Accepted as poster #THU0056 at the Annual European Congress of Rheumatology 2020 for the European League Against Rheumatism (EULAR), June 3–6, 2020

SM04755, a Potential Disease-Modifying Treatment for Tendinopathy, Modulates the Wnt Pathway via Inhibition of CLKs and DYRK1A

Vishal Deshmukh, PhD, Alyssa Lauren O'Green, MS, Timothy Seo, MS, Yusuf Yazici, MD

Samumed, LLC, San Diego, CA

Background: Tendinopathy is an inflammatory and degenerative disorder of tendons caused by injuries and/or overuse. Left untreated, tendinopathy can lead to pain and rupture. Current therapeutic options only treat symptoms. Stem cell- and growth factor-based treatments are under investigation but have not established safety or efficacy, leaving considerable unmet need. The Wnt pathway is upregulated in chronic tendinopathy, affecting inflammation and tenocyte differentiation. SM04755, a novel, topical, small-molecule Wnt pathway inhibitor, has previously been shown to inhibit inflammation, protect tenocytes, and increase tenocyte differentiation in nonclinical models.[1]

Objectives: To identify molecular targets of SM04755 and its associated mechanism of action.

Methods: Wnt pathway inhibition was measured using a cell-based luciferase reporter assay controlled by a β-catenin/TCF-responsive promoter in SW480 colon cancer cells. A kinome screen (318 kinases) and kinase assays were performed. Effects of SM04755 on phosphorylation of proteins, including serine/arginine-rich splicing factor (SRSF) proteins in rat tendon-derived stem cells (rTDSCs) and peripheral blood mononuclear cells (PBMCs), were measured using western blot. SiRNA-mediated knockdown of CDC-like kinases (CLKs) and dual-specificity tyrosine kinase (DYRK1A) were performed in human mesenchymal stem cells, rTDSCs, and rat tenocytes. Effects of SM04755 and siRNA knockdowns on Wnt pathway gene expression and catabolic enzymes (MMPs) were measured using qPCR. SM04755 and siRNA effects on tenocyte marker expression were assessed by qPCR and immunostaining. Effects of SM04755 on LPS-induced expression of inflammatory cytokines in PBMCs were measured by MSD-based ELISA. Statistical analyses used one-way ANOVA for multiple group comparisons and t-tests for comparison between 2 groups.

Results: SM04755 was a potent inhibitor (EC_{50}=156 nM) of Wnt signaling. Biochemical assays identified CLKs and DYRK1A as molecular targets of SM04755. SM04755 potently inhibited CLK-mediated phosphorylation of SRSF proteins compared with DMSO controls. Knockdowns of CLKs and DYRK1A led to inhibition of Wnt pathway genes (*AXIN2*, *LEF1*, *TCF4*, *TCF7*, etc.) compared with siRNA controls (siCtrl). CLK1, 2, and 4 and DYRK1A knockdowns also induced expression of tenocyte markers in rTDSCs and inhibited IL-1β-induced expression of catabolic enzymes (MMP1, 3, 9, 13) in tenocytes compared with siCtrl. SM04755 treatment of LPS-stimulated PBMCs resulted in reduced phosphorylation of NF-κB and STAT3 and inhibited production of inflammatory cytokines compared with DMSO.

Conclusion: SM04755 inhibited CLKs and DYRK1A, which led to Wnt pathway modulation. Knockdowns of CLKs and DYRK1A, compared with control siRNA, induced tenocyte

differentiation and reduced tendon-destroying proteases in tenocytes. This supports the potential disease modification of tendinopathy with SM04755. Furthermore, the anti-inflammatory effects of SM04755 are mechanistically supported by the decreased phosphorylation of STAT3 and NF-κB. These data support that SM04755, as a single agent, may potentially improve symptoms and provide disease modification in tendinopathy. Human tendinopathy trials are planned.

References: 1. Deshmukh V, et al. *Arthritis and Rheum*. 2016.