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

Male C57BL/6JRj mice (12 weeks old) received a single intratracheal instillation (II) of BLEO (2 mg/kg, 50 µL) or saline (CTRL) at study day 1. BLEO-IPF animals were randomized into study groups based on relative (%) body weight loss at study day 7 post-BLEO followed by 21 days of treatment.
Terminal pulmonary endpoints included spirometry (Flexivent) for expiratory/inspiratory capacity, hydroxyproline (HP), quantitative histological markers of inflammation (galectin-3), fibrosis (PSR, Col1a1, Col3) and fibrogenesis (α-SMA).
An automated deep learning-based digital imaging analysis pipeline (Gubra Histopathological Objective Scoring Technique, GHOST) was developed for automated Ashcroft scoring of fibrosis severity.

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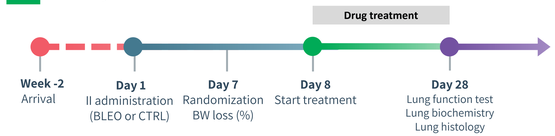
1 Study outline



Group	Animal model	Number of animals	Treatment	Dose	Route of administration	Dosing frequency
1	CTRL	10	Vehicle	NA	PO	BID
2	BLEO-IPF	18	Vehicle	NA	PO	BID
3	BLEO-IPF	18	ALK5i	30 mg/kg	PO	BID

Figure 1. Study outline, groups and treatments.
Abbreviations: II: Intratracheal instillation; BLEO: Bleomycin; BW: Body weight; CTRL; Control; PO: Per oral; BID: twice daily.

2 Metabolic and biochemical parameters

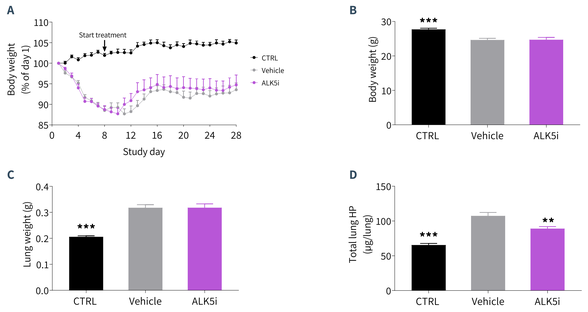


Figure 2. ALK5i reduces total lung hydroxyproline levels in BLEO-IPF mice.
(A) Body weight change relative to baseline (day 1). **(B)** Terminal body weight (g). **(C)** Terminal lung weight (g). **(D)** Terminal total lung hydroxyproline (HP). **p<0.01, ***p<0.001 compared to BLEO-IPF Vehicle group (Dunnett's test one-factor linear model).

3 Pulmonary function

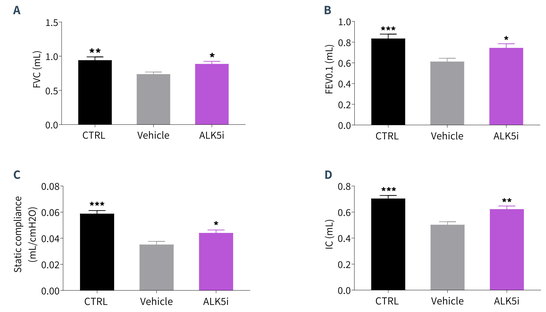


Figure 3. ALK5i improves pulmonary function in BLEO-IPF mice.
(A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). *p<0.05, **p<0.01, and ***p<0.001 compared to BLEO-IPF Vehicle group (Dunnett's test one-factor linear model).

4 Histopathological Ashcroft scoring

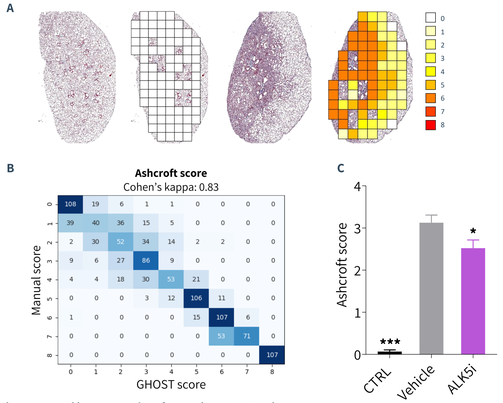


Figure 4. ALK5i improves Ashcroft score in BLEO-IPF mice.
Histopathological Ashcroft scores were determined by GHOST deep learning-based image analysis. **(A)** Representative Masson's Trichome photomicrographs used for GHOST evaluation. **(B)** Ashcroft compared by GHOST assessment and manual scoring. **(C)** Ashcroft score by GHOST. Mean ± SEM. *p<0.05 and ***p<0.001 compared to BLEO-IPF Vehicle group (Dunnett's test one-factor linear model).

5 Histological markers of inflammation, fibrosis, and fibrogenesis

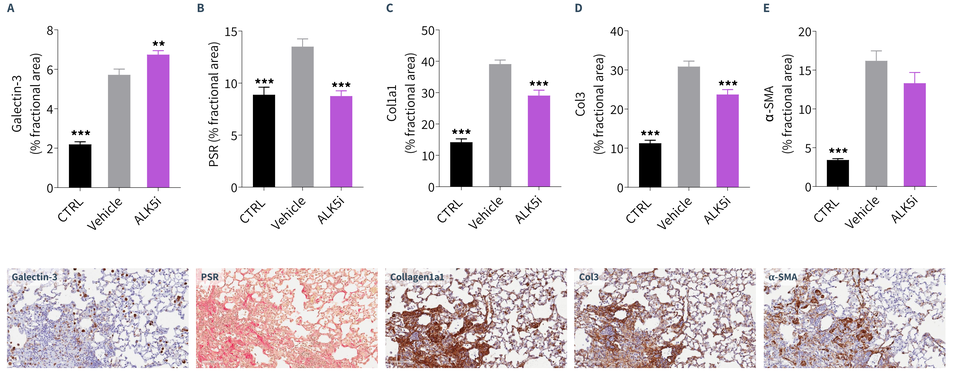


Figure 5. ALK5i decreases histological markers of fibrosis in BLEO-IPF mice.
Histomorphometric assessments were performed by conventional IHC image analysis (panels A-E). **(A)** % area of Galectin-3. **(B)** % area of PSR. **(C)** % area of collagen-1a1 (Col1a1). **(D)** % area of collagen 3 (Col3). **(E)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. **p<0.01, and ***p<0.001 compared to BLEO-IPF Vehicle group (Dunnett's test one-factor linear model). Lower panels: Representative galectin-3, PSR, collagen 1a1, collagen 3, and α-SMA photomicrographs for BLEO-IPF Vehicle group (scale bar, 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.