

Potential Disease Modifying Treatment of Knee Osteoarthritis by SM04690, a Small Molecule Wnt Pathway Inhibitor

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Poster 47

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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 by modulating inflammation, cartilage breakdown, and bone/cartilage formation. Increased Wnt signaling induces stem cell differentiation into osteoblasts and inhibition shifts lineage fate towards chondrogenesis.²
- Samumed is developing a small molecule, intra-articular (IA) Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug (DMOAD).
- Preclinical evidence of SM04690 effects on chondrogenesis, cartilage protection, inflammation, and joint health are reported.

Methods

- Chondrogenesis and osteogenesis were evaluated in human mesenchymal stem cells (hMSCs) treated for 21 days, by immunocytochemistry and qRT-PCR. Matrix production in chondrocytes was measured as sulfated glycosaminoglycan (sGAG) with a dimethylmethylene blue (DMMB) assay. Cytokine induced GAG breakdown in chondrocytes was measured with a DMMB assay.
- Pharmacokinetics were evaluated following a single IA injection of SM04690 into Sprague-Dawley rats and measurement in cartilage, bone and plasma by LC-MS.
- *In vivo* activity of a single IA injection of SM04690 was evaluated in 2 rat OA models:
 - Anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMx): One Week after surgery, vehicle or SM04690 (0.3ug) was injected. Protease and chondrogenic gene expression in cartilage was measured by qRT-PCR and sGAG by DMMB at Week 5. Cartilage was evaluated using Osteoarthritis Research Society International (OARSI) histology scoring and thickness measurements at Week 13. Biomarkers were measured in plasma by ELISA.
 - Monosodium iodoacetate (MIA) injection-induced model: 3 days after IA MIA (300 µg) injection, vehicle or SM04690 (0.3 µg) was injected. Joint inflammation was evaluated histologically and by qRT-PCR measurement of pro-inflammatory markers (TNF-α, IL-1β, IL-6) on day 11. Pain was measured as paw withdrawal threshold using Von Frey apparatus on days 0-22.

Results

SM04690 induced chondrocyte differentiation in hMSCs and protected chondrocytes from catabolic breakdown *in vitro*

