Therapeutic effects of ALK5i on pulmonary function and fibrosis in a bleomycin-induced and spirometry-confirmed mouse model of IPF

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BACKGROUND & AIM

Idiopathic pulmonary fibrosis (IPF) is a chronic and fatal interstitial lung disease, characterized by progressive lung fibrosis and decline in pulmonary function. TGFβ-ALK5 signalling is critical in the progression of fibrosis. The aim of the present study was to characterize the effects of ALK5 inhibition (ALK5i) on pulmonary function as well as metabolic, biochemical, and histological endpoints in a bleomycin-induced (BLEO) mouse model of spirometry-confirmed IPF.

METHODS

12 weeks-old male C57BL6/JRJ mice were acclimatized for 2 weeks before receiving a single intratracheal instillation (2 mg/kg – 3 U/kg) of bleomycin (BLEO) or sterile saline. BLEO-IPF were randomized into study groups based on enhanced pause (PenH) and body weight at study day -1, followed by 21 days of treatment (see figure 1). Vehicle-treated mice served as normal controls (CTRL). Terminal pulmonary endpoints included spirometry (flexiVent) for lung function assessment, hydroxyproline content, AI-assisted Ashcroft scoring using Gubra Histopathological Objective Scoring Technique (GHOST) and quantitative histological markers of inflammation (galectin-3) and fibrosis (PSR, Col1a1, Col3, α-SMA).

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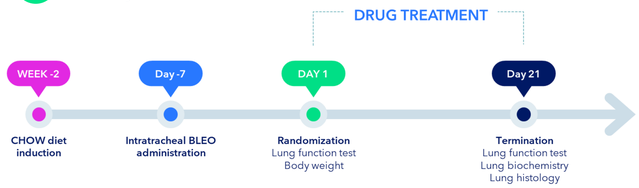
1 Study outline



Group	Animal model	Number of animals	Treatment	Dose	Route of administration	Frequency
1	CTRL	10	**Vehicle**	NA	PO	BID
2	BLEO-IPF	14	**Vehicle**	NA	PO	BID
3	BLEO-IPF	13	**ALK5i**	30 mg/kg	PO	BID

Figure 1. Study overview. Study outline and groups table.

2 Metabolic and biochemical parameters

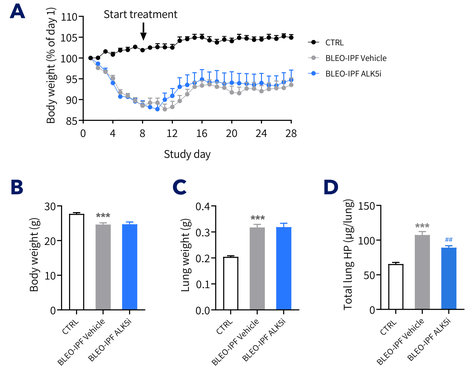


Figure 2. Metabolic and biochemical parameters. (A) Body weight change relative to baseline (% of day 1). **(B)** Terminal body weight (g). **(C)** Terminal lung weight (g). **(D)** Terminal lung total hydroxyproline (HP) levels. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL; ##p<0.01 vs. vehicle group.

3 Pulmonary function testing

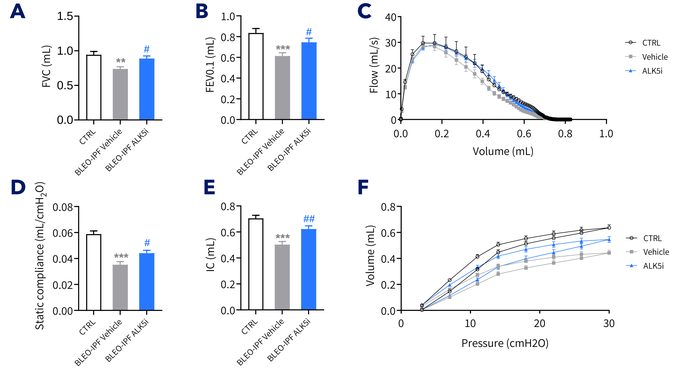


Figure 3. Pulmonary function testing. (A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Flow-volume curve. **(D)** Static compliance. **(E)** Inspiratory capacity (IC). **(F)** Pressure-volume curve. Dunnett's test one-factor linear model. **p<0.01, ***p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

