Retrospective assessment of nintedanib in the mice model of pulmonary fibrosis: alignment with patient outcomes in idiopathic pulmonary fibrosis

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Abstract

Pulmonary function tests (PFTs) routinely implemented in clinics are the first step in the diagnosis of idiopathic pulmonary fibrosis. Evaluation of PFTs in the mouse model of pulmonary fibrosis accompanied by histological readouts may improve the clinical predictability of new therapeutic candidates. Forced vital capacity (FVC) is considered the most predictive of restrictive pulmonary disorders. This study aimed to test the improvement of PFT in mice lung fibrosis induced by treatment with an approved substance nintedanib, considered the gold standard. The hypothesis that treatment in animal models will demonstrate similar effects as in humans in the most relevant clinical outcomes was tested. Two experimental designs were enrolled in this study, a preventive regimen, with treatment initiation from the day of the challenge; and a therapeutic regimen, starting on day 7 post-challenge when fibrotic changes are present in the lungs. Experiments were terminated at two different time points, at 14 and 21 days post-challenge. C57BL/6 mice were administered with bleomycin (BLM) intranasally and treated with nintedanib from day 0 to day 14 or from day 7 until day 21. Fourteen or 21 days after the BLM challenge, PFTs were assessed using the in vivo invasive lung function measurement system Buxco® Pulmonary Function Test (PFT) (DSI™, New Brighton, USA). Histological evaluation was performed as a modified Ashcroft score. The bleomycin challenge induced a significant decrease of FVC in both experiments. However, nintedanib treatment given in both regimens significantly improved lung functionality. These findings were confirmed with histological analysis of the Ashcroft scoring system, modified by Matsuse. In conclusion, a good correlation between functional test parameters and the clinical effect of nintedanib was shown in both experiments: the preventive regimen was sampled 14 days post-challenge and the therapeutic regimen 21 days post-challenge. Based on these findings, the implementation of PFTs could be a good platform to increase the translational value of the model and potential new treatments.

Key words: *idiopathic pulmonary fibrosis; bleomycin; mice model; pulmonary functions; forced vital capacity; nintedanib*

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Introduction

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease, characterized as a chronic, progressive and ultimately fatal lung disease leading to the death of the patient within 2-3 years of diagnosis. The disease is characterised by usual interstitial pneumonitis (UIP) and progressive interstitial fibrosis caused by the formation of fibroblasts and myofibroblast clusters. The terminal stage is scarring and destruction of the lung architecture, stiffness of lung tissue and loss of tissue elastance with a decline in lung function and progressive respiratory failure (Moeller et al., 2008; Wilson and Wynn, 2009; Barrat et al., 2018). After decades of unsuccessful trials to develop an effective therapy, finally in 2014, nintedanib and the equally effective treatment pirfenidone received global approval (Raghu et al., 2015, Sgalla et al, 2018). Nintedanib is an intracellular multiple inhibitor of tyrosine kinase receptors. It is an orally available small molecule, primarily designed as an anti-angiogenic entity for cancer treatment (Wollin et al., 2015). Antifibrotic activity of nintedanib was observed through one phase 2 and two phase 3 randomised clinical studies, demonstrating a significant reduction in the rate of lung function decline over 52 weeks (Karimi-Shah and Chowdhury, 2015). Nintedanib was developed in the animal model and its effect on the reduction of lung fibrosis was reported (Wollin et al., 2014). Nevertheless, the effect of nintedanib on lung functionality in the mice model of BLM induced fibrosis has not previously been fully described. Though they improve lung functionality and partially fibrotic lesions, the available treatments are not optimal. Therefore, the development of novel, more effective therapeutic entities is required.