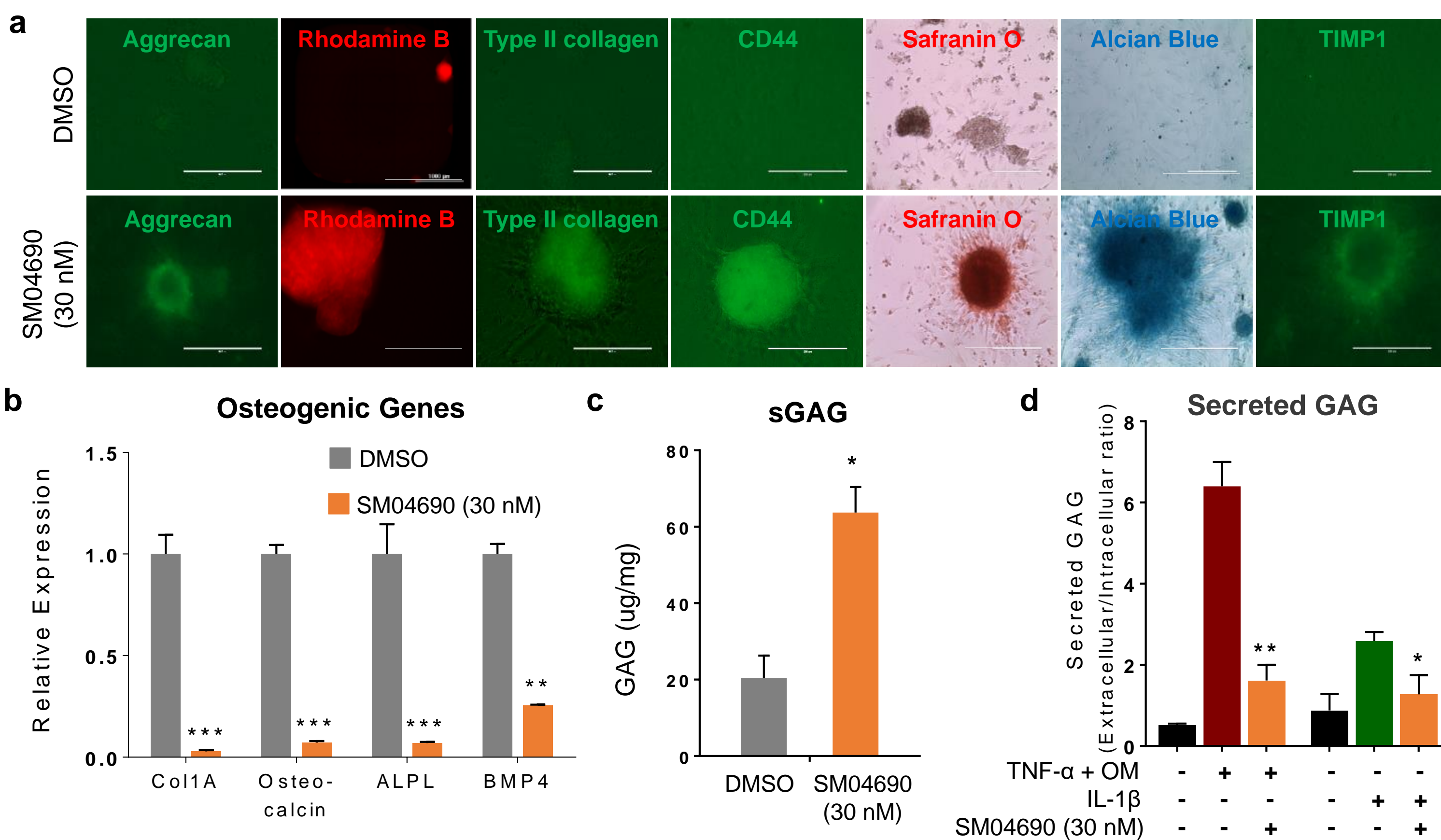


Figure 1. hMSCs treated with DMSO or SM04690 (30 nM). (a) Staining markers for mature chondrocytes. (b) Osteogenic gene expression. (c) Quantification of sulphated GAG in chondrocytes. (d) Cytokine-induced catabolic matrix breakdown measured as levels of secreted GAG. (n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

