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

samumed

Vishal Deshmukh, PhD, Maureen Ibanez, MS, Charlene Barroga, PhD, John Hood, PhD, and Yusuf Yazici, MD
Samumed, LLC, San Diego, CA

Poster# 2143

Background

- Knee osteoarthritis (OA) is characterized by the destruction of articular cartilage, subchondral bone alterations, and varying degrees of synovitis.[1]
- Amongst many cellular processes, inflammation has been associated with OA.[2]
- 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.[3]
- 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.[4]
- 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 SW480 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 monocyte 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 IgM, 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 NFkB in THP-1 cells was evaluated by qPCR and Western Blot.

Results

SM04690 demonstrated specific and potent inhibition of Wnt signaling

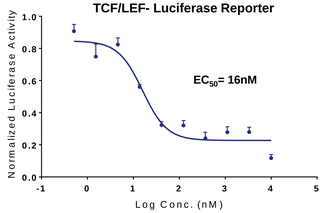

Figure 1. Dose response of SM04690 treatment of SW480 cells transduced with the TCF/LEF promoter-driven luciferase reporter. n=4, Mean ± SEM.

SM04690 inhibited TLR4 dependent inflammatory cytokine secretion *in vitro* in human monocytes

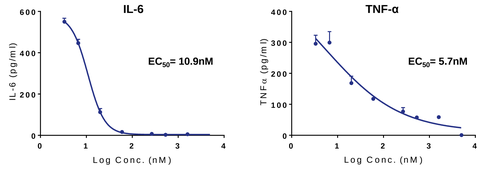

Figure 2. Inhibition of IL-6 and TNF-α secretion in THP-1 cells stimulated with LPS and treated with SM04690 for 24hrs as measured by ELISA. n=4, Mean ± SD.

SM04690 inhibited TLR4 dependent inflammatory cytokine secretion *in vitro* in human PBMCs

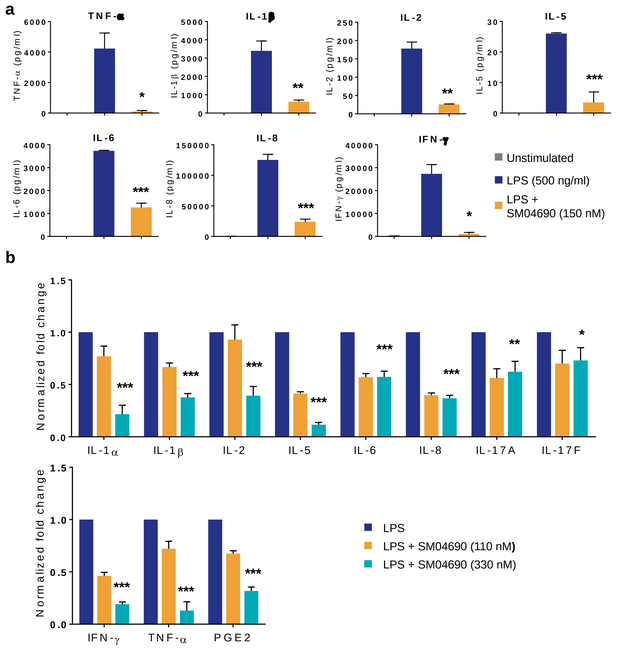

Figure 3. (**a**) Inhibition of pro-inflammatory cytokine secretion in human PBMCs stimulated with LPS and treated with SM04690 for 24hrs as measured using the MSD platform. (**b**) Inhibition of pro-inflammatory cytokine secretion in human PBMCs stimulated with LPS and treated with SM04690 for 24hrs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

Results

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

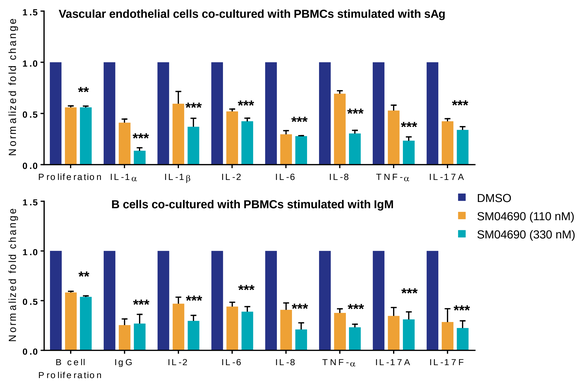

Figure 4. Inhibition of pro-inflammatory cytokine secretion by SM04690 in (**a**) vascular endothelial cells co-cultured with human PBMCs, stimulated with super antigen (sAg) and (**b**) B cells co-cultured with human PBMCs and stimulated with IgM, as measured using the DiscoverX BioMAP® platform. n=3, Mean ± SEM, **p<0.01, ***p<0.001.

