SM04690, a Wnt Pathway Inhibitor: Anti-Inflammatory and Cartilage Protective Effects in Preclinical OA Models

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Disclosures

Vishal Deshmukh	Samumed, LLC, employee and shareholder
Tim Seo	Samumed, LLC, employee and shareholder
Christopher Swearingen	Samumed, LLC, employee and shareholder
Yusuf Yazici	Samumed, LLC, employee and shareholder

The Wnt pathway, osteoarthritis (OA), and inflammation

- Increased Wnt signaling drives bone formation, cartilage breakdown, and inflammation[1-4]
- Wnt pathway mutations (e.g., FrzB, DOT1L) are associated with OA[5,6]
- Wnt proteins are over-expressed in OA joints[7,8]

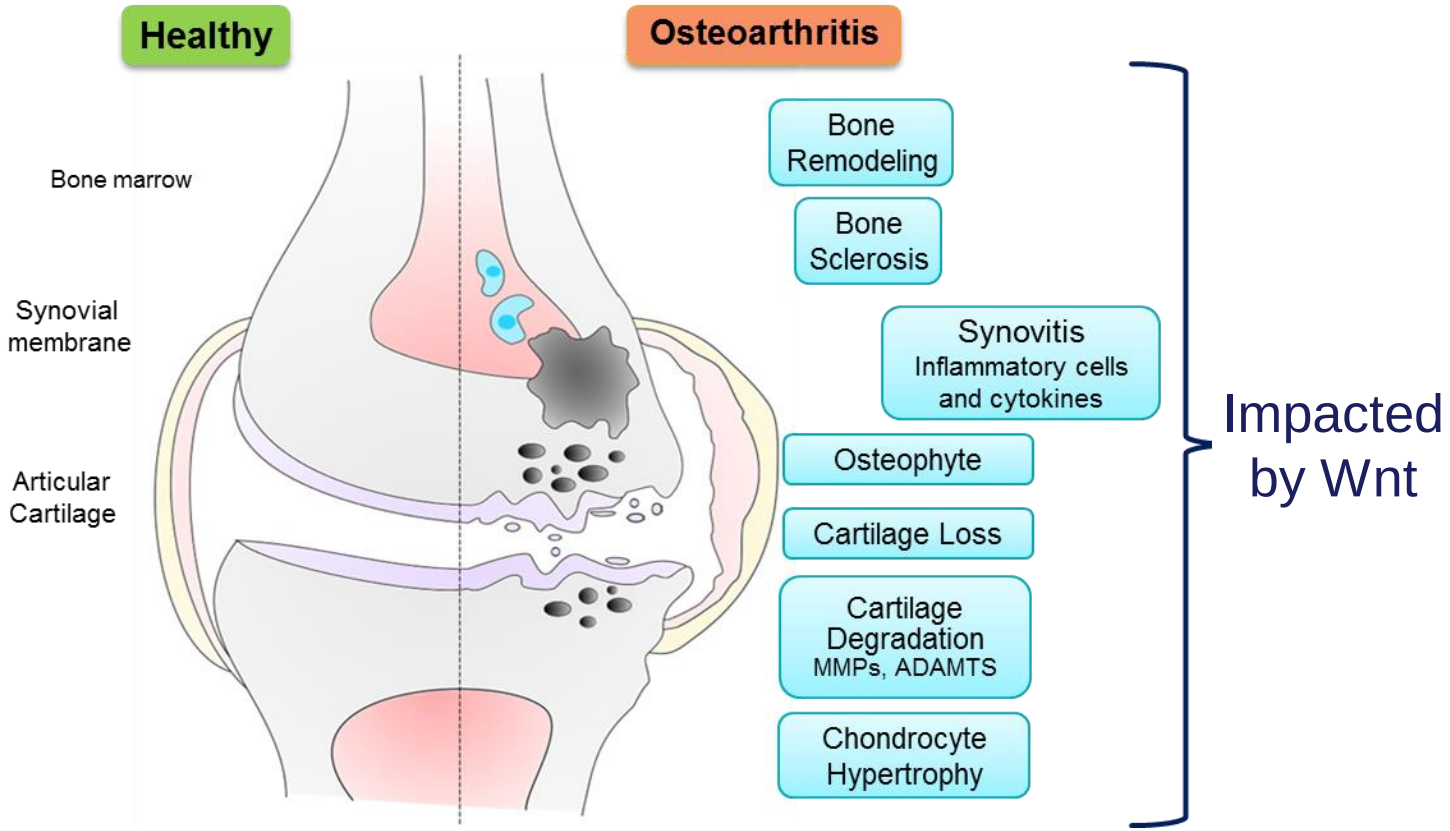


Figure adaptation: Bush and Beier. (2013) *Nature Medicine.*

1. Hamerman D. (1993) *N Engl J Med.*
2. Yuasa, t et al. (2008) *Lab Invest.*
3. Ma B and Hottiger MO (2016) *Frontiers Immun.*
4. Sokolove J and Lepus CM. (2013) *Ther Adv Musculoskelet Dis.*
5. Blom AB, et al. (2009) *Arthritis Rheum.*
6. Monteagudo S, et al. (2017) *Nat Commun.*
7. Rudnicki JA and Brown AM. (1997) *Dev Biol.*
8. Thomas RS, et al. (2011) *Arthritis Res Ther.*

SM04690: A Wnt pathway inhibitor for knee OA

- SM04690 is a small molecule, intra-articular (IA), Wnt pathway inhibitor in development for treatment of knee OA[1,2]
- In previous preclinical studies, SM04690:
  - Inhibited inflammation[1]
  - Decreased cartilage degradation[1]
  - Regenerated cartilage[1]
  - Demonstrated sustained local exposure and no observable systemic toxicity[1,2]
- In previous phase 1 and phase 2a clinical studies, a single IA SM04690 injection appeared well-tolerated and showed potential for improving symptoms and maintaining joint space width in knee OA subjects[3]

