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

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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 3 intratracheal instillations every other week 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 (WBP) at baseline for randomization, and termination at week 8. Terminal pulmonary endpoints included spirometry, hydroxyproline (HP), quantitative histological markers of inflammation (Gal-3), fibrogenesis (α-SMA) and fibrosis (PSR, Col1a1, Col3), Ashcroft Score using Gubra Histopathological Objective Scoring Technique (GHOST), as well as transcriptome signatures using bulk RNAsequencing.

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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

Figure 1. Study outline and group overview

2 Metabolic and biochemical parameters

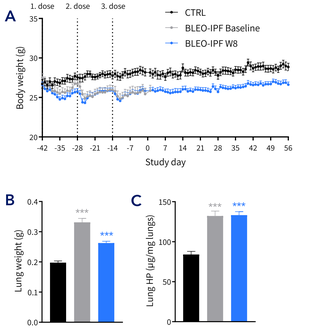


Figure 2. Metabolic and biochemical parameters.
(A) Body weight profile (g). **(B)** Terminal lung weight (g). **(C)** Terminal lung total hydroxyproline (HP) levels. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

3 Pulmonary function

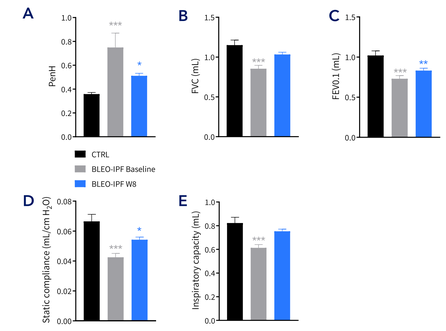


Figure 3. Pulmonary function testing.
(A) PenH measured with whole body plethysmography at baseline and week 8. **(B)** Forced vital capacity (FVC). **(C)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(D)** Static compliance. **(E)** Inspiratory capacity (IC). Dunnett's test one-factor linear model. *p<0.05, **p<0.01, ***p<0.001 vs. CTRL group.

4 Histopathological Ashcroft scoring

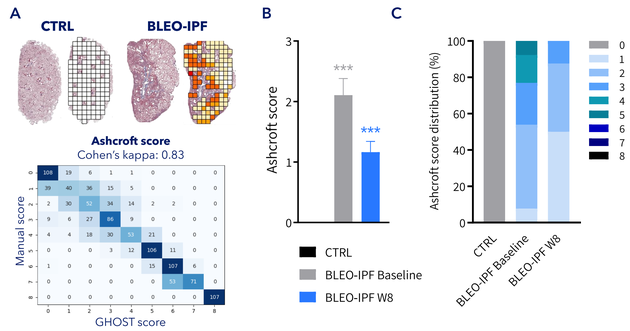
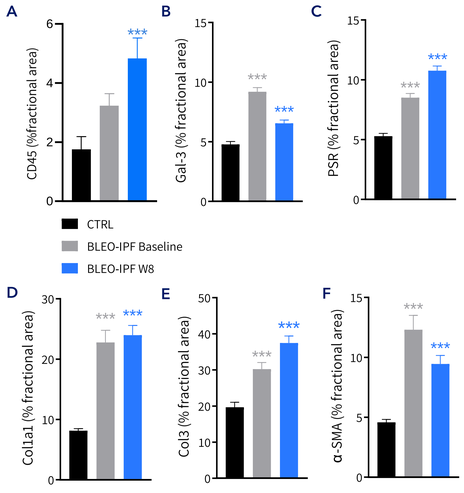


Figure 4. Automated deep learning-assisted Ashcroft scoring of lung fibrosis.
(A) GHOST-based Ashcroft scoring applied to the entire left lung and correlation of manual versus GHOST-based assessment of Ashcroft score, with the kappa value (0.83) **(B)** GHOST-based Ashcroft scoring of mice included in the present study. **(C)** Group-wise distribution of Ashcroft scores. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

5 Histological markers of inflammation, fibrosis, and fibrogenesis



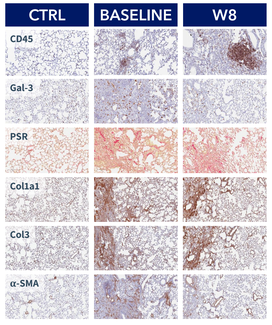


Figure 5. Lung quantitative histological markers of inflammation, fibrosis and fibrogenesis.
Histomorphometric assessments were performed by conventional IHC image analysis. Left panels: **(A)** %-area of CD45. **(B)** %-area of galactin-3 (gal-3) **(C)** %-area of PSR-stained fibers. **(C)** %-area 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).

6 Transcriptomic profile of inflammation and fibrosis

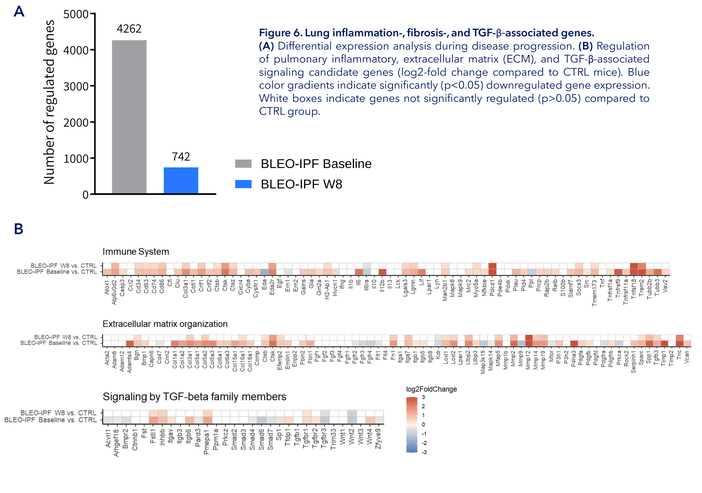


Figure 6. Lung inflammation-, fibrosis-, and TGF-β-associated genes.
(A) Differential expression analysis during disease progression. **(B)** Regulation of pulmonary inflammatory, extracellular matrix (ECM), and TGF-β-associated signaling candidate genes (log2-fold change compared to CTRL mice). Blue color gradients indicate significantly (p<0.05) downregulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to CTRL group.

Conclusion

Repetitive bleomycin installations demonstrated:

+ Persistent increases in lung weight and pulmonary inflammation (CD45, Gal-3)
+ Persistent lung functional impairment (PenH, FEV0.1, static compliance)
+ Persistent (HP, Col1a1) and progressive (PSR, Col3) pulmonary fibrosis
+ Marked lung transcriptomic regulation and increased fibrosis- and inflammation-associated gene expression.

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

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