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

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Poster# 489

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 local 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 NFkB 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-1β, 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

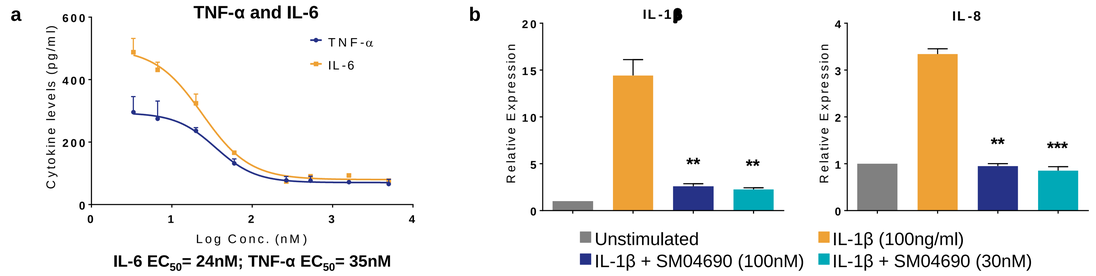

Figure 1. (a) Inhibition of IL-6 and TNF-α secretion in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs as measured by ELISA. (**b**) Inhibition of inflammatory cytokine secretion in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs 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

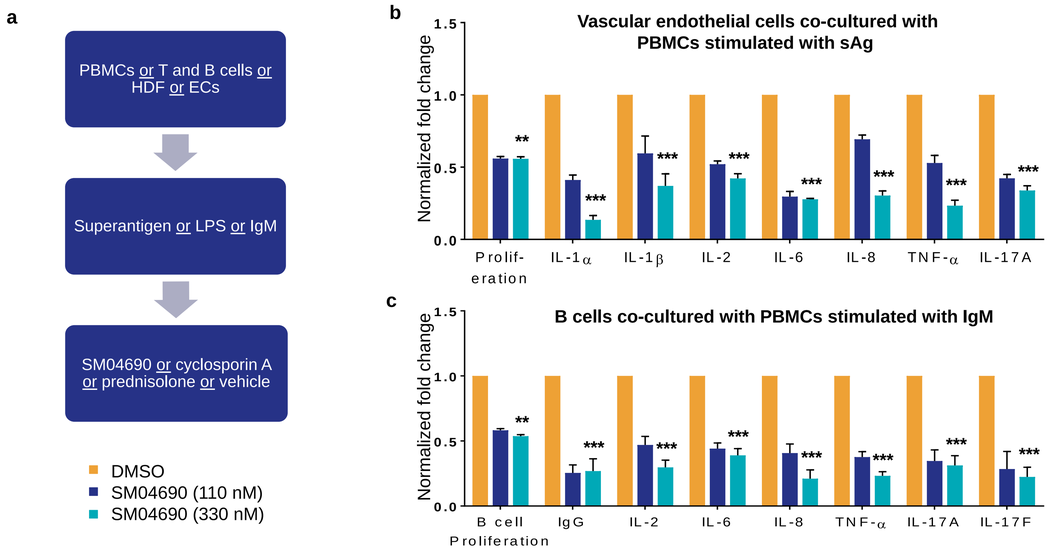

Figure 2. (**a**) *In vitro* assay schematic. (**b, c**) Inhibition of pro-inflammatory cytokine secretion by SM04690 in (**b**) vascular endothelial cells co-cultured with human PBMCs, stimulated with super antigen (sAg) and (**c**) B cells co-cultured with human PBMCs and stimulated with IgM, as measured 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

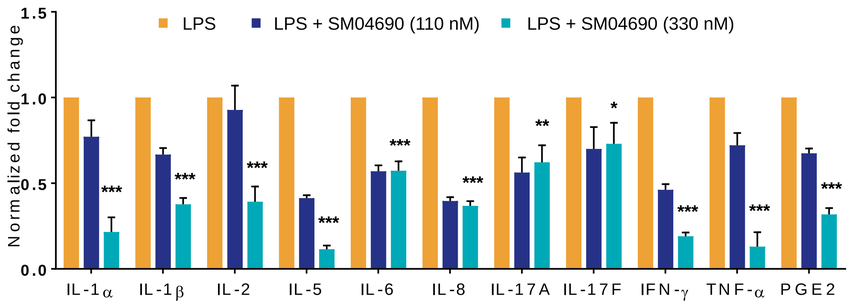

Figure 3. Inhibition of pro-inflammatory cytokine secretion in human PBMCs stimulated with LPS and treated with SM04690 for 24hrs as measured using the DiscoverX BioMAP® platform. n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Results

SM04690 inhibited inflammatory responses in co-culture systems 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 (120 nM)	2	3	2	2	2	0	0	0	0
Prednisolone (120 nM)	0	0	1	1	1	0	0	0	0

