Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04690) as a Potential Disease Modifying Treatment for Knee Osteoarthritis

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Background

- Knee osteoarthritis (OA) is characterized by destruction of articular cartilage, subchondral bone alterations, and synovitis.[1]
- At a cellular level, Wnt signaling affects OA pathogenesis in joints by influencing inflammation, cartilage breakdown, and bone / cartilage formation. Increased Wnt signaling induces stem cells to differentiate into osteoblasts, and decreased signaling induces chondrogenesis.[2]
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug (DMOAD) injected into the knee.
- Preclinical studies of SM04690 were conducted to evaluate chondrogenesis, anti-inflammation, cartilage protection, and joint health.

Methods

- Wnt pathway inhibition was measured by qPCR in human mesenchymal stem cells (hMSCs).
- Chondrogenesis was evaluated using hMSCs by qRT-PCR and immunocytochemistry.
- Cytokine induced protease release and glycosaminoglycan (GAG) breakdown in chondrocytes was measured by qRT-PCR and dimethylmethylene blue (DMMB) assay.
- Anti-inflammatory activity was evaluated by measuring TNF-α and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL-1β. Pro-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 in T and B cell co-cultures stimulated with superantigen or LPS, compared to vehicle or two benchmark immunosuppressants (cyclosporin A and prednisolone).
- Pharmacokinetics of SM04690 in plasma and joint were evaluated following intra-articular (IA) injection in rats.
- *In vivo* activity of SM04690 was evaluated in a rat model: anterior cruciate ligament transection with medial meniscal tear (ACLT+pMMx) using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in knee and plasma by qPCR and ELISA.

Results

SM04690 inhibited Wnt signaling and induced chondrocyte differentiation in hMSCs *in vitro*

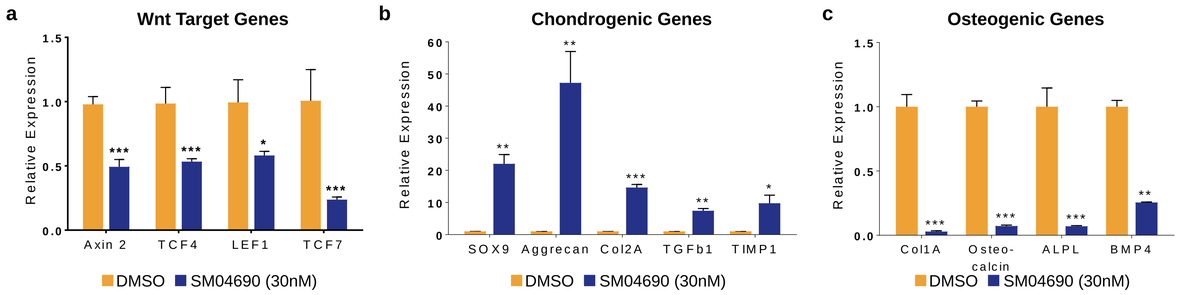


Figure 1. hMSCs treated for 21 days with DMSO or SM04690 (30nM). (**a**) qRT-PCR Wnt pathway gene expression (**b, c**) Gene expression of (**b**) mature chondrocyte markers and (**c**) osteocyte and tendon/ligament markers (qRT-PCR). Fold change relative to DMSO control is shown. (**d**) Staining for various markers for mature chondrocytes (scale bars, 200 μm). (**e**) Quantification of total sulphated GAG levels relative to cell weight aggregates (DMMB assay). (n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

