SM04646 inhibited Wnt pathway gene expression stimulated in response to Transforming Growth Factor-β and was effective in a chronic model of bleomycin-induced pulmonary fibrosis

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Rationale

- The Wnt/β-catenin pathway is associated with Transforming Growth Factor-β (TGF-β) mediated fibrosis, and aberrant activation is implicated in idiopathic pulmonary fibrosis (IPF) pathophysiology.[1]
- TGF-β1 modulates expression of Wnt pathway genes Frizzled-8 (FZD8) and WNT1 inducible signaling pathway protein 1 (WISP1).[2,3] Endoplasmic reticulum (ER) stress can also induce TGF-β mediated fibrosis.[4]
- SM04646, a novel small-molecule Wnt pathway inhibitor in development as an inhaled IPF therapy, inhibited TGF-β1-stimulated fibrotic gene expression *in vitro*, and attenuated pulmonary fibrosis in an acute bleomycin mouse model.[5]
- The ability of SM04646 to inhibit TGF-β-stimulated fibrotic and Wnt pathway gene expression in combination with anti-fibrotics (nintedanib [NIN] and pirfenidone [PIRF]) was investigated.
- A chronic bleomycin-induced pulmonary fibrosis model, more closely resembling IPF than the acute model, was used to investigate SM04646 efficacy and expression of fibrosis and ER stress genes.[6]

Methods

Wnt pathway and fibrotic gene expression

- Normal primary human lung fibroblasts (NHLF) and a normal lung fibroblast cell line (MRC-5) were treated for 24 hours with DMSO (vehicle) or TGF-β1 (10 ng/mL). TGF-β1-stimulated cells were treated with SM04646 (1 μM), PIRF (1600 μM), or NIN (1 μM) alone and in combinations. Effects on extracellular matrix (smooth muscle α-2 actin [*ACTA2*], collagen type 1 α1 [*COL1A1*], and fibronectin 1 [*FN1*]) and Wnt pathway (*FZD8*, *WISP1*) gene expression were evaluated by TaqMan RT-qPCR and normalized to the housekeeping gene GAPDH.

Chronic 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 intermittent "2 weeks on/2 weeks off" aerosol dosing for 16 weeks with vehicle or SM04646 (~0.1 mg/kg QD x 5 days/week during 2-week-on period) (n=12/group) (Oro-Nasal and Respiratory Exposure System [CH Technologies]). Lung fibrosis was blindly evaluated by Ashcroft scoring post-mortem (n=8/group). Mouse lung expression of fibrotic, Wnt and ER stress (heat shock protein 5 [*Hspa5*] and 1 [*Hspb1*]) genes were measured by RT-qPCR.

Results

SM04646 alone or combined with PIRF or NIN inhibited fibrotic gene expression in human lung fibroblasts

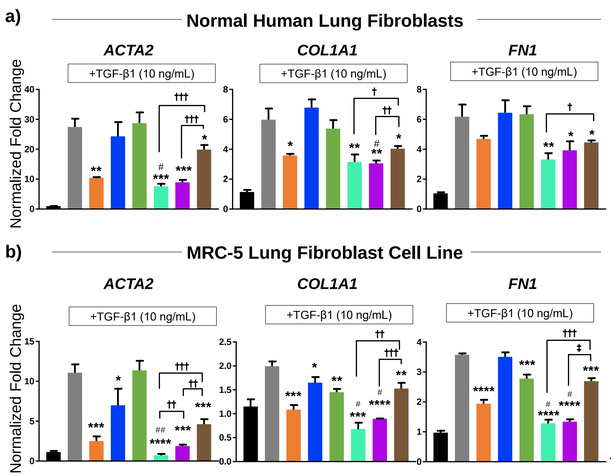

Figure 1. Expression of *ACTA2*, *COL1A1* and *FN1* in **(a)** NHLF, and **(b)** MRC-5 cells treated for 24 hours with monotherapies or combinations as indicated. Fold changes are relative to DMSO control. Y-axes adjusted to fold-change degree. (*p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 vs. DMSO; #p<0.05, ##p<0.01 vs SM04646; †p<0.05, ††p<0.01, †††p<0.001, ‡p<0.0001; n=3-4 experiments; mean ± SEM)

SM04646 alone or combined with PIRF or NIN inhibited TGF-β-stimulated Wnt pathway genes in lung fibroblasts

