Lung protective effects of TGFßR1/ALK5 inhibitor 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 two independent BLEO-IPF mouse studies.

Methods

Male C57BL/6JRj mice (12-14 weeks old) received a single intratracheal instillation of BLEO (2 mg/kg, 50 µL) or saline (CTRL, 50 µL) on study day -8. BLEO-IPF animals were randomized into study groups based on body weight (BW) and whole-body plethysmography (WBP) measurements (enhanced pause, PenH) on day -1. Treatment intervention started at day 1, followed by 14 days of ALK5i (SB525334, 30 mg/kg, BID or 60 mg/kg, QD) treatment or vehicle (BID/QD). Control group received vehicle (BID/QD). All dosing per oral (PO) administrations.

Terminal pulmonary endpoints included spirometry (flexiVent), hydroxyproline (HP), quantitative histological markers of fibrosis (PSR, Col1a1, Col3), fibrogenesis (α-SMA), and inflammation (Gal-3), including Ashcroft score using Gubra Histopathological Objective Scoring Technique (GHOST).

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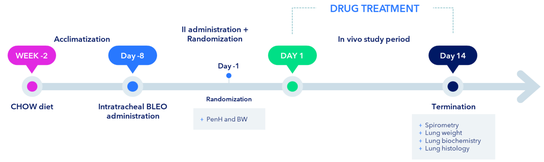
1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Dose (mg/kg)	Administration route	Dosing Frequency	Dosing volume
1	CTRL	Male	10	**Vehicle**	NA	PO	BID or QD	5
2	BLEO-IPF	Male	15/18	**Vehicle**	NA	PO	BID or QD	5
3	BLEO-IPF	Male	15/18	**ALK5i**	30 or 60	PO	BID or QD	5

Figure 1. Study outline and group overview

2 Metabolic and biochemical parameters

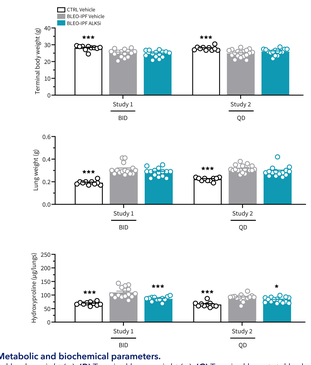


Figure 2. Metabolic and biochemical parameters.
(A) Terminal body weight (g). **(B)** Terminal lung weight (g). **(C)** Terminal lung total hydroxyproline (HP) levels. Data in individual mice according to group and study. *p<0.05, ***p<0.001 vs. BLEO-IPF Vehicle, one-way ANOVA with Dunnett's test for multiple comparisons.

3 Pulmonary function

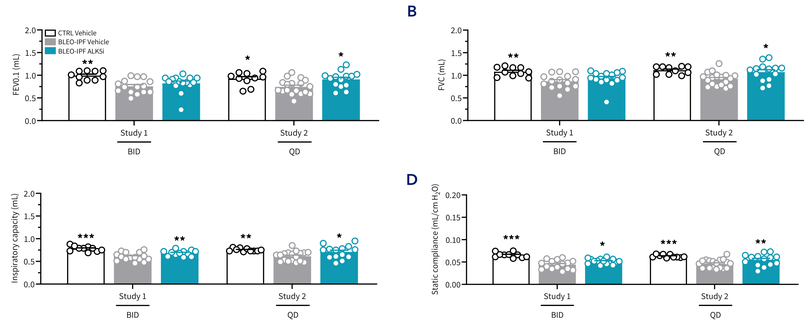


Figure 3. Pulmonary function testing.
(A) Forced expiratory volume in 0.1 seconds (FEV0.1). **(B)** Forced vital capacity (FVC). **(C)** Inspiratory capacity (IC). **(D)** Static compliance. Data in individual mice according to group and study. *p<0.05, **p<0.01, ***p<0.001 vs. BLEO-IPF Vehicle, one-way ANOVA with Dunnett's test for multiple comparisons.

