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### Discovery of a Small Molecule Inhibitor of the Wnt pathway (SM04646) 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 Transforming Growth Factor- $\beta$  (TGF- $\beta$ )–mediated fibrosis, and aberrant activation of the Wnt/ $\beta$ -catenin pathway is implicated in IPF pathophysiology. SM04646, a novel, small molecule Wnt pathway inhibitor, was evaluated in preclinical studies to assess its ability to inhibit fibrotic activity and ameliorate fibrosis.

**METHODS:** To evaluate Wnt inhibition, SM04646 was tested in a Wnt/ $\beta$ -catenin promoter-driven reporter assay using human bronchial epithelial cells (NL-20). The ability of SM04646 and benchmark compounds (pirfenidone, nintedanib) to inhibit TGF- $\beta$ 1-stimulated  $\alpha$ -SMA expression was tested in a high content screening assay (HCS) in an IPF fibroblast cell line (LL29). The effect on TGF- $\beta$ 1-stimulated extracellular matrix (ECM) gene expression was evaluated by qPCR in normal primary human lung fibroblasts (NHLF), a lung fibroblast cell line (MRC-5), and LL29 cells. SM04646 was also tested in BioSeek’s BioMAP-MyoF system, a primary cell model of fibrosis. The bleomycin-induced pulmonary fibrosis model in mice was used to assess efficacy of SM04646 delivered by aerosol inhalation. Mice were treated with vehicle or with SM04646 delivered by aerosol at 0.021 mg/kg or 0.063 mg/kg. Lung fibrosis was blindly evaluated by the Ashcroft scoring system. Plasma was obtained to determine concentration of MMP-7 by ELISA. Lung homogenates were processed to detect cyclin D1 by immunoblotting.

**RESULTS:** In the NL-20 Wnt reporter cell line, SM04646 demonstrated an EC<sub>50</sub>= ~0.4 $\mu$ M, while pirfenidone had minimal effect (EC<sub>50</sub>= >10  $\mu$ M). As summarized in Table 1, SM04646 was also more effective in inhibiting TGF- $\beta$ -induced  $\alpha$ -SMA and ECM gene expression in normal fibroblasts (NHLF and MRC-5) and in LL29 cells compared to both pirfenidone and nintedanib.

Cell Type	% Inhibition of TGF- $\beta$ -stimulated Gene Expression $\pm$ SEM								
	$\alpha$ -Smooth Muscle Actin (ACTA2)			alpha-1 type I Collagen (COL1A1)			Fibronectin-1 (FN1)		
	1 $\mu$ M SM04646	1600 $\mu$ M Pirfenidone	1 $\mu$ M Nintedanib	1 $\mu$ M SM04646	1600 $\mu$ M Pirfenidone	1 $\mu$ M Nintedanib	1 $\mu$ M SM04646	1600 $\mu$ M Pirfenidone	1 $\mu$ M Nintedanib
Human Lung Fibroblasts (Normal)	73 $\pm$ 5 <sup>1,2,3</sup>	30 $\pm$ 3 <sup>1</sup>	1 $\pm$ 12	56 $\pm$ 8 <sup>1,2,3</sup>	25 $\pm$ 4 <sup>1</sup>	22 $\pm$ 8 <sup>1</sup>	46 $\pm$ 8 <sup>1,2,3</sup>	11 $\pm$ 4	17 $\pm$ 8 <sup>1</sup>
MRC-5 Lung Fibroblast Cell Line (Normal)	82 $\pm$ 2 <sup>1,2,3</sup>	34 $\pm$ 4	0.1 $\pm$ 2	47 $\pm$ 3 <sup>1,2,3</sup>	-1 $\pm$ 5	21 $\pm$ 2	48 $\pm$ 1 <sup>1,2,3</sup>	3 $\pm$ 3	21 $\pm$ 2
LL29 Lung Fibroblasts (IPF)	83 $\pm$ 2 <sup>1,2,3</sup>	-10 $\pm$ 11 <sup>1</sup>	-47 $\pm$ 44	54 $\pm$ 5 <sup>1,2,3</sup>	-4 $\pm$ 5	-3 $\pm$ 11 <sup>1</sup>	42 $\pm$ 8 <sup>1,2,3</sup>	2 $\pm$ 14	-9 $\pm$ 10 <sup>1</sup>

<sup>1</sup> vs vehicle-TGF- $\beta$ -treated cells, p<0.05

<sup>2</sup> vs pirfenidone-TGF- $\beta$ -treated cells, p<0.05

<sup>3</sup> vs nintedanib-TGF- $\beta$ -treated cells, p<0.05

Table 1. SM04646 Inhibits TGF- $\beta$ -stimulated Gene Expression in Normal and IPF Lung Fibroblasts

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Inhibition of myofibroblast differentiation by SM04646 was confirmed by HCS quantitative fluorescent microscopy, whereby SM04646 significantly inhibited TGF- $\beta$ 1-induced  $\alpha$ -SMA protein expression in LL29 cells ( $EC_{50}=0.082\mu M$ ). When tested in a primary cell model of fibrosis, SM04646 demonstrated a  $\geq 50\%$  reduction of type-I and -III collagen. In the bleomycin-induced fibrosis model, SM04646 (0.063mg/kg) significantly decreased Ashcroft scoring of lung fibrosis compared to vehicle alone ( $p<0.01$ ). SM04646 also mediated a significant reduction of MMP-7 compared to vehicle-treated animals ( $p<0.05$ ). Functional inhibition of Wnt/ $\beta$ -catenin activity was demonstrated by a reduction of cyclin D1 levels in SM04646-treated lungs.

**CONCLUSIONS:** SM04646 is a novel, small molecule, Wnt pathway inhibitor that demonstrates therapeutic potential to treat IPF. A Phase 1 study of SM04646 in subjects with IPF is planned.