5 Highly potent — 0 Weakly active

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

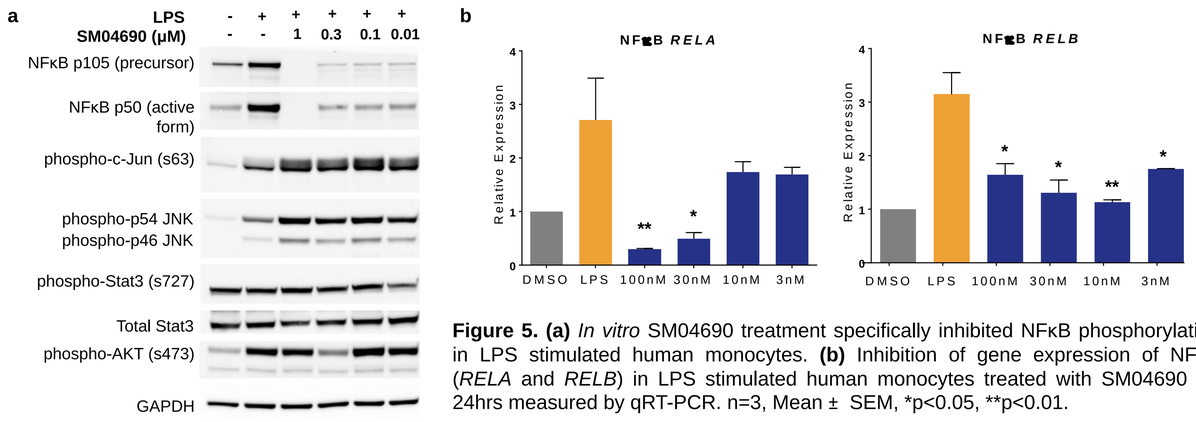

Figure 5. (a) *In vitro* SM04690 treatment specifically inhibited NFκB phosphorylation in LPS stimulated human monocytes. **(b)** Inhibition of gene expression of NFκB (*RELA* and *RELB*) in LPS stimulated human monocytes treated with SM04690 for 24hrs measured by qRT-PCR. n=3, Mean ± SEM, *p<0.05, **p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

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

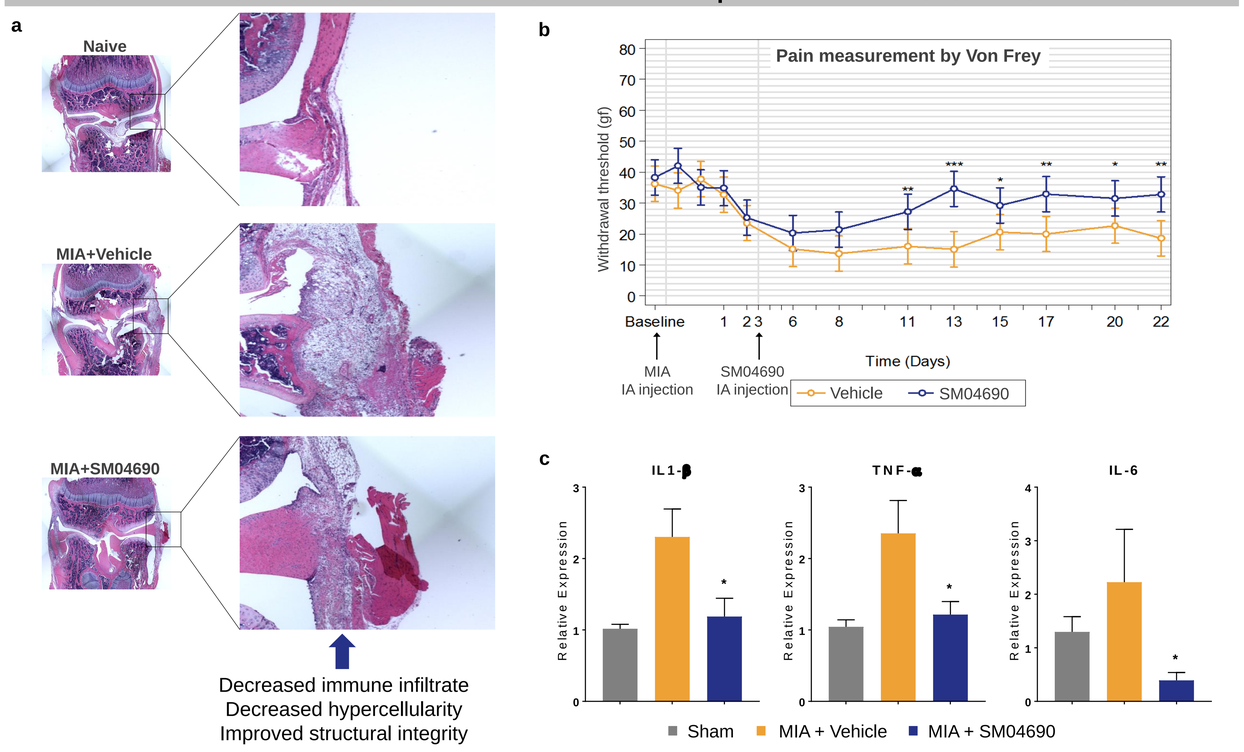

Figure 6. Intra-articular MIA injection-induced OA in treated rats (single IA injection of vehicle or SM04690 [0.3μg]). (**a**) Representative images of H&E stained section of the knee on Day 11. (**b**) Pain in the MIA-injected limb measured as paw withdrawal threshold using the Von Frey apparatus (n=10 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, t-test).

Discussion and Conclusions

- SM04690, a small molecule, previously shown to regenerate and protect cartilage[4] in an OA animal model, demonstrated potent anti-inflammatory activity in various cell types, with inhibition of NFkB 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.
- SM4690 has potential for the treatment of OA signs and symptoms and as a DMOAD.
- Human clinical trials with SM04690 are ongoing.

References

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