SM04690 showed sustained local and low systemic exposure in rats

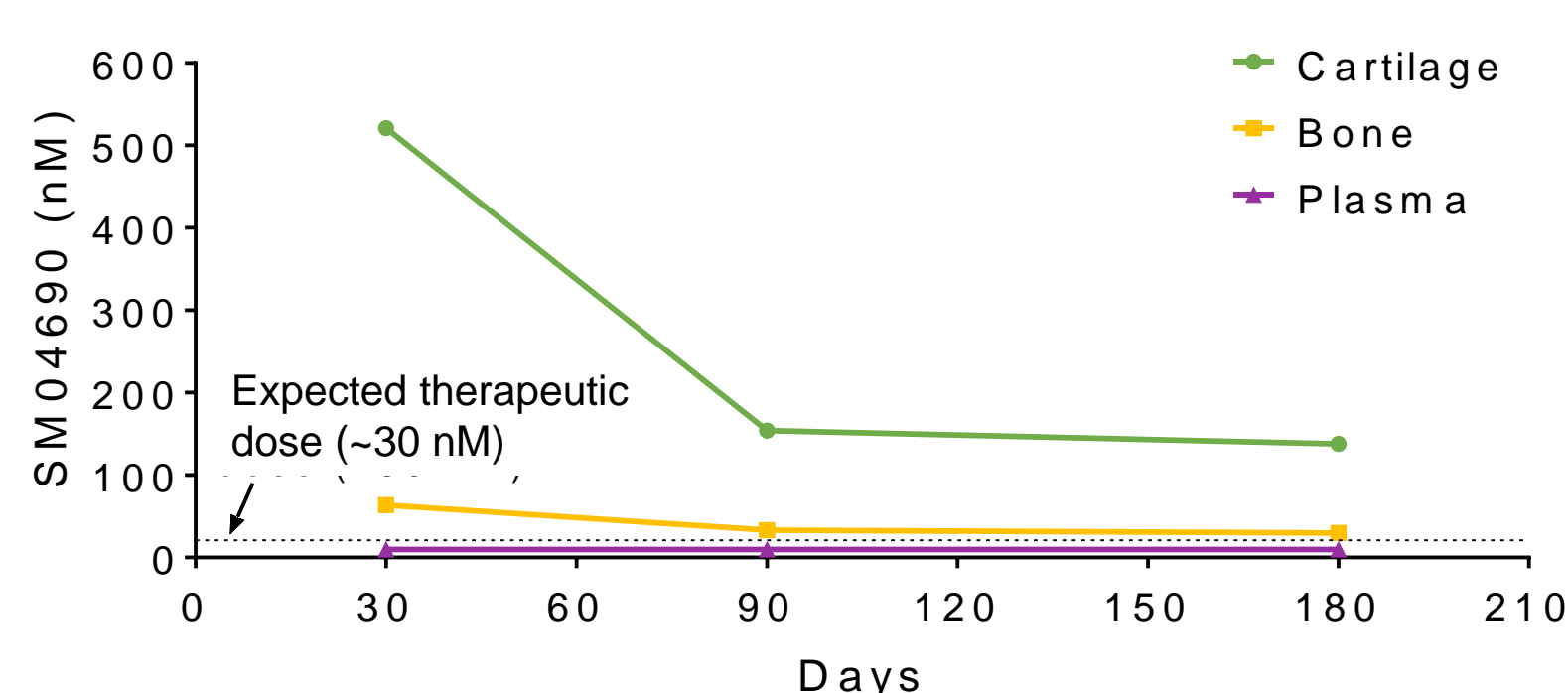


Figure 2. Pharmacokinetics in rat cartilage, bone, and plasma following single IA injection of SM04690 (0.3 µg).

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

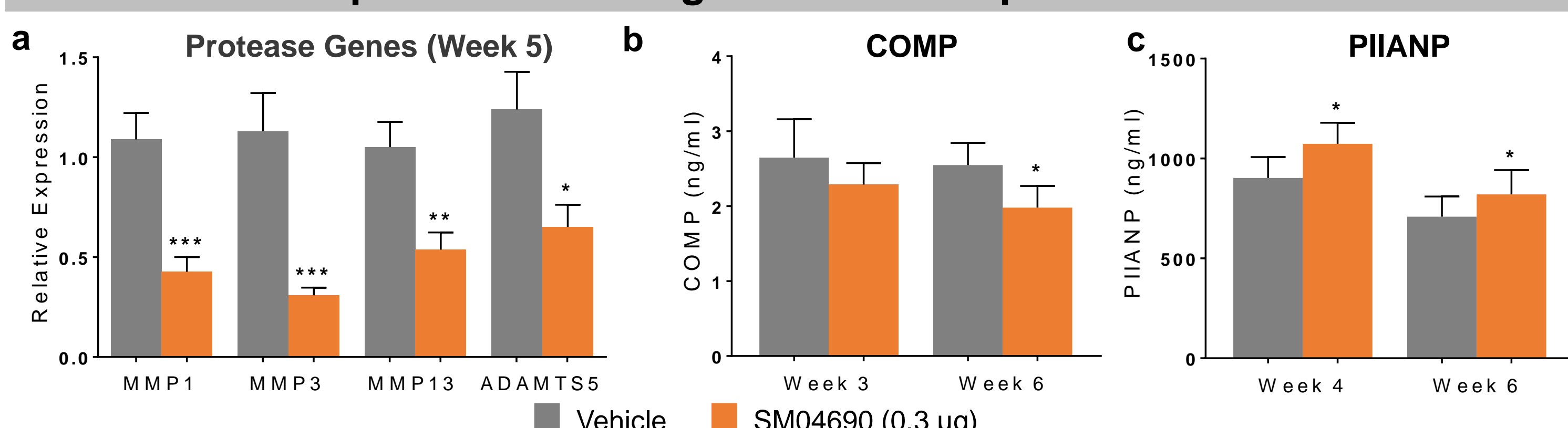


Figure 3. ACLT+pMMx induced OA in rats treated with IA vehicle or SM04690 (0.3 µg) at one week. (a) Protease gene expression in rat cartilage at Week 5 (b, c) Circulating COMP and PIIANP measured by ELISA. (n=12 rats, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