Animal models play an important role in the understanding of disease pathogenesis and the development of current therapies (Izbicki et al., 2002; Barrios, 2008; Scotton and Chambers, 2010, Reinert et al., 2013; Yanagihara et al., 2020). Bleomycin induced fibrosis is the most commonly used and best-characterised animal model available for preclinical testing, employing mice as a preferable species (Moeller et al., 2008; Jenkins et al., 2017). However, when observed retrospectively, a high number of tested molecules that yielded success in the preclinical mice model of fibrosis showed no expected effect in clinical trials (Moeller et al., 2008). Therefore, in 2017, the American Thoracic Society published an official workshop report entitled "Use of animal models for the preclinical assessment of potential therapies for pulmonary fibrosis" (Jenkins et al., 2017). The most accurate analysis for lung fibrosis involves collagen and histological assessment in *post-mortem* lung tissue. However, the discrepancies between preclinical and clinical tests highlight the need for additional readouts. The alignment of readouts in models to those in patients is one step closer to increasing translatability of the results. Spirometry, as a first-step diagnostic tool in patients with respiratory disease symptoms, indicates that pulmonary function tests (PFTs) in animal models have the potential to be included as a relevant readout in corroborating fibrotic changes and/or therapeutic response to interventions. However, it presents a considerable technical challenge, which is likely the main reason why this technique is rarely employed in preclinical animal models (Vanoirbeek et al., 2010; Peng et al., 2013).

This study hypothesised that using the standard treatment nintedanib, as

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the gold standard, in preventive and therapeutic regimens will improve the values of forced vital capacity (FVC) as the relevant lung function parameter, as FVC correlates with a reduction in pathological changes in mice lungs induced by a single administration of bleomycin. The tested assumption was that two applied treatment regimens of nintedanib will demonstrate a therapeutic effect on the most relevant clinical outcomes in the mouse bleomycin model that is comparable to those in humans.

Materials and methods

Two BLM induced fibrosis in mice experiments were performed, including different treatment regimens and sampling time points.

Animals

Experiments were conducted at the company Selvita Ltd., Zagreb, Croatia in compliance with European Union Directive 2010/63/EU and the national legislation (Official Gazette 55/13) regulating the use of laboratory animals in scientific research, with oversight of the Institutional Committee on Animal Research Ethics (CARE-Zg). All efforts were made to minimise suffering.

C57BL strains of SPF (specific pathogen-free) mice were used and purchased from Charles River Laboratories, Germany. All mice were housed in conventional polysulfone cages (TECNIPLAST S.p.A., Buguggiate VA, Italy, cages, type III) with 3–4 cm thick ALPHA-dri® dust free bedding (pure cellulose fibre, uniform particle size 5 mm² highly absorbent, LBS Serving Biotechnology, London, UK). Each cage was enriched with cotton nestlets for nest making and paper shelter (Lillico Serving Biotechnology, London, UK). Cages were supplied with high-fat pelleted food for mice (SDS VRF 1 (P), LBS Serving Biotechnology, London, UK), and water from the municipal water main (bottles TECNIPLAST S.p.A., Buguggiate VA Italy); *ad libitum*. A light cycle of 12 hours light/dark was maintained. Animals were tested during the light period. Room conditions were set to a temperature of 22±2°C, relative humidity of 55±10%, and a cycle of 15–20 air changes per hour.

Experimental design

Male mice (9–10 weeks old) were allocated into three groups (*n*=8 per group) per experiment: phosphate buffered saline (PBS) challenged control (ctrl) group, bleomycin challenged and vehicle administered positive ctrl group (BLM/Vehicle); and bleomycin challenged and nintedanib treated (BLM/Nintedanib). In the first experiment, nintedanib treatment was administered in the preventive regimen, starting on the day of the bleomycin challenge, and PFT and lung sampling was performed on day 14 of the study. In the second experiment, the nintedanib treatment regimen was applied starting on day 7 post- challenge, and PFT measurements and lung sampling was performed on day 21.

Pulmonary fibrosis in mice was developed by intranasal instillation of bleomycin sulfate (Enzo Life Sciences, Inc., New York, USA) at the dose of approx. 1 mg/kg on the first day of the experiment. In each experiment, animals in the first control group received phosphate-buffered saline (PBS, Merck KGaA, Darmstadt, Germany) intranasally (*i.n.*), while animals in the positive control group and testing group were challenged with bleomycin. Prior to *i.n* administration, mice were anesthetised with a combination of ketamine

hydrochloride (Bioveta, a.s. the Ivanovice na Hané, Czech Republic) and xylazine hydrochloride (Alfasan International B.V, Woerden, Netherlands). On the last day of the experiment, day 14 (D14) for the first experiment or day 21 (D21) for the second experiment, PFT measurements were performed and lungs were sampled for histopathological analysis.

