Reproducibility and 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 declining pulmonary function. The bleomycin (BLEO)-induced mouse model of pulmonary fibrosis is the most commonly model applied in preclinical drug discovery for IPF. In addition to presenting a robust disease phenotype, it is pertinent that the BLEO-IPF mouse model demonstrates reproducible effects of drug intervention regimens.

Transforming growth factor-beta (TGFβ) is critically involved in IPF pathogenesis. We therefore profiled therapeutic outcomes of TGFβ1R/ALK5 inhibitor (ALK5i) treatment in four independent BLEO-IPF mouse studies.

Methods

BLEO-IPF study design were identical in all four studies (Fig. 1). In brief, male C57BL/6JRj mice (12 weeks old) received a single intratracheal instillation of BLEO (2 mg/kg, 50 µL) or saline (CTRL) on study day 1. BLEO-IPF animals were randomized into study groups based on enhanced pause (PenH) and body weight on day 6 after BLEO administration, followed by 21 days of oral ALK5i (SB525334, 30 mg/kg, BID) treatment. Terminal pulmonary endpoints included spirometry (flexiVent), lung weight, 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	Gender	Number of animals	Treatment	Dose (mg/kg)	Administration route	Dosing Frequency	Dosing volume
1	CTRL	Male	10	**Vehicle**	NA	PO	BID	5
2	BLEO-IPF	Male	16	**Vehicle**	NA	PO	BID	5
3	BLEO-IPF	Male	16	**ALK5i**	30	PO	BID	5

Figure 1. Study outline.

2 Metabolic and biochemical parameters

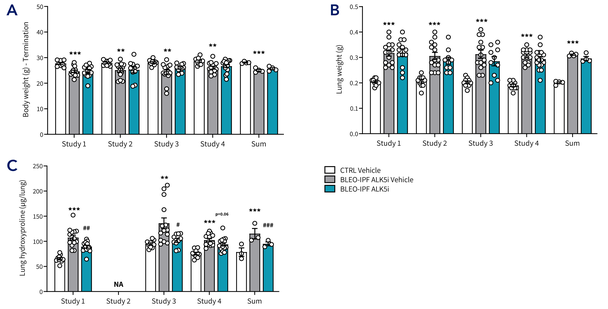

Figure 2. Metabolic and biochemical parameters. (A) Terminal body weight (g). **(B)** Terminal lung weight (g). **(C)** Terminal lung total hydroxyproline (HP) levels. The Sum graph shows group mean value in each individual ALK5i study. Dunnett's test one-factor linear model (study 1-4). Repeated-measure 1-way ANOVA (Sum). **p<0.01, ***p<0.001 (vs. corresponding CTRL Vehicle group), #p<0.05, ##p<0.01, ###p<0.001 (vs. corresponding ALK5i Vehicle group).

3 Pulmonary function

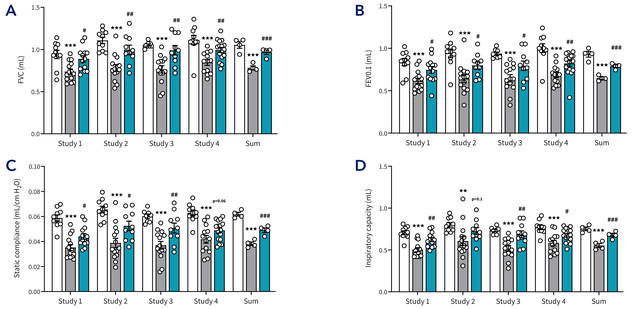

Figure 3. Pulmonary function testing. (A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). The Sum graph shows group mean value in each individual ALK5i study Dunnett's test one-factor linear model (study 1-4). Repeated-measure 1-way ANOVA (Sum). **p<0.01, ***p<0.001 (vs. corresponding CTRL Vehicle group), #p<0.05, ##p<0.01, ###p<0.001 (vs. corresponding ALK5i Vehicle group).

