Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SMO4646) Delivered as an Inhaled Aerosol for the Treatment of Idiopathic Pulmonary Fibrosis (IPF)

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Rationale

- IPF is a chronic, usually fatal, disease characterized by fibrosis-induced deterioration of lung architecture.
- Wnt signaling is associated with TGF-β-mediated fibrosis, and excess activation of the Wnt-β-catenin pathway is implicated in IPF pathophysiology.
- SMO4646, a novel, small molecule Wnt pathway inhibitor, was evaluated in preclinical studies to assess its ability to inhibit fibrotic activity and attenuate fibrosis.

Methods

Inhibition of Wnt pathway signaling
- SMO4646 was tested in a Wnt-β-catenin promoter-driven reporter assay using a human bronchial epithelial cell line (NL-25).

Antibiotic activity
- Effects on extracellular matrix (ECM) gene expression were evaluated by TaqMan real-time qPCR and normalized to housekeeping gene GAPDH in normal primary human lung fibroblasts (NHFL), a normal lung fibroblast cell line (MRC-5), and an IPF lung fibroblast cell line (L29). Cells were treated for 24 hours with DMSO, or SM04646 or NINT (at the presence of SMO4646, PI3K, or NINT (NINT).

Myofibroblast differentiation was tested in LL29 cells treated with TGF-β (20ng/mL) and treated for 96 hours with SMO4646, PI3K, or NINT. o SMA expression was measured by high content immunofluorescence screening.

Attenuation of fibrosis in a bleomycin-induced pulmonary fibrosis mouse model
- Bleomycin (2U/kg) or PBS was instilled in the lungs of C57BL/6 mice 7 days before initiation of once-daily aerosol dosing for 13 days with vehicle or SMO4646 (0.021 mg/kg or 0.063 mg/kg) (n=12/group) (Ors-Nasal and Respiratory Exposure System; DiscoveRx, San Diego, CA). Lung fibrosis was blindly evaluated by Ashcroft scoring post-mortem. Sections from top, middle, and bottom lung regions were scored from the PBS + air control (n=32). Bleo + vehicle (n=64), Bleo + SMO4646 (0.021 mg/kg) (n=78), and Bleo + SMO4646 (0.063 mg/kg) (n=63) groups. MMP-7 and MMP-3 plasma concentrations were measured by ELISA.

Results

Figure 1. Dose response of SMO4646 in NL-20 cells transfected with a Wnt-β-catenin promoter-driven luciferase reporter. (n=4; experiments, Mean ± SEM).

Figure 2. Expression of ACT2, COL1A1 and FN1 in (a) NHFL, (b) MRC-5, and (c) LL-29 cells treated with TGF-β alone or with SMO4646, PI3K, or NINT for 24 hours. Data presented as relative fold changes to DMSO-treated controls. Scale of Y-axes adjusted to degree of fold-change and to reflect variability of gene expression across all cell lines. (p<0.05, *p<0.01, **p<0.001, ***p<0.0001 vs. TGF-β treatment alone; n=3-4; experiments, Mean ± SEM).

Figure 3. Fibrosis-related marker expression in Myofib chronic lung fibrosis model following SMO4646 treatment indirectly compared to historical data for (a) NIN 10 μM and 3 μM) or (b) PRF (500 μM). (n=3, Mean ± SEM); *BiMAPP=Myofib.

Figure 4. o-SMA protein expression in TGF-β1-stimulated LLC2 cells treated with SMO4646, PI3K, or NINT for 4 days measured by immunofluorescence. (n=3; experiments, Mean ± SEM)

Figure 5. (a) Representative HA staining images of bleomycin-induced pulmonary fibrosis in C57BL/6 mice lungs. (n=12/group) (b) Blinded Ashcroft scoring of fibrosis. (p<0.01; Mean ± SEM) (c) Plasma levels of MMP-7 and MMP-3 from treated mice. (n=12/group, samples ran in duplicate)

Conclusions

- SMO4646 demonstrated dose-dependent inhibition of the Wnt signaling pathway in an in vitro reporter assay.
- SMO4646 showed greater antifibrotic properties in vitro compared to Pi3K and NINT as measured by:
  - Greater inhibition of fibrogenic genes in TGF-β1-stimulated normal and IPF lung fibroblasts.
  - Greater inhibition of α-SMA in IPF lung fibroblasts, an indication of prevention of myofibroblast differentiation.
  - Indirect comparison to historical data showing that SMO4646 demonstrated stronger reduction of VCAM-1 and type-1 and type-III collagen in a primary cell model of fibrosis.
- Aerosolized SMO4646 attenuated pulmonary fibrosis in vivo in the bleomycin mouse model of pulmonary fibrosis.
- SMO4646 is being investigated in a phase 1 study of IPF patients.

References


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