A Small Molecule, SM04690, has Inhibitory Effects on the Wnt Pathway and Inflammation In Vitro, With Potential Implications For the Treatment of Osteoarthritis

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Poster# 2143

SM04690 is a small molecule Wnt pathway inhibitor, with potent anti-inflammatory and anti-proliferative activity in human PBMCs and synovial fibroblasts. It was found to inhibit the Wnt pathway and inflammatory cytokine secretion in vitro, and to be effective in the treatment of various diseases.

**Background**

- Knee osteoarthritis (OA) is characterized by the destruction of articular cartilage, subchondral bone alterations, and varying degrees of synovitis.
- Amongst many cellular processes, inflammation has been associated with OA.
- In addition to the critical role it plays in tissue repair and regeneration, the Wnt signaling pathway has been linked with inflammation and inflammatory diseases.
- Samumed is developing a small molecule inhibitor of the Wnt pathway, SM04690, as a potential OA therapeutic to be administered in the form of a local injection into the affected joint.

SM04690 has previously been shown to regenerate and protect cartilage in an animal model of knee OA.

SM04690 was evaluated in a series of preclinical studies to determine its potential to inhibit inflammation.

**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 SW808 colon cancer cells.
- **Anti-inflammatory activity** was evaluated by measuring TNF-α and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL-1β. In THP-1 monocytic cells stimulated with lipopolysaccharides (LPS), and in peripheral blood mononuclear cells (PBMCs) stimulated with anti-CD3/anti-CD28.
- 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, and PGE2) was 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 LPS or IFN-γ compared to vehicle, immunosuppressant or benchmark steroid (cyclosporin A and prednisolone) using the DiscoverX BioMAP® platform.
- The effect of SM04690 on the LPS-induced expression and phosphorylation of NFκB in THP-1 cells was evaluated by qPCR and Western Blot.

SM04690 demonstrated potent inhibitory effects on Wnt signaling and inflammatory cytokine secretion in vitro.

**Results**

- SM04690 inhibits TLR4 dependent inflammatory cytokine secretion in vitro in human monocytes
- SM04690 inhibits T and B cell inflammatory responses in co-culture systems in vitro
- SM04690 inhibited NfκB phosphorylation and expression in vitro in human PBMCs

**Discussion**

- SM04690, a small molecule Wnt pathway inhibitor, was a potent anti-inflammatory agent in various cell types, with inhibition of NFκB-signaling in vitro.
- These anti-inflammatory properties of SM04690 may provide beneficial effects in the treatment of various diseases.
- Human clinical trials with SM04690 are ongoing.

**References**


Figure 1: Dose response of SM04690 treatment of SW808 cells transduced with the TCF/LEF promoter-driven luciferase reporter. n=4, mean ± SEM.

Figure 2: Inhibition of the Wnt pathway by SM04690 in THP-1 cells stimulated with LPS and treated with SM04690 for 24hrs as measured by ELISA. n=4, mean ± SD.

Figure 3: Inhibition of pro-inflammatory cytokine secretion in human PBMCs stimulated with LPS and treated with SM04690 for 24hrs as measured using the MSD platform. (a) 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.

Figure 4: Inhibition of pro-inflammatory cytokine secretion by SM04690 in (a) vascular endothelial cells co-cultured with human PBMCs, stimulated with super-antigen (sAg) and β; (b) B cells co-cultured with human PBMCs and stimulated with IFN-γ, as measured using the DiscoverX BioMAP® platform. n=3, Mean ± SEM. *p<0.05, **p<0.001.

Figure 5: Inhibition of NFκB phosphorylation in human PBMCs stimulated with LPS and treated with SM04690 for 24hrs as measured by IFN-γ-ELISA. n=3, Mean ± SEM. *p<0.05, **p<0.01.

Figure 6: Comparison of in vitro anti-inflammatory activity of SM04690 with cyclosporin A and prednisolone (administered on the DiscoverX BioMAP® platform using an empirical scale (SLT), with Gwede activity and Schlaght potency study. SM04690 demonstrated comparable or better activity than the two standard-of-care drugs across several anti-inflammatory assays.

Figure 7: (a) Inhibition of IL-6 and TNF-α secretion in human synovial fibroblasts stimulated with LPS and treated with SM04690 for 24hrs as measured by ELISA. Inhibition of inflammatory cytokine secretion in human synovial fibroblasts stimulated with LPS and treated with SM04690 for 24hrs as measured by qPCR. n=3, mean ± SEM. *p<0.05, **p<0.01. ***p<0.001.