Anti-inflammatory Properties of SM04690, a Small Molecule Inhibitor of the Wnt Pathway as a Potential Treatment for Knee Osteoarthritis

Vishal Deshmukh, PhD, Charlene Barroga, PhD, Maureen Ibanez, MS, Timothy Seo, MS, Sunil KC, PhD, Luis Dellamary, BS, Josh Stewart, BS, and Yusuf Yazici, MD
Samumed, LLC, San Diego, CA

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Background

• Knee osteoarthritis (OA) is characterized by destruction of articular cartilage, subchondral bone alterations, synovitis, and inflammation. 1,2
• In addition to its role in tissue repair and regeneration, the Wnt signaling pathway has also been linked to inflammation.3
• Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential OA therapeutic administered as a oral joint injection.
• SM04690 has previously been shown to regenerate and protect cartilage in an animal model of knee OA.4
• SM04690 was evaluated in a series of preclinical studies to determine its potential to inhibit inflammation.

Methods

• Anti-inflammatory activity was evaluated by measuring TNF-α, IL-6 secretion using ELISA and IL-1β and IL-8 by qRT-PCR in synovial fibroblasts stimulated with IL-1β.
• A panel of pro- and anti-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-17A, IL-17F, IFN-γ, PGE2) were evaluated by ELISA, T and B cell proliferation by flow cytometry in PBMCs, and T and B cell co-cultures stimulated with super-antigen (sAg) or lipopolysaccharides (LPS) or IgM, compared to vehicle, immunosuppressant or benchmark steroid (cyclosporin A and prednisolone) using DiscoverX BioMAP® platform.
• The effects of SM04690 on LPS-induced expression and phosphorylation of NFκB in THP-1 cells were evaluated by qPCR and Western Blot.
• In vivo activity of SM04690 was evaluated in a rat monosodium iodoacetate (MIA) injection-induced model of OA, followed by single intra-articular (IA) SM04690 or vehicle injection at day 3. Joint inflammation was evaluated by qPCR measurement of pro-inflammatory markers (TNF-α, IL-18, IL-6). Pain was measured as paw withdrawal threshold using Von Frey apparatus in this 28 day study.

Results

SM04690 inhibited inflammatory cytokine secretion in human synovial fibroblasts stimulated with IL-1β

Figure 1. (a) Inhibition of IL-6 and TNF-α secretion in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs as measured by ELISA. (b) Inhibition of inflammatory cytokine secretion in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs as measured by qRT-PCR. n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

SM04690 inhibited T and B cell inflammatory responses in co-culture systems

Figure 2. (a) In vitro assay schematic. (b, c) Inhibition of pro-inflammatory cytokine secretion by SM04690 in (b) vascular endothelial cells co-cultured with human PBMCs, stimulated with super antigen (sAg) and (c) B cells co-cultured with human PBMCs and stimulated with IgM, as measured using the DiscoverX BioMAP® platform. n=3, Mean ± SEM, **p<0.01, ***p<0.001.

SM04690 inhibited LPS stimulated inflammatory cytokine secretion in human PBMCs

Figure 3. Inhibition of pro-inflammatory cytokine secretion in human PBMCs stimulated with LPS and treated with SM04690 for 24hrs as measured using the DiscoverX BioMAP® platform. n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

SM04690 inhibited inflammatory responses in co-culture systems with comparable to or greater potency than Cyclosporin A and Prednisolone

Figure 4. Comparison of in vitro anti-inflammatory activity of SM04690 with cyclosporin A and prednisolone as performed on the DiscoverX BioMAP® platform using an empirical scale (0-5), with 0=weak activity and 5=highly potent activity. SM04690 demonstrated comparable or better activity than the two standard-of-care drugs across several anti-inflammatory assays.

SM04690 inhibited NFκB phosphorylation and expression in human monocytes stimulated with LPS

Figure 5. (a) In vitro SM04690 treatment specifically inhibited NFκB phosphorylation in LPS stimulated human monocytes. (b) Inhibition of expression of NFκB (RELA and RELB) in LPS stimulated human monocytes treated with SM04690 for 24hrs measured by qRT-PCR, n=3, Mean ± SEM, *p<0.05, **p<0.01.

SM04690 attenuated acute inflammation and reduced pain in the MIA model for rat knee OA

Figure 6. Intra-articular MIA injection-induced OA in treated rats (single IA injection of vehicle or SM04690 [0.3μg]). (a) Representative images of H&E stained section of the knee on Day 11. (b) Pain in the MIA-injected limb measured as paw withdrawal threshold using the Von Frey apparatus (n=10 rats, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001). (c) Gene expression of inflammatory markers in the rat knee on Day 11, measured by qRT-PCR (n=10 rats, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001).

Discussion and Conclusions

• SM04690, a small molecule, previously shown to regenerate and protect cartilage in an OA animal model, demonstrated potent anti-inflammatory activity in various cell types, with inhibition of NFκB signaling in vitro.
• In the MIA model of OA, SM04690 attenuated inflammation and structural damage to the knee and improved pain in treated rats as compared to placebo.
• SM04690 treatment addressed 3 major pathologic processes in OA through increased cartilage regeneration, reduced cartilage breakdown and reduced inflammation.
• SM04690 has potential for the treatment of OA signs and symptoms as a DMOAD.
• Human clinical trials with SM04690 are ongoing.

References