4 Histopathological Ashcroft scoring

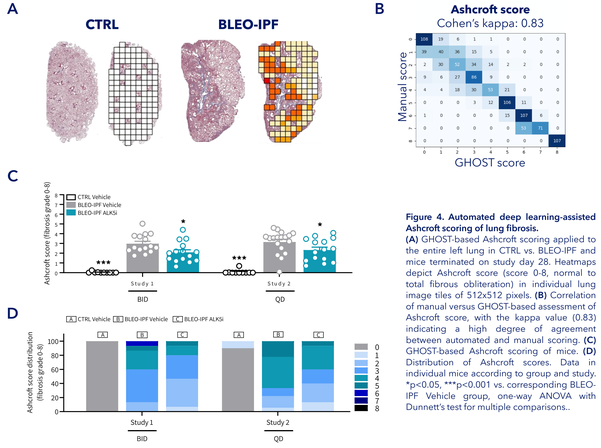
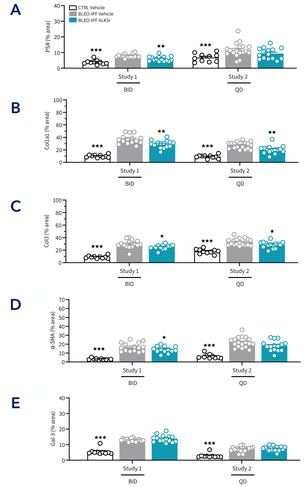


Figure 4. Automated deep learning-assisted Ashcroft scoring of lung fibrosis.
(A) GHOST-based Ashcroft scoring applied to the entire left lung in CTRL vs. BLEO-IPF and mice terminated on study day 28. Heatmaps depict Ashcroft score (score 0-8, normal to total fibrous obliteration) in individual lung 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. **(D)** Distribution of Ashcroft scores. Data in individual mice according to group and study. *p<0.05, ***p<0.001 vs. corresponding BLEO-IPF Vehicle group, one-way ANOVA with Dunnett's test for multiple comparisons..

5 Histological markers of fibrosis, fibrogenesis, and inflammation



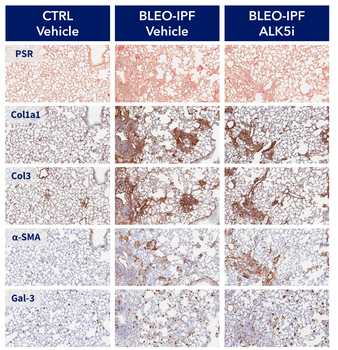


Figure 5. Lung quantitative histological markers of fibrosis, fibrogenesis, and inflammation.
Histomorphometric assessments of fibrosis (PSR, Col1a1, Col3), fibrogenesis (α-SMA) and inflammtion (Gal-3) were performed by conventional image analysis. Data were calculated as proportionate (%) area of histological staining (mean ± SEM). **(A)** % fractional area of PSR-stained fibers; **(B)** % fractional area of Collagen-1α1; **(C)** % fractional area of Collagen-3; **(D)** % fractional area of alpha-smooth muscle actin (α-SMA); **(E)** % fractional area of Galectin-3. Data in individual mice according to group and study. *p<0.05, **p<0.01, ***p<0.001 vs. corresponding BLEO-IPF Vehicle group, one-way ANOVA with Dunnett's test for multiple comparisons. **(F)** Representative photomicrographs of lung histological stainings. Histological staining from selected ALK5i treatment study (study 1) in BLEO-IPF mice.

Conclusion

- BLEO-IPF mouse model demonstrates consistent lung disease phenotype with impaired pulmonary function and lung inflammation and fibrosis
- 14 days of treatment with ALK5i:
  - improves pulmonary inspiratory and expiratory function.
  - reduces total lung HP levels.
  - has a consistent effect on histopathological Ashcroft score.
  - improves quantitative histological markers of fibrosis.

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

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