Repetitive installations of bleomycin induce persistent progressive lung fibrosis in a 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 (CD45, CD3, CD20, 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.

Conclusio

Repetitive bleomycin installations demonstrated:

+ Persistent increases in lung weight and pulmonary inflammation (CD45, CD3, CD20, 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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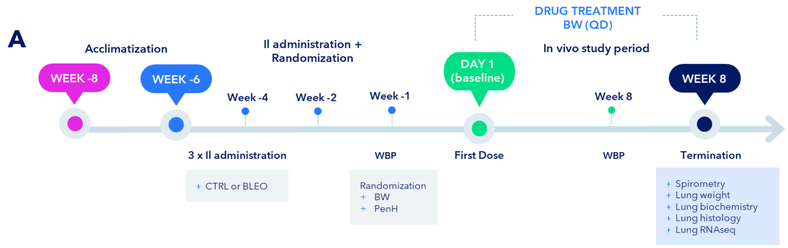
1 Study outline



B

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. (A) Study outline and **(B)** group overview.

2 Metabolic and biochemical parameters

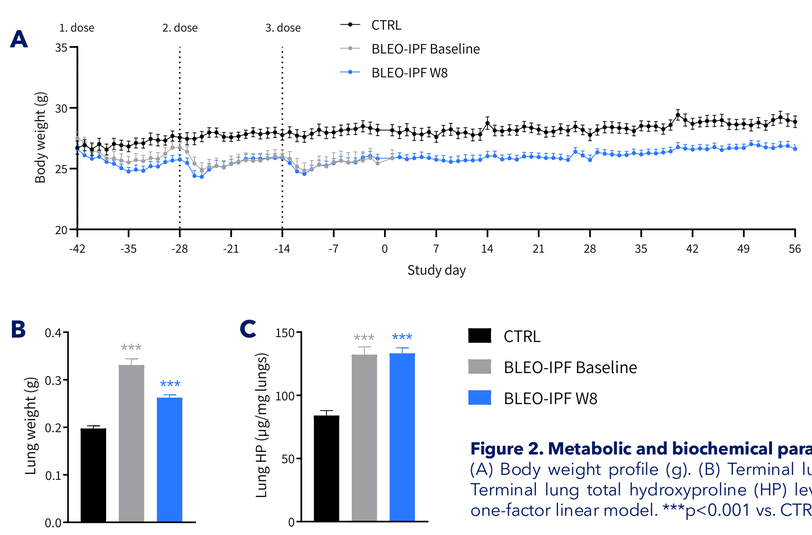


Figure 2. Metabolic and biochemical parameters.
(A) Body weight profile (g). (B) Terminal lung weight (g). (C) Terminal lung total hydroxyproline (HP) levels. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

3 Pulmonary function

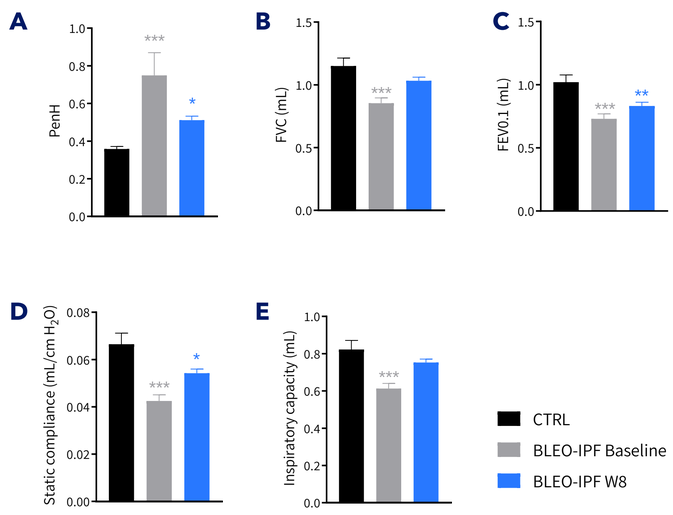


Figure 3. Pulmonary function testing.
(A) PenH measured with whole body plethysmography at baseline and week 8. (B) Forced vital capacity (FVC). (C) Forced expiratory volume in 0.1 seconds (FEV0.1). (D) Static compliance. (E) Inspiratory capacity (IC). Dunnett's test one-factor linear model. *p<0.05,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

