# Molecular Hallmarks of Lung Cellular Senescence in the Repetitive Bleomycin-induced and Spirometry-confirmed Mouse Model of IPF


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## Background & Aim

Growing evidence suggests that senescent cells contribute to the development and progression of idiopathic pulmonary fibrosis (IPF).

This study aimed to identify and characterize key markers of lung senescence using a repetitive bleomycin-induced (BLEO), spirometry-confirmed mouse model of IPF.

## Methods

Twelve-week-old male C57BL/6JRj mice received three intratracheal instillations of either bleomycin (1.75 mg/kg, 50 µL) or saline (control, 50 µL) administered every other week over a six-week period. Following this induction phase, a subset of bleomycin-treated mice was euthanized to establish the BLEO-IPF Baseline group. The remaining bleomycin-treated (BLEO-IPF W8) and control (CTRL) mice were subsequently dosed with oral saline (BID, PO) for an additional eight weeks. Body weight was recorded daily, and enhanced pause (PenH) was assessed using whole-body plethysmography (WBP) at baseline (for randomization) and at the eight-week endpoint.

Terminal pulmonary endpoints included spirometry, hydroxyproline (HP) quantification, quantitative histological markers of cellular senescence (p21), fibrosis (PSR), and inflammation (Gal-3), including Ashcroft Score using Gubra Histopathological Objective Scoring Technique (GHOST), as well as transcriptomic profiling through bulk RNA sequencing.

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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

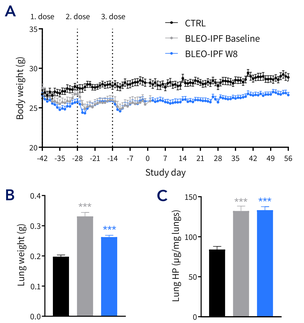


**Figure 2. Metabolic and biochemical parameters.**
**(A)** Body weight profile (g). **(B)** Terminal lung weight (g). **(C)** Terminal lung total hydroxyproline (HP) levels. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

## 3 Pulmonary function

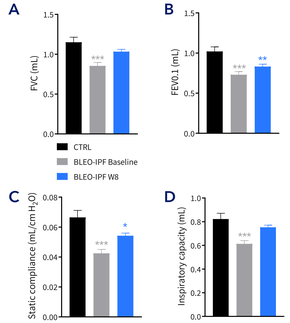


**Figure 3. Pulmonary function testing.**
**(A)** Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). Dunnett's test one-factor linear model. *p<0.05, **p<0.01, ***p<0.001 vs. CTRL group.

## 4 Histological markers for inflammation and fibrosis

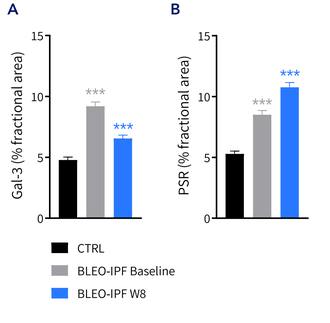


**Figure 4. Lung histology.**
**(A)** Galectin-3. **(B)** PSR-stained fibers. Histomorphometric assessments were performed by conventional image analysis. Data were calculated as proportionate (%) area of histological staining (mean ± SEM). Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

## 5 Histopathological Ashcroft scoring

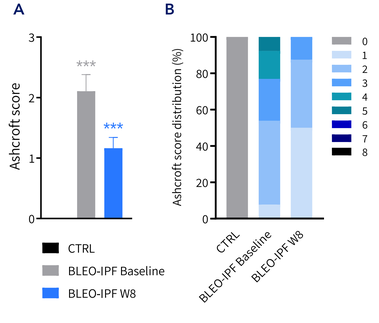


**Figure 5 Automated deep learning-assisted Ashcroft scoring of lung fibrosis.**
**(A)** GHOST-based Ashcroft scoring of mice. **(D)** Distribution of Ashcroft scores. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

## 6 Histological markers of lung senescence

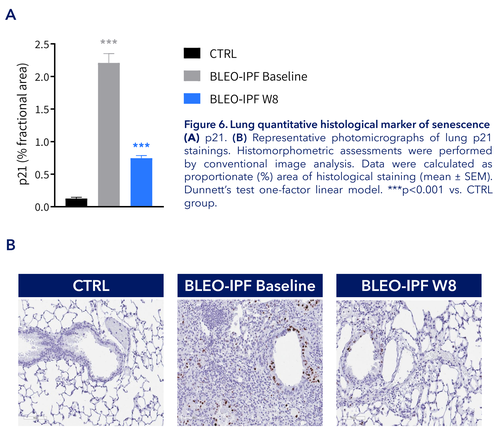


**Figure 6. Lung quantitative histological marker of senescence**
**(A)** p21. **(B)** Representative photomicrographs of lung p21 stainings. Histomorphometric assessments were performed by conventional image analysis. Data were calculated as proportionate (%) area of histological staining (mean ± SEM). Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

## 7 Transcriptome signature of lung senescence

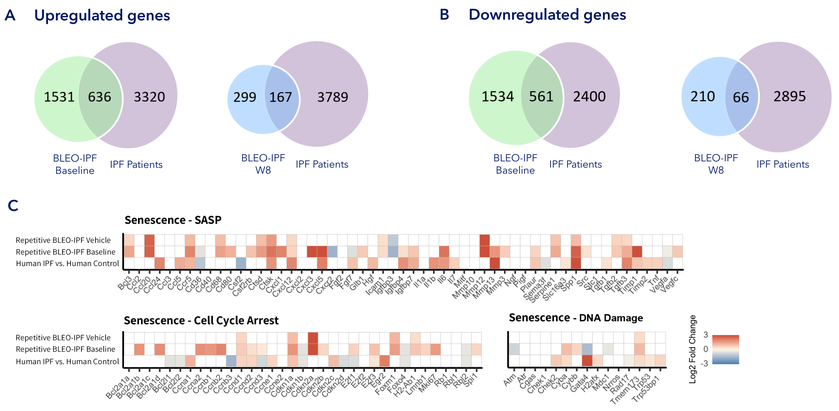


**Figure 7. Lung senescence-associated genes in BLEO-IPF mice.**
Lung transcriptome changes in BLEO-IPF mice validated against lung RNA sequencing data from IPF patients. **(A)** and **(B)** Venn diagrams depicting shared and separate differentially expressed genes (DEGs; false discovery rate<0.05) in BLEO IPF mice vs. patients with advanced IPF (Sivakumar et al., 2019). **(C)** Regulation of senescence-associated secretory phenotype (SASP), cell cycle arrest and DNA damage candidate genes (log2-fold change compared to CTRL mice). Blue color gradients indicate significantly (p<0.05) down-regulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to CTRL group.

## Conclusion

The repetitive bleomycin-induced model of IPF is characterized by:

+ Sustained increases in lung weight and pulmonary inflammation (Gal-3)
+ Persistent lung functional impairment (FEV0.1, static compliance)
+ Persistent (HP) and progressive (PSR) pulmonary fibrosis
+ Lung cellular senescence detected by quantitative histology (p21)
+ Pronounced transcriptome perturbations involving upregulation of cell senescence-associated gene markers

**The repetitive BLEO-IPF mouse model serves as a translational preclinical platform for evaluating senotherapeutics in IPF.**

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