The current studies evaluated SM04690 effects in an inflammatory model of OA

1. Deshmukh V, et al. (2017) *OAC.*
2. Yazici Y, et al. (2017) *OAC.*
3. Yazici Y, et al. (2017) *Arthritis Rheum.*

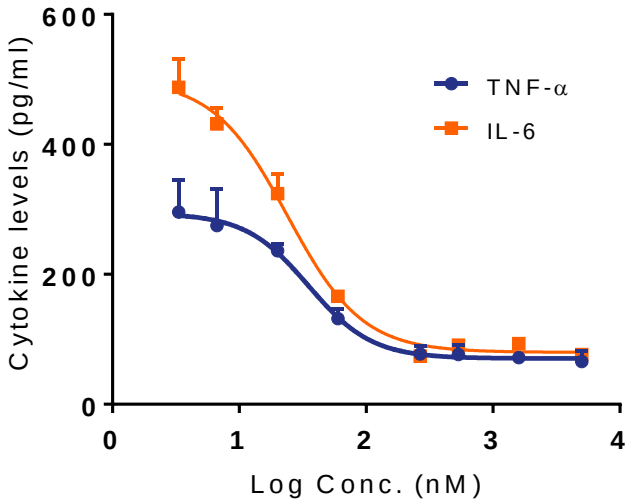
SM04690 anti-inflammatory activity - *In vitro*

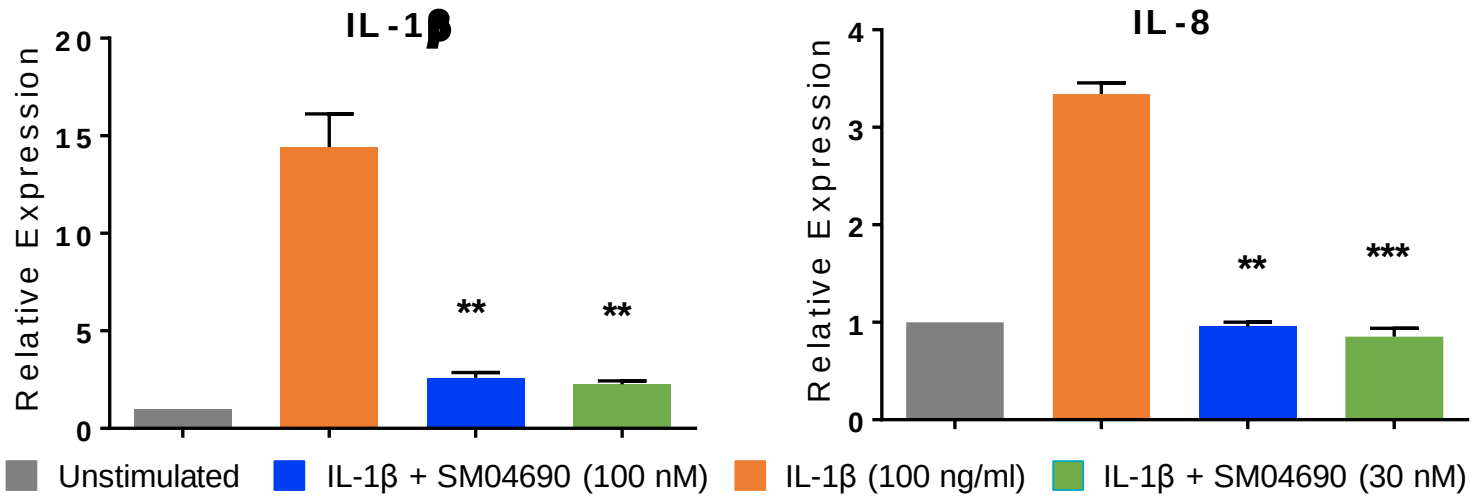
Decreased inflammation: SM04690 suppressed inflammatory cytokines

Cellular assay:

- Synovial fibroblasts were stimulated with IL-1β to induce cytokine production, then treated with SM04690
- Cytokine production was quantified by ELISA and qRT-PCR
- Dose dependent inhibition of IL-1β, IL-6, IL-8, and TNF-α production was demonstrated

