < 0.05$). Patients receiving nintedanib presented with higher Gal-3 serum levels compared with those who did not receive nintedanib ($p < 0.05$).

Conclusion: Nintedanib elevates Gal-3 levels in both experimental models, along with patient samples. These findings highlight the possibility of using combined inhibition therapy for patients with IPF.

Keywords: galectin-3, idiopathic pulmonary fibrosis, *in vivo* models, nintedanib, signal transducer and activator of transcription 3 (STAT3)

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Introduction

Idiopathic pulmonary fibrosis (IPF) is considered the most common and severe form of pulmonary fibrosis, with a 5-year survival rate of only 20% reflecting the lack of effective therapies.[1] The pathology of IPF is characterized by scar tissue formation (fibrosis) and excessive extracellular matrix deposition, resulting in the loss of lung function.

Galectin-3 (Gal-3) is currently regarded as a potential inflammatory marker identified as a molecule that functions to drive the inflammatory response and oxidative stress.[2,3] It was shown to modulate cell–receptor interactions, immune response, cancer cell behavior, inflammation, as well as scarring processes.[4–8] Accumulating evidence indicates that Gal-3 activates a variety of profibrotic factors, promotes fibroblast

proliferation and transformation, and mediates collagen production.[9] There are several known ligands for Gal-3 that are involved in its profibrotic functions, in particular various glycosylated matrix proteins (i.e. laminin, fibronectin, and integrins).[10] Numerous studies have defined the key role of Gal-3 in fibrogenesis in diverse organ systems, including the liver, lung, heart, and kidney.[11–13]

There are multiple pathways implicated in fibrosis progression.[14,15] Currently, nintedanib and pirfenidone are the only FDA-approved IPF treatments.[14,15] Nintedanib is a triple angiokinase inhibitor that has been shown to block platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF).[16] However, the precise mechanism of nintedanib in preventing fibrosis is not completely understood.

Although proven effective in several clinical trials for the reduction of forced vital capacity (FVC) decline,[17–19] patients experience frequent adverse events (e.g. diarrhea and increased aminotransferase levels). Thus, new treatment options are currently being explored including the Gal-3 inhibitor, TD139 (Galecto, Copenhagen, Denmark). Since this treatment is given 'on top' of nintedanib, it is important to evaluate its effect on Gal-3 levels.

This study assessed the impact of nintedanib treatment on Gal-3 levels using both *in vitro* and *in vivo* models. In addition, serum Gal-3 levels were evaluated in patients with IPF receiving nintedanib.

Materials and methods

Primary human lung fibroblast culture

Primary human lung fibroblasts (HLFs) were isolated by the explant culture method from lung tissue samples of 9 patients with IPF (histologically confirmed) and 20 normal control donors (N-HLF) (histologically normal lung distant from a resected tumor). The isolation was performed as described previously.[20] Following extraction, the HLFs were cultured in Dulbecco's modified Eagle's medium supplemented with 15% FCS, L-glutamine (2 mM), and antibiotics (Biological Industries, Beit-Haemek, Israel). Cells were maintained in 5% CO_2 at 37°C. Nintedanib (10–100 nM, provided by Avalyn Pharma, USA) was dissolved in DMSO. A similar DMSO concentration served as control.

Study population and serum sampling

The study included sera samples from 41 patients with interstitial lung disease (ILD), above 18 years of age. The medical records of all participants were reviewed to gather data including age, gender, comorbidities, and pulmonary function test results. Blood samples were taken only from participants who had previously consented and authorized the use of their samples for research purposes. Serum was harvested after low-speed centrifugation (3000 g for 10 min) and stored at –80°C before analysis.

Biomarker analysis

Gal-3 was measured using a validated competitive Enzyme-Linked Immunosorbent Assay (ELISA) produced by BG Medicine (Waltham, MA, USA) according to the manufacturer's instructions.

In vivo *silica mouse model*

Mouse studies were performed in collaboration with McMaster University using established protocols, as previously described.[21] Briefly, IPF was induced on day 1 in 20–22 g female C57BL/6 mice by a single intratracheal intubation of silica (2.5 mg/kg) or phosphate-buffered saline (PBS) control while animals were under isoflurane anesthesia. Nintedanib was delivered using intranasal (IN) administration of 50 µl formulation directly to the lung.[21,22] After acclimation, animals were randomized based upon body weight. On days 10–29, animals were lightly anesthetized with isoflurane and dosed once a day with 0.021, 0.21, or 2.1 mg/kg nintedanib (35 µl per dose) or inhaled vehicle. Doses and number of animals were chosen based on previous reports,[23,24] A total of 10 mice were used for the control, and 13 were enrolled into each IN dose group. Treatment/controls were given in parallel, and thus the order of treatments and measurements was similar between the groups. The left lobe of each lung was inflated to 30 cmH2O for 3–5 min in a 10% formalin solution for paraffin block preparation.

