Nintedanib does not improve lung function and fibrosis in a spirometry-confirmed and bleomycin-induced 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. Nintedanib is currently one of only two drugs that are clinically approved for treating IPF patients.

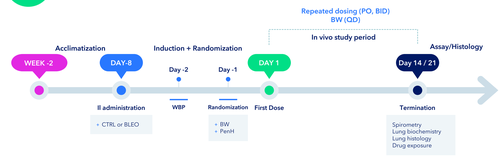
The present study aimed to characterize Nintedanib in a bleomycin-induced (BLEO) and spirometry-confirmed mouse model of IPF.

Methods

Male C57BL/6JRj mice (16-19 weeks old) received a single intratracheal instillation of BLEO (2 mg/kg, 50 µL) or saline (CTRL). BLEO-IPF animals were randomized and stratified to treatment based on whole-body plethysmography (WBP; enhanced pause, PenH) and body weight (BW) on day 6 after BLEO administration. Nintedanib (50 or 60 mg/kg, PO, BID) or vehicle (PO, BID) was administered for 14 days (study 1) or 21 days (study 2 and 3), starting on day 1. The TGFβ receptor inhibitor ALK5 (ALK5i, SB525334, 60 mg/kg, QD for 21 days) was included as comparator drug.

Terminal pulmonary endpoints included spirometry (flexiVent), hydroxyproline (HP), Ashcroft score using Gubra Histopathological Objective Scoring Technique (GHOST), and quantitative histological markers of fibrosis (PSR, MT), and inflammation (Gal-3). Terminal plasma exposure of Nintedanib and lung RNA sequencing was performed in study 2.

1 Study outline



Study	Group	Animal	Gender	Number of animals	Treatment	Route of administration	Dosing frequency	Dose [mg/kg]	Treatment duration (days)
1	1	CTRL	Male	10	**Vehicle**	PO	BID	**NA**	14
	2	BLEO-IPF	Male	18	**Vehicle**	PO	BID	**NA**	
	3	BLEO-IPF	Male	16	**Nintedanib**	PO	BID	**50**	
2	1	CTRL	Male	10	**Vehicle**	PO	BID	**NA**	21
	2	BLEO-IPF	Male	16	**Vehicle**	PO	BID	**NA**	
	3	BLEO-IPF	Male	16	**Nintedanib**	PO	BID	**50**	
3	1	CTRL	Male	10	**Vehicle**	PO	BID	**NA**	21
	2	BLEO-IPF	Male	16	**Vehicle**	PO	BID	**NA**	
	3	BLEO-IPF	Male	15	**Nintedanib**	PO	BID	**60**	
	4	BLEO-IPF	Male	17	**ALK5i**	PO	QD	**60**	

Figure 1. Study outline and group overview

2 Randomization

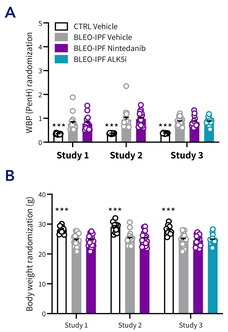


Figure 2. Baseline randomization. (A) WBP (PenH). **(B)** Body weight. One-way ANOVA with Dunnett's test for multiple comparisons. ***p<0.001 vs. BLEO-IPF Vehicle

3 Metabolic and biochemical parameters

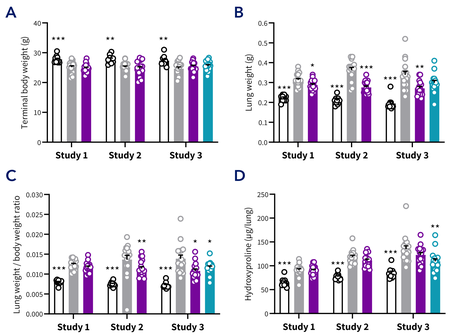


Figure 3. Terminal metabolic and biochemical parameters. (A) Terminal body weight (g). **(B)** Lung weight (g). **(B)** Lung to body weight ratio. **(D)** Lung total hydroxyproline (HP). One-way ANOVA with Dunnett's test for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001 vs. BLEO-IPF Vehicle.