Synovial fibroblasts



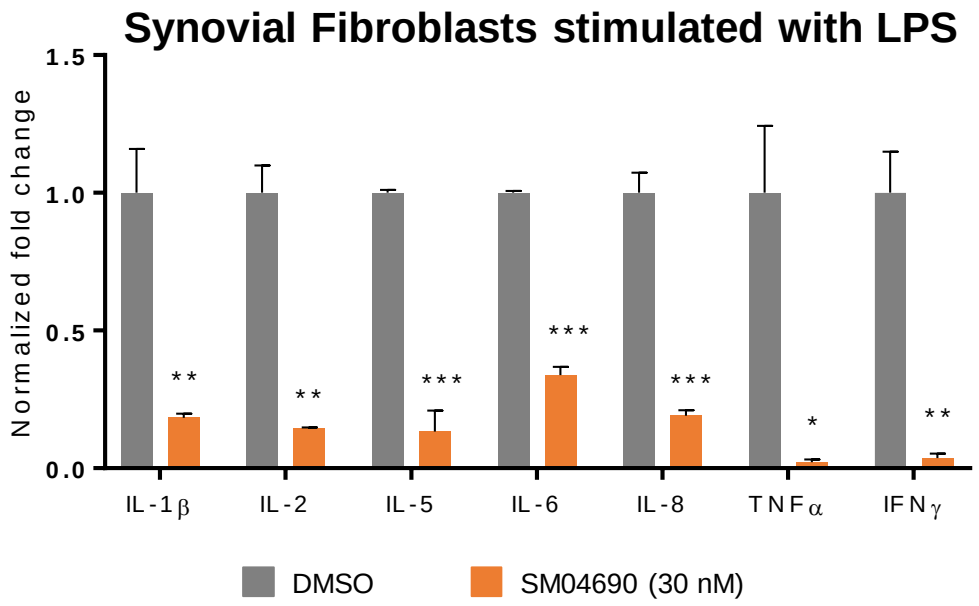


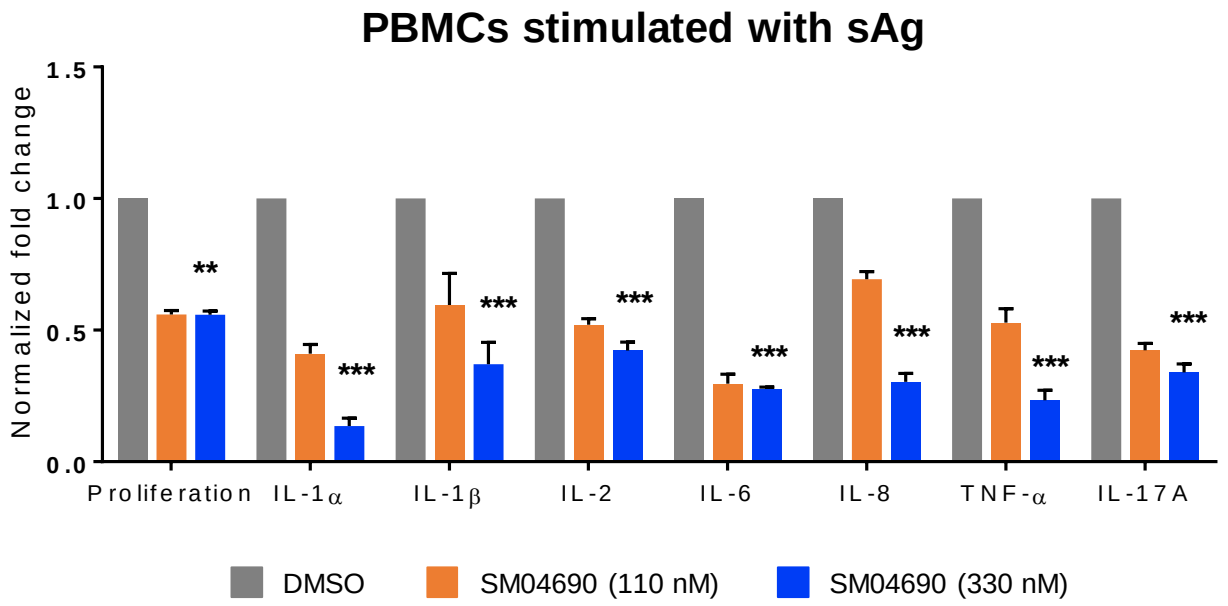
IL-6 EC_{50} = 24 nM; TNF-α EC_{50} = 35 nM
n=3 replicates, Mean ± SEM, **p<0.01, ***p<0.001

Decreased inflammation: SM04690 suppressed inflammatory cytokines

Cellular assays:

- Synovial fibroblasts were stimulated with LPS and peripheral blood mononuclear cells (PBMCs) were stimulated with super antigen (sAg)
- SM04690 inhibited pro-inflammatory cytokine secretion compared to vehicle





n=3 replicates, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001

SM04690 exhibited broad anti-inflammatory properties

- *In vitro* anti-inflammatory activity of SM04690 was measured on the DiscoverX BioMAP® platform using an empirical scale (0-5), where 0=weak activity and 5=highly potent activity
- SM04690 demonstrated comparable or better activity than prednisolone and cyclosporin A across several anti-inflammatory assays