Immunohistochemistry

Sections prepared from mice lungs were deparaffinized in xylene and alcohol, rinsed in PBS and immersed in citrate buffer (pH 6). The samples were stained using the Mouse on Mouse ImmPRESS polymer kit (Vector Laboratories, Burlingame, CA, USA) according to manufacturers' instructions. Slides were incubated overnight at 4°C with the anti-Gal-3 primary antibody (1:200) (Abcam, Cambridge, MA, USA), and developed using ImmPACT DAB (Vector Laboratories) according to manufacturers' instructions. Use of the isotype-matched control antibody (negative control) eliminated nonspecific staining. The expression level of Gal-3 was measured using QuPath in a blinded measurement.[25]

Real-time quantitative polymerase chain reaction

Following cultures, RNA was extracted from HLFs using the RNeasy kit (QAIGEN, Hilden, Germany). Extracted RNA was converted to cDNA using GeneAmp (Applied Biosystems, Waltham, MA, USA USA). Quantitative polymerase chain reaction was performed using Power SYBR Green (Applied Biosystems) to validate the expression pattern of Gal-3 (LGALS3): forward :5′-CAGAATTGCTTTAGATTTCCAA-3′; reverse:5′-TTATCCAGCTTTGTATTGCAA-3′, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene: forward: 5′-CTCTGCTCCTCCTGTTCGAC-3′, reverse: 5′ TTAAAAGCAGCCCTGGTGAC 3′. IL-8 forward: 5′-CTCTTGGCAGCCTTCCTG ATTT-3′ and reverse: 5′-TGGGGTGGAAAGG TTTGGAGTA -3′.

Western blot

N-HLF, IPF-HLF, or 10 mg of lung tissue samples were lysed and western blot was performed as previously described.[26] The following rabbit/mouse antihuman antibodies were used: Gal-3 (ab2785) and GAPDH (ab9484) (Abcam, USA), phospho-signal transducer and activator of transcription 3 (pSTAT3) S727 (#9134) and STAT3 (#9139) (Cell Signaling Technologies, USA), alpha-tubulin (T5168) from Sigma-Aldrich (Burlington, MA, USA). Bound antibodies were visualized using goat peroxidase-conjugated secondary antibodies (Millipore, Burlington, MA, USA), anti-mouse IgG #AP308P and anti-rabbit IgG #AP307P) followed by enhanced chemiluminescence detection (Millipore, USA). LAS-3000 (Fujifilm, Tokyo, Japan) was used to quantify protein expressions. Results were normalized to tubulin and GAPDH.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA) and by SPSS (IBM). ANOVA was performed to compare differences between multiple cohorts. Paired Student's *t*-tests were employed to analyze differences between two groups. An effect was considered significant when the p value was <0.05. All experiments were repeated at least three times.

Ethical approval

The HLF and serum sample studies were approved by the Ethics Committee of the Meir Medical Center (MMC-016-16, MMC-001-20, respectively). Informed consent was obtained from all patients. The nintedanib mouse studies were approved by the Animal Research Ethics Board of McMaster University (IRB Protocol #160414) and carried out in accordance with the Guidelines of the Canadian Council on Animal Care. Animals were monitored for weight loss and other signs of physical distress throughout these experiments.

Results

Nintedanib exposure elevates Gal-3 levels in HLF cells

In order to test the effect of nintedanib on Gal-3 expression, N-HLFs were exposed to nintedanib (10–100 nM) for 24 h, and Gal-3 levels were tested. In fact, exposure to nintedanib resulted in a significant elevation in Gal-3 protein and mRNA levels (Figure 1(a) and (b)). Since Gal-3 is known to be activated by the STAT3 pathway, we also tested STAT3 phosphorylation, which was also found to be elevated by nintedanib following 1 h of exposure in N-HLFs ($p<0.05$) (Figure 1(c-d)). IL-8 that was previously shown to be induced by Gal-3 and linked to STAT3 (Figure 1(e))[27] was also significantly elevated by the nintedanib treatment ($p<0.05$) (Figure 1(f)).