4 Histopathological Ashcroft scoring

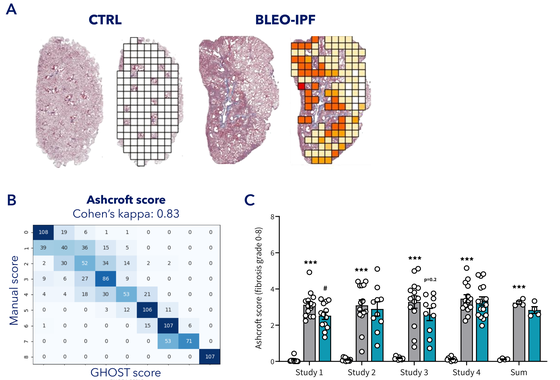

Figure 4. Automated deep learning-assisted Ashcroft scoring of lung fibrosis. (A) GHOST-based Ashcroft scoring applied to the entire left lung in CTRL vs. BLEO-IPF and mice terminated on study day 28. Heatmaps depict Ashcroft score (score 0-8, normal to total fibrous obliteration) in individual lung 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)** GHOST-based Ashcroft scoring of mice included in the present study. The Sum graph shows group mean value in each individual ALK5i study. Dunnett's test one-factor linear model (study 1-4). Repeated-measure 1-way ANOVA (Sum). ***p<0.001 (vs. corresponding CTRL Vehicle group), #p<0.05 (vs. corresponding ALK5i Vehicle group).

5 Histological markers of inflammation, fibrosis, and fibrogenesis

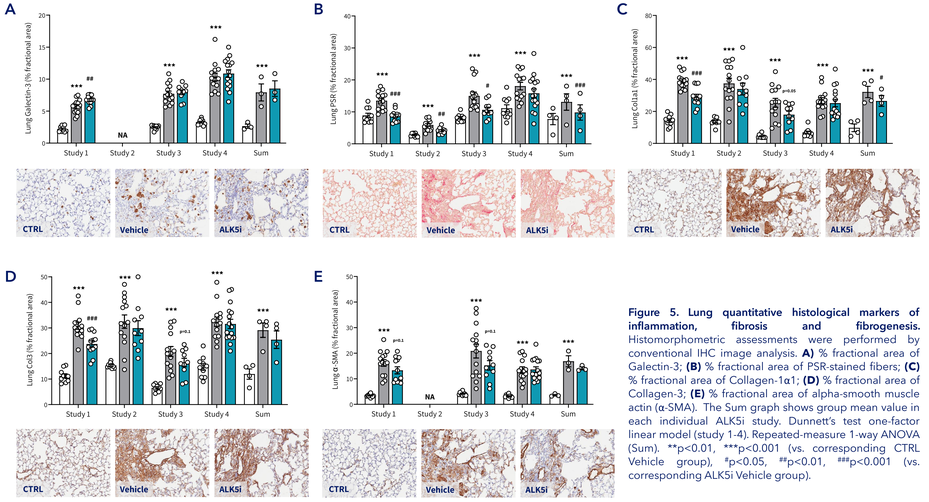

Figure 5. Lung quantitative histological markers of inflammation, fibrosis and fibrogenesis. Histomorphometric assessments were performed by conventional IHC image analysis. **A)** % fractional area of Galectin-3; **(B)** % fractional area of PSR-stained fibers; **(C)** % fractional area of Collagen-1α1; **(D)** % fractional area of Collagen-3; **(E)** % fractional area of alpha-smooth muscle actin (α-SMA). The Sum graph shows group mean value in each individual ALK5i study. Dunnett's test one-factor linear model (study 1-4). Repeated-measure 1-way ANOVA (Sum). **p<0.01, ***p<0.001 (vs. corresponding CTRL Vehicle group), #p<0.05, ##p<0.01, ###p<0.001 (vs. corresponding ALK5i Vehicle group).

Conclusion

- BLEO-IPF mice consistently demonstrate impaired lung function and pulmonary fibrosis
- ALK5i reproducibly improves pulmonary inspiratory and expiratory function
- ALK5i reproducibly lowers total lung HP levels
- ALK5i reproducibly improves quantitative histological markers of fibrosis
- ALK5i has no consistent effect on histopathological Ashcroft score

ALK5i serves an applicable reference compound in BLEO-IPF mouse model studies

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