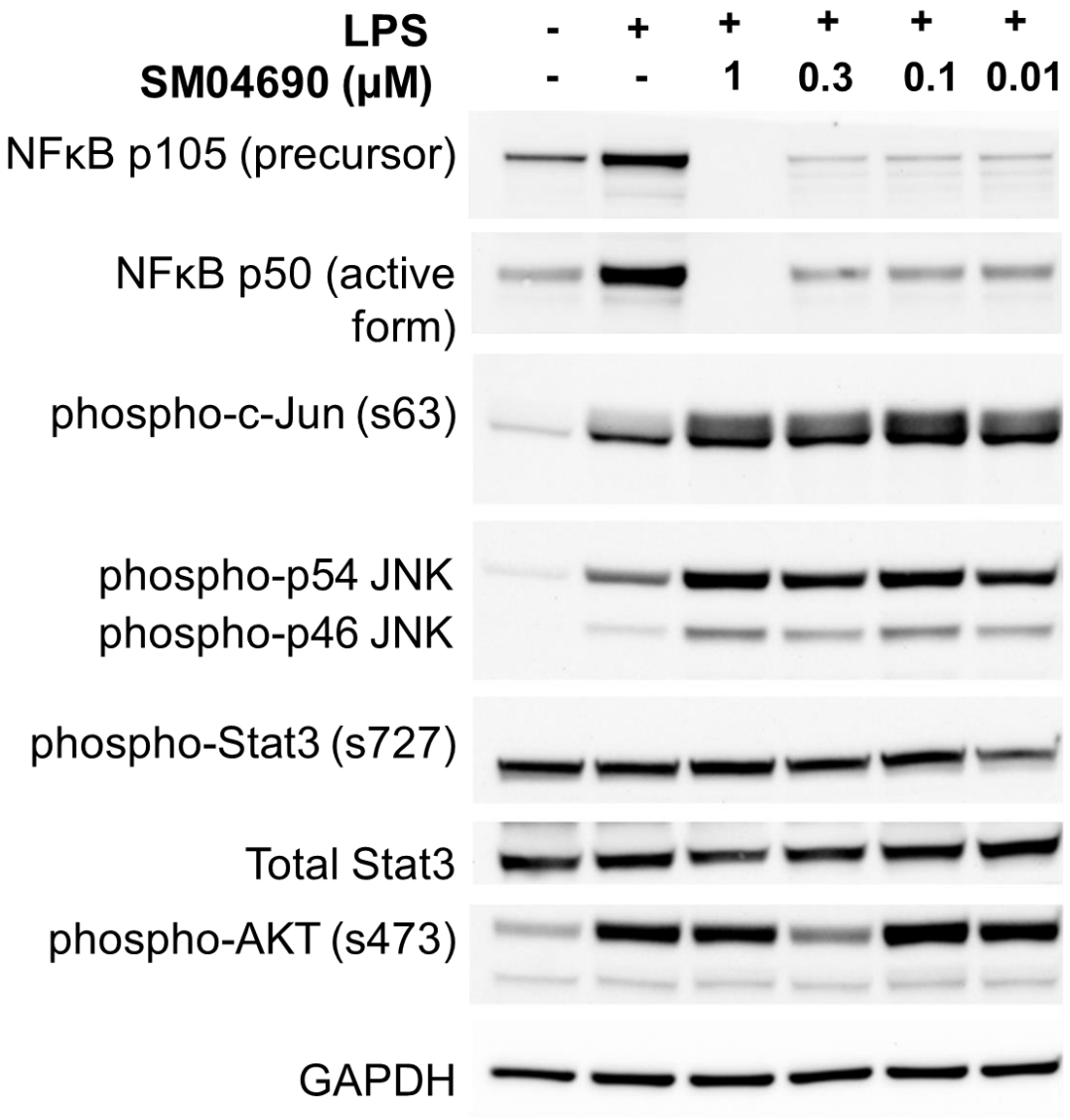
Compound	Immuno-suppression		Anti-Inflammatory	Th1/Th2/Th17 Inhibition			Cell Cytotoxicity		
	T Cell	B cell		Th17	Th1	Th2	PBMC	HDF	EC
SM04690 (37 nM)	5	3	3	3	3	2	0	0	1
Cyclosporin A (120nM)	2	3	2	2	2	0	0	0	0
Prednisolone (120nM)	0	0	1	1	1	0	0	0	0

Abbreviations: PBMC, peripheral blood mononuclear cells; HDF, human dermal fibroblasts; EC, endothelial cells; LPS, lipopolysaccharide

SM04690 inhibited LPS-stimulated inflammation in human monocytes via NFκB

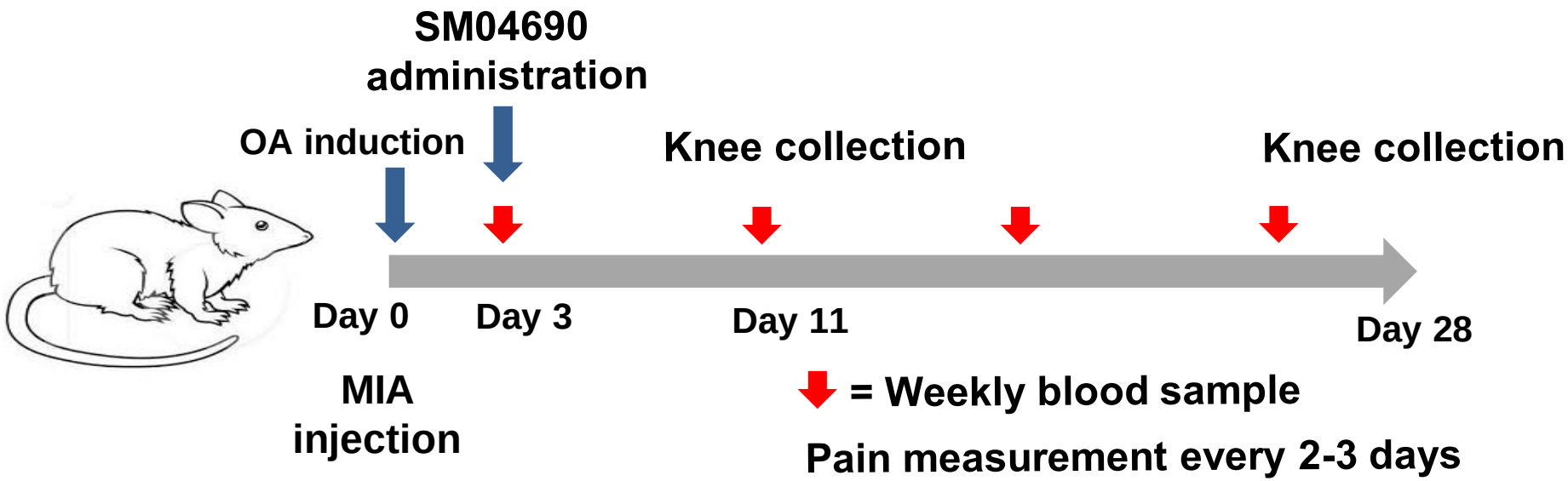
Cellular assay:

- Human monocytes were stimulated with LPS and treated with SM04690 for 4hrs
- Levels of proteins were measured by Western blot
- SM04690 specifically inhibited NFκB phosphorylation *in vitro* and had no effects on other pathways



SM04690 anti-inflammatory activity - *In vivo*

Inflammatory model of rat OA: Monosodium Iodoacetate (MIA) injection



Rat MIA model:

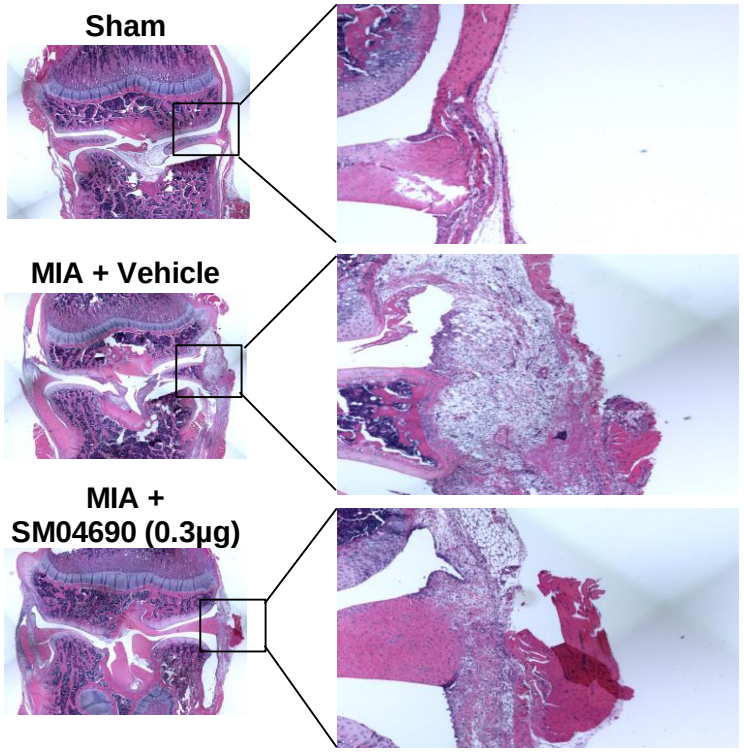
Inflammation within 2 hours and cartilage degeneration within 1-2 weeks

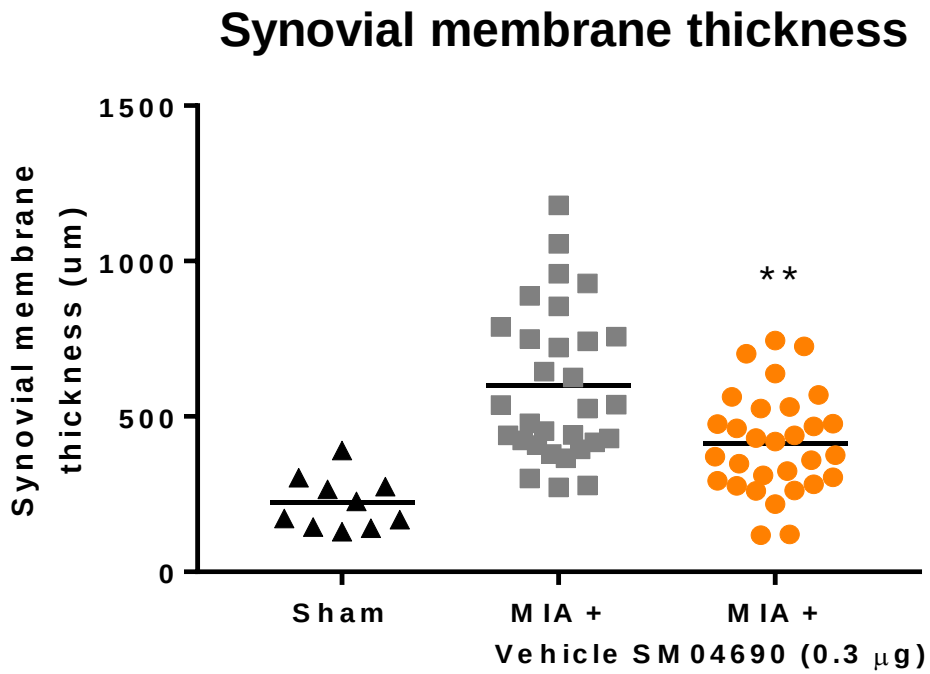
- Monosodium iodoacetate (MIA) intra-articular (IA) injection on Day 0
- SM04690 IA injection on Day 3 (0.3 µg)
- Joint histology performed on Day 11 for histology and Day 28 for joint health

SM04690 attenuated acute inflammation in the rat MIA knee OA model compared to vehicle

- H&E staining after a single IA injection of SM04690 decreased inflammatory infiltrates, decreased hypercellularity, and improved structural integrity, compared to vehicle treatment at Day 11
- Synovial membrane thickness was significantly decreased in SM04690 joints compared to vehicle at Day 11