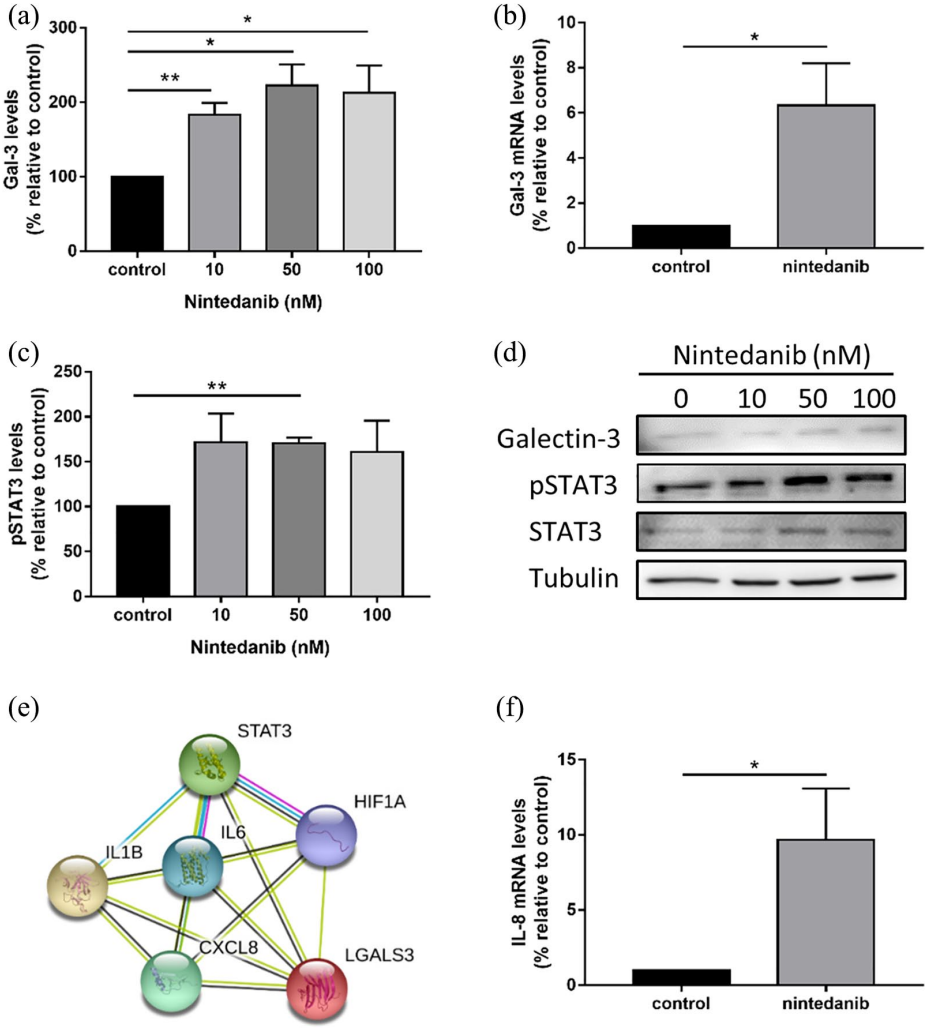

Figure 1. Nintedanib exposure elevates Gal-3 levels in N-HLFs.
N-HLFs were exposed to nintedanib (10–100 nM). Gal-3 levels were measured by (a) western blot and (b) qPCR after 24 h. (c) The effect of nintedanib on N-HLF pSTAT3-S727 (60 min) was analyzed by western blotting. (d) Representative western blots for (a) and (c). Protein interaction networks were constructed using STRING (http://string-db.org/) (e). IL-8 mRNA levels were measured by qPCR after 24 h (f).
*$p < 0.05$, **$p < 0.01$. ($n \geq 4$).
CXCL8, C-X-C motif chemokine ligand 8; Gal-3, galectin-3; HLF, human lung fibroblast; IL, interleukin; N-HLF, human lung fibroblast from control donors; qPCR, quantitative polymerase chain reaction; pSTAT, phospho-signal transducer and activator of transcription 3; STAT, signal transducer and activator of transcription 3.

Gal-3 is overexpressed in IPF lung tissue

Following these results, Gal-3 baseline expression was evaluated in primary HLFs derived from patients with IPF (IPF-HLF) and control N-HLF. Although Gal-3 expression was higher in the IPF-HLF compared with N-HLF cells, it did not reach significance (Figure 2(a) and (c)). Nevertheless, when analyzing whole tissue samples from IPF and non-IPF controls, we found that total Gal-3 levels were significantly elevated in IPF tissue samples (Figure 2(b) and (d)) ($p < 0.05$). These results suggest that although fibroblast involvement is observed, the increase in Gal-3 levels results primarily from other cell types. Immunohistochemical staining of IPF tissue samples demonstrated stromal involvement with strong staining mainly in the alveolar macrophages (AM) (Figure 2(e) and (f)).