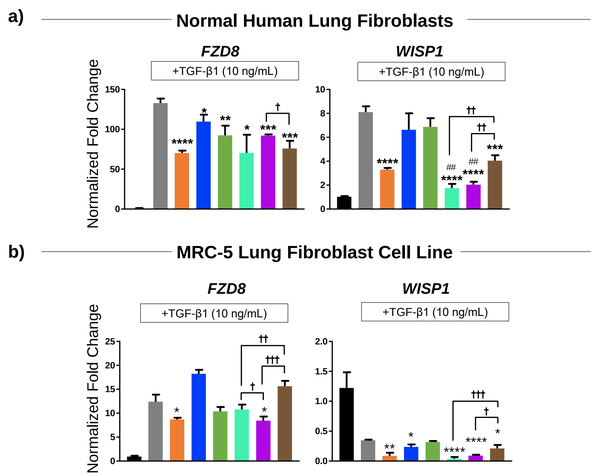

Figure 2. Expression of *FZD8* and *WISP1* in **(a)** NHLF and **(b)** MRC-5 cells treated for 24 hours with monotherapies or combinations as indicated. Fold changes are relative to DMSO control. Y-axes adjusted to fold-change degree. (p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 vs. DMSO; #p<0.05, ##p<0.01 vs SM04646; †p<0.05, ††p<0.01, †††p<0.001; n=3-4 experiments; mean ± SEM)

■ DMSO – TGF-β1 ■ DMSO ■ SM04646 ■ PIRF ■ NIN ■ SM04646 + PIRF ■ SM04646 + NIN ■ PIRF + NIN

SM04646 attenuated bleomycin-induced upregulation of ER stress vs. vehicle and showed trends in reducing fibrotic and Wnt markers

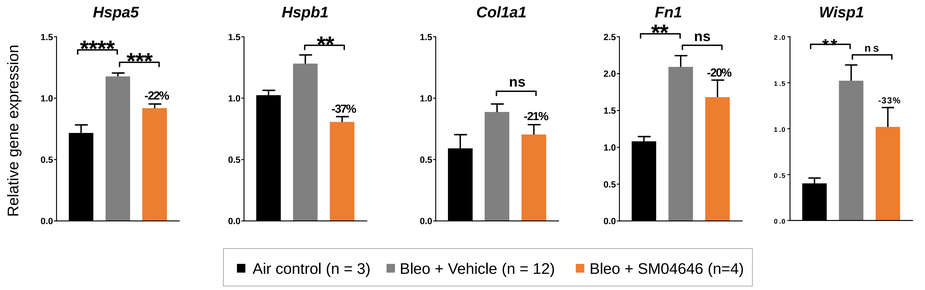

Figure 3. Expression of *Hspa5*, *Hspb1 Col1a1*, *Fn1*, and *Wisp1* in mouse lungs following 16-week chronic bleomycin treatment. Fold changes are relative to 0. Percentage values are degree of decline of SM04646 vs. vehicle. Y-axes adjusted to fold-change degree. (**p<0.01; ***p<0.001; ****p<0.0001; ns = not significant; mean ± SEM)

SM04646 attenuated fibrosis vs. vehicle in a chronic bleomycin model of pulmonary fibrosis

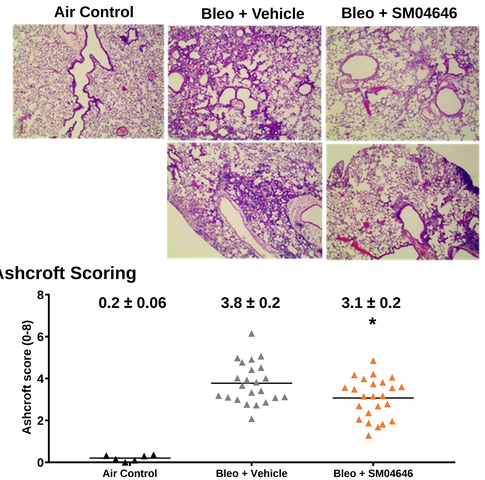

Figure 4. (**a**) Representative H&E staining images of bleomycin-induced pulmonary fibrosis in C57BL/6 mouse lungs (n=6/group) (10x objective) (**b**) Blinded Ashcroft scoring of fibrosis (n=23-24 sections from 8 mice/group) (mean ± SEM; *p=0.015; t-test vs. vehicle)

Conclusions

- SM04646, alone or in combination with pirfinedone or nintedanib, inhibited profibrotic and Wnt pathway gene expression stimulated by TGF-β1, demonstrating potential crosstalk reduction between these pathways.
- Compared with vehicle, SM04646 reduced ER stress markers, showed trends (ns) in reducing fibrotic and Wnt markers, and attenuated pulmonary fibrosis in a chronic bleomycin model.
- These data show potential for SM04646 as a therapy for IPF.
- A phase 1 study of SM04646 in IPF subjects is ongoing.

References

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