H&E Staining



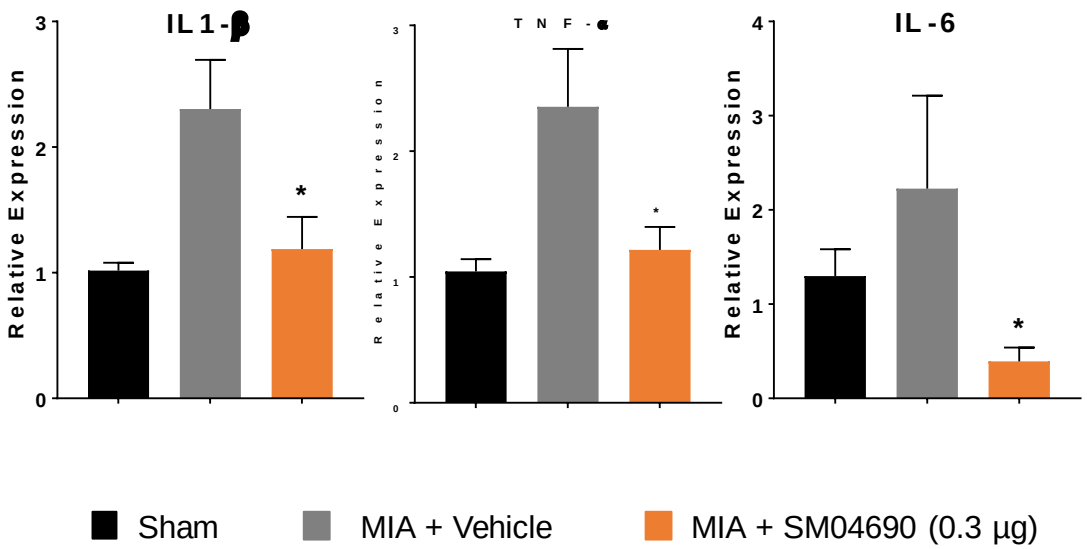


n=30 sections, Mean ± SEM, **p<0.01, one-way ANOVA

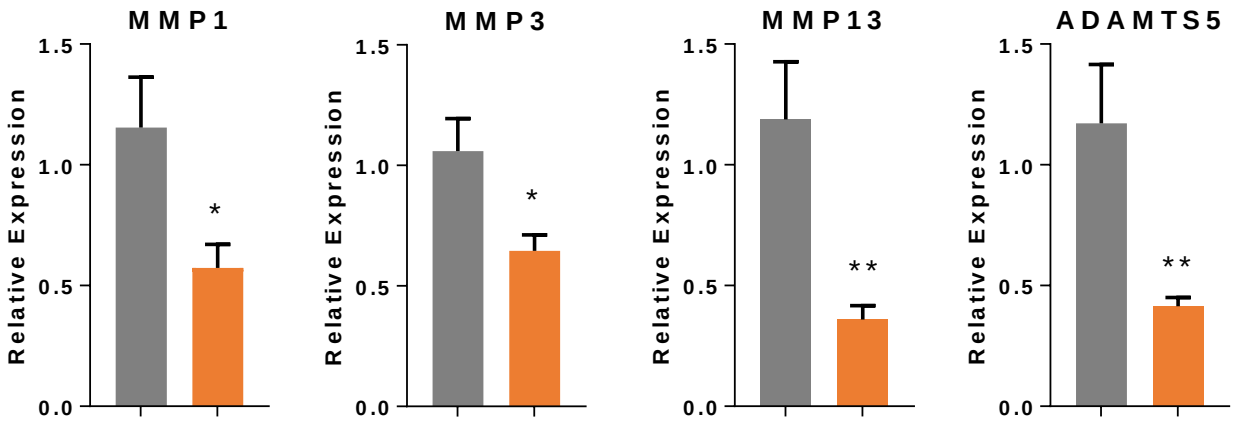
SM04690 attenuated acute inflammation and protected cartilage in the rat MIA knee OA model

- A single IA injection of SM04690 decreased inflammatory cytokines and matrix metalloproteinases (MMPs), compared to vehicle treatment at Day 11

Cytokine gene expression



Protease gene expression



Sham MIA + Vehicle MIA + SM04690 (0.3 µg)

n=10 rats/group, Mean ± SEM, *p<0.05, one-way ANOVA

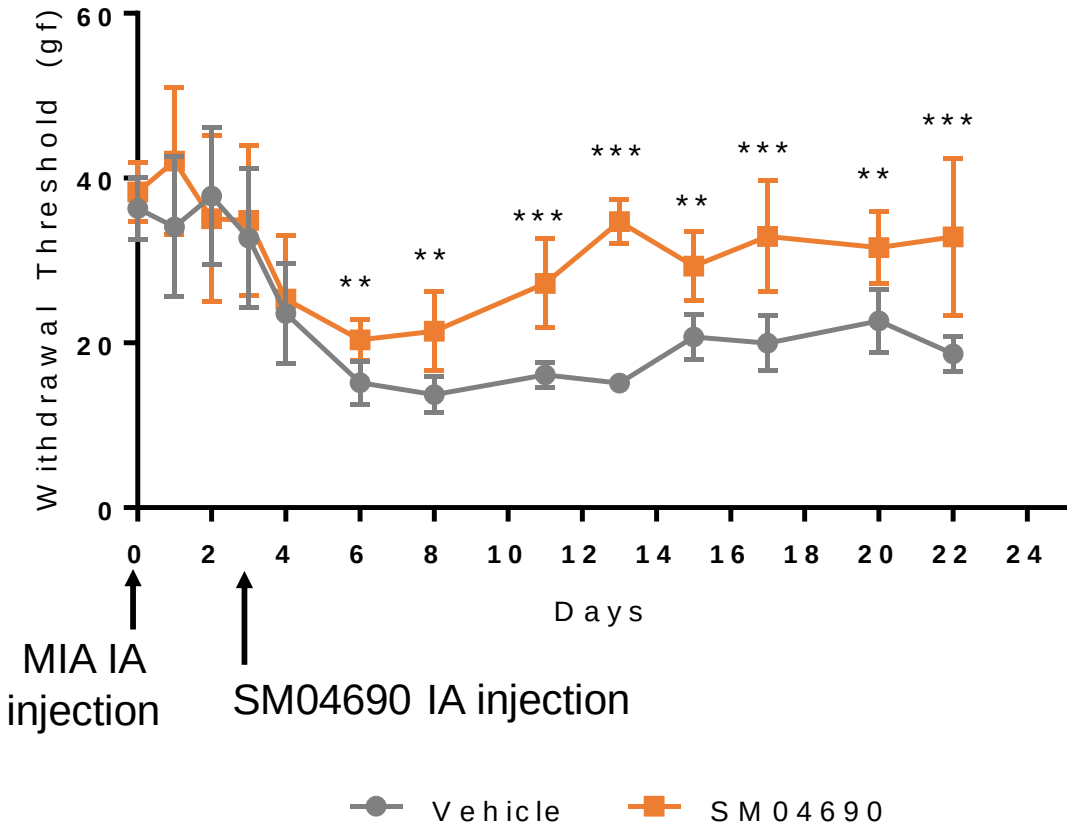
MIA + Vehicle MIA + SM04690 (0.3 µg)