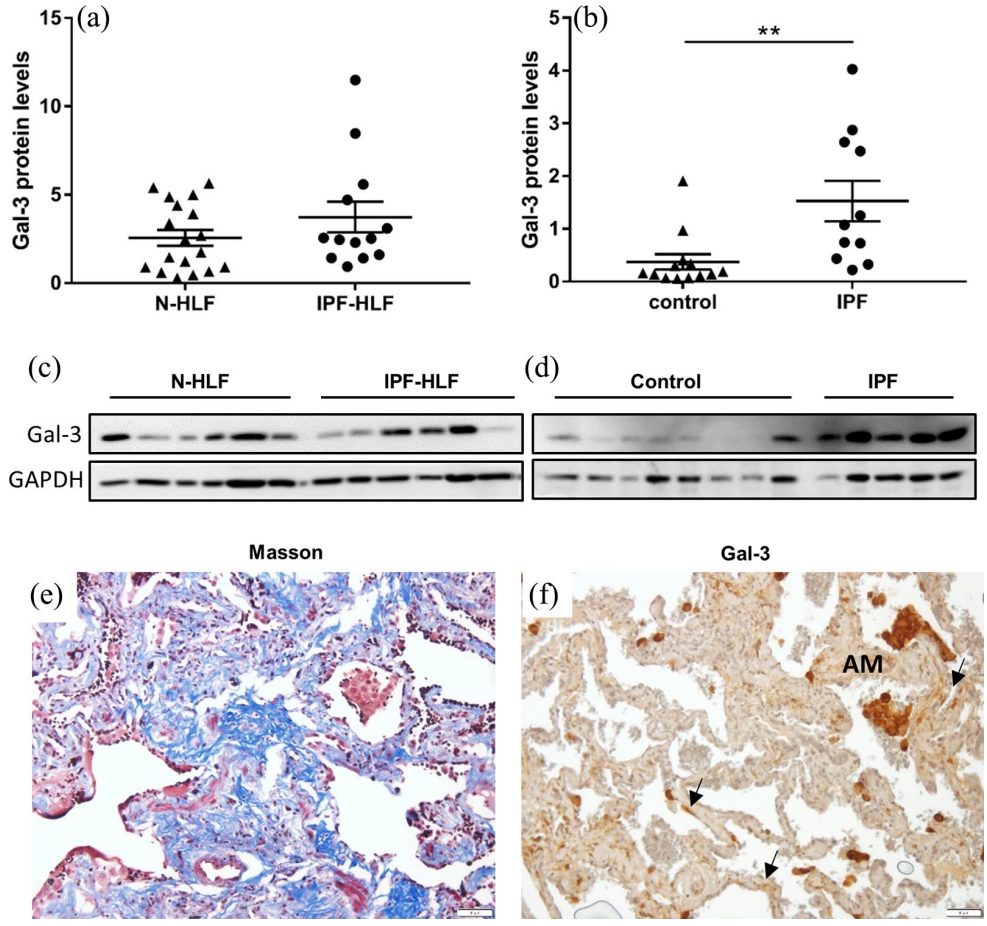

Figure 2. Gal-3 is overexpressed in IPF lung tissue.
Proteins were extracted from HLFs from (a) patients with IPF (IPF-HLF) or control donors (N-HLF) and (b) from lung tissue lysates. Gal-3 protein levels were measured by western blotting. (c) and (d) Representative western blots for (a) and (b) $^{**}p < 0.01$ ($n \geq 10$). IPF tissue samples were fixed in formaldehyde and paraffin embedded. Following deparaffinization, slides were stained with (e) Masson's trichrome stain, and (f) anti-Gal-3 antibody. Black arrows indicate stromal involvement.
AM, alveolar macrophages; Gal-3, galectin-3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HLF, human lung fibroblast; IPF, idiopathic pulmonary fibrosis; N-HLF, human lung fibroblast from control donors.

Nintedanib exposure elevates Gal-3 levels in silica-induced fibrosis mice model

In our recent work,[21] nintedanib was given to silica-exposed mice in three doses (0.021–2.1 mg/kg) and was shown to inhibit the silica-induced expression of fibrotic markers (e.g. smooth muscle actin, collagen, and IL-1β). However, inhibition was not complete thus suggesting activation of parallel mechanisms, possibly Gal-3. Therefore, left lungs were stained to assess parenchymal Gal-3 levels. Compared with sham animals, silica exposure stimulated a significant increase in lung Gal-3 expression (Figure 3). Positive cells were mostly AMs, epithelial cells, as well as some fibroblasts. Interestingly, nintedanib treatment (0.21–2.1 mg/kg) further elevated Gal-3 levels, mostly in the AMs ($p < 0.05$) (Figure 3).

Patients receiving nintedanib show elevated Gal-3 serum levels

Serum was collected from 41 consecutive patients with ILD routinely visiting the pulmonary clinic enrolled in the study. A total of 11 patients were receiving nintedanib. The time patients had received nintedanib varied between several months and up to 3 years. In the non-nintedanib group, there was one subject with rheumatoid