Nintedanib treatment

Mice were administered with nintedanib (eNovation Chemicals LLC, New Jersey, USA) in a dose of 60 mg/kg twice daily (*bid*). The nintedanib formulation was prepared by dissolving the dry compound in a 0.1% hydroxyethyl-cellulose solution (Merck KGaA, Darmstadt, Germany).

Lung function measurements

PFTs were assessed by using the *in vivo* invasive lung function measurement system Buxco® Pulmonary Function Test (PFT) (DSI™, New Brighton, USA). Mice were anesthetised and instrumented before airway function measurements. Animals were intubated extra-orally and placed on a mechanical ventilator. A tracheal tube provided direct access to the lungs. Parameters measured using this technique include lung volumes, capacities, airway pressure, pulmonary resistance and dynamic lung compliance. Mice were anesthetised for surgery and after approximately 10 minutes, mice were tracheotomised. Tracheostomy was performed by cutting the skin in the neck area and releasing the trachea from the surrounding tissue. Tracheal tubes were placed into the trachea and fixed with a tied suture. Standard 18-gauge stainless steel tubes were used. Mice were then placed into a plethysmograph. Four test sequences were applied to each animal. Three semiautomatic manoeuvres were performed: Boyle's law functional residual capacity (FRC), quasistatic Pressure-Volume test (PV) and fast flow volume manoeuvre. During these first three measuring sequences, the animal was breathing spontaneously. After it was completed, mechanical ventilation was applied for the FRC measurements. Ventilation was conducted with 120 breaths/min, a max mouth pressure and max deep breath mouth pressure of 30 cm H_2O and a max stroke volume of 0.3 mL. Data were calculated using automated data acquisition software Buxco® FinePointe™.

Histopathology

Excised lungs were placed immediately into marked bottles containing 10% buffered formalin (Shandon™ Formal-Fixx™ Neutral Buffered Formalin, Thermo Fisher Scientific, Rockford, IL, USA) for further histopathological evaluation. After 24 hours of fixation in formalin, lungs were placed in tissue embedding cassettes (Thermo Fisher Scientific, Rockford, IL, USA). After fixation, all tissue cassettes were subjected to dehydration and further paraffin block embedding. Paraffin tissue sections were cut to a thickness of 1 µm and transferred to a glass slide. Histological slides underwent deparaffinisation and hydration procedures and finally were stained subsequently according to the model protocol.

Crossman's Trichrome for muscle and collagen staining

Prepared histological slides were placed into Tissue-Tek® DRS™ 2000 (Sakura Finetek Europe, Netherlands) for the processes of deparaffinization. Lung tissue sections were prepared and stained manually according to the Crossman's Trichrome for muscle and collagen staining protocol as follows: slides were stained in Mayer's hemalaum

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solution for three minutes, rinsed and placed for three minutes in a solution of acid fuchsin (Sigma-Aldrich Chemie GmbH, Munich, Germany), orange G, ACROS organics™ (Thermo Fisher Scientific, Rockford, IL, USA), acetic acid glacial (Merck KGaA, Darmstadt, Germany) and distilled water. Then samples were rinsed in distilled water and immersed in a solution of dodeca molybdophosphoric acid (Kemika, Zagreb, Croatia) and distilled water for five minutes, and then placed in the final solution of light green SF yellowish (Merck KGaA, Darmstadt, Germany), acetic acid glacial and distilled water for five minutes. Slides were dehydrated in 96% (2x for two minutes) and 100% ethanol (2x for three minutes) and xylene accordingly, and finally mounted with Shandon™ Consul-Mount™ (Thermo Fisher Scientific, Rockford, IL, USA) in a ClearVue™ Coverslipper (Thermo Fisher Scientific, Rockford, IL, USA).

Matsuse modification of the Ashcroft score

Slides were examined under a light microscope Axio Imager.A1 (Carl Zeiss, Jena, Germany). Matsuse modification of the Ashcroft score (Ashcroft et al., 1988; Matsuse et al., 1999) was employed for fibrosis evaluation in lung specimens. Samples were examined under 50x magnification and ten fields were scored according to Table 1. Slides were scanned using the Axio Scan.Z1 slide scanner (Zeiss).