SM04690 protected chondrocyte from catabolic breakdown *in vitro*

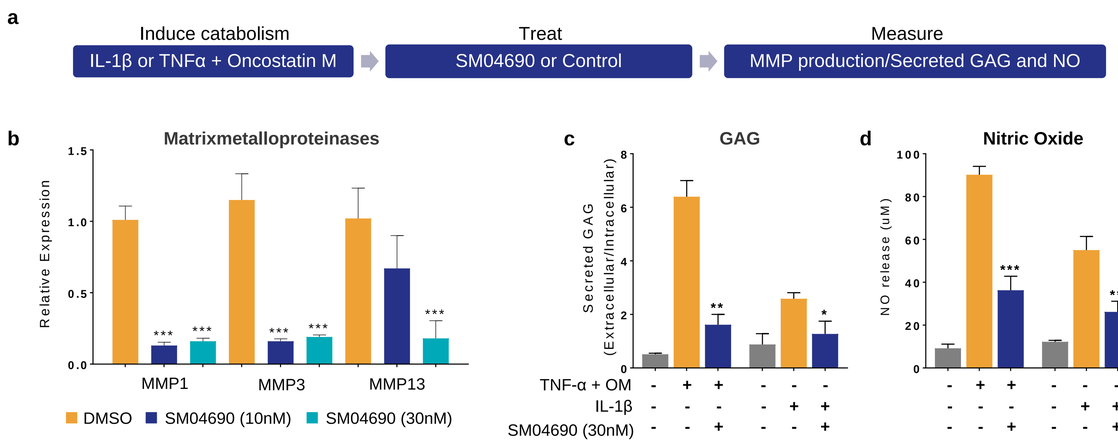


Figure 2. (**a**) Schematic of chondrocytes treated with cytokines TNF-α (20ng/ml) + Oncostatin M (10ng/ml) or IL-1β (10ng/ml) and SM04690 (30nM) or vehicle for 72hrs. (**b**) Gene expression of proteases (MMP1, MMP3, MMP13, IHH), measured by qRT-PCR. (**c**) Levels of secreted GAG expressed as ratio of intracellular GAG measured by DMMB assay. (n=3, Mean ± SEM) (**d**) Levels of secreted Nitric Oxide measured using Griess reagent assay. (n=6, Mean ± SD, *p<0.05, **p<0.01, ***p<0.001)

