Correlation of nintedanib efficacy on fibrotic lesion deposition and lung function within a rat IPF model

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease characterised by increased deposition of extra cellular matrix and scarring of lung tissue over an extended period of time. The deposition of extra cellular matrix and scarring leads to a decline in lung function which is ultimately fatal. The aim of this study was to assess whether decreases in lung function capacity in a bleomycin-induced rat model of IPF correlated with the lung fibrotic lesion area and whether nintedanib treatment could attenuate these changes.

Methods

Male SD rats were administered bleomycin or saline i.t. on Day 0. Treatment with nintedanib (100 mg/kg q.d.) commenced either prophylactically prior to bleomycin challenge on Day 0 or therapeutically from Day 7 post bleomycin challenge. On Day 21 invasive airway mechanics – forced vital capacity (FVC), peak expiratory flow (PEF) and forced expiratory volume in 100 msec (FEV 100) – were assessed using the eSpira Forced Manoeuvres System (EMMS) in anesthetised rats. On Day 21 lungs were removed, and the left lung fixed in 10% neutral buffered formalin and histologically stained with Masson's trichrome. Lung sections were examined histopathologically and scored according to a modified Ashcroft method. Whole section images were analysed by OracleBio using Indica Labs HALO® platform to quantify distinct regions of interest (ROI) per section including lesion and parenchyma (Figure 1). Statistical analysis was carried out using GraphPad Prism software. Data was analysed by ANOVA with Tukey post hoc test. Correlation data was analysed by Pearson correlation analysis.

Results

Bleomycin administration resulted in a statistically significant decrease of 51% in forced vital capacity (FVC) (Figure 2). This decline in FVC was significantly attenuated by both prophylactic and therapeutic treatment with nintedanib (24 and 14% improvement respectively).

Bleomycin administration resulted in a significant increase in % fibrotic lesion area in the lungs (Figure 3). This increase in lesion area was reduced by both prophylactic and therapeutic treatment with nintedanib (38% decrease with both dosing regimens).

Bleomycin administration also induced a significant increase in Ashcroft score (Figure 4). The increase in Ashcroft score was reduced by nintedanib dosing both prophylactically and therapeutically (23 and 13% decrease respectively). Nintedanib was also able to reduce TGF-β levels both prophylactically and therapeutically, significantly reduce lung hydroxyproline levels prophylactically, and significantly reduce wet lung weights both prophylactically and therapeutically (data not shown).

There were significant correlations between FVC and % fibrotic lesion area, FVC and Ashcroft score and % fibrotic lesion area and Ashcroft score (r2 of 0.48, 0.62 and 0.70 respectively, with p<0.0001 for each comparison) (Figure 5).

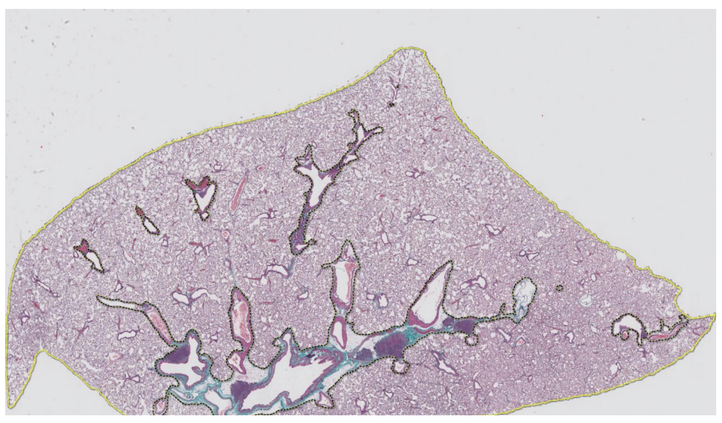

Image Source: External (approved by OracleBio Ltd.)

Figure 1A. Saline control animal scanned Masson's trichrome stained left lung section.