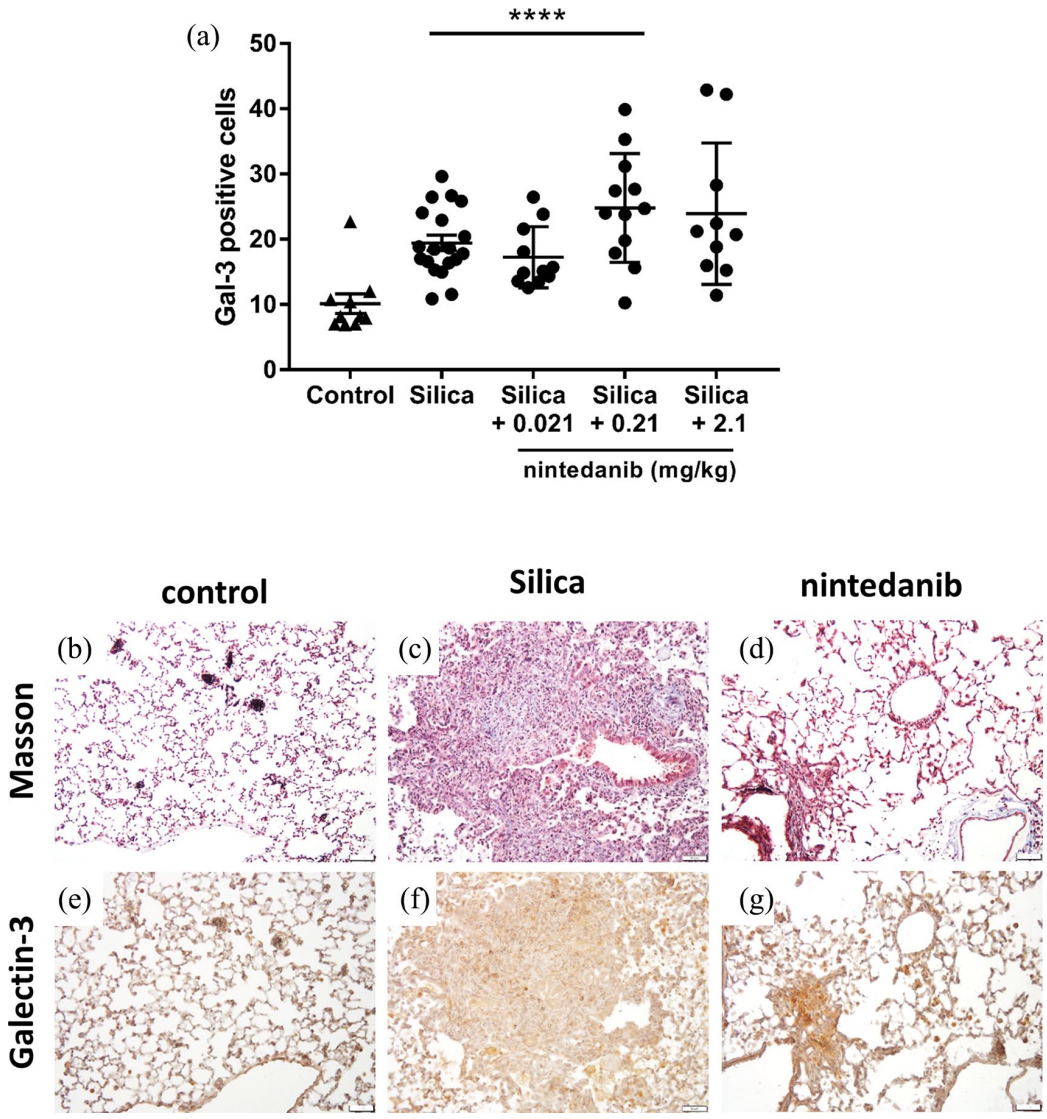

Figure 3. Nintedanib exposure elevates Gal-3 levels in silica-induced fibrosis mice model.
Mice were exposed to silica with/without nintedanib treatment (0.021–2.1 mg/kg) for 30 days. Paraffin blocks were prepared from the left lobes of each lung. (a) Slides were stained with Masson and for Gal-3 and quantified by QuPath. One-way ANOVA was used within treatments ****$p < 0.0001$. Representative images for Masson's trichrome staining of (b) control, (c) silica, and (d) silica + nintedanib 0.21 mg/kg (nintedanib). Representative images for Gal-3 staining of (e) control, (f) silica, and (g) silica + nintedanib 0.21 mg/kg (nintedanib). Number of sections used: $n = 10$ control, $n = 19$ silica vehicle, $n = 11$ silica + 0.021 nintedanib, $n = 12$ silica + 0.021 nintedanib, $n = 11$ silica + 0.21 nintedanib.
ANOVA, analysis of variance; Gal-3, galectin-3.

arthritis and two subjects with systemic sclerosis. Each group had one subject who previously had had a myocardial infarction. We evaluated Gal-3 levels in those receiving nintedanib *versus* those not receiving nintedanib. Of these patients, 21 were diagnosed with IPF and 20 with non-IPF-ILD (e.g. nonspecific interstitial pneumonia, sarcoidosis). Most patients receiving nintedanib had IPF. As shown in Table 1, other demographic parameters between the groups were comparable. Nevertheless, nintedanib-receiving patients had significantly higher levels of serum Gal-3 (15.6 ± 0.8 ng/ml *versus* 21.2 ± 3 ng/ml, $p < 0.05$) (Figure 4). When analyzing only the subgroup of patients with IPF ($n = 21$), Gal-3 levels were similarly elevated (16.3 ± 3.4 ng/ml *versus* 22.2 ± 11 ng/ml, $p = 0.37$), yet did not reach significance due to the low number of samples.

Table 1. Patient demographic data.

	No nintedanib *n* = 30	Nintedanib *n* = 11	*p* value
Age, years	61.7 ± 13	69.4 ± 7.9	0.08
Gender (% male)	15 (50%)	9 (81.8%)	0.07
BMI	27.7 ± 5.9	27.6 ± 4	0.94
Smoker	13 (43.3%)	2 (18.2%)	0.24
Steroid treatment	10 (33.3%)	2 (18.2%)	0.38
FEV1 (%)	75.6 ± 23.1	80 ± 20.2	0.60
FVC (%)	75 ± 21.7	75.5 ± 14.5	0.96
DLCO (%)	52.9 ± 18.4	46.8 ± 13.8	0.37
Resting O2 (%)	95.3 ± 3.1	93.35 ± 5.2	0.31
Diabetes	9 (30%)	5 (45.5%)	0.35
COPD	2 (6.7%)	1 (9.1%)	0.79
IHD	4 (13.3%)	3 (27.3%)	0.29
IPF	12 (40%)	10 (90.9%)	0.01[$]
NSIP	13 (43%)	0	
Other ILDs*	5 (16.7)		

*Other ILDS included sarcoidosis, vasculitis, fibrocystic lung, and common variable immunodeficiency with pulmonary fibrosis.
[$]Chi-square analysis for IPF, NSIP, and other ILDs.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IHD, ischemic heart disease; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia.

