Comparison of ALK5i treatment efficacy in chow or high-fat diet + bleomycin-induced and spirometry-confirmed mouse models of IPF

Authors
Asbjørn Graver Petersen[1], Jamal Bousamaki[1], Laura Jul Andreasen[1], Denise Oró[1], Jonas Damgård Schmidt[1], Yaohui Nie[2], Jordan Butts[2], Manuel Roqueta-Rivera[2], Henrik H Hansen[1], Michael Feigh[1]

[1]Gubra, Hørsholm, Denmark
[2]Enanta Pharmaceuticals, Massachusetts, United States

Corresponding author
Michael Feigh - **mfe@gubra.dk**

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 most commonly model applied in preclinical drug discovery for IPF. We have recently reported accelerated disease onset in BLEO-IPF mice fed a high-fat diet (HFD) compared to normal diet (CHOW).

Transforming growth factor-beta (TGFβ) is critically involved in IPF pathogenesis. This study aimed to compare therapeutic effects of a TGFβ/ALK5 inhibitor (ALK5i) in CHOW-BLEO-IPF and HFD-BLEO-IPF mice.

METHODS

12 weeks-old male C57BL6/JRJ mice were fed CHOW or HFD (60% kcal of fat) for 2 weeks before receiving a single intratracheal instillation (1.5 mg/kg – 2.25 U/kg) of bleomycin (BLEO) or sterile saline. Mice were kept on the respective diets throughout the study. CHOW-BLEO-IPF and 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 (see figure 1). Untreated CHOW or HFD 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	CHOW-CTRL	10	**Vehicle**	NA	PO	BID
2	CHOW-BLEO-IPF	14	**Vehicle**	NA	PO	BID
3	CHOW-BLEO-IPF	13	**ALK5i**	30 mg/kg	PO	BID
4	HFD-CTRL	10	**Vehicle**	NA	PO	BID
5	HFD-BLEO-IPF	15	**Vehicle**	NA	PO	BID
6	HFD-BLEO-IPF	13	**ALK5i**	30 mg/kg	PO	BID

Figure 1. Study overview. Study outline and groups table for CHOW-BLEO-IPF and HFD-BLEO-IPF.

2 Metabolic and biochemical parameters

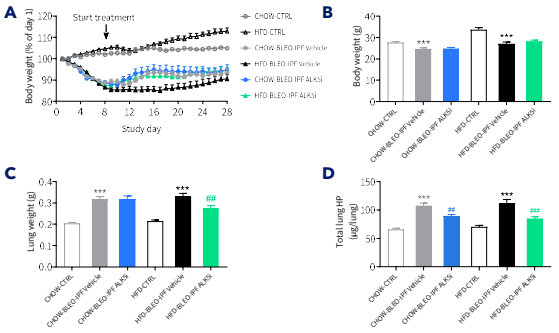

Figure 2. Metabolic and biochemical parameters. (A) Body weight change relative to baseline (% of day 1). **(B)** Terminal body weight (g). **(C)** Terminal lung weight (g). **(D)** Terminal lung total hydroxyproline (HP) levels. Dunnett's test one-factor linear model. ***p<0.001 (vs. corresponding CTRL),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

3 Pulmonary function testing

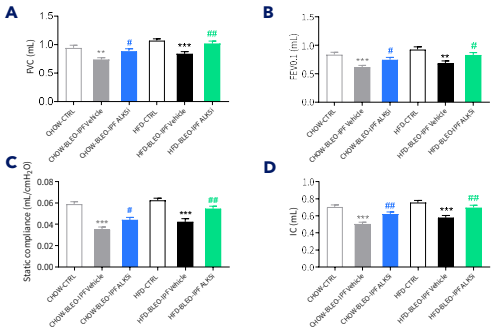

Figure 3. Pulmonary function testing. (A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). Dunnett's test one-factor linear model. **p<0.01, ***p<0.001 (vs. corresponding CTRL), #p<0.05, ##p<0.01 (vs.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

4 Histopathological Ashcroft scoring

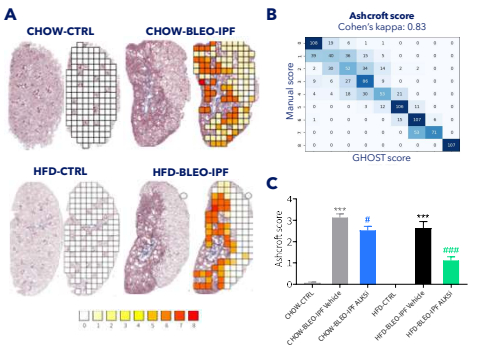

Figure 4. Automated deep learning-assisted Ashcroft scoring of lung fibrosis. Ashcroft score was determined by GHOST deep learning-based image analysis and validated against manual scoring on lung sections stained with Masson´s trichome. **(A)** GHOST-based Ashcroft scoring applied to the entire left lung in CHOW vs. CHOW-BLEO-IPF and HFD-BLEO-IPF 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. Dunnett's test one-factor linear model. ***p<0.001 (vs. corresponding CTRL), *p<0.05, ###p<0.001 (vs. corresponding vehicle group).

5 Histological markers of inflammation and fibrosis

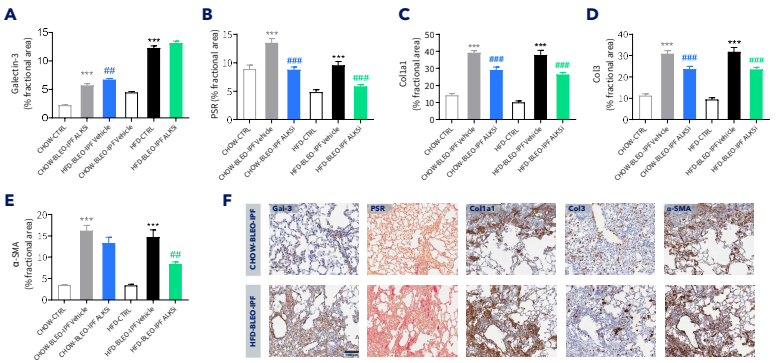

Figure 5. Lung quantitative histological markers of inflammation, fibrosis and fibrogenesis. Histomorphometric assessments were performed by conventional IHC image analysis. **A)** % fractional area of Galectin-3; **(B)** % fractional area of PSR-stained fibers; **(C)** % fractional area of Collagen-1α1; **(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. corresponding CTRL), **p<0.01, ###p<0.001 (vs. corresponding vehicle group). **(F)** Representative photomicrographs of CHOW-BLEO-IPF and HFD-BLEO-IPF vehicle groups (scale bar x 20, 100 µm).

Conclusion

- ALK5i treatment improves total lung HP content in both CHOW-BLEO-IPF and HFD-BLEO-IPF models.
- ALK5i treatment improves pulmonary inspiratory and expiratory function, including FVC, in both CHOW-BLEO-IPF and HFD-BLEO-IPF models.
- ALK5i treatment improves Ashcroft fibrosis score in both CHOW-BLEO-IPF and HFD-BLEO-IPF models, albeit to a greater degree in HFD-BLEO-IPF mice.
- ALK5i treatment reduces quantitative histological markers of fibrosis in both CHOW-BLEO-IPF and HFD-BLEO-IPF models.
- The CHOW-BLEO-IPF and HFD-BLEO-IPF mouse models are highly relevant for efficacy profiling of novel test drugs with potential therapeutic effects in IPF.