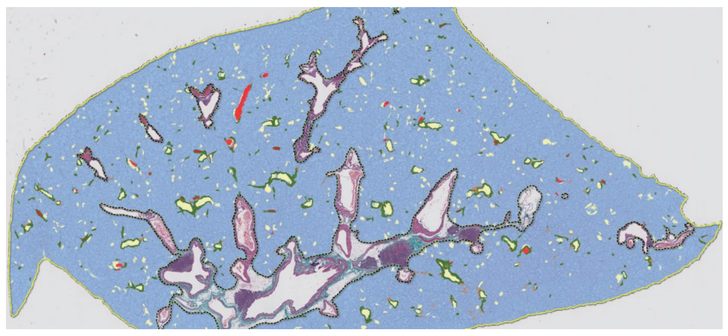

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Figure 1B. Saline control animal scanned Masson's trichrome stained left lung section with ROI overlay.

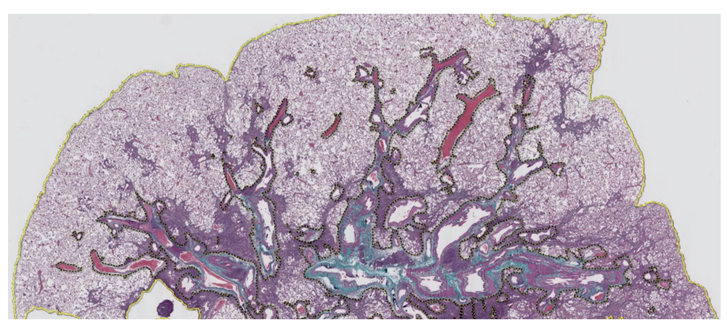

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Figure 1C. Bleomycin control animal scanned Masson's trichrome stained left lung section.

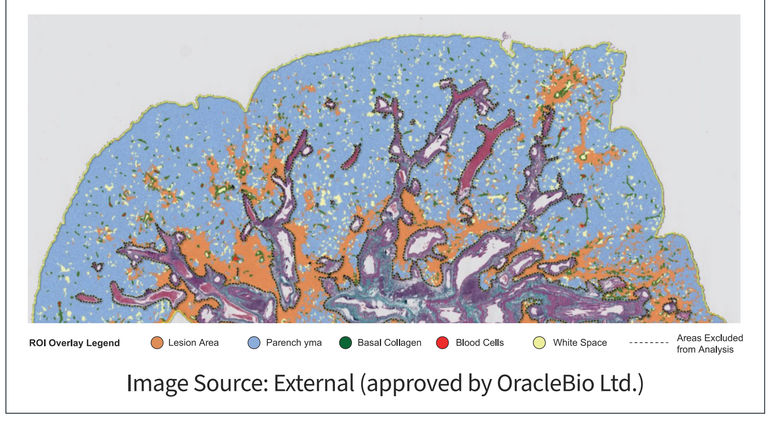

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Figure 1D. Bleomycin control animal scanned Masson's trichrome stained left lung section with ROI overlay.

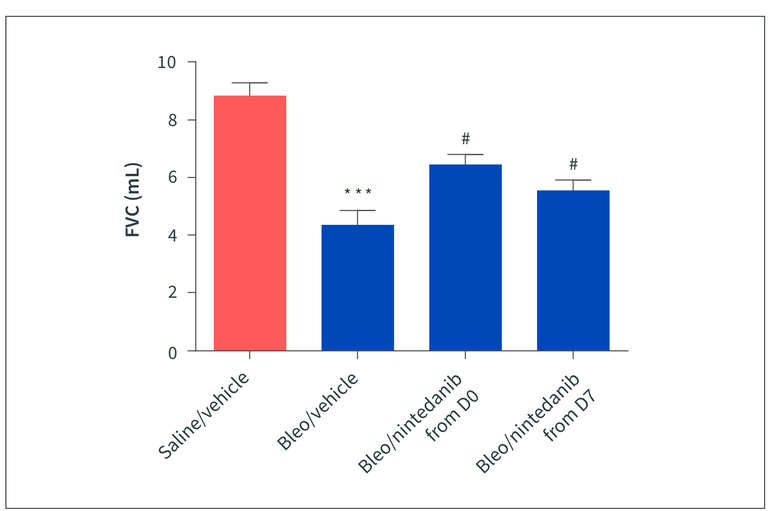

Figure 2. Forced Vital Capacity (FVC) at Day 21 post bleomycin. Bleomycin-induced a significant decline in FVC which is significantly attenuated by both prophylactic and therapeutic nintedanib dosing.

