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 signaling pathway is associated with Transforming Growth Factor-β (TGF-β)–mediated fibrosis, and aberrant activation is implicated in the pathophysiology of idiopathic pulmonary fibrosis (IPF).1 Expression of Wnt pathway genes such as Frizzled-8 (FZD-8) and Wnt1 inducible signaling pathway protein 1 (WISP-1) are stimulated by TGF-β1.2,3 SM04646, a novel small-molecule Wnt pathway inhibitor being developed as an inhaled treatment for IPF, was shown to inhibit TGF-β-stimulated expression of fibrotic genes.4 The ability of SM04646 to block TGF-β1-stimulated expression of Wnt pathway genes, and its effectiveness in a chronic model of bleomycin-induced pulmonary fibrosis, which resembles IPF more closely than acute models5, were investigated.

Methods. TGF-β1-induced expression of FZD-8, WISP-1 and smooth muscle actin (α-sma) in normal primary human lung fibroblasts (NHLF) were measured by RT-qPCR following treatment with SM04646 or benchmark compounds (pirfenidone and nintedanib) alone or in combination for ~24 hours. A 16-week chronic bleomycin-induced pulmonary fibrosis model was used in which mice (n=12/group) intermittently received aerosolized vehicle, SM04646 (~0.1 mg/kg QD x 5 days/week for 2 weeks, which resumed after a 2-week off-period), or air control (n=5). Blinded histological scoring of lung fibrosis (Ashcroft; n=8), and RT-qPCR measurement (n=4) of Wnt pathway and fibrotic gene expression were performed.

Results: In NHLF cells, TGF-β1 strongly induced gene expression of FZD-8 (>100-fold) and WISP-1 (>5-fold). Treatment with SM04646 (1 μM) significantly inhibited TGF-β1-induced expression of FZD-8 (-55%, p<0.001) and WISP-1 (-74%, p<0.001) compared to TGF-β1-treated cells, whereas pirfenidone (1600 μM) or nintedanib (1 μM) had limited effects. Inhibition of TGF-β1-stimulated α-sma and WISP-1 gene expression by SM04646 (1 μM) combined with pirfenidone (1600 μM) or nintedanib (1 μM) was significantly greater than either SM04646 alone (p<0.05) or pirfenidone plus nintedanib (p<0.05). In the chronic bleomycin-induced fibrosis model, SM04646 significantly decreased Ashcroft scores vs. vehicle (p<0.05). Evaluation of biomarkers demonstrated that, cyclin D1 (p<0.05), fibronectin-1 (p<0.01), WISP-1 (p<0.01), Wnt-5a (p<0.05) and heat shock protein 70 (HSP70) (p<0.001) gene expression were elevated in vehicle-treated lung samples vs. air control. SM04646-treated samples demonstrated a trend in reducing all markers, and significantly reduced expression of HSP70 (-22%, p<0.001) and HSP27 (-37%, p<0.01) vs. vehicle.
CONCLUSIONS: SM04646 ameliorated pulmonary fibrosis induced by chronic bleomycin administration, and showed ability to reduce TGF-β–Wnt crosstalk by inhibiting Wnt pathway genes stimulated in response to TGF-β1. SM04646 demonstrated potential to treat IPF alone or in combination with pirfenidone or nintedanib. A phase 1 study IPF subjects is ongoing.

REFERENCES:

2. Spanjer et al. (2016). FASEB J 5: 1823
4. Tam et al. (2017) ATS Conference: A5139