ORJ-001, a novel β1 integrin agonist, reduces fibrosis and induces lung epithelial regeneration in a pulmonary fibrosis model.

Introduction

ORJ-001 (previously known as NP-201) is a novel, synthetic peptide and a potent and highly selective agonist of β1 integrin. ORJ-001 is in clinical development for the treatment of patients with idiopathic pulmonary fibrosis (IPF), a chronic, progressive interstitial lung disease (ILD) with unknown etiology that is characterized by the gradual fibrotic scarring of the lungs. The biological processes that trigger the onset of IPF are still incompletely understood, though repetitive alveolar epithelial injury, in concert with an aberrant regenerative response, are critical underlying events. The ability of injured Alveolar Epithelial Cells (AECs) to affect local fibroblast behavior in a paracrine fashion has been demonstrated in vitro, through the release of cytokines that may either stimulate or suppress fibroblast activity. Recent evidence has emerged to suggest that stimulation, rather than inhibition, of β1 integrin promotes the repair and regeneration of damaged pulmonary epithelium and reverses the fibrotic process associated with IPF.

Objectives:

Characterize ORJ-001, a β1 integrin binding agonistic peptide, and investigate its anti-fibrotic and pro-regenerative effects, to support development for idiopathic pulmonary fibrosis (IPF).

Methods

Drug discovery used in vitro phage-display screening against human collagen types 4 and 7 and β1 integrin, followed by in vivo phage screening in fibrotic mouse lungs. Affinity and specificity to β1 integrin was tested in Biacore assays using human lung LL29 cells. Phage clones were sequenced and those binding to nontarget organs or to healthy lungs were excluded from the collection. Subsequent optimization efforts led to ORJ-001, a fully synthetic, 19 amino acid peptide agonist of β1 integrin.

Anti-fibrotic effect of ORJ-001 was assessed in vitro in TGFβ1 treated IPF lung-derived fibroblasts and bleomycin (BLM)-treated A549 lung epithelial cells. Expression of tight junction proteins (E-cadherin, ZO-1 and Occludin) were used as markers of epithelial regeneration. In vivo anti-fibrotic effects were evaluated in the BLM-induced fibrosis model (mice received intra-tracheal BLM on Day 1 and then treated, from Days 7-21, with ORJ-001 or nintedanib). Cy5.5-labeled ORJ-001 was used to evaluate tissue-binding in BLM-induced fibrosis in mice. Immunofluorescence staining of T1α podoplanin was utilized to demonstrate regeneration of type I AEC and Sirius Red staining was used to quantify fibrotic areas of the lungs.

Results – in vivo **model of IPF**

The scope of nonclinical pharmacology studies was designed to characterize the biological properties of ORJ-001, including the specificity and selectivity of the peptide for the intended target, β1 integrin, and the consequences of β1 integrin activation by ORJ-001 in a therapeutic model of pulmonary fibrosis in mice with bleomycin-induced fibrosis. Nintedanib was used as a comparator in the studies. At the cellular level, treatment of human lung fibroblasts in vitro with ORJ-001 inhibited gene expression of pro-fibrotic factors (Figure 6) and, in epithelial cells, activated signaling pathways involved in cell migration and tissue repair and regeneration (Figure 4). ORJ-001 consistently showed a substantial effect on reversal of fibrosis and on markers of epithelial regeneration in these models, to an extent that was equivalent or superior to the comparator agents.