n=8 rats/group, Mean ± SEM, *p<0.05, **p<0.01, t-test

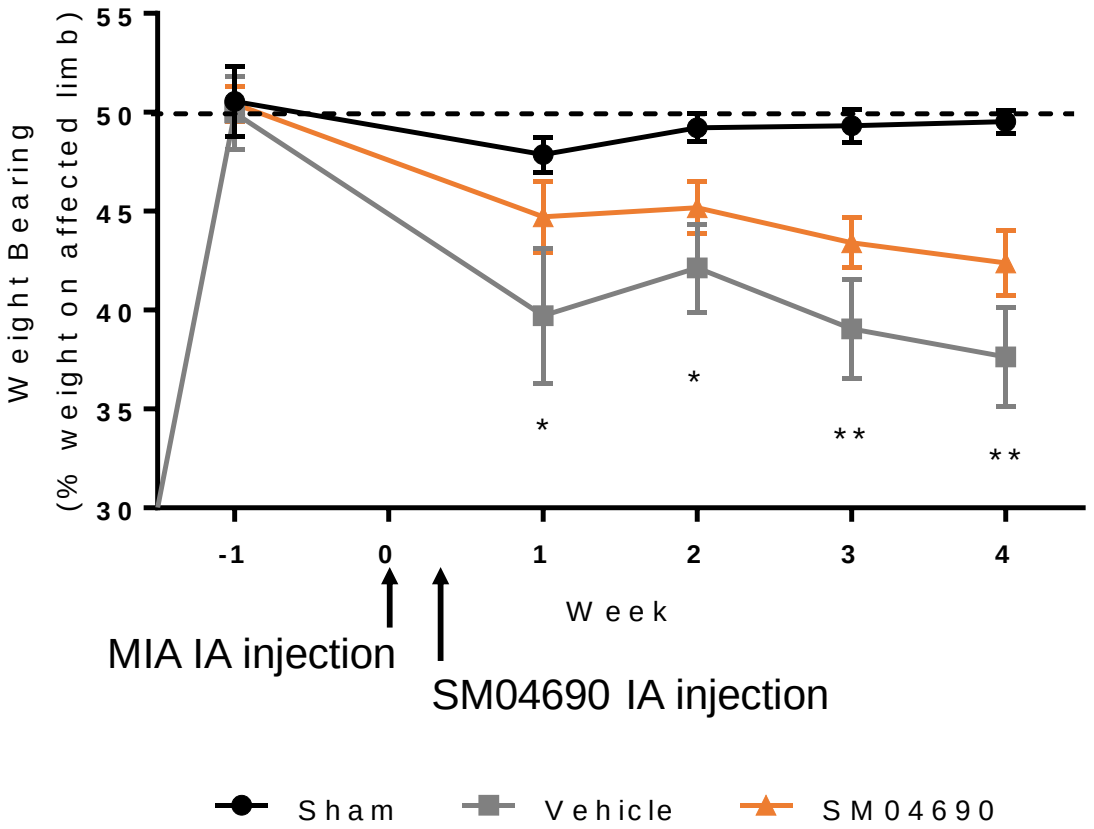
SM04690 attenuated pain in the rat MIA knee OA model

- A single IA injection of SM04690 decreased pain (measured by Von Frey) and improved gait (measured as weight distribution), compared to vehicle treatment

