SM0464 Inhibited TGF-β1-stimulated Expression of Fibrotic Genes in Normal and IPF Lung Fibroblasts

**Methods**

**Inhibition of Wnt pathway signaling**
- SM0464 was tested in a Wnt/β-catenin promoter-driven reporter assay using a human bronchial epithelial cell line (N-L25).
- **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 (NHLF), a normal lung fibroblast cell line (MRC-5), and an IPF lung fibroblast cell line (LL29). Cells were treated for 24 hours with DMSO, or TGF-β1 alone or in the presence of SM0464, Pirfenidone, or Nintedanib (NIN).
- **SM0464 was evaluated in a primary fibroblast cell model (BioScreen Biopharm, Myofibroblast differentiation) and indirectly compared to historical Pirfenidone (PIRF) or NIN data.**
- **Myofibroblast differentiation** was tested in LL29 cells stimulated with TGF-β1 (20ng/mL) and treated for 96 hours with SM0464, Pirfenidone, or NIN. α-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 SM0464 (0.021 mg/kg or 0.063 mg/kg) (n=12/group) or (Oro-Nasal and Respiratory Exposure System [CH Technologies]). 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=324), Bleo + SM0464 (0.021 mg/kg) (n=77), and Bleo + SM0464 (0.063 mg/kg) (n=632) groups. MMP-7 and MMP-3 plasma concentrations were measured by ELISA.

**Results**

**SM0464 Inhibited the Wnt Signaling Pathway in NL-20 Cells**

**Figure 2.** Expression of ACTA2, COL1A1 and FN1 in (a) NHLF, (b) MRC-5, and (c) LL-29 cells treated with TGF-β1 alone or with SM0464, Pirfenidone, or NIN 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 cell lines. *(p<0.05, **p<0.01, ***p<0.001; n=3-4 experiments; Mean ± SEM)*

**Figure 3.** Fibrosis-related marker expression in Myofibroblast chronic lung fibroblast model following SM0464 treatment indirectly compared to historical data for α-SMA (n=10 μM and 3 μM) or (b) PRF (500 μM); (n=3, Mean ± SEM) *BioScreen Biopharm, Myofibroblast differentiation.*

**Figure 4.** α-SMA protein expression in TGF-β1-stimulated LL-29 cells treated with SM0464, Pirfenidone, or NIN for 4 days measured by immunofluorescence; (n=3 experiments, Mean ± SEM)

**SM0464 Attenuated Fibrosis in a Bleomycin Model of Pulmonary Fibrosis**

**Figure 5.** (a) Representative H&E staining images of bleomycin-induced pulmonary fibrosis in C57Bl/6 mice lungs, (n=12/group) (b) Blinded Ashcroft scoring post-mortem of fibrosis. *(p<0.01; Mean ± SEM) (c) Plasma levels of MMP-7 and MMP-3 from treated mice. (n=12/group, samples run in duplicate)*

**Conclusions**
- **SM0464 demonstrated dose-dependent inhibition of the Wnt signaling pathway in an in vitro reporter assay.**
- **SM0464 showed greater anti-fibrotic properties in vitro compared to Pirfenidone and Nintedanib as measured by:**
  1. Greater inhibition of fibrotic genes in TGF-β1-stimulated normal and IPF lung fibroblasts.
  2. Greater inhibition of α-SMA in IPF lung fibroblasts, an indication of prevention of myofibroblast differentiation
  3. Indirect comparison to historical data showing 25% reduction in VCAM-1 and type-I and -III collagen in a primary cell model of fibrosis
- **Aerosolized SM0464 attenuated pulmonary fibrosis in vivo in the bleomycin mouse model of pulmonary fibrosis.**
- **SM0464 is being investigated in a phase 1 study of IPF patients.**

**References**


**Disclosures**

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