Statistical analysis

Statistical analysis and graphical presentation were performed using Graph Pad Prism software (version 9) (GraphPad Software, Inc., La Jolla, CA, USA). The mean rank of each group was compared to the mean rank of the positive control group (BLM/Vehicle group).

Pulmonary function test values were analysed with the unpaired t-test using Fine Pointe™ software and presented graphically with Graph Pad Prism.

For the evaluation of the Ashcroft score results, nonparametric statistics (Wilcoxon Signed Rank Test, Mann-Whitney test) were performed using a group median. Spearman correlation for the comparison of histological and PFT data was also performed using Graph Pad Prism software. The r correlation coefficient and P-value were also expressed. Differences between groups were considered statistically significant when $P < 0.05$.

Table 1. Matsuse modification of Ashcroft score (Ashcroft et al., 1988; Matsuse et al., 1999)

	ASHCROFT SCORE
1	Normal lung (no fibrosis)
2	Minimal fibrotic thickening of alveolar or bronchial walls (network of fine collagen fibrils)
3	Moderate fibrotic thickening of walls without obvious damage to lung architecture
4	Fibrosis with damage of pulmonary structure (coarse fibrous band or small fibrous masses, intra-alveolar collagen fibrils)
5	Large fibrous area with severe distortion of lung structure

Results

Sampling was performed on day 14 or day 21 post BLM challenge. On D14, measurements of two animals from the BLM/Nintedanib group were assessed as invalid due to technical issues and were excluded from further evaluation.

Lung function measurements

On the final day of the experiment, D14 in the preventive regimen and D21 in the therapeutic regimen, animals were anesthetised, instrumented and PFTs were performed. Results of the PFT parameter pressure-volume curve and forced vital capacity measured on D14 and D21 are presented in Figures 1 and 2. The flow volume curve (F-V curve) presented a smaller AUC of the animals challenged with BLM and vehicle administered (BLM/Vehicle), while the nintedanib treated group showed improvement, with a similar AUC to the PBS group in both experiments. To define the F-V difference between groups, FVC parameter analysis, calculated from the F-V curve, was performed and results revealed a significant decline in the volume of mice from the positive control group BLM/Vehicle, when compared to the healthy, non-challenged PBS group. The decline on D14 was 30%, while on D21 it was 38%. Nintedanib treatment starting on the day of the BLM challenge and the effect evaluated on D14 of the experiment resulted in a significant improvement of the FVC parameter, 26% when compared to the positive control group BLM/Vehicle. The therapeutic regimen with nintedanib treatment significantly improved the FVC parameter (21%) as compared to BLM/Vehicle ctrl group evaluated on D21 of the experiment.

Histology

Histology analysis of the lungs sampled immediately after the PFT measurements were performed on formalin fixed tissues and Crossman's Trichrome stained slides. There were no pathological changes or fibrotic lesions in lung tissue samples from the negative control PBS groups. Lung fibrosis induced by intranasal bleomycin application was characterised by respiratory epithelium damage with reactive epithelial hyperplasia and bronchiolisation, pulmonary inflammation, accumulation of fibroblasts/myofibroblasts and collagen deposition, as well as distortion of pulmonary structure on both D14 and D21 of the experiment. (Figure 4).

Results of the Matsuse modified Ashcroft score analysis are presented in Figure 3. The BLM challenged positive control group score exhibited a significantly higher Ashcroft score when compared to the unchallenged BLM/Vehicle control. The group treated with nintedanib in both treatment regimens significantly reduced the Ashcroft score in comparison to the positive control BLM/Vehicle group.

The correlation between the PFT parameter FVC and the Ashcroft score was evaluated using simple linear regression and Spearman correlation (Figure 5). Ashcroft score mean values demonstrated a significant correlation to lung function as observed in both evaluated experiments (D14: r= -0.6926 P=0.0004; D21: r=-0.5794 P=0.003).