Results

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

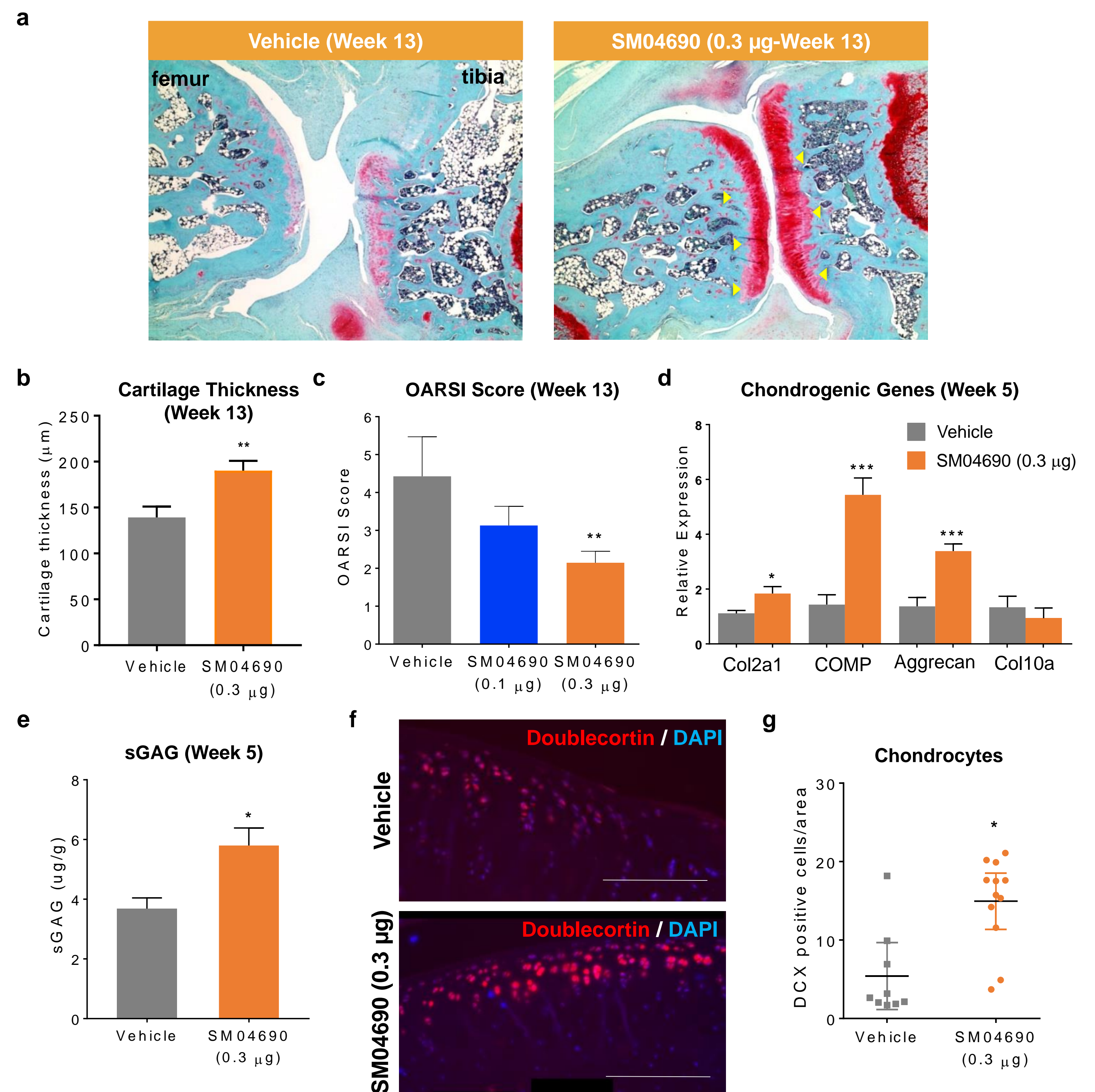


Figure 4. ACLT+pMMx induced OA in rats treated with IA vehicle or SM04690 (0.3 µg) at one week. (a) Representative images of rat knee stained with Safranin O-Fast Green. (b) Cartilage thickness from Safranin O stained sections. (c) OARSI Joint scores (d) Gene expression of chondrocyte markers in rat cartilage. (e) Total sGAG levels relative to tissue weight. (f) Representative images of rat knee stained for Doublecortin (Dcx)-expressing chondrocytes in the superficial zone of the articular cartilage. (g) Quantification of Dcx-positive chondrocytes in (f). (n=12, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

