ORJ-001, a Selective β1 Integrin Agonist Peptide, Has Long Tissue Target Engagement and Lowers Tenascin-C Levels in the Therapeutic Model of Bleomycin-induced Lung Fibrosis.

Oorja Bio

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Introduction

In the therapeutic bleomycin model of pulmonary fibrosis, treatment with ORJ-001, a β1 integrin agonist peptide, reduced established lung fibrosis and repaired the alveolar epithelium. ORJ-001 binds with high selectivity to the primed configuration of β1 integrin in damaged tissues following activation by cytokines and growth factors, with negligible binding to normal tissues. Tenascin-C (TN-C), an extracellular matrix protein expressed during wound healing, correlates to changes in FVC in IPF patients. Surfactant protein-D (SP-D), a marker for alveolar epithelial injury and lung inflammation was also evaluated. This study aimed to characterize ORJ-001 in vivo target engagement and the suitability of TN-C and SP-D as PD biomarkers for ORJ-001.

Conclusions

Target Engagement

- ORJ-001 demonstrated preferential localization and prolonged retention in fibrotic lung tissue following a single SC administration.
- Target engagement in fibrotic tissue persisted up to 7 days post-dose
- These findings support further clinical evaluation of ORJ-001 in fibrotic lung disease.

Biomarker Effects

- ORJ-001 treatment reduced circulating TN-C and SP-D level in the bleomycin induced pulmonary fibrosis model.
- These biomarker findings support the use of TN-C and SP-D as biomarkers of ORJ-001's response in future studies in IPF patients.

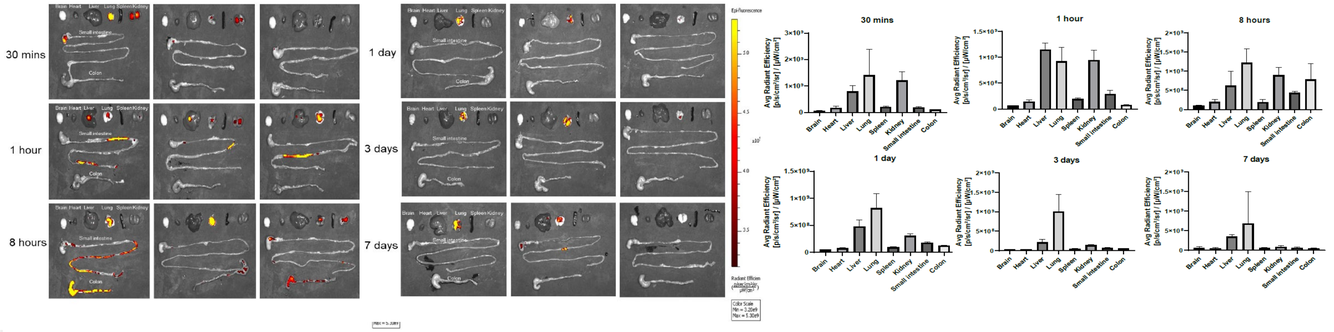
Lung Histology and Ashcroft Score

- ORJ-001 treatment attenuated bleomycin-induced pulmonary fibrosis, with improved preservation of alveolar architecture, reduced septal thickening, and decreased collagen deposition compared with untreated bleomycin controls
- Histology analysis demonstrated consistently lower Ashcroft fibrosis scores and reduced collagen-positive area in ORJ-001 treated mice across all timepoints, supporting the ability of once-weekly SC ORJ-001 to reduce lung fibrosis.

Results

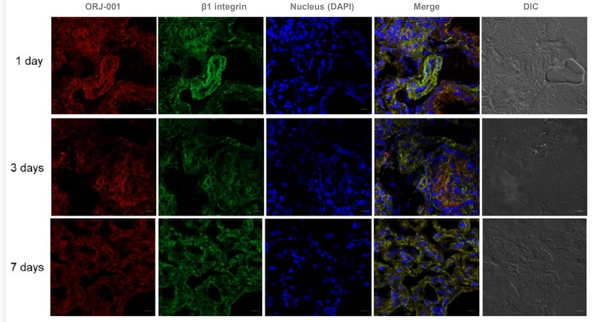
Target Engagement

Ex vivo imaging indicated early distribution to liver, intestine, kidney, and lung (30 min–8 h), shifting to predominantly lung signal from Days 1 - 7.