Discussion

Animal models of human diseases have been developed to better understand the pathophysiology of a disease, for target validation, and as a tool for the development of new therapies. A successful animal model has to reproduce the key characteristics that resemble the disease in humans (Mouratis and Aidinis, 2011). However, a model by definition is not a perfect replication of the clinical condition.
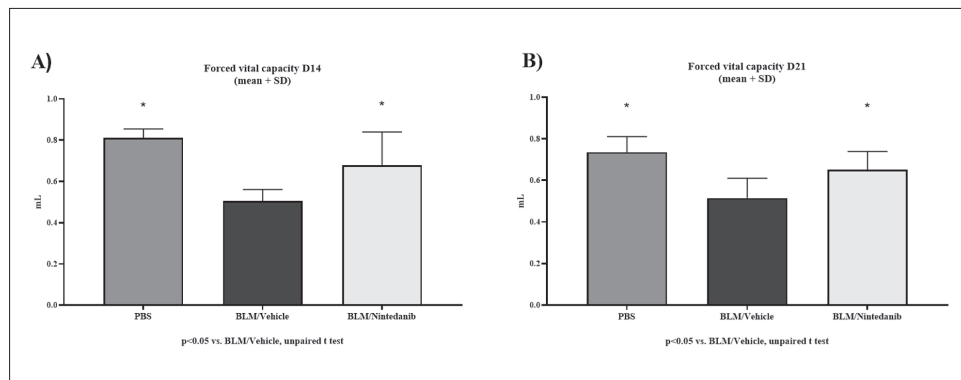
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Figure 1. The forced vital capacity parameter was evaluated on day 14 (A) and day 21 (B). *$P < 0.05$, unpaired t-test, Buxco® Fine Pointe™.
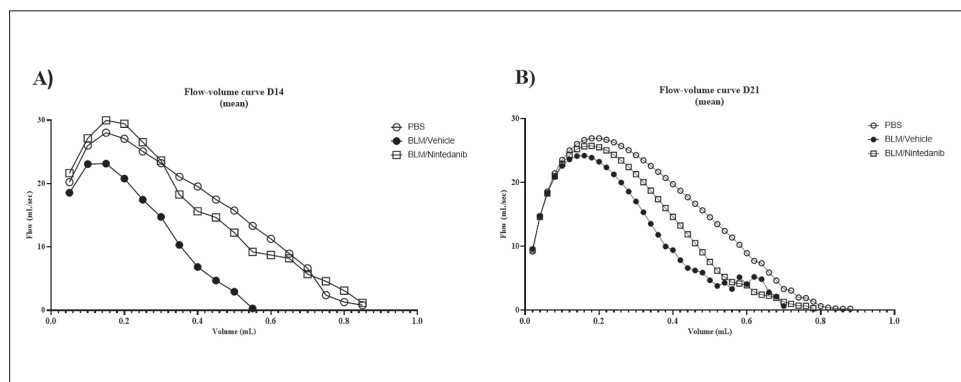

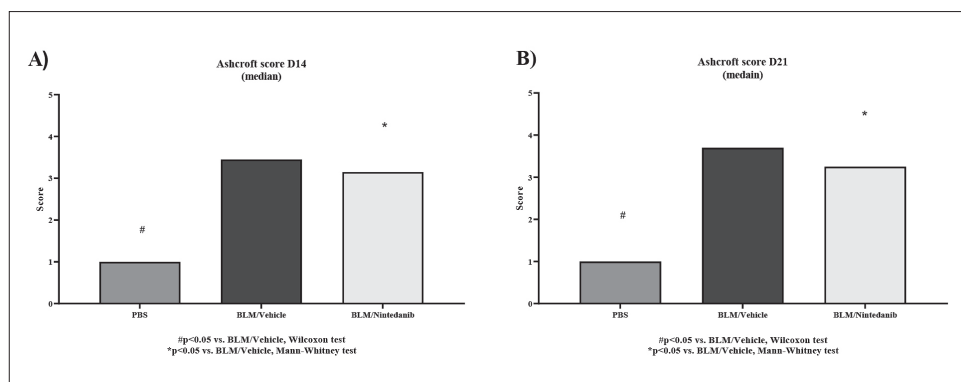**Figure 2.** The flow volume curve was evaluated on day 14 (A) and day 21 (B).

