Therapeutic effects of ALK5i on pulmonary function and fibrosis in high-fat diet + 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 fibrotic development within the lungs 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 in addition to metabolic, biochemical, and histological changes in a combined high-fat diet (HFD) + bleomycin-induced (BLEO) and spirometry-confirmed mouse model of IPF.

METHODS

Mature (12 weeks old) male C57BL/6JRj mice were fed HFD (60% kcal fat) for two weeks prior to receiving a single intratracheal instillation (II) of BLEO (1.5 mg/kg, 50 µL) or saline (CTRL) at study day 1. Animals remained on HFD for the entire study period. HFD-BLEO-IPF animals were randomized into study groups based on body weight loss at study day 7 post-BLEO followed by 21 days of treatment. Terminal pulmonary end-points included spirometry (Flexivent) for expiratory/inspiratory capacity, biochemical analysis for hydroxyproline (HP) content, quantitative histomorphometry for markers of inflammation and fibrosis. Gubra Histopathological Objective Scoring Technique (GHOST) was used for performing pathological Ashcroft scoring.

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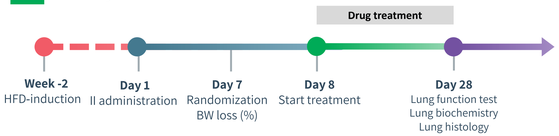
1 Study outline



Group	Animal model	Number of animals	Treatment	Dose	Route of administration	Dosing frequency
1	HFD-CTRL	10	Vehicle	NA	PO	QD
2	HFD-BLEO-IPF	15	Vehicle	NA	PO	QD
3	HFD-BLEO-IPF	15	ALK5i	30 mg/kg	PO	BID

Figure 1. Study outline, groups and treatments. HFD: High-fat diet; II: Intratracheal instillation; BW: Body weight; PO: Per oral; QD: once daily; BID: twice daily.

2 Metabolic and biochemical parameters

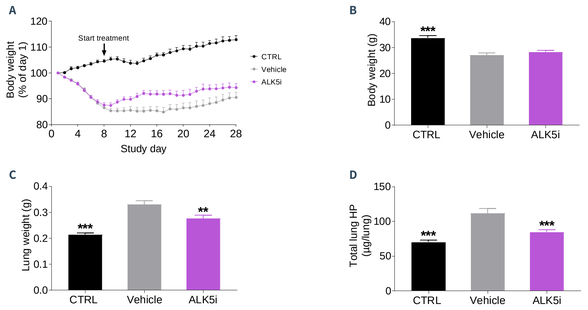


Figure 2. ALK5i improves lung weight and total lung hydroxyproline in HFD-BLEO-IPF mice. (A) Body weight change relative to baseline (day 1). **(B)** Terminal body weight (g). **(C)** Terminal lung weight (g). **(D)** Terminal lung total HP. **p<0.01 and ***p<0.001 compared to HFD-BLEO-IPF Vehicle group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

3 Pulmonary function testing

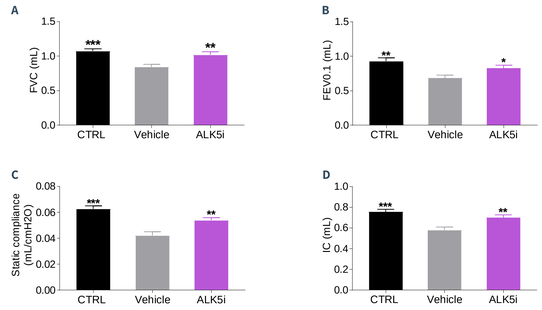


Figure 3. ALK5i improves pulmonary function testing in HFD-BLEO-IPF mice. (A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). *p<0.05, **p<0.01, and ***p<0.001 compared to HFD-BLEO-IPF Vehicle group (Dunnett's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

4 Histopathological Ashcroft scoring

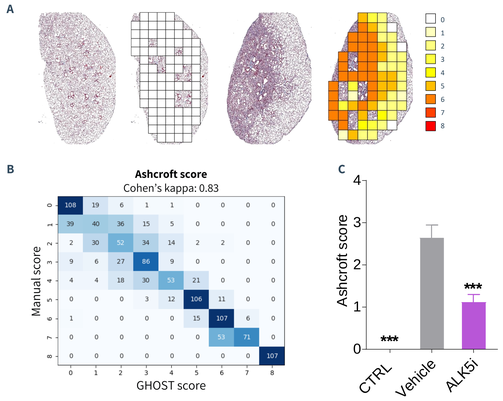


Figure 4. ALK5i improves Ashcroft score in HFD-BLEO-IPF mice. Histopathological Ashcroft scores were determined by GHOST deep learning-based image analysis. **(A)** Representative Masson's Trichome photomicrographs used for GHOST evaluation. **(B)** Ashcroft compared by GHOST assessment and manual scoring.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

5 Histological markers of inflammation, fibrosis, and fibrogenesis

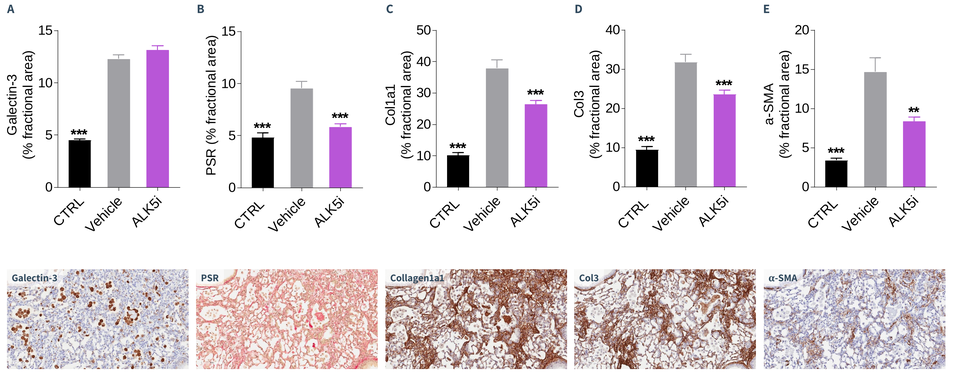


Figure 5. ALK5i decreases histological markers of fibrosis and fibrogenesis in HFD-BLEO-IPF mice. Histomorphometric assessments were performed by conventional IHC image analysis (panels A-D). **(A)** % area of Galectin- 3 content. **(B)** % area of PSR content. **(C)** % area of collagen-1a1 (Col3) content. **(D)** % area of collagen 3 (Col3) content. **(E)** % area of alpha-smooth muscle actin (α-SMA) content. Mean ± SEM. *p<0.05, *p<0.01, and ***p<0.001 compared to HFD-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 HFD-BLEO-IPF Vehicle group (scale bar, 100 µm).

CONCLUSION

- HFD-BLEO-IPF mice demonstrate progressive increase in lung weight and markers of inflammation and fibrosis in conjunction with pulmonary dysfunction including FVC decline. Furthermore, HFD-BLEO-IPF mice demonstrate clinical relevant Ashcroft histopathological score.
- ALK5i treatment reduced lung weight and total lung HP.
- ALK5i treatment improves pulmonary inspiratory and expiratory function, including FVC.
- ALK5i treatment improves histopathological Ashcroft score.
- ALK5i treatment decreases quantitative histological markers of fibrosis and fibroblast cell activation.

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