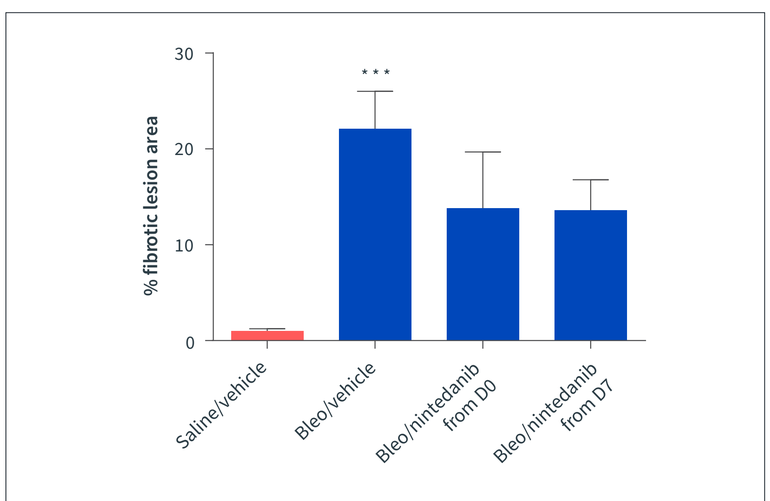

Figure 3. Percent fibrotic lesion area administration as assessed by OracleBio using Indica Labs HALO platform. Bleomycin administration resulted in a significant increase in % fibrotic lesion area which was reduced by both prophylactic and therapeutic nintedanib.

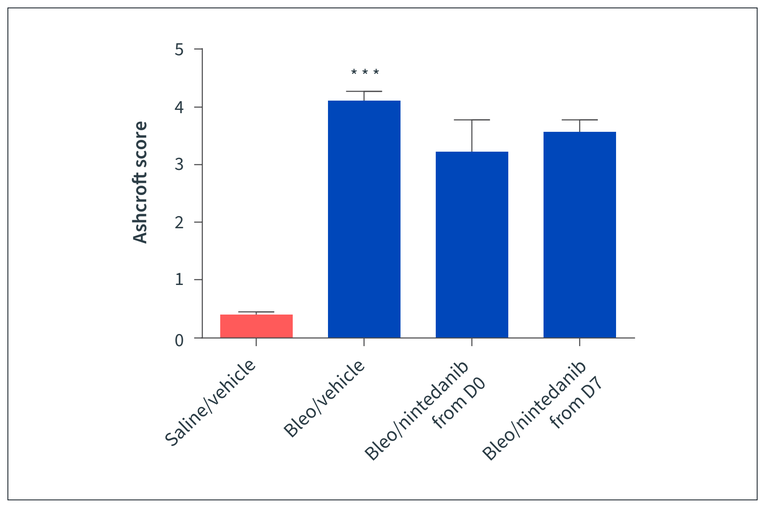

Figure 4. Ashcroft score at Day 21 post bleomycin administration. Bleomycin administration induced a significant increase in Ashcroft score which was reduced by nintedanib administration both prophylactically and therapeutically.