4 Histopathological Ashcroft scoring

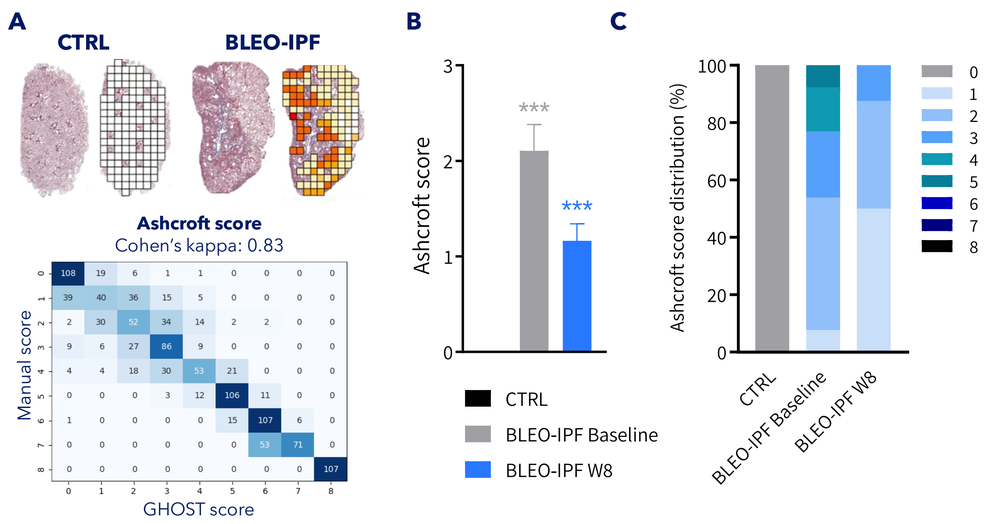


Figure 4. Automated deep learning-assisted Ashcroft scoring of lung fibrosis.
(A) GHOST-based Ashcroft scoring applied to the entire left lung and correlation of manual versus GHOST-based assessment of Ashcroft score, with the kappa value (0.83) (B) GHOST-based Ashcroft scoring of mice included in the 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