Discussion

IPF is a progressive disease with poor survival and limited treatment options.[28] Treatment options currently available show some reduced disease progression, yet their effect is still rather limited. Newly discovered pathways in IPF, such as Gal-3, are now being tested in clinical trials on top of nintedanib. Thus, we explored the effect of nintedanib on Gal-3 expression. Using *in vitro* and *in vivo* models, we showed that Gal-3 expression was significantly elevated by nintedanib. These findings highlight the possibility of combined inhibition for patients with progressive ILD.

Nintedanib is a small tyrosine kinase inhibitor (TKI) that acts primarily, but not exclusively, downstream of PDGF, FGF, and VEGF, all of which are major factors in IPF pathogenesis, as

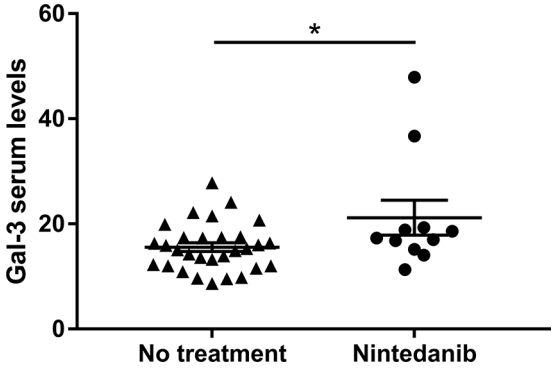

Figure 4. Patients receiving nintedanib show elevated Gal-3 serum levels.
Serum samples were analyzed for Gal-3 using ELISA. Values are means ± SE.
*$p \leq 0.05$, $n = 41$.
ELISA, enzyme-linked immunosorbent assay; Gal-3, galectin-3; SE, standard error.

well as angiogenesis. These factors are related to antifibrotic and anti-inflammatory pathways in animal models.[28,29] Nintedanib was previously shown to inhibit STAT3 signaling in IPF models.[30,31] However, TKIs may also lead to activation of various bypass signaling pathways, leading to resistance. Activation of STAT3 as the mechanism for resistance to TKIs has been previously suggested. Li *et al.* who showed that STAT3 activation was associated with gefitinib resistance in colorectal cancer cells.[32] Moreover, Wang *et al.* showed that epidermal growth factor receptor (EGFR)-TKIs lead to activation of Src/IL-6/STAT3 even in EGFR-TKI-sensitive lung cancer cells.[33] In another work, afatinib was shown to induce STAT3 activation through autocrine IL-6 production.[34] Since IPF and cancer share multiple signaling pathways,[35] and since Gal-3 was already shown to be elevated in cancer,[36] targeting both STAT3/Gal-3 and TKI activation may be an effective strategy for ILD treatment. IPF diagnosis is based on a combination of radiological or histological findings, which often include the usual interstitial pneumonia (UIP) pattern. In this pattern, the fibrosis must be heterogeneous, with normal lung adjacent to established fibrosis. Thus, the effect of IPF treatments on normal HLFs needs to be considered. Here, we show that HLFs exposed to nintedanib, without any additional stimulus, show STAT3 pathway activation. Thus, since STAT3 physically binds to the Gal-3 (*LGALS3*) promoter and induces its transcription,[37] this could serve as part of a potential mechanism for our findings. Moreover, IL-8 in HLFs exposed to nintedanib was also elevated. These results support the work by Nishi *et al.*,[38] which showed that increased Gal-3 in lungs may be related to fibrosis and disease resistance to immunosuppressive therapies by induction of angiogenesis and IL-8.

The UIP pattern cannot be replicated in animal models of pulmonary fibrosis, which is probably the reason that despite the significant progress in our understanding of the molecular events underlying pulmonary fibrosis in animals, we have little knowledge about what regulates the progression of IPF in the human lung.[39] Gal-3 was previously shown to be increased in a mouse model of bleomycin-induced lung fibrosis.[40] Here, we show that our silica-induced fibrosis model also caused a similar increase, in parallel with elevation in other fibrotic markers. Interestingly, in spite of a significant reduction in fibrotic markers, nintedanib further elevated Gal-3 levels. This elevated expression was primarily in the AMs. MacKinnon *et al.* showed increased Gal-3 levels in bleomycin-treated mice lungs, in which the staining was also primarily observed in the AMs.[41] Therefore, it is reasonable to assume that the antifibrotic treatment may cause an increase in inflammatory cells that can later promote the fibrotic process. Since our animal study, as well as most others,[41] was limited to about 1 month, it is difficult to predict long-term outcomes. These findings should be studied in a long-term model.