4 Spirometry

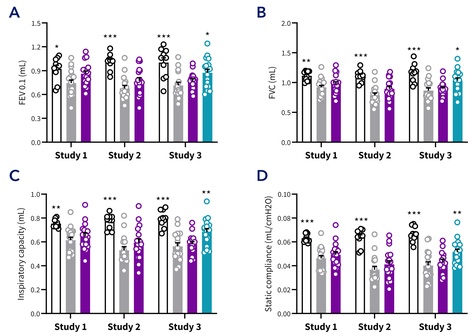


Figure 4. Spirometry. (A) Forced expiratory volume in 0.1 seconds (FEV0.1). **(B)** Forced vital capacity (FVC). **(C)** Inspiratory capacity (IC). **(D)** Static compliance. One-way ANOVA with Dunnett's test for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001 vs. BLEO-IPF Vehicle

5 Histology

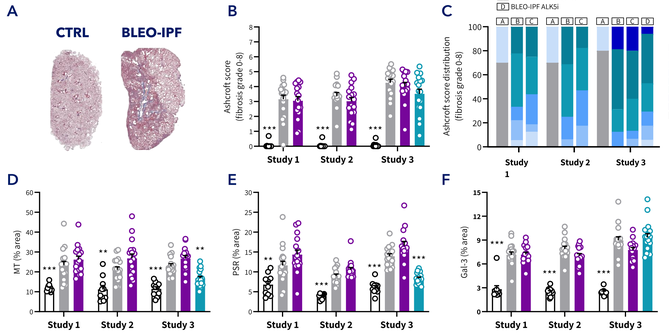


Figure 5. Lung histology. Histomorphometric assessments were performed by conventional image analysis. Quantitative data were calculated as proportionate (%) area of histological staining (mean ± SEM). **(A)** Entire MT-stained left lung in CTRL vs. BLEO-IPF terminated on study day 21. **(B)** GHOST-based Ashcroft score. **(C)** Distribution of Ashcroft scores. **(D)** MT-stained fibers. **(E)** PSR-stained fibers. **(F)** Galectin-3. One-way ANOVA with Dunnett's test for multiple comparisons. **p<0.01, ***p<0.001 vs. corresponding BLEO-IPF Vehicle group.

6 Nintedanib exposure

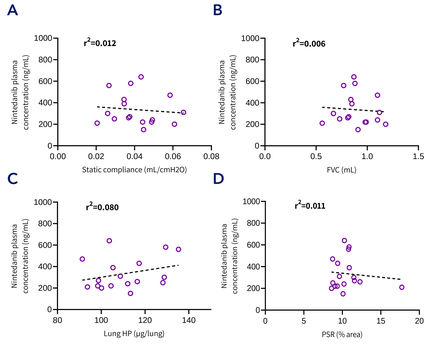


Figure 6. Nintedanib exposure. Terminal plasma concentrations of Nintedanib measured 2.5 hours after last dose vs. **(A)** static compliance, **(B)** FVC, **(C)** total lung HP levels, and **(D)** %-area of PSR staining. Simple linear regression analysis.

7 Lung transcriptome profiling

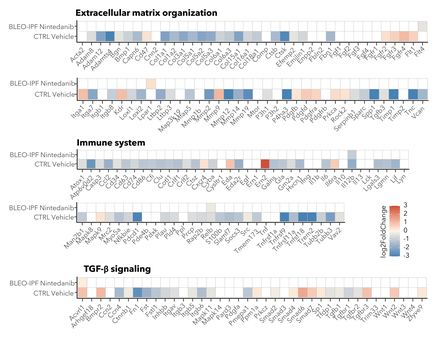


Figure 7. Lung transcriptome signatures. Heatmap on gene candidate markers of Extracellular matrix organization, Immune system and TGF-β signalling (log2-fold change vs. BLEO-IPF Vehicle, p_{adj}<0.05 after correcting for multiple testing. CTRL Vehicle, n=6; BLEO-IPF Vehicle, n=10; BLEO-IPF Nintedanib, n=12). Red and blue colours indicate up- and down-regulation as compared to BLEO-IPF Vehicle. Unregulated genes are indicated in white. Compared to BLEO-IPF Vehicle mice, the total number of differentially expressed genes was 6,294 (CTRL Vehicle mice) and 305 (BLEO-IPF Nintedanib mice), respectively.

Conclusion

- Nintedanib significantly reduces lung weight in BLEO-IPF mice
- Nintedanib has no effect of lung functional outcomes in BLEO-IPF mice
- Nintedanib has no effect on lung HP levels in BLEO-IPF mice
- Nintedanib has no effect on histological and transcriptional markers of fibrosis, fibrogenesis and inflammation in BLEO-IPF mice
- Nintedanib plasma exposure shows no correlation to key endpoints in BLEO-IPF mice
- Nintedanib is not an applicable reference drug in BLEO-IPF mouse studies

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