*** p< 0.001 compared to saline/vehicle animals,
# p<0.05 compared to bleo/vehicle.

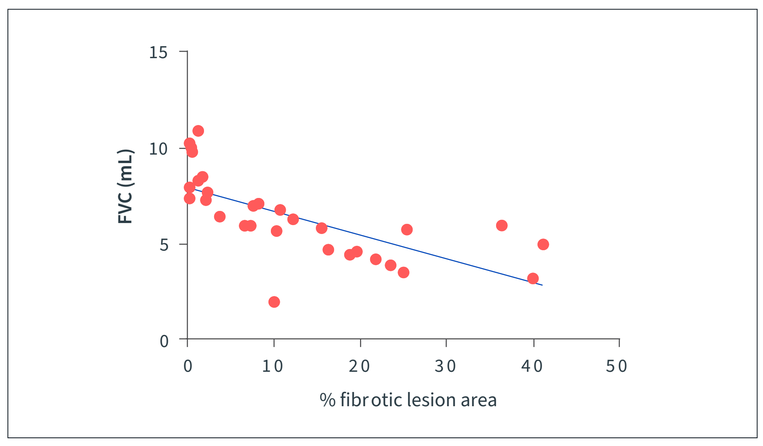

Figure 5A. Correlation between FVC and % lesion area. p<0.0001.

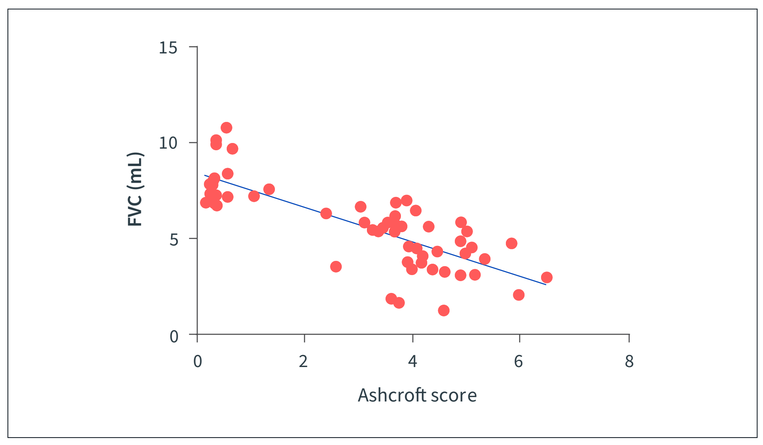

Figure 5B. Correlation between FVC and Ashcroft score. p<0.0001.

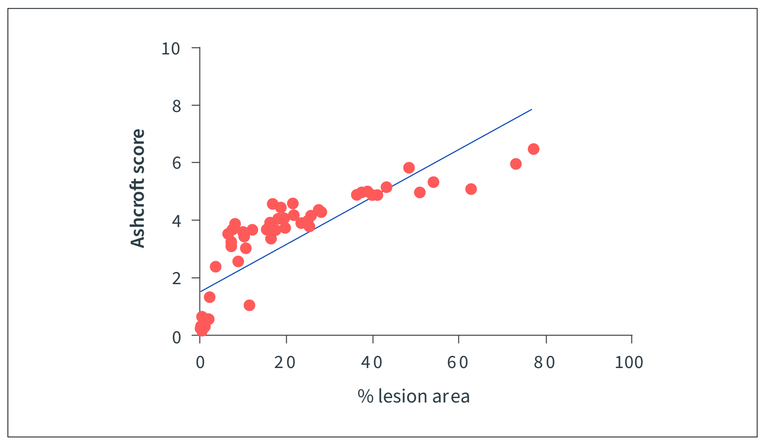

Figure 5C. Correlation between % lesion area and Ashcroft score. p<0.0001.

Discussion

These results show lung fibrosis induced by bleomycin was attenuated by 100 mg/kg q.d. nintedanib both prophylactically and therapeutically from Day 7. The correlations between the decline in FVC and % fibrotic lesion area and Ashcroft score suggest that the degree of lung function decline is driven by % of fibrotic lesion area in the lungs. Furthermore, the attenuation of the fibrotic response by nintedanib partly reversed lung function decline.

Animal experiments were conducted according to the Animals (Scientific Procedures) Act, 1986, and 2012 amendments following local ethical approval. Work was conducted in an AAALAC-accredited facility.

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