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

samumed

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Background

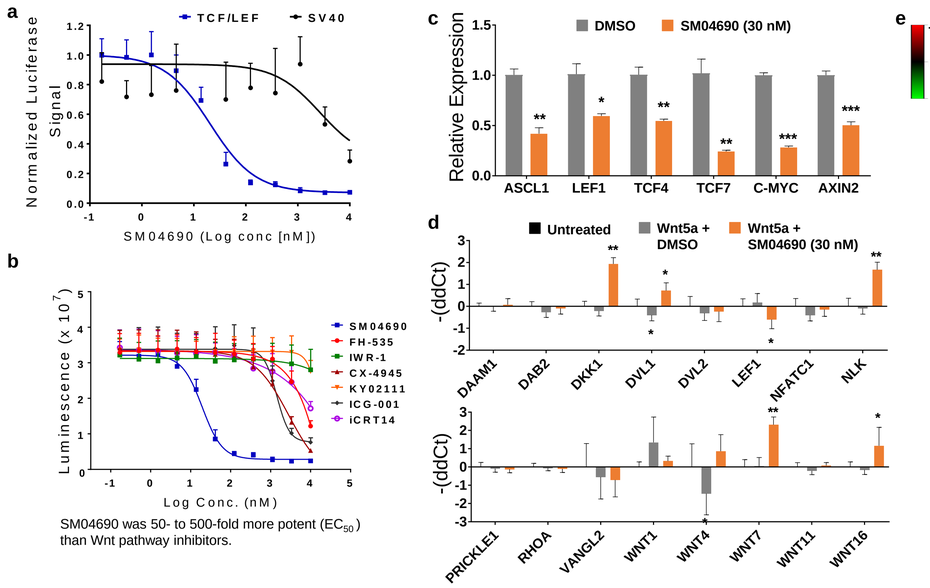
- Knee osteoarthritis (OA) is characterized by articular cartilage destruction, subchondral bone alterations, and synovitis.[1]
- Wnt signaling affects OA pathogenesis by modulating inflammation, cartilage breakdown, and bone/cartilage formation. Increased Wnt signaling induces stem cell differentiation into osteoblasts while a decrease shifts lineage fate towards chondrogenesis.[2]
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug.
- Preclinical evidence of SM04690 effects on Wnt signaling, chondrogenesis, cartilage protection, and joint health are reported.

Methods

- A small molecule library was screened using a cell-based TCF/LEF-luciferase reporter assay and hits were counter-screened using an SV-40-luciferase reporter. Expression of Wnt target genes (Ascl1, Lef-1, TCF4, TCF7, c-myc, Axin2) were measured by qPCR in human mesenchymal stem cells (hMSCs).
- Global gene expression in response to 16hr treatment with SM04690 was measured in hMSCs using RNA-sequencing (RNA-seq).
- Chondrogenesis and osteogenesis were evaluated in hMSCs treated with SM04690 by immunocytochemistry and qPCR.
- Anti-inflammatory activity was evaluated by measuring TNF-α and IL-6 secretion using ELISA in IL1-β-stimulated synovial fibroblasts. Cytokine-induced GAG breakdown in chondrocytes was measured by DMMB assay.
- Pharmacokinetics & toxicology were evaluated in rats & dogs [single or multiple IA or IV injections (Q 30 days for up to 9 months)].
- *In vivo* activity of SM04690 was evaluated in a rat anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMx) model of OA. A single SM04690 (0.3 µg) IA injection was administered 1 week after transection.
  - Week 5 after transection: Wnt pathway modulation in cartilage measured by a panel of 84 Wnt pathway genes using qPCR and β-catenin nuclear localization by immunohistochemistry (IHC). Protease and chondrogenic gene expression measured by qPCR.
  - Week 13 after transection: Cartilage was evaluated using Osteoarthritis Research Society International (OARSI) histology scoring, and thickness measurements and Doublecortin (Dcx) positive chondrocytes were measured by IHC.
- Statistics - Parametric data: t-test for 2 groups, one-way ANOVA for >2 groups. Non-parametric data: Mann-Whitney U test.