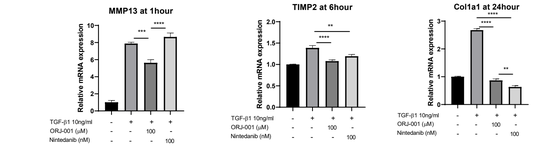


Figure 6: Effect on ORJ-001 on markers of fibrosis in vitro. Diseased Human Lung Fibroblasts, Idiopathic Pulmonary Fibrosis (DHLF-IPD). Treatment with ORJ-001 decreased mRNA expression of MMP13, TIMP2 and Col1a1 within 1 hour, 6 hours, and 24 hours, respectively. **p<0.01; ***p<0.001; ****p<0.00001

Lung fibrosis was established in mice for 1 week by intra-tracheal instillation of bleomycin. On Day 7, treatment was initiated with ORJ-001 at 30, 60, or 90 mg/kg SC twice weekly x 2 weeks or nintedanib 100 mg/kg PO once daily x 2 weeks. Results demonstrate a substantial reduction in fibrotic area and return to near normal lung morphology that was superior to nintedanib treatment (Figures 7a and 7b).

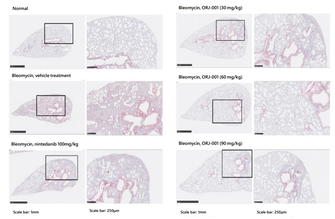
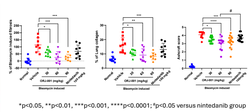


Figure 7a: Images of mouse lung histologic sections (Sirius Red stain). Fibrosis was induced following once daily administration of bleomycin x 7 days and treatment with ORJ-001 or nintedanib were given from Days 7-21. Sirius Red staining highlights fibrotic areas. ORJ-001 consistently showed a substantial effect on reversal of fibrosis.



*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; †p<0.05 versus nintedanib group

Figure 7b: Quantitation of fibrosis from lungs of BLM model, expressed as a percentage of the untreated control group, and the Ashcroft score, evaluated using Sirius Red staining. The percentage of BLM-induced fibrosis correlates with the lung collagen content and ORJ-001 treatment resulted in lower levels of fibrosis compared to the no treatment group, indicating its effectiveness in reducing fibrosis. Animals treated with nintedanib showed a decrease in the level of bleomycin-induced fibrosis compared to the no treatment group, however, this reduction was not statistically significant, suggesting that while nintedanib may reduce fibrosis, its impact in this study was less pronounced than that of ORJ-001.

Results – Alveolar epithelial regeneration

ORJ-001 was superior to nintedanib in promoting alveolar epithelial regeneration based on immunofluorescence and image analysis of T1α podoplanin, a cell surface marker for type I alveolar epithelial cells (Figure 8).

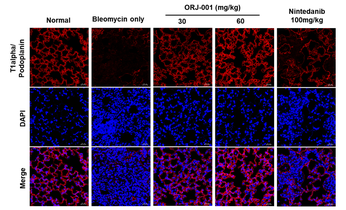


Figure 8: Immunofluorescence staining of type I AEC. ORJ-001 was superior to nintedanib in promoting alveolar epithelial regeneration based on immunofluorescence and image analyses of podoplanin, a cell surface marker for type I alveolar epithelial cells.

In vivo target validation was demonstrated using ORJ-001 labeled with the fluorescent dye, Sulfo-Cyanine 5.5 (Cy5.5) administered (60mg/kg) to mice with established pulmonary fibrosis (bleomycin model), Using IVIS™ Optical Imaging, ORJ-001 was found to localize almost exclusively in fibrotic lungs of mice within 1 hour following administration and lasting for up to 7 days after a single administration of ORJ-001 (Figure 9).

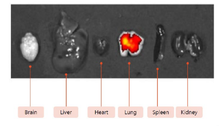


Figure 9: Localization of ORJ-001 in mouse fibrotic lung. ORJ-001 bound preferentially for the fibrotic lung as early as 1-hour post-administration.

Results – Discovery and characterization

Computer-assisted structural modeling revealed that ORJ-001 preferentially binds to β1 integrin when it is associated with an α integrin subunit in the primed configuration (Figure 1). ORJ-001 bound β1 integrin with high specificity and affinity ~1,000-fold higher than to other integrins (Figure 2).It is believed that binding of ORJ-001 to β1 integrin in this manner acts as an agonist and promotes conversion to the active/extended conformation of the integrin heterodimer to trigger the intracellular signal activating the phosphorylation of focal adhesion kinase (FAK), and induction of tight junction proteins.