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

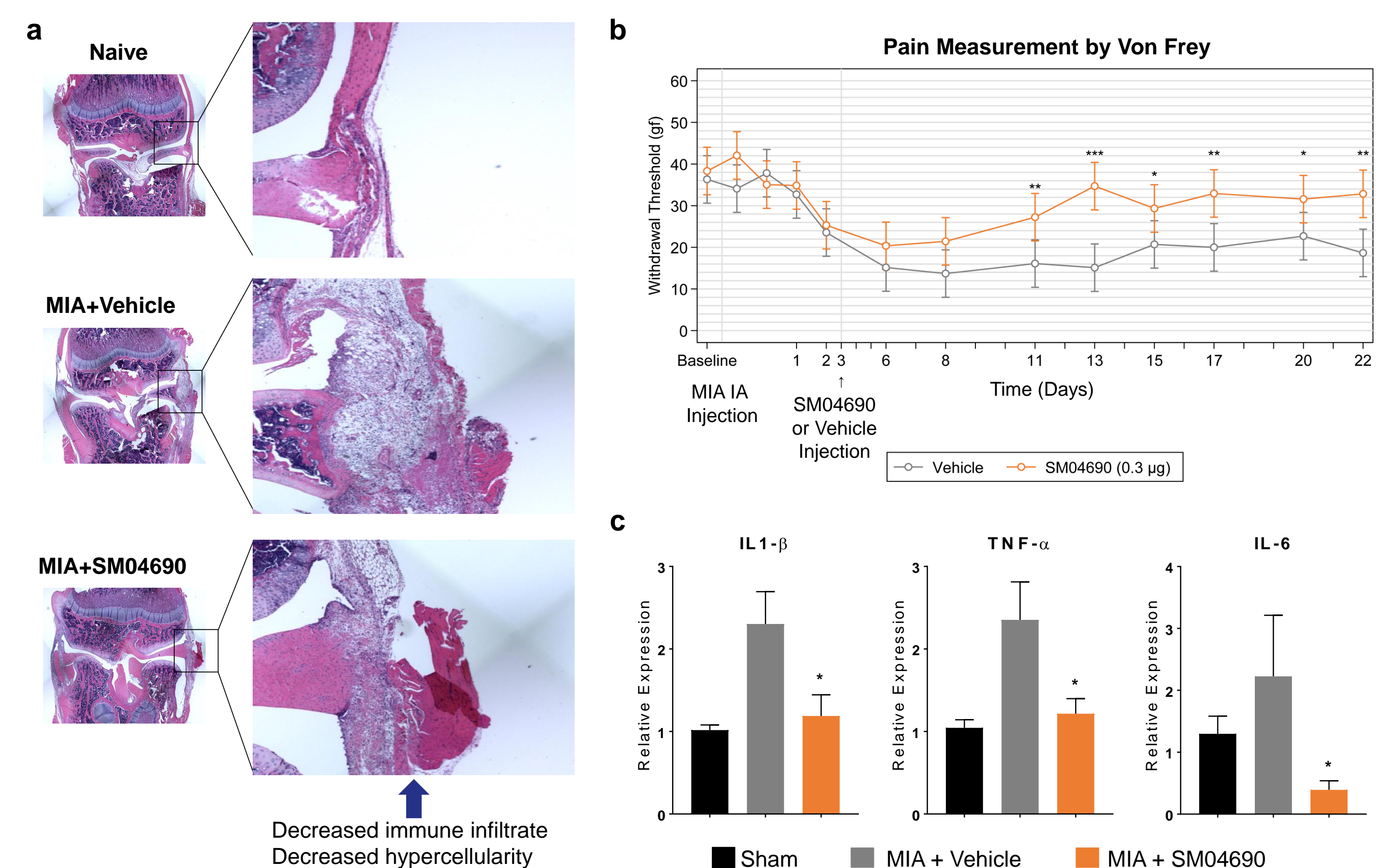


Figure 5. IA MIA injection-induced OA in rats treated with IA vehicle or SM04690 (0.3 µg) at Day 3 (a) Representative images of H&E stained knee sections on Day 11. (b) Pain in the MIA-injected limb measured as Von Frey paw withdrawal threshold. (c) Gene expression of inflammatory markers in the rat knee on Day 11. (n=10 rats, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

Conclusions

- SM04690, a Wnt pathway inhibitor, induced chondrogenesis, protected chondrocytes from catabolic breakdown, increased cartilage thickness, and improved joint health in a rat injury model of knee OA.
- In the MIA model, SM04690 showed potent anti-inflammatory effects that improved pain responses and structure.
- SM04690 has potential as a DMOAD. Human clinical trials 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.