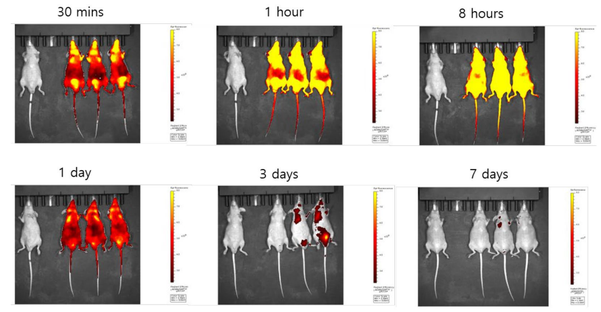


ORJ-001 colocalized with β1 integrin in fibrotic lungs from Days 1–7.

Whole-body fluorescence was seen up to 24 h, then declined by Days 3–7.



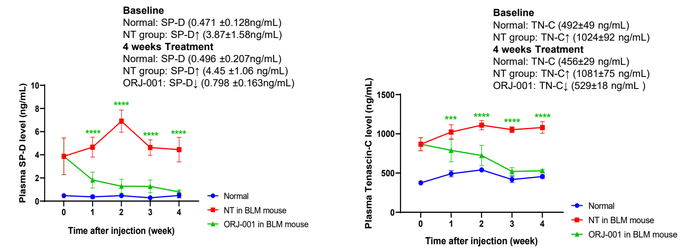
Cy5.5-ORJ-001 (red), β1 integrin (green), DAPI (blue). Extensive yellow overlap in merged panel shows colocalization of ORJ-001 with β1 integrin.



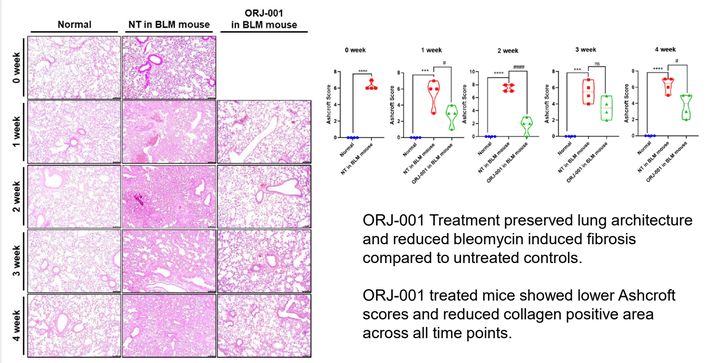
Images shown are from ventral side.

Biomarker Effects

Plasma TN-C and SP-D levels were elevated in the bleomycin-induced group treated with vehicle compared with normal controls at baseline. Treatment with ORJ-001 induced decline of TN-C and SP-D while TN-C and SP-D levels remained unchanged or elevated in the NT groups, respectively.



Lung Histology & Ashcroft Score



ORJ-001 Treatment preserved lung architecture and reduced bleomycin induced fibrosis compared to untreated controls.

ORJ-001 treated mice showed lower Ashcroft scores and reduced collagen positive area across all time points.

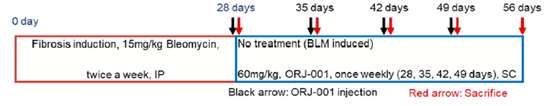
Methods

Target Engagement Model

60mg/kg, Cy5.5 labeled ORJ-001, SC. Sacrifice at 30 min, 1h, 8h, 1 day, 3 days, 7 days post injection

- Lung fibrosis was induced in mice for 28 days with 15 mg/kg bleomycin injected intraperitoneally (IP) twice a week.
- ORJ-001 was labeled with Cy5.5 and administered as single SC dose (60mg/kg) on Day 28.
- Whole-body and organ fluorescence images were acquired using IVIS Lumina III in vivo system
- Tissue sections were stained for β1 integrin, incubated with Alexa Fluor 488-conjugated secondary antibody and counterstained with DAPI.

Model for Biomarkers and Ashcroft Scores



Lung Histology

- At day 28, 35, 42, 49 and 56, mice were euthanized, and the lungs were isolated. Samples were stained with Hematoxylin & Eosin Y (H&E). The fibrotic tissue deposition was observed microscopically with images acquired at 100x magnification.

Ashcroft Scoring

- The Ashcroft score was employed to assess pathological changes in pulmonary fibrosis (PF). Scoring was performed by examining at least five sites per specimen under a microscope.

PD biomarker Assessment

- Mice were treated with saline or ORJ-001 (60mg/kg, SC) on Days 28, 35, 42 and 49 and sacrificed on Day 56.
- Plasma TN-C and SP-D levels were measured using ELISA.