IPF patient serum, as well as broncho-alveolar lavage, samples, were previously shown to contain more Gal-3 than those from control subjects.[38] Here, we show that IPF tissue samples show increased levels of Gal-3, supporting previous findings. Elevated Gal-3 serum level was also found to be associated with ILD severity.[42] In the last part of our work, we tested serum samples from patients with ILD receiving nintedanib *versus* those that did not. Our results support previous findings and show that the average Gal-3 level in patients with ILD is above the control range. Moreover, according to a large meta-analysis for heart failure, the value for low risk was set at 17.8 ng/ml.[43] In our study, the nintedanib-receiving group had an average value of 21.2 ng/ml. It is worth noting that myocardial infarction was reported in five patients in the nintedanib group (1.6%) and one patient in the placebo group (0.5%) in INPULSIS-1, and in five patients in the nintedanib group (1.5%) and one patient in the placebo group (0.5%) in INPULSIS-2.[17] Thus, it would be of interest to study the long-term effects of nintedanib, as well as other antifibrotic treatments (e.g. pirfenidone), on Gal-3 levels in patients with ILD.

Study limitations include a relatively low number of subjects in the patient serum analyses. In addition, it could be interesting to test the 'before' and 'after' nintedanib treatment for each patient.

In conclusion, we showed that nintedanib elevated Gal-3 levels in two experimental models, as well as in ILD patient samples. These findings warrant further study for establishing a possible future treatment combination.

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Authors' contributions

GES and TZG drafted the manuscript, designed the experiments, and analyzed the results. AP and BB-W performed the experiments and analyzed the results. KA performed the *in vivo* studies. NR, BS and YL recruited patients and helped in drafting the manuscript. EE and MS revised the manuscript for critically important intellectual content. DS contributed to conception and design, drafting the manuscript for important intellectual content, and revised the final version. All authors approved the final version.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Any data can be supplemented on demand.

References

1. Gribbin J, Hubbard RB, Le Jeune I, *et al.* Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Thorax* 2006; 61: 980–985.
2. Gao Z, Liu Z, Wang R, *et al.* Galectin-3 is a potential mediator for atherosclerosis. *J Immunol Res* 2020; 2020: 5284728.
3. Dong R, Zhang M, Hu Q, *et al.* Galectin-3 as a novel biomarker for disease diagnosis and a target for therapy (Review). *Int J Mol Med* 2018; 41: 599–614.
4. Ochieng J, Fridman R, Nangia-Makker P, *et al.* Galectin-3 is a novel substrate for human matrix metalloproteinases-2 and -9. *Biochemistry* 1994; 33: 14109–14114.
5. Yang RY, Hsu DK and Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc Natl Acad Sci U S A* 1996; 93: 6737–6742.
6. Jeng KC, Frigeri LG and Liu FT. An endogenous lectin, galectin-3 (epsilon BP/Mac-2), potentiates IL-1 production by human monocytes. *Immunol Lett* 1994; 42: 113–116.
7. Li LC, Li J and Gao J. Functions of galectin-3 and its role in fibrotic diseases. *J Pharmacol Exp Ther* 2014; 351: 336–343.
8. Henderson NC and Sethi T. The regulation of inflammation by galectin-3. *Immunol Rev* 2009; 230: 160–171.
9. Dang Z, MacKinnon A, Marson LP, *et al.* Tubular atrophy and interstitial fibrosis after renal transplantation is dependent on galectin-3. *Transplantation* 2012; 93: 477–484.
10. Nabi IR, Shankar J and Dennis JW. The galectin lattice at a glance. *J Cell Sci* 2015; 128: 2213–2219.
11. Desmedt V, Desmedt S, Delanghe JR, *et al.* Galectin-3 in renal pathology: more than just an innocent bystander. *Am J Nephrol* 2016; 43: 305–317.
12. Ho JE, Liu C, Lyass A, *et al.* Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol* 2012; 60: 1249–1256.
13. de Boer RA, Lok DJ, Jaarsma T, *et al.* Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med* 2011; 43: 60–68.
14. Lederer DJ and Martinez FJ. Idiopathic pulmonary fibrosis. *N Engl J Med* 2018; 379: 797–798.
15. Lederer DJ and Martinez FJ. Idiopathic pulmonary fibrosis. *N Engl J Med* 2018; 378: 1811–1823.
16. Hilberg F, Roth GJ, Krssak M, *et al.* BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res* 2008; 68: 4774–4782.
17. Richeldi L, du Bois RM, Raghu G, *et al.* Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014; 370: 2071–2082.
18. Yoon HY, Park S, Kim DS, *et al.* Efficacy and safety of nintedanib in advanced idiopathic pulmonary fibrosis. *Respir Res* 2018; 19: 203.
19. Richeldi L, Costabel U, Selman M, *et al.* Efficacy of a tyrosine kinase inhibitor in idiopathic

pulmonary fibrosis. *N Engl J Med* 2011; 365: 1079–1087.