Pain Measurement by Von Frey



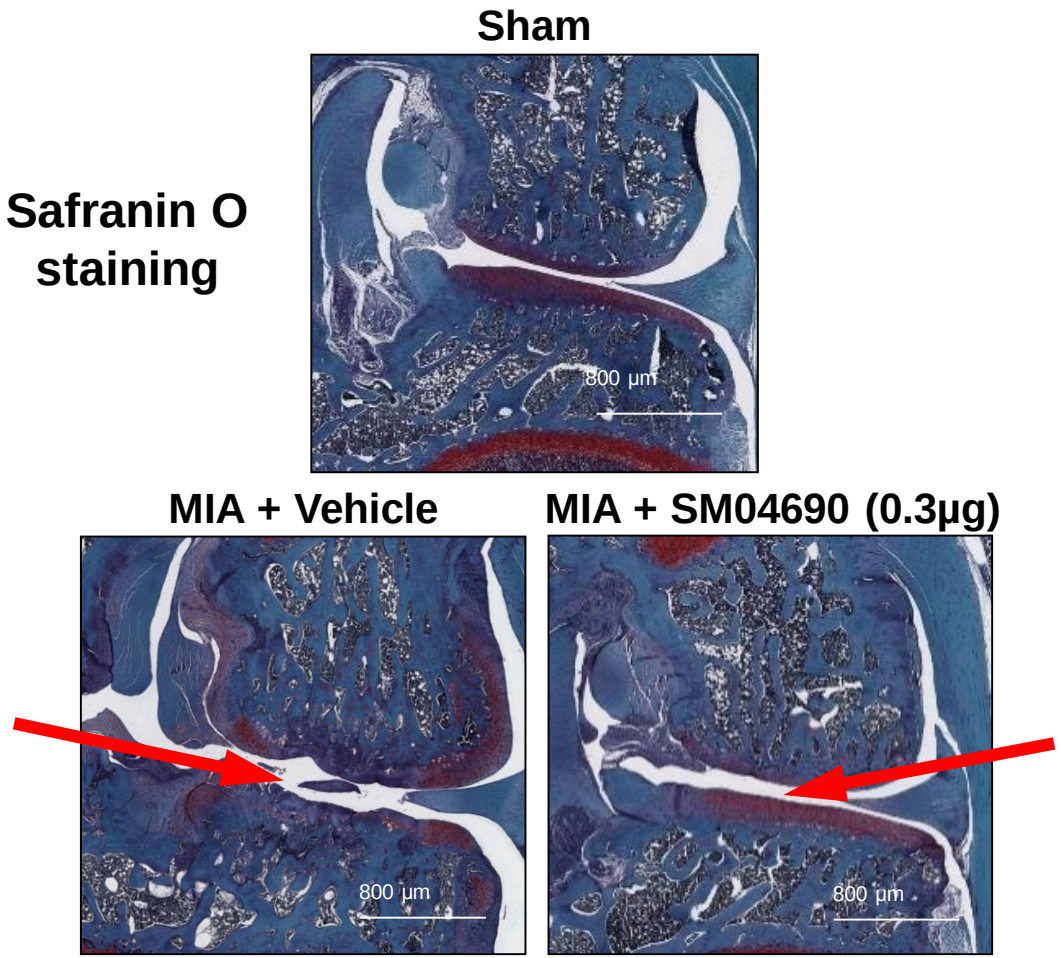
Weight Distribution by Incapacitance Meter

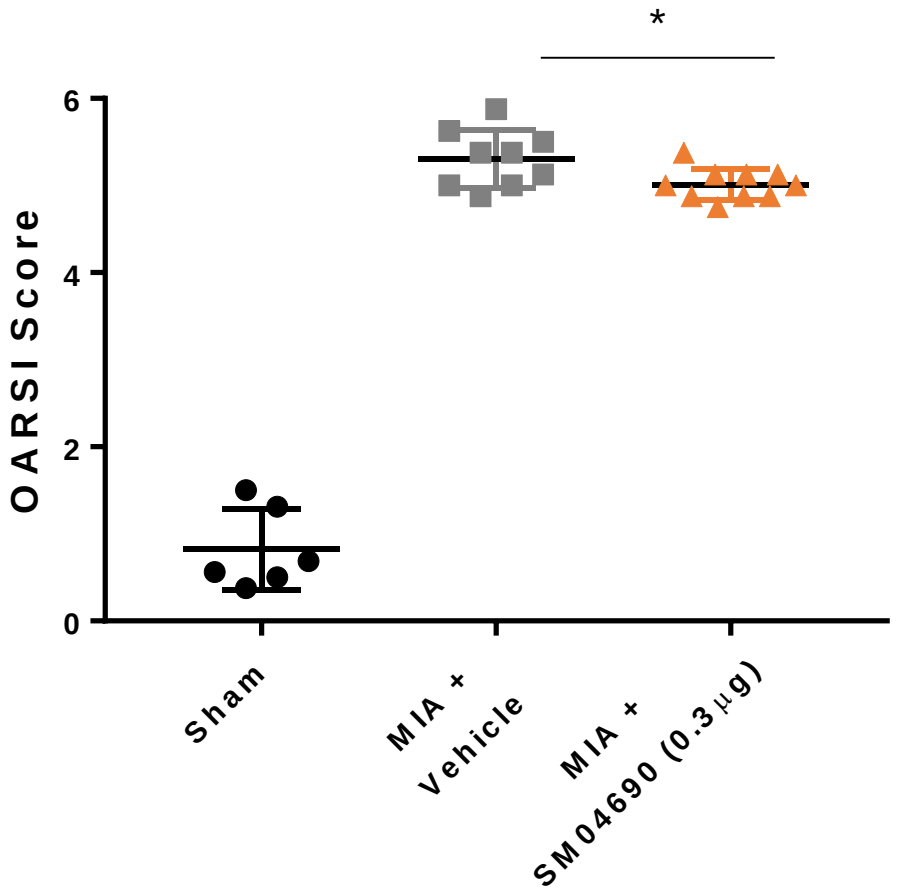


n=10, estimated treatment effect ± 95% CI, *p<0.05, **p<0.01, ***p<0.001, generalized estimating equation regression

SM04690 protected cartilage in the rat MIA knee OA model

- A single IA injection of SM04690 improved Safranin O staining and OARSI scores compared to vehicle at Day 28





n= 10, Mean ± SD, *p<0.05, Mann-Whitney U test

Conclusions

From *in vitro* models:

- SM04690 demonstrated potent anti-inflammatory effects across a broad range of cytokines
- These effects appeared to be mediated via NFκB

In the MIA rat knee OA model, SM04690, compared to vehicle:

- Attenuated inflammation and structural damage to the knee
- Improved pain in treated rats
- Protected cartilage from catabolic breakdown
- Limitations: inflammatory / degenerative responses exaggerated compared to man

- Further studies elucidating the role of SM04690 in inflammatory pathways are ongoing
- A human Phase 2b clinical trial is in progress

Thank you