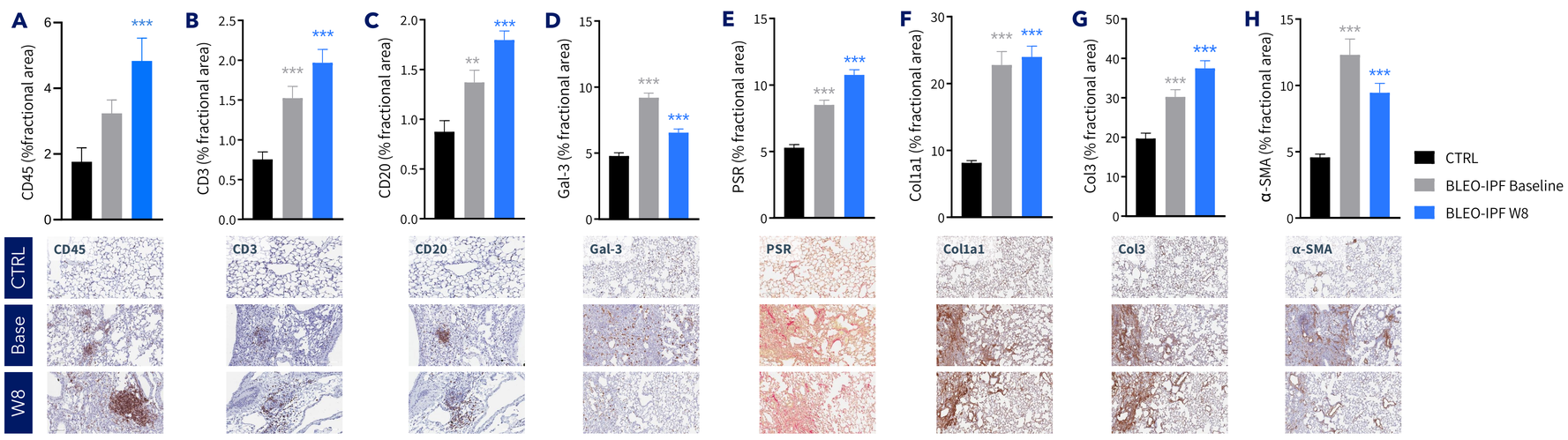


Figure 5. Lung quantitative histological markers of inflammation, fibrosis and fibrogenesis.
Histomorphometric assessments were performed by conventional IHC image analysis. Upper panels: (A) %-area of CD45. (B) %-area of CD3 **(C)** %-area of CD20 **(D)** %-area of galactin-3 (gal-3) (E) %-area of PSR-stained fibers. (F) %-area of collagen-1α1 (Col1a1). (G) %-area of collagen-3 (Col3). (H) %-area of alpha-smooth muscle actin (α-SMA). Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group. Lower panels: Representative photomicrographs (scale bar, 100 µm).

6 Transcriptomic profile of inflammation and fibrosis

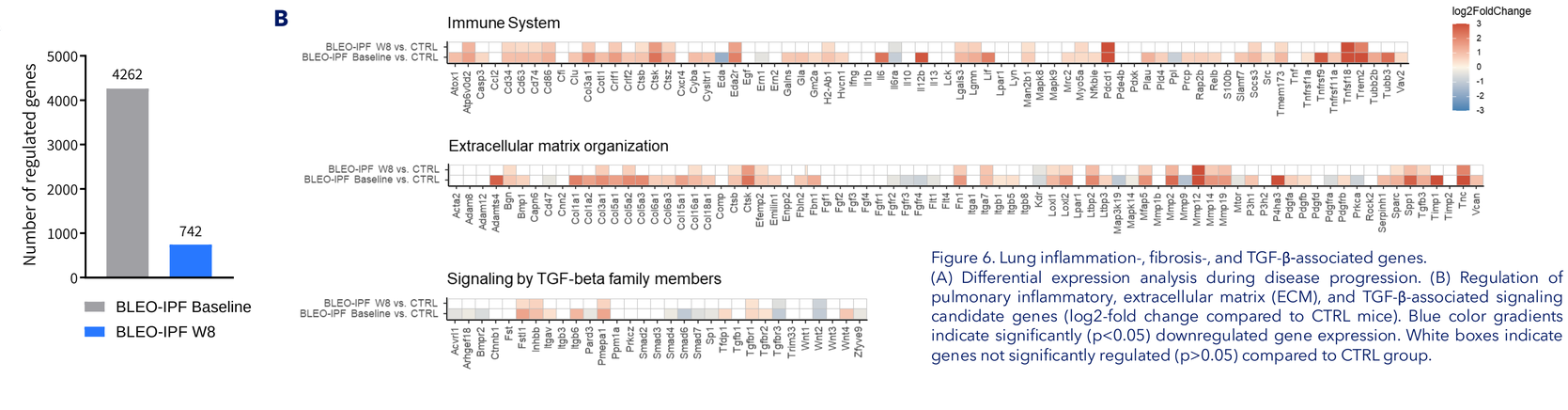


Figure 6. Lung inflammation-, fibrosis-, and TGF-β-associated genes.
(A) Differential expression analysis during disease progression. (B) Regulation of pulmonary inflammatory, extracellular matrix (ECM), and TGF-β-associated signaling candidate genes (log2-fold change compared to CTRL mice). Blue color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