20. Epstein Shochet G, Brook E, Israeli-Shani L, *et al*. Fibroblast paracrine TNF-α signaling elevates integrin A5 expression in idiopathic pulmonary fibrosis (IPF). *Respir Res* 2017; 18: 122.
21. Epstein-Shochet G, Pham S, Beck S, *et al*. Inhalation: a means to explore and optimize nintedanib's pharmacokinetic/pharmacodynamic relationship. *Pulm Pharmacol Ther* 2020; 63: 101933.
22. Ayaub EA, Kolb PS, Mohammed-Ali Z, *et al*. GRP78 and CHOP modulate macrophage apoptosis and the development of bleomycin-induced pulmonary fibrosis. *J Pathol* 2016; 239: 411–425.
23. Wollin L, Maillet I, Quesniaux V, *et al*. Antifibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor nintedanib in experimental models of lung fibrosis. *J Pharmacol Exp Ther* 2014; 349: 209–220.
24. Li C, Lu Y, Du S, *et al*. Dioscin exerts protective effects against crystalline silica-induced pulmonary fibrosis in mice. *Theranostics* 2017; 7: 4255–4275.
25. Bankhead P, Loughrey MB, Fernández JA, *et al*. QuPath: open source software for digital pathology image analysis. *Sci Rep* 2017; 7: 16878.
26. Epstein Shochet G, Drucker L, Pomeranz M, *et al*. First trimester human placenta prevents breast cancer cell attachment to the matrix: the role of extracellular matrix. *Mol Carcinog* 2017; 56: 61–74.
27. Weinmann D, Schlangen K, Andre S, *et al*. Galectin-3 induces a pro-degradative/inflammatory gene signature in human chondrocytes, teaming up with galectin-1 in osteoarthritis pathogenesis. *Sci Rep* 2016; 6: 39112.
28. Wollin L, Wex E, Pautsch A, *et al*. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *Eur Respir J* 2015; 45: 1434–1445.
29. Wollin L, Distler JHW, Redente EF, *et al*. Potential of nintedanib in treatment of progressive fibrosing interstitial lung diseases. *Eur Respir J* 2019; 54: 1900161.
30. Liu F, Wang L, Qi H, *et al*. Nintedanib, a triple tyrosine kinase inhibitor, attenuates renal fibrosis in chronic kidney disease. *Clin Sci (Lond)* 2017; 131: 2125–2143.
31. Liu CY, Huang TT, Chu PY, *et al*. The tyrosine kinase inhibitor nintedanib activates SHP-1 and induces apoptosis in triple-negative breast cancer cells. *Exp Mol Med* 2017; 49: e366.
32. Li Q, Zhang D, Chen X, *et al*. Nuclear PKM2 contributes to gefitinib resistance via upregulation of STAT3 activation in colorectal cancer. *Sci Rep* 2015; 5: 16082.
33. Wang J, Wang Y, Zheng C, *et al*. Tyrosine kinase inhibitor-induced IL-6/STAT3 activation decreases sensitivity of EGFR-mutant non-small cell lung cancer to icotinib. *Cell Biol Int* 2018; 42: 1292–1299.
34. Kim SM, Kwon OJ, Hong YK, *et al*. Activation of IL-6R/JAK1/STAT3 signaling induces de novo resistance to irreversible EGFR inhibitors in non-small cell lung cancer with T790M resistance mutation. *Mol Cancer Ther* 2012; 11: 2254–2264.
35. Pietras K and Ostman A. Hallmarks of cancer: interactions with the tumor stroma. *Exp Cell Res* 2010; 316: 1324–1331.
36. Cay T. Immunhistochemical expression of galectin-3 in cancer: a review of the literature. *Turk Patoloji Derg* 2012; 28: 1–10.
37. Guha P, Bandyopadhyaya G, Polumuri SK, *et al*. Nicotine promotes apoptosis resistance of breast cancer cells and enrichment of side population cells with cancer stem cell-like properties via a signaling cascade involving galectin-3, α9 nicotinic acetylcholine receptor and STAT3. *Breast Cancer Res Treat* 2014; 145: 5–22.
38. Nishi Y, Sano H, Kawashima T, *et al*. Role of galectin-3 in human pulmonary fibrosis. *Allergol Int* 2007; 56: 57–65.
39. McDonough JE, Ahangari F, Li Q, *et al*. Transcriptional regulatory model of fibrosis progression in the human lung. *JCI Insight* 2019; 4: e131597.
40. Kasper M and Hughes RC. Immunocytochemical evidence for a modulation of galectin 3 (Mac-2), a carbohydrate binding protein, in pulmonary fibrosis. *J Pathol* 1996; 179: 309–316.
41. Mackinnon AC, Gibbons MA, Farnworth SL, *et al*. Regulation of transforming growth factor-β1-driven lung fibrosis by galectin-3. *Am J Respir Crit Care Med* 2012; 185: 537–546.
42. Ho JE, Gao W, Levy D, *et al*. Galectin-3 is associated with restrictive lung disease and interstitial lung abnormalities. *Am J Respir Crit Care Med* 2016; 194: 77–83.
43. Meijers WC, Januzzi JL, deFilippi C, *et al*. Elevated plasma galectin-3 is associated with near-term rehospitalization in heart failure: a pooled analysis of 3 clinical trials. *Am Heart J* 2014; 167: 853–860.e854.