Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04755) as a Potential Topical Treatment for Scleroderma

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Background

- Scleroderma is an autoimmune fibrotic disease which can present with skin manifestations amongst other signs and symptoms.[1]
- The Wnt pathway plays an important role in inflammation, skin fibrosis, and vasculopathy, and is upregulated in scleroderma.[2]
- TGF-β and upregulation of the Wnt pathway promote differentiation of fibroblasts into myofibroblasts and secretion of excessive extracellular matrix proteins from myofibroblasts, which drive fibrosis and dermal thickening.[3]
- Samumed is developing SM04755, a potent small molecule Wnt signaling inhibitor, as a potential topical therapeutic for the treatment of scleroderma.
- SM04755 was evaluated in a series of preclinical studies to determine its potential to reduce inflammation, dermal fibrosis, and vasculopathy, thereby improving skin health in scleroderma.

Methods

- To identify small molecule Wnt signaling inhibitors, a small molecule chemical library was screened in a cellular Wnt pathway-based assay using a β-catenin/TCF responsive reporter in SW480 colon cancer cells. Wnt pathway inhibition was further confirmed by qRT-PCR for Wnt target genes (Axin2, TCF4, TCF7, and LEF1) in SW480 cells.
- Anti-inflammatory activity was evaluated by measuring IL-6 and TNF-α secretion using ELISA in lipopolysaccharides (LPS)-stimulated THP-1 monocytes and anti-CD3/anti-CD28-stimulated peripheral blood mononuclear cells (PBMCs).
- Effects on fibrosis were assessed in TGF-β-stimulated human dermal fibroblasts by measuring smooth muscle actin (αSMA), plasminogen activator inhibitor (PAI-1), connective tissue growth factor (CTGF), and collagen expression by qRT-PCR. The effect on myofibroblast differentiation and reversion was measured by immunocytochemistry for αSMA.
- Pharmacokinetics were evaluated following topical application in rats by analysis of compound concentrations in skin and plasma.
- *In vivo* efficacy was evaluated in a subcutaneous bleomycin (50 μg)-induced mouse model of scleroderma[4] by histological measurements of the thickness of the layers of the skin, CD31 immunohistochemistry for vasculopathy, and qRT-PCR based expression of Wnt signaling and fibrotic markers.

Results

SM04755 demonstrated specific and potent inhibition of Wnt signaling

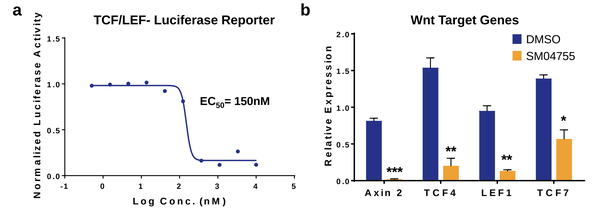


Figure 1. (**a**) Dose response of SM04755 treatment of SW480 cells transduced with the TCF/LEF promoter-driven luciferase reporter. (**b**) Expression of Wnt pathway genes following treatment with SM04755 (1 μM) or DMSO for 24hrs as measured by qRT-PCR. n=3, Mean ± SD, *p<0.05, **p<0.01, ***p<0.001, t-test.

SM04755 inhibited inflammatory cytokine secretion *in vitro*

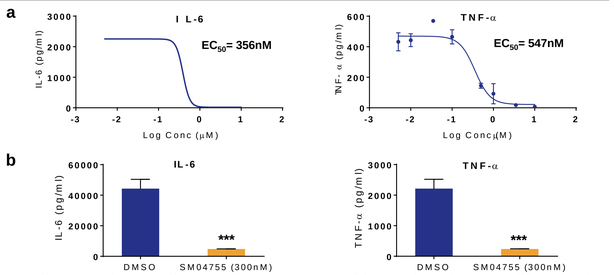


Figure 2. (**a**) Inhibition of IL-6 and TNF-α secretion in THP-1 cells stimulated with LPS and treated with SM04755 for 24hrs. (**b**) Inhibition of IL-6 and TNF-α secretion in human PBMCs stimulated with anti-CD3/anti-CD28 and treated with SM04755 for 24hrs. n=3, Mean ± SD, ***p<0.001, t-test.

SM04755 prevented and reversed fibrosis *in vitro*

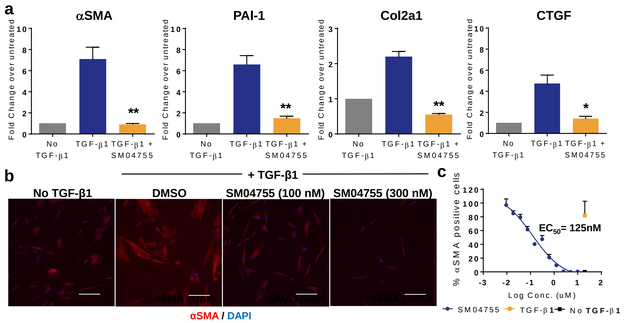
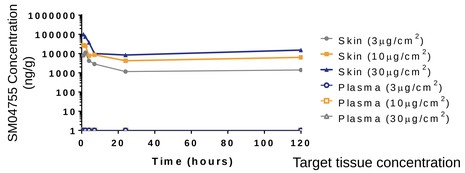


Figure 3. (**a**) HDFα cells treated with TGF-β1 (10 ng/mL) and SM04755 (1 μM) for 48hrs. Gene expression of αSMA, PAI-1, Col2a1, and CTGF measured by qRT-PCR. (**b**) HDFα cells treated with TGF-β1 (10 ng/mL) for 48hrs to induce fibrosis, followed by treatment with various doses of SM04755 for 48hrs. Bars=100 μm. (**c**) Quantification of the number of cells positive for αSMA in (**a**). n=3, Mean ± SEM, *p<0.05, **p<0.01, t-test.