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

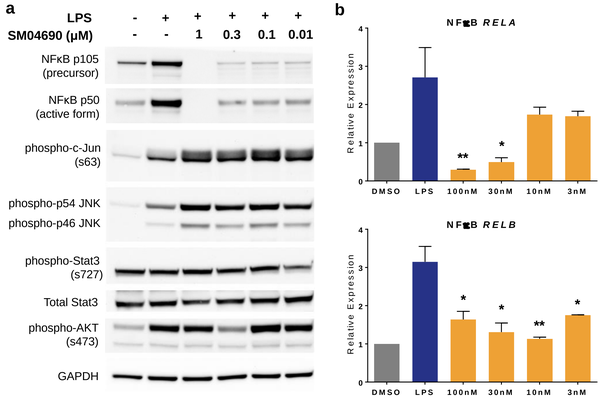

Figure 5. **(a)** SM04690 treatment (4hrs) specifically inhibited NFκB phosphorylation in LPS stimulated human monocytes. **(b)** Inhibition of gene expression of NFκB subunits (*RELA* and *RELB*) in human monocytes stimulated with LPS and treated with SM04690 for 24hrs as measured by qRT-PCR. n=3, Mean ± SEM, *p<0.05, **p<0.01.

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

Compound	Immuno-suppression		Anti-Inflammatory	Th1/Th2/Th17 Inhibition			Cell Cytotoxicity		
	T Cell	B cell		Th17	Th1	Th2	PBMC	HDF	EC
SM04690 (37 nM)	5	3	3	3	3	2	0	0	1
Cyclosporin A (120nM)	2	3	2	2	2	0	0	0	0
Prednisolone (120nM)	0	0	1	1	1	0	0	0	0

5 Highly potent — 0 Weakly active

Figure 6. 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 inflammatory cytokines IL-6 and TNFα secretion *in vitro* in human synovial fibroblasts stimulated with IL-1β

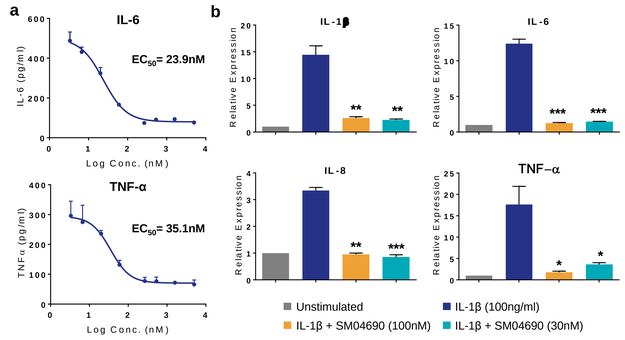

Figure 7. **(a)** Inhibition of IL-6 and TNF-α secretion in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs as measured by ELISA. (**b**) Inhibition of inflammatory cytokine secretion in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs as measured by qRT-PCR. n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Discussion

- SM04690, a small molecule Wnt pathway inhibitor, was a potent anti-inflammatory agent in various cell types, with inhibition of NFkB signaling *in vitro*.
- These anti-inflammatory properties of SM4690 may provide beneficial effects in the treatment of various diseases.
- Human clinical trials with SM04690 are ongoing.

References

1. Hamerman D. *N Engl J Med.* 1989;320(20):1322-30.
2. Sokolove J and Lepus CM. *Ther Adv Musculoskelet Dis.* 2013;5(2):77-94.
3. Sliva-Garcia O, et al. *Mediators Inflamm.* 2014;2014:310183.
4. Barroga C, et al. *Arthritis Rheumatol.* 2015:67(suppl 10).

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