Repetitive bleomycin installations promote persistent progressive lung fibrosis in a spirometry-confirmed mouse model of IPF

Authors
Asbjørn Graver Petersen, Susanne Pors, Mikkel Christensen-Dalsgaard, Yessica Wouters-Van Looy, Henrik H Hansen, Michael Feigh

Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

Background & Aim

The bleomycin (BLEO) mouse model of pulmonary fibrosis is extensively used in preclinical drug discovery for idiopathic pulmonary fibrosis (IPF). A major limitation of the single-dose BLEO-IPF model is spontaneous resolution of lung fibrosis.

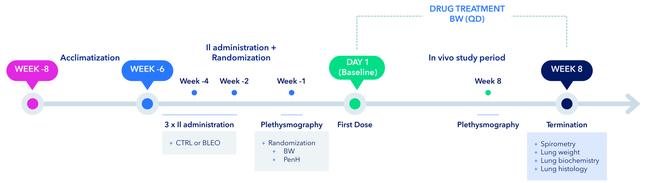
The aim of the present study was to establish a novel BLEO-IPF mouse model with persistent progressive pulmonary fibrosis using repetitive bleomycin instillations.

Methods

12-week-old C57BL/6JRj male mice received bi-weekly intratracheal instillations of either bleomycin (1.75 mg/kg, 50 µL) or saline (control, 50 µL) for a total of six weeks. After the bleomycin instillation lead-in period, a BLEO-IPF baseline group was terminated (BLEO-IPF Baseline). Other BLEO-IPF (BLEO-IPF W8) and control mice (CTRL) were administered saline (BID, PO) for additional 8 weeks. Body weight was monitored daily, and enhanced pause (PenH) was measured by whole-body plethysmography at baseline for randomization, and termination at week 8. Terminal pulmonary endpoints included spirometry, hydroxyproline (HP), severity of fibrosis using Ashcroft score, quantitative histological markers of inflammation (Gal-3), fibrogenesis (α-SMA) and fibrosis (PSR, Col1a1, Col3).

www.gubra.dk

1 Study outline



Group	Animal	Group	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume
1	CTRL	W8	Male	10	**Saline**	PO	BID	5 mL/kg
2	BLEO-IPF	Baseline	Male	13	**NA**	NA	NA	NA
3	BLEO-IPF	W8	Male	17	**Saline**	PO	BID	5 mL/kg

2 Metabolic and biochemical parameters

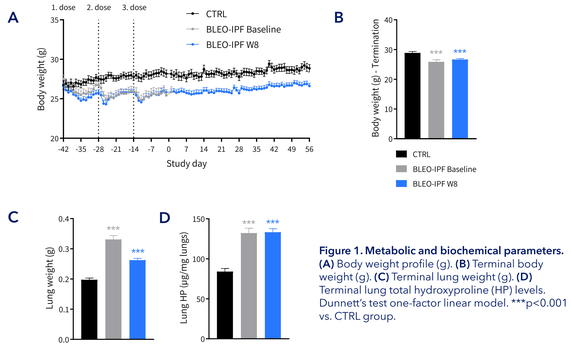


Figure 1. Metabolic and biochemical parameters. **(A)** Body weight profile (g). **(B)** Terminal body weight (g). **(C)** Terminal lung weight (g). **(D)** Terminal lung total hydroxyproline (HP) levels. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

3 Pulmonary function

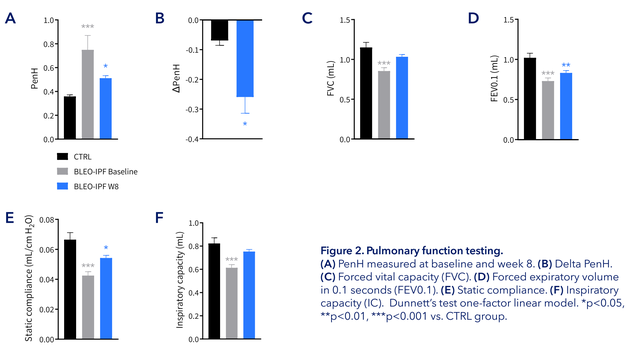


Figure 2. Pulmonary function testing. **(A)** PenH measured at baseline and week 8. **(B)** Delta PenH. **(C)** Forced vital capacity (FVC). **(D)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(E)** Static compliance. **(F)** Inspiratory capacity (IC). Dunnett's test one-factor linear model. *p<0.05, **p<0.01, ***p<0.001 vs. CTRL group.

