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

samumed

Vishal Deshmukh, PhD, Timothy Seo, MS, Maureen Ibanez, MS, Luis Dellamary, PhD, Josh Stewart, BS, John Hood, PhD, and Yusuf Yazici, MD
Samumed, LLC, San Diego, CA

Poster# 1104

Background

- Chronic tendinopathy is an inflammatory, degenerative, and fibrotic condition caused by injuries or overuse. It is characterized clinically by pain, swelling, and impaired performance.[1-3]
- Current therapeutic options focus on alleviating the symptoms and pain rather than treatment of the underlying pathology, therefore presenting an unmet medical need. [4]
- The Wnt pathway plays an important role in tenocyte differentiation. It is upregulated in chronic tendinopathy, in ossified deposits in animal tendons, as well as in clinical tendinopathy samples. Altered Wnt signaling may contribute to tissue metaplasia and failed healing in some cases of tendinopathy.[5]
- Samumed is developing SM04755, a potent small molecule Wnt signaling inhibitor, as a potential topical therapeutic for the treatment of tendinopathy.

Methods

- To identify 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 in SW480 colon cancer cells.
- Effects on fibrosis were assessed in TGF-β-stimulated human dermal fibroblasts (HDFα) by measuring smooth muscle actin (αSMA), plasminogen activator inhibitor (PAI-1), connective tissue growth factor (CTGF), and collagen expression by qRT-PCR.
- *In vitro* and *in vivo* tendon regeneration were evaluated by differentiation of human mesenchymal stem cells (hMSCs) into tenocytes expressing scleraxis A (SCXA), tenomodulin, and tenascin C as measured by high-content imaging and qRT-PCR using rat tendons.
- Pharmacokinetics were evaluated by topical application in rats, followed by analysis of compound concentrations in tendon and plasma by LC-MS.
- *In vivo* efficacy of topical SM04755 was evaluated in single or repeat intra-tendon collagenase injection-induced rodent tendinopathy models by scoring (range 5-20) histological indicators of tendon health.
- *In vivo* inflammation was measured by chemokine ligand 1 (CXCL1) levels in plasma by ELISA and other inflammatory markers (IL-1β, TNF-α, IFN-γ, IL-6 and IL-8) in the tendon by qRT-PCR.

Results

SM04755 demonstrated specific and potent inhibition of Wnt signaling

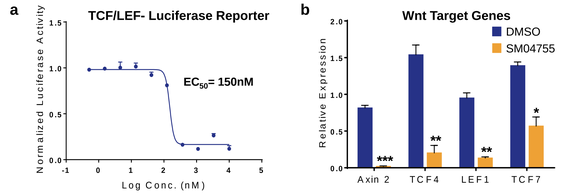

Figure 1. (**a**) Dose response of SM04755 treatment of SW480 cells transduced with the TCF/LEF promoter-driven luciferase reporter. (**b**) Expression of Wnt pathway genes following treatment with SM04755 (1 μM) or DMSO for 24hrs as measured by qRT-PCR. n=3, Mean ± SD, * p<0.05, ** p<0.01, ***p<0.001,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

SM04755 prevented and reversed fibrosis *in vitro*

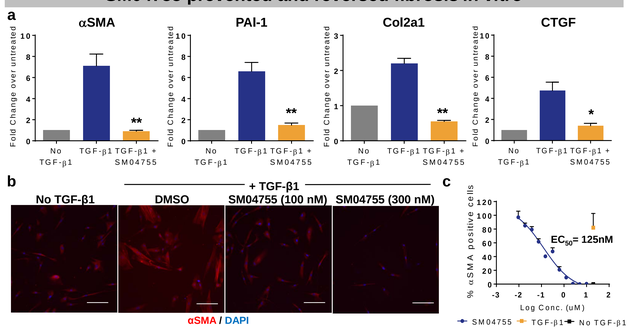

Figure 2. (**a**) HDFα cells treated with TGF-β1 (10 ng/mL) and SM04755 (1 μM) for 48hrs. Gene expression of αSMA, PAI-1, Col2a1, CTGF measured by qRT-PCR. (**b**) HDFα cells treated with TGF-β1 (10 ng/mL) for 48hrs to induce fibrosis, followed by treatment with various doses of SM04755 for 48hrs. Bars=100 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 induced tenocyte differentiation from hMSCs *in vitro*

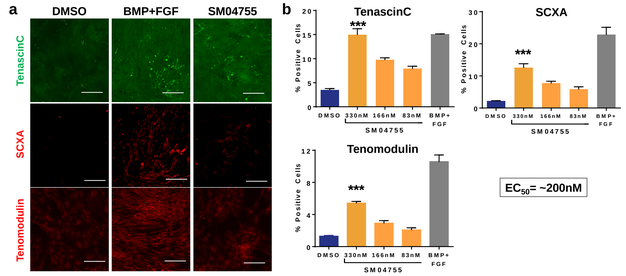

Figure 3. (**a**) hMSCs treated with either DMSO or SM04755 (330nM) for 7 days and stained for tenomodulin, SCXA and tenascin C. BMP-12 + FGF-2 was used as a positive control. Bars=50 μm. (**b**) Quantification of the number of tenocytes in (**a**). n=9, Mean ± SEM, ***p<0.001, t-test

SM04755 demonstrated sustained local and minimal systemic exposure

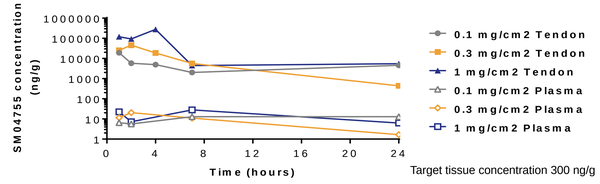

Figure 4. Pharmacokinetics of SM04755 in rat tendon and plasma following a single topical application. Target concentration achieved and retained in the tendon for up to 24hrs with minimal systemic exposure.