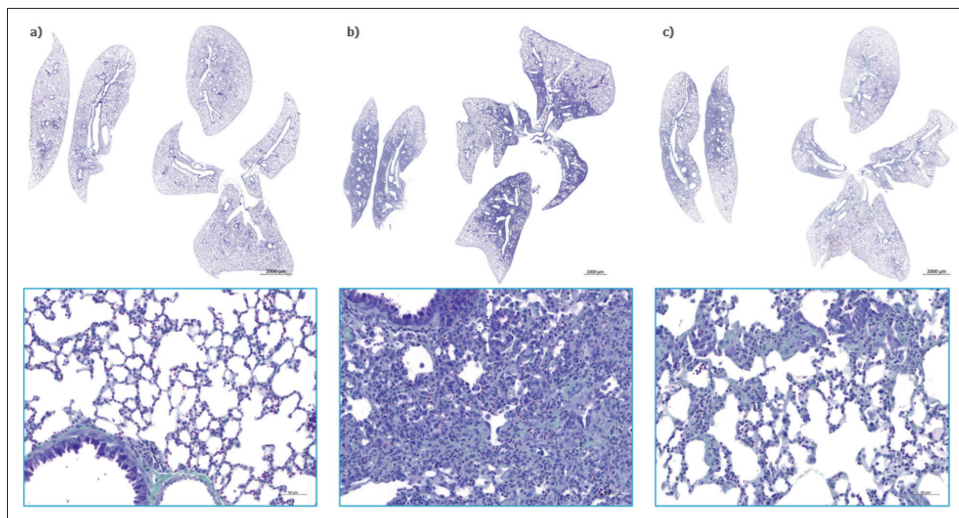**Figure 3.** Modified Ashcroft score evaluated on D14 and D21

Figure 4. Histology of the lungs on D21 of the experiment (Crossman's Trichrome staining) showed at the high magnification (2000 µm, upper figure) and low magnification (50 µm, lower figure).

a) Negative control PBS group; normal lung, b) Positive control BLM/Vehicle group; marked multifocal deposition of de novo synthesized collagen within the alveolar septa and alveoli with severe distortion of lung structure, c) Nintedanib treated group; moderate multifocal deposition of de novo synthesized collagen within the alveolar septa
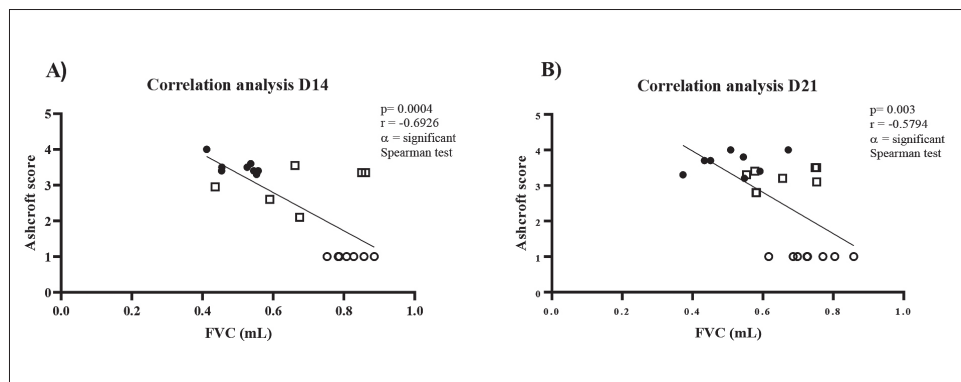

Figure 5. Spearman correlation between the modified Ashcroft score and forced vital capacity parameter of pulmonary function test measurement evaluated on day 14 (A) and day 21 (B) ○ PBS group ● BLM/Vehicle group □ BLM/Nintedanib

Therefore, to achieve better translation, Denayer et al. (2014) listed several factors that should be considered when conducting animal studies. Among the criteria, they noted that animal models should achieve as many similarities as possible in the biology and symptoms of disease,

the demonstration of similar effects of a known treatment should be achieved in models and treatment initiation should be initiated at the right time.

The bleomycin model is well characterised, easy to perform, has clinical relevance, is widely accessible and repro-

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ducible, and therefore fulfils important criteria expected from a good animal model. Another add-on to the clinical relevance of the model is the finding of significant histological hallmarks of IPF in the animal model, including intra-alveolar buds, mural incorporation of collagen and obliteration of the alveolar space (Moeller et al., 2008; Degryse and Lawson, 2011; Moore et al., 2013).