4 Histopathological Ashcroft scoring

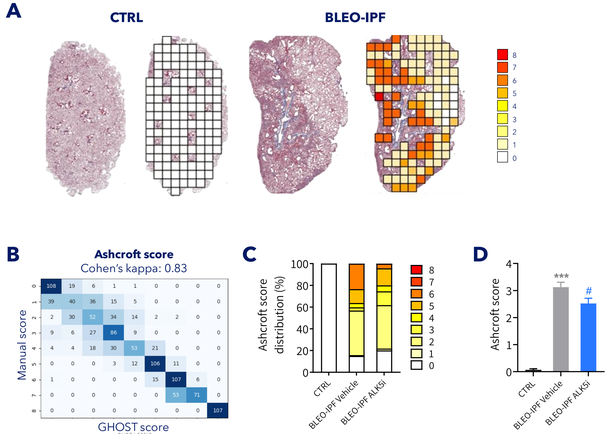


Figure 4. Automated deep learning-assisted Ashcroft scoring of lung fibrosis. (A) GHOST-based Ashcroft scoring applied to the entire left lung in BLEO-IPF mice terminated on study day 21. Heatmaps depict Ashcroft score (score 0-8, normal to total fibrous obliteration) in individual lung image tiles of 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)** Distribution of GHOST-based Ashcroft scores. **(D)** Average GHOST-based Ashcroft scoring. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL; #p<0.05 vs. vehicle group.

5 Histological markers of inflammation and fibrosis

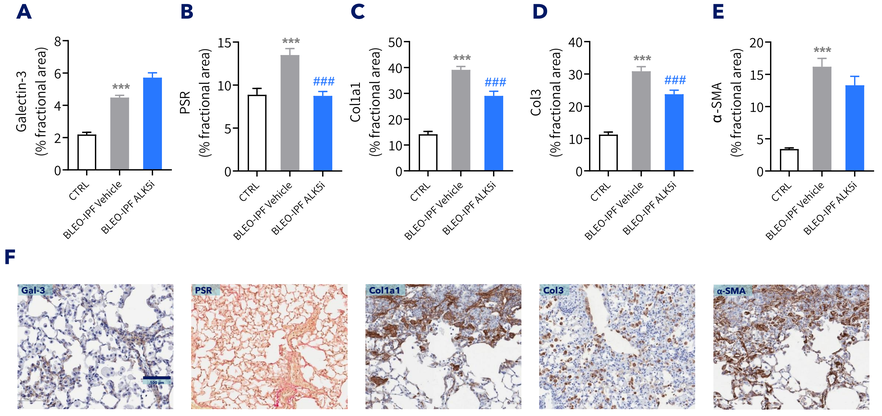


Figure 5. Lung quantitative histological markers of inflammation, fibrosis and fibrogenesis. Histomorphometric assessments were performed by conventional IHC image analysis. **A)** % fractional area of Galectin-3; **(B)** % fractional area of PSR-stained fibers; **(C)** % fractional area of Collagen-1α1; **(D)** % fractional area of Collagen-3; **(E)** % fractional area of alpha-smooth muscle actin (α-SMA). Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL; ##p<0.01, ###p<0.001 vs. vehicle group. **(F)** Representative photomicrographs of BLEO-IPF vehicle groups (scale bar x 20, 100 µm).

Conclusion

- BLEO-IPF mice demonstrate progressive increase in lung weight and impaired lung function, including decline in FVC.
- BLEO-IPF mice demonstrate significant pulmonary inflammation and fibrosis, including clinically relevant Ashcroft histopathological scores.
- ALK5i treatment reduces total lung HP levels
- ALK5i treatment improves pulmonary inspiratory and expiratory function, including FVC
- ALK5i treatment improves histopathological Ashcroft score and decreases quantitative histological markers of fibrosis

The BLEO-IPF mouse represents a translational preclinical model for exploring novel therapeutic agents for IPF.