SM04755 promoted *in vivo* tendon healing in acute and chronic collagenase-induced tendinopathy models in rats

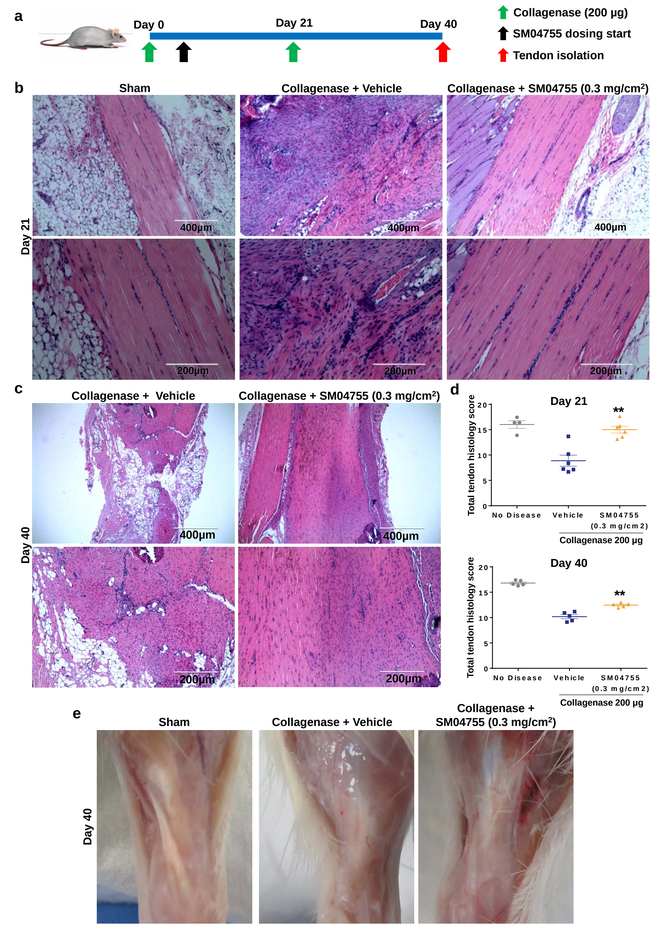

Figure 5. (**a**) Schematic of the collagenase-induced tendinopathy model in rats. (**b, c**) Images of rat tendons stained with H&E from sham or collagenase-injected and vehicle- or SM04755 (0.3 mg/cm^2) treated rats on (**b**) day 21 and (**c**) day 40. (**d**) Histological score of inflammation, linearity and density of tendon fibers, shape of tenocytes and hemorrhage for the rat tendons. Mean ± SEM, day 21: n=4 sham, n=6 vehicle & SM04755; day 40: n=5, **p<0.01, t-test. (**e**) Images of rat tendons on day 40.

SM04755 promoted *in vivo* tendon regeneration in the acute collagenase-induced tendinopathy model in rats

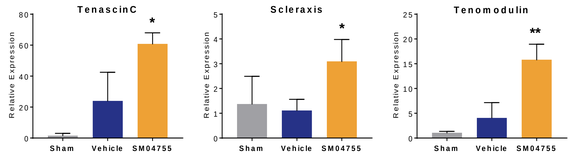

Figure 6. Expression of tenocyte markers in the tendon following sham or collagenase injection and treatment with either vehicle or SM04755 (0.3 mg/cm^2) for 21 days as measured by qRT-PCR. Fold change relative to sham control is shown. n=6, Mean ± SEM, * p<0.05, ** p<0.01, t-test.

SM04755 inhibited *in vivo* inflammation in the acute collagenase-induced tendinopathy model in rats

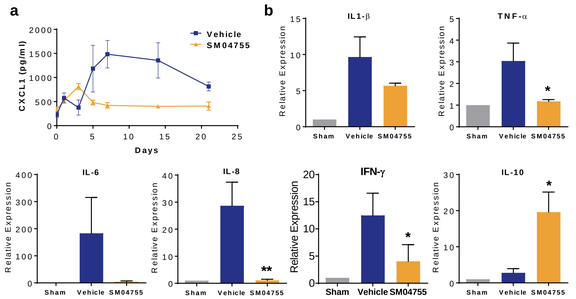

Figure 7. (**a**) Levels of circulating CXCL1 in peripheral blood following treatment as measured by ELISA. (**b**) Expression of inflammatory genes in the tendon following sham or collagenase injection and treatment with either vehicle or SM04755 (0.3 mg/cm^2) for 21 days as measured by qRT-PCR. Fold change relative to sham control is shown. n=6, Mean ± SEM, * p<0.05, ** p<0.01, t-test.

Discussion

- Topical SM04755 reduced markers of tendon inflammation and an inflammatory marker (CXCL1) in plasma, showed evidence of tendon regeneration, and increased tendon health scores compared to vehicle in a rodent tendinopathy model.
- Plasma and systemic exposure were minimal in rats.
- SM04755 demonstrates potential to promote tendon healing in chronic tendinopathy.
- A Phase 1 trial with healthy volunteers is planned to start in 2016.

References

1. Khan KM, et al. *BMJ*. 2002;324:2.
2. Kountouris A and Cook J. *Pract Res Clin Rheumatol*. 2007;21(2):295-316.
3. Maffulli N, et al. *Arthroscopy: The J of Arthro and Rel Surg*. 1998;14(8):4.
4. Rees JD, et al. *Br J Sports Med*. 2014;48(21):1553-1557.
5. Lui PP, et al. *Rheumatology (Oxford)*. 2013;52(9):1609-1618.

9381 Judicial Drive ● San Diego, CA 92121 ● Email: info@samumed.com