A report on the characterisation of pathological changes over time upon a single *i.t.* bleomycin challenge was published by Izbicki et al. (2002). A single challenge in the early days (days 3 and 6 post instillation) resulted in slight inflammatory changes in lung tissue as an increased amount of alveolar cellularity, mainly macrophages, tissue infiltration with neutrophils and macrophages and findings of perivascular and peribronchiolar lymphocytes. On day 14, findings in the lungs consisted of multifocal or diffuse changes as a combination of thickened alveolar septa, intra-alveolar fibrosis with myofibroblasts within the lumen, occasional foci of dense fibrosis, increased alveolar macrophages, and focal dilatation of respiratory bronchioles and alveolar ducts. On day 21, observed changes in severely affected animals were diffuse and included intra-alveolar fibrosis, focally dense fibrosis, often subpleural, and epithelial hyperplasia in alveolar ducts. Another report on disease phases was published by Peng et al. (2013). They divided the model into three phases: the inflammation phase (days 1-7), active fibrosis phase (days 7-14) and late fibrosis phase (days 21-35).

This paper examines two different study set-ups: a preventive regimen affecting the inflammatory and active phase of the disease, and a therapeutic regimen initiating the treatment in the active and late fibrosis phase. According to ATS guidelines, scientists advocate a therapeutic regimen as a more translatable set-up (Jenkins et al., 2017). However, we wanted to evaluate the effects of nintedanib on all three phases of the disease, and therefore both regimen studies were reported in this paper.

Although the *i.t.* route of BLM application is most usually applied, this study applied the intranasal route as less invasive and technically less demanding. A single intranasal administration in our model resulted in damage of lung tissue characteristic for fibrotic changes induced by BLM instillation which according to Degryse and Lawson (2011) recreates the characteristic histological features of UIP in a reasonably short time frame. The histopathological score is the most frequently reported outcome in the model of BLM induced fibrosis (Gilhodes et al., 2017). About 40 years ago, Ashcroft and colleagues designed a scoring system based on a scale of lung biopsy histological changes from patients with IPF, popularly known as the Ashcroft score, to enable correlation between the severity of pulmonary fibrosis with pulmonary function tests performed in the clinic (Ashcroft et al., 1988). Since its initial publication, several modifications of the score have been published to better reflect histological changes in the bleomycin induced mouse model of lung fibrosis (Matsuse et al., 1999; Hübner et al., 2008). In this study, animals from the BLM/Vehicle group showed severe multifocal deposition of *de novo* synthesized collagen within the alveolar septa and distortion of lung structure, while in animals treated with nintedanib *de novo* synthesized collagen is also positioned multifocally but in a moderate manner. Since the scoring system was used to indicate differences between groups, results are expressed as the median and the non-parametric

Mann-Whitney t-test was used for comparison between the BLM/Vehicle group and Nintedanib group. On the other hand, Wilcoxon signed-rank test was used to compare the negative control PBS and BLM/Vehicle group since the negative control column is not identical by a matter of chance (Bowers, 2008).

PFTs were performed on the last day of the study, i.e., on day 14 in the preventive and day 21 in the therapeutic regimen set-up. Despite the routine employment of spirometry in patient care, PFTs measurements are not frequently used diagnostic tools in BLM mice models.

The invasive method of PFT as used in these studies is considered the gold standard for the precise determination of lung functions in mice. Direct access to the lungs through an endotracheal tube enables avoidance of any influences on the upper airways, while mechanically ventilation ensures the control and constant of both tidal volume and respiratory rate (Irvin and Bates, 2003). The Buxco® PFT system provides clinically relevant parameters, such as FEV1 (in mice in 100 ms), VC, FVC, Tiffeneau index, RV and TLC by measuring standard and maximal P-V and F-V curves, as well as lung mechanistic parameters resistance (Ri) and compliance (Cdyn) (Vanoirbeek et al., 2010). Given that the clinical evaluation of IPF and treatment efficacy most commonly relies on repeated physiological lung function measurements of FVC, we wanted to investigate the correlation of this parameter and histology in a preclinical mice model of BLM induced fibrosis. To evaluate the translatability of these findings to clinical outcomes, a retrospective evaluation of nintedanib treatment, as the gold standard drug, was performed here.