Results

SM04690 was a potent and specific inhibitor of Wnt signaling *in vitro*



SM04690 was 50- to 500-fold more potent (EC_{50}) than Wnt pathway inhibitors.

Figure 1. (**a, b**) SW480 cells with TCF/LEF promoter-driven or SV40 promoter-driven luciferase reporters treated with (**a**) SM04690 or (**b**) SM04690 or several Wnt pathway inhibitors for 24hrs. (**c**) Wnt pathway gene expression in hMSCs measured by qPCR at 24hrs. (**d**) Non-canonical Wnt pathway gene expression in hMSCs after DMSO or SM04690 (30 nM) treatment for 24hrs. (**e**) Wnt pathway gene expression measured by RNA-seq at 16hrs. (n=6, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

SM04690 induced chondrocyte differentiation in hMSCs, demonstrated anti-inflammatory properties, and protected chondrocytes from catabolic breakdown *in vitro*

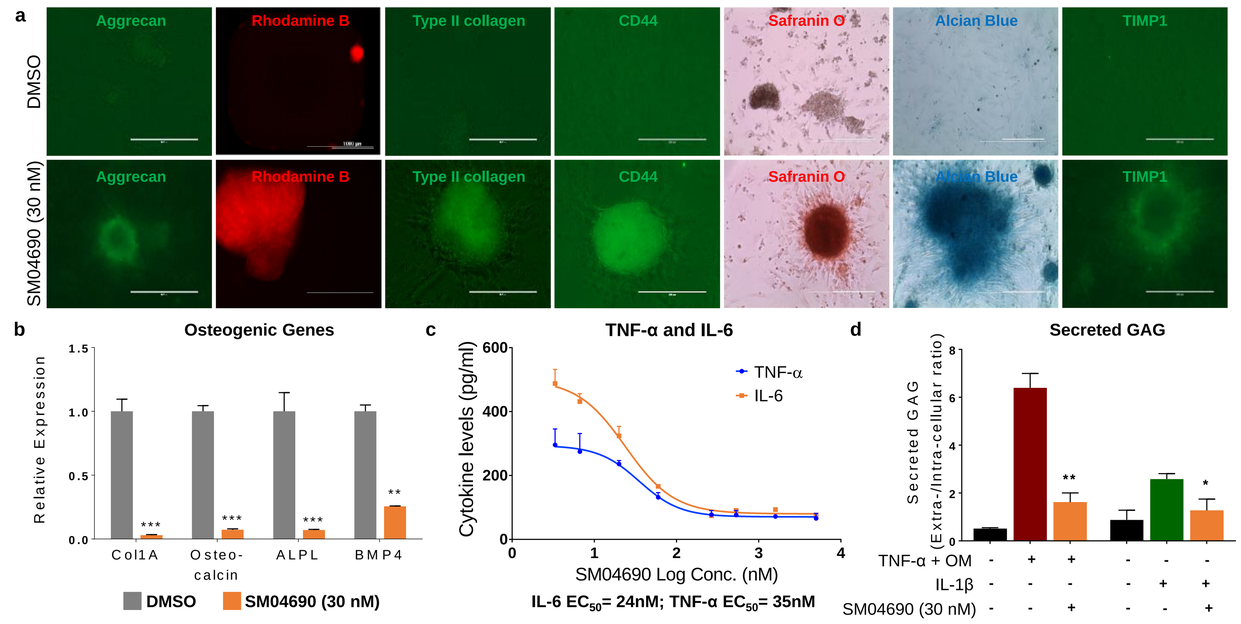
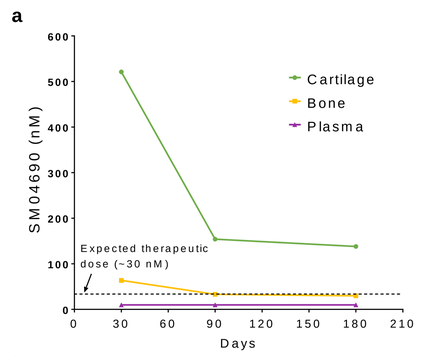