4 Histopathological Ashcroft scoring

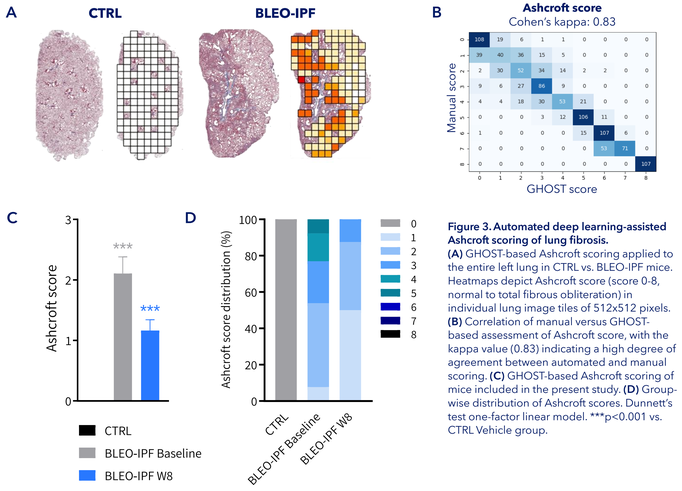


Figure 3. Automated deep learning-assisted Ashcroft scoring of lung fibrosis. **(A)** GHOST-based Ashcroft scoring applied to the entire left lung in CTRL vs. BLEO-IPF mice. Heatmaps depict Ashcroft score (score 0-8, normal to total fibrous obliteration) in individual lung image tiles of 512x512 pixels. **(B)** Correlation of manual versus GHOST-based assessment of Ashcroft score, with the kappa value (0.83) indicating a high degree of agreement between automated and manual scoring. **(C)** GHOST-based Ashcroft scoring of mice included in the present study. **(D)** Group-wise distribution of Ashcroft scores. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL Vehicle group.

5 Histological markers of inflammation, fibrosis and fibrogenesis

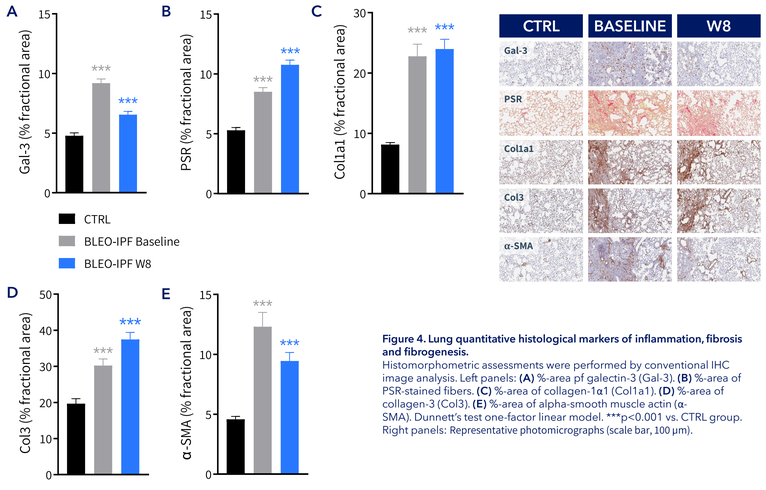


Figure 4. Lung quantitative histological markers of inflammation, fibrosis and fibrogenesis. Histomorphometric assessments were performed by conventional IHC image analysis. Left panels: **(A)** %-area pf galectin-3 (Gal-3). **(B)** %-area of PSR-stained fibers. **(C)** %-area of collagen-1α1 (Col1a1). **(D)** %-area of collagen-3 (Col3). **(E)** %-area of alpha-smooth muscle actin (α-SMA). Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group. Right panels: Representative photomicrographs (scale bar, 100 µm).

Conclusion

Repetitive bleomycin installations promote:

+ Persistent increases in lung weight and pulmonary inflammation (Gal-3)
+ Persistent lung functional impairment (PenH, FEV0.1, static compliance)
+ Persistent (HP, Col1a1) and progressive (PSR, Col3) pulmonary fibrosis

The chronic BLEO-IPF mouse model is suitable for testing novel IPF-targeted drug therapies

Scan the QR code to see the poster online