In a phase 3 clinical trial, nintedanib reduced the annual rate of decline in FVC when compared to placebo patients with IPF. In the referred study, 24.8% of patients showed an improvement of 77 mL FVC in a 52-week trial together with a reduction of the deposition of extracellular matrix in the lungs. These results accompanied by the positive outcome in preclinical research contributed to the FDA approval of nintedanib as a treatment for IPF (Karimi-Shah and Chowdhury, 2015; Flaherty et al., 2018).

In the present study, nintedanib given prophylactically starting from the day of the BLM challenge and administered during the inflammatory and active fibrotic phase resulted in an improvement of the FVC parameter by 26% over the control BLM challenged group. Results were evaluated on day 14 post BLM challenge at the fibrotic peak according to Izbicki et al. (2002). Likewise, when given therapeutically during the active and late fibrotic phase (from D7 until D21), on D21 post-challenge, the 21% FVC improvement in the nintedanib treated mice was significant over the BLM control group . Correlations between pathological changes and the values of PFTs were significant on both observed days.

Conclusions

In summary, the present study provides an important contribution to the establishment of the most relevant values for laboratory pulmonary functions in BLM-induced pulmonary fibrosis in both treatment regimens. We demonstrate that with the use of invasive pulmonary function methods, restrictive respiratory diseases such as lung fibrosis in mice could be distinguished based on the same functional parameters as the forced vital capacity parameter and flow volume curve that are applied in humans. By using the gold standard treatment nintedanib, a trend of improvement in

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both functional and structural parameters of lung evaluation was observed. These results are comparable to improvements reported in IPF patients. In conclusion, the implementation of PFTs could be a good platform to increase the predictive translational value of the model and potential new treatments.

Acknowledgments

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Retrospektivna procjena učinka nintedaniba u mišjem modelu plućne fibroze – usporedba učinkovitosti s kliničkim ishodom idiopatske plućne fibroze

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Testovi funkcije pluća prvi su klinički postupak u dijagnostici respiratornih bolesti kao što je idiopatska plućna fibroza (IPF). Mišji modeli plućne fibroze su vrlo važni u razvoju novih terapija. Uvođenje testova u životinjski model, zajedno s histološkom procjenom omogućit će bolji probir novih molekula i njihovo daljnje kliničko istraživanje. Cilj je ovog istraživanja bio odrediti precizne vrijednosti parametara plućnih funkcija korištenjem standardne humane terapije za IPF, nintedaniba na mišjim modelima u svrhu postavljanja platforme za učinkovitiji razvoj novih potencijalnih terapija. Testiranje je vršeno pod pretpostavkom da će odabrani tretman u mišjem modelu dati sličan efekt kao i kod ljudi u odabranoj dijagnostičkoj metodi. Testirana su dva eksperimentalna protokola: preventivno uvođenje terapije prije razvoja fibrotičnih promjena i terapijski protokol aplikacije terapije u vrijeme nastanka plućne fibroze. C57Bl/6 miševima je bleomicin apliciran intranazalno prvog dana studije, a terapija nintedanibom primijenjena je od prvog dana do 14. u preventivnom te od sedmog dana do 21. u terapijskom protokolu. Četrnaestog ili 21. dana nakon početka pokusa nakon bleomicinske primjene, testovi funkcije pluća su provedeni koristeći Buxco® Pulmonary Function Test (PFT) (DSI™, New Brighton, SAD) u *in vivo* invazivni sustav. Histološka procjena je provedena koristeći modificirani Ashcroft sustav ocjenjivanja količine patoloških promjena u plućima. Primjena bleomicina u plućima miševa je utjecala na značajno smanjenje parametra forsiranog vitalnog kapaciteta (FVC) u oba ispitana protokola, dok je terapija nintedanibom značajno poboljšala nastale promjene. Ovi rezultati su potvrđeni i ocjenom histoloških promjena u tkivu pluća. Testovi funkcionalnosti pluća u mišjem modelu značajno koreliraju s kliničkim efektom istraživane terapije u obje studije. U preventivnom protokolu istraženom 14. dana te u terapijskom 21. dana eksperimenta. Na temelju ovih saznanja, možemo zaključiti kako uvođenje ovog testa, kao relevantne kliničke dijagnostike, možemo poboljšati translacijsku vrijednost životinjskih modela u razvoju nove terapije.

Ključne riječi: *idiopatska plućna fibroza, bleomicin, mišji model plućne fibroze, testovi funkcije pluća, nintedanib*