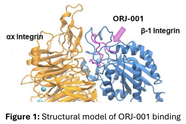


Figure 1: Structural model of ORJ-001 binding to β1 integrin

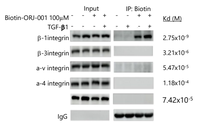


Figure 2: Specificity and affinity of ORJ-001 to β1 integrin

To demonstrate the downstream effects of ORJ-001 signaling though FAK and induction of tight junction proteins to repair the epithelium, A549 human lung epithelial cells were treated with BLM and 100 μM ORJ-001; an increase in phosphorylated FAK and AKT (pFAK and pAKT) levels were observed at 1 hour and then decreased to baseline values without any change in total FAK or AKT protein levels (Figure 3). Within 24h of treatment with ORJ-001, expression of E-Cadherin, ZO-1, and Occludin increased and were sustained at least until 72h post- treatment, indicating long duration of effect after a single treatment (Figure 4).

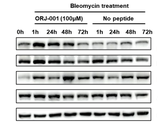


Figure 3: Phosphorylation of FAK and AKT following treatment with ORJ-001

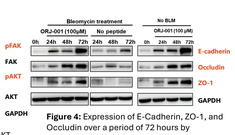
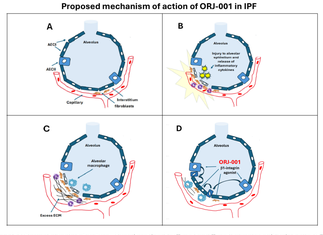


Figure 4: Expression of E-Cadherin, ZO-1, and Occludin over a period of 72 hours by immunoblot analyses

Proposed mechanism of action of ORJ-001 in IPF



Panel A: Normal alveolar structure. Alveoli are lined by Type I and Type II alveolar epithelial cells (AECI and AECII, respectively). AECI comprise ~95% of the alveolar surface and facilitate efficient gas exchange. AECII are cuboidal in shape and comprise ~5% of the alveolar surface. AECII are the source of pulmonary surfactant and play a crucial role in alveolar repair and regeneration, as they can differentiate into AECI when needed.
Panel B: Injury to the alveolar epithelium. Injury to the alveolar epithelium is thought to be the initiating event in the development of IPF. Injured epithelial cells release signal molecules that promote the migration, proliferation, and activation of immune cells and fibroblasts.
Panel C: Uncontrolled extracellular matrix (ECM) deposition and fibrosis formation. Dysregulated tissue repair of the alveolar epithelium leads to uncontrolled, persistent differentiation of fibroblasts into myofibroblasts and excessive deposition of ECM. The abnormal wound healing response results in the irreversible destruction of lung architecture, leading to impaired gas exchange and, eventually, respiratory failure.
Panel D: β1 Integrin drives epithelial cell regeneration. β1 integrin is essential for the differentiation of AECII into AECI and for the normal function of lung fibroblasts to regenerate and repair the alveolar epithelium. ORJ-001 is a highly potent and specific agonist of β1 integrin. ORJ-001 binds to β1 integrin to upregulate Focal Adhesion Kinase (FAK) signaling, which drives the differentiation of AECII into AECI. The regenerated epithelium allows for normal tissue repair processes to take place, including the degradation of ECM by tissue macrophages.

Conclusions

ORJ-001 demonstrated high binding affinity and specificity to β1 integrin, as well as preferential localization to fibrotic tissue.

ORJ-001 effectively inhibited the expression of profibrotic genes and reduced lung fibrosis, while promoting alveolar epithelial regeneration in nonclinical models of IPF.

ORJ-001 could be a potent therapeutic agent for treatment of IPF, having both anti-fibrotic and pro-regenerative benefits.

References

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