SM04755 had sustained local and minimal systemic exposure



Target tissue concentration 300 ng/g

Figure 4. Pharmacokinetics of SM04755 following a single topical application at various doses.

Results

SM04755 reduced dermal & deep fascia thicknesses and increased adipose tissue thickness in a mouse bleomycin scleroderma model

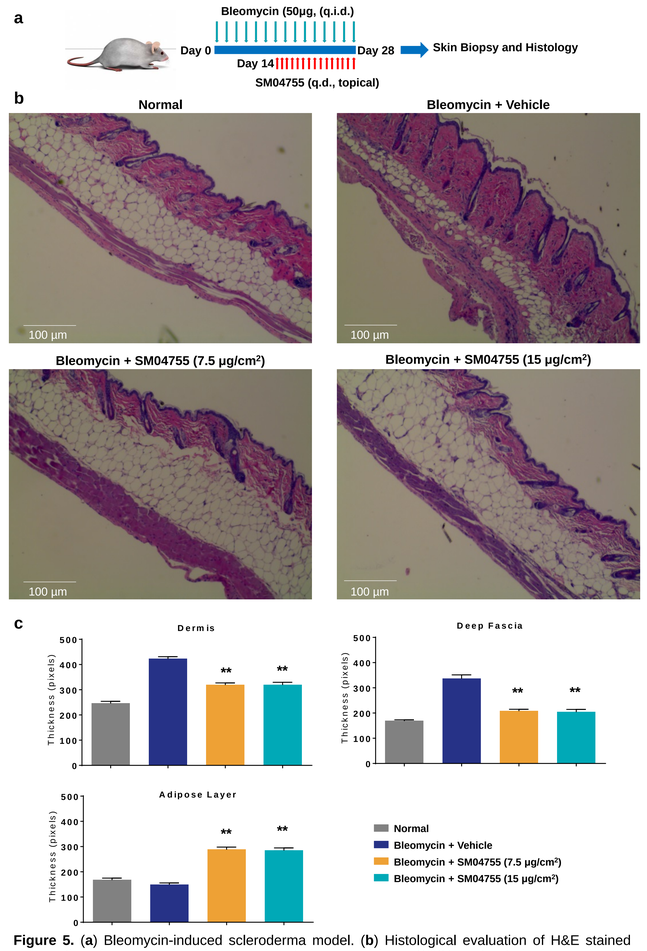


Figure 5. (**a**) Bleomycin-induced scleroderma model. (**b**) Histological evaluation of H&E stained skin sections from normal or bleomycin-injected and vehicle- or SM04755-treated mice on day 28. (**c**) Quantification of the thickness measured in pixels of the layers of skin in (**b**). Mean ± SEM, n=7 mice/group for treatment, 6 mice/group for vehicle, and 3 mice/group for normal, **p<0.01, t-test.

SM04755 reduced vasculopathy in a mouse bleomycin scleroderma model

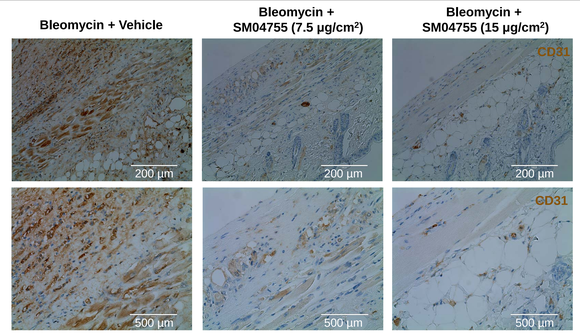


Figure 6. Histological evaluation of endothelium in CD31-stained skin sections from bleomycin-injected and vehicle- or SM04755-treated mice.

SM04755 inhibited Wnt signaling and reversed fibrosis in a mouse bleomycin scleroderma model

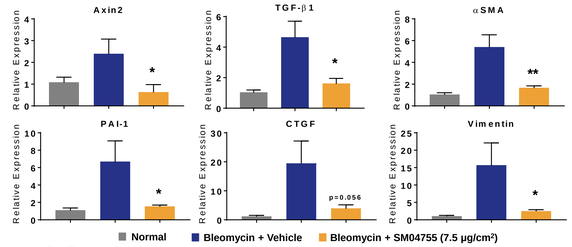


Figure 7. Expression of genes in the Wnt signaling pathway and fibrotic markers as measured by qRT-PCR. Mean ± SEM, n=7 mice/group for treatment, 6 mice/group for vehicle, and 3 mice/group for normal, *p<0.05, **p<0.01, t-test.

Discussion

- SM04755, a potent and specific inhibitor of Wnt signaling, inhibited inflammation and reversed dermal fibrosis *in vitro.*
- In an *in vivo* bleomycin-induced mouse scleroderma model, topically applied SM04755 reversed dermal fibrosis, increased adipose tissue, and reduced vasculopathy compared to vehicle, with minimal exposure in the plasma.
- SM04755 has potential as a topical therapy for scleroderma.
- A Phase 1 trial with healthy volunteers is planned to start in 2016.

References

1. Stern EP and Denton CP. *Rheumatic Disease Clinics of North America.* 2015;41(3):367-382.
2. Beyer C, et al. *Ann Rheum Dis.* 2012;71:761-767.
3. Akhmetshina A, et al. *Nat Comm.* 2012;3:735.
4. Yamamoto T, et al. *Arch Dermatol Res.* 2000;292(11):535-41.

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