Figure 2. (**a, b**) hMSCs treated with DMSO or SM04690 (30 nM) for 21 days. (**a**) Staining markers for mature chondrocytes. (**b**) Osteogenic gene expression. (**c**) TNF-α and IL6 secretion in synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 48hrs (**d**) Cytokine-induced catabolic matrix breakdown measured as levels of secreted GAG at 72hrs. (n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, scale bars = 200µm)

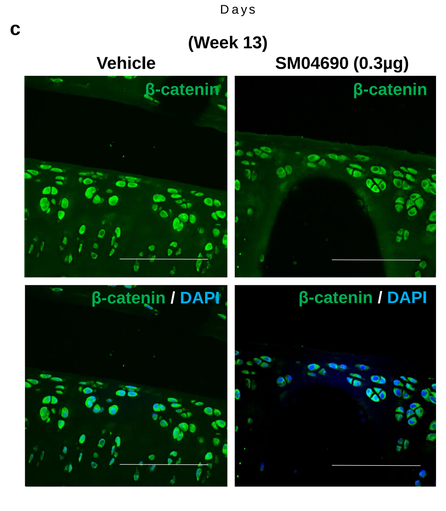
Results

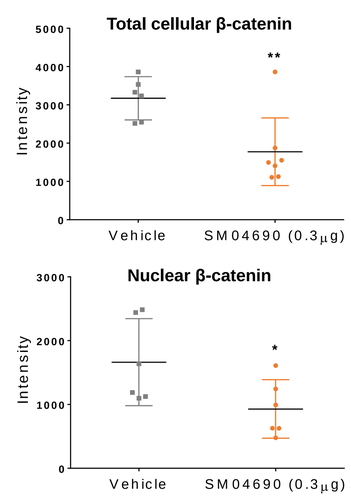
Pharmacokinetics and Pharmacodynamics: SM04690 had sustained local exposure in the joint and inhibited Wnt signaling in cartilage *in vivo*



b

IA and IV injection in rats and dogs	
Results	No systemic toxicity. No measured effects on body weight, target/non-target organs effects, clinical pathology, CNS, respiratory or cardiovascular functions. Macroscopic findings limited to the increased lung weight (IV injection only).
Therapeutic Index	Up to 400X lowest therapeutic dose. Local inflammation at injection site at >1400X lowest therapeutic dose.





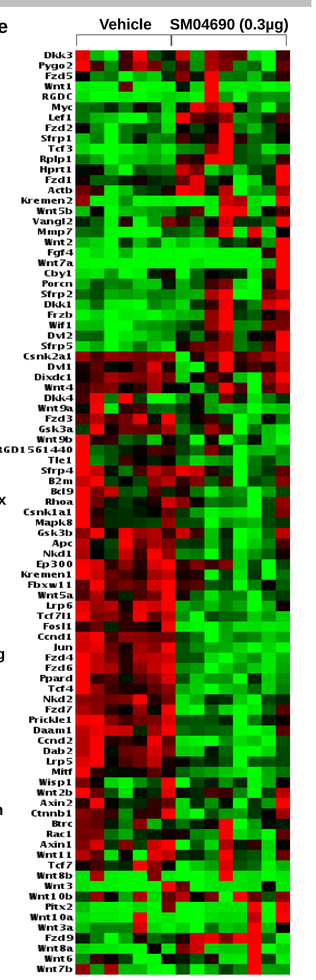
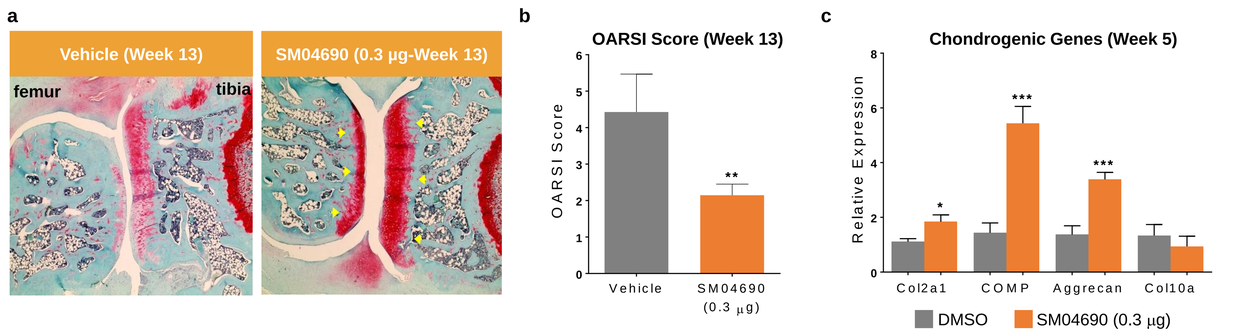