Results

SM04690 regenerated cartilage in the ACLT+pMMx model of rat OA

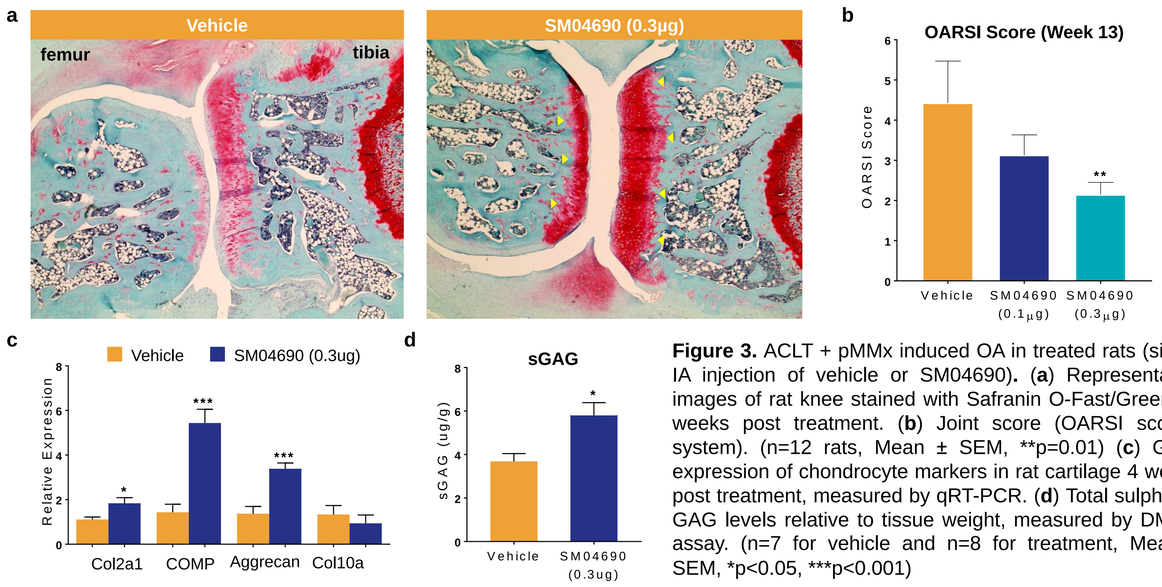


Figure 3. ACLT + pMMx induced OA in treated rats (single IA injection of vehicle or SM04690)**.** (**a**) Representative images of rat knee stained with Safranin O-Fast/Green 12 weeks post treatment. (**b**) Joint score (OARSI scoring system). (n=12 rats, Mean ± SEM, **p=0.01) (**c**) Gene expression of chondrocyte markers in rat cartilage 4 weeks post treatment, measured by qRT-PCR. (**d**) Total sulphated GAG levels relative to tissue weight, measured by DMMB assay. (n=7 for vehicle and n=8 for treatment, Mean ± SEM, *p<0.05, ***p<0.001)

SM04690 protected cartilage in the ACLT+pMMx model of rat OA

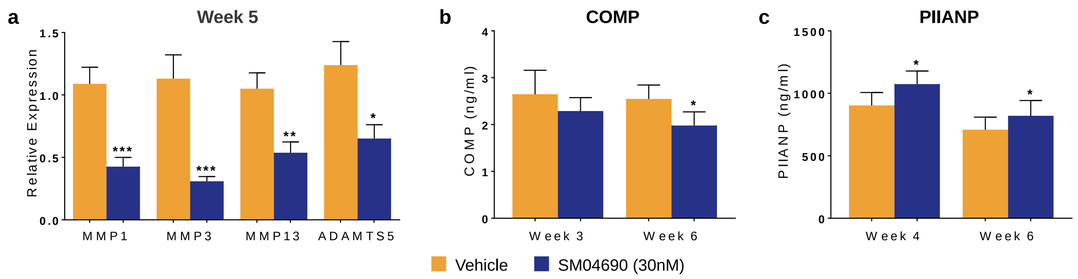
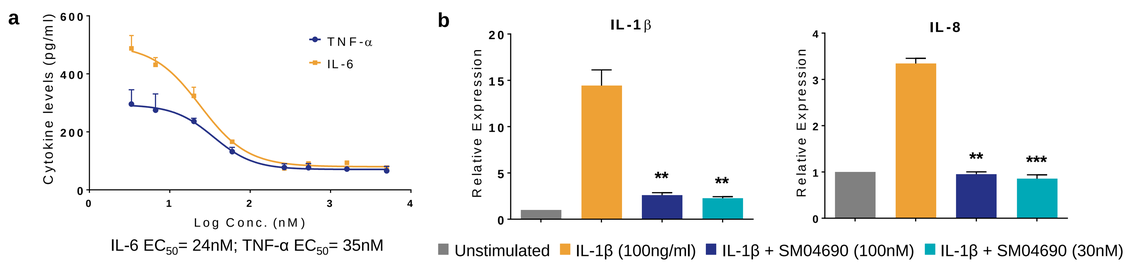


Figure 4. ACLT + pMMx induced OA in treated rats (single IA injection of vehicle or SM04690). **(a)** Protease gene expression in rat cartilage 4 weeks post treatment (qRT-PCR). (n=7 vehicle, n=8 treatment, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001) (**b, c**) Circulating COMP and PIIANP measured by ELISA. (n=12 rats, Mean ± SEM, *p<0.05)

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



c

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 5. (a) IL-6 and TNF-α inhibition in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs measured by ELISA. (**b**) Inflammatory cytokine inhibition in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs (qRT-PCR). (n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001) **(c)** Comparison of *in vitro* anti-inflammatory activity of SM04690 with cyclosporin A and prednisolone performed on DiscoverX BioMAP® platform (scale 0-5; 0=weak and 5=highly potent activity). SM04690 demonstrated comparable or greater potency than the two standard-of-care drugs across several anti-inflammatory assays.

Conclusions

- SM04690, a small molecule Wnt pathway inhibitor, induced chondrogenesis, protected chondrocytes from catabolic breakdown, increased cartilage thickness and improved joint health in a rat model of knee OA.
- Additionally, potent anti-inflammatory effects of SM04690 observed in various cell types may provide beneficial effects in the treatment of OA.
- Human clinical trials with SM04690 are ongoing.

References

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