Figure 3. (**a**) Pharmacokinetics of SM04690 in rats. (**b**) Systemic toxicology results (**c, d**) ACLT+pMMx induced OA in rats treated with IA vehicle or SM04690 (0.3 µg) at one week after surgery (**c**) Representative images of superficial zone articular cartilage stained for β-catenin (**d**) Staining intensity quantification of β-catenin in the total cell and cell nuclei, from (**c**). (n= 6 rats/group, Mean ± SEM, *p<0.05, **p<0.01, scale bars = 200 µm) (**e**) Expression of Wnt pathway genes in rat cartilage. Color scale represented. (n= 7 for vehicle, n=8 for SM04690)

SM04690 regenerated cartilage *in vivo* in an ACLT+pMMx model of rat OA



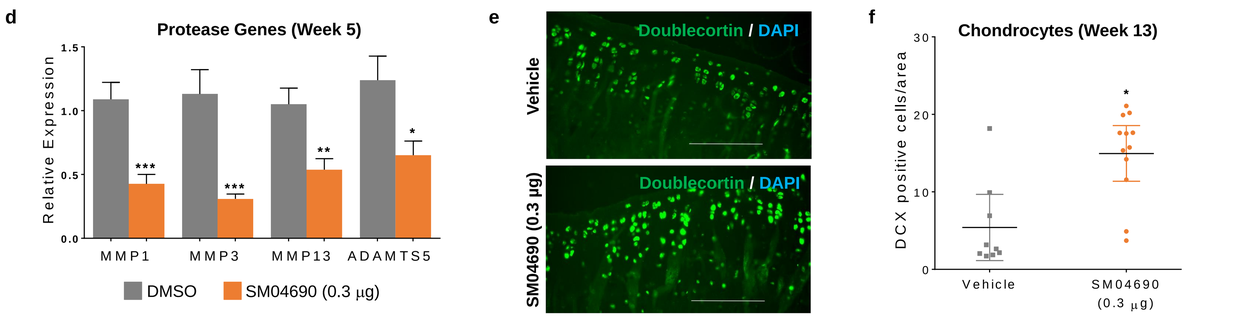


Figure 4. ACLT+pMMx induced OA in rats treated with IA vehicle or SM04690 (0.3 µg) at one week after surgery. (**a**) Representative images of rat knee stained with Safranin O-Fast Green. (**b**) OARSI Joint scores. (**c,d**) Gene expression of (**c**) chondrocyte markers (**d**) protease markers in rat cartilage. (n=12 rats/group, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001) (**e, f**) Dcx stained chondrocytes in articular cartilage. (n=9 for vehicle, n=12 for SM04690, Mean ± SEM, *p<0.05, scale bars = 200 µm)

Conclusions

- SM04690 was a potent and specific inhibitor of canonical Wnt signaling.
- SM04690 induced chondrogenesis, inhibited cytokine production, and protected chondrocytes from catabolic breakdown.
- Following IA injection, SM04690 had prolonged residence time in the joint, no systemic exposure, and no systemic toxicity.
- Treatment with SM04690 inhibited Wnt signaling *in vivo*, increased cartilage thickness, increased chondrocyte numbers, and improved joint